Evasion of Innate Immunity by the Rhesus Cytomegalovirus (RhCMV) and development of a RhCMV based Vaccine Vector

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“An inefficient virus kills its host.

A clever virus stays with it.”

James Lovelock

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%: percent

°C: degree Celsius

AA: amino acid

AAV: adeno-associated virus

ABI: Applied Biosystems

Ad5: adenovirus vector based on the human adenovirus type 5 (HAdV-5).

AIDS: acquired immune deficiency syndrome

APOBEC3G: apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G

ATP: adenosine triphosphate

AtriCMV: owl monkey (*Aotus trivirgatus*) cytomegalovirus

ATRX: alpha thalassemia/mental retardation syndrome X-linked

BAC: bacterial artificial chromosome

BaCMV: babbon (*Papio*) cytomegalovirus

BAL: bronchoalveolar lavage

Bcl-AF1: Bcl-2-associated transcription factor 1

BHK-21: baby hamster kidney fibroblasts

bp: base pair

BTM: basal transcriptional machinery

CCMV: chimpanzee (*Pan troglodytes*) cytomegalovirus

CCR7: C-C chemokine receptor type 7

CD: cluster of differentiation

CD3: T-cell co-receptor

CD3ζ: T-cell receptor zeta chain

CD4: T-cell surface glycoprotein

CD8: T-cell surface glycoprotein

CD28: T-cell-specific surface glycoprotein

CD34: hematopoietic progenitor cell antigen

CD46: complement regulatory protein

CD49d: Integrin, alpha 4

CD55: complement decay-accelerating factor

CD56: Neural Cell Adhesion Molecule (NCAM)

CD59: MAC-inhibitory protein (MAC-IP)

CD69: early T-cell activation antigen P60

CD94: killer cell lectin-like receptor subfamily D, member 1 (KLRD1)

CD95: FAS receptor (FasR) or apoptosis antigen 1 (APO-1)

CD96: T cell activation, increased late expression (TACTILE)

CD112: herpesvirus entry mediator B (HVEM)

CD155: poliovirus receptor

CD226: DNAX Accessory Molecule-1 (DNAM-1)

cDNA: complementary DNA

CgueCMV: mantled guereza (*Colobus guereza)* cytomegalovirus

cm: centimeter

Cm: chloramphenicol

CMV: cytomegalovirus

CNPRC: California National Primate Research Center

COX-2: cyclooxygenase-2

CPE: cytopathic effect

CT: computed tomography

CTL: cytotoxic T lymphocyte

CyCMV: cynomolgus (*Macaca fascicularis)* cytomegalovirus

d: day

Da: dalton

DAI: Z-DNA binding protein 1

DAXX: death-associated protein 6

ddH_2O: double-distilled water

DMEM: Dulbecco's modified Eagle's medium

DNA: deoxyribonucleic acid

DOE: Department of Energy

dpi: days post infection

dsRNA: Double-stranded RNA

DTT: dithiothreitol

E. coli: *Escherichia coli*

E: early

EBV: Epstein–Barr virus

EC: endothelial cell

ECL: enhanced chemiluminescence

EDTA: ethylenediaminetetraacetic acid

EF1α: eukaryotic translation elongation factor 1 alpha

ELISA: enzyme-linked immunosorbent assay

EM: electron microscopy

emPAI: exponentially modified protein abundance index

ER: endoplasmic reticulum

ERGIC: ER-Golgi intermediate compartment

ExPASy: Expert **P**rotein **A**nalysis **Sy**stem

FBS: fetal bovine serum

Fc region: fragment crystallizable region

FCICA: flow cytometry intracellular cytokine assay

FLP recombinase: flippase recombinase

FTICR: Fourier transform ion cyclotron resonance

g: gram

gag: group-specific antigen

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

GgorCMV: gorilla *(Gorilla gorilla)* cytomagalovirus

H: histones

h: hour

H60: histocompatibility antigen 60

HCF: host-cell factor

HCMV: human (*homo sapiens*) cytomegalovirus

HDAC: histone deacetylase

HHV: human herpes virus

HIV: human immunodeficiency virus

HLA: human leukocyte antigen

HRP: horseradish peroxidase

HSV: herpes simplex virus

IACUC: Institutional Animal Care and Use Committee

ICP0: infected cell polypeptide 0

ICP34.5: infected cell polypeptide 34.5

IE: immediate-early

IE1 (IE72): immediate early protein 1 (72kDa)

IE2 (IE86): immediate early protein 2 (86kDa)

IFI16: gamma-interferon-inducible protein IFI-16

IFN: interferon

IFNAR: interferon α/β receptor

IFNβ: interferon β

IgA: immunoglobulin A

IgG: immunoglobulin G

IgM: immunoglobulin M

IKK-α: inhibitor of nuclear factor kappa-B kinase subunit alpha

IKK-β: inhibitor of nuclear factor kappa-B kinase subunit beta

IL6: interleukin 6

IL8: interleukin 8

IOM: Institute of Medicine

IRF3: interferon regulatory factor 3

IRF9: interferon regulatory factor 9

ISG: interferon stimulated gene

ISGF3: interferon stimulated gene factor 3

ISRE: interferon-stimulated response element

IκB: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor

J Clin Invest.: Journal of Clinical Investigation

J Virol.: Journal of Virology

JAK1: Janus kinase 1

Kan: kanamycin

Kan^{r}: kanamycin resistance

kDa: kilodalton

KIR: killer-cell immunoglobulin-like receptor

L: late

lb/in^2: pound-force per square inch

LB: lysogeny broth (a.k.a. Luria-Bertani medium)

LC-MS/MS: liquid chromatography-tandem mass spectrometry

LIR-1: leukocyte immunoglobulin-like receptor-1

LPS: lipopolysaccharides

Ly49: killer cell lectin-like receptor, subfamily A, member 2

Ly49H: killer cell lectin-like receptor, subfamily A, member 8

M: molar

MAC: membrane attack complexes

MCMV: murine (*Mus musculus*) cytomegalovirus

MDA-5: melanoma differentiation-associated protein 5

mg: milligram

MHC class I: major histocompatibility complex class I

MHC class II: major histocompatibility complex class II

MICA: MHC class I polypeptide-related sequence A

MICB: MHC class I polypeptide-related sequence B

MIEP: major immediate-early promoter

min: minute

miR: microRNA

ml: millilitre

mM: nanomolar

MOI: multiplicity of infection

mol%: molar percentage

MOPS: 3-(N-morpholino) propanesulfonic acid

MRI: magnetic resonance imaging

mRNA: messenger RNA

MS/MS: tandem mass spectrometry

MS-GF: mass spectrum-generating function)

MsphCMV: mandrill (*Mandrillus sphinx*) cytomegalovirus

MULT1: murine ULBP-Like Transcript 1

MVA: Modified Vaccinia Ankara

MVSC: Molecular Virology Support Core

NAS: National Academy of Sciences

NCBI: National Center for Biotechnology Information

NCLDV: nucleocytoplasmic large DNA viruses

NCRR: National Center for Research Resources

ND10: nuclear domain 10

NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells

NGS: next-genration sequencing

NHP: non-human primate

NIEP: noninfectious enveloped particle

NIGMS: National Institute of General Medical Sciences

NIH: National Institutes of Health

NK cell: natural killer cell

NKG2A: killer cell lectin-like receptor subfamily C, member 1 (a.k.a. CD159a)

NKG2D: killer cell lectin-like receptor subfamily K, member 1 (a.k.a. CD314)

NKp40: activating natural cytotoxicity receptor (NCR)

NKp46: natural cytotoxicity triggering receptor 1 (a.k.a. CD335)

nl/min: nanolitre per minute

NQ: not quantified

NWM: new world monkey

OD: optical density

OHSU: Oregon Health and Science University

ONPRC: Oregon National Primate Research Center

ORF: open reading frame

ORIP: Office of Research Infrastructure Programs

OSU: Oregon State University

OWM: old world monkey

PAGE: polyacrylamide gel electrophoresis

PAMP: pathogen-associated molecular pattern

PBMC: peripheral blood mononuclear cell

PBS: phosphate buffered saline

PCR: polymerase chain reaction

PFU: plaque-forming unit

pH: negative decadic logarithm of H_3O^+ concentration

PID: postinoculation day

PKR: protein kinase R

PML: promyelocytic leukaemia protein

PNNL: Pacific Northwest National Laboratory

pp65: 65kDa phosphoprotein

pp71: 71kDa phosphoprotein

ppm: part per million

PpygCMV: Bornean orangutan (*Pongo pygmaeus*) cytomegalovirus

PRR: pattern recognition receptor

RAE-1: ribonucleic acid export 1

Rb: retinoblastoma protein

RCA: regulators of complement activation

ref/tat/nef (retanef, RTN): SIV_{mac239} ref, tet and nef fusion protein

RhCMV: rhesus (*Macaca mulatta)* cytomegalovirus

RIG-I: retinoic acid-inducible gene 1

RM: rhesus macaques

rMVA: recombinant Modified Vaccinia Ankara

RNA: ribonucleic acid

RT-PCR: reverse transcription polymerase chain reaction

s.c.: subcutaneous

SCMV: simian (African green monkey, *Chlorocebus*), cytomegalovirus

SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

SHP2: Src homology region 2 domain-containing phosphatase 2

SIV: simian immunodeficiency virus

SIV_{MAC239}: highly pathogenic SIV strain mac239

SP100: nuclear dot-associated speckled 100 kDa protein

SPF: specific-pathogen free

SsciCMV: squirrel monkey (*Saimiri sciureus)* cytomegalovirus

STAT1: Signal transducer and activator of transcription 1, 91kDa

STAT2: Signal transducer and activator of transcription 2, 113kDa

STDEV (or SD): standard deviation

SUMO1: small ubiquitin-like modifier 1

TAP: transporter for antigen processing

Taq: DNA polymerase from *Thermus aquaticus*

TBST: Tris-buffered saline plus 0.2% Tween

T_{CM}: central memory T-cell

T_{EM}: effector memory T-cell

TF: transcription factor

TLR: toll like receptor

TNF: tumor necrosis factors

tRFs: telomerized rhesus fibroblasts

TRIM5α: tripartite interaction motif five, splice variant α

Tyk2: tyrosine kinase 2

U/ml: units per milliliter

UCD: University of California, Davis

U_L: unique long

ULBP1: UL16 binding protein 1

ULBP2: UL16 binding protein 2

ULBP3: UL16 binding protein 3

U_S: unique short

USA: United States of America

V: volt

vCOX-2: viral cyclooxygenase-2

vCXCL2: viral Chemokine (C-X-C motif) ligand 2

vCXCL3: viral Chemokine (C-X-C motif) ligand 3

vCXCL4: viral Chemokine (C-X-C motif) ligand 4

VGTI: Vaccine Gene Therapy Institute

vICA: Viral inhibitor of caspase-8-induced apoptosis (UL36)

VIHCE: viral inhibitor of heavy chain expression

VV: vaccinia virus

VZV: Varicella zoster virus

WB: Western blot

WSSV: white spot syndrome virus

WT: wildtype

µF: microfarad

µg/ml: microgram per millilitre

µl: microlitre

Kurzzusammenfassung

Das humane Zytomagalovirus (HCMV) ist weit verbreitet, und mehr als die Hälfte der Bevölkerung in den entwickelten Ländern und fast die gesamte Bevölkerung in den Entwicklungsländern ist mit dem Virus infiziert. Es ist somit eines der erfolgreichsten und am weitesten verbreiteten humanen Pathogene. Obwohl sehr viel Arbeit und Energie in die Erforschung des Viruses investiert wurden, sind sehr viele Fragen bezüglich seiner Biologie noch immer offen, welches teilweise durch sein für Viren riesiges Genom und damit einhergehende immense Kodierungskapazität und Komplexität erklärt werden kann. Ein großer Teil der vom Virus kodierten Proteine sind bisher noch nicht funktionell charakterisiert worden, allerdings haben vorhergehende Studien gezeigt, dass nur eine Minderheit aller CMV Proteine essentiell für die virale Replikation sind, wohingegen die überwältigende Mehrheit der viralen open reading frames (ORFs), besonders in den terminalen Regionen des Genoms, für Proteine kodieren, deren Aufgabe es ist, dem Wirtsimmunsystem entgegen zu wirken. Das Virus ist durch Millionen von Jahren von Ko-evolution so gut an den Wirt angepasst, dass das Überstehen der Infektion und die Beseitigung des Virus durch das Immunsystem dem Wirt keinen Schutz gegen Reinfektion verleihen. Wegen dieser langen Ko-evolution sind CMVs, isoliert von verschiedenen Spezies, außerdem ausgesprochen speziesspezifisch und HCMV kann keine Versuchstiere infizieren. Deshalb müssen die Zytomegalievieren der entsprechenden Versuchstiermodelle als Modell System für *in vivo* Studien verwendet werden. In dieser Studie präsentieren wir eine umfassende Charakterisiereng des Rhesus-Zytomegalovirus (RhCMV), welches das am nächsten verwandte, verwendbare Model zur Untersuchung von HCMV darstellt. Zur genaueren Beschreibung des publizierten RhCMV BAC (bacterial artifical chromosom) (Stamm 68-1), welcher die Grundlage für alle hergestellten RhCMV Mutanten darstellt, wurde dieser von uns unter Einsatz von next-generation sequencing (NGS) sequenziert und die kodierten ORFs mittels vergleichender Genomuntersuchungen neu bestimmt. Hierdurch waren wir in der Lage zu zeigen, dass dieses CMV deutlich größere Homologie zur humanen Variante hat als bisher angenommen, da sich die meisten ORFs ohne Homologie zu HCMV ORFs nicht bestätigen ließen. Darüber hinaus konnten wir nun, auf unseren vorherigen Studien zur Etablierung von RhCMV als viralem Vektor zur Entwicklung von T-Zell Vakzinen aufbauend, das neu annotierte RhCMV 68-1 Genom zur Konstruktion von mehreren Deletionsmutanten mit erhöhter *in vitro* und *in vivo* Attenuierung verwenden, um einen Impfvektor der zweiten Generation zu entwickeln. Das Ziel war, weiterhin

eine starke und andauernde Immunantwort, besonders gegen die eingefügten fremden Antigene, zu induzieren, aber zur gleichen Zeit eine reduzierte Pathogenität und Ausbreitung des Impfvektors zu erreichen. Unsere ersten Kandidaten waren die RhCMV Homologe des HCMV pp65 Proteins, pp65 a und b. Unglücklicher Weise führte eine Deletion dieser ORFs nicht zu einer erhöhten Sicherheit des Vektors. Ganz im Gegenteil, diese Mutante replizierte *in vivo* zu deutlich höheren viralen Genomkopien verglichen mit dem Wildtyp, die Deletionsmutante zeigte also eine höhere Virulenz verglichen mit dem parentalen Virus. Dieser Unterscheid lässt sich höchstwahrscheinlich mit der Abwesenheit von pp65 spezifischen $CD8^+$ T-Zellen nach Infektion mit der Mutante erklären. pp65 ist das Hauptantigen für T-Zellen, und die Mehrheit der CMV spezifischen T-Zellen erkennen Epitope dieses Proteins. In Abwesenheit von pp65 spezifischen T-Zellen in der frühen Phasen der Infektion ist es dem Immunsystems des Wirtes unmöglich die virale Replikation zu kontrollieren und die Deletionsmutants kann ungehemmt replizieren. Ein weiterer potentieller Kandidat für einen attenuierten RhCMV Vektor war ein anderes wichtiges Tegumentprotein, pp71, ein Protein, welches in der Evasion der intrinsischen Immunantwort eine Rolle spielt, indem es die in den PML-bodies enthaltenden Proteine Daxx, Bcl-AF1, Rb und ATRX degradiert. Diese Mutante zeigte eindeutig höhere Attenuierung verglichen mit der pp65 Deletionsmutante und unsere *in vivo* Resultate deuten an, dass, obwohl dieses Virus eine sehr starke T-Zell Antwort gegen die eingefügten SIV Antigene induziert, es nicht von den geimpften Affen im Urin ausgeschieden wird und dass es auch nicht von einem CMV-positiven auf einen naiven Rhesus Makaken mittels adoptivem Leukozyten Transfer übertragen werden kann. All diese Ergebnisse implizieren pp71 als vielversprechenden Kandidaten für die Entwicklung eines auf attenuiertem HCMV basierenden HIV-Impfstoffs für klinische Studien in der näheren Zukunft.

Schlagworte:
Zytomegalievirus, CMV, Rhesusmakaken, Impfstoff, Vektoren

Abstract

Human cytomegalovirus (HCMV) infects half of the population in the developed world and nearly every person of the developing world, making it one of the most successful and widespread human pathogens. Although a lot of research has been conducted to unravel the biological properties of this virus, there is still a lot of work to be done given the high complexity of the virus due to its huge genome size and coding capacity. While most virally encoded proteins have not been functionally characterized or properly mapped, it is nevertheless apparent that only a minority of all CMV proteins are essential for viral replication *in vitro*. However, most viral open readings frames (ORFs), especially in the terminal regions of the genome, encode for proteins involved in evading the host adaptive and innate immune systems. This results in a universal inability of the infected host to clear the virus despite ongoing immunological responses directed at CMV as well as no protective immunity against re-infection. These immune evasion mechanisms are the product of millions of year of virus-host coevolution that have additionally resulted in tight species specificity that thoroughly precludes infection of non-host species and complicates *in vivo* studies. Here we further characterize the rhesus cytomegalovirus (RhCMV) as the closest, usable *in vivo* model presently available for understanding HCMV. We resequenced the RhCMV strain 68-1 BAC and re-annotated the entire genome using comparative genomics. By doing so, we were able to show that this CMV shows substantially higher homology to the human version than previously assumed, because most ORFs without homologues in HCMV could not be confirmed by our system. Furthermore, given our previous work establishing RhCMV as a delivery vector for the development of T-cell vaccines, we now used our newly annotated genome of RhCMV 68-1 to construct several deletion mutants with increasing *in vitro* and *in vivo* attenuation with the goal to create a 2nd generation vaccine vector that retains high and long lasting immunogenicity, especially against the inserted foreign antigens, but at the same time shows reduced pathogenicity and spread. Our first deletion candidates, the RhCMV homologues of HCMV pp65, RhCMV pp65 a and b, did not achieve the goal of superior safety. On the contrary, the virus replicated *in vivo* to significantly higher viral copy number compared to the WT, describing the first observed case in RhCMV where a deletion mutant showed higher virulence than the parental virus. This increase is most likely connected to the absence of pp65 specific CD8+ T-cells in the deletion mutant. Pp65 is the major T cell antigen and the majority of CMV specific T-cells are directed against this very protein, underlying its importance for the generation of a strong anti-CMV immune response and

explaining why the virus is more virulent in the absence of pp65 specific T-cells early in infection. Our second deletion candidate was another important tegument protein, pp71, a protein involved in intrinsic immune evasion through degradation of the PML-body associated proteins DAXX, Bcl-AF1, Rb and ATRX. This virus showed substantially higher attenuation than the pp65 deletion mutant and our *in vivo* results indicate that although the virus induces a very strong T-cell response against the inserted SIV antigens, it is not shed in the urine of vaccinated monkeys and cannot be transferred to CMV naïve animals by adoptively transferring leukocytes from a vaccinated monkey. All these results imply pp71 as a promising potential candidate for the development of an attenuated HCMV based HIV-vaccine for clinical trials in the foreseeable future.

Key words:
Cytomegalovirus, CMV, Rhesus macaques, Vaccine, Vectors

Extended Summary

1. General Introduction

1.1 Herpesviruses

Herpesviruses form the family *herpesviridae* within the order *herpesvirales*, an order of large double stranded DNA viruses with a broad host spectrum ranging from molluscs (*malacoherpesviridae*) over fish and amphibians (*alloherpesviridae*) to birds, reptiles and mammals (*herpesviridae*), including humans [1, 2] (for a recent review see [3]). The origin of the family is not fully certain, but genetic similarities point to a relationship with the order *caudovirales*, a diverse order of double stranded DNA bacteriophages, indicating that the earliest ancestors of herpesviruses might have evolved more than 2 billion years ago [4, 5].

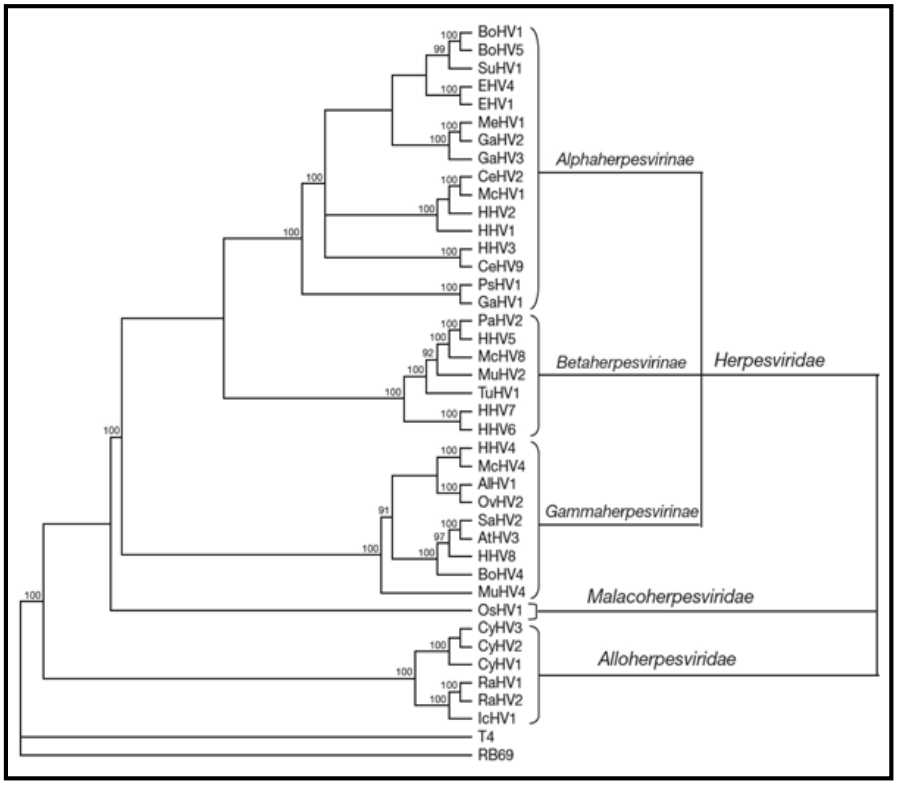

Fig.1: Cladogram depicting relationships among viruses in the order *Herpesvirales*, based on the conserved regions of the terminase gene. The Bayesian maximum-likelihood tree was rooted by using bacteriophages T4 and RB69. Numbers at each node represent the posterior probabilities (values >90 shown) of the Bayesian analysis. (Figure taken from Michel et al., Emerg Infect Dis 16(12), 1835-1843 (2010) [6]).

The *herpesviridae* can be further subdivided into 3 subfamilies, the *alpha-*, *beta-* and *gammaherpesvirinae*, which in turn are each made up of 4 genera. All in all, there are 8 known

human herpes viruses (HHV1 – HHV8) to date, members of which can be found in all subfamilies. These subfamilies show significant differences in cell tropism, latency and pathogenesis, whereas the genome organization, general virion composition and appearance and the core proteins are largely conserved throughout the entire family. With a genome size ranging from 124 – 230 kb in length [7], herpesviruses contain some of the largest viral genomes for eukaryotic viruses and are surpassed only by some members of the nucleocytoplasmic large DNA viruses (NCLDVs, e.g. *pox- or mimiviridae*) and white spot syndrome virus (WSSV) a member of the family *nimaviridae* [8]. The genome exists as a monopartite, linear, dsDNA, made up of repeated sequences in direct or inverted orientation separated by non-repeated (unique) segments. For most alpha- and beta-herpesviruses, the genome structure presents in the classical herpesviral form with two unique regions (U_L, unique long) and (U_S, unique short) divided by terminal and internal repeats, whereas the genome of gammaherpesviruses contains variable numbers of internal repeats [9].

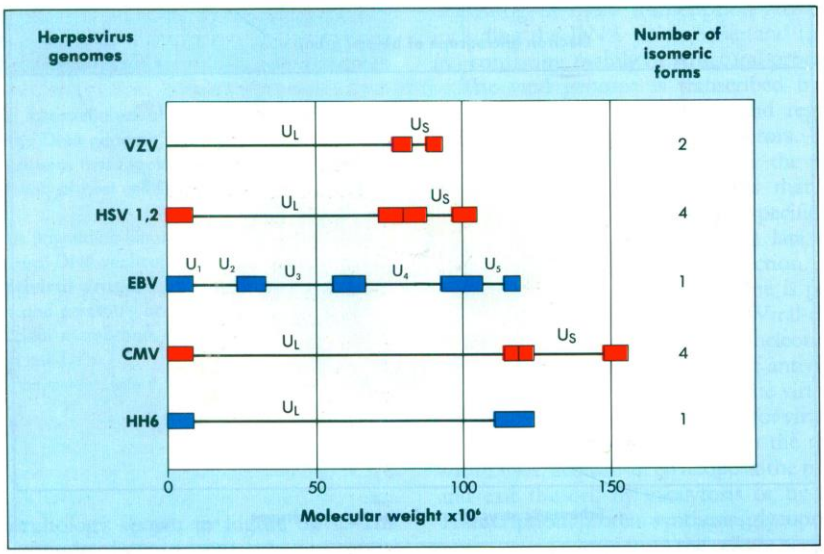

Fig.2: Herpesvirus genomes. Large genetic repeat sequences are boxed. Direct repeat DNA sequences are shown in blue, indirect repeat DNA sequences are shown in red; U_L (long unique region); U_S (short unique region). The genome of HSV and CMV have two sections, the unique long (U_L) and the unique short (U_S), each of which is bracketed by two sets of inverted repeats of DNA. The inverted repeats facilitate the replication of the genome but also allow the UL and the US regions to invert independently of each other to give four different genome configurations or isomers. VZV has only one set of inverted repeats and can form two isomers. EBV exists in only one configuration with several unique regions surrounded by direct repeats. (Figure taken from Prof. Dr. Gehan Aly El-Sherbeny, Part (3): Medical Virology, Chapter (10) Laboratory Diagnosis of Viral Disease, http://dc231.4shared.com/doc/1OJVQm4w/preview.html).

The orientation of the unique segments within the genome is variable, and during the viral genome replication, up to 4 different isomers can be produced. Depending on the virus these

different isomers can be produces in equal (e.g. HSV-1) (stoichiometric) or varying (e.g. VZV) ratios [10-15]. The members of this viral family form enveloped virions of spherical to pleomorphic appearance which are 150-200 nm in diameter. The envelope contains numerous glycoproteins necessary for budding and entry and surrounds a capsid with T=16 icosahedral symmetry consisting of 162 capsomers [16] and a layer of an amorphous mixture of proteins called the tegument separating the envelop and the capsid [17]. All in all, these virions are a highly intricate structure comprising potentially more than 60 different structural proteins [18].

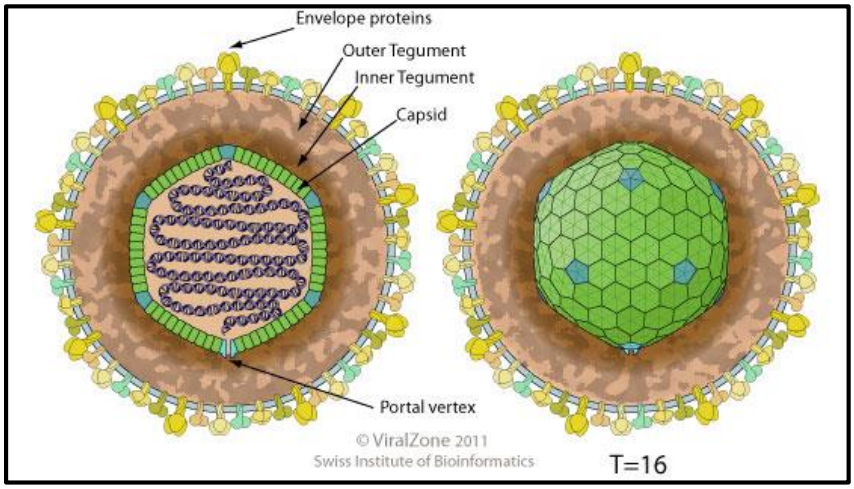

Fig.3: Structure of a herpesviral particle. (Figure taken from the from ExPASy's web-resource ViralZone, subsection Herpesviridae, http://viralzone.expasy.org/all_by_species/176.html).

Herpesviruses have co-evolved with their respective hosts for millions of years, maybe even since the evolution of their host species, so they show a high degree of species specificity and zoonotic infection are rare [19-21].

1.2 HHV5 (human cytomegalovirus, HCMV)

The prototypic member of the β-herpesvirus subfamily is HHV5, commonly known as the human cytomegalovirus (HCMV). This virus is highly widespread reaching seroprevalence levels of around 50% in the United States [22] and close to 100% in the developing world [23, 24]. Seroprevalence increases with age, and more that 90% of tested individuals aged 80 or older in the US are CMV positive [25].

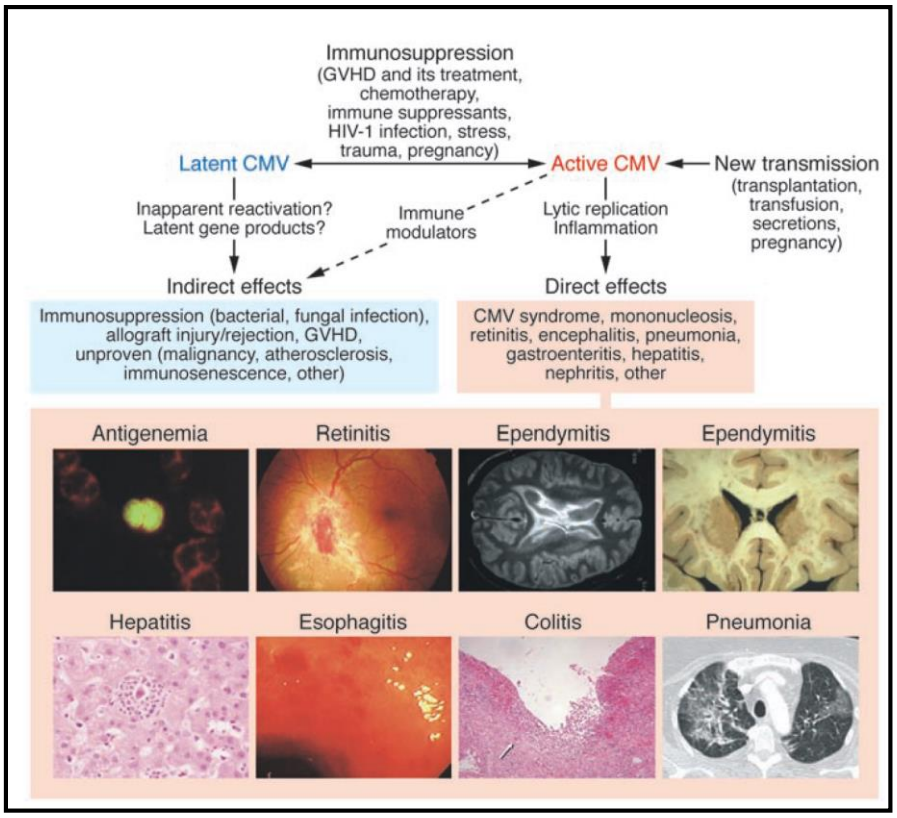

Fig.4: CMV disease mechanisms. In addition to CMV antigenemia, a common indicator of active infection, examples of end CMV organ disease commonly occurring in AIDS patients and in transplant recipients are shown. Image credits: antigenemia, pp65+ cell in a leukocyte cytospin preparation (M. Boeckh); retinitis, ophthalmoscopic view of retinal hemorrhage and inflammation (E. Chuang); ependymitis, periventricular inflammation detected by MRI (left; reproduced from Drew and Bates: Cytomegalovirus. In: Sexually Transmitted Diseases, Holmes K., Sparling P., Mardh P.A. et al. (Eds). McGraw-Hill Professional, New York, NY, 313-326 (1999) [26] with permission from McGraw Hill) and postmortem brain specimen (right; C. Marra); hepatitis, microabscesses associated with CMV hepatitis (A. Limaye); esophagitis, endoscopic view of shallow esophageal ulcers (G. McDonald); colitis, deep ulcer in a colonic biopsy (G. McDonald); pneumonia, chest CT scan of CMV pneumonia (M. Boeckh). (Figure taken from Boeckh and Geballe, J Clin Invest 121(5), 1673-1680 (2011) [27]).

While the primary infection is generally asymptomatic, some patients can present with mononucleosis-like clinical symptoms, including fatigue, fever and myalgia [28, 29]. The threat this virus poses is not to the fully immunocompetent, but to individuals with an immunocompromised immune system like AIDS patients and transplant recipients. Here the symptoms are significantly more severe and can include retinitis even as severe as to cause blindness, pneumonia, diarrhea, ulcers in the digestive tract, hepatitis, encephalitis, behavioral changes, seizures and coma [27]. Complications resulting from these symptoms can be fatal [30, 31]. Additionally, the virus poses a severe threat to transplant recipients, because primary CMV infection or CMV re-activation from either the patient or the transplant can lead to graft rejection [32]. Another group of immunocompromised individuals that are at high risk of suffering from

severe consequences from CMV infection are neonates. Here, the virus is the leading environmental cause of childhood hearing loss, the most common sequelae following congenital CMV infection [33-35], accounting for approximately 15%-21% of all hearing loss at birth in the United States [36, 37]. CMV-related hearing does not manifest immediately after birth, and children with potential congenital CMV infection have to be continually monitored for progressive or late-onset hearing defects. Additionally, congenital CMV infection is as common a cause of serious developmental disability as are Down syndrome and neural tube defects [38]. Given that Cytomegalovirus (CMV) is one of the most frequently transmitted intrauterine infections, detectable in an estimated 0.64%-0.70% of live births worldwide [39, 40], the number of families impacted by this virus is enormous and the cost put on the US health care system astronomical, estimated to be upwards of 2 billion dollars annually [41]. Given the hardship caused by the virus, it is not surprising that the Institute of Medicine (IOM) of the National Academy of Sciences (USA) has declared the development of a CMV vaccine the highest priority in 1999 [42], but so far none of the multiple studies performed resulted in the successful development of an effective vaccine candidate [43-56].

1.3 Cell Entry and Gene Expression

The viral lifecycle of the cytomegalovirus begins with the attachment to the target cell via the glycoproteins on the viral membrane. Multiple glycoproteins are involved in this step and the exact details are still elusive [57]. The same is true for the actual receptor the virus uses to enter the host cell. Multiple different proteins have been proposes as CMV receptors and the downregulation of many of these candidates indicated a role in virus entry as the amount of virus that was able to enter the cell was significantly diminished in these cells compared to control cells, but virus entry was not completely abrogated, identifying the proteins as potential co-receptors, but not as the main receptor for virus entry [58-63]. This situation is complicated by the fact that the virus seems to employ seperate strategies to enter different cell types. Whereas a complex formed by the glycoproteins gH/gL/gO together with gB is needed for entry into fibroblasts through attachment, receptor binding and subsequent membrane fusion [64-66], the mechanism by which the virus enters endothelial cells utilizes a pentameric complex made up of gH/gL/UL128/UL130/UL131A

to attach to the target cell and enter it by receptor mediated endocytosis and subsequent membrane fusion of the viral membrane with the endosome [60, 65, 67]. In either case, the virus enters the cell and the viral capsid and tegument are released into the cytoplasm [64]. Multiple early immune evasive functions are connected to the tegument proteins that are brought into the infected cell by the virus, so the release of the viral tegument represents an immediate early viral step to avoid detection by manipulating the host's intrinsic immunity [68]. After the capsid is released into the cytosol, it is further transported along microtubules to the nucleus, where the viral DNA is injected through the nuclear pore [69, 70], and viral gene expression can occur. In all herpesviruses, gene expression is a highly regulated process in which the viral genes are expressed in three subsequent cascades (Immediate Early (IE, α), Early (E, β) and Late (L, γ)). The immediate early genes are the first genes to be expressed. Their expression is amplified by tegument proteins brought into the cell within the virion, but not solely dependent on them [71-73]. The IE genes have multiple functions including evading the innate immune response (see below), arresting the cell cycle at a point favorable for the viral replication [74-77] and, last but not least, transactivating the expression of the next stage of viral gene expression, expression of the E genes [78-83]. The E genes comprise the majority of the viral genes expressed throughout the lytic viral replication. Generally, the definition for an E gene is a gene that is not expressed without prior IE gene expression, but is not dependent on viral DNA replication like L genes [84]. Functionally E genes are highly diverse, and almost every nonstructural function known for herpesviruses is carried out by one or even multiple genes expressed with early kinetics. Additionally, genes involved in viral DNA replication will also be expressed with early kinetics, and the hereby initiated viral genome replication will in turn induce L gene expression [85, 86]. This last phase of viral genes expression can be subdivided into an early (E-L) and a late (L) phase (also termed γ1 and γ2). Most of the structural genes are expressed with late kinetics, and the assembly of new capsids will start in the nucleus at this point. These newly synthesized capsids, termed A capsids will mature to B capsids by incorporating the assembly protein (UL80.5), through which the viral DNA will be channeled into the immature capsid [87-92]. The now fully mature C capsid is exported by the nuclear egress complex through the nuclear membrane into the cytosol, where tegument proteins have accumulated [93, 94]. The capsid is coated in an inner and an outer layer of different tegument protein and further matures through the endoplasmic reticulum (ER). All viral glycoprotein expressed during viral replication have been synthesized into the ER and further mature through

the Golgi until they are anchored into trans-Golgi cisternae [95] or early endosomes [96-98]. When the virus now enters the ER, the immature capsids are transported through the ER and Golgi and the mature virions are enveloped in the trans-Golgi or the early endosomes from where they will be released through exocytosis [96].

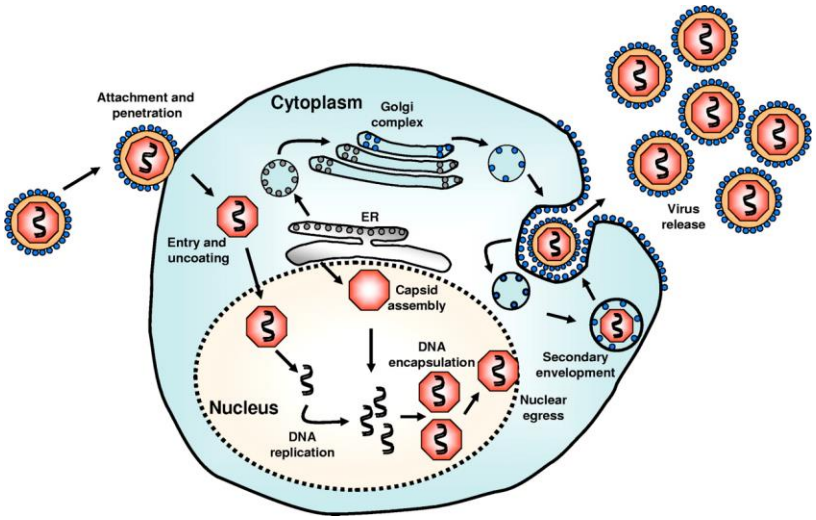

Fig.5: Life cycle of HCMV in a human cell. HCMV enters human cells either through direct fusion or through the endocytic pathway. The virus attaches to the cell via interactions between viral glycoproteins (e.g., gB and gH) and a specific surface receptor(s) (e.g., platelet-derived growth factor α), followed by the fusion of the envelope with the cellular membrane to release nucleocapsids into the cytoplasm. These nucleocapsids are translocated into the nucleus, where viral DNA is released. This initiates the expression of IE-1/IE-2 genes. Viral replication and maturation follow the stimulation and parallel accumulation of viral synthesis function. This process involves the encapsulation of replicated viral DNA as capsids, which are then transported from the nucleus to the cytoplasm. Secondary envelopment occurs in the cytoplasm at the endoplasmic reticulum (ER)-Golgi intermediate compartment. This is followed by a complex two-stage final envelopment and egress process that leads to virion release by exocytosis at the plasma membrane. (Figure taken from Crough and Khanna, Clin Microbiol Rev 22(1), 76-98, (2009) [99]).

1.4 CMV Latency

Besides this lytic replication in which the virus will eventually kill the host cell, herpesviruses can also enter a latent phase in which the viral genome will persist in the host. The pattern of viral gene expression will shift substantially from genes that are highly expressed in lytic infection to specific latency associated genes expressed during latency [100]. All Herpesviruses have the ability to persist in their respective host for the entire lifetime of the host after primary infection [101], but the appearance, the location and extend of latency varies greatly between the different subfamilies. Whereas gammaherpesviruses enter latency shortly after infection of B cells [102-104], alphaherpesviruses will enter latency as efficiently in neurons like the trigeminal ganglia in case

of HSV-1 and VZV [105-107]. The Cytomegalovirus, the prototypical betaherpesviruse on the other hand, is thought to have a very different form of latency in which it infects monocytes, white blood cells produced in the bone marrow, that serve as precursors to macrophages and dendritic cells [108-110]. CMV is capable of infecting these cells, but it is not able to fully replicate in monocytes and to produce virus progeny, so the viral genome is maintained as an episome in the infected cell and the virus remains latent. When these infected monocytes are activated and mature to macrophages, the cells become fully permissible to lytic viral replication and the virus reactivates and produces new infectious viral particles, so the infected monocytes serve as a reservoir for constant viral re-activation [111, 112]. Although a change in gene expression patterns has not been reported in this instance, CMV does express genes that will be preferentially expressed during latency as was shown for a clinical isolate of CMV infecting $CD34^+$ stem cells [113-116]. In this experiment, the expression pattern changed towards the expression of UL133–UL138, genes encoded in the viral ULb' region, a region not present in some fibroblast adapted strains of HCMV like AD169. This constant switch between lytic replication and latency is a characteristic and important feature for all herpesviruses, because it characterizes their biological niche as opportunistic pathogens.

1.5 Immune evasion by Cytomegalovirus

The development of an effective CMV vaccine proved to be more challenging than initially assumed due to the fact that even a pre-existing primary CMV infection does not confer protective immunity against re-infection, not against the same clade, the same strain or even the same isolate [117-120]. This interesting phenomenon severely complicates the creation of an effective vaccine, because if successfully fighting the infection does not generate protective immunity, how can an attenuated life virus or a subunit vaccine change this outcome? The reason for not establishing a sufficient immune response to avoid reinfection is even more curios given that the virus will indeed induce a very strong B- and T-cell response [121, 122], but although up to 10% of all T-cells in an individual can be directed against various CMV epitopes [121], it still does not prevent re-infection or re-activation. This can lead to individuals being infected by multiple strains of CMV either simultaneously or seqeuentially, something that has been observed not only in humans [117-120], but also in wild mice infected with multiple strains of MCMV [123]. After decades of research the

explanation for the efficient evasion of the host immune system by the virus was found in dozens of viral genes expressed during the viral life cycle, directed against every single aspect of the immune response to insure viral survival and persistence [124]. Although all herpesviruses encode for immune evasion genes, many of these genes are encoded in the terminal regions of the viral genome. Whereas the genes encoded in the central part of the genome in a given herpesvirus show fairly well conservation not only between members of the same subfamily but to a certain degree even between all herpesviruses [125] this does not hold true for viral proteins encoded in the terminal regions of the genome. These genes are mostly virus specific and are not conserved even throughout members of the same subfamily. As a result of this, every single member of the herpesvirus family encodes for its own set of viral immune evasion genes.

1.5.1 Evasion of the innate immune response

One of the earliest steps for the host to fight entering viruses is the innate immunity, a collection of broad, pathogen unspecific defense mechanism. These defense mechanisms are triggered by the pathogen either by interacting with a pattern recognition receptor (PRR) on the cell surface, like certain toll like receptors (TLRs), or by triggering a response by engaging an intracellular toll like receptors receptor or other intracellular receptors like MDA-5 and RIG-I [126]. All these receptors will recognize pathogen-associated molecular patterns (PAMPs) including DNA, RNA, Glycoproteins or LPS, so that the entire range of potential pathogen trying to infiltrate the cell will be detected by the cellular immunity [126]. Triggering a PRR will activate different, very intricate signaling cascades resulting in the activation of several pathways including the NF-κB-pathway [127], the IRF3- pathway [128] and eventually the JAK/STAT-pathway [128], all leading to the specific expression of cellular genes with anti-viral, anti-microbial or pro-inflammatory activity. Furthermore, some of the proteins synthesized by the cell in response to the detection of an infecting pathogen will be secreted and can signal in either an autocrine- or paracrine fashion to alert the neighboring cells and to attract different cells of the immune system [129].

This unspecific arm of the immune system is essential for the host in controlling viral infections and pathogenesis. As can be shown in several *in vivo* models and in humans with certain mutations of the IFN receptors or associated signaling molecules, lack of an effective interferon

response will lead to a higher susceptibility, and to more severe viral infections shown for various different herpes viruses [130-133]. On the other hand, pretreatment of cells with interferon will put the cells into an antiviral state, impairing the ability of the virus to replicate to high titers [134, 135]. As a result of this finding, recombinant IFN α has been used therapeutically to successfully control HCMV-induced retinitis during AIDS [136] and to control viremia following congenital infections [137].

To activate the NF-κB-pathway, triggering a PRR will lead to the phosphorylation of IKK-α and IKK-β, two serine/threonine protein kinase whose activation will in turn lead to the phosphorylation and subsequent ubiquitination of the IκB protein complex [138]. IκB binds to both NF-κB subunits and inhibits their nuclear localization [139]. Ubiquitination of IκB will result in its proteosomal degradation [138], allowing NF-κB to translocate into the nucleus and to induce the transcription of important chemokines and cytokines like IL6, IL8 and IFNβ, all involved in activating and alerting other cell of the innate and adaptive immune system [140-144]. This protein expression is inhibited in the presence of HCMV, and further research revealed the immediate early protein 2 (IE2, IE86) was needed and sufficient for this blockage [145, 146], so HCMV developed a mechanism to efficiently undermine signaling through this important host defense pathway.

NF-κB signaling can not only be inhibiting at its last step by inhibiting the synthesis of newly synthesized antiviral proteins, but also by interfering with cellular PRRs siganlling through the NF-κB-pathway. Protein Kinase R is an intracellular receptor activated by dsRNA which will be synthesized during transcription of complementary strands of the CMV genome. HCMV not only encodes for one, but two proteins (TRS1 and IRS1) with the capability to bind dsRNA. By hiding the dsRNA from the cellular receptor, the virus can ensure viral gene expression without triggering the PRR and alarming the innate immune response. TRS1 and IRS1 can substitute for the vaccinia virus (VV) RNA binding protein [147, 148], however, the role of these proteins in the context of HMCV infection has not been evaluated [149].

Similar to the NF-κB-pathway, signaling through the IRF3-pathway also needs prior triggering of a PRR by the entering pathogen. The resulting phosphorylation cascade will lead to the phosphorylation of IRF3, which can now homodimerize and relocate to the nucleus where the dimer binds to its recognition site on the DNA, inducing the expression of a whole range of different proteins, including many with shown antiviral activity and the important cytokine IFNβ

[150]. Although reports exists describing HCMV pp65 as a potential inhibitor of IRF3 signaling [151, 152], these results were called into question, because the mutant virus used in both studies was a full deletion of the entire UL83 open reading frame effecting the expression of the upstream UL82 (pp71) ORF and resulting in an attenuated virus with significantly delayed gene expression [153]. A second pp65 deletion mutant with introduced stop codons instead of a full deletion did not inhibit IFNβ expression [153]. RhCMV on the other hand has been shown to effectively inhibit IRF3 phosphorylation, dimerization and nuclear localization completely abrogating any IRF3 dependent signaling [148]. The viral protein for this phenotype has not been identified, but interestingly RhCMV is only capable to inhibit IRF3 signaling in rhesus fibroblasts, whereas human fibroblast infected with RhCMV show no inhibition of IRF3 nuclear localization or IRF3 dependent gene expression (Malouli et al., unpublished data).

After the infected cell synthesizes IFNβ, either through the NF-κB- or the IRF3-pathway, this cytokine will be released from the cell where it can now signal either in a paracrine (to a different cell) or in an autocrine (to itself) fashion [144]. The interferon will bind the interferon-α/β receptor (IFNAR), a cell surface protein that will bind all type I interferons and which is associated with the JAK1 and Tyk2 tyrosine kinases [144]. Upon ligand receptor binding, these two kinases autophosphorylate and phosphorylate the IFNAR, which will lead to the phosphorylation of two different Signal Transducer and Activator of Transcription (STAT) proteins, STAT1 and STAT2. Upon phosphorylation these two proteins dimerize and complex with IRF9 to form the ISGF3 (Interferon Stimulated Gene Factor 3) complex [144]. This protein complex relocates to the nucleus where it can bind to multiple ISRE (Interferon-Stimulated Response Element) promoters to induce the expression of interferon stimulated genes (ISGs) [144]. Like the genes expressed upon IRF3 signaling, many of these ISGs have proven antiviral activity [154], and successful JAK-STAT signaling transfers the cell into an anti-viral state [155]. This underlines the importance for CMV to inhibit JAK-STAT signalling, because if the virus was not inhibiting this signaling cascade, the infected cell would alarm all neighboring cells through the release of type I interferon rendering them less infectious and prepared for a potential infection. The human CMV immediate early protein 1 (IE1, IE72) can inhibit the binding of ISGF3 to the ISRE and by that block signaling through the JAK/STAT pathway [156]. The region of IE1 responsible for this inhibition has been mapped, although different studies disagree about the exact

location of the active region [157, 158] and this function of IE1 is also conserved in RhCMV (Malouli et al, unpublished data).

Besides blocking of ISG synthesis by inhibiting the binding of the ISGF3 to the ISRE, CMV has developed multiple independent mechanisms to undermine signaling through the JAK/STAT pathway. Impaired Interferon signaling in cells infected with cytomegalovirus has been described independently by multiple groups [156, 159, 160], but different phenotypes explaining the compromised interferon response were found. Degradation [160-162] as well as absence of phosphorylation [160] has been described for many kinases and signaling molecules in the pathway, including JAK1, IRF9, STAT1 and STAT2. Interestingly, proteasome-dependent STAT2 degradation late in HCMV infection has been described for multiple different viral strains except HCMV Towne [161], but an explanation for this interstrain difference has not yet been found. Because the various signaling molecules and kinases are part of multiple independent JAK/STAT signaling pathways, degradation of single signaling molecules can effect stimulation by diffident cytokines. Type II interferon (IFNγ) induced JAK/STAT signaling through the IFNGR is also blocked by HCMV through the inhibition of phosphorylation and proteasomal degradation of multiple important players in the signaling pathway [162]. The viral proteins responsible for all the described phenotypes have not been identified so far.

Finally, there are two more described mechanisms utilized by the virus to derail the innate immune response. One involves the feedback loop that the JAK-STAT pathway has evolved in response to newly synthesized interferon that can signal in an autocrine fashion to restimulate the pathway. The pathway is negatively regulated by various cellular proteins [163], among them protein tyrosine phosphatases that will dephosphorylate proteins in the pathway, effectively shutting down the signaling. Lack of phosphorylation has been shown after HCMV infection and IFNγ stimulation [164] and the cellular tyrosine phosphatase SHP2 (Src homology region 2 domain-containing phosphatase 2) has been shown to be involved in this phenomenon [164, 165]. What this implies is that HCMV evolves the capability to selectively activate the cellular tyrosine phosphatases SHP2 to manipulate IFN-induced phosphorylation of JAK1 and by that to diminish or shut down all JAK/STAT signaling pathways involving this kinase.

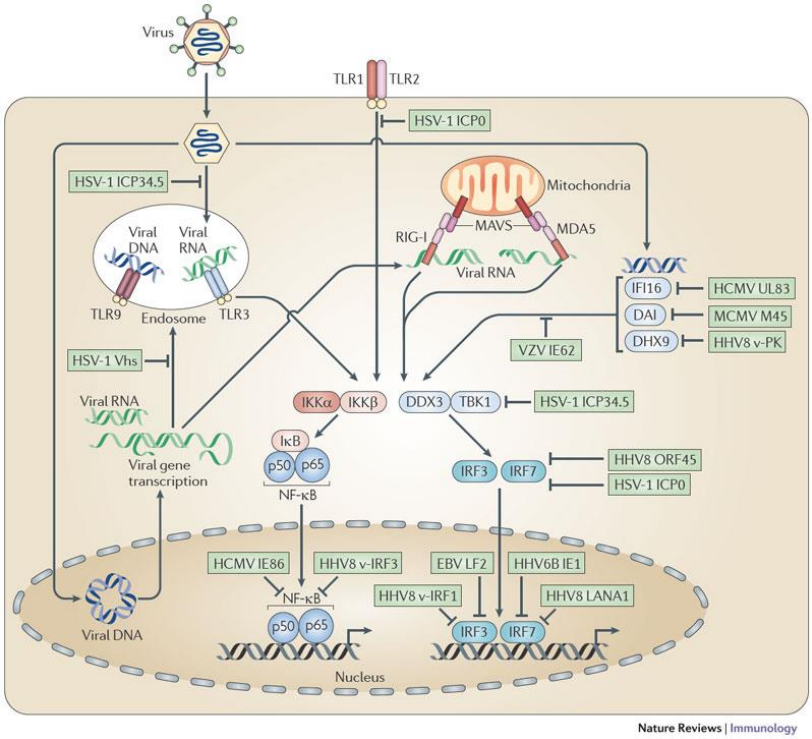

Fig.6: Herpesviruses encode proteins that help them to evade detection by pattern recognition receptors (PRRs). For example, the herpes simplex virus type 1 (HSV-1) proteins ICP34.5 and virion host shut-off protein (Vhs) prevent the recognition of viral nucleic acids by inhibiting autophagy and degrading viral RNA, respectively. Herpesviruses also inhibit signalling through PRRs using multiple mechanisms. Some are specific to individual PRRs; for example: HSV-1 ICP0 protein inhibits Toll-like receptor 2 (TLR2) signalling by stimulating the degradation of TLR adaptor molecules; and murine cytomegalovirus (MCMV) M45 protein inhibits the recruitment of receptor-interacting protein 1 (RIP1) to DNA-dependent activator of IFN-regulatory factors (DAI). There are also more general mechanisms that target all PRRs; for example, human herpesvirus 8 (HHV8) ORF45 protein interacts with IFN regulatory factor 7 (IRF7) and inhibits its phosphorylation and nuclear translocation. Finally, several herpesvirus-encoded proteins (such as HHV8 v-IRF3) inhibit transcription by interacting with nuclear factor-κB (NF-κB) and IRF3 and/or IRF7 in the nucleus. This prevents the interaction of these transcription factors with DNA, and the assembly of functional transcriptional complexes. (Figure taken from Paludan et al., Nature reviews. Immunology 11(2), 143-154 (2011) [166]).

1.5.2 Evasion of the intrinsic immune response

The very first line of defense against any intracellular infection is always the intrinsic immunity. This immunity differs from the innate immunity, in that the proteins of the intrinsic immunity are constantly expressed and ready within a cell [167], whereas the expression of anti-viral interferon stimulated genes (ISGs) for instance, has to be induced after a given pathogen triggers a pattern recognition receptors (PRRs) like a toll like receptor [168, 169]. The best described members of

the intrinsic immunity have been found in connection with anti-HIV research, where cellular proteins like TRIM5α (Tripartite interaction motif five, splice variant α), a cellular protein capable of preventing retroviral uncoating, and APOBEC3G (Apolipoprotein editing complex 3 G), a cytidine deaminase that will randomly introduce cytidine to uracil nucleotide changes into the viral genome during retroviral reverse transcription leaving the resulting provirus inviable due to the multitude on nonsense mutations [170, 171], have been found to possess substantial antiviral activity.

In connection with herpesviruses, the part of the intrinsic immunity that has been shown to effectively inhibit viral propagation and spread is a protein complex that forms small dot like structure in the cellular nucleus, and that is known as ND10- or PML bodies [172, 173]. This protein complex consists permanently or transiently of more than 50 different cellular proteins [174], and many of them are involved in such diverse cellular functions as the DNA damage response, chromatin modification, the stress response, senescence, and protein stability, and there is growing scientific evidence linking ND10 function and protein modification with small ubiquitin-like modifier (SUMO) family members [173, 175, 176]. After intensive research, some of these proteins have been shown to directly interact with intruding viruses, including the promyelocytic leukaemia protein (PML), the small ubiquitin-like modifier 1 (SUMO1), the SP100 nuclear antigen, the Death-associated protein 6 (DAXX) and ATRX, the protein responsible for alpha-thalassemia X-linked mental retardation when mutated [177]. The assembly of ND10-bodies is coordinated by sumoylated PML, and by SUMO interaction motif (SIM)-dependent interactions between PML and other sumoylated proteins [178], which in turn will lead to the recruitment of other important protein complex constituents like DAXX and ATRX [179-183]. These two proteins will locate to histones, where they are involved in chromatin modification functions. This is also the site were herpesvirus genomes are located immediately after infection and where virus transcription and DNA replication are initiated [184-190]. Experimental RNA knockdown of PML or DAXX or a combination of both proteins lead to a significantly increased HCMV of HSV-1 replication [191-194], indicating why it is advantageous for CMV to disrupt ND10 bodies during infection [195-199]. The virus employs multiple strategies to ensure dispersal and degradation of the host proteins with intrinsic antiviral activity. PML and Sp100 will be dispersed and degraded by the immediate early protein 1 (IE1) [198-203], which will locate to PML bodies shortly after infection, whereas the immediate early protein 2 (IE2) will co-localize with CMV genomes

adjacent to ND10-bodies [204]. Additionally, the tegument protein pp71 interacts with Daxx, which leads to its dispersal and partial degradation [205-208]. Other ND10 body components directly affected by pp71 include ATRX [192], BclAF1 [209] and Rb protein family members [210]. Due to this importance of pp71 in degrading or delocalizing multiple proteins involved in anti-viral intrinsic immunity, it is not surprising that viral propagation and spread are substantially reduced in human fibroblasts after low MOI infection with an HCMV pp71 deletion mutant, given that the mutant virus is no longer able to ensure efficient viral DNA replication [211].

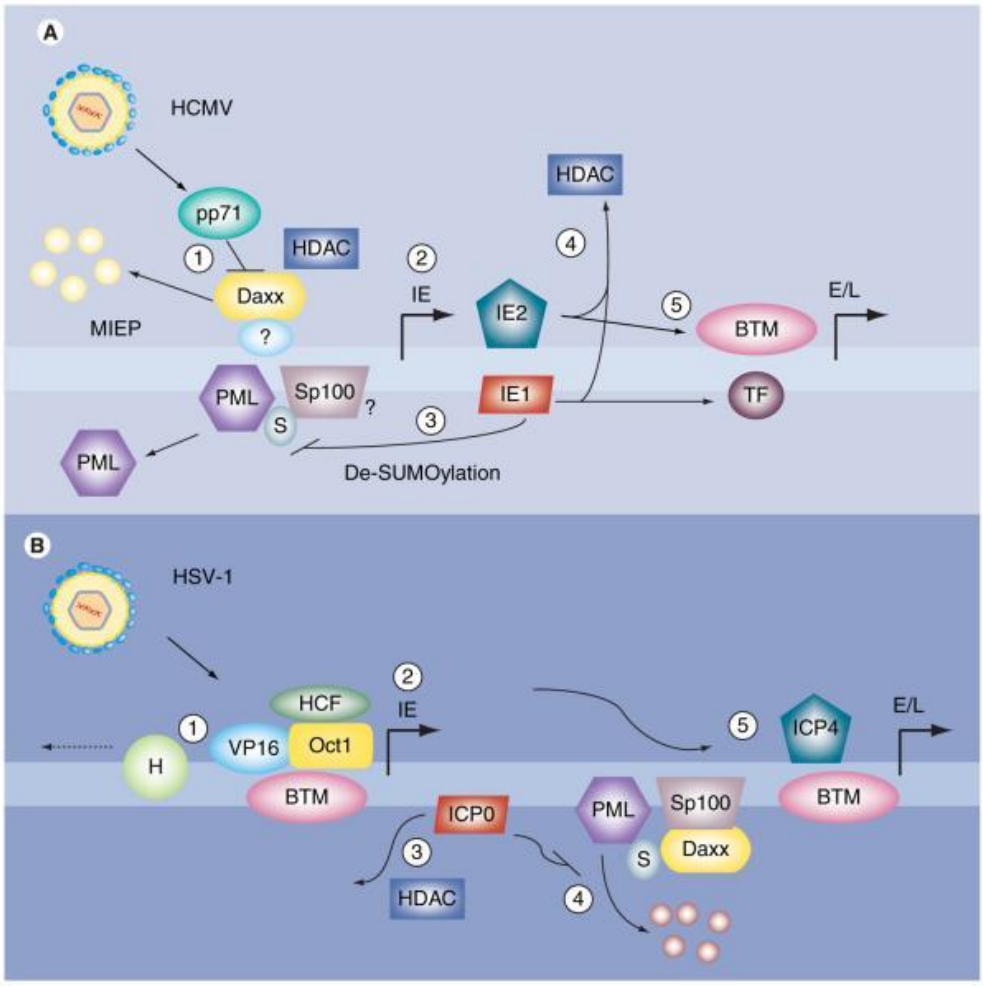

Fig.7: Activation of HCMV and HSV-1 viral gene expression. (A) Upon HCMV fusion and content delivery to the infected cell, the tegument protein pp71 binds to and induces the degradation of Daxx (1). This de-represses the viral MIEP and promotes the expression of the IE genes (2). IE1 disrupts the remaining PML-NB proteins by preventing/disrupting the SUMOylation status of PML, and possibly Sp100, further increasing IE gene expression (3). Both IE1 and IE2 negate the effect of HDACs by binding to and sequestering them away from viral promoters (4). Finally, the IE proteins can recruit BTM and TFs to early and late viral promoters to activate their respective genes (5). (B) Upon HSV-1 fusion and content delivery to the infected cells, the tegument protein VP16 binds to the cellular Oct1 and HCF proteins and targets to the viral IE promoter, where it displaces cellular H (1), and activates viral gene

expression by recruiting the BTM (2). ICP0 has multiple functions to activate subsequent viral gene expression, including dissociating HDAC complexes (3) and inducing the degradation of PML and Sp100 (4). ICP4 promotes the expression of early and late viral genes by recruiting BTM to targeted promoters (5). BTM: Basal transcriptional machinery; E/L: Early/late; H: Histones; HCF: Host-cell factor; HCMV: Human cytomegalovirus; HDAC: Histone deacetylase; HSV: Herpes simplex virus; IE: Immediate-early; MIEP: Major immediate-early promoter; PML: Promyelocytic leukemia; PML-NB: PML-nuclear body; TF: Transcription factor. (Figure taken from Saffert and Kalejta, Future Virol. 1;3(3):265-277 (2008) [212]).

1.5.3 CMV evasion of natural killer (NK) cells

One very important part of the innate immune response are natural killer (NK) cells, which can be classified as a heterologous group of $CD3^-$ and $CD56^+$ cells and which represent about 15% of peripheral blood lymphocytes [213, 214]. As members of the innate immune response their activity is generally unspecific, but studies in mice indicated that an NK cell subset with immunological memory might exist [215, 216]. NK cells area activated in response to type I interferons and induce the lysis of malignant or virally infected cells, thereby containing the viral replication early in infection. Although they act at a very early stage of infection before B- or T-cells can mount an immune response, NK cells play a pivotal role in bridging the innate and adaptive immune response by regulating the development of the adaptive immunity [217, 218] through the secretion of cytokines and chemokines [219]. Unlike members of the adaptive immune response, NK cells do not detect pathogenic antigens through specific receptors but express a complex network of activation and inhibitory receptors that interact with different cellular molecules expressed on the target cell [220]. The overall combined stimulus transmitted through this signaling network determines the activation status of the NK cell and the fate of the target cell, and many pathogens have developed mechanisms to alter the signaling to NK cells in their favor to ensure their replication and survival. HCMV is a prime example of this as it encodes for multiple genes interfering with NK signaling and activation [221, 222].

The need for viruses to interfere with NK cell activity becomes apparent when case studies of humans with NK cell deficiencies are considered [223, 224]. These patients suffer from numerous viral infections including multiple herpes viruses and similar effects were seen in patients suffering from immunodeficiencies with impaired NK cell numbers and function, where infections with HSV, VZV and CMV among others where frequent [225-227].

The first set of activation receptors expressed on NK cells is a family of protein comprising

three members, NKp46, NKp40 and NKp30 [228]. Their cellular ligand is unknown, although several viral and tumor associated interaction partners have been identified [229]. One of them is the HCMV tegument protein pp65, which can interact with NKp30, leading to the dissociation of the linked CD3ζ from NKp30 and subsequently the inhibition of NK cell mediated killing [230].

A second important set of receptors are the killer immunoglobulin-like receptors (KIRs), the human homologues to the mouse Ly49 C-type lectin-like receptors. KIRs interact with specific allotypes of the classical (HLA-A, HLA-B and HLA-C) and nonclassical (HLA-G) human leukocyte antigen class I receptors and interaction of the target cell MHC class I with the NK-cell KIR will deliver either an activating or inhibitory signal depending on the KIR involved [231]. HCMV encodes for multiple proteins with structural homology to MHC-class I that can bind to inhibitory KIRs and prohibit NK cell activation. Furthermore, the HCMV protein UL18 can serve as a ligand for the leukocyte immunoglobulin-like receptor 1 (LIR-1), another related inhibitory NK cell receptor, which will also prevent NK cell activation [232, 233].

One last, very well described group, of NK cell receptors is a family of C-type lectins abundantly expressed on the cell surface of many lymphocytes. Members of this receptor family can deliver activating or inhibitory signals. For instance NKG2A binds to the non-classical HLA-E molecule loaded with peptides derived from other HLA class I molecules. By doing so the NK cell is controlling for "missing self", for the downregulation of MHC class I molecules during viral infection. If the level of MHC class I signal peptides presented on HLA-E goes below a certain threshold, then the inhibitory signal to the NK cell will not be strong enough anymore to prevent NK cell activation and the activated NK cell will lyse the target cell. HCMV has developed a very interesting mechanism to counter this immune defense. The virus encodes for a proteins (UL40) that contains a signal sequence with high homology to the HLA-A signal sequence, so that the UL40 signal sequence can be loaded onto HLA-E molecules instead of the HLA-A signal sequence, tricking the NKG2A receptor into believing that the cell is still expressing the classical MHC class I molecules at normal levels on the cell surface [234]. Even the viral proteins involved in CD8+ T-cell evasion do not interfere with the transport of the UL40 signal peptide loaded HLA-E protein complex to the cell surface, so that even in the midst of a full blown lytic infection, the outer appearance of the cell to the inhibitory NKG2A receptor is still normal.

Another member of the same NK cell receptor family is the highly expressed NKG2D protein, which forms a heterodimer with CD94 [235]. The ligands for the human version of this

receptor are a group of cellular proteins including MICA, MICB and the UL16 binding proteins ULBP1-6, all of which are upregulated during the cellular stress response in malignant cells or after viral infection [221, 222]. HCMV encodes for several proteins that will interfere with the expression of the NKG2D ligands of the cell surface of infected cells. UL16 can bind to MICB, ULBP1 and ULBP2, leading to the intracellular retention of these proteins [236, 237]. MICA is also retained in the same compartment, albeit by a different viral protein, UL142, a protein that was initially missed because it was lost during passage in HCMV laboratory strains like AD169 [222, 238]. In addition to viral proteins, HCMV also encodes for a viral microRNA termed miR-UL112 that can downregulate the expression of MICB leading to decreased binding of NKG2D and reduced killing by NK cells [239, 240].

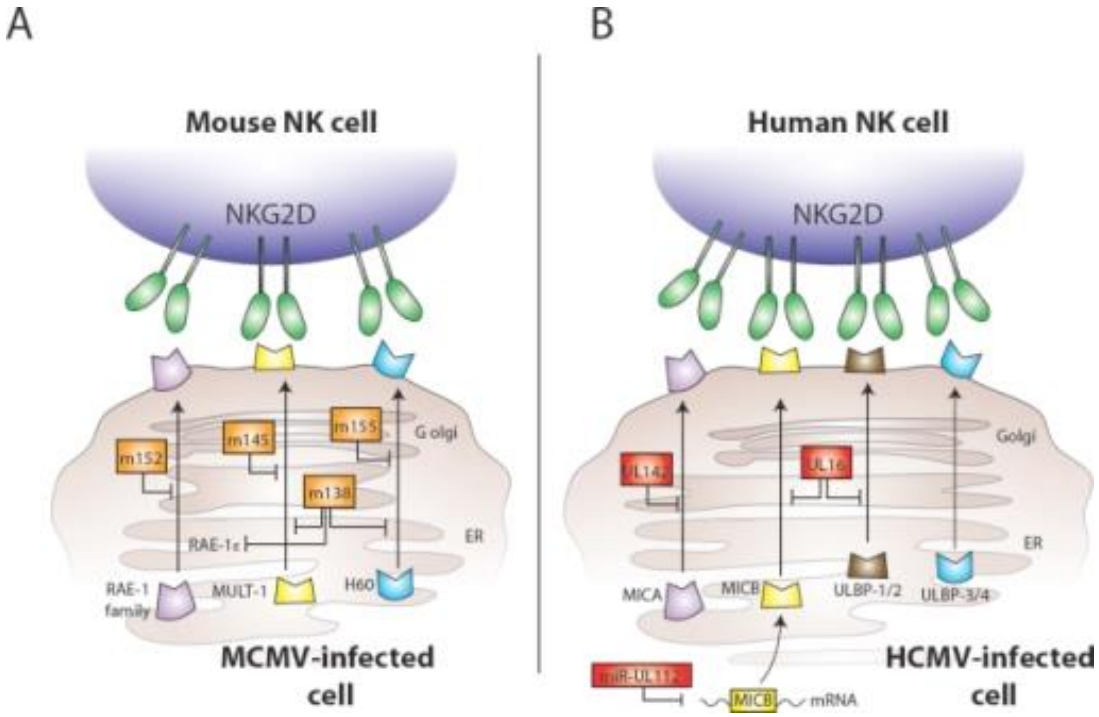

Fig.8: MCMV and HCMV proteins interfere with expression of NKG2D ligands and host recognition of virally infected cells. (A) MCMV-encoded glycoproteins (shown in orange) inhibit the expression of mouse NKG2D ligands: m152 interferes with expression of all five members of the RAE-1 family, m145 prevents surface expression of MULT1, m155 causes degradation of H60, and m138 assists to block expression of RAE-1ε, MULT1, and H60. (B) HCMV-encoded components (shown in red) also inhibit the expression of NKG2D ligands: UL142 inhibits MICA expression, UL16 binds MICB, ULBP1, and ULBP2 in the Golgi, and the miR-UL112 microRNA targets MICB mRNA for degradation leading to diminished cell surface expression of MICB. (Figure taken from Sun and Lanier, Viruses 1(3), 362 (2009) [222]).

Viral NK cell evasion is a field of research that has gotten a lot of attention in the

past due to its impact on pathogenesis and vaccine development and reviews summarizing the vast amount of research data surrounding this topic have been published over the years [241].

1.5.4 Evasion of the complement system

Another integral part of the innate immunity is the complement system [242], a network of plasma proteins involved in direct lysis of infected cells and in amplifying the inflammatory response effectively bridging the innate and the adaptive immune response [243-247]. The complement system can be activated through different pathways (see Fig.9) either by binding antibody antigen complexes or by directly binding to the surface of pathogens or infected cells. In either case, this activation will ultimately lead to the formation of membrane attack complexes (MACs), which will integrate into the lipid cellular membranes and disrupt the cell by increasing the osmotic pressure [248]. Besides the formation of the MACs, activation of the complement will also lead to the expression of several anaphylatoxins (C3a, C4a and C5a), which are involved in the recruitment of antibodies, further complement proteins and leukocytes to the site of infection, and even direct interaction of complement proteins with viral virions has been reported and has a neutralizing effects [249-253].

Multiple viral families have evolved means to evade the complement system, and HCMV-infected cells have been shown to resist complement mediated cell lysis shortly after infection suggesting a viral mechanism to evade this host defense mechanism [254, 255]. One way the virus is able to achieve this, is by inhibiting complement activation through the classical pathway by avoiding complement binding to antibody–antigen complexes through the expression of viral Fc receptors on the surfaces of virally infected cells. As will be described later (see evasion of the humoral immune response), HCMV encodes for at least four functional viral Fc receptors which not only impair the humoral response, but also the activation of the complement system and so ultimately complement mediated cell lysis [256-258].

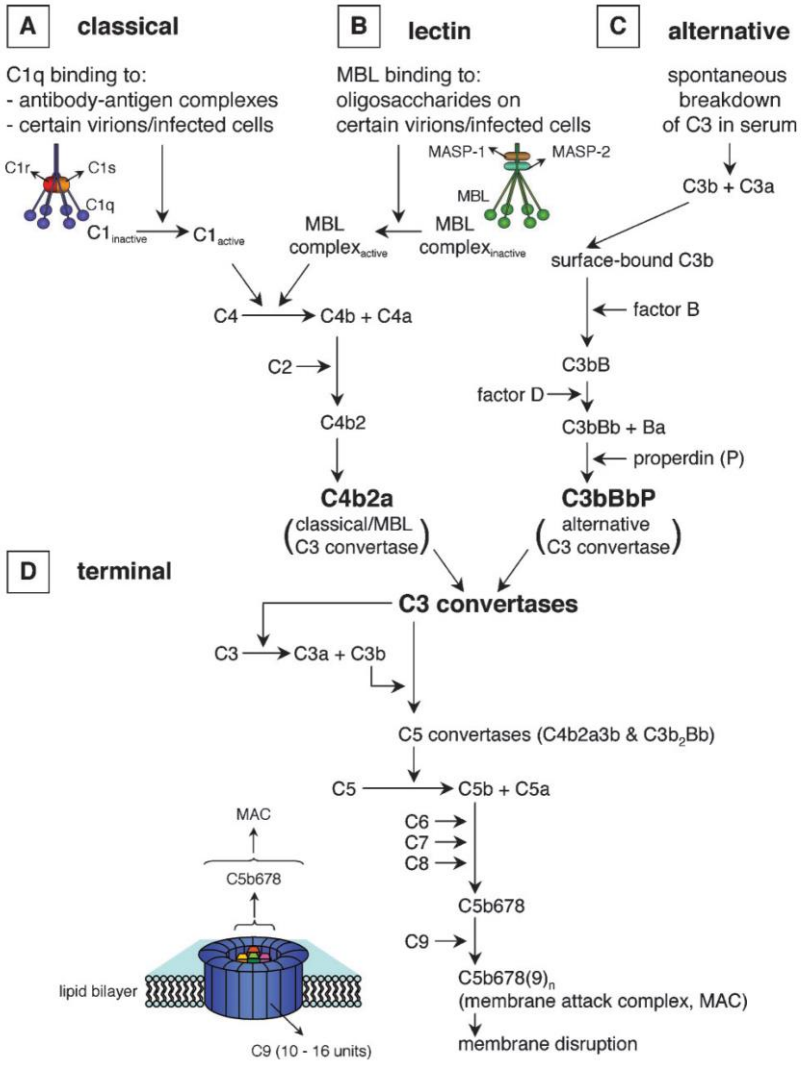

Fig.9: Activation of the complement cascade via the classical (A), lectin (B) or alternative (C) pathway results in the initiation of the terminal complement pathway (D), leading to the formation of membrane attack complexes. (Taken from Favoreel et al., J Gen Virol 84(Pt 1), 1-15 (2003) [259]).

Cells on the other hand will express 'regulators of complement activation' (RCA) like the membrane cofactor protein CD46, complement decay-accelerating factor CD55, and CD59 (protectin), which will allow them to inhibit the complement system to a certain extent [260-262]. While CMV does not encode for a viral homologues of any of these RCAs like other viruses, it does incorporate cellular CD55 and CD59 into its virion [263] and it will also upregulate the expression of CD46 and CD55 on the surface of infected cells [264]. Both of these viral strategies have been shown to inhibit complement mediated cell lysis of HCMV infected fibroblasts [263-265] increasing viral replication and survival. Members of the viral US6 family known to be

involves in $CD8^+$ T cell evasion were examined as potential candidates for HCMV proteins responsible for the upregulation of CD59 and other RCA proteins, and all family members were shown to either increase the total expression or the number of cells that express at least one RCA. Furthermore, overexpression of US2 was shown to reduce cellular lysis by the complement system in a functional assay indicating a role of the protein in *in vivo* complement evasion [266].

1.5.5 Evasion of the adaptive T-cell response

The second important arm of the immune response countering invading pathogens is the adaptive immunity. Here, T-cells, and for the defense against intracellular parasites like viruses, especially $CD8^+$ cytotoxic T lymphocytes (CTLs), which patrol the body in search in infected cells, play a vital part in controlling the spread of previously encountered pathogens. Infected cells will be recognize by the CTLs through the peptides presented by the host cells on the plasma membrane by MHC-class I complexes. These peptides are derived from proteins degraded in the proteasomes, and virally infected cells will present peptides derived from virus proteins in addition to host peptides [267-269]. These foreign peptides will be recognized by the CTLs, which in turn will force the infected host cell into apoptosis to prevent spread of the infection and to avoid harm to the host. To counteract this host defense mechanism, CMV encodes for an entire family of proteins, termed the US6 family of proteins that will prevent viral recognition and secure survival of the virally infected cell. The four members of the US6 family are US2, US3, US6 and US11, and they interfere at different time points post infection with different steps of the MHC class I antigen presentation pathway [270]. Expression of US2 and US11 will lead to relocation of major histocompatibility complex class I heavy chains from the ER to the cytosol, where they are deglycosylated and subsequently degraded by the proteasome [271], whereas US6 acts as a TAP inhibitor, preventing the loading of MHC class I complexes with proteasomally derived peptides prevents ATP hydrolysis [272]. Finally, US3, the only US6 family member expressed with IE kinetics [273], retains MHC class I complexes in the ER [274]. Interestingly, RhCMV, the rhesus macaques counterpart of HCMV, contains locational and functional homologues to all four HCMV US6 family members [275], which enables *in vivo* research into the importance of these proteins in an animal infection model. When the US6 homologues of RhCMV (Rh182-Rh189) were deleted

from the viral genome, the mutant virus was still able to persistently infect naïve animals, but was incapable of infecting CMV positive macaques [276].

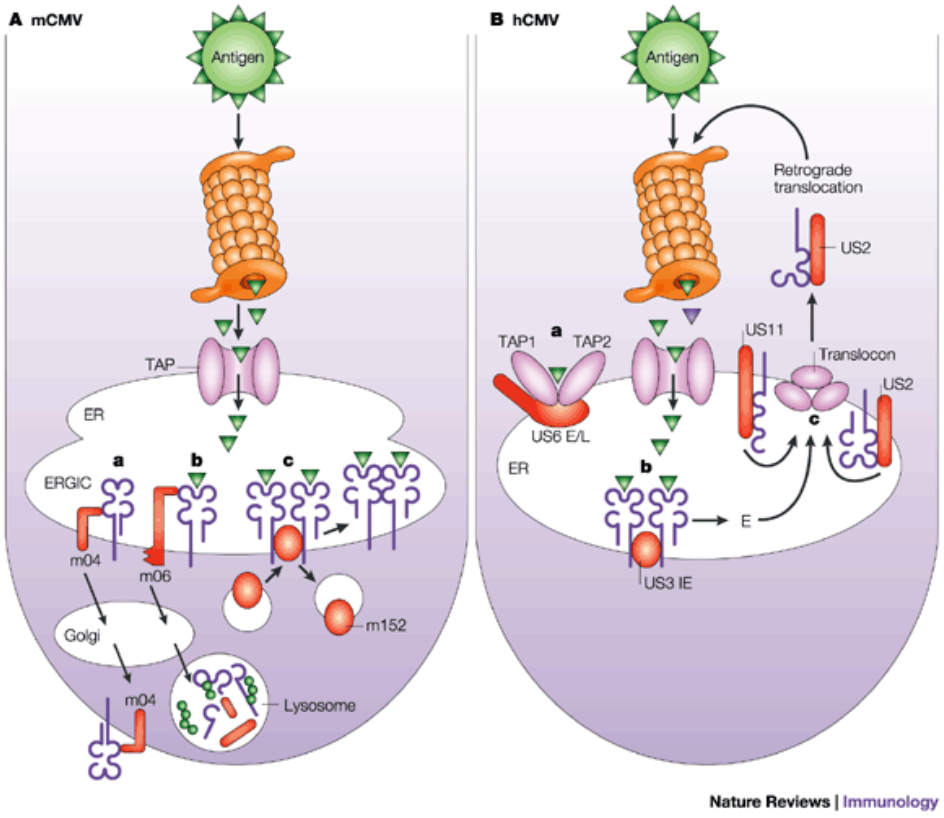

Fig.10: Immunoevasins of the murine- and the human cytomegalovirus. A) Immunoevasins of murine cytomegalovirus (mCMV). m04 binds to MHC class I molecules in the endoplasmic reticulum (ER) and escorts them to the cell surface. The complex does not seem to be recognized by $CD8^+$ T cells. It is unclear whether the complexed MHC molecules do not present peptide or whether the bound m04 prevents recognition. m04-mediated surface display of MHC class I molecules might silence natural killer (NK) cells. m06 binds through a lumenal domain to peptide-loaded MHC class I molecules in the ER and reroutes the resulting complex to a late endosomal/lysosomal compartment for degradation. m152 triggers the retention and accumulation of peptide-loaded MHC class I molecules in the ER Golgi intermediate compartment (ERGIC). B) Immunoevasins of human cytomegalovirus (hCMV). US6 blocks peptide translocation through the transporter for antigen processing (TAP) by interaction with the lumenal surfaces of both subunits of the TAP1–TAP2 heterodimer in the transient peptide-loading complex — which consists of TAP, the MHC class I complex and ER-resident chaperones. US3 is an immediate-early (IE) protein that causes retention of peptide-loaded MHC class I molecules in the ER. US3-bound complexes are degraded in the early (E) phase by US2- and/or US11-mediated mechanisms. US2 and US11 both induce rapid proteasomal degradation of MHC class I α-chains by mediating retrograde translocation from the ER to the cytosol. Unlike US11, which remains in the ER membrane, US2 seems to be co-dislocated and to escort the MHC class I α-chain to the cytosol. (Figure taken from Reddehase, Nature reviews. Immunology 2(11), 831-844 (2002) [277]).

This underlines the importance of the virally encoded T-cell evasion genes, especially in the context of re-activation and re-infection, when the virus has to overcome a pre-existing T-cell response in order to establish a productive infection and persistence. This was additionally proven

when CMV positive rhesus macaques were $CD8^+$ T-cell depleted, which now enabled the US6 family deleted mutant to establish an infection [276]. Additionally, the viral Rh189 (US11) protein is responsible for a change in the T-cell epitope presentation. Whereas infection of macaques with SIV or SIV protein containing commonly used viral vectors (Ad5, MVA, etc.) leads to a presentation of canonical SIV epitopes, well characterized epitopes presented by all monkeys of a given MHC I allotype, RhCMV vectors will lead to the complete absence of canonical epitopes and the presentation of new, non-canonical SIV epitopes [278] Deletion of US11 from the RhCMV vaccine vector will restore the presentation of canonical epitopes, suggesting another active mechanism of the virus in derailing CTL response through inhibiting the presentation of highly active epitopes on the infected cell. One significant difference between RhCMV and HCMV is that RhCMV encodes for a fifth T-cell evasion gene that does not have a homologue in HCMV called Rh178 or VIHCE (Viral Inhibitor of Heavy Chain Expression) [279, 280]. This protein binds to the signal peptide of newly synthesized MHC class I heavy chains and inhibits the translation of the protein on ER bound ribosomes into the ER.

1.5.6 Evasion of the humoral immune response

Besides the T-cell response, B-cells and the antibodies they produce represent the second important arm of the adaptive immune response to fight invading pathogens and to develop an immunological memory to prevent reinfection with most formally encountered viruses. In the case of the human cytomegalovirus, protective immunity does not exist, and that even in the face of not only and incredible strong T-cell response to the virus [121] but also a strong and broad B-cell response against multiple glycoproteins and proteins complexes on the viral membrane [281-285]. An infected human will even produce neutralizing antibodies, mostly against the gB glycoprotein, but also against the pentameric complex needed for viral endocytosis into endothelial cells [286-293], not resulting in complete sterilizing immunity, but in significantly reduced infectivity [56, 294-298]. There is clinical evidence for a role of antibodies in limiting HCMV infection *in vivo* [299], which is why nowadays most proposed subunit vaccines have a T-cell (pp65 or IE1)- and a B-cell (gB or the pentameric complex) stimulating component, and this also explains why it is advantageous for the virus to encode for proteins counteracting the humoral immune response.

Like other viruses, HCMV does this by encoding for its own Fc-receptors [300, 301], which are thought to prevent antiviral immunoglobulin G (IgG) from neutralizing free virus and engaging in antibody-dependent cellular cytotoxicity (ADCC) against infected cells [302]. Initially, two such receptor have been described for HCMV, first the RL11 protein, which encodes for a 34kDa glycoprotein expressed with early kinetics.[256], and second the spliced UL119/UL118 protein which encodes for a glycoprotein of 68kDa [257]. Interestingly, although both well described virally encoded Fc-receptors show strong homology to human Fc-receptors, they resemble different host Fc-receptors. UL119-UL118 relates most closely to the third domain of Fcγ-receptor I, whereas RL11 is reminiscent of the second domain of Fcγ-receptor II/III [257], again emphasizing the importance to effectively evade the neutralizing antibody response, given that the virus independently integrated homologues to two different human Fcγ-receptor into its genome. Recently, further Fcγ-receptors have been discovered in the viral genome. RL13, a protein described to limit viral replication of primary isolates on human fibroblasts *in vitro* [303] has been shown to function as an Fcγ-receptor, as well as a third RL11 family member, RL12 [258].

1.6 *In vivo* models for HCMV

Due to the strict species specificity of CMV and most herpesviruses, animal model have to be applied to study the virus *in vivo* [304]. Fortunately, CMVs are widespread and it is plausible that most species co-evolved with their own form of the virus, so establishing a wide array of different animal models is theoretically possible. In reality, only very few animal models have been pursued, the mouse model with its version of CMV the murine Cytomegalovirus (MCMV) being the most widely explored and utilized [305]. MCMV has been first isolated in 1954 by Margaret Smith from the salivary gland tissue of infected laboratory mice [306] and has since then been extensively examined *in vitro* and *in vivo* [307-311]. The virus also has been cloned as a bacterial artificial chromosome (BAC) [312], which enabled research groups to design and construct mutants for further characterization of the encoded viral proteins. Additionally, the mouse host has also been extensively characterized and might represent the mammal with the best described and analyzed molecular biology and biochemistry other than humans, and, moreover, mice are available as inbred mouse strains, increasing the reproducibility of achieved research data by decreasing the

background of variability in the host. Furthermore, the production of a range of genetically engineered mice enables the dissection of viral functions *in vivo* by selectively knocking out single pathways and by analyzing the effect these pathways have on the virus. The major problem of the mouse-model as a model system for HCMV is the distant relationship between the hosts and the viruses alike. While the two viral species share high homology in their coding content and in the pathogenicity they cause in their respective host, the differences are most apparent and important findings cannot be extrapolated without verification. Furthermore, although the two viruses show similar behavior regarding infection, replication and pathogenesis, MCMV will confer protective immunity against re-infection, at least under laboratory conditions using inbred mouse strains and laboratory MCMV strains, a result not achievable with HCMV. Also, the biggest threat to humans posed by HCMV is to unborn children through congenital infection, leading to severe neurological damage (see above), something that has never been observed in newborn mice, so the model cannot be applied to study this route of infection, which, unfortunately, is also true for another widely used animal model established for HCMV, rat CMV. RCMV is grouped into the same genus as MCMV (Muromegalovirus). Genome organization and infectious behavior mirror MCMV. One significant different between the two rodent CMV species is, that two of the isolated and sequenced RCMV strains differ so substantially that they are considered different species, which would mean that the rat has co-evolved with two different but equally virulent species of CMV [313, 314]. Still, for the investigation of congenital HCMV infection another *in vivo* model had to be developed, and it was finally found in the guinea pig and the guinea pig cytomegalovirus (GPCMV), a virus that does cross the placenta causing infection and disease *in utero* in unborn guinea pigs [315]. Although the commonly used strain of GPCMV is resistant to Ganciclovir, the drug of choice for treatment of CMV infections [316, 317], the model is widely usable for the *in vivo* testing of potential anti-herpesviral compounds, as well as for toxicity studies.

1.7 Great Ape and Non-Human Primate (NHP) CMVs

Besides the few rodent isolates mentioned above, cytomegaloviruses have been preferentially isolated from great apes and nonhuman primates (NHP) because of the close evolutionary connection to humans [318-328].

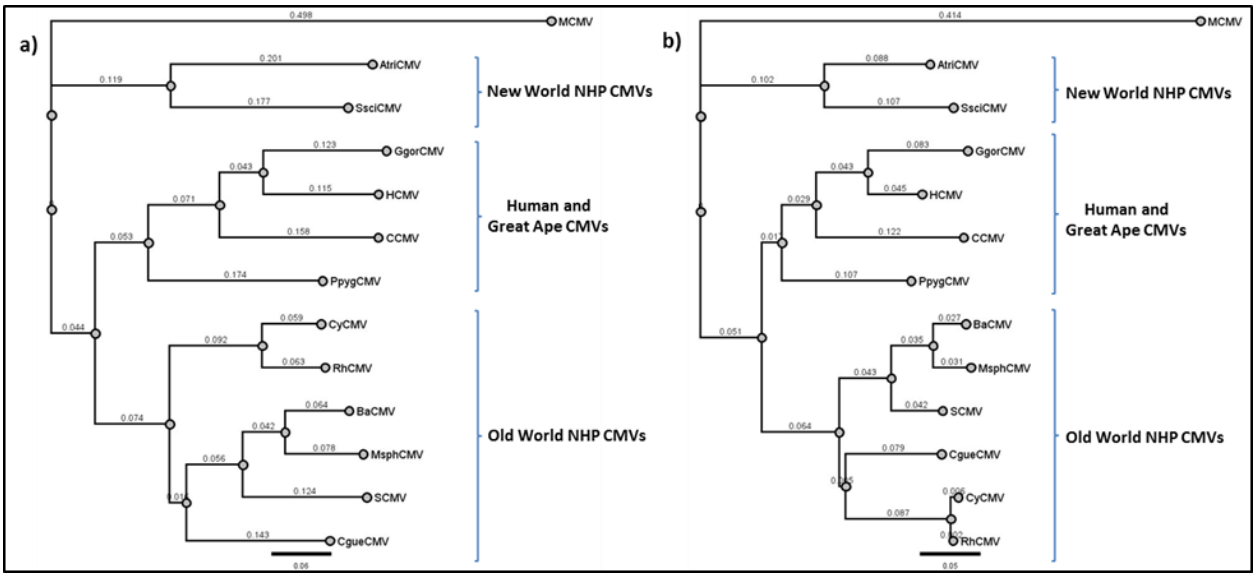

Fig.11: Phylogenetic tree of (A) gB and (B) the viral DNA polymerase for great ape and monkey CMVs. Accession numbers of the protein sequences used to make this figure are HCMV (X17403), CCMV (NC_003521), GgorCMV (FJ538490), PpygCMV (AY129396), RhCMV (AY186194), BaCMV (AC090446), SCMV (FJ483968), CyCMV (AY728171), MsphCMV (AY129399), CgueCMV (AY129397), AtriCMV (FJ483970) and SsciCMV (FJ483967). MCMV (GU305914) was used as an outgroup in both graphs. The phylogenetic trees were made using Geneious Pro 5.5.2. (Figure taken from Früh et al., In: Cytomegaloviruses: From Molecular Pathogenesis to Intervention, Caister Academic Press, 463-496 (2013) [329]).

As can be seen in Fig.11, CMVs have been characterized from many different species of Great Apes as well as old- and new world NHP. Phylogenetically, the old world NHP CMVs are closer related to HCMV then the new world NHP CMVs which mirrors the relationship between the host species where the separation point between apes (*Hominidea*) and old world monkeys (*Cercopithecidae*, the superfamilies *Hominidea* and *Cercopithecidae* form the parvorder *Catarrhini*) on one side and new world monkeys (*Platyrrhini*) on the other was estimated around about 35 million years ago [330-334]. Interestingly, this would be substantially after the African and the South American continents divided, indicating that the two higher primates parvorders did not separated geographically with the continental divide, but that the new world NHP migrated to the American continent at a later timepoint [335, 336]. The divide within the parvorder *Catarrhini* between Old World monkeys and apes occurred more recently at about 23 million years ago [337] and the latest separation between the genera *homo* (humans) and *pan* (chimpanzees) occurred roughly 5 – 7.5 million years ago [338-341].

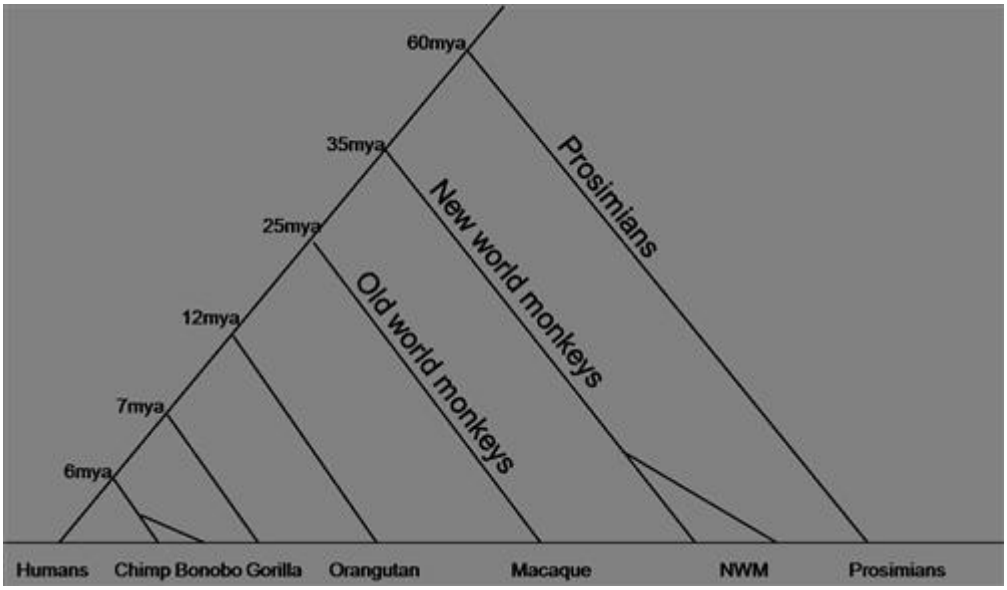

Fig.12: Phylogenetic tree of some primates. The Old world monkeys split from the New world monkeys +/-35mya. The Human-Chimp-Gorilla-Orangutan lineage split from that of the rhesus macaque lineage after the last major genomic infection (+/-25mya). Then the Human-Chimp-Gorilla lineage split from the Orangutan lineage (+/-12mya), and then the Human-Chimp lineage split from the Gorilla lineage (+/-7mya), and finally Humans and Chimps diverged +/-6mya. (Taken from Blogs24 (http://blogs.24.com/insilico/2011/10/page/2/#_edn3), but adapted from Polavarapu et al., Genome Biol. 2006;7(6):R51.Nov;81(22):12210-7 [342]).

The closest relatives to HCMV can be found in the closest relatives to man, the chimpanzees and gorillas as well as the Orang-Utans, but due to ethical and economical concerns and the fact that many of these species are endangered and protected, it is impossible to establish *in vivo* models in Great Apes. This is different for old world monkeys, which are widely utilized in biomedical research in primate centers all around the world. The most commonly kept species are rhesus macaques, cynomolgus macaques, mangabeys and baboons, all of which have been used in SIV models for HIV and AIDS and other related areas. Out of these monkey species, the rhesus macaque has the most extensively characterizes CMV, the rhesus cytomegalovirus RhCMV. In fact, two independent strain of RhCMV have been isolated and fully sequenced (68-1 [343] and 180.92 [319]), and RhCMV 68-1 has been cloned as a BAC [344], so it can now be used to create mutants through homologous recombination. The coding potential of both isolated RhCMV strains differ, because both strains seem to have acquired deletions during serial passage on rhesus fibroblast [345], leading to the loss of multiple kilobases of coding DNA and to deletions similar to what has been described earlier for fibroblast adapted strains of HCMV like AD169 or Towne [346]. The published coding potential of RhCMV is estimated around 230 - 260 ORFs [319, 343, 345], which exceeds the coding potential for all other published CMV genomes by at least 60

ORFs [346-351]. If all of the to date annotated RhCMV ORFs are indeed coding, then 60% of the encoded proteins have homologues to HCMV proteins, whereas 40% of all ORFs are old world monkey or even RhCMV specific [343]. To date only very few RhCMV genes not found in HCMV have been verifies and described (vCOX-2 [352], VIHCE [279, 280]).

1.8 Utilizing CMV as a vaccine vector

Given the remarkable feature of RhCMV to induce a strong B- and T- cell response without generating a sterilizing immunity opens up another interesting application for the virus, namely as a vector for the development of new vaccines. It is well established that T-cell epitopes introduced into CMV can generate strong *in vivo* T-cell responses [353] and given the non-sterilizing immunity, at least in HCMV and in NHP model systems, the same CMV vectors could be used over and over again to boost the initially primed immune responses to achieve an even greater number of cells of the adaptive immune response directed against a selected foreign antigen introduced into the CMV vector. The idea of using one virus as a vaccine vector to vaccinate against a second pathogen is not new and different viral families have been considered prospective candidates. Extensive research has been conducted using Adenovirus [354] or Parvoviruses (AAV, Adeno associated viruses) [355], and they proofed promising for some aspects of vaccine development, but they failed for the development of T-cell based vaccines against important human pathogens like HIV (Step study, Phambili study (HVTN 503) and HVTN 505 study) [356-358]. It became apparent that CMV has some important attributes that makes it superior to all previously considered viral vectors for the development of T-cell based vaccines. First of all, CMV establishes a persistent infection. But it reactivates from this latency constantly, challenging the existing anti-CMV immune response over and over again, precluding the CTLs from reaching their central memory phenotype (T_{CM}) and keeping them as effector memory T-cells (T_{EM}) [353]. This means, that the T-cells against CMV and the inserted foreign antigen will be constantly activated and alarmed, and ready to fight the real infection immediately should they ever encounter it, whereas with other vaccine strategies, the CTLs would move on to central memory T-cells, from where they will have to be re-activated upon stimulation, and this delay of 1-2 week compared to the CMV vaccine strategy makes all the difference. *In vivo* experiments in rhesus macaques

showed no protective effect when using Ad5 based α-SIV vaccine vectors [359], but RhCMV 68-1 based vaccine vectors enables 50% of the monkeys to control the viral infection and the associated viremia even after repeated SIV challenge [359], showing the first successful vaccine approach against SIV in a monkey model. Additionally, even though these monkeys initially were infected with SIV and then moved on to show elite controller phenotype in that they were able to control the infection with no significant viral load without any treatment, further experiments showed that they actually cleared the virus and adoptive transfer of leukocytes from these monkeys to SIV negative monkeys did not results in seroconversion [360].

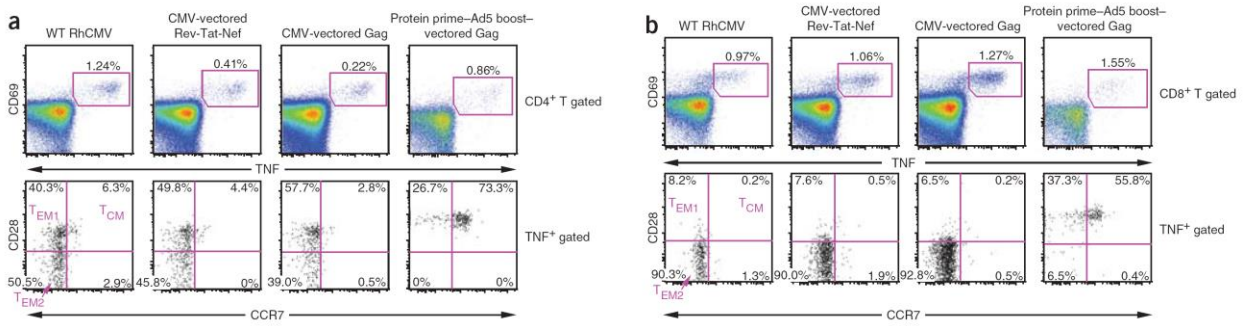

Fig.13: (a,b) Combined FCICA and surface phenotype analysis of CD4$^+$ (a) and CD8$^+$ (b) peripheral blood T cells responding to wild-type (WT) RhCMV lysate, SIV Gag or Rev-Tat-Nef–overlapping 15-mer peptides. The graphs compare the CD28 versus CCR7 phenotype of RhCMV and SIV antigen-responsive CD4$^+$ or CD8$^+$ T cells (CD69$^+$TNF$^+$) in a representative initially RhCMV-positive rhesus macaque that was inoculated 595 d and 330 d earlier with RhCMV-Retanef and RhCMV-Gag, respectively (left and middle). They also compare the SIV Gag response of this rhesus macaque to another rhesus macaque that received a Gag protein prime and Ad5(Gag) boost (105 d after the boost; right). (Figure taken from Hansen et al., Nat Med 15(3), 293-299 (2009) [353]).

Additionally, when SIV gag was introduced into several different viral delivery vectors and rhesus macaques were infected with all these different constructs, it was noticed that the T-cell epitopes presented by RhCMV 68-1 were substantially different than in any other examined construct (Ad5, MVA, DNA, SIV) [278]. Our RhCMV vectors based on the laboratory adapted strain 68-1 that does not have a functional pentameric complex, presented substantially more and different, non-overlapping epitopes than were observed in all control samples (32 compared to 14) and 2/3 of these epitopes were actually MHC class II dependent [278], which had not been observed before. To top everything off, some of the epitopes were presented in all examined monkeys independent either by MHC class I or MHC class II and that independent of the MHC alleles encoded by the individual monkeys. These highly promiscuous peptides are extremely unusual and have not been described in connection with viral infections before. Because these newly discovered epitopes where so promiscuous and where found in all examined animals, they

were termed "supertopes" and they, in combination with the MHC class II dependent CD8+ T cells generated by the RhCMV 68-1 vaccine vector, could play a crucial role in the protection seen in SIV challenge experiments with SIV after RhCMV vector vaccination. A broader T-cell response against more epitopes essentially spanning the entire protein makes it substantially harder for the virus to evade the pressure of the immune system by mutation, even in the case of a highly variable and highly mutagenic virus like SIV (or HIV). The overall impact of the altered epitope repertoire being presented in regard to protection against challenge is still the matter of intensive research and preliminary data suggests, that restoration of the pentameric complex in 68-1 abrogated the generation of supertopes and MHC class II dependent CD8+ T cells [278], but deletion of single members of this complex did not lead to the restoration of the RhCMV 68-1 phenotype, although the tropism specificity induced by a fully functional pentameric complex is lost (unpublished data). This indicates that it is not the entire complex or the tropism that is responsible for the appearance of supertopes and MHC class II dependent CD8+ T cells, but a combination of single members of the complex.

As mentioned above, Rh189 (US11) also has an effect on the T cell epitopes that are being presented by the infected cell. In the presence on US11, the epitopes that are presented do not resemble the epitopes found in a natural SIV infection or after vaccination with any viral vector expression SIV gag other than RhCMV. Because these epitope are so common in SIV infection, they are termed canonical epitopes and the epitopes that are presented in the presence of US11 are termed non-canonical epitopes [278]. Interestingly, if US11 is deleted, the canonical epitopes will also be presented in the context of RhCMV as the delivery vector for gag [278], indicating that the exact design of the RhCMV vector has a deep and profound impact on the T cell response that will be generated against the viral vector as well as against the inserted foreign antigen. All these incredible findings enable us now to exactly customize the T-cell response we wish to achieve by constructing vectors containing or lacking certain genes involved in T-cell epitope generation and presentation. This immense flexibility has only been achieved with RhCMV vectors so far, and further steps are being undertaken right now to characterize the different T-cell responses generated to determine what vector configuration works best for the generation of a strong and broad immune response without sacrificing the viability and infectivity of the viral vector. With further animal studies planned, we hope that the protection achieved in our first published RhCMV vector study where 50% of the challenged rhesus macaques were protected from SIV challenge

[359] can be extended so that the number of protected animals, and further down the road humans, can be maximizes to protect as many individual as possible.

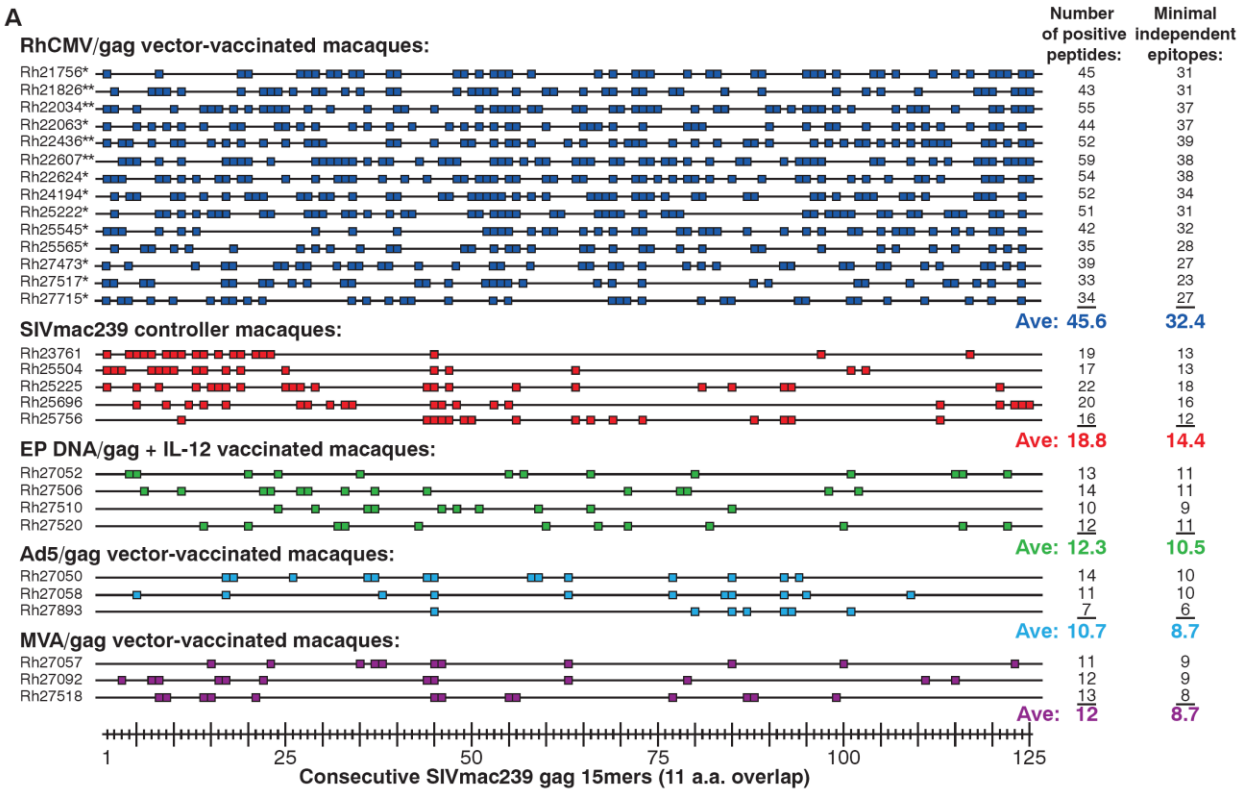

Fig.14: RhCMV vector–elicited and conventional SIVgag-specific CD8$^+$ T cell responses differ in epitope breadth and promiscuity. CD8$^+$ T cell responses to SIVgag were epitope-mapped using flow cytometric ICS to detect recognition of 125 consecutive 15mer gag peptides (with an 11–amino acid overlap) in macaques vaccinated with strain 68-1 RhCMV/gag vectors [*BAC-derived RhCMV/gag; **non–BAC-derived RhCMV/gag(L); n = 14], electroporated DNA/gag + IL-12 vectors (n = 4), Ad5/gag vectors (n = 3), and MVA/gag vectors (n = 3) and in SIV$^+$ macaques with controlled infection (n = 5). Peptides resulting in above-background CD8$^+$ T cell responses are indicated by a colored box, with the total number of these positive responses and the minimal number of independent epitopes potentially contained within these reactive peptides in each macaque designated at right. $P < 0.0001$, epitope breadth of RhCMV/gag-vaccinated macaques compared to macaques pooled over the other groups, using two-tailed Wilcoxon rank sum tests. (Figure taken from Hansen et al., Science 340(6135), 1237874 (2013) [278].

2. Main aims of this thesis

In our previous studies, we were able to establish RhCMV as an *in vivo* model system for HCMV infection, and more importantly, we were able to establish RhCMV as a new viral delivery vector for T-cell vaccines against SIV and potentially against a wide range of different other deadly human pathogens [353, 359]. Our generated data was so promising, that the main focus now shifted towards the generation of an attenuated viral vaccine vector. This RhCMV mutant should still have the capability of inducing a strong T-cell response against the virus as well as against the inserted transgenes and should still re-activate occasionally to re-stimulate the virally induced T-cells to keep them in effector memory phenotype (T_{EM}) instead of allowing them to progress to central memory T-cells (T_{CM}). On the other hand, it should demonstrate reduced viral shedding to preclude transmission from animal to animal and most importantly, should not display any signs of pathogenesis even in the immunocompromised host, a point highly important for FDA approval of a potential HCMV counterpart of our RhCMV model virus for future human clinical trials. Attenuation of our viral delivery vectors should be achieved by generating deletion mutants lacking genes needed for efficient viral replication, effectively slowing down the viral replication and spread *in vivo*. As a result, the host's immune system should be able to control the virus better, and should be capable to control the virus before it can cause any detectable medical condition. The reduced viral replication should also lead to reduced shedding of the virus in saliva and urine leading to significantly diminished horizontal transmission between animals. The first step in generating targeted viral deletions is actually a step back. Given the relative novelty of the RhCMV model system and the very limited use in the scientific community due to money restraints and lack of accessibility, the virus is not well characterized and further detailed characterization of the viral genome and the viral coding capacity is needed to generate a reliable genome map of RhCMV that can be used as a starting point for all further recombineering steps. To achieve this, the only existing RhCMV BAC of strain 68-1 has to be fully sequenced and the preliminary open reading frame (ORF) annotation has to be re-analyzed and either verified or dismissed for every single ORF. After generating the new viral genome map, target proteins can be chosen for deletion to create attenuated viral mutants. An import prerequisite for every potential RhCMV candidate ORF is, that it has to have an HCMV homologue, because RhCMV naturally only serves as a model system for HCMV and all the data generated in our model system must be translatable into HMCV

for future human clinical trials, which would exclude 40% of all annotated RhCMV ORFs [343]. Many different levels of attenuation can be achievable. Slight attenuation should lead to a vector that should be easily propagated in tissue culture to high viral titers, but this level of attenuation might not be sufficient to achieve enhance safety *in vivo*. This could be achieved by deleting a gene with higher importance during viral replication leading to a more attenuated virus, but the problem here might be the viral propagation *in vitro*, which might have to be achieved by complementing the virus *in trans* on a stable, complementing cell line. Lastly, the highest level of attenuation could be achieved by deleting a gene essential for viral replication. Such a mutant would have to be grown on a complementing cell line, creating a single step virus that generates infectious viral particles on complementing cells with high efficiency but is unable to generate infectious particles on non-complementing cells or *in vivo*. Although examples for single step viruses in CMV exist (i.e. gL deletion mutants in MCMV [361] and RhCMV [362]) it is not known whether these viruses are capable of creating a persistent infection *in vivo*, or if this level of attenuation will lead to an immediate clearance of the virus from the infected host. In a first step we will focus on the generation of mildly and moderately attenuated mutant viruses by deleting the major viral tegument proteins pp65a and b or pp71. These viral protein serve not only as structural proteins in the viral tegument, but also play an important role in the evasion of the intrinsic- and innate immune response immediately after viral infection of the host cell [363]. The resulting deletion mutants should show significant attenuation *in vivo* hopefully leading to better control of the viral vectors by the host immune response leading to improved safety.

3. Reevaluation of the Coding Potential and Proteomic Analysis of the BAC-Derived Rhesus Cytomegalovirus Strain 68-1

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3.1 Abstract

Cytomegaloviruses are highly host restricted, resulting in cospeciation with their hosts. As a natural pathogen of rhesus macaques (RM), rhesus cytomegalovirus (RhCMV) has therefore emerged as a highly relevant experimental model for pathogenesis and vaccine development due to its close evolutionary relationship to human CMV (HCMV). Most *in vivo* experiments performed with RhCMV employed strain 68-1 cloned as a bacterial artificial chromosome (BAC). However, the complete genome sequence of the 68-1 BAC has not been determined. Furthermore, the gene content of the RhCMV genome is unknown, and previous open reading frame (ORF) predictions relied solely on uninterrupted ORFs with an arbitrary cutoff of 300 bp. To obtain a more precise picture of the actual proteins encoded by the most commonly used molecular clone of RhCMV, we reevaluated the RhCMV 68-1 BAC genome by whole-genome shotgun sequencing and determined the protein content of the resulting RhCMV virions by proteomics. By comparing the RhCMV genome to those of several related Old World monkey (OWM) CMVs, we were able to filter out many unlikely ORFs and obtain a simplified map of the RhCMV genome. This comparative genomics analysis suggests a high degree of ORF conservation among OWM CMVs, thus decreasing the likelihood that ORFs found only in RhCMV comprise true genes. Moreover, virion proteomics independently validated the revised ORF predictions, since only proteins that were conserved across OWM CMVs could be detected. Taken together, these data suggest a much higher conservation of genome and virion structure between CMVs of humans, apes, and OWMs than previously assumed.

3.2 Introduction

Human cytomegalovirus (HCMV), a member of the Herpesviridae subfamily Betaherpesvirinae, is a large DNA virus of about 235 kb [346] encoding approximately 165 different proteins [364]. It is widespread in the developing world, with infection rates of close to 100%, but is also very common in the developed world, where about 60% of the population is CMV positive [365]. CMV infection is generally asymptomatic in healthy, immunocompetent individuals but can cause severe diseases in immunodeficient patients. It is the major viral cause for congenital birth defects such as mental retardation and deafness in the United States [366]. HCMV can also cause disease in solid organ and bone marrow transplant recipients, where the virus can reactivate from either the transplanted organ or the CMV-positive recipient and lead to disease or graft rejection [367]. The Institute of Medicine (IOM) of the U.S. National Academy of Sciences declared the development of a CMV vaccine a high priority in 1999 [42]. However, attempts to develop a vaccine against HCMV have had limited success so far (for a recent review, see reference [368]. One of the challenges of vaccine development is that HCMV is strictly species specific and cannot infect immunocompetent animals that could serve as a model system. In fact, reports of CMVs infecting species other than their natural hosts are very rare and occur only under artificial circumstances such as laboratory infections or xenotransplantations [369-372]. There are no reports of proven natural zoonotic infections of humans by animal CMVs.

Due to this species specificity, most current animal models employ CMVs that naturally infect the respective animal. Since the relatedness of CMVs generally mirrors the evolutionary relatedness of their hosts, many of the genes and gene families present in HCMV are absent in CMVs of small animals, thus limiting their usefulness in predicting the *in vivo* function of HCMV genes. The closest relatives to HCMV are CMVs of human primates. However, due to ethical and financial reasons, gorilla or chimpanzee models of CMV are not practical. Therefore, CMVs infecting nonhuman primate (NHP) species are the best alternative to those infecting small animals to study CMV pathogenesis and the establishment and maintenance of persistent infection [373]. In particular, rhesus CMV (RhCMV) has emerged as an attractive model for studying CMV infection, pathogenesis, and immunology [374, 375]. This model was further used for the development of CMV as a new vaccine vector platform against HIV [353, 359, 374]. In order to correlate results from *in vivo* studies using wild-type (WT) and recombinant RhCMVs with potential outcomes for HCMV, it is important to accurately predict the locations and potential

functions of open reading frames (ORFs) carried by RhCMV and their relationship to HCMV-carried ORFs, as well as to have exact knowledge of the genome content of RhCMV used in each of the studies.

Two different strains of RhCMV, 68-1 and 180.92, have been fully sequenced previously [319, 343], and partial sequences of various regions of the genome from low-passage-number isolates have been published [345]. However, many of the *in vivo* experiments performed to date, particularly those involving recombinant CMVs, employed RhCMV strain 68-1 cloned as a self-excisable bacterial artificial chromosome (BAC) [344]. The previously determined RhCMV 68-1 sequence was obtained by cosmid cloning from the original strain [343]. Since viable virus was not reconstructed from the individual cosmids, it is not known whether the respective genome sequence is infectious. During BAC cloning, a molecular clone of a selected virus strain is fixed as a genome and thus might differ in its genome content from the parental strain, which likely contains a mixture of molecular clones. For instance, the 68-1 BAC is known to contain a mutation in Rh61/Rh60, the RhCMV homologue of UL36, which is not present in the published RhCMV 68-1 sequence [376]. Moreover, the extensive *in vitro* propagation required for BAC cloning might select for additional tissue culture adaptations compared to the original strain.

For these reasons, we determined the full genome sequence of the RhCMV 68-1 BAC by whole-genome shotgun sequencing. Compared to the previously determined 68-1 sequence, several mutations in ORFs distributed across the entire genome were found and confirmed. To determine the potential impact of these mutations on the predicted function of RhCMV ORFs, we further reannotated the RhCMV genome by comparative genomics using recently completed CMVs of closely related Old World monkeys (OWM), thus creating a prototypical wild-type RhCMV sequence. We further experimentally verified the expression of a number of ORFs by proteomics. Our analysis revealed that RhCMV, as well as all other OWM CMVs, is much more closely related to ape and human CMVs than previously assumed, since almost all of the ORFs previously categorized as "RhCMV specific," i.e., found exclusively in RhCMV, were rendered unlikely by this combined genomics, *in silico*, and proteomics approach. Most of the remaining ORFs are conserved in HCMV, and almost all RhCMV ORFs have closely related orthologues in OWM CMV genomes. The close relatedness to HCMV is also reflected in the fact that BAC-derived RhCMV shows fibroblast adaptations that are remarkably similar to those of HCMV. In addition to the previously noted mutations in the RhCMV 68-1 orthologues of the endothelial cell

(EC) tropism-determining glycoprotein complex gH/gL/UL128/UL130/UL131A and the apoptosis inhibitor UL36, our analysis also suggests that all known RhCMV sequences and most OWM CMV sequences contain independent mutations in genes homologous to RL13, which was recently shown to limit HCMV growth in fibroblasts [303]. Despite these mutations, however, BAC-derived RhCMV is able to establish and maintain persistent infections upon experimental inoculation of CMV-naïve or CMV-positive rhesus macaques (RM) suggesting that persistent viral infection occurs despite multiple attenuating mutations.

3.3 Materials and Methods

3.3.1 Cells, viruses, and reagents. Telomerized rhesus fibroblasts (TRFs) [377] were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and were grown at 37°C in humidified air with 5% CO_2. Generation of the RhCMV 68-1 BAC was previously described [344]. To derive the virus, TRFs were transformed via electroporation (250 V, 950 μF) with BAC DNA and cytopathic effect (CPE) was observed after 7 to 10 days.

3.3.2 454 sequencing and annotation of the BAC-cloned RhCMV 68-1 genome. RhCMV 68-1 BAC DNA was prepared using the NucleoBond AX kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. 454 sequencing was carried out on a GS FLX instrument using titanium series chemistry by Eurofins MWG Operon (Huntsville, AL). Gap closure was performed by Sanger sequencing on an ABI 3730XL sequencer. The other genomes used in this study to annotate the RhCMV 68-1 BAC sequence were RhCMV 68-1 (accession number AY186194), RhCMV 180.92 (DQ120516), RhCMV CNPRC (EF990255), cynomolgus CMV (CyCMV) Ottawa (JN227533), SCMV GR2715 (FJ483968), simian CMV (SCMV) Colburn (FJ483969), SCMV Stealth virus 1 (U27883, U27627, U27469, U27770, U27471, and U27238), baboon CMV (BaCMV) OCOM4-37 (AC090446), chimpanzee CMV (CCMV) Heberling (AF480884), and HCMV Merlin (AY446894).

3.3.3 RhCMV particle purification procedures. RhCMV 68-1 BAC-derived particles were purified as described before[18]. The virus was isolated from the culture medium of infected TRFs when the cells displayed maximal cytopathic effect. The cellular supernatants were first clarified by centrifugation at 7,500 × *g* for 15 min. The clarified medium was layered over a sorbitol cushion (20% d-sorbitol, 50 mM Tris [pH 7.4], 1 mM $MgCl_2$), and virus was pelleted by centrifugation at 64,000 × *g* for 1 h at 4°C in a Beckman SW28 rotor. The virus pellet was resuspended in TNE buffer (50 mM Tris [pH 7.4], 100 mM NaCl, and 10 mM EDTA). The virus particles were further purified by layering them over a discontinuous 5% to 50% Nycodenz (Sigma) gradient in TNE buffer and centrifuged at 111,000 × *g* for 2 h at 4°C in a Beckman SW 41 Ti rotor. The virion band in the gradient was isolated with a syringe through the side of the centrifuge tube, and the particles were pelleted in a Beckman TLA-45 rotor in a Beckman Optima TL 100 ultracentrifuge at 40,000 × *g* for 1 h and washed twice with TNE buffer. The pellet was resuspended in TNE buffer, and

electron microscopy was performed to confirm the purity of the sample. In order to assess the protein content of the purified virions, a denatured protein preparation was separated on a NuPAGE morpholinepropanesulfonic acid (MOPS) gradient gel (Invitrogen, Carlsbad, CA) and visualized by Coomassie brilliant blue and silver staining.

3.3.4 Tryptic digestion of RhCMV particles. RhCMV particles were denatured in 8 M urea–100 mM NH_4HCO_3–5 mM dithiothreitol (DTT) at 56°C for 30 min. The cysteine residues were then alkylated by adding iodoacetamide to a final concentration of 10 mM and incubating in the dark at room temperature for 2 h. The sample was then diluted 4-fold with 25 mM NH_4HCO_3, and $CaCl_2$ was added to 1 mM. Methylated, sequencing-grade porcine trypsin (Promega, Madison, WI) was added at a substrate-to-enzyme ratio of 20:1 (mass/mass) and incubated at 37°C for 15 h. The digested peptides were cleaned up with C_{18} cartridges, as previously described [378].

3.3.5 Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Peptides were loaded onto capillary columns (75 μm by 65 cm; Polymicro) packed with C_{18} beads (3-μm particles; Phenomenex) connected to a custom-made 4-column liquid chromatography system. The elution was performed in an exponential gradient from 0 to 100% solvent B (solvent A, 0.1% formaldehyde; solvent B, 90% acetonitrile–0.1% formaldehyde) over 100 min with a constant pressure of 10,000 lb/in^2 and a flow rate of ~400 nl/min. Alternatively, the separation was performed in a nanoAcquity instrument (Waters) using a longer capillary column (75 μm by 100 cm; Polymicro) with a gradient of 0 to 45% solvent B over 10 h at a constant flow rate of 150 nl/min. Eluted peptides were analyzed online in a linear ion trap Orbitrap mass spectrometer (LTQ Orbitrap XL; Thermo Scientific, San Jose, CA). Peptides were measured over a 400 to 2000 *m/z* range, and the 6 most intense ions were selected for collision-induced dissociation (isolation width of 3 Da and 35% normalized collision energy) in the linear ion trap. Each parent mass was fragmented once before being dynamically excluded for 60 s.

3.3.6 Data analysis. LC-MS/MS spectra were converted into DTA files using default parameters and submitted for SEQUEST (v27.12) [379] searches against the RhCMV open reading frames (275 sequences) or stop-to-stop reads (4,304 sequences, ≥30 amino acid residues), the Macaca mulatta Ensembl database (21,905 sequences, downloaded from www.ensembl.org on 15

November 2010), and 186 common contaminant sequences (downloaded from www.ncbi.nlm.nih.gov/protein in August 2006). All sequences were searched in both the correct and reverse orientations (i.e., for a total of 44,732 or 52,790 searched sequences). Parameters employed for searches were as follows: (i) 50 ppm and 1 Da for peptide and fragment mass tolerance, respectively; (ii) tryptic digestion; (iii) maximum of two missed cleavage sites; and (iv) cysteine carbamidomethylation and methionine oxidation as static and variable modifications, respectively. Peptide-to-spectrum matches were then filtered with a mass spectrum-generating function (MS-GF) score of $\leq 1 \times 10^{-8}$, and each protein was required to have at least one peptide with an MS-GF score of $\leq 1 \times 10^{-10}$, which resulted in less than 1% of reverse sequences. Protein abundances were estimated with the exponentially modified protein abundance index (emPAI) as previously described [380].

3.3.7 Nucleotide sequence accession number. The final BAC sequence determined in this work was submitted to GenBank under accession number JQ795930.

3.4 Results

3.4.1 Determining the full-length sequence of the RhCMV 68-1 BAC.

The rhesus cytomegalovirus strain 68-1 was cloned as a self-excisable bacterial artificial chromosome [344]. The BAC-derived virus showed essentially the same growth kinetics as the parental strain *in vitro* and retained pathogenicity *in vivo* [344]. Furthermore, BAC-derived viruses used as vaccine vectors against simian immunodeficiency virus (SIV) established and maintained persistent infection as indicated by shedding from infected animals as well as long-term effector memory T cell responses indicative of the continuous presence of viral antigens ([359],[353]). Previously, a full-length sequence of RhCMV 68-1 was assembled from individually sequenced cosmids. To determine whether the molecular clone preserved as a BAC was identical to this parental strain, DNA of the RhCMV BAC was obtained from Escherichia coli (EL250) ([381]) and subjected to shotgun sequencing on a GS FLX instrument using titanium series chemistry (Eurofins MWG Operon, Huntsville, AL). The resulting DNA contigs were assembled *in silico*, and gap closure was performed by Sanger sequencing technology on an ABI 3730XL sequencer. The final BAC sequence was aligned against the published RhCMV 68-1 sequence (accession number AY186194), and the differences in the DNA sequences between the viral genomes were resequenced for independent confirmation. By this analysis, a total of 39 DNA changes in the 68-1 BAC compared to the published 68-1 sequence were confirmed, of which 18 were substitutions, 9 were deletions, and 12 were insertions (Table 1). Out of the 39 mutations detected, 31 were located in regions predicted to encode a viral protein, whereas 8 mutations were in noncoding regions. To distinguish between true mutations present in the BAC but not in the parental 68-1 sequence and sequence differences due to errors in the published 68-1 sequence, we also compared the BAC sequence to the second fully sequenced RhCMV genome of strain 180.92 (DQ120516)[319]. If the BAC sequence was identical to that of 180.92 but different from that of 68-1, the change was considered a sequencing error in the previous 68-1 sequence. In contrast, if the BAC sequence differed from both the 68-1 sequence and the 180.92 sequence, the new sequence was considered a mutation acquired during molecular cloning of the BAC. According to this analysis, only 13 true mutations are present in the BAC-derived genome compared to the parental genome, i.e., 10 point mutations, 1 insertion of a single base, 1 insertion of two bases, and 1 deletion of a single base. These mutations cause amino acid changes in the following ORFs: Rh08 (RL11 family), Rh13.1 (RL13), Rh61/Rh60 (UL36), Rh67.1 (UL41A), Rh72 (UL45),

R152/Rh151 (UL119/UL118), Rh164 (UL141), Rh194 (US14), and Rh197 (US14) (Table 1; see Fig.S1A to I in the supplemental material). Overall, this result suggests that only a very limited number of changes occurred in the RhCMV genome despite extensive tissue culture and repeated plaque purification that occurred during BAC cloning.

Change No.	Nucleotide No.	Change	ORF	AA Change	Cause
1	2487	G → A	Rh01	G → E	Sequencing Error
2	3931	C → T	Rh05	A → V	Sequencing Error
3	4642	G → A	none	none	Sequencing Error
4	4650	G → A	none	none	Sequencing Error
5	5369	C → T	Rh07	silent	Sequencing Error
6	6126	G → A	Rh08	silent	Sequencing Error
7	6327	A → -	Rh08	Frameshift	Mutation
8	9869	- → C	Rh10	Frameshift	Sequencing Error
9	9877	G → -	Rh10	Frameshift	Sequencing Error
10	9958	- → A	none	none	Sequencing Error
11	10846	- → A	none	none	Sequencing Error
12	10951	T → -	none	none	Sequencing Error
13	12415	A → G	Rh13.1	Stop → W	Mutation
14	12820	- → CT	Rh13.1	Frameshift	Mutation
15	45935	C → -	Rh57	Frameshift	Sequencing Error
16	48651	- → T	Rh61/Rh60	Frameshift	Mutation
17	52900	C → A	Rh67.1	W → C	Mutation
18	57844	C → T	Rh72	E → K	Mutation
19	57848	C → T	Rh72	C → Y	Mutation
20	78626	C → G	Rh89	C → S	Sequencing Error
21	78687	G → T	Rh89	Q → K	Sequencing Error
22	95620	C → -	Rh100.1	Frameshift	Sequencing Error
23	98911	A → G	none	none	Mutation
24	153493	C → T	Rh152/Rh151	W → Stop	Mutation
25	162589 – 162610	- → AGACTAATTTGACCCGTCTCTC	none	none	Sequencing Error
26	169753	G → A	Rh164	P → L	Mutation
27	184466	T → G	none	none	Mutation
28	202215	G → A	Rh194	S → F	Mutation
29	204412	C → T	Rh197	W → Stop	Mutation
30	206264	- → A	Rh199	Frameshift	Sequencing Error

31	**219638**	**T → -**	**Rh216**	**Frameshift**	**Sequencing Error**
32	**219641**	**A → -**	**Rh216**	**Frameshift**	**Sequencing Error**
33	**219642**	**C → -**	**Rh216**	**Frameshift**	**Sequencing Error**
34	**219645**	**C → -**	**Rh216**	**Frameshift**	**Sequencing Error**
35	**219728**	**- → G**	**Rh216**	**Frameshift**	**Sequencing Error**
36	**219729**	**- → C**	**Rh216**	**Frameshift**	**Sequencing Error**
37	**219730**	**- → T**	**Rh216**	**Frameshift**	**Sequencing Error**
38	**219732**	**- → A**	**Rh216**	**Frameshift**	**Sequencing Error**
39	**219733**	**- → C**	**Rh216**	**Frameshift**	**Sequencing Error**

Table 1: Changes in the nucleotide sequence of the RhCMV 68-1 BAC compared to the parental virus

3.4.2 BAC-carried ORFs containing mutations.

Mutations in BAC ORFs could be divided into two categories: terminal mutations that changed the length of a given ORF and internal mutations that resulted in single amino acid changes within protein sequences (Table 2). As described previously[376], a point mutation in Rh61/Rh60 of the BAC introduces a premature stop codon resulting in a truncated, nonfunctional protein. Rh61/60 encodes the RhCMV homologue of UL36, also called viral inhibitor of caspase-8-induced apoptosis (vICA). The full-length RhCMV protein was shown to be fully functional, whereas the shortened mutant is not, leaving the virus vulnerable to induced premature apoptosis *in vitro*.

ORF (RhCMV)	ORF (HCMV)	bp RhCMV 68-1	AA RhCMV 68-1	bp RhCMV 68-1 BAC	AA RhCMV 68-1 BAC
Rh08	RL11 family	516	171	507	168
Rh13.1	RL13	300	99	969	322
Rh61/Rh60	UL36	1416	471	765	254
Rh67.1	UL41A	240	79	240	79
Rh72	UL45	2550	849	2550	849
Rh152/Rh151	UL119/UL118	1272	423	1212	403
Rh164	UL141	1293	430	1293	430
Rh194	US14	834	277	834	277
Rh197	US14	726	241	702	233

Table 2: Nucleotide changes affecting the lengths or sequences of predicted ORFs in BAC-derived RhCMV 68-1

Rh08 and Rh13.1, two members of the viral RL11 family, contain mutations in the RhCMV 68-1 BAC. Since the RL11 family of proteins shows a very high degree of sequence variability even within a CMV species [382], the most closely related HCMV protein for Rh08 is difficult to define. The highest degree of homology is displayed by HCMV UL1, a predicted glycoprotein with unknown function. Figure S1A in the supplemental material shows a comparison of the Rh08 proteins encoded by the RhCMV 68-1 BAC, the RhCMV 68-1 virus, and the RhCMV 180-92 strain. The N-terminal portion of the protein is almost completely conserved between all sequences, whereas the C-terminal tail is entirely different in all published sequences. This finding suggests that the N terminus is functionally more important.

Rh13.1 contains an A → G substitution in the BAC, which mutates the ORF's stop codon into a tryptophan codon, prolonging the entire ORF to 969 bp, more than three times the length of the ORF carried by the original strain 68-1. The BAC-encoded Rh13.1 now displays a significant homology to HCMV RL13 in both length and sequence, whereas both RhCMV 68-1 and RhCMV 180.92 seem to carry truncated versions of this ORF. A closer inspection of the 68-1 and 180.92 Rh13.1 sequences suggests that each virus contains a premature stop codon due to a single-base-pair substitution, since the Rh13.1 ORF continues beyond the stop codon, encoding a highly conserved protein sequence (Fig.15). Similarly, SCMV strain GR2715 contains a full-length RL13 homologue, whereas SCMV Colburn encodes a truncated version of the protein due to a premature stop codon. Similar to the case for RhCMV, the Colburn ORF continues beyond the stop codon, encoding an RL13-homologous protein fragment that is almost identical to the protein sequence encoded by strain GR2715. For HCMV, it was recently demonstrated that full-length RL13 protein inhibits viral replication in fibroblasts [303], resulting in the rapid selection of nonfunctional RL13 variants *in vitro*. Therefore, it seems likely that the truncated RL13 ORFs found in several OWM CMVs represent such fibroblast adaptation mutants. Interestingly, despite the fact that the RL13 homologue carried by the BAC seems to be full length, it is likely nonfunctional since it contains multiple mutations that frameshift part of the protein's C terminus. As a result, the predicted amino acid sequence of this protein part differs substantially from those of all other RL13 proteins encoded by OWM CMVs (Fig.15). It was previously shown that the BAC-derived virus shows the same growth characteristics in fibroblasts as the parental 68-1 strain [344]. The fact that all three currently sequenced RhCMV genomes contain independent mutations in this ORF that likely

render it nonfunctional suggests that, similar to the case for HCMV RL13, native Rh13.1 likely limits growth in fibroblasts.

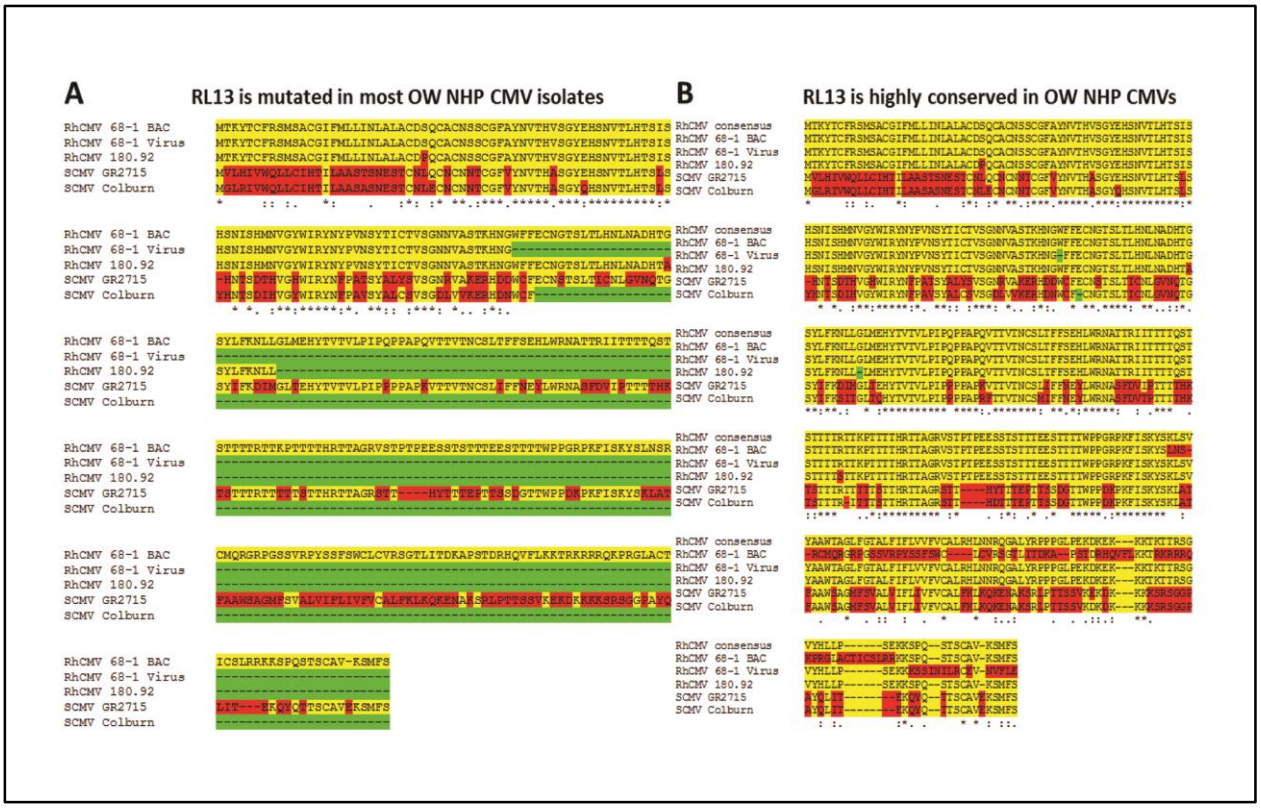

Fig.15: Alignment of Rh13.1 (RL13) from different species of Old World monkey cytomegaloviruses. (A) RL13 is mutated in most Old World NHP CMV isolates. The Rh13.1 (RL13) ORFs of the RhCMV 68-1 BAC, RhCMV 68-1, RhCMV 180.92, SCMV GR2715, and SCMV Colburn were aligned. Conserved amino acids are shown in yellow, whereas nonconserved amino acids are shown in red. Truncations due to premature stop codons are shown in green. (B) RL13 is highly conserved in Old World NHP CMVs. Stop codons were ignored for *in silico* translation, and an alignment of the resulting sequences is shown. A hypothetical consensus sequence for full-length Rh13.1 of RhCMV is also included. Both CLUSTAL format alignments were generated using MAFFT L-INS-i (v6.860b).

Two members of the US12 family of proteins, Rh194 and Rh197, show mutations in the BAC compared to either 68-1 or 180.92 (see Fig.S1H and I in the supplemental material). Whereas the mutation in Rh194 is a single point mutation leading to a serine-to-phenylalanine substitution, Rh197 is truncated by 8 amino acids (aa) in the BAC due to point mutations resulting in a premature stop codon. Whether these mutations affect protein function is uncertain. The US12 family is found only in primate CMVs [383] and encodes predicted seven-transmembrane-domain proteins that have some features in common with G-protein-coupled receptors [384]. Both Rh194 and Rh197 are homologues of HCMV US14 which is additionally homologous to Rh195 and Rh196. Since US14 and other US12 family members locate to the cytoplasmic virion assembly

compartment (AC) in some cells [385], a possible role for these proteins in the process of virion maturation and egress is suspected.

The ORFs Rh152/Rh151 encode the RhCMV homologue of the spliced viral Fcγ receptor UL119-UL118 (gp68) of HCMV [257]. In the BAC, a C → T substitution results in a premature stop codon, thus truncating the predicted Rh152/Rh151 protein by 20 aa. It is possible that this truncation affects the function of BAC-derived Rh152/Rh151, which is expected to be involved in counteracting the humoral immune response, similarly to gp68 of HCMV [300, 301].

Three additional ORFs in the RhCMV 68-1 BAC show internal point mutations: Rh67.1, Rh72, and Rh164, the homologues of HCMV UL41A, UL45, and UL141, respectively (see Fig.S1D, E, and G in the supplemental material). UL41A is a protein of unknown function with a potential transmembrane domain [346]. UL45 of HCMV is homologous to the R1 subunit of the cellular ribonucleotide reductases and forms a complex with the cellular R2 subunit, effectively forming a mixed viral-cellular enzyme [386]. HCMV lacking UL45 showed a growth defect at low multiplicity of infection (MOI) in fibroblasts [387] but not at high MOI in endothelial cells [388], whereas the MCMV homologue of M45 is essential for viral replication in endothelial cells [389]. UL141 mediates NK cell immune evasion by downregulating CD155 and CD112, ligands of the NK cell receptors DNAM-1 (CD226) and TACTILE (CD96) [390, 391]. The UL141 homologue Rh164 is absent in RhCMV 180.92 due to genomic rearrangements during fibroblast adaptation [319, 345]. Whether any of the point mutations found in the BAC-derived RhCMV genome affects protein function remains to be determined.

3.4.3 Reevaluating the RhCMV genome.

We further wanted to determine which ORFs were mutated in the BAC compared to a prototypical wild-type RhCMV sequence. To assemble the ORF map of such a RhCMV prototype, we initially recapitulated previous ORF identification methods using a 300-bp cutoff and the NCBI software ORF Finder [392] for full-length RhCMV genomes (strain 68-1 [343] and strain 180.92 [319]) and the ULb′ region of low-passage-number isolate RhCMV CNPRC (accession number EF990255) [345]. By this count, RhCMV 68-1 had 268 predicted ORFs, RhCMV 180.92 had 261 predicted ORFs, and the ULb′ region from the low-passage-number isolate of RhCMV had 19 ORFs (see Fig.S2 in the supplemental material). This analysis predicted 12 new genes, including Rh00 and Rh00.1 in RhCMV 180.92 and Rh142.4 and Rh220.1 in RhCMV 68-1. The new ORFs Rh00.2,

Rh94.1, Rh96.1, Rh228.1, and Rh231 are predicted for both RhCMV strains, whereas ORFs Rh163.1, Rh165.1, and Rh166.1 are conserved between RhCMV 68-1 and the ULb′ region of the low-passage-number isolate RhCMV CNPRC as described previously [345]. However, the total number of ORFs predicted by this method for either RhCMV strain was substantially higher than that for any other published CMV genome (HCMV [347], CCMV [346], mouse CMV [MCMV] [348], rat CMV [RCMV] [349], and guinea pig CMV [GPCMV] [350, 351]). Moreover, this annotation predicted that eight ORFs were unique to RhCMV 68-1 (Rh09, Rh39, Rh61, Rh93, Rh94, Rh142.4, Rh153, and Rh220.1) and that four ORFs were unique to RhCMV 180.92 (Rh13.1, Rh106.1, Rh142.3, and Rh178.2). This analysis raised the question whether RhCMV indeed had a higher number of ORFs than other primate CMVs and whether different strains of RhCMV differed substantially in their ORF content or whether these RhCMV-specific and strain-specific ORFs were, in fact, artifacts of the annotation method used and in reality do not encode proteins.

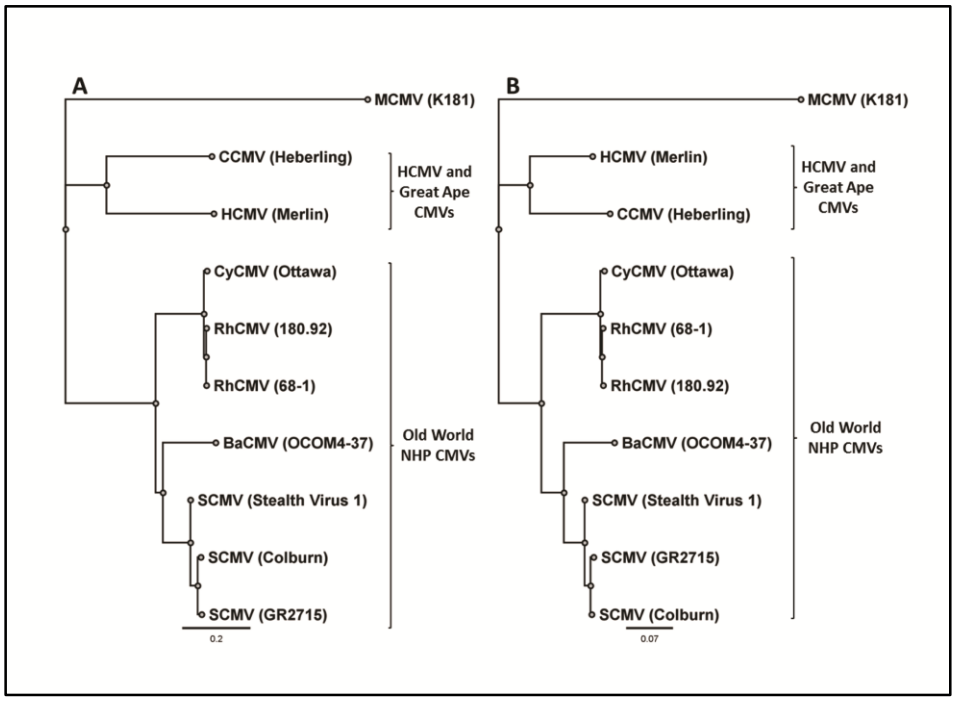

Fig .16: Phylogenetic trees of the major capsid protein (UL48) and the viral DNA polymerase (UL54) of Old World primate cytomegaloviruses. MCMV K181 M48 (A) (CAP08095) and M54 (B) (CAP08103) were used for comparison in the graphs. The phylogenetic trees were generated using Geneious Pro 5.5.2.

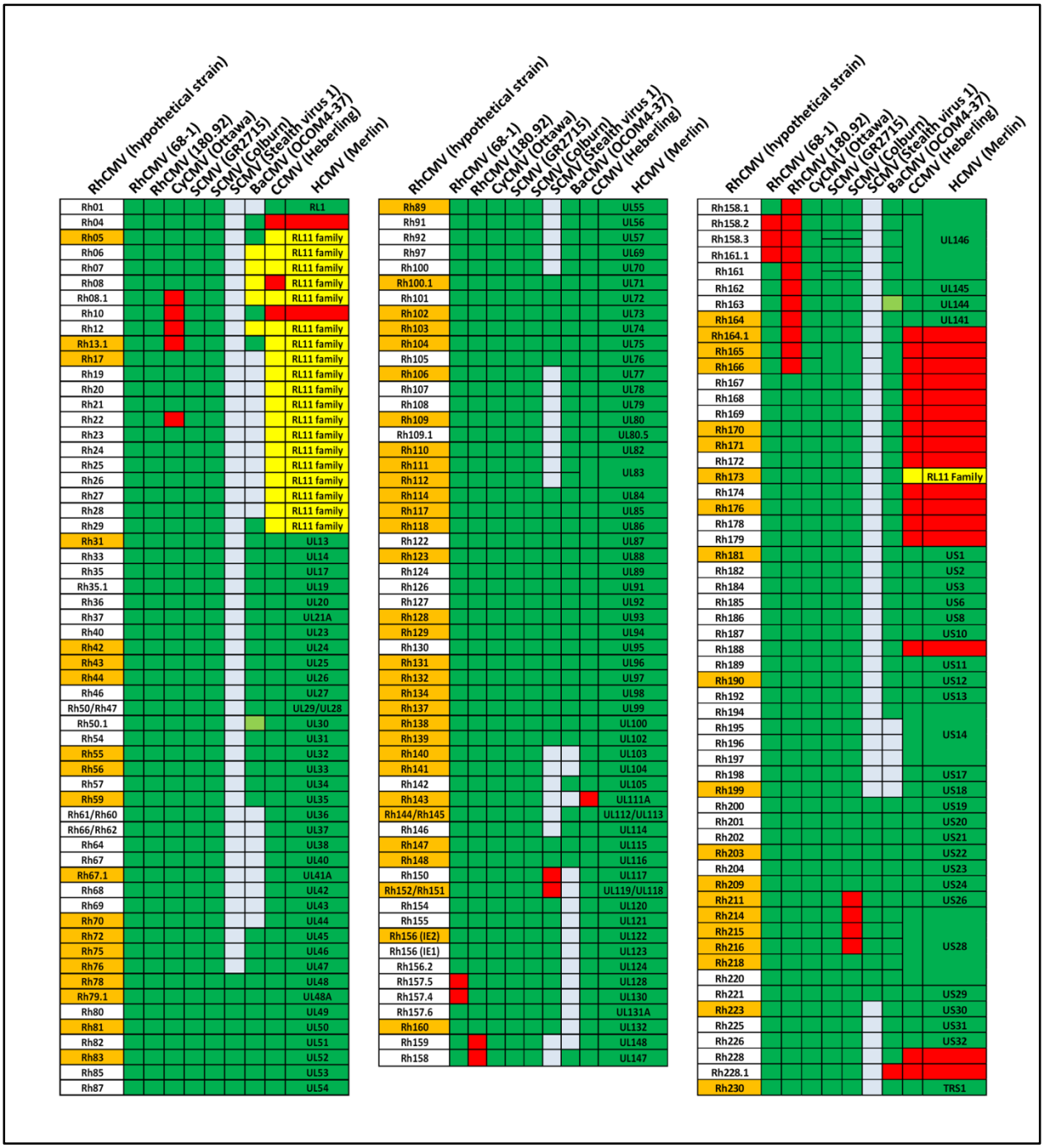

Fig.17: Conservation of RhCMV ORFs compared to those of OWM, chimpanzee, and human CMVs. Comparison of different RhCMV and OWM CMV genomes reveals the hypothetical ORF composition of wild-type RhCMV. Conserved ORFs are shown in green. ORFs in yellow are members of the RL11 family in HCMV, a gene family that is conserved albeit highly polymorphic so that homologies between single family members cannot be clearly assigned. Genes in red are either absent from the indicated OWM CMVs or not conserved in human and great ape CMVs. Gray indicates missing sequence information. Shown in light green are two ORFs (BaCMV UL30 and UL144) that show strong sequence homology to their respective RhCMV and HCMV homologues but lack a start codon. ORFs highlighted in orange in the leftmost column were identified by proteomics in RhCMV virions.

To obtain a higher level of confidence in our ORF predictions, we took advantage of the recently determined full-length genome sequences for CMVs from OWM species that are evolutionarily closely related to rhesus macaques: cynomolgus CMV Ottawa (JN227533), simian CMV GR2715 (FJ483968), simian CMV Colburn (FJ483969), simian CMV Stealth virus 1 (U27883, U27627, U27469, U27770, U27471, and U27238), and baboon CMV OCOM4-37 (AC090446) (Fig.16). When the ORF predictions for the two RhCMV strains were compared to the ORF predictions for these other OWM CMVs (Fig.17), it immediately became apparent that almost all of the "RhCMV-specific" ORFs (i.e., ORFs only found in RhCMV) were not conserved in other OWM CMVs. Moreover, these RhCMV-specific ORFs were mostly or entirely carried within other ORFs on either the same or the opposite DNA strand of the RhCMV genome (Fig.17; see Fig.S2 in the supplemental material). Furthermore, most of these RhCMV-specific ORFs were rather small, with an average size of 417 bp. Therefore, we conclude that these ORFs most likely do not represent true genes, i.e., that they do not encode unique proteins. Removal of these small and overlapping ORFs from the ORF list results in a prediction of 167 ORFs for RhCMV strain 68-1 and 160 ORFs for RhCMV 180.92. Taking this together with the BAC sequence and the RhCMV CNPRC sequence, it was now possible to generate a new ORF map for a prototypical wild-type RhCMV that contains 172 ORFs (Fig.18). Two different nomenclatures are in use for the various ORFs annotated in RhCMV [319, 343]. In Fig.18 we used the nomenclature introduced by Hansen et al. [343] for all ORFs. For ORFs first described by Rivailler et al. [319] or by us in this study, we chose names according to the original nomenclature by Hansen et al. to simplify the nomenclature. Additionally, a nomenclature is used that was recently introduced by Davison et al. [3] to underline the close relationship of most RhCMV proteins to their HCMV or OWM CMV homologues. Comparison of the prototypical RhCMV genome to those of chimpanzee CMV (Heberling, AF480884) and human CMV (Merlin, AY446894) reveals that 80% of the RhCMV ORFs have identifiable homologues in HCMV or CCMV and that 90% of the RhCMV ORFs show conservation at least at the protein family level (RL11 family) (Fig.17). In fact, almost every ORF carried between Rh31 (UL13) and Rh164 (UL141) of RhCMV is homologous to a corresponding gene in ape and human CMVs. Only two regions within the RhCMV genome diverged substantially from CMVs of humans and apes. The first region comprises the RL11 gene family, which shows an extremely high sequence variation even within a given CMV species such as HCMV [382]. The second unique genome region in RhCMV is homologous to the ULb′ region of

HCMV. This region contains a number of genes found only in OWM CMVs between the ORFs encoding homologues of UL141 and US1. In HCMV, this region also contains internal repeats that are lacking in RhCMV (e.g., IRS1) (Table 3). As previously described, laboratory strains of both HCMV and RhCMV display multiple deletions and mutations in this region due to fibroblast adaption [345]. Although diverging substantially between RhCMV and great ape CMVs, this area of the genome is highly conserved within the OWM CMVs with the exception of Rh165. The ORFs Rh165 and Rh166 are conserved among all RhCMV strains and also among the closely related CyCMV strain Ottawa, whereas SCMV and BaCMV carry only one copy of this gene, which shows stronger homology to Rh166 than to Rh165. As shown in Fig.19, Rh166 and Rh165 are closely related and most likely represent the result of a recent gene duplication event. The Rh165 ORF is thus the only bona fide RhCMV-specific ORF when our filtering criteria are applied. Rh166 shows weak homology to UL133 and UL138 of HCMV [393]. The UL133-UL138 locus in HCMV has been implicated in latency [113], and it has been hypothesized that the region Rh166-Rh171 performs a similar function in RhCMV [393]. However, the functions of these genes as well as those of most OWM CMV-specific genes in the ULb′ region remain to be determined. The only gene product in this region that has been characterized is Rh178 (VIHCE), which was shown to inhibit expression of the major histocompatibility complex class I (MHC-I) heavy chain [279, 280]. This region also carries several ORFs encoding predicted chemokines [345, 394].

HCMV ORF	Function	Reference
RL5A	no known function	
RL6	no known function	
RL8A	no known function	
RL9A	no known function	
RL10	no known function	
RL11	type 1 membrane protein; binds IgG Fc; involved in immune regulation	Lilley et al., [256]; Atalay et al., [257]
RL12	RL11 family, no known function	
UL1	RL11 family, no known function	
UL2	no known function	
UL4	RL11 family, no known function	
UL5	RL11 family, no known function	
UL7	RL11 family, no known function	
UL8	RL11 family, no known function	
UL9	RL11 family, no known function	
UL10	RL11 family, no known function	
UL15A	no known function	

UL16	NK-cell evasion through intracellular retention of NKG2D ligands MICB, ULBP1, and ULBP2	Dunn et al., [236]; Welte et al., [395]
UL18	MHC-I homologue, NK cell inhibition through binding to the inhibitory NK cell receptor LIR-1	Cosman et al., [396]; Reyburn et al., [397]; Prod'homme et al., [233]
UL22A	binds CC chemokine RANTES; involved in immune regulation (predicted)	
UL30A	no known function	
UL74A	no known function	
UL147A	no known function	
UL142	NK-cell evasion through intracellular retention of NKG2D ligands MICA and ULBP3	Chalupny et al., [238]; Bennett et al., [398]
UL140	no known function	
UL139	highly polymorphic type I membrane glycoprotein; shared sequence homology with human CD24, a signal transducer modulating B-cell activation responses	Qi et al., [399]; Bradley et al., [400]
UL138	needed to establish latency *in vitro* in CD34 hematopoietic progenitor cells	Goodrum et al., [113]
UL136	support of pUL138 expression	Grainger et al., [114]
UL135	support of pUL138 expression	Grainger et al., [114]
UL133	support of pUL138 expression	Grainger et al., [114]
UL148A	no known function	
UL148B	no known function	
UL148C	no known function	
UL148D	no known function	
UL150	no known function	
UL150A	no known function	
IRS1	transcriptional activator; blocks phosphorylation of eIF2alpha and host shutoff of protein synthesis; binds dsRNA; involved in gene regulation; involved in translational regulation	Hakki et al., [401]
US7	no known function	
US9	cytoplasmic glycoprotein dispensable for growth in tissue culture, no undisputed function	Huber et al., [402]; Mandic et al., [403]
US15	type 3 membrane protein; 7 transmembrane domains; possibly involved in virion morphogenesis (predicted)	
US16	type 3 membrane protein; 7 transmembrane domains; possibly involved in virion morphogenesis (predicted)	
US27	7 transmembrane domains; putative chemokine receptor; possibly involved in intracellular signaling (predicted)	
US33A	no known function	
US34	Expressed, no known function	Scott et al., [404]
US34A	no known function	

Table 3: HCMV ORFs not found in rhesus or OWM CMVs

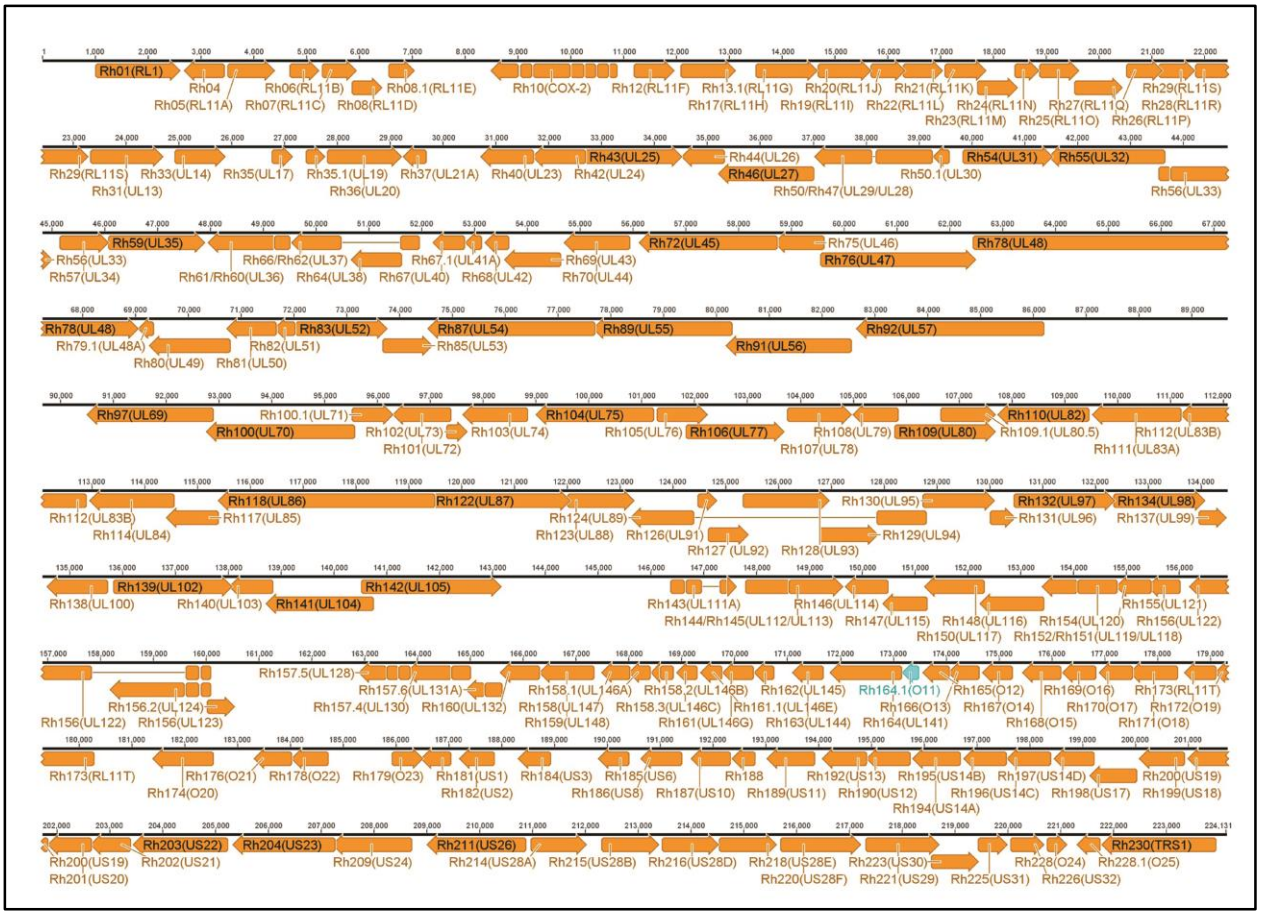

Fig.18: Annotated genome map of a hypothetical low-passage-number RhCMV isolate. The genome of this hypothetical RhCMV strain was generated as a consensus sequence between the fully sequenced strains 68-1 and 180.92 and the ULb′ region of low-passage-number isolate RhCMV CNPRC. It contains all ORFs found in a low-passage-number RhCMV isolate in their correct order. The nomenclature is based on the original nomenclature introduced by Hansen et al. ([359]), whereas the nomenclature in parentheses was newly introduced by Davison et al. ([3]) and was slightly modified by us. The genomic map was generated using Geneious Pro 5.5.2.

Besides the RL11 and ULb′ regions, only a small number of predicted genes are unique to the OWM CMV subfamily. One characterized gene is Rh10 (vCOX-2), a viral homologue to the gene for the host cyclooxygenase-2 enzyme (COX-2), which is a member of the eicosanoid synthetic pathway. Unlike HCMV ([405], [406],[407]), RhCMV does not induce cellular COX-2 expression, suggesting that the virus encodes its own copy of the protein to compensate for this lack. vCox-2 was shown to facilitate growth in endothelial cells ([352]). Other ORFs not found in great ape and human CMVs are Rh04, Rh188, Rh228, and Rh228.1, which have not been characterized to date.

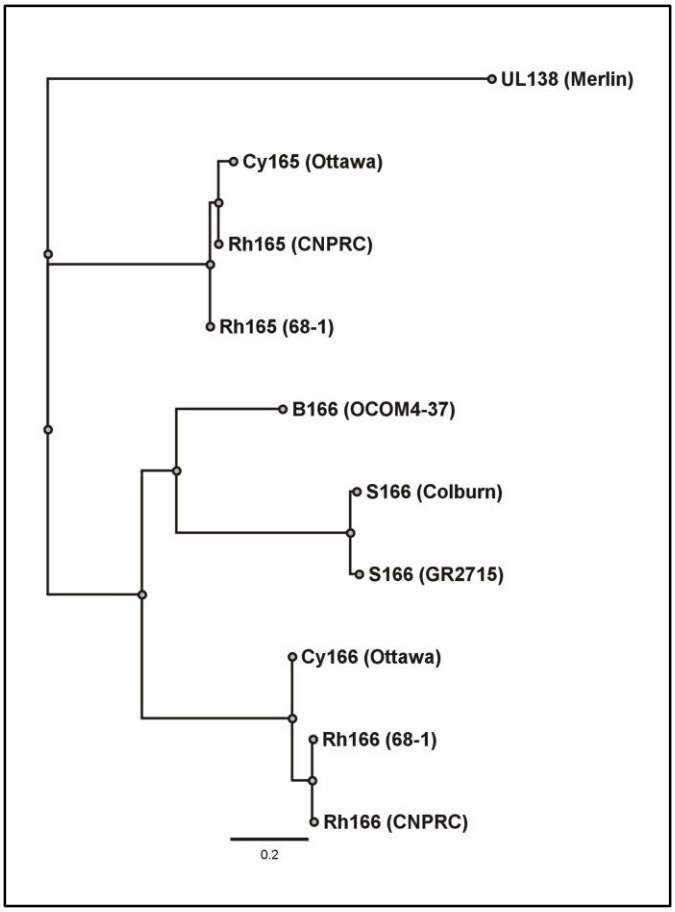

Fig.19: Phylogenetic tree of the OWM CMV Rh165/Rh166 gene family. UL138 of HCMV strain Merlin was used as an outgroup because it showed some homology to the Rh165/Rh166 gene family. The phylogenetic tree was generated in Geneious Pro 5.5.2.

HCMV-specific ORFs that are absent in RhCMV are summarized in Table 3, using the HCMV strain Merlin as the prototype HCMV strain [347, 364]. While most gene families are conserved between ape CMVs and monkey CMVs, individual family members might differ. Examples are the RL11, UL146/147, and US12 families of proteins. The latter comprises 10 members in HCMV from US12 to US21, of which the ORFs UL15 and UL16 have no homologue in RhCMV. No function is known for the two proteins encoded by these ORFs.

Interestingly, all OWM CMVs lack several known HCMV antagonists of NK cell function, including UL16, which retains the NKG2D ligands MICB, ULBP1, and ULBP2 [236, 395], UL141, which retains the NKG2D ligands MICA and ULBP3 [238, 398, 408], and UL18, which encodes an MHC-I homologue that binds to the inhibitory NK cell receptor LIR-1 [233, 396, 397]. Since evasion of NK cell immunity is conserved in MCMV [409], it is likely that OWM CMV-specific genes will encode NK cell evasins.

In addition to the HCMV-specific ORFs absent from WT RhCMV, the RhCMV 68-1 BAC lacks the endothelial cells tropism genes Rh157.5 (UL128) and Rh157.4 (UL130) [345], the above-mentioned UL36, and the viral chemokine genes Rh158.2 (vCXCL4), Rh158.3 (vCXCL3), and Rh161.1 (vCXCL2) (Fig.20).

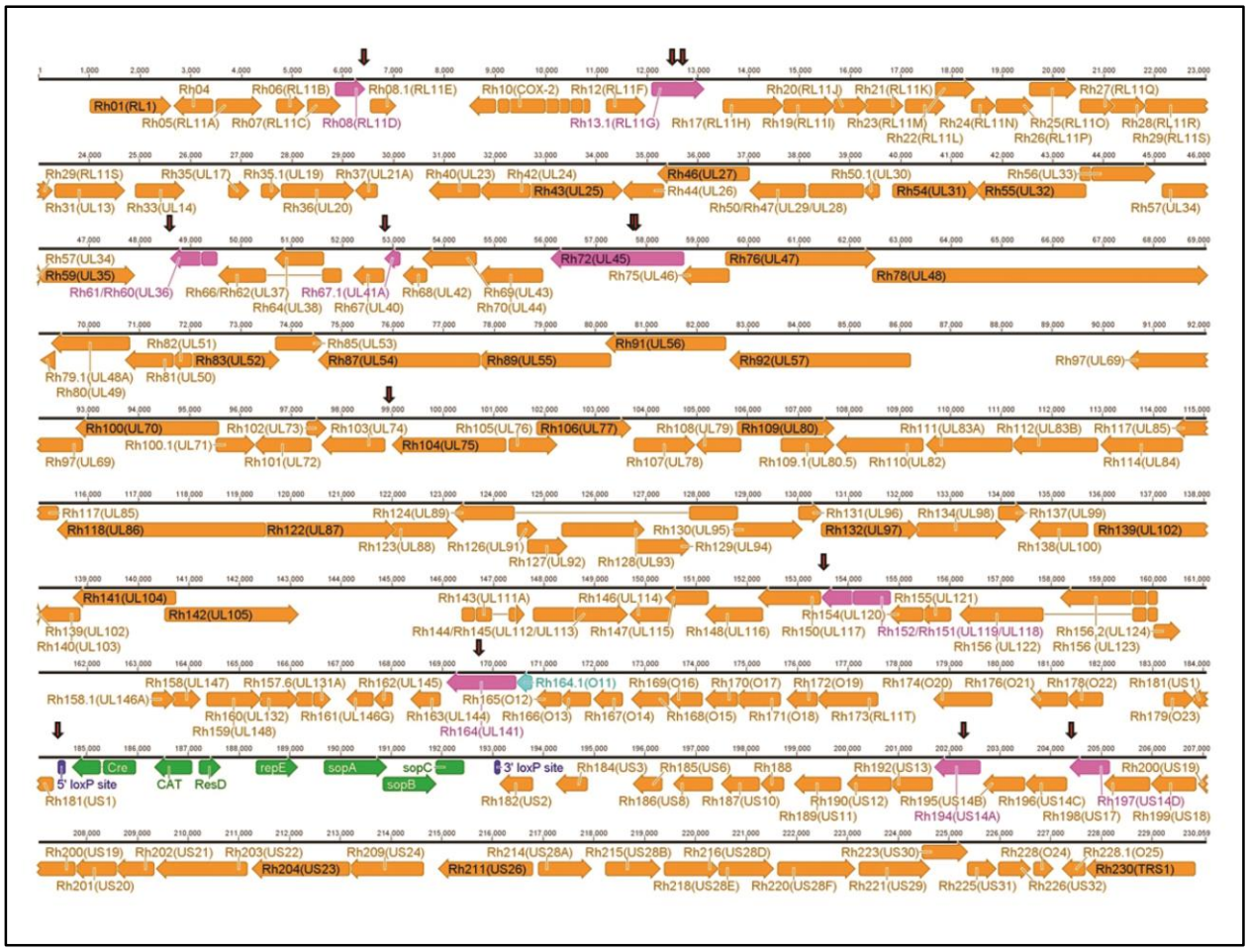

Fig.20: Annotated genome map of the RhCMV 68-1 BAC. The genome map shows the genomic organization of the RhCMV 68-1 BAC. Viral ORFs are shown in orange, and BAC genes are shown in green. Approximate locations of mutations described in Table 1 are shown as red arrows, and RhCMV ORFs mutated compared to the RhCMV 68-1 parental virus sequence are shown in pink. The ORF annotation is based on the filtered ORF analysis discussed in the text. The sequence is available in GenBank (accession number JQ795930). The genome map was generated using Geneious Pro 5.5.2.

3.4.4 Validating ORF predictions by proteomics.

To verify our ORF predictions in an unbiased manner, we determined the proteome of RhCMV virions. While this approach is limited to verifying ORFs that are incorporated into virions,

previous analysis of HCMV (59 proteins) ([18]) and MCMV (58 proteins) ([410]) virions revealed that more than a third of all predicted ORFs are contained in virus particles.

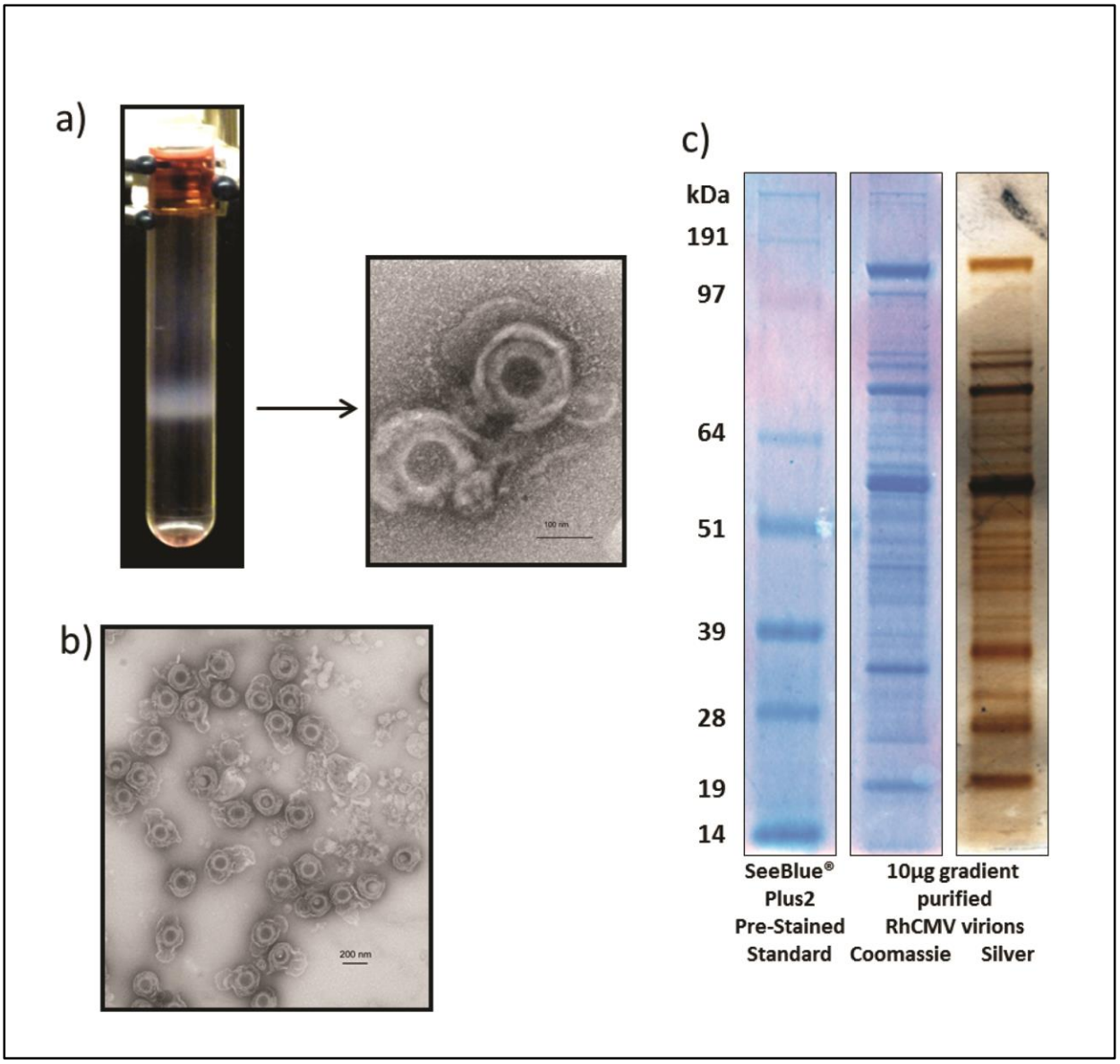

Fig.21: Isolation and purification of RhCMV virions. (a) RhCMV virions were purified from the supernatant of telomerized rhesus fibroblasts infected with BAC-derived RhCMV 68-1 (MOI of 0.01) until complete cytopathic effect was reached (10 days). Virus particles were separated and concentrated using a Nycodenz gradient as described in Materials and Methods. The single band isolated from the gradient was enriched for herpesvirus-like particles of 170 nm to 200 nm with an icosahedral capsid of 80 nm to 90 nm in diameter as revealed by electron microscopy. (b) Gradient-purified virus particles were highly enriched and contained only minor contaminations of cellular debris. No dense bodies or NIEPs could be identified. (c) Purified virions were lysed, and 10 µg of proteins were electrophoretically separated using NuPAGE MOPS gradient gels and visualized by Coomassie blue or silver staining.

A

RhCMV ORF	HCMV ORF	Description	Mol % WT Virions	STDEV
Rh112	UL83	Tegument protein pp65b	7.24	1.16
Rh118	UL86	major capsid protein (MCP)	7.22	0.30
Rh117	UL85	minor capsid protein (mCP)	6.62	1.21
Rh129	UL94	Tegument protein	4.89	0.44
Rh102	UL73	Envelope glycoprotein N	4.84	0.13
Rh44	UL26	Tegument protein	4.15	0.68
Rh59	UL35	Tegument protein	3.81	0.23
Rh111	UL83	Tegument protein pp65a	3.76	0.30
Rh138	UL100	Envelope glycoprotein M	3.69	0.26
Rh75	UL46	Triplex capsid protein, subunit 1	3.45	0.36
Rh104	UL75	Envelope glycoprotein H	3.17	0.21
Rh137	UL99	Myristylated tegument protein	2.97	0.55
Rh43	UL25	Tegument protein	2.87	0.28
Rh55	UL32	Tegument protein pp150	2.86	0.12
Rh215	US28	G-protein coupled receptor homologue (vGPCR2)	2.75	0.33
Rh100.1	UL71	Tegument protein	2.74	0.78
Rh110	UL82	Tegument protein pp71	2.73	0.23
Rh230	TRS1	Tegument protein	2.44	0.25
Rh89	UL55	Envelope glycoprotein B	2.38	0.24
Rh79.1	UL48A	Small capsid protein	2.34	0.06
Rh203	US22	Tegument protein	2.17	0.11
Rh72	UL45	Ribonucleotide reductase subunit 1 (R1)	2.10	0.13
Rh148	UL116		1.65	0.51
Rh76	UL47	Tegument protein	1.51	0.16
Rh78	UL48	Large tegument protein	1.42	0.06
Rh140	UL103	Tegument protein	1.35	0.30
Rh123	UL88	potential Tegument protein	1.32	0.07
Rh106	UL77	Virion-packaging protein	1.21	0.08
Rh147	UL115	Envelope glycoprotein L	1.03	0.11
Rh164	UL141	Membrane glycoprotein	0.92	0.18
Rh132	UL97	Phosphotransferase	0.87	0.13
Rh211	US26		0.85	0.21
Rh160	UL132	Envelope glycoprotein	0.82	0.31
Rh56	UL33	G-protein coupled receptor homolog, Envelope glycoprotein	0.78	0.21
Rh152/Rh151	UL119/UL118	Membrane glycoprotein, Viral Fc-gamma receptor	0.70	0.85
Rh128	UL93	capsid-associated protein	0.65	0.11
Rh141	UL104	Capsid portal protein	0.47	0.04
Rh216	US28	G-protein coupled receptor homologue (vGPCR3B)	0.36	0.31
Rh131	UL96	Tegument protein	0.35	0.33
Rh17	RL11 family		0.32	0.01
Rh42	UL24	Tegument protein	0.29	0.17
Rh214	US28	G-protein coupled receptor homologue (vGPCR1)	0.28	0.01
Rh173	RL11 family		0.27	0.01
Rh218	US28	G-protein coupled receptor homologue (vGPCR4)	0.27	0.12
Rh164.1			0.22	0.39
Rh103	UL74	Envelope glycoprotein O	0.20	0.07
Rh165			0.18	0.32
Rh13.1	RL13	Membrane protein	0.14	0.24
Rh109	UL80	Capsid maturation protease	0.10	0.07
Rh70	UL44	DNA polymerase processivity factor	0.10	0.00
Rh05	RL11 family		0.09	0.16
Rh156 (IE2)	UL122	immediate-early protein 2, pp86	0.08	0.08
Rh81	UL50	Nuclear egress membrane protein	0.04	0.06
Rh31	UL13		NQ	NQ
Rh67.1	UL41A		NQ	NQ
Rh83	UL52	DNA Packaging protein	NQ	NQ
Rh114	UL84	Initiator protein	NQ	NQ
Rh134	UL98	Deoxyribonuclease	NQ	NQ
Rh139	UL102	DNA helicase/primase complex-associated protein	NQ	NQ
Rh143	UL111A	Viral interleukin 10, cmvIL-10	NQ	NQ
Rh144/Rh145	UL112/UL113	Early phosphoprotein p84	NQ	NQ
Rh166			NQ	NQ
Rh170			NQ	NQ
Rh171			NQ	NQ
Rh176			NQ	NQ
Rh181	US1		NQ	NQ
Rh190	US12	Membrane protein	NQ	NQ
Rh199	US18	Transmembrane protein	NQ	NQ
Rh209	US24	Tegument protein	NQ	NQ
Rh223	US30	potential Membrane protein	NQ	NQ
Total			100.00	

B

Capsid		
RhCMV ORF	HCMV ORF	Mol % WT Virions
Rh118	UL86	7.22
Rh117	UL85	6.62
Rh75	UL46	3.45
Rh79.1	UL48A	2.34
Rh106	UL77	1.21
Rh128	UL93	0.65
Rh141	UL104	0.47
Rh109	UL80	0.10
Total		22.03

Tegument		
RhCMV ORF	HCMV ORF	Mol % WT Virions
Rh112	UL83	7.24
Rh129	UL94	4.89
Rh44	UL26	4.15
Rh59	UL35	3.81
Rh111	UL83	3.76
Rh137	UL99	2.97
Rh43	UL25	2.87
Rh55	UL32	2.86
Rh100.1	UL71	2.74
Rh110	UL82	2.73
Rh203	US22	2.17
Rh76	UL47	1.51
Rh78	UL48	1.42
Rh140	UL103	1.35
Rh131	UL96	0.35
Rh42	UL24	0.29
Rh209	US24	NQ
Total		45.09

Envelope and Glycoproteins		
RhCMV ORF	HCMV ORF	Mol % WT Virions
Rh102	UL73	4.84
Rh138	UL100	3.69
Rh104	UL75	3.17
Rh215	US28	2.75
Rh89	UL55	2.38
Rh147	UL115	1.03
Rh164	UL141	0.92
Rh160	UL132	0.82
Rh56	UL33	0.78
Rh152/Rh151	UL119/UL118	0.70
Rh216	US28	0.36
Rh214	US28	0.28
Rh218	US28	0.27
Rh103	UL74	0.20
Rh13.1	RL13	0.14
Rh81	UL50	0.04
Rh67.1	UL41A	NQ
Rh190	US12	NQ
Total		22.38

Uncharacterized		
RhCMV ORF	HCMV ORF	Mol % WT Virions
Rh148	UL116	1.65
Rh123	UL88	1.32
Rh211	US26	0.85
Rh17	RL11 family	0.32
Rh173	RL11 family	0.27
Rh164.1		0.22
Rh165		0.18
Rh05	RL11 family	0.09
Rh31	UL13	NQ
Rh166		NQ
Rh170		NQ
Rh171		NQ
Rh176		NQ
Rh181	US1	NQ
Rh199	US18	NQ
Rh223	US30	NQ
Total		4.92

Transcription-replication machinery		
RhCMV ORF	HCMV ORF	Mol % WT Virions
Rh230	TRS1	2.44
Rh72	UL45	2.10
Rh132	UL97	0.87
Rh70	UL44	0.10
Rh156 (IE2)	UL122	0.08
Rh83	UL52	NQ
Rh114	UL84	NQ
Rh134	UL98	NQ
Rh139	UL102	NQ
Rh144/Rh145	UL112/UL113	NQ
Total		5.58

Secreted proteins		
RhCMV ORF	HCMV ORF	Mol % WT Virions
Rh143	UL111A	NQ
Total		0.00

Fig.22: Protein composition of BAC-derived RhCMV 68-1 virions. (A) Shown are all proteins identified in at least one of six MS/MS runs (two biological repeats and four technical repeats; see Fig.S2 in the supplemental material). Red bars indicate the ranked average abundance relative to total virion proteins of all virus proteins identified in three repeat runs of the same sample (repeats B01, B02, and B03 of biological repeat B). The standard deviation (STDEV) is shown for the mean abundance of the three technical repeats. Proteins identified in other experiments are included but were not quantified (NQ). If applicable, the HCMV nomenclature for the identified proteins is shown in addition to the RhCMV nomenclature. (B) The proteins shown in panel A were grouped according to function and/or localization within the virion.

The RhCMV BAC-derived virions were isolated from the supernatant of infected telomerized rhesus fibroblasts as described earlier for HCMV ([18]). Virions formed a single band on the Nycodenz gradient (Fig.21a), and the most abundant proteins contained in this fraction migrated at very similar molecular weights as and displayed abundances comparable to those of previously reported HCMV virion proteins upon NuPAGE (Fig.21c) ([18]). Additionally, we performed electron microscopy (EM) to visualize the particles and to verify the purity of the sample (Fig.21a and b). RhCMV virions displayed a diameter ranging from 170 nm to 200 nm, with an icosahedral capsid of about 80 nm to 90 nm in diameter. While some cell debris contamination was visible (Fig.21b), all viral particles were highly similar in appearance, indicating that the virion preparation was rather homogeneous.

The samples were then analyzed by one-dimensional (1D) LC-MS/MS and searched against stop-to-stop translated sequences of the RhCMV 68-1 BAC genome for ORFs encoding proteins of ≥30 aa. The virion preparation was analyzed by MS/MS upon elution from a 0 to 100% acetonitrile gradient over 100 min. This analysis was repeated three times, and the protein content in each experiment was quantified. The average percentage of these results is shown in Fig.22 (red bars). Additional proteins that were identified in independent virion preparations or upon MS/MS from samples eluted over a longer time period (10 h) from a 0 to 40% nanoAcquity gradient are also shown, but these proteins were not included in the quantification. In total, peptides of 70 different viral proteins could be identified in at least one of the MS/MS analyses (Fig.22A; see Tables S1 and S2 in the supplemental material). All of these proteins were predicted by the filtered ORFs (Fig.20), with the exception of Rh164.1, which was not included in our prediction due to its small size. However, similar to the case for other predicted ORFs, Rh164.1 is highly conserved in OWM CMVs (O11). In contrast, "RhCMV-specific" ORFs predicted by ORFinder (see Fig.S2 in the supplemental material) were notably absent from the virion proteome. The proteomics analysis thus further supported our conclusion that most, if not all, RhCMV ORFs are shared among OWM CMVs. Of the 70 proteins identified in RhCMV virions, 61 are homologous to HCMV, and these homologues corresponded to 99 mol% of virion proteins. Thus, RhCMV virions consist largely of proteins conserved in HCMV. The most abundant protein was pp65b (Rh112, UL83b). With 7.24 mol%, pp65b was almost twice as abundant as the second pp65 homologue, pp65a (Rh111, UL83a), with 3.76 mol%. Interestingly, this correlates with the finding that Rh112 induces stronger humoral and cellular immune responses *in vivo* than Rh111 [343, 411]. Overall, 22.03 mol% of the proteins can be categorized as capsid proteins, 45.09 mol% as tegument proteins, 22.38 mol% as envelope or glycoproteins, and 5.58 mol% as proteins of the transcription-replication machinery, and 4.92 mol% are uncharacterized proteins with unknown function and localization (Fig.22B). These ratios are remarkably similar to ratios determined by Varnum et al. [18] for HCMV AD169, where the virion was comprised of 30% capsid proteins, 50% tegument proteins, 13% envelope proteins, and 7% undefined proteins. The only major difference between HCMV and RhCMV is that capsid proteins were comprised of larger amounts of UL48A and UL80 (12.6% and 7.7%, respectively) in HCMV, whereas among the RhCMV capsid proteins they comprised only 2.34 mol% and 0.10 mol%.

3.5 Discussion

By combining genomic sequencing, comparative genomics, and virion proteomics, we generated a simplified ORF map of rhesus CMV in general and of a molecular clone derived from strain 68-1 in particular. This approach filtered out many ORFs previously annotated as RhCMV specific, resulting in a much larger percentage of RhCMV ORFs being homologous to HCMV ORFs than previous ORF annotations had indicated. Our results thus further support the notion that infection of rhesus macaques by RhCMV is the closest animal model for infection of humans with HCMV.

Molecular cloning of viral strains as a BAC singles out a viral genome as the starting point for the generation of all subsequent recombinant viruses. Thus, any initial genomic variability that was present in the original isolate will be lost upon BAC cloning. Moreover, the *in vitro* manipulation and selection occurring during BAC cloning might further result in divergence from the BAC-derived genome of the parental sequence. However, the sequence relationship between parental CMV isolates and their BAC offspring has, so far, been studied in only one example: the HCMV isolate Merlin. In that instance, the BAC clone of Merlin contained 12 differences compared to the parental isolate [303]. For RhCMV, we concluded that the BAC-derived sequence contained 13 nucleotide differences compared to strain 68-1. Thus, it seems that the genetic variability present within a given CMV strain or isolate is rather limited and that BAC-derived molecular clones contain a very limited set of mutations. In contrast, recent deep-sequencing results for CMV genomes *ex vivo* revealed the presence of an unexpectedly complex mixture of viral genomes within a given host [412]. However, once an isolate is established *in vitro*, the genomic variability seems to be quite limited, consistent with a low error rate during genome replication.

Interestingly, the adaptations that do occur as a consequence of adaptation to *in vitro* culture of CMV isolates in fibroblasts are remarkably similar in HCMV and RhCMV. Five of the 12 mutations occurring in the Merlin BAC clustered in UL36 [303]. Similarly, the UL36 homologue Rh61/Rh60 is mutated in the RhCMV BAC compared to the parental 68-1 strain ([376]). The independent mutation of this gene upon BAC cloning in two different CMV species indicates that expression of UL36 might represent a growth disadvantage *in vitro* despite its antiapoptotic function. In addition, the Merlin BAC contained mutations in RL13, and repairing this gene decreased growth kinetics in fibroblasts [303]. The RhCMV BAC contained frameshift mutations in the RL13 homologue Rh13.1. Since this gene is truncated in the parental 68-1 strain,

it seems that several independent Rh13.1 mutants were present in the original isolate. A detailed analysis of HCMV adaptations to tissue culture revealed that RL13 is rapidly mutated ([413]) even when starting with a molecular clone [303]. Thus, RL13 seems to be rapidly selected against in both HCMV and RhCMV. Finally, the gene UL128 is mutated even prior to BAC cloning both in RhCMV and in many HCMV isolates, including Merlin, as discussed elsewhere [414]. Thus, compared to their original wild-type strains, BAC clones of both HCMV and RhCMV share mutations in three loci: RL13, UL36, and UL128. In previous work, two of these genes, UL36 and UL128, were repaired in RhCMV [415], and it was demonstrated that the repaired virus grew better in epithelial cells. In contrast, repair of RL13 would be expected to decrease the ability of RhCMV to grow in tissue culture as reported for HCMV.

The impact of these tissue culture adaptations on viral pathogenesis *in vivo* is not known for HCMV, but a recent side-by-side comparison of BAC-derived RhCMV and a low-passage-number isolate of RhCMV revealed clear signs of attenuation, such as lower plasma titers and shedding [416]. However, these attenuations do not prevent BAC-derived RhCMV vectors from establishing persistent infections, even in CMV-positive hosts, as evident from persistent immune stimulation and long-term, low-level shedding of RhCMV expressing heterologous antigens [353, 359]. Moreover, RhCMV 68-1 and BAC-derived vectors retain their pathogenicity in fetal macaques [417]; P. A. Barry, unpublished data). Therefore, it seems that while tissue culture adaptations render BAC-derived viruses less fit, they still seem to retain the ability to establish and maintain persistent infection.

With a total of 173 ORFs, the number of ORFs in the simplified ORF map of RhCMV is now in the same range as that reported for other primate CMVs. As shown previously for CCMV and HCMV [346], the comparison of closely related CMV genomes greatly facilitates genome annotations. By taking into account recently completed full genome sequences of CMVs from Old World monkey (OWM) species such as African green monkey (Cercopithecus aethiops), baboon (Papio anubis), and cynomolgus macaque (Macaca fascicularis) [321, 418], we constructed a hypothetical WT genome. This was facilitated by the fact that the genomes of OWM CMVs are highly conserved across different species, with only very minor differences. Most previously annotated RhCMV-specific genes are now absent from our prediction. We believe that these ORFs are unlikely to encode unique proteins, since most of them are shorter than average and are carried within other ORFs on either the same or the opposite DNA strand. Moreover, all ORFs identified

by proteomics analysis are conserved among OWM CMVs despite the fact that the peptides were compared to an unbiased database consisting of stop-to-stop translated sequences of the RhCMV 68-1 BAC genome for ORFs encoding ≥30 aa. While it is conceivable that virion proteomics shows a bias toward highly conserved ORFs compared to proteomics of the infected cell, our analysis detected more than one-third of the predicted ORFs, including many ORFs not conserved in ape or human CMV or with low homology. Thus, while the proteomics analysis does not formally rule out that RhCMV-specific ORFs exist, the results suggest that such ORFs must be rather rare.

Taken together, this analysis suggested a high degree of conservation between OWM CMVs and HCMV, not only with respect to genome structure but also for the virion composition, since the relative abundances of many proteins and the abundance rankings of the proteins were very similar in RhCMV 68-1 and HCMV AD169. Moreover, some of the differences detected might be due to experimental differences in virion preparations and data analysis. For instance, the large amounts of UL80.5 detected in HCMV virions might indicate the presence of noninfectious particles (NIEPs) in the HCMV virion preparations, since NIEPs were not separated by the gradient used. Moreover, the method we used to determine the relative abundance of proteins in our sample was different from the technique used in the previous study. Whereas Varnum et al. [18] averaged the intensities of the Fourier transform ion cyclotron resonance (FTICR) MS spectra of the most abundant peptides for each protein to determine the relative quantities of the viral proteins, we transformed our raw peptide data into molarities and ranked the proteins as moles percent of the entire protein amount, taking into account only viral proteins.

In summary, the combined analysis of BAC sequence, comparative genome analysis, and virion proteomics performed here revealed a much closer evolutionary relationship between rhesus and human CMVs than previously assumed. These results thus further validate the importance of RhCMV as a model system for HCMV, because the close genomic relationship facilitates deciphering the role of individual genes and gene families found only in primate CMVs for infection, spread, and pathogenesis as well as for shaping and evading the host's immune response. Together with the close relationship of the host species, this similarity aids the development of therapeutic and preventative approaches for HCMV as well as the development and evaluation of novel CMV-based vaccine vector systems.

3.6 Acknowledgements

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4. Cytomegalovirus pp65 limits dissemination but is dispensable for persistence

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4.1 Abstract

The most abundantly produced virion protein in human cytomegalovirus (HCMV) is the immunodominant phosphoprotein 65 (pp65), which is frequently included in CMV vaccines. Although it is nonessential for in vitro CMV growth, pp65 displays immunomodulatory functions that support a potential role in primary and/or persistent infection. To determine the contribution of pp65 to CMV infection and immunity, we generated a rhesus CMV lacking both pp65 orthologs (RhCMVΔpp65ab). While deletion of pp65ab slightly reduced growth in vitro and increased defective particle formation, the protein composition of secreted virions was largely unchanged. Interestingly, pp65 was not required for primary and persistent infection in animals. Immune responses induced by RhCMVΔpp65ab did not prevent reinfection with rhesus CMV; however, reinfection with RhCMVΔUS2-11, which lacks viral-encoded MHC-I antigen presentation inhibitors, was prevented. Unexpectedly, induction of pp65b-specific T cells alone did not protect against RhCMVΔUS2-11 challenge, suggesting that T cells targeting multiple CMV antigens are required for protection. However, pp65-specific immunity was crucial for controlling viral dissemination during primary infection, as indicated by the marked increase of RhCMVΔpp65ab genome copies in CMV-naive, but not CMV-immune, animals. Our data provide rationale for inclusion of pp65 into CMV vaccines but also demonstrate that pp65-induced T cell responses alone do not recapitulate the protective effect of natural infection.

4.2 Introduction

Human cytomegalovirus (HCMV) persistently infects most of humanity [419]. While the vast majority of these infections are asymptomatic and not associated with any pathologic consequence, HCMV can cause serious disease in the setting of immune deficiency or immaturity, including late-stage AIDS, iatrogenic immune suppression (particularly, organ and stem cell transplantation), and fetal infection (where infection can cause hearing loss and mental retardation) [420, 421]. In maternal-to-fetal transmission and, to a certain extent, with transplantation, the most serious disease appears to arise in the setting of primary HCMV infection [421-423]. Therefore, vaccination has been proposed as potential intervention to ameliorate these poor outcomes [41]. Although it was initially thought that an effective HCMV vaccine might prevent acquisition of HCMV altogether, accumulating data indicate that even the potent natural immunity elicited by persistent HCMV infection of healthy subjects is, at best, only partially protective against superinfection [424]. Thus, conceptually, the most realistic goal of an HCMV vaccine would be to establish a similar level of immunity as present in typical $HCMV^+$ individuals, in $HCMV^-$ females prior to pregnancy, or all $HCMV^-$ subjects prior to transplantation with $HCMV^+$ cells or tissue, so as to prevent the potentially severe consequences of primary infection in these subjects. Indeed, due to the importance of HCMV in causing congenital disease and complications in transplant recipients, vaccine development efforts have been given high priority by the Institute of Medicine of the National Academy of Sciences [42].

While initial approaches to CMV vaccines focused on the development of an attenuated strain of HCMV (Towne) [425, 426], more recently, the focus has shifted toward the development of subunit vaccines [427], either single antigen vaccines [56] or cocktails of antibody-inducing and T cell–inducing subunits [428]. A frequently used T cell–inducing subunit in the development of CMV vaccines is the phosphoprotein 65 (pp65), which is consistently a major target for the T cell response in infected individuals [121, 429-431]. HCMV pp65 is part of the viral tegument and the most abundant virion protein [18]. Multiple functions in modulation of innate and intrinsic immunity [151, 152, 432] as well as adaptive immune responses [230, 433, 434] have been assigned to HCMV pp65. Moreover, pp65 has been shown to modulate the activity of serine/threonine kinases [435-437], Polo-like kinase 1 [438], and the viral UL97 serine/threonine kinase [439]. Nevertheless, pp65 is dispensable for viral replication in HCMV-infected fibroblasts

[440], but pp65-deleted HCMV showed decreased virus production in monocyte-derived macrophages [441].

The role of pp65 for the establishment and maintenance of persistent infection by HCMV is unknown, due to the strict species specificity of HCMV that does not infect immunocompetent experimental animals. Thus, animal CMVs are generally used as models to study CMV and CMV vaccines [374, 442, 443]. Since host restriction resulted in coevolution of CMVs with their respective hosts, infection of rhesus macaques (RMs) with rhesus CMV (RhCMV) represents an animal model that closely resembles infection of humans with HCMV [375]. We therefore used this model to study the role of RhCMV pp65 in infection and immunity. RhCMV encodes 2 ORFs, Rh111 and Rh112, with comparable homology to HCMV pp65 (pp65a ~34%, pp65b ~40%) and 40% identity to each other [343, 411]. The 2 proteins combined comprise approximately 11% of the entire viral proteome in RhCMV virions [444], which is similar to HCMV, in which the single pp65 protein makes up 15% of the virion proteins [18]. To examine the function of pp65 in vitro and in vivo, we deleted both pp65 homologs from the genome of RhCMV. We characterized the impact of pp65 deletion on viral growth in vitro and on the composition of the virion proteome. We then determined the role of pp65 for the ability of RhCMV to establish primary or secondary persistent infection in $RhCMV^+$ or $RhCMV^-$ animals, respectively. By challenging with recombinant RhCMV lacking the immunoevasins US2, 3, 6, and 11, a virus incapable of superinfecting, we further evaluated whether pp65-specific T cells are required for the protective effect of preexisting CMV infection or sufficient to recapitulate T cell–mediated protection induced by natural infection. Our observations demonstrate a unique physiologic role for pp65 in CMV biology and, moreover, have implications for the use of pp65 as a subunit vaccine.

4.3 Methods

4.3.1 Cells and reagents. TRFs [377] were maintained in DMEM with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin and were grown at 37°C in humidified air with 5% CO_2.

4.3.2 Viruses and construction of recombinant mutants. BAC-derived RhCMV 68-1 [344] was reconstituted by electroporating BAC DNA into TRFs (250 V, 950 μF), and cytopathic effect was observed after 7 to 10 days. Recombinant RhCMV mutants were created by homologous recombination [445, 446] in *E. coli* strain EL250, which contains heat-inducible λ-recombination (rec) genes and an arabinose-inducible FLP recombinase [381]. Bacterial cultures were grown in LB at 30°C until an OD of 0.35 at 600 nm was reached, and the rec genes were induced through heat induction by shaking the culture at 42°C in a water bath for 15 minutes. The bacteria were subsequently chilled on ice for 10 minutes and made electrocompetent by washing them 4 times with cold, deionized water. Electrocompetent EL250 were always made and used fresh to increase the recombination efficiency.

To construct the pp65a and pp65b (RhCMV-Δpp65ab) double-deletion virus, recombination primers containing 50 bp homology to regions flanking the pp65 ORFs (forward mutagenesis primer 5′-GAAATAAGTGTGCGGTCTCGGGGGATTGGGGTTTTTATATAGGTATGGGT-3′ and reverse mutagenesis primer 5′-ATGAGCCAAGTTGCGCAGCTCAGTCGGCGGTGTCGCCAAAGTCAGACAAC-3′) were used to amplify a kanamycin (Kan) resistance cassette from plasmid pCP015 [447]. The pCP015 forward primer binding site (5′gtaaaacgacggccagt) and reverse primer binding site (5′gaaacagctatgaccatg) were added to the 3′ end of the mutagenesis primers.

Competent EL250 bacteria containing WT RhCMV BAC were then electroporated with the PCR product for recombination using a MicroPulser (Bio-Rad) and selected for Kan and chloramphenicol (Cm) resistance at 30°C on LB agar. To induce the FLP recombinase excising the Kan^r cassette, clones were grown in LB with Cm until they reached an OD of 0.5 at 600 nm and incubated with 1 mg/ml arabinose for 1 hour. The bacteria were streaked out on an LB plate with Cm selection using an inoculation loop and incubated overnight at 30°C. After colonies were visible, clones were replica plated on LB agar with Kan and Cm and LB agar with Cm only, and

colonies were selected that had lost Kan^r and characterized by restriction digest, Southern blot, and partial sequencing. Virus was reconstituted by electroporation of TRFs with 5 to 10 μg of BAC DNA.

To construct the second pp65ab double-deletion mutant containing the SIV ref/tat/nef (retanef) fusion protein, which was driven by the EF1α promoter and inserted in place of the pp65ab proteins, recombination primers containing the same 50 bp homology to regions flanking the pp65 ORFs as before were used to amplify a Kan resistance cassette from plasmid pCP015rtndx [353]. The pCP015rtndx forward primer binding site (5′gtaaaacgacggccagt) and reverse primer binding site (5′gtatgttgtgtggaattgtgag) were added to the 3′ end of the mutagenesis primers. All subsequent steps to generate the final mutant virus were the same as described above. The lack of expected genes in the recombinant viruses and lack of WT contamination was confirmed by Western blot analysis of purified viral stocks (Fig.24D) and RT-PCR of cDNA from virally infected cells (Fig.23A). The antibodies used in this study to confirm the presence or absence of RhCMV viral proteins were generated at the VGTI Monoclonal Antibody Core (mouse α-RhCMV pp65a clone 3H3.1.2, mouse α-RhCMV pp65b clone 19C12.2, and mouse α-RhCMV clone 6H7.3).

4.3.3 RhCMV particle purification procedures. RhCMVΔpp65ab virions were purified over a discontinuous Nycodenz gradient, as described before for HCMV AD169 [18] and RhCMV 68-1 BAC-derived WT [444]. The virus was isolated from the culture medium of infected TRFs when the cells displayed maximal cytopathic effect. The cellular supernatants were first clarified by centrifugation at 7,500 *g* for 15 minutes. The clarified medium was layered over a sorbitol cushion (20% D-sorbitol, 50 mM Tris [pH 7.4], 1 mM $MgCl_2$), and virus was pelleted by centrifugation at 64,000 *g* for 1 hour at 4°C in a Beckman SW28 rotor. The virus pellet was resuspended in TNE buffer (50 mM Tris [pH 7.4], 100 mM NaCl, and 10 mM EDTA). The virus particles were further purified by layering them over a discontinuous 5% to 50% Nycodenz gradient (Sigma-Aldrich) in TNE buffer and centrifuging at 111,000 *g* for 2 hours at 4°C in a Beckman SW41 Ti rotor. The virion bands in the gradient were isolated with a syringe through the side of the centrifuge tube, and the particles were pelleted in a Beckman TLA-45 rotor in a Beckman Optima TL 100 Ultracentrifuge at 40,000 *g* for 1 hour and washed twice with TNE buffer. The pellet was resuspended in TNE buffer, and electron microscopy was performed to confirm the purity of the

sample. In order to assess the protein content of the purified RhCMVΔpp65ab virions, especially in comparison to a parental RhCMV WT sample, a denatured protein preparation was separated on a NuPAGE morpholine propanesulfonic acid (MOPS) gradient gel (Invitrogen) and visualized by Coomassie brilliant blue staining (Fig.24C).

4.3.4 Quantitative proteomic analysis. The quantitative proteomic analysis was performed as previously described in detail [444]. Briefly, RhCMV particles were denatured in 8 M urea, 100 mM NH_4HCO_3, and 5 mM DTT, and the cysteine residues were alkylated with 10 mM iodoacetamide. Then, the samples were 4-fold diluted with 25 mM NH_4HCO_3 and 1 mM $CaCl_2$ and digested overnight with a 1:20 (mass/mass) trypsin-to-protein ratio. The digested peptides were desalted with C18 cartridges and dried in a vacuum centrifuge before being separated in capillary columns (75 μm × 65 cm capillary [Polymicro] packed with 3-μm C18 particles [Phenomenex]) connected to a custom-made 4-column liquid chromatography LC system [448] or a longer capillary column (75 μm × 100 cm) connected to a nanoAcquity system (Waters). Eluting peptides were analyzed directly in a linear ion-trap orbitrap mass spectrometer (LTQ Orbitrap XL, Thermo Scientific).

Collected MS/MS spectra were searched against forward and reverse sequences of the RhCMV ORFs (275 sequences), *Macaca mulatta* Ensembl database (21,905 sequences, downloaded from http://www.ensembl.org on November 15, 2010), and 186 common contaminants (downloaded from http://www.ncbi.nlm.nih.gov/protein on August, 2006). Identified peptides were first filtered with a mass spectrum–generating function (MS-GF) [449] probability of $\leq 1 \times 10^{-8}$ and, to ensure a low false discovery rate, each protein was required to have at least one peptide with MS-GF $\leq 1 \times 10^{-10}$. Protein abundances were estimated by exponentially modified protein abundance index as previously described [380]. Data are available at Pacific Northwest National Laboratory (http://omics.pnl.gov) and in the PeptideAtlas online database (http://www.peptideatlas.org; dataset identifier: PASS00367).

4.3.5 RMs. A total of 9 male and 4 female purpose-bred juvenile RMs (*M. mulatta*) of Indian genetic background were used in this study. All RMs were specific-pathogen free (SPF), as defined by being free of cercopithecine herpesvirus 1, D-type simian retrovirus, simian T lymphotrophic virus type 1, SIV, rhesus rhadinovirus, *Mycobacterium tuberculosis*, and RhCMV infection at the

start of the study. The Δpp65ab-deleted RhCMV vector was tested in vitro by administering the deletant virus s.c. at a dose of 1×10^7 PFUs to 2 SPF (defined above) RMs. To test whether the immune responses generated by the primary infection with Δpp65ab were sufficient to prevent superinfection, the same 2 RMs were inoculated with the same 1×10^7 PFUs dose with ΔVIHCEΔUS2-11gag, followed by superinfection with RhCMVgag and later with Δpp65ab(retanef).

To determine whether vaccine-induced pp65-specifc T cells could prevent CMV infection, 3 SPF RMs were vaccinated intramuscularly with 1.0 mg pND/pp65b DNA followed by an intramuscular boost using 5×10^8 PFUs pp65b-MVA at week 6 and 12 after DNA vaccination. For a control in these experiments, 3 additional SPF RMs were vaccinated following the same vaccine strategy using pND (empty) and MVA (empty), respectively [450, 451]. The plasmids pND and pND/pp65b were provided by Peter A. Barry, UCD, Davis, California, USA [450]; the empty MVA as well as the pp65b-expressing recombinant MVA were provided by Don J. Diamond, City of Hope, Duarte, California, USA. As a first step, the gene encoding RhCMV 68-1 pp65-2 was amplified from previously described plasmid expression vectors and engineered into the pZWIIA MVA transfer vector using established protocols [451]. rMVA expressing RhCMV pp65-2 (Rhpp65-MVA) was generated on BHK-21 cells via homologous recombination. The protein expression levels for RhCMV pp65-2 in infected BHK-21 cells were confirmed by Western blot using polyclonal antibodies to RhCMV pp65-2 by chemiluminescence detection (ECL, Amersham Pharmacia Biotech). The plasmid DNA of pND and pND/pp65-2 was isolated using the EndoFree Plasmid Mega Kit (Qiagen) to avoid any endotoxin contamination of the DNA. Vaccinated animals were challenged s.c. 18 weeks after the initial DNA vaccination with 10^7 PFUs RhCMV ΔUS2-11gag.

BAL fluid, peripheral blood, and urine samples were collected at specified time points (see Fig.25-27) throughout the entire experiment. Isolated $CD4^+$ and $CD8^+$ T cells from BAL fluid and peripheral blood were stimulated with antigen-specific peptides to examine the immune response induced by the initial vaccination and the subsequent viral challenge.

4.3.6 Nested real-time PCR. To determine the viral copy numbers of RhCMV 68-1 GAG and RhCMVΔpp65ab retanef, 3 naive and 2 CMV^+ RMs were infected s.c. with 10^7 PFUs of each virus in the opposite arm on the same day. Blood samples were taken once a week to monitor $CD4^+$ and

$CD8^+$ T cell responses and to determine the presence of cell-free virus in PBMCs. At the indicated time points after infection, the macaques were necropsied and tissues were harvested. DNA was isolated from the samples by ONPRC's Molecular Virology Support Core (MVSC). Tissue samples (<100 mg) were prepared using FastPrep (MP Biomedicals) in 1 ml TriReagent (Molecular Research Center Inc.). 100 µl bromochloropropane (MRC Inc.) was added to each homogenized tissue sample to enhance phase separation. 0.5 ml DNA back-extraction buffer (4 M guanidine thiocyanate, 50 mM sodium citrate, and 1 M Tris) was added to the organic phase and interphase materials, which was then mixed by vortexing. The samples were centrifuged at 14,000 *g* for 15 minutes, and the aqueous phase was transferred to a clean microfuge tube containing 240 µg glycogen and 0.4 ml isopropanol and centrifuged for 15 minutes at 14,000 *g*. The DNA precipitate was washed twice with 70% ethanol and resuspended in 100 to 500 µl ddH_2O. Nested real-time PCR was performed with primer and probe sets for the inserted SIV proteins GAG (first round: for-GAAACCATGCCGAAGACCTCTC and rev-CTCGTTGATGATGTCACGGATG; second round: for-CAACTACGTCCACCTGCCACTGTC, rev-TCCAACGCAGTTCAGCATCTGG, and probe-CCGAGAACCCTGAACGCTTGGGTCAAGC-FAM) and SIVretanef (first round: for-CGGAAGCAGAACATGGACGAC and rev-CCCCTTCTCCTTGATGAAGTGC; second round: for-CGACGAGGAGGACGACGACTTA, rev-CCAACTTGTACGACATCGTCCG, and probe-TCTCAGTGCGGCCGAAGGTCCC-FAM). For each DNA sample, 10 individual replicates (5 µg each) were amplified by first-round PCR synthesis (12 cycles of 95°C for 30 seconds and 60°C for 1 minute) using Platinum Taq in 50 µl reactions. Then, 5 µl of each replicate was analyzed by nested quantitative PCR (45 cycles of 95°C for 15 seconds and 60°C for 1 minute) using Fast Advanced Master Mix (ABI Life Technologies) in an ABI StepOnePlus Real-Time PCR machine. The results for all 10 replicates were analyzed by Poisson distribution and expressed as copies per cell equivalents [452].

4.3.7 Viral detection in urine by coculture. We centrifuged filter-sterilized (0.4 mm) urine at 16,000 *g* for 1 hour at 4°C to concentrate virus for coculture on rhesus fibroblasts. Cell lysates were prepared after we observed extensive cytopathic effects or after 42 days of coculture if cytopathic effects were minimal or not observed. The prepared cell lysates were assessed for the

presence of RhCMV on the basis of expression of RhCMV- or SIV-specific antigens by Western immunoblotting.

4.3.8 Immunologic assays. RhCMV- and SIV-specific $CD4^+$ and $CD8^+$ T cell responses were measured in mononuclear cell preparations from blood and BAL fluid by flow cytometric intracellular cytokine analysis, as previously described [353]. Briefly, sequential 15-mer peptides (overlapping by 11 amino acids) comprising the SIV_{MAC239}Gag, or Rev/Nef/Tat and RhCMV 68.1pp65ab, or IE1/2 were used in the presence of costimulatory CD28 and CD49d monoclonal antibodies (BD Biosciences). Cells were incubated with antigen and costimulatory molecules alone for 1 hour, followed by addition of Brefeldin A (Sigma-Aldrich) for an additional 8 hours. Costimulation without antigen served as a background control. Cells were then stained with fluorochrome-conjugated monoclonal antibodies, flow cytometric data were collected on a LSR II (BD Biosciences), and data were analyzed using the FlowJo software program (version 8.8.7; Tree Star). Responses frequencies ($CD69^+/TNF^+$ and/or $CD69^+/IFN^+$) were first determined in the overall $CD4^+$ and $CD8^+$ population and then memory corrected (with memory T cell subset populations delineated on the basis of CD28 and CD95 expression).

4.3.9 Measuring RhCMV-specific antibody responses by ELISA. Antibody levels to RhCMV were measured in circulating plasma of RMs by standard ELISA using plates coated with lysates of fibroblasts infected with either WT-RhCMV or RhCMV-Δpp65 at 10 μg total protein per well. Nonspecific binding sites were blocked with 2% milk powder resuspended in PBS. Serial 2-fold dilutions of plasma were incubated for 1.5 hours prior to washing 3 times with ELISA wash buffer (PBS with 0.1% Tween-20; 200 μl per well). Primary antibody binding was detected and quantified with HRP-conjugated anti-rhesus IgG/IgA/IgM secondary antibody and addition of o-phenylenediamine chromogen substrate. A log-log transformation was performed on the linear portion of the curve and end point titers were calculated using 0.1 OD units as the cutoff point. Each plate contained a positive control sample to normalize ELISA titers between assays and a negative control sample to ensure assay specificity and to subtract background. Graphical data was generated using Prism GraphPad software.

4.3.10 Measuring RhCMV-specific antibody responses by SDS-PAGE and Western blotting. Rhesus fibroblasts infected with WT-RhCMV or RhCMV-Δpp65 were solubilized in 2× Laemmeli's sample buffer, and 200 μg total protein per lane were loaded onto NuPAGE 4%–12% Bis-Tris gradient gels (Invitrogen, Life Technologies) and run in MOPS buffer. Proteins were transferred to Immobilon-P blotting membrane, and nonspecific binding sites were blocked in 2% milk powder in Tris-buffered saline with 0.02% Tween-20 for 60 minutes. Primary rhesus monkey polyclonal antisera was added at 1:700 dilution in blocking buffer for 60 minutes, and membranes were washed 3 times for 10 minutes each in Tris-buffered saline plus 0.2% Tween (TBST). Secondary goat anti-rhesus-HRP conjugate was added at 1:5,000 in blocking buffer for 30 minutes, and membranes were washed 3 times for 10 minutes each in TBST. Membranes were developed using ECL Advance Lumigen-TNA (GE Healthcare) for 1 minute and exposed to Biomax Light Film (Kodak) at various exposure times.

4.3.11 Ethics statement. All RMs were handled in accordance with good animal practice, as defined by relevant national and/or local animal welfare bodies. The use of nonhuman primates was approved by the ONPRC Institutional Animal Care and Use Committee (IACUC no. 0691). The ONPRC is fully accredited by the Assessment and Accreditation of Laboratory Animal Care International. For blood and BAL collection, monkeys were anesthetized with ketamine by intramuscular injection. Monkeys were humanely euthanized by the veterinary staff at ONPRC in accordance with end point policies. Euthanasia was conducted under anesthesia with ketamine, followed by overdose with sodium pentobarbital. This method is consistent with the recommendation of the American Veterinary Medical Association.

4.3.12 Statistics. All *P* values in this study were determined using a 1-tailed unpaired Student's *t* test. The cutoff for statistical significance was defined as $P < 0.05$ unless otherwise specified. All error bars shown in all presented figures represent mean ± SD.

4.3.13 Study approval. All animals in the presented study were used with approval of the ONPRC Animal Care and Use Committee, under the standards of the US NIH Guide for the Care and Use of Laboratory Animals.

4.4 Results

4.4.1 RhCMVΔpp65ab shows delayed growth kinetics at low MOI. To study the function of pp65 in RMs, we deleted the pp65a- and pp65b-encoding genes *Rh111* and *Rh112* in the RhCMV strain 68-1–derived BAC [344] to generate Δpp65ab. Upon reconstitution of recombinant virus in telomerized rhesus fibroblasts (TRFs), we verified that genes *Rh111* and *Rh112* were absent, whereas the neighboring genes *Rh110* (UL82 [pp71] homolog) and *Rh114* (UL84 homolog) were still expressed (Fig.23A).

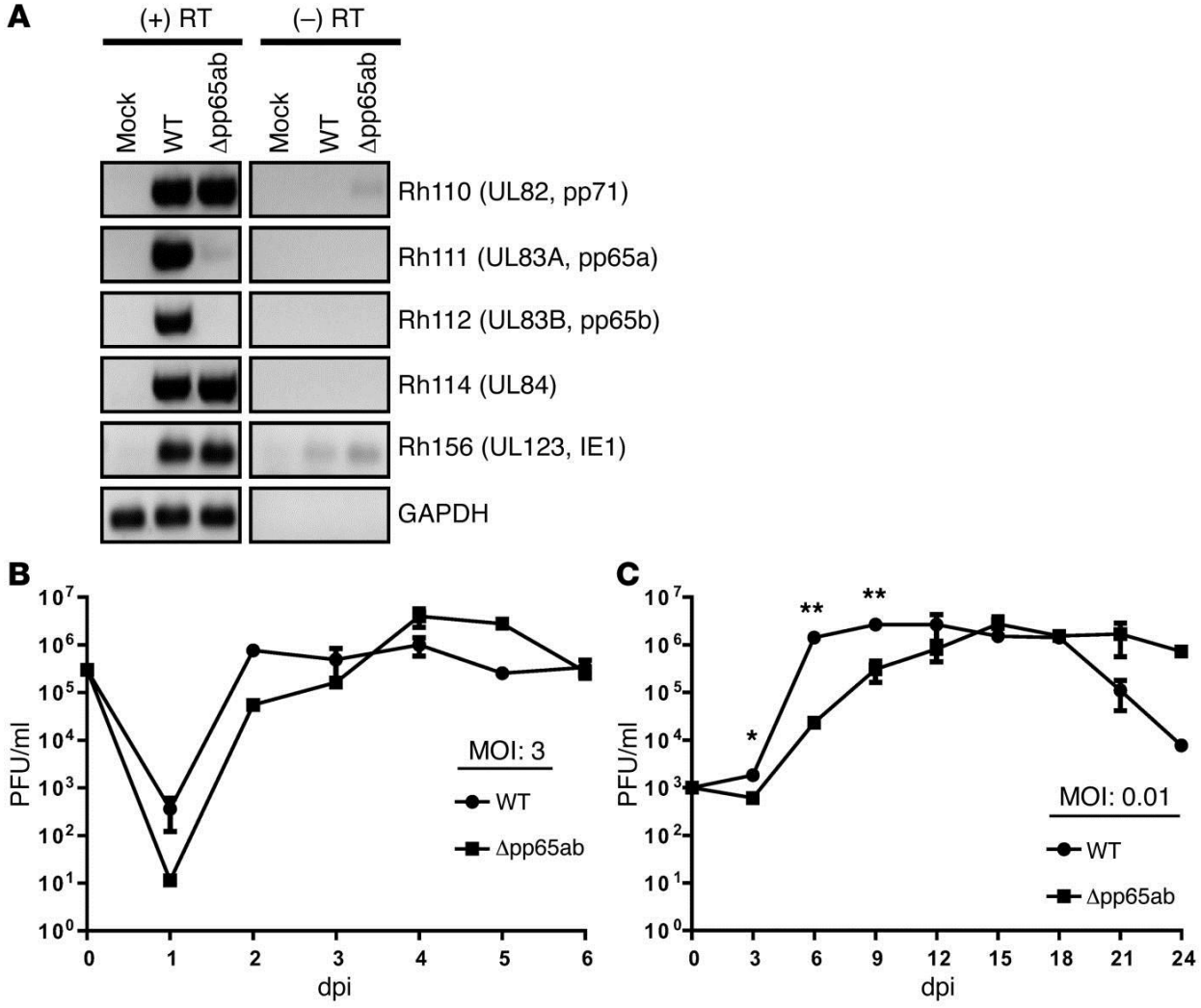

Fig.23: Characterization of RhCMVΔpp65ab in vitro. (**A**) RT-PCR results for cDNA of infected TRFs showing the expression of pp65a (Rh111) and pp65b (Rh112) and their neighboring ORFs Rh110 (pp71) and Rh114. IE1 and GAPDH were included as controls. (**B**) Single-step (MOI of 3) and (**C**) multistep (MOI of 0.01) growth curves of RhCMV WT and RhCMVΔpp65ab on TRFs. **P* < 0.05, Student's *t* test; ***P* < 0.01, Student's *t* test.

To determine whether pp65 deletion affected in vitro growth properties of RhCMV, we compared the growth kinetics of Δpp65ab with those of BAC-derived RhCMV 68-1 (herein referred to as WT control). TRFs were infected with Δpp65ab or WT either at a high MOI of 3 to generate a single-step growth curve or at a low MOI of 0.01 to measure multistep growth. Supernatants collected at high MOI contained similar titers of the 2 viruses, with a peak titer reached on and after 4 days postinfection (dpi) (Fig.23B). However, when multiple rounds of infection were measured, Δpp65ab displayed a modest, but significant, delay in viral growth, ultimately reaching the same peak titer as WT (Fig.23C).

4.4.2 Characterization of Δpp65ab virions. Since previous reports suggested that pp65 in HCMV affected viral assembly and thus the incorporation of other viral proteins [441], we studied the structure and protein composition of Δpp65ab virions. Using mass spectrometry, we demonstrated recently a remarkable similarity between the predominant viral proteins found in both RhCMV and HCMV, with respect to protein ratios and protein abundance [444]. To similarly determine the proteome of Δpp65ab virions, we concentrated viral particles from the supernatant of infected TRFs followed by purification over a discontinuous Nycodenz gradient (see Methods). Compared with WT, we observed an increased appearance of particles that sedimented with higher density in virion preparation of Δpp65ab (Fig.24A). Electron microscopy of this high-density band revealed abnormal structures consistent with capsidless (defective) viral particles. However, the lower density virion band contained an essentially pure preparation of particles with the same general structure as WT RhCMV, including an icosahedral capsid containing the viral DNA as the core of the virion surrounded by a tegument layer and enveloped by a lipid membrane (Fig.24B). NuPAGE and Western blot analysis of gradient-purified WT and Δpp65ab mutant virions demonstrated the absence of pp65a and pp65b in the deletion mutant (Fig.24, C and D). Comparison of the dimensions of the WT versus Δpp65ab virions revealed an overall reduced diameter of Δpp65ab virions (173.4 nm) compared with that of WT virions (222.5 nm) (Fig.24E). This reduction in particle size was primarily due to a significant reduction of the viral tegument layer (38.4 nm [Δpp65ab] compared with 61.2 nm [WT]), consistent with the fact that pp65a and pp65b constitute a major portion of the viral tegument in WT (approximately 24.4%; ref.[444]). In addition, the capsid appeared to be diminished in size, although to a lesser degree (89.7 nm

[Δpp65ab] compared with 100.2 nm [WT]). Thus, both phenotypically normal, but smaller, virions and defective particles were recovered from the supernatant of cells infected with Δpp65ab.

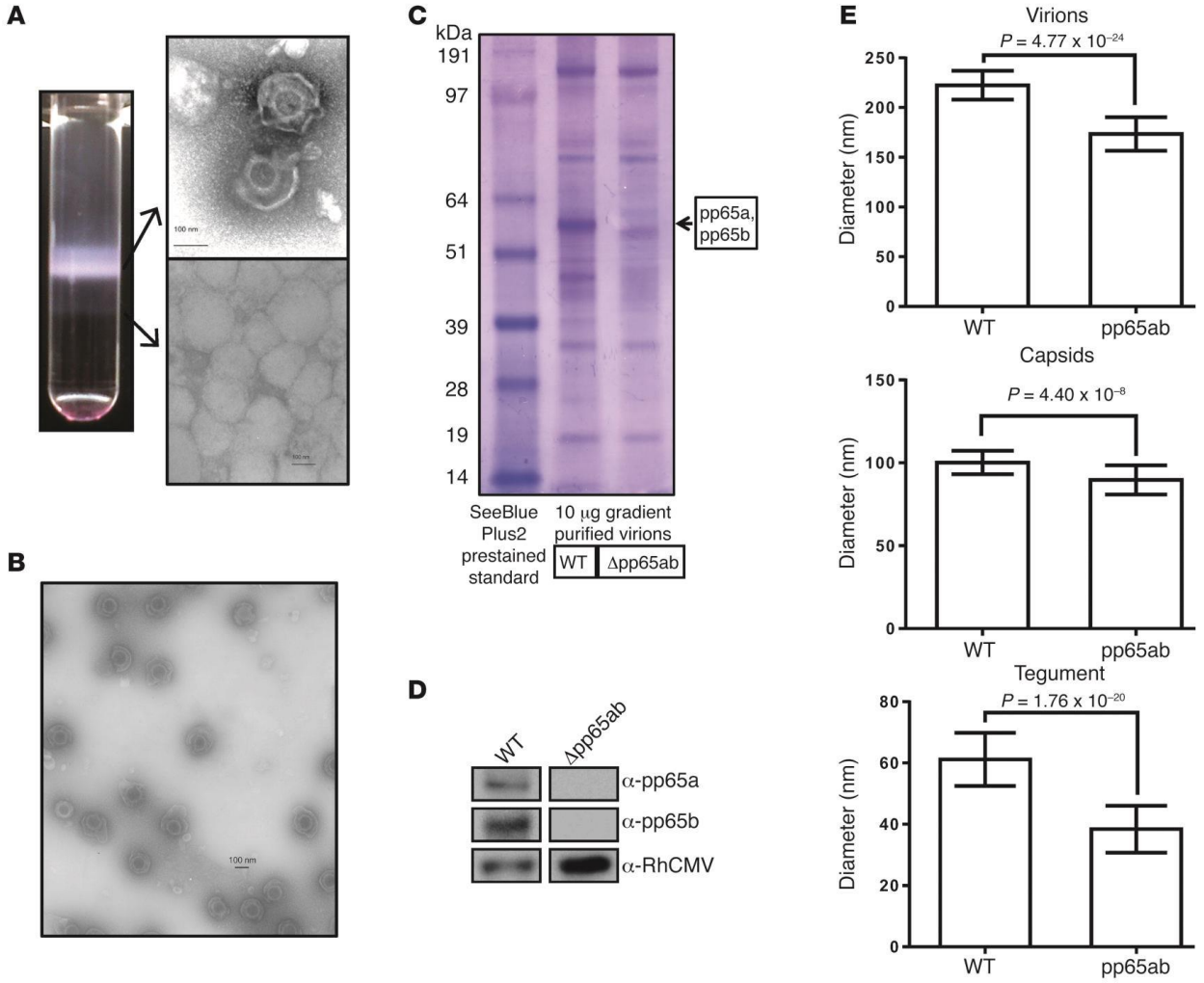

Fig.24: Intact and defective viral particles are secreted from fibroblasts infected with Δpp65ab. (**A**) Image of a Nycodenz gradient loaded with RhCMVΔpp65ab, and electron microscope images of virions (top image) and defective particles (bottom image) contained in the visible bands of the gradient. (**B**) Electron microscope image of purified RhCMVΔpp65ab virions showing the purity of the sample. (**C**) Purified RhCMV WT and Δpp65ab virions were lysed, and 10 μg protein was electrophoretically separated using NuPAGE MOPS gradient gels and visualized by Coomassie blue staining. (**D**) Western blots of 5 μg gradient-purified RhCMV 68-1 WT and viral mutant Δpp65ab stained for RhCMV pp65a, pp65b, or a RhCMV-specific antibody. (**E**) Various electron microscopy images of purified WT and Δpp65ab virions were taken, and the diameters of virions, capsids, and the tegument were determined in multiple images and magnifications (WT, $n = 39$; Δpp65ab, $n = 45$). The mean diameters with their respective SDs are shown, and Student's t tests were performed to determine the P values. Scale bars: 100 nm.

To further characterize the proteome of the virions contained in the upper band by mass spectrometry, the recovered material was digested with trypsin and analyzed by 1D LC-MS/MS. The resulting mass spectra were initially searched against stop-to-stop translated sequences of the RhCMV 68-1 BAC genome for ORFs ≥30 amino acids. Since all proteins identified by this method

corresponded to proteins contained in our recently published reannotation of the RhCMV 68-1 BAC genome [444], we also used a protein library based on the revised annotation for further analysis of protein abundance. The virion preparation was analyzed by LC-MS/MS upon elution from 0% to 100% acetonitrile gradient over 100 minutes, and 5 technical repeats were performed. 68.5 mol% of all identified proteins and 69.9 mol% of all identified peptides corresponded to viral proteins, whereas 31.5 mol% of the proteins and 30.1% of the peptides were derived from the host (Supplemental Figure 3B; supplemental material available online with this article; doi: 10.1172/JCI67420DS1). This result is similar to that previously obtained for WT (64.8 mol% viral proteins, 63.4% viral peptides and 35.2 mol% host proteins, 36.6% host peptides), suggesting a similar level of sample purity. As expected, peptides corresponding to pp65a or pp65b were not detected in the Δpp65ab virion preparation (Table 4). However, a total of 50 different viral proteins could be identified for Δpp65ab, which is comparable to that for WT virions, for which 53 different viral proteins were identified. Every capsid protein found in WT virions was found in the deletion mutant, and besides the deleted pp65 proteins, this also holds true for the tegument proteins (Table 4). Similarly, all major glycoproteins were present in Δpp65ab in equal abundance compared to WT. In fact, most proteins that differed in their abundance between Δpp65ab virions compared with WT were low-abundance proteins, suggesting that these proteins might not be consistently part of the virions or that they were missed in our analysis due to low abundance. If an abundance threshold of 0.25 mol% is applied, 8 proteins differ between Δpp65ab and WT: Rh17 (RL11 family), Rh131 (UL96), Rh211 (US26), and Rh214 (US28) are decreased in the Δpp65ab mutant compared with the WT, whereas Rh05 (RL11 family), Rh13.1 (RL13), Rh173 (RL11 family), and Rh218 (US28) were increased in the Δpp65ab mutant compared with the WT (Fig.25). Of those, Rh211 is the only protein with a substantial presence with 0.85 mol% in WT virions that is completely absent in mutant virions. Rh211 is the homolog of HCMV US26, whose function is unknown. Thus, our proteomics analysis revealed that only 8 proteins with a higher abundance than 0.25 mol% showed marked changes of more than 2-fold between the WT and the pp65ab deletion mutant, with most of these being low-abundance proteins.

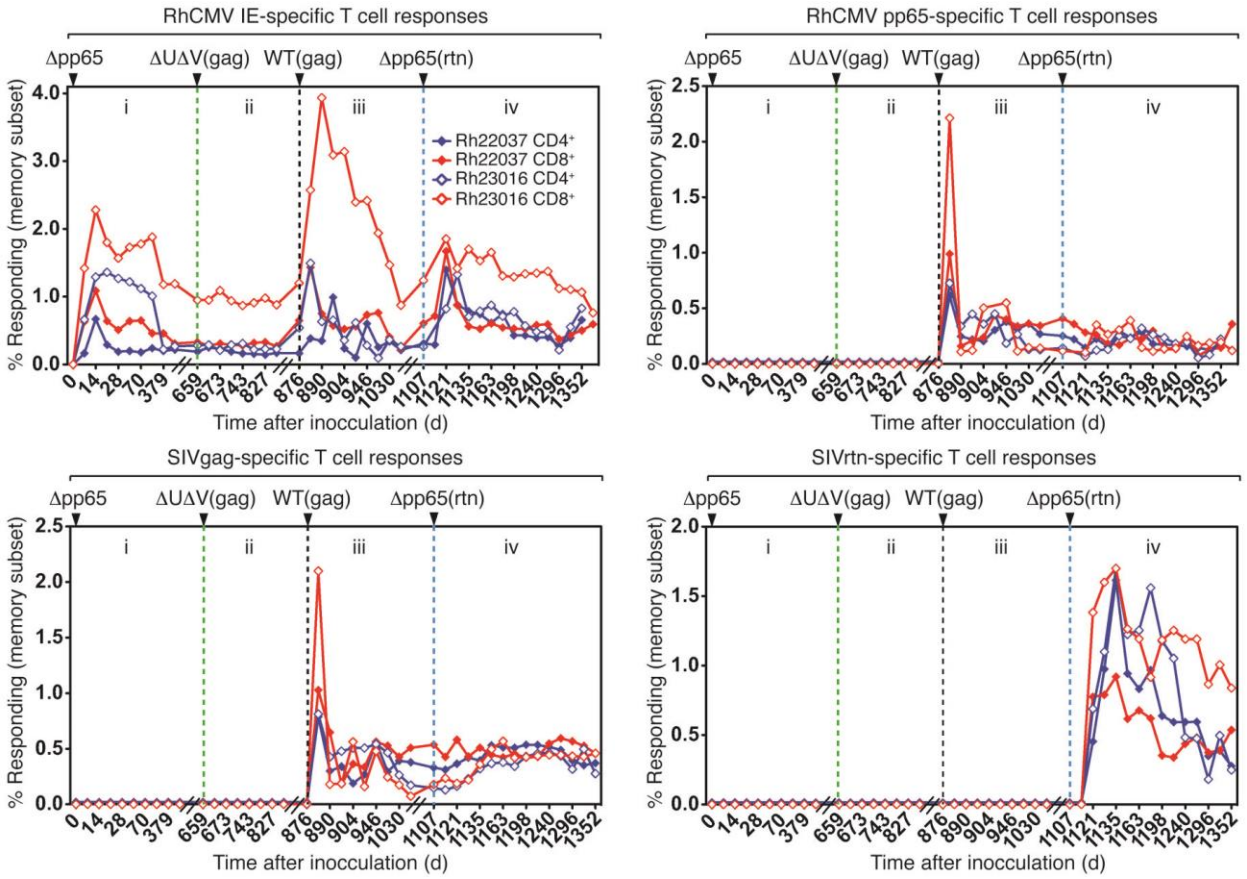

Fig.25: Δpp65ab establishes primary and secondary infections and protects against superinfection with ΔUS2-11. (i) Two RhCMV seronegative male RMs (filled circles, Rh22037; open circles, Rh23016) were infected s.c. with 10^7 PFUs of Δpp65ab at day 1. $CD4^+$ (blue) and $CD8^+$ (red) T cell responses were monitored in peripheral blood (PBMCs) by intracellular cytokine staining at the indicated days using overlapping peptides of pp65ab and IE1/2. (ii) On day 659, the 2 animals were inoculated s.c. with 10^7 PFUs of ΔUS2-11gag (green dotted line), and the T cell response to SIVgag was measured in addition. Note the absence of a T cell response to SIVgag or pp65 and a lack of boosting of responses to IE1. (iii) On day 876, the 2 RMs were inoculated with 10^7 PFUs of WTgag (black dotted line), and the T cell response was monitored by intracellular cytokine staining. Note the appearance of de novo responses to SIVgag and pp65 and a boosting of the T cell response to IE1. (iv) On day 1,107, the 2 RMs were inoculated with 10^7 PFUs of Δpp65ab-rtn (blue dotted line). Using overlapping 15-mer peptides, a de novo response to SIVretanef was detectable, indicating superinfection. Also note a boosting of the IE1 response but not of pp65- or SIVgag-specific responses. The corresponding T cell responses obtained from BAL fluid are shown in Supplemental Figure 4.

Table 1

Comparison of viral proteins contained in WT and Δpp65ab virions

ORF RhCMV	ORF HCMV	Description	Group[A]	Mol% in virion (RhCMV WT)[B]	Mol% in virion (Δpp65ab)	Mol% in virion (Δpp65ab adjusted)	Fold change	Direction
Rh112	UL83	Tegument protein pp65b	A	7.23	0.00	0.00	*	↓
Rh118	UL86	Major capsid protein (MCP)	C	7.22	10.21	9.09	1.26	↑
Rh117	UL85	Minor capsid protein (mCP)	C	6.63	7.99	7.11	1.07	↑
Rh129	UL94	Tegument protein	A	4.88	5.24	4.67	1.05	↓
Rh102	UL73	Envelope glycoprotein N	B	4.83	7.28	6.48	1.34	↑
Rh44	UL26	Tegument protein	A	4.16	2.85	2.54	1.64	↓
Rh59	UL35	Tegument protein	A	3.81	4.34	3.86	1.01	↑
Rh111	UL83	Tegument protein pp65a	A	3.76	0.00	0.00	*	↓
Rh138	UL100	Envelope glycoprotein M	B	3.70	3.92	3.49	1.06	↓
Rh75	UL46	Triplex capsid protein, subunit 1	C	3.45	3.09	2.75	1.25	↓
Rh104	UL75	Envelope glycoprotein H	B	3.17	2.69	2.39	1.32	↓
Rh137	UL99	Myristoylated tegument protein	A	2.97	3.20	2.85	1.04	↓
Rh43	UL25	Tegument protein	A	2.86	2.90	2.58	1.11	↓
Rh55	UL32	Tegument protein pp150	A	2.86	3.06	2.73	1.05	↓
Rh100.1	UL71	Tegument protein	A	2.75	2.44	2.17	1.27	↑
Rh215	US28	G protein–coupled receptor homolog (vGPCR2)	B	2.75	3.21	2.85	1.04	↓
Rh110	UL82	Tegument protein pp71	A	2.73	3.72	3.31	1.22	↑
Rh230	TRS1	Tegument protein	D	2.43	1.66	1.47	1.65	↓
Rh89	UL55	Envelope glycoprotein B	B	2.38	2.34	2.08	1.14	↓
Rh79.1	UL48A	Small capsid protein	C	2.34	2.25	2.00	1.17	↓
Rh203	US22	Tegument protein	A	2.17	3.37	3.00	1.38	↑
Rh72	UL45	Ribonucleotide reductase subunit 1 (R1)	D	2.10	2.01	1.79	1.18	↓
Rh148	UL116		E	1.66	2.04	1.82	1.09	↑
Rh76	UL47	Tegument protein	A	1.51	1.98	1.77	1.17	↑
Rh78	UL48	Large tegument protein	A	1.42	2.11	1.88	1.33	↑
Rh140	UL103	Tegument protein	A	1.34	1.20	1.07	1.26	↓
Rh123	UL88	potential Tegument protein	E	1.32	1.91	1.70	1.28	↑
Rh106	UL77	Virion-packaging protein	C	1.21	1.48	1.32	1.09	↑
Rh147	UL115	Envelope glycoprotein L	B	1.03	1.04	0.93	1.11	↓
Rh164	UL141	Membrane glycoprotein	B	0.92	1.02	0.91	1.01	↓
Rh132	UL97	Phosphotransferase	D	0.87	1.00	0.89	1.02	↑
Rh211	US26		E	0.85	0.00	0.00	*	↓
Rh160	UL132	Envelope glycoprotein	B	0.81	1.05	0.93	1.14	↑
Rh56	UL33	GPCR homolog, envelope glycoprotein	B	0.78	0.45	0.40	1.93	↓
Rh152/Rh151	UL119/UL118	Membrane glycoprotein, viral Fc-γ receptor	B	0.70	0.73	0.65	1.08	↓
Rh128	UL93	Capsid-associated protein	C	0.64	0.74	0.66	1.02	↑
Rh141	UL104	Capsid portal protein	C	0.47	0.66	0.59	1.25	↑
Rh216	US28	GPCR homolog (vGPCR3B)	B	0.36	0.59	0.53	1.47	↑
Rh131	UL96	Tegument protein	A	0.35	0.20	0.17	1.99	↓
Rh17	RL11 family		E	0.32	0.00	0.00	*	↓
Rh214	US28	GPCR homolog (vGPCR1)	B	0.28	0.00	0.00	*	↓
Rh42	UL24	Tegument protein	A	0.28	0.38	0.33	1.18	↑
Rh173	RL11 family		E	0.27	0.69	0.62	2.25	↑
Rh218	US28	GPCR homolog (vGPCR4)	B	0.27	0.88	0.78	2.88	↑
Rh164.1			E	0.23	0.15	0.14	1.71	↓
Rh103	UL74	Envelope glycoprotein O	B	0.20	0.35	0.31	1.56	↑
Rh165			E	0.18	0.00	0.00	*	↑
Rh13.1	RL13	Membrane protein	B	0.14	0.51	0.46	3.14	↑
Rh109	UL80	Capsid maturation protease	C	0.10	0.03	0.02	4.27	↓
Rh70	UL44	DNA polymerase processivity factor	D	0.10	0.12	0.10	1.07	↑
Rh05	RL11 family		E	0.09	0.49	0.44	4.68	↑
Rh156 (IE2)	UL122	Immediate-early protein 2, pp86	D	0.08	0.17	0.15	1.85	↑
Rh81	UL50	Nuclear egress membrane protein	B	0.04	0.02	0.02	1.63	↓
Rh83	UL52	Packaging protein	D	0.00	0.17	0.15	*	↑
Rh134	UL98	Deoxyribonuclease	D	0.00	0.07	0.07	*	↑
Rh114	UL84		D	0.00	0.02	0.02	*	↑

[A]The identified proteins were separated into 5 different groups dependent on their subcellular localization or function:A, tegument; B, envelope and glycoproteins; C, capsid; D, transcription/replication machinery; and E, uncharacterized. [B]These data are based on WT proteomics results published by Malouli et al. (38). Asterisks indicate proteins that are not present in either the WT or Δpp65ab sample, so fold changes cannot be calculated.

Table 4: Comparison of viral proteins contained in WT and Δpp65ab virions.

In general, there was surprisingly little impact of pp65ab deletion on the presence of other proteins in the virions. There was no substantial difference among nonstructural proteins (transcription/replication machinery or uncharacterized category), whereas the quantities of most structural proteins (capsid, envelope, glycoproteins) were, in fact, slightly elevated in Δpp65ab compared with those in WT (Table 5). Moreover, we did not observe a decrease in specific, non-pp65 tegument proteins, but rather, we saw a decrease in the abundance of all non-pp65 tegument proteins in Δpp65ab virions. This is in contrast to a previous report for HCMV, describing selective lack of specific tegument proteins in pp65-deleted virions [441]. Thus, it seems that RhCMV virions assembled normally but with an overall reduced tegument. Indeed, when virion protein abundance is adjusted for the absence of pp65ab by normalizing to a total of 89% (11% of the WT virion is made up by pp65a and pp65b combined), protein quantities are very similar to those of WT (Tables 4 and 5). Despite the lack of major tegument proteins that normally represent 11% of the viral particle mass, there was little change in virion composition.

Table 2
Relative abundance of the 5 functionally different groups of viral proteins in RhCMV WT and Δpp65ab

	RhCMV 68-1	SD	RhCMVΔpp65ab	SD	*P* value compared to 68-1	RhCMVΔpp65ab adjusted	SD	*P* value compared to 68-1
Tegument	45.09	2.26	37.03	1.58	0.0005	32.95	1.40	0.00004
Envelope and glycoproteins	22.38	1.30	26.00	2.37	0.03	23.14	2.11	0.30
Capsid	22.03	1.58	26.46	1.07	0.001	23.55	0.95	0.07
Transcription/replication machinery	5.58	0.42	5.24	0.84	0.27	4.67	0.75	0.05
Uncharacterized	4.92	1.14	5.28	0.59	0.28	4.70	0.52	0.36
Total	100.00		100.00			89.00		

Table 5: Relative abundance of the 5 functionally different groups of viral proteins in RhCMV WT and Δpp65ab.

In contrast to the limited impact of pp65ab deletion on virion proteins, a number of host proteins were substantially different between WT and Δpp65ab (Supplemental Figure 3). 279 host proteins were identified in WT, whereas 240 host proteins were identified in Δpp65ab. Only 172 host proteins were identified in both viral samples, with the remaining proteins being unique to each sample. The role of host cell proteins in CMV virions is unknown, but it seems likely that these proteins reflect the source or host cell membrane used for envelopment. The differential presence of host cell proteins could thus indicate that envelopment of pp65ab-deleted viruses differs somewhat from that of WT virus. This would be consistent with the increased production

of defective particles described above. The differential incorporation of host cell proteins likely reflects changes in viral assembly pathways but is less likely of consequence for virion function.

4.4.3 Infection of RMs with Δpp65ab. To determine whether pp65ab-deleted viruses would be infectious, we inoculated 2 seronegative male RMs with 10^7 PFUs of Δpp65ab and monitored the CMV-specific T cell response using overlapping peptides to the RhCMV proteins IE1/2 and, as control, to pp65ab for about 22 months. We also monitored viral shedding by coculture of urine samples with rhesus fibroblasts. In previous experiments, we showed that infection of RhCMV-negative RMs results in the appearance of peak T cell responses within the first 2 weeks of infection, followed by a contraction and stabilization of the T cell response at a level that remains more or less constant for the duration of the life of the animal [353, 453]. The maintenance of such a long-lived effector memory T cell response reflects the establishment of persistent infection. Similarly, both animals infected with Δpp65ab responded vigorously to IE1/2, with a peak $CD4^+$ and $CD8^+$ T cell response in PBMCs and bronchoalveolar lavage (BAL) fluid at 2 weeks, followed by a slow decline and stabilization of the response that lasted for the entire time (Fig.25, i, and Supplemental Figure 4A). Importantly, T cell responses to pp65ab were not observed, which is consistent with the IE1/2 responses being induced by the pp65ab-deleted virus. The stable T cell response to IE1/2 suggested that the pp65-deleted virus established persistent infection. Persistence was further confirmed by coculture of urine samples with TRFs, in which IE1 was detected in urine cocultures of Δpp65ab-infected animals but pp65 was not detected, confirming that there was no contamination with WT virus (Fig.26). Thus, these data suggest that RhCMV is able to establish and maintain a persistent infection despite the absence of pp65ab.

Although pp65 is one of the major targets of the CMV-specific T cell response in both humans and monkeys [121, 411, 429-431], the contribution of pp65-specific T cells to control of CMV replication is not known. Indeed, the experimental determination of the efficacy of RhCMV-specific T cell responses is complicated by the fact that RhCMV readily superinfects $RhCMV^+$ RMs, overcoming preexisting T cell responses due to the presence of viral proteins that inhibit MHC-I antigen presentation [276]. However, RhCMV lacking the genes encoding for homologs of the HCMV US2, 3, 6, and 11 immunoevasins is unable to superinfect CMV^+ RMs but is capable of establishing persistent infection in CMV-naive animals or upon depletion of $CD8^+$ T cells from CMV-immune animals [276]. Thus, the ability to protect against superinfection with ΔUS2-11

RhCMV is a convenient surrogate marker for the quality of T cell responses, i.e., a T cell response that is as efficient as that induced by natural infection. To test whether the T cell responses induced by Δpp65ab to antigens other than pp65 would be sufficient to prevent superinfection with immunoevasins-deleted virus, we inoculated the 2 Δpp65ab-infected RMs with ΔVIHCEΔUS2-11gag, a previously described recombinant virus that expresses the SIVgag as immunological marker. In addition to US2-11, this virus lacks the RhCMV-specific viral inhibitor of heavy chain expression (VIHCE), which is not required for superinfection [276]. As observed for RMs naturally infected with RhCMV, ΔVIHCEΔUS2-11 was unable to superinfect Δpp65ab-infected animals, as evident from the absence of an immune response to SIVgag as well as a lack of a boosting response to IE or a de novo response to pp65 (Fig.25, ii, and Supplemental Figure 4B). In fact, T cell responses to IE1/2 remained stable for the entire duration of this experiment (200 days). Thus, the T cell responses generated by Δpp65ab were as efficacious as T cell responses induced by WT in protecting against immunoevasins-deleted virus challenge, indicating that a pp65-specific T cell response is not required for an effective anti-RhCMV immune response and that T cells specific for other codominant or subdominant antigens are sufficient for protection.

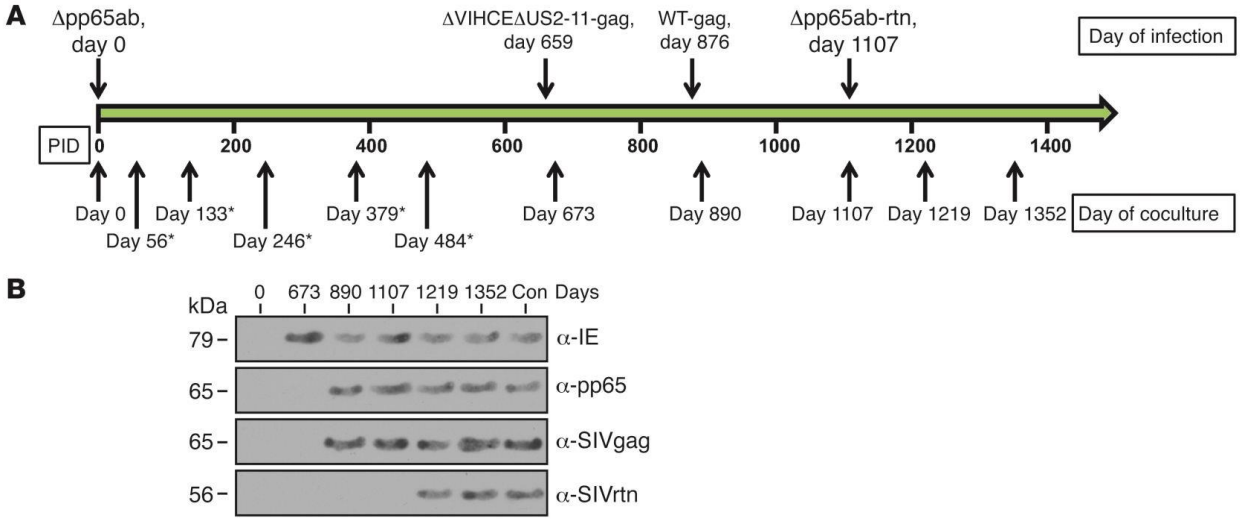

Fig.26: **RhCMVΔpp65ab is persistently secreted from infected animals.** (**A**) The time line depicts the time points of inoculation with different RhCMV constructs and the days when cocultures were started from urine. Time points marked with asterisks indicate additional days in which cocultures were positive for Δpp65ab, but the data are not shown. PID, postinoculation day. (**B**) Immunoblot for the indicated antigens in lysates from representative viral cocultures with urine collected on the indicated dpi. The presence of RhCMV-IE1, RhCMV-pp65b, SIVgag, and SIVretanef in cell lysates was detected by immunoblot using antibodies specific for the respective antigens (IE, pp65) or for epitope tags fused to SIVgag or SIVretanef. Note that, initially, secreted RhCMV expressed IE, but not pp65, whereas superinfection with WTgag and Δpp65retanef is indicated by the appearance of pp65-containing virus expressing the respective antigens. As positive control (Con), coculture lysates from a RM inoculated with WTgag and WTretanef is included.

To determine whether animals infected with Δpp65ab are resistant to superinfection by WT RhCMV, we inoculated both RMs with WT-gag, a previously described virus that carries SIVgag inserted into the ORF Rh211 between hypothetical ORFs 213 and 214 [353]. Upon inoculation of 10^7 PFUS WT-gag, both animals displayed clear signs of superinfection, as evident by the development of de novo responses to SIVgag and pp65ab and by boosting of the preexisting T cell response to IE1/2 (Fig.25, iii, and Supplemental Figure 4C). Moreover, cocultures of urine samples from these animals contained SIVgag-expressing virus (Fig.26). These data thus demonstrate that the immune responses induced by Δpp65ab, like those elicited by WT RhCMV, are unable to protect against superinfection with WT RhCMV.

Given the role of HCMV pp65 as modulator of several immune response pathways (including protecting against IE-specific T cells [23] and NK cells [22]), it was possible that pp65 itself contributed to the ability of WT to overcome preexisting immune responses. In fact, our previous finding that evasion of T cell responses plays a central role in overcoming preexisting immune responses does not rule out that evasion of other immune response components, e.g., B cells and NK cells, might also contribute to superinfection [276]. To examine whether RhCMV lacking pp65ab would be able to superinfect CMV-positive animals, we inserted an expression cassette for SIVretanef (a fusion protein of rev, int, tat, and nef; refs. [353, 454]) into the RhCMV genome by replacing the pp65-encoding genes *Rh111* and *Rh112*. After confirming pp65 deletion, in vitro growth properties, and expression of SIVretanef (data not shown), we inoculated the 2 RMs previously infected with Δpp65ab and WT-gag with Δpp65ab-retanef and monitored the immune response to SIVretanef. As shown in Fig.25, iv (Supplemental Figure 4D), both animals showed clear signs of superinfection, as evident from the development of a de novo T cell response to SIVretanef and a boosting of the preexisting IE1/2 response. Note that the T cell responses to pp65ab and SIVgag were not boosted, confirming the lack of pp65ab and SIVgag. We thus conclude that pp65ab is dispensable for the establishment of both primary and secondary persistent infections.

4.4.4 Vaccine-induced pp65-specific T cells do not recapitulate the protective effect of T cells induced by natural infection. In the RM model, it was previously demonstrated that vaccination with subunit vaccines consisting of pp65b (with or without IE1) as T cell–inducing components and gB as neutralizing antibody-inducing component reduced RhCMV viremia and

shedding [450, 451, 455]. However, our data also suggest that T cell responses to antigens other than pp65 play an important role in the protective effect of RhCMV infection against ΔUS2-11 challenge. We were therefore wondering whether induction of a T cell response to pp65 alone would be sufficient to recapitulate the protective effect of preexisting infections against challenge with RhCMV lacking the US2-11 immunoevasins [276]. Therefore, we used a previously described heterologous prime-boost regimen to induce pp65b-specific T cell responses [450, 451]. Three animals were vaccinated with DNA encoding pp65b, followed by 2 boosts with pp65b-expressing modified vaccinia Ankara (MVA). For control, we vaccinated 3 animals with antigen-free plasmid and MVA. As shown in Fig.27A (Supplemental Figure 5), all 3 pp65b-vaccinated animals developed a robust $CD4^+$ and $CD8^+$ T cell response to pp65b after this prime-boost vaccination regimen that, in the 6 weeks following the final boost, was similar in magnitude and phenotype to pp65-specific T cell responses that develop in the context of RhCMV infection (Fig.25, iii and iv, and Supplemental Figure 4). As expected, pp65b-specific T cells were not observed in the control MVA-vaccinated group. Six weeks after the final MVA/pp65 versus control MVA boost, all animals were challenged with RhCMV lacking US2-11 and expressing SIVgag (ΔUS2-11gag). Similar to ΔVIHCEΔUS2-11gag, this virus is unable to overcome preexisting T cell immunity, despite the presence of the RhCMV-specific MHC-I inhibitor VIHCE [276]. All 3 control-vaccinated animals developed the expected T cell response to pp65 as well as SIVgag consistent with infection. However, the pp65-vaccinated animals also developed T cell responses to SIVgag with similar kinetics and magnitude compared with those in the control group. We further observed a boost of the T cell response to pp65b consistent with infection by ΔUS2-11gag. These data indicate that pp65-specific T cells alone are unable to provide the level of protection against CMV afforded by natural infection or experimental infection with whole virus.

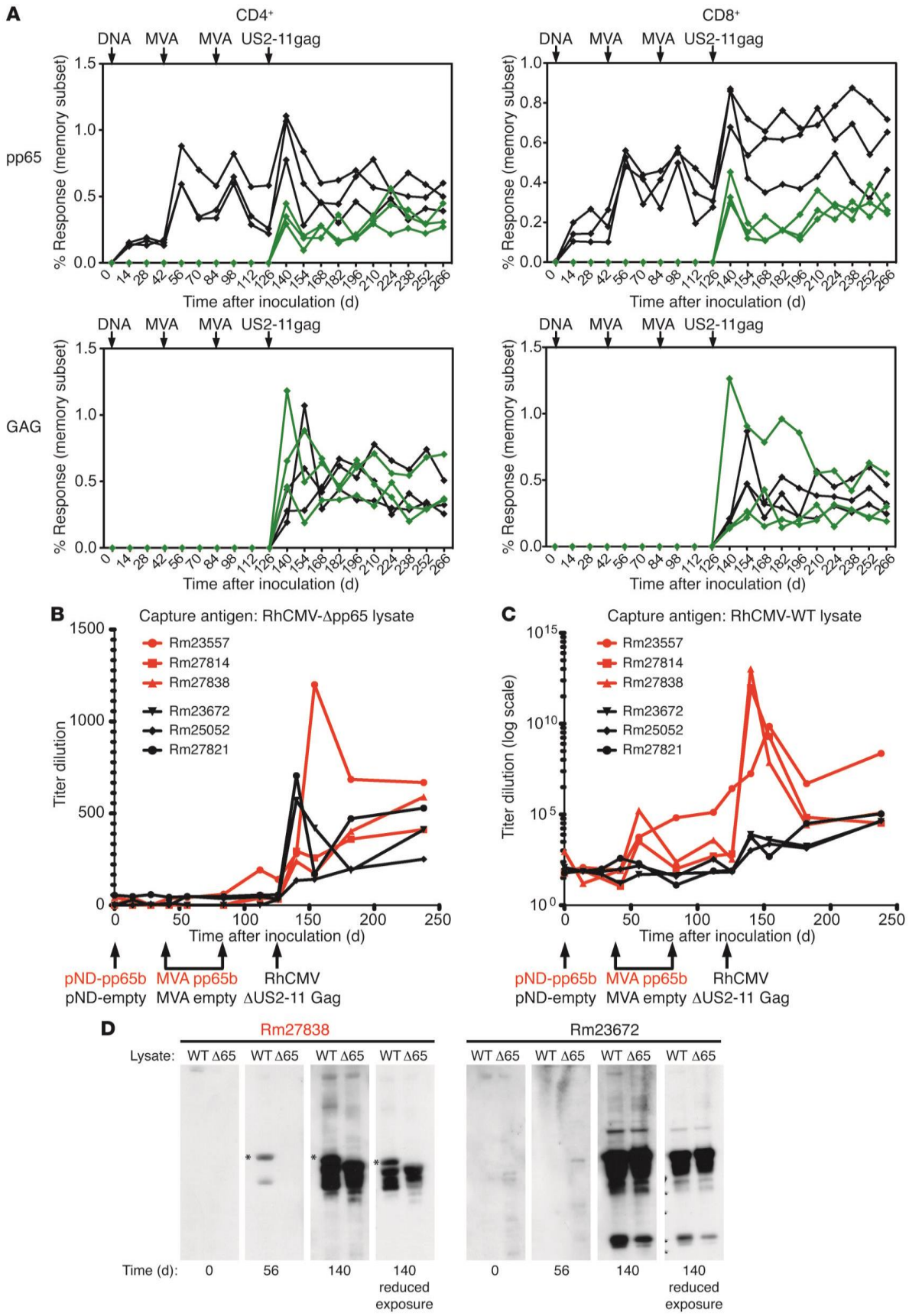
A
CD4+
CD8+
DNA MVA MVA US2-11gag
pp65
GAG
% Response (memory subset)
Time after inoculation (d)
B
Capture antigen: RhCMV-Δpp65 lysate
C
Capture antigen: RhCMV-WT lysate
Rm23557
Rm27814
Rm27838
Rm23672
Rm25052
Rm27821
Titer dilution
Titer dilution (log scale)
pND-pp65b MVA pp65b RhCMV
pND-empty MVA empty ΔUS2-11 Gag
D
Rm27838
Rm23672
Lysate: WT Δ65 WT Δ65 WT Δ65 WT Δ65
Time (d): 0 56 140 140 reduced exposure

Fig.27: T cells induced by heterologous prime-boost vaccination with pp65b do not protect against superinfection with ΔUS2-11. (**A**) Three CMV-negative RMs were vaccinated with 1 mg pND/pp65b and boosted with 5×10^8 PFUs MVApp65b at 6 and 12 weeks after the initial vaccination (black). As controls, 3 CMV-negative RMs were vaccinated with the parental pND plasmid not expressing any antigen and boosted with WT MVA at 6 and 12 weeks after the initial vaccination (green). At 18 weeks after the initial DNA vaccination, both groups of animals were challenged with 10^7 PFUs ΔUS2-11gag. The top row shows the specific T cell responses to pp65, whereas the bottom row shows specific T cell responses to SIVgag. T cells were isolated from peripheral blood (PBMCs). The corresponding T cell responses obtained from BAL fluid are shown in Supplemental Figure 5. The production of anti-RhCMV antibodies in pp65-vaccinated animals (Rm23557, Rm27814, Rm27838) was compared to that in control-vaccinated animals (Rm23672, Rm25052, Rm27821) prior to and upon challenge with RhCMV-ΔUS2-11. At the indicated time points, RhCMV-specific end point antibody (IgG, IgA, IgM) titers were measured in plasma from each animal by ELISA using lysates from fibroblasts infected with either (**B**) RhCMV-Δpp65 or (**C**) WT-RhCMV as the capture antigen. (**D**) Viral proteins recognized by the antibodies were detected by Western blotting. Lysates of cells infected with WT-RhCMV or RhCMV-Δpp65 were separated by SDS-PAGE and immunoblotted with antisera from the pp65-vaccinated animal (Rm27838) or a control-vaccinated animal (Rm23672). Asterisks denote the pp65 proteins. The results from these 2 animals are representative of the responses observed in the other animals of each group.

Conceivably, pp65 vaccination could affect the viral loads of ΔUS2-11gag, as has been reported for pp65/gB-vaccinated animals challenged with RhCMV [455]. Since primary infection with RhCMV 68-1 (the parental strain of ΔUS2-11gag) does not result in robust viremia (see below), direct measurements of viral loads were unlikely to be informative. Instead, we used the development of anti-CMV antibodies as a surrogate for CMV antigen load, because it was shown previously in the murine model that reduced viral spread correlates with reduced antibody responses but does not affect T cell responses [456]. However, when lysates of Δpp65-infected cells were used as antigen, a very modest antibody response was observed in all animals challenged with ΔUS2-11gag, and there was no difference in the kinetics or magnitude specificity of this response between pp65-vaccinated and control-vaccinated animals (Fig.27B), and we did not observe a difference in the specific antigens recognized by immunoblot (Fig.27D). Remarkably, when pp65-containing CMV lysate was used as antigen for our ELISA or immunoblot, we observed an extraordinary increase in the titers of pp65-specific antibodies induced by DNA/MVA vaccination. As shown in Fig.27B, pp65-specific antibodies were above background levels upon boosting with MVApp65. Moreover, subsequent challenge with ΔUS2-11gag increased these pp65-specific antibodies by several orders of magnitude. pp65 antigen was recognized in sera from pp65-vaccinated animals by immunoblot, and this response was strongly increased upon challenge. In contrast, control-vaccinated animals did not recognize the corresponding 65-kDa band even after ΔUS2-11 challenge (Fig.27D). These data indicate that pp65 vaccination did not affect ΔUS2-11 viral load to a level that would affect the induction of CMV-specific antibodies, although a

modest reduction, as reported previously, cannot be ruled out. In addition, these observations suggest that antibody responses to pp65, and potentially to other antigens as well, are substantially boosted upon infection with ΔUS2-11 virus.

Table 3

Copy numbers of RhCMV WT-gag and Δpp65ab-retanef genomes in CMV+ RMs

	14 dpi (Rh29036)		28 dpi (Rh25999)	
Tissue type[A]	**RhCMV 68-1**	**Δpp65ab**	**RhCMV 68-1**	**Δpp65ab**
Skin injection site (WT)	ND	18	ND	21
Skin injection site (Δpp65ab)	11	32	ND	23
Axillary lymph node (WT)	ND	ND	ND	ND
Axillary lymph node (Δpp65ab)	ND	ND	ND	ND
Iliosacral lymph node (WT)	ND	1	ND	ND
Iliosacral lymph node (Δpp65ab)	ND	ND	ND	ND
Inguinal lymph node (WT)	ND	ND	ND	ND
Inguinal lymph node (Δpp65ab)	ND	ND	ND	ND
Inferior mesenteric lymph nodes	ND	ND	ND	ND
Medial mesenteric lymph nodes	ND	ND	ND	ND
Superior mesenteric lymph nodes	ND	ND	ND	ND
Duodenum	ND	5	ND	ND
Jejunum	ND	ND	ND	ND
Ileum	ND	ND	ND	ND
Colon	ND	2	ND	ND
Ileocecal	ND	ND	ND	ND
Submandibular salivary gland (WT)	ND	ND	ND	ND
Submandibular salivary gland (Δpp65ab)	ND	ND	ND	ND
Sublingual salivary gland (WT)	ND	122	ND	12
Sublingual salivary gland (Δpp65ab)	ND	ND	ND	ND
Parotid salivary gland (WT)	ND	ND	ND	ND
Parotid salivary gland (Δpp65ab)	ND	3	ND	ND
BAL pellet	ND	ND	ND	ND
Lung	ND	ND	ND	ND
Spleen	ND	5	ND	ND
Liver	ND	2	ND	ND
Urinary bladder	ND	12	ND	ND
Urine	ND	ND	ND	ND
Brain	ND	9	ND	ND
Spinal cord (lumbar)	ND	ND	ND	ND
Spinal cord (thoracic)	ND	ND	ND	ND
Spinal cord (cervical)	ND	ND	ND	ND
Bone marrow	ND	ND	ND	ND
PBMC	ND	ND	ND	ND
Plasma	ND	ND	ND	ND

Genome copy numbers in tissue samples are given per 10^7 cell equivalents, whereas genome copies in urine and plasma are shown per ml. [A]When tissues were harvested from both the left and right side, it is indicated in brackets whether the sample was derived from the side of WT or Δpp65ab infection. ND, not detected.

Table 6: Copy numbers of RhCMV WT-gag and Δpp65ab-retanef genomes in CMV+ RMs.

Table 4
Genome copies of RhCMV 68-1 WT-gag in CMV+ RMs

	14 dpi		21 dpi		28 dpi	
Tissue type	**Rh22461**	**Rh25976**	**Rh26147**	**Rh26463**	**Rh26508**	**Rh26471**
Skin injection site (WT)	47	541	16,502	23,793	376	4,480,600
Skin injection site (mutant)	11	12	7	193	355	36,110
Axillary lymph node (WT)	117	4	7	25	509	3,429
Axillary lymph node (mutant)	ND	ND	ND	ND	ND	1,600
Iliosacral lymph node	1,434	ND	1	ND	ND	18
Submandibular salivary gland	12	ND	ND	2	ND	4
Parotid salivary gland	11	ND	ND	2	ND	ND
Lung	4	ND	ND	ND	ND	3
Spleen	6	6	ND	ND	ND	1
Liver	1	ND	ND	ND	ND	3
Urinary bladder	3	ND	ND	3	12	9
Brain	ND	3	ND	ND	ND	ND
Spinal cord	ND	ND	3	ND	3	3
Bone marrow	9	ND	ND	ND	ND	7

Genome copy numbers in tissue samples are given per 10^7 cell equivalents, whereas genome copies in urine are shown per ml.

Table 7: Genome copies of RhCMV 68-1 WT-gag in CMV+ RMs.

Table 5
Copy numbers of RhCMV WT-gag and Δpp65ab-retanef genomes in CMV-naive RMs

	14 dpi (Rh23351)		21 dpi (Rh25047)		28 dpi (Rh25815)	
Tissue type	**RhCMV 68-1**	**Δpp65ab**	**RhCMV 68-1**	**Δpp65ab**	**RhCMV 68-1**	**Δpp65ab**
Skin injection site (WT)	658	78,337	353	583	560	23,723
Skin injection site (Δpp65ab)	1	633,469	ND	549	ND	5,880
Axillary lymph node (WT)	2,167	932,433	5,160	207,594	500	7,598
Axillary lymph node (Δpp65ab)	ND	52,360,075	ND	3,711,480	ND	8,918
Iliosacral lymph node (WT)	ND	240,741	ND	20,763	ND	262
Iliosacral lymph node (Δpp65ab)	ND	77,134	ND	1,726,199	ND	291
Inguinal lymph node (WT)	ND	12,693	12	1,560,278	ND	13
Inguinal lymph node (Δpp65ab)	ND	4,254	ND	1,716,316	185	98,240
Inferior mesenteric lymph nodes	ND	71,711	ND	1,316,668	ND	17
Medial mesenteric lymph nodes	ND	14,213	ND	9,183,921	ND	7
Superior mesenteric lymph nodes	ND	319,333	3	24,513,856	ND	3,086
Duodenum	ND	1,835	ND	32	ND	12,143
Jejunum	ND	1,737,984	ND	ND	ND	168,019
Ileum	9	3,037,728	ND	9	ND	150,184
Colon	15,779	94,282	ND	4,285,540	ND	112
Ileocecal	ND	1,107,878	1	914,011	ND	16
Submandibular salivary gland (WT)	ND	301	ND	ND	ND	ND
Submandibular salivary gland (Δpp65ab)	ND	ND	ND	319	–	–
BAL pellet	ND	4,080	–	–	ND	ND
Lung	ND	3,697	ND	156,596	ND	12
Spleen	4	659,632	4	546,874,241	ND	11
Liver	ND	18,601	ND	40,219	ND	34
Urinary bladder	4,522	15,150	ND	3,336	ND	93
Urine	ND	ND	ND	ND	ND	ND
Brain	–	–	ND	ND	ND	120
Spinal cord (lumbar)	ND	ND	ND	ND	ND	1,343
Spinal cord (thoracic)	ND	7	ND	ND	ND	3
Spinal cord (cervical)	ND	12	ND	ND	ND	7
Bone marrow	ND	3,810	ND	67,120	–	–
PBMC	ND	44,496	ND	562,307	ND	7
Plasma 0 dpi	ND	ND	ND	ND	ND	ND
Plasma 7 dpi	ND	8,142	ND	14,389	ND	ND
Plasma 14 dpi	ND	60	ND	48,471	ND	80,856
Plasma 21 dpi	–	–	ND	ND	ND	17
Plasma 28 dpi	–	–	–	–	ND	ND

Genome copy numbers in tissue samples are given per 10^7 cell equivalents, whereas genome copies in urine and plasma are shown per ml.

Table 8: Copy numbers of RhCMV WT-gag and Δpp65ab-retanef genomes in CMV-naive RMs.

4.4.5 pp65 limits dissemination of RhCMV *in vivo*. Taken together, these data suggest that neither pp65 itself nor the T cell response to pp65 have a major impact on the overall course of either primary or secondary RhCMV infection. However, it remained possible that the extent of RhCMV replication is affected by the presence or absence of pp65. If lack of pp65 delays RhCMV replication kinetics in vivo, as it does in vitro, infections with pp65-deleted RhCMV would be expected to proceed more slowly and/or manifest reduced spread or peak viral production relative to WT virus. On the other hand, if pp65-induced immune responses have superior efficacy, infections with pp65-deleted RhCMV would manifest greater viral replication and spread than WT RhCMV. To address these possibilities, we performed experiments in which genetically marked WT and Δpp65ab RhCMV constructs (using SIVgag and SIVretanef as the identifying markers, respectively) were simultaneously, but separately (right arm vs. left arm), inoculated into either RhCMV seropositive (n = 2) or RhCMV seronegative RMs (n = 3). These RMs were sacrificed and taken to necropsy at 14, 21, or 28 days after inoculation; DNA was isolated from the sites of inoculation and distant tissues; and the extent and magnitude of viral spread was determined by an ultrasensitive, nested quantitative PCR analysis using primers that specifically amplify fragments of the SIVgag versus SIVretanef inserts [359]. In the setting of superinfection, little tissue-associated viral DNA was detected for either the WT or Δpp65ab constructs, with the former only identified in one of the inoculation sites in the RM analyzed at day 14 and the latter identified at very low level in inoculation sites and scattered distant tissues in both the RMs analyzed at day 14 and day 28 after inoculation (Table 6). Although, in these 2 RMs, the extent of spread by the Δpp65ab RhCMV was greater than that of the coadministered WT RhCMV, the level of tissue-associated virus observed in the Δpp65ab RhCMV superinfection was still within the range of that found in WT RhCMV superinfection in other RMs (Table 7), and thus, deletion of pp65 does not seem to significantly affect viral dissemination during superinfection. In striking contrast, the extent of Δpp65ab RhCMV replication in RhCMV-naive RMs was 3 to 4 logs higher than the simultaneously administered WT RhCMV (Table 8). Indeed, the degree of Δpp65ab RhCMV replication during primary infection was astonishing, reaching almost 10^8 DNA copies in the LN draining the injection site at day 14 after inoculation. Analyses of viral loads in blood indicate that the difference in WT versus Δpp65ab RhCMV replication by can be observed by day 7 after inoculation (Table 8). The levels of Δpp65ab RhCMV in blood and tissue declined dramatically at later time points, indicating that this virus is eventually brought under immune control. Taken

together, these data unequivocally indicate that expression of pp65 strongly limits primary viral dissemination over several orders of magnitude and suggest that, while RhCMV-specific T cells can control RhCMV in the absence of pp65, a rapid immune response to pp65 is necessary to limit viral spread during the early days of primary infection.

4.5 Discussion

The goal of this study was to assess the role of one of the major structural components of the CMV virion in infection and immunity. Similar to HCMV, a large percentage (approximately 11%) of the protein mass of RhCMV virions consists of the 2 pp65 homologs [444]. Nevertheless, in both HCMV and RhCMV, pp65 is not essential for growth in vitro although increased production of defective particles occurs during infection with RhCMVΔpp65ab. This could be reflective of assembly defects due to the lack of pp65ab. For HCMV, it has been reported that pp65 is required for the incorporation of other virion proteins, most notably UL25, UL69, and UL97 [441]. However, we did not observe a major skewing in the protein composition of the viral tegument as would have been expected if pp65 selectively controls the incorporation of other viral proteins. Instead, the tegument composition seemed normal but without pp65 present. Conceivably, this could be due to a difference in virion assembly between HCMV and RhCMV. However, the overall virion proteome of RhCMV is highly similar to that of HCMV [444], and UL25, UL69, UL97 are highly conserved in RhCMV. Therefore, it seems more likely that effects of pp65 on incorporation of other tegument proteins are nonselective. The fact that virions are assembled, carrying the same ratios of viral proteins as WT, while lacking pp65, suggests that although pp65 might facilitate virus assembly, once the virus is assembled, the lack of pp65 does not affect the overall viral structure, except for a reduction on overall virion size due to a reduced tegument protein layer.

In addition to viral assembly, pp65 has an immediate function upon release of the tegument into cells during membrane fusion. Similar to other tegument proteins (e.g., pp71 and UL35), pp65 is thought to contribute to setting the stage for optimal viral replication by counteracting intrinsic and innate antiviral host response mechanisms [68]. HCMV lacking pp65 showed increased induction of IFN-stimulated genes (ISGs) [151, 152]. We reported previously that RhCMV particles inhibited ISG expression [148] and pp65 was a possible candidate for this inhibition. However, Δpp65ab did not induce ISGs, suggesting that inhibitory mechanisms mediated by other RhCMV proteins perform this function (data not shown). It has also been reported that HCMV pp65 binds to and induces the major immediate early promoter (MIEP) in conjunction with the cellular protein IFI16 [432]. We have not investigated in detail the impact of pp65 on IE expression in RhCMV. However, in this study, we did observe a delay in virus production in multistep growth curves (Fig.23C), consistent with this effect. Thus, RhCMV pp65 proteins appear to facilitate

optimal expression of viral genes in the early stages of cellular infection but are not required for productive infection.

The role of HCMV pp65 for the establishment and maintenance of infection in vivo is unknown due to the strict species specificity of HCMV. The rationale for studying RhCMV pp65 in the RM model was therefore the close evolutionary relationships of both the host to human and of the virus to HCMV. Given the multiple functions assigned to HCMV pp65, it was completely unexpected that deletion of both homologs in RhCMV did not only not affect the ability of RhCMV to establish and maintain a long-term infection in the rhesus host but, in fact, strongly increased the ability of RhCMV to replicate and disseminate during primary infection. In contrast, lack of the pp65 homologous genes *M83*/*M84* and *GP83* in murine CMV (MCMV) and guinea pig CMV, respectively, reduced peak viremia during primary infection [457-459]. In contrast to primary infection, differences in the replication and dissemination of Δpp65ab versus WT RhCMV were minimal, if not absent, in the setting of superinfection of CMV^+ RMs, suggesting that once established, adaptive immune responses to antigens other than pp65 can effectively control the infection. These data suggest that pp65 likely acts as an "immunological brake" during the initial stages of primary infection to limit viral replication and dissemination. We therefore hypothesize that the main function of pp65 is not that of immune evasion, but immune induction, i.e., eliciting a rapid immune response that controls viremia. The most likely candidate mechanism for this effect is the pp65-specific effector T cell response, which, due to the abundance and immunogenicity of pp65 proteins, might appear earlier in primary infection than the response to other CMV proteins. Alternatively or additionally, pp65 might induce innate immune responses that limit viral replication in primary infection. This immune induction function of pp65 is reminiscent of the NK cell–stimulating protein m157 of MCMV, whose deletion or mutation increases viral replication and titers in mice carrying the NK cell receptor Ly49H for which m157 is a ligand [460, 461].

The parental strain used to generate Δpp65ab, RhCMV 68-1, shows reduced secretion from infected animals, most likely due to the lack of genes in the ULb′-homology region required for tissue tropism [345]. Since RhCMV 68-1 does not generate robust plasma viremia in infected animals, the appearance of RhCMV-Δpp65ab in plasma samples became particularly striking. Thus, it is conceivable that the increased dissemination of Δpp65ab might be less pronounced in viruses carrying an intact ULb′ region. However, in preliminary observations, we did not observe increased dissemination of RhCMV 68-1.2, a virus that is repaired for tissue tropism [415]. Thus,

it is likely that pp65 deletion will have a similar effect on a repaired or low-passage viral background, although this still needs to be verified experimentally.

T cells from HCMV-infected individuals recognize a broad spectrum of viral ORFs that are highly variable between individuals [121]. Although no ORFs are recognized by all seropositive people, pp65 is one of the most consistently recognized CMV proteins by both $CD4^+$ and $CD8^+$ T cells [121], a level of immunogenicity that has led vaccine developers to include pp65 in HCMV vaccines [427, 462-466]. However, relatively little is known about the protective effect of pp65-specific T cells in humans, since vaccine trials generally involve a cocktail of proteins and efficacy cannot be directly correlated to pp65 alone [428]. The most direct evidence for a protective effect of pp65-specific T cells comes from adoptive T cell transfer experiments that used pp65-derived peptides to expand HCMV-specific T cells [467-472]. In these studies, transfer of pp65-specific T cells accelerated the restoration of antiviral immunity posttransplantation, without graft versus host side effects associated with nonspecific T cell transfer. Our finding that pp65-specific immunity seems to curtail viral dissemination in the early stages of infection would support the inclusion of pp65 in subunit vaccines, provided it is indeed the pp65-specific T cells that are responsible for this effect.

On the other hand, our data also indicate that pp65-specific T cell responses are not sufficient to recapitulate the level of protective immunity generated by actual viral infection. To examine the protective effect of pp65-specific T cells or T cells specific to other CMV antigens we developed a novel challenge strategy. Our approach relies on our previous observation that viral genes encoding the RhCMV homologs of HCMV immunoevasins US2, 3, 6, and 11 are essential for RhCMV to superinfect RhCMV-positive animals [276, 278]. The ability to establish secondary persistent infections is also a common occurrence in HCMV, resulting in frequent coinfection with different strains of HCMV [424]. Since depletion of $CD8^+$ T cells restores the ability of US2-11–deleted RhCMV to infect seropositive animals, infection with ΔUS2-11 viruses can be used to monitor the quality of a vaccine-induced T cell response. Moreover, the clear protection observed by natural infection allows these studies to take place in a very small group of animals, since the outcome of superinfection is binary. The results shown in Fig.25 are typical: both animals inoculated with Δpp65ab were clearly protected against superinfection with US2-11–deleted RhCMV but not with WT. (In fact, we observed superinfection in more than 200 animals

inoculated with recombinant RhCMV.) Evidently, the T cell responses to antigens other than pp65 are protective in this challenge model.

Using the US2-11 challenge approach we were able to examine whether pp65-specific T cells elicited by heterologous prime-boost vaccination were sufficient to recapitulate the protective effect of T cells elicited by preexisting infection. We used a DNA-prime/MVA-boost protocol employed previously to vaccinate animals with a combination of pp65, IE1, and gB [455]. In this previous work, it was shown that this vaccination regimen, while unable to protect against superinfection with RhCMV, reduced local and systemic viremia as well as viral shedding. Moreover, reduction in shedding correlated with the magnitude of pp65-specific T cell responses [455]. In our hands, the heterologous prime-boost vaccination induced a robust $CD4^+$ and $CD8^+$ T cell response to pp65b that in the blood was similar in magnitude to pp65-specific T cell responses elicited by RhCMV infection. Although prime-boost vaccination would not be expected to maintain the effector-memory–biased T cells responses elicited by RhCMV infection over the long term [278, 353, 359], at the time of challenge (6 weeks after the final MVA boost), the responses generated by the prime-boost vaccine still manifested a predominant effector memory phenotype. Despite this, these vaccine-generated pp65-specific T cell responses were insufficient to protect against infection by ΔUS2-11gag virus, as shown by the induction of Gag-specific T cell responses and CMV-specific antibody responses. This suggests that T cells induced by pp65 alone do not reproduce the protective effect of T cells induced by ongoing persistent infections. Since the T cell response to pp65 was substantial in all 3 animals, it seems unlikely that a different vaccination strategy would have induced a better protection. Rather, it seems more likely that additional antigens might be required to recapitulate the protective effect of natural infection. Thus, our results caution against the use of pp65 as the only T cell stimulatory subunit in a CMV vaccine.

The ΔUS2-11 challenge used in this study provides an excellent tool to evaluate the T cell component of subunit vaccines. Conceivably, a similar approach could be used in human clinical trials to specifically evaluate the T cell immunity generated by a given vaccine. Recently, challenge with the Towne strain was used to evaluate the efficacy of subunit vaccines by monitoring an anamnestic HCMV-specific immune response [465]. Conceivably, a US2-11–deleted Towne strain would not generate an anamnestic response, similar to our observation that IE1-specific responses were not boosted when RhCMVΔUS2-11 failed to superinfect (Fig.25). In this case, a second challenge with WT-Towne could be used to monitor protection and T cell boosting as

described previously [465]. In contrast, a boosting of the pp65-specific T cell and antibody responses, as observed in pp65-vaccinated animals (Fig.27), would be a clear indication of infection by US2-11–deleted Towne and lack of protection by the T cell component of a given vaccine. Furthermore, our observation that vaccine-induced antibody responses against pp65 were strongly boosted by inoculation with ΔUS2-11 virus suggests that a safe, US2-11–deleted HCMV could be used to boost antibody levels induced by a given vaccine. Since high levels of antibodies are a desired feature of many vaccine regimens, inclusion of CMV as a new tool to enhance antibodies should be considered.

In summary, our work revealed a novel and surprising function of pp65, suggesting that this viral protein acts as an immune inducer that generates an immune response that stringently restricts viral replication during primary infection but that has little impact on long-term maintenance, immunogenicity, or viral shedding. A likely explanation for this finding is that the highly abundant and immunogenic protein pp65 induces a rapid T cell response that limits viral dissemination. Since an intact immune system is required for this "immunological brake" mechanism to function, the lack of pp65-mediated control likely contributes to the high level of dissemination observed in immunocompromised individuals, such as transplant recipients, or in fetuses with immature immune systems. In RMs, RhCMV can cause severe sequelae, including spontaneous abortions, when injected into the developing fetus [473, 474]. Since the immune-dominance of pp65 is conserved in HCMV, it is likely that this "antivirulence" function is conserved as well. HCMV and RhCMV thus seem to use the adaptive immune response to limit their dissemination during primary infection. It is not immediately obvious why CMV would choose such a self-imposed restriction. However, since the establishment and maintenance of persistent infection, as well as persistent shedding from the infected host, is not affected by the presence or absence of pp65, it seems that the ultimate "goal" of CMV, to establish a benign infection that easily spreads through the human population, is unaffected by pp65. The pp65-mediated immunological control of CMV dissemination might thus serve to soften the impact of primary infection on the host to ensure a healthy host that is able to maintain and transmit the virus for a long time.

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5. Discussion and Future Perspectives

In the study presented here, we tried to shed light on a fascinating new vaccine platform which showed promising results in protecting rhesus macaques against challenge with highly virulent SIVmac239 [359, 360]. RhCMV has been shown to induce unique T-cell responses substantially different from any other vaccine vector examined previously [278]. Due to the nature of the virus as a persistent pathogen, CMV will remain with the infected host for life and reactivate continuously from latency [475]. This chronic infection induces a massive T-cell response to proteins encoded by the CMV vector backbone as well as any introduced foreign antigens. T-cell frequencies can comprise as much as 10% or more of circulating memory T-cells in the host [121]**.** These T-cells control the lytic viral infection and limit viral reactivation from latency. Upon depletion of T-cells from test animals or if the host becomes immunosuppressed, either due to disease or medication, the virus can reactivate and cause disseminated infections, observations that underline the importance of a persistent T-cell response to control cytomegalovirus. Due to repeated reactivation and the resulting constant challenge, the T-cells cannot progress to central memory (T_{CM}) phenotype as they would after infection with non-persistent viruses (e.g. adenoviruses), but display effector memory (T_{EM}) phenotype [353]. As such they remain polyfunctional and capable of CTL activity immediately upon encounter of their target antigen. Since effector memory T-cells do not proliferate in response to pathogen challenge they provide an immediate defense at the entry or reactivation sites of potential infections. Whereas CMV evolved mechanism to evade clearance by the host's immune response [124], SIV and other pathogens do not have this capability, and by inserting SIV antigens into the RhCMV backbone the highly pathogenic SIV strain mac239 was eventually cleared by the CMV-induced immune response [360]. The significance of this finding cannot be stressed enough since for the first time these results suggest that a HIV vaccine might be possible which would save the life of millions of people. CMV is normally a benign infection causing only mild pathology in the naïve host [28]. Seropositivity in a population increases with age and is dependent on the location and the living conditions of an individual. Close to 100% of individuals will become seropositive during childhood in most developing countries whereas seronegative individuals can still be found in the highest age brackets in developed nations [22-24]. Given the almost universal distribution of the virus, utilizing this virus as a new vaccine delivery vector does not introduce a never before encountered pathogen into the human population. Thus, vaccinating with a live, un-attenuated

vaccine vector would not be different than exposing an individual to wildtype CMV. However, while this might be acceptable in individuals that are already infected by CMV, non-attenuated vectors would likely not be acceptable in CMV-negative vaccine recipients due to the risk of causing pathology upon immunosuppression or upon fetal infection. Therefore, current work in RhCMV focuses on modeling possible attenuation strategies by selecting potential candidate genes for deletion in the viral genome for future development of attenuated HCMV vaccine vectors.

5.1 Characterization of the protein coding content of RhCMV

To design a functional *in vivo* monkey model for HCMV, it is important to determine the exact coding potential of our RhCMV vaccine vector in comparison to HCMV. Multiple studies have tried to estimate the number of potential ORFs encoded by this virus, but some predictions increased the total number of putative viral proteins well above experimentally determined results achieved for other CMV family members like HCMV and MCMV [347, 348]. Therefore, it seemed likely that a significant number of predicted ORFs were false and non-coding. These predictions were based on very simple computer algorithms that only searched for potential proteins spanning more than 100 amino acids and encoding for a start and a stop codon, without further examining the validity of these proposed ORFs. For HCMV, one possible method to elucidate the true coding potential is to compare multiple different fully sequenced genomes and determine the ORFs conserved among all of them, since it is very unlikely that different full length isolates encode for vastly different proteins. The first step is to eliminate all strain-specific predictions. However, this approach cannot be used for RhCMV since only two RhCMV strains have been fully sequenced, and both appear to have undergone major tissue culture adaptations and deletion of ORFs [345]. Therefore, we started with the assumption that NHP CMV members are closely related so that sequence comparisons of isolates from different NHP species would reveal the likely coding content of a prototypical RhCMV isolate. This assumption is based on the fact that HCMV and CCMV show remarkable coding similarity [346] and that several NHP species are so closely related that they can produce fertile offspring [476-478].

Alignment of several genomes of NHP CMVs of Asian and African origin showed high sequence similarity of the genomic DNA sequence, particularly in the central genome region encoding most of the structural proteins and proteins responsible for DNA replication. However, even the less-conserved terminal genome regions were remarkably conserved, not only among

NHP CMVs but also between human and NHP viruses, with 90% of all HCMV ORFs having homologues in NHP CMVs, at least on the protein family level. This conservation is substantially higher than predicted previously [343] supporting a close genetic relationship between non-human and human primate cytomegaloviruses. In contrast, MCMV, the most widely used animal model system for HCMV, shows very little homology to the human virus outside the core region with many genes having functional but not sequence or locational homologues. Our finding that RhCMV has true homologues to almost all HCMV ORFs thus highlights the potential of the virus as a valuable tool to determine *in vivo* functions of important HCMV proteins. A further difference between primate and murine CMV is the fact that MCMV provides its host with protective immunity after sublethal challenge of naïve mice [305], so the boosting of initially generated T-cell responses with another MCMV based vaccine vector is impossible, although superinfections have been demonstrated in wild mice [479].

Interestingly, there are small differences between CMVs derived from different primate species with evolutionary distances between the host species reflecting evolutionary distances between the corresponding CMVs (see Fig.11). In addition to differences in sequences of homologous proteins, there are also differences in the number of viral proteins encoded by individual genomes. For instance, NWM CMVs as well as great ape and human CMVs encode only one copy of the major tegument protein pp65, whereas all OWM CMV genomes, both Asian or African monkeys CMVs, encode two copies of the ORF [444]. These two copies are not fully equal, since we were able to show that the pp65b protein is twice as abundant in the viral virion as the pp65a protein [444].

A further difference can be found in the number of viral CXCL chemokines encoded by the different viruses. HCMV encodes for two of these proteins (UL146 and UL147), whereas CCMV encodes for three (UL146, UL146A and UL147) [346]. OWM of Asian origin have six chemokine family members (UL146B, UL146C, UL146D, UL146F, UL146H and UL147) while OWM of African origin have eight of these proteins (UL146B, UL146C, UL146D, UL146E, UL146F, UL146G, UL146H and UL147) [444]. Finally, NWM, like HCMVs, only encode two viral CXCL chemokines [3].

Besides differences in coding capability, homologous viral proteins from different CMV species can also exhibit altered functions. In HCMV, pp71 is an almost essential viral protein [211]. The virus does not replicate at low MOIs in the absence of this protein, since pp71 is

responsible for, among other things, the degradation of DAXX [480]. By doing so the viral protein enables viral gene expression and viral genome replication by hijacking the cellular DNA replication machinery. In an HCMV pp71 deletion mutant, DAXX is not degraded, leading to a severe attenuation of the virus at low MOI [194, 211]. RhCMV also encodes for a pp71 protein with significant identity to the HCMV protein. Like its HCMV homologue, it also degrades DAXX and other ND-10 body components (data not shown), but the virus only exhibits a moderate attenuation at low MOI in non-complementing cells (Fig.29-30). The probable explanation for this difference is that, other than in HCMV, a RhCMV pp71 deletion mutant still delocalizes and degrades DAXX efficiently (data not shown), indicating that RhCMV might encode a second DAXX-targeting protein that, in the absence of pp71, can partially compensate for this defect.

Another example can be found in the viral NK cell evasion. For HCMV the viral proteins UL16 and UL142 as well as the viral miRNA miR-UL112 have been shown to interfere with the activating NKG2D ligands (MICA, MICB and the UL16-binding protein (ULBP) 1-6) by sequestering them in the ER or downregulating their expression [481]. Interestingly, neither of these proteins, nor the described miRNA is conserved in RhCMV [444]. In fact, only HCMV and CCMV have been described to encode for these proteins whereas OWM CMVs as well as NWM CMVs do not contain homologues of these proteins. This implies that these viruses must use other mechanisms to evade NK cells, since work in MCMV suggests that NK cell evasion is important for the viral replication *in vivo* [224, 482-485]. Work performed in our lab led to the idetification of Rh159 as the viral protein responsible for downregulating NKG2D ligands from the cell surface of infected cells by sequestering them in the ER (Sturgill et al, manuscript in preparation). This protein is conserved in all OWM and NWM CMVs and also has a homologue in HCMV and CCMV termed UL148 [444]. This conservation does not extend to rodent CMVs, indicating that this protein has been part of the primate CMV genome for at least before the separation of NWM (*Platyrrhini)* and OWM (*Catarrhini*) about 35 million years ago [330-334]. As mentioned above, UL16 and UL142 are specific to the human- and great ape CMVs which indicates that these genes entered the viral genome after the separation of OWM and apes about 23 million years ago [337] and before the separation of the genera *homo* (humans) and *pan* (chimpanzees) roughly 5 – 7.5 million years ago [338-341]. This finding leads us to speculate, that the common ancestor of all primate CMVs contained a UL148 homologue as its main mean of NKG2D evasion whereas over

the course of millions of years of co-evolution with its host, the common ancestor of human and chimpanzee CMV acquired UL16 and UL142 after the species separated from the OWM.

To further determine which potential ORFs actually encode for proteins we decided to determine the protein content of purified RhCMV virions using mass spectrometry comparable to what has been published by Varnum et al. for HCMV AD169 [18]. Admittedly, this only enabled us to detect structural proteins of the viral virion and we were not able to detect nonstructural proteins involved in immune evasion since they are not integrated into the viral virion, but we nevertheless obtained insight into a substantial part of the viral proteome and, more importantly, we were able to determine the validity of our ORF prediction. If we had found multiple proteins in the virion that were not predicted by our prior bioinformatics analysis, this would have indicated that our analysis did not lead to a better and more reliable annotation of the viral genome as compared to the stop-to-stop analysis used previously. However, only a single protein (Rh164.1) identified in the virion was not predicted during our initial analysis (Fig.22) due to our arbitrary cutoff of 300bp. In addition, Rh164.1 does not have a homologue in HCMV, so it was excluded in our preliminary annotation. All other proteins identified in the virion were predicted by our analysis. Nevertheless, it is still possible that some of the genes we predicted will not give rise to proteins so that further work will be needed to generate the final finished genome map of the virus. Yet, the overall match between predicted and confirmed protein sequences indicates that our approach showed reliable results and that the predicted genes are likely to be coding. It is possible that we might have missed several viral ORFs, especially very small ORFs that were recently found in HCMV by ribosomal profiling [486]. However, since our predicted total of 175 ORFs is consistent with the number of ORFs predicted for all other CMVs we have a high degree of confidence in our new annotation.

5.2 Use of attenuated RhCMV vectors in vaccine development

To further improve our previously established RhCMV vaccine vectors for potential use in humans against HIV, tuberculosis (Tb) or any other potential pathogen, we had to confirm that the vaccine backbone itself was safe. To ensure this, we encountered the problem to design a vector construct that would generate a high and specific T cell response against the targeted organism without exhibiting any pathogenicity by itself in immunocompetent adult macaques. In the long run this vector should even have the potential to be used safely in immunocompromised individuals and

neonates, since these two target groups are most affected by the virus. Furthermore, it is of the utmost importance that our final vaccine vector cannot be transmitted either horizontally or vertically between individuals, but remains solely in the vaccinated individual.

As a first target for attenuation, we chose the two RhCMV homologues of the HCMV pp65 protein (pp65a and pp65b). The HCMV pp65 protein is involved in the evasion of the innate [151, 152, 432] as well as the adaptive immune response [230, 433, 434], but deletion of the protein from the virus does not affect virus growth in fibroblasts [440], so viral stocks of the deletion mutant can be grown on non-complementing cells, saving us the effort to generate a complementing cell line. Furthermore, as we have shown earlier, pp65 a and b are two of the most abundant members of the viral tegument [444], so deleting these ORFs and inserting a foreign antigen in its place under the endogenous viral promoter should lead to comparably high levels of targeted antigen expression while at the same time attenuating the virus *in vivo*. Generating the RhCMV deletion mutants proved to be unproblematic and the reconstituted virus showed similar *in vitro* growth characteristic as its previously described HCMV and MCMV counterparts [440, 457]. The growth defect *in vitro*, even at low MOIs was minor, and only a short delay in peak viral titer was noticed whereas the viral peak titers were unchanged.

To evaluate the impact of the absence of both pp65 proteins on the viral virion, we gradient purified mutant virus and performed mass spectrometry on in 8M urea denatured virions. It has been noted for HCMV pp65 deletion mutants, that the absence of this major tegument protein affects the levels of other virion protein and that pp65 might play a significant role in the loading of proteins into the viral virion [441]. Contrary to this data and to data published in another study performed and published after the publication of our work [487], the total absence of both pp65 a and b had only a minor impact on the protein levels of other virion components. When we adjusted the relative protein abundances of all proteins found in the RhCMV virion to the absence of pp65 a and b by adjusting the total relative amount of proteins to 89% (11% representing the absence pp65 proteins) to render the two datasets of the WT and the deletion mutant more comparable, we obsered that none of the more abundant proteins showed a significant increase or decrease in relative abundance between our two independent experiments. Significant changes can be found in proteins that show very low abundance, but it is unclear if theses changes reflect real differences in protein composition, or technical difficulties picking up trace amount of low abundance proteins using mass spectrometry. Additionally, when we examined the viral virions of RhCMV WT and

our pp65 deletion mutant by electron microscopy, no obvious structural differences were observed, A more detailed measure of the different virion dimensions (diameter of the virion, the capsid and the tegument layer) revealed that the overall size of the mutant virions was significantly reduced, and that this reduction is mostly due to a significantly smaller tegument layer, This data indicates that the virus does not compensate for the absence of the major tegument components pp65 a and b by inserting more of the other dominant tegument proteins into the virion, but that the absence of pp65 a and b simple leads to an overall decrease of tegument. The relative decrease of the tegument layer by ≈25% is in the same range as the percentage of tegument normally taken up by pp65a and b. Thus, the deletion of these proteins does not affect other tegument or virion protein significantly, but leads to a reduced tegument layers.

To determine the overall strength of the T-cell response generated by our potential vaccine vector *in vivo* in comparison to the RhCMV WT, we challenged the vaccinated animals with a previously described RhCMV 68-1 ΔUS2-ΔUS11 deletion mutant [276]. This mutant exhibits an interesting phenotype in that it infects naïve animals normally and establishes persistent infections *in vivo* in these animals, but is incapable of infecting seropositive animals, since these animals posses a pre-existing anti-RhCMV T-cell response. The proteins encoded within the US2-US11 region are known T-cell immune evasion genes [124], and the virus cannot overcome the preexisting adaptive host T-cell response in the absence of these proteins [276]. We expected our pp65 deleted vaccine vector to induce a T-cell response with similar amplitude and efficacy as the T-cell response induce by the 68-1 WT, but since pp65 is a major target for CD8+ T-cells, its absence might weaken or misdirect the host immune response to the vector. Initial results in naïve rhesus macaques showed that the amplitude of the anti-CMV CD4+ and CD8+ T-cell responses in BAL as well as PBMCs were comparable to the overall levels achieved in WT infections. A RhCMV mutant lacking the US-US11 region was not able to overcome the T-cell response induced by the pp65 deletion mutant, indicating that the T-cells directed to proteins other than pp65 are capable of controlling RhCMV in re-infection or, potentially, re-activation. Vaccination with our deletion mutant did not protect against challenge with a 68-1 WT vaccine vector. This finding is not surprising, as vaccination with a WT vaccine vector does not protect against re-challenge with the same virus either [353]. Re-infections are common in the human as well as animal populations, and protective immunity does not exist in humans and primates after infection with their respective CMVs. Lastly, RhCMV 68-1 Δpp65ab was able to superinfect CMV positive animals. This

attribute is essential for every potential CMV vaccine vector, since superinfection is needed for its use as a vaccine vector, not only for the purpose of boosting established immune responses, but also to inoculate naturally HCMV positive individuals with CMV based vaccine vectors. Pre-existing immunity can be an issue in vaccine trials as has been encountered in the use of HAdV-5 based vaccine vectors, human vectors based on the human adenovirus type 5, a type widely common in the human population [488-490].

Since pp65 is such a prominent target for the T cell response, the protein by itself has been proposed a potential subunit vaccine [491]. Several *in vivo* animal and human trials with pp65 as the sole constituent, or with pp65 as the T cell component or part of the T cell component in an anti-CMV subunit vaccine have been tested [455, 463, 465, 492, 493]. The results were in many cases promising, and future trials with different compositions are under way or have been proposed. Given that our RhCMV deletion mutant still protected against re-infection with a ΔUS2-ΔUS11 deletion mutant, we wanted to examine whether pp65 responses by themselves could achieve similar results. Interestingly, although we were able to generate strong pp65b specific immune responses with our DNA prime MVA boost strategy, the T cell responses were not good enough to protect against our subsequent RhCMV ΔUS2-ΔUS11 challenge, indicating that not only the amplitude, but also a certain breadth in T-cell targets is needed to generate an effective anti-CMV T-cell response. This very interesting finding suggest, that subunit vaccine based on pp65 as their sole T-cell target, or even vaccines with two or three of the most prominent T-cell targets might not generate a T-cell response with the same breadth and hence the same efficacy as the WT infection does. Thus, it might be better to use a live attenuated or single cycle CMV vaccine vector, since these vectors, if constructed properly, should generate a broader and superior immune response.

Since viremia is the single-most determining factor in the pathogenesis in CMV-related diseases [494], we wanted to compare the virus load in tissues in infected animals. For this purpose, the same CMV naïve rhesus macaques were infected with a WT vaccine vector and Δpp65 deletion mutant expressing different SIV markers, and the animals were sacrificed at predetermined intervals after vaccination. Because of their different foreign antigens, the two vaccine vectors could be detected by nested PCR in the same samples and viral loads could be determined. RhCMV 68-1 is a laboratory strain of RhCMV, and as such, has adapted to culture in rhesus fibroblasts. Similar to what has been observed in HCMV AD169 or HCMV Towne [495], genomic regions in

the co-called ULb' region of HCMV have been lost in RhCMV 68-1 due to *in vitro* passaging. As a result, the virus lost its pentameric complex needed to infect cells through endocytosis and successively its ability to infect cells other than fibroblasts effectively [415]. Consequently, the virus is barely detectably in the saliva and urine of naïve rhesus macaques infected with the BAC derived virus early after infection compared to low passage clinical isolates [416], and we see very low copy numbers of vaccine vectors based on 68-1 in animal tissues, even the injection site, 2-3 weeks post infection. Deleting pp65a and b from the 68-1 WT revealed a very different picture. The virus was easily detectable in almost all tissues of infected animals, whereas the WT was mostly restricted to the injection site and the nearest draining lymph node. Moreover, the viral loads of the pp65 deletion mutant were in some cases 6-7 logs higher than measured for the 68-1 WT in the very same animals. Our data indicate that *in vivo* pp65 acts as a silencer or damper of its own viral replication in the context of a primary infection, probably limiting the virus to a viral load that will ensure the survival and well being of the host, so that the virus can establish a persistent infection and spread to other individuals who are in contact with the infected individual. This is the first time we encountered that a deletion mutant in on a somewhat benign parental virus led to a highly significant increase of viral replication and dissemination *in vivo*. Therefore, deleting pp65 from a potential vaccine vector is not an option.

5.3 Developing a pp71 deletion virus into a 2nd generation CMV vaccine vector.

Given that the pp65 deletion mutant did not achieve the expected results, we had to explore other options to create a safer vaccine vector platform with high immunogenicity. The second potential candidate was pp71, another structural virion protein found in the tegument [18, 444]. Like pp65, this protein was also shown to have immediate intrinsic immune evasion functions by degrading multiple proteins within ND10 bodies including DAXX and ATRX [192, 480], enabling the viral replication utilizing the host DNA replication machinery [208]. It also appears to be involved in viral reactivation, as the protein remains in the cytosol during latency, not affecting the endogenous DAXX levels, whereas re-entry into the nucleus seems to shift the virus from latency into lytic replication [68]. Preliminary data from our institute indicates that HCMV lacking pp71 can infect humanized mice normally and establishes latency but cannot reactivate from this state (Crawford et al., unpublished data).

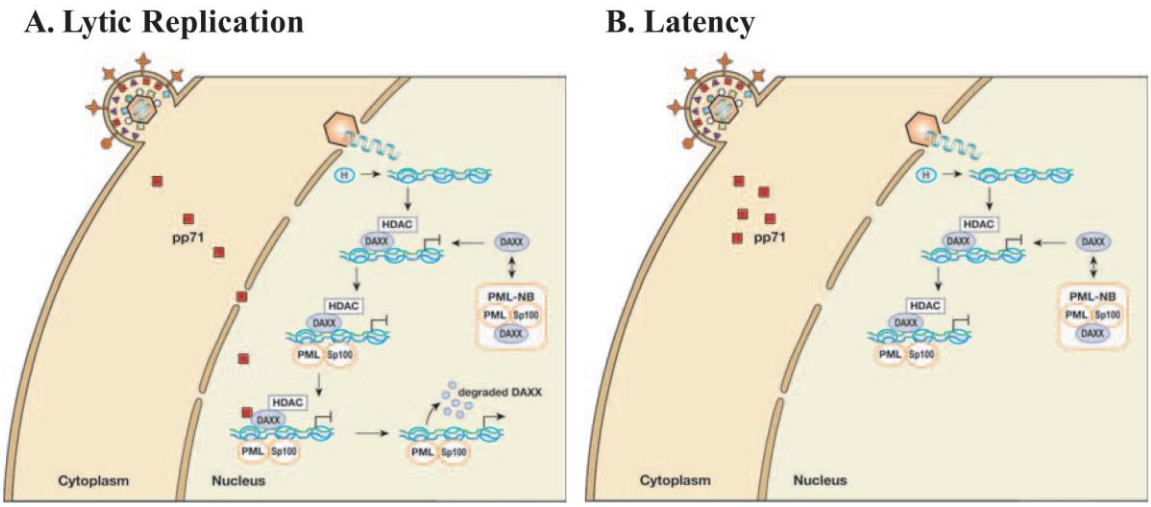

Fig.28: Subcellular localization of tegument-delivered pp71 determines whether HCMV initiates lytic replication or establishes quiescent, latent-like infections. (A) Lytic replication initiates when tegument-delivered pp71 is allowed access to the nucleus. Capsids docked at nuclear pores release their DNA into the nucleus, and viral genomes associate with cellular histones (H). The Daxx protein, which rapidly dissociates from, and reassociates with, PML-NBs, accumulates around viral genomes, recruits an HDAC, and silences viral IE gene expression. Other PML-NB components are also recruited and participate in the silencing of viral genomes. pp71 binds to Daxx in these newly formed PML-NBs, induces Daxx degradation, derepresses viral IE gene expression, and thus initiates the lytic replication cycle. (B) In cells where quiescent or latent infections are established, tegument-delivered pp71 remains in the cytoplasm. Daxx (and presumably other PML-NB proteins) silences viral gene expression in these cells (taken from Kalejta, Microbiol Mol Biol Rev. 2008 Jun;72(2):249-65.).

One fundamental problem with generating a pp71 deletion mutant is that this virus was shown to have a severe replication defect in HCMV at low MOIs. As a matter of fact, the virus barely replicated at all at an MOI of 0.01 in primary human fibroblasts, whereas viral replication an a high MOI of 3 did not seem to be affected as much (Fig.29) [211]. When we repeated these experiments ourselves with the same virus provided to us by the authors of the study, we achieved similar results (Fig.30). Interestingly, when we constructed a pp71 deletion mutant based on the RhCMV laboratory train 68-1, we were able to reconstitute the virus in non-complementing tRFs. To generate high titers of the virus, we established a complementing cell line conditionally expressing RhCMV pp71 controlled by a *Tet-On* operon [496]. Although expression of the protein should be inhibited in the absence of doxycycline, the regulation of the operon turned out to be leaky, and even uninduced cells expressed small amounts of the viral proteins, even enough to complement a full deletion mutant. Using this cell line, high titer stocks of the pp71 deletion mutant could be generated. Because the attenuation of the pp71 deletion is alleviated at high MOIs, stocks generated on complementing cell lines can subsequently be used to create new stocks of the deletion mutant on non-complementing cells by infected these cells at a high MOI. The hereby

generated virus is negative for pp71 in the genome as well as in its virion, since virus grown on complemented cells still contains pp71 expressed by the host cells *in trans* in its virion, giving it a replication advantage and WT appearance during its first round of replication.

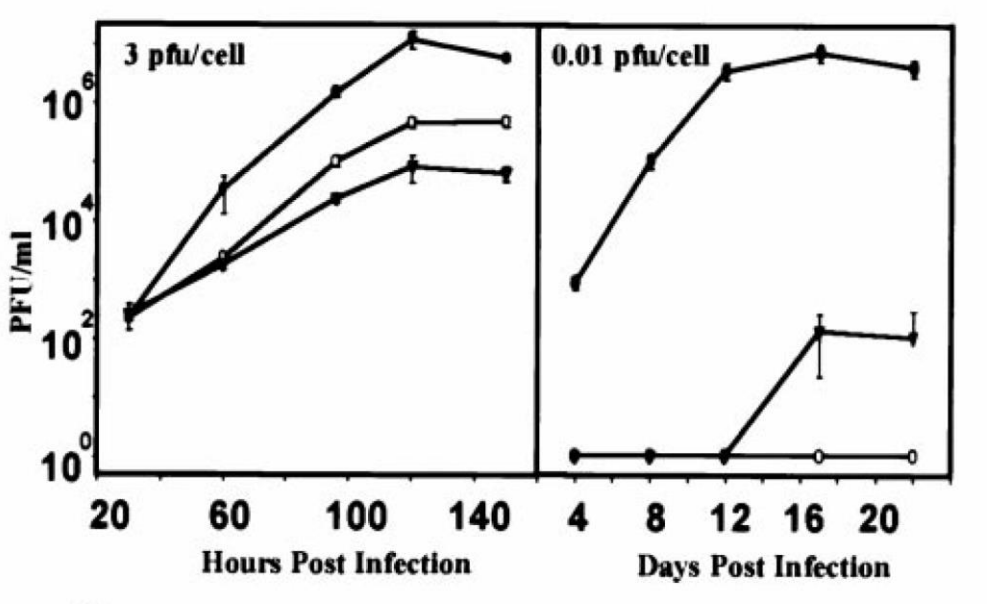

Fig.29. Growth kinetics of wt and mutant viruses. Human fibroblasts were infected (3 or 0.01 pfu per cell) with wt (closed circles), AD Sub UL82^{+UL82} (open circles), or AD Sub UL82^{-UL82} (closed triangles) virus. Cultures were harvested at the indicated times after infection, and infectious virus was quantified by plaque assay on WF28-71-HA cells (taken from Bresnahan and Shenk, Proc Natl Acad Sci U S A. 2000 Dec 19;97(26):14506-11.).

Single and multistep growths curves performed at different MOIs on tRFs revealed that the RhCMV Δpp71 deletion mutant only showed a minor delay in viral titers at high MOI, independent of the virus being grown on complementing or non-complementing cells, whereas both version of the mutant showed significant attenuation at lower initial MOIs (Fig.31). It was surprising that the virus showed grew at low MOIs, even in the absence of pp71, given that a similar HCMV deletion mutant did not grow under these conditions [211].

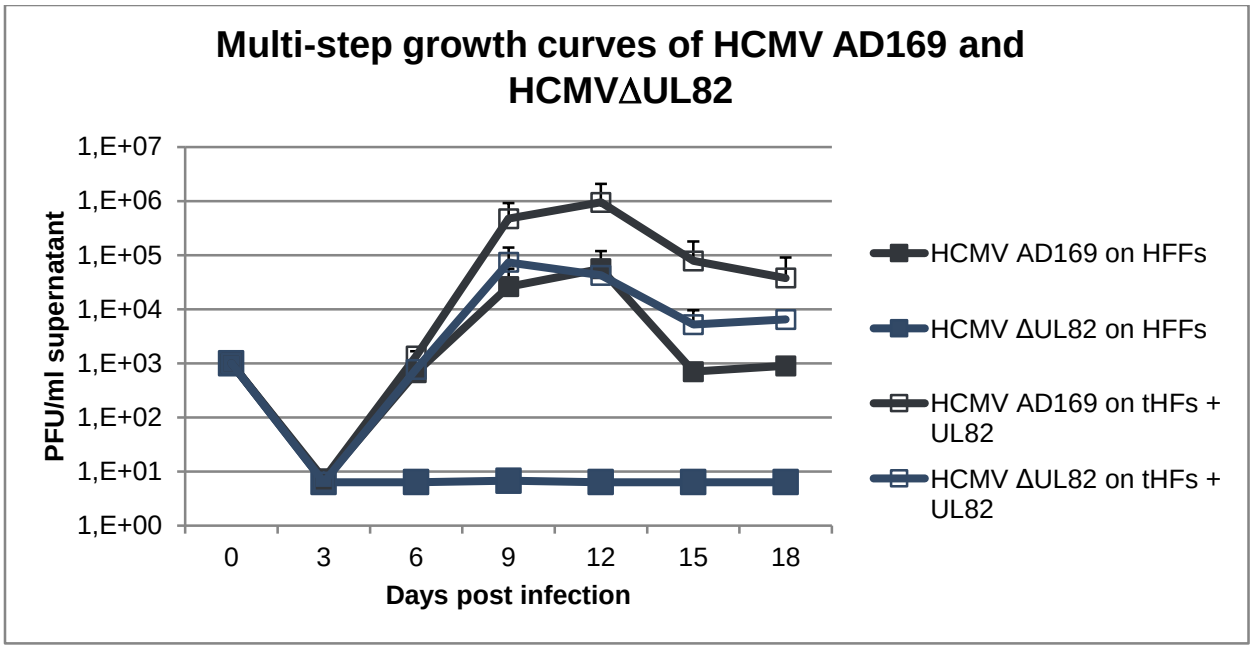

Fig.30: Multi-step growth curve of HCMV AD169 ΔUL82 (pp71). Primary human foreskin fibroblasts (1°HFFs, solid squares) or telomerized human fibroblasts stably expressing HCMV UL82 (pp71) (tHFs + UL82, open squares) were infected with HCMV AD169 WT or HCMV AD169 ΔUL82 (pp71) at an MOI of 0.01. Supernatant was harvested in 3 day intervals, and the viral titers were determined by limited dilution assay.

Further examination of the RhCMV pp71 protein revealed, that the protein is sufficient to delocalize DAXX and ATRX from the nucleus of transfected cells in a fashion similar to the HCMV pp71 protein. Interestingly, when we examined tRFs infected with our RhCMV Δpp71 deletion mutant, DAXX as well as ATRX were still delocalized and DAXX was still degraded, indicating that RhCMV must encode for a second viral protein with a similar function as pp71 (Marshall et al., unpublished data). This protein is, at least functionally, not conserved between RhCMV and HCMV, explaining why an HCMV Δpp71 deletion mutant can no longer degrade DAXX to transactivate viral gene expression and viral genome replication and why a deletion mutant in the human virus is more growth deficient in the absence of pp71 compared to homologues RhCMV constructs. While this indicates that pp7-deleted RhCMV vectors are likely less attenuated *in vivo* compared to the corresponding HCMV mutant, any *in vivo* attenuation observed for RhCMV will most likely be conserved in HCMV since the HCMV version will be even more attenuated than the comparable RhCMV version.

Although our deletion mutant only showed a moderate growth reduction *in vitro* at low MOIs compared to the HCMV Δpp71 construct, the level of *in vivo* attenuation achieved with this mutant was remarkable. After infection of naïve RMs with the construct, urine was collected from

the animals on a regular basis to detect secreted virus by co-culturing the urine on tRFs stably expressing RhCMV pp71. RhCMV 68-1 based WT vaccine vectors can be detected by immunoblot from urine co-cultures by day 28, whereas we were not able to detect our RhCMV pp71 deletion at any time point up to day 231 in co-cultures on complementing cells (Fig.32). This indicates that the virus is not shed by the infected animals, which could indicate that the pp71 deletion mutant, if used as the backbone for a future vaccine vector, probably cannot be transmitted horizontally between humans, since transmission generally occurs through secretion in bodily fluids. Given the more severe attenuation of the human deletion virus *in vitro*, it is very likely that we will see a similar effect for an HCMV Δpp71 deletion vector backbone, fulfilling one essential requirement for future use in the human population.

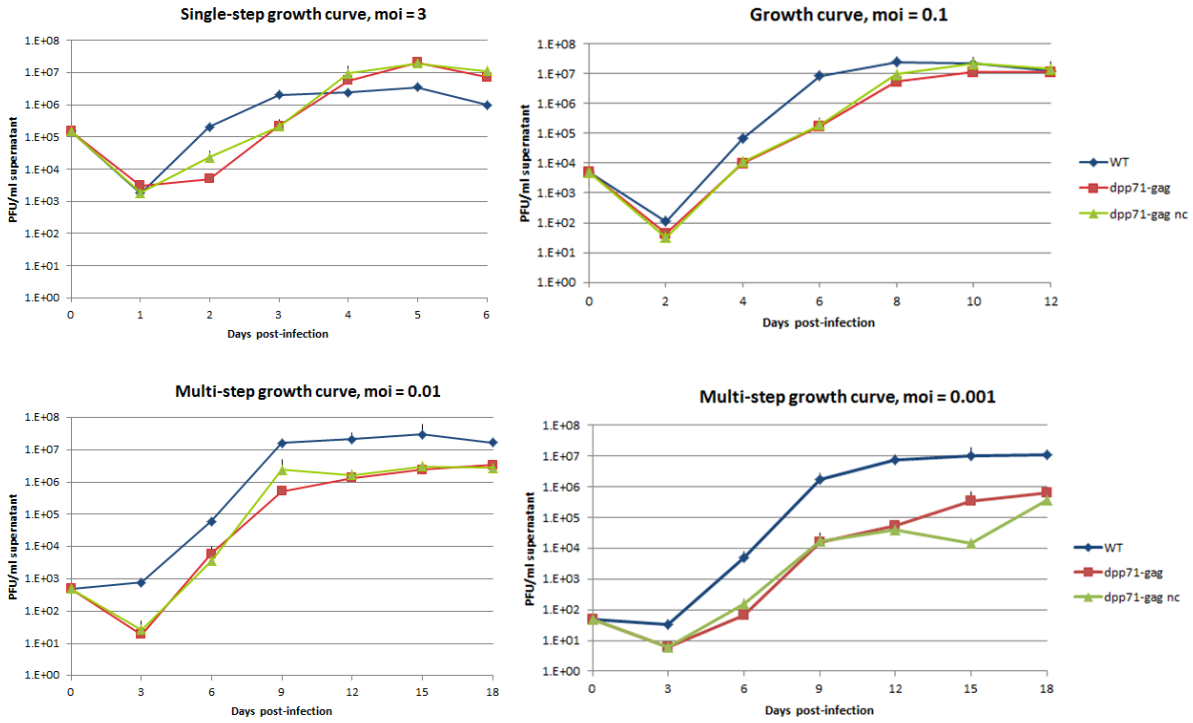

Fig.31: Growths kinetics of complemented and uncomplemented RhCMV 68-1 Δpp71 deletion mutants compared to 68-1 WT. Telomerized rhesus fibroblasts (tRFs) were infected with RhCMV 68-1, RhCMV 68-1 Δpp71 grown on complementing tRFs stably expressing pp71, or RhCMV 68-1 Δpp71 grown on non-complementing tRFs at an MOI of 3 (A), 0.1 (B), 0.01 (C) and 0.001 (D). Cell free supernatant was collected on the indicated time points and the viral titers were determined with limited dilution assays and graphed over time.

Interestingly, while the deletion mutant was not shed by the infected naïve animals, indicating *in vivo* spread-deficiency compared to the WT, the immune response induced by the vector exhibited the same kinetics, amplitude and duration as the immune responses induced by

RhCMV 68-1 WT vectors (Fig.33). $CD4^+$ and $CD8^+$ T-cell responses against the immunodominant CMV proteins IE and pp65 (Fig.33A) or against complete CMV lysates (Fig.33B) are detectable in PBMC as well as BAL 7-14dpi and peek around 21dpi. Thereafter, the amplitude of the response declines and plateaus at a steady level around 56dpi where it remains ad infinitum. Similar to data generated with our pp65ab deletion vector, infection with a pp71 deletion vector induces an adaptive immune response strong enough to protect against re-infection with a ΔUS2-ΔUS11 deleted RhCMV mutant. This shows a breadth in CD8+ T cells responses broader than achieved with pp65 alone (Fig.27) and potentially equally protective as responses generated by the WT vectors.

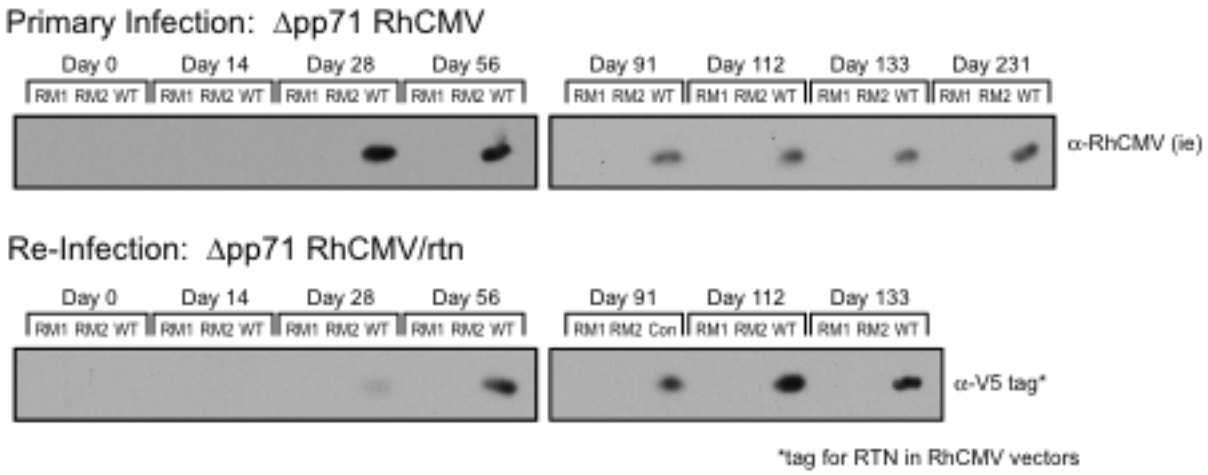

Fig.32: RhCMV 68-1 Δpp71 is not shed by infected animals. (Upper panel) Naïve RMs were infected with RhCMV 68-1 Δpp71 and urine was collected in 14 days intervals after infection. The urine samples were co-cultured on pp71 expressing telomerized rhesus fibroblasts. 28 days after inoculation, the cells were lysed and Western blots were performed to detect the RhCMV IE protein. (Lower panel) CMV positive RMs were superinfected with RhCMV 68-1 Δpp71 SIV RTN. Like described above, Urine was collected in 14 day intervals and co-cultures were performed on complementing cells. After 28 days of co-culture, cells were lysed and Western blots were performed to detect the V5 tagged RTN transgene.

The quality of the immune response is impressive especially considering the spread-deficiency observed *in vivo*, but it indicates that this vector could work as a T-cell inducing vaccine vector. Moreover, also considering the required safety of the vaccine backbone, especially in CMV vulnerable populations like women of childbearing age and immunocompromised individuals, the combination of high immunogenicity paired with high attenuation and probably low pathogenicity would make this vectors design perfect for future human applications.

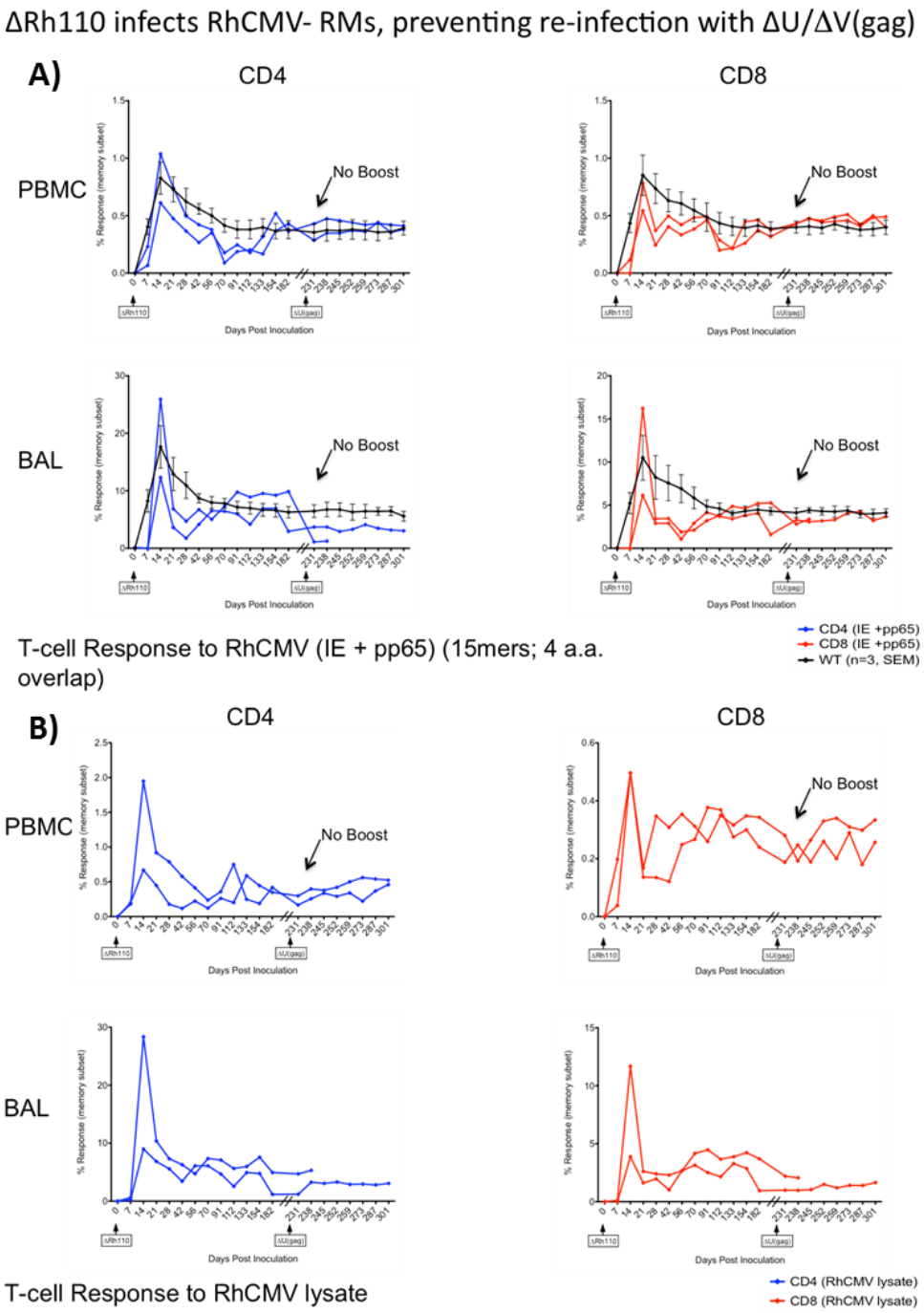

Fig.33: RhCMV 68-1 ΔRh110 (pp71) infects naïve RMs normally and protects against RhCMV 68-1 ΔRh182 (US2)-Δ189 (US11) GAG + ΔRh178 superinfection. A) Two naïve RMs were infected with infected with RhCMV 68-1 ΔRh110 on day 0. PBMCs (upper panel) and BAL (lower panel) were harvested in 7 day intervals and RhCMV IE and pp65 specific $CD4^+$ (left panel, blue lines) and $CD8^+$ (right panel, red lines) T-cell responses were determined by co-culturing isolated T-cells with APCs presenting specific peptides for IE and pp65. Results of prior experiments infecting naïve RMs with RhCMV 68-1 WT are shown for comparison (black lines). B) To determine broader RhCMV specific T cells responses, peptides from whole RhCMV lyses were loaded onto APCs and presented to T-cells isolated from PBMCs (upper panel) or BAL (lower panel). On day 231 post infection, the RMs were superinfected with RhCMV 68-1 ΔRh182 (US2)-Δ189 (US11) GAG + ΔRh178. As can be seen in all panels, no boost in $CD4^+$ or $CD8^+$ T cell responses was detected after superinfection, indicating, that the virus did not successfully superinfect.

To determine just how powerful the level of attention of the pp71 deletion *in vivo* really is, we constructed a mutant virus lacking pp71 replaced with SIV GAG. On top of it, we subsequently deleted pp65 a and b from the viral genome creating a GAG expressing triple deletion mutant. The absence of pp65ab alone from the RhCMV backbone leads to a massive increase of viral replication in naïve animals with 3-6 logs increased viral loads of the mutants compared to the 68-1 WT vectors in almost all tissues in the same animals (Table 8). This massive replication and the connected higher probability of increased CMV induced pathology led us to exclude pp65 as a potential candidate for attenuation of the WT vector. When we now co-infected naïve juvenile RMs with RhCMV Δpp65ab RTN and RhCMV Δdpp71 GAG + Δpp65ab deletion mutants and compared viral loads in tissues at 14 dpi, it became apparent, that while the pp65 deletion showed increased viral loads in the test animals, this phenotype was overcome by the additional deletion of pp71 (Table 9), reducing the viral loads to viral titers seen in similar experiments performed with pp71 deletion mutants (data not shown). Furthermore, deletion of pp71 also reduced the dissemination of the vaccine vector. Whereas the pp65ab deletion virus spreads to all major organs in the infected animals, the pp71 deletion restricts the viral spread mostly to the injection site and the nearest draining lymph nodes. All these factors further underline the potential of our pp71 deletion to serve as an attenuation factor to create a safer CMV vaccine vector that retains the full CMV potential to induce a strong and lasting adaptive immune response against the vector as well as against any inserted foreign protein. In addition to the data presented here, we were also able to show that a pp71 deleted vector is still fully capable to re-infect CMV positive animals and that the virus induces T cells with T_{EM} phenotype.

As mentioned above, the T_{EM} phenotype of the $CD8^+$ T cells is likely the reason for the superior efficacy of our CMV-based T cell vaccine vectors compared to any other previously studied vaccine platforms, since these T cells do not have to proliferate like T_{CM} after re-encountering their target antigen, giving them a faster response time and a kinetic advantage against the initial encounter of invading pathogens. Additionally, we were able to demonstrate, that pp71 deleted vectors cause substantially reduced pathogenesis when directly injected into RM fetuses compared to WT vectors and especially clinical isolates with fewer incidents of spontaneous abortions and less severe or even total absence of virus induced pathology and viral titers in tissues. As shown in Fig.32 the vectors are not shed by infected animals, and by observing

a nursing mother vaccinated with multiple different pp71-based vaccine vectors, we were able to demonstrate that our vectors were not transmitted to the baby, and the young animals remained seronegative for all vaccine vectors for the entire time it was monitored (Marshall et al., unpublished data). Additionally, when we tried to adoptively transfer the RhCMV Δpp71 deletion mutant through isolated PBMCs from a vaccinated macaque to naïve animals, we failed, and the recipient animals never showed immune responses against SIV (Hansen et al, unpublished data). Using PBMCs from animals vaccinated with vaccine vectors based on the RhCMV 68-1 WT, horizontal transmission through adoptive transfer is easily achievable.

Tissue Type	Rh21112		Rh21979	
	Δpp65ab	Δpp65ab/Δpp71	Δpp65ab	Δpp65ab/Δpp71
Skin Injection Site (Left)	54	ND	ND	2,080
Skin Injection Site (Right)	31,767	ND	173	ND
Axillary LN (Left)	1,338	34,044	ND	68,146
Axillary LN (Right)	6,289	ND	15,032	ND
Iliosacral LN	44,447	ND	ND	12
Tonsil	ND	5	ND	ND
Tracheobroncial LN	69,901	ND	ND	ND
Submandibular Salivary Gland (Left)	3	600	23	ND
Submandibular Salivary Gland (Right)	3,867	ND	ND	ND
Thyroid	3	ND	3	ND
Lung	7,843	ND	5	ND
Spleen	88,148	ND	528	ND
Kidney	4,475	ND	ND	ND
Esophagus	ND	ND	7	ND
Ileum	5	ND	1,725	ND
Colon	2,230	ND	72	ND
Liver	38,320	ND	946	ND
Ileocaecal	ND	ND	3	ND
Inguinal LN (Left)	417	452	ND	ND
Inguinal LN (Right)	2,699	ND	ND	ND
Inferior Mesenteric LN	491	ND	ND	ND
Medial Mesenteric LN	3	ND	ND	ND
Superior Mesenteric LN	1,147	ND	3	4,465
Urinary Bladder	32	ND	1,871	ND
Brain	17	ND	ND	ND
Spinal Cord (Thoracic)	ND	ND	4	ND
Spinal Cord (Cervical)	9	ND	ND	ND
Bone Marrow	7	ND	ND	ND
PBMC	100	ND	ND	ND

Table 9: Genome copy numbers of RhCMV Δpp65ab RTN and RhCMV Δdpp71 GAG + Δpp65ab CMV-naive RMs.

Taken all these data into consideration, this indicates that although pp71 deletion mutant are only moderately growth-deficient *in vitro* they are attenuated *in vivo* in regards to shedding

and horizontal transmission, they still induce T-cell responses that seem to be as frequent and effective as responses observed in animals infected with vaccine vectors based on the RhCMV 68-1 WT virus. In fact, preliminary challenge studies resulted in similar levels of protection as observed previously [359] indicating that our pp71 deletion vectors achieve similar if not higher levels of protection and clearance in monkeys challenged with the virulent SIVmac239 strain.

5.4 The bright future (or where to go from here).

Our preliminary results shown here for vaccine vectors based on pp71 deletion mutants are very encouraging and warrant further studies and closer examination. But to push our vaccine platform from the bench and the animal model system into the real world and the human patient, it is also essential, in parallel to conducting further animal trials and studies, to generate a human version of our vaccine platform to facilitate future clinical trials. Several issues have to be overcome to achieve this goal. First and foremost, as was shown in previous publications and by us, a HCMV pp71 deletion mutant is severely attenuated and does not show spreading through tissue cultures at low MOIs [211], so generating stocks of vaccine vectors in HCMV is more challenging that it has been for the RhCMV Δpp71 mutant, since this virus still retains a medium level of spreading at low MOIs. Additionally, since the HCMV vaccine mutants are meant to be generated for future human trials, all steps of virus reconstitution from the BAC and generation of viral stocks have to comply with FDA guidelines and will have to be approved by this institution, further complicating an already challenging task. To this end, only MRC-5 fibroblast as host cells to grow our vaccine stocks for further *in vivo* trials in humans are FDA approved, whereas FDA approved pp71 complementing cell lines currently do not exist. Thankfully due to the innovative efforts of multiple scientists in our group, we have succeeded in generating high titer stocks of HCMV pp71 deletion mutants without the use of complementing cells, simple by inhibiting DAXX translation with specific siRNAs, a function performed by pp71 in the WT (van den Worm et al., unpublished data). These stocks can now be produced in our own facility under GRP conditions to generate research virus seed stocks. Final clinical trial material will be generated by contract manufacturing organizations specialized on generating GMP products.

In addition to solving the production issues with our vaccine platform, we also have to demonstrate immunogenicity of antigens introduced into our HCMV vectors, since data generated with RhCMV in rhesus macaques cannot be simply extrapolated. This is very problematic, since

all CMVs are considered to species specific, and no animal model other than humanized mice can be employed to test HCMV infections *in vivo*. This model system is used in our institute by engrafting human CD34+ stem cells into Nod/SCID/IL2Rγ k.o. mice. As mentioned above, HCMV WTs as well as pp71 deletion mutants based on the low passage HCMV strain TR (repaired for its US2-US11 deletion) are able to infect humanized mice and establish viral latency. More importantly, whereas all tested HCMV low passage WTs are capable of reactivating from latency, pp71 deletion mutants are not, indicating *in vivo* attenuation. However, this model is not suitable to study immunogenicity of HCMV-based vectors. To study immunogenicity an immunocompetent animal model is required that can be infected with HCMV. Jurak and Brune [497] were able to show that MCMV does not replicate in human cells, because the virus cannot counteract the virus-induced apoptosis like it would in mouse cells through its antiapoptotic proteins. Interestingly, when the antiapoptotic UL37x1 protein from HCMV is introduced into MCMV, the generated mutant is able to inhibit apoptosis and to replicate in human cells. Importantly, HCMV vectors based on clinical isolates containing full-length genomes, apart from deletions and mutations indroduced to attenuate the virus *in vivo* (like pp71), can infect rhesus macaques (Caposio et al., unpublished data). These data indicate that the species specificity is not absolute, and the virus can cross the species barrier, at least in the context of a forced *s.c.* injection. These infections do, in all likelihood, not occur naturally by co-hosting two different species together in close quarters. Prior reports show the isolation of African green monkey derived simian CMV (SCMV) from humans in two independent cases [370, 498, 499], without any examination regarding the source of the virus, but these cases were generally regarded to be anecdotal at best. We are now able to study the immunogenicity, and potentially efficacy against pathogen challenge, of our HCMV vaccine vectors *in vivo* in rhesus macaques, and we are able to determine the immunogenicity of attenuated mutants to select those most likely to succeed in humans prior to clinical trial, making our final construct more like lo prevail in this rigorous process.

As a second essential step to construct a new, marketable vaccine platform, it is more than desirable to have a technology that is applicable to more than one, although very important, pathogen. Most of our studies so far have been focused on SIV, and by extension HIV, but we have also explored other important human pathogens. RhCMV vector expressing *Mycobacterium tuberculosis* antigens have been generated and tested *in vivo* in several trials, and preliminary data indicates that the protection achieved by our vectors in the rhesus macaque model is superior to

the protection generated by the licensed BCG vaccine (Xu, Hansen and Picker, unpublished data). This is even more interesting given that in SIV, large regions of the viral genome were integrated into the different RhCMV vaccine vectors to generate broad T-cell responses against most of the major viral proteins, whereas in Tb, only a small fraction of the pathogen genome can be inserted given the immense size of the bacterial genome (4 million bp, 4000 genes, compared with 10000 bp and 19 proteins in SIV). Still, by selecting the right combination of T-cell targets, protections can be achieved, even in the rhesus macaque model where infection with 25 CFU of Tb is lethal within months. Similarly, preliminary studies using vaccine vectors generated in RhCMV encoding for immunodominant protein of the liver stage (sporozoite) of the protozoan parasite *Plasmodium*, the infectious cause of malaria, showed a significant effect *in vivo* against challenge with *P. knowlesi* sporozoites (Scholz et al., unpublished data). However, given the immense size of the parasite's genome (14 chromosomes, varying in size from 500 kb to 3500 kb, combined accumulating to 23Mb encoding for about 5300 genes), the major challenge is to find the right combination of potential T-cell targets to generate sterile immunity and further studies are needed to optimize antigen and vector backbone combinations. In addition to the aforementioned pathogens, a wide array of other human pathogenic viruses, bacteria and parasites as well as cancers are studied right now to broaden the applicability of our vaccine platform, and we are confident that our work will help to save and improve the lives of millions of people all around the globe in the future.

Finally, the last point of improvement we have to achieve to increase the applicability of our vaccine platform is its efficacy. In our SIV challenge studies, we were able to generate T-cell responses against SIV that enabled 50% of the vaccinated animals to not only control, but eventually clear the virus altogether [359, 360]. This is an impressive result and it is superior to all prior studies using other vaccine platforms, but it also means that 50% of the animals were not protected and that the vaccine was ineffective in these animals. We are still investigating the immunological differences between the animals that were protected and the animals in which the vaccine proved to be ineffective. Given the high virulence of SIV, it is important to stress, however, that 50% protection in animals does not necessarily predict 50% protection in humans. In addition to studying genetic or stochastic paramerters for this outcome, we are also evaluating the role of various vector backbones on this outcome because it became apparent over the course of our investigation that the vector design of our CMV vaccine construct has a tremendous effect on the

generated T-cell response and probably subsequently on the vaccine efficacy. As we were able to show, RhCMV vaccine vectors induce broad T-cell responses against foreign antigens with total numbers of specific, independent epitopes well above what has been achieve in natural SIV infections or with any other vaccine platform [278]. The T-cell epitope coverage of foreign antigens in the context of RhCMV is so high, that in the case of SIV-GAG epitopes spanning large parts of the viral protein are presented to T-cells [278]. This breadth alone makes it hard, even for a highly mutagenic pathogen like a retrovirus, to evade the T-cell response by mutating epitopes, since dozens of epitopes all across the genome would have to be mutated simultaneously. Furthermore, the T-cells responses in the context of a RhCMV infection differ significantly from the T-cell response seen with other vaccine approaches. RhCMV 68-1 does not induce canonical MHC class I restricted epitope normally seen in SIV infection or after vaccination with DNA or common vaccine vectors, but it introduces a collection of very unique and never before described responses [278]. About 2/3 of the epitopes presented to CD8+ T-cells are actually presented by MHC class-II molecules, violating an immunological dogma in which MHC class I presents to CD8+ T-cells and MHC class-II presents to CD4+ T-cells. The remaining 1/3 of epitopes are presented by MHC-class I, but not by classical HLA molecules, but by the non-classical HLA-E molecules (Hansen, Sacha and Picker, unpublished data). These molecules are known to present signal sequences of classical MHC class I alleles to NK-cells to counteract the "missing self" recognition and thereby preventing NK-cell mediated killing. In addition, some of the epitopes presented either on MHC class-I or class-II molecules are presented in all or almost all tested animals. This would not be that surprising in inbred mouse strains, but rhesus macaques represent an outbred population with a wide array of MHC haplotypes, and it should be impossible for an outbred population with different MHC alleles to present the same epitopes. We termed theses widely presented epitopes "supertopes". In the context of MHC-II, these promiscuous epitopes can be presented by multiple different MHC class-II alleles (Sacha et al, unpublished data). In the context of MHC class I, the non-polymorphic nature of HLA E permits the universal presentation of the same peptides (Hansen, Sacha and Picker, unpublished data). In addition, deletion of the US11 protein from the vaccine backbone further increases the number of epitopes presented, since the deletion of this MHC class-I evasion gene now enables the presentation of canonical epitopes recognized by T cells in SIV infected animals, but not elicited in the context of US11-containing RhCMV. All theses different responses can be either generated or inhibited depending on the

makeup of the vaccine backbone. As mentioned above, canonical T cell responses can be generated by the deletion of US11, whereas MHC class-II restricted epitopes only appear in the absence of a functional pentameric complex. Supertopes and HLA-E responses are inhibited by simultaneously expressing functional UL128 and UL130 proteins, possibly due to their chemokine activity (Hansen, Malouli, Früh and Picker, unpublished data). Taken together, we are now able to generate custom vaccine vectors generating exactly the immune responses needed for a specific target pathogen. To date, it is not clear which of the aforementioned unique T-cell responses generated by CMV vector are responsible for the protection of our animals in the vaccine studies, but in ongoing studies we will determine the optimal vector combinations to fight SIV infections, which will hopefully further increase the effectiveness of our vaccine platform.

We conclude that our new and innovative approach might help save the lives of millions of peoples suffering from infectious diseases like HIV and Tb. Protecting the exposed population from these pathogen is a challenging task, but we are confident, that our vaccine platform will provide new hope to contain and hopefully eradicate HIV. Further steps have to be undertaken before human testing can begin, but our animal results show that we are able to not only enable vaccinated animals to control the viral infection, but also enable them to clear the virus altogether suggesting that our approach might be beneficial for already HIV positive individuals ultimately curing HIV.

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Supplementary Material

Supplemental Figure 1: Alignment of ORFs Rh08, Rh13.1, Rh61/Rh60, Rh67.1, Rh72, Rh152/Rh151, Rh164, Rh194 and Rh197 from RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92. The amino acid sequences of the Rh08 (A), Rh13.1 (B), Rh61/Rh60 (C), Rh67.1 (D), Rh72 (E), Rh152/Rh151 (F), Rh164 (G), Rh194 (H) and Rh197 (I) from our BAC derived RhCMV 68-1 sequence discussed in this paper and from the two fully sequenced RhCMV strains 68-1 (AY186194) and 180.92 (DQ120516) (A-F and H-I) or RhCMV isolate CNPCR (EF990255) (G) were aligned using a CLUSTAL W (1.83) multiple sequence alignment.

Supplemental Figure 1A: Alignment of ORF Rh08 from RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh08 (RL11 family)

```
68-1_BAC_       MNAYHTLISCLCFFILYAYITESTRVVTLQYVYNVSIGENVTLSKPDNLSFQLHSWFCNN
68-1_Virus_     MNAYHTLISCLCFFILYAYITESTRVVTLQYVYNVSIGENVTLSKPDNLSFQLHSWFCNN
180.92          MNAYHTLISCLCFFILYAYITESTRVVTLQYVYNVSIGENVTLSKPDNLSFQLHSWLCNN
                ************************************************************:***

68-1_BAC_       RASACNNPIMKLCEETAGNNKPNSYTRFQNNCHPPTFTCNTTGLYLYNVQETDPTTYTLT
68-1_Virus_     RASACNNPIMKLCEETAGNNKPNSYTRFQNNCHPPTFTCNTTGLYLYNVQETDPTTYTLT
180.92          RASACNNPIMKLCEETAGNNKPNSYTRFQNNCHPPTFTCNTTGLYLYNVQDTDPATYTLT
                ************************************************:***:*****

68-1_BAC_       QRAGNGNITDRNTTYIIHFITSTTPPPVTNYICNLSSTSCTNTSNYQL------------
68-1_Virus_     QRAGNGNITDRNTTYIIHFITSTTPPPVTKLHMQFIFNQLHKHKQLPTIAT---------
180.92          QRAGNGNITDRNTTYIIHFITSTTPPPPLQTTYAIYLQPVAQTHATTNYSYITITLTVIT
                ***************************  :    :      :

68-1_BAC_       ----------------------------------------
68-1_Virus_     ----------------------------------------
180.92          LILFMLGAGYLKHRRSLKHYKQNTHKCTSLGESRYPESSI
```

Supplemental Figure 1B: Alignment of ORF Rh13.1 from RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh13.1 (RL13)

```
68-1_BAC_       MTKYTCFRSMSACGIFMLLINLALACDSQCACNSSCGFAYNVTHVSGYEHSNVTLHTSIS
68-1_Virus_     MTKYTCFRSMSACGIFMLLINLALACDSQCACNSSCGFAYNVTHVSGYEHSNVTLHTSIS
180.92          MTKYTCFRSMSACGIFMLLINLALACDPQCACNSSCGFAYNVTHVSGYEHSNVTLHTSIS
                **************************.******************************

68-1_BAC_       HSNISHMNVGYWIRYNYPVNSYTICTVSGNNVASTKHNGWFFECNGTSLTLHNLNADHTG
68-1_Virus_     HSNISHMNVGYWIRYNYPVNSYTICTVSGNNVASTKHNG----------------------
180.92          HSNISHMNVGYWIRYNYPVNSYTICTVSGNNVASTKHNGWFFECNGTSLTLHNLNADHTA
                ************************************

68-1_BAC_       SYLFKNLLGLMEHYTVTVLPIPQPPAPQVTTVTNCSLTFFSEHLWRNATTRIITTTTQST
68-1_Virus_     ------------------------------------------------------------
180.92          SYLFKNLL------------------------------------------------------


68-1_BAC_       STTTTRTTKPTTTTHRTTAGRVSTPTPEESSTSTTTEESTTTTWPPGRPKFISKYSLNSR
68-1_Virus_     ------------------------------------------------------------
180.92          ------------------------------------------------------------
```

Supplemental Figure 1C: Alignment of ORFs Rh61/Rh60 from RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh61/Rh60 (UL36)

```
68-1_BAC_       MSYRLMSGMDDLRDTLMAYGCIAVRAQDPASLYTFVDQECGTKLHLAWPDNGYIQLRPRT
68-1_Virus_     MSYRLMSGMDDLRDTLMAYGCIAVRAQDPASLYTFVDQECGTKLHLAWPDNGYIQLRPRT
180.92          MSYRLMSGMDDLRDTLMAYGCIAVRAQDPASLYTFVDQECGTKLHLAWPDNGYIQLRPRT
                ************************************************************

68-1_BAC_       LMGPFSSKYYDVCCQGKYVCCNELMEPFGVVELSKLGFYQLVMMIGRSGAIYCYEETEKC
68-1_Virus_     LMGPFSSKYYDVCCQGKYVCCNELMEPFGVVELSKLGFYQLVMMIGRSGAIYCYEETEKC
180.92          LMGPFSSKYYDVCCQGKYVCCNELMEPFGVVELSKLSFYQLVMMIGRSGAIYCYEETEKC
                ***********************************.************************

68-1_BAC_       VYCLAPDMKSFIQLGLRRCDYLQKMELYQEPVIDCDEIIKELMIFNWDVDRISDVVAKNG
68-1_Virus_     VYCLAPDMKSFIQLGLRRCDYLQKMELYQEPVIDCDEIIKELMIFNWDVDRISDVVAKNG
180.92          VYCLAPDMKSFIQLGLRRCDYLQKMELYQEPVIDCDEIIKELMIFNWDVDRISDVVAKNG
                ************************************************************

68-1_BAC_       YRVYDIRDPLGEQVDSHFALWSSDSAVANFQDTSFSLMSPSGLRSFEIMVRCVARIVCVN
68-1_Virus_     YRVYDIRDPLGEQVDSHFALWSSDSAVANFQDTSFSLMSPSGLRSFEIMVRCVARIVCVN
180.92          YRVYDIRDPLGEQVDSHFALWSSDSAVANFQDTSFSLMSPSGLRSFEIMVRCVARIVCVN
                ************************************************************

68-1_BAC_       QLLGVLGCFRKEKK----------------------------------------------
68-1_Virus_     QLLGVLGCFRKEKNEFLVRLYVLVDKFGTIYGFDPALNSIYRLAENMRMFTCMMGKKGYR
180.92          QLLGVLGCFRKEKNEFLVRLYVLVDKFGTIYGFDPALNSIYRLAENMRMFTCMMGKKGYR
                ************:

68-1_BAC_       ------------------------------------------------------------
68-1_Virus_     NHRHDRRRTAIVRLEKVPYCMHGEEPSDPMIMFNDDSEDEKPPKTEADVVVGIYEAIKAD
180.92          NHRHDRRRTAIVRLEKVPYCMHGEEPSDPMIMFNDDSEDEKPPKTEADVVVGIYEAIKAD


68-1_BAC_       ------------------------------------------------------------
68-1_Virus_     IRFGVDMMMRDSSVTQKFWPQHLEALSDSPLLPSLIYDMEDVRSKMLGNIADMRAFDMSF
180.92          IRFGVDMMMRDSSVTQKFWPQHLEALSDSPLLPSLIYDMEDVRSKMLGNIADMRAFDMSF


68-1_BAC_       ------------------------------------------------
68-1_Virus_     VGLAEDNDSDREETVRGYLFDDTVCTRCVSSRRLRLFRSGRGMGRARVSYV
180.92          VGLAEDNDSDREETVRGYLFDDTVCTRCVSSRRLRLFRSGRGMGRARVSYV
```

Supplemental Figure 1D: Alignment of ORF Rh67.1 from RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh67.1(UL41a)

```
68-1_BAC_       MMLLWCNASFTELRQNYFLPCPWLVGVGCFVLGLFLLIFACLMKTVWSRKKYHHLLTTDE
68-1_Virus_     MMLLWCNASFTELRQNYFLPCPWLVGVGCFVLGLFLLIFACLMKTVWSRKKYHHLLTTDE
180.92          MMLLWCNASFTELRQNYFLPCPWLVGVGCFVLGLFLLIFACLMKTVWSRKKYHHLLTTDE
                ************************************************************

68-1_BAC_       EEEDIVCEKKPLSTKDIEF
68-1_Virus_     EEEDIVWEKKPLSTKDIEF
180.92          EEEDIVWEKKPLSTKDIEF
                ****** ************
```

Supplemental Figure 1E: Alignment of ORF Rh72 from RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh72 (UL45)

```
68-1_BAC_       MAQASLRNTGAGGLEAVMQEGSEGGDGGTEENGVEAMEVATSSPDAEQQQAQQQQQQPQV
68-1_Virus_     MAQASLRNTGAGGLEAVMQEGSEGGDGGTEENGVEAMEVATSSPDAEQQQAQQQQQQPQV
180.92          MAQASLRNTGAGGLEAVMQEGSEGGDGGTEENGVEAMEVATSSPDAEQQQAQQQQQQPQV
                ************************************************************

68-1_BAC_       GVHACWSLADQGTATSCRPDASSSLVQHMPAMNTVQLLMGKKCHCHGRWGKFRFCGVPDP
68-1_Virus_     GVHACWSLADQGTATSCRPDASSSLVQHMPAMNTVQLLMGKKCHCHGRWGKFRFCGVPDP
180.92          GVHACWSLADQGTATSCRPDASSSLVQHMPAMNTVQLLMGKKCHCHGRWGKFRFCGVPDP
                ************************************************************

68-1_BAC_       VKHVEDRATLWRDIDSASRQSGIRGAYRLFQMLMRYGPALIRQIPRSDLLIGRFYLKVNW
68-1_Virus_     VKHVEDRATLWRDIDSASRQSGIRGAYRLFQMLMRYGPALIRQIPRSDLLIGRFYLKVNW
180.92          VKHVEDRATLWRDIDSASRQSGIRGAYRLFQMLMRYGPALIRQIPRSDLLIGRFYLKVNW
                ************************************************************

68-1_BAC_       LRESRNALNYTSSMCEGPLRDFVMRHSEDLPKILADITRYLDLAGCWGFYGAIVLTDKVS
68-1_Virus_     LRESRNALNYTSSMCEGPLRDFVMRHSEDLPKILADITRYLDLAGCWGFYGAIVLTDKVS
180.92          LRESRNALNYTSSMCEGPLRDFVMRHSEDLPKILADITRYLDLAGCWGFYGAIVLTDKVS
                ************************************************************

68-1_BAC_       RQIYGQDESLGGIFLRISMAITLAIVSSPCARVYRFHMDCRHKYEVLESVVKRCRDGQLS
68-1_Virus_     RQIYGQDESLGGIFLRISMAITLAIVSSPCARVYRFHMDCRHECEVLESVVKRCRDGQLS
180.92          RQIYGQDESLGGIFLRISMAITLAIVSSPCARVYRFHMDCRHECEVLESVVKRCRDGQLS
                ******************************************: *****************

68-1_BAC_       LTPFSMSNIGFVELPQYDYLISCDLYSREVDWLALHKWLYENLTRGVSLSINVTRFNVEA
68-1_Virus_     LTPFSMSNIGFVELPQYDYLISCDLYSREVDWLALHKWLYENLTRGVSLSINVTRFNVEA
180.92          LTPFSMSNIGFVELPQYDYLISCDLYSREVDWLALHKWLYENLTRGVSLSINVTRFNVEA
                ************************************************************

68-1_BAC_       ISVIRCIGGFCDMIREKEVHRPIVRIFVDLWDVAAIRVLNFVLKETDIIGIHYAFNIPSV
68-1_Virus_     ISVIRCIGGFCDMIREKEVHRPIVRIFVDLWDVAAIRVLNFVLKETDIIGIHYAFNIPSV
180.92          ISVIRCIGGFCDMIREKEVHRPIVRIFVDLWDVAAIRVLNFVLKETDIIGIHYAFNIPSV
                ************************************************************

68-1_BAC_       LMKRYRAQDSHYSLFGRTVSRKLSECGNEFAFEKEYVRYETTVPKVTVKASEFMRNMLFC
68-1_Virus_     LMKRYRAQDSHYSLFGRTVSRKLSECGNEFAFEKEYVRYETTVPKVTVKASEFMRNMLFC
180.92          LMKRYRAQDSHYSLFGRTVSRKLSECGNEFAFEKEYVRYETTVPKVTVKASEFMRNMLFC
                ************************************************************

68-1_BAC_       ALKGKCALVFVHHIVKYSVLTGNMPLPPCLGPDMASCHFGESDLPLQRLSINLTRCLFTR
68-1_Virus_     ALKGKCALVFVHHIVKYSVLTGNMPLPPCLGPDMASCHFGESDLPLQRLSINLTRCLFTR
180.92          ALKGKCALVFVHHIVKYSVLTGNMPLPPCLGPDMASCHFGESDLPLQRLSINLTRCLFTR
                ************************************************************

68-1_BAC_       TDDDVLCRDNVVLGNTRRYFDMQVLRTLVTEAVVWGNARLDALIRSGDWPLESAICKMRS
68-1_Virus_     TDDDVLCRDNVVLGNTRRYFDMQVLRTLVTEAVVWGNARLDALIRSGDWPLESAICKMRS
180.92          TDDDVLCRDNVVLGNTRRYFDMQVLRTLVTEAVVWGNARLDALIRSGDWPLESAICKMRS
                ************************************************************

68-1_BAC_       LNIGVTGLHTVLMRLGFTYFASWDLIERIFENMYYAALRTSVDLCKSGLPPCEWFDRTIY
68-1_Virus_     LNIGVTGLHTVLMRLGFTYFASWDLIERIFENMYYAALRTSVDLCKSGLPPCEWFDRTIY
180.92          LNIGVTGLHTVLMRLGFTYFASWDLIERIFENMYYAALRTSVDLCKSGLPPCEWFDRTIY
                ************************************************************

68-1_BAC_       KEGKFIFELYRKPHLSLPVAQWETLRTEMQEYGVRNAQLLSIAADEETAFLWNVTPSIWA
68-1_Virus_     KEGKFIFELYRKPHLSLPVAQWETLRTEMQEYGVRNAQLLSIAADEETAFLWNVTPSIWA
180.92          KEGKFIFELYRKPHLSLPVAQWETLRTEMQEYGVRNAQLLSIAADEETAFLWNVTPSIWA
                ************************************************************

68-1_BAC_       ARDRIVDEETVLPVSPPSDECYFPTVMQKHLKVPIINYAWIEHHDEVKAKSITQGTVQRA
68-1_Virus_     ARDRIVDEETVLPVSPPSDECYFPTVMQKHLKVPIINYAWIEHHDEVKAKSITQGTVQRA
180.92          ARDRIVDEETVLPVSPPSDECYFPTVMQKHLKVPIINYAWIEHHDEVKAKSITQGTVQRA
                ************************************************************

68-1_BAC_       DVPSCVFQRAAELQADVEMASVNVSMFVDQCVPLPFYYESSMTPDLLMKRMLKWYHLRCK
68-1_Virus_     DVPSCVFQRAAELQADVEMASVNVSMFVDQCVPLPFYYESSMTPDLLMKRMLKWYHLRCK
180.92          DVPSCVFQRAAELQADVEMASVNVSMFVDQCVPLPFYYESSMTPDLLMKRMLKWYHLRCK
                ************************************************************

68-1_BAC_       VGVYKYCAS
68-1_Virus_     VGVYKYCAS
180.92          VGVYKYCAS
                *********
```

Supplemental Figure 1F: Alignment of ORFs Rh152/Rh151 from RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh152/Rh151 (UL119/UL118)

```
68-1_BAC_       MLGTGHVLALAAAVLIAQQVIGGTSTTTAANTTSTTTAPSTSTVTSSATSVTTSLTSSSA
68-1_Virus_     MLGTGHVLALAAAVLIAQQVIGGTSTTTAANTTSTTTAPSTSTVTSSATSVTTSLTSSSA
180.92          MLGTGHVLALAAAVLIAQQVIGGTSTTTAANTTSATTAPSTSTVTSSPTSVTTSVASSSA
                **********************************:************.******::****

68-1_BAC_       AASSVTSSNAASSSTSGTATSTATSTQKTSTSNSSTDTGTQTTSSNTTTAPATTESATTS
68-1_Virus_     AASSVTSSNAASSSTSGTATSTATSTQKTSTSNSSTDTGTQTTSSNTTTAPATTESATTS
180.92          TASSVTSSSAAASTTSGTVTSTSK---------SSTDSSTQTTSSNTTTAPATTESATTS
                :*******.**:*:****.***:.         ****:.**********************

68-1_BAC_       SNASDNSTTENSTVTTTADTT----------SDTSTAATSTTANKPRVPDIYVTCESAYS
68-1_Virus_     SNASDNSTTENSTVTTTADTT----------SDTSTAATSTTANKPRVPDIYVTCESAYS
180.92          SNASENSTTENSTVTTNATDSNATTDTTTAETTITAATNITTTTKPRVPDIYVTCESAYS
                ****:***********.*  :          :  ::*:. **:.****************

68-1_BAC_       YNYLVLQTTCQIHNMSHAQNVSRDLISIECFEQVGCDGNLTSIGSVTTSNTSHGMLYNIT
68-1_Virus_     YNYLVLQTTCQIHNMSHAQNVSRDLISIECFEQVGCDGNLTSIGSVTTSNTSHGMLYNIT
180.92          YNYLVLQTTCQIHNMSHAQNVSRDLISIECFEQVGCDGNLTSIGSVTTSNTSHGMLYNIT
                ************************************************************

68-1_BAC_       TQTFTMYRQAPNVTTQYSCRFIATGQTLNKSWEFLVMPIKAVFASPTNDSMIQLRVLVND
68-1_Virus_     TQTFTMYRQAPNVTTQYSCRFIATGQTLNKSWEFLVMPIKAVFASPTNDSMIQLRVLVND
180.92          TQTFTMYRQAPNVTTQYSCRFIATGQTLNKSWEFLVMPIKAVFASPTNDSMIQLRVLVND
                ************************************************************

68-1_BAC_       HPCTNETVYSSSKAFVYFGNTNHSSHKVQNITRHNQSLWEYIFHFTNHDLPNTAHMKILL
68-1_Virus_     HPCTNETVYSSSKAFVYFGNTNHSSHKVQNITRHNQSLWEYIFHFTNHDLPNTAHMKILL
180.92          HPCTNETVYSSSKAFVYFGNTNHSSHKVQNITRHNQSLWEYIFHFTNHDLPNTAHMKILL
                ************************************************************

68-1_BAC_       GDRYSVSTHVFIKRDPDEWPIIGTLGYIVLAFLLFMLFALLYITYVLMRQRNP-------
68-1_Virus_     GDRYSVSTHVFIKRDPDEWPIIGTLGYIVLAFLLFMLFALLYITYVLMRQRNPWAYRRLD
180.92          GDRYSVSTHVFIKRDPDEWPIIGTLGYIVLAFLLFMLFALLYITYVLMRQRNPWAYRRLD
                ************************************************

68-1_BAC_       -------------
68-1_Virus_     EEKPYPVPYFKQW
180.92          EEKPYPVPYFKQW
```

Supplemental Figure 1G: Alignment of ORF Rh164 form RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV CNPRC

Rh164 (UL141)

```
68-1_BAC_       MSYTVRFRKGFGRVSEEAETVQLLAEGQEGADSADAESASKRTIHDGPLRVKACTPVSAP
68-1_virus_     MSYTVRFRKGFGRVSEEAETVQLLAEGQEGADSADAESASKRTIHDGPLRVKACTPVSAP
clinical        MSYTVRFRKGFGRVSEEAETVQLLAEGQEGADSADAESASKRTIHDGPLRVKACTPVSAP
                ************************************************************

68-1_BAC_       RAAMWVRRAMVAITIVMVSLTPRVRGGSIDHTMWDECYEHNSPAPLIMPIGSQVTVPCAF
68-1_virus_     RAAMWVRRAMVAITIVMVSLTPRVRGGSIDHTMWDECYEHNSPAPLIMPIGSQVTVPCAF
clinical        RAAMWVRRAMVAITIVMVSLTPRVRGGSIDHTMWDECYEHNSPAPLIMPIGSQVTVPCAF
                ************************************************************

68-1_BAC_       LPHSWPMVSIRARFCQSEYGGYELKINATNGTVVDDDLTYRLINASWKFHDLAISHYVTL
68-1_virus_     LPHSWPMVSIRARFCQSEYGGYELKINATNGTVVDDDLTYRLINASWKFHDLAISHYVTL
clinical        LPHSWPMVSIRARFCQSEYGGYELKINATNGTVVDDDLTYRLINASWKFHDLAISHYVTL
                ************************************************************

68-1_BAC_       TMNISDNTTGMFDCVLRNATHGFLMTRFTIVTQIETLHRLGDPDCAPKLGFHADGKKIWS
68-1_virus_     TMNISDNTTGMFDCVLRNATHGFLMTRFTIVTQIETLHRPGDPDCAPKLGFHADGKKIWS
clinical        TMNISDNTTGMFDCMLRNATHGFLMTRFTIVTQIETLHRPGDPDCAPKLGFHADGKKIWS
                **************:********************** ********************

68-1_BAC_       AEYNEWQRHQCGTFYGFDRLYYYLAASNQSNTKPPCPPSEPDRCWPVLQQYVLDGDCFRS
68-1_virus_     AEYNEWQRHQCGTFYGFDRLYYYLAASNQSNTKPPCPPSEPDRCWPVLQQYVLDGDCFRS
clinical        AEYNEWQRHQCGTFYGFDRLYYYLAASNQSNTKPPCPPSEPDRCWPVLQQYVLDGDCFRS
                ************************************************************

68-1_BAC_       QNFRREPPLPTEKTPVPIFVIDWQWVSLGLTMMVIGGVCLGLVLVVRCACGEMCRNRERF
68-1_virus_     QNFRREPPLPTEKTPVPIFVIDWQWVSLGLTMMVIGGVCLGLVLVVRCACGEMCRNRERF
clinical        QNFRREPPLPTEKTPVPIFVIDWQWVSLGLTMMVIGGVCLGLVLVVRCACGEMCRNRERF
                ************************************************************

68-1_BAC_       QKKMNAYRPMSTHFMRPPGYEELYSVVDDESDSGYFEKEDRSESYNDLVDENVYDEVAVP
68-1_virus_     QKKMNAYRPMSTHFMRPPGYEELYSVVDDESDSGYFEKEDRSESYNDLVDENVYDEVAVP
clinical        QKKMNAYRPMSTHFMRPPGYEELYSVVDDESDSGYFEKEDRSESYNDLVDENVYDEVAVP
                ************************************************************

68-1_BAC_       PLYSKIKRRL
68-1_virus_     PLYSKIKRRL
clinical        PLYSKIKRRL
                **********
```

Supplemental Figure 1H: Alignment of ORF Rh194 form RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh194(US14)

```
68-1_BAC_       MPFAPRLQPFTVHRPPAPMIQLDLDERSSLSWLRQHLPLASVYLCLLFVIAVCICSYGAF
68-1_Virus_     MPFAPRLQPFTVHRPPAPMIQLDLDERSSLSWLRQHLPLASVYLCLLFVIAVCICSYGAF
180.92          MPFAPRLQPFTVHRPPAPMIQLDLDERSSLSWLRQHLPLASVYLCLLFVIAVCICSYGAF
                ************************************************************

68-1_BAC_       KSQFHCMVFNTEICRMEPAFILIIVPVLLMFVWNMFDHRQDDMIHMGNGLLYIVVFACIG
68-1_Virus_     KSQFHCMVFNTEICRMEPAFILIIVPVLLMFVWNMFDHRQDDMIHMGNGLLYIVVFACIG
180.92          KSQFHCMVFNTEICRMEPAFILIIVPVLLMFVWNMFDHRQDDMIHMGNGLLYIVVFACIG
                ************************************************************

68-1_BAC_       FTLISFCTDGITAGLSLLFTATFFLTCSGLALWSSRPLPSKCRYVATLVSTFLLLLFYFG
68-1_Virus_     STLISFCTDGITAGLSLLFTATFFLTCSGLALWSSRPLPSKCRYVATLVSTFLLLLFYFG
180.92          STLISFCTDGITAGLSLLFTATFFLTCSGLALWSSRPLPSKCRYIATLVSTFLLLLFYFG
                 ***********************************************:************

68-1_BAC_       QLSHSVMRNGLSIILHGSMGIIIWENIYITKFNLTMKHVVSACIVYVDILIVMYYMYVYL
68-1_Virus_     QLSHSVMRNGLSIILHGSMGIIIWENIYITKFNLTMKHVVSACIVYVDILIVMYYMYVYL
180.92          QLSHSVMRNGLSIILHGSMGIIIWENIYITKFNLTMKHVVSACIVYVDILIVMYYMYVYL
                ************************************************************

68-1_BAC_       LTPSLWTLDPHKMLTGVSQLWNGSFNRTFCSPSSVYG
68-1_Virus_     LTPSLWTLDPHKMLTGVSQLWNGSFNRTFCSPSSVYG
180.92          LTPSLWTLDPHKMLTGVSQLWNGSFNRTFCSPSSVYG
                ************************************
```

Supplemental Figure 1I: Alignment of ORF Rh197 form RhCMV 68-1 (BAC), RhCMV 68-1 and RhCMV180.92

Rh197 (US14)

```
68-1_BAC_       MVSKMVTKLTNHVIWLNRSIHVWSVYGWLAFQVSITVLVYGLVRCQQYLFDTCAQEPVRQ
68-1_Virus_     MVSKMVTKLTNHVIWLNRSIHVWSVYGWLAFQVSITVLVYGLVRCQQYLFDTCAQEPVRQ
180.92          MVSKMVTKLTNHVIWLNRSIHVWSVYGWLAFQVSITVLVYGLVRCQQYLFDTCAQEPVRQ
                ************************************************************

68-1_BAC_       IMITSPALVFIQESYINRVIRKVSLWKNCGVALFCVIHIAFSHVWFSGCVATWTVIQSWI
68-1_Virus_     IMITSPALVFIQESYINRVIRKVSLWKNCGVALFCVIHIAFSHVWFSGCVATWTVIQSWI
180.92          IMITSPALVFIQESYINRAIRKGSLWKNCGVALFCVIHIAFSHVWFSGCVATWTVIQSWI
                ******************.*** ************************************

68-1_BAC_       ATFCLFILMIYVSDGSNWKPFIERQVLSDMLCAGALAANCFVHSVTQPSVTLWWIAQTLY
68-1_Virus_     ATFCLFILMIYVSDGSNWKPFIERQVLSDMLCAGALAANCFVHSVTQPSVTLWWIAQTLY
180.92          ATFCLFILMIYVSDGSNWKPFIERQVLSDMLCAGALAANCFVHSVTQPSVTLWWIAQTLY
                ************************************************************

68-1_BAC_       IIGTVGFMNAMCLQLSNVRRQQRSNERAMSISLLLYCIFHLVHYNNVIMWSFP-------
68-1_Virus_     IIGTVGFMNAMCLQLSNVRRQQRSNERAMSISLLLYCIFHLVHYNNVIMWSFPWKAEDPW
180.92          IIGTVGFMNAMCLQLSNVRRQQRSNERAMSISLLLYCIFHLVHYNNVIMWSFPWKAEDPW
                ************************************************

68-1_BAC_       -
68-1_Virus_     L
180.92          L
```

Supplemental Figure 2: List of all predicted ORFs in the fully sequenced RhCMV strains 68-1 and 180.92 and the partial sequence for the ULb'-region from RhCMV CNPRC. The sequences of the two fully sequenced genomes for RhCMV 68-1 and 180.92 and the partial sequence for RhCMV CNPRC were analyzed for potential ORFs by entering the sequences into NCBI's Open Reading Frame Fonder (*ORF Finder*). As a cutoff point we selected ORFs ≥300bp. The three different sequences are listed next to each other and the predicted ORFs are in the order they would be found in a low passage isolate. The nomenclature used for the different sequences was chosen according to their original publication, so the nomenclature for 68-1 is based on Hansen et al. (30), the nomenclature for 180.92 is based on Rivailler at al. (63) and the nomenclature for RhCMV CNPRC is based on Oxford et al.(50). ORFs not annotated by the original authors were named using the nomenclature created by Hansen et al.

RhCMV strain 68-1					RhCMV strain 180.92					RhCMV low passage isolate (ULb' region, partial sequence)				
ORF	**Frame**	**from**	**to**	**Length**	**ORF**	**Frame**	**from**	**to**	**Length**	**ORF**	**Frame**	**from**	**to**	**Length**
					Rh00	1	37	480	444					
					Rh00.1	2	164	637	474					
Rh00.2	3	864	1223	360	Rh00.2	2	866	1225	360					
Rh01	2	1040	2566	1527	RhRL1	1	1042	2568	1527					
Rh02	1	1486	2037	552	Rh02	3	1488	2039	552					
Rh02.1	3	2280	2606	327	Rh02.1	2	2282	2608	327					
Rh03	2	2618	3082	465	Rh03	1	2620	3084	465					
Rh04	-2	2703	3401	699	Rh04	-2	2705	3403	699					
Rh03.1	3	2751	3053	303	Rh03.1	2	2753	3055	303					
Rh03.2	1	3088	3465	378	Rh03.2	3	3090	3467	378					
Rh05	3	3528	4349	822	Rh05	2	3530	4351	822					
Rh06	3	4710	5198	489	Rh06	2	4712	5356	645					
Rh07	3	5325	5906	582	Rh07	3	5343	5921	579					
Rh08	1	5881	6396	516	Rh08	1	5896	6558	663					
Rh08.1	2	6578	7000	423	Rh08.1	3	6600	7148	549					
Rh09	-1	6724	7029	306										
Rh10(1)	-1	8521	9027	507	Rh10(1)	-2	8555	9061	507					
Rh10(2)	-2	9267	9845	579	Rh10(2)	-3	9301	9906	606					
Rh11	3	9462	9902	441	Rh11	1	9496	9936	441					
Rh10(3)	-3	9806	10126	321	Rh10(3)	-3	9979	10302	324					
Rh12	2	11225	11920	696	Rh12	1	11260	11952	693					
Rh13	-1	11233	11547	315	Rh13	-1	11268	11582	315					
					Rh13.1	1	12148	12534	387					
Rh14	2	12506	13087	582	Rh14	1	12538	13113	576					
Rh15	-1	12556	12879	324	Rh15	-1	12588	12911	324					
Rh16	-1	12937	13458	522	Rh16	-3	13165	13488	324					
Rh17	1	13525	14625	1101	Rh17	1	13555	14652	1098					
Rh18	-2	13557	13922	366	Rh18	-1	13587	13949	363					
Rh19	1	14707	15642	936	RhUL7	1	14734	15681	948					
Rh20	1	15700	16293	594	Rh20	3	15708	16337	630					

					Rh21(1)	3	16371	16712	342
Rh21	1	16327	17004	678	Rh21(2)	2	16580	17038	459
Rh22	2	17111	17818	708	Rh22	3	17145	17852	708
Rh23	1	17716	18411	696	RhUL11	2	17750	18445	696
Rh24	2	18428	18817	390	Rh24	3	18462	18851	390
Rh25	2	18896	19570	675	Rh25	3	18930	19604	675
Rh26	3	19554	20402	849	Rh26	1	19588	20436	849
Rh27	3	20547	21152	606	Rh27	1	20581	21186	606
Rh28	1	21154	21777	624	Rh28	2	21188	21811	624
Rh29	2	21854	23239	1386	Rh29	2	21887	23245	1359
Rh30	-3	23315	23638	324	Rh30	-2	23321	23632	312
Rh31	3	23355	24662	1308	Rh31	3	23361	24671	1311
Rh32	-2	23577	24029	453	Rh32	-1	23583	24035	453
Rh33	3	24942	25838	897	RhUL14	1	24949	25860	912
Rh34	-2	26718	27041	324	Rh34	-2	26726	27049	324
Rh35	3	26778	27101	324	Rh35	2	26786	27109	324
Rh36	1	27826	29172	1347	RhUL20	2	27833	29179	1347
Rh37	-1	29275	29640	366	RhUL21a	-2	29282	29647	366
Rh38	3	29898	30251	354	Rh38	1	29905	30258	354
Rh39	-2	29916	30380	465					
Rh40	-3	30731	31669	939	RhUL23	-2	30737	31675	939
Rh41	3	31416	31796	381	Rh41	3	31422	31802	381
Rh42	-1	31726	32652	927	RhUL24	-3	31732	32658	927
Rh43	1	32719	34476	1758	RhUL25	1	32725	34485	1761
Rh44	-1	34537	35283	747	RhUL26	-3	34546	35295	750
Rh45	1	34897	35328	432	Rh45	1	34909	35340	432
Rh46	-3	35237	36976	1740	RhUL27	-2	35249	36988	1740
Rh47	-3	37064	38077	1014	RhUL28	-2	37076	38089	1014
Rh48	2	37289	37786	498	Rh48	2	37301	37798	498
Rh49	3	38190	38588	399	Rh49	3	38202	38600	399
Rh50	-2	38208	39218	1011	RhUL29	-1	38220	39230	1011
Rh51	-3	38351	38755	405	Rh51	-2	38363	38767	405
Rh52	3	38925	39395	471	Rh52	3	38937	39407	471
Rh53	-3	39548	39979	432	Rh53	-2	39560	39991	432
Rh54	1	39865	41487	1623	RhUL31	1	39877	41499	1623
Rh55	-3	41498	43615	2118	RhUL32	-2	41510	43627	2118
Rh56	3	43983	44972	990	RhUL33	3	43995	44981	987
Rh57	3	45177	45977	801	RhUL34	3	45186	46043	858
Rh58	2	45491	45877	387	Rh58	2	45500	45886	387
Rh59	3	46098	47870	1773	RhUL35	2	46106	47878	1773
Rh60	-2	47988	49151	1164	RhUL36	-2	47996	49159	1164
Rh61	-3	49115	49480	366					
Rh62	-2	49578	50396	819	RhUL37(1)	-1	49587	50405	819

Rh63	3	50598	50981	384	Rh63	3	50607	50990	384
Rh64	-3	50702	51583	882	rhUL38	-2	50711	51592	882
Rh65	2	50768	51133	366	Rh65	2	50777	51142	366
Rh66	-1	51625	51930	306	RhUL37(2)	-3	51634	51939	306
Rh67	-1	52255	52785	531	Rh67	-3	52264	52794	531
Rh68	-2	53229	53621	393	RhUL42	-1	53238	53627	390
Rh69	-1	53605	54606	1002	RhUL43	-3	53611	54612	1002
Rh70	-1	54730	55902	1173	RhUL44	-3	54736	55908	1173
Rh71	1	55132	55608	477	Rh71	1	55138	55614	477
Rh72	-1	56143	58692	2550	RhUL45	-3	56149	58698	2550
Rh73	1	56404	56829	426	Rh73	1	56410	56835	426
Rh74	2	58181	58483	303	Rh74	2	58187	58489	303
Rh75	-1	58711	59583	873	RhUL46	-3	58717	59589	873
Rh76	2	59582	62458	2877	RhUL47	2	59588	62464	2877
Rh77	-2	62052	62357	306	Rh77	-1	62058	62363	306
Rh78	1	62479	69012	6534	RhUL48	1	62485	69018	6534
Rh78.1	2	62978	63370	393	Rh78.1	2	62984	63376	393
Rh79	1	69181	69846	666	Rh79	1	69187	69852	666
Rh80	-1	69295	70764	1470	RhUL49	-3	69301	70770	1470
Rh81	-3	70754	71629	876	RhUL50	-2	70760	71638	879
Rh82	-2	71655	71990	336	RhUL51	-1	71664	71999	336
Rh83	3	72069	73724	1656	RhUL52	3	72078	73733	1656
Rh84	-3	72179	72484	306	Rh84	-2	72188	72493	306
Rh85	1	73717	74583	867	RhUL53	1	73726	74592	867
Rh86	-1	74128	74559	432	Rh86	-3	74137	74568	432
Rh87	-3	74561	77668	3108	RhUL54	-2	74570	77677	3108
Rh88	2	77489	77866	378	Rh88	2	77498	77875	378
Rh89	-3	77687	80251	2565	RhUL55	-2	77696	80266	2571
Rh90	2	79376	79822	447	Rh90	2	79322	79837	516
Rh91	-2	80217	82523	2307	RhUL56	-1	80232	82538	2307
Rh91.1	-1	81220	82587	1368	Rh91.1	-3	81235	82602	1368
Rh92	-3	82670	86161	3492	RhUL57	-2	82685	86176	3492
Rh93	-3	87380	87694	315					
Rh94	2	87758	88315	558					
Rh94.1	-2	87846	88319	474	Rh94.1	-1	87858	88331	474
Rh95	-3	88049	88750	702	Rh95	-2	88061	88762	702
Rh96	3	88116	89006	891	Rh96	3	88128	89018	891
Rh96.1	1	88186	88560	375	Rh96.1	1	88198	88572	375
Rh97	-2	90525	92858	2334	RhUL69	-1	90537	92867	2331
Rh98	1	91522	91830	309	Rh98	1	91531	91839	309
Rh99	3	92073	92855	783	Rh99	3	92082	92864	783
Rh100	-3	92792	95644	2853	RhUL70	-2	92801	95539	2739
Rh99.1	2	94067	94390	324	Rh99.1	2	94076	94399	324

Rh100.1	3	95610	96248	639	RhUL71	2	95552	96256	705
Rh101	-1	96316	97347	1032	RhUL72	-1	96324	97355	1032
Rh102	1	97342	97656	315	RhUL73	3	97350	97664	315
Rh103	-3	97637	98806	1170	RhUL74	-3	97645	98814	1170
Rh104	-3	99023	101185	2163	RhUL75	-3	99031	101193	2163
Rh105	2	101318	102202	885	RhUL76	1	101326	102210	885
Rh106	3	101871	103658	1788	RhUL77	2	101879	103666	1788
					Rh106.1	-2	101975	102358	384
Rh107	3	103785	104924	1140	RhUL78	2	103793	104932	1140
Rh108	-3	105020	105820	801	RhUL79	-3	105028	105828	801
Rh109	3	105819	107663	1845	RhUL80	2	105827	107671	1845
Rh110	-1	107776	109422	1647	RhUL82	-1	107784	109430	1647
Rh111	-1	109552	111171	1620	RhUL83a	-1	109560	111179	1620
Rh112	-2	111240	112868	1629	RhUL83b	-2	111248	112879	1632
Rh113	3	112041	112487	447	Rh113	2	112052	112498	447
Rh114	-1	112990	114528	1539	RhUL84	-1	112998	114533	1536
Rh115	2	113051	113419	369	Rh115	1	113059	113424	366
					Rh115.1	-3	113125	113484	360
Rh116	3	114270	114728	459	Rh116	2	114275	114733	459
Rh117	-3	114443	115369	927	RhUL85	-3	114448	115374	927
Rh118	-3	115430	119461	4032	RhUL86	-3	115435	119466	4032
Rh119	3	115995	116309	315	Rh119	2	116000	116314	315
Rh120	-2	116922	117656	735	Rh120	-2	116927	117661	735
Rh121	2	117836	118309	474	Rh121	1	117841	118314	474
Rh122	1	119476	122022	2547	RhUL87	3	119481	122027	2547
Rh123	1	122035	123237	1203	RhUL88	3	122040	123242	1203
Rh124(1)	-2	123234	124181	948	RhUL89(1)	-2	123239	124186	948
Rh125	3	123897	124469	573	Rh125	2	123902	124477	576
Rh126	1	124501	124803	303	RhUL91	3	124509	124820	312
Rh127	2	124697	125401	705	RhUL92	1	124705	125418	714
Rh128	3	125367	126932	1566	RhUL93	2	125384	126949	1566
Rh128.1	2	125528	125848	321	Rh128.1	1	125545	125865	321
Rh129	1	126808	127848	1041	RhUL94	3	126825	127865	1041
Rh124(2)	-2	127845	128768	924	RhUL89(2)	-2	127862	128785	924
Rh130	1	128767	130047	1281	RhUL95	3	128784	130061	1278
Rh131	3	130044	130433	390	RhUL96	2	130058	130447	390
Rh132	3	130491	132323	1833	RhUL97	2	130505	132331	1827
Rh133	-2	131142	131456	315	Rh133	-2	131150	131464	315
Rh134	2	132374	134044	1671	RhUL98	1	132382	134052	1671
Rh135	-1	132886	133287	402	Rh135	-1	132894	133295	402
Rh136	-3	133325	133918	594	Rh136	-3	133333	133926	594
Rh137	1	133981	134436	456	RhUL99	3	133989	134444	456
Rh138	-3	134603	135673	1071	RhUL100	-3	134611	135681	1071

Rh139	1	135862	138036	2175	RhUL102	3	135870	138044	2175					
Rh140	-1	138058	138813	756	RhUL103	-1	138066	138821	756					
Rh141	-3	138740	140707	1968	RhUL104	-3	138748	140715	1968					
Rh142	3	140544	143123	2580	RhUL105	2	140552	143131	2580					
Rh142.1	2	140693	140995	303	Rh142.1	1	140701	141003	303					
Rh142.2	-2	142185	142514	330	Rh142.2	-2	142193	142522	330					
Rh142.4	-2	142776	143243	468										
					Rh142.3	2	143999	144301	303					
Rh143	1	146491	146958	468	RhUL111a	3	146505	146966	462					
Rh144	1	147820	148620	801	RhUL112(1)	1	147838	148629	792					
Rh145	3	148719	149600	882	RhUL112(2)	3	148728	149609	882					
Rh146	-3	149714	150457	744	RhUL114	-2	149723	150466	744					
Rh147	-2	150420	151196	777	RhUL115	-1	150429	151205	777					
Rh148	-1	151207	152277	1071	RhUL116	-1	151503	152066	564					
Rh147.1	2	151808	152299	492	Rh147.1	1	151594	152088	495					
Rh149	-3	151952	152416	465	Rh149	-3	151726	152205	480					
Rh150	-2	152259	153407	1149	RhUL117	-2	152048	153196	1149					
Rh149.1	3	153243	153545	303	Rh149.1	2	153032	153334	303					
Rh151	-2	153432	154031	600	RhUL119(1)	-2	153221	153820	600					
Rh152	-1	154111	154782	672	RhUL119(2)	-1	153900	154574	675					
Rh153	-2	154260	154577	318										
					Rh151.1	1	154123	154524	402					
Rh154	-1	154831	155427	597	Rh154	-1	154623	155219	597					
Rh155	-3	155429	155977	549	Rh155	-3	155221	155769	549					
Rh156(1)	-1	156229	157689	1461	RhUL122	-1	156027	157757	1731					
Rh156(2)	-2	158214	159383	1170	RhUL123	-3	158017	159192	1176					
Rh156.1	1	159898	160200	303	Rh156.1	2	159707	160009	303					
Rh156.2	2	160052	160495	444	Rh156.2	3	159861	160307	447					
Rh156	-2	161559	162053	495	Rh157.1	-3	161371	161865	495					
Rh157	1	161947	162489	543	Rh157.3	-1	161571	162098	528					
Rh157.3	-3	161966	162286	321	Rh157	2	161759	162337	579					
Rh157.2	3	162081	162404	324	Rh157.2	1	161893	162198	306					
					RhUL128(1)	-1	162726	163052	327	RhUL128(1)	-1	722	1048	327
					RhUL128(2)	-1	163293	163631	339	RhUL128(2)	-2	1288	1626	339
					rhUL130	-3	163675	164364	690	RhUL130	-1	1628	2359	732
Rh160	3	165417	166082	666	rhUL132	-3	165406	166071	666	RhUL132	-2	3403	4068	666
Rh159	1	164371	165351	981						RhUL148	-3	4134	5114	981
Rh158	1	163705	164166	462						RhUL147	-3	5319	5780	462
Rh158.1	2	163289	163654	366						RhUL146	-1	5831	6175	345
										RhUL146b	-3	6738	7064	327
										Rh161.1	-3	7188	7520	333
Rh161	-2	167130	167570	441						Rh161.2	-3	7602	8117	516
Rh162	-2	167655	167960	306						RhUL145	-3	8202	8507	306

Rh163	-2	168378	168893	516						RhUL144	-3	8925	9440	516
Rh163.1	3	168912	169268	357						Rh163.1	3	9459	9815	357
Rh164	-1	169096	170388	1293						RhUL141	-2	9643	10935	1293
Rh165	-1	170857	171288	432						Rh165	-1	11396	11827	432
Rh165.1	1	170872	171312	441						Rh165.1	2	11411	11851	441
Rh166	-2	171333	171857	525						Rh166	-2	11872	12396	525
Rh166.1	1	171772	172080	309						Rh166.1	2	12311	12619	309
Rh167	-1	171988	172491	504	Rh167	-1	166137	166766	630	Rh167	-1	12527	13030	504
Rh168	-3	172745	173401	657	Rh168	-3	167020	167676	657					
Rh168.1	2	173495	173926	432	Rh168.1	1	167773	168204	432					
Rh169	-3	173501	174064	564	Rh169	-3	167779	168342	564					
Rh170	-2	174198	174764	567	Rh170	-2	168476	169042	567					
Rh171	-2	174768	175607	840	Rh171	-2	169046	169885	840					
Rh172	-2	175806	176336	531	Rh172	-3	170083	170613	531					
Rh171.1	3	175839	176213	375	Rh171.1	1	170116	170490	375					
Rh173	-2	176388	177515	1128	Rh173	-3	170665	171786	1122					
Rh174	-2	178695	179774	1080	Rh174	-2	172913	173992	1080					
Rh175	2	180470	180922	453	Rh175	1	174688	175140	453					
Rh176	-1	180619	181260	642	Rh176	-1	174837	175478	642					
Rh177	-3	181226	181669	444	Rh177	-3	175444	175887	444					
Rh178	-2	181320	182060	741	Rh178	-2	175538	176278	741					
					Rh178.2	-3	176224	176544	321					
Rh178.1	3	182280	182642	363	Rh178.1	2	176498	176932	435					
Rh178.3	2	182609	182971	363	Rh178.3	1	176827	177189	363					
Rh179	3	183231	183746	516	Rh179	2	177449	177964	516					
Rh180	-3	183347	183670	324	Rh180	-3	177565	177888	324					
Rh181	-1	183766	184272	507	RhUS1	-3	177988	178623	636					
Rh180.1	2	184121	184447	327	Rh180.1	2	178343	178735	393					
Rh182	-3	184502	185092	591	Rh182	-1	178725	179315	591					
Rh183	1	185590	185952	363	Rh183	1	179818	180180	363					
Rh184	-1	185617	186159	543	Rh184	-3	179845	180387	543					
Rh185	-3	187133	187645	513	Rh185	-1	181392	181904	513					
Rh186	-3	187934	188638	705	Rh186	-1	182193	182897	705					
Rh187	-3	188879	189559	681	Rh187	-1	183138	183821	684					
Rh188	-2	189657	190031	375	Rh188	-3	183913	184287	375					
Rh189	-1	190318	191163	846	RhUS11	-2	184574	185416	843					
Rh190	-2	191367	192149	783	RhUS12	-2	185621	186403	783					
Rh191	-1	191524	191856	333	Rh191	-1	185778	186110	333					
Rh192	-2	192207	192971	765	RhUS13	-2	186461	187225	765					
Rh193	-3	192977	193462	486	Rh193	-3	187231	187716	486					
Rh194	-1	193084	193917	834	Rh194	-1	187338	188171	834					
Rh195	-3	194048	194791	744	Rh195	-3	188302	189045	744					
Rh196	-3	194864	195622	759	RhUS14	-3	189118	189876	759					

Rh197	-3	195728	196453	726	Rh197	-3	189982	190707	726
					Rh196.1	-2	190262	190576	315
Rh198	-2	196431	197255	825	RhUS17	-2	190685	191509	825
Rh199	-1	197605	198216	612	RhUS18	-2	191615	192415	801
Rh200	-2	198336	199121	786	Rh200	-3	192535	193320	786
Rh201	-2	199182	199943	762	RhUS20	-3	193381	194142	762
Rh202	-3	199991	200677	687	RhUS21	-1	194190	194876	687
Rh203	-1	200797	202521	1725	RhUS22	-2	194996	196720	1725
Rh204	-2	202680	204548	1869	RhUS23	-3	196879	198750	1872
Rh205	3	202743	203504	762	Rh205	1	196942	197706	765
Rh206	-3	202979	203326	348	Rh206	-1	197178	197528	351
Rh208	3	204333	204650	318	Rh208	1	198535	198852	318
Rh209	-3	204572	206002	1431	RhUS24	-1	198774	200204	1431
Rh210	2	205019	205594	576	Rh210	3	199221	199796	576
Rh211	-3	206363	208156	1794	RhUS26	-1	200565	202358	1794
Rh212	-1	206818	207132	315	Rh212	-2	201020	201334	315
Rh213	2	206909	207424	516	Rh213	3	201111	201626	516
Rh214	2	208328	209314	987	Rh214	3	202530	203516	987
Rh215	2	209660	210673	1014	Rh215	3	203862	204875	1014
Rh216(1)	2	210809	211138	330	Rh216	3	205011	206012	1002
Rh216(2)	1	211078	211809	732					
Rh217	-2	211704	212006	303	Rh217	-2	205907	206209	303
Rh218	1	211882	212901	1020	Rh218	3	206085	207104	1020
Rh219	-1	212671	212976	306	Rh219	-1	206874	207179	306
					RhUS28(1)	3	207249	207635	387
Rh220	1	213046	214497	1452	RhUS28(2)	2	207794	208711	918
Rh220.1	-2	213093	213440	348					
Rh221	3	214653	215978	1326	RhUS29	1	208867	210192	1326
Rh222	1	215128	215451	324	Rh222	2	209342	209665	324
Rh223	1	215896	216717	822	Rh223	2	210110	210931	822
Rh224	-2	216579	217196	618	Rh224	-3	210793	211410	618
Rh225	1	216793	217278	486	RhUS31	2	211007	211492	486
Rh226	1	217405	217965	561	RhUS32	2	211619	212179	561
Rh227	-3	217463	217879	417	Rh227	-1	211677	212093	417
Rh228	1	218098	218403	306	Rh228	2	212312	212623	312
Rh228.1	-2	218655	219032	378	Rh228.1	-1	212880	213257	378
Rh229	-2	219051	219506	456	Rh229	-1	213276	213731	456
Rh230	-1	219127	221214	2088	RhTRS1	-3	213352	215439	2088
Rh231	-2	220326	220631	306	Rh231	-1	214551	214856	306

Supplemental Table 1. Identfication and quantification of RhCMV viron proteins.
*Whereas biological replicates are named in letters, the technical ones are stated by numbers.

				Unique peptide count						emPAI						emPAI (mol %)					
				100 min runs				600 min runs		100 min runs				600 min runs		100 min runs				600 min runs	
Protein	Min_MSGF	Unique peptides	Spectra	A01	B01	B02	B03	B04	B05	A01	B01	B02	B03	B04	B05	A01	B01	B02	B03	B04	B05
Rh05	1.05243E-14	3	5	2			1	1	1	0.178769	0	0	0.085711	0.085711	0.085711	0.837664	0	0	0.273199	0.145095	0.153876
Rh100.1	3.57848E-23	17	81	4	5	7	8	14	13	0.44544	0.584893	0.905461	1.089296	2.630781	2.311311	2.087216	1.925198	2.821118	3.472065	4.453485	4.149475
Rh102	8.48229E-24	4	31	1	2	2	2	4	3	0.584893	1.511886	1.511886	1.511886	5.309573	2.981072	2.740659	4.97643	4.710541	4.819045	8.988248	5.35189
Rh103	2.15332E-18	6	28	3	2	2	1	6	5	0.115884	0.075836	0.075836	0.037225	0.245197	0.200508	0.543003	0.249617	0.23628	0.118653	0.415079	0.35997
Rh104	3.91838E-26	44	440	9	26	28	25	38	37	0.265531	0.974488	1.080568	0.923494	1.702827	1.633022	1.244209	3.207564	3.366693	2.943581	2.88261	2.93175
Rh106	1.73264E-24	33	171	6	16	17	15	29	26	0.127649	0.377623	0.405485	0.350314	0.787203	0.683011	0.59813	1.242961	1.263357	1.116604	1.332607	1.226203
Rh109	5.70237E-17	8	17	1	1	1	3	4	6	0.018152	0.018152	0.018152	0.05545	0.074608	0.113974	0.085054	0.059747	0.056555	0.176742	0.126299	0.204616
Rh110	9.3771E-29	46	566	25	25	23	25	34	28	0.882458	0.882458	0.789564	0.882458	1.363885	1.030918	4.134971	2.904643	2.460022	2.81278	2.308837	1.850797
Rh111	3.64234E-31	55	652	28	29	27	28	46	45	1.17445	1.235618	1.114955	1.17445	2.582753	2.484726	5.50317	4.067082	3.473834	3.743487	4.372182	4.460806
Rh112	3.0477E-27	70	1533	44	40	47	39	55	59	2.440213	2.074715	2.742577	1.989577	3.685239	4.242183	11.43421	6.829001	8.544967	6.341655	6.238512	7.615951
Rh114	2.26072E-16	3	5	2				2	1	0.062468	0	0	0	0.062468	0.030761	0.292708	0	0	0	0.105748	0.055225
Rh117	1.13106E-25	39	748	20	25	25	30	34	35	1.31013	1.848036	1.848036	2.511192	3.151278	3.328761	6.13893	6.082878	5.757872	8.00427	5.334603	5.976094
Rh118	4.03416E-33	150	2816	82	98	101	104	136	129	1.607616	2.143851	2.256045	2.372242	3.901797	3.516711	7.532875	7.056564	7.029093	7.561377	6.605111	6.313519
Rh123	1.2207E-20	30	172	7	15	15	16	25	25	0.17119	0.403004	0.403004	0.435036	0.75832	0.75832	0.802153	1.326502	1.255627	1.38665	1.283714	1.361406
Rh128	8.60986E-19	17	75	2	10	11	8	13	12	0.038793	0.209608	0.232847	0.164436	0.280672	0.256532	0.181773	0.689932	0.725474	0.52413	0.475133	0.460549
Rh129	1.08727E-28	32	639	21	22	22	20	30	28	1.490155	1.600724	1.600724	1.384287	2.68161	2.375218	6.982483	5.268844	4.987331	4.412329	4.539531	4.264207
Rh13.1	1.5219E-21	4	18	1			2	4	4	0.064209	0	0	0.132541	0.28265	0.28265	0.300868	0	0	0.422467	0.47848	0.507439
Rh131	8.41065E-20	4	16	1	3	2		4	3	0.062468	0.199354	0.128838	0	0.274275	0.199354	0.292708	0.656181	0.401416	0	0.464303	0.357898
Rh132	1.8301E-24	25	110	5	11	12	9	18	19	0.119488	0.281868	0.311134	0.22528	0.501311	0.535587	0.559892	0.927777	0.969391	0.718066	0.848638	0.961535
Rh134	1.04985E-17	10	13	8				1	4	0.173722	0	0	0	0.020224	0.083384	0.814016	0	0	0	0.034236	0.149699
Rh137	3.14221E-22	17	202	4	8	11	10	14	15	0.311134	0.719072	1.106345	0.968419	1.580862	1.7617	1.457894	2.366853	3.447005	3.086778	2.676143	3.162763
Rh138	1.09863E-16	13	172	4	8	9	9	12	9	0.425103	1.030918	1.218982	1.218982	1.894266	1.218982	1.991922	3.393303	3.797948	3.885431	3.206686	2.188427
Rh139	3.08508E-13	1	1	1						0.019705	0	0	0	0	0	0.092333	0	0	0	0	0
Rh140	1.33514E-23	17	109	2	10	9	7	15	17	0.084146	0.497747	0.43845	0.326805	0.832981	0.987218	0.394286	1.638354	1.366066	1.04167	1.410101	1.772344
Rh141	3.87518E-26	20	78		9	9	8	11	16	0	0.152508	0.152508	0.134474	0.18944	0.287031	0	0.501985	0.475164	0.428627	0.320691	0.515304
Rh143	6.91292E-17	4	7	4					1	0.373824	0	0	0	0	0.082637	1.751642	0	0	0	0	0.148357
Rh144_Rh145	1.82222E-15	2	2	2						0.059254	0	0	0	0	0	0.277648	0	0	0	0	0
Rh147	2.70467E-24	14	71	2	5	6	5	11	10	0.110336	0.299081	0.368875	0.299081	0.778279	0.687612	0.517008	0.984437	1.149292	0.953304	1.317501	1.234464
Rh148	2.96631E-21	3	38	1	2	2	3	3	3	0.193777	0.425103	0.425103	0.701254	0.701254	0.701254	0.907987	1.399241	1.32448	2.235205	1.18711	1.258955
Rh151	5.85938E-14	4	13	1	3		1	4	3	0.145048	0.501311	0	0.145048	0.719072	0.501311	0.679656	1.650083	0	0.46233	1.217273	0.899998
Rh156.IE2	6.20433E-19	6	11	5	2		1	2	1	0.128838	0.04967	0	0.024534	0.04967	0.024534	0.603701	0.163489	0	0.0782	0.084083	0.044045
Rh160	5.31858E-17	5	34	2	2	2	1	5	4	0.311134	0.311134	0.311134	0.145048	0.968419	0.719072	1.457894	1.024109	0.969391	0.46233	1.639377	1.290944
Rh164	1.10596E-20	10	124	5	8	6	8	9	9	0.187482	0.316446	0.229001	0.316446	0.362474	0.362474	0.87849	1.041593	0.713492	1.008652	0.61361	0.650747
Rh164.1	1.36719E-13	1	5				1	1	1				0.211528	0.211528	0.211528				0.674231	0.358082	0.379754
Rh165	1.09863E-14	1	2		1				1	0	0.165914	0	0	0	0.165914	0	0.546113	0	0	0	0.297865
Rh166	1.33031E-15	2	4	1				1	2	0.145048	0	0	0	0.145048	0.311134	0.679656	0	0	0	0.245542	0.558576
Rh17	2.19264E-14	1	10		1	1	1	1	1	0	0.100694	0.100694	0.100694	0.100694	0.100694	0	0.331439	0.31373	0.320956	0.170459	0.180775
Rh170	2.56348E-21	3	3	3						0.43845	0	0	0	0	0	2.054463	0	0	0	0	0
Rh171	6.73066E-11	1	1	1						0.074608	0	0	0	0	0	0.349593	0	0	0	0	0
Rh173	1.95313E-15	2	13	1	1	1	1	2	2	0.085711	0.085711	0.085711	0.085711	0.178769	0.178769	0.40162	0.282121	0.267048	0.273199	0.302626	0.320942
Rh176	5.41225E-11	1	2					1	1	0	0	0	0	0.068	0.068	0	0	0	0	0.115114	0.122081
Rh181	2.28926E-11	1	2					1	1	0	0	0	0	0.074608	0.074608	0	0	0	0	0.126299	0.133943
Rh190	1.11226E-15	2	3	1				1	1	0.082637	0	0	0	0.082637	0.082637	0.387214	0	0	0	0.139891	0.148357
Rh199	5.87628E-11	1	1	1						0.232847	0	0	0	0	0	1.09106	0	0	0	0	0
Rh203	4.1332E-29	42	394	20	25	28	26	34	38	0.482314	0.635593	0.735067	0.668101	0.952533	1.112449	2.259999	2.092077	2.290227	2.129529	1.612483	1.99717
Rh209	1.49464E-14	1	1					1		0	0	0	0	0.01787	0	0	0	0	0	0.030251	0
Rh211	5.12695E-21	29	108	10	12	8	11	19	19	0.253254	0.311134	0.19793	0.281868	0.535587	0.535587	1.186685	1.024109	0.616684	0.898435	0.906663	0.961535
Rh214	5.85938E-14	2	8	1	1	1	1	2	2	0.089023	0.089023	0.089023	0.089023	0.185971	0.185971	0.417139	0.293022	0.277366	0.283755	0.314819	0.333872
Rh215	3.06829E-21	12	104	4	9	8	8	10	10	0.34596	0.951293	0.811609	0.811609	1.101748	1.101748	1.621081	3.131217	2.528707	2.586955	1.865081	1.977958
Rh216	9.76563E-17	9	25	2	3	3		6	6	0.110336	0.169989	0.169989	0	0.368875	0.368875	0.517008	0.559525	0.52963	0	0.624445	0.662237
Rh218	1.46484E-18	6	21		1	1	2	6	6	0	0.062468	0.062468	0.128838	0.43845	0.43845	0	0.205615	0.194629	0.410663	0.742225	0.787145
Rh223	8.36415E-11	1	1	1						0.059254	0	0	0	0	0	0.277648	0	0	0	0	0
Rh230	4.71454E-28	39	409	20	23	27	23	33	33	0.592283	0.707353	0.873817	0.707353	1.154435	1.154435	2.775285	2.328277	2.722527	2.254643	1.954271	2.072546
Rh31	9.76563E-16	3	3	2					1	0.048113	0	0	0	0	0.023774	0.225446	0	0	0	0	0.042681
Rh42	1.37929E-18	12	35	3	3	4	1	10	9	0.100694	0.100694	0.136464	0.032497	0.376857	0.333521	0.471827	0.331439	0.425176	0.103583	0.637958	0.598768
Rh43	1.90735E-26	44	394	16	25	27	23	36	39	0.505836	0.895736	0.995262	0.801174	1.511886	1.712273	2.370219	2.948347	3.10091	2.553691	2.559379	3.074027
Rh44	8.02875E-32	30	437	14	15	19	19	25	23	0.930698	1.02359	1.442053	1.442053	2.237458	1.947052	4.36101	3.369183	4.492963	4.596456	3.787653	3.495524
Rh55	3.69533E-36	72	950	26	42	42	44	65	62	0.475133	0.873817	0.873817	0.930698	1.642883	1.526954	2.226351	2.876202	2.722527	2.966542	2.781135	2.741327

Rh56	3.07344E-16	12	55	1	4	7	6	8	8	0.039122	0.165914	0.308177	0.258925	0.359356	0.359356	0.183317	0.546113	0.96018	0.825309	0.608332	0.645149
Rh59	5.89955E-22	46	505	22	30	33	30	33	36	0.744851	1.13634	1.304822	1.13634	1.304822	1.486591	3.490178	3.740304	4.065395	3.622013	2.208852	2.668863
Rh67.1	3.77398E-19	1	3					1	1	0	0	0	0	0.258925	0.258925	0	0	0	0	0.438319	0.464846
Rh70	6.77356E-24	6	12	6	1	1	1	1	1	0.193777	0.02996	0.02996	0.02996	0.02996	0.02996	0.907987	0.098616	0.093347	0.095497	0.050718	0.053788
Rh72	4.35602E-22	71	550	21	42	42	39	51	51	0.295089	0.677254	0.677254	0.616427	0.873817	0.873817	1.382709	2.229208	2.110102	1.964824	1.479231	1.568756
Rh75	4.09431E-22	27	310	12	20	18	20	26	26	0.572973	1.127496	0.972795	1.127496	1.668268	1.668268	2.684805	3.711196	3.030909	3.593826	2.824108	2.995026
Rh76	3.1232E-23	49	306	9	23	28	24	45	43	0.149219	0.426798	0.541415	0.449018	1.004534	0.943527	0.6992	1.404821	1.68687	1.431218	1.700513	1.693905
Rh78	1.38456E-32	128	839	24	58	63	64	109	107	0.153494	0.412117	0.454758	0.463439	0.91273	0.890104	0.719235	1.3565	1.416876	1.477184	1.545105	1.597996
Rh79.1	8.66389E-23	10	75	1	5	5	5	9	10	0.115884	0.730196	0.730196	0.730196	1.682696	1.993577	0.543003	2.403466	2.27505	2.327454	2.848531	3.57905
Rh81	1.50796E-11	1	1		1					0	0.032497	0	0	0	0	0	0.106966	0	0	0	0
Rh83	9.20053E-11	1	1						1	0	0	0	0	0	0.027791	0	0	0	0	0	0.049893
Rh89	1.83444E-24	59	552	19	36	39	33	49	47	0.341272	0.744258	0.827027	0.665239	1.132353	1.067456	1.599112	2.449753	2.576744	2.120408	1.91689	1.916393

Supplemental Table 2. Detailed information of peptide identification of RhCMV viron proteins.
MSGF - mass spectrum generating function probability
Xcorr - Sequest cross-correlation score
DCn - Normalized difference of top two highest Xcorrs
Sp -Sequest preliminary score

Protein	Peptide	Number of spectra	M+H	Average mass error (ppm)	Min_MSGF	Max_XCorr	Max_DCn	Tryptic termini	Max_Sp	Rank_Sp
Rh05	K.EHPHVTVIEPTK.S	1	1386.73759	-6.25	2.82E-14	2.63	0.34	2	551.3	1
Rh05	K.SADGTVVGLSAVSDDKPATLWLSR.-	1	2445.26199	-7.23	1.05E-14	3.55	0.50	2	598.1	1
Rh05	R.PQDSGLYVLR.D	2	1147.6106	0.19	7.81E-14	2.92	0.46	1	889.1	1
Rh100.1	K.AFANDQEVTK.H	7	1122.54258	0.52	1.26E-13	3.21	0.54	2	1044.1	1
Rh100.1	K.FSTDQVALCEVQK.V	4	1524.73631	-0.21	1.09E-17	4.64	0.55	2	1755.3	1
Rh100.1	R.ASVGADYVLLR.P	4	1163.6419	0.93	5.86E-15	3.35	0.39	1	1536.1	1
Rh100.1	R.ASVGADYVLLRPSEDVNM*QEMQSFFEENFGTLGITPEDLK.A	1	4493.11631	0.82	9.62E-20	4.74	0.06	2	1062	1
Rh100.1	R.ASVGADYVLLRPSEDVNMQEMQSFFEENFGTLGITPEDLK.A	5	4477.12141	-0.68	1.33E-22	5.78	0.62	2	1813.8	1
Rh100.1	R.IM*QALDVM*ILK.L	1	1306.71088	0.41	3.04E-10	1.98	0.24	2	286.5	1
Rh100.1	R.IM*QALDVMILK.L	2	1290.71598	1.10	6.13E-13	3.26	0.34	2	912.1	1
Rh100.1	R.IMQALDVM*ILK.L	2	1290.71598	0.62	6.25E-14	3.24	0.35	2	1203.2	1
Rh100.1	R.IMQALDVMILK.L	2	1274.72108	1.46	3.60E-09	2.11	0.16	2	386.1	2
Rh100.1	R.LWGFLSCQR.R	7	1166.57756	0.27	1.72E-12	2.92	0.32	2	1063.2	1
Rh100.1	R.M*LQDYLADSCQPHTR.P	8	1850.81601	-0.63	1.58E-16	3.80	0.51	1	1103.5	1
Rh100.1	R.MLQDYLADSCQPHTR.P	3	1834.82111	-0.24	2.28E-17	4.43	0.54	1	1153.5	1
Rh100.1	R.MLQDYLADSCQPHTRPAAEVENQK.S	5	2801.29817	-3.07	1.02E-15	3.97	0.36	2	987.4	1
Rh100.1	R.PAAEVENQKSQR.I	2	1356.68662	1.19	2.15E-16	3.56	0.47	1	1429.9	1
Rh100.1	R.RLWGFLSCQR.R	3	1322.67867	-2.28	1.06E-11	2.95	0.15	2	1341.1	1
Rh102	K.HAEDDFYTAHCTSHM*YQVSLR.S	5	2584.098	-0.90	1.58E-16	3.11	0.50	2	970.9	1
Rh102	K.HAEDDFYTAHCTSHMYQVSLR.S	6	2568.1031	-0.18	8.48E-24	6.34	0.73	2	2469.9	1
Rh102	R.HCCFQNFTTTTVAGY.-	11	1806.75749	-0.68	8.54E-13	4.26	0.72	2	745.5	1
Rh102	R.KHAEDDFYTAHCTSHMYQVSLR.S	1	2696.19807	-0.33	3.09E-12	2.26	0.34	2	480.8	2
Rh103	K.QLQDM*VTWLYTTIR.Y	1	1783.90472	1.41	5.26E-16	3.12	0.57	2	473.4	1
Rh103	K.QLQDMVTWLYTTIR.Y	7	1767.90982	-1.11	2.89E-18	4.73	0.65	2	1760.4	1
Rh103	K.SFYLTNAM*SR.N	4	1205.56192	-0.37	1.70E-11	2.31	0.30	2	316.6	1
Rh103	K.SFYLTNAMSR.N	5	1189.56702	-0.22	2.25E-14	2.57	0.49	2	1025.4	1
Rh103	R.SYAYTIPMPK.F	2	1170.58636	0.67	1.93E-12	1.90	0.36	2	330.6	1
Rh103	R.YNQEPFCDK.S	4	1200.49904	-1.54	1.01E-10	2.82	0.32	2	792.9	1
Rh104	K.DHELLFTTR.E	17	1131.5793	-0.19	7.81E-13	3.05	0.58	2	1113.8	1
Rh104	K.DHIHYHTYIK.T	4	1326.65895	0.05	2.91E-12	2.62	0.33	2	224	1
Rh104	K.EQASTVPPPENLR.L	9	1437.73323	-0.17	8.24E-13	2.91	0.49	2	269.9	1
Rh104	K.HDM*EQIR.N	2	944.42543	0.28	1.79E-09	1.94	0.37	2	198.8	2
Rh104	K.HDMEQIR.N	10	928.43053	0.11	3.13E-10	2.85	0.34	2	893.4	1
Rh104	K.HDTLPIVM*IR.N	7	1210.66124	0.00	2.78E-13	2.64	0.58	2	959.8	1
Rh104	K.HDTLPIVMIR.N	14	1194.66634	-0.43	7.81E-14	3.04	0.56	2	1083.8	1
Rh104	K.KDHIHYHTYIK.T	1	1454.75391	-43.56	2.82E-10	2.33	0.09	2	292	3
Rh104	K.NYM*DLDVLLSVWNR.H	4	1753.85777	1.41	1.84E-14	4.17	0.49	2	1500.2	1
Rh104	K.NYMDLDVLLSVWNR.H	5	1737.86287	1.16	1.36E-15	4.75	0.50	2	2103.8	1
Rh104	K.SFAAFTVSHATTYLVSTEYIAR.G	6	2435.22415	3.22	3.03E-19	5.02	0.64	2	1063	1
Rh104	K.TALSVLETLRPQTLATFPEISCVSTTK.S	13	2963.57584	0.33	1.17E-18	6.92	0.71	2	1939.7	1
Rh104	K.TFQQYASTLNTYATVSQDNIQYK.Y	5	2684.28385	-1.62	3.92E-26	5.90	0.72	2	1563.9	1
Rh104	K.THISSFLSR.F	9	1047.55817	-0.42	8.59E-13	2.50	0.52	2	599.4	1
Rh104	K.THYLM*FLK.N	2	1068.55465	-0.52	6.18E-10	1.79	0.20	2	434.8	1
Rh104	K.THYLMFLK.N	4	1052.55975	-0.67	1.56E-11	1.98	0.42	2	625.5	1
Rh104	K.TTDFADSLLSK.N	13	1197.59976	0.03	1.95E-15	3.63	0.54	2	1345.9	1
Rh104	K.YYGQNLSYQNNLK.E	2	1604.77035	-0.77	9.82E-16	3.95	0.48	2	861.9	1
Rh104	R.AIFAFETGLCSLAELSHWSQLIGSEEHNHVSDLYSPCAGSGR.R	6	4633.15116	-0.91	1.72E-17	4.51	0.57	2	925.2	1
Rh104	R.CLFTGEFADNLLNQVDLSK.T	6	2184.06418	-0.27	5.79E-23	6.72	0.71	2	4193.3	1
Rh104	R.DHALEHLQK.M	7	1090.56398	-3.74	1.56E-12	2.64	0.48	2	840.6	1
Rh104	R.DHALEHLQKM*FPR.A	2	1637.82166	0.36	3.58E-13	2.45	0.37	2	371.8	1
Rh104	R.DHALEHLQKMFPR.A	3	1621.82676	-0.08	1.32E-15	3.66	0.54	2	947.1	1
Rh104	R.ENAITFNFYESANHYTVFQLPR.C	29	2661.27323	0.20	8.86E-24	4.87	0.66	2	820.8	1
Rh104	R.ENFILKQTK.R	2	1120.63609	0.16	1.86E-10	2.25	0.19	2	194.7	1
Rh104	R.ETPLLLIFGDTK.R	12	1346.75659	0.53	3.91E-16	4.10	0.65	2	1342.7	1
Rh104	R.ETPLLLIFGDTKR.I	3	1502.85771	0.30	3.91E-17	3.53	0.52	2	1380.7	1
Rh104	R.GTSFPVLTTVVGR.S	31	1333.74743	-0.02	2.44E-18	4.45	0.62	2	1051.5	1
Rh104	R.HAVNVLQNGQCR.S	11	1395.69103	-4.50	3.91E-16	3.91	0.64	2	2398.4	1

Rh104	R.HQLIVLVK.K	8	949.61931	-0.35	6.25E-11	3.13	0.42	2	1321.6	1
Rh104	R.HQLIVLVKK.D	1	1077.71428	0.33	7.54E-09	1.89	0.34	2	140	1
Rh104	R.IM*FKAPYR.R	1	1041.55498	-1.78	6.48E-11	2.13	0.15	2	582.9	1
Rh104	R.IMFKAPYR.R	1	1025.56008	-0.71	6.56E-09	1.70	0.09	2	386.5	5
Rh104	R.LIYILVK.Q	1	861.5808	-0.23	6.23E-09	1.73	0.20	2	281.7	1
Rh104	R.LQTTTSYAK.P	6	1012.53095	0.31	7.81E-13	2.68	0.35	1	228.2	1
Rh104	R.NLILLLHK.T	3	963.63496	0.10	1.36E-10	2.26	0.32	2	669.2	1
Rh104	R.PQTLATFPEISCVSTTK.S	3	1879.94703	-0.50	1.23E-14	3.84	0.58	1	541.4	1
Rh104	R.QELYLASGIIHSM*LHHSAER.R	11	2308.15026	0.30	5.27E-17	3.97	0.54	2	634	1
Rh104	R.QELYLASGIIHSMLHHSAER.R	17	2292.15536	0.24	9.62E-21	4.69	0.64	2	1492.2	1
Rh104	R.QELYLASGIIHSMLHHSAERR.A	2	2448.25647	0.72	1.32E-12	2.50	0.19	2	258.4	4
Rh104	R.TTGHISSVLTISR.L	17	1371.75905	0.17	9.77E-18	3.88	0.61	2	969.6	1
Rh104	R.TTLLMYPNAVQLANSTLR.T	1	2006.07392	-1.63	3.71E-10	2.62	0.45	2	515.4	1
Rh106	K.EEVVANQVR.K	5	1043.548	0.42	3.91E-13	1.85	0.35	2	163.1	1
Rh106	K.EIYSSGGGPEAPPQK.V	3	1516.72781	-0.33	3.24E-14	2.95	0.50	2	352.7	1
Rh106	K.FTLADILGNDALPR.T	2	1515.81657	0.13	5.47E-12	3.57	0.36	2	945.4	1
Rh106	K.LSDEVGR.E	1	775.39446	0.32	1.56E-10	1.72	0.06	2	142.5	2
Rh106	K.VNPVADFM*FAQSSK.Q	1	1556.74134	0.62	9.54E-09	2.97	0.46	2	234.7	1
Rh106	K.VNPVADFMFAQSSK.Q	2	1540.74644	-0.41	3.44E-19	3.57	0.52	2	1080.7	1
Rh106	K.VVNSESVFAPR.A	5	1204.63206	-0.29	3.04E-11	2.85	0.37	2	577	1
Rh106	K.YYMPLSSGR.H	1	1073.50844	0.14	8.03E-10	2.03	0.45	2	136.3	1
Rh106	R.CPPHVLQR.L	1	1006.52513	0.75	3.13E-11	2.31	0.17	2	164.6	16
Rh106	R.DDGLSVWCR.R	2	1107.48881	-0.12	6.38E-12	2.09	0.26	2	257.2	1
Rh106	R.DLPTSIQGVLTELPHLLNK.L	3	2088.16993	0.44	2.41E-19	4.25	0.40	2	1539.7	1
Rh106	R.DPTVTISQLFPGIM*LLAITESVR.S	8	2517.36327	0.62	1.13E-14	4.88	0.64	2	803.3	1
Rh106	R.DPTVTISQLFPGIMLLAITESVR.S	6	2501.36837	-2.03	2.58E-19	5.76	0.70	2	1495.5	1
Rh106	R.DVPIFVHEQQYLR.S	3	1643.85402	-1.48	1.95E-17	4.09	0.56	2	901	1
Rh106	R.EELSDLNQR.I	7	1103.53274	0.17	1.56E-12	2.48	0.30	2	464.9	1
Rh106	R.GQAATWVAQCSDQEK.E	3	1678.749	-1.14	7.54E-18	5.05	0.65	2	1888	1
Rh106	R.HYSPGTFDR.H	5	1079.49048	0.05	1.75E-10	2.20	0.25	2	252.1	1
Rh106	R.IQTYCEDLESR.V	6	1413.63151	-0.61	3.91E-15	4.06	0.61	2	906.1	1
Rh106	R.LELHDALLFHCEHGLGR.L	5	2017.00728	-0.18	4.38E-21	4.70	0.61	2	1222.9	1
Rh106	R.LLSVALPR.H	1	868.56146	-1.08	3.13E-11	1.61	0.10	2	496.5	1
Rh106	R.LPIVLFEPHAENILR.C	8	1761.00577	-0.72	1.18E-13	4.26	0.50	2	1427.8	1
Rh106	R.LVDDSVAGLR.K	4	1044.5684	-0.47	5.86E-14	2.23	0.41	2	379.6	1
Rh106	R.NDGQAGDGSGTVLM*QISK.V	2	1793.8334	0.71	2.83E-21	4.12	0.63	2	778.1	1
Rh106	R.NDGQAGDGSGTVLMQISK.V	2	1777.8385	-0.78	8.71E-22	4.73	0.62	2	1163.1	1
Rh106	R.QVIQSLPGYGAQTAAVVQER.M	7	2115.11929	-1.81	1.73E-24	6.83	0.64	2	1298.6	1
Rh106	R.RQVIQSLPGYGAQTAAVVQER.M	3	2271.2204	-0.10	3.90E-15	3.63	0.51	2	419.7	1
Rh106	R.SGLTCISALLLIWK.V	2	1574.8975	1.38	1.95E-13	3.64	0.38	2	999.3	1
Rh106	R.SLYTQPR.W	4	864.45739	-0.28	4.84E-10	2.17	0.41	2	173	1
Rh106	R.TSSEDDSYSYGHR.V	3	1503.59826	-2.18	1.51E-16	3.49	0.58	2	1324.1	1
Rh106	R.VSEAEALLK.Q	1	959.54079	-0.43	7.81E-13	2.28	0.16	2	231.4	14
Rh106	R.YLREELSDLNQR.I	3	1535.78125	-2.70	6.34E-14	3.43	0.39	2	519	1
Rh109	K.DAFFSLIGASR.H	1	1183.6106	-0.89	4.65E-13	2.93	0.42	2	1301.9	1
Rh109	R.FPDLTEADR.E	2	1063.50547	1.05	8.59E-12	2.42	0.32	2	586.9	1
Rh109	R.GFDYTAWLR.R	3	1128.54727	0.80	1.40E-11	2.54	0.38	2	485.3	1
Rh109	R.RGTLAVYGR.E	2	992.56359	-0.75	1.76E-11	2.19	0.34	2	283.9	1
Rh109	R.SVYPAAPPHHAYPVM*NYEDPSR.G	1	2514.15065	-1.18	9.86E-13	2.51	0.35	2	275.6	2
Rh109	R.SVYPAAPPHHAYPVMNYEDPSR.G	2	2498.15575	-0.50	5.70E-17	3.00	0.48	2	414.8	1
Rh109	R.YDEAPEEAELVLPSDVVDR.W	1	2146.01864	-0.57	1.80E-12	3.47	0.51	2	528.7	1
Rh110	K.GHCSSTFR.D	5	951.41016	1.12	3.91E-12	2.22	0.62	2	224.5	1
Rh110	K.ITINVQNLTANAITLAHM*QMLGFIHLFR.R	2	3196.71218	1.39	1.49E-13	3.65	0.38	2	1051.3	2
Rh110	K.ITINVQNLTANAITLAHMQM*LGFIHLFR.R	2	3196.71218	1.39	5.18E-13	3.71	0.10	2	1066.7	1
Rh110	K.ITINVQNLTANAITLAHMQMLGFIHLFR.R	1	3180.71728	1.07	2.02E-14	3.77	0.32	2	735.7	1
Rh110	K.NTSYLR.S	4	753.38898	-0.67	3.13E-09	1.77	0.30	2	116.1	1
Rh110	K.NTSYLRSELDGDICTAADLK.T	1	2242.06564	-7.18	6.68E-15	3.54	0.34	2	848.8	1
Rh110	K.REGTAAPVTHR.E	1	1194.63379	-0.35	4.50E-09	2.25	0.23	2	178.8	5
Rh110	K.TAM*LLVVPSWNM*YIHPDLLMPLTAR.I	1	2914.50275	0.93	4.47E-09	2.74	0.20	2	240.7	1
Rh110	K.TAM*LLVVPSWNMYIHPDLLM*PLTAR.I	1	2914.50275	-0.34	1.15E-10	2.66	0.17	2	226.8	2
Rh110	K.TAM*LLVVPSWNMYIHPDLLMPLTAR.I	3	2898.50785	-2.03	1.77E-11	2.93	0.31	2	306.4	1
Rh110	K.TAMLLVVPSWNM*YIHPDLLMPLTAR.I	1	2898.50785	-0.84	5.15E-11	2.68	0.11	2	150.4	1
Rh110	K.TAMLLVVPSWNMYIHPDLLMPLTAR.I	2	2882.51295	-5.57	2.27E-18	4.31	0.59	2	608.9	1
Rh110	K.TTLQALAQVPGR.R	13	1254.71646	-0.31	9.67E-16	3.25	0.53	2	741.1	1

Rh110	K.VNVSAITWTR.K	13	1146.62658	-0.13	1.95E-14	3.67	0.61	2	1586	1
Rh110	R.ALAPAVTFER.L	14	1074.59422	-1.34	1.95E-14	2.94	0.59	2	859.7	1
Rh110	R.AQYTWTVK.V	6	996.51491	-0.32	3.70E-11	1.92	0.24	2	259.9	1
Rh110	R.AVICAFQEM*K.S	11	1212.57516	-0.29	1.56E-13	3.06	0.53	2	958.6	1
Rh110	R.AVICAFQEMK.S	13	1196.58026	-0.33	4.00E-14	3.73	0.54	2	1102.5	1
Rh110	R.AVICAFQEMKSSR.D	1	1526.74543	0.48	7.60E-15	2.70	0.50	2	352.2	1
Rh110	R.DALQLTDLNLK.G	16	1243.68924	0.75	2.05E-14	4.08	0.52	2	1729.4	1
Rh110	R.DSLRTDACNYANR.R	2	1555.69182	-3.55	4.99E-15	3.85	0.45	2	1001.8	1
Rh110	R.DSLRTDACNYANRR.L	1	1711.79293	-7.60	3.84E-09	3.05	0.41	2	94.1	3
Rh110	R.EAHKAKR.E	1	839.48461	3.49	1.71E-09	1.53	0.08	2	156.4	4
Rh110	R.EGTAAPVTHR.E	10	1038.53268	-0.46	2.05E-13	2.53	0.34	2	568.4	1
Rh110	R.GRGNSQNRPPR.A	1	1238.64609	-4.14	1.02E-11	2.76	0.27	2	780.6	1
Rh110	R.GSVGVLPNK.T	7	870.50434	-0.20	3.91E-13	2.43	0.42	2	427.5	1
Rh110	R.GSVGVLPNKTETPR.C	10	1454.79617	-0.76	1.13E-15	3.02	0.57	2	1066.1	1
Rh110	R.HNPNPFFER.H	26	1157.54867	-1.67	1.51E-12	2.25	0.52	2	606.8	1
Rh110	R.HSENGFLVK.C	7	1030.53162	-0.70	1.19E-12	2.89	0.31	2	451.1	1
Rh110	R.IAEEAVK.N	2	759.4247	-1.17	1.56E-10	1.55	0.10	2	502.7	1
Rh110	R.IAEEAVKNTSYLR.S	4	1493.79583	-0.14	1.03E-17	4.08	0.54	2	1146.7	1
Rh110	R.LSPGSQTSAM*LVFALPIVR.V	14	2003.09939	-1.85	1.75E-15	4.79	0.52	2	1254.4	1
Rh110	R.LSPGSQTSAMLVFALPIVR.V	21	1987.10449	-0.24	5.53E-20	4.87	0.61	2	1572.9	1
Rh110	R.LSPGSQTSAMLVFALPIVRVPVTGIHLFR.G	3	3106.75979	0.20	5.17E-18	2.58	0.51	2	399.1	1
Rh110	R.LTCQILR.L	3	903.50808	-0.30	1.25E-09	2.44	0.19	2	494.3	2
Rh110	R.LVCTQHSPLDVDAVQTM*NWHTSVEVANR.A	12	3223.52593	0.40	1.01E-23	7.06	0.70	2	2909.8	1
Rh110	R.LVCTQHSPLDVDAVQTMNWHTSVEVANR.A	59	3207.53103	0.07	9.38E-29	7.78	0.68	2	2243.4	1
Rh110	R.PPEEEEEPRPSTSR.A	11	1639.75582	-1.00	2.46E-13	3.94	0.53	1	501	1
Rh110	R.RAQYTWTVK.V	4	1152.61602	-0.53	1.55E-10	2.72	0.27	2	698.5	1
Rh110	R.RGSVGVLPNKTETPR.C	1	1610.89728	-7.12	3.37E-11	3.53	0.45	2	630	1
Rh110	R.SELDGDICTAADLK.T	72	1507.6945	-0.33	4.88E-19	5.10	0.59	2	3245.5	1
Rh110	R.SELDGDICTAADLKTTLQALAQVPGR.R	1	2743.39312	-5.46	6.42E-19	4.13	0.54	2	1358.2	1
Rh110	R.TDACNYANR.R	16	1084.44767	-0.37	2.55E-13	3.48	0.57	2	1584.2	1
Rh110	R.VPVTGIHLFR.G	25	1138.67314	0.62	3.91E-14	3.59	0.50	2	906.6	1
Rh111	K.DVYLHVHNPTDKPITPADEPM*SFFLYALPLR.H	3	3612.81954	-0.09	1.67E-19	6.29	0.62	2	1228.5	1
Rh111	K.DVYLHVHNPTDKPITPADEPMSFFLYALPLR.H	9	3596.82464	0.12	1.79E-19	8.03	0.72	2	4019.9	1
Rh111	K.HM*TFEDQELK.D	8	1293.57796	-0.20	1.17E-12	3.60	0.44	2	1061.5	1
Rh111	K.HMTFEDQELK.D	14	1277.58306	-1.15	3.91E-14	3.13	0.53	2	964.6	1
Rh111	K.KTDHSIFR.D	2	1003.53195	0.09	4.01E-10	2.14	0.20	2	411.1	1
Rh111	K.LYKPLVQR.K	6	1016.62513	-0.38	2.04E-10	2.09	0.21	2	650.7	1
Rh111	K.PFLTPQQR.N	18	986.54179	-0.92	4.78E-11	2.70	0.19	1	694.5	1
Rh111	K.PITPADEPMSFFLYALPLR.H	1	2178.13037	0.44	2.96E-14	4.00	0.41	1	547.1	1
Rh111	K.RFTEDDAAK.K	1	1052.50071	0.05	8.34E-10	2.33	0.25	2	952.7	1
Rh111	K.SSSDDENDDFFPTLYWGIWQFGVR.A	1	2881.27402	-3.15	4.44E-17	4.54	0.70	2	755.7	1
Rh111	K.SVPGCSISCNPIM*STQCIFM*EIR.S	4	2719.20174	-0.08	2.94E-16	3.93	0.61	2	141.6	1
Rh111	K.SVPGCSISCNPIM*STQCIFMEIR.S	14	2703.20684	0.25	1.15E-18	5.46	0.53	2	1952	1
Rh111	K.SVPGCSISCNPIMSTQCIFM*EIR.S	17	2703.20684	0.23	6.24E-19	5.35	0.63	2	1475	1
Rh111	K.SVPGCSISCNPIMSTQCIFMEIR.S	18	2687.21194	0.22	4.97E-22	6.55	0.73	2	3169.8	1
Rh111	K.TDHSIFR.D	11	875.43699	-0.69	1.56E-10	1.91	0.41	2	207.8	1
Rh111	K.VFIHLAWEQSSGIITMNR.N	1	2102.08516	2.15	1.25E-09	3.20	0.40	2	173.7	1
Rh111	K.VIQSVFDEAR.E	18	1163.60551	0.15	3.91E-14	3.86	0.56	2	1124	1
Rh111	K.VIQSVFDEAREPVKPHETK.I	2	2209.16116	-3.19	9.81E-21	4.29	0.38	2	989.6	1
Rh111	K.YIGFICPK.S	7	997.51759	-0.48	1.56E-11	2.35	0.27	2	569	1
Rh111	R.ATELTPM*IASVCGDQLPHQEFSWEGDDDLR.I	4	3433.53113	-2.14	3.58E-21	4.88	0.49	2	609.3	1
Rh111	R.ATELTPMIASVCGDQLPHQEFSWEGDDDLR.I	14	3417.53623	-0.52	8.68E-24	4.98	0.66	2	1236.3	1
Rh111	R.EHANVM*IDTYFESDK.Y	3	1814.79014	-0.91	1.88E-18	4.41	0.71	2	945.3	1
Rh111	R.EHANVM*IDTYFESDKYIGFICPK.S	1	2793.28989	-0.16	7.56E-15	2.24	0.26	2	268.2	1
Rh111	R.EHANVMIDTYFESDK.Y	4	1798.79524	-0.01	2.19E-17	4.06	0.66	2	1156.3	1
Rh111	R.EHANVMIDTYFESDKYIGFICPK.S	9	2777.29499	-0.48	1.85E-19	4.59	0.64	2	1056.1	1
Rh111	R.EKLYKPLVQR.K	2	1273.76268	-0.99	4.09E-13	2.24	0.20	2	361.7	1
Rh111	R.FNAYHLQWTQR.Y	34	1463.71786	-1.17	4.45E-15	4.33	0.52	2	1583.5	1
Rh111	R.FTEDDAAK.K	8	896.3996	-0.13	3.91E-12	2.37	0.29	2	416.4	1
Rh111	R.FTEDDAAKK.S	3	1024.49457	-1.33	3.91E-13	2.31	0.35	2	934.2	1
Rh111	R.HLVPADLTLHPGGTYNPELPTYDAAVQAFAQGYHTR.F	58	3920.93546	0.10	2.04E-29	6.66	0.68	2	1614.3	1
Rh111	R.HYSPSEQLPSTSGESVSK.R	13	1919.89813	-1.33	3.12E-22	6.24	0.68	2	1990.9	1
Rh111	R.HYSPSEQLPSTSGESVSKRPCE.-	1	2462.12528	-6.24	3.82E-22	4.33	0.60	2	981.2	1
Rh111	R.IFLESFQEETPDDK.V	15	1697.79047	-0.55	6.73E-17	5.30	0.52	2	2360.3	1

Rh111	R.IFLESFQEETPDDKVFIHLAWEQSSGIITM*NR.N	3	3796.85269	0.11	3.00E-09	4.44	0.41	2	1452.1	1
Rh111	R.IFLESFQEETPDDKVFIHLAWEQSSGIITMNR.N	3	3780.85779	-0.70	7.92E-11	4.04	0.42	2	1077.5	1
Rh111	R.IFSGLSGSWHM*YQAPK.R	3	1824.87375	-0.38	1.68E-13	3.50	0.49	2	711.1	1
Rh111	R.IFSGLSGSWHMYQAPK.R	7	1808.87885	-0.58	2.11E-21	4.26	0.60	2	1868.7	1
Rh111	R.ITTSFEYHQGK.G	14	1310.63754	-0.57	2.85E-15	4.03	0.59	2	1672.1	1
Rh111	R.KIQLVDK.V	3	843.52983	0.29	1.25E-09	2.05	0.10	2	359.3	1
Rh111	R.KIQLVDKVK.G	1	1070.69321	-8.19	1.01E-10	1.67	0.13	2	137.6	31
Rh111	R.LDYVSELGPIAGK.V	10	1361.73111	-0.68	1.95E-17	4.79	0.59	2	2241.7	1
Rh111	R.LDYVSELGPIAGKVIQSVFDEAR.E	3	2506.31878	1.63	2.06E-23	4.07	0.53	2	853.8	1
Rh111	R.NGYTILNPK.R	2	1019.55202	-0.41	1.56E-12	1.85	0.24	2	245.7	5
Rh111	R.NLFFTYK.K	13	932.48763	-1.17	1.56E-10	2.31	0.39	2	372.9	1
Rh111	R.NLFFTYKK.T	2	1060.58259	-1.84	7.47E-10	2.11	0.20	2	354.8	1
Rh111	R.NPKPFLTPQQR.N	9	1325.73245	-0.83	5.69E-14	2.50	0.47	2	456.8	1
Rh111	R.PSIIFLTQFAR.N	17	1292.73613	0.01	7.81E-15	4.61	0.59	1	1508.3	1
Rh111	R.SLHDSVYIEPFQAIASLHFFDR.N	39	2592.28815	-0.55	5.41E-23	7.70	0.70	2	2636.6	1
Rh111	R.VQVHRPSIIFLTQFAR.N	16	1912.09156	-2.12	5.47E-17	5.76	0.59	2	2400.8	1
Rh111	R.WMPHGVHHTASFYVNTSPMPLWSINVANELVCSLR.S	1	4050.95645	2.79	6.40E-09	3.23	0.23	2	537.7	1
Rh111	R.YEHNTLQIK.H	13	1145.59495	-0.13	1.56E-12	3.42	0.44	2	739.5	1
Rh111	R.YEHNTLQIKHMTFEDQELK.D	1	2404.16017	0.59	2.39E-10	2.55	0.12	2	668.5	1
Rh111	R.YRLDYVSELGPIAGK.V	10	1680.89555	-0.66	3.80E-15	4.98	0.57	2	2828	1
Rh111	R.YRLDYVSELGPIAGKVIQSVFDEAR.E	1	2825.48322	0.76	4.65E-15	2.61	0.36	2	380.9	1
Rh112	K.AETSDESEEDDDDRETLVFSWPNWQCGIK.S	6	3458.46015	-2.16	8.71E-17	5.37	0.61	2	1299	1
Rh112	K.AVHESLYIEQYEELGWLYFFDR.K	5	2807.33516	0.32	3.74E-13	3.88	0.55	2	1348.6	1
Rh112	K.AVHESLYIEQYEELGWLYFFDRK.M	3	2935.43012	-4.10	3.60E-18	4.05	0.57	2	950.6	1
Rh112	K.CFVHLAWENGNHDIVM*NR.N	13	2228.01242	4.75	2.60E-21	5.18	0.62	2	1776.2	1
Rh112	K.CFVHLAWENGNHDIVMNR.N	17	2212.01752	3.81	2.84E-22	5.73	0.66	2	1870.5	1
Rh112	K.DEVKVYLECLQEDTPETK.C	1	2196.03769	-5.29	1.17E-13	3.02	0.05	2	1240.3	1
Rh112	K.EHLNPHTTYIADTHLNVR.V	24	2131.06793	-2.34	3.06E-24	5.22	0.68	2	1349.8	1
Rh112	K.GTATEPAQAR.L	30	1001.50105	-0.47	1.95E-14	3.01	0.43	2	480.2	1
Rh112	K.HTAFEPR.S	12	857.42643	0.22	7.81E-11	2.07	0.47	2	313.8	1
Rh112	K.ISKDEVK.V	4	818.46181	0.09	2.11E-09	1.78	0.00	2	298.1	1
Rh112	K.ISKDEVKVYLECLQEDTPETK.C	2	2524.24875	-5.84	1.43E-15	5.79	0.44	2	1482.6	1
Rh112	K.LEYHHVYGDQADDESGSSASDSDM*EVFR.H	2	3162.28649	-0.84	9.44E-22	7.46	0.71	2	1553.9	1
Rh112	K.LEYHHVYGDQADDESGSSASDSDMEVFR.H	10	3146.29159	-1.70	3.05E-27	8.57	0.75	2	4350.6	1
Rh112	K.LIFNANK.E	2	819.47231	-0.86	4.36E-10	1.74	0.15	2	209.6	9
Rh112	K.LIFNANKEHLNPHTTYIADTHLNVR.V	15	2931.5224	-3.80	2.09E-16	3.81	0.47	2	761.4	1
Rh112	K.M*ILTKGTATEPAQAR.L	2	1603.8472	-0.51	1.45E-17	3.68	0.34	2	1089.1	1
Rh112	K.MILTKGTATEPAQAR.L	6	1587.8523	0.11	1.16E-15	4.92	0.58	2	1294.2	1
Rh112	K.PSSSAASSTM*TTR.S	19	1299.58451	1.62	1.48E-17	4.12	0.68	1	1428.5	1
Rh112	K.PSSSAASSTMTTR.S	49	1283.58961	-0.86	1.22E-18	3.97	0.64	1	1665.2	1
Rh112	K.STSLVPIVASAHGDR.L	29	1509.80198	-0.20	5.02E-17	3.92	0.56	2	883.9	1
Rh112	K.STSLVPIVASAHGDRLPYQDFPWGEGDDYR.T	10	3348.592	-0.83	7.36E-24	4.31	0.65	2	501	1
Rh112	K.TSHLM*FDVCFESDK.Y	4	1731.7353	0.37	6.90E-16	4.14	0.65	2	1274.8	1
Rh112	K.TSHLMFDVCFESDK.Y	10	1715.7404	-0.03	8.47E-18	4.41	0.66	2	1913	1
Rh112	K.TSHLMFDVCFESDKYVAIICPR.T	25	2688.26195	-2.82	1.44E-19	4.85	0.65	2	1660.6	1
Rh112	K.VYLECLQEDTPETK.C	23	1724.80478	-0.98	2.03E-17	5.58	0.49	2	2433.6	1
Rh112	K.YVAIICPR.T	17	991.53938	-0.85	1.64E-11	3.15	0.37	2	953.5	1
Rh112	R.ETLVFSWPNWQCGIK.S	6	1864.9051	0.09	4.30E-15	4.52	0.58	2	890.8	1
Rh112	R.FPDLVTDLGPTSGHLVK.L	36	1795.95888	-0.66	9.16E-23	5.60	0.62	2	2929.4	1
Rh112	R.FPDLVTDLGPTSGHLVKLIFNANK.E	11	2596.41335	1.40	2.58E-18	5.45	0.61	2	1241.8	1
Rh112	R.HPPSGSSAR.R	1	895.43805	1.57	3.16E-11	1.55	0.11	2	157.3	3
Rh112	R.HTRLEPIPESCTVSVPK.K	1	1950.01136	-1.74	6.58E-12	3.78	0.44	2	746	1
Rh112	R.HTRLEPIPESCTVSVPKK.H	2	2078.10632	-3.54	1.26E-11	3.37	0.33	2	1161.6	1
Rh112	R.KISKDEVK.V	5	946.55677	-0.91	3.13E-11	2.73	0.25	2	851.3	1
Rh112	R.KMILTKGTATEPAQAR.L	2	1715.94727	10.91	1.08E-10	1.96	0.38	2	144.8	1
Rh112	R.KPSSSAASSTM*TTR.S	26	1427.67947	1.99	3.40E-15	3.88	0.56	2	507.7	1
Rh112	R.KPSSSAASSTMTTR.S	31	1411.68457	0.51	2.50E-19	4.34	0.58	2	1425.3	1
Rh112	R.LEPIPESCTVSVPK.K	28	1555.80366	-0.61	1.25E-17	3.32	0.42	2	916.8	1
Rh112	R.LEPIPESCTVSVPKK.H	2	1683.89862	-2.56	1.37E-10	2.99	0.32	2	351.6	2
Rh112	R.LPYQDFPWGEGDDYR.T	87	1857.80785	-0.21	1.22E-20	4.80	0.58	2	1511.5	1
Rh112	R.LPYQDFPWGEGDDYRTFSGIEIDLQPYQTQR.R	1	3734.73978	-5.72	6.04E-20	4.34	0.41	2	1374.2	1
Rh112	R.NGFTILCPQTLHLKPGK.T	4	1924.04735	-2.39	3.41E-14	3.78	0.51	2	400.3	1
Rh112	R.NPKPYLR.A	7	887.50976	0.53	4.73E-10	2.20	0.12	2	418.2	1
Rh112	R.PHRFPDLVTDLGPTSGHLVK.L	2	2186.17166	-0.20	1.01E-10	4.00	0.49	1	860.1	1

Rh112	R.PIPTTAEPLSM*YVYALPLDPVTPPELILR.Q	1	3222.73704	0.15	5.10E-10	2.79	0.16	1	235.1	1
Rh112	R.PIPTTAEPLSMYVYALPLDPVTPPELILR.Q	8	3206.74214	0.66	3.18E-18	4.52	0.63	1	1439.4	1
Rh112	R.PSQPPASSSR.K	10	1013.50105	-0.20	3.91E-14	2.70	0.44	1	452.6	1
Rh112	R.RPSQPPASSSR.K	27	1169.60216	2.23	9.77E-16	4.09	0.44	2	511.1	1
Rh112	R.RTTVADAVVQQIDDK.K	13	1658.87079	-0.01	1.00E-13	5.29	0.57	2	2926.9	1
Rh112	R.RTTVADAVVQQIDDKK.W	8	1786.96575	1.40	4.66E-17	6.49	0.56	2	2592.2	1
Rh112	R.RTTVADAVVQQIDDKKWHTR.L	1	2367.25277	-2.27	8.71E-16	2.47	0.14	2	809.1	1
Rh112	R.SLEPLVVHVHNPTDR.P	3	1712.90784	-0.63	1.49E-16	3.80	0.53	1	782.7	1
Rh112	R.SLEPLVVHVHNPTDRPIPTTAEPLSM*YVYALPLDPVTPPELILR.Q	51	4916.62704	0.13	7.91E-18	5.04	0.69	2	2101.6	1
Rh112	R.SLEPLVVHVHNPTDRPIPTTAEPLSMYVYALPLDPVTPPELILR.Q	71	4900.63214	-0.24	6.49E-20	5.16	0.70	2	2586.1	1
Rh112	R.TFSGIEIDLQPYQTQR.R	76	1895.94977	-0.69	1.65E-15	5.34	0.58	2	1373.3	1
Rh112	R.TIPGISM*SCNPLLPLQNLFM*EIK.A	25	2648.34964	0.89	1.51E-18	5.36	0.61	2	1347	1
Rh112	R.TIPGISM*SCNPLLPLQNLFMEIK.A	20	2632.35474	-0.14	1.61E-18	5.75	0.67	2	1130.1	1
Rh112	R.TIPGISMSCNPLLPLQNLFM*EIK.A	22	2632.35474	0.68	3.61E-19	5.31	0.65	2	1334.4	1
Rh112	R.TIPGISMSCNPLLPLQNLFMEIK.A	29	2616.35984	-0.74	3.49E-21	5.88	0.70	2	1282.7	1
Rh112	R.TTVADAVVQQIDDK.K	34	1502.76968	-0.76	1.25E-18	5.18	0.57	2	2013.9	1
Rh112	R.TTVADAVVQQIDDKK.W	13	1630.86464	0.34	1.07E-19	5.07	0.57	2	2250.6	1
Rh112	R.TTVADAVVQQIDDKKWHTR.L	2	2211.15166	-3.70	2.89E-19	2.75	0.52	2	698	1
Rh112	R.VNQPSVILATQFTPESQPCQR.Y	47	2400.19766	-1.41	5.98E-24	6.35	0.66	2	2670.4	1
Rh112	R.VTKAETSDESEEDDDDRETLVFSWPNWQCGIK.S	1	3786.67121	-8.70	1.22E-23	5.54	0.52	2	1996.1	1
Rh112	R.YDTNLQIK.H	31	994.52039	-1.20	3.13E-11	2.89	0.34	2	698.2	1
Rh112	R.YDTNLQIKHTAFEPR.S	10	1832.92897	0.83	4.95E-16	4.13	0.54	2	1124.9	1
Rh112	R.YTANSVYHTTSFIFNSQQM*PLGSM*NTASELVCSIPHTHVTNIR.K	9	4886.29711	-0.82	2.29E-23	5.07	0.57	2	1322.9	1
Rh112	R.YTANSVYHTTSFIFNSQQM*PLGSMNTASELVCSIPHTHVTNIR.K	13	4870.30221	-0.80	1.70E-21	6.24	0.20	2	1766.4	1
Rh112	R.YTANSVYHTTSFIFNSQQMPLGSM*NTASELVCSIPHTHVTNIR.K	9	4870.30221	-0.68	2.96E-21	4.37	0.20	2	698.6	1
Rh112	R.YTANSVYHTTSFIFNSQQMPLGSMNTASELVCSIPHTHVTNIR.K	22	4854.30731	-0.55	2.12E-24	5.69	0.60	2	1172.7	1
Rh114	R.AIIEEQLTQMSIVR.L	1	1630.88327	-5.37	4.87E-13	3.27	0.38	2	1131.6	1
Rh114	R.DPEEQGLLCAVANPDSDIFPILSPFPAQAGSCNIIR.A	2	3911.89432	0.15	2.26E-16	4.75	0.57	2	575.1	1
Rh114	R.QELNMPVTIWLPR.T	2	1596.85666	-1.92	1.13E-13	3.03	0.42	2	578.7	1
Rh117	K.AAVNQLQHVTFR.D	23	1383.74916	0.40	1.95E-16	4.13	0.62	2	1771.4	1
Rh117	K.CQELLM*R.L	1	965.45432	-1.42	2.72E-09	2.26	0.22	2	366.9	2
Rh117	K.CQELLMR.L	14	949.45942	-0.85	7.81E-10	3.07	0.32	2	678.6	1
Rh117	K.GDALCQLPPLFSGPLVR.E	18	1839.9786	-0.11	4.69E-18	5.00	0.56	2	1307.4	1
Rh117	K.HHHLGLHQYIDSTR.G	23	1713.85681	-0.49	4.35E-17	4.14	0.58	2	2064.2	1
Rh117	K.LALEDSSM*LLTK.C	5	1336.70283	-0.01	7.81E-16	3.72	0.51	2	1597.7	1
Rh117	K.LALEDSSMLLTK.C	10	1320.70793	0.17	3.91E-16	3.85	0.53	2	1851.9	1
Rh117	K.LSLTDVGK.L	3	832.47746	-1.37	1.74E-10	1.83	0.29	2	425.8	1
Rh117	K.LSLTDVGKLAK.L	2	1144.6936	0.42	1.83E-12	2.77	0.26	2	633.2	1
Rh117	K.LTGAIIPIPQR.H	27	1178.72557	0.03	6.97E-13	3.64	0.57	2	940.3	1
Rh117	K.SATCIFK.G	1	826.41279	-1.71	3.24E-10	1.74	0.23	2	292.7	2
Rh117	K.SATCIFKG.-	1	883.43425	-0.59	1.36E-09	1.94	0.30	2	231.5	1
Rh117	K.TACLSM*SM*VANLASDLTVNYVR.K	2	2448.15675	0.27	3.78E-15	3.90	0.57	2	872.3	1
Rh117	K.TACLSM*SMVANLASDLTVNYVR.K	18	2432.16185	0.43	3.68E-16	4.13	0.61	2	1626.1	1
Rh117	K.TACLSMSM*VANLASDLTVNYVR.K	10	2432.16185	0.03	1.26E-18	4.17	0.56	2	1637.5	1
Rh117	K.TACLSMSMVANLASDLTVNYVR.K	163	2416.16695	0.92	1.89E-17	5.49	0.65	2	1609.4	1
Rh117	K.TACLSMSMVANLASDLTVNYVRK.L	1	2544.26191	-0.84	1.15E-13	3.36	0.39	2	598.5	1
Rh117	R.DATFTIPDPVIENHLLLDM*K.T	8	2298.1686	0.18	2.30E-15	3.28	0.50	2	641.6	1
Rh117	R.DATFTIPDPVIENHLLLDMK.T	11	2282.1737	-0.66	2.43E-17	3.14	0.58	2	364.6	1
Rh117	R.EHPVSVGNWNLVLPWLLPAPLAVEINQR.I	15	3161.72585	0.29	1.07E-21	5.93	0.71	2	1874.3	1
Rh117	R.GRPSHVSPDEEISR.L	15	1565.76666	-1.61	9.05E-17	4.15	0.46	2	1375.6	1
Rh117	R.IEGNQLAM*QVPTHGHLYTVLNTGPVLWEK.G	9	3261.67248	-0.44	9.12E-22	5.61	0.61	2	1366.2	1
Rh117	R.IEGNQLAMQVPTHGHLYTVLNTGPVLWEK.G	15	3245.67758	-0.64	1.40E-21	7.21	0.68	2	2485.1	1
Rh117	R.ILIM*ALYSLDR.S	8	1323.73407	-5.54	3.13E-14	3.72	0.59	2	1889.9	1
Rh117	R.ILIMALYSLDR.S	17	1307.73917	0.80	3.91E-14	3.92	0.51	2	1562.5	1
Rh117	R.KLALEDSSM*LLTK.C	1	1464.79779	1.66	1.50E-13	4.32	0.43	2	771.7	1
Rh117	R.KLALEDSSMLLTK.C	5	1448.80289	-0.63	1.80E-16	3.96	0.58	2	1940.7	1
Rh117	R.LNRDDDGTGGNNSNR.L	3	1604.70076	3.05	1.91E-11	3.75	0.49	2	481.5	1
Rh117	R.LSALFVM*LR.Q	4	1065.6125	-0.24	4.93E-11	2.68	0.34	2	676.3	1
Rh117	R.LSALFVMLR.Q	8	1049.6176	-0.09	1.56E-12	3.87	0.48	2	1252.1	1
Rh117	R.NM*TPTILR.R	7	961.51351	-0.96	7.71E-11	2.05	0.21	2	300.1	3
Rh117	R.NMTPTILR.R	17	945.51861	-1.46	5.29E-11	2.02	0.37	2	409.8	1
Rh117	R.PSHVSPDEEISR.L	13	1352.64408	-0.99	1.04E-14	3.98	0.46	1	1118.8	1
Rh117	R.QLDDIIHEQVM*FTVCDVSPDNK.S	12	2619.20655	0.81	5.28E-19	4.00	0.65	2	725.1	1
Rh117	R.QLDDIIHEQVMFTVCDVSPDNK.S	14	2603.21165	0.21	1.30E-22	5.52	0.65	2	1563.2	1

Rh117	R.RIEGNQLAM*QVPTHGHLYTVLNTGPVLWEK.G	3	3417.77359	-0.83	8.18E-13	4.87	0.61	2	1401.1	1
Rh117	R.RIEGNQLAMQVPTHGHLYTVLNTGPVLWEK.G	6	3401.77869	-0.21	3.00E-16	6.79	0.68	2	2760.9	1
Rh117	R.SYEEVK.A	2	754.36176	-3.19	4.69E-09	1.74	0.07	2	498.1	1
Rh117	R.SYEEVKAAVNQLQHVTFR.D	7	2119.09308	0.18	9.71E-23	5.88	0.64	2	2634.7	1
Rh118	K.AACELIITPVSTDINYFK.I	9	2055.04674	-0.15	5.71E-15	5.41	0.60	2	1515.9	1
Rh118	K.AACELIITPVSTDINYFKIPNNPR.G	1	2746.4233	-3.41	5.02E-12	2.88	0.45	2	467.8	1
Rh118	K.AGQNSQTSLPM*DILQLGEHAVALEHHR.H	16	2968.46936	-0.22	1.09E-18	4.50	0.52	2	1483.8	1
Rh118	K.AGQNSQTSLPMDILQLGEHAVALEHHR.H	31	2952.47446	-0.06	4.01E-23	5.90	0.66	2	2472.7	1
Rh118	K.APPYFVVQTLVENATLCR.Q	55	2078.07396	1.50	4.27E-21	7.39	0.68	2	4714	1
Rh118	K.DNALPTSVYLLNR.D	16	1475.78527	0.10	2.34E-15	4.78	0.62	2	887.6	1
Rh118	K.FDDLNK.L	1	751.3621	-0.41	3.13E-09	1.78	0.01	2	647.2	1
Rh118	K.FDDLNKLTTGK.M	5	1251.65794	-1.13	3.91E-15	2.75	0.42	2	742.5	1
Rh118	K.IFYLCLLPAM*TNNR.A	7	1741.87643	-0.07	6.84E-18	4.88	0.59	2	1083.8	1
Rh118	K.IFYLCLLPAMTNNR.A	16	1725.88153	-2.37	1.49E-18	4.95	0.64	2	1303.9	1
Rh118	K.IGNLSLYTELHPFFDFAHHQENGETATLCTPR.I	8	3715.75984	0.23	2.21E-24	6.29	0.60	2	1494.6	1
Rh118	K.IKLTETKDNALPTSVYLLNR.D	1	2289.28127	-4.95	1.14E-15	3.67	0.51	2	702.3	1
Rh118	K.ILAEYCIVVPFHR.F	4	1616.86178	-0.38	2.16E-15	4.38	0.54	2	730.9	1
Rh118	K.ILNIEAIHTVLR.V	19	1391.83691	0.16	7.81E-16	4.56	0.54	2	1913.9	1
Rh118	K.ISPVSIALM*AK.S	7	1145.65984	1.22	2.15E-14	2.81	0.42	2	688.5	1
Rh118	K.ISPVSIALMAK.S	10	1129.66494	-0.42	3.91E-15	3.13	0.57	2	718.7	1
Rh118	K.LKNPSGSLATSETHFGNYIIGETIPLHQHMLFNS.-	2	3753.86936	-1.07	2.54E-12	3.26	0.31	2	406.9	1
Rh118	K.LTETKDNALPTSVYLLNR.D	1	2048.10225	-6.78	8.03E-18	5.52	0.48	2	1194	1
Rh118	K.LTTGKM*LFHIQVPR.V	1	1656.92539	0.01	1.49E-11	2.67	0.25	2	308.2	1
Rh118	K.LTTGKMLFHIQVPR.V	2	1640.93049	0.13	3.02E-12	2.99	0.33	2	878	1
Rh118	K.M*ISTIFLLNR.T	4	1223.68164	4.71	1.56E-12	3.16	0.46	2	892.1	1
Rh118	K.M*LFHIQVPR.V	7	1156.62955	0.10	6.25E-12	3.19	0.46	2	1207.7	1
Rh118	K.M*VESTTNSILENPATYVTASGQQLR.G	11	2726.33014	-0.54	9.01E-19	6.03	0.64	2	2121.8	1
Rh118	K.MISTIFLLNR.T	19	1207.68674	0.00	1.56E-13	4.23	0.51	2	1451.9	1
Rh118	K.MLFHIQVPR.V	12	1140.63465	-0.49	1.56E-12	3.67	0.37	2	1548.3	1
Rh118	K.MVESTTNSILENPATYVTASGQQLR.G	18	2710.33524	-0.34	8.60E-18	5.98	0.66	2	1726.3	1
Rh118	K.NPSGSLATSETHFGNYIIGETIPLHQHM*LFNS.-	14	3528.68523	-0.48	1.40E-21	6.03	0.63	2	1532.4	1
Rh118	K.NPSGSLATSETHFGNYIIGETIPLHQHMLFNS.-	19	3512.69033	-1.13	2.15E-21	6.62	0.62	2	1262.5	1
Rh118	K.NTADAM*ER.G	1	923.38871	2.97	1.65E-10	1.88	0.29	2	321.9	1
Rh118	K.NTADAMER.G	21	907.39381	0.02	3.91E-12	2.98	0.39	2	356.2	1
Rh118	K.NTDTKNPLER.N	8	1187.60149	0.16	5.06E-13	3.38	0.25	2	461.1	1
Rh118	K.QDYTPSDFYK.I	9	1263.55281	-0.48	9.77E-14	2.57	0.40	2	320.8	1
Rh118	K.SCTSLIINNPQVTK.E	16	1574.8207	-0.90	2.11E-18	4.63	0.62	2	2562.9	1
Rh118	K.SCTSLIINNPQVTKEER.D	2	1989.007	-5.59	1.20E-15	4.27	0.51	2	1582.2	1
Rh118	K.SGVHPGFALTAVR.T	27	1311.7168	-0.22	7.41E-16	3.55	0.63	2	1143.1	1
Rh118	K.SPITIPFELSAACLTHLR.E	26	2026.07904	0.12	1.11E-20	5.41	0.66	2	1235.5	1
Rh118	K.TIDEYLLR.A	27	1022.55169	-1.50	3.13E-11	3.03	0.50	2	527	1
Rh118	K.TKMISTIFLLNR.T	2	1436.82938	1.70	3.13E-15	3.41	0.48	2	1357.9	1
Rh118	K.TLFKTIDEYLLR.A	4	1511.84681	1.43	7.09E-15	3.59	0.58	2	1120.7	1
Rh118	K.TLM*VDLFYR.P	4	1173.59724	0.68	3.13E-12	2.60	0.40	1	961.7	1
Rh118	K.TLMVDLFYR.P	14	1157.60234	0.11	7.81E-13	2.88	0.46	1	1118.1	1
Rh118	K.TLYDHTEPDAQTFASTHNPWASNR.G	32	2759.24445	-0.49	2.21E-26	7.28	0.69	2	2978.6	1
Rh118	K.TSAGEEM*FDSLR.I	17	1358.58926	-0.59	1.95E-16	3.40	0.62	2	1282.9	1
Rh118	K.TSAGEEMFDSLR.I	28	1342.59436	0.27	9.77E-17	3.85	0.61	2	1968.4	1
Rh118	K.VGIPADFLTHVK.T	14	1296.73105	-0.11	9.77E-17	2.84	0.52	2	576.7	1
Rh118	K.YSEKSPITIPFELSAACLTHLR.E	3	2533.31196	1.21	1.33E-22	5.28	0.56	2	1633.8	1
Rh118	R.ACGIGLNLK.T	18	945.51865	-1.28	1.56E-12	3.07	0.44	2	1014.3	1
Rh118	R.AQLDPAQR.H	12	898.47411	0.27	3.13E-11	2.32	0.45	2	632.7	1
Rh118	R.ASHIAEM*VR.L	12	1029.51458	-0.26	7.81E-13	2.60	0.44	2	983.1	1
Rh118	R.ASHIAEMVR.L	36	1013.51968	-0.76	3.91E-13	2.80	0.51	2	1164.5	1
Rh118	R.AYTTVENK.I	12	925.46254	-0.63	2.34E-11	2.14	0.38	2	327.3	1
Rh118	R.AYTTVENKIK.L	3	1166.64156	-0.68	5.59E-12	2.62	0.43	2	389.2	1
Rh118	R.CIVSAFQNR.Q	24	1094.54118	-0.94	8.20E-13	3.80	0.45	2	1443.5	1
Rh118	R.DINTTYHVTQNINTVDM*GLGYTSATCVAYLNR.S	36	3621.69484	-0.05	8.66E-30	7.16	0.74	2	3747.7	1
Rh118	R.DINTTYHVTQNINTVDMGLGYTSATCVAYLNR.S	130	3605.69994	-0.01	4.03E-33	6.67	0.77	2	2147.5	1
Rh118	R.EGVQIIADR.Q	37	1000.54219	-0.90	1.56E-12	3.09	0.32	2	1062.1	1
Rh118	R.EGVQIIADRQPLNTANIESR.H	4	2224.16803	-3.87	1.34E-19	4.64	0.59	2	1252	1
Rh118	R.ELFLVLQFVPEHAK.I	12	1669.9312	-0.03	4.26E-18	5.06	0.54	2	1176.1	1
Rh118	R.ERLGYNSQFYSPCAQFFNTEEIINANK.T	1	3240.50552	-5.03	7.97E-18	6.34	0.51	2	1481	1
Rh118	R.ETFENTILDK.I	14	1209.59976	0.34	3.22E-13	2.76	0.26	2	796.2	1

Rh118	R.ETFENTILDKILNIEAIHTVLR.V	6	2582.41883	2.56	2.71E-22	5.95	0.66	2	1140.4	1
Rh118	R.FQQESM*AGIAR.R	37	1253.59428	-1.38	1.62E-14	3.48	0.52	2	1256.9	1
Rh118	R.FQQESMAGIAR.R	34	1237.59938	-0.71	4.46E-15	3.73	0.52	2	999.1	1
Rh118	R.FVTDFPHYHR.H	23	1318.63273	-0.43	9.77E-15	3.42	0.50	2	1358.9	1
Rh118	R.FYSDPTICAALNDDIK.R	16	1842.85788	0.03	1.22E-21	5.71	0.66	2	2417.4	1
Rh118	R.FYSDPTICAALNDDIKR.F	10	1998.95899	-0.43	3.39E-22	5.69	0.59	2	1941.1	1
Rh118	R.GDTDTQYICVEGTEQLIECPCR.M	68	2644.13249	-0.08	1.25E-20	5.90	0.73	2	2181.9	1
Rh118	R.GLIHCFIQVLLR.K	26	1468.84574	-1.23	7.41E-15	3.63	0.58	2	1315.8	1
Rh118	R.GLIHCFIQVLLRK.A	4	1596.9407	-4.97	7.90E-14	3.81	0.45	2	1352.1	1
Rh118	R.GSLADIM*YNIK.N	8	1240.62419	0.08	1.72E-13	3.70	0.46	2	1560.1	1
Rh118	R.GSLADIMYNIK.N	12	1224.62929	-0.19	3.91E-15	4.35	0.55	2	1646	1
Rh118	R.GSLADIMYNIKNR.E	2	1494.77332	1.08	7.93E-12	3.21	0.44	2	419.8	1
Rh118	R.GVM*VSTAQVIQTLM*TLLK.S	18	1965.07587	-0.01	2.00E-16	6.28	0.63	2	2056.3	1
Rh118	R.GVM*VSTAQVIQTLMTLLK.S	22	1949.08097	0.18	2.13E-16	6.51	0.61	2	3718.7	1
Rh118	R.GVMVSTAQVIQTLM*TLLK.S	17	1949.08097	0.04	4.16E-19	5.89	0.65	2	2765.4	1
Rh118	R.GVMVSTAQVIQTLMTLLK.S	10	1933.08607	0.54	2.10E-19	5.90	0.60	2	3144.9	1
Rh118	R.GVMVSTAQVIQTLMTLLKSNISK.Q	3	2462.37208	0.71	6.13E-13	3.13	0.39	2	362.7	1
Rh118	R.GVPTDPAVAR.L	7	982.53162	-0.16	2.05E-13	1.93	0.39	2	312.9	1
Rh118	R.HAAGVDRPQFLDTETISM*LTFGSM*SEK.N	9	3000.40772	-0.46	1.15E-14	5.40	0.63	2	1139.1	1
Rh118	R.HAAGVDRPQFLDTETISM*LTFGSMSEK.N	8	2984.41282	0.22	3.37E-15	5.68	0.59	2	1233.9	1
Rh118	R.HAAGVDRPQFLDTETISMLTFGSM*SEK.N	6	2984.41282	-0.22	1.39E-16	5.45	0.59	2	1661.8	1
Rh118	R.HAAGVDRPQFLDTETISMLTFGSMSEK.N	14	2968.41792	1.08	1.11E-17	6.18	0.72	2	2145.2	1
Rh118	R.HDEVDRWVR.H	1	1211.59159	-5.73	3.35E-10	2.12	0.03	2	506.8	1
Rh118	R.HDGGFPLATAFAHEYWNWLR.S	40	2388.13086	0.21	4.40E-26	6.01	0.73	2	2574.2	1
Rh118	R.HGATDFTSLQHVLYNGCCAITAPK.I	16	2661.25489	-0.41	8.84E-23	6.70	0.68	2	3396.2	1
Rh118	R.HHGVSDVPR.M	18	1003.5068	0.79	1.95E-13	2.54	0.40	2	851.6	1
Rh118	R.HVYKNTDTK.N	3	1105.56365	1.71	2.01E-11	1.95	0.32	2	296.4	1
Rh118	R.HVYKNTDTKNPLER.N	3	1714.88711	0.03	5.89E-15	3.24	0.50	2	1191.2	1
Rh118	R.IVTGNIPDGLAPSAFQELR.A	47	1998.06547	-0.79	3.92E-20	5.31	0.60	2	1285.7	1
Rh118	R.IYFGDDPER.Y	20	1111.50547	-0.71	3.91E-13	3.05	0.53	2	562.1	1
Rh118	R.IYFGDDPERYNIHFEAIFGTFCNR.L	2	2981.36757	1.14	9.28E-16	3.38	0.41	2	1274.2	1
Rh118	R.KAPPYFVVQTLVENATLCR.Q	20	2206.16892	0.67	6.29E-24	5.52	0.70	2	1303.4	1
Rh118	R.LDFVDALQCLCHPVVHEATPCLQVFTER.G	64	3354.60691	0.99	1.52E-25	7.96	0.69	2	3022.7	1
Rh118	R.LEWVYFLQTALASAAHAIK.F	11	2132.15389	-0.69	2.53E-23	5.82	0.66	2	3159.9	1
Rh118	R.LEWVYFLQTALASAAHAIKFDDLNK.L	1	2864.49814	-2.42	5.31E-15	3.46	0.47	2	520.1	1
Rh118	R.LGYNSQFYSPCAQFFNTEEIINANK.T	17	2955.36182	-0.81	3.78E-20	6.63	0.65	2	1829.6	1
Rh118	R.LRPPPDYEATLQLFK.S	19	1787.96905	-0.20	1.46E-14	4.60	0.57	2	1686.5	1
Rh118	R.LTHFYR.V	6	836.44135	-0.11	3.13E-09	2.06	0.18	2	311.4	1
Rh118	R.LWPPFVCHLPR.N	17	1421.75111	1.43	1.95E-15	2.53	0.47	2	446.3	1
Rh118	R.M*AAM*DADEPLFVDDYQATDDEWTLQK.I	3	3050.29175	-0.16	6.06E-24	5.23	0.69	2	1567.9	1
Rh118	R.M*AAMDADEPLFVDDYQATDDEWTLQK.I	18	3034.29685	-0.08	1.70E-21	6.63	0.08	2	2187.5	1
Rh118	R.M*M*QEALPILSTTTQALM*ESK.L	3	2271.09164	0.75	1.18E-13	4.69	0.62	2	1220.2	1
Rh118	R.M*M*QEALPILSTTTQALMESK.L	5	2255.09674	-0.51	1.15E-14	5.51	0.67	2	1483.4	1
Rh118	R.M*MQEALPILSTTTQALM*ESK.L	18	2255.09674	-0.19	1.98E-15	4.81	0.63	2	850.7	1
Rh118	R.M*MQEALPILSTTTQALMESK.L	26	2239.10184	0.18	2.16E-19	5.41	0.68	2	1510.7	1
Rh118	R.MAAM*DADEPLFVDDYQATDDEWTLQK.I	16	3034.29685	-0.14	2.19E-21	6.46	0.70	2	2139.9	1
Rh118	R.MAAMDADEPLFVDDYQATDDEWTLQK.I	15	3018.30195	-0.74	1.46E-23	6.67	0.76	2	2178.9	1
Rh118	R.MM*QEALPILSTTTQALM*ESK.L	18	2255.09674	-0.19	1.63E-15	4.81	0.64	2	850.7	1
Rh118	R.MM*QEALPILSTTTQALMESK.L	26	2239.10184	0.18	2.16E-19	5.41	0.66	2	1416.1	1
Rh118	R.MMQEALPILSTTTQALM*ESK.L	18	2239.10184	-0.01	2.53E-20	5.66	0.69	2	1730	1
Rh118	R.MMQEALPILSTTTQALMESK.L	19	2223.10694	-0.49	6.76E-20	5.85	0.61	2	1391	1
Rh118	R.NIDLTFFFPVGLYIPESR.A	14	2128.11135	-0.27	4.04E-17	5.90	0.62	2	3041.6	1
Rh118	R.NREGVQIIADR.Q	3	1270.68622	-2.34	2.38E-11	2.39	0.28	2	456.9	1
Rh118	R.NREGVQIIADRQPLNTANIESR.H	3	2494.31207	-3.13	1.57E-17	4.69	0.58	2	3246.6	1
Rh118	R.PAFTM*PTEPESVGEM*LTQLVDEIATNEDVNLFEACR.E	14	4085.86645	0.08	6.32E-19	7.20	0.68	1	3365.5	1
Rh118	R.PAFTM*PTEPESVGEMLTQLVDEIATNEDVNLFEACR.E	2	4069.87155	0.20	1.05E-16	6.39	0.53	1	1475.3	1
Rh118	R.PAFTMPTEPESVGEM*LTQLVDEIATNEDVNLFEACR.E	13	4069.87155	0.23	1.18E-21	6.95	0.20	1	2609	1
Rh118	R.PAFTMPTEPESVGEMLTQLVDEIATNEDVNLFEACR.E	7	4053.87665	0.46	2.39E-20	6.91	0.62	1	2336.4	1
Rh118	R.PPPDYEATLQLFK.S	2	1518.78387	0.53	1.36E-14	3.33	0.40	1	827.4	1
Rh118	R.QM*LAFAHSYTM*IAM*IVEHLGDGLLSSQIHTHYR.T	3	3818.84509	1.85	1.37E-11	2.74	0.17	2	699.2	1
Rh118	R.QM*LAFAHSYTMIAM*IVEHLGDGLLSSQIHTHYR.T	3	3802.85019	0.36	2.64E-13	2.68	0.05	2	502.9	1
Rh118	R.QMLAFAHSYTM*IAM*IVEHLGDGLLSSQIHTHYR.T	3	3802.85019	0.52	1.15E-13	4.44	0.16	2	1146.5	1
Rh118	R.QMLAFAHSYTMIAMIVEHLGDGLLSSQIHTHYR.T	1	3770.86039	0.92	1.49E-14	3.64	0.33	2	894.8	1
Rh118	R.QPLNTANIESR.H	18	1242.64369	-0.12	3.91E-15	2.46	0.41	2	350.5	1

Rh118	R.QTTIM*VTK.Y	4	937.50228	0.28	2.11E-10	1.99	0.24	2	53.8	1
Rh118	R.QTTIMVTK.Y	8	921.50738	0.14	1.56E-11	2.11	0.46	2	306.3	1
Rh118	R.RLTHFYR.V	3	992.54246	-1.42	3.44E-10	2.37	0.22	2	369.6	1
Rh118	R.SNILQSFK.T	18	936.51491	-0.75	3.13E-11	3.16	0.27	2	983.8	1
Rh118	R.SNILQSFKTK.M	2	1165.65755	1.09	2.08E-13	2.60	0.41	2	586.1	1
Rh118	R.SRSDMGVR.V	2	907.44143	0.70	1.33E-10	2.49	0.15	2	507.3	1
Rh118	R.TDTFEVDM*LLYSGK.S	21	1634.7618	-0.05	1.37E-17	4.49	0.70	2	2000	1
Rh118	R.TDTFEVDMLLYSGK.S	32	1618.7669	1.06	2.93E-18	4.88	0.66	2	1806.6	1
Rh118	R.TIMAILR.L	3	817.49642	-0.85	6.25E-10	2.21	0.23	2	448.2	1
Rh118	R.TPNEVKQDYTPSDFYK.I	2	1931.90215	-4.42	3.13E-16	5.56	0.67	2	1458.6	1
Rh118	R.TSCM*LGVDPYDTDAAIK.T	15	1872.83541	-1.13	5.30E-15	5.42	0.66	2	1683	1
Rh118	R.TSCMLGVDPYDTDAAIK.T	23	1856.84051	-0.27	8.19E-16	5.43	0.65	2	1666.4	1
Rh118	R.TSDRDYILK.L	5	1110.57896	-0.36	1.83E-11	1.85	0.09	2	200.9	4
Rh118	R.TSDRDYILKLLTK.M	4	1565.88973	0.16	2.90E-13	2.89	0.46	2	612.6	1
Rh118	R.VASGAGM*PTSR.Q	27	1049.5044	1.32	9.77E-16	3.58	0.46	2	933.6	1
Rh118	R.VASGAGMPTSR.Q	19	1033.5095	-0.52	4.88E-16	3.46	0.52	2	1219.3	1
Rh118	R.VFPM*HVHR.H	4	1038.53017	0.18	1.56E-11	2.15	0.24	2	367	1
Rh118	R.VFPMHVHR.H	10	1022.53527	0.13	3.91E-12	2.67	0.47	2	483.2	1
Rh118	R.VLKNTADAM*ER.G	5	1263.63615	-0.44	2.73E-13	3.20	0.34	2	390.1	1
Rh118	R.VLKNTADAMER.G	9	1247.64125	-0.47	9.77E-15	3.41	0.39	2	846.1	1
Rh118	R.VSSLPGLNNGQLVEEPLSAYANALHDHR.L	8	3001.51262	0.14	4.35E-20	3.78	0.48	2	894.7	1
Rh118	R.YNIHFEAIFGTFCNR.L	7	1888.87995	0.61	1.46E-18	5.17	0.61	2	2394.7	1
Rh118	R.YTAGCPNTLQSVLTLACM*LYK.I	21	2420.16591	0.17	9.09E-21	5.23	0.63	2	1596.5	1
Rh118	R.YTAGCPNTLQSVLTLACMLYK.I	20	2404.17101	-0.04	2.45E-24	5.17	0.62	2	1159.4	1
Rh123	K.AGVSVSSDEELPGPGWR.D	5	1742.8344	-0.39	6.73E-17	4.81	0.58	2	868.3	1
Rh123	K.FLTDYSESFSPFK.S	1	1567.7315	-0.91	1.51E-16	4.29	0.50	2	1761.7	1
Rh123	K.ILLLDLPR.L	2	952.61898	-0.94	1.25E-10	2.67	0.45	2	655.3	1
Rh123	K.IVVECGQVGSDSVER.Q	7	1633.78505	-1.71	1.22E-20	5.04	0.61	2	2275.7	1
Rh123	K.QLVAVLR.S	1	798.5196	0.49	4.90E-09	1.73	0.12	2	58.9	7
Rh123	K.QPFSEDLCPCPPDVEDR.L	7	2060.86889	-0.12	6.87E-18	4.43	0.70	2	1051.9	1
Rh123	K.STESDSTFLLHR.K	4	1392.67538	-0.90	9.77E-17	3.07	0.57	2	910.9	1
Rh123	K.VAACVAFILNCFYK.H	2	1675.83355	0.65	1.08E-12	2.92	0.45	2	486.2	1
Rh123	R.AFVSLFR.N	1	839.4774	-2.90	6.41E-10	1.68	0.04	2	701.5	1
Rh123	R.AIAMAQK.F	1	732.40727	0.26	5.13E-09	1.62	0.11	2	154.6	2
Rh123	R.DAALIMHNGM*VR.E	4	1343.65584	-0.78	4.96E-11	2.32	0.24	2	313.6	1
Rh123	R.DAALIMHNGMVR.E	4	1327.66094	-0.85	4.99E-13	3.09	0.48	2	906	1
Rh123	R.EHM*FYHPDVAEIVR.R	4	1758.8268	-0.73	3.08E-16	3.92	0.56	2	657.4	1
Rh123	R.EHMFYHPDVAEIVR.R	6	1742.8319	0.07	2.88E-17	4.06	0.65	2	853.1	1
Rh123	R.GDIEVFDK.K	1	922.45164	0.26	7.81E-11	1.52	0.15	2	225.1	2
Rh123	R.GDIEVFDKK.V	4	1050.5466	1.27	7.81E-13	2.13	0.35	2	503.6	1
Rh123	R.KQLVAVLR.S	1	926.61456	-1.51	8.65E-10	1.60	0.03	2	254.5	6
Rh123	R.LFPLLNLSR.G	5	1072.65134	-0.04	6.25E-12	2.59	0.29	2	510.6	1
Rh123	R.LLDHCVTLAASK.K	2	1327.70388	-1.23	2.44E-16	3.55	0.55	2	1528.4	1
Rh123	R.LPEVNNR.V	5	841.45264	0.47	3.13E-10	2.08	0.22	2	358.1	1
Rh123	R.LPEVNNRVDECDLAVIALR.R	2	2196.14416	0.24	3.75E-15	3.36	0.44	2	514.9	1
Rh123	R.M*EVQVPLDAAVTR.K	5	1444.74643	-0.07	3.13E-17	4.02	0.48	2	1667.9	1
Rh123	R.MEVQVPLDAAVTR.K	8	1428.75153	0.80	8.35E-16	4.25	0.50	2	1019	1
Rh123	R.MIPPPPDAEEGCVFASELAFYTSGR.C	8	2741.2586	-0.20	4.64E-18	4.14	0.63	2	1013.5	1
Rh123	R.NVVWPGTSVR.W	4	1114.60037	0.21	1.53E-10	2.31	0.32	2	356.2	1
Rh123	R.NYLFWTK.Q	2	971.49853	0.40	9.94E-10	1.97	0.31	2	280.9	1
Rh123	R.QFYDSPGIYLIR.V	5	1471.75799	3.07	1.24E-15	3.46	0.38	2	705.5	1
Rh123	R.VDECDLAVIALR.R	9	1373.70936	-0.19	2.77E-14	4.26	0.53	2	2150.5	1
Rh123	R.VSDGTVVPR.N	8	929.50507	-0.28	7.81E-13	3.14	0.40	2	811	1
Rh123	R.WSKEVGIR.V	1	974.54179	-1.65	1.03E-10	1.93	0.09	2	565.2	1
Rh128	K.TNPSETWYADVVR.C	1	1537.72815	-1.00	2.12E-16	3.04	0.60	2	713	1
Rh128	R.DAGLTVAPTFR.V	5	1147.6106	-0.35	2.63E-12	2.96	0.41	2	1000.3	1
Rh128	R.EEMSVCQSTEPADRPDPSLLMTDAMLELAK.S	2	3364.54158	4.94	6.69E-18	3.48	0.40	2	182.9	1
Rh128	R.FRDWEFLVR.D	2	1267.65822	0.14	3.38E-10	2.58	0.26	2	431.6	1
Rh128	R.GHEIVQPR.I	2	935.50574	0.77	1.56E-11	2.36	0.11	2	675.8	1
Rh128	R.IEVNPDVIYASGPHEDDR.T	6	2025.95122	-1.14	8.61E-19	4.92	0.63	2	1800.7	1
Rh128	R.LGSVWDVR.Q	1	931.49959	-4.18	1.94E-11	2.57	0.29	2	529.4	1
Rh128	R.LSGAQM*LYLR.L	1	1167.61904	0.37	4.93E-10	2.48	0.41	2	565.6	1
Rh128	R.LSGAQMLYLR.L	5	1151.62414	-0.46	1.56E-13	3.40	0.45	2	1366	1
Rh128	R.LSVEYQLLR.E	5	1120.63609	0.17	3.13E-12	2.92	0.28	2	1080	1

Rh128	R.LTLDSYFDR.L	6	1129.55242	-0.08	7.81E-13	2.45	0.46	2	692	1
Rh128	R.MLLETGWTR.A	2	1106.56629	0.71	8.20E-13	2.55	0.24	2	976.9	1
Rh128	R.VFLRPFELLTYLR.D	2	1666.96792	0.65	7.25E-14	3.19	0.39	2	635.8	1
Rh128	R.VPSCGL.-	1	632.30726	-1.27	5.23E-09	1.78	0.50	2	308.4	1
Rh128	R.VVCHHALFTTLGIR.C	4	1623.87883	0.04	1.50E-15	3.48	0.50	2	837.9	1
Rh129	K.DHGGHVDPTGCYIGPVPDK.G	9	2020.91819	-0.93	3.43E-19	5.17	0.63	2	2218.8	1
Rh129	K.GCSGSYEPGVEK.A	23	1269.54163	0.65	2.30E-15	4.19	0.59	2	1595.3	1
Rh129	K.GNCLCYTLCNSPTM*NPITNENPVAFFCDVER.A	20	3709.58495	-0.82	3.42E-27	6.87	0.74	2	2289.7	1
Rh129	K.GNCLCYTLCNSPTMNPITNENPVAFFCDVER.A	20	3693.59005	-0.72	1.09E-28	7.53	0.78	2	3072.2	1
Rh129	K.HREFQAVACR.S	2	1273.62189	-0.75	1.66E-13	3.33	0.38	2	1359.2	1
Rh129	K.LVFLGQLGPAR.V	26	1170.69935	-0.22	7.81E-15	3.66	0.48	2	1908.1	1
Rh129	K.NETGDWLPLNESAWQLVR.V	19	2128.04579	-0.16	1.71E-19	5.69	0.65	2	1516.7	1
Rh129	K.RGGENLICFSCNGNYM*GTFCCPR.I	2	2786.13617	-0.40	4.84E-14	4.20	0.63	2	598.7	1
Rh129	K.RGGENLICFSCNGNYMGTFCCPR.I	6	2770.14127	-1.19	3.16E-19	5.75	0.74	2	2691	1
Rh129	K.SKVTLADSLDYHIGAK.N	6	1717.91193	15.09	5.16E-16	4.92	0.53	2	1777.1	1
Rh129	K.VTLADSLDYHIGAK.N	14	1502.78493	0.45	4.71E-18	4.67	0.50	2	2287.6	1
Rh129	R.ASYLCAVGSK.T	18	1055.51904	-1.24	1.95E-14	3.26	0.56	2	1295.5	1
Rh129	R.ASYLCAVGSKTK.S	2	1284.66168	-0.42	9.84E-15	2.34	0.45	2	366.1	1
Rh129	R.CSITPSLM*YEVCTLVPPEEAEK.I	2	2569.1871	-0.70	1.22E-09	3.23	0.46	2	332.6	1
Rh129	R.CSITPSLMYEVCTLVPPEEAEK.I	20	2553.1922	-0.25	1.00E-12	5.94	0.63	2	766.7	1
Rh129	R.CSITPSLMYEVCTLVPPEEAEKIR.V	3	2822.37737	-3.87	9.38E-17	4.49	0.57	2	1060.8	1
Rh129	R.EFQAVACR.S	27	980.46186	-0.57	7.81E-12	2.64	0.37	2	1032.4	1
Rh129	R.GGENLICFSCNGNYM*GTFCCPR.I	6	2630.03506	-0.12	1.23E-20	4.60	0.78	2	1363.2	1
Rh129	R.GGENLICFSCNGNYMGTFCCPR.I	12	2614.04016	-0.81	2.04E-21	5.97	0.80	2	1446.9	1
Rh129	R.LM*ALATACGQVGCTYCK.D	25	1919.84831	-0.75	2.19E-20	6.32	0.70	2	3517.3	1
Rh129	R.LMALATACGQVGCTYCK.D	70	1903.85341	-0.85	4.93E-19	6.82	0.72	2	3020	1
Rh129	R.M*IVCACPVLK.N	11	1206.60439	-0.25	3.21E-13	2.95	0.51	2	1724.6	1
Rh129	R.MIVCACPVLK.N	18	1190.60949	-0.76	3.13E-13	3.24	0.46	2	1348.7	1
Rh129	R.MIVCACPVLKNLVH.-	4	1653.86381	0.60	2.72E-16	2.98	0.53	2	509.3	1
Rh129	R.QDLVGLHTTYK.L	7	1274.67393	-0.31	3.91E-15	3.50	0.46	2	459.2	1
Rh129	R.QFLQKECCWR.Q	1	1454.66682	0.72	3.45E-11	2.06	0.35	2	150.6	3
Rh129	R.SAIFSPPGGDASCLACQLLLFK.R	25	2352.17272	0.66	8.39E-25	6.01	0.60	2	1667.8	1
Rh129	R.VDFVPAFSSM*TSVVPR.C	18	1754.87817	-0.66	3.97E-21	4.47	0.58	2	1558.6	1
Rh129	R.VDFVPAFSSMTSVVPR.C	45	1738.88327	-0.22	9.83E-20	4.67	0.63	2	1857.3	1
Rh129	R.VEEPVSR.M	9	815.42576	-0.26	7.81E-11	2.10	0.20	2	673.6	1
Rh129	R.VKGCSGSYEPGVEK.A	5	1496.70501	-1.43	5.60E-16	3.98	0.54	2	1754.7	1
Rh129	R.VRQDLVGLHTTYK.L	11	1529.84345	-2.20	5.79E-15	3.88	0.46	2	1077.3	1
Rh13.1	R.GLACTICSLR.R	4	1150.5708	1.65	9.45E-13	2.46	0.32	2	592.1	1
Rh13.1	R.IITTTTQSTSTTTTR.T	6	1612.83882	-1.62	3.14E-20	4.63	0.71	2	1721.3	1
Rh13.1	R.SGTLITDK.A	3	834.45672	-0.69	9.14E-10	2.36	0.26	2	366.7	1
Rh13.1	R.YNYPVNSYTICTVSGNNVASTK.H	3	2452.14495	-0.16	1.52E-21	4.28	0.63	2	860.9	1
Rh131	K.AADVDNALDSLIELK.D	3	1586.82719	1.62	1.78E-15	4.79	0.48	2	2000	1
Rh131	K.DTVEDVR.D	1	833.39994	0.42	2.07E-09	1.53	0.06	2	152.8	3
Rh131	R.VTSHHSLQLMR.V	2	1308.68412	-0.02	4.79E-12	1.67	0.25	2	182	1
Rh131	R.YSQAVVGQVTDNVVR.D	5	1634.84966	-1.00	8.41E-20	5.69	0.66	2	2259.4	1
Rh132	K.AGPEAEEMPGVYHGLLTATGCCLMHNLTVYQR.F	2	3575.6539	-0.24	4.59E-20	4.39	0.61	2	1025.8	1
Rh132	K.GLVCDASLFSR.M	4	1224.60417	0.51	8.79E-15	2.24	0.43	2	438.8	1
Rh132	R.AFHHLANAIK.F	2	1121.62144	-0.26	5.21E-13	2.11	0.32	2	453.1	1
Rh132	R.CVVVFQETGTAR.R	5	1366.6784	-1.07	6.63E-16	3.68	0.50	2	1593.3	1
Rh132	R.ESQAAAAAAAAAPAASSTR.N	7	1672.8249	-0.40	1.83E-24	4.66	0.70	2	1547.9	1
Rh132	R.FHSDLFHFENWDLTSVPSYR.R	1	2497.15713	1.71	8.76E-12	2.97	0.45	2	577.9	1
Rh132	R.FPVSNSPR.R	9	903.46829	0.17	1.29E-11	2.26	0.32	2	430.3	1
Rh132	R.GIEGVHNGPESLLFK.H	1	1596.83803	-0.14	1.43E-13	3.73	0.47	2	966.4	1
Rh132	R.IGCGSFGEIWPIDR.R	5	1606.76827	0.06	1.46E-18	3.66	0.49	2	879	1
Rh132	R.LAANISTEEDLDGDCR.Q	4	1778.78617	-0.76	3.69E-21	5.26	0.69	2	1596.5	1
Rh132	R.LLAMADGVYLYNSFR.L	7	1732.8727	-0.01	3.66E-20	5.62	0.61	2	1791.1	1
Rh132	R.M*TQPELCEMAVTYLLVYVPK.D	2	2401.18521	1.09	6.59E-16	4.39	0.40	2	1081.8	1
Rh132	R.MTQPELCEM*AVTYLLVYVPK.D	1	2401.18521	1.02	2.84E-11	2.31	0.23	2	61.6	5
Rh132	R.MTQPELCEMAVTYLLVYVPK.D	2	2385.19031	-0.01	1.28E-19	5.03	0.63	2	1505.5	1
Rh132	R.QAAMAAAVR.E	3	888.472	-0.07	1.74E-10	2.54	0.30	2	102.2	1
Rh132	R.QSSIDGPVLYAADPQLQCCEDSHWSFEYDEDAPTQNTGIVQPR.A	1	4924.1738	0.66	3.67E-09	3.61	0.46	2	348.4	1
Rh132	R.SASAESGRPWQPAPLR.R	2	1709.87179	0.04	3.55E-16	3.17	0.46	2	452.2	1
Rh132	R.SASAESGRPWQPAPLRR.S	4	1865.9729	-1.66	6.59E-17	3.82	0.43	2	519.3	1
Rh132	R.VCYSVDM*TDASR.R	4	1419.58791	0.13	5.63E-13	3.19	0.59	2	1177.6	1

Rh132	R.VCYSVDMTDASR.R	8	1403.59301	-0.78	2.44E-17	3.96	0.65	2	1714.4	1
Rh132	R.YYHECLGALPFDYVR.N	3	1902.88437	-0.31	4.29E-18	5.02	0.64	2	2132.9	1
Rh134	R.DLPNAHLVSAIFR.E	1	1452.79577	-4.95	1.46E-17	3.59	0.54	2	606.6	1
Rh134	R.HDADIPSGR.E	2	967.45918	0.01	4.26E-11	2.43	0.46	2	526.6	1
Rh134	R.HVVSTVVDR.W	2	1011.55817	-1.78	3.95E-10	2.07	0.23	2	457.4	1
Rh134	R.QLELYQILER.E	1	1304.72088	-2.00	6.64E-13	2.78	0.31	2	304	2
Rh134	R.SEVIVFDAK.N	1	1007.54079	-3.06	1.29E-09	1.77	0.32	2	598.9	1
Rh134	R.SFHDEDTPDLLGQLTLYEVSR.F	1	2435.17251	0.82	7.01E-11	2.23	0.27	2	199.5	1
Rh134	R.TSLHISPCASVYEIK.C	1	1704.86257	-7.25	1.55E-09	2.93	0.41	2	716.4	1
Rh134	R.TYGLAVAEEFVSAVAALAQDELLR.D	2	2536.32935	-1.30	4.59E-13	4.10	0.49	2	769.8	1
Rh134	R.TYGLAVAEEFVSAVAALAQDELLRDILTNR.Y	1	3248.71613	-2.93	1.05E-17	3.13	0.27	2	421.3	1
Rh134	R.VFSCDHIPLLIIVTPVVLDPK.F	1	2375.34074	-3.26	2.85E-09	4.02	0.38	2	806.8	1
Rh137	K.AQLTLALM*EDGDGYHSPPR.V	6	2086.98622	-0.67	3.22E-18	5.59	0.72	2	1802.2	1
Rh137	K.AQLTLALMEDGDGYHSPPR.V	10	2070.99132	0.46	2.58E-18	5.77	0.56	2	1896.3	1
Rh137	K.IAMRPPTPKNQR.R	1	1408.78416	0.18	3.08E-12	1.72	0.01	2	145.6	12
Rh137	K.KTQSAQHTTQK.A	1	1257.65459	3.15	3.84E-09	2.43	0.36	2	391.2	1
Rh137	K.TQSAQHTTQK.A	12	1129.55963	2.07	4.31E-12	2.72	0.51	2	363.2	1
Rh137	R.AEKIAMRPPTPK.N	1	1338.75622	-0.98	1.49E-10	2.49	0.21	2	351.6	4
Rh137	R.DTMGRPVCLR.S	4	1204.59256	-0.86	9.00E-11	2.26	0.09	2	341.9	1
Rh137	R.ICCFFGASSGK.P	13	1233.53916	-0.44	4.30E-15	3.35	0.51	1	1104.7	1
Rh137	R.ICCFFGASSGKPLR.D	14	1599.7771	0.94	8.01E-18	4.06	0.58	2	1958.7	1
Rh137	R.LSLASQDSM*APAPK.P	35	1431.71479	-0.44	3.14E-16	3.91	0.55	1	1429.2	1
Rh137	R.LSLASQDSM*APAPKPK.K	4	1656.86252	-0.21	2.69E-17	3.57	0.49	2	489.7	1
Rh137	R.LSLASQDSMAPAPK.P	16	1415.71989	-0.22	1.25E-17	4.51	0.55	1	2340.7	1
Rh137	R.LSLASQDSMAPAPKPK.K	6	1640.86762	-1.86	2.70E-20	4.27	0.59	2	1235.7	1
Rh137	R.SFADIDNTSSEEDEADLISINSDR.L	13	2643.15402	-0.78	3.30E-19	5.64	0.70	2	1282.2	1
Rh137	R.TYNRPSPPSK.A	8	1146.5902	0.81	7.50E-13	2.48	0.44	2	805.7	1
Rh137	R.TYNRPSPPSKAK.T	1	1345.72227	-0.45	1.61E-09	2.24	0.19	2	310.4	1
Rh137	R.TYNRPSPPSKAKTPR.A	2	1699.92383	-2.49	4.33E-12	2.81	0.27	2	282.9	1
Rh138	K.EASM*ATSDAEEM*A.-	5	1374.50352	0.24	8.86E-15	3.68	0.81	2	716.7	1
Rh138	K.EASM*ATSDAEEMA.-	6	1358.50862	-0.06	3.11E-11	3.50	0.73	2	618.4	1
Rh138	K.EASMATSDAEEM*A.-	7	1358.50862	1.88	1.10E-16	3.49	0.84	2	915.2	1
Rh138	K.EASMATSDAEEMA.-	14	1342.51372	-0.29	3.42E-12	2.91	0.80	2	522.5	1
Rh138	K.EEGLNLNQR.T	31	1072.53816	-1.44	1.56E-12	2.72	0.34	2	735.9	1
Rh138	K.HLPATDELASLK.E	16	1294.70014	-0.40	9.28E-16	3.60	0.54	2	1257.7	1
Rh138	K.HLPATDELASLKEASMATSDAEEMA.-	1	2618.19602	-5.00	1.85E-12	3.05	0.42	2	602.1	1
Rh138	K.LKGTIQYR.T	1	978.57309	0.14	6.25E-11	2.06	0.13	2	528.9	1
Rh138	K.QNLFNLQR.I	11	1032.5585	-0.32	6.25E-11	2.28	0.22	2	469	1
Rh138	K.VDWM*NSR.I	2	923.40396	0.93	1.64E-10	1.85	0.36	2	385.4	1
Rh138	K.VDWMNSR.I	1	907.40906	-0.38	8.59E-11	1.88	0.28	2	338.7	1
Rh138	K.YKHLPATDELASLK.E	1	1585.85843	-0.44	3.92E-10	2.48	0.24	2	285.4	1
Rh138	R.KEEGLNLNQR.T	22	1200.63313	-0.07	1.56E-13	3.64	0.49	2	1458.7	1
Rh139	R.QASETLFSHLSTR.A	1	1476.74413	-1.64	3.09E-13	2.33	0.37	2	761.2	1
Rh140	K.CLLDLLGYLK.V	2	1207.67554	0.79	4.69E-13	2.98	0.42	2	1222.2	1
Rh140	K.LICSTLYLFFDDK.T	3	1634.81349	0.83	4.88E-17	4.12	0.57	2	1320.7	1
Rh140	K.LVEVALIEVLNSNFPSLPLCDPNML.-	2	2797.45149	0.66	2.05E-11	3.94	0.47	2	451.8	1
Rh140	K.SSFVGYSTIFLLENEDVM*K.N	1	2195.05765	-0.33	1.09E-13	3.70	0.39	2	430.9	1
Rh140	K.SSFVGYSTIFLLENEDVMK.N	2	2179.06275	0.90	3.58E-15	3.81	0.59	2	424.4	1
Rh140	K.TTEIVEQVPK.V	5	1143.62558	-0.02	2.39E-13	3.12	0.38	2	819.6	1
Rh140	K.VFILFYESR.Q	3	1173.63027	0.99	4.43E-12	3.25	0.48	2	1472.5	1
Rh140	R.DQFSTLLR.I	5	979.52072	-0.39	6.25E-11	2.60	0.30	2	609.1	1
Rh140	R.GVLEVHTDETSR.N	6	1342.65973	-0.51	1.03E-15	3.88	0.62	2	1126.4	1
Rh140	R.ITRQDESYK.V	1	1139.56913	0.54	8.63E-09	1.84	0.15	2	181.3	11
Rh140	R.KCLLDLLGYLK.V	2	1335.77051	1.11	3.16E-14	3.42	0.39	2	560.5	1
Rh140	R.LWVHTDHGM*LCSISEYR.G	2	2119.96883	-0.53	8.04E-21	3.43	0.63	2	1102.4	1
Rh140	R.LWVHTDHGMLCSISEYR.G	7	2103.97393	-0.09	5.04E-21	5.24	0.68	2	2057.8	1
Rh140	R.NVIVTTPQIVDITVSNDR.L	14	1984.07095	-0.92	2.15E-23	5.40	0.69	2	1626.1	1
Rh140	R.QSFLHTILR.F	2	1114.63675	-0.29	3.13E-12	2.44	0.42	2	117.8	5
Rh140	R.TSNLM*HFTLCTILSCVENLTLTR.K	7	2740.34673	0.17	3.13E-15	4.98	0.63	2	1014.3	1
Rh140	R.TSNLMHFTLCTILSCVENLTLTR.K	8	2724.35183	0.19	1.95E-14	5.42	0.61	2	1618.8	1
Rh141	K.AQEVAFNPLAIEDNR.V	1	1686.84457	-0.41	7.27E-18	3.70	0.40	2	1550.1	1
Rh141	K.ICQLLNTYPVK.A	2	1348.72937	-0.17	2.13E-13	2.44	0.29	2	277	2
Rh141	K.IHSTLFPYQALDDEDLENYLLVWSASVR.Q	1	3294.63173	-0.22	9.76E-11	2.76	0.42	2	471.7	1
Rh141	K.LENLLTELWENEYFR.T	2	1968.97017	0.56	6.38E-17	4.49	0.51	2	1445.6	1

Rh141	K.PAEIAQR.A	2	784.43118	0.10	3.13E-10	1.52	0.16	1	150.1	1
Rh141	K.STETHPLAQDLLNSNLHLLQQVVTHLCGVR.I	5	3393.76962	0.47	1.85E-22	6.02	0.66	2	1267.8	1
Rh141	R.DDDTVIDIFPTAQTLSYIR.L	3	2183.08666	-0.86	2.93E-12	4.25	0.62	2	891.1	1
Rh141	R.IGIEDSVESFLQDLTPSLVDQSR.L	7	2548.2777	0.96	9.15E-16	5.32	0.65	2	987.5	1
Rh141	R.LLHGFIGTCR.G	2	1173.61976	0.75	1.56E-13	2.69	0.50	2	622.7	1
Rh141	R.LVTAQGAEEAIVYSNYTVER.V	1	2213.10845	-0.31	1.20E-14	4.63	0.61	2	717.4	1
Rh141	R.NQLFFTTPISHLHEEILR.Y	2	2195.16076	0.43	2.71E-16	3.60	0.58	2	373.4	1
Rh141	R.QSVQTGVLGTLR.D	2	1258.71138	0.32	2.13E-12	2.27	0.26	2	311.1	1
Rh141	R.QYVELICNR.E	3	1194.5936	-0.03	2.62E-12	2.39	0.42	2	489.2	1
Rh141	R.SAQTFER.E	1	838.40536	0.05	4.69E-10	1.80	0.25	2	128.9	1
Rh141	R.SILAADER.L	1	874.46287	1.45	1.56E-11	2.05	0.26	2	297.8	1
Rh141	R.TPGGGPSASAPPVTNATQDR.D	6	1880.90969	-0.08	2.17E-17	5.24	0.63	2	1003.4	1
Rh141	R.VVANSFFSQFIPNTEK.L	1	1827.92758	-1.23	4.40E-09	1.81	0.28	2	155.9	4
Rh141	R.YIVTEPLAYER.N	5	1353.70489	0.25	1.95E-15	2.87	0.44	2	843.8	1
Rh143	K.DTVYYTSLFNDR.V	1	1493.6907	-7.87	2.25E-15	2.96	0.39	2	775.3	1
Rh143	K.TPAWMYFLEVEHK.L	1	1650.79848	-4.24	6.91E-17	4.61	0.64	2	1288.1	1
Rh143	R.VLHEMLSPMGCR.V	1	1429.67491	-6.78	1.20E-12	3.42	0.37	2	863.5	1
Rh143	R.VTNELMEHYLDGVLPR.A	4	1885.94766	-1.56	6.07E-14	3.04	0.52	2	1113.3	1
Rh144_Rh145	K.SGDSSLLTVISEPDTLQVTFIR.H	1	2378.24495	-3.93	1.82E-15	3.58	0.43	2	1050.7	1
Rh144_Rh145	R.TYWTFR.G	1	873.42536	-3.37	3.79E-09	1.78	0.43	2	338.4	1
Rh147	K.GQFAALVVVR.H	2	1059.63094	1.80	3.77E-10	2.53	0.29	2	413.1	1
Rh147	K.HAASTIPLDDTFM*DFVALLHNNPAQLR.T	3	3023.50435	0.73	5.05E-18	3.47	0.52	2	1229.5	1
Rh147	K.HAASTIPLDDTFMDFVALLHNNPAQLR.T	9	3007.50945	0.28	9.71E-22	4.65	0.61	2	1406.9	1
Rh147	K.IM*QGYSECGDTGSIYTCVDNVCR.A	3	2701.09979	-0.07	1.46E-19	5.45	0.76	2	1057.4	1
Rh147	K.IMQGYSECGDTGSIYTCVDNVCR.A	4	2685.10489	-0.25	2.56E-21	5.28	0.70	2	1812.9	1
Rh147	K.YYLDIPDEWK.Q	2	1341.63614	0.56	7.30E-12	1.71	0.10	2	234.8	1
Rh147	R.ADRNGLQLFYALYNLVR.E	1	2026.08687	-7.95	3.18E-09	2.80	0.24	2	422.9	1
Rh147	R.CFTGETFSPEDDSWAK.P	3	1876.76946	0.24	1.28E-12	3.93	0.55	1	718.2	1
Rh147	R.HDLDTALIDR.L	13	1168.59568	-0.63	7.81E-14	3.17	0.51	2	1111.2	1
Rh147	R.HDLDTALIDRLDK.Y	1	1524.80165	-0.16	5.32E-14	2.97	0.39	2	807.9	1
Rh147	R.IPVATR.A	1	656.40899	-7.05	6.25E-09	1.52	0.12	2	151.7	1
Rh147	R.NGLQLFYALYNLVR.E	2	1683.9217	1.22	1.10E-13	3.99	0.44	2	1239.8	1
Rh147	R.YLTYSNSIFTENVLGFDFGHK.G	3	2452.18195	0.49	7.64E-13	2.62	0.31	2	214.1	1
Rh148	K.LLGLPDVNSDFLNQLGR.Y	10	1871.00214	-0.52	2.97E-21	5.44	0.72	2	1445.5	1
Rh148	R.DWYTTE.-	4	814.32538	-0.36	1.72E-09	1.74	0.67	2	350.9	1
Rh148	R.YHPIILQGQIEYR.D	17	1629.87475	-0.57	1.00E-16	5.03	0.63	2	1682.6	1
Rh151	K.SWEFLVMPIK.A	1	1249.66494	1.27	1.78E-12	2.03	0.16	2	209.5	11
Rh151	R.FIATGQTLNK.S	3	1092.60479	-1.99	9.25E-13	2.17	0.21	2	566.1	1
Rh151	R.QAPNVTTQYSCR.F	2	1424.65872	-0.32	1.02E-11	1.95	0.43	2	203.6	1
Rh151	R.YSVSTHVFIK.R	3	1180.63609	0.30	5.86E-14	2.59	0.47	2	616.6	1
Rh156.IE2	K.IPNLSISTPFLVEHTMPNVYPAEVVR.K	2	2923.53863	-1.94	6.20E-19	4.13	0.51	2	1205.1	1
Rh156.IE2	K.TAEACASGIK.Q	1	1007.48266	-9.19	2.78E-13	2.85	0.52	2	986.4	1
Rh156.IE2	R.IFTNENSNSFMLPIYDQAAK.M	1	2303.10126	-5.54	1.35E-10	3.89	0.41	2	415.8	1
Rh156.IE2	R.KTAEACASGIK.Q	1	1135.57762	-5.33	6.20E-10	2.35	0.37	2	396.9	1
Rh156.IE2	R.NMIVHAATPVDFLGSLQVLVPLVQR.F	2	2717.5171	0.75	6.77E-11	3.03	0.37	2	327.8	1
Rh156.IE2	R.VRADEVSR.M	1	931.49557	-5.75	2.32E-10	2.53	0.25	2	631.2	2
Rh160	K.GNLTSFVNPQYGHR.S	7	1589.78191	-1.68	8.22E-17	4.27	0.46	2	951.4	1
Rh160	K.MLQVSTR.E	5	834.4502	-0.68	8.95E-10	1.97	0.16	2	675.6	2
Rh160	K.TKYSEQEGAK.L	1	1140.55314	2.02	3.79E-10	1.78	0.10	2	226	2
Rh160	K.YSEQEGAK.L	4	911.4105	0.56	3.37E-10	2.26	0.20	2	317.2	1
Rh160	R.EVTLKEPEYDN.-	7	1336.6267	-1.00	1.62E-14	3.87	0.66	2	1354	1
Rh164	K.IWSAEYNEWQR.H	8	1481.6808	-1.23	1.95E-15	3.91	0.60	2	1325.3	1
Rh164	K.KIWSAEYNEWQR.H	1	1609.77577	-6.20	1.45E-11	4.18	0.21	2	1309.2	1
Rh164	R.CACGEMCR.N	6	1043.35266	0.14	1.65E-10	3.09	0.68	2	769.8	1
Rh164	R.CWPVLQQYVLDGDCFR.S	22	2055.9416	-0.44	1.98E-20	5.98	0.67	2	3481.4	1
Rh164	R.FCQSEYGGYELK.I	10	1480.64134	-0.70	9.77E-17	4.36	0.57	2	2205	1
Rh164	R.FTIVTQIETLHR.L	14	1457.81109	-0.16	3.91E-16	3.93	0.58	2	1461.5	1
Rh164	R.HQCGTFYGFDR.L	12	1387.58483	-1.00	9.77E-16	3.26	0.68	2	984.4	1
Rh164	R.LGDPDCAPK.L	5	972.44554	-0.02	3.91E-13	3.04	0.60	2	888.9	1
Rh164	R.REPPLPTEK.T	9	1066.58914	0.37	2.05E-10	3.07	0.37	2	627.3	1
Rh164	R.SESYNDLVDENVYDEVAVPPLYSK.I	7	2745.27776	0.11	1.04E-17	5.27	0.65	2	435.6	1
Rh165	R.VEITSNSSGDR.A	1	1164.54912	0.68	2.15E-12	2.34	0.33	2	268.9	1
Rh166	R.EGDNEGLVPEVQNVQPVDTVVTIEGNQPVTR.V	1	3332.66047	-0.41	3.27E-12	3.28	0.44	2	403.6	1
Rh166	R.TLTSLNSGELFTIGGWITDR.F	3	2181.11862	-0.88	1.33E-15	5.54	0.60	2	1496.2	1

Rh167	R.QAEGVLMEIDYLK.E	2	1508.76651	1.68	5.12E-10	1.88	0.17	2	178.9	1
Rh17	K.LCFLSSYVPQR.R	7	1369.69332	0.44	2.89E-14	3.14	0.52	2	830	1
Rh170	K.QSQCTQNTATINSMVK.T	1	1810.84224	-4.97	2.56E-21	3.84	0.60	2	820.6	1
Rh170	K.VLVSNCPAALAEIMDDEGFSEIMNSK.F	1	2840.31513	-4.05	1.19E-09	3.63	0.51	2	515.7	1
Rh170	K.VQSVLFSVQAINPYQSSAEAK.V	1	2266.17139	-5.24	1.46E-16	4.98	0.68	2	781.1	1
Rh171	R.CCSQISAR.N	1	981.42413	2.39	6.73E-11	2.41	0.44	2	654.8	1
Rh173	R.HSDDTEQLVVR.Y	8	1298.63352	-1.27	1.95E-15	4.18	0.64	2	1042.2	1
Rh173	R.LTTGYEQMYISS.-	2	1392.63516	0.16	1.70E-09	2.15	0.52	2	235.1	1
Rh176	K.TPAAGANNCCQQK.G	2	1419.61043	2.95	5.41E-11	1.93	0.52	2	256.3	1
Rh181	R.SLTLDSDSSR.S	2	1080.51676	-0.90	2.29E-11	2.00	0.14	2	229.8	1
Rh187	R.TPAPADLGR.I	1	897.47886	-0.33	9.27E-10	1.65	0.21	2	267.4	1
Rh190	K.DTLPIWMMIVPTVLR.F	2	1784.98015	0.43	5.60E-11	2.75	0.31	2	345.6	1
Rh190	R.IQTPYLKDTLPIWMMIVPTVLR.F	1	2628.46558	-4.44	1.11E-15	4.61	0.47	2	853.9	1
Rh199	R.SGSATFMCMLIETGTCVMTGFCCPR.Q	1	2874.18814	-5.86	5.88E-11	4.52	0.62	2	835.1	1
Rh200	R.NTASSPFDLDFSLGR.W	2	1626.77583	0.54	3.03E-10	2.64	0.36	2	470.9	1
Rh203	K.ELAHHGLLTCESVYR.H	7	1784.87486	-0.47	2.42E-15	3.64	0.56	2	702.1	1
Rh203	K.EWQNEEPVTNER.E	8	1530.68193	-0.89	7.78E-17	4.06	0.56	2	1015.7	1
Rh203	K.IPTSCCWGR.S	4	1136.49763	-0.85	1.76E-10	2.96	0.58	2	472.6	1
Rh203	K.LEALGILGVQLR.D	7	1281.7889	0.03	1.56E-15	4.46	0.48	2	1951.5	1
Rh203	K.LLCLDPTDAR.G	10	1173.59327	-0.58	8.25E-14	2.98	0.39	2	1385.4	1
Rh203	K.NSGTIIPLR.N	5	970.56801	0.07	6.25E-12	2.50	0.27	2	619.7	1
Rh203	K.NSGTIIPLRNPCSWFLVLR.S	2	2243.21179	-4.00	1.36E-12	3.67	0.40	2	588.8	1
Rh203	K.QDLSWCSMVNTVTAEQLCGAYDWLTR.S	1	3104.39112	0.40	2.45E-15	3.24	0.58	2	507.8	1
Rh203	K.TAEEFHGVNVVVK.T	5	1428.74816	4.27	5.37E-16	3.28	0.28	2	672.3	1
Rh203	K.TAEEFHGVNVVVKTPGR.S	1	1839.97117	0.42	1.89E-13	3.21	0.29	2	656.9	1
Rh203	K.TPGRSEADPLLLLGTVEK.L	1	1896.04367	-0.74	1.37E-11	3.56	0.40	2	596.3	1
Rh203	K.TSDGTVMFR.V	6	1013.47206	-0.60	1.95E-13	2.96	0.58	2	541.3	1
Rh203	R.ADNFSNLIK.Y	3	1021.53129	-1.50	1.82E-11	2.99	0.33	2	832.9	1
Rh203	R.AHDKIPTSCCWGR.S	4	1587.71556	-0.33	1.29E-12	3.12	0.48	2	648.5	1
Rh203	R.CDYGVFSLR.N	3	1116.51429	-0.16	5.96E-13	3.39	0.24	2	1242.1	1
Rh203	R.CGFLKLR.G	1	893.5026	0.31	7.46E-10	1.95	0.27	2	375.1	1
Rh203	R.CLTM*LQGVYLR.Q	4	1369.69667	0.49	2.90E-14	3.53	0.52	2	1571.5	1
Rh203	R.CLTMLQGVYLR.Q	13	1353.70177	0.17	8.93E-15	3.97	0.52	2	1909.5	1
Rh203	R.DERPEDVIR.C	12	1128.56438	0.01	6.25E-13	2.70	0.37	2	527.7	1
Rh203	R.FQVEKLLCLDPTDAR.G	2	1804.92623	0.21	2.67E-12	3.54	0.40	2	643.8	1
Rh203	R.GLAKTAEEFHGVNVVVK.T	2	1797.98576	0.61	1.42E-16	4.31	0.43	2	915.1	1
Rh203	R.HPQTPFSTTEPR.F	12	1397.6808	-1.56	4.50E-13	3.08	0.52	2	449.4	1
Rh203	R.IDESDVFR.L	11	980.46835	-1.21	7.81E-12	2.84	0.50	2	827.7	1
Rh203	R.KQDLSWCSM*VNTVTAEQLCGAYDWLTR.S	3	3248.48099	-0.18	9.45E-12	4.85	0.57	2	812.8	1
Rh203	R.KQDLSWCSMVNTVTAEQLCGAYDWLTR.S	10	3232.48609	-0.60	2.68E-21	6.51	0.72	2	2323.8	1
Rh203	R.LADNIQM*LFR.C	6	1236.6405	0.53	6.25E-13	3.60	0.40	2	1368.1	1
Rh203	R.LADNIQMLFR.C	13	1220.6456	-0.37	1.74E-13	3.88	0.43	2	1472	1
Rh203	R.LESECYCYHGAR.K	6	1544.62575	-0.55	1.24E-15	3.76	0.63	2	1027.1	1
Rh203	R.NLVFQR.C	2	776.44135	-0.84	6.41E-09	2.02	0.20	2	134.3	1
Rh203	R.NPCSWFLVLR.S	17	1291.66162	0.28	1.73E-13	3.24	0.52	2	739.3	1
Rh203	R.PEDVIR.C	2	728.39373	0.34	3.13E-09	1.85	0.13	1	259.3	1
Rh203	R.QYDQPCLR.E	8	1079.49389	-0.96	3.13E-11	2.01	0.30	2	206.1	1
Rh203	R.SAAEAWGTYLK.Q	10	1196.59461	-0.45	3.71E-15	3.52	0.54	2	1291.2	1
Rh203	R.SDLM*PTGVLQENQNWCFPWSSVTVLQGPQSGAWQENDELTEK.T	6	4822.2036	0.24	2.42E-21	5.15	0.62	2	897.7	1
Rh203	R.SDLMPTGVLQENQNWCFPWSSVTVLQGPQSGAWQENDELTEK.T	7	4806.2087	0.54	3.17E-27	5.48	0.68	2	1129.3	1
Rh203	R.SEADPLLLLGTVEK.L	12	1484.82065	0.13	1.59E-18	5.01	0.62	2	2268.1	1
Rh203	R.SFDEYLCCK.E	11	1221.49154	-0.36	1.56E-12	2.95	0.59	2	975.7	1
Rh203	R.SGIDIPQVETR.S	16	1214.63754	-0.30	1.21E-13	3.48	0.40	2	788.2	1
Rh203	R.VSIYDPLNANIHCFD.-	14	1777.82143	0.25	1.50E-17	4.74	0.64	2	1395.6	1
Rh203	R.WYVLGVTGR.Y	14	1050.57309	-1.09	3.91E-13	3.45	0.39	2	1363.5	1
Rh209	R.DQQLVQAQHAAR.K	1	1364.70294	0.57	1.49E-14	3.36	0.43	2	524.9	1
Rh211	K.AAILEGCPYPR.P	2	1246.6249	-0.45	6.75E-13	2.54	0.38	1	573.8	1
Rh211	K.DLFSHQESQSSLR.I	1	1533.72921	0.05	3.30E-17	3.57	0.30	2	1218.2	1
Rh211	K.LICCEEPLEVLGVLQYWPGR.R	6	2431.21492	0.05	3.13E-12	4.09	0.60	2	1778.4	1
Rh211	R.AQAASLSSPSR.S	4	1074.55381	0.58	1.12E-13	2.78	0.38	2	410.8	1
Rh211	R.CDFPQATEVEAAHNFWR.R	4	2077.91852	0.04	3.92E-17	4.59	0.55	2	1223.6	1
Rh211	R.CYSIWNQMNPVLER.A	3	1809.84112	-0.20	1.40E-18	4.79	0.67	2	1736.4	1
Rh211	R.DQNVLYFLASGLDDFAR.H	4	1943.94977	0.80	4.39E-20	5.43	0.63	2	2425.3	1
Rh211	R.FQWLVR.R	3	848.47773	-1.27	6.88E-09	1.92	0.15	2	595	1

Rh211	R.FSLALSDAER.L	6	1108.56331	0.28	2.56E-12	2.60	0.40	2	655.1	1
Rh211	R.GQCIPLR.T	1	843.45057	-2.40	3.25E-09	1.70	0.12	2	463.9	2
Rh211	R.HGLLHCESIYSGK.F	3	1500.72641	-0.90	2.26E-15	3.80	0.47	2	914	1
Rh211	R.LADDIDSLLTM*GLLK.I	1	1633.87169	2.32	1.60E-13	4.16	0.45	2	333.9	1
Rh211	R.LADDIDSLLTMGLLK.I	2	1617.87679	1.33	8.77E-17	4.80	0.56	2	2369.6	1
Rh211	R.LNPGGCYSR.N	1	1023.46768	-2.22	1.16E-09	1.55	0.44	2	181.1	1
Rh211	R.M*PMLTTQPDDVISHLR.M	2	1869.91972	0.99	2.19E-15	3.68	0.08	2	651.2	1
Rh211	R.MNDYSLCQLQR.A	3	1427.64063	1.44	3.59E-14	4.06	0.49	2	1246.5	1
Rh211	R.MPM*LTTQPDDVISHLR.M	3	1869.91972	-0.10	5.13E-21	4.22	0.16	2	888	1
Rh211	R.MPMLTTQPDDVISHLR.M	4	1853.92482	-0.31	6.24E-21	5.25	0.61	2	1774.2	1
Rh211	R.MVFATFPSVFGEDTPCQGPGSPWSK.L	8	2729.23747	0.47	5.38E-19	4.59	0.64	2	855.4	1
Rh211	R.NSHHEVSGAADGSWEPHVR.C	3	2071.93289	0.01	7.85E-14	3.98	0.45	2	1038.3	1
Rh211	R.QSMSAEGHASEPESASASNDAK.P	1	2190.92039	1.64	7.70E-13	3.97	0.60	1	684.2	1
Rh211	R.RFSLALSDAER.L	2	1264.66443	0.01	2.24E-11	2.37	0.24	2	580.6	1
Rh211	R.TPGEMTRPMLICAEADDLK.S	1	2148.01341	0.26	3.65E-13	3.28	0.35	2	842.5	1
Rh211	R.TYQFWDR.Y	1	1015.46321	-1.52	2.60E-10	1.91	0.31	2	633.4	1
Rh211	R.YASGPAVR.R	1	820.43118	-4.14	3.91E-11	2.11	0.28	2	202.3	5
Rh211	R.YESLWVLDR.D	2	1180.5997	0.33	1.93E-12	2.51	0.49	2	1150.6	1
Rh211	R.YESLWVLDRDQNVLYFLASGLDDFAR.H	1	3105.53163	-5.11	1.49E-11	4.19	0.58	2	924.7	1
Rh211	R.YRGQCIPLR.T	1	1162.61501	-5.92	6.10E-09	1.91	0.05	2	282.4	3
Rh214	R.ASPDQLHELYR.C	2	1328.65934	0.13	8.30E-14	2.80	0.38	2	162.9	2
Rh214	R.TNSFSDVLFR.H	3	1185.58986	-0.77	5.86E-14	3.03	0.48	2	534.4	1
Rh215	K.DCWYLQLR.H	8	1153.54593	0.03	3.20E-11	2.75	0.40	2	959.6	1
Rh215	K.GVPVSDPAPHDCECFL.-	11	1799.7728	1.07	9.74E-19	3.56	0.73	2	1004	1
Rh215	R.HLCSLMTETLVFLR.S	7	1719.89209	-0.13	9.77E-19	4.93	0.63	2	2051	1
Rh215	R.NESNEHQCIM*R.N	8	1433.58964	0.25	7.81E-15	4.21	0.56	2	583.1	1
Rh215	R.NESNEHQCIMR.N	8	1417.59474	-0.60	1.17E-14	4.16	0.56	2	532	1
Rh215	R.SVFNPYIYM*IISYK.F	1	1753.88694	1.73	2.93E-10	2.23	0.32	2	100.4	6
Rh215	R.SVFNPYIYMIISYK.F	1	1737.89204	-0.25	4.74E-12	2.75	0.41	2	288.6	1
Rh215	R.TQYDALDTTQLAETM*QLK.A	4	2086.00086	-0.31	3.97E-21	6.16	0.64	2	2695	1
Rh215	R.TQYDALDTTQLAETMQLK.A	9	2070.00596	-1.11	8.33E-21	6.79	0.66	2	3632.2	1
Rh215	R.WSTFGYR.N	4	916.43118	-0.55	4.31E-10	1.91	0.22	2	260	1
Rh215	R.YGSLVWIAPITR.N	7	1375.77325	0.94	4.70E-14	3.70	0.55	2	962.1	1
Rh216	R.AIFNPFMYMCVSTR.L	1	1736.79575	6.97	1.51E-09	1.96	0.29	2	208.5	1
Rh216	R.DIVSDTSEDNK.D	1	1222.54337	0.11	4.58E-14	2.42	0.49	2	264.6	1
Rh216	R.IPYETLDAEHAK.L	5	1386.68997	0.05	9.77E-17	3.75	0.53	2	1305.5	1
Rh216	R.IYVCTSGNK.K	1	1041.50339	0.19	3.52E-10	1.86	0.24	2	247.8	1
Rh216	R.MVGVALVYGR.A	1	1064.59211	0.04	5.08E-13	2.16	0.25	2	641.9	1
Rh216	R.NANVPDPKPR.E	2	1107.59053	-2.75	6.25E-14	2.33	0.41	2	1045.7	1
Rh216	R.RIYVCTSGNK.K	1	1197.6045	-0.43	2.08E-12	2.58	0.40	2	285.4	1
Rh216	R.TTM*DASINIWSFVVPAVTTLLIAR.R	3	2635.41637	1.18	1.51E-14	4.29	0.56	2	722.2	1
Rh216	R.TTMDASINIWSFVVPAVTTLLIAR.R	4	2619.42147	0.90	1.22E-15	4.55	0.67	2	1127.3	1
Rh218	R.FREEIYSLFR.R	2	1359.70556	0.03	3.33E-13	2.96	0.36	2	935	1
Rh218	R.GITAPIIYVGISGR.F	2	1416.82093	0.94	1.46E-18	4.00	0.66	2	1153.6	1
Rh218	R.QPYNDLDPDANQFMIELTSQGR.S	3	2552.17219	0.28	1.13E-16	4.32	0.58	2	526.9	1
Rh218	R.QSESNVPQPEECFW.-	2	1736.72211	0.30	2.01E-17	2.66	0.58	2	501	1
Rh218	R.RQPYNDLDPDANQFMIELTSQGR.S	4	2708.2733	-0.02	2.65E-12	3.88	0.44	2	825.8	1
Rh218	R.YYDTTNCDVEK.I	2	1407.57332	-1.24	5.13E-16	3.33	0.59	2	835.8	1
Rh223	R.LISEQHVPAR.H	1	1149.63748	-8.46	8.36E-11	2.19	0.23	2	284.2	1
Rh230	K.DEFLLM*ATAWADAFIANTTAR.A	8	2344.12779	0.95	1.81E-14	5.88	0.65	2	1975	1
Rh230	K.DEFLLMATAWADAFIANTTAR.A	3	2328.13289	0.85	3.16E-15	5.77	0.52	2	1516	1
Rh230	K.LPSGPEEDEDPGEDDSFTLLPYR.T	4	2578.14675	-1.73	2.92E-18	5.03	0.64	2	880.4	1
Rh230	K.WPHAGSIVAAVR.N	2	1263.69567	-1.41	3.37E-10	1.90	0.27	2	295.5	1
Rh230	R.AEVDDDGRLLEK.N	1	1359.67505	-0.29	2.40E-13	2.68	0.25	2	354.6	2
Rh230	R.ANWFDTR.S	6	909.42134	-0.48	2.54E-10	2.38	0.39	2	740.7	1
Rh230	R.DAQALALPAEAGQEPIPQSDQALVSR.A	10	2675.3635	-1.22	6.85E-21	5.35	0.66	2	2198	1
Rh230	R.DLASQFAFLTR.P	12	1268.66336	0.52	3.91E-15	4.33	0.49	1	2402.2	1
Rh230	R.DLASQFAFLTRPGR.W	5	1578.8387	0.25	6.90E-13	3.01	0.43	2	377.9	1
Rh230	R.DRDPSVFPK.Y	3	1060.54219	-0.50	1.18E-11	1.76	0.09	2	439.8	1
Rh230	R.DVGATDLR.Q	8	846.43157	-0.59	7.81E-12	2.65	0.39	2	550.3	1
Rh230	R.GPNTVM*QR.R	3	918.44616	2.84	7.81E-11	2.02	0.39	2	267.7	1
Rh230	R.GPNTVMQR.R	6	902.45126	0.10	1.56E-11	2.77	0.50	2	536.6	1
Rh230	R.HEGLFLAIGCHFR.V	9	1556.77911	-0.30	9.77E-18	4.73	0.53	2	2179.4	1
Rh230	R.HGIGDLPR.V	1	864.46863	-0.32	5.99E-09	2.04	0.19	2	376.2	1

Rh230	R.HNAPASDVPPAATPGPAAAAAAATVTHSPK.C	7	2746.39072	-0.88	2.08E-21	5.60	0.68	2	1044.5	1
Rh230	R.HTLHLAATDADDFFR.H	18	1729.82926	-0.70	2.44E-20	5.03	0.62	2	2901.4	1
Rh230	R.LAAYQGSTVK.R	8	1037.56259	-1.22	1.76E-13	2.41	0.29	2	506.9	1
Rh230	R.LDTDTSTCQSR.D	15	1283.55326	0.62	1.95E-15	4.25	0.63	2	1421.9	1
Rh230	R.LLPDQDPYVWER.P	8	1530.75872	-0.88	1.95E-16	3.00	0.52	1	406.8	1
Rh230	R.NFISER.L	1	765.38898	0.14	9.38E-09	2.01	0.17	2	126.7	3
Rh230	R.PANFYAVVTPR.S	17	1234.65788	2.44	3.13E-15	4.28	0.64	1	2138.4	1
Rh230	R.PDALYLWPR.P	1	1130.59931	0.27	8.20E-13	2.73	0.22	0	635.6	1
Rh230	R.PLPIQPR.D	2	820.50395	-0.24	3.87E-09	1.58	0.10	1	169.8	1
Rh230	R.PRPDALYLWPR.P	9	1383.75318	0.14	6.19E-15	3.29	0.45	0	1157.8	1
Rh230	R.QLSPTDDWIVLVATLVHEM*SPSPQDPPTLCR.H	8	3518.72944	0.52	1.27E-22	3.78	0.55	2	950.3	1
Rh230	R.QLSPTDDWIVLVATLVHEMSPSPQDPPTLCR.H	12	3502.73454	0.76	4.71E-28	3.86	0.62	2	1081.1	1
Rh230	R.QTGQLLPLGR.P	5	1082.63167	-0.11	3.13E-13	2.94	0.52	1	537.3	1
Rh230	R.QTGQLLPLGRPANFYAVVTPR.S	2	2298.27171	-4.29	6.16E-16	2.81	0.44	2	343.3	1
Rh230	R.RPLPIQPR.D	3	976.60506	-0.43	3.28E-11	2.68	0.24	2	451.2	1
Rh230	R.SPHSWQHLR.Y	1	1147.57555	0.86	6.43E-09	1.82	0.20	2	297.1	1
Rh230	R.TWAAGDYNPHWDPQSVR.G	11	1999.90455	-0.63	1.22E-18	5.45	0.56	2	1410.4	1
Rh230	R.TWAAGDYNPHWDPQSVRGPNTVMQR.R	1	2883.33797	-4.56	2.38E-14	2.56	0.30	2	319.7	1
Rh230	R.TWPNAAAAAAAIGHTAAGQTICLQTPGRPPQPCLLTTCWEEVER.W	4	4772.31313	-0.36	1.58E-13	4.34	0.44	2	276.4	1
Rh230	R.VFLFDLPR.H	5	1006.57203	-0.79	7.81E-11	2.48	0.39	2	582.7	1
Rh230	R.VGLLAALTAGYAAASPDQAAVR.L	19	2086.12913	0.08	4.55E-21	6.25	0.62	2	2803.1	1
Rh230	R.VRDAQALALPAEAGQEPIPQSDQALVSR.A	5	2930.53302	-0.66	1.59E-18	6.44	0.65	2	2732.6	1
Rh230	R.VYACPTLPPLTVHPPEPVELLSR.T	19	2585.37964	-0.56	8.49E-18	3.73	0.56	2	642.4	1
Rh230	R.WRLDTDTSTCQSR.D	3	1625.73368	-2.36	3.79E-15	3.29	0.56	2	739.9	1
Rh31	R.NAITLTGSTDSLDLTR.L	1	1677.86537	-1.12	1.47E-13	3.14	0.43	2	547.6	1
Rh31	R.NSMEVTDACNR.E	1	1296.53075	-6.24	9.77E-16	3.69	0.56	2	1236.3	1
Rh31	R.SASGSQPVPNHR.I	1	1236.60797	-5.75	2.34E-12	2.42	0.13	2	473.6	1
Rh42	K.CFEVTSTSGFMLR.S	2	1534.7029	-0.33	1.13E-11	2.76	0.44	2	457.4	1
Rh42	K.GEMPSSPCYVR.W	1	1282.5555	-0.37	1.67E-11	2.01	0.48	2	336.9	1
Rh42	R.AVDVLYCPR.H	3	1092.55068	-0.10	2.89E-12	2.05	0.35	2	223	1
Rh42	R.FTGDCELHDTVPECPR.G	2	1932.82154	-0.64	2.48E-15	3.98	0.55	2	796.3	1
Rh42	R.LADDLTAFMCLGLR.Q	2	1595.79205	1.38	2.21E-14	4.10	0.55	2	1253	1
Rh42	R.LSYYLCCQTR.L	2	1363.61339	0.31	3.52E-12	2.46	0.46	2	503.3	1
Rh42	R.NHGNYLQLPYPEGACLR.L	1	2001.95999	-4.62	7.22E-10	2.28	0.26	2	670.8	1
Rh42	R.RFTGDCELHDTVPECPR.G	1	2088.92266	-0.03	1.78E-13	2.26	0.29	2	524.2	1
Rh42	R.SHFGDQVICLGTVDLK.G	2	1788.89493	-0.01	1.38E-18	3.92	0.49	2	999.5	1
Rh42	R.VFCLDLVNLR.Y	6	1248.67694	0.13	1.37E-13	3.58	0.53	2	1508.1	1
Rh42	R.VPIVLVDGGR.V	2	1024.61496	-0.20	9.38E-13	2.94	0.49	2	560.6	1
Rh42	R.VVGEPVLVHGQR.K	3	1289.73245	-3.22	7.37E-15	2.88	0.47	2	830.9	1
Rh43	K.AVMTKQYQACLR.R	2	1468.73995	-0.77	8.33E-13	2.92	0.44	2	633.3	1
Rh43	K.FLLYDFSVQHITR.V	1	1638.86385	-1.37	2.28E-13	2.65	0.38	2	601	1
Rh43	K.QYQACLR.R	6	938.4513	0.20	8.98E-10	2.12	0.37	2	206.3	1
Rh43	K.SSFEVVSETDSGSEAEAER.G	4	2015.86761	-0.18	3.81E-26	6.13	0.64	2	3552.2	1
Rh43	K.TTSAGERDLTR.G	1	1206.6073	-8.09	4.08E-10	2.59	0.11	2	281	8
Rh43	R.AAALALHFLM*PQK.A	3	1426.7875	-0.50	6.18E-11	3.03	0.43	2	555	1
Rh43	R.AAALALHFLMPQK.A	3	1410.7926	0.34	2.04E-12	3.60	0.38	2	1154.3	1
Rh43	R.APANELDEM*DLM*EAGLLSSSSQSDNK.S	3	2784.21858	0.09	8.26E-16	5.56	0.73	2	1140.7	1
Rh43	R.APANELDEM*DLMEAGLLSSSSQSDNK.S	7	2768.22368	-0.53	1.55E-18	5.22	0.42	2	1171.3	1
Rh43	R.APANELDEMDLM*EAGLLSSSSQSDNK.S	6	2768.22368	-0.27	8.14E-19	5.52	0.60	2	1191.6	1
Rh43	R.APANELDEMDLMEAGLLSSSSQSDNK.S	10	2752.22878	-1.05	5.88E-22	6.34	0.69	2	2404.1	1
Rh43	R.ATTSHGDEPLFQVNTWLFDYLR.A	13	2610.26233	-0.15	1.77E-19	6.28	0.72	2	2856.5	1
Rh43	R.CGDTQTKLLVR.N	2	1290.68348	-0.33	1.41E-11	2.74	0.37	2	497.8	1
Rh43	R.CNSTEEREVNSHR.N	1	1617.70344	-6.26	1.24E-18	2.58	0.50	2	873.4	1
Rh43	R.DYNLIFPIAPYTNR.G	16	1696.86933	-0.25	6.87E-16	4.58	0.56	2	1367.1	1
Rh43	R.GDYIQVTEAIYR.A	17	1427.71652	0.93	6.30E-16	4.43	0.57	2	2187.6	1
Rh43	R.GIVTHAVLVNYFYQAK.A	2	1822.98503	-0.26	7.92E-14	4.43	0.55	2	1464.3	1
Rh43	R.LSEELR.K	1	746.40429	-1.46	8.63E-09	1.72	0.00	2	133.3	13
Rh43	R.LSEELRK.R	3	874.49926	-0.70	6.72E-10	2.18	0.04	2	544.2	1
Rh43	R.LYTFDDLDDEVFLDPIPR.A	21	2183.05429	0.95	1.70E-16	4.80	0.69	2	1578.2	1
Rh43	R.NLEAVVCPDYM*M*CALR.Q	4	1973.85883	0.39	1.00E-12	4.41	0.66	2	858.3	1
Rh43	R.NLEAVVCPDYM*MCALR.Q	7	1957.86393	-0.73	2.59E-13	4.64	0.67	2	1135.1	1
Rh43	R.NLEAVVCPDYMM*CALR.Q	6	1957.86393	-0.85	8.08E-15	4.62	0.64	2	1337.2	1
Rh43	R.NLEAVVCPDYMMCALR.Q	6	1941.86903	0.53	5.08E-17	4.99	0.59	2	1497.9	1
Rh43	R.NLGFEPTLISPPNTEFLK.F	10	2017.06407	-1.44	3.30E-16	5.17	0.58	2	1297	1

Rh43	R.NVLKLLSSEK.L	2	1130.67795	-0.42	2.25E-13	2.43	0.31	2	336.1	1
Rh43	R.QTPVEHVLVPTASLNR.P	1	1760.96536	-1.05	2.20E-12	2.60	0.38	1	271.9	1
Rh43	R.QTPVEHVLVPTASLNRPYLR.C	4	2290.26663	0.06	1.79E-12	2.36	0.43	2	149.2	3
Rh43	R.QWTTVHEDNPITDYM*FHDPAIQDSR.I	7	3032.34791	-0.50	2.00E-20	3.58	0.55	2	618.6	1
Rh43	R.QWTTVHEDNPITDYMFHDPAIQDSR.I	16	3016.35301	0.90	2.17E-20	4.01	0.60	2	670.9	1
Rh43	R.RLSEELR.K	4	902.50541	0.00	1.23E-09	2.55	0.10	2	592.9	3
Rh43	R.RVTESPSTLEILETIR.D	4	1844.01237	-0.50	5.92E-13	3.68	0.41	2	774.7	1
Rh43	R.RVTESPSTLEILETIRDYNLIFPIAPYTNR.G	2	3521.86386	-1.87	3.43E-10	4.09	0.45	2	1021.4	1
Rh43	R.SFLETNGNHPM*SQLQ.-	5	1718.78025	1.76	9.12E-14	4.62	0.46	2	575.2	1
Rh43	R.SFLETNGNHPMSQLQ.-	8	1702.78535	-0.96	1.06E-13	4.13	0.49	2	974.2	1
Rh43	R.SM*QCSLNATIHGQTM*NNIATYVTNLTPGGR.A	3	3282.53001	-0.45	3.27E-12	3.57	0.49	2	537.7	1
Rh43	R.SM*QCSLNATIHGQTMNNIATYVTNLTPGGR.A	7	3266.53511	0.05	1.79E-16	4.67	0.52	2	1239.5	1
Rh43	R.SMQCSLNATIHGQTM*NNIATYVTNLTPGGR.A	3	3266.53511	-0.07	5.78E-14	3.76	0.10	2	634.8	2
Rh43	R.SMQCSLNATIHGQTMNNIATYVTNLTPGGR.A	8	3250.54021	0.06	7.11E-21	4.29	0.66	2	631.1	1
Rh43	R.VNQCIPM*PTFLLTSLINPIAK.T	14	2386.28731	1.10	1.31E-19	4.89	0.62	2	1569.9	1
Rh43	R.VNQCIPMPTFLLTSLINPIAK.T	10	2370.29241	-0.40	8.62E-18	5.07	0.59	2	971.8	1
Rh43	R.VTESPSTLEILETIR.D	20	1687.91126	1.97	2.29E-17	5.01	0.51	2	1791.5	1
Rh43	R.VTESPSTLEILETIRDYNLIFPIAPYTNR.G	6	3365.76275	-0.13	1.66E-17	4.77	0.56	2	983.2	1
Rh44	K.AANSLSELLK.E	2	1045.5888	-0.34	7.81E-14	2.37	0.40	2	406.6	1
Rh44	K.AANSLSELLKEGVR.E	3	1486.82238	-3.13	1.81E-14	3.60	0.47	2	1154.4	1
Rh44	K.EGVREGYFDDVR.R	1	1441.67063	-7.85	3.64E-10	2.79	0.33	2	264.3	6
Rh44	K.EGVREGYFDDVRR.A	1	1597.77174	-3.44	7.71E-11	3.03	0.22	2	399.8	17
Rh44	R.AGALQHSQR.R	3	967.5068	0.34	3.16E-12	3.22	0.38	2	764.1	1
Rh44	R.DFSYWR.L	8	873.38898	-0.72	1.65E-09	2.18	0.25	2	197.6	1
Rh44	R.EAGCVFNVEDYVR.R	22	1557.70025	0.17	1.22E-18	4.10	0.63	2	1185.9	1
Rh44	R.EAGCVFNVEDYVRR.N	1	1713.80137	-1.47	3.37E-13	2.17	0.36	2	186.9	1
Rh44	R.EGYFDDVR.R	4	1000.43705	-1.09	6.05E-12	1.96	0.23	2	765	1
Rh44	R.EGYFDDVRR.A	8	1156.53816	0.06	1.95E-13	2.08	0.27	2	182.6	1
Rh44	R.GYTLFVCDEDDTILTPR.D	39	2014.94267	-0.03	4.67E-18	5.58	0.69	2	1712.7	1
Rh44	R.HFDLPSPR.G	30	968.49484	0.03	2.42E-11	2.89	0.31	2	1103.4	1
Rh44	R.LLPLM*KGQLR.V	1	1184.71836	1.98	1.76E-10	1.96	0.31	2	273.6	1
Rh44	R.LLPLMKGQLR.V	3	1168.72346	0.63	9.38E-13	3.24	0.37	2	598.5	1
Rh44	R.PVAAIGGCGGIFCYEIGEHNYVVK.A	3	2610.24801	0.78	4.51E-17	3.67	0.60	1	494	1
Rh44	R.QNAVVEGEDPEELTQEM*FASGIM*SSSLFTPALDYSLQPESGAR.K	5	4663.13379	0.29	8.03E-32	6.11	0.66	2	1287.5	1
Rh44	R.QNAVVEGEDPEELTQEM*FASGIMSSSLFTPALDYSLQPESGAR.K	12	4647.13889	0.61	1.20E-30	6.42	0.62	2	1061.9	1
Rh44	R.QNAVVEGEDPEELTQEMFASGIM*SSSLFTPALDYSLQPESGAR.K	25	4647.13889	0.53	1.08E-31	6.89	0.61	2	1601.4	1
Rh44	R.QNAVVEGEDPEELTQEMFASGIMSSSLFTPALDYSLQPESGAR.K	14	4631.14399	-1.45	5.43E-31	7.17	0.71	2	1637.5	1
Rh44	R.RQNAVVEGEDPEELTQEM*FASGIM*SSSLFTPALDYSLQPESGAR.K	2	4819.23491	-0.30	6.71E-20	5.15	0.71	2	1093.6	1
Rh44	R.RQNAVVEGEDPEELTQEM*FASGIMSSSLFTPALDYSLQPESGAR.K	17	4803.24001	0.27	2.73E-21	5.83	0.67	2	990.2	1
Rh44	R.RQNAVVEGEDPEELTQEMFASGIM*SSSLFTPALDYSLQPESGAR.K	35	4803.24001	0.08	1.22E-26	5.90	0.71	2	1424.4	1
Rh44	R.RQNAVVEGEDPEELTQEMFASGIMSSSLFTPALDYSLQPESGAR.K	26	4787.24511	-0.23	1.33E-26	6.71	0.74	2	1755.7	1
Rh44	R.SESDASASLVTR.P	7	1222.59099	-0.10	4.88E-17	4.12	0.55	1	2567.7	1
Rh44	R.SESDASASLVTRPCLR.R	13	1748.85961	-1.77	3.42E-16	2.63	0.55	2	391.4	1
Rh44	R.SESDASASLVTRPCLRR.Q	1	1904.96072	-5.47	2.54E-14	2.34	0.28	2	318.7	1
Rh44	R.VIGTIGTDEM*FTWDR.P	11	1756.82105	-0.34	1.08E-16	3.35	0.59	1	601.1	1
Rh44	R.VIGTIGTDEM*FTWDRPVAAIGGCGGIFCYEIGEHNYVVK.A	6	4348.05122	-0.11	4.39E-22	4.32	0.44	2	592.7	1
Rh44	R.VIGTIGTDEMFTWDR.P	8	1740.82615	-0.21	5.86E-20	4.70	0.62	1	1195.6	1
Rh44	R.VIGTIGTDEMFTWDRPVAAIGGCGGIFCYEIGEHNYVVK.A	12	4332.05632	-1.10	2.82E-28	5.60	0.61	2	705.1	1
Rh55	K.AEPLTSPR.N	16	870.46796	-0.64	3.95E-11	2.02	0.38	2	170.1	1
Rh55	K.AQTASSTEVPGGR.A	12	1260.61787	0.41	5.13E-17	4.04	0.63	2	1296.9	1
Rh55	K.ATPLTVDGSVLKPASFSAPIPGK.D	4	2253.24891	-0.14	4.85E-16	3.88	0.59	2	484.4	1
Rh55	K.ATPLTVDGSVLKPASFSAPIPGKDVTIVK.V	1	2908.63939	-4.26	1.85E-21	5.63	0.59	2	420.7	1
Rh55	K.CGEKFLHR.R	5	1046.52005	-0.33	1.88E-11	2.63	0.32	2	444.1	1
Rh55	K.DDDDDNGQLLTTK.K	2	1449.63397	-0.54	5.16E-13	3.45	0.54	2	808.4	1
Rh55	K.DETDDVK.V	1	821.35232	0.74	2.34E-10	2.22	0.24	2	385.2	1
Rh55	K.DETDDVKVDTLVDFDGGLGSLK.L	2	2338.12964	-0.27	2.42E-17	4.60	0.57	2	879.6	1
Rh55	K.DLLDESSGTIPVPDNVQAIIEK.I	12	2353.21331	0.31	8.28E-18	6.02	0.57	2	2861.5	1
Rh55	K.DVM*ALEDSLLAISNNM*FEIR.P	1	2313.11008	1.56	2.13E-10	3.25	0.43	1	411.8	1
Rh55	K.DVM*ALEDSLLAISNNMFEIR.P	11	2297.11518	0.25	3.95E-14	4.73	0.61	1	2017.7	1
Rh55	K.DVM*ALEDSLLAISNNMFEIRPVK.L	6	2621.33132	0.83	7.94E-14	4.27	0.54	2	783.3	1
Rh55	K.DVMALEDSLLAISNNMFEIR.P	12	2281.12028	0.59	4.94E-17	4.63	0.64	1	1699.2	1
Rh55	K.DVMALEDSLLAISNNMFEIRPVK.L	6	2605.33642	-2.82	1.11E-16	4.86	0.60	2	1150.5	1
Rh55	K.DVTIVK.V	3	674.40832	0.11	3.13E-09	1.86	0.22	2	444.7	1
Rh55	K.ENVDLEQHPK.I	6	1208.59059	-0.16	3.91E-14	3.03	0.35	2	602.9	1

Rh55	K.ENVDLEQHPKILR.K	2	1590.85983	0.03	2.30E-12	2.93	0.19	2	303.4	2
Rh55	K.EVSGLDSISDYETK.T	10	1542.71697	-4.39	2.44E-19	5.00	0.71	2	1694.4	1
Rh55	K.FIGLPPR.K	4	799.48249	0.33	9.69E-10	1.79	0.17	2	315.2	1
Rh55	K.FIQTPILSSPFATQFSNPLFATGSSETIPGLETPLK.E	28	3836.99968	0.33	3.70E-36	7.36	0.74	2	2078.9	1
Rh55	K.LDDDDKPSTSTYK.F	6	1484.67511	-0.37	2.28E-16	4.08	0.50	2	1375.3	1
Rh55	K.LKDLLDESSGTIPVPDNVQAIIEK.I	11	2594.39234	-0.88	1.33E-17	5.19	0.63	2	1461.3	1
Rh55	K.LSDEAK.L	2	662.33555	0.03	4.69E-09	1.94	0.15	2	346.1	2
Rh55	K.LSECQPLGTGEIEFIQLR.Q	19	2090.0587	-0.19	6.73E-18	5.56	0.59	2	2488.1	1
Rh55	K.M*KAEPLTSPR.N	3	1145.59831	0.59	2.38E-09	2.28	0.18	2	406.8	1
Rh55	K.MKAEPLTSPR.N	6	1129.60341	-0.18	4.17E-12	2.74	0.20	2	1152	1
Rh55	K.PASFSAPIPGK.D	4	1071.58332	-0.16	7.08E-11	2.06	0.32	1	319.1	1
Rh55	K.PKPSFGPFTSVTSPSTSVLKPR.K	3	2317.25506	-1.51	2.78E-19	3.85	0.52	1	1282.7	1
Rh55	K.PSFGPFTSVTSPSTSVLKPR.K	10	2092.10733	-0.80	8.81E-23	5.81	0.65	1	1347.3	1
Rh55	K.QSSQFWDTKPK.T	3	1351.66409	-2.93	4.29E-13	2.56	0.34	2	726.4	1
Rh55	K.RVTFWSDDENQGR.A	3	1609.73536	-0.49	7.79E-14	4.27	0.56	2	1489.8	1
Rh55	K.TLPTKENVDLEQHPK.I	2	1748.91774	-4.36	9.86E-18	4.12	0.50	2	607.9	1
Rh55	K.TPPASDYSDR.E	27	1108.49054	0.33	1.95E-14	3.44	0.70	2	936.3	1
Rh55	K.VDTLVDFDGGLGSLK.L	10	1535.79516	-0.01	2.44E-20	5.29	0.59	2	1919.5	1
Rh55	K.VIDGESADM*LLEM*LMEDFAIYEDIFPR.S	4	3194.46179	1.45	1.43E-17	4.44	0.09	2	971.4	1
Rh55	K.VIDGESADM*LLEMLM*EDFAIYEDIFPR.S	2	3194.46179	1.38	2.73E-11	3.81	0.13	2	902.6	1
Rh55	K.VIDGESADMLLEMLMEDFAIYEDIFPR.S	2	3162.47199	1.46	5.59E-13	3.47	0.44	2	684.6	1
Rh55	K.VPTTIQTFTPGLTTTTSTK.A	7	1995.06446	-2.22	5.87E-15	5.54	0.65	2	1827.1	1
Rh55	K.VTAITNFLK.T	6	1006.59316	-0.88	5.70E-12	2.60	0.42	2	694.2	1
Rh55	R.AHM*YPFR.D	5	937.43487	0.13	6.25E-10	1.95	0.46	2	612	1
Rh55	R.AHM*YPFRDPVK.A	3	1376.67795	-0.35	1.01E-12	2.86	0.52	2	748.1	1
Rh55	R.AHMYPFR.D	19	921.43997	-2.77	7.81E-11	2.10	0.53	2	616.4	1
Rh55	R.AHMYPFRDPVK.A	3	1360.68305	0.58	1.63E-12	2.79	0.49	2	566.7	1
Rh55	R.AKDETDDVKVDTLVDFDGGLGSLK.L	10	2537.26172	0.01	1.21E-22	6.29	0.69	2	3214.6	1
Rh55	R.ESSFSPNK.R	5	895.41559	-0.25	3.91E-12	2.47	0.43	2	136.9	1
Rh55	R.EWQKWLR.G	1	1045.55778	0.12	6.41E-10	1.73	0.04	2	84.7	27
Rh55	R.HSNSSLVNATNK.L	12	1271.63385	0.00	4.88E-17	4.98	0.57	2	957.5	1
Rh55	R.HSNSSLVNATNKLIYLGK.V	2	1959.0658	0.58	6.21E-18	3.92	0.52	2	603	1
Rh55	R.KVPDKSER.Q	2	958.53162	1.33	1.56E-11	2.29	0.13	2	296.6	1
Rh55	R.KVTAITNFLK.T	7	1134.68812	0.10	7.81E-14	2.84	0.51	2	984.5	1
Rh55	R.NTPKLSDEAK.L	2	1102.57388	0.21	5.70E-10	2.28	0.19	2	289.5	5
Rh55	R.PVKLHLER.H	4	991.60473	-0.54	3.13E-11	2.73	0.48	1	1062.3	1
Rh55	R.QKDVM*ALEDSLLAISNNM*FEIR.P	2	2569.26362	1.32	1.37E-09	2.94	0.28	1	374.6	1
Rh55	R.QKDVM*ALEDSLLAISNNMFEIR.P	8	2553.26872	1.09	6.25E-15	3.58	0.51	1	1030	1
Rh55	R.QKDVM*ALEDSLLAISNNMFEIRPVK.L	5	2877.48486	0.51	1.52E-15	3.40	0.35	2	347.8	1
Rh55	R.QKDVMALEDSLLAISNNMFEIR.P	8	2537.27382	1.97	7.04E-17	4.67	0.61	1	1008.1	1
Rh55	R.QKDVMALEDSLLAISNNMFEIRPVK.L	8	2861.48996	-0.39	1.61E-20	4.73	0.55	2	667.2	1
Rh55	R.QTKDDDDDNGQLLTTK.K	11	1806.83519	-0.89	3.04E-20	3.48	0.61	2	542.6	1
Rh55	R.QYAFSLLSPTK.Q	14	1254.67286	0.26	1.17E-14	3.19	0.45	2	461	1
Rh55	R.RAHMYPFR.D	2	1077.54108	-0.26	5.34E-11	2.46	0.21	2	626.9	1
Rh55	R.SCLFNQLVLWLAYYR.E	1	1945.99934	1.17	3.35E-12	3.83	0.41	2	789.8	1
Rh55	R.SFESNYCSNIIK.H	13	1461.66789	-0.45	2.13E-15	3.83	0.49	2	1290.5	1
Rh55	R.SVSQSLGDPFSLEYDDEELHR.E	31	2423.09974	-0.03	1.04E-20	4.74	0.67	2	823.5	1
Rh55	R.SVSQSLGDPFSLEYDDEELHREWQK.W	4	2994.37519	-1.14	4.08E-14	2.92	0.33	2	446.7	1
Rh55	R.TDVTVIQNAM*VGATALAK.L	31	1818.96296	0.24	2.86E-19	5.55	0.58	2	2635.1	1
Rh55	R.TDVTVIQNAMVGATALAK.L	44	1802.96806	0.13	1.38E-22	6.30	0.61	2	3307.8	1
Rh55	R.TLEATVFGDDSR.T	56	1310.62229	-0.06	4.88E-17	4.79	0.67	2	1414.7	1
Rh55	R.TLEATVFGDDSRTDVTVIQNAMVGATALAK.L	2	3094.57251	-3.63	3.53E-15	5.76	0.54	2	1465.1	1
Rh55	R.TSWDILAEK.C	11	1062.5466	-0.35	8.20E-13	3.17	0.34	2	689.1	1
Rh55	R.VTFWSDDENQGR.A	30	1453.63425	0.17	4.88E-17	3.86	0.61	2	1337.2	1
Rh55	R.YHIPDLSSLLDEFEVNANAVTR.R	44	2503.24634	0.42	1.16E-21	6.08	0.68	2	3459.4	1
Rh56	K.RLSMSHQNLR.L	2	1241.65315	0.22	3.56E-12	2.87	0.30	2	197.5	1
Rh56	R.LSM*SHQNLR.L	4	1101.54694	0.99	3.13E-12	2.78	0.38	2	385.4	1
Rh56	R.LSMSHQNLR.L	9	1085.55204	-0.17	1.56E-12	3.02	0.36	2	684	1
Rh56	R.LVPNLHCM*VNPILYALM*GNDFVSK.V	2	2777.38233	0.80	5.83E-12	3.50	0.48	2	659	1
Rh56	R.LVPNLHCM*VNPILYALMGNDFVSK.V	2	2761.38743	1.78	7.46E-11	2.74	0.22	2	550.3	1
Rh56	R.LVPNLHCMVNPILYALM*GNDFVSK.V	4	2761.38743	0.53	3.55E-14	3.87	0.60	2	887.7	1
Rh56	R.LVPNLHCMVNPILYALMGNDFVSK.V	1	2745.39253	0.47	8.53E-10	2.50	0.08	2	426.3	1
Rh56	R.NSDDVPTIVSQQPATPTIVNKPEK.N	6	2578.33589	-1.00	3.07E-16	4.76	0.54	2	744	1
Rh56	R.RVINTFSR.L	1	992.56359	-0.53	5.51E-11	2.31	0.18	2	201.3	5

Rh59	K.CNYLCNDLYFAYVQVPECR.G	6	2484.07821	-0.25	1.87E-14	4.70	0.66	2	1325.2	1
Rh59	K.LHNGTLSEESFPM*R.N	5	1633.76387	-0.53	1.88E-15	3.56	0.55	2	535.7	1
Rh59	K.LHNGTLSEESFPMR.N	4	1617.76897	1.23	5.23E-18	4.17	0.63	2	1264	1
Rh59	K.NPFGNSEIFR.I	12	1180.57455	-0.15	1.95E-14	3.01	0.55	2	957.9	1
Rh59	K.NPFGNSEIFRIPEQASR.Y	2	1961.9828	-4.46	3.11E-16	3.74	0.39	2	597.3	1
Rh59	K.NSQDLLVDLQANLDEINSGANK.Q	7	2371.17357	0.99	5.90E-22	6.28	0.66	2	2267.5	1
Rh59	K.QIANSVIR.I	1	900.52614	0.27	3.13E-11	1.52	0.05	2	114.1	40
Rh59	K.RADYVVNDVLFLLSAR.H	4	1851.01231	0.51	1.89E-16	4.80	0.59	2	1591.2	1
Rh59	R.ADYVVNDVLFLLSAR.H	26	1694.9112	0.38	5.34E-17	5.21	0.56	2	2495.9	1
Rh59	R.ALTEALDLNR.E	15	1115.60551	-0.32	7.81E-14	3.72	0.32	2	1050.3	1
Rh59	R.DSLAEELEQL.-	1	1146.55247	0.68	5.18E-09	2.27	0.29	2	432.5	1
Rh59	R.EGDLDLEVDPTSTPLQNFYFAK.N	6	2499.19258	0.13	9.07E-17	5.15	0.66	2	1333.7	1
Rh59	R.EGDSSDSDSENQEAAEDRDTLLR.R	3	2539.06627	-2.77	3.02E-21	3.91	0.63	2	719.6	1
Rh59	R.EGDSSDSDSENQEAAEDRDTLLRR.F	1	2695.16738	-6.24	3.14E-17	3.59	0.47	2	472.1	1
Rh59	R.FREGDLDLEVDPTSTPLQNFYFAK.N	7	2802.3621	-0.02	1.82E-17	5.79	0.65	2	1582.3	1
Rh59	R.GTFCNFVSGLQVVR.K	15	1583.79991	0.01	7.22E-18	4.59	0.57	2	1735.6	1
Rh59	R.HRLELQILETWIVNK.C	8	1892.07524	0.89	3.98E-12	4.75	0.49	2	2283.6	1
Rh59	R.ICILANYLK.N	5	1107.62311	-0.34	3.13E-12	2.88	0.25	2	788.9	1
Rh59	R.IPEQASR.Y	6	800.42609	0.99	3.13E-10	2.05	0.31	2	264.6	1
Rh59	R.LGPEQPPR.Q	22	893.48394	-0.40	1.56E-11	2.51	0.56	2	489.1	1
Rh59	R.NAWIDR.S	3	774.38931	-1.95	3.13E-09	1.83	0.18	2	108.8	1
Rh59	R.NLYSLIM*EGASR.Q	6	1369.67801	0.82	7.26E-16	4.14	0.45	2	2027	1
Rh59	R.NLYSLIMEGASR.Q	17	1353.68311	-0.41	1.99E-16	4.46	0.48	2	2370.3	1
Rh59	R.NM*SQLLDAGDVTM*GVYR.Q	8	1901.87314	-0.66	6.62E-20	5.34	0.60	2	2048.4	1
Rh59	R.NM*SQLLDAGDVTMGVYR.Q	6	1885.87824	-2.72	2.42E-20	5.10	0.64	2	2194.6	1
Rh59	R.NMSQLLDAGDVTM*GVYR.Q	13	1885.87824	-0.58	2.85E-18	5.44	0.66	2	2789.4	1
Rh59	R.NMSQLLDAGDVTMGVYR.Q	18	1869.88334	-0.07	6.16E-20	5.21	0.70	2	2492.6	1
Rh59	R.PAQPSSGSSASLSTPSTSSAPSGQPSPSR.L	7	2700.27072	0.14	6.26E-22	5.08	0.65	1	1913.7	1
Rh59	R.QFMAALQQAPLGHDEPGAADHSDLFADVDIR.P	1	3335.57496	0.51	5.10E-14	2.20	0.07	1	453.8	1
Rh59	R.QGLTQMR.A	3	833.4298	-0.10	6.25E-10	1.96	0.34	2	402.7	1
Rh59	R.QGPDIAASASASGGEDPR.D	44	1685.77253	-0.35	7.45E-21	4.71	0.68	2	1845.7	1
Rh59	R.QGPDIAASASASGGEDPRDSLAEELEQL.-	8	2813.30717	-1.54	9.82E-17	5.27	0.52	2	1005.7	1
Rh59	R.QTNM*M*DVYR.N	2	1189.49759	0.69	2.30E-11	2.14	0.51	2	262.1	1
Rh59	R.QTNM*MDVYR.N	1	1173.50269	0.09	1.25E-11	1.90	0.06	2	135.3	3
Rh59	R.QTNMM*DVYR.N	4	1173.50269	-0.14	9.01E-13	2.16	0.20	2	281.6	1
Rh59	R.QTNMMDVYR.N	2	1157.50779	-3.41	3.91E-13	2.22	0.55	2	264.6	1
Rh59	R.RNAWIDR.S	2	930.49042	-0.67	9.83E-10	2.77	0.29	2	567.3	1
Rh59	R.SFNLQTQINR.S	13	1220.63821	-0.59	3.42E-13	3.75	0.37	2	1450.3	1
Rh59	R.SNLTYISYK.L	11	1088.56225	-0.51	7.81E-13	2.92	0.44	2	1190.4	1
Rh59	R.VREGDSSDSDSENQEAAEDRDTLLR.R	3	2794.23579	-6.47	4.41E-21	4.04	0.48	2	1022.6	1
Rh59	R.VREGDSSDSDSENQEAAEDRDTLLRR.F	1	2950.3369	-7.14	4.04E-20	4.56	0.55	2	1569	1
Rh59	R.YLSLSNFSPYSVAR.H	13	1603.81148	-0.34	3.71E-17	4.98	0.64	2	1401.9	1
Rh67.1	K.YHHLLTTDEEEEDIVCEK.K	3	2260.00745	0.12	3.77E-19	6.23	0.65	2	2769.4	1
Rh68	R.NDSEDVRPPAYDEIVGSPPRPR.D	1	2466.20079	-6.04	1.41E-09	2.98	0.24	2	173.3	18
Rh70	K.IHMCAPDFNMEFSSPCVHGQDLMR.E	1	2879.21911	-6.11	6.29E-13	2.35	0.15	2	644	1
Rh70	K.NLYQTLMNCAVTK.L	1	1555.76075	-4.19	1.17E-16	4.35	0.57	2	1798.4	1
Rh70	K.TINNSTPMLGNFMYLTSSK.D	1	2119.01984	-4.86	2.15E-16	4.38	0.62	2	496.4	1
Rh70	R.AVAVTNSGAMHDDGCGSGLDSEMMSEPVSR.K	1	3067.28603	-6.41	2.43E-20	6.11	0.68	2	1576.7	1
Rh70	R.ESDNSAVHVDLDHSVVSELIK.W	1	2293.13065	-5.51	6.77E-24	3.50	0.46	2	498.3	1
Rh70	R.LPEPPTLALR.L	4	1106.65682	-0.21	3.93E-12	2.42	0.48	2	748	1
Rh72	K.AKSITQGTVQR.A	1	1188.66951	-2.00	3.36E-13	2.53	0.39	2	895	1
Rh72	K.ASEFMR.N	1	740.33959	0.05	4.69E-09	1.51	0.07	2	136.4	1
Rh72	K.CALVFVHHIVK.Y	1	1322.74021	0.53	1.97E-13	3.34	0.50	2	487.9	1
Rh72	K.ETDIIGIHYAFNIPSVLMK.R	3	2161.13619	1.31	5.77E-15	2.99	0.46	2	402.5	1
Rh72	K.FIFELYR.K	1	987.52983	0.08	3.83E-09	1.89	0.22	2	242.9	1
Rh72	K.ILADITR.Y	1	801.48288	-4.30	1.25E-09	1.91	0.14	2	202.1	2
Rh72	K.LSECGNEFAFEK.E	3	1430.62569	0.35	4.88E-17	3.98	0.55	2	1566.5	1
Rh72	K.SGLPPCEWFDR.T	6	1363.60998	0.37	1.01E-13	2.36	0.41	2	209.1	1
Rh72	K.SITQGTVQR.A	7	989.53743	0.17	1.56E-12	2.73	0.23	2	918.7	1
Rh72	K.VPIINYAWIEHHDEVK.A	4	1963.00722	0.16	2.08E-17	3.77	0.58	2	1077.8	1
Rh72	K.VTVKASEFMR.N	1	1167.61906	-0.03	1.92E-09	2.04	0.11	2	125.7	36
Rh72	K.WLYENLTR.G	5	1094.56292	-1.07	1.64E-11	2.86	0.33	2	735.9	1
Rh72	K.YEVLESVVK.R	2	1065.58265	-0.90	6.71E-11	2.06	0.34	2	428.4	1
Rh72	K.YSVLTGNM*PLPPCLGPDMASCHFGESDLPLQR.L	2	3575.64265	-0.34	1.14E-15	3.91	0.25	2	300.4	1

Rh72	K.YSVLTGNMPLPPCLGPDM*ASCHFGESDLPLQR.L	1	3575.64265	0.95	8.14E-17	4.50	0.51	2	279.5	1
Rh72	K.YSVLTGNMPLPPCLGPDMASCHFGESDLPLQR.L	11	3559.64775	-0.07	4.36E-22	4.96	0.60	2	627.3	1
Rh72	R.ADVPSCVFQR.A	10	1178.5623	-0.15	4.20E-13	3.06	0.38	2	682.7	1
Rh72	R.AQDSHYSLFGR.T	11	1280.60183	-0.05	1.95E-15	3.69	0.55	2	1684.6	1
Rh72	R.CIGGFCDM*IR.E	4	1244.52212	0.09	9.08E-12	2.56	0.21	2	564	1
Rh72	R.CIGGFCDMIR.E	8	1228.52722	0.09	3.32E-13	3.18	0.42	2	796.1	1
Rh72	R.DNVVLGNTR.R	8	987.52178	-0.40	3.91E-13	2.62	0.50	2	324.3	1
Rh72	R.DNVVLGNTRR.Y	1	1143.6229	-0.02	5.52E-10	1.97	0.00	2	231	16
Rh72	R.EVDWLALHK.W	2	1110.59422	0.11	2.60E-12	2.23	0.30	2	239.8	1
Rh72	R.FCGVPDPVK.H	8	1018.50266	-0.64	3.91E-13	2.36	0.41	2	827.9	1
Rh72	R.FCGVPDPVKHVEDR.A	1	1654.80064	-7.30	9.08E-12	2.22	0.27	2	457.4	1
Rh72	R.FHMDCR.H	1	865.34439	-2.22	1.72E-09	1.98	0.46	2	746.5	1
Rh72	R.FNVEAISVIR.C	12	1147.64698	-0.03	1.04E-13	3.64	0.46	2	1904	1
Rh72	R.GVSLSINVTR.F	7	1045.60003	-0.63	8.98E-13	3.42	0.42	2	1312.9	1
Rh72	R.HKYEVLESVVK.R	2	1330.73653	-0.06	9.38E-12	2.73	0.32	2	567.8	1
Rh72	R.HSEDLPKILADITR.Y	1	1607.87515	0.17	2.78E-14	2.51	0.44	2	401.7	1
Rh72	R.IFENM*YYAALR.T	4	1406.67728	-0.07	9.38E-15	3.92	0.52	2	1767.4	1
Rh72	R.IFENMYYAALR.T	5	1390.68238	0.21	2.20E-15	3.97	0.50	2	1803.6	1
Rh72	R.IFVDLWDVAAIR.V	10	1417.78381	0.81	4.92E-16	4.96	0.49	2	2447.3	1
Rh72	R.ISM*AITLAIVSSPCAR.V	1	1705.89756	0.23	3.22E-09	2.14	0.07	2	276.4	1
Rh72	R.ISMAITLAIVSSPCAR.V	3	1689.90266	0.18	2.30E-10	2.74	0.40	2	359	1
Rh72	R.IVDEETVLPVSPPSDECYFPTVM*QK.H	2	2896.36311	0.26	1.28E-09	2.93	0.51	2	270.8	1
Rh72	R.IVDEETVLPVSPPSDECYFPTVMQK.H	7	2880.36821	0.81	7.60E-14	4.88	0.55	2	435.6	1
Rh72	R.KLSECGNEFAFEK.E	2	1558.72066	0.40	4.09E-16	4.05	0.51	2	1316.6	1
Rh72	R.KLSECGNEFAFEKEYVR.Y	1	2105.9961	31.93	3.94E-15	3.30	0.38	2	274.1	1
Rh72	R.KPHLSLPVAQWETLR.T	7	1774.99626	1.51	4.27E-14	5.34	0.52	2	2066.3	1
Rh72	R.LFQM*LMR.Y	1	954.48994	-5.71	4.15E-09	2.15	0.09	2	495.2	1
Rh72	R.LFQMLM*R.Y	2	954.48994	-0.19	2.75E-09	1.97	0.15	2	630.3	1
Rh72	R.LFQMLMR.Y	4	938.49504	-1.18	6.41E-10	2.73	0.22	2	639.8	1
Rh72	R.LGFTYFASWDLIER.I	8	1717.85843	1.00	6.99E-17	4.64	0.53	2	2211.5	1
Rh72	R.LSINLTR.C	1	816.49378	-0.75	9.84E-09	1.51	0.11	2	110.9	3
Rh72	R.NALNYTSSM*CEGPLR.D	7	1728.768	-1.09	1.05E-19	4.80	0.69	2	1997.3	1
Rh72	R.NALNYTSSMCEGPLR.D	10	1712.7731	-0.80	6.10E-20	4.79	0.70	2	2104	1
Rh72	R.NALNYTSSMCEGPLRDFVMR.H	1	2361.07847	-4.97	1.71E-14	2.89	0.39	2	552	1
Rh72	R.NAQLLSIAADEETAFLWNVTPSIWAAR.D	32	2987.52615	0.06	3.36E-20	5.77	0.67	2	1313.3	1
Rh72	R.QIYGQDESLGGIFLR.I	8	1695.87006	0.16	1.95E-19	4.58	0.50	2	1392.3	1
Rh72	R.RYFDMQVLR.T	5	1227.63029	0.31	8.07E-13	3.62	0.40	2	912.1	1
Rh72	R.SDLLIGR.F	6	773.45158	-1.81	6.25E-10	1.95	0.19	2	176.6	1
Rh72	R.SGDWPLESAICK.M	11	1362.63586	0.20	4.98E-15	3.60	0.51	2	873.3	1
Rh72	R.SLNIGVTGLHTVLM*R.L	7	1626.89957	-0.41	1.00E-19	4.14	0.63	2	1288.4	1
Rh72	R.SLNIGVTGLHTVLMR.L	5	1610.90467	-0.47	3.23E-19	4.29	0.64	2	1625.4	1
Rh72	R.TDDDVLCR.D	15	993.43062	-0.48	7.81E-12	3.08	0.52	2	1275.8	1
Rh72	R.TDDDVLCRDNVVLGNTR.R	2	1961.93456	-5.38	4.76E-16	3.73	0.45	2	1082.6	1
Rh72	R.TDDDVLCRDNVVLGNTRR.Y	1	2118.03568	-7.79	7.28E-12	3.02	0.52	2	265	3
Rh72	R.TEM*QEYGVR.N	2	1128.49899	0.20	7.81E-13	3.07	0.50	2	960.1	1
Rh72	R.TEMQEYGVR.N	7	1112.50409	-0.77	3.91E-13	3.04	0.48	2	1041.3	1
Rh72	R.TLVTEAVVWGNAR.L	17	1415.76414	0.23	2.44E-18	4.32	0.57	2	2213.6	1
Rh72	R.TSVDLCK.S	5	822.40262	-1.55	1.56E-10	1.75	0.25	2	428.1	1
Rh72	R.YETTVPK.V	4	837.43526	-1.26	7.81E-11	2.26	0.35	2	355.5	1
Rh72	R.YFDM*QVLR.T	1	1087.52408	0.25	6.25E-11	2.66	0.36	2	681.5	1
Rh72	R.YFDMQVLR.T	10	1071.52918	0.03	1.56E-11	3.31	0.45	2	873.1	1
Rh72	R.YGPALIR.Q	1	789.46175	-5.49	5.00E-09	1.57	0.21	2	179.8	2
Rh75	K.AALAWLDVGSK.V	4	1130.62044	-0.41	1.95E-15	3.11	0.58	2	1412.8	1
Rh75	K.ATNVSMATK.C	5	922.46624	-2.04	2.05E-13	2.67	0.45	2	757.7	1
Rh75	K.CQLEGIYTSGPAK.A	7	1423.68863	-0.08	7.21E-17	3.58	0.55	2	1693.5	1
Rh75	K.DM*YM*TLEETQGR.L	5	1505.62464	-0.73	2.64E-15	4.41	0.55	2	1251.7	1
Rh75	K.DM*YMTLEETQGR.L	4	1489.62974	-1.13	2.94E-16	4.70	0.26	2	1791.8	1
Rh75	K.DMYM*TLEETQGR.L	8	1489.62974	-0.09	1.86E-15	4.09	0.40	2	1293	1
Rh75	K.DMYMTLEETQGR.L	12	1473.63484	-0.83	2.22E-16	4.94	0.65	2	1789.3	1
Rh75	K.LGWCLADDIHTSVLTHQEVK.L	4	2322.15473	-0.97	1.06E-15	4.04	0.52	2	1674.1	1
Rh75	K.REVNTLAVR.Y	2	1057.61127	-0.24	6.06E-13	3.10	0.19	2	667.1	1
Rh75	K.SVSLGITSLLTCVLSGYLYNTLK.T	3	2502.35242	1.35	8.86E-17	5.03	0.56	2	1438.9	1
Rh75	K.TEIFSLWIPK.D	6	1233.68779	0.11	2.17E-13	3.63	0.49	2	1347.3	1
Rh75	K.VAIYVATSSISNR.H	12	1380.74816	-0.72	6.34E-16	4.44	0.56	2	2405.2	1

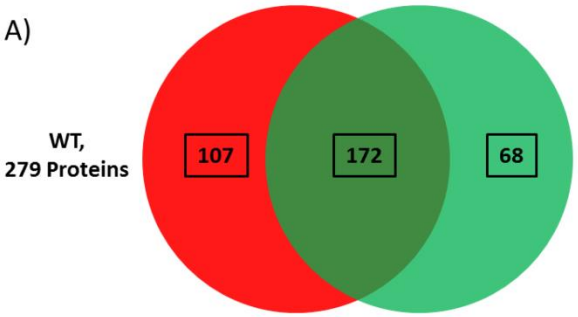
Rh75	R.CSFVNK.R	1	754.35527	0.19	6.41E-09	1.66	0.22	2	150.2	3
Rh75	R.CSFVNKR.I	1	910.45638	0.11	1.56E-10	1.63	0.24	2	141.4	7
Rh75	R.DPNDEDNELVLAVK.L	12	1570.75951	0.60	2.93E-19	5.10	0.59	2	2986.7	1
Rh75	R.EVNTLAVR.Y	15	901.51016	-0.73	2.85E-11	2.16	0.37	2	720.6	1
Rh75	R.FFVPEGLVEFEVHPGSLM*FR.M	4	2353.16854	-0.68	1.00E-12	3.16	0.54	2	307.3	1
Rh75	R.FFVPEGLVEFEVHPGSLMFR.M	6	2337.17364	-1.07	4.09E-22	5.68	0.67	2	1579.9	1
Rh75	R.HKFIAER.C	5	900.50501	-13.50	3.13E-10	1.99	0.15	2	452.5	1
Rh75	R.HTLTDLVR.H	13	954.53671	-0.96	1.56E-11	2.42	0.33	2	288.8	1
Rh75	R.LEHFNVELSEFTEFV.-	26	1839.87996	-1.16	1.30E-15	4.69	0.69	2	2063.1	1
Rh75	R.LQNVYLIM*VYDYEVLETK.V	4	2249.14098	0.22	4.26E-16	4.69	0.61	2	1602.3	1
Rh75	R.LQNVYLIMVYDYEVLETK.V	5	2233.14608	-0.57	6.04E-17	5.94	0.59	2	2705.7	1
Rh75	R.M*ETGAESPR.Y	16	993.43057	1.51	3.91E-13	3.12	0.54	2	650.1	1
Rh75	R.METGAESPR.Y	10	977.43567	0.52	5.86E-13	2.62	0.38	2	463.9	1
Rh75	R.QISLCTGVIQK.L	10	1246.68242	0.10	1.56E-14	3.33	0.27	2	663.7	1
Rh75	R.YLYEADHQAITSR.F	12	1566.7547	-0.52	9.77E-18	5.02	0.68	2	2254.5	1
Rh76	K.AVNSELNTEFQTFITSDK.R	2	2043.98694	-0.91	3.56E-18	5.30	0.67	2	1116.9	1
Rh76	K.DQAENDILTSIYPEAR.H	4	1834.88175	0.31	1.05E-15	4.96	0.53	2	1779	1
Rh76	K.DQQLSDSFHR.I	3	1232.56544	-0.39	1.29E-13	3.21	0.41	2	1379.2	1
Rh76	K.FTSLGLNR.Q	1	907.49959	-5.35	1.64E-11	1.67	0.20	2	238.5	5
Rh76	K.GATELEKDYLYR.D	3	1457.72709	-0.07	1.33E-12	3.09	0.38	2	687.3	1
Rh76	K.HDQELDTILK.M	3	1211.62664	-0.18	2.96E-12	3.12	0.43	2	469.7	1
Rh76	K.LFDTNQFLAATVPDR.K	5	1707.87006	-0.19	5.96E-15	4.73	0.56	2	1610	1
Rh76	K.LFDTNQFLAATVPDRK.N	2	1835.96502	-0.85	4.05E-14	2.98	0.42	2	322	1
Rh76	K.LTPDAQSPSLDNVTTADVIK.R	4	2085.07101	0.29	1.45E-19	5.26	0.65	2	1002.3	1
Rh76	K.LVSLYNLTTFISEIDAGVFR.T	5	2258.20671	0.88	5.00E-19	5.63	0.71	2	2448.3	1
Rh76	K.NIEEHGIR.Y	3	967.49557	-0.13	3.95E-11	1.77	0.36	2	383.2	1
Rh76	K.QITEDVIQLNEYSK.N	2	1679.84866	0.24	4.57E-16	3.69	0.46	2	627.3	1
Rh76	K.STEVQAVLNTLSQIEIGALDVETVNATK.I	8	2943.55209	-0.27	2.77E-18	5.13	0.62	2	1585.7	1
Rh76	K.TCMVLSSK.N	2	925.44819	0.19	2.42E-09	1.88	0.26	2	224.5	1
Rh76	K.VFNANLNDR.D	4	1062.53268	-0.47	1.11E-12	3.02	0.37	2	1023.8	1
Rh76	K.YLEHTNLCYSFAHTIGQLSADMSTVELTLQSK.T	2	3657.7564	0.54	1.99E-19	6.26	0.64	2	1232.9	1
Rh76	R.DISDNGTQYLIR.S	8	1394.69103	-0.93	1.77E-15	4.02	0.52	2	1159.2	1
Rh76	R.DSLSSAQELLK.E	5	1190.62631	1.25	2.27E-14	3.10	0.32	2	1132.2	1
Rh76	R.EAGVEHSPLQM*SGNTDIQQQIPIPEVLTNR.Y	6	3317.64303	-0.17	2.69E-15	5.59	0.52	2	612.6	1
Rh76	R.EAGVEHSPLQMSGNTDIQQQIPIPEVLTNR.Y	9	3301.64813	0.13	9.63E-20	5.77	0.56	2	881.7	1
Rh76	R.HAITSPCEYLNNQTTLTHLQTFM*DK.M	2	2979.39754	0.12	1.25E-14	4.69	0.51	2	254.3	1
Rh76	R.HAITSPCEYLNNQTTLTHLQTFMDK.M	5	2963.40264	-1.66	5.47E-22	6.40	0.63	2	1336.6	1
Rh76	R.KFTSLGLNR.Q	1	1035.59455	-0.65	1.79E-09	2.54	0.18	2	365.2	4
Rh76	R.KIILEQQR.S	3	1027.62586	-0.16	2.61E-10	2.16	0.08	2	363.7	2
Rh76	R.LDLIAQM*CSQVSNEFFR.T	3	2073.97324	0.45	9.46E-17	4.91	0.60	2	2177.4	1
Rh76	R.LDLIAQMCSQVSNEFFR.T	9	2057.97834	0.28	1.19E-18	5.72	0.61	2	2100.1	1
Rh76	R.LIIGSLK.R	1	743.50255	0.01	1.56E-09	1.57	0.28	2	233.5	1
Rh76	R.LILIM*EQTQK.S	2	1232.69187	0.35	2.03E-12	2.69	0.31	2	771.1	1
Rh76	R.LILIMEQTQK.S	3	1216.69697	0.77	1.15E-12	2.92	0.31	2	1200.8	1
Rh76	R.LLSEESTPSASEM*QR.L	6	1680.77449	-0.69	7.32E-20	4.68	0.62	2	1109	1
Rh76	R.LLSEESTPSASEMQR.L	8	1664.77959	-1.34	2.44E-20	5.06	0.62	2	1020.1	1
Rh76	R.LLVPDQIALYNK.L	3	1386.79913	0.70	4.09E-15	3.12	0.44	2	964.2	1
Rh76	R.LVAELIDM*LYHNTLR.W	4	1816.96257	0.92	1.32E-13	3.52	0.48	2	1709.7	1
Rh76	R.LVAELIDMLYHNTLR.W	8	1800.96767	-1.38	6.54E-18	4.33	0.52	2	1427.4	1
Rh76	R.NLGPFM*VSDADGDQQIGAEHIR.D	6	2386.10918	-0.06	2.85E-20	4.58	0.59	2	791.2	1
Rh76	R.NLGPFMVSDADGDQQIGAEHIR.D	8	2370.11428	0.69	4.69E-18	3.87	0.59	2	493	1
Rh76	R.STLIGSR.R	1	733.42028	0.01	7.81E-10	1.72	0.30	2	107.7	3
Rh76	R.SVFTQIEEATEHIR.P	2	1659.83368	1.09	1.03E-17	3.09	0.43	1	866.9	1
Rh76	R.SVFTQIEEATEHIRPFSNCR.L	3	2421.1616	-0.09	4.89E-15	2.74	0.37	2	870.8	1
Rh76	R.SVSAIPASAVDYLPDNAESQLR.T	13	2303.15138	-0.62	3.12E-23	5.60	0.71	2	1310.2	1
Rh76	R.TFLKGFR.E	1	868.50395	-0.17	1.68E-09	1.56	0.33	2	289.4	1
Rh76	R.TVDFQVILNELK.Q	8	1418.78896	2.06	1.28E-15	4.19	0.38	2	1932.6	1
Rh76	R.TYAQM*ALLQPSR.T	4	1394.70965	-0.06	3.67E-13	3.44	0.38	2	1140.7	1
Rh76	R.TYAQMALLQPSR.T	10	1378.71475	0.06	7.81E-16	4.17	0.52	2	1572.6	1
Rh76	R.WAMGLMQYHAFIEK.H	3	1724.82873	0.87	4.65E-17	3.86	0.42	2	1405.2	1
Rh76	R.YCIAESR.G	10	898.40876	-0.08	1.56E-10	2.52	0.23	2	661.4	1
Rh76	R.YEPSNQTNDLLNWYITSK.D	4	2186.04004	-0.43	2.93E-19	5.18	0.67	2	691.6	1
Rh78	K.APPTAGWILFSSSGM*PFDEAFLTDR.M	4	2729.29156	0.94	6.14E-15	5.20	0.65	2	1058.5	1
Rh78	K.APPTAGWILFSSSGMPFDEAFLTDR.M	4	2713.29666	1.36	1.70E-16	5.52	0.59	2	1377.8	1

Rh78	K.AQAFDM*INALINQIPHASTIIK.D	5	2425.29078	1.16	1.62E-14	4.76	0.54	2	607	1
Rh78	K.AQAFDMINALINQIPHASTIIK.D	6	2409.29588	0.94	1.46E-19	6.23	0.64	2	922.8	1
Rh78	K.ASTQM*SYLDAEK.T	3	1359.60966	-0.03	2.79E-12	2.92	0.44	2	291	1
Rh78	K.ASTQMSYLDAEK.T	2	1343.61476	-0.27	1.18E-12	2.35	0.40	2	315.1	1
Rh78	K.DIVQEAINNVVQMPPAYNADEIK.G	2	2571.27593	-0.33	1.75E-12	4.10	0.46	2	458.7	1
Rh78	K.DLHTANAAISQLSK.Q	2	1468.77543	-0.43	9.77E-19	3.97	0.45	2	1276.3	1
Rh78	K.DTLDLILTEGAQLDSLVETILQQK.R	4	2656.42912	1.24	1.43E-21	6.93	0.62	2	3336.6	1
Rh78	K.EIQNITIK.A	1	958.55677	-0.27	5.64E-09	1.61	0.04	2	182.1	2
Rh78	K.FAQVKNVVTGR.Y	1	1218.69533	-0.97	8.87E-12	2.68	0.48	2	671	1
Rh78	K.GFCVNPK.I	2	821.39747	-0.28	6.83E-10	1.82	0.23	2	210.7	1
Rh78	K.HLILNPVAALTELLAK.A	5	1716.04182	0.01	2.01E-12	3.42	0.59	2	1060.7	1
Rh78	K.HNQIISGTLPVQDM*QALQR.R	2	2165.11315	-0.06	1.30E-16	4.59	0.64	2	811.3	1
Rh78	K.HNQIISGTLPVQDMQALQR.R	2	2149.11825	1.13	1.78E-18	5.73	0.54	2	2060.3	1
Rh78	K.IHDLM*VHIEDTVQNLTSR.S	4	2137.07061	4.38	6.72E-15	3.09	0.45	2	594.8	1
Rh78	K.IHDLMVHIEDTVQNLTSR.S	7	2121.07571	0.24	6.13E-18	5.58	0.67	2	2313	1
Rh78	K.ILDIFNTILER.L	6	1346.76783	0.73	1.64E-14	4.17	0.38	2	1605.1	1
Rh78	K.IWSSVNLSR.V	1	1061.57382	-0.86	5.37E-11	1.81	0.00	2	84.5	7
Rh78	K.KNDTYYEVIDAFGLHQLM*SR.T	1	2416.16016	1.85	6.63E-17	4.25	0.53	2	596.8	1
Rh78	K.KNDTYYEVIDAFGLHQLMSR.T	2	2400.16526	0.82	3.37E-19	5.65	0.54	2	1320.5	1
Rh78	K.LDLESAGPFQR.S	7	1232.62698	0.29	4.88E-15	3.70	0.45	2	1009.9	1
Rh78	K.LESLQDVIASLGSGAR.D	8	1615.86498	1.15	1.34E-16	4.84	0.64	2	2653.5	1
Rh78	K.LPSSSSSSER.A	4	1036.49054	-0.19	9.77E-14	2.29	0.44	2	704.5	1
Rh78	K.LQTFLEQEAK.K	4	1206.63648	-0.14	2.43E-11	2.79	0.33	2	414.8	1
Rh78	K.LSAAIYGIDSR.Y	6	1165.62116	0.01	5.86E-15	3.82	0.56	2	1564.4	1
Rh78	K.LTVTSAEMVHQFMQTAPSATAAELARPELSAK.L	2	3386.70829	-0.49	7.25E-22	5.21	0.61	2	1274.1	1
Rh78	K.M*TSLSEQWSDLVTR.Q	1	1668.78975	-0.27	2.02E-16	3.37	0.45	2	904.3	1
Rh78	K.MDAM*EVATPDAK.H	1	1294.56535	-1.21	9.08E-10	2.42	0.24	2	266.1	1
Rh78	K.MTSLSEQWSDLVTR.Q	4	1652.79485	-0.17	6.49E-17	4.16	0.54	2	1350.3	1
Rh78	K.NDTYYEVIDAFGLHQLMSR.T	3	2272.07029	0.14	5.69E-22	4.32	0.66	2	1205.6	1
Rh78	K.NQIENHPGHPCYAQLPNFPVLIQQAQGR.L	5	3226.59635	0.38	1.23E-16	5.85	0.59	2	1062.1	1
Rh78	K.NVNTSSLTVLQR.A	6	1331.72775	0.14	3.91E-16	4.06	0.45	2	1555.5	1
Rh78	K.QQDFSTIVNAVNSR.R	5	1578.78706	-1.09	1.02E-18	4.38	0.61	2	510	1
Rh78	K.QQHLNTSEEVPFPMPPIPGNDITVASPLSFAK.G	4	3461.74097	-0.07	2.79E-14	5.17	0.62	2	640.9	1
Rh78	K.TLHGLYQEEQATR.I	3	1545.7656	-0.12	2.00E-17	4.35	0.51	2	1082.8	1
Rh78	K.TLKDETELMNTK.L	1	1422.71447	-0.43	1.78E-11	3.09	0.40	2	641.3	1
Rh78	K.VNYTHLPIR.L	3	1112.6211	-0.79	1.60E-12	2.94	0.38	2	821.2	1
Rh78	K.VQELLLR.L	3	870.54073	-0.18	1.25E-09	2.15	0.12	2	554.8	1
Rh78	K.WLQDAK.K	1	760.39882	0.38	8.05E-09	1.61	0.01	2	127.8	5
Rh78	K.YENLPNDQTM*K.R	1	1368.60999	0.47	7.02E-11	2.30	0.28	2	440.8	1
Rh78	K.YENLPNDQTMK.R	1	1352.61509	-0.07	7.91E-09	1.84	0.32	2	272.3	1
Rh78	K.YLALHHDEK.H	1	1125.56873	-1.23	2.81E-11	2.26	0.12	2	469.3	1
Rh78	R.AGSQCVSNCFMYLHALHLHGAHTTLSK.D	1	3040.43393	-0.43	4.58E-13	2.43	0.16	2	303.6	3
Rh78	R.AVFAQYSSSQGPK.V	6	1369.67466	-0.08	4.88E-18	3.80	0.56	2	1429	1
Rh78	R.AVILLDHDIFVFDPHASER.S	8	2194.12913	-1.00	3.20E-22	4.68	0.64	2	2702.5	1
Rh78	R.DAM*TVFSNSDYLEDYQR.Y	2	2069.87566	-0.23	2.21E-17	4.23	0.63	2	616.3	1
Rh78	R.DAMTVFSNSDYLEDYQR.Y	7	2053.88076	-1.13	7.03E-20	6.03	0.70	2	2522.4	1
Rh78	R.DDDNLHEVFIEK.I	4	1473.68561	-0.82	3.34E-14	3.91	0.38	2	1610.2	1
Rh78	R.DPDFLHVQR.A	2	1126.56398	0.48	7.82E-13	2.92	0.17	2	398.7	1
Rh78	R.EDPPPISR.P	1	910.46287	1.73	3.52E-11	1.87	0.25	1	271.2	1
Rh78	R.EDVTTVSPSAASSSFNNPPITTLTQNVISAIQILSSVR.V	20	3945.04513	0.11	4.07E-27	6.48	0.67	2	1848.8	1
Rh78	R.ELSNQLDYLITGQASSSNAM*SFSDELM*QLR.S	2	3380.56205	0.41	9.37E-15	3.79	0.48	2	490.3	1
Rh78	R.ELSNQLDYLITGQASSSNAM*SFSDELMQLR.S	2	3364.56715	1.39	2.21E-14	4.23	0.28	2	218.3	1
Rh78	R.ELSNQLDYLITGQASSSNAMSFSDELM*QLR.S	2	3364.56715	0.15	3.60E-16	3.81	0.33	2	474.6	1
Rh78	R.ELSNQLDYLITGQASSSNAMSFSDELMQLR.S	7	3348.57225	-0.37	3.40E-29	6.40	0.68	2	1895.7	1
Rh78	R.ESNTNTHLNTGEPIM*QTISIR.H	5	2372.15105	0.17	9.02E-17	5.51	0.65	2	1171.6	1
Rh78	R.ESNTNTHLNTGEPIMQTISIR.H	8	2356.15615	0.72	2.06E-20	5.42	0.65	2	958.5	1
Rh78	R.ETIMTVETVWATLEPK.R	8	1847.94593	1.29	3.03E-13	3.78	0.50	2	1156.6	1
Rh78	R.FDQTHSR.F	1	890.41151	1.43	1.64E-10	1.63	0.16	2	396.9	1
Rh78	R.GFSESIDR.F	4	910.42649	-1.00	2.34E-11	2.22	0.19	2	187.1	1
Rh78	R.HLEYTNR.D	2	932.45846	0.01	2.23E-09	1.84	0.25	2	312.4	1
Rh78	R.HLEYTNRDPDFLHVQR.A	4	2040.0046	-0.66	4.63E-17	3.25	0.46	2	1556.6	1
Rh78	R.IDDIPAVLPPTDIDHK.K	5	1758.92724	-0.29	1.95E-12	2.78	0.50	2	388.7	1
Rh78	R.IM*VENPFPTVPLIDVPESDLISFTR.V	16	2845.46919	0.47	6.63E-20	7.25	0.70	2	2511.9	1
Rh78	R.IMVENPFPTVPLIDVPESDLISFTR.V	13	2829.47429	-0.01	3.99E-22	6.13	0.64	2	1779	1

Rh78	R.IPIQDLCITITTFYPEYIYGIVK.H	1	2760.45689	0.02	1.76E-09	3.33	0.35	2	491.5	1
Rh78	R.LCDVNFTIPFR.E	10	1381.69332	0.15	2.21E-15	3.76	0.52	2	1045.5	1
Rh78	R.LEQAVNLAQEM*SELR.L	3	1746.86906	-1.01	2.59E-17	3.90	0.56	2	893	1
Rh78	R.LEQAVNLAQEMSELR.L	2	1730.87416	0.30	5.51E-17	4.17	0.61	2	1673.2	1
Rh78	R.LGEEIPNIITSSFGK.T	4	1604.85301	0.57	3.32E-16	4.03	0.49	2	728.4	1
Rh78	R.LM*EYVEEHPLDIDETFVCLK.Q	5	2496.16731	0.39	3.52E-20	4.55	0.51	2	938.8	1
Rh78	R.LMEYVEEHPLDIDETFVCLK.Q	4	2480.17241	0.22	1.88E-20	5.37	0.59	2	1470.3	1
Rh78	R.LNALTELVQYFVSNGK.V	4	1795.95888	-0.86	2.31E-15	4.44	0.61	2	1115.5	1
Rh78	R.M*EQLVMSSIEHASCLHTR.W	4	2144.98858	0.96	5.23E-13	3.47	0.22	2	940.7	1
Rh78	R.MEQLVM*SSIEHASCLHTR.W	1	2144.98858	-0.46	2.83E-12	2.74	0.13	2	318.5	3
Rh78	R.MEQLVMSSIEHASCLHTR.W	5	2128.99368	-0.33	7.54E-22	5.65	0.64	2	1787.9	1
Rh78	R.NQLSQETLR.T	10	1088.56946	-0.38	3.13E-12	2.98	0.33	2	946.8	1
Rh78	R.PFNGTAETR.D	4	992.47958	0.23	7.81E-13	2.68	0.39	1	340.9	1
Rh78	R.PGEKLPVFR.L	3	1042.60439	-0.05	1.46E-10	2.15	0.24	1	421.3	2
Rh78	R.PLQTIVR.R	1	826.51451	0.05	1.28E-09	1.52	0.10	1	102.4	2
Rh78	R.PTTPMYQQVR.Q	1	1220.60922	1.18	1.21E-10	1.50	0.10	1	70.9	66
Rh78	R.QGLTYIGTLM*R.N	4	1268.66672	-0.03	2.90E-13	2.22	0.45	2	354.9	1
Rh78	R.QGLTYIGTLMR.N	8	1252.67182	-0.37	7.81E-15	3.20	0.52	2	479.7	1
Rh78	R.QGM*VTSELAR.I	5	1107.54627	-0.10	1.01E-13	2.52	0.41	2	321.2	1
Rh78	R.QGMVTSELAR.I	7	1091.55137	-0.69	6.85E-13	2.35	0.40	2	342.7	1
Rh78	R.QICAVGPR.K	6	900.47203	-0.39	4.79E-11	2.52	0.32	2	99.4	1
Rh78	R.QISDLLER.K	2	973.53129	-1.34	1.27E-10	1.68	0.06	2	208	1
Rh78	R.QITTQQQEQVQQALQK.L	2	1898.99303	-1.01	2.45E-14	4.76	0.50	2	1393.8	1
Rh78	R.QLEM*QIQEAR.M	5	1261.6205	-0.29	6.49E-13	2.96	0.35	2	584.3	1
Rh78	R.QLEMQIQEAR.M	6	1245.6256	-0.29	3.13E-13	3.34	0.40	2	905.4	1
Rh78	R.QRPLQTIVR.R	3	1110.6742	-2.02	1.35E-11	2.25	0.23	2	202.1	1
Rh78	R.REDVTTVSPSAASSSFNNPPITTLTQNVISAIQILSSVR.V	35	4101.14624	-0.68	3.15E-32	7.52	0.72	2	3140.8	1
Rh78	R.RLEQAVNLAQEM*SELR.L	6	1902.97017	0.04	2.10E-13	3.75	0.40	2	1135.9	1
Rh78	R.RLEQAVNLAQEMSELR.L	7	1886.97527	0.45	3.87E-18	5.37	0.60	2	2482.1	1
Rh78	R.RQITTQQQEQVQQALQK.L	4	2055.09414	-1.21	6.34E-15	5.14	0.51	2	1558.5	1
Rh78	R.SLLATLSNLVK.L	5	1158.70925	0.32	1.95E-14	4.08	0.47	2	1608.4	1
Rh78	R.SQFTYATQTK.E	8	1174.57388	2.07	5.86E-14	3.21	0.46	2	971.8	1
Rh78	R.SSNITMSMIQEQLSELQQLGGGNM*PDIAK.R	1	3136.4959	1.49	2.17E-15	3.49	0.45	2	283.8	1
Rh78	R.SSNITMSMIQEQLSELQQLGGGNMPDIAK.R	4	3120.501	0.98	1.28E-18	4.70	0.59	2	1039.2	1
Rh78	R.SVFATVLTQQLETHK.K	4	1701.91701	-0.59	1.10E-18	3.74	0.39	2	518.4	1
Rh78	R.SVNETINR.L	3	932.47958	0.20	6.35E-11	1.75	0.31	2	279.1	1
Rh78	R.TFVEPVFR.Q	8	994.53564	-0.50	3.91E-12	2.30	0.34	2	540.4	1
Rh78	R.TGTPLHYLLSYGNVLFK.Y	6	1923.03746	0.96	1.81E-17	4.39	0.56	2	1068.3	1
Rh78	R.TIHSILRPPTTAER.D	11	1591.89147	-0.93	3.78E-15	2.67	0.48	2	608.7	1
Rh78	R.TLSNLVLQSM*FVEK.C	4	1624.86146	0.70	4.42E-13	3.62	0.45	2	1219.7	1
Rh78	R.TLSNLVLQSMFVEK.C	5	1608.86656	0.69	7.57E-16	4.49	0.52	2	1976.5	1
Rh78	R.TPLQLDFIPLIIYNAHDR.T	15	2139.1597	0.33	1.26E-21	7.28	0.61	2	3057.9	1
Rh78	R.VDAKNLTVGK.D	2	1044.60479	0.18	3.87E-12	1.96	0.16	2	236.1	20
Rh78	R.VHTVGSSVDVPEVVSEGETTSGDETEGEDFTHLPSEEEQQSLPSTR.T	9	4914.21954	-0.94	1.49E-29	7.33	0.72	2	1310.4	1
Rh78	R.VHVNNNASCENFVAQHESGGAITDR.E	5	2726.2336	-0.41	1.04E-21	6.02	0.72	2	1936.2	1
Rh78	R.VLWDHNLVR.Q	6	1151.632	-0.83	7.81E-13	3.07	0.38	2	764.6	1
Rh78	R.VPINTDFLR.E	7	1074.59422	-0.04	7.81E-13	2.94	0.46	2	740.4	1
Rh78	R.VSFDDKETK.D	2	1068.52078	0.13	6.35E-13	2.43	0.33	2	608.1	1
Rh78	R.VSLCIQDVLTGQDTHYQQYQEDAR.A	8	2867.3265	-0.78	1.73E-21	4.88	0.60	2	2048.1	1
Rh78	R.VSTTAPYNCLEALPALTR.Y	11	1977.01102	-0.49	3.21E-17	4.28	0.46	2	1437	1
Rh78	R.WRPTTPMYQQVR.Q	3	1562.78964	-2.82	1.77E-13	2.39	0.23	2	447.2	1
Rh78	R.YQKVQELLLR.M	1	1289.7576	0.31	4.15E-12	2.26	0.34	2	305.2	1
Rh78	R.YVLAGYQYLTECINR.Q	7	1862.91058	-0.10	5.32E-18	5.94	0.62	2	3137.5	1
Rh78	R.YVLAGYQYLTECINRQPLICQR.I	1	2758.38042	-5.96	1.98E-20	4.25	0.55	2	858.8	1
Rh79.1	K.YAFKLDLLR.M	3	1138.66191	0.46	3.28E-12	2.42	0.44	2	826.9	1
Rh79.1	R.HVCTNVLDLPQESM*EHPVTNTM*LAK.Y	2	2896.36378	-0.29	1.53E-10	2.48	0.35	2	190.7	1
Rh79.1	R.HVCTNVLDLPQESM*EHPVTNTMLAK.Y	4	2880.36888	-0.61	4.33E-18	5.41	0.27	2	653.3	1
Rh79.1	R.HVCTNVLDLPQESMEHPVTNTM*LAK.Y	6	2880.36888	-0.55	5.13E-18	5.47	0.42	2	1012.7	1
Rh79.1	R.HVCTNVLDLPQESMEHPVTNTMLAK.Y	13	2864.37398	-0.59	8.66E-23	6.84	0.63	2	1521.1	1
Rh79.1	R.M*SSYFTDK.Y	8	994.41861	-0.32	1.56E-11	2.31	0.44	2	274.8	1
Rh79.1	R.M*SSYFTDKYAFK.L	1	1503.68243	-0.47	2.63E-09	2.37	0.20	2	344.2	1
Rh79.1	R.MLAVAR.T	5	660.38614	-0.45	4.69E-09	1.88	0.19	2	319.3	1
Rh79.1	R.MSSYFTDK.Y	8	978.42371	-0.95	3.91E-12	2.31	0.47	2	737.7	1
Rh79.1	R.MSSYFTDKYAFK.L	3	1487.68753	0.50	4.88E-17	3.32	0.47	2	862.3	1

Rh83	K.NQTNTVCLLCELMACSHYDNLVLR.N	1	2924.35227	-0.91	9.20E-11	2.42	0.00	2	179.6	4
Rh88	K.RLRHGALGDQIR.R	1	1391.79784	-48.45	9.54E-09	2.08	0.22	2	256.6	6
Rh89	K.AASATKEFSLEEAYQMLLALQKLDQEK.R	1	3055.56563	-4.96	1.41E-16	2.89	0.21	2	840.6	1
Rh89	K.AEADDEDFASNGQSAGFLDR.L	1	2114.88975	-0.78	7.26E-20	5.44	0.68	2	1396.4	1
Rh89	K.ALELYSEDELR.S	20	1337.65834	1.22	3.91E-15	3.80	0.59	2	935.7	1
Rh89	K.ALGVAIGAVGGAVASFVEGVVGFIK.N	1	2288.30128	29.95	8.77E-13	5.27	0.73	2	907.9	1
Rh89	K.CVEVDQNSVK.V	6	1177.5518	-0.08	1.17E-11	2.86	0.41	2	1124.7	1
Rh89	K.EAPPSYEQSQYENIK.E	6	1782.81809	-0.82	1.31E-17	3.51	0.53	2	476.9	1
Rh89	K.EAPPSYEQSQYENIKEK.A	1	2039.95564	-6.09	4.89E-20	3.57	0.29	2	416.1	1
Rh89	K.EELNESDPSFQCIK.D	3	1695.75308	-0.50	4.88E-19	4.19	0.57	2	1122.9	1
Rh89	K.EFSLEEAYQM*LLALQK.L	7	1928.96738	0.40	5.13E-13	4.04	0.45	2	1251.1	1
Rh89	K.EFSLEEAYQMLLALQK.L	9	1912.97248	-0.31	6.66E-18	5.90	0.53	2	2629.9	1
Rh89	K.EFSLEEAYQMLLALQKLDQEK.R	1	2526.27962	-3.31	2.60E-14	2.40	0.36	2	322.4	1
Rh89	K.ESGVCYSR.P	10	957.40949	-0.20	1.00E-10	2.11	0.54	1	98.7	1
Rh89	K.ESGVCYSRPVVLYTFK.N	1	1904.95753	-4.81	1.69E-15	2.37	0.38	2	347	1
Rh89	K.INPSAM*LSAIYDKPIAAR.F	1	1947.03679	-0.52	4.74E-15	3.82	0.55	2	291.4	1
Rh89	K.INPSAMLSAIYDKPIAAR.F	5	1931.04189	-0.83	8.92E-18	4.73	0.65	2	1432.9	1
Rh89	K.PFEHFFPYVVPPTTVK.E	3	1904.99453	0.41	7.91E-12	3.36	0.53	1	704.1	1
Rh89	K.SIVYAQLQYTYDTLR.N	16	1833.93814	-0.11	5.50E-19	6.12	0.60	2	2959.7	1
Rh89	K.TSEEVLK.S	1	805.43018	-1.19	9.96E-09	1.77	0.06	2	353.5	5
Rh89	K.VFDKVPGYLR.G	5	1193.66772	-0.24	3.44E-11	2.77	0.30	2	780.3	1
Rh89	K.YPYDFFVTSDGK.V	7	1438.65252	0.15	4.50E-16	3.34	0.53	2	1416.7	1
Rh89	R.AYEKPFEHFFPYVVPPTTVK.E	14	2396.23253	-0.21	5.84E-16	4.82	0.61	2	1839	1
Rh89	R.CYNSVVR.N	14	897.42475	-0.71	7.81E-11	2.53	0.45	2	792.7	1
Rh89	R.DIRPYTFK.V	2	1039.55711	-0.54	3.13E-11	2.03	0.18	2	150.3	12
Rh89	R.DKFSVR.K	1	751.40971	-0.91	6.41E-09	1.74	0.12	2	162	1
Rh89	R.DNAPEVAHPLVGFFER.P	1	1797.89186	0.94	3.59E-14	3.06	0.55	1	353.6	1
Rh89	R.EFNTYK.Q	6	801.37775	-0.17	1.56E-09	1.95	0.22	2	196.8	1
Rh89	R.EFNTYKQR.M	2	1085.53743	-0.49	1.64E-11	1.98	0.28	2	104.4	11
Rh89	R.ENHLLGFSQEHLAVPM*WEVHYINK.L	3	2907.42464	-0.05	1.41E-15	3.23	0.34	2	611.3	1
Rh89	R.ENHLLGFSQEHLAVPMWEVHYINK.L	3	2891.42974	-1.08	1.09E-23	4.59	0.51	2	1226.9	1
Rh89	R.FEQNINCDSFK.P	1	1401.61038	0.16	7.86E-12	2.88	0.31	1	461.6	1
Rh89	R.FEQNINCDSFKPTK.E	4	1727.80578	-1.06	2.18E-17	3.87	0.51	2	1211.3	1
Rh89	R.FVGDVISLAK.C	9	1048.60372	-0.34	3.91E-14	2.98	0.33	2	1025.8	1
Rh89	R.GLDDM*M*SGLGSAGK.A	5	1370.59261	-0.16	1.40E-17	4.57	0.66	2	1633.9	1
Rh89	R.GLDDM*MSGLGSAGK.A	14	1354.59771	0.02	2.33E-17	4.20	0.63	2	1288.8	1
Rh89	R.GLDDMM*SGLGSAGK.A	8	1354.59771	-0.37	1.29E-17	3.99	0.60	2	1131	1
Rh89	R.GLDDMMSGLGSAGK.A	12	1338.60281	0.00	2.44E-19	4.42	0.59	2	2740.8	1
Rh89	R.HRTEACEYPSLK.I	5	1490.70567	1.80	7.93E-13	4.40	0.50	2	1826.6	1
Rh89	R.KAEADDEDFASNGQSAGFLDR.L	5	2242.98471	-1.05	3.14E-18	6.02	0.69	2	2293.3	1
Rh89	R.M*IPLDSISTVDTM*ISLDIDPLENTDFK.A	2	3055.47373	0.49	3.59E-12	4.58	0.49	2	529.9	1
Rh89	R.M*IPLDSISTVDTMISLDIDPLENTDFK.A	6	3039.47883	0.61	1.57E-19	7.22	0.29	2	2548.3	1
Rh89	R.MIHVEGK.V	4	813.42874	0.03	1.56E-10	1.77	0.29	2	434.9	1
Rh89	R.MIHVEGKVFDK.V	1	1302.68747	0.71	8.60E-12	2.40	0.31	2	336.6	1
Rh89	R.MIPLDSISTVDTM*ISLDIDPLENTDFK.A	4	3039.47883	-0.25	3.54E-20	5.48	0.25	2	1221.6	1
Rh89	R.MIPLDSISTVDTMISLDIDPLENTDFK.A	9	3023.48393	0.62	1.83E-24	6.94	0.67	2	1595.9	1
Rh89	R.NVAGATYVNYHR.D	19	1364.67057	-0.02	5.50E-17	4.07	0.64	2	1349.5	1
Rh89	R.PVVLYTFK.N	7	966.56588	0.18	7.81E-12	2.77	0.50	1	709.9	1
Rh89	R.QIAEAWCK.D	7	1005.48226	-0.70	1.64E-11	2.38	0.49	2	268.2	1
Rh89	R.QSYSFIR.E	1	900.45739	-1.07	4.69E-10	1.61	0.20	2	150	1
Rh89	R.SEADDTYHYTSSSM*TATFLTSK.E	8	2459.05548	-0.64	6.84E-18	5.15	0.77	2	1741.5	1
Rh89	R.SEADDTYHYTSSSMTATFLTSK.E	10	2443.06058	-0.16	1.01E-22	5.25	0.70	2	2143.7	1
Rh89	R.SKYPYDFFVTSDGK.V	4	1653.77951	-0.70	6.82E-17	3.79	0.60	2	2048.9	1
Rh89	R.SSNVFDLEDIM*R.E	12	1441.66276	0.41	3.91E-16	4.23	0.57	2	1351.1	1
Rh89	R.SSNVFDLEDIMR.E	20	1425.66786	-0.34	1.95E-16	4.31	0.51	2	1385.3	1
Rh89	R.TEACEYPSLK.I	10	1197.54565	-0.44	1.95E-14	3.26	0.59	2	1119.8	1
Rh89	R.TKESGVCYSR.P	4	1186.55213	0.10	3.28E-12	2.79	0.48	1	806.8	1
Rh89	R.TLEVFK.E	3	736.42397	-0.51	3.13E-09	1.94	0.08	2	442.4	1
Rh89	R.VCSM*AQGTDLLR.F	16	1366.64537	-1.04	8.01E-16	4.02	0.57	2	1273.1	1
Rh89	R.VCSMAQGTDLLR.F	17	1350.65047	-0.55	2.05E-16	3.84	0.59	2	1186.1	1
Rh92	R.LNDCDAVR.V	1	962.43604	-6.42	1.39E-10	2.17	0.20	2	494.9	1

A)



B)

	RhCMV 68-1		RhCMV Δpp65ab	
Source	viral	host	viral	host
% peptides	63.4	36.6	69.9	30.1
mol% protein	64.8	35.2	68.5	31.5

C)

Protein ID	wt	Δpp65
s100 calcium-binding protein a11	1.64	0.00
s100 calcium-binding protein a11	1.14	0.00
annexin a5	1.04	0.00
cd81	0.80	0.00
programmed cell death protein 6	0.65	0.00
annexin a4	0.56	0.00
gtp-binding protein smg p21b	0.55	0.00
lrrn4 c-terminal-like protein	0.50	0.00
40s ribosomal protein s26	0.47	0.00
autosomal recessive hypercholesterolemia protein	0.45	0.00
s100 calcium-binding protein a4	0.41	0.00
cytokeratin-16	0.39	0.00
chronic lymphocytic leukemia deletion region gene 6 protein homolog	0.33	0.00
copine i	0.32	0.00
annexin a11	0.32	0.00
60s ribosomal protein l13	0.31	0.00
elongation factor 1-alpha-like 3	0.31	0.00
60s ribosomal protein l34	0.29	0.00

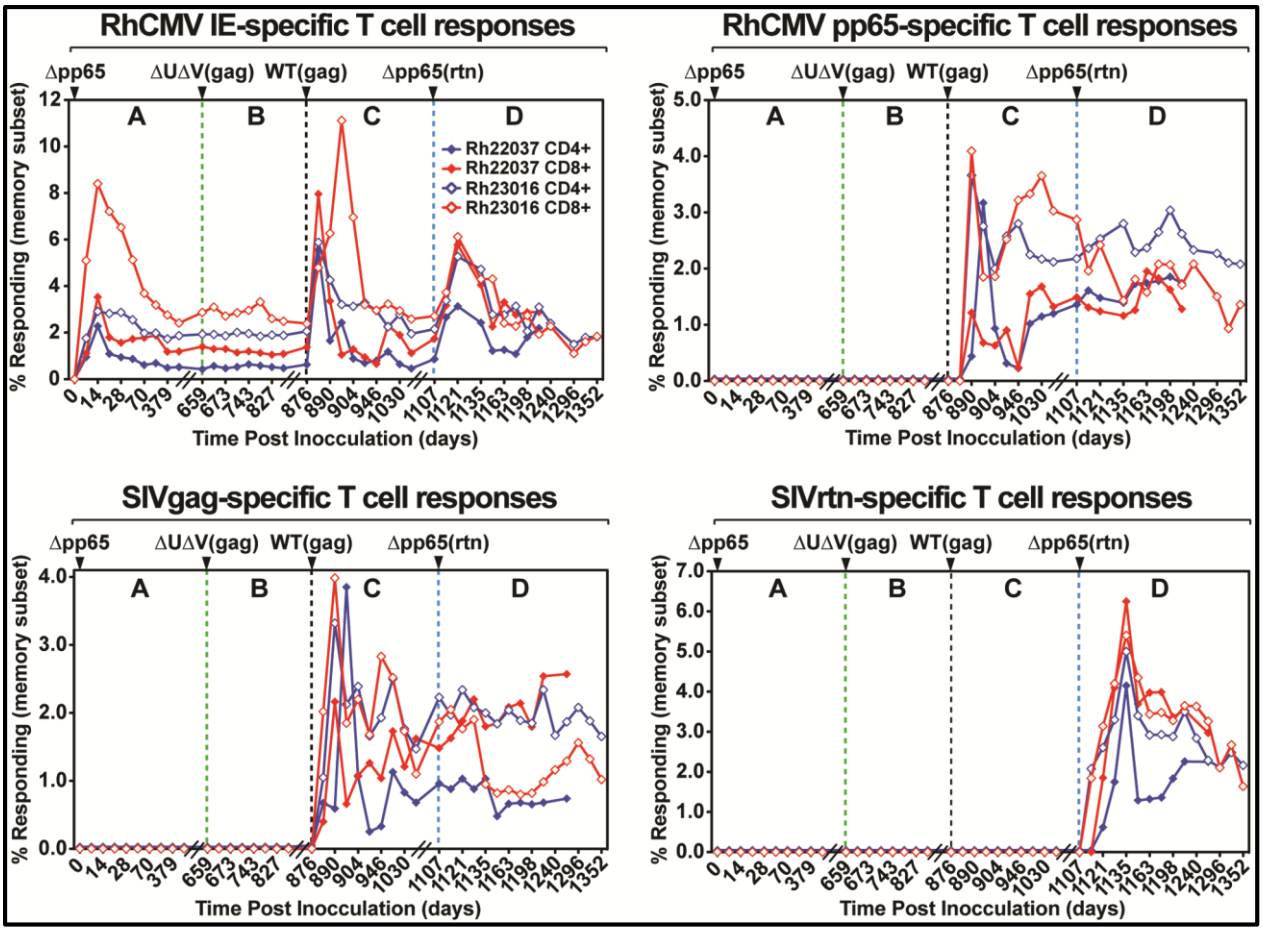
Protein ID	wt	Δpp65	Fold Change
60s ribosomal protein l6	0.33	0.04	8.68
sodium potassium-transporting atpase subunit alpha-1	0.48	0.06	7.59
4f2 cell-surface antigen heavy chain	0.45	0.07	6.54
40s ribosomal protein s27a	0.90	0.18	5.13
hemoglobin alpha chain	1.02	0.21	4.75
cytokeratin-9	1.09	0.26	4.22
glucose transporter type erythrocyte brain	0.41	0.11	3.73
elongation factor 1-alpha 2	0.36	0.10	3.71
40s ribosomal protein s27a	0.33	0.09	3.64
annexin a1	0.46	0.14	3.33
cytokeratin-10	1.88	0.67	2.79
annexin a2	1.41	0.51	2.78
casein kinase ii subunit alpha	0.50	0.19	2.57
cytokeratin-2e	0.53	0.23	2.36
fibronectin	0.36	0.17	2.10
vesicle transport through interaction with t-snares homolog 1a	0.82	0.40	2.04

D)

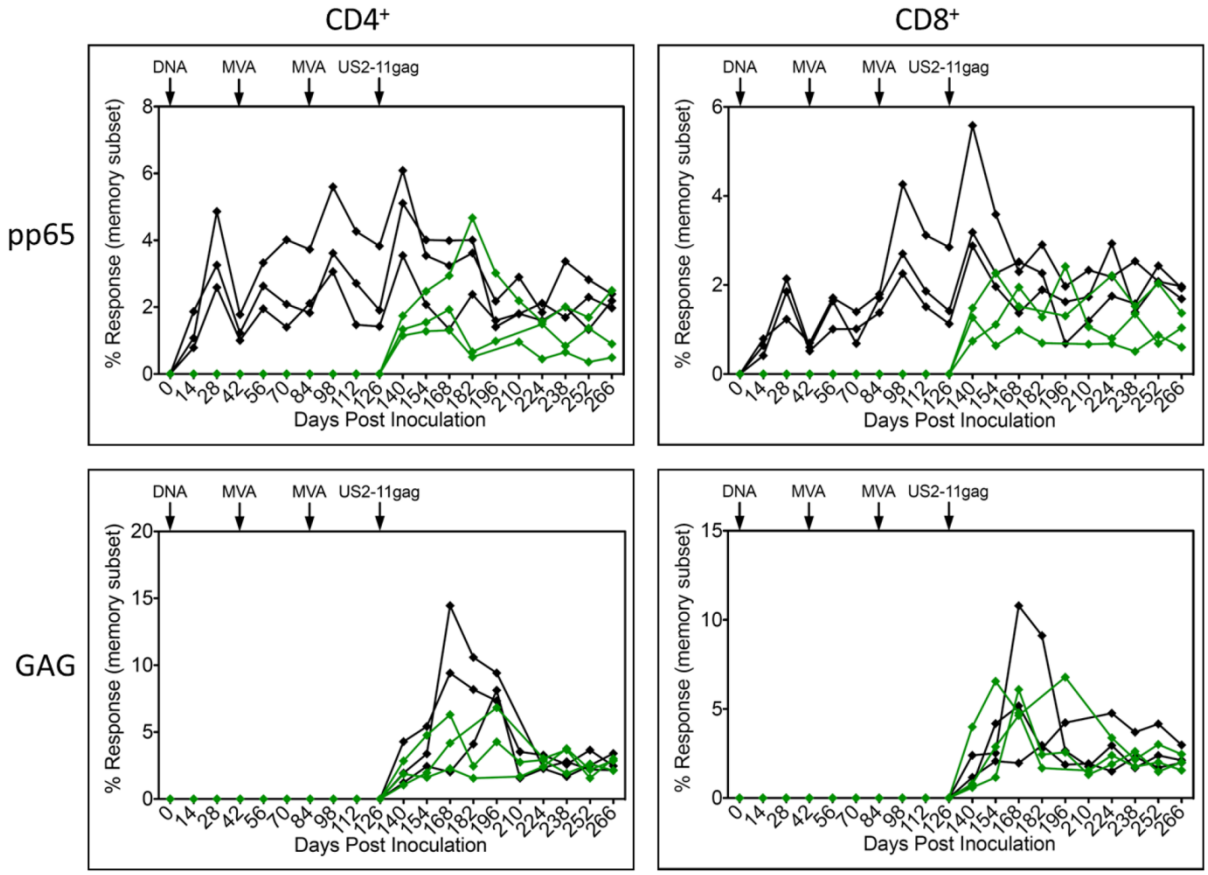
Protein ID	wt	Δpp65
marvel domain-containing protein 1	0.00	0.85
intestinal membrane a4 protein	0.00	0.61
class i histocompatibility gogo-b*0101 alpha chain	0.00	0.57
trypsinogen c	0.00	0.44
heterogeneous nuclear ribonucleoprotein x	0.00	0.33
transmembrane protein 179b	0.00	0.33
lim mineralization protein	0.00	0.32
aurora ipl1-related kinase 1	0.00	0.28
integral membrane protein 2b	0.00	0.27
vitronectin	0.00	0.26
glyceraldehyde-3-phosphate dehydrogenase	0.00	0.25
hla-b-associated transcript 3	0.00	0.25

Protein ID	wt	Δpp65	Fold Change
cd155	0.17	0.86	5.06
peptidyl-prolyl cis-trans isomerase a	0.24	1.19	4.89
l-lactate dehydrogenase b chain	0.07	0.31	4.44
l-lactate dehydrogenase c chain	0.06	0.26	4.44
l-lactate dehydrogenase a chain	0.06	0.25	4.44
mapk-activating protein pm28	0.08	0.35	4.23
syntaxin-12	0.08	0.28	3.38
histone h3	0.23	0.76	3.33
histone h3	0.22	0.74	3.33
mhc class i antigen patr-a*0401	0.20	0.62	3.10
eukaryotic initiation factor 4a-ii	0.10	0.27	2.76
mhc class i antigen a*69	0.19	0.47	2.45
mhc class i antigen e	1.93	4.61	2.39
sorting nexin-17	0.47	1.10	2.33
beta-2-microglobulin	0.26	0.60	2.31
histone h2b type 1-j	0.25	0.54	2.18
histone h2b type 1-f j l	0.25	0.54	2.18
histone h2b 291b	0.25	0.54	2.18
mhc class i antigen e	1.11	2.39	2.16
lysosomal-associated membrane protein 3	0.53	1.11	2.11
histone h2a q	0.75	1.57	2.10
serine protease 11	0.22	0.45	2.05

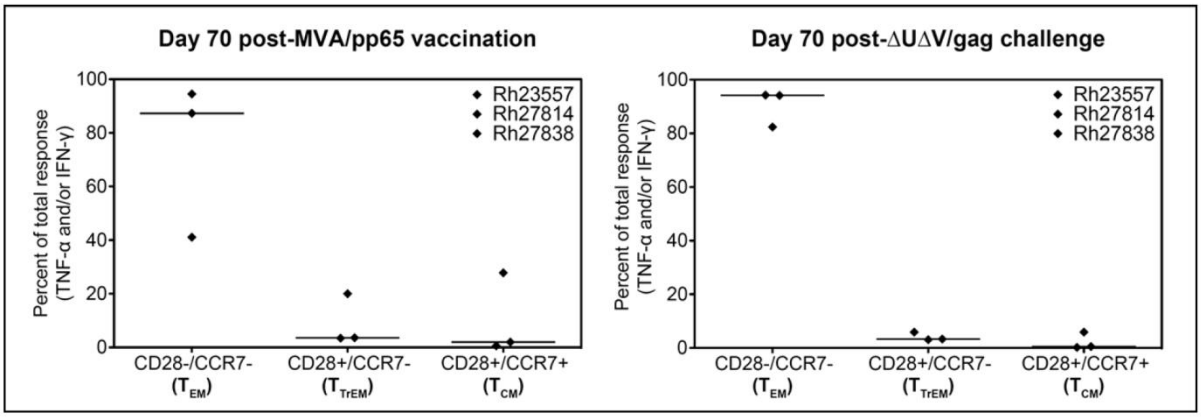
Supplemental Figure 3. Comparison of host proteins contained in WT and Δpp65ab virions. A) The total number of host proteins found in RhCMV WT and Δpp65ab virions and the overlapping proteins found in both samples are shown. B) All peptides and proteins found in WT and Δpp65ab virions as shown here separated into either host or viral proteins dependent on their origin. C) Host proteins with a minimum abundance of 0.25mol% of the total amount of host proteins were ranked by abundance into two groups, proteins that had significant abundance in WT virions and were not found in Δpp65ab virions (upper panel) and host proteins that were found in both virions, but with at least two fold higher abundance in the WT. D) Similar to C), host proteins were ranked by abundance, but only proteins are shown that were either present in Δpp65ab virions and not in the WT (upper panel) or host proteins that were found in both virions, but with at least two fold higher abundance in Δpp65ab.



Supplemental Figure 4. Δpp65ab establishes primary and secondary infections and protects against super-infection with ΔUS2-11.. A) Two RhCMV sero-negative male RM (RM1 and RM2) were infected s.c with 10^7 PFU of Δpp65ab at day 1. $CD4^+$ (blue) and $CD8^+$ (red) T-cell responses were monitored in broncho-alveolar lavages (BAL) by intracellular cytokine staining (ICCS) at the indicated days using overlapping peptides of pp65ab and IE1/2) On day 659 the two animals were inoculated s.c. with 10^7 PFU of ΔUS2-11gag (green dotted line) and the T cell response to SIVgag was measured in addition. Note the absence of a T cell response to SIVgag or pp65 and a lack of boosting of responses to IE1. C) On day 876, the two RM were inoculated with 10^7 PFU of WTgag (black dotted line) and the T cell response was monitored by ICCS. Note the appearance of *de novo* responses to SIVgag and pp65 and a boosting of the T cell response to IE1. D) On day 1107 the two RM were inoculated with 10^7 PFU of Δpp65ab-rtn (blue dotted line). Using overlapping 15mer peptides a *de novo* response to SIVrev/tat/nef was detectable indicating super-infection. Also note a boosting of the IE1 response but not of pp65 or SIVgag-specific responses.



Supplemental Figure 5. T cells induced by heterologous prime/boost vaccination with pp65b do not protect against super-infection with ΔUS2-11. Three CMV-negative RM were vaccinated with 1mg of pND/pp65b and boosted with MVApp65b at 6 and 12 weeks after the initial vaccination (black). As controls three CMV-negative RM were vaccinated with the parental pND plasmid not expressing any antigen and boosted with WT MVA at 6 and 12 weeks after the initial vaccination (green). At 18 weeks after the initial DNA vaccination both groups of animals were challenged with 10^7 PFU of ΔUS2-11gag. The left two panels show the specific T-cell responses to pp65 whereas the right two panes show specific T-cell responses to SIV gag. T-cells were isolated from broncho-alveolar lavages (BAL).



Supplemental Figure 6. Pp65b-specific T cells induced in naïve RM after DNA prime and MVA boost vaccination show mostly effector memory (T_{EM}) phenotype at the time of RhCMV ΔUΔV challenge. T cells were isolated from peripheral blood drawn from the three RM described in Figure 6 (Supplemental Figure 3) at the times indicated above each dot plot. The memory phenotype of the total pp65b response was determined by flow cytometry using the cell surface markers CD28 and CCR7 as previously described [353].

Curriculum Vitae

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Declaration

according to §6(1) of the doctoral degree regulations of the Faculty of Natural Science, Gottfried Wilhelm Leibniz Universität Hannover I declare that the work presented is my own and has not been submitted previously in any form for another degree at any university or institution. All additional help by others and sources of information used have been acknowledged within individual chapters.

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Daniel Malouli

Erklärung

gemäß §6(1) der Promotionsordnung der Naturwissenschaftlichen Fakultät der Gottfried Wilhelm Leibniz Universität Hannover für die Promotion zum Dr. rer. nat. Hierdurch erklare ich, dass ich meine Dissertation selbststandig verfasst und die benutzten Hilfsmittel und Quellen sowie gegebenenfalls die zu Hilfeleistungen herangezogenen Institutionen vollstandig angegeben habe. Die Dissertation wurde nicht schon als Masterarbeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

……

Daniel Malouli

List of Publications

1. Verweij M.C., Wellish M., **Malouli D.**, Lapel M., Jonjić S., Haas J., DeFilippis V.R., Mahalingam R. Früh K. Simian Varicella Virus Inhibits Interferon-Stimulated JAK-STAT Signaling through Multiple Mechanisms. *PLoS Pathog*. 2014 (submitted).

2. Viswanathan K.*, Verweij M.C.*, **Malouli D.***, Früh K., Quantitative membrane proteomics reveals a role for Tetraspanin Enriched Microdomains during Entry of Human Cytomegalovirus. *J Virol*. 2014 (in revision).

3. **Malouli D.**, Howell G.L., Legasse A.W., Kahl C., Axthelm M.K., Hansen S.G., Früh K. Full genome sequence analysis of a novel adenovirus of rhesus macaque origin indicates a new simian adenovirus type and species. *Virology Reports.* 2014 Sep–Dec; 3:18–29.

4. **Malouli D.***, Hansen S.G.*, Nakayasu E.S., Marshall E.E., Hughes C.M., Ventura A.B., Gilbride R.M., Lewis M.S., Xu G., Kreklywich C., Whizin N., Fischer M., Legasse A.W., Viswanathan K., Siess D., Camp D.G. 2nd, Axthelm M.K., Kahl C., DeFilippis V.R., Smith R.D., Streblow D.N., Picker L.J.[#], Früh K..[#] Cytomegalovirus pp65 limits dissemination but is dispensable for persistence. *J Clin Invest.* 2014 May 1;124(5):1928-44.

5. Hansen, S.G., Sacha, J.B., Hughes, C.M., Ford, J.C., Burwitz, B.J., Scholz, I., Gilbride, R.M., Lewis, M.S., Gilliam, A.N., Ventura, A.B., **Malouli D.**, Xu G., Richards R., Whizin N., Reed J.S., Hammond K.B., Fischer M., Turner J.M., Legasse A.W., Axthelm M.K., Edlefsen P.T., Nelson J.A., Lifson J.D., Früh K., Picker L.J. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. *Science.* 2013 May 24; 340:1237874.

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10. Powers C., DeFilippis V., **Malouli D.**, and Früh K.. Cytomegalovirus immune evasion. *Current topics in microbiology and immunology.* 2008; 325:333-359.

*Shared First Authorship, #Shared Corrsponding Authorship

Conference Presentations

Oral Presentations

1. **Malouli D.**, Burwitz B.J., Ventura A.B., Hancock M.H., Bhusari A., Reed J.S., Hammond K.B., Espinosa Najera R.G., Bimber B.N., Legasse A.W., Axthelm M.K., Nelson J.A., Hansen S.G., Picker L.J., Früh K., Sacha J.B. Characterization of cytomegalovirus isolated from cynomolgus macaques (Macaca fascicularis) and identification of viral factors controlling crossspecies infection with rhesus cytomegalovirus. 39th Annual International Herpesvirus Workshop, *Kobe*, Japan, July 2014

2. **Malouli D.**, Hansen S.G., Nakayasu E.S., Marshall E.E., Hughes C.M., Ventura A.B., Gilbride R.M., Lewis M.S., Xu G., Kreklywich C., Whizin N., Fischer M., Legasse A.W., Viswanathan K., Siess D., Camp D.G. 2nd, Axthelm M.K., DeFilippis V.R., Smith R.D., Streblow D.N., Picker L.J., Früh K. Rhesus Cytomegalovirus pp65 Homologues are Dispensable for Persistence and Immunity. 38th Annual International Herpesvirus Workshop, *Grand Rapids*, Michigan, USA, July 2013

3. **Malouli D.**, Nakayasu E.S., Viswanathan K., Camp D.G. 2nd, Chang W.L., Barry P.A., Smith R.D., Früh K., Re-evaluation of the coding potential and proteomic analysis of the BAC derived Rhesus cytomegalovirus strain 68-1. 37th Annual International Herpesvirus Workshop, Calgary, Alberta, Canada, August 2012

4. **Malouli D.**, Hansen SG., Marshall EE., Hughes CM., Lewis MS., Axthelm MK., Siess D., DeFilippis VR., Picker LJ., Früh K., The Rhesus Cytomegalovirus major tegument proteins pp65a and pp65b are dispensable for replication *in vitro* and persistent infection *in vivo*. 36th Annual International Herpesvirus Workshop, Gdańsk, Poland, July 2011

Poster Presentations

1. **Malouli D.**, Burwitz B.J., Ventura A.B., Hancock M.H., Bhusari A., Reed J.S., Hammond K.B., Espinosa Najera R.G., Bimber B.N., Legasse A.W., Axthelm M.K., Nelson J.A., Hansen S.G., Picker L.J., Früh K., Sacha J.B. Characterization of cytomegalovirus isolated from cynomolgus macaques (Macaca fascicularis) and identification of viral factors controlling crossspecies infection with rhesus cytomegalovirus. 39th Annual International Herpesvirus Workshop, *Kobe*, Japan, July 2014

2. **Malouli D.**, Hansen S.G., Nakayasu E.S., Marshall E.E., Hughes C.M., Ventura A.B., Gilbride R.M., Lewis M.S., Xu G., Kreklywich C., Whizin N., Fischer M., Legasse A.W., Viswanathan K., Siess D., Camp D.G. 2nd, Axthelm M.K., DeFilippis V.R., Smith R.D., Streblow D.N., Picker L.J., Früh K. Rhesus Cytomegalovirus pp65 Homologues are Dispensable for Persistence and Immunity. 38th Annual International Herpesvirus Workshop, *Grand Rapids*, Michigan, USA, July 2013

3. **Malouli D.**, Nakayasu E.S., Viswanathan K., Camp D.G. 2nd, Chang W.L., Barry P.A., Smith R.D., Früh K., Re-evaluation of the coding potential and proteomic analysis of the BAC derived Rhesus cytomegalovirus strain 68-1. 37th Annual International Herpesvirus Workshop, Calgary, Alberta, Canada, August 2012

4. **Malouli D.**, Hansen SG., Marshall EE., Hughes CM., Lewis MS., Axthelm MK., Siess D., DeFilippis VR., Picker LJ., Früh K., The Rhesus Cytomegalovirus major tegument proteins pp65a and pp65b are dispensable for replication *in vitro* and persistent infection *in vivo*. 36th Annual International Herpesvirus Workshop, Gdańsk, Poland, July 2011

5. **Malouli D.**, DeFilippis VR., Früh KJ.. Rhesus Cytomegalovirus inhibits Type I Interferon dependent signaling through the JAK/STAT pathway. 35th Annual International Herpesvirus Workshop, Salt Lake City, Utah, USA, July 2010