

Defining and Characterising the anti-HCV Actions of the Interferon-Induced Transmembrane proteins

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Table of Contents

List of Figures and Tables	ix
Abstract	xiii
Declaration	xv
Acknowledgements	xvi
Publications Arising from this PhD	xvii
Awards Received During PhD	xvii
Presentations Arising From PhD	xvii
Materials Providers	xix
Abbreviations Used	xxi
Chapter 1	1
Introduction	1
1.1 Hepatitis C Virus	1
1.1.1 Epidemiology	1
1.1.2 Transmission	2
1.1.3 Pathogenesis.....	2
1.1.4 Treatment	3
1.1.5 The HCV genome	5
1.1.6 Classification of genotypes	6
1.1.7 HCV proteins	7
1.1.8 HCV life cycle	11
1.1.9 HCV model systems	13
1.2 Innate Immune Response to HCV	17
1.2.1 Activation of the innate immune response.....	17
1.2.2 Cellular Recognition of HCV	19
1.2.3 Interferon Transduction Pathway.....	20

1.2.4 Interferon-stimulated genes	21
1.2.5 HCV Evasion of Innate Immune Response	26
1.3 Interferon-Induced Transmembrane (IFITM) Protein	28
1.3.1 IFITMs: protein structure and cellular distribution	29
1.3.2 Biological functions of the IFITM proteins	32
1.3.3 Antiviral Actions of the IFITM proteins.....	34
1.3.4 IFITM proteins in hepatocytes and HCV.....	39
1.4 Hypothesis and Aims	40
Chapter 2	42
Materials and Methods.....	42
2.1 General Reagents	42
2.1.1 Transient transfection of plasmid DNA	42
2.1.2 Lentiviral packaging of IFITM protein to generate overexpression cell lines	42
2.2 Tissue Culture Techniques.....	44
2.2.1 Tissue culture medium.....	44
2.2.2 Maintenance of cell lines	45
2.2.3 Cryopreservation of cultured cells	45
2.2.4 Resuscitation of frozen cells	46
2.2.5 Trypan blue exclusion.....	46
2.3 Cultured Cell Lines.....	46
2.3.1 Huh-7	46
2.3.2 NNeoC-5B (RG)	46
2.3.3 Huh-7.5	47
2.3.4 Huh-7+IFITM	47
2.3.5 Huh-7+Empty	47
2.3.6 Huh-7+shIFITM1.....	47
2.4 HCVcc Infectious System	48

2.4.1	Generation of HCVcc viral stock.....	48
2.4.2	General infection protocol for HCVcc.....	51
2.4.3	HCVpp Assay	51
2.4.4	Extracellular:Intracellular Infectivity Assay.....	51
2.5	General Molecular Biology Methods	52
2.5.1	Synthetic oligonucleotides	52
2.5.2	Bacterial transformation.....	53
2.5.3	Mini-preparation (small scale) of plasmid DNA	54
2.5.4	Maxi-preparation (large scale) of plasmid DNA	54
2.5.5	Restriction endonuclease digestion.....	54
2.5.6	Agarose gel electrophoresis	55
2.5.7	DNA ligation.....	55
2.5.8	Gel purification	55
2.5.9	DNA sequencing.....	56
2.5.10	Extraction of total RNA.....	56
2.5.11	<i>DNAseI</i> treatment of RNA samples	57
2.5.12	Nucleic acid quantification	57
2.5.13	cDNA preparation.....	57
2.5.14	Polymerase Chain Reaction	58
2.5.15	High Fidelity PCR	58
2.5.16	Real-Time Quantitative PCR.....	59
2.5.17	Extraction of cellular protein	60
2.5.18	SDS PAGE and protein transfer	60
2.5.19	Western blotting.....	61
2.5.20	Immunoprecipitation.....	62
2.5.21	Dual Renilla luciferase assay	62
2.5.22	Site-Directed Mutagenesis	62
2.5.23	Immunofluorescence microscopy	63
2.6	Data Analysis	66

Chapter 3	67
The impact of the IFITM proteins on HCV replication.....	67
3.1 Introduction.....	67
3.2 Characterisation of endogenous IFITM proteins <i>in vitro</i>	68
3.3 Regulation of IFITM1 by IFN in hepatocytes	69
3.3.1 IFITM1 is upregulated by IFN- α in Huh-7 cells.....	69
3.3.2 IFITM1 is upregulated by type I and III IFNs in primary human hepatocytes (PHH).....	70
3.4 Knockdown of endogenous IFITM1 reduces the anti-HCV activity of IFN-α	70
3.5 Cloning and characterisation of IFITM1, IFITM2 and IFITM3.....	72
3.5.1 Cloning of IFITM1, IFITM2 and IFITM3 into a lentiviral expression vector	72
3.5.2 Transient expression of IFITM proteins decreases HCV replication	73
3.5.3 Producing lentiviral particles encoding IFITM1, IFITM2 and IFITM3	74
3.5.4 IFITM proteins may have a role at the early stages of HCV infection <i>in vitro</i>	75
3.6 Generation of stable IFITM Huh-7 cell lines	76
3.6.1 Stable expression of IFITM1, IFITM2 and IFITM3 decreases Jc1 replication	77
3.7 Characterising the role of the IFITM proteins on the different stages of the HCV lifecycle	78
3.7.1 IFITM1, IFITM2 and IFITM3 decrease HCV entry into Huh-7 cells.....	78
3.7.2 The IFITM proteins have no effect on HCV RNA replication.....	79
3.7.3 The IFITM proteins have no effect on HCV IRES activity.....	80
3.7.4 The IFITM proteins have no effect on HCV egress	81
3.8 Discussion	82
Chapter 4	87

Characterising the anti-HCV effect of IFITM1 at the Molecular Level.....	87
4.1 Introduction.....	87
4.2 Localisation of the IFITM proteins in the context of the essential host entry factors for HCV.....	88
4.2.1 The IFITM proteins do not co-localise with the tight junction protein Occludin (OCLN)	88
4.2.2 The IFITM proteins do not co-localise with the tight junction protein Claudin-1 (CLDN1) at the cell surface.....	88
4.2.3 The IFITM proteins do not co-localise with the cell surface protein SR-BI	89
4.2.4 IFITM1 co-localises with the HCV entry factor CD81 on the hepatic cell surface	90
4.3 IFITM1 interacts with CD81 on the hepatic cell surface.....	90
4.4 Mutagenic analysis reveals important regions of IFITM1 for anti-HCV activity.....	92
4.4.1 Characterisation of a panel of IFITM1 mutants.....	92
4.4.2 The N-terminal region of IFITM1 is important for its anti-HCV activity....	93
4.4.3. The C-terminal extension of IFITM1 is important for localisation within the hepatocyte	95
4.5 Discussion	96
Chapter 5	101
Cellular localisation of IFITM2 and IFITM3	101
5.1 Introduction.....	101
5.2 Localisation of IFITM2 and IFITM2 within Huh-7 cells.....	102
5.2.1 IFITM2 and IFITM3 do not localise to the endoplasmic reticulum (ER) ..	102
5.2.2 IFITM2 and IFITM3 do not localise to the Golgi complex.....	102
5.2.3 IFITM2 and IFITM3 do not localise to lipid droplets	103
5.2.4 IFITM3 partially co-localises with the early endosome	103
5.2.5 IFITM2 partially co-localises with the late endosome	104

5.2.6 IFITM2 and IFITM3 localise to the lysosome, and IFITM1 partially co-localises with the lysosome.....	104
5.2.7 The IFITM proteins do not associate with VAP-A in the hepatocyte	105
5.3 Discussion	106
Chapter 6	111
Post-translational modifications of the IFITM proteins are essential for anti-HCV activity.....	111
6.1 Introduction.....	111
6.2 N-terminal tyrosine phosphorylation of IFITM2 and IFITM3 is essential for cellular localisation but not for anti-HCV activity	112
6.2.1 IFITM1, IFITM2 and IFITM3 undergo tyrosine phosphorylation in Huh-7 cells.	112
6.2.2 Identification of conserved and non-conserved tyrosine residues between the IFITM proteins.....	113
6.2.3 Y19 and Y20 are responsible for IFITM2 and IFITM3 phosphorylation, while the conserved Y78 is responsible for IFITM1 phosphorylation.	114
6.2.4 The N-terminal tyrosine residue is required for the endosomal localisation of IFITM2 and IFITM3	115
6.2.5 IFITM2:Y19A and IFITM3:Y20A enhances anti-HCV activity compared to wildtype.....	115
6.2.6 IFITM2:Y19A and IFITM3:Y20A co-localise with CD81 on the hepatic cell surface	117
6.3 S-palmitoylation of the IFITM proteins is crucial for anti-HCV activity ..	118
6.3.1 Generation of IFITM mutants targeting S-palmitoylation sites.....	118
6.3.2 A single conserved cysteine residue in the CIL is important for the anti-HCV properties of the IFITM proteins.....	119
6.3.3 Mutation of conserved cysteine residues that undergo S-palmitoylation localise the IFITM proteins predominantly to the lysosome	119

6.4 Discussion	120
Chapter 7	126
Conclusions and Future Directions	126
Appendices.....	137
Appendix I. General Solutions and Buffers.....	137
Appendix II. Infectious HCV Constructs.	140
Appendix III. pGem-T Easy	141
Appendix IV. pLenti6/V5-D-TOPO	142
Appendix V. pLenti6/V5-D-TOPO/IFITM (IFITM1, IFITM2, IFITM3).....	143
Appendix VI. PRL-HL	144
References.....	145

List of Figures and Tables

Figure Number		On page:
Chapter 1		
Figure 1.1	Clinical spectrum of HCV infection	3
Figure 1.2	Progression of HCV-induced liver disease	3
Figure 1.3	HCV Genome and Polyprotein processing	6
Figure 1.4	Global HCV genotype distribution	6
Figure 1.5	Model of HCV entry	11
Figure 1.6	Schematic representation of the HCV lifecycle	12
Figure 1.7	HCV Model Systems	15
Figure 1.8	Cellular recognition pathways of HCV	19
Figure 1.9	IFN- α/β signal transduction pathway	21
Figure 1.10	HCV evasion of the innate immune response	26
Figure 1.11	Schematic representation of IFITM1, IFITM2 and IFITM3 and proposed topological models	29
Table 1.1	Summary of post-translational modifications undergone by IFITM3	30
Table 1.2	Summary of viruses inhibited by IFITM proteins	34
Chapter 2		
Table 2.1	Cell lines and culture conditions used in this study	44
Table 2.2	Primer Sequence	52
Table 2.3	Western Blot Antibody Concentrations	61
Table 2.4	Immunofluorescence Antibody concentrations	64
Chapter 3		
Figure 3.1	IFN- α induces the expression of IFITM1 in Huh-7 cells	68
Figure 3.2	High level of sequence conservation prevents specific detection of IFITM2 and IFITM3 mRNA in Huh-7 cells	69
Figure 3.3	IFN- α induces the expression of IFITM1 in Huh-7 cells	69
Figure 3.4	IFN- α and IFN- λ induces IFITM1 expression in PHH	70
Figure 3.5	shRNA mediated knockdown of endogenous IFITM1	71

	in Huh-7 cells	
Figure 3.6	IFITM1 knockdown reduces the anti-HCV activity of IFN- α	72
Figure 3.7	Analysis of IFITM clones following ligation into the lentiviral expression vector	72
Figure 3.8	Transient detection of IFITM1, IFITM2 and IFITM3 in Huh-7 cells	73
Figure 3.9	Transient expression of IFITM proteins in Huh-7 cells can decrease HCV replication	73
Figure 3.10	Transient transduction detection of IFITM1, IFITM2 and IFITM3 in Huh-7 cells	74
Figure 3.11	The IFITM proteins have an effect on the early stages of HCV infection <i>in vitro</i>	75
Figure 3.12	Characterisation of Huh-7 cell lines stably expressing IFITM1, IFITM2 and IFITM3	76
Figure 3.13	The IFITM proteins exhibit anti-HCV activity	77
Figure 3.14	The IFITM proteins significantly inhibit HCV entry into Huh-7 cells	78
Figure 3.15	The IFITM proteins have no effect on HCV RNA replication	79
Figure 3.16	The IFITM proteins have no effect on HCV RNA replication	80
Figure 3.17	The IFITM proteins do not modulate HCV IRES activity	81
Figure 3.18	The IFITM proteins have no effect on HCV egress	81
Chapter 4		
Figure 4.1	Model of HCV entry	87
Figure 4.2	The IFITM proteins do not co-localise with HCV entry receptor Occludin	88
Figure 4.3	The IFITM proteins do not co-localise with HCV entry receptor Claudin-1	89
Figure 4.4	The IFITM proteins do not co-localise with HCV entry receptor SR-BI	89
Figure 4.5	The IFITM proteins co-localises with the HCV entry receptor CD81 on the hepatic cell surface	90
Figure 4.6	IFITM1 interacts with the HCV entry receptor CD81 on the hepatic cell surface	91
Figure 4.7	Schematic representation of Proximity Ligation Assay	91

Figure 4.8	IFITM1 interacts with the HCV entry receptor CD81 on the hepatic cell surface	91
Figure 4.9	Panel of IFITM1 mutants	92
Figure 4.10	The N-terminal region of IFITM1 is important for its anti-HCV activity	94
Figure 4.11	The N-terminal region of IFITM1 is important for its anti-HCV activity	95
Figure 4.12	The C-terminal region of IFITM1 is important for its localisation within the hepatocyte	95
 Chapter 5		
Figure 5.1	The IFITM proteins do not localise to the ER within the hepatocyte	102
Figure 5.2	The IFITM proteins do not localise to the Golgi apparatus within the hepatocyte	102
Figure 5.3	The IFITM proteins do not localise to lipid droplets within the hepatocyte	103
Figure 5.4	IFITM3 partially co-localises with the early endosome marker Rab5a	103
Figure 5.5	IFITM3 partially co-localises with the early endosome marker Rab5a	103
Figure 5.6	IFITM2 partially co-localises with the late endosome marker Rab7	104
Figure 5.7	IFITM2 partially co-localises with the late endosome marker Rab7	104
Figure 5.8	IFITM1, IFITM2 and IFITM3 co-localise with the lysosomal marker Lamp1	105
Figure 5.9	IFITM1, IFITM2 and IFITM3 co-localise with the lysosomal marker Lamp1	105
Figure 5.10	The IFITM proteins do not localise to Vap-A within the hepatocyte	105
Table 1.1	Summary of IFITM1, IFITM2 and IFITM3 localisation within Huh-7 cells	107
 Chapter 6		
Figure 6.1	Wildtype IFITMs undergo phosphorylation in Huh-7 cells	112
Figure 6.2	Identification of conserved and non-conserved tyrosine residues between IFITM1, IFITM2 and IFITM3	113

Figure 6.3	Mutation of a conserved tyrosine residue in the N-terminus of IFITM2 and IFITM3	113
Figure 6.4	IFITM1 undergoes tyrosine phosphorylation at the conserved Y78 residue	114
Figure 6.5	IFITM2 and IFITM3 N-terminal tyrosine mutants do not undergo phosphorylation in Huh-7 cells	114
Figure 6.6	Tyrosine mutated IFITM2 and IFITM3 have different cellular localisation	115
Figure 6.7	The anti-HCV activity of IFITM2 and IFITM3 is independent of tyrosine residues Y19 and Y20	116
Figure 6.8	The anti-HCV activity of IFITM2 and IFITM3 is independent of tyrosine residues Y19 and Y20	116
Figure 6.9	The IFITM2 and IFITM3 N-terminal tyrosine mutants co-localise with CD81 on the hepatic cell surface	117
Figure 6.10	Identification of conserved cysteine residues between IFITM1, IFITM2 and IFITM3	118
Figure 6.11	Mutation of conserved cysteine residues in IFITM1, IFITM2 and IFITM3	118
Figure 6.12	A single conserved cysteine residue in the CIL is essential for the anti-HCV activity of the IFITM proteins	119
Figure 6.13	Palmitoylation IFITM1 mutants re-localise to the lysosome	120
Figure 6.14	Palmitoylation IFITM2 mutants partially co-localise to the late endosome but primarily re-localise to the lysosome	120
Figure 6.15	Palmitoylation IFITM1 mutants re-localise to the lysosome	120
 Chapter 7		
Figure 7.1	Schematic representation of the localisation and potential molecular mechanism of IFITM2 and IFITM3 against HCV	129

Abstract

Hepatitis C virus (HCV) is a significant human pathogen of the liver that in the majority of infected individuals causes a chronic infection of the liver. This may over time culminate in the development of severe liver disease such as cirrhosis and hepatocellular carcinoma. Prior to 2012, the only treatment option available for HCV infection was combination therapy with pegylated interferon- α (IFN- α) and ribavirin. However, the recent development and addition of direct acting antivirals (DAAs) into treatment regimes has significantly improved sustained virological response rates. While the ultimate aim is for IFN- α free therapy, IFN- α is often required in combination with the DAAs to reduce the development of viral resistance and due to cost. It is clear that IFN (either exogenous or endogenous) can induce an antiviral state in HCV infected cells; however, the exact mechanisms that underpin this action remain unclear.

The interferon-induced transmembrane (IFITM) family of proteins - IFITM1, IFITM2 and IFITM3 has recently been identified as important host effector molecules of the type I IFN response against a broad range of RNA viruses. During the course of this PhD study, a number of investigations identified the IFITM proteins to be potent antiviral effectors against HCV; however, the mechanism(s) for this antiviral activity remains contradictory. In this thesis, we demonstrate that IFITM1, IFITM2 and IFITM3 play an integral role in the IFN response against HCV and act specifically to inhibit early and late stages of HCV entry to inhibit infection. We reveal that IFITM1 localises to the cell surface in hepatocytes and interacts with the host entry factor CD81 to limit HCV entry. Furthermore, the N-terminus, in particular amino acids 21-28, of

IFITM1 plays an important role in this anti-HCV activity, while the C-terminus is found to be important for localisation to the cell surface.

We also established that in hepatocytes, IFITM2 and IFITM3 localise to the late and early endosomes respectively, as well as the lysosome, indicating that IFITM2 and IFITM3 follow the established paradigm of targeting the late entry stages of HCV infection. Furthermore, we have demonstrated that S-palmitoylation of all three IFITM proteins is essential for both anti-HCV activity and cellular localisation, while the conserved tyrosine residue in the N-terminus of IFITM2 and IFITM3 plays a significant role in protein localisation. However, this tyrosine was found to be dispensable for anti-HCV activity, with mutation of the tyrosine resulting in an IFITM1-like phenotype with the retention of anti-HCV activity and co-localisation of IFITM2 and IFITM3 with CD81.

In conclusion, we propose that the IFITM proteins act in a coordinated manner to restrict HCV infection by targeting the endocytosed HCV virion for lysosomal degradation and demonstrate that the actions of the IFITM proteins are indeed virus and cell-type specific. We believe we have significantly added to our understanding of the interplay between HCV and the host innate immune response and that in the long term these findings will aid in the generation of novel and targeted anti-HCV therapeutics for patients chronically infected with HCV.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work, in the future, will be used in a submission in my name for any other degree or diploma in any other university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Sumudu Kumari Narayana

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Awards Received During PhD

- 2012 School of Molecular and Biomedical Science School Symposium Poster Prize – 2nd Place - \$200
- 2012 Nomination by the Golden Key International Honour Society to attend the 2013 International Scholar Laureate Program (ISLP) Delegation on Medicine
- 2012 Australian Centre for Hepatitis Virology Travel Award for HCV Research – \$2500
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K.J. Helbig, J.M. Carr, **S.K. Narayana**, E.M. McCartney, N.S. Eyre and M.R. Beard. The ISG viperin has novel anti-hepatitis C virus activity through interaction with a

HCV pro-host cell factor. Keystone Symposia 2012 - Innate Immunity, Colorado, USA, 2012. (poster presentation)

McCartney E.M., Helbig, K.J., Eyre, N.S, **Narayana, S.K.** and Beard, M.R. The role of STAT3 in the life cycle of HCV. 18th International Symposium on Hepatitis C Virus and Related Viruses, Seattle, USA, 2011. (poster presentation)

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Materials Providers

Abcam	Cambridge, UK
Ambion	Texas, USA
Amersham Pharmacia Biotech	Birminghamshire, UK
Amrad Biotech	Boronia, VIC, Australia
Anogen	Ontario, Canada
Applied Biosystems	Warrington, UK
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SPSS Inc	Illinois, USA
Stratagene	California, USA
UVP Inc	California, USA
Vector Laboratories	California, USA
Vision Systems	Mount Waverley, VIC, Australia

Abbreviations Used

A	adenosine
aa	amino acids
bp	base pairs
BSA	bovine serum albumin
BVDV	bovine viral diarrhoea virus
C	cytosine
° C	degrees Celsius
cDNA	complimentary deoxyribosenucleic acid
CLDN1	claudin-1
CHC	chronic hepatitis C
CIL	cytoplasmic intracellular loop
CMV	cytomegalovirus
dATP	deoxyadenosine-5'-triphosphate
dCTP	deoxycytosine-5'-triphosphate
DEPC	diethyl pyrocarbonate
DAA	direct acting antiviral
dGTP	deoxyguanosine-5'-triphosphate
dH ₂ O	deionised water
DMEM	Dulbecco's Modified Eagle Medium with HEPES
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
dTTP	deoxythymidine-5'-triphosphate
DENV	Dengue virus
EBoV	Ebola virus
EDTA	ethylene diamine tetra acetic acid
ER	endoplasmic reticulum

FCS	foetal calf serum
FITC	fluorescein isothiocyanate
FFU	focus forming unit
g	grams
G	guanosine
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	gamma-activated sequence
GFP	green fluorescent protein
HCC	hepatocellular carcinoma
HBV	hepatitis B virus
HCV	hepatitis C virus
hr	hour(s)
HRP	horse radish peroxidase
HIV	human immunodeficiency virus ^{○○○○}
HPV	human pappilomavirus
IAV	influenza virus
IDU	infecting drug use
IFITM	interferon-induced transmembrane protein
IFN- α	interferon alpha
IFN- β	interferon beta
IFN- γ	interferon gamma
IFN- λ	interferon lambda
IRES	internal ribosome entry site
ISRE	interferon stimulated response element
JAK	janus kinase
kb	kilobase
kDa	kilo Dalton
L-Agar	LB + agar
LD	lipid droplet

LB	Luria Bertani broth
LDL	low density lipoproteins
Luc	luciferase
µg	micrograms
µl	microlitres
µM	micromolar
mA	milliamps
mg	milligrams
ml	millilitres
mM	millimolar
MCS	Multiple Cloning Site
MEM	Minimum Essential Medium
min	minute(s)
mRNA	messenger RNA
MW	molecular weight
MLV	murine leukemia virus
ng	nanograms
nM	nanomolar
N/A	not applicable
nt	nucleotide
NTD	N-terminal domain
NF-κB	nuclear factor-kappa-light chain enhancer
OCLN	occludin
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline; 0.15M NaCl, 6M K ₂ HPO ₄ , 2mM KH ₂ PO ₄ (pH 7)
PAMP	pathogen associated membrane patterns

PCR	polymerase chain reaction
PHH	primary human hepatocyte
PRR	pathogen recognition receptors
PTM	post-translational modification
pg	picograms
pmol	picomolar
RNA	ribonucleic acid
rpm	revolutions per minute
RFP	red fluorescent protein
RdRp	RNA-dependent-RNA polymerase
RC	replication complex
RIG-I	retinoic acid inducible gene I
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
sd	standard deviation
SDS	sodium dodecyl sulfate
sec	second(s)
SEM	standard error of mean
ss	single stranded
siRNA	small interfering RNA
SR-BI	scavenger receptor class B member I
STAT	signal transducer and activator of transcription
SVR	sustained virological response
T	thymidine
TAE	0.04M Tris (pH 8), 0.04M Acetic Acid, 1mM EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine

Tris	3,3,5,5-tetramethylbenzidine
TLR	Toll-like receptor
TYK2	tyrosine kinase 2
U	units
UTR	untranslated region
V	volts
VSV	vesicular stomatis virus
w/v	weight per volume
WNV	West Nile Virus