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A multi-component prime-boost vaccination regimen with a consensus MOMP antigen enhances Chlamydia trachomatis clearance.

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A multi-component prime-boost vaccination regimen with a consensus MOMP antigen enhances *Chlamydia trachomatis* clearance.

Running title: Prime-boost regimens against *C. trachomatis*.

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23 **Abstract**

24 Background: A vaccine for *Chlamydia trachomatis* is of urgent medical need. We explored
25 bioinformatic approaches to generate an immunogen against *C. trachomatis* that would
26 induce cross-serovar T cell responses as (i) $CD4^+$ T cells have been shown in animal models
27 and human studies to be important in chlamydial protection, and (ii) antibody responses may
28 be restrictive and serovar-specific.

29 Methods: A consensus antigen based on over 1,500 MOMP sequences provided high epitope
30 coverage against the most prevalent *C. trachomatis* strains *in silico*. Having designed the T
31 cell immunogen, we assessed it for immunogenicity in prime-boost regimens. This consensus
32 MOMP transgene was delivered using plasmid DNA, Human Adenovirus-5 (HuAd5) or
33 modified vaccinia Ankara (MVA) vectors with or without MF59® adjuvanted recombinant
34 MOMP protein.

35 Results: Different regimens induced distinct immune profiles. The DNA-HuAd5-MVA-
36 Protein (DAMP) vaccine regimen induced a cellular response with a Th1 biased serum
37 antibody response, alongside high serum and vaginal MOMP-specific antibodies. This
38 regimen significantly enhanced clearance against intravaginal *C. trachomatis* serovar D
39 infection in both BALB/c and B6C3F1 mouse strains. This enhanced clearance was shown to
40 be $CD4^+$ T cell dependent. Future studies will need to confirm the specificity and precise
41 mechanisms of protection.

42 Conclusions: A *C. trachomatis* vaccine needs to induce a robust cellular response with broad
43 cross-serovar coverage and a heterologous prime-boost regimen may be an approach to
44 achieve this.

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Introduction

Genital chlamydial infection is the most common cause of bacterial sexually transmitted diseases (STDs) worldwide, accounting for more than 90 million cases of STDs globally each year (WHO, 2001). Over US$3 billion is spent annually on an estimated 4 million reported clinical cases of genital chlamydial infections in the US alone (CDC, 2010), thus development of a vaccine against *Chlamydia trachomatis* represents a significant public health priority. A promising vaccine antigen of *C. trachomatis* is the major outer membrane protein (MOMP). The MOMP antigen has been used in a range of previous pre-clinical vaccine studies with a mixture of encouraging (Pal et al., 2001;Farris et al., 2010) and disappointing results (Igietseme and Murdin, 2000;Sun et al., 2009). This may reflect the high level of amino acid variability in the MOMP antigen, the basis for *C. trachomatis* serotypes (Yuan et al., 1989).

New bioinformatic approaches have been developed to account for amino acid diversity and aid in the design of immunogens to induce cross-serovar T cell responses: these include the design of mosaic or conserved antigen sequences. Mosaic vaccine antigens are designed with the intent to be used in polyvalent combinations to optimise the coverage of potential T cell epitopes (Fischer et al., 2007), for example HIV mosaic antigens increased the breadth and potency of vaccine elicited immune responses (Barouch et al., 2010), conferring protective responses in non-human primate models (Barouch et al., 2013). Consensus vaccine antigens rely on a single centralised antigen designed to reduce sequence distances between the vaccine and circulating strains by using the most common amino acid at each position of the protein (Gaschen et al., 2002). By reducing the genetic differences between the vaccine and the primary isolate, consensus antigens can increase the breadth of immune response (Eugene et al., 2013).

Here we computationally design and *in silico* assess both consensus and mosaic MOMP antigens for broad T cell coverage against the *C. trachomatis* genital serovars D-K, for the reasons that (a) chlamydial infections in both animal models and humans suggest a strong protective role for $CD4^+$ Th1-biased immune responses (Su and Caldwell, 1995;Li et al., 2008;Farris et al., 2010) and (b) that these may be supplemented by MOMP specific antibodies able to mediate antibody-dependent cellular cytotoxicity (ADCC) (Moore et al., 2002).

We assess the quantity and quality of the antibody and cellular response to MOMP following different prime-boost combinations using DNA vaccines, recombinant viral vectors and protein-in-adjuvant formulations (McKay et al., 2014). Both DNA and recombinant viral vectors preferentially induce cellular immunity (Tregoning and Kinnear, 2014) and subunit proteins humoral immunity: the use of different prime boost combinations can be tailored to influence the phenotype of both arms of induced adaptive immunity. DNA vaccine vectors, human Adenovirus 5 and MVA viral vectors have already entered phase I clinical trials, showing safety and tolerability (Hayton et al., 2014;Nilsson et al., 2015). Research groups have reported enhanced T cell induction through heterologous prime-boost vaccination strategies in a range of disease models (including tuberculosis (McShane et al., 2001), HIV (Hanke et al., 1998), HPV (van der Burg et al., 2001) and Ebola (Sullivan et al., 2000)) but these strategies have yet to be comprehensibly investigated for *C. trachomatis*.

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93 We demonstrate that different vaccination regimens when used to deliver the same MOMP
94 antigen via differing platforms can be configured to induce distinct immune outcomes. We
95 further investigate which distinct MOMP-specific immune responses are required for
96 enhanced *C. trachomatis* clearance following genital challenge in mice. We observe that a
97 regimen using DNA-HuAd5-MVA-Protein (DAMP) vaccines reduced bacterial load early
98 after infection regardless of mouse strain used, and that this enhanced clearance while
99 dependent upon $CD4^+$ T cell responses may have been augmented by induced MOMP-
100 specific antibody responses.

101

102 Material and Methods

103 Bioinformatic antigen design

104 1,464 serovar E *ompA* sequences, surveyed from a total of 5,026 *C. trachomatis* strains
105 isolated in 33 distinct geographic regions from five continents were compared (Nunes et al.,
106 2010). Phylogenetic analysis was based on the protein sequence alignment derived from
107 (Nunes et al., 2010), and the maximum likelihood tree was created using FastTree (Price et
108 al., 2009) using default settings; the figure was generated using Rainbow Tree
109 (www.hiv.lanl.gov). 49 distinct *ompA* variants were present within the serovar E sequences,
110 and were used in the *in silico* generation of the consensus MOMP (Con E) antigen sequence
111 for the experimental studies. The generated consensus and mosaic antigens were assessed for
112 their coverage against different Chlamydia MOMP variants using the Epitope Coverage
113 Assessment Tool EPICOVER. Full bioinformatic tool settings, a detailed description of the
114 method, and consensus and mosaic antigen sequence information is provided in the
115 Supplemental Methods and Materials (Supplementary File 1).

116

117 Plasmid, viral vectors and recombinant protein

118 Mammalian codon optimised MOMP Con E antigen was synthesised by GeneArt (Invitrogen,
119 UK) and cloned into pcDNA3.1 (Invitrogen, UK). Con E was homologously recombined into
120 the E1 and E3 deleted HuAd5 genome plasmid, pAL1112 (kindly provided by Prof. Gavin
121 Wilkinson, Cardiff University). Con E was recombined into the MVA pox vector by the Viral
122 Vector Core Facility, The Jenner Institute (University of Oxford, UK). *E. coli* codon
123 optimised Con E expressed at too low a yield in BL21 *E. coli,* and as such a recombinant
124 MOMP matching *C. trachomatis* from serovar D/UW/Cx expressed in BL21 *E. coli* was
125 used.

126

127 Chlamydia

128 *C. trachomatis* serovar D (strain UW-3/Cx) was provided by Dr. Frank Follmann (Statens
129 Serum Institut) and propagated in McCoy cells as described previously (Scidmore, 2005).
130 Chlamydial EBs were harvested, purified and quantified as described in (Scidmore, 2005),
131 and stored at -80°C in SPG buffer (Sucrose/Phosphate/Glutamic acid: 0.2 M sucrose, 20 mM
132 sodium phosphate and 5 mM glutamic acid).

133

134 Mice immunisations and infections

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135 Female 6-8 weeks old, BALB/c mice (Harlan, Stornoway, UK) and female 6-8 weeks old
136 B6C3F1 mice (Charles River, Italy) were kept in specific-pathogen-free conditions in
137 accordance with the UK Home Office guidelines. All work was approved by the Imperial
138 College Ethical Review Process (ERP) Committee. Mice received immunisations at three-
139 week intervals (Table 1). DNA vaccinations were at 10 µg doses, intramuscularly into the
140 hind quadriceps muscle in a volume of 50 µl with electroporation. Electroporation was with 5
141 mm electrodes at the immunisation site using an ECM 830 Square Wave Electroporation
142 System (BTX), with three pulses of 100 V each, followed by three pulses of the opposite
143 polarity with each pulse (P_{ON}) lasting 50 ms and an interpulse (P_{OFF}) interval of 50 ms. All
144 HuAd5 and MVA vaccinations were at dosages of 10^7 PFU and 10^6 PFU respectively.
145 rMOMP was administered at a dose of 10 µg in a 1:1 mixture with MF59® (an oil-in-water
146 emulsion adjuvant) (Novartis, Sienna, Italy) in a final volume of 50 µl for intramuscular
147 immunisations. Because of the multi-component immunisation regime, vehicle and vector-
148 alone controls were not included to reflect reduce, replace and refinement practice. Seven
149 days prior to intravaginal infection, mice were injected subcutaneously with 2mg of DMPA
150 (Depo-Provera, Pfizer). For intravaginal infections, purified *C. trachomatis* D/UW-3/Cx EBs
151 were dissolved in SPG buffer to a concentration of $4x10^7$ IFU/ml, mice were anaesthetised,
152 and 10 µl of the EB solution pipetted into the mouse vagina. The optimal infective dose of
153 $4x10^5$ IFU of *C. trachomatis* D/UW-3/Cx EBs per mouse was previously determined by Dr.
154 Frank Follmann, SSI (unpublished data) and consistent with (O'Connell et al., 2011;Picard et
155 al., 2012). Furthermore, bacterial clearance profiles were consistent between naïve BALB/c
156 and B6C3F1 mice at this infective dose (Fig S2). Unfortunately *C. trachomatis* E/Bour could
157 not be propagated to a high enough infectious titre for intravaginal infection.

158

159 **Intravaginal *C. trachomatis* load quantification**

160 Vaginal swabs were obtained at 3, 7, 10 and 14 days after infection. Swabs were vortexed
161 with glass-beads in 500 µl SPG buffer and stored at −80°C until analysis. Infectious load was
162 determined as described in (Hansen et al., 2008). Inclusions were visualised by staining with
163 polyclonal rabbit anti-MOMP serum (provided by Dr. Frank Follmann, SSI), followed by an
164 Alexa 594-conjugated goat anti-rabbit H+L (Life Technologies, UK).

165

166 **Mice sampling**

167 Tail bleeds were collected before regimen, and two weeks post each immunisation. Blood
168 was collected and centrifuged at 1,000 g for 10 min. The serum was harvested and stored at -
169 20°C. To assess IFN-γ T cell responses, lymphocyte cultures from spleens were prepared as
170 described previously (McKay et al., 2014). Vaginal lavage was performed at the same time
171 points as tail bleeds, using three 25 µl washes/mouse with sterile Phosphate buffered saline
172 (PBS) that were later pooled. Lavage samples were incubated with protease inhibitor (Roche
173 Diagnostics, Germany) before centrifuging at 1,000 g for 10 min. The fluid supernatant from
174 these samples was harvested and stored at -20°C.

175

176 **Semi-quantitative MOMP-specific ELISA, avidity assay, and MOMP-specific IFN-γ**
177 **ELISpot**

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A semi-quantitative immunoglobulin ELISA protocol described previously (Badamchi-Zadeh et al., 2015) was followed. The avidity indices of serum samples were determined by their antibody-antigen binding resistance to 8 M urea. Serum samples were pre-diluted to give an $OD_{450\ nm}$ readout between 1.0 and 1.5 in an ELISA and were added to MOMP antigen coated plates. Plates were then washed three times with either PBS-T or 8 M urea in PBS-T, before incubating with anti-mouse IgG-HRP. Samples were developed with TMB as described above. The avidity index was calculated as the percentage of urea treated $OD_{450\ nm}$/ PBS-T $OD_{450\ nm}$. IFN-γ ELISpot assays (Mabtech, UK) were carried out on mouse splenocytes as to manufacturer's instructions.

Depletion of $CD4^+$ T-cells

Mice were depleted of $CD4^+$ T-cells by the i.p. route with injections of 500 μg monoclonal anti-mouse CD4 IgG2b (clone GK1.5) (BioXcell, Cat: BE0003-1) on days -1 and +1 with respect to day of challenge being day 0. The depletion of $CD4^+$ T cells was verified by FACS analysis on murine PBMC, splenocytes and vaginal tissue on day +2 using anti-CD3e PE, anti-CD4 APC and anti-CD8a eFluor605NC antibodies (All BD Biosciences, UK).

Statistical Analysis

All statistical analyses were carried out using Prism 6.0 (GraphPad, USA). Normality of the data distribution was assessed using the Kolmogorov Smirnov normality test. For non-parametric data the Kruskal-Wallis test with Dunn's multiple comparison post-test was used to compare more than two groups, or the two-tailed Mann-Whitney test to compare two groups. For parametric data, a one-way ANOVA was used for multiple comparisons, with Bonferroni's multiple comparison post-test for comparison of specific groups. $P<0.05$ was considered significant (* $p<0.05$, ** $p<0.01$ and *** $p<0.001$).

Results

Design and cross-serovar coverage assessment of consensus and mosaic MOMP antigens

The worldwide prevalence of specific urogenital *C. trachomatis* serovars has not been fully characterised. A literature review (PubMed) identified 13 publications describing the country or regional serovar-specific prevalence (Lan et al., 1995;Morre et al., 2000;Suchland et al., 2003;Yamazaki et al., 2005;Gao et al., 2007;Suarkia et al., 2007;Bandea et al., 2008;Hafner et al., 2008;Donati et al., 2009;Petrovay et al., 2009;Smelov et al., 2009;Gallo Vaulet et al., 2010;Papadogeorgakis et al., 2010). In 10 out of the 13 *C. trachomatis* serovar surveys published, serovar E emerged as the most prevalent (Fig 1A). MOMP is a lead vaccine antigen candidate for *C. trachomatis*, and with worldwide MOMP sequence data available (Nunes et al., 2010) for the differing genital serovars (D-K, Da, Ia, and Ja) it was possible to bioinformatically perform MOMP based immunogen design. We found sequences within all serovars to be conserved, with only sporadic amino acid substitutions (Fig 1B). From our phylogenetic analyses, *C. trachomatis* has quite distant species, but high levels of conservation within a serovar. Thus this kind of phylogenetic profile lends itself more towards a consensus antigen design approach as opposed to a single mosaic antigen design (Fischer et al., 2007). Based on 49 published serovar E sequence variants (Nunes et al., 2010)

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we generated a novel MOMP consensus sequence (Con E), which fully matched the solution for a single mosaic and was identical to the circulating *C. trachomatis* strain E-Bour, as well as to 8 additional partial MOMP protein sequences from a wide variant of geographic origins (Nunes et al., 2010). Using a single consensus antigen the potential epitope coverage of the E serovar led to extremely high coverage (~95%) (Fig 1C). For even broader coverage, our analyses suggest multiple mosaic antigens may be more appropriate (Fig 1C and Supplementary File 1).

Differences in humoral and cellular immunogenicity of *C. trachomatis* MOMP vaccines following intramuscular multi-component prime-boost regimen screen

The immunogenicity of the consensus MOMP antigen was assessed in BALB/c mice immunised in multi-component prime-boost regimens with DNA (D), HuAd5 (A), MVA (M) and protein with the oil-in-water emulsion adjuvant MF59® (P) vaccines (Table 1). The MF59® adjuvant has been demonstrated to induce IL-5 and IL-10 responses to the MOMP antigen and was therefore used as a comparator to the more Th1 skewing DNA and viral vector approaches (Knudsen et al., 2016). Serum and vaginal washes were sampled two weeks after final immunisation. The highest MOMP-specific serum IgG concentrations were observed after the PPP regimen (mean + SEM = 1.27 +/- 0.16 mg/ml) and the lowest from the DDD regimen (mean + SEM = 15.4 +/- 2.54 µg/ml) (Fig 2A). Protein (+ MF59®) immunisation significantly increased MOMP-specific serum IgG concentrations compared to prime-boost regimen without two protein boosts. MOMP-specific vaginal IgG concentrations were measured following the multi-component prime-boost regimens (Fig 2B). The PPP regimen induced significantly higher MOMP-specific vaginal IgG than DDDAM, DDDA, DDDM, DDD, AM or the naïve group ($p \leq 0.05$). MOMP-specific IgA was not detectable in the sera or vaginal washes following any of the prime-boost regimens (data not shown).

Viral vector vaccines have been shown to induce high and long lasting cytophilic, Th1 skewed, antibody responses (Douglas et al., 2010). A correlate for Th1 and Th2 skewing of the immune response in mice is the antigen-specific IgG2a to IgG1 ratio. MOMP-specific serum IgG2a and IgG1 concentrations were measured by ELISA, and their ratios calculated (Fig 2C). The highest MOMP-specific serum IgG2a:IgG1 ratio was induced following the DAMP regimen (mean = 14.2), with the lowest ratio induced following PP (+ MF59®) vaccination (mean = 0.0036). There was a significant statistical difference in the IgG2a:IgG1 ratios between DAMP and PPP ($p \leq 0.005$) and DAMP and PP ($p \leq 0.0005$, one-way ANOVA with Bonferroni's multiple comparison post-test). Protein only vaccine regimens induced Th2-biased immune environments indicated by IgG2a:IgG1 ratios of less than 1 caused by high IgG1 concentrations. Vaccine regimens with a vector-based vaccine prime consistently induced a Th1-biased, cytophilic antibody response as indicated by IgG2a:IgG1 ratios of greater than 1.

To differentiate and qualitatively evaluate the humoral responses an avidity assay was performed (Fig 2D). All protein free regimens had mean avidity indices of <30%, with the DAMP and PP regimens also having low mean avidity indices. Regimens involving two protein boosts all had avidity indices >40%, with the APP regimen inducing MOMP-specific serum IgGs with the highest avidity (mean = 51.8%).

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268 T cell responses were assessed one week after the final immunisation. MOMP specific IFN-
269 γ+ T cell responses as assessed by ELISpot were induced by all prime-boost regimens, with
270 the DDD and AM regimens inducing the strongest T cell responses (a median of 504 and 502
271 SFU/10^6 splenocytes respectively, Fig 2E). T cell responses induced by the DDD and AM
272 regimens were significantly higher than those induced in the PP regimen ($p \leq 0.05$). From
273 this we conclude that there were significant differences in both the quantity and quality of the
274 antibody and cellular response following the different regimens.

275

276 **Multi-component prime-boost regimens induce analogous immunogenicity profiles in**
277 **both BALB/c and B6C3F1 mice**

278 As we wished to test the effect of altering the immune response on Chlamydial protection, the
279 following groups were chosen for further investigation as they gave distinct, skewed immune
280 responses in the BALB/c screen: AM and DDD (T cell, low antibody), PPP (Th2 skewed
281 antibody, no Th1 T cells), and DAMP (Th1 skewed antibody and T cell). These vaccine
282 regimens induced comparable responses in B6C3F1 mice as they did in BALB/c mice for
283 MOMP-specific serum IgG concentrations (Fig 3A), MOMP-specific vaginal IgG
284 concentrations (Fig 3B), MOMP-specific serum IgG2a to IgG1 ratios (Fig 3C), and MOMP
285 specific IFN-γ T cell responses (Fig 3D).

286

287 **The DAMP vaccine regimen enhances the clearance of *C. trachomatis*, regardless of**
288 **mouse strain, and is $CD4^+$ T dependent**

289 Immunised BALB/c mice were challenged with *C. trachomatis* D/UW-3/Cx intravaginally.
290 The DAMP regimen significantly reduced chlamydial IFU per swab at day 3 after challenge
291 (median = 354 IFU/swab) compared to unvaccinated controls (median = 22,688 IFU/swab)
292 (DAMP: * $p = 0.0359$, 2-tailed Mann-Whitney test) (Fig 4A). There were no statistical
293 differences at the later sampling points of 7, 10 and 14 days (data not shown) after challenge
294 reflecting the natural clearance of *C. trachomatis* in mice. The DDD, AM, and PPP regimens
295 did not significantly reduce chlamydial shedding at any time points sampled after challenge
296 in BALB/c mice.

297

298 Immunised B6C3F1 mice were challenged with *C. trachomatis* D/UW-3/Cx intravaginally.
299 Similar to BALB/c mice, the DAMP vaccination regimen significantly reduced chlamydial
300 IFU per swab at day 3 after challenge (median = 6,531 IFU/swab) compared to unvaccinated
301 controls (median = 34,788 IFU/swab) (* $p = 0.0303$, 2-tailed Mann-Whitney test) in
302 B6C3F1s (Fig 4B). In addition, the PPP regimen significantly reduced chlamydial IFU per
303 swab at day 3 after challenge (median = 8,095 IFU/swab) compared to unvaccinated controls
304 (median = 34,788 IFU/swab) (* $p = 0.0451$, 2-tailed Mann-Whitney test) (Fig 4B). There
305 were no statistical differences at the later sampling points of day 7, day 10 or day 14 after
306 challenge, and consistent with this challenge model, there was no oviduct pathology observed
307 (hydrosalpinx) in challenged BALB/c or B6C3F1 mice (data not shown). Of the four tested
308 prime-boost regimens, only the DAMP regimen consistently enhanced the clearance of
309 intravaginal *C. trachomatis*.

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310

311 We assessed the mechanism of the vaccine-induced enhanced clearance. Monoclonal
312 antibodies were used to deplete $CD4^+$ T cells in DAMP vaccinated and unvaccinated control
313 B6C3F1 mice prior to intravaginal challenge. There was no difference between the DAMP
314 immunised $CD4^+$ depleted groups and the naïve $CD4^+$ depleted group in chlamydial loads,
315 indicating that the DAMP vaccine induced enhanced clearance is $CD4^+$ T cell dependent (Fig
316 4C).

317

318 **Discussion**

319 New bioinformatic strategies have been developed as an approach to elicit broad immune
320 responses against the more intractable antigenically variable pathogens, such as *C.*
321 *trachomatis*. Two such approaches, consensus and mosaic, were used to design a MOMP
322 antigen(s) to provide broad cellular cross-serovar coverage. We performed phylogenetic
323 analyses revealing *C. trachomatis* MOMP to have quite distant species but high levels of
324 amino acid conservation within a serovar. This phylogenetic structure lends itself more
325 towards a consensus-based antigen design as opposed to a mosaic-based approach (Fischer et
326 al., 2007). Therefore a single consensus MOMP antigen (Con E), constructed from variant
327 sequences of the most common *C. trachomatis* serovar, was used. Nevertheless, combining
328 the Con E antigen with additional mosaic antigens (that we have designed (Supplementary
329 Materials)) would provide complementary coverage to all other serovars. This would be a
330 well-justified approach to theoretically achieve comprehensive coverage of all serovars in
331 one vaccine, with no loss of coverage of the E serovar (Fig 1C), however assessing these
332 additional antigens immunologically was out of the scope of this initial study.

333

334 Having designed our broad-coverage T cell immunogen, we then assessed its
335 immunogenicity using a range of prime-boost regimens. Few multi-component prime-boost
336 vaccine regimens have been tested for the generation of immune responses against
337 Chlamydia (Brown et al., 2012), with the majority focussing on homologous prime-boost
338 strategies (Pal et al., 2005;Schautteet et al., 2011). The use of different vectors within prime-
339 boost regimen can help to avoid anti-vector immunity and improve vaccine-elicited immune
340 responses. Previous uses of DNA vaccines expressing Chlamydia transgenes have not been
341 adjuvanted by electroporation as in this study (Dong-Ji et al., 2000;Penttila et al., 2004), and
342 only one previous use of an adenovirus-vectored vaccine against *Chlamydia muridarum*
343 (expressing CPAF) has been reported (Brown et al., 2012).

344

345 Following an initial screen of eleven prime-boost regimens, four immunologically distinct
346 regimens were assessed in both BALB/c and B6C3F1 mice. The AM, DDD, PPP and DAMP
347 regimens showed no mouse-strain specific discrepancies in the humoral or cellular responses
348 induced. Of these four regimens, only the DAMP vaccination regimen enhanced the
349 clearance of intravaginal *C. trachomatis,* regardless of mouse strain. This enhanced
350 chlamydial clearance afforded by the DAMP vaccination regimen was dependent on $CD4^+$ T
351 cells, as shown by $CD4^+$ T cell depletion following vaccination. Previous chlamydia vaccine
352 studies in mice have also revealed a role for $CD4^+$ T cells in their protection, including in the

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353 liposomal delivery of rMOMP (Hansen et al., 2008) and the subcutaneous delivery of CTH1
354 (Olsen et al., 2010).

355

356 The importance of IFN-γ *in vivo* to chlamydia control has been demonstrated previously
357 (Cotter et al., 1997;Ito and Lyons, 1999). In this respect it appears somewhat surprising that
358 the DDD and AM regimens, inducing stronger T cell IFN-γ responses than DAMP, failed to
359 show any evidence for enhanced clearance. However, a distinct characteristic of the DAMP
360 vaccination regimen was the co-induction of significantly higher levels of MOMP-specific
361 IgG2a (with a high IgG2a/IgG1 ratio). This was in spite of the use of the protein adjuvant
362 MF59®, which has previously been shown to skew T cell responses against MOMP towards
363 IL-5 and IL-10 (Knudsen et al., 2016); this may be explained by the order of immunisations,
364 with the initial MOMP antigen exposures in the context of DNA and viral vector delivery
365 skewing the response, as the protein-adjuvant boosted regimens resulted in a strong IgG1
366 bias. Chlamydial-specific IgG2a antibody concentrations have previously correlated with
367 protection in animal studies (Pal et al., 2001). Thus although enhanced clearance was
368 dependent upon CD4 T cell responses, these data suggest induced IgG2a responses may have
369 played a contributory role in the enhanced clearance of *C. trachomatis* infection. In this
370 respect, EB opsonisation by IgG isotypes is known to affect the uptake of chlamydia into
371 cells (Peterson et al., 1997). EB opsonisation with a high IgG1 and low IgG2a anti-MOMP
372 polyclonal sera has been shown to enhance infection of a cell line through the FcRn mediated
373 uptake of these IgG-coated EBs, suggesting a negative role for MOMP-specific IgG1
374 antibodies (Armitage et al., 2014). The IgG2a isotype by contrast mediates effector functions
375 including antibody-dependent cellular cytotoxicity (ADCC), with evidence suggesting this
376 effector function may facilitate the early clearance of a chlamydial infection (Moore et al.,
377 2002), furthermore ADCC is associated with enhanced antigen presentation with the potential
378 to amplify CD4 T cell responses (Rafiq et al., 2002). Collectively, these suggest that the anti-
379 MOMP IgG isotype may have a contributory role in the protection observed from the DAMP
380 regimen, though without a DAM comparator arm this would require further investigation.

381

382 We cannot rule out that the DAMP vaccination regimen itself is in fact lowering the
383 establishment of infection, and thus resulting in the lower IFU observed, as our earliest
384 sampling point is day 3 after challenge. Moreover, we cannot exclude the possibility that non-
385 specific effects of the vaccine components in the DAMP regimen may impact general T-cell
386 function. Future work should assess the potential non-specific immunologic effects of
387 antigen-delivery by viral vectors alongside adjuvantation during prime-boost vaccinations.

388

389 In this study we undertook a bioinformatic approach to generate an immunogen that would
390 induce cross-serovar Chlamydial T cell responses. We have revealed a capability to induce an
391 array of MOMP-specific immune responses, both cellular and humoral, using four differing
392 MOMP-based vaccine modalities in multi-component prime-boost regimens. The comparison
393 of the same antigen by different modalities gives us insight into the distinct immune profiles
394 induced by these vaccines. Based on our focus of developing vaccine candidates to progress
395 towards clinical testing we opted to use *C. trachomatis* for our challenged studies, rather than
396 the more conventional use of *C. muridarium* that is pathogenic in mice. Though *C.*
397 *trachomatis* is not a natural pathogen of mice, it has been argued that that intravaginal
398 infection with *C. trachomatis* mimics in many ways both the course and outcome of infection

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399 in most women as asymptomatic and self-limiting (Lyons et al., 2005). In this context, the
400 observed significant reduction in shedding following DAMP vaccination observed 3 days
401 post infection is particularly encouraging given rapid natural clearance in naïve animals. We
402 would anticipate a greater impact on *C. trachomatis* clearance in transcervical infection
403 models, in non-human primate models, and in humans, where the infection is slow to clear
404 and/or may establish chronic infection and this will form the focus of our future studies.

405 **Conflict of Interest Statement:**
406 The authors declare no commercial or financial conflict of interest.
407
408 **Author Contributions:**
409 AB, PM, BK, GB, AW performed the experiments; AB, PM, JT & RS designed the studies;
410 AN, JG, FF provided data sources. AB& JT wrote the paper.
411
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424
425
426 **References**

427 Armitage, C.W., O'meara, C.P., Harvie, M.C., Timms, P., Blumberg, R.S., and Beagley,
428 K.W. (2014). Divergent outcomes following transcytosis of IgG targeting intracellular
429 and extracellular chlamydial antigens. *Immunol Cell Biol* 92**,** 417-426.
430 Badamchi-Zadeh, A., Mckay, P.F., Holland, M.J., Paes, W., Brzozowski, A., Lacey, C.,
431 Follmann, F., Tregoning, J.S., and Shattock, R.J. (2015). Intramuscular Immunisation
432 with Chlamydial Proteins Induces Chlamydia trachomatis Specific Ocular Antibodies.
433 *PLoS One* 10**,** e0141209.
434 Bandea, C.I., Debattista, J., Joseph, K., Igietseme, J., Timms, P., and Black, C.M. (2008).
435 Chlamydia trachomatis serovars among strains isolated from members of rural
436 indigenous communities and urban populations in Australia. *J Clin Microbiol* 46**,**
437 355-356.
438 Barouch, D.H., O'brien, K.L., Simmons, N.L., King, S.L., Abbink, P., Maxfield, L.F., Sun,
439 Y.H., La Porte, A., Riggs, A.M., Lynch, D.M., Clark, S.L., Backus, K., Perry, J.R.,
440 Seaman, M.S., Carville, A., Mansfield, K.G., Szinger, J.J., Fischer, W., Muldoon, M.,
441 and Korber, B. (2010). Mosaic HIV-1 vaccines expand the breadth and depth of
442 cellular immune responses in rhesus monkeys. *Nat Med* 16**,** 319-323.
443 Barouch, D.H., Stephenson, K.E., Borducchi, E.N., Smith, K., Stanley, K., Mcnally, A.G.,
444 Liu, J., Abbink, P., Maxfield, L.F., Seaman, M.S., Dugast, A.S., Alter, G., Ferguson,
445 M., Li, W., Earl, P.L., Moss, B., Giorgi, E.E., Szinger, J.J., Eller, L.A., Billings, E.A.,
446 Rao, M., Tovanabutra, S., Sanders-Buell, E., Weijtens, M., Pau, M.G., Schuitemaker,
447 H., Robb, M.L., Kim, J.H., Korber, B.T., and Michael, N.L. (2013). Protective

Provisional

448 efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in
449 rhesus monkeys. *Cell* 155**,** 531-539.
450 Brown, T.H.T., David, J., Acosta-Ramirez, E., Moore, J.M., Lee, S., Zhong, G., Hancock,
451 R.E.W., Xing, Z., Halperin, S.A., and Wang, J. (2012). Comparison of immune
452 responses and protective efficacy of intranasal prime-boost immunization regimens
453 using adenovirus-based and CpG/HH2 adjuvanted-subunit vaccines against genital
454 Chlamydia muridarum infection. *Vaccine* 30**,** 350-360.
455 Cdc (2010). Sexually Transmitted Disease Surveillance 2010. *Atlanta, GA: Centers for*
456 *Disease Control and Prevention*.
457 Cotter, T.W., Ramsey, K.H., Miranpuri, G.S., Poulsen, C.E., and Byrne, G.I. (1997).
458 Dissemination of Chlamydia trachomatis chronic genital tract infection in gamma
459 interferon gene knockout mice. *Infect Immun* 65**,** 2145-2152.
460 Donati, M., Di Francesco, A., D'antuono, A., Pignanelli, S., Shurdhi, A., Moroni, A.,
461 Baldelli, R., and Cevenini, R. (2009). Chlamydia trachomatis serovar distribution and
462 other concurrent sexually transmitted infections in heterosexual men with urethritis in
463 Italy. *Eur J Clin Microbiol Infect Dis* 28**,** 523-526.
464 Dong-Ji, Z., Yang, X., Shen, C., Lu, H., Murdin, A., and Brunham, R.C. (2000). Priming
465 with Chlamydia trachomatis major outer membrane protein (MOMP) DNA followed
466 by MOMP ISCOM boosting enhances protection and is associated with increased
467 immunoglobulin A and Th1 cellular immune responses. *Infect Immun* 68**,** 3074-3078.
468 Douglas, A.D., De Cassan, S.C., Dicks, M.D., Gilbert, S.C., Hill, A.V., and Draper, S.J.
469 (2010). Tailoring subunit vaccine immunogenicity: maximizing antibody and T cell
470 responses by using combinations of adenovirus, poxvirus and protein-adjuvant
471 vaccines against Plasmodium falciparum MSP1. *Vaccine* 28**,** 7167-7178.
472 Eugene, H.S., Pierce-Paul, B.R., Cragio, J.K., and Ross, T.M. (2013). Rhesus macaques
473 vaccinated with consensus envelopes elicit partially protective immune responses
474 against SHIV SF162p4 challenge. *Virol J* 10**,** 102.
475 Farris, C.M., Morrison, S.G., and Morrison, R.P. (2010). CD4+ T cells and antibody are
476 required for optimal major outer membrane protein vaccine-induced immunity to
477 Chlamydia muridarum genital infection. *Infect Immun* 78**,** 4374-4383.
478 Fischer, W., Perkins, S., Theiler, J., Bhattacharya, T., Yusim, K., Funkhouser, R., Kuiken, C.,
479 Haynes, B., Letvin, N.L., Walker, B.D., Hahn, B.H., and Korber, B.T. (2007).
480 Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1
481 variants. *Nat Med* 13**,** 100-106.
482 Gallo Vaulet, L., Entrocassi, C., Corominas, A.I., and Rodriguez Fermepin, M. (2010).
483 Distribution study of Chlamydia trachomatis genotypes in symptomatic patients in
484 Buenos Aires, Argentina: association between genotype E and neonatal conjunctivitis.
485 *BMC Res Notes* 3**,** 34.
486 Gao, X., Chen, X.S., Yin, Y.P., Zhong, M.Y., Shi, M.Q., Wei, W.H., Chen, Q., Peeling,
487 R.W., and Mabey, D. (2007). Distribution study of Chlamydia trachomatis serovars
488 among high-risk women in China performed using PCR-restriction fragment length
489 polymorphism genotyping. *J Clin Microbiol* 45**,** 1185-1189.
490 Gaschen, B., Taylor, J., Yusim, K., Foley, B., Gao, F., Lang, D., Novitsky, V., Haynes, B.,
491 Hahn, B.H., Bhattacharya, T., and Korber, B. (2002). Diversity considerations in
492 HIV-1 vaccine selection. *Science* 296**,** 2354-2360.
493 Hafner, L., Beagley, K., and Timms, P. (2008). Chlamydia trachomatis infection: host
494 immune responses and potential vaccines. *Mucosal Immunol* 1**,** 116-130.
495 Hanke, T., Blanchard, T.J., Schneider, J., Hannan, C.M., Becker, M., Gilbert, S.C., Hill,
496 A.V., Smith, G.L., and Mcmichael, A. (1998). Enhancement of MHC class I-

Provisional

497 restricted peptide-specific T cell induction by a DNA prime/MVA boost vaccination
498 regime. *Vaccine* 16**,** 439-445.
499 Hansen, J., Jensen, K.T., Follmann, F., Agger, E.M., Theisen, M., and Andersen, P. (2008).
500 Liposome delivery of Chlamydia muridarum major outer membrane protein primes a
501 Th1 response that protects against genital chlamydial infection in a mouse model. *J*
502 *Infect Dis* 198**,** 758-767.
503 Hayton, E.-J., Rose, A., Ibrahimsa, U., Del Sorbo, M., Capone, S., Crook, A., Black, A.P.,
504 Dorrell, L., and Hanke, T. (2014). Safety and Tolerability of Conserved Region
505 Vaccines Vectored by Plasmid DNA, Simian Adenovirus and Modified Vaccinia
506 Virus Ankara Administered to Human Immunodeficiency Virus Type 1-Uninfected
507 Adults in a Randomized, Single-Blind Phase I Trial. *PLoS ONE* 9**,** e101591.
508 Igietseme, J.U., and Murdin, A. (2000). Induction of protective immunity against Chlamydia
509 trachomatis genital infection by a vaccine based on major outer membrane protein-
510 lipophilic immune response-stimulating complexes. *Infect Immun* 68**,** 6798-6806.
511 Ito, J.I., and Lyons, J.M. (1999). Role of gamma interferon in controlling murine chlamydial
512 genital tract infection. *Infect Immun* 67**,** 5518-5521.
513 Knudsen, N.P., Olsen, A., Buonsanti, C., Follmann, F., Zhang, Y., Coler, R.N., Fox, C.B.,
514 Meinke, A., U, D.O., Casini, D., Bonci, A., Billeskov, R., De Gregorio, E., Rappuoli,
515 R., Harandi, A.M., Andersen, P., and Agger, E.M. (2016). Different human vaccine
516 adjuvants promote distinct antigen-independent immunological signatures tailored to
517 different pathogens. *Sci Rep* 6**,** 19570.
518 Lan, J., Melgers, I., Meijer, C.J., Walboomers, J.M., Roosendaal, R., Burger, C., Bleker,
519 O.P., and Van Den Brule, A.J. (1995). Prevalence and serovar distribution of
520 asymptomatic cervical Chlamydia trachomatis infections as determined by highly
521 sensitive PCR. *J Clin Microbiol* 33**,** 3194-3197.
522 Li, W., Murthy, A.K., Guentzel, M.N., Seshu, J., Forsthuber, T.G., Zhong, G., and
523 Arulanandam, B.P. (2008). Antigen-specific CD4+ T cells produce sufficient IFN-
524 gamma to mediate robust protective immunity against genital Chlamydia muridarum
525 infection. *J Immunol* 180**,** 3375-3382.
526 Lyons, J.M., Ito, J.I., Jr., Pena, A.S., and Morre, S.A. (2005). Differences in growth
527 characteristics and elementary body associated cytotoxicity between Chlamydia
528 trachomatis oculogenital serovars D and H and Chlamydia muridarum. *J Clin Pathol*
529 58**,** 397-401.
530 Mckay, P.F., Cope, A.V., Mann, J.F., Joseph, S., Esteban, M., Tatoud, R., Carter, D., Reed,
531 S.G., Weber, J., and Shattock, R.J. (2014). Glucopyranosyl Lipid A Adjuvant
532 Significantly Enhances HIV Specific T and B Cell Responses Elicited by a DNA-
533 MVA-Protein Vaccine Regimen. *PLoS One* 9**,** e84707.
534 Mcshane, H., Brookes, R., Gilbert, S.C., and Hill, A.V. (2001). Enhanced immunogenicity of
535 CD4(+) t-cell responses and protective efficacy of a DNA-modified vaccinia virus
536 Ankara prime-boost vaccination regimen for murine tuberculosis. *Infect Immun* 69**,**
537 681-686.
538 Moore, T., Ananaba, G.A., Bolier, J., Bowers, S., Belay, T., Eko, F.O., and Igietseme, J.U.
539 (2002). Fc receptor regulation of protective immunity against Chlamydia trachomatis.
540 *Immunology* 105**,** 213-221.
541 Morre, S.A., Rozendaal, L., Van Valkengoed, I.G., Boeke, A.J., Van Voorst Vader, P.C.,
542 Schirm, J., De Blok, S., Van Den Hoek, J.A., Van Doornum, G.J., Meijer, C.J., and
543 Van Den Brule, A.J. (2000). Urogenital Chlamydia trachomatis serovars in men and
544 women with a symptomatic or asymptomatic infection: an association with clinical
545 manifestations? *J Clin Microbiol* 38**,** 2292-2296.

Provisional

546 Nilsson, C., Hejdeman, B., Godoy-Ramirez, K., Tecleab, T., Scarlatti, G., Bråve, A., Earl,
547 P.L., Stout, R.R., Robb, M.L., Shattock, R.J., Biberfeld, G., Sandström, E., and
548 Wahren, B. (2015). HIV-DNA Given with or without Intradermal Electroporation Is
549 Safe and Highly Immunogenic in Healthy Swedish HIV-1 DNA/MVA Vaccinees: A
550 Phase I Randomized Trial. *PLoS ONE* 10**,** e0131748.
551 Nunes, A., Nogueira, P.J., Borrego, M.J., and Gomes, J.P. (2010). Adaptive evolution of the
552 Chlamydia trachomatis dominant antigen reveals distinct evolutionary scenarios for
553 B- and T-cell epitopes: worldwide survey. *PLoS One* 5.
554 O'connell, C.M., Abdelrahman, Y.M., Green, E., Darville, H.K., Saira, K., Smith, B.,
555 Darville, T., Scurlock, A.M., Meyer, C.R., and Belland, R.J. (2011). Toll-like receptor
556 2 activation by Chlamydia trachomatis is plasmid dependent, and plasmid-responsive
557 chromosomal loci are coordinately regulated in response to glucose limitation by C.
558 trachomatis but not by C. muridarum. *Infect Immun* 79**,** 1044-1056.
559 Olsen, A.W., Theisen, M., Christensen, D., Follmann, F., and Andersen, P. (2010). Protection
560 against Chlamydia promoted by a subunit vaccine (CTH1) compared with a primary
561 intranasal infection in a mouse genital challenge model. *PLoS One* 5**,** e10768.
562 Pal, S., Peterson, E.M., and De La Maza, L.M. (2005). Vaccination with the Chlamydia
563 trachomatis major outer membrane protein can elicit an immune response as
564 protective as that resulting from inoculation with live bacteria. *Infect Immun* 73**,** 8153-
565 8160.
566 Pal, S., Theodor, I., Peterson, E.M., and De La Maza, L.M. (2001). Immunization with the
567 Chlamydia trachomatis mouse pneumonitis major outer membrane protein can elicit a
568 protective immune response against a genital challenge. *Infect Immun* 69**,** 6240-6247.
569 Papadogeorgakis, H., Pittaras, T.E., Papaparaskevas, J., Pitiriga, V., Katsambas, A., and
570 Tsakris, A. (2010). Chlamydia trachomatis serovar distribution and Neisseria
571 gonorrhoeae coinfection in male patients with urethritis in Greece. *J Clin Microbiol*
572 48**,** 2231-2234.
573 Penttila, T., Tammiruusu, A., Liljestrom, P., Sarvas, M., Makela, P.H., Vuola, J.M., and
574 Puolakkainen, M. (2004). DNA immunization followed by a viral vector booster in a
575 Chlamydia pneumoniae mouse model. *Vaccine* 22**,** 3386-3394.
576 Peterson, E.M., Cheng, X., Motin, V.L., and De La Maza, L.M. (1997). Effect of
577 immunoglobulin G isotype on the infectivity of Chlamydia trachomatis in a mouse
578 model of intravaginal infection. *Infect Immun* 65**,** 2693-2699.
579 Petrovay, F., Balla, E., Nemeth, I., and Gonczol, E. (2009). Genotyping of Chlamydia
580 trachomatis from the endocervical specimens of high-risk women in Hungary. *J Med*
581 *Microbiol* 58**,** 760-764.
582 Picard, M.D., Cohane, K.P., Gierahn, T.M., Higgins, D.E., and Flechtner, J.B. (2012). High-
583 throughput proteomic screening identifies Chlamydia trachomatis antigens that are
584 capable of eliciting T cell and antibody responses that provide protection against
585 vaginal challenge. *Vaccine* 30**,** 4387-4393.
586 Price, M.N., Dehal, P.S., and Arkin, A.P. (2009). FastTree: computing large minimum
587 evolution trees with profiles instead of a distance matrix. *Mol Biol Evol* 26**,** 1641-
588 1650.
589 Rafiq, K., Bergtold, A., and Clynes, R. (2002). Immune complex-mediated antigen
590 presentation induces tumor immunity. *J Clin Invest* 110**,** 71-79.
591 Schautteet, K., Stuyven, E., Beeckman, D.S., Van Acker, S., Carlon, M., Chiers, K., Cox, E.,
592 and Vanrompay, D. (2011). Protection of pigs against Chlamydia trachomatis
593 challenge by administration of a MOMP-based DNA vaccine in the vaginal mucosa.
594 *Vaccine* 29**,** 1399-1407.

Provisional

Scidmore, M.A. (2005). Cultivation and Laboratory Maintenance of Chlamydia trachomatis. *Curr Protoc Microbiol* Chapter 11**,** Unit 11A 11.

Smelov, V., Quint, K.D., Pleijster, J., Savelkoul, P.H., Shalepo, K., Shipitsyna, E., Domeika, M., Gorelov, A., Savicheva, A., Quint, W.G., De Vries, H.J., Ouburg, S., and Morre, S.A. (2009). Chlamydia trachomatis serovar distributions in Russian men and women: a comparison with Dutch serovar distributions. *Drugs Today (Barc)* 45 Suppl B**,** 33-38.

Su, H., and Caldwell, H.D. (1995). CD4+ T cells play a significant role in adoptive immunity to Chlamydia trachomatis infection of the mouse genital tract. *Infect Immun* 63**,** 3302-3308.

Suarkia, D.L., Mgone, C.S., Lehmann, D., Passey, M.E., Lupiwa, T., Paniu, M.M., Kono, J., Kakazo, M., Yeka, W., and Alpers, M.P. (2007). Chlamydia trachomatis infection and distribution of serovars in the Eastern Highlands Province, Papua New Guinea. *P N G Med J* 50**,** 134-144.

Suchland, R.J., Eckert, L.O., Hawes, S.E., and Stamm, W.E. (2003). Longitudinal assessment of infecting serovars of Chlamydia trachomatis in Seattle public health clinics: 1988-1996. *Sex Transm Dis* 30**,** 357-361.

Sullivan, N.J., Sanchez, A., Rollin, P.E., Yang, Z.Y., and Nabel, G.J. (2000). Development of a preventive vaccine for Ebola virus infection in primates. *Nature* 408**,** 605-609.

Sun, G., Pal, S., Weiland, J., Peterson, E.M., and De La Maza, L.M. (2009). Protection against an intranasal challenge by vaccines formulated with native and recombinant preparations of the Chlamydia trachomatis major outer membrane protein. *Vaccine* 27**,** 5020-5025.

Tregoning, J.S., and Kinnear, E. (2014). Using Plasmids as DNA Vaccines for Infectious Diseases. *Microbiology Spectrum* 2.

Van Der Burg, S.H., Kwappenberg, K.M., O'neill, T., Brandt, R.M., Melief, C.J., Hickling, J.K., and Offringa, R. (2001). Pre-clinical safety and efficacy of TA-CIN, a recombinant HPV16 L2E6E7 fusion protein vaccine, in homologous and heterologous prime-boost regimens. *Vaccine* 19**,** 3652-3660.

Who (2001). Global Prevalence and Incidence of Selected Curable Sexually Transmitted Diseases: Overview and Estimates. *Geneva, Switzerland: World Health Organisation*.

Yamazaki, T., Hagiwara, T., Kishimoto, T., Sasaki, N., Takahashi, S., Ishihara, O., Wangroongsarb, P., Kusum, M., and Sirivongrangsan, P. (2005). Distribution of Chlamydia trachomatis serovars among female prostitutes and non-prostitutes in Thailand, and non-prostitutes in Japan during the mid-90s. *Jpn J Infect Dis* 58**,** 211-213.

Yuan, Y., Zhang, Y.X., Watkins, N.G., and Caldwell, H.D. (1989). Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 Chlamydia trachomatis serovars. *Infect Immun* 57**,** 1040-1049.

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Table 1. Multi-component prime-boost vaccine regimens.

In vaccine regimen nomenclature, D represents DNA (+ electroporation), A represents HuAd5, M represents MVA, and P represents recombinant MOMP protein adjuvanted with MF59®.

Regimen	d0	d21	d42	d63	d84
DDDAM	DNA	DNA	DNA	HuAd5	MVA
DDDA	DNA	DNA	DNA	HuAd5	
DDDM	DNA	DNA	DNA	MVA	
DDD	DNA	DNA	DNA		
AM	HuAd5	MVA			
DAMP	DNA	HuAd5	MVA	Protein	
AMPP	HuAd5	MVA	Protein	Protein	
DDPP	DNA	DNA	Protein	Protein	
APP	HuAd5	Protein	Protein		
PPP	Protein	Protein	Protein		
PP	Protein	Protein			

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Figure 1. Global *C. trachomatis* serovar prevalence, phylogeny and theoretical epitope coverage of consensus and mosaic MOMP antigens.

(**A)**The serovar prevalence of *C. trachomatis* worldwide compiled from a literature review and represented in descending prevalence at global locations. **(B)** A phylogenetic maximum likelihood tree based on the *ompA* sequence alignments derived from (Nunes et al., 2010) was created using FastTree and the graphic generated by Rainbow Tree. **(C)** Potential epitope coverage against all serovars (total) and individual serovars (serovars D-K) were analysed for a monovalent Con E antigen, Con E and Con F antigens, 2 mosaic antigens, a Con E antigen and a mosaic antigen, three mosaic antigens and a Con E antigen with 2 additional mosaic antigens using EPICOVER. Mean 9-mer coverage presented against individual and total combined serovars D to K, with exact (red), off-by-1 (orange) and off-by-2 (yellow) epitope matching.

Figure 2. Antibody and cellular responses following multi-component prime-boost vaccination regimens.

BALB/c mice (n = 8 per group) were intramuscularly immunised in various prime-boost regimens, with sera and vaginal wash collected two weeks after final boost. MOMP-specific IgG concentrations were measured in serum **(A)** and vaginal washes **(B)**, expressed as the

mean + SEM concentrations. **(C)** Serum MOMP-specific IgG2a and IgG1 isotype concentrations were measured by ELISA, and the mean + SEM IgG2a:IgG1 ratios plotted. The dotted line indicates the IgG2a:IgG1 ratio of 1, demonstrating Th1-skewing above this line or Th2-skewing below it. **(D)** Serum antibody avidity was measured by MOMP-specific IgG ELISA with non-reducing (H_2O) and reducing (8 M urea) washes after sample addition. Results are shown as percentage (%) change in binding ($^{\text{reducing OD 650}}/_{\text{non-reducing OD}}$ x 100). IgG concentrations, avidities and IgG2a:IgG1 ratio represented as group means and SEM. **(E)** Vaccinated BALB/c mice (n = 8 per group) were sacrificed one-week post-final immunisation and splenocytes assessed by IFN-γ ELISpot for MOMP-reactive T cells stimulated by a peptide pool consisting of 15-mers overlapping by 11 amino acids. Data expressed as group medians (+ interquartile range) (SFU/million antigen stimulated cells). * $p \leq 0.05$ (yellow), ** $p \leq 0.005$ (orange), *** $p \leq 0.0005$ (red) and **** $p \leq 0.0001$ (dark red) by one-way ANOVA with Bonferroni's multiple comparison post-test on logged values **(A-D)** and by Kruskal-Wallis with Dunn's multiple comparison test **(E)**.

Figure 3. Prime-boost vaccine regimens induce comparable immune responses in both BALB/c and B6C3F1 mouse strains.

MOMP-specific IgG concentrations were measured in serum **(A)** and vaginal washes **(B)** for both BALB/c and B6C3F1 vaccinated mice (n = 8 per group), expressed as individual concentrations with bars representing the means. **(C)** Serum MOMP-specific IgG2a and IgG1 isotype concentrations were measured by ELISA for both BALB/c and B6C3F1 vaccinated mice (n = 5-8 per group), and individual points and bars representing the means IgG2a:IgG1 ratios plotted. The dotted line indicates the IgG2a:IgG1 ratio of 1, demonstrating Th1-skewing above this line or Th2-skewing below it. **(D)** Splenocytes were assessed by IFN-γ ELISpot for MOMP-reactive T cells stimulated by a peptide pool consisting of 15-mers overlapping by 11 amino acids for both vaccinated BALB/c and B6C3F1 mice. Data expressed as individual values with bars representing group medians (+ interquartile range) (SFU/million antigen stimulated cells).

Figure 4. The DAMP vaccine regimen enhances the clearance of intravaginal *C. trachomatis* in BALB/c and B6C3F1 mice, in a $CD4^+$ T cell dependent manner.

Six weeks after the final vaccination and 1 week after 2 mg/mouse subcutaneous Depo-Provera treatment, BALB/c and B6C3F1 mice (n = 7 to 10 per group) were infected intravaginally with $4x10^5$ IFU of *C. trachomatis* D/UW-3/Cx. The vaginal vault of mice were sampled using individual swabs at day 3 (**(A)** BALB/c; **(B)** B6C3F1) after challenge, and vaginal Chlamydial loads quantified by infection assay and immunoflorescent microscopy. The fold reduction in median Chlamydial load compared to naïve BALB/c **(A)** and B6C3F1 **(B)** mice at day 3 after infection is also represented. **(C)** B6C3F1 mice (n = 8 per group) were immunised with the DAMP regimen or left unvaccinated and subsequently depleted of $CD4^+$ T cells by i.p. injections of 500 µg/mouse of anti-mouse CD4 monoclonal antibody (clone GK1.5) on days -1 and +1 with respect to day of challenge day 0. *C. trachomatis* load was measured in the vaginal vault at day 3 after infection. Individual and median values are represented. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, 2-tailed Mann-Whitney U test.

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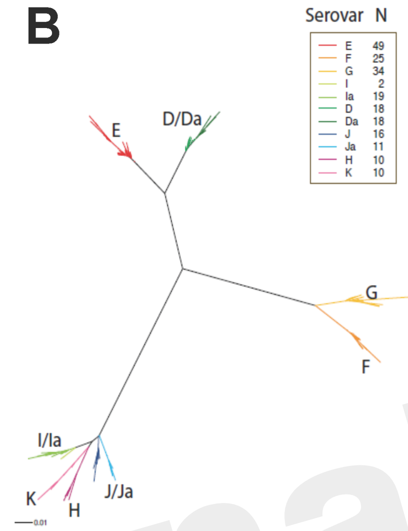
Figure 1

A

B



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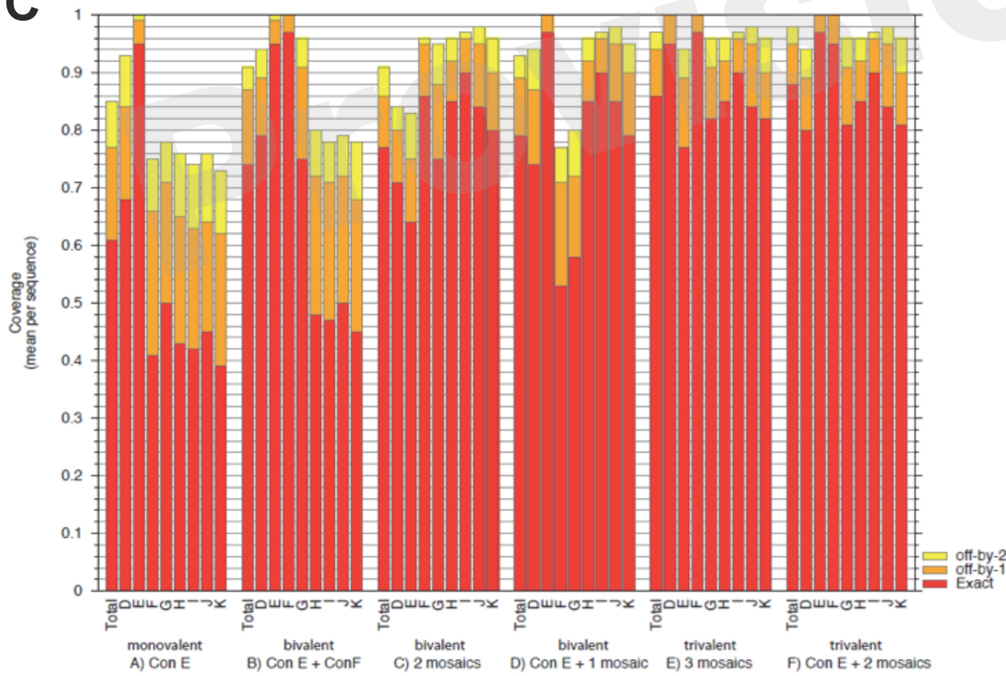

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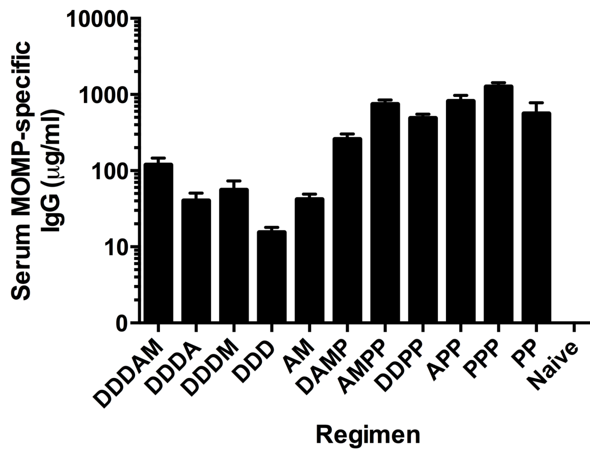
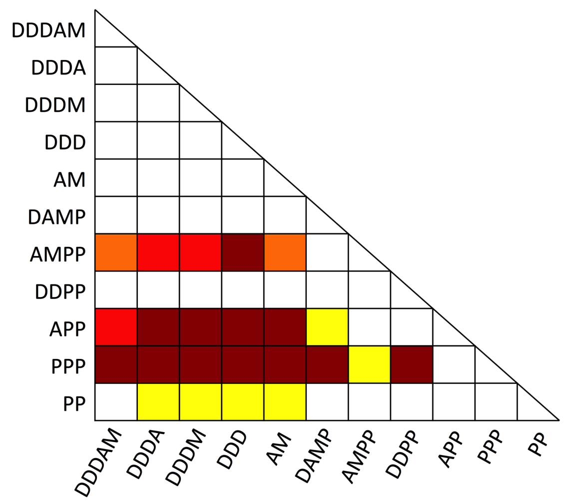
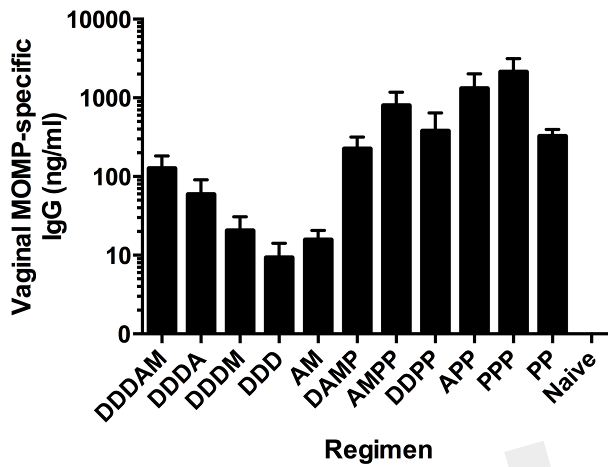
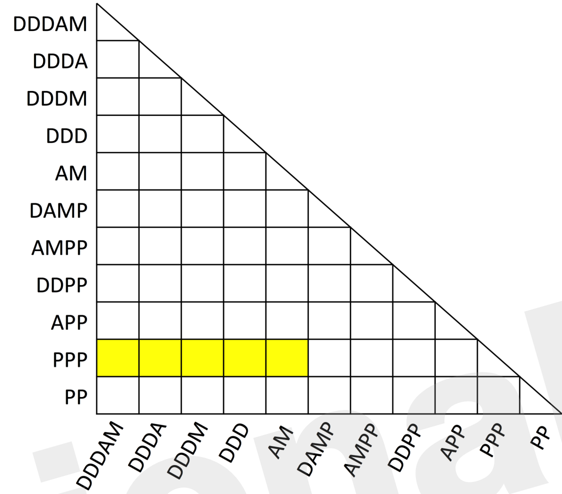
Figure 2

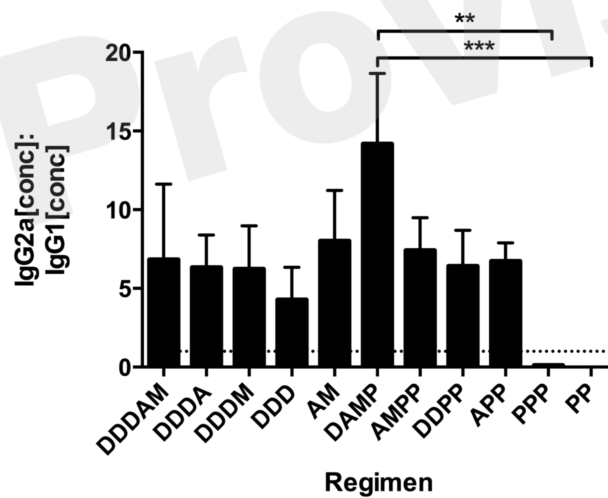
Figure 02.TIFF

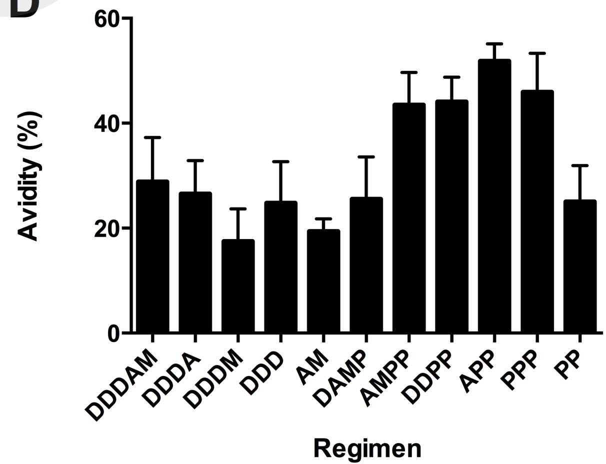


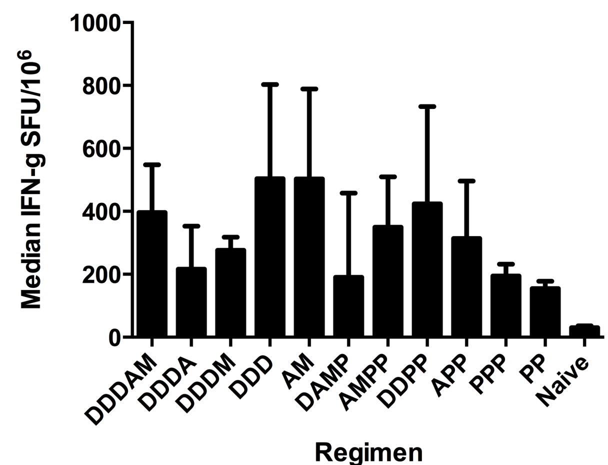


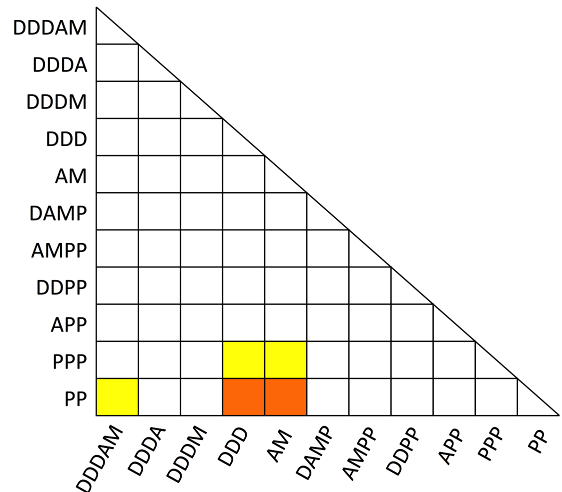








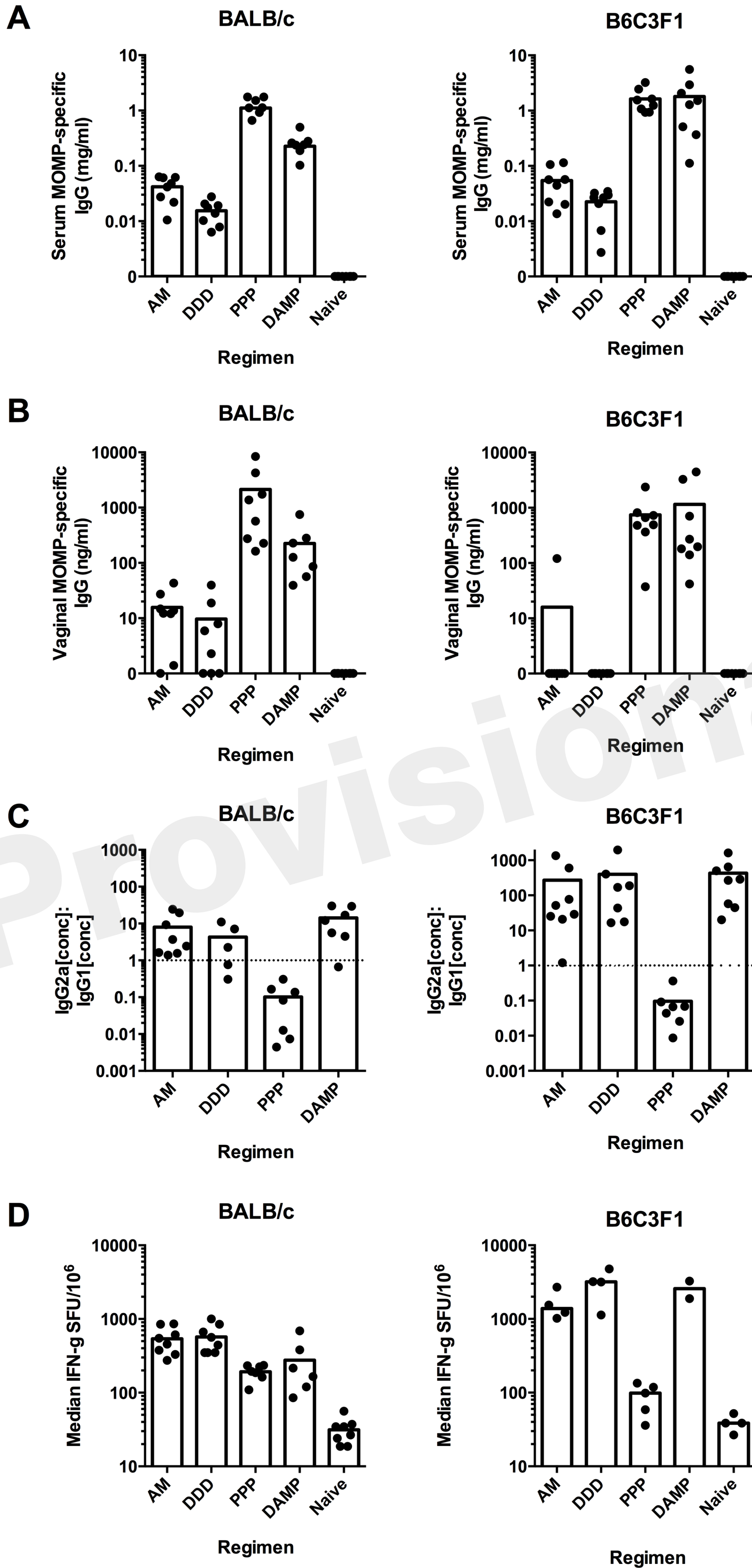




Provisional

Figure 3

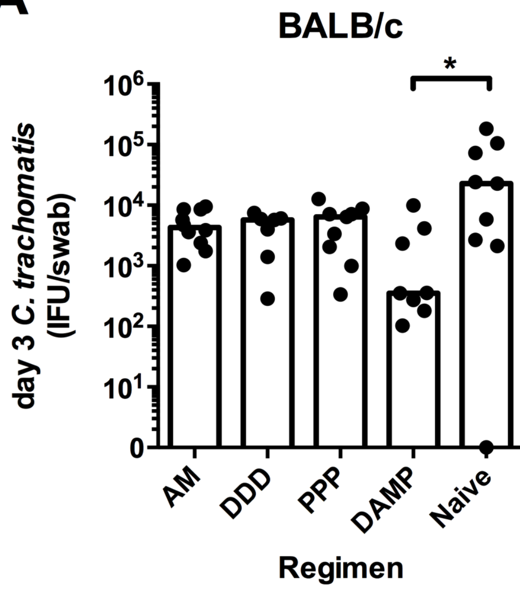
Figure 03.TIFF

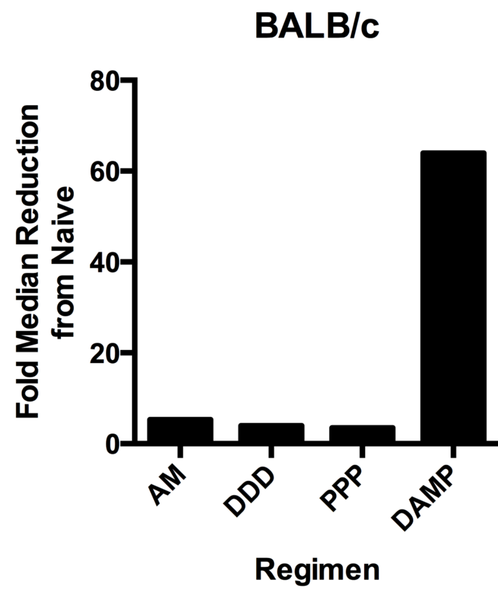


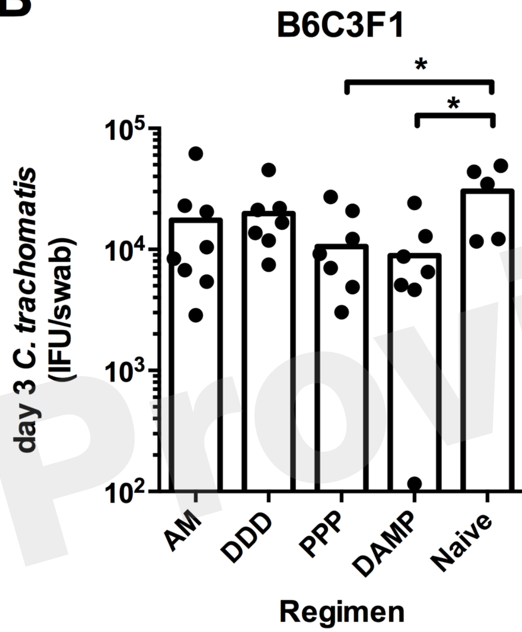
Provisional

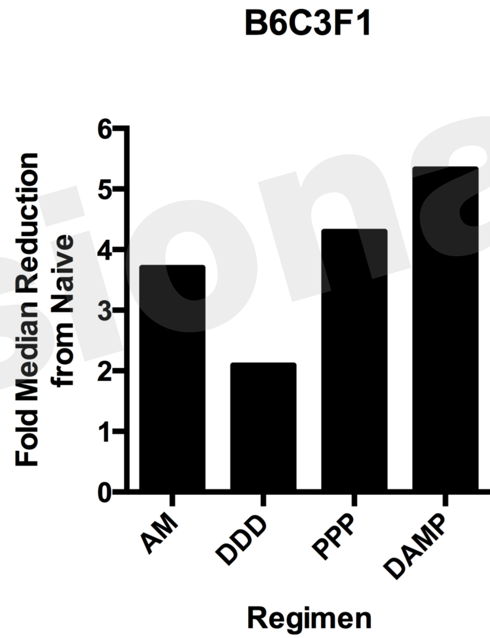
Figure 4

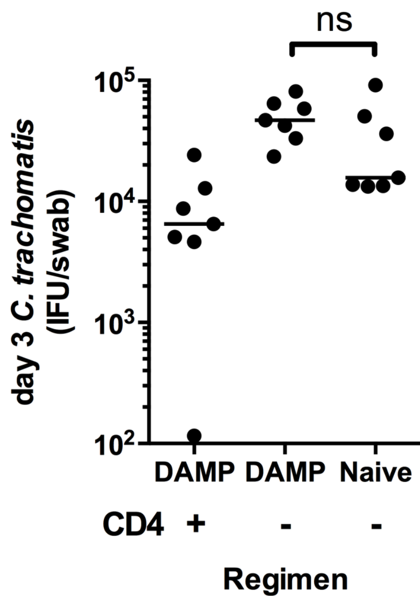
Figure 04.TIFF











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