**The utility of *Plasmodium berghei* as a rodent model for anti-merozoite malaria vaccine assessment.**

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**Supplemental Figure 1: Efficacy of AdHu5-MVA PbMSP1$_{42}$, PbAMA1, and PbMSP9 immunization against *P. berghei* NK65 pRBC infection.**

BALB/c mice (*n* = 6 / group) were immunized i.d. with 1 x $10^{10}$ vp AdHu5 and boosted 8 weeks later with 1 x $10^{7}$ pfu MVA encoding either **(A)** PbMSP1$_{42}$, **(B)** PbAMA1 or **(C)** PbMSP9; or **(D)** mice were co-administered i.d. in the same manner a mixture of all three AdHu5 vaccines (dose of each virus = 1 x $10^{10}$ vp; total 3 x $10^{10}$ vp) followed by a boost with a mixture of all three MVA vaccines (dose of each virus = 1 x $10^{7}$ pfu; total 3 x $10^{7}$ pfu). Two weeks post-boost vaccinated mice as well as non-immunized controls **(E)** were challenged with 5 x $10^{2}$ *P. berghei* NK65 pRBC and monitored for parasitemia. Lines represent individual mice. † indicates that mice were culled and the number culled from the total number in the group is shown in brackets.

**Supplemental Figure 2: PfMSP1$_{19}$-specific IgG following PfM115 or PfM128 immunization of rabbits, and efficacy of purified and passively transferred anti-PfMSP1 polyclonal rabbit IgG.**

**(A)** New Zealand white rabbits (*n* = 3-4 / group) were immunized with vaccines expressing PfM128. Rabbits were primed i.m. with AdHu5 or ChAd63 and then boosted i.m. eight weeks later with MVA. Two weeks post-boost serum was collected and analysed for anti-PfMSP1$_{19}$ (3D7/ETSR allele) total IgG responses. Points indicate individual rabbits and bars are median titers (same rabbits as used in (B)). Results from two similar and previously published studies are shown for comparison (samples re-run here at the same time). Study 1 [1] used AdHu5 and simian adenoviral vaccines as well as MVA encoding PfM115 administered i.d. or i.m. (five groups of *n* = 3 pooled, total

*n*=15). Study 2 used AdHu5 and MVA vaccines encoding PfM128 administered i.d. (*n* = 8) [1]. **(B)** Total IgG was purified from sera of New Zealand white rabbits prior to immunization (d0) and two weeks post-boost (d70) following prime-boost immunization with adenovirus (AdHu5 or ChAd63) and MVA expressing PfM128 or no antigenic insert (control). 0.5mg/day of purified IgG from a single rabbit was used to passively immunize i.v. a single BALB/c mouse on three consecutive days. On the second day all mice were challenged i.v. with 5 x $10^2$ Pb-PfM19 pRBC. Naïve mice did not receive any IgG. Parasitemia on day 5 post-challenge is shown. Points indicate individual mice and bars indicate median parasitemia.

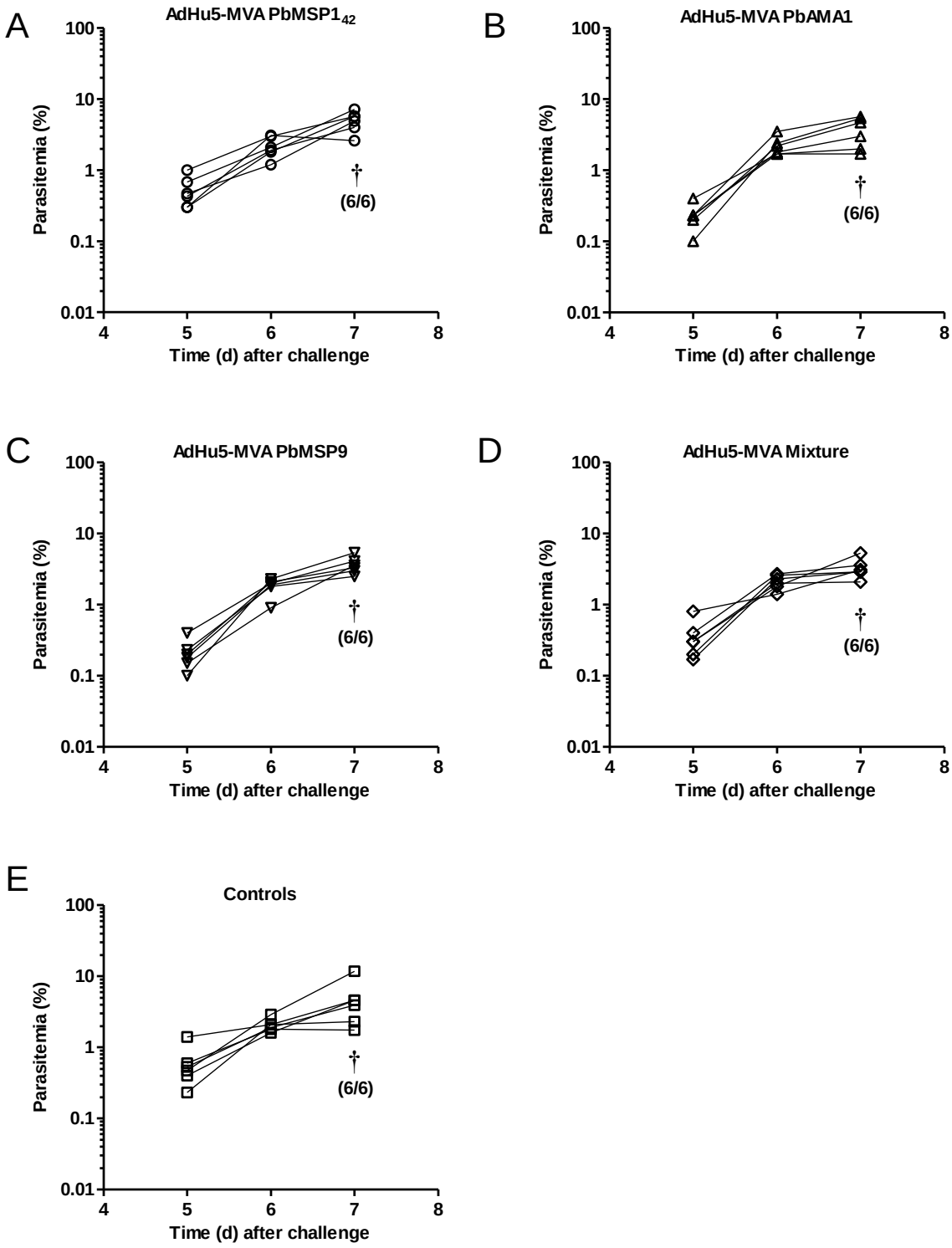
**Supplemental Figure 3: Efficacy of AdHu5-MVA anti-merozoite vaccines against Δ*smac P. berghei*.**

BALB/c mice (*n* = 5-6 / group) were immunized i.m. with 1 x $10^{10}$ vp AdHu5 and boosted ten weeks later i.m. with 1 x $10^7$ pfu MVA encoding either OVA (control) or the antigens PbMSP1$_{42}$, PbAMA1 or PbMSP9. In the case of the triple antigen mixture, vaccines were administered i.m. as per the doses in Supplemental Figure 1. Two weeks post-boost mice were challenged i.v. with 5 x $10^2$ *P. berghei* ANKA Δ*smac* pRBC and monitored for parasitemia. Day 9 parasitemias are shown for individual mice in each group. Lines represent the medians.

**Supplemental References:**

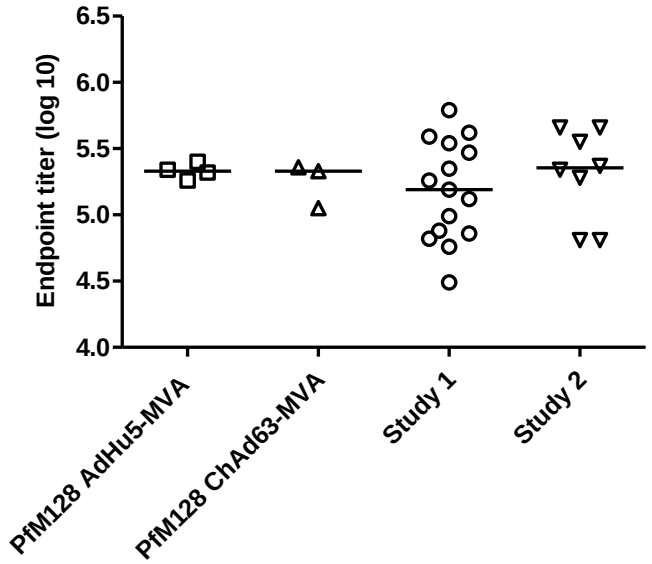
1 Goodman, A.L. *et al.*, New candidate vaccines against blood-stage Plasmodium falciparum malaria: prime-boost immunization regimens incorporating human and simian adenoviral vectors and poxviral vectors expressing an optimized antigen based on merozoite surface protein 1. *Infect Immun* 78 (11), 4601-4612 (2010).

# Supplemental Figure 1

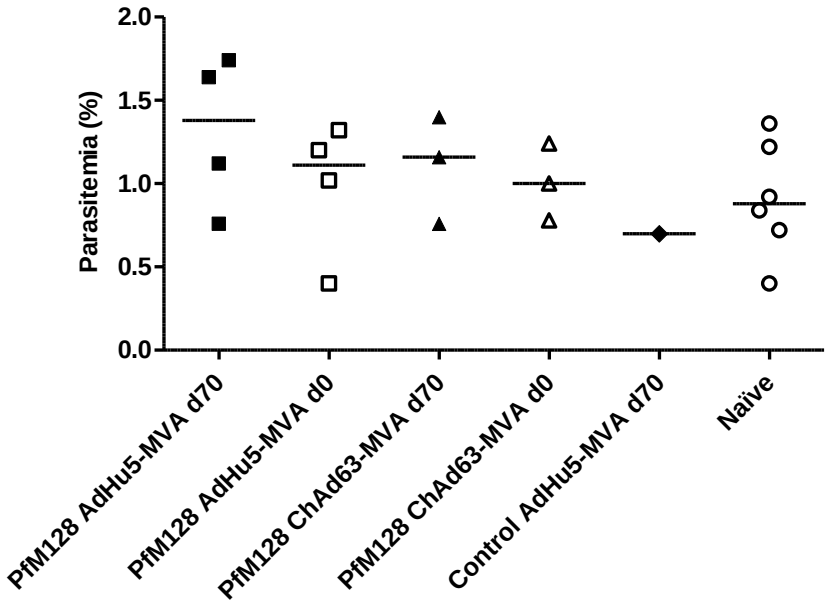


# Supplemental Figure 2

A




B



# Supplemental Figure 3