

Adenovirus-prime and baculovirus-boost heterologous immunization achieves sterile protection against malaria sporozoite challenge in a murine model

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Supplementary Information

Immunoblotting and IFA assay

For immunoblotting, HEK293A cells (6×10^4) were infected with AdHu5-PfCSP (MOI = 1) or transduced with emBDES-PfCSP (MOI = 100). After 48h incubation, the infected or transduced cells were prepared in reducing Laemmli lysis buffer and boiled for 5 min. The proteins were separated by 10% SDS-PAGE, transferred to an Immobilon-FL PVDF membrane (Merck Millipore, Guyancourt, France), and then probed with an anti-PfCSP monoclonal Ab (mAb) 2A10 (MR4, Manassas, VA). After incubation with IRDye-800-conjugated anti-mouse IgG Ab (Rockland Immunochemicals, Gilbertsville, PA, USA), the bound Abs were detected by electrochemiluminescence (GE Healthcare, Waukesha, WI), as described previously¹. For IFA, HEK293A cells (3×10^4) were infected with AdHu5-PfCSP (MOI = 1) or transduced with emBDES-PfCSP (MOI = 100). After 48h incubation, the infected or transduced cells were fixed with 5% paraformaldehyde and after blocking with 10% normal goat serum, were incubated with anti-PfCSP mAb 2A10 conjugated with Alexa Fluor 594 for 1 h, washed five times in PBS, and mounted with Vectashield containing 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA). A BZ-X700 fluorescence microscope (Keyence, Tokyo, Japan) with a 100× objectives was used for image acquisition.

- 1 Mizutani, M. *et al.* Development of a *Plasmodium berghei* transgenic parasite expressing the full-length *Plasmodium vivax* circumsporozoite VK247 protein for testing vaccine efficacy in a murine model. *Malar J* **15**, 251 (2016).

Supplementary Figures and Table:

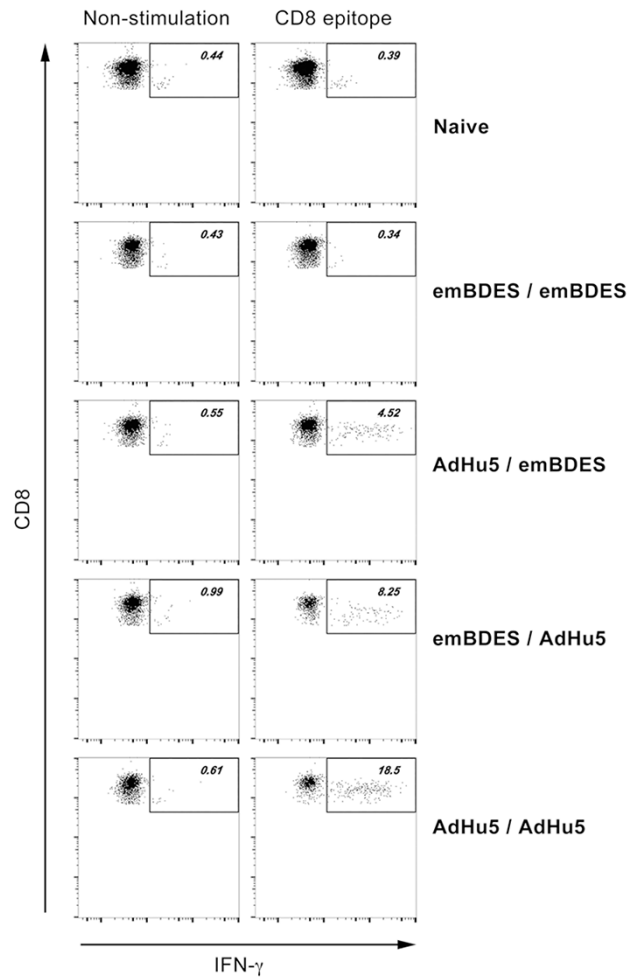


Figure S1. PfCSP-specific cellular immune responses after heterologous prime–boost immunization regimens using AdHu5-PfCSP and emBDES-PfCSP/IL12. BALB/c mice were immunized with the indicated regimens. AdHu5-PfCSP and emBDES-PfCSP/IL12 are shown as AdHu5 and emBDES, respectively. Two weeks after boosting, splenocytes were stimulated with the synthetic PfCSP-specific CD8 epitope. Percentages of IFN- γ -secreting cells in the CD8⁺CD4[−] T-cell population were determined after the subtraction of the percentages of cells that stained with an isotype control Ab.

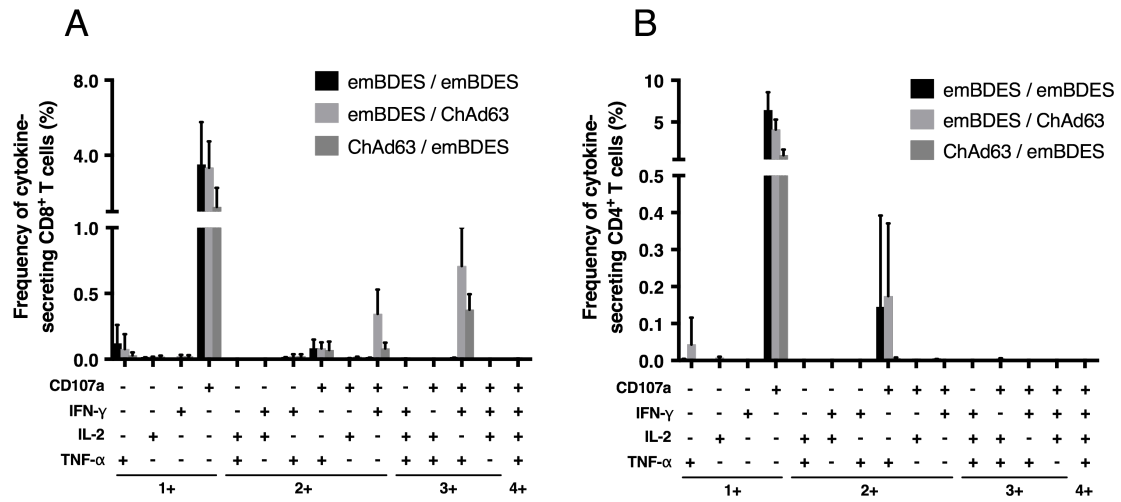


Figure S2. T-cell multifunctionality after heterologous prime-boost immunization regimens using ChAd63-PfCSP and emBDES-PfCSP/IL12. BALB/c mice were immunized with the indicated regimens. ChAd63-PfCSP and emBDES-PfCSP/IL12 are shown as ChAd63 and emBDES, respectively. Single cells from the mouse spleens were stimulated with the peptide pool of PfCSP and stained as described in Fig. 6 and the *Materials and Methods*. Graphs represent the percentages of CD8⁺ T cells (A) or CD4⁺ T cells (B) secreting either one, two, or all three cytokines and/or expressing CD107a. Data were analysed with FlowJo.

Supplementary Table S1. The mean numbers of infectious bites per mouse in Table 1.

Prime	Boost	Mean numbers of infectious bites $\pm$ S.D. (min number – max number)		
		Expt 1 ^a	Expt 2 ^b	Expt 3 ^b
BES-GL3	BES-GL3	2.9 $\pm$ 1.0 (2 - 5)	3.6 $\pm$ 0.8 (3 - 5)	3.3 $\pm$ 0.8 (2 - 4)
emBDES	emBDES	3.1 $\pm$ 1.2 (2 - 5)	-	-
emBDES	AdHu5	3.8 $\pm$ 1.1 (2 - 6)	-	-
AdHu5	emBDES	2.9 $\pm$ 0.6 (2 - 4)	3.9 $\pm$ 1.2 (3 - 7)	2.7 $\pm$ 0.7 (2 - 4)
AdHu5	AdHu5	3.5 $\pm$ 1.4 (2 - 6)	-	-

^a No significant difference among the groups by a Kruskal-Wallis test.

^b No significant difference between the groups by a Mann-Whitney test.