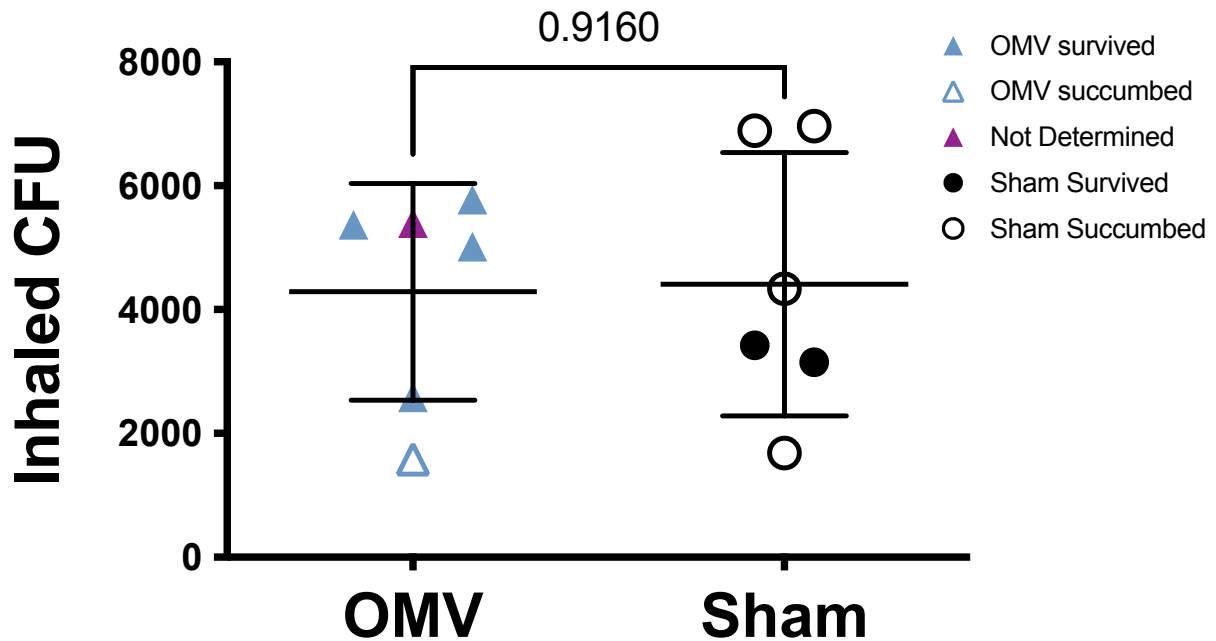
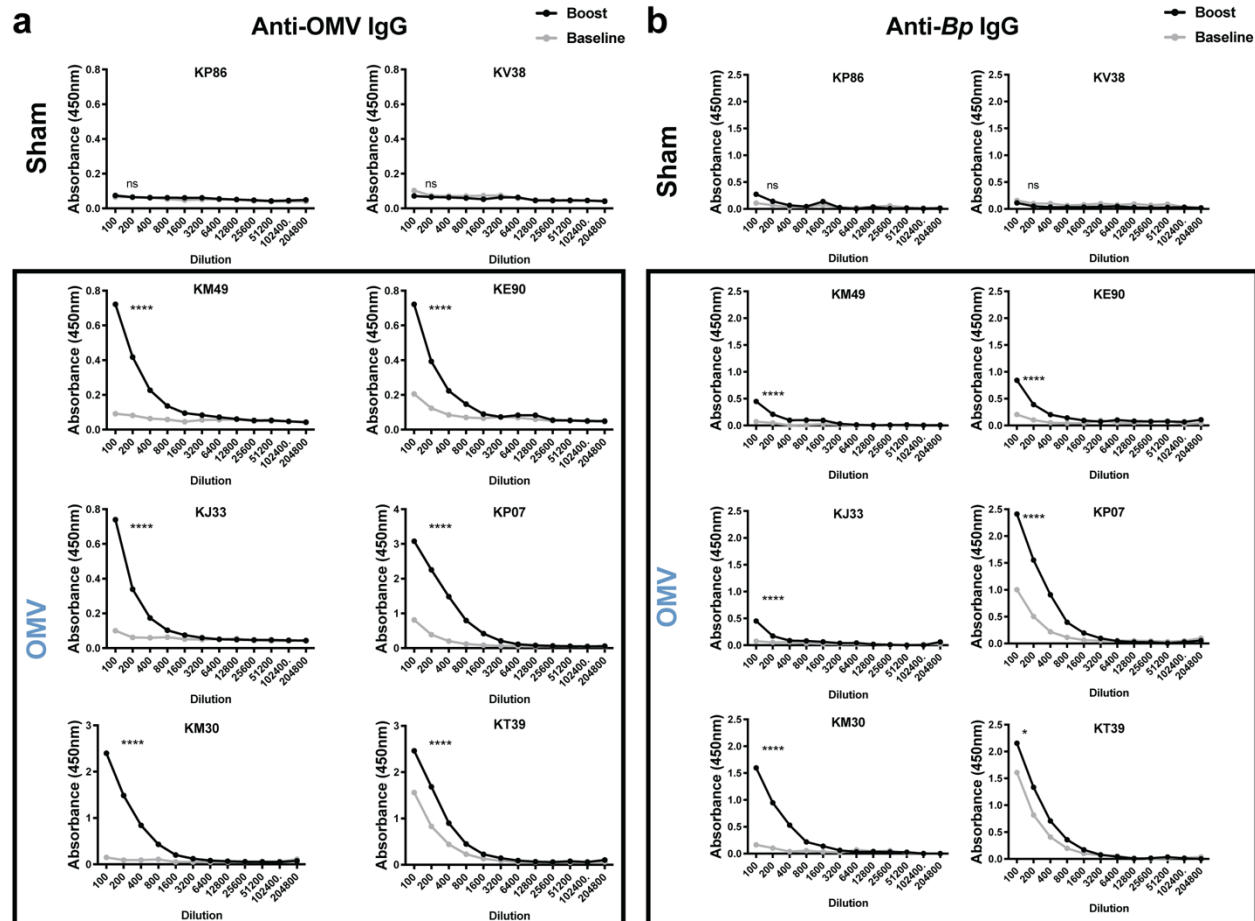


An outer membrane vesicle vaccine prevents lung pathology in a macaque model of pneumonic melioidosis

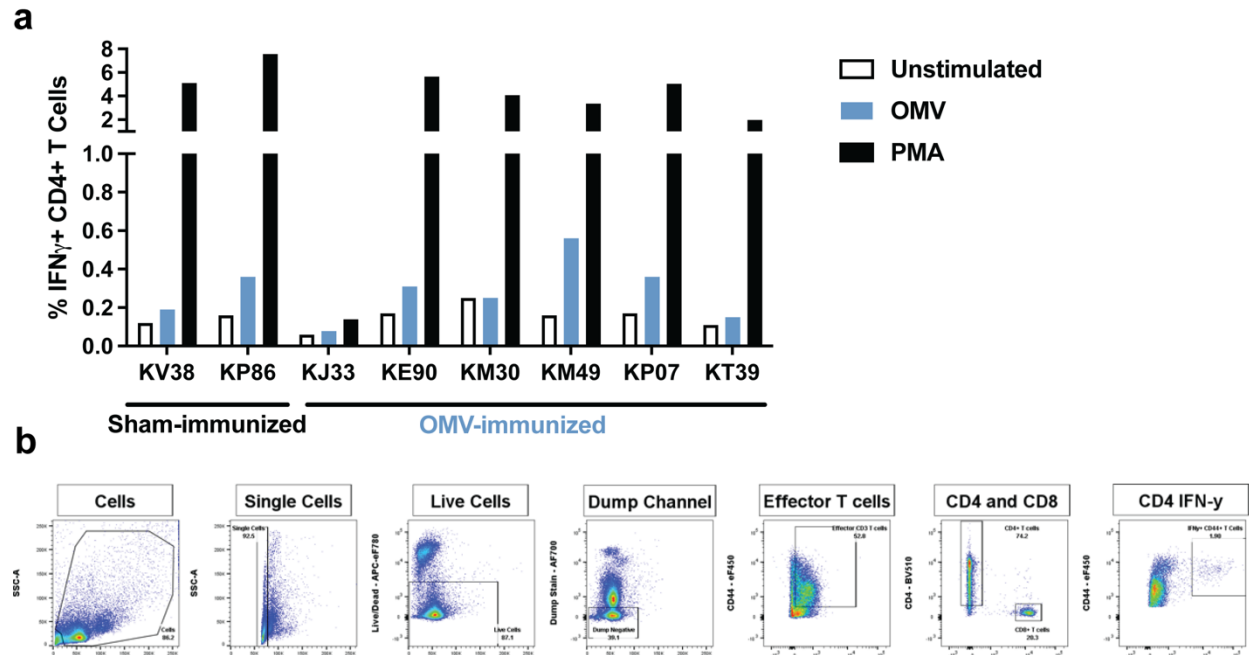
Supplementary Information File



Supplementary Figure 1. Aerosol challenge of Rhesus macaques with *B. pseudomallei*. NHPs were challenged with *B. pseudomallei* by small particle aerosol. There was no difference in *B. pseudomallei* aerosol challenge doses for rhesus macaques immunized with sham or OMV vaccine (ns = not significant, $p=0.92$ by t-test). Individual NHPs are indicated as blue open triangles for OMV-immunized succumbed, blue filled triangles for OMV-immunized survived, purple filled triangle for not determined, black open circles for sham-immunized succumbed and black filled circles for sham-immunized survived.

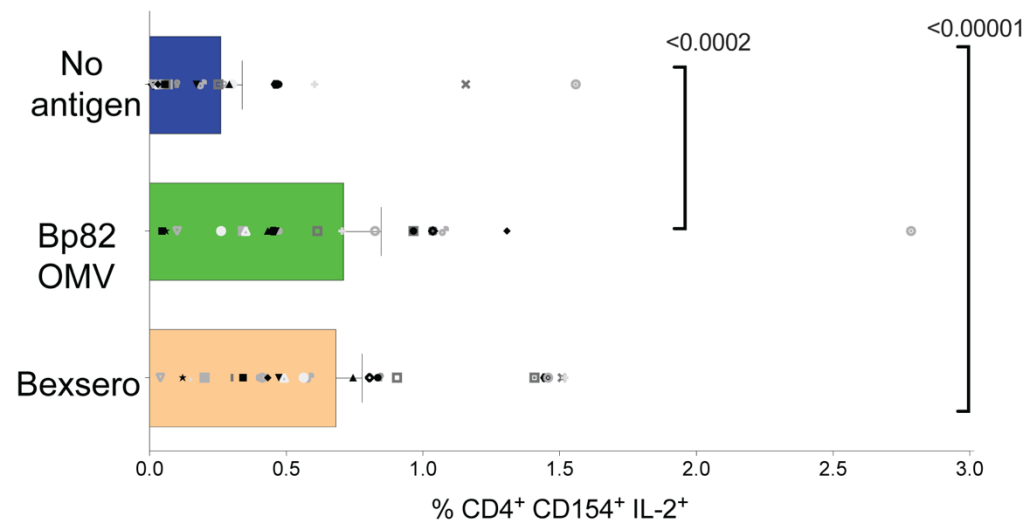
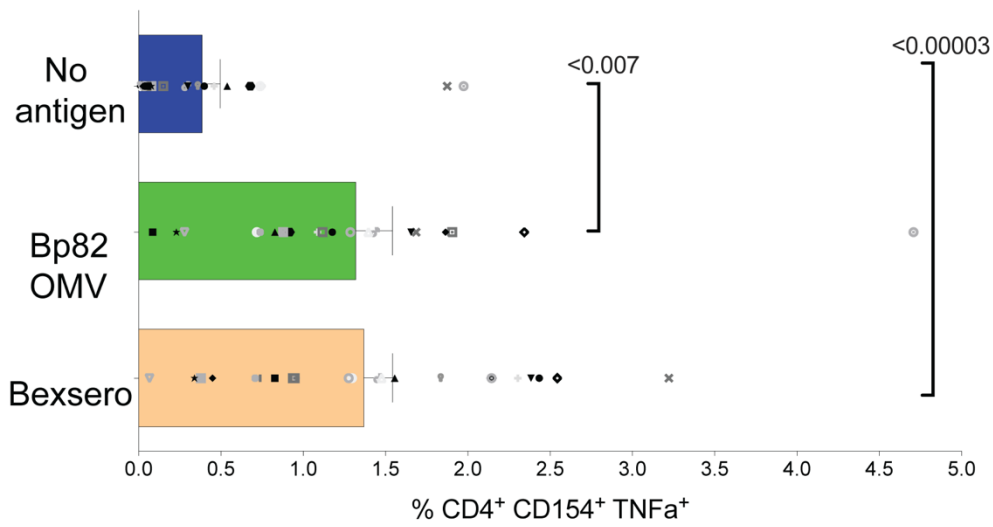
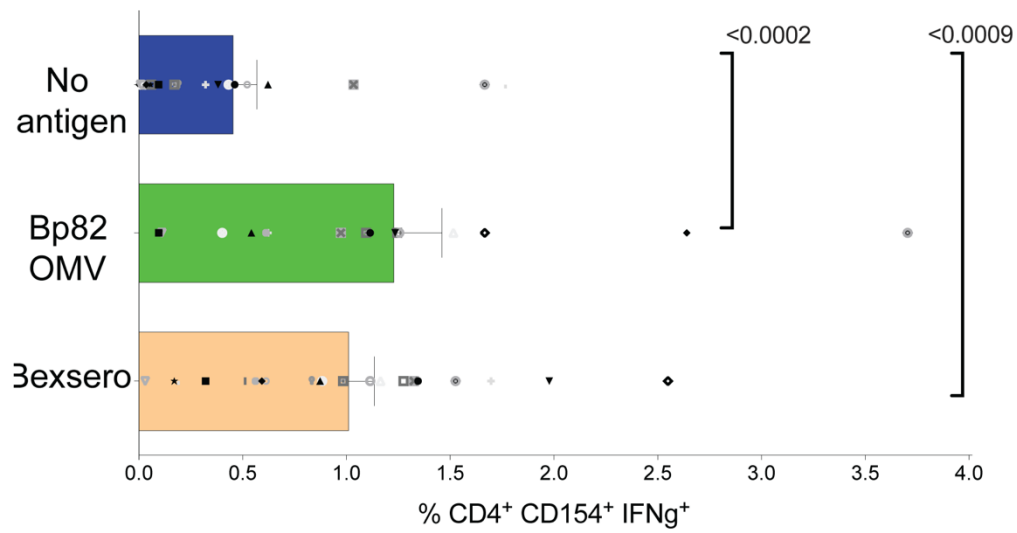


Supplementary Figure 2. OMV-immunized macaques demonstrate increases in IgG specific for the *Bp* and the OMV vaccine in bronchoalveolar lavage fluid. (a) OMV- and (b) *B. pseudomallei*-specific bronchoalveolar lavage (BAL) fluid IgG were measured in sham-immunized and OMV-immunized (boxed) rhesus macaques by ELISA. Antibodies were measured prior to immunization (baseline) and two weeks after the second dose (boost). Reciprocal titers are presented for each dilution. Pairwise comparison of baseline versus boost sample curves of OD450 vs dilution number was done by the modified chi-squared test. ns= not significant, * = <0.05, ** = p<0.0001.**

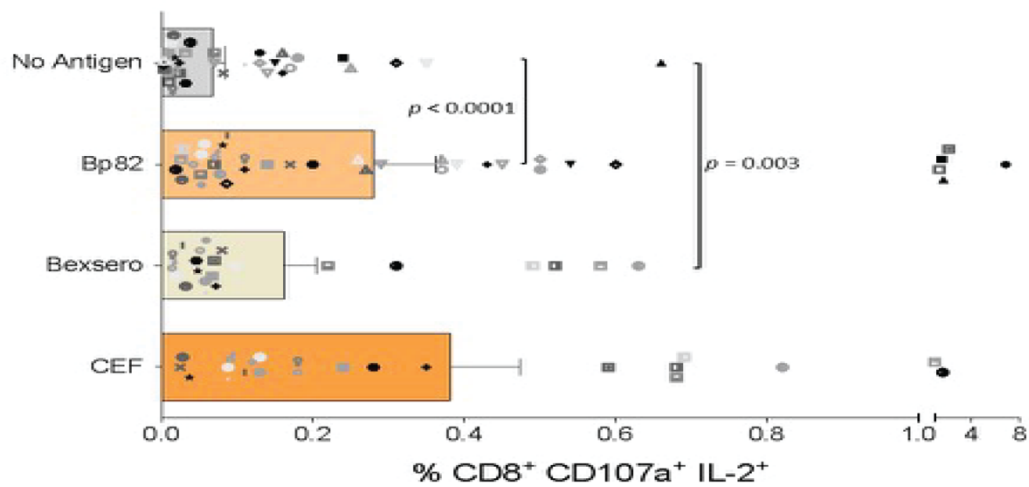
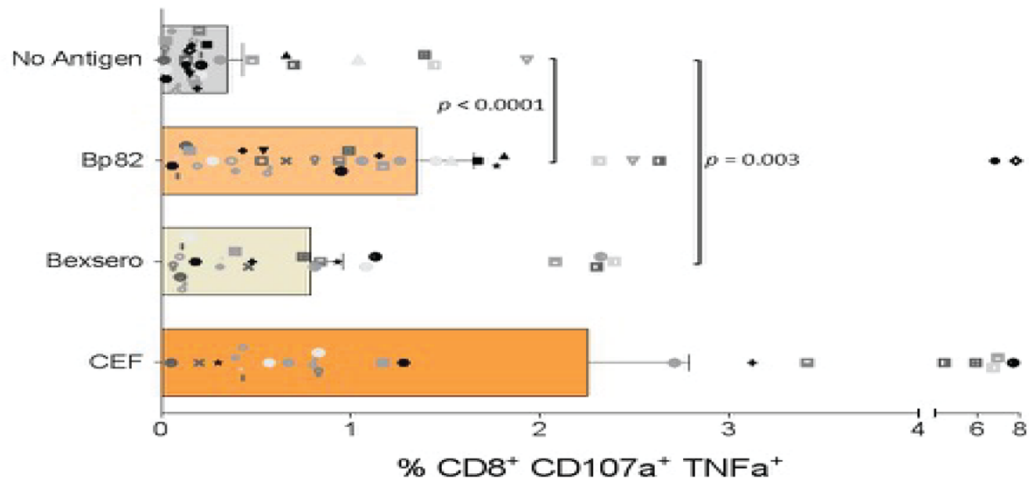
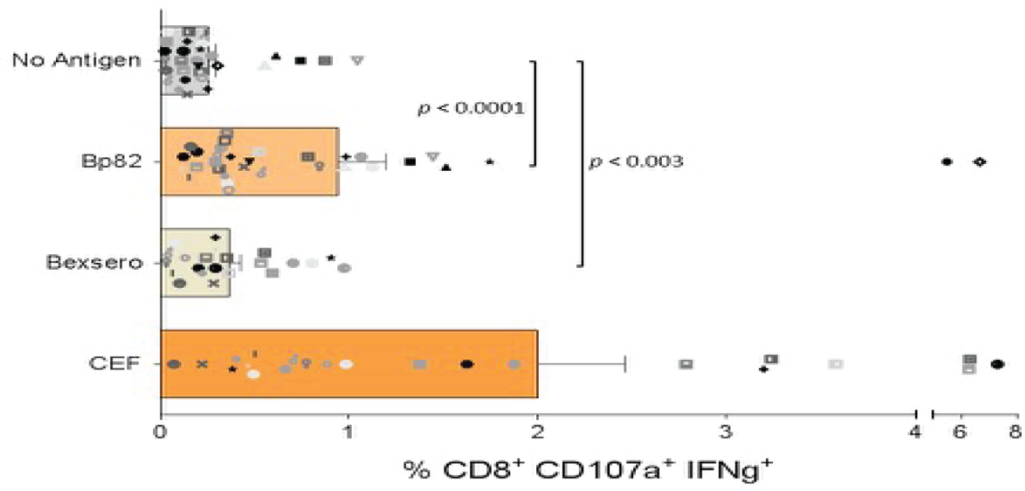


Supplementary Figure 3. Differences in cellular memory responses were not detected in peripheral blood after OMV immunization. (a) Peripheral Blood Mononuclear Cells (PBMCs) from immunized and control macaques were restimulated with OMVs *ex vivo* and the % of IFN- γ (PE, Clone B27, BD) producing CD4 T cells (V510, Clone L200, BD) measured by flow cytometry. PMA stimulation was used as a positive control. **(b)** Gating strategy to evaluate CD4 T cells.

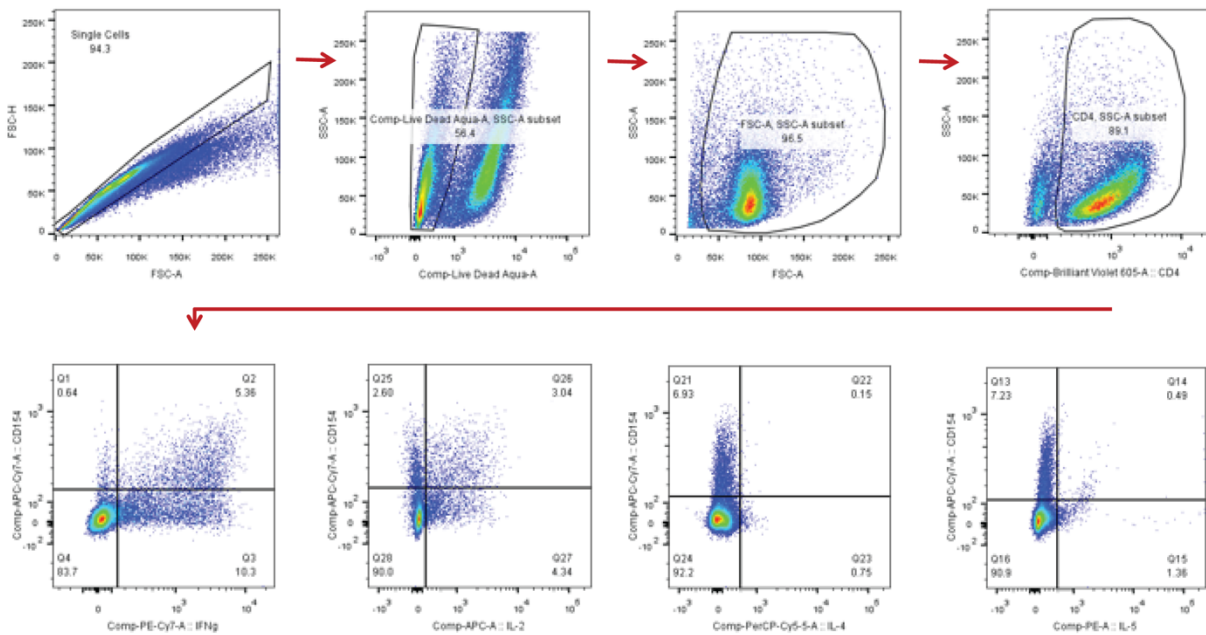
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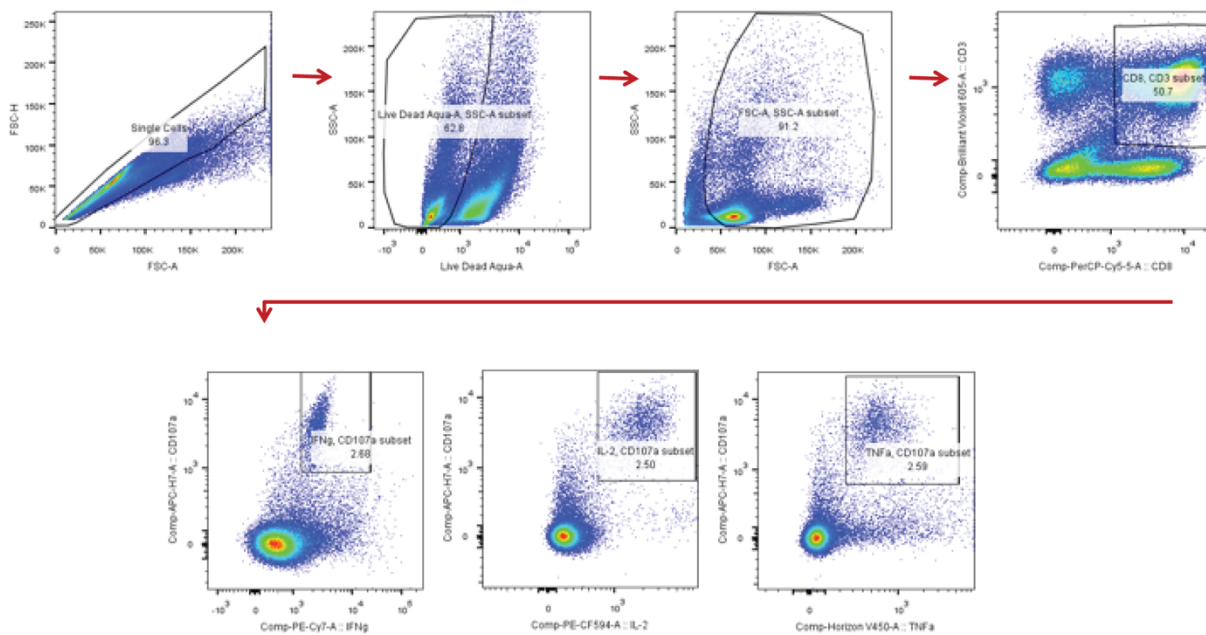
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c



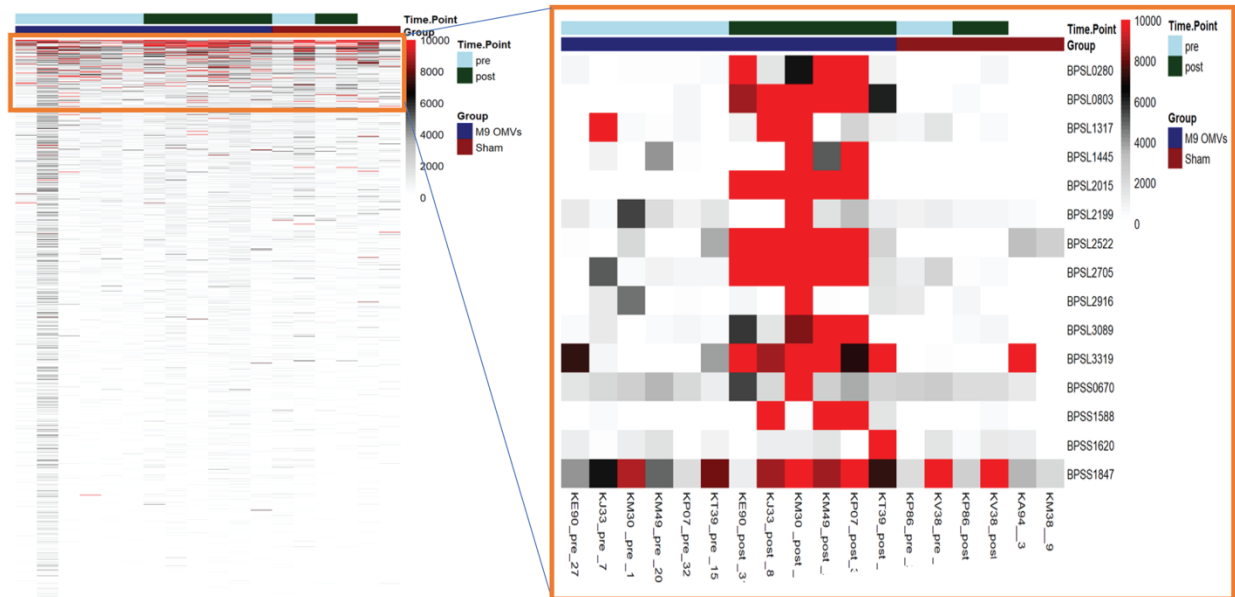
d



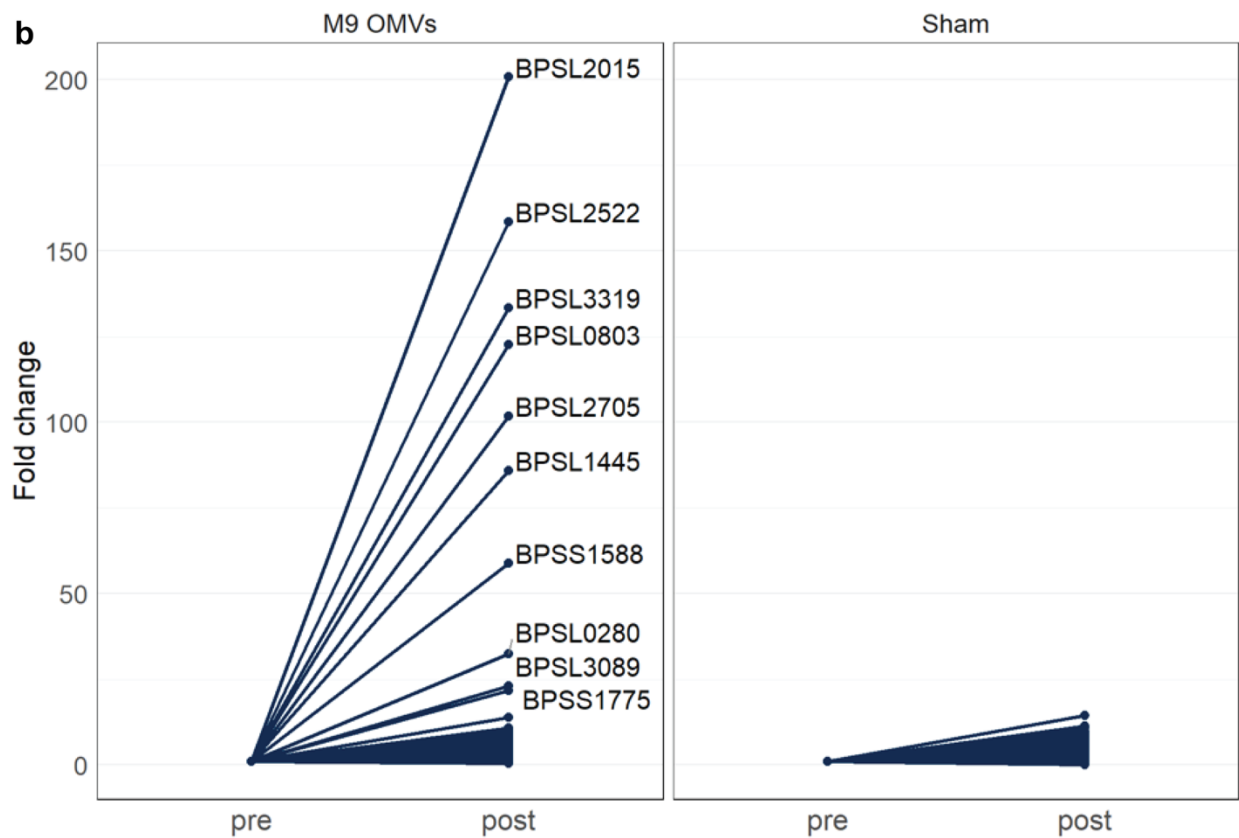
Supplementary Figure 4. OMVs drive vaccine-specific CD4 and CD8 T cells in the human MIMIC system. Individual human donor cells (n=20) were stimulated with Bp82 OMVs, Bexsero (Neisseria meningitidis OMVs), CEF (HLA Class I-restricted T cell epitopes from Cytomegalovirus, Epstein-Barr virus, and Influenza virus, Cellular Technology LTD) or

mock-stimulated in the lymphoid tissue equivalent module¹⁹. After 10-14 days, the % of IFN- γ , TNF- α , and IL-2 producing a) CD4 and b) CD8 T cells were measured by intracellular cytokine staining and flow cytometry. The gating strategies are shown in panels c (CD4) and d (CD8). To enumerate the number and percentages of CD4+ and CD8+ T cells which were activated and producing cytokines, the flow cytometry data were evaluated in this manner: 1) using FSC-H vs FSC-A, doublets were eliminated from the analysis, 2) live cells were selected using the dye LDA, 3) cellular debris were eliminated using FSC vs SSC plots, 4) gates were placed on CD4+ or CD8+ populations, and 5) these populations were assessed for expression of T cell activation markers CD154 (for the CD4+ T cell analysis) or CD107a (for CD8+ T cells) along with a panel of intracellular cytokine staining. Statistical analysis was performed using a paired T test.

a

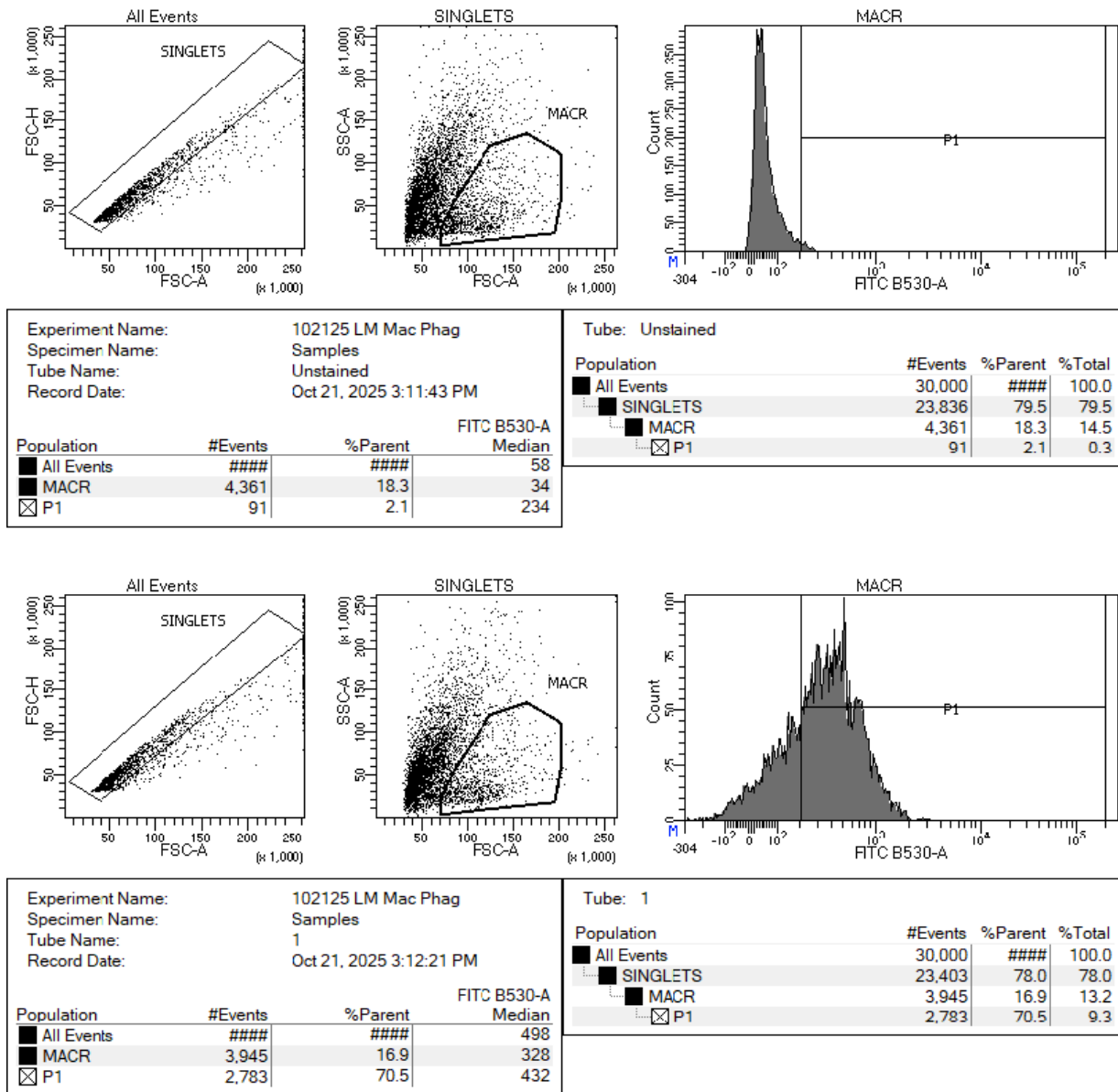


b



Supplementary Figure 5. *Burkholderia* Immuno-Proteome Array (BIPA) detects serum reactivity to immunodominant antigens. (a) *Burkholderia* Immuno-Proteome Array (BIPA)

containing approx. 3000 ORFs from *B. pseudomallei* was used to examine sero-reactivity of pre and post immune NHP sera. The enlarged image to the right demonstrates some of the ORFs with increased MFI between pre and post sera for an individual NHP. **(b)** Serum reactivity to identified immunodominant antigens undergo a significant fold change of > 50 MFI following vaccination, whereas antigen reactivity with sham serum remains < 10 MFI. The top 10 seroreactive proteins in OMV vaccinated animals are identified.



Supplementary Figure 6. Gating scheme for Opsonophagocytosis Assay. Forward

scatter, side scatter (linear) and fluorescence (log) parameters were collected and CSFE fluorescence intensity of the gated macrophages was measured as a parameter for phagocytosis of labelled bacteria. Top panel shows cells only control to establish baseline macrophage population with fluorescence (P1). Bottom panel demonstrates experimental sample.

Group	Animal ID	Baseline weight (kg)	Endpoint weight (kg)	% loss or gain
Sham	KV38	7.10	6.10	-14%
	KF36	9.34	8.6	-9%
OMV	KT39	8.60	8.30	-3%
	KP07	7.10	7.60	+7%
	KM30	7.85	7.60	-3%

Supplementary Table 1. Body weights in sham versus OMV vaccinated

macaques. Weights (kg) of surviving NHPs compared at challenge (baseline) and at euthanasia. *Animal KM49 was euthanized at day 14 due to an unrelated event.