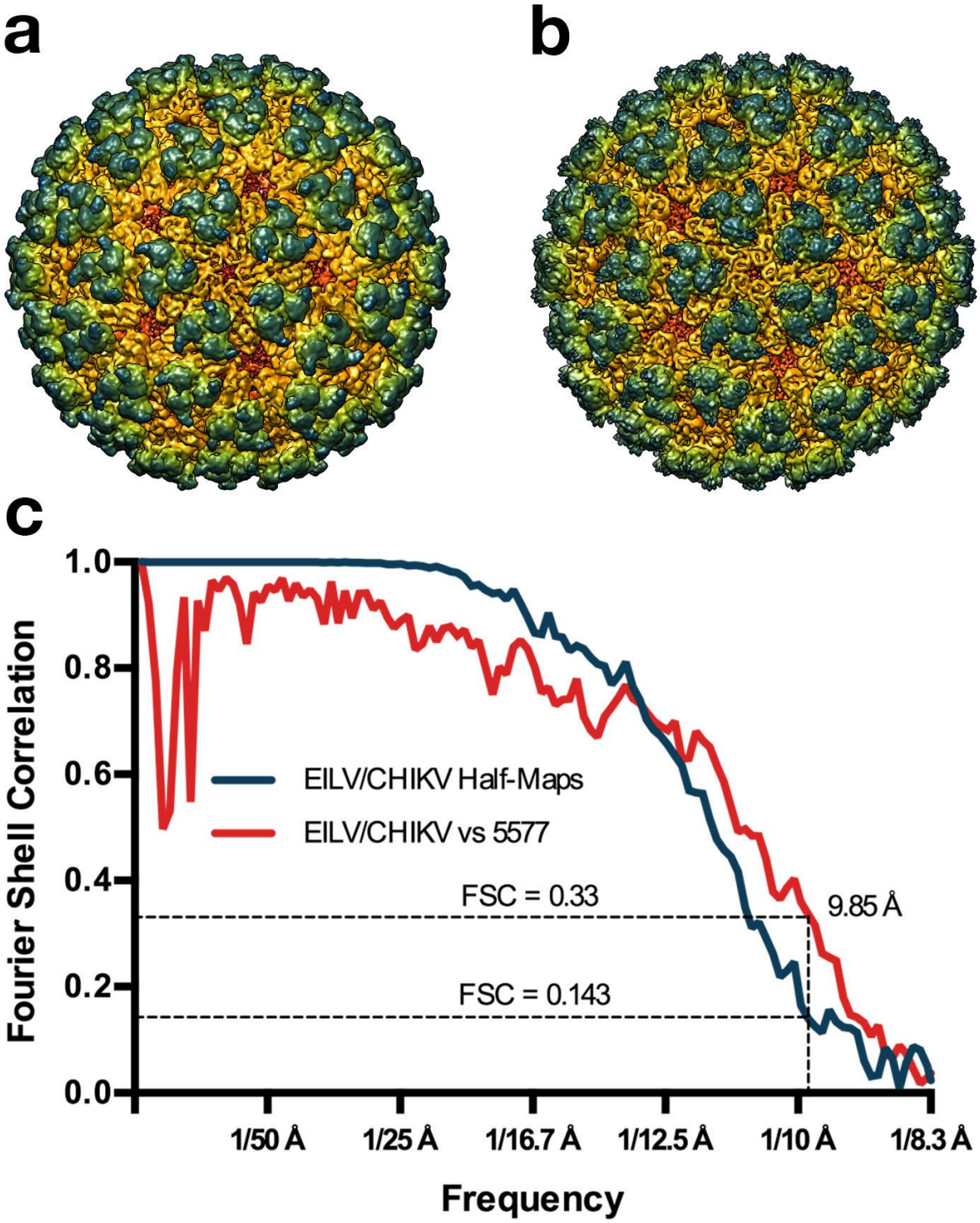
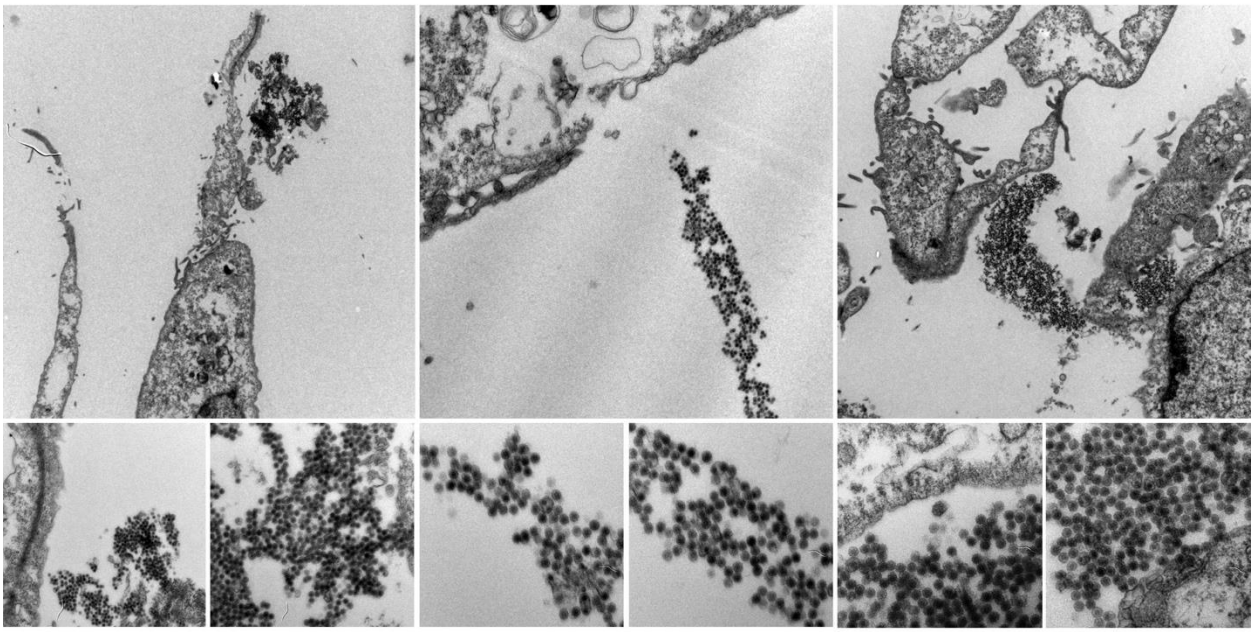
		Cross-Lineage Neutralization $PRNT_{80}$ Titers			
		Asian	Asian	West African	Indian Ocean
Group	**NHP**	**Strain 181/25**	**Strain 99659**	**Strain 37997**	**Strain LR**
EILV/CHIKV	**142590**	80	80	20	40
	150844	640	640	160	320
	150849	320	320	40	40
Mock	**142839**	<20	<20	<20	<20
	150821	<20	<20	<20	<20

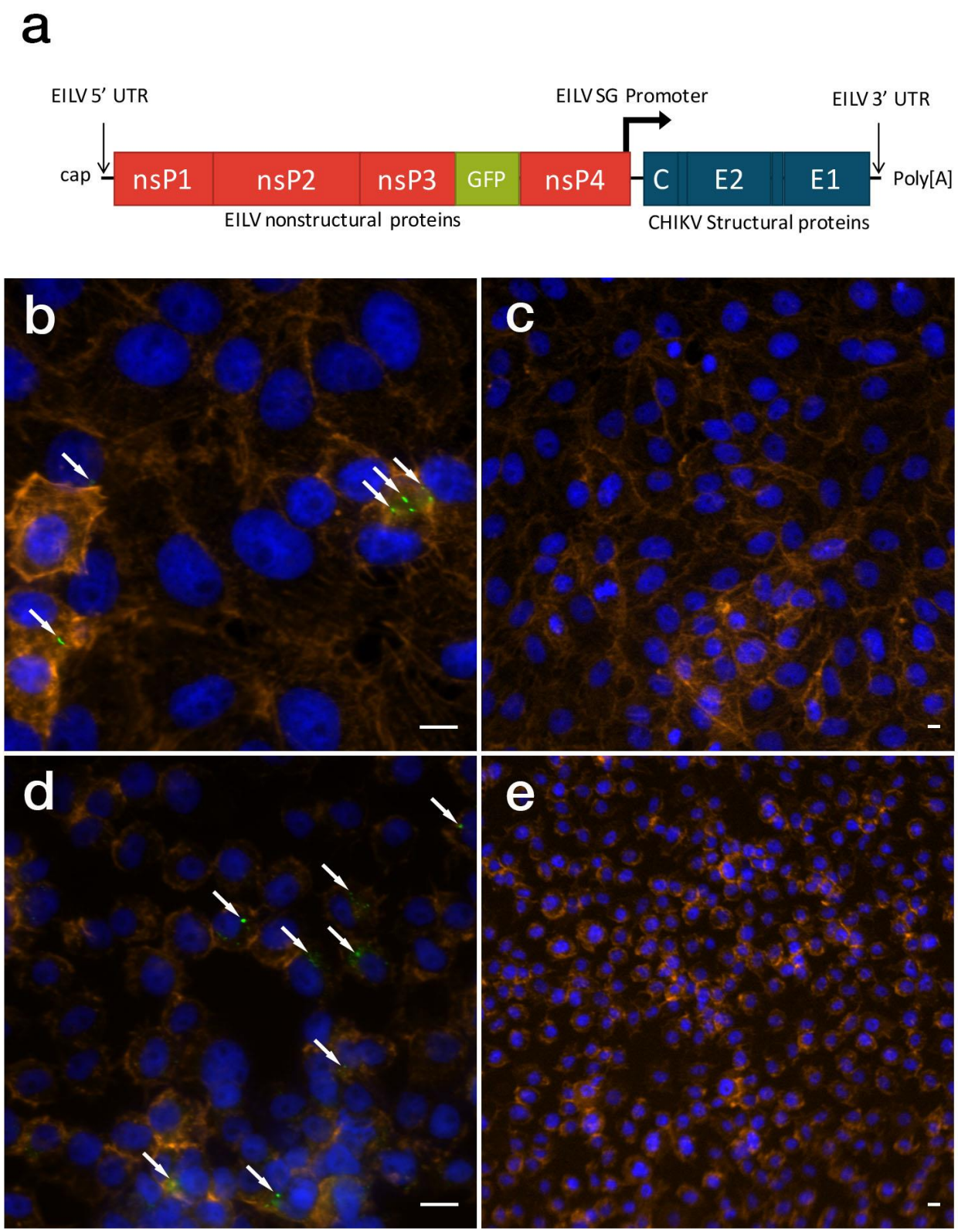
Supplementary Table 1. Cross-neutralizing (80%) titers of nonhuman primate sera collected 28 days after vaccination with either EILV/CHIKV or mock-vaccination with PBS



Supplementary Figure 1. Structural comparison of EILV/CHIKV and CHIKV VLP EMD-5577. (a) Cryo-EM reconstruction of EILV/CHIKV. (b) Previously published cryo-EM reconstruction of CHIKV VLP-5577 (18) low-pass filtered to 8Å. (c) Fourier shell correlation of EILV/CHIKV and CHIKV VLP-5577 and their agreement at the resolution of EILV/CHIKV (9.85Å).

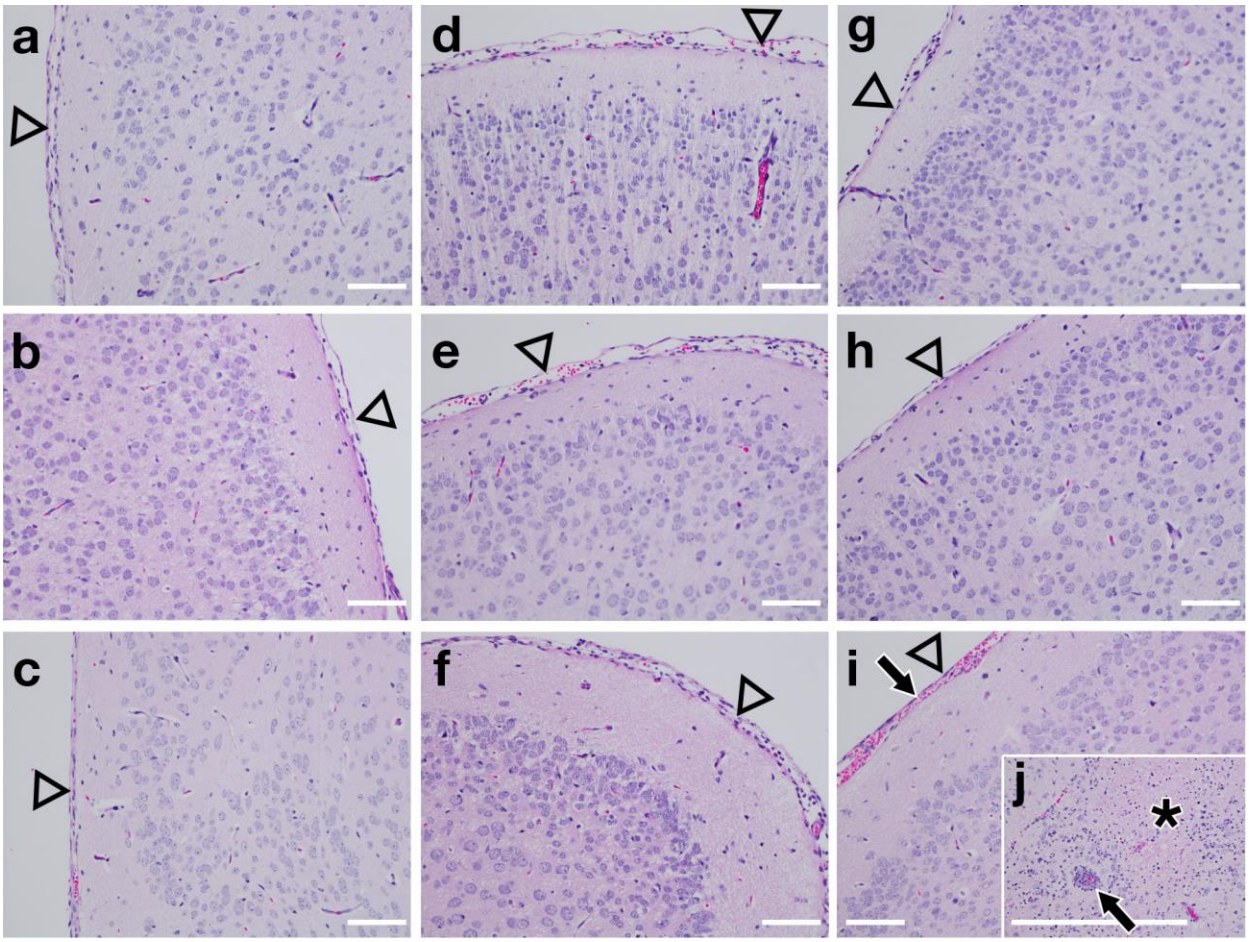


Supplementary Figure 2. Formalin-inactivation causes virus particle aggregation. Formalin inactivated EILV/CHIKV was bound to Vero cells for 1hr at 4°C and monolayers were washed and then incubated at 37°C. At 5 min post-binding, no particles were observed within endosomes while large aggregates of virus particles can be seen at cell surfaces.

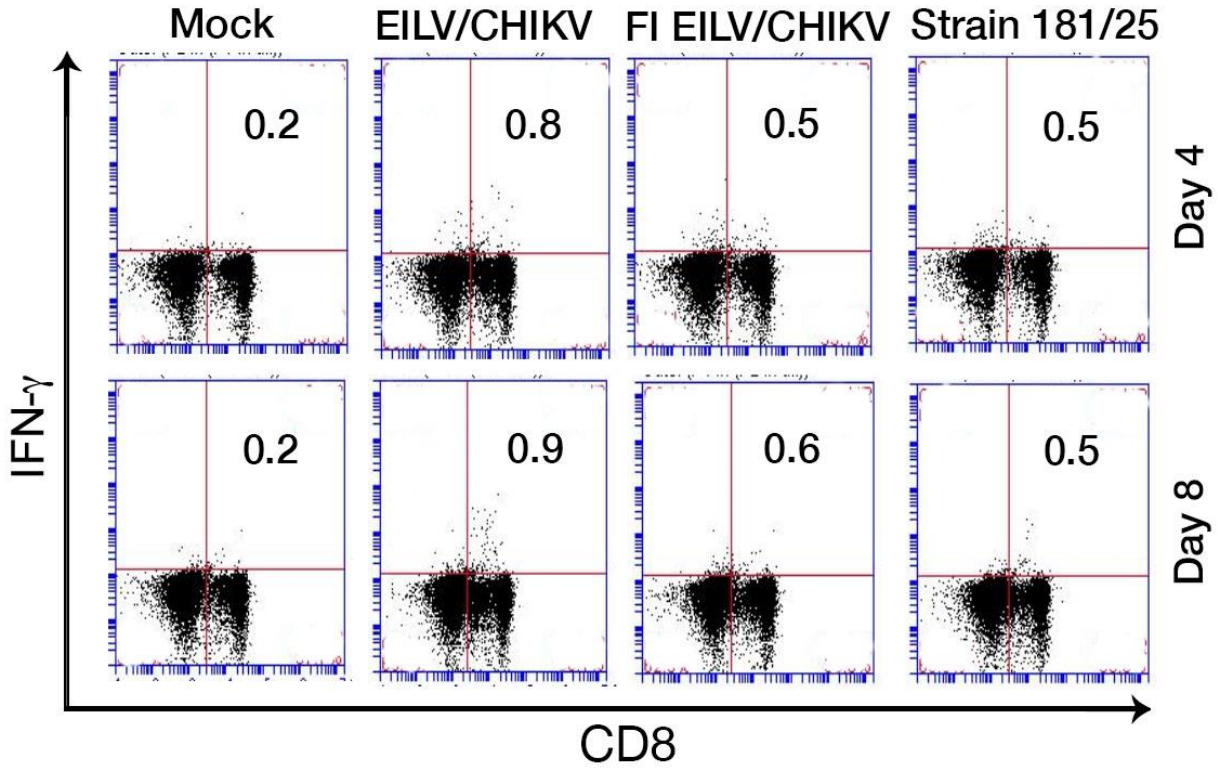


Supplementary Figure 3. Infection of Vero or C7/10 cells with EILV/nsP3-GFP/CHIKV (a) Genome organization of EILV/nsP3-GFP/CHIKV, generated by fusing GFP directly after the end of nsP3 of EILV/CHIKV before the opal stop codon. (b) Vero cells infected with

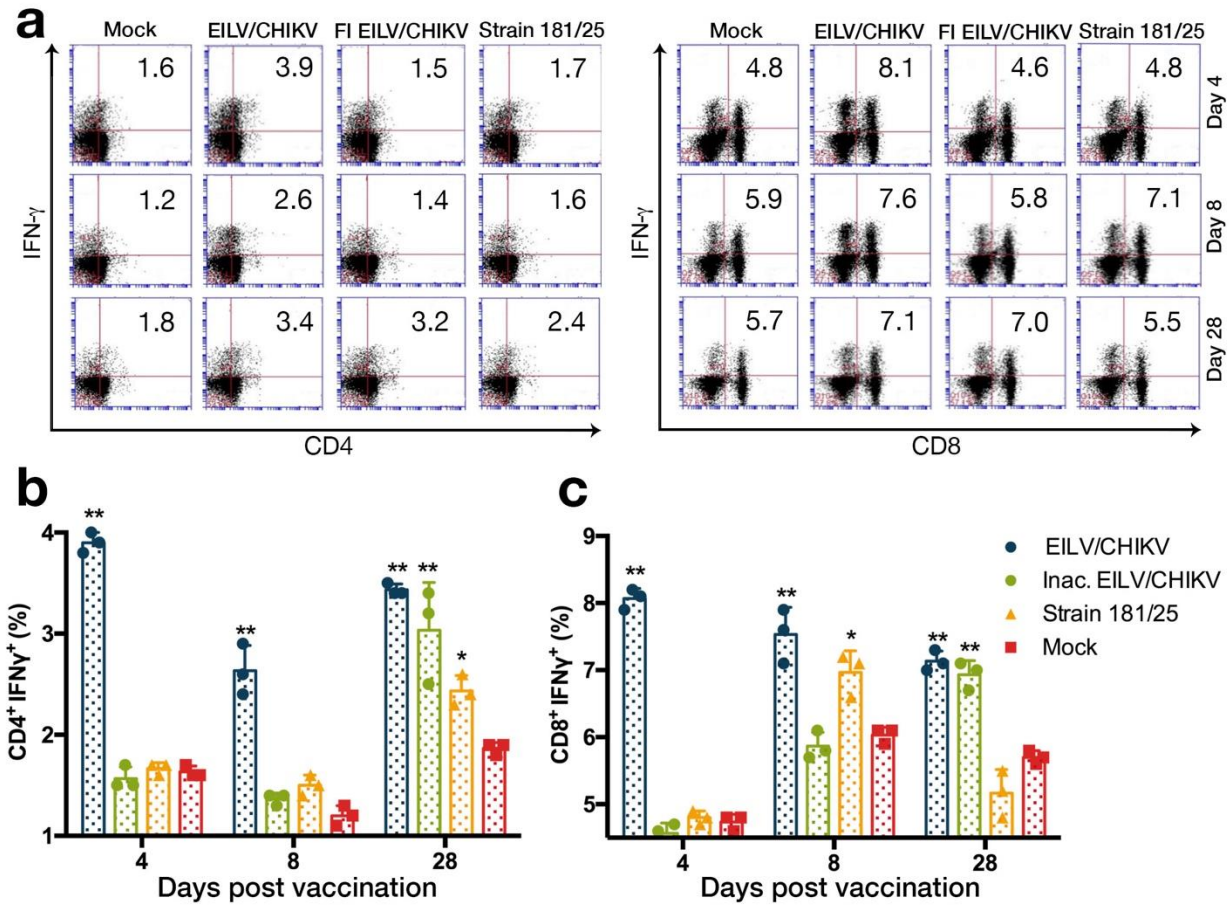
EILV/nsP3-GFP/CHIKV or (c) mock infected and fixed 8 hours post-infection (HPI). (d) C7/10 cells infected with EILV/nsP3-GFP/CHIKV or (e) mock infected and fixed 8 HPI. (b-e) after fixation, all groups were permeabilized and stained with DAPI and phalloidin conjugate to visualize nucleus and cytoskeleton, respectively, and then analyzed by fluorescence microscopy. Arrows indicate GFP foci (scale bar = 10 μm). Representative images from one of three independent experiments.



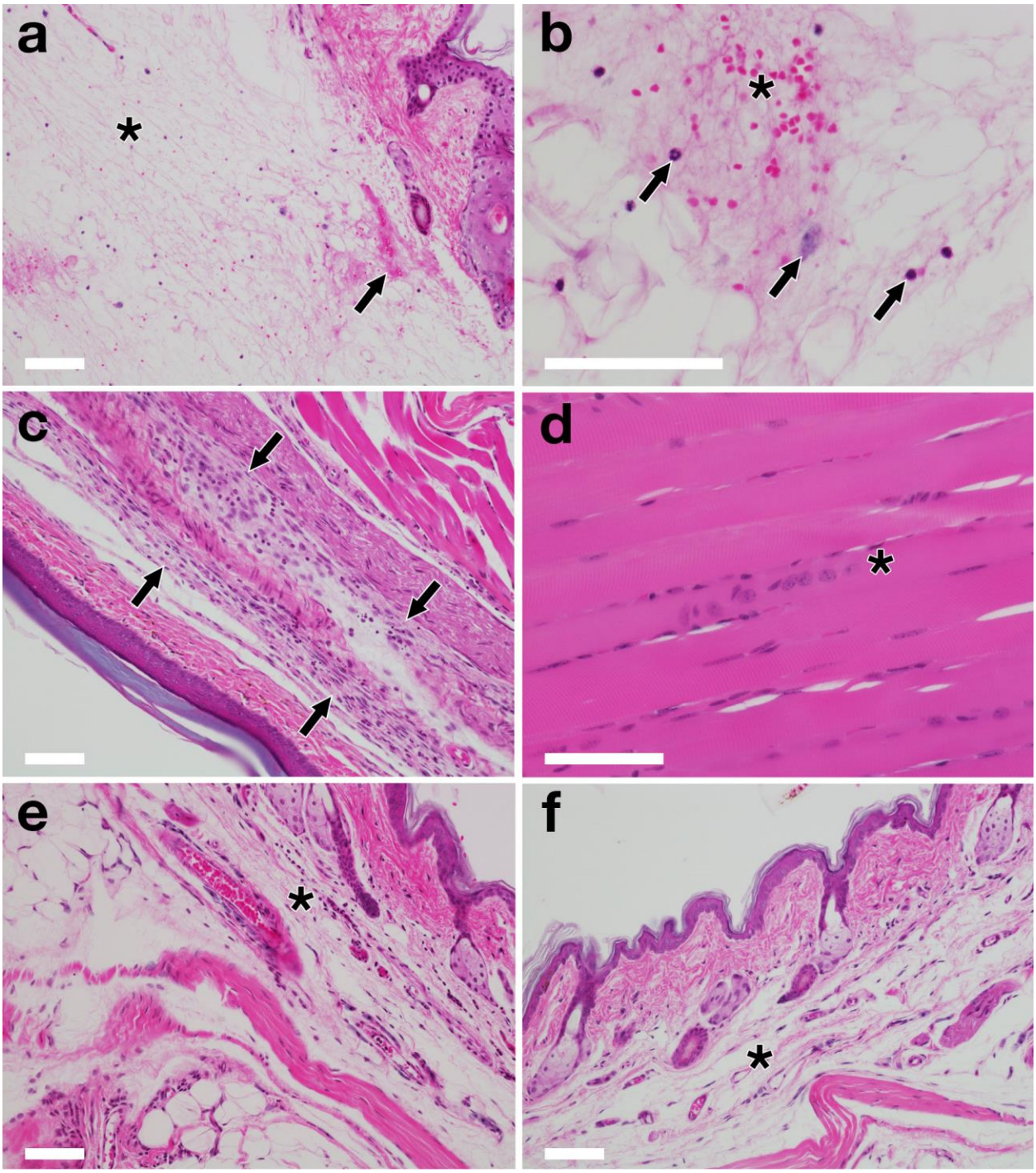
Supplementary Figure 4. Representative brain histopathologic examinations. Newborn CD1 mice (7 days old, n=3/group/time point) were mock infected (a-c), or infected with EILV/CHIKV (d-f), or strain 181/clone25 (g-j). Triangles indicate meninges for orientation purposes. (a,d,g) 0 days post-infection. (b,e,h) 3 days post-infection. (c,f,i) 7 days post-infection, arrow indicates region of hemorrhage. (j) 7 days post-infection, arrow indicates perivascular cuffing, *indicates area of necrosis in neuronal parenchyma and neutrophilic infiltrate (scale bar = 200 μm).



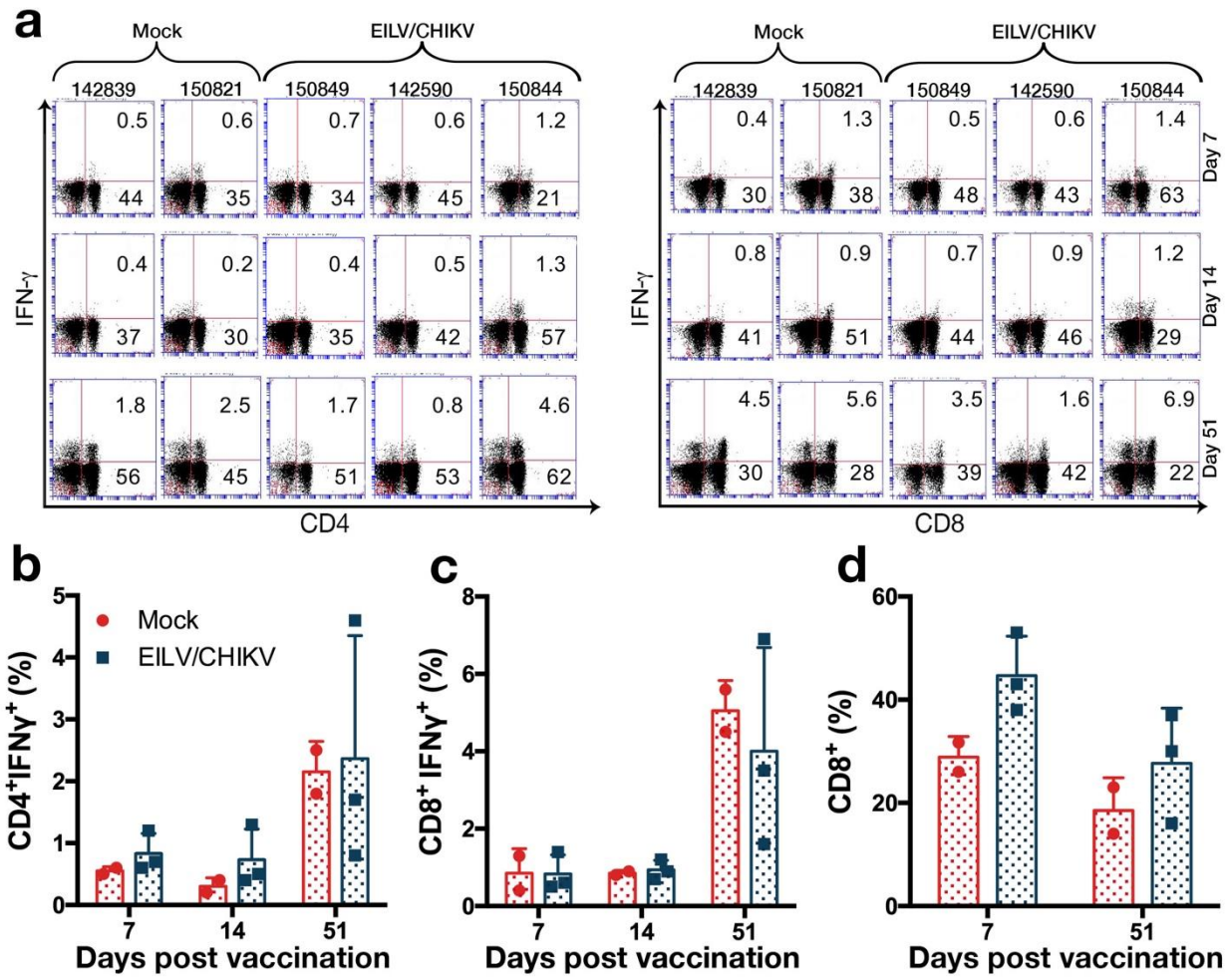
Supplementary Figure 5. Representative flow cytometry plots of CD3+-gated splenocytes. Immunocompetent C57BL/6 mice were mock vaccinated or vaccinated with EILV/CHIKV, formalin inactivated (FI) EILV/CHIKV, or live-attenuated strain 181/clone25 and splenocytes were collected on days 4 or 8 post-vaccination and stimulated with a peptide pool containing CHIKV CD8 T-cell epitopes (26).



Supplementary Figure 6. CD4 and CD8 T-cell activation following non-specific stimulation with PMA and ionomycin. (a-d) Immunocompetent C57BL/6 mice were mock-vaccinated or vaccinated with EILV/CHIKV, formalin inactivated (FI) EILV/CHIKV, or live-attenuated strain 181/clone25 and splenocytes were collected on days 4, 8, or 28 post-vaccination, stimulated with PMA/ionomycin, stained for CD3, CD4, CD8, and IFN-γ, and analyzed by flow cytometry. (a) Representative flow cytometry plots of CD3+ gated splenocytes. (b,c) Summary data depicted as mean + s.d. of each group (n=3/group/timepoint). (Statistical analysis: two-way ANOVA with Tukey's multiple comparison test, (c,d) compared to mock ** $p < 0.0001$, * $p < 0.001$)



Supplementary Figure 7. Representative pelvic limb histopathologic evaluation of IFNα/βR-/- mice challenged with CHIKV 292 days post-vaccination. (a) Mock vaccinated animal, arrow indicates hemorrhage, *indicates subcutaneous edema. (b) Mock vaccinated animal, arrows indicate leukocytes, *indicates hemorrhage. (c) formalin-inactivated EILV/CHIKV vaccinated animal, arrows outline inflammatory infiltrate. (d) formalin-inactivated EILV/CHIKV vaccinated animal, *indicates regenerative muscle fiber. (e) Strain 181/clone25 vaccinated animal, *indicates normal subcutis. (f) EILV/CHIKV vaccinated animal, *indicates normal subcutis (scale bar = 100 μm).



Supplementary Figure 8. CD4 and CD8 T-cell activation following non-specific stimulation with PMA and ionomycin. (a-d) Cynomolgus macaques were mock vaccinated or vaccinated with EILV/CHIKV and peripheral blood mononuclear cells (PBMCs) were harvested from whole blood on days 7, 14, and 51 post-vaccination (macaques were challenged with CHIKV-LR on day 30 post-vaccination), stimulated with PMA/ionomycin, stained for CD3, CD4, CD8, and IFN-γ, and analyzed by flow cytometry. (a) Representative flow cytometry plots of CD3+ gated PBMCs. (b) Activated CD4 T-cells. (c) Activated CD8 T-cells. (d) CD8+ T-cells as a percentage of total live PBMCs. Summary data depicted as mean + s.d. of each group [n=3 (EILV/CHIKV) or n=2 (mock) per timepoint].