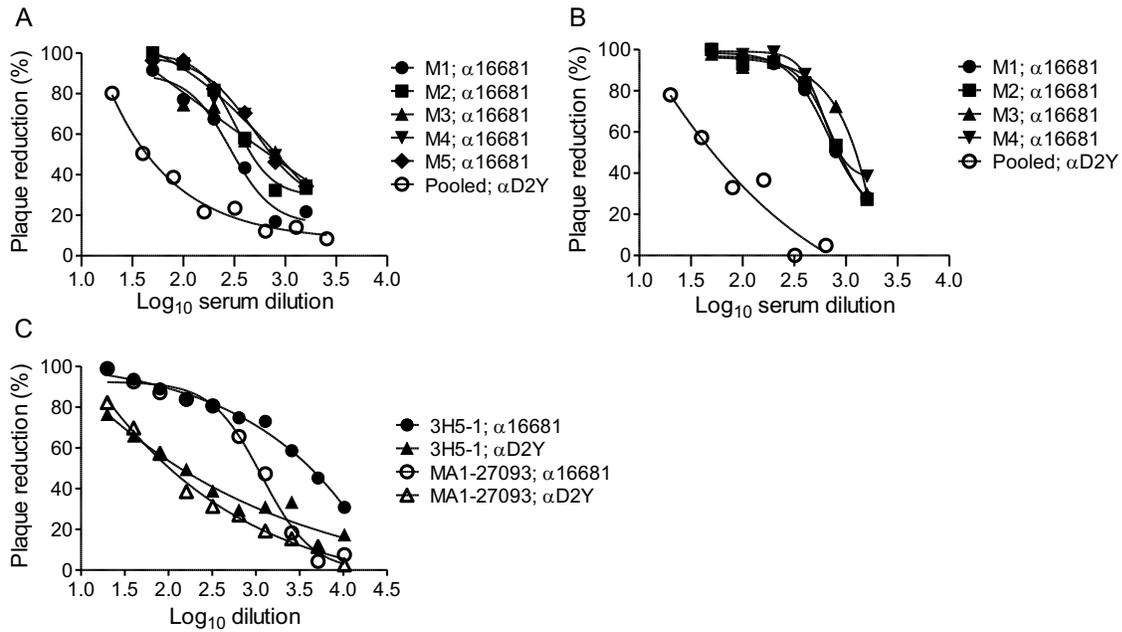


## Supplemental data



**FIG. S1. Representative neutralization curves.** (A) Adult A129 immunized with PDK53. Neutralization curves of sera collected three weeks after immunization are shown. Samples were individually assessed (M1 to M5) on their neutralization of strain 16681 and pooled to assess neutralization of strain D2Y98P-PP1. (B) Pups born to PDK53-immunized dams. Curves of sera from 3 wko mice are shown. (C) 3H5-1 and MA1-27093 mAb.

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16681      MRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYC
PDK53      MRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYC
D2Y98P-PP1 MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKHPATLRKYC
*****

16681      IEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFR
PDK53      IEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFR
D2Y98P-PP1 IEAKLTNTTTASRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFT
*****

16681      CKKNMEGKVVQPENLEYTIVITPHSGEEHAVGNDTGKKGKEIKITPQSSITEAELTGYGT
PDK53      CKKNMEGKVVQPENLEYTIVITPHSGEEHAVGNDTGKKGKEIKITPQSSITEAELTGYGT
D2Y98P-PP1 CKKNMEGKIVQPENLEYTIVITPHSGEENAVGNDTGKKGKEIKVTPQSSITEAELTGYGT
*****

16681      VTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTF
PDK53      VTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTF
D2Y98P-PP1 VTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTF
*****

16681      KNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYS
PDK53      KNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYS
D2Y98P-PP1 KNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYS
*****

16681      MCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTE
PDK53      MCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTE
D2Y98P-PP1 MCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTE
*****

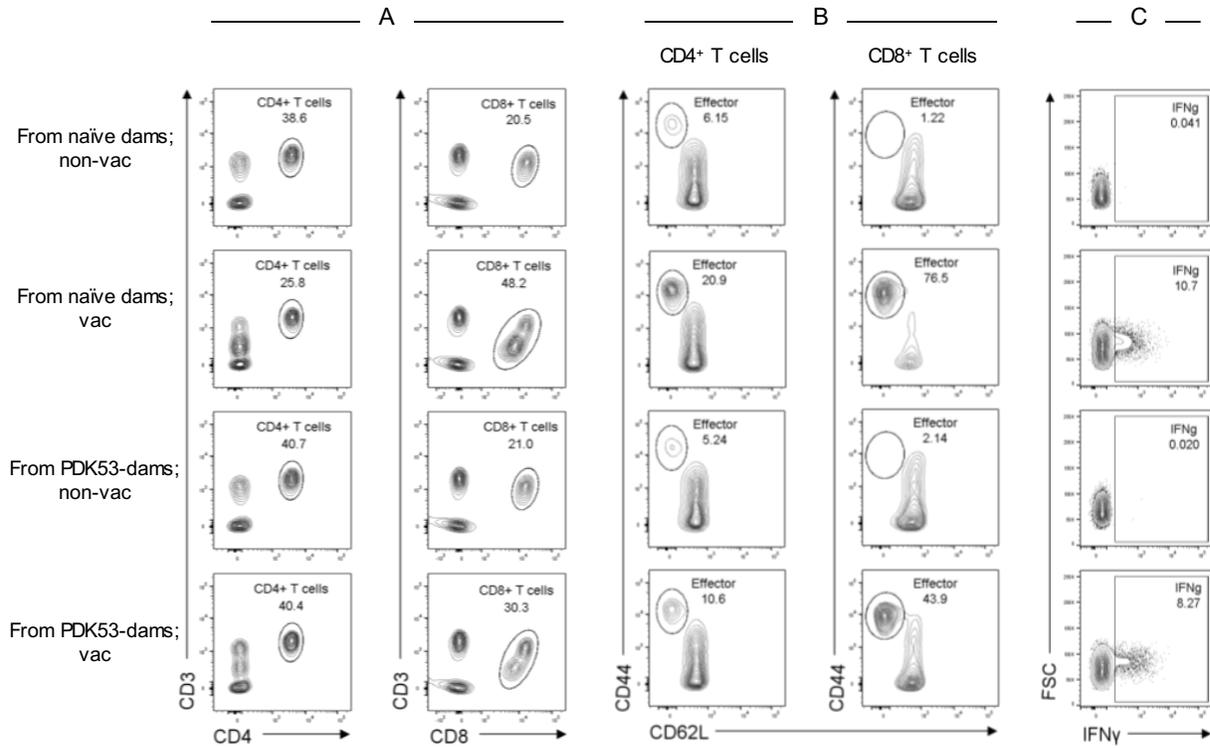
16681      KDSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFFKKGSSIGQMFETMRGAKRMAILGDTAW
PDK53      KDSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFFKKGSSIGQMFETMRGAKRMAILGDTAW
D2Y98P-PP1 KDSPVNIEAEPPFGDSYIIIGVEPGQLKLSWFFKKGSSIGQMFETMRGAKRMAILGDTAW
*****

16681      DFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVITWIGMNSRSTSLSVTLV
PDK53      DFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVITWIGMNSRSTSLSVTLV
D2Y98P-PP1 DFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVITWIGMNSRSTSLSVSLV
*****

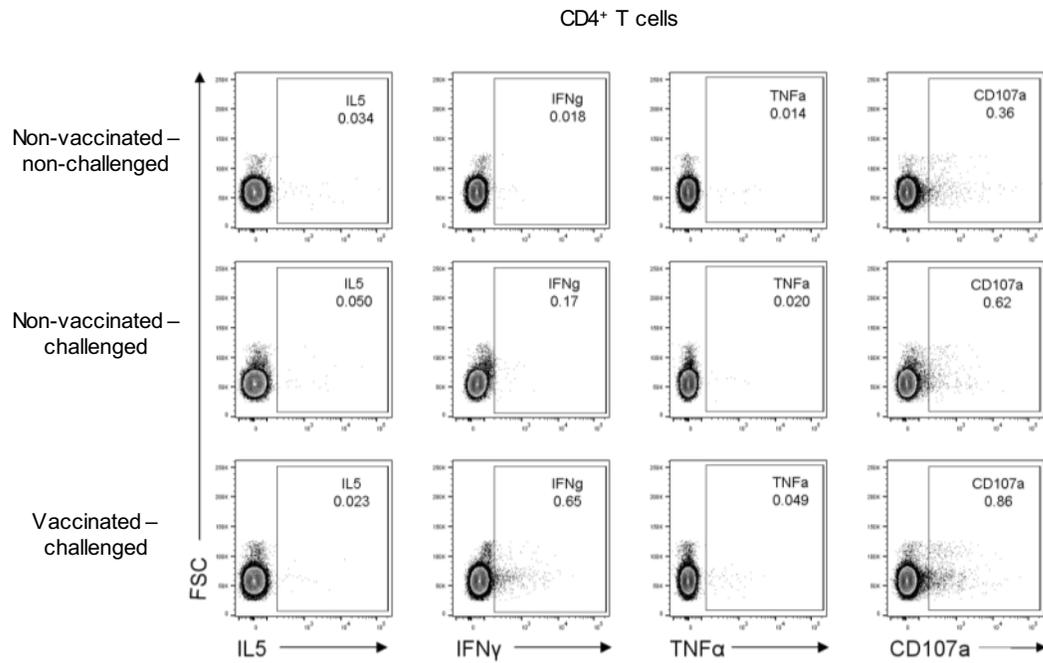
16681      LVGIVTLYLGVMVQA
PDK53      LVGIVTLYLGVMVQA
D2Y98P-PP1 LVGVVTLYLGVMVQA
***

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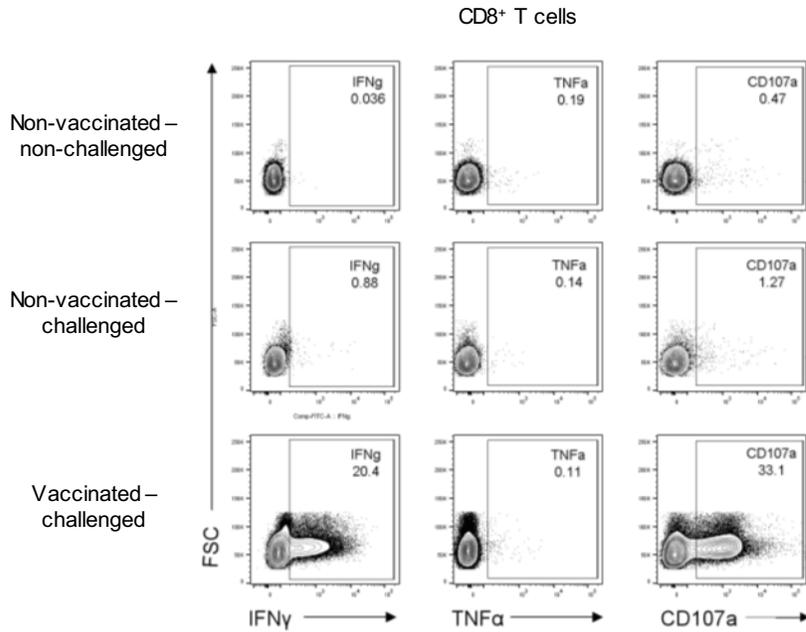
**FIG. S2. Envelope (E) protein sequences of DENV2 strains PDK53, 16681 and D2Y98P-PP1.** Sequences were aligned using Clustal Omega (EMBL-EBI). Domain III (EDIII) sequence is underlined; variable position is highlighted.



**FIG. S3. T cell response to PDK53 vaccine.** Pups were immunized with  $10^5$  PFU of PDK53 at three weeks of age and blood was collected seven days later for analysis of T cell populations. (A) Live CD4<sup>+</sup> and CD8<sup>+</sup> T cells were identified using a lymphocyte SSC vs. FSC gate followed by surface expression of CD3 and CD4, or CD3 and CD8. Numbers in FACS plots denote % of lymphocytes. (B) Effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells were identified by gating on the respective CD44<sup>hi</sup>CD62L<sup>lo</sup> subpopulations. Numbers in plots denote % of respective T cell subset. (C) Proportions of IFN $\gamma$ <sup>+</sup>CD8<sup>+</sup> T cells. PBMC were stimulated with DENV2 NS4B<sub>99-107</sub> peptide for 5 h in the presence of brefeldin A and monensin. Expression of IFN $\gamma$  was determined by intracellular cytokine staining. Numbers in plots denote % of CD8<sup>+</sup> T cells. Plots from one of two independent experiments are shown.



**FIG. S4. Effector functions of CD4<sup>+</sup> T cells.** Splenocytes were incubated with DENV2 NS3<sub>198-212</sub> and NS4B<sub>99-107</sub> peptides for 5 h in the presence of brefeldin A and monensin. Expression of IL5, IFN $\gamma$ , TNF $\alpha$  and CD107a by CD4<sup>+</sup> T cells was examined by flow cytometry. Numbers in FACS plots denote % of CD4<sup>+</sup> T cells. Plots from one of two independent experiments are shown.



**FIG. S5. Effector functions of CD8<sup>+</sup> T cells.** Splenocytes were incubated with DENV2 NS3<sub>198-212</sub> and NS4B<sub>99-107</sub> peptides for 5 h in the presence of brefeldin A and monensin. Expression of IFN $\gamma$ , TNF $\alpha$  and CD107a by CD8<sup>+</sup> T cells was examined by flow cytometry. Numbers in FACS plots denote % of CD8<sup>+</sup> T cells. Plots from one of two independent experiments are shown.