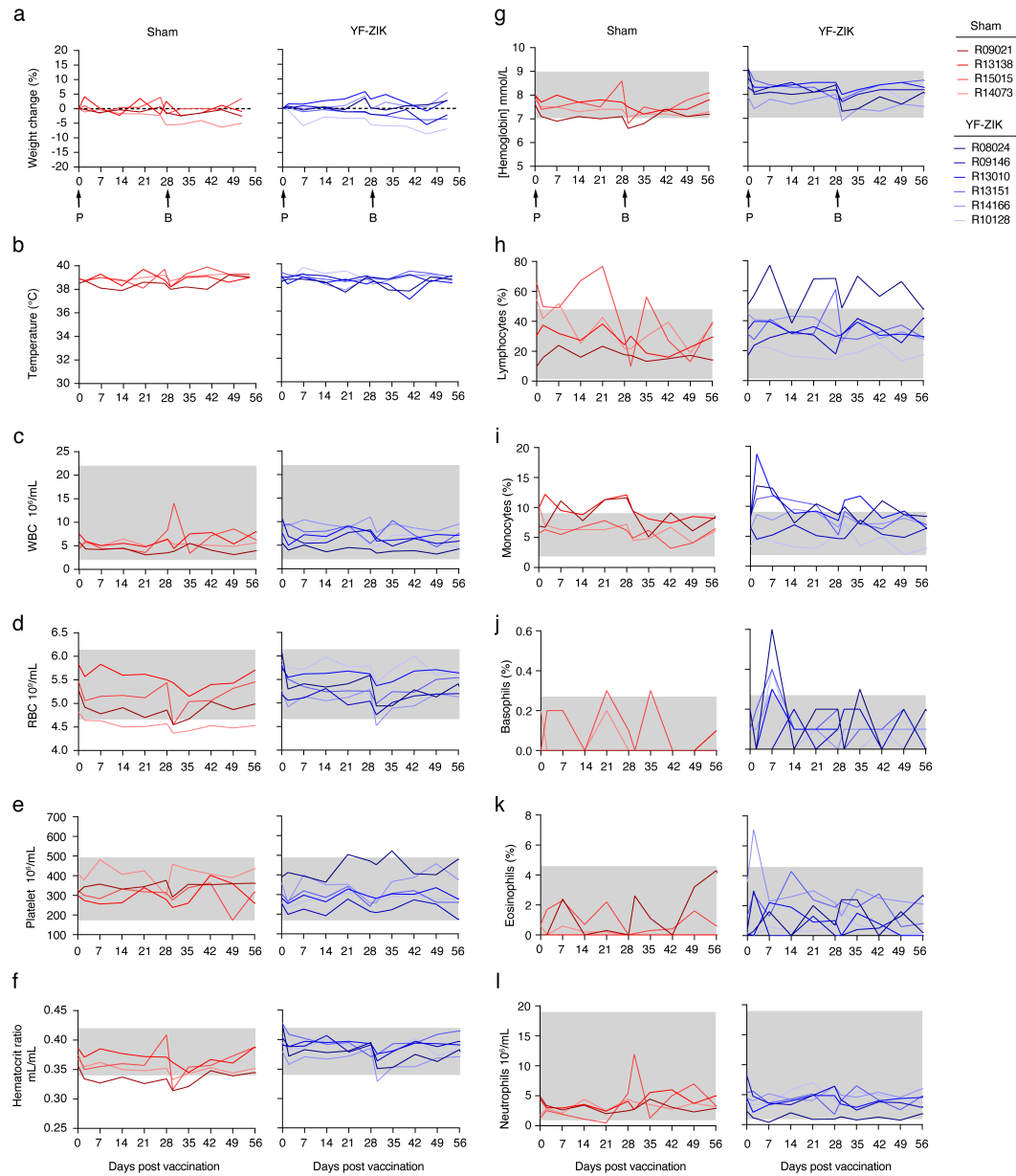


SUPPLEMENTARY INFORMATION

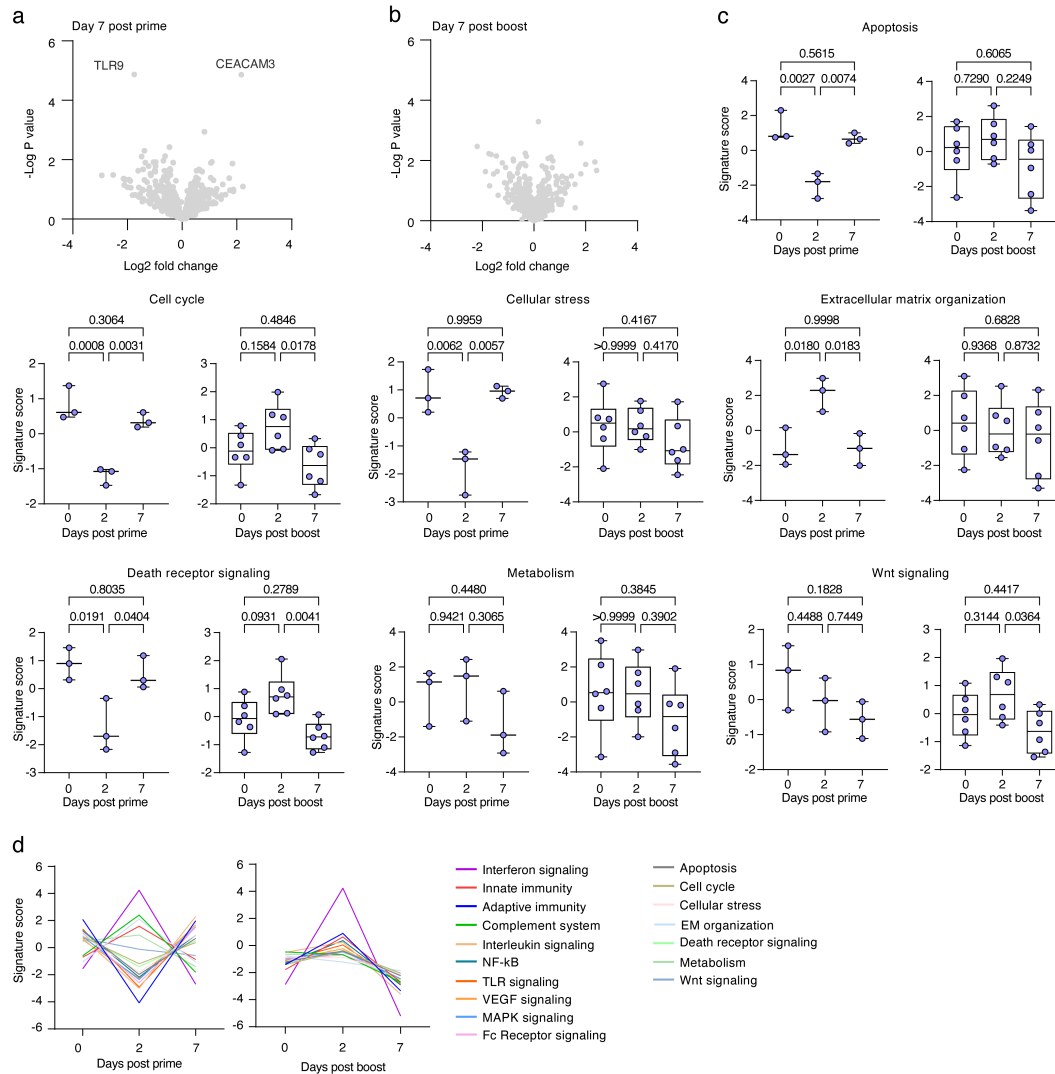
Immunological and transcriptomic profile of chimeric live-attenuated Zika vaccine linked to protection in non-human primates

Ji Ma, Bert Malengier-Devlies, Babs E. Verstrepen, Yeranddy A. Alpizar, Thomas Vercruysse, Mahadesh Prasad Arkalagud Javarappa, Lorena Sanchez-Felipe, Gerrit Koopman, Natasja G. de Groot, Patrick Matthys, Johan Neyts, Ernst J. Verschoor, Lotte Coelmont, Hendrik Jan Thibaut, Johan Van Weyenbergh, Kai Dallmeier*

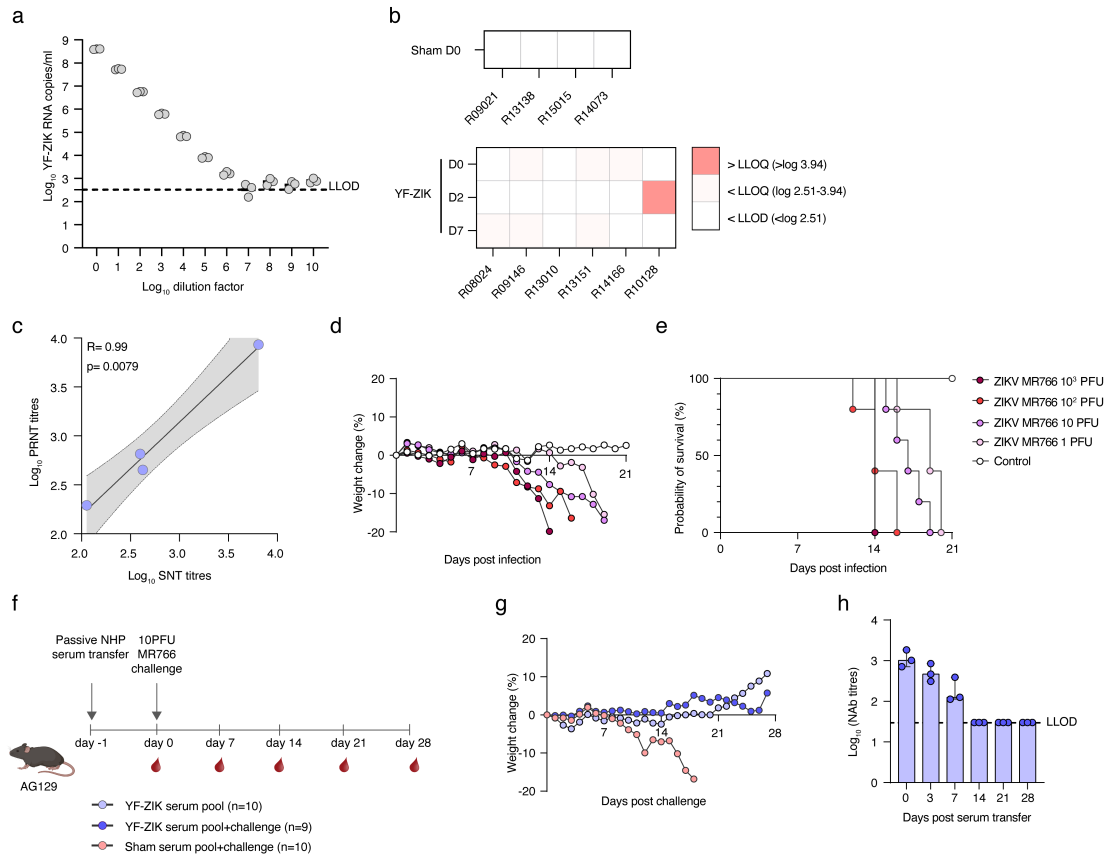
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Supplementary Fig. 1. Clinical monitoring of immunized macaques. (a, b) Weight evolution (relative to the weight before vaccination) and body temperature after vaccination (Prime ‘P’, and Boost ‘B’). Individual data are presented for each animal. (c-l) Hematological parameters after vaccination. Individual data of white blood cells (WBC) count, red blood cells (RBC) count, platelets count, hematocrit, hemoglobin, neutrophils count, and percent of lymphocytes, monocytes, basophils, eosinophils are presented for each animal according to the time. Reference range for healthy individuals (grey shaded area) is indicated in each panel.

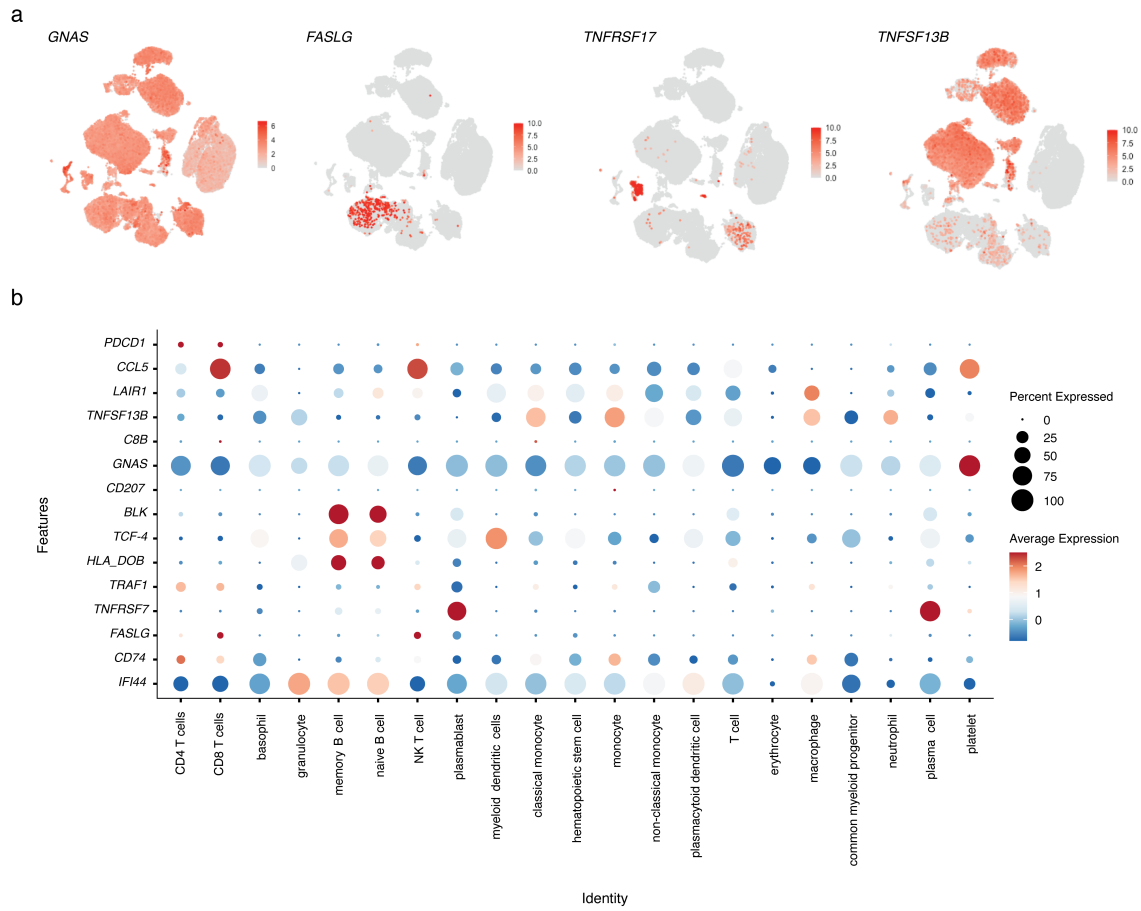


Supplementary Fig. 2. Transcriptional analysis after YF-ZIK prime and boost. Volcano plot of DEGs at day 7 post-prime vaccination (as compared with the baseline of day 0 pre-prime vaccination) **(a)** and day 7 post-boost vaccination (as compared with the baseline of day 28 pre-boost vaccination) **(b)**. Log P -values are shown in y-axis; dotted lines represent cut-off values based on the adjusted P -value. **(c)** Immune pathways score in individual macaques. Symbols represent individual animals. Horizontal bars in post-prime subsets indicate the median; boxes in post-boost subsets represent the interquartile range (25th–75th percentile) with the median. Whiskers indicate the minimum and maximum values ($n = 3$ YF-ZIK post prime vaccination due to missing samples, $n = 6$ post boost). Data were analyzed by one-way ANOVA with Tukey's multiple comparisons test. **(d)** Kinetics of immune pathways score in YF-ZIK immunized macaques.

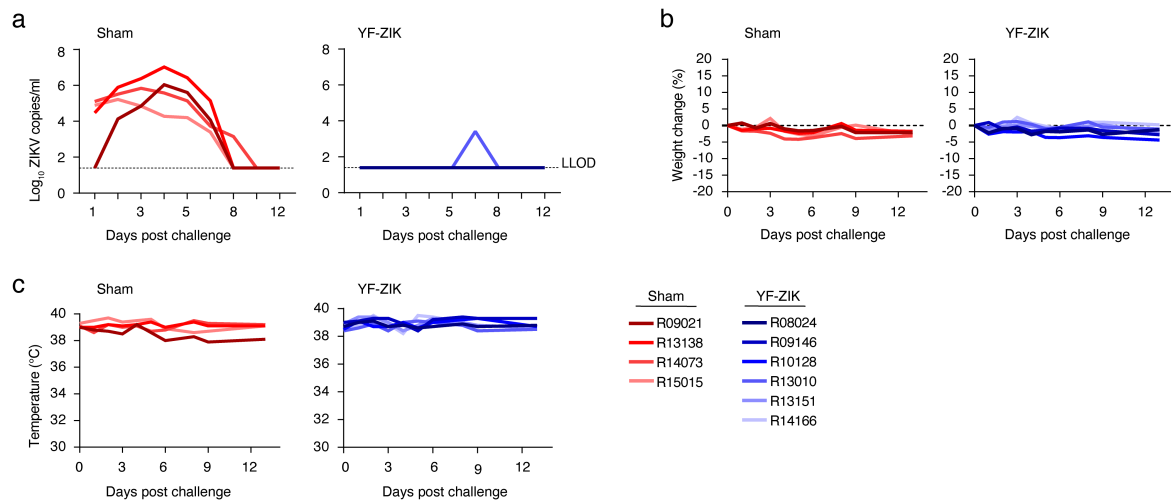


Supplementary Fig. 3. ZIKV MR766 titration in AG129 mice and ZIKV-specific NAb kinetics after passive serum transfer. (a) Titration of standard viral stock YF-ZIK to determine the lower limit of detection (LLOD). (b) Viral RNA levels of YF-ZIK before (D0) and after (D2, D7) vaccination with YF-ZIK. Viral RNA levels are color-coded based on the LLOD and the lower limit of quantification (LLOQ). (c) Correlation analysis between the in-house developed SNT and standard PRNT assay. NAb titres (PRNT₉₀ and SNT₉₀) were determined by using the same serum samples from YF-ZIK immunized macaques. For both the SNT and PRNT assay, each data point represents three technical replicates. The solid line indicates the mean of the simple linear regression; the shaded area representing the 95% confidence interval. The goodness of fit (R) and corresponding *p* values are shown in each panel. (d, e) **Challenge virus titration.** AG129 mice were injected intraperitoneally (i.p.) with 1 PFU, 10 PFU, 10² PFU, 10³ PFU of ZIKV MR766 or medium (*n* = 5 from single experiment). Weight change (d) and survival (e) were monitored until day 21 after infection. (f) **Passive serum transfer study scheme.** AG129 mice received a single i.p. dose of 450 µl of NHPs serum pool from either YF-ZIK-immunized macaques, or sham-immunized macaques. Mice were challenged i.p. 24 h later with 10 PFU of ZIKV MR766 (*n* = 9-10 combined from two independent experiments). (g) Mean weight change after ZIKV MR766 challenge. (h) AG129 mice that received a single dose of 450 µl of NHP serum pool from YF-ZIK immunized macaques were bled at day 3, 7 and then weekly afterwards to determine NAb decay kinetics (*n* = 3 from single experiment). Data presented as median ± IQR; LLOD, lower limit of

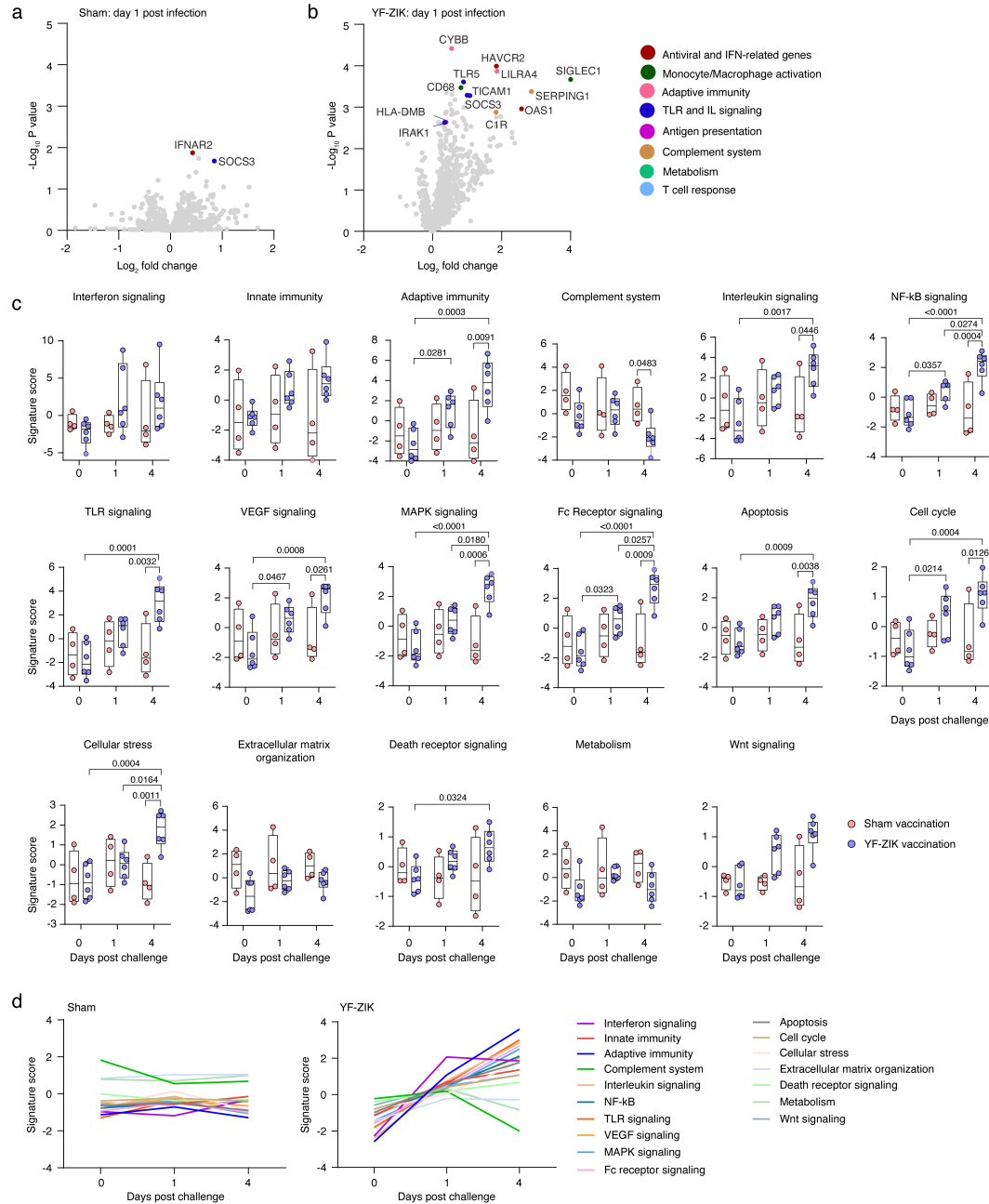
detection. Supplementary Figure 3f was created in BioRender. Ma, J. (2025)
<https://BioRender.com/owo395w>



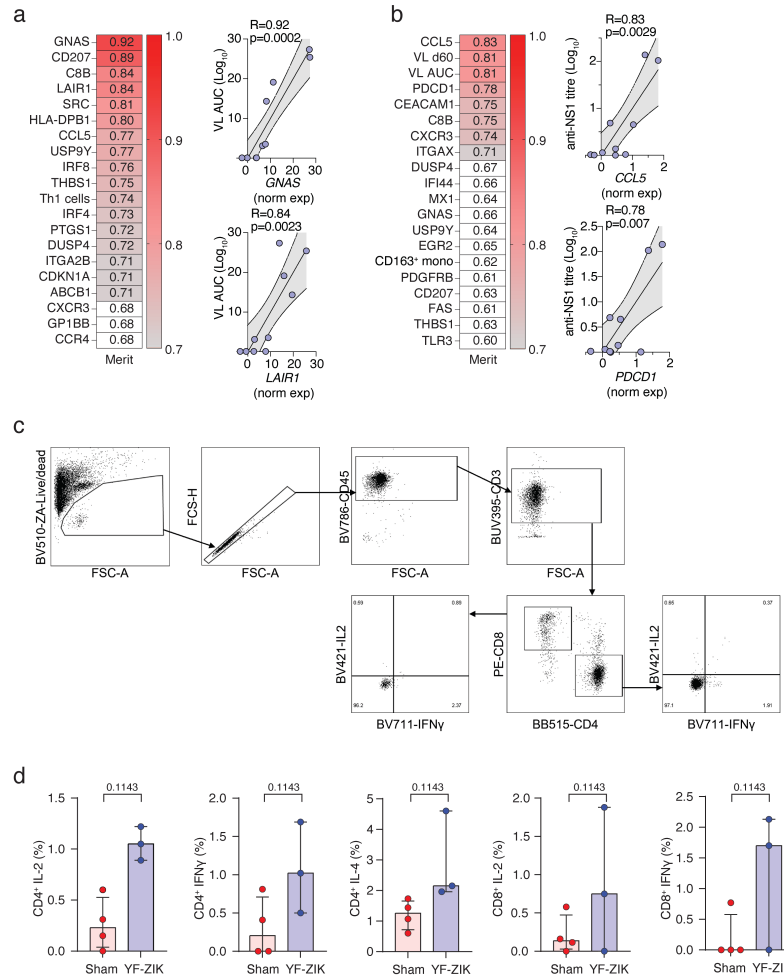
Supplementary Fig. 4. Expression levels of selected most predictive genes in the UMAP space of circulating human blood cells. (a, b) UMAP (a) and dot plot (b) visualizing expression of selected most predictive genes (merit score >0.8) for NABs at day 56 after vaccination (*FASLG*, *TNFRSF17*, *TRAF1*, *HLA-DOB*, *TCF-4*, *BLK*), for peak viral load in plasma at day 60 and for viral load across all time points (*CD207*, *GNAS*, *C8B*, *LAIR1*), and for viral antigen expression after challenge (*CCL5*, *PDCD1*).



Supplementary Fig. 5. ZIKV RNAemia and clinical parameters after ZIKV infection (a) Blood plasma ZIKV RNA kinetics after challenge of Sham ($n = 4$) or YF-ZIK ($n = 6$) immunized macaques. LLOD, the lower limit of detection. **(b)** Weight and **(c)** temperature change after ZIKV challenge in macaques. Colour-coded lines represent individual animals.



Supplementary Fig. 6. Transcriptional analysis after ZIKV (PD1 strain) challenge. Volcano plot of DEGs at day 1 post challenge (as compared with the baseline of day 0 before challenge) in sham ($n = 4$) **(a)** and YF-ZIK ($n = 6$) **(b)** immunized macaques. Log P -values are shown in y-axis; dotted lines represent cut-off values based on the adjusted P -value. P -values were calculated using a negative binomial generalized linear model (NanoString's method) with Benjamini–Hochberg false discovery rate correction. **(c)** Immune pathways score in individual macaques. Symbols represent individual macaques, centre line represents the median value of each immune pathway, the lower and upper hinges represent the first and third quartiles, and the whiskers represent the minimum and maximum. Data were analysed by two-way variance (ANOVA) with Tukey multi-comparison tests. **(d)** Kinetics of immune pathways score after challenge.



Supplementary Fig. 7. Predictive gene analysis and ZIKV-specific T cell responses (a, b) Univariate linear regression and ranking (attribute selection by machine learning) of top 20 most predictive genes for viral load area under the curve (VL AUC) across all time points ($n = 6$, panel **a**), and for ZIKV NS1-specific antibody at day 70 as readout for viral antigen expression after challenge ($n = 10$, panel **b**). Individual data points represent independent animals. The solid line indicates the mean of the simple linear regression; the shaded area representing the 95% confidence interval. The goodness of fit (R) and corresponding p values are shown in each panel. **(c)** Gating strategy for ICS. First, dead cells were gated out as Zombie Aqua (ZA)-positive; doublets were eliminated in an FSC-H versus FSC-A plot and T cells ($CD45^+CD3^+$) were stratified into $CD4^+$ T cells and $CD8^+$ T cells. Thresholds defining positive and negative populations for intracellular markers were set based on non-stimulated control samples. **(d)** ZIKV-specific cellular responses at day 42 were assessed by flow cytometry with intracellular staining (ICS) after stimulating PBMCs with ZIKV E protein peptide. Percent of IL-2, IFN- γ and IL-4 producing $CD4^+$ T cells and percent of IL-2 and IFN- γ producing $CD8^+$ T cells ($n = 4$ for sham and $n = 3$ for YF-ZIK). Data presented as median $\pm$ interquartile range (IQR). Data were analysed by the nonparametric two-tailed Mann-Whitney U -test.