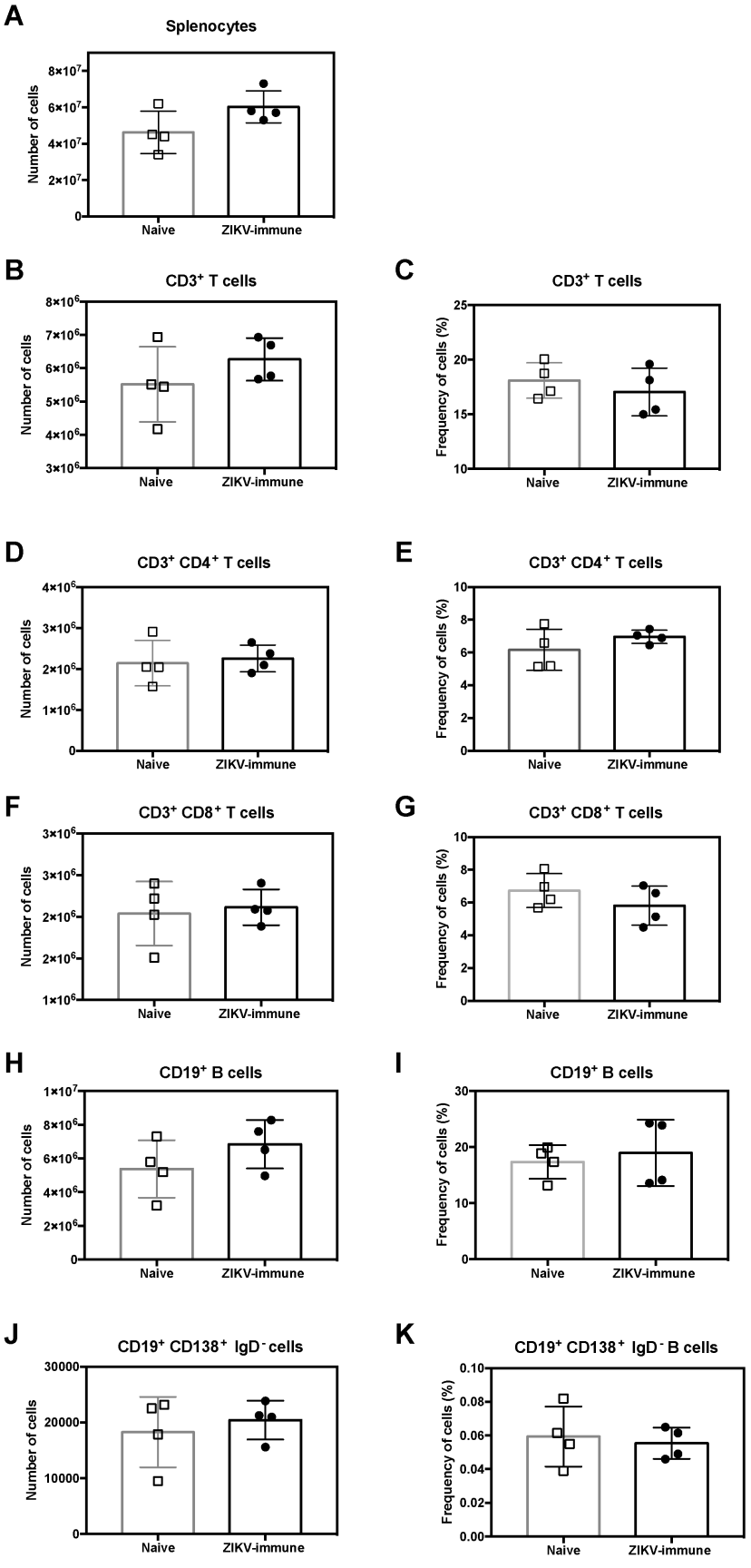
A
Splenocytes
Number of cells
8×10^7
6×10^7
4×10^7
2×10^7
0
Naive
ZIKV-immune
B
CD3+ T cells
Number of cells
8×10^6
7×10^6
6×10^6
5×10^6
4×10^6
3×10^6
Naive
ZIKV-immune
C
CD3+ T cells
Frequency of cells (%)
25
20
15
10
Naive
ZIKV-immune
D
CD3+ CD4+ T cells
Number of cells
4×10^6
3×10^6
2×10^6
1×10^6
0
Naive
ZIKV-immune
E
CD3+ CD4+ T cells
Frequency of cells (%)
10
8
6
4
2
0
Naive
ZIKV-immune
F
CD3+ CD8+ T cells
Number of cells
3×10^6
3×10^6
2×10^6
2×10^6
1×10^6
Naive
ZIKV-immune
G
CD3+ CD8+ T cells
Frequency of cells (%)
10
8
6
4
2
0
Naive
ZIKV-immune
H
CD19+ B cells
Number of cells
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
Naive
ZIKV-immune
I
CD19+ B cells
Frequency of cells (%)
30
20
10
0
Naive
ZIKV-immune
J
CD19+ CD138+ IgD- cells
Number of cells
30000
20000
10000
0
Naive
ZIKV-immune
K
CD19+ CD138+ IgD- B cells
Frequency of cells (%)
0.10
0.08
0.06
0.04
0.02
0.00
Naive
ZIKV-immune

Figure S1, Related to Figure 1. Immune cell populations in the spleen from pups born to ZIKV-immune mothers and naïve mothers. Spleens were harvested from 4- to 5-week-old *LysMCre*+*Ifnar1*$^{fl/fl}$ mice born to mothers infected with ZIKV strain SD001 (10^6 FFU via retro-orbital route) for 8 months (*n*=2) or naïve mothers (*n*=1). Splenocytes were analyzed for CD3, CD4, CD8, CD19, CD138, and IgD expression via flow cytometry. (**A**) Total number of live splenocytes, (**B**) Number of $CD3^+$ splenocytes, (**C**) Frequency of $CD3^+$ splenocytes, (**D**) Number of $CD4^+$ T cells, (**E**) Frequency of $CD4^+$ T cells (**F**) Number of $CD8^+$ T cells, (**G**) Frequency of $CD8^+$ T cells, (**H**) Number of $CD19^+$ B cells, (**I**) Frequency of $CD19^+$ B cells, (**J**) Number of $CD19^+CD138^+IgD^-$ plasma cells, (**K**) Frequency of $CD19^+CD138^+IgD^-$ plasma cells. An unpaired student's t test was performed and no statistically significant differences were observed.

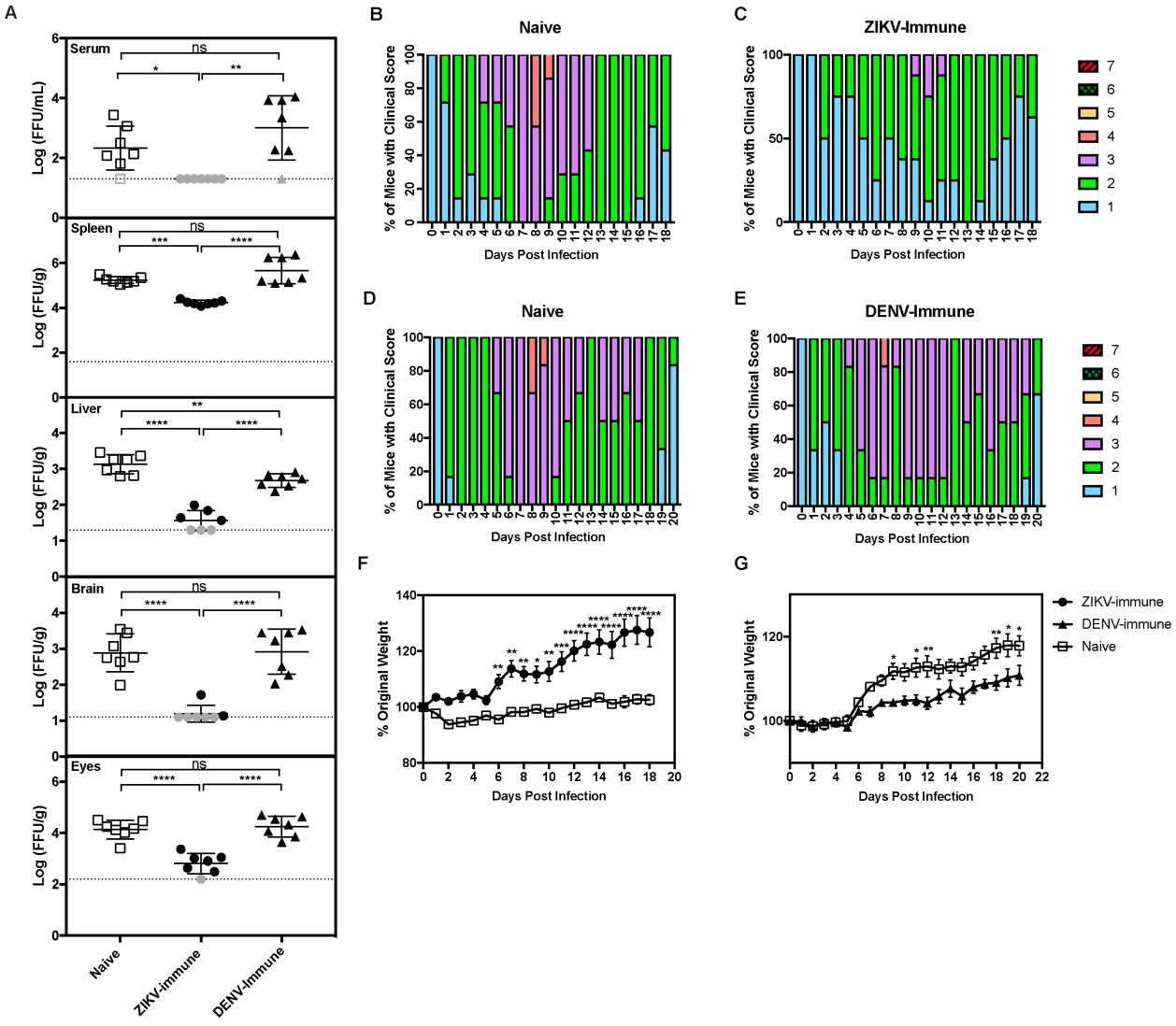

Figure S2, Related to Figures 1. Decreased ZIKV tissue burden and ZIKV-induced clinical disease manifestations in mice born to ZIKV-immune mothers but not DENV-immune mothers. Four- to 5-week-old *LysMCre*$^{+}$*Ifnar1*$^{fl/fl}$ mice born to mothers previously infected with ZIKV strain SD001 (10^6 FFU via retro-orbital route), mothers previously infected with DENV2 strain S221 (5 x 10^5 FFU via tail vein), or naïve mothers were challenged via retro-orbital route with 10^5 FFU of ZIKV strain SD001. (**A**) Mice were sacrificed 3 days p.i. and serum, spleen, liver, brain, and eyes were harvested. Samples were titrated for infectious ZIKV via focus forming assay. Open squares represent pups

(*n*=7) from naïve mothers (*n*=2), closed circles represent pups (*n*=7) born to ZIKV-immune mothers infected for 2 months (*n*=2), and closed triangles represent pups (*n*=7) born to DENV-immune mothers infected for 2-3 months (*n*=2). Dotted line indicates limit of detection. (**B**) Clinical scores of pups (*n*=7) from naïve mothers (*n*=2), (**C**) Clinical scores of pups (*n*=6) born to ZIKV-immune mothers infected for 6-7 months (*n*=2), (**D**) Clinical scores of pups (*n*=7) from naïve mothers (*n*=2), (**E**) Clinical scores of pups (*n*=8) born to DENV2-immune mothers infected for 2-3 months (*n*=2), (**F**) Weight loss of pups born to ZIKV-immune mothers, and (**G**) Weight loss of pups born to DENV-immune mothers. Data represent pooled data from two independent experiments and are expressed as mean ± standard error of mean. One-way ANOVA was used for all comparisons in (**A**). Unpaired Student's t test of groups for each day in (**F**) and (**G**). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

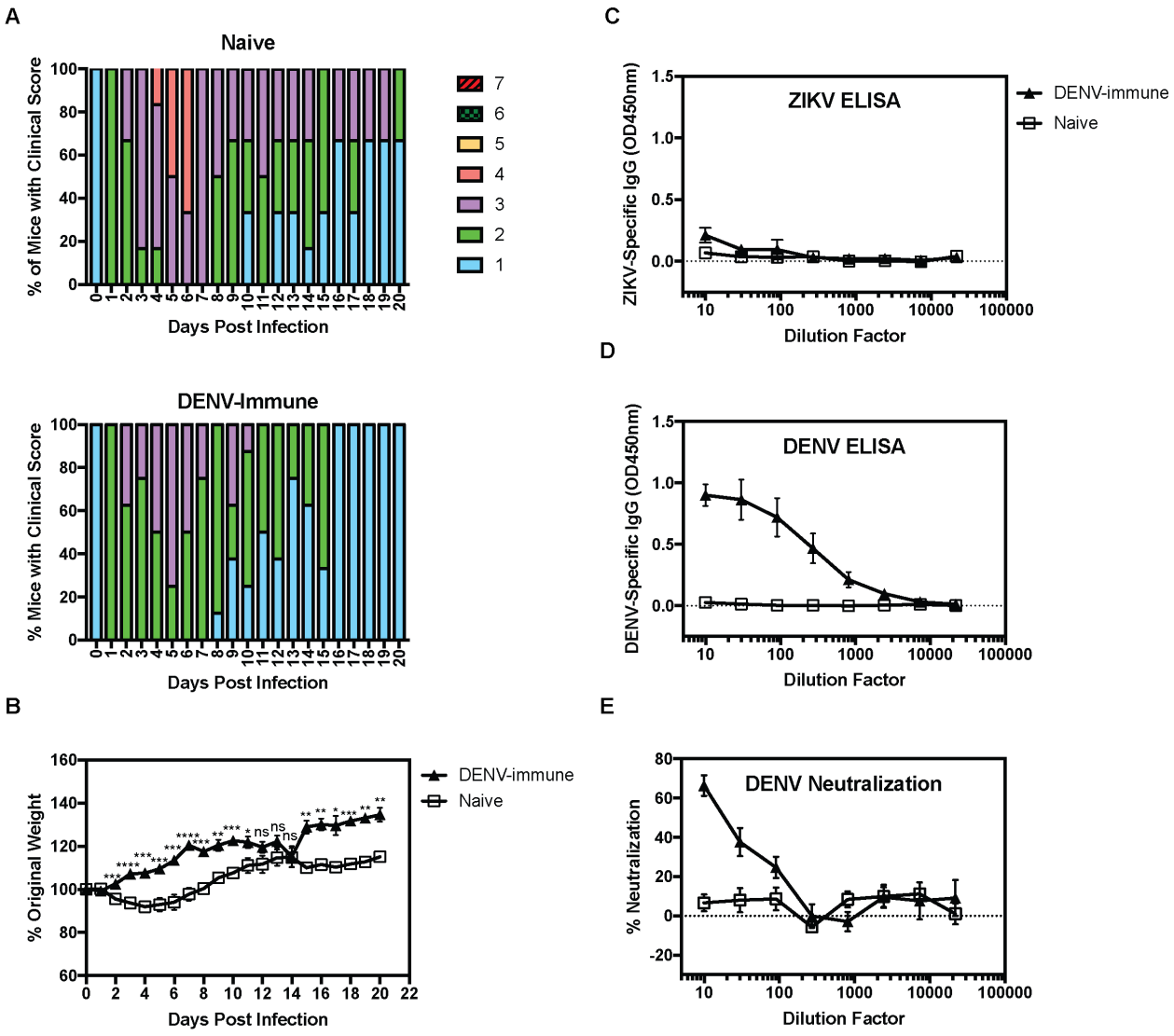

Figure S3, Related to Figures 1 and 2. Mice born to DENV-immune mothers have reduced DENV disease compared to mice born to naïve mothers, and sera from mice born to DENV-immune mothers do not bind ZIKV. Four- to 5-week-old *LysMCre*$^{+}$*Ifnar1*$^{fl/fl}$ mice born to mothers previously infected with DENV2 strain S221 (5x10^{5} FFU via tail vein) or to naïve mothers were challenged with 10^{6} FFU of DENV2 strain S221 via tail vein injection. (**A**) Clinical scores of pups (*n*=6) from naïve mothers (*n*=2), Clinical scores of pups (*n*=12) born to DENV2-immune mothers infected for 8-11 months (*n*=3), and (**B**) Weight loss of pups born to DENV2-immune mothers. Serum

samples were collected from 4- to 5-week-old *LysMCre+Ifnar1*$^{fl/fl}$ mice born to mothers previously infected with DENV2 strain S221 ($5x10^5$ FFU via tail vein) or naïve mothers. Open squares represent pups (*n*=10) from naïve mothers (*n*=2) and closed triangles represent pups (*n*=12) born to DENV2-immune mothers infected for 8-11 months (*n*=3). (**C**) anti-ZIKV IgG and (**D**) anti-DENV IgG were detected via ELISA. (**E**) Neutralization capacity was assessed against DENV2 S221 via U937-DC-SIGN cells and a flow cytometry-based assay (Wen et al., 2017). Dotted line indicates limit of detection for ELISA and 0% neutralization for the neutralization assay. Data represent pooled data from two independent experiments and are expressed as mean ± standard error of mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Unpaired Student's t test of groups for each day in (**B**).

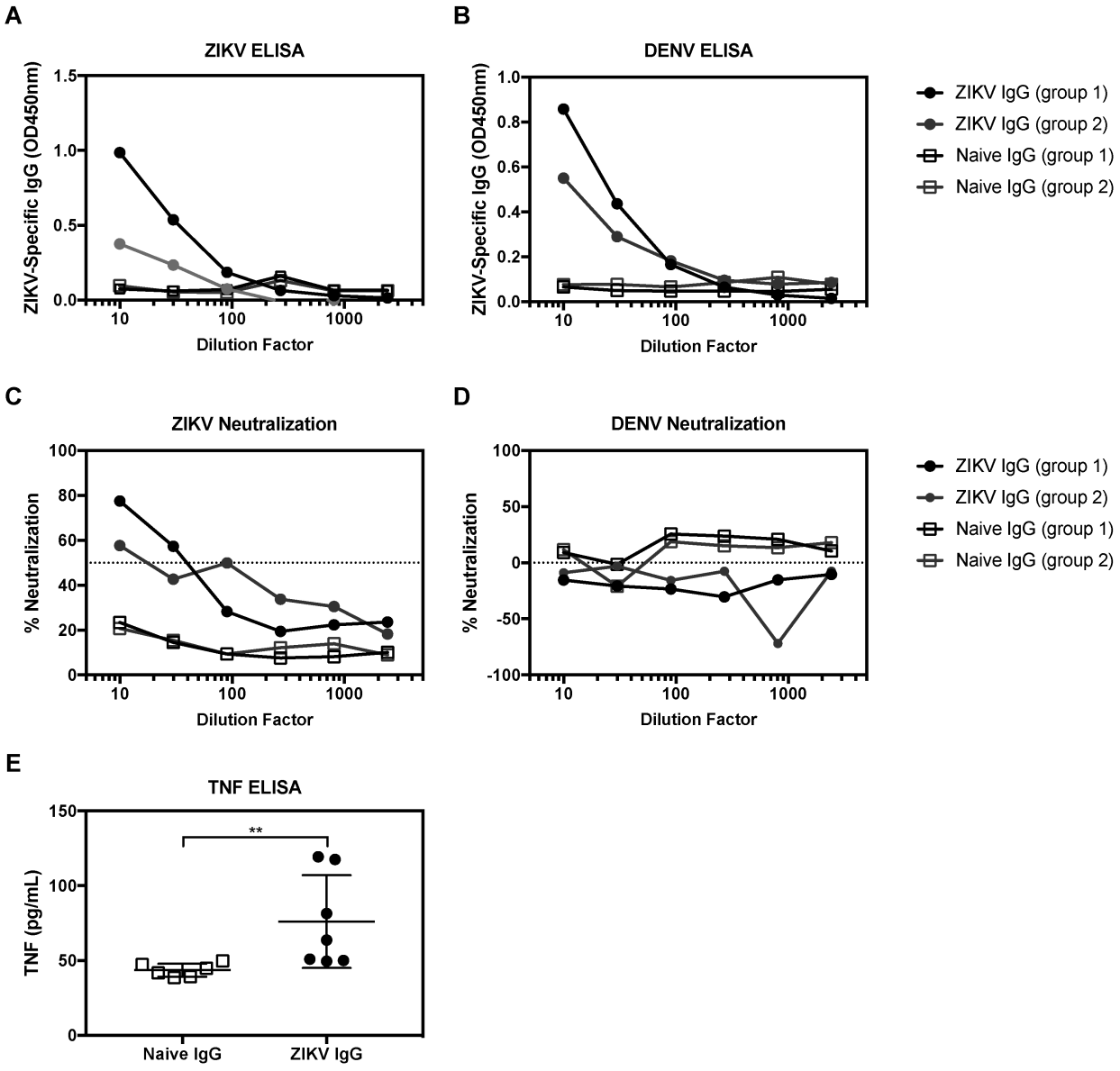

Figure S4, Related to Figure 3. IgG isolated from pups born to ZIKV-immune mothers bind both ZIKV and DENV2 but neutralize ZIKV and not DENV2. IgG isolated and pooled from 23 mice born to ZIKV-immune mothers or 23 mice born to naïve mothers (group 1) and 13 mice born to ZIKV-immune mothers and 9 mice born to naïve mothers (group 2) for the IgG preparations used in passive transfer experiments in Figure 3 was assessed for binding and neutralization capabilities. (**A**) anti-ZIKV IgG and (**B**) anti-DENV IgG were detected via ELISA. Neutralization capacity was assessed against (**C**) ZIKV

SD001 and (**D**) DENV S221 via U937-DC-SIGN cells and a flow cytometry-based assay (Wen et al., 2017). Dotted line indicates limit of detection for ELISA and 50% or 0% neutralization for the neutralization assay. Data are expressed as mean +/- standard error of mean. (**E**) Day 3 p.i. serum samples from mice that received 145 μg of IgG and then challenged with 2×10^5 FFU of DENV2 strain S221 via tail vein injection (same mice used in Figure 3) were assessed for TNF levels using a R&D systems Quantikine ELISA kit for mouse TNF. Data are pooled from 2 independent experiments with n=6-8 mice group, and are expressed as mean +/- standard error of mean. Mann-Whitney test, $^{**}P < 0.01$.