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Supplemental Information

Oral Antibiotic Treatment of Mice Exacerbates the Disease Severity of Multiple Flavivirus Infections

Larissa B. Thackray, Scott A. Handley, Matthew J. Gorman, Subhajit Poddar, Prachi Bagadia, Carlos G. Briseño, Derek J. Theisen, Qing Tan, Barry L. Hykes Jr., Hueylie Lin, Tiffany M. Lucas, Chandni Desai, Jeffrey I. Gordon, Kenneth M. Murphy, Herbert W. Virgin, and Michael S. Diamond

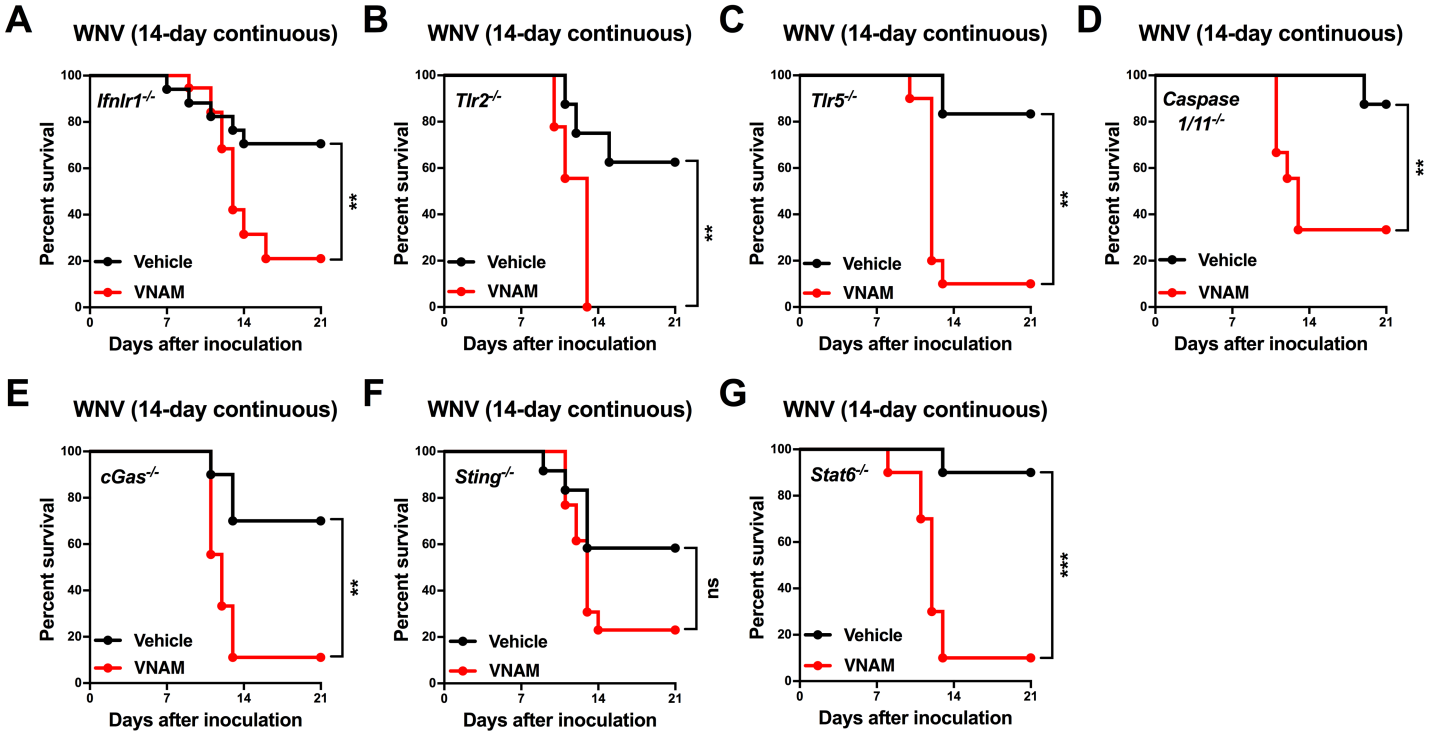

Figure S1. The effect of oral Abx treatment on severe WNV infection does not require the IFN-lambda receptor, Tlr2, Tlr5, Caspase 1, Caspase 11, cGas, Sting or Stat6. Related to Figure 1.

A-G. Mice deficient in the IFN-lambda receptor (*Ifnlr1*$^{-/-}$) **(A)**, Toll-like receptor (Tlr) 2 (*Tlr2*$^{-/-}$) **(B)**, Tlr5 (*Tlr5*$^{-/-}$) **(C)**, caspase 1 and 11 (*Caspase 1/11*$^{-/-}$) **(D)**, the cyclic GMP-AMP synthase (*cGas*$^{-/-}$) **(E)**, the Stimulator of interferon genes (*Sting*$^{-/-}$) **(F)**, and the Signal transducer and activator of transcription 6 (*Stat6*$^{-/-}$) **(G)** were treated with vehicle or VNAM, inoculated with WNV at 12 weeks of age, and survival was analyzed as in **Fig 1B**. Results were combined from two to three independent experiments: vehicle (n = 17), VNAM (19) **(A)**; vehicle (9), VNAM (9) **(B)**; vehicle (6), VNAM (10) **(C)**; vehicle (8), VNAM (9) **(D)**; vehicle (10), VNAM (9) **(E)**; vehicle (12), VNAM (13) **(F)**; vehicle (10), VNAM (10) **(G)**.

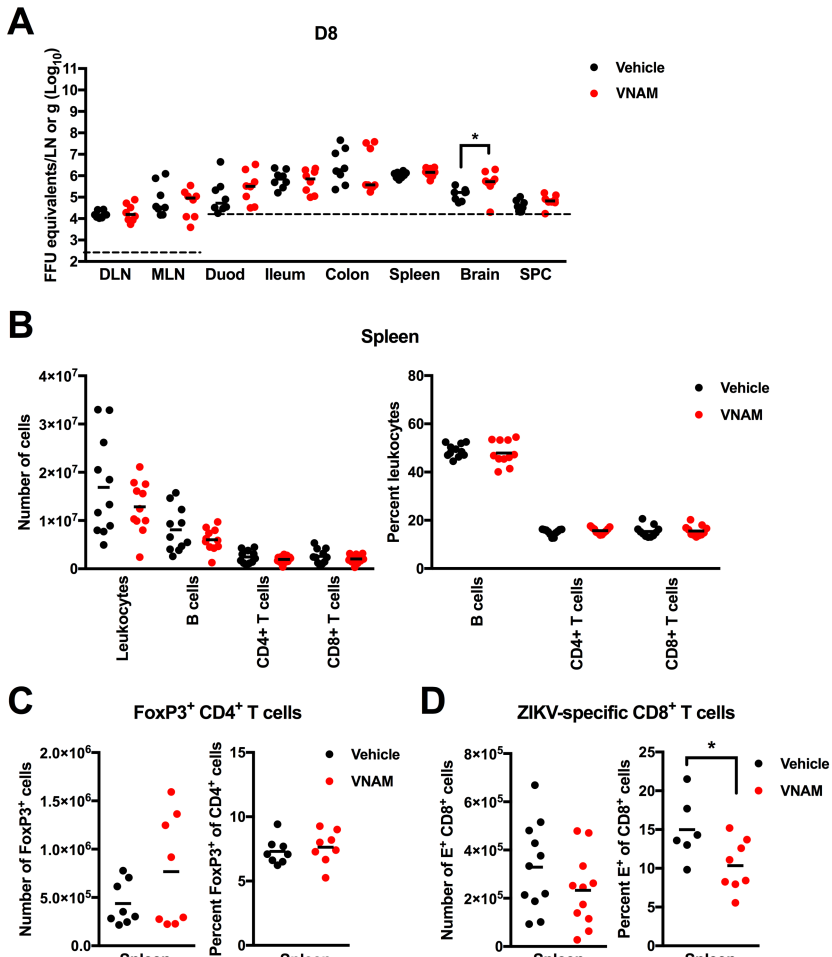

Figure S2. The effect of oral Abx treatment on ZIKV infection. Related to Figures 2 and 3.
A, B. *Ifnar1*$^{f/f}$*LysMCre*$^{+/+}$ mice were treated with vehicle or VNAM (n = 6 to 8) and inoculated with ZIKV as in **Fig 1G**. Tissues were harvested at eight days after ZIKV inoculation. **A.** ZIKV burden was assessed by qRT-PCR. Statistical significance was determined using the Mann-Whitney test (* $P < 0.05$). Results were combined from two independent experiments. Duod = duodenum, SPC = spinal cord. **B-D.** The numbers (*left panel*) and percentages (*right panel*) of leukocytes, B cells, and CD4$^+$ and CD8$^+$ T cells **(B)**, Tregs **(C)**, and ZIKV-specific CD8$^+$ T cells **(D)**. Statistical significance was determined using an unpaired t-test, all comparisons were not significant unless indicated (* $P < 0.05$). Results were combined from two independent experiments.

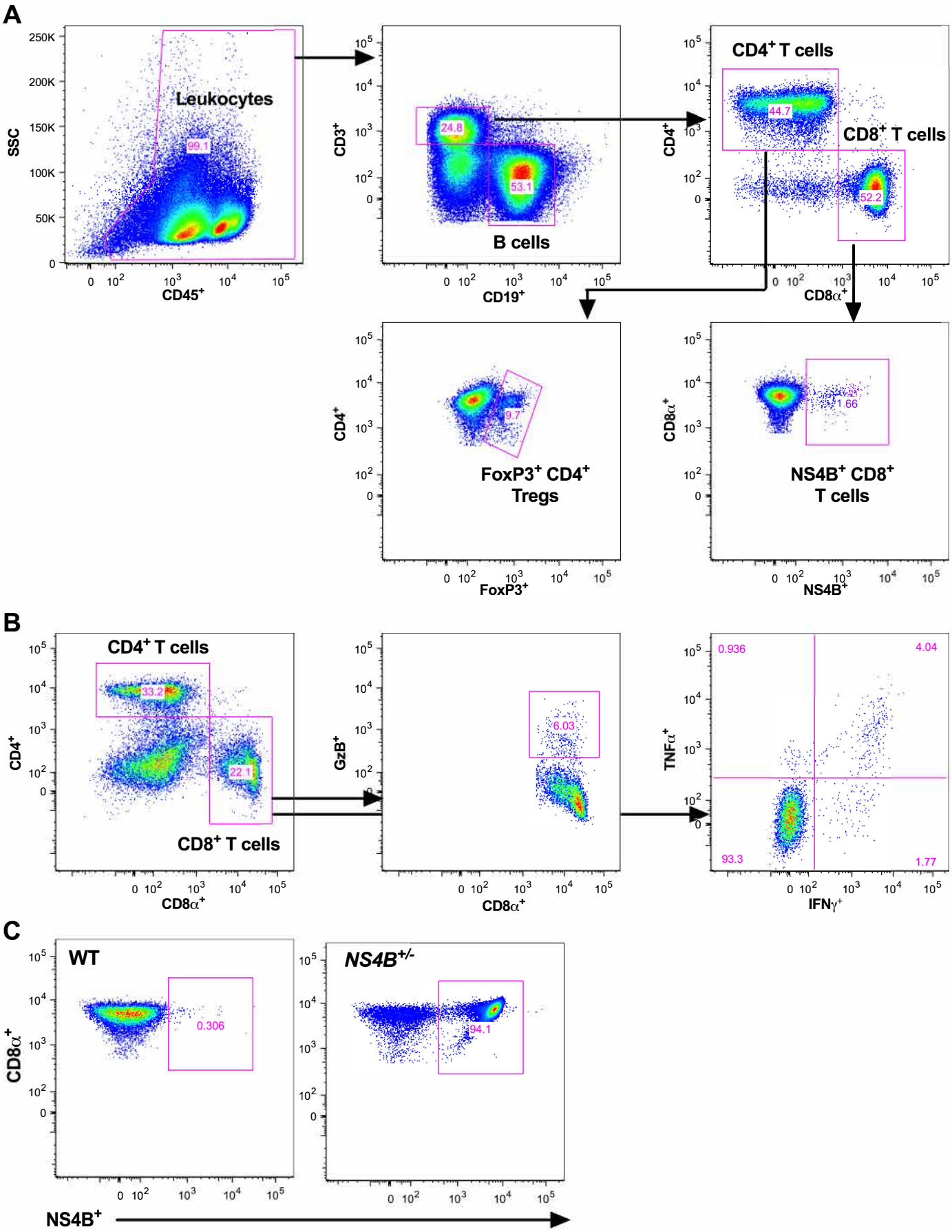

Figure S3. Flow cytometry gating schemes of WNV-infected mice. Related to Figures 3 and 4.
A-C. Representative flow cytometry plots showing gating schemes. **A.** Percentages of cell populations: leukocytes, B cells, $CD4^+$ and $CD8^+$ T cells, Tregs, and WNV-specific $CD8^+$ T cells. **B.** Percentages of CD8+ T cells expressing GzB, or IFN-γ and/or TNF-α after *ex vivo* NS4B peptide stimulation. **C.** Percentages of WNV-specific $CD8^+$ T cells from litter-mate WT or $NS4B^{+/-}$ transgenic mice.

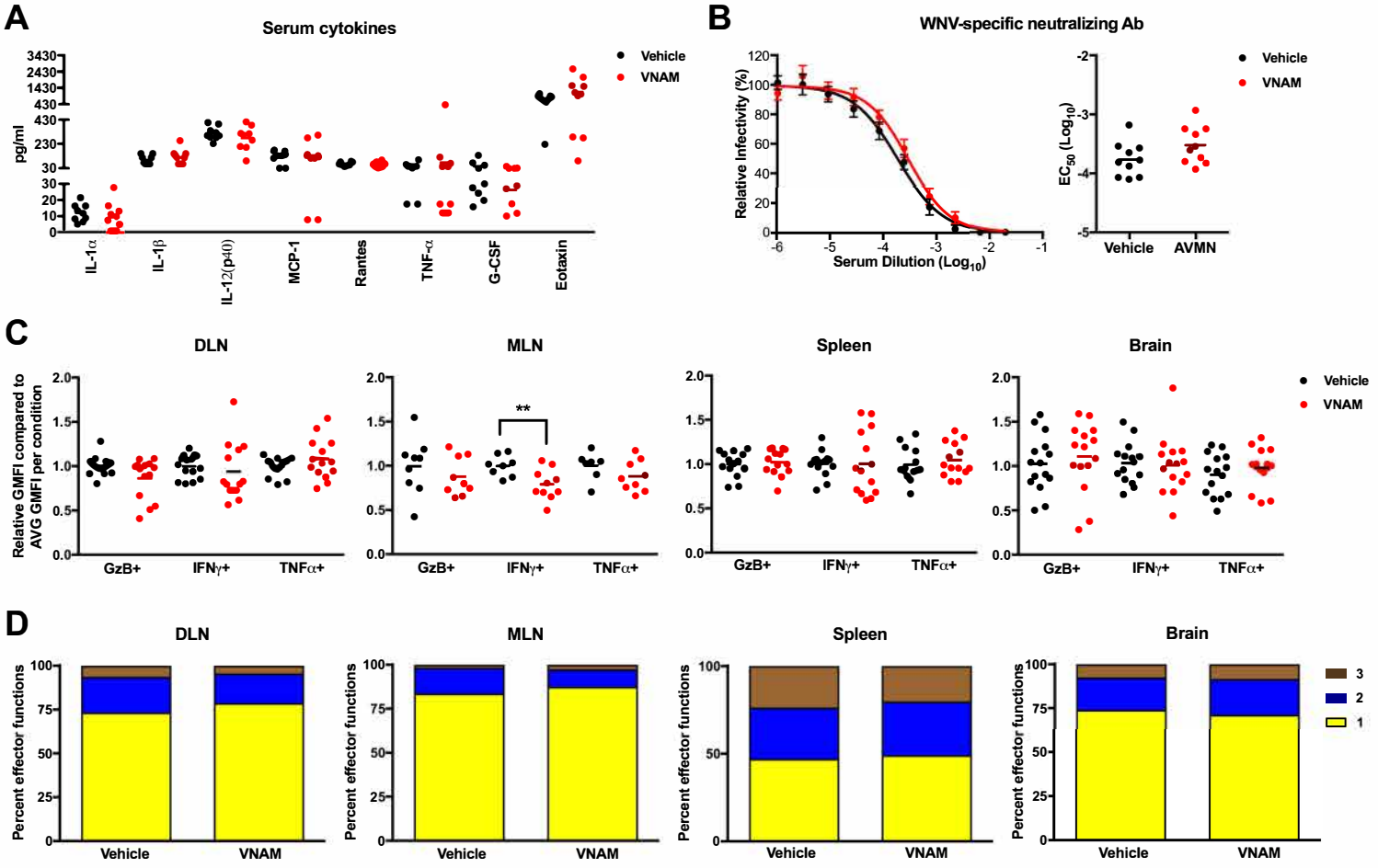

Figure S4. The effect of oral Abx treatment on immunity during WNV infection. Related to Figure 3.
A-D. Mice were treated with vehicle or VNAM (n = 10 to 15), inoculated with WNV, and tissues were harvested as in **Fig 3**. **A.** Serum cytokine response during WNV infection (all comparisons not significant, $P > 0.05$; unpaired t-test) **B.** Serum neutralizing antibody response against WNV (comparison of EC_{50} values not significant, $P > 0.05$; unpaired t-test). **C.** Geometric mean fluorescent intensity (GMFI) of GzB^+, and $IFN\gamma^+$ and $TNF\alpha^+$ $CD8^+$ T cells after *ex vivo* NS4B peptide stimulation. Statistical significance was determined using an unpaired t-test (** $P < 0.01$, all other comparisons not significant, $P > 0.05$). **D.** Representation of the average percentage of $CD8^+$ T cells with one (1), two (2) or three (3) effector functions (GzB^+, and IFN-γ^+ and/or TNF-α^+ after ex vivo NS4B peptide stimulation) (comparisons between vehicle and VNAM for each effector function not significant, $P > 0.05$, unpaired t test). Results were combined from two to three independent experiments.

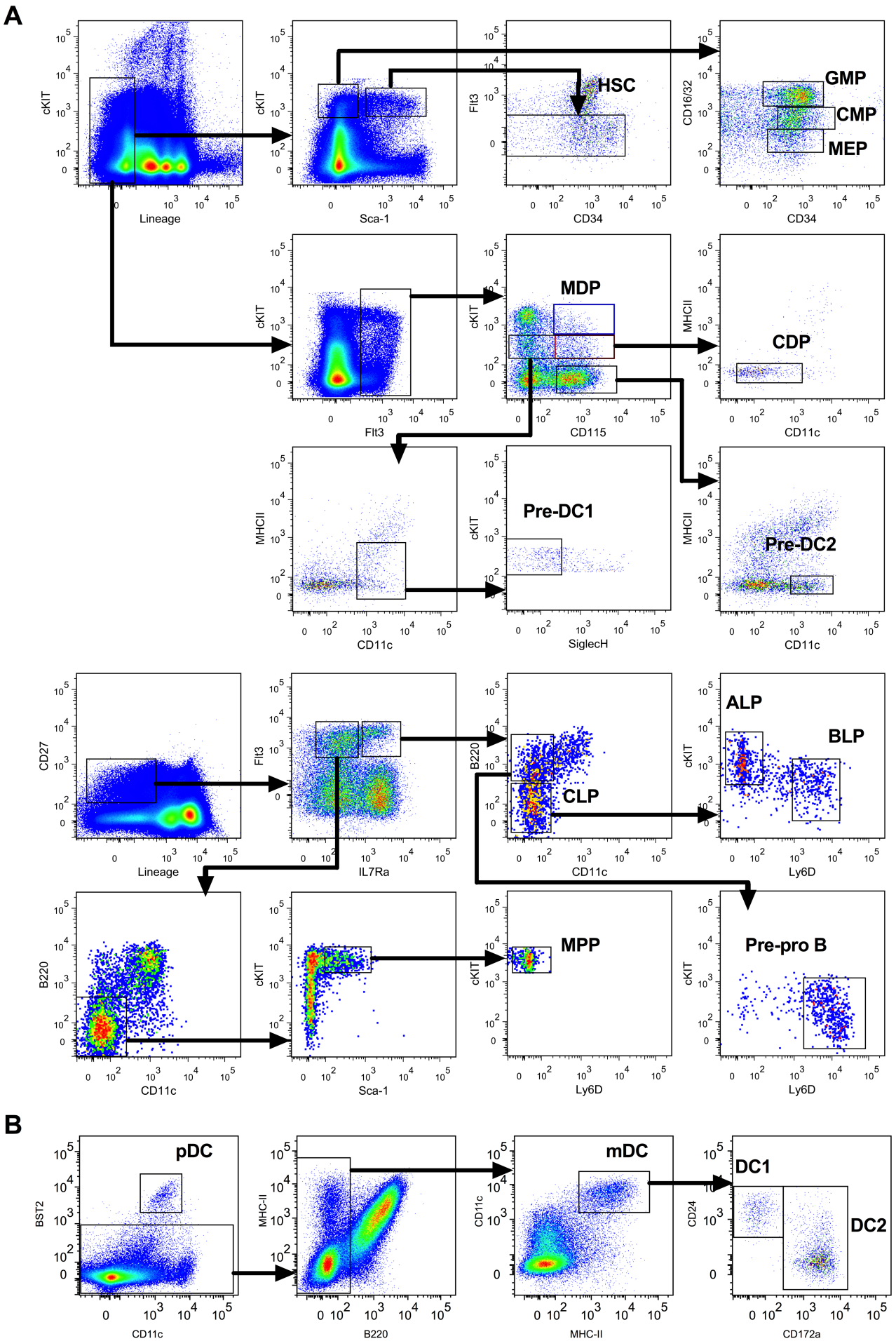

Figure S5. Flow cytometry gating schemes of bone marrow cell populations and dendritic cell subsets from naïve mice. Related to Figure 5.
A, B. Representative flow cytometry plots showing gating schemes for bone marrow **(A)** and dendritic cell **(B)** analyses.

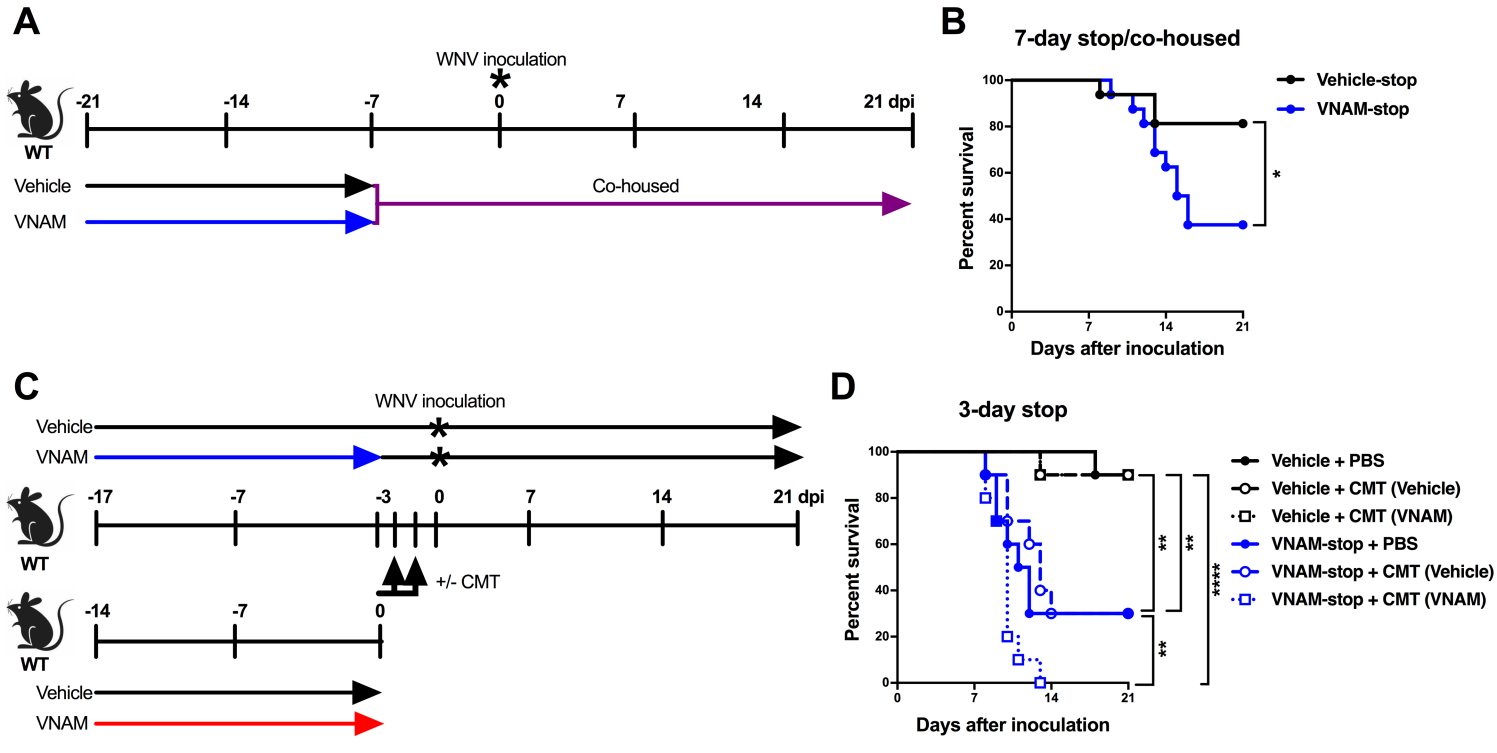

Figure S6. The effect of microbiota transfer on increased susceptibility to WNV infection following Abx treatment. Related to Figure 6. A-D. WT mice were treated with vehicle or VNAM as illustrated in panels **A** and **C**. Vehicle- and VNAM-treated mice were co-housed for one week after cessation of treatment and during infection **(B)**. Vehicle- and VNAM-treated mice were gavaged twice with cecal contents [cecal microbial transfer (CMT)] from vehicle- or VNAM-treated mice after cessation of VNAM **(D)**. Mice were inoculated with WNV as in **Fig 1B**. Survival curves were compared using the log-rank test with a Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$). Results were combined from two independent experiments: vehicle-stop (n = 16), VNAM-stop (16) **(B)**; Vehicle + PBS (10), Vehicle + CMT (Vehicle) (10), Vehicle + CMT (VNAM) (10), VNAM-stop + PBS (10), VNAM-stop + CMT (Vehicle) (10), VNAM-stop + CMT (VNAM) (10) **(D)**.

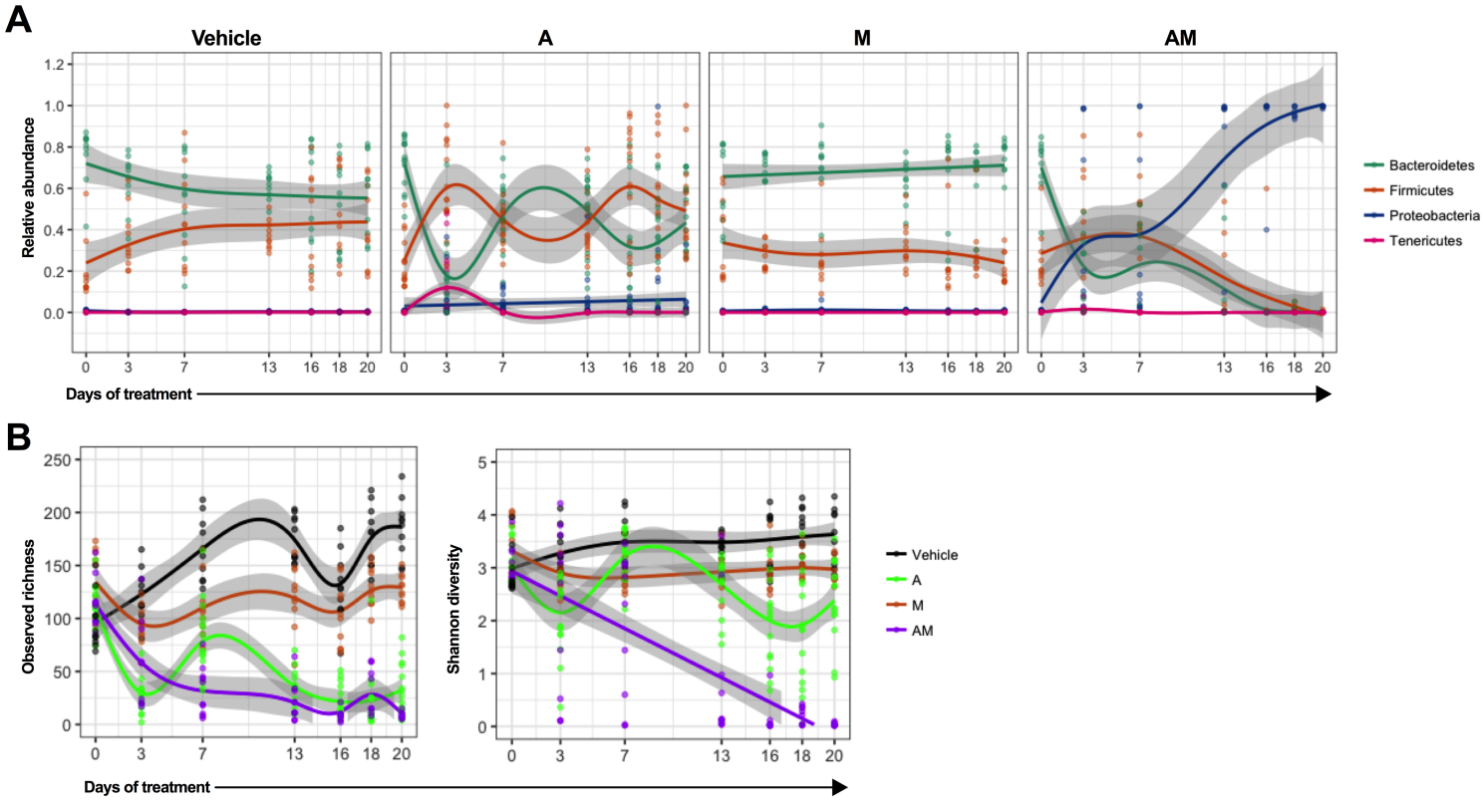

Figure S7. Oral Abx treatment alters the gut bacterial community. Related to Figure 6.
A-B. Female mice were vehicle- or Abx-treated, inoculated with WNV, and fecal samples harvested as in **Fig 6E-H**. **A.** Effects of treatment on the relative abundance of the most abundant (> 3%) phyla. **B.** The effect of treatment on alpha-diversity as defined by observed richness (*left panel*) and Shannon diversity (*right panel*). The line represents the general additive model smoother for each treatment with the standard error represented in gray.