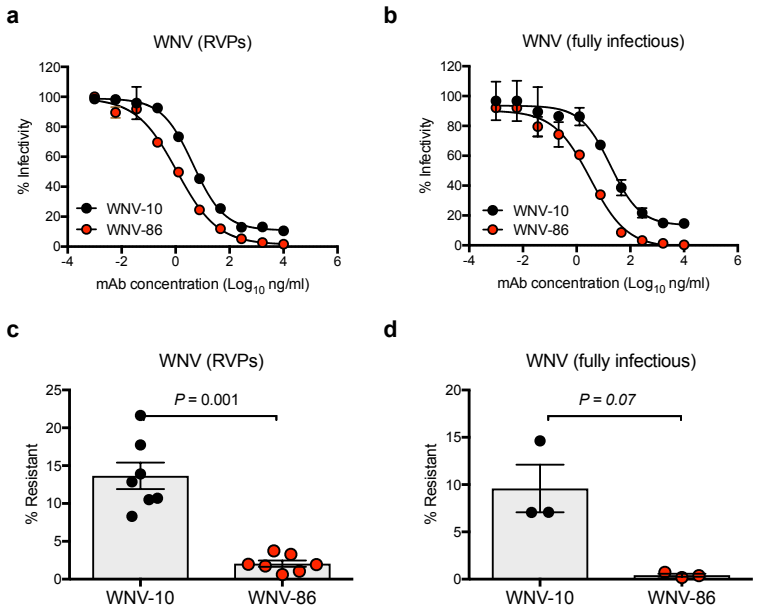
A potently neutralizing human monoclonal antibody targeting an epitope in the West Nile virus E protein preferentially recognizes mature virions

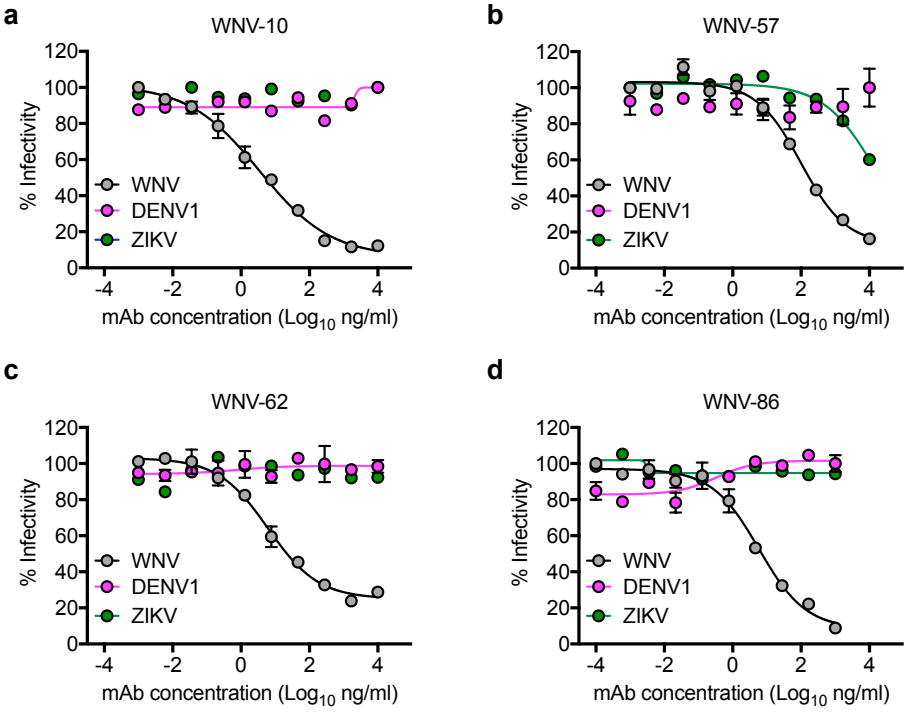
Supplementary Table 1. Human monoclonal antibodies isolated from WNV-survivors

WNV mAbs	IgG subclass[a]	Light chain
WNV-6	1	κ
WNV-10	1	κ
WNV-13[b]	1 and 3	κ
WNV-15	1	λ
WNV-57	1	κ
WNV-61	1	λ
WNV-62	1	λ
WNV-39	1	λ
WNV-18	1	κ
WNV-86	1	κ

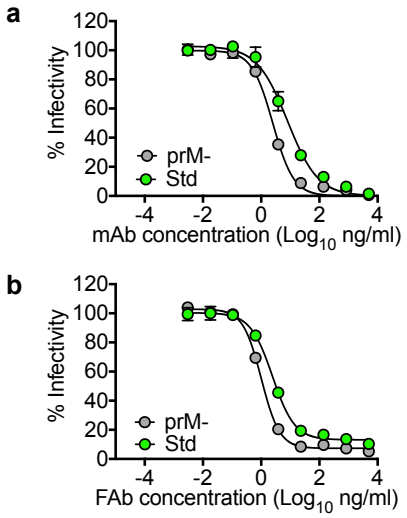
[a] Antibody subclass determined by ELISA in two independent experiments performed with technical replicates.
[b] Testing for WNV-13 gave ambiguous results, as it reproducibly bound both IgG1- and IgG3-specific reagents in ELISA experiments.



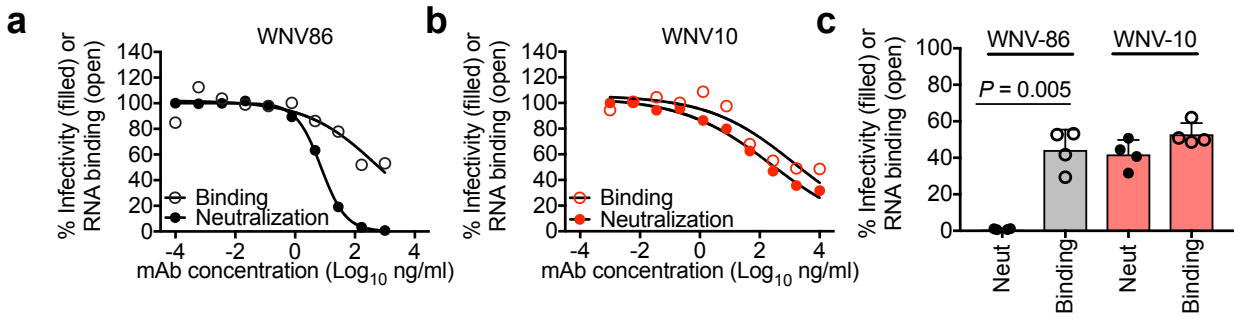
Supplementary Figure 1. Incomplete neutralization by mAb WNV-10. Neutralization of WT WNV **(a)** RVPs or **(b)** fully infectious viruses by mAbs WNV-10 or WNV-86. Infectivity was normalized to levels observed in the absence of antibody. Data points and error bars indicate the mean and range of duplicate infections, respectively. Curves shown are representative of 7 or 3 independent experiments for panel a and b, respectively. Mean percentages of WNV **(c)** RVPs or **(d)** fully infectious viruses that remain resistant to neutralization at the highest concentration of mAb WNV-10 or WNV-86 tested (10 μg/mL) were obtained from 7 or 3 independent experiments with RVPs or infectious viruses, respectively. Error bars represent the standard error of the mean. The indicated *P* values were obtained from two-sided paired t-tests. The mean difference between groups in panels **c** and **d** (and the 95% confidence interval of this difference) was -11.6 (-16.7 to -6.63) and -9.1 (-20.1 to 1.8), respectively.



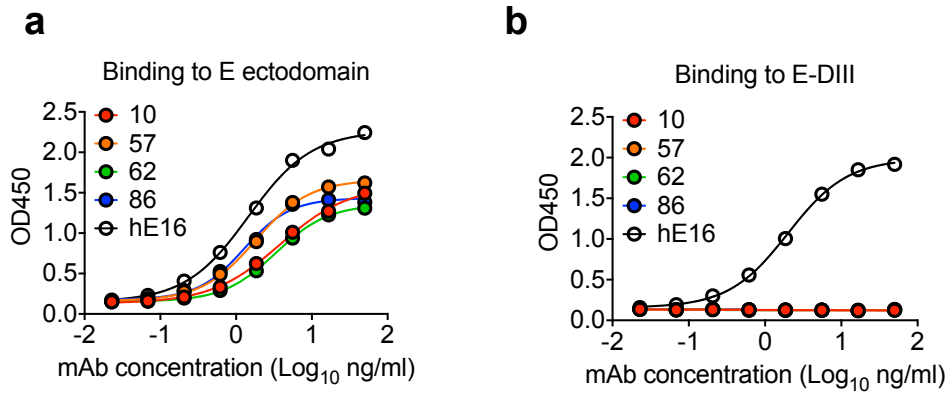
Supplementary Figure 2. MAb cross-reactivity against flaviviruses. Standard preparations of WNV, DENV1, or ZIKV RVPs were tested for sensitivity to neutralization by mAbs **(a)** WNV-10, **(b)** WNV-57, **(c)** WNV-62, or **(d)** WNV-86. Shown are dose-response curves representative of three independent experiments. Infectivity was normalized to levels observed in the absence of antibody. Data points and error bars indicate the mean and range of duplicate infections, respectively.



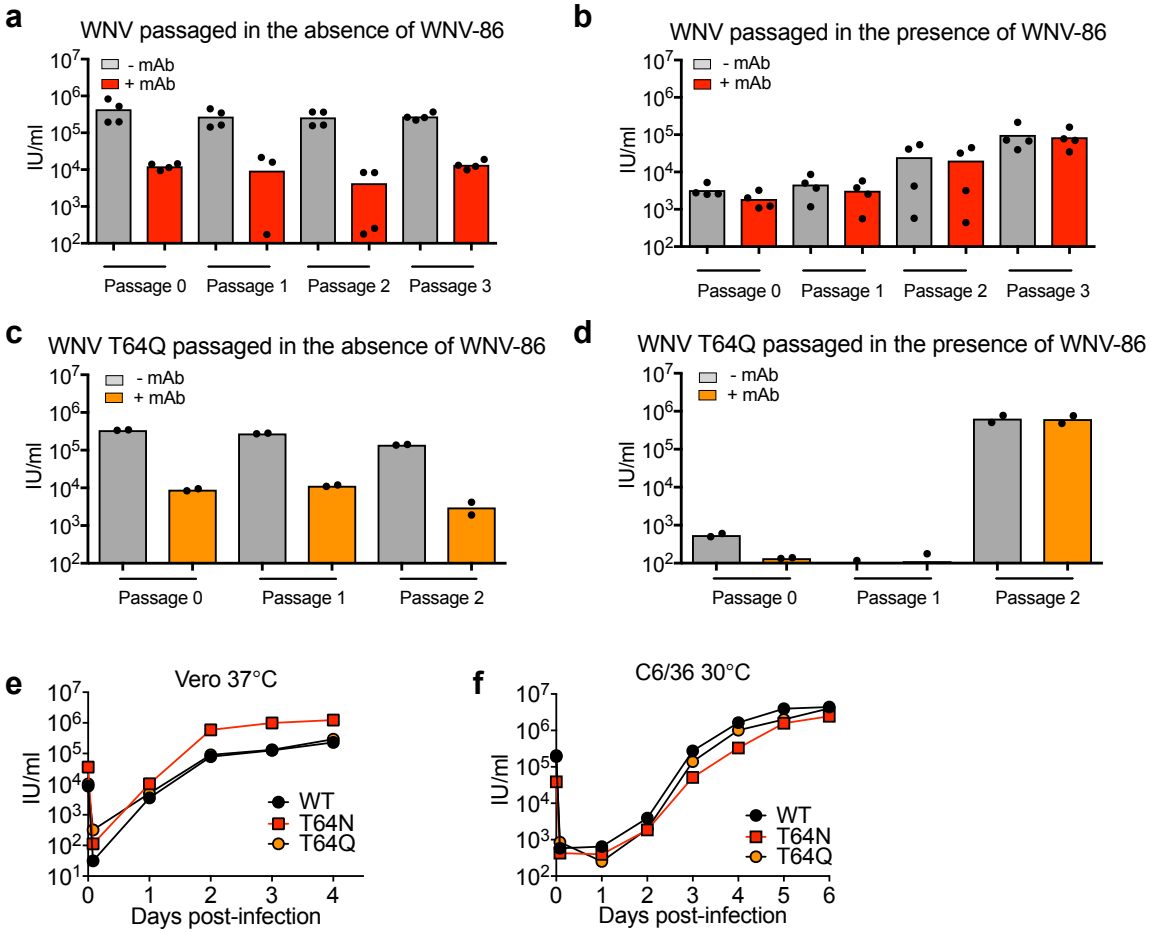
Supplementary Figure 3. Inhibition of WNV by Fab fragments of WNV-86. Neutralization of WT standard (std) and mature prM- WNV RVPs by intact mAb **(a)** or Fab fragments **(b)** of WNV-86. Infectivity was normalized to levels observed in the absence of antibody. Data points and error bars indicate the mean and range of duplicate infections, respectively. Shown are dose-response curves representative of two independent experiments. Mature preparations of RVPs were more sensitive to neutralization by both intact antibody and Fab fragments of WNV-86 as compared to preparations produced under standard conditions that retain uncleaved prM. While neutralization titres with Fab fragments were similar to those with intact antibody, these studies revealed a fraction of virions insensitive to neutralization even at the highest concentrations tested.



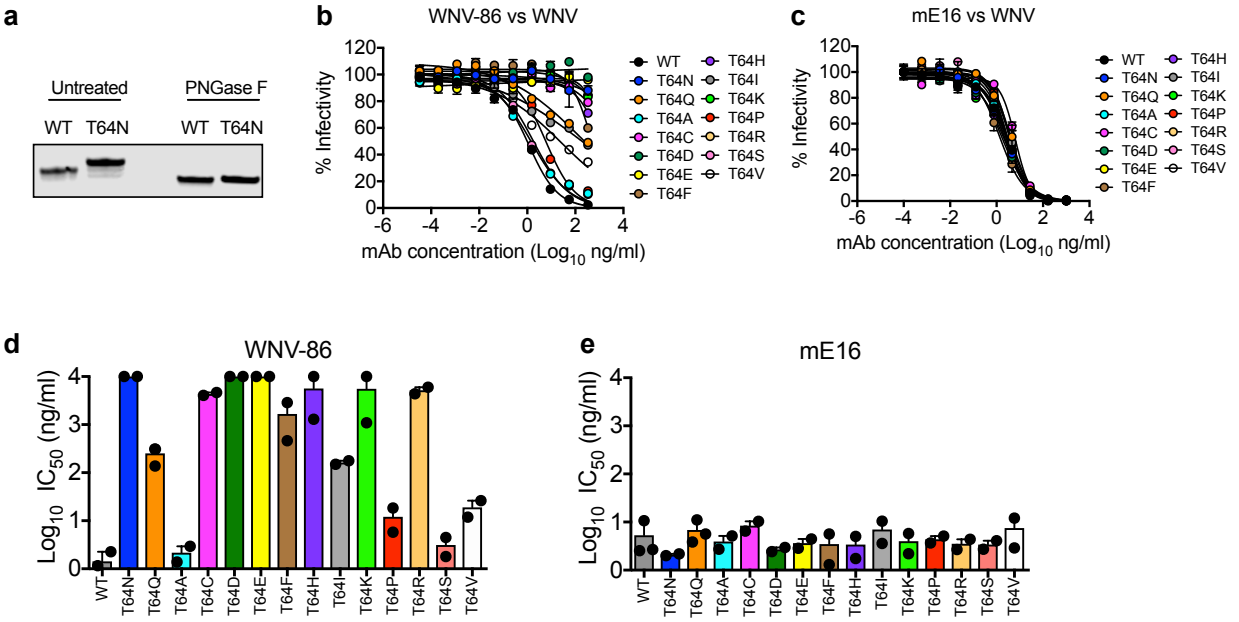
Supplementary Figure 4. WNV-86 blocks infection at a pre- and post-attachment step in the virus entry pathway. The sensitivity of standard preparations of WNV RVPs to neutralization by serial dilutions of mAb WNV-86 **(a)** or WNV-10 **(b)** was measured (filled circles). The ability of antibody to block attachment of RVPs to DC-SIGNR-expressing cells was evaluated in parallel wells using quantitative reverse-transcriptase PCR. The relative levels of cell-associated RNA are shown as the mean of triplicate technical replicates (open circles). Panels **a** and **b** depict dose-response curves representative of four independent experiments. Infectivity or viral RNA levels were normalized to levels observed in the absence of antibody. **(c)** The mean infectivity or binding levels at the highest mAb concentration of antibody tested obtained from four independent experiments are depicted. Error bars represent standard error of the mean. The indicated *P* value was obtained from a two-sided paired t-test. The mean difference between these groups (and the 95% confidence interval of this difference) was 43.3 (25 to 61), respectively.



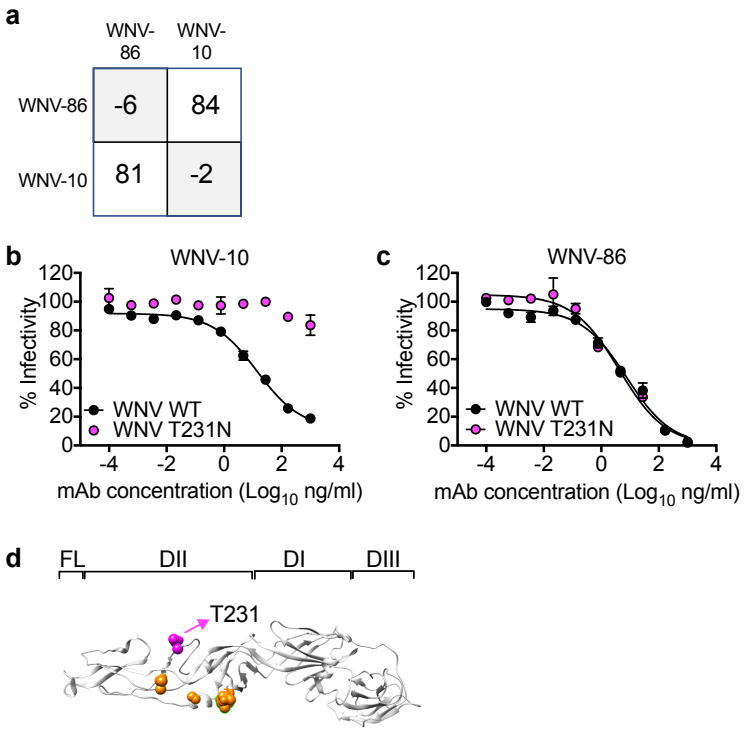
Supplementary Figure 5. Binding properties of human mAbs. Monoclonal antibody binding to recombinant **(a)** WNV E protein ectodomain or **(b)** WNV E protein DIII fragment was determined by ELISA. DIII-specific humanized murine mAb E16 (hE16) was used as a control. Data points indicate the mean OD450 value obtained from triplicate wells from a single experiment.



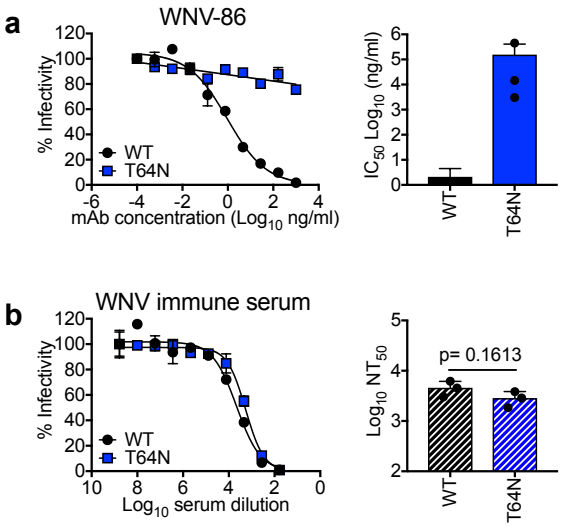
Supplementary Figure 6. Selection of WNV resistant to neutralization by WNV-86. Vero cells were inoculated with WT WNV or a T64Q WNV variant in duplicate in the presence of mAb WNV-86 or medium. Continuous viral replication was maintained by serial passaging of virus in medium with or without mAb WNV-86 on fresh Vero cells. At each passage, an aliquot of viral supernatant from **(a, c)** medium only or **(b, d)** mAb WNV-86 selection was serially diluted and used to inoculate Raji-DCSIGNR cells in the presence (colored bars) or absence (black bars) of WNV-86 to monitor the growth of escape variants. Average viral titres expressed as infectious units per ml (IU/ml) in panels **a** and **b** were obtained from duplicate wells in two independent virus selection experiments, whereas those in panels **c** and **d** were obtained from duplicate wells in a single virus selection experiment. (**e, f**) Replication of WT, T64N, and T64Q viruses was established in a single experiment performed on Vero cells or the mosquito cell line C6/36. The infectious titre was calculated using the linear portion of the virus dose-infectivity curves using the following formula: Infectious units (IU) per virus volume = (% GFP-positive cells) x (dilution factor) x (number of cells).



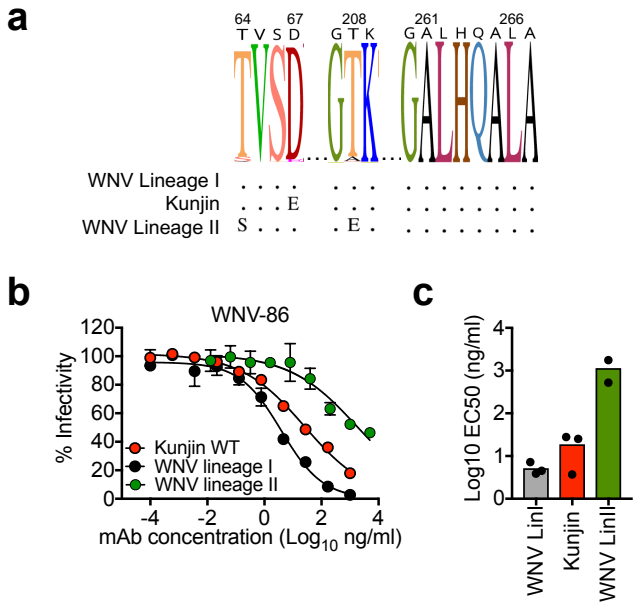
Supplementary Figure 7. Characteristics of WNV E T64 RVP variants. (a) The E protein glycan occupancy of standard preparations of WNV WT and T64N RVPs was assessed by SDS-PAGE followed by western blotting of virus lysates that were either untreated or treated with PNGaseF. E protein was detected using murine mAb 4G2. Data are representative of two independent experiments. WT WNV RVPs or variants containing a mutation at E residue 64 were tested for sensitivity to neutralization by **(b)** mAb WNV-86 or **(c)** murine mAb E16. Dose-response curves representative of two independent experiments are shown. Infectivity was normalized to levels observed in the absence of antibody. Data points and error bars indicate the mean and range of duplicate infections, respectively. Average IC_{50} values of **(d)** WNV-86 or **(e)** mE16 against WNV E T64 RVP variants obtained from two independent experiments. Data points and error bars indicate the mean and range of duplicate infections, respectively. A value of 10ug/ml was assigned in instances where an IC_{50} could not be calculated with confidence due to limited neutralization activity.



Supplementary Figure 8. WNV-10 and WNV-86 bind distinct epitopes. (a) Antibody competition binding studies were performed using bio-layer interferometry to determine if WNV-86 and WNV-10 bind to overlapping epitopes. The numbers indicate the percent of uncompeted binding for the antibodies listed on top observed when competition was tested by applying the antibody to the left, followed by the antibody listed on the top of the table. (**b, c**) Mutation at E DII residue T231 confers escape from WNV-10. Neutralization of WNV WT or T231N RVPs by mAb **(b)** WNV-10 or **(c)** WNV-86. Data points and error bars indicate the mean and range of duplicate infections, respectively. Neutralization curves shown are representative of three independent experiments. **(d)** Crystal structure of the WNV E protein monomer (PDB 2HG0) with DI, DII, DIII, and DII-FL indicated above the structure. Residues that reduced the neutralization potency of mAb WNV-86 or WNV-10 are highlighted in orange or magenta, respectively.



Supplementary Figure 9. Effect of WNV T64N mutation on serum neutralizing activity. WNV WT and T64N RVPs were tested for sensitivity to neutralization by **(a)** mAb WNV-86 or **(b)** WNV-immune serum from which mAb WNV-86 was isolated. Left panels show dose-response curves representative of three independent experiments. Data points and error bars indicate the mean and range of duplicate infections, respectively. Right panels show mean **(a)** IC_{50} or **(b)** NT_{50} values obtained from three independent experiments. Error bars indicate the standard error of the mean. The *P* value shown was obtained from a two-sided paired t-test. The mean difference between these groups (and the 95% confidence interval of this difference) was -1730 (-5148 to 1688), respectively.



Supplementary Figure 10. Impact of strain variation on sensitivity to neutralization by WNV-86. (**a**) WNV E protein amino acid sequences were aligned to reveal conservation of residues shown to be involved in WNV-86 recognition. Results of the analysis of ~2,000 strains are shown in a Logos format. The identity of residues in the RVPs used in our neutralization studies are shown. (**b**) Standard preparations of WNV RVPs produced using structural genes of the lineage I strains NY99 and Kunjin virus, and a GFP-expressing lineage II virus (strain WN 956 D117 3B) were tested for neutralization sensitivity to WNV-86 on Raji-DCSIGNR cells. Infectivity was normalized to levels observed in the absence of antibody. Data points and error bars indicate the mean and range of duplicate infections, respectively. Neutralization curves shown are representative of three (lineage I strains) and two (lineage II strain) independent experiments, and mean neutralization potencies are summarized in **(c)**.