## Supplementary Materials

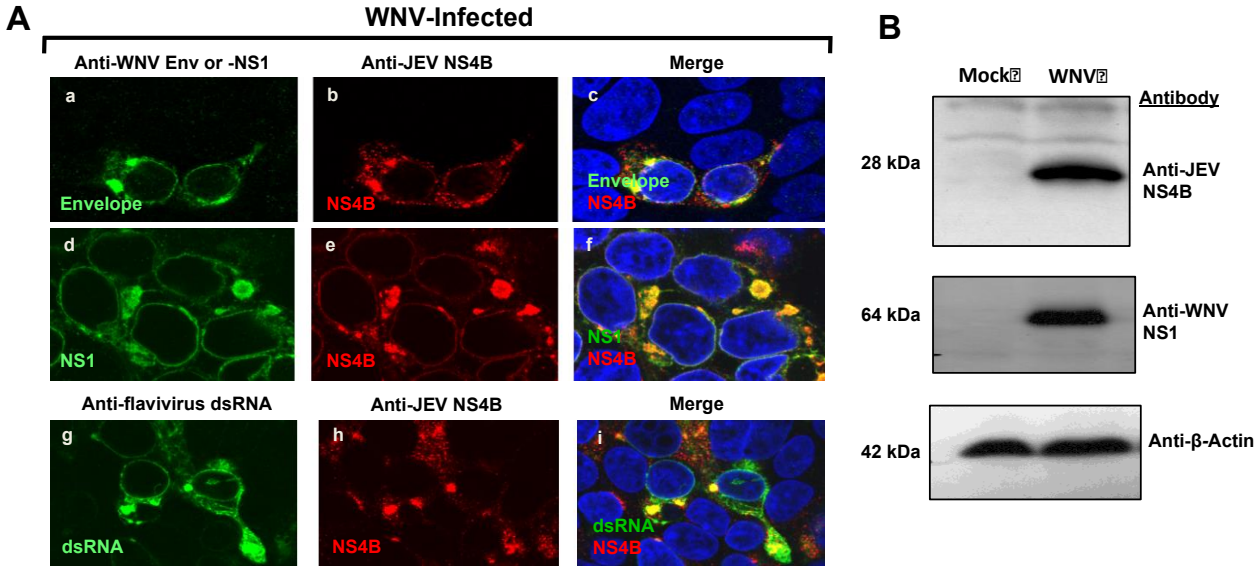


**Figure S1**. Detection of $WNV_{NY99}$ NS4B in WNV infected HEK293 cells with the anti-JEV NS4B antibody. HEK293 cells were infected with $WNV_{NY99}$ at a MOI of 1. Twenty-four hours after infection, cells were fixed for immunofluorescence or lysed for Western blot analysis. (A) Confocal microscopy images showing the colocalization of $WNV_{NY99}$ NS4B with WNV Env and NS1 proteins (a and d) and dsRNA (g) in infected HEK293 cells. The antibodies used for immunolabeling are depicted on the top of each panel and the detected viral components (Env, NS1 or dsRNA) are depicted in the lower left of each image. DAPI was used to stain nuclear DNA. Merged pictures are shown on the right (c, f, and i). Confocal images were of optical slice thickness ~1 μm. (B) Western blot detection of $WNV_{NY99}$ NS4B by the anti-JEV NS4B antibody in infected HEK293 cells. The antibodies used for immunoblotting are shown on the right side of each panel. An anti-WNV NS1 antibody was used to confirm infection. ß-actin served as the loading control. Molecular weight markers (kDa) are given on the left side of each panel. Abbreviations: WNV: West Nile virus NY99 strain ($WNV_{NY99}$); JEV: Japanese encephalitis; NS4B: Non-structural 4B protein.

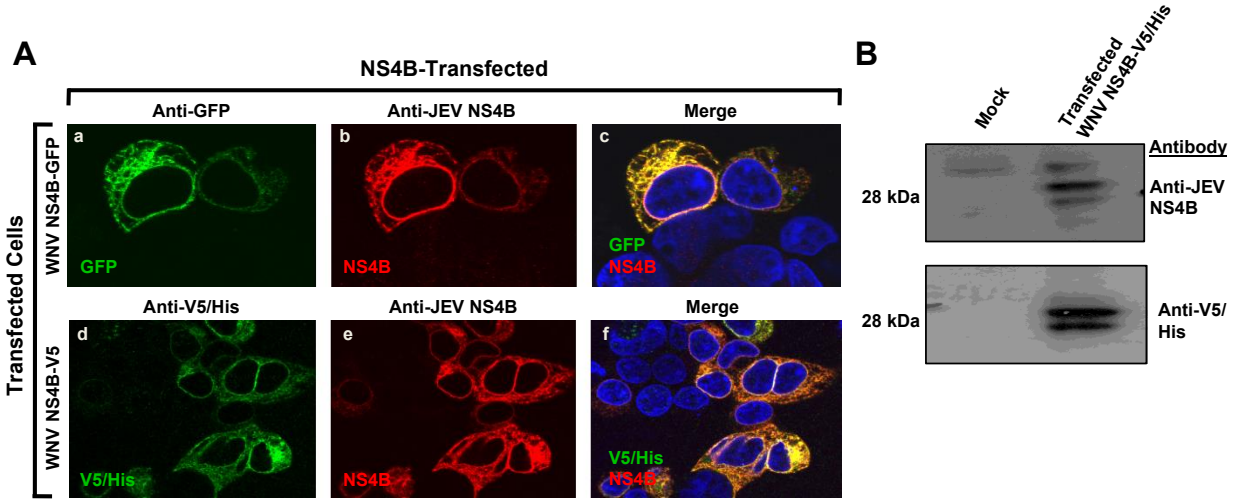


**Figure S2**. Detection of $WNV_{NY99}$ NS4B with the anti-JEV NS4B antibody in transfected HEK293 cells. (A) Cells were transfected with $WNV_{NY99}$ NS4B-GFP fusion (a, b and c) or NS4B-V5/His fusion (d, e and f) plasmids. Twenty-four hours after transfection, cells were fixed and processed for immunofluorescence. Antibodies used in this study are depicted on the top of each panel and the detected fusion (GFP or V5/His) or viral (NS4B) proteins are indicated in the lower left of each image. Nuclear DNA was counterstained with DAPI. Merged images are shown on the right (c and f). Slides were analyzed by confocal laser scanning microscopy. Confocal IF images were of optical slice thickness ~1 μm. B) HEK293 cells transfected with $WNV_{NY99}$ NS4B-V5/His fusion plasmid were harvested 24 hours after transfection. Cell lysates (50 μg) were subjected to SDS-PAGE and Western blot analysis using anti-JEV NS4B and anti-V5/His antibodies. Molecular weight (kDa) is given on the left side of each panel. Abbreviations: V5/His: V5/Histidine tags; GFP: Green fluorescent protein tag.

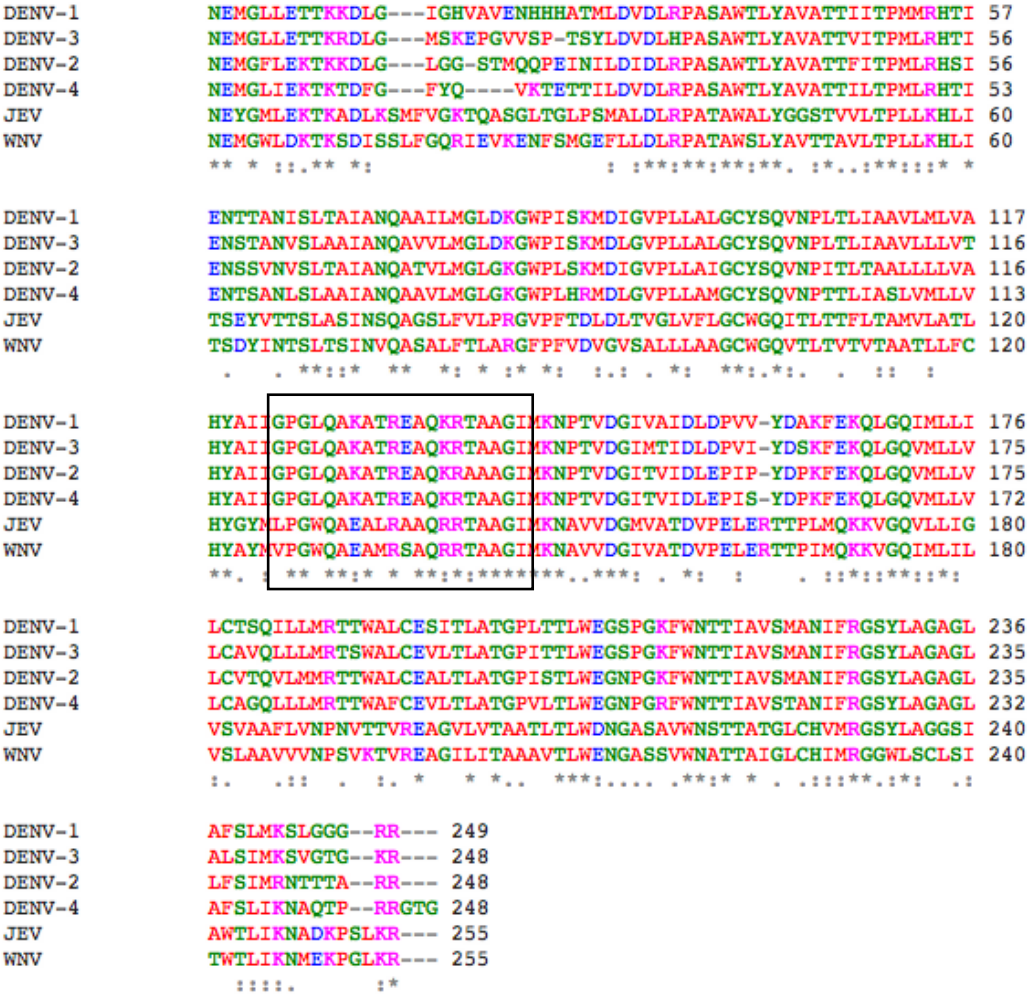


**Figure S3.** Multiple sequence alignment of the primary amino acid sequence of NS4B of different flaviviruses. Complete NS4B amino acid alignments of the selected flaviviruses used in our study showing conserved regions within the DENV serotypes, WNV, and JEV as depicted by the boxed residues. Virus abbreviations (Genbank accession number: DENV-1: Dengue 1 (EU848545.1); DENV-2: Dengue 2 (EF457904.1); DENV-3: Dengue 3 (M93130.1); DENV-4: Dengue 4 (AY947539.1); WNV: West Nile virus NY99 strain (YP_001527886); JEV: Japanese encephalitis (NP_775673).