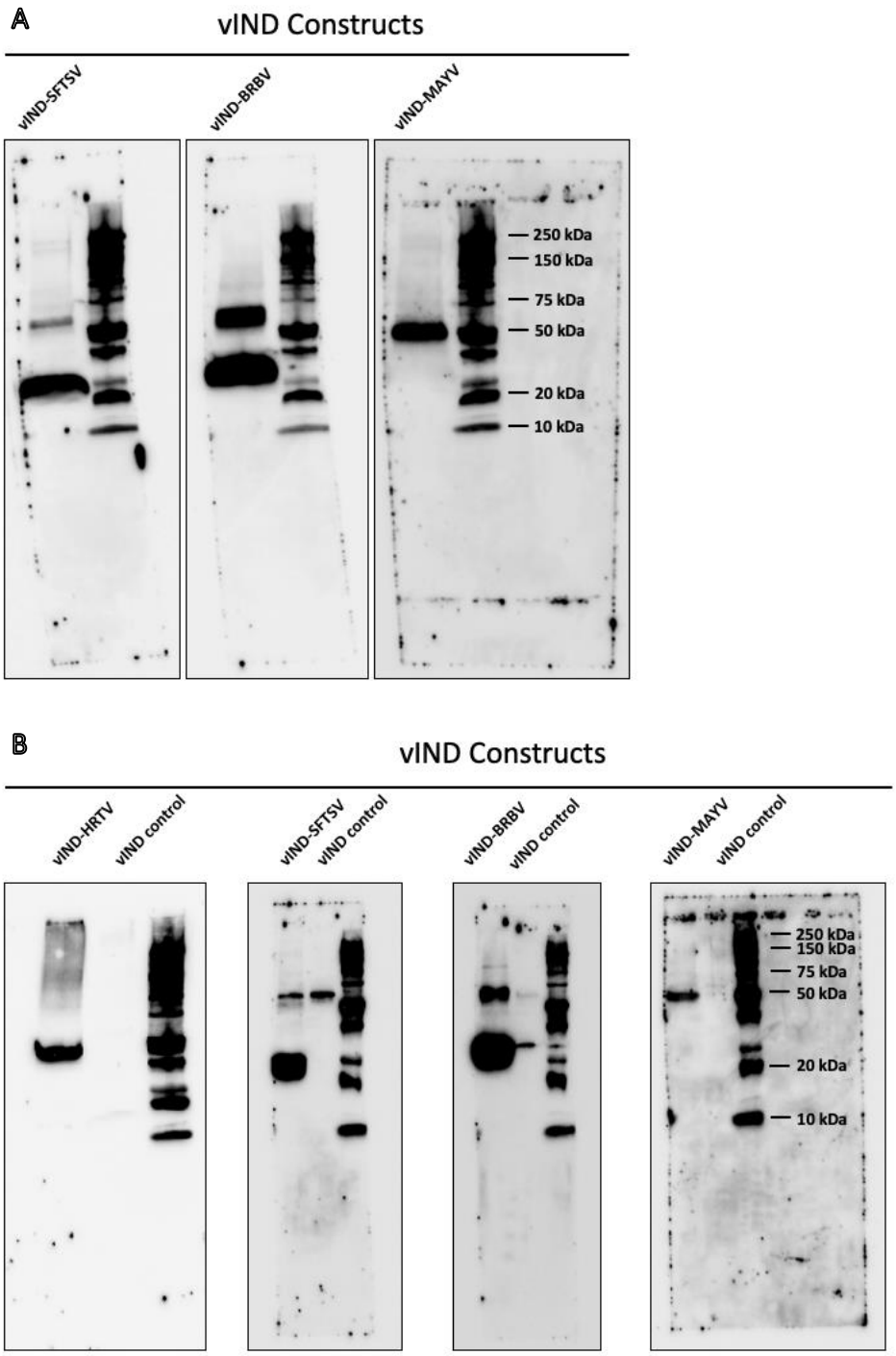
Supplementary Materials

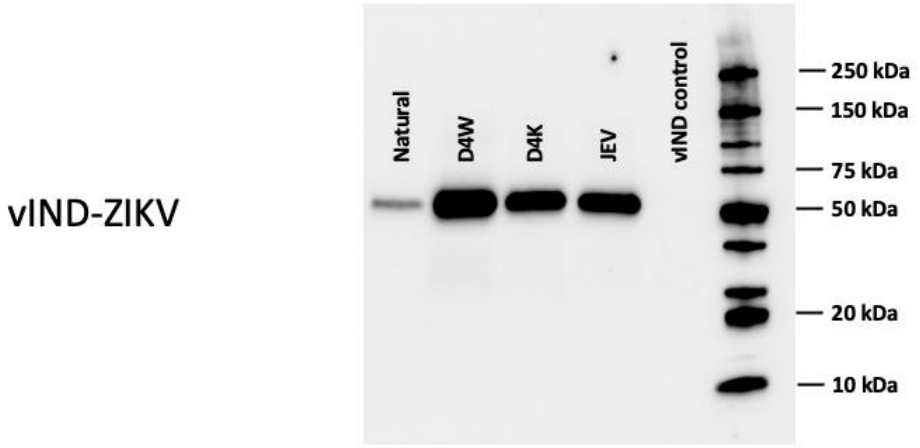
Supplementary Table S1. Cloning and sequencing primers for POWV constructs

Purpose	Sequence (5′ → 3′)
vIND-POWV (prototype lineage) PCR amplification, forward	TCTCAATGGCTAAGGCGTC
vIND-POWV (prototype lineage) PCR amplification, reverse	AGATCCAGCTTGCCTTCCAC
vIND-POWV (prototype lineage) sequencing	ACGTCAACGGGTTCTTCCTC
vIND-POWV (DTV lineage) PCR amplification, forward	TCTCAATGGCTAAGGCGTC
vIND-POWV (DTV lineage) PCR amplification, reverse	CGGGATGACCACAGACCTTC
vIND-POWV (DTV lineage) sequencing	ACACCATTCTCCCATGTCCG



Supplementary Figure S1. Detection of protein expression (vIND-HRTV, vIND-SFTSV, vIND-BRBV, and vIND-MAYV) in western blots. PEG precipitated VLP samples were harvested from Vero cells infected with each vIND construct. A vIND that does not encode any gene of interest was used to infect Vero cells to generate a negative control. The negative control was PEG precipitated in the same manner as the test vINDs. (A) Western blots without a negative control. (B) Western blots with a negative control included.

Supplementary Figure S2. Detection and comparison of protein expression (vIND-POWV) in western blots. PEG precipitated VLP samples were harvested from Vero cells infected with each vIND construct. A vIND that does not encode any gene of interest was used to infect Vero cells to generate a negative control. The negative control was PEG precipitated in the same manner as the test vINDs. Whole protein concentration of each POWV VLP sample was quantitated by Qubit 4 fluorometer (Invitrogen) to ensure equal loading amount. Two sets of Vero cells were infected, with one set in the presence of doxycycline inducer, thus allowing replication of the vINDs.



Supplementary Figure S3. Detection and comparison of protein expression (vIND-ZIKV) in western blots. Detailed information of ZIKV genes, VLP production, and western blot conditions can be found in our previous study [30]. PEG precipitated VLP samples were harvested from Vero cells infected with each vIND construct in the absence of doxycycline inducer. A vIND that does not encode ZIKV genes was used to infect Vero cells to generate a negative control. The negative control was PEG precipitated in the same manner as the test vINDs. TGX stain-free gel (Bio-Rad) was imaged before membrane transfer to ensure equal amount of protein was loaded to each well.