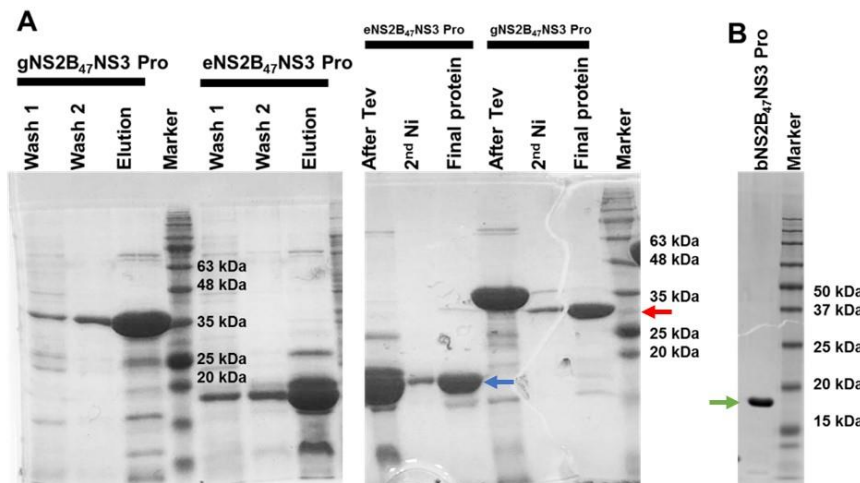
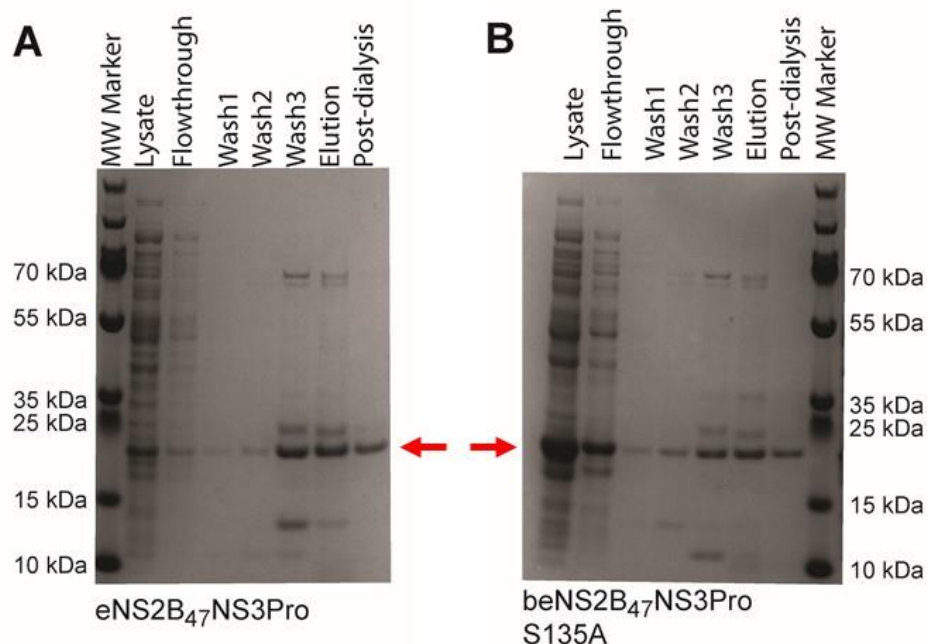
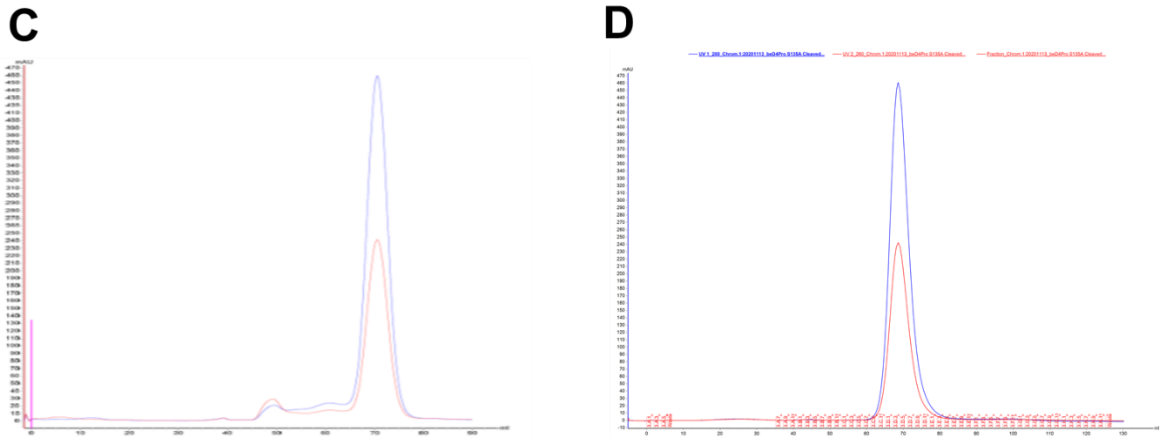


## Supplementary Figures

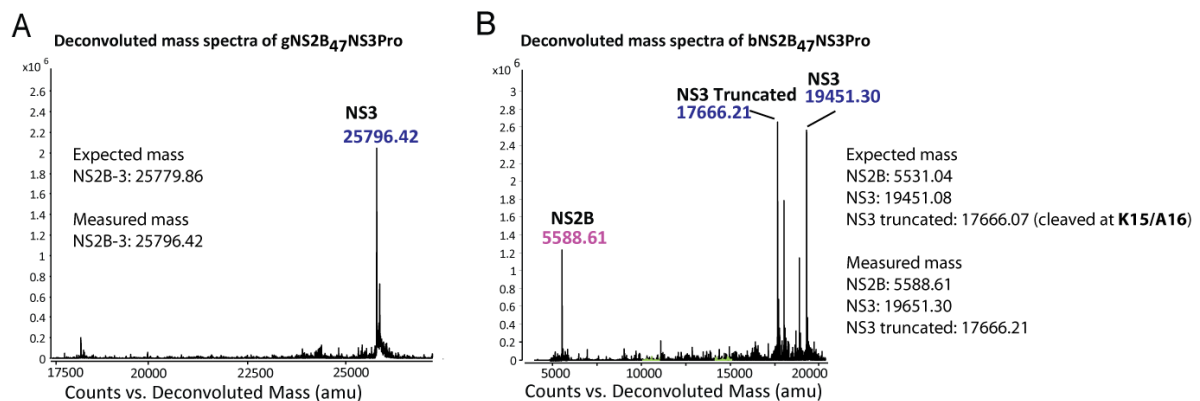


**Supplementary Figure S1.** SDS PAGE analysis of purified g-, e-, and b-NS2B<sub>47</sub>NS3Pro proteins. The g-NS2B<sub>47</sub>NS3Pro proteins, NS3Pro of e-NS2B<sub>47</sub>NS3Pro proteins and NS3 of b-NS2B<sub>47</sub>NS3Pro proteins have expected molecular weights of 25.7 kDa, 19.7 kDa and 19.4kDa respectively. The g-NS2B<sub>47</sub>NS3Pro proteins, NS3Pro of e-NS2B<sub>47</sub>NS3Pro proteins and NS3 of b-NS2B<sub>47</sub>NS3Pro are indicated by the red, blue and green arrows respectively.

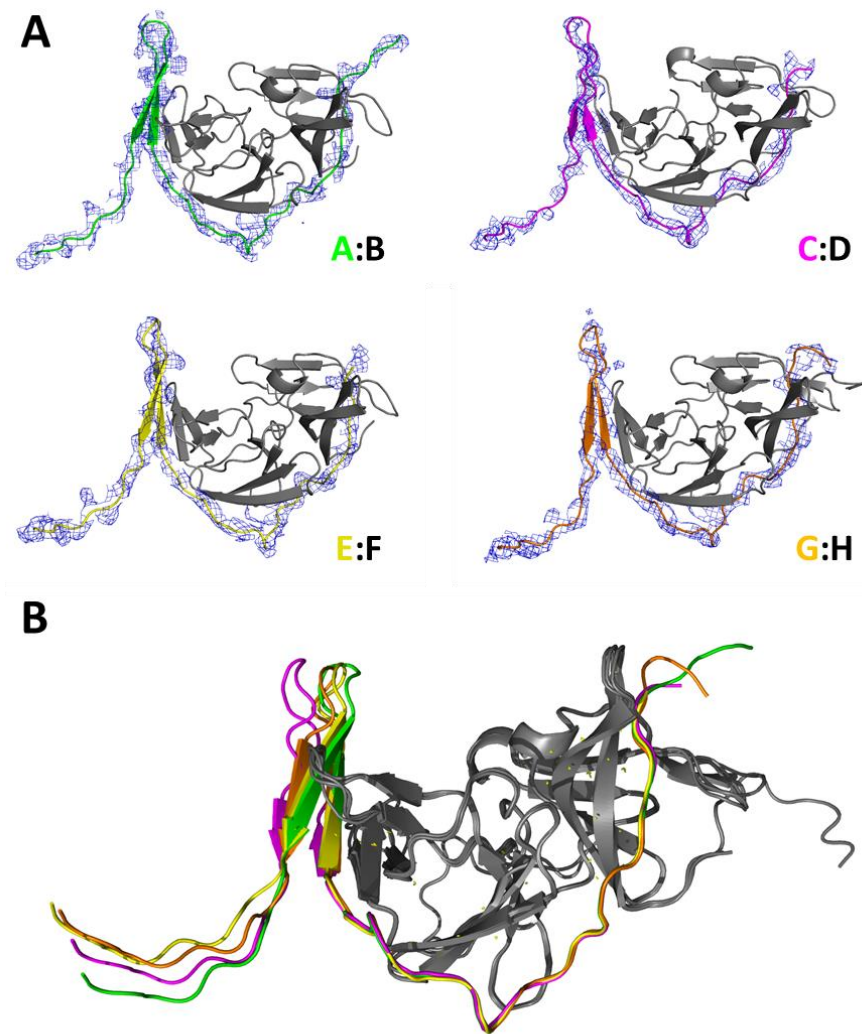




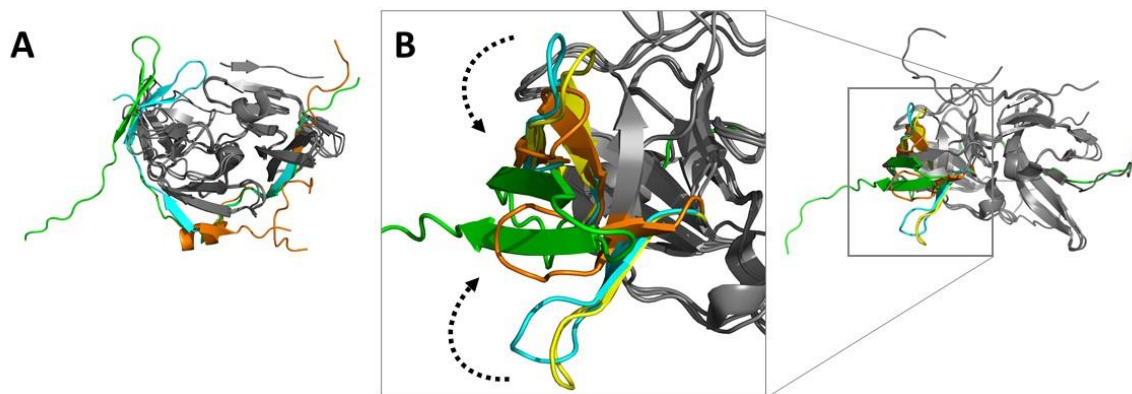
**Supplementary Figure S2.** SDS PAGE analysis of (A) eNS2B<sub>47</sub>NS3Pro and (B) beNS2B<sub>47</sub>NS3Pro S135A. The expected molecular weight of the NS3Pro is 19.7kDa. The bands of interest are indicated by the arrows. Gel filtration profiles of (C) eNS2B<sub>47</sub>NS3Pro and (D) beNS2B<sub>47</sub>NS3Pro S135A.



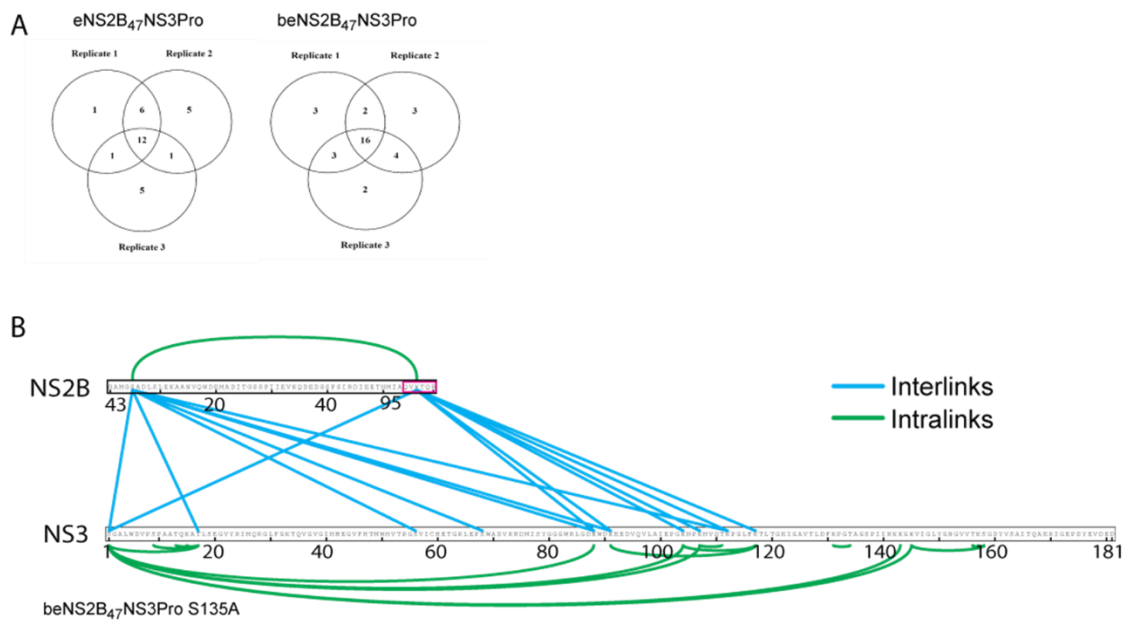
**Supplementary Figure S3.** Molecular weight determination of DENV4 recombinant NS2B-NS3 protease. (A) Deconvoluted mass spectra of gNS2B<sub>47</sub>NS3Pro. No internal cleavage of observed. (C) Deconvoluted mass spectra of bNS2B<sub>47</sub>NS3Pro. No truncated NS2B was observed. A portion of NS3 protease was truncated at N-terminus.



**Supplementary Figure S4.** (A) Electron density map of NS2B co-factor for the four eNS2B<sub>47</sub>NS3Pro molecules. The NS3 domains are in grey and NS2B in green, magenta, yellow and orange. The electron density map (2Fo-Fc) are coloured in blue and contoured at 1 $\sigma$ . (B) Superimposition of four molecules of eNS2B<sub>47</sub>NS3Pro.



**Supplementary Figure S5.** Comparison of eNS2B<sub>47</sub>NS3Pro with published structure of Flavivirus NS2B-NS3 protease. (A) Conformation of NS2B cofactor in various conformation. NS2B cofactor of eNS2B<sub>47</sub>NS3Pro in partially closed conformation in green, NS2B cofactor of DENV2 gNS2B<sub>47</sub>NS3 Pro (PDB:2FOM) in open conformation in orange and NS2B cofactor of DENV3 gNS2B<sub>47</sub>NS3 Pro (PDB:3U1I) in closed conformation in cyan. (B) Two loops (residues 111-126 and residues 151-162) in the NS3 act as hinges to mediate the conformation changes of NS2B-NS3 protease. The NS2B cofactor of eNS2B<sub>47</sub>NS3Pro are shown in green. While the loops are shown in yellow for eNS2B<sub>47</sub>NS3Pro, orange for DENV2 gNS2B<sub>47</sub>NS3 Pro (PDB:2FOM) in open conformation, cyan for DENV3 gNS2B<sub>47</sub>NS3 Pro (PDB:3U1I) in closed conformation.



**Supplementary Figure S6.** Mapping of dynamic interactions between NS2B and NS3 via crosslinking mass spectrometry. (A) Venn diagram showing overlap of cross-links identified between three replicates. (B) Residues from NS2B and NS3 that are mapped as intra and inter molecular crosslinks in beNS2B<sub>47</sub>NS3Pro S135A.