

**Figure S1. N-terminal amino acids 39 to 150 of the NP are critical for NP oligomerization.**

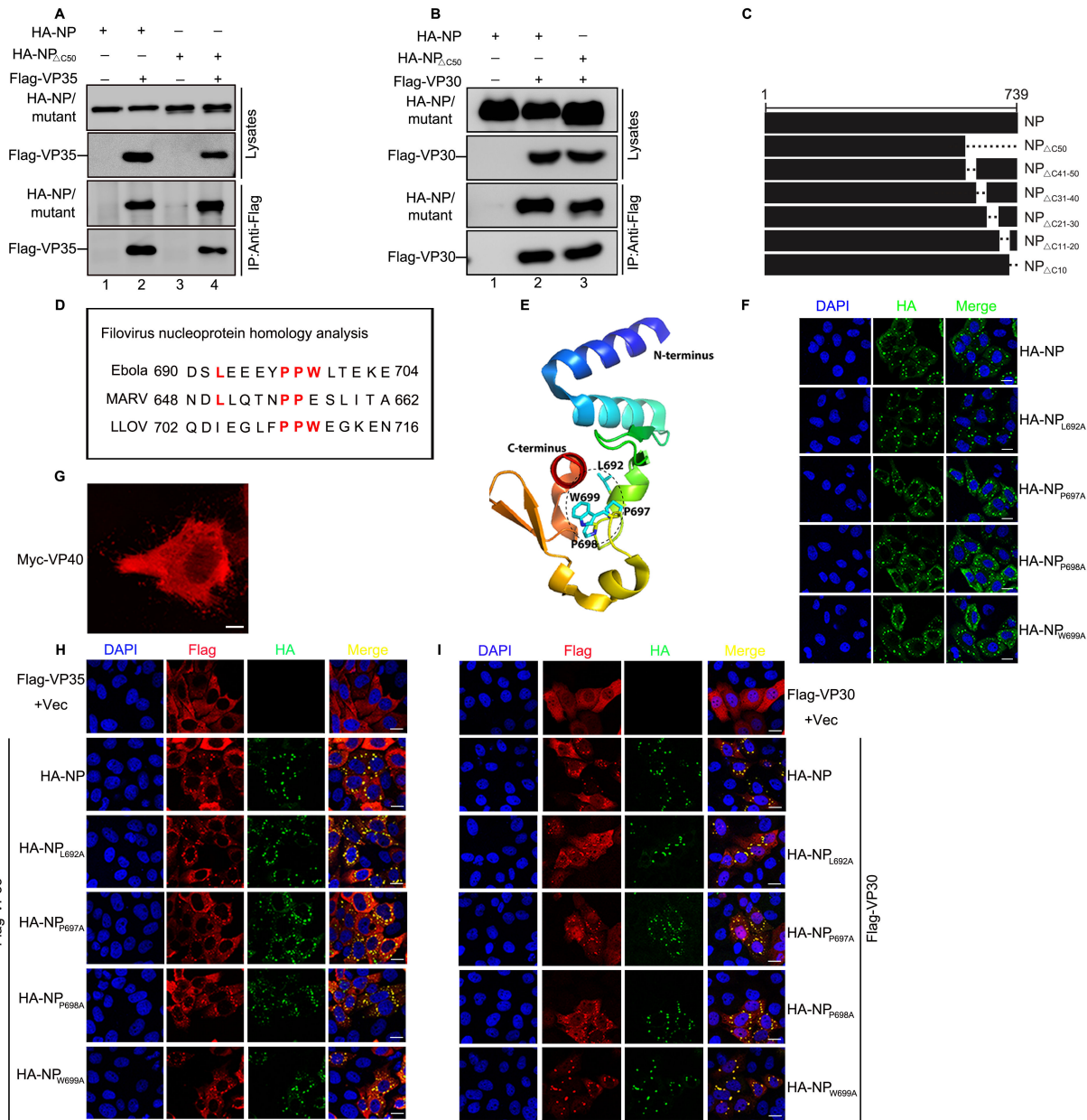
(A) coIP of NP with VP40. HEK293T cells were co-transfected with HA-NP and VP40-Flag. At 30 h p.t, IP was performed with anti-Flag antibodies.

(B) VLP budding assay of VP40 with NP. HEK293T cells were co-transfected with HA-NP and Myc-VP40 for 36 h. Cell lysates and VLPs were subjected to WB assay with anti-Myc, anti-HA antibodies.

(C) Protease protection assay for NP-VP40. HEK293T cells were transfected with HA-NP and Myc-VP40. The prepared VLP samples were divided into four aliquots, left untreated or treated with TritonX-100, trypsin, or Triton X-100 plus trypsin and then analyzed via WB.

(D) The subcellular localization of NP N-terminally truncated mutants. Hela cells were transfected with HA-NP/NP mutants for 26 h, and then their distribution was analyzed by Leica microscopy. Scale bars=10 $\mu$ m.

(E-F) HEK293T cells were co-transfected with Myc-NP and HA-NP/NP N-terminal mutants for 30 h. Cell lysates were subjected to IP with anti-Myc antibodies. The coIP of NP with NP $\Delta$ N25 or NP $\Delta$ N26-38 (E); the coIP of NP with NP $\Delta$ N39-90, NP $\Delta$ N91-120, or NP $\Delta$ N121-150 (F).



**Figure S2. The characteristics of NP $\Delta$ C50, NP<sub>L692A</sub>, NP<sub>P697A</sub>, NP<sub>P698A</sub> and NP<sub>W699A</sub>.**

(A) coIP of NP $\Delta$ C50 with VP35. HEK293T cells were co-transfected with HA-NP/ NP $\Delta$ C50 and Flag-VP35 for 30 h. Cell lysates were subjected to IP with anti-Flag antibodies.

(B) coIP of NP $\Delta$ C50 with VP30. HEK293T cells were co-transfected with HA-NP/ NP $\Delta$ C50 and Flag-VP30 for 30 h. Cell lysates were subjected to IP with anti-Flag antibodies.

(C) Schematic diagrams of wild type NP and its C-terminally truncated mutants.

(D) The homology analysis of NP C-terminus among the filoviruses.

(E) The structure analysis of NP C-terminus. The side chains of L692, P697, P698 and W699 are shown by a stick model, and the elliptical dotted line indicates the hydrophobic core formed by their side chains (PDB:4QB0).

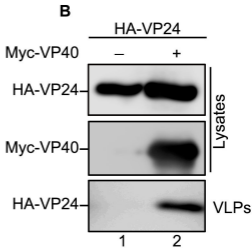
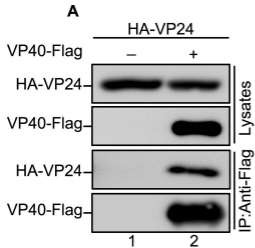
(F) HeLa cells were transfected with HA-NP/ NP C-terminal point mutations for 26 h, and analyzed for the distribution of NP/ NP C-terminal mutants by Leica microscopy. Scale bars=10 $\mu$ m.

(G) HeLa cells were transfected with Myc-VP40 for 26 h and analyzed for the distribution of VP40 by superresolution microscopy (Zeiss LSM 800). Scale bars= 5 $\mu$ m.

(H) HeLa cells were transfected with Flag-VP35, individually or co-transfected with HA-NP/ NP C-terminal point mutations for 26 h. The co-localization of VP35 with NP/ NP C-terminal mutants was analyzed on Leica microscopy. Scale bars=10 $\mu$ m.

(I) HeLa cells were transfected with Flag-VP30, individually or co-transfected with HA-NP/ NP C-terminal point mutations for 26 h. The co-localization of VP30 with NP/ NP

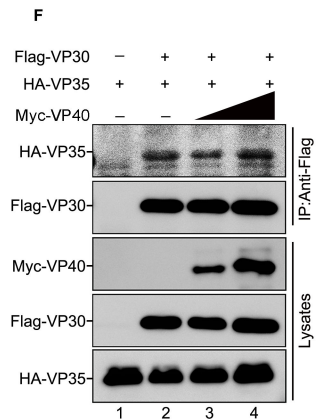
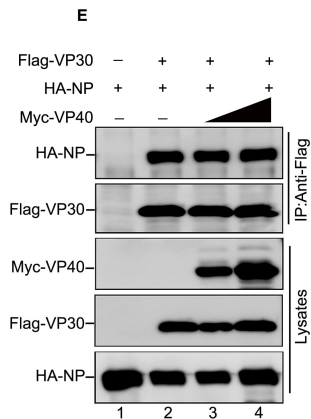
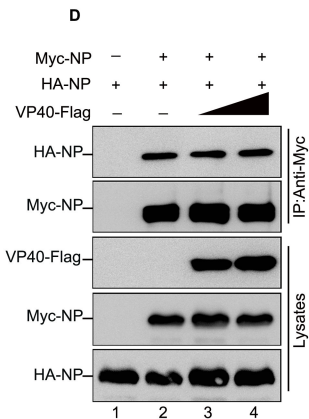
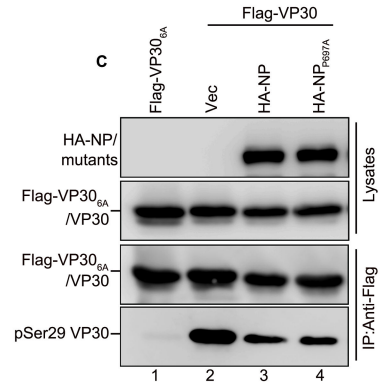
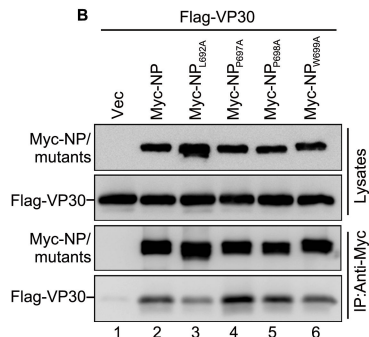
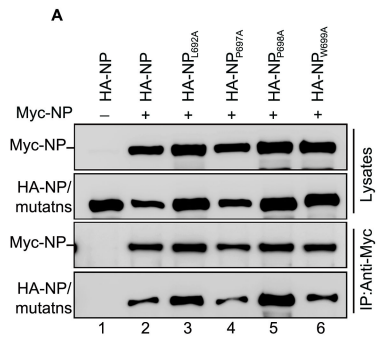
C-terminal mutants was analyzed on Leica microscopy. Scale bars=10 $\mu$ m.



**Figure S3. VP24 can interact with VP40 and be incorporated into VP40–VLP.**

(A) coIP of VP24 with VP40. HEK293T cells were co-transfected with HA-VP24 and VP40-Flag. At 30 h p.t, IP was performed with anti-Flag antibodies.

(B) VLP budding assay of VP40 with VP24. HEK293T cells were co-transfected with HA-VP24 and Myc-VP40 for 36 h. Cell lysates and VLPs were subjected to WB assay with anti-Myc, anti-HA antibodies.



**Figure S4. The functions of NP<sub>L692A</sub>, NP<sub>P697A</sub>, NP<sub>P698A</sub> and NP<sub>W699A</sub> and the effects of VP40 on nucleocapsid formation.**

(A) coIP assay of the NP with NP<sub>L692A</sub>, NP<sub>P697A</sub>, NP<sub>P698A</sub> or NP<sub>W699A</sub>. HEK293T cells were co-transfected with HA-NP/NP C-terminal mutants and Myc-NP for 30 h. Cell lysates were subjected to IP with anti-Myc antibodies and analyzed via WB using anti-HA, anti-Myc antibodies.

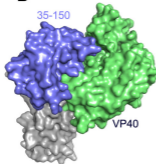
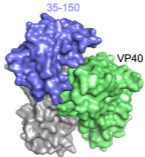
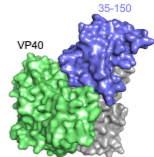
(B) coIP assay of the Flag-VP30 with Myc-NP/NP<sub>L692A</sub>, NP<sub>P697A</sub>, NP<sub>P698A</sub> or NP<sub>W699A</sub>. HEK293T cells were transfected with the indicated plasmids. Cell lysates were subjected to IP with anti-Myc antibodies and analyzed via WB using anti-Flag, anti-Myc antibodies.

(C) The effect of NP<sub>P697A</sub> on the levels of VP30 phosphorylation. HEK293T cells were co-transfected with HA-NP/NP<sub>P697A</sub> and Flag-VP30 for 30 h. Flag-VP30<sub>6A</sub> (six serines at N-terminus were mutated to alanines) was transfected as a negative control. IP was performed with anti-Flag antibodies. The levels of VP30 phosphorylation were detected using VP30 pSer29 antibodies.

(D) The effect of VP40 on NP oligomerization. HEK293T cells were co-transfected with Myc-NP, HA-NP, and increasing amounts of VP40-Flag for 30 h. Cell lysates were subjected to IP with anti-Myc antibodies and analyzed via WB.

(E-F) The effect of VP40 on the interactions of NP-VP30 or VP30-VP35. (E) HEK293T cells were co-transfected with Flag-VP30, HA-NP, and increasing amounts of Myc-VP40. IP was performed with anti-Flag; (F) Flag-VP30, HA-VP35, and increasing amounts of Myc-VP40 were co-transfected into HEK293T cells. IP was performed with

anti-Flag.

**A****B****C****D**

**Figure S5. The structural analysis of the NP N-terminus and VP40 interacting regions.**

(A) The structural analysis was performed using the HDOCK server, based on the NP core structure (PDB: 4Z9P; residues from 35-350; as the receptor molecular), and the VP40 structure (PDB: 1ES6; residues from 31-326; as the ligand molecular). Residues 35-150 of NP that form one face of the N-lobe was specified as the binding site. Blue indicates the N-lobe of NP; gray indicates the C-lobe of NP; green indicates VP40.

(B-D) The top three predicted results were analyzed. (B) VP40 binds to N-lobe of NP, and this model with the third highest docking score, but exhibited the largest interacting area of the three; (C-D) The top two models have interacting region with the C-lobe.