

Supplementary Materials for

A Pseudovirus Nanoparticle-Based Trivalent Rotavirus Vaccine Candidate Elicits High and Cross P
Type Immune Response

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Figures. S1 to S3

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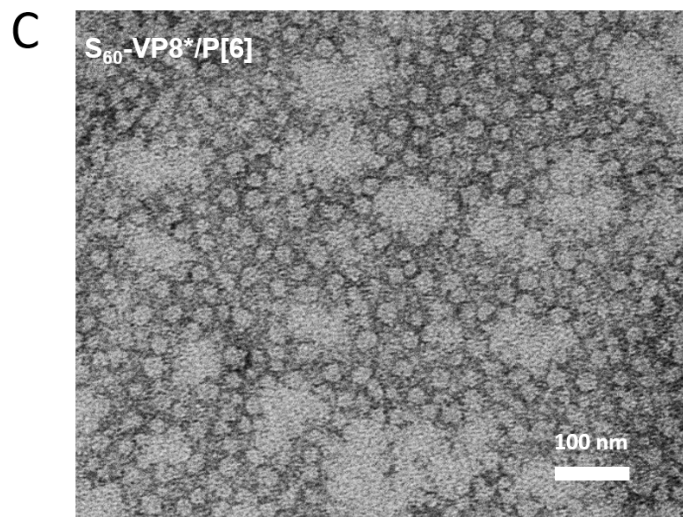
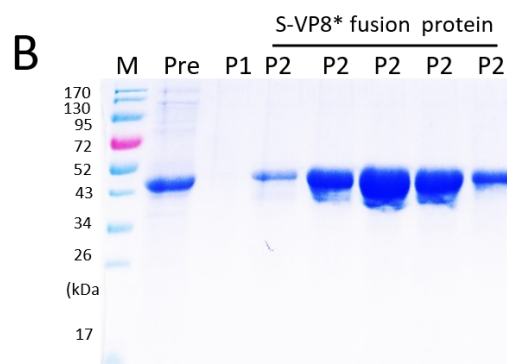
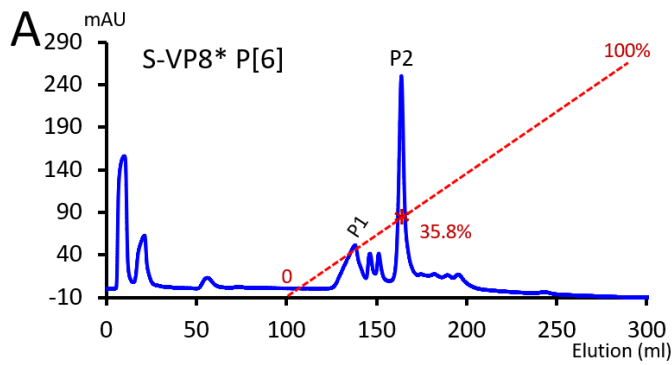


Figure S1. Purification of tag-free S-VP8* P[6] protein and its self- assembly into pseudovirus nanoparticles (PVNPs). (A) An anion exchange elution curve of the ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ precipitated S-VP8* P[6] protein. X-axis indicates elution volume (mL), whereas Y-axis shows UV (A_{280}) absorbances (mAU). The red dashed line indicates the linear increase of elution buffer B (0-100%) with a red star symbol indicating the percentage of buffer B at the elution peak of the S-VP8* P[6] protein (35.8%). Two major elution peaks after applying buffer B that were analyzed by SDS-PAGE are indicated as P1 (peak 1) and P2 (peak 2). (B) SDS-PAGE analysis of the pre-loaded protein (Pre) and samples of P1 and P2 from the ion exchange chromatography. Pre is the $(\text{NH}_4)_2\text{SO}_4$ precipitated protein sample before loading to the column. M is prestained protein standards with indicated molecular weights in kDa. The S-VP8* P[6] protein was eluted in P2. (C) A micrograph of negative staining transmission electron microscopy (TEM) of the protein from P2 shows spheric-shaped PVNPs.

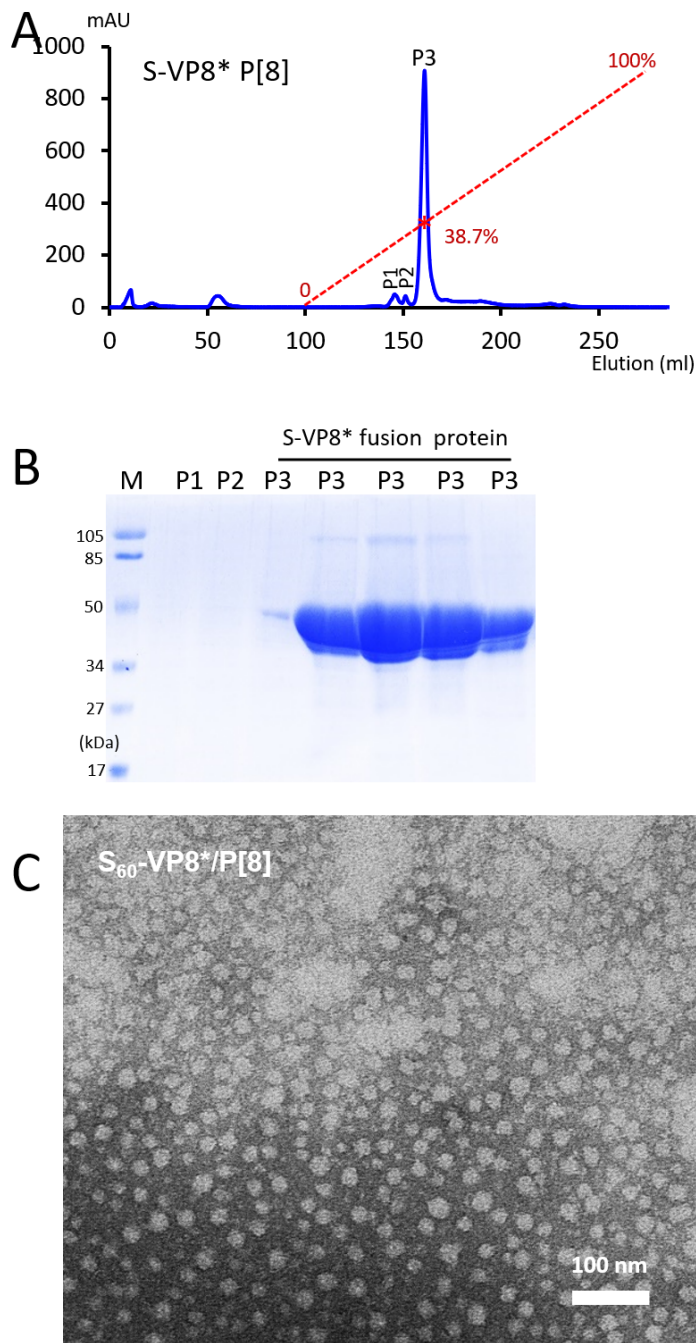


Figure S2. Purification of tag-free S-VP8* P[8] protein and its self-assembly into pseudovirus nanoparticles (PVNPs). (A) An anion exchange elution curve of the ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ precipitated S-VP8* P[8] protein. X-axis indicates elution volume (mL), whereas Y-axis shows UV (A_{280}) absorbances (mAU). The red dashed line indicates the linear increase of the elution buffer B (0-100%) with a red star symbol indicating the percentage of buffer B at the elution peak of the S-VP8* P[8] protein (38.7%). Three elution peaks (P1, P2, and P3) after applying buffer B that were analyzed by SDS-PAGE are indicated. (B) SDS-PAGE analysis of the P1, P2, and P3 elution peaks from the ion exchange chromatography. M is prestained protein standards with indicated molecular weights in kDa. The S-VP8* P[8] protein was eluted in P3. (C) A micrograph of negative staining transmission electron microscopy (TEM) of the protein from P3 peak shows spheric-shaped PVNPs.

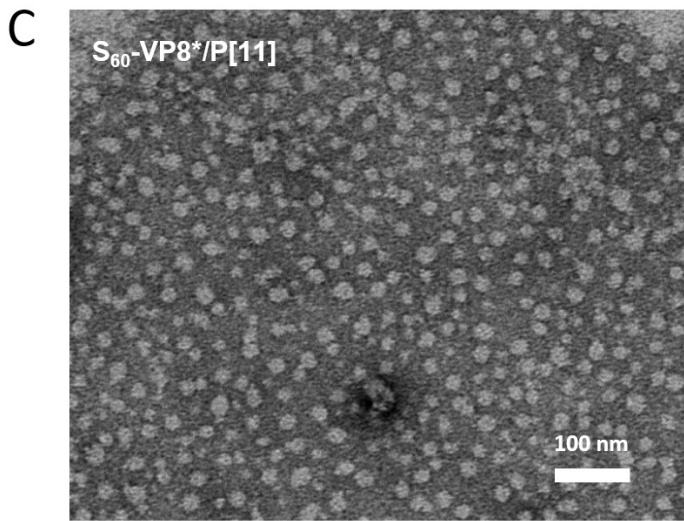
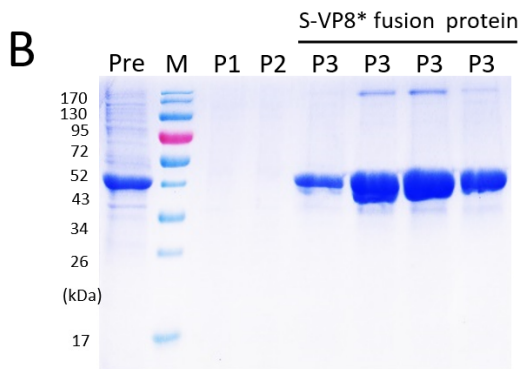
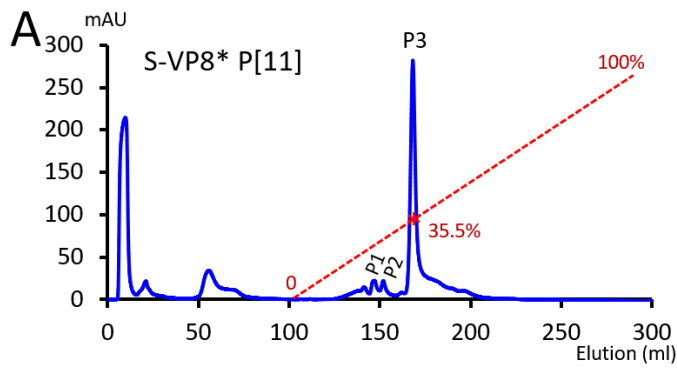


Figure S3. Purification of tag-free S-VP8* P[11] protein and its self-assembly into pseudovirus nanoparticles (PVNPs). (A) An anion exchange elution curve of the ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ precipitated S-VP8* P[11] protein. X-axis indicates elution volume (mL), whereas Y-axis shows UV (A_{280}) absorbances (mAU). The red dashed line indicates the linear increase of the elution buffer B (0-100%) with a red star symbol indicating the percentage of buffer B at the elution peak of the S-VP8* P[8] protein (35.5%). Three elution peaks (P1, P2, and P3) after applying buffer B that were analyzed by SDS-PAGE are indicated. (B) SDS-PAGE analysis of the pre-loaded protein (Pre) and the P1, P2, and P3 elution peaks from the ion exchange chromatography. Pre is the $(\text{NH}_4)_2\text{SO}_4$ precipitated protein sample before loading to the column. M is prestained protein standards with indicated molecular weights in kDa. The S-VP8* P[11] protein was eluted in P3. (C) A micrograph of negative staining transmission electron microscopy (TEM) of the protein from P3 peak shows spheric-shaped PVNPs.

Statistical analyses for Figure 4A:

Coat GST-VP8 P4 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.5294$

Trivalent vs. S_{60} -VP8 P6: $P=0.0079$

Trivalent vs. S_{60} -VP8 P8: $P=0.1689$

Coat GST-VP8 P6 protein:

Trivalent vs. S_{60} -VP8 P4: $P<0.0001$

Trivalent vs. S_{60} -VP8 P6: $P=0.5490$

Trivalent vs. S_{60} -VP8 P8: $P<0.0001$

Coat GST-VP8 P8 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.0055$

Trivalent vs. S_{60} -VP8 P6: $P=0.0004$

Trivalent vs. S_{60} -VP8 P8: $P=0.7560$

Coat GST-VP8 P4 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P<0.0001$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.5349$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P=0.4523$

Coat GST-VP8 P6 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.0003$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.0951$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P<0.0001$

Coat GST-VP8 P8 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.4543$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.0181$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P=0.0025$

Statistical analyses for 4B:

Coat GST-VP8 P4 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.4835$

Trivalent vs. S_{60} -VP8 P6: $P=0.0006$

Trivalent vs. S_{60} -VP8 P8: $P=0.0238$

Coat GST-VP8 P6 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.0003$

Trivalent vs. S_{60} -VP8 P6: $P=0.9992$

Trivalent vs. S_{60} -VP8 P8: $P<0.0001$

Coat GST-VP8 P8 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.0006$

Trivalent vs. S_{60} -VP8 P6: $P=0.0001$

Trivalent vs. S_{60} -VP8 P8: $P=0.9992$

Coat GST-VP8 P4 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.0085$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.1095$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P=0.3687$

Coat GST-VP8 P6 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.0003$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.1390$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P<0.0001$

Coat GST-VP8 P8 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.2656$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.0098$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P=0.0040$

Statistical analyses for Figure 5:

Coat GST-VP8 P4 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.1965$

Trivalent vs. S_{60} -VP8 P6: $P=0.0085$

Trivalent vs. S_{60} -VP8 P8: $P=0.0444$

Coat GST-VP8 P6 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.0042$

Trivalent vs. S_{60} -VP8 P6: $P=0.2491$

Trivalent vs. S_{60} -VP8 P8: $P=0.0054$

Coat GST-VP8 P8 protein:

Trivalent vs. S_{60} -VP8 P4: $P=0.0158$

Trivalent vs. S_{60} -VP8 P6: $P=0.0080$

Trivalent vs. S_{60} -VP8 P8: $P=0.2181$

Coat GST-VP8 P4 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.0008$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.0041$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P=0.3668$

Coat GST-VP8 P6 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.0151$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.7384$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P=0.0170$

Coat GST-VP8 P8 protein:

S_{60} -VP8 P4 vs. S_{60} -VP8 P6: $P=0.0391$

S_{60} -VP8 P4 vs. S_{60} -VP8 P8: $P=0.0340$

S_{60} -VP8 P6 vs. S_{60} -VP8 P8: $P=0.0097$

Statistical analyses for Figure 6A:

Trivalent vs. S₆₀-VP8 P4: $P=0.0234$

Trivalent vs. S₆₀-VP8 P6: $P=0.0028$

Trivalent vs. S₆₀-VP8 P8: $P=0.6510$

S₆₀-VP8 P4 vs. S₆₀-VP8 P6: $P=0.1473$

S₆₀-VP8 P4 vs. S₆₀-VP8 P8: $P=0.0304$

S₆₀-VP8 P6 vs. S₆₀-VP8 P8: $P=0.0019$

Statistical analyses for Figure 6B:

Trivalent vaccine:

G1P8 Wa vs. G2P4 DS1: $P=0.7560$

G1P8 Wa vs. G4P6 ST3: $P=0.3195$

G2P4 DS1 vs. G4P6 ST3: $P=0.5413$

S₆₀-VP8* P4 vaccine:

G1P8 Wa vs. G2P4 DS1: $P=0.0246$

G1P8 Wa vs. G4P6 ST3: $P=0.0008$

G2P4 DS1 vs. G4P6 ST3: $P=0.0043$

S₆₀-VP8* P8 vaccine:

G1P8 Wa vs. G2P4 DS1: $P=0.0205$

G1P8 Wa vs. G4P6 ST3: $P=0.0039$

G2P4 DS1 vs. G4P6 ST3: $P=0.0012$

S₆₀-VP8* P6 vaccine:

G1P8 Wa vs. G2P4 DS1: $P=0.4295$

G1P8 Wa vs. G4P6 ST3: $P=0.0009$

G2P4 DS1 vs. G4P6 ST3: $P=0.0009$