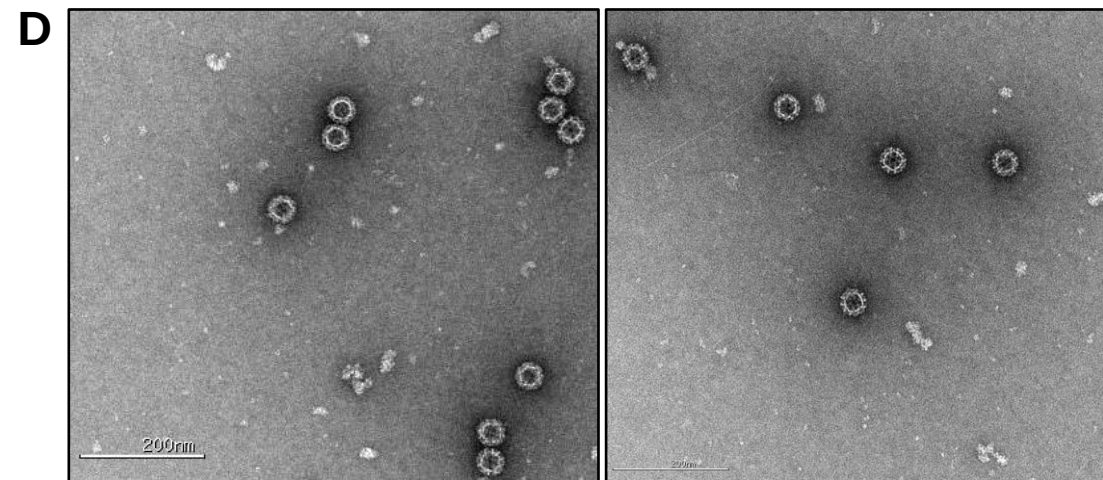
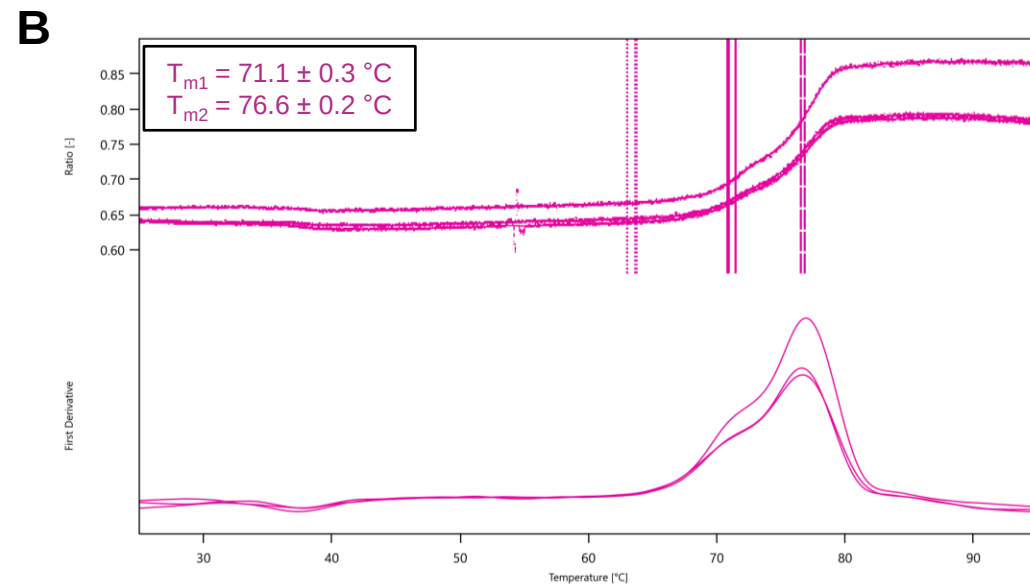
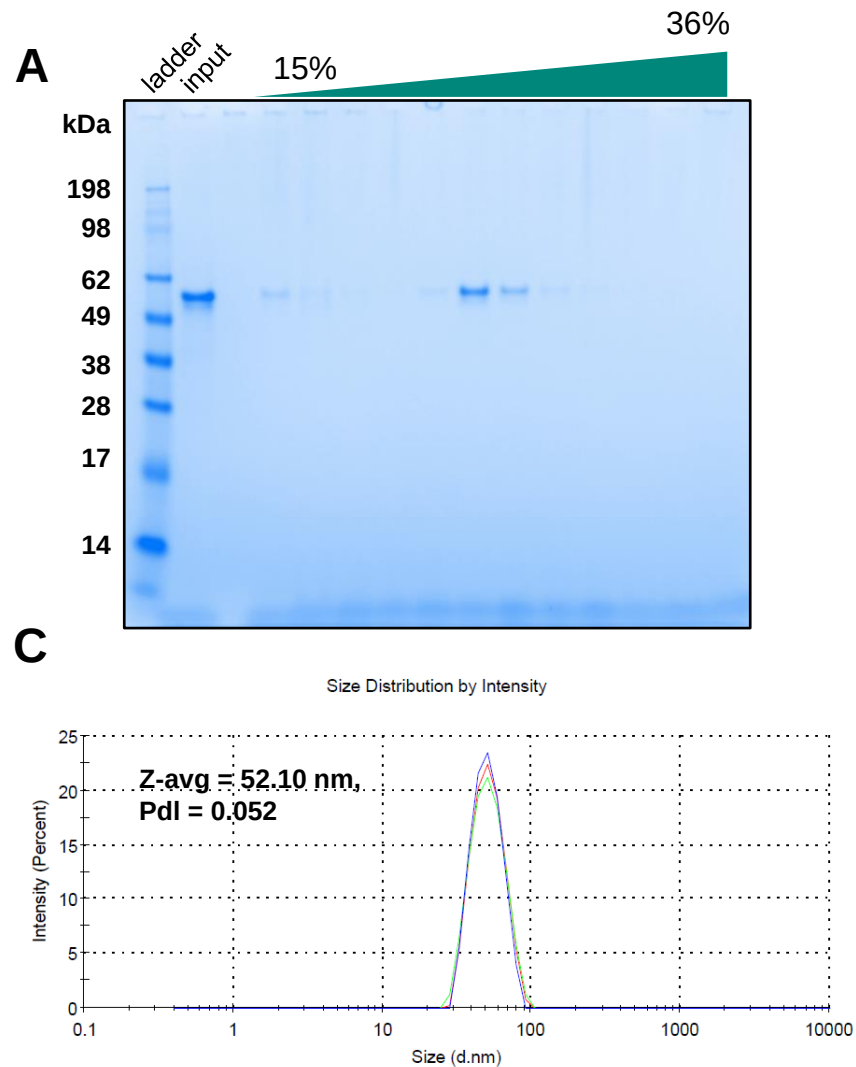
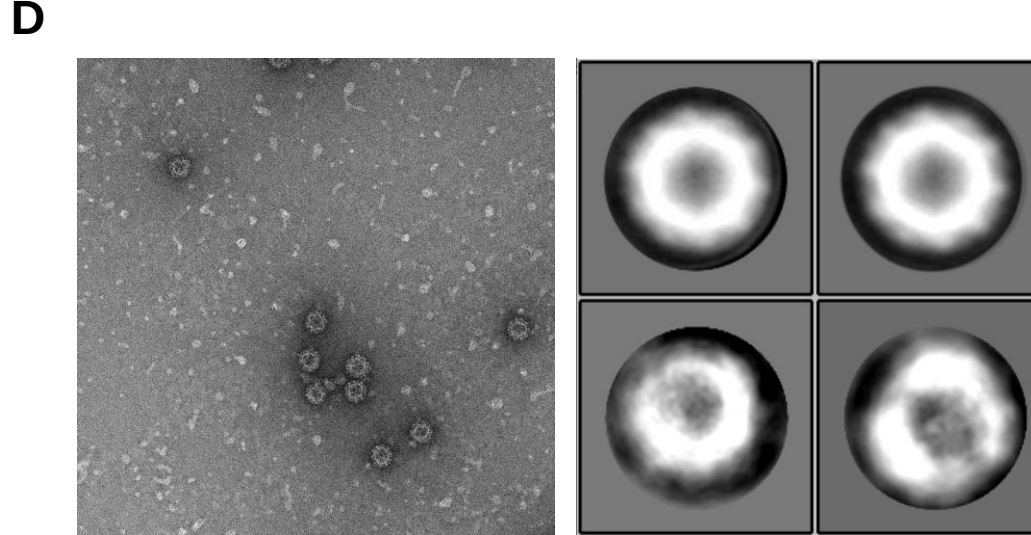
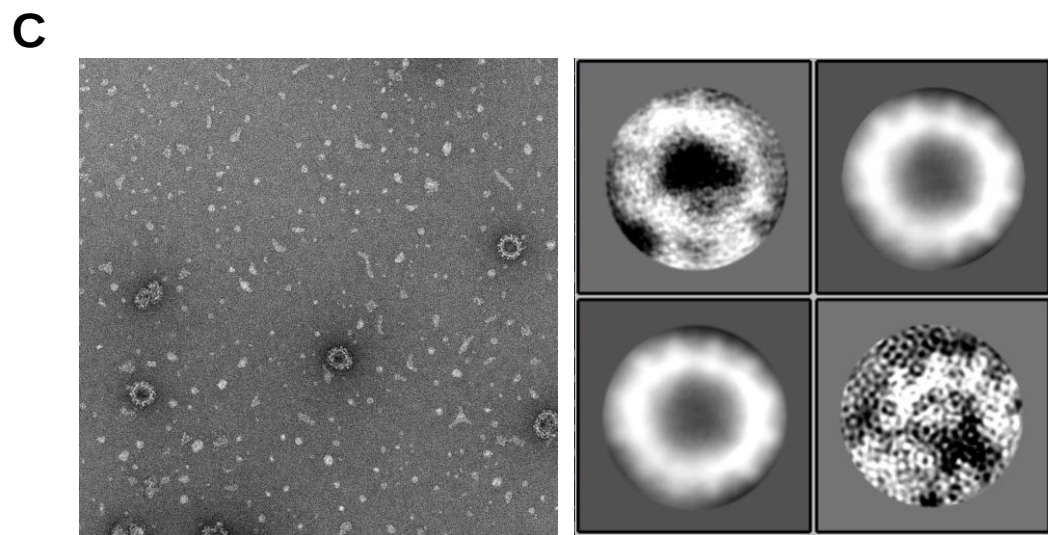
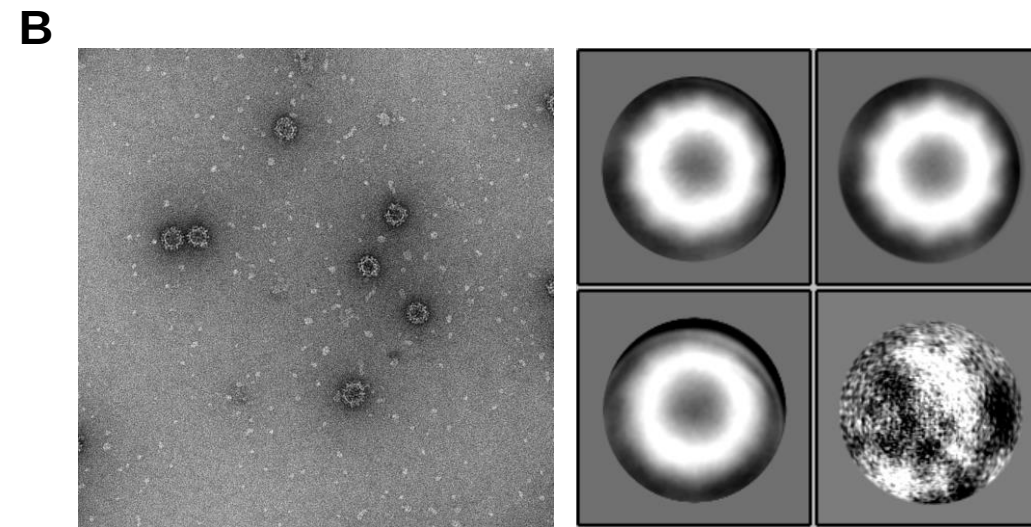
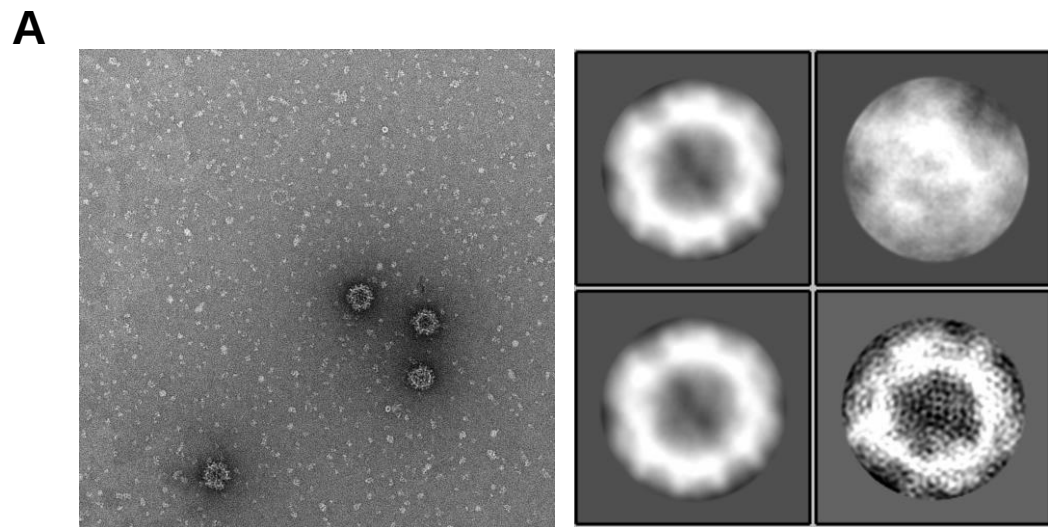




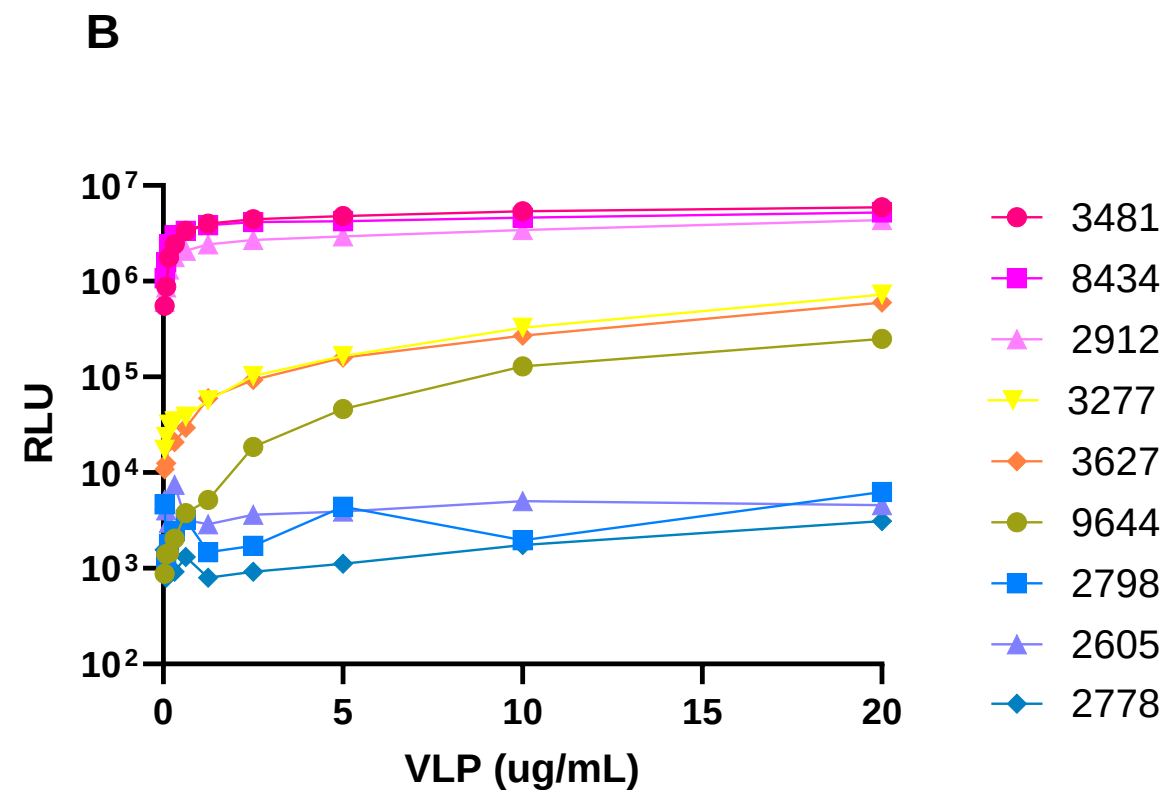
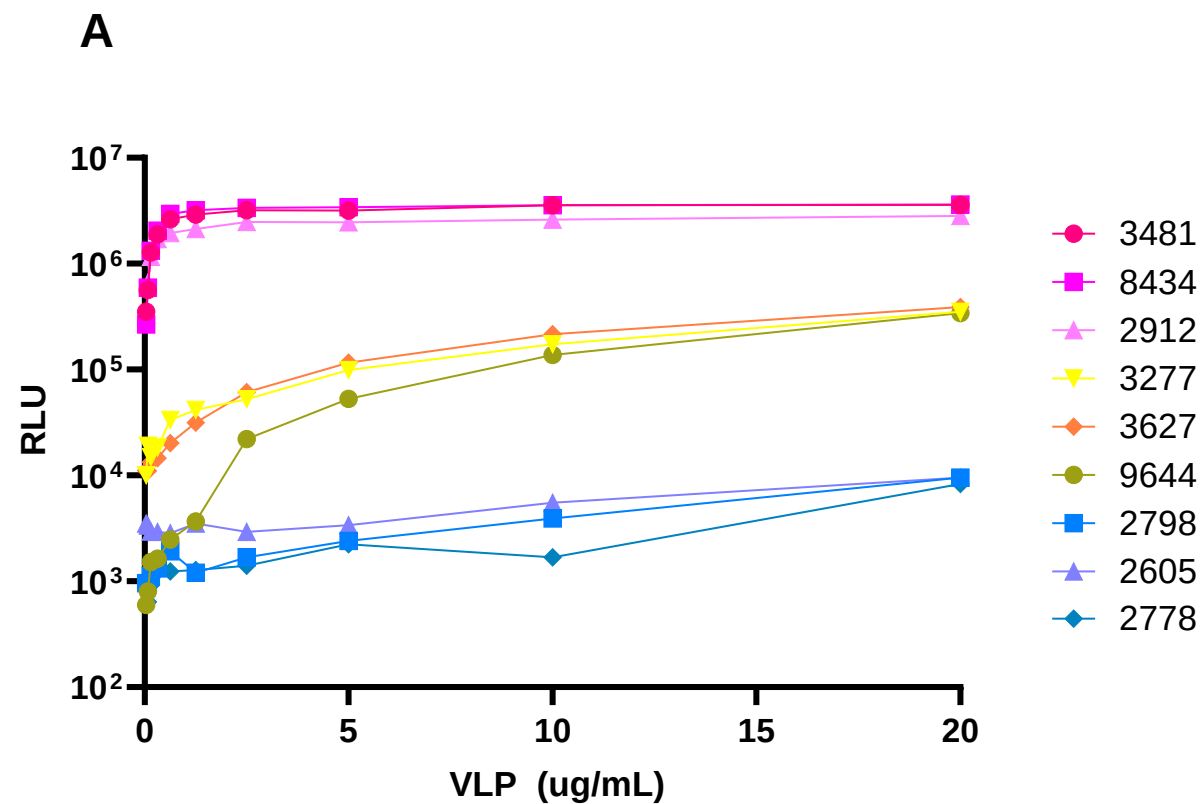
Supplemental Figure 1– GII.4 VLPs are stable without the DS1 mutation addition.

A Sucrose gradient fractionation of fully purified GII.4. VP1 protein observed in fractions 5-7 at ~ 25% sucrose, the percentage at which VLPs are known to fractionate. Gradient input denoted inp. **B** Differential scanning fluorimetry (DSF) was used to determine the thermal stability of GII.4 VLP. GII.4 VLP shows a biphasic transition with melting temperatures (T_m) of 71.1 °C and 76.6 °C. **C** Dynamic light scattering (DLS) of GII.4 VLP. Samples were run in triplicate. GII.4 VLP was determined to be homogeneous, compact, and monodisperse (polydispersity index of 0.052) with a hydrodynamic radius of 52.10 nm. **D** Representative negative-stain electron micrograph (nsEM) of GII.4 VLP. nsEM shows intact VLPs with few breakdown or partially assembled VP1 protomers.

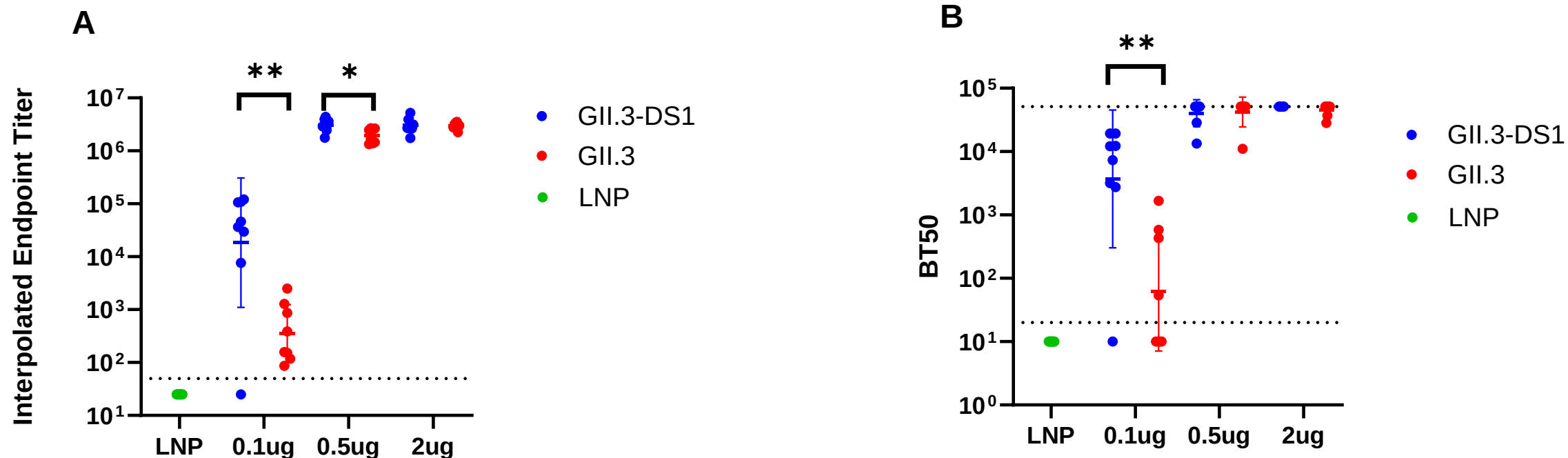


Supplemental Figure 3 - DS1 mutation leads to more uniform VLPs.

Representative negative-stain electron micrographs (nsEM) and 2D classes of GII.3 (**A**), GII.3-DS1 (**B**), GII.6 (**C**), and GII.6-DS1 (**D**) VLPs. 2D class averages of intact VLPs are in the panel set on the right. nsEM magnification is 58,000X. VLPs were stored at 4°C until processed for imaging. **A** GII.3 2D class averages derived from 66 particles picked from 22 micrographs. **B** GII.3-DS1 2D class averages derived from 295 particles picked from 23 micrographs. **C** GII.6 2D class averages derived from 141 particles picked from 27 micrographs. **D** GII.6-DS1 2D class averages derived from 197 particles picked from 26 micrographs.

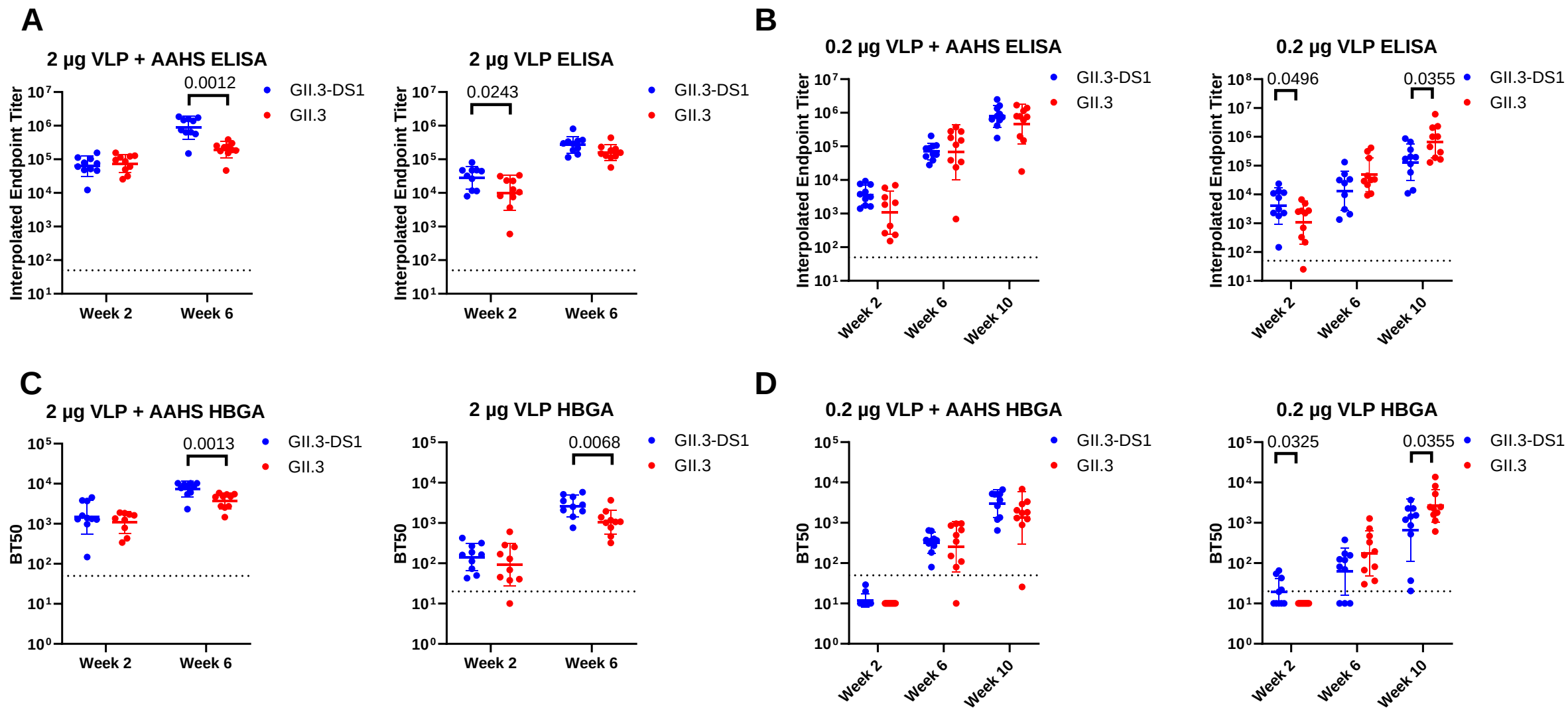


Supplemental Figure 4, GII.3 and GII.3-DS1 VLPs bind to human saliva samples. ELISA plates were coated with human saliva samples and incubated with either GII.3 (A) or GII.3-DS1 (B) VLPs in a two-fold serial dilution for 10 points starting from 20 $\mu\text{g/ml}$. Bound VLPs were detected using polyclonal sera against GII.3. The legend includes donor numbers for each individual saliva sample. Relative luminescent units (RLU) are plotted.

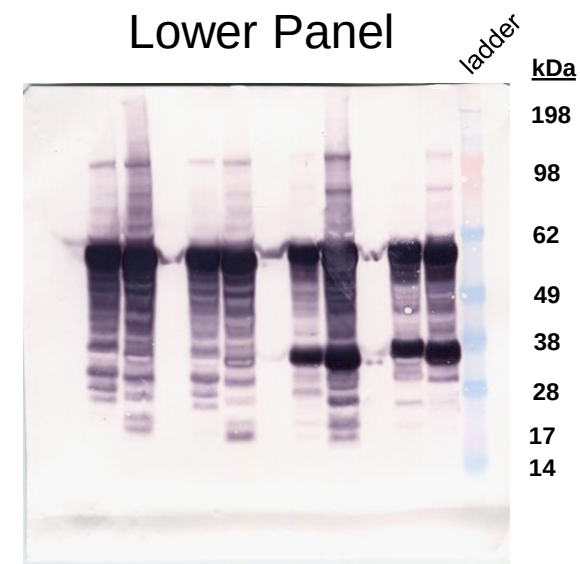
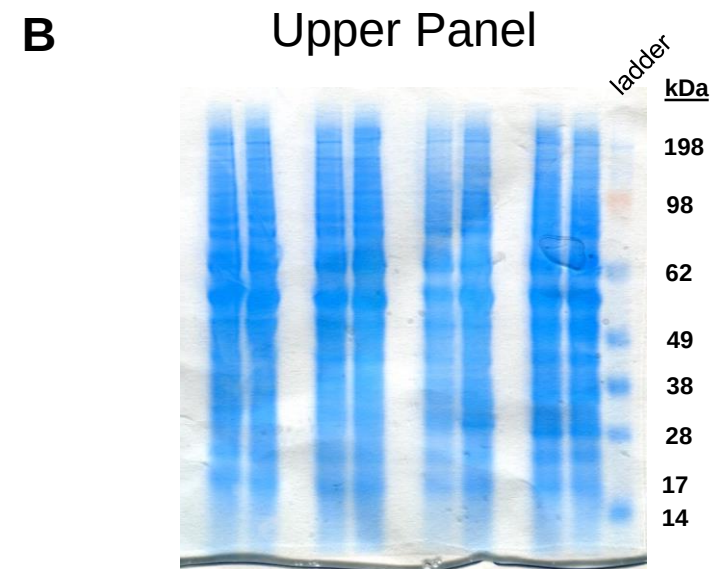
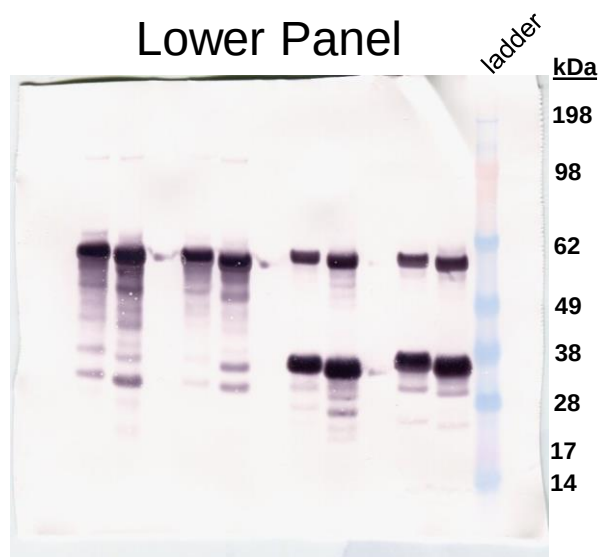
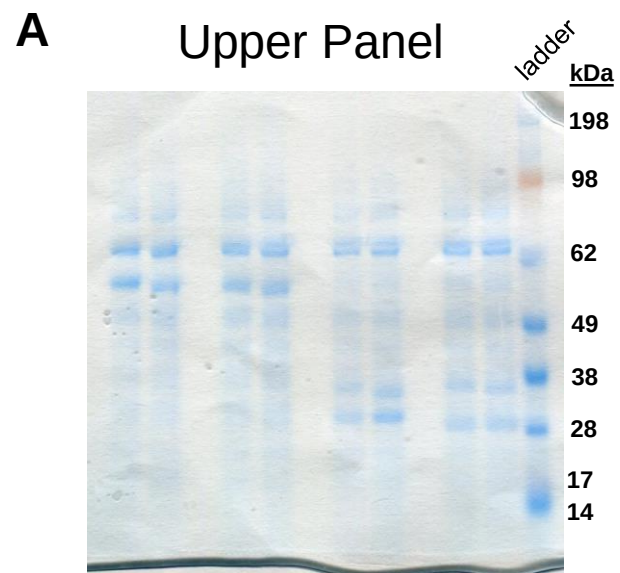


Supplemental Figure 5 - Mouse immunogenicity study of mRNAs encoding either GII.3 or GII.3-DS1, using assay reagent GII.3 VLP.

Week 6 sera shown in Fig VI were assayed for the presence of GII.3 binding antibodies (A) or HBGA blockade antibodies (B). Limits of detection are indicated with horizontal dotted lines (titer = 50 in A and 20 in B). Empty lipid nanoparticle (LNP) was immunized as a negative control. Geometric mean titers with geometric standard deviation are shown in scatter dot plot. P values were determined using GraphPad Prism software ($p < 0.01 = **$, $p < 0.05 = *$).



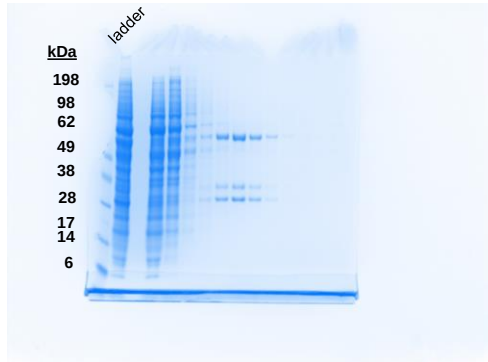
Supplemental Figure 6 - Mouse immunogenicity study of either GII.3 or GII.3-DS1 VLPs. BALB/c mice (N = 10 per group) were immunized with 2 μ g (A and C) or 0.2 μ g (B and D) of GII.3-DS1 (blue dots) or GII.3 VLPs (red dots) with and without AAHS at weeks 0 and 4. Blood was drawn at weeks 2, 6 and 10. Serum antibody ELISA titers (A and B) and HBGA blockade antibody titers (C and D) against GII.3-DS1 VLP were tested. Limits of detection are indicated with horizontal dotted lines (lower limit titer = 50 in A and B and 20 in C and D). Geometric mean titers with geometric standard deviation are shown in scatter dot plots. P values were determined using GraphPad Prism software and P values less than 0.05 are shown.



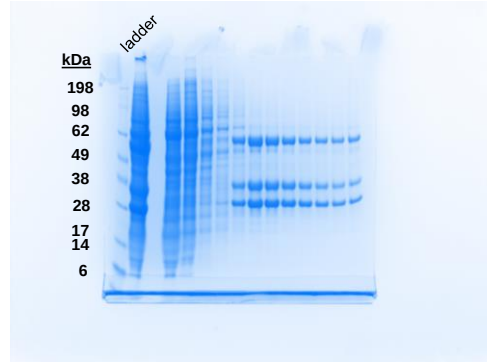
Supplemental Figure 7: Uncropped SDS-PAGE and western blot images related to main figure 2

A

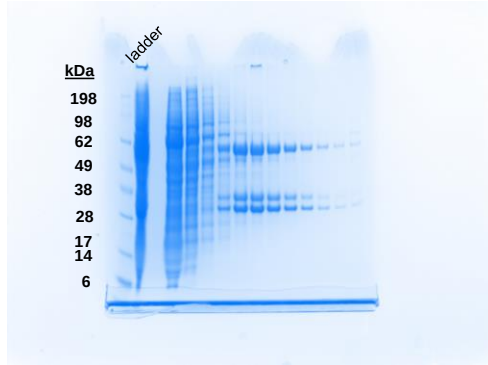
First
(GII.3)



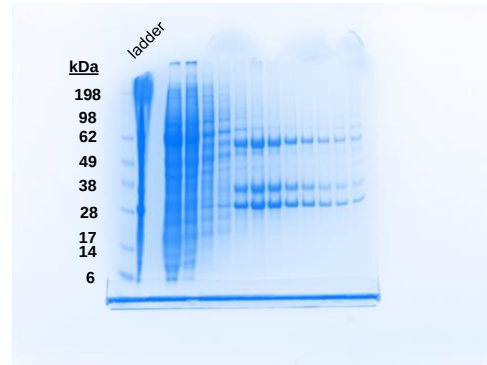
Third
(GII.6)



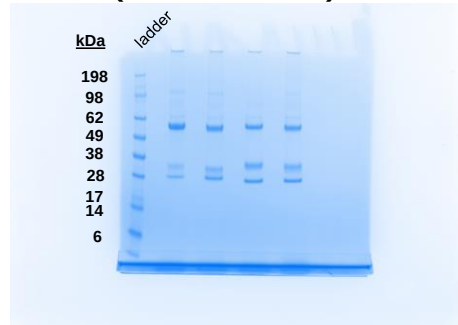
Second
(GII.3-DS1)



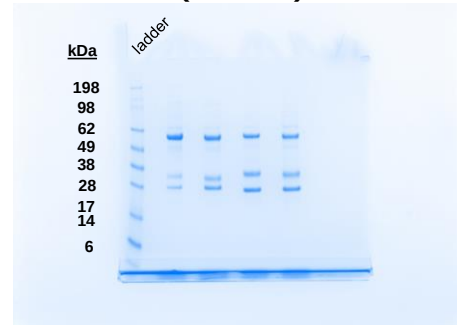
Fourth
(GII.6-DS1)



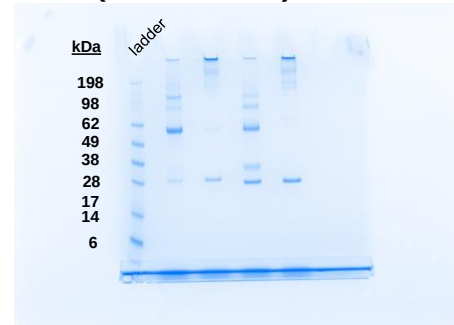
First
(untreated)



Second
(BME)

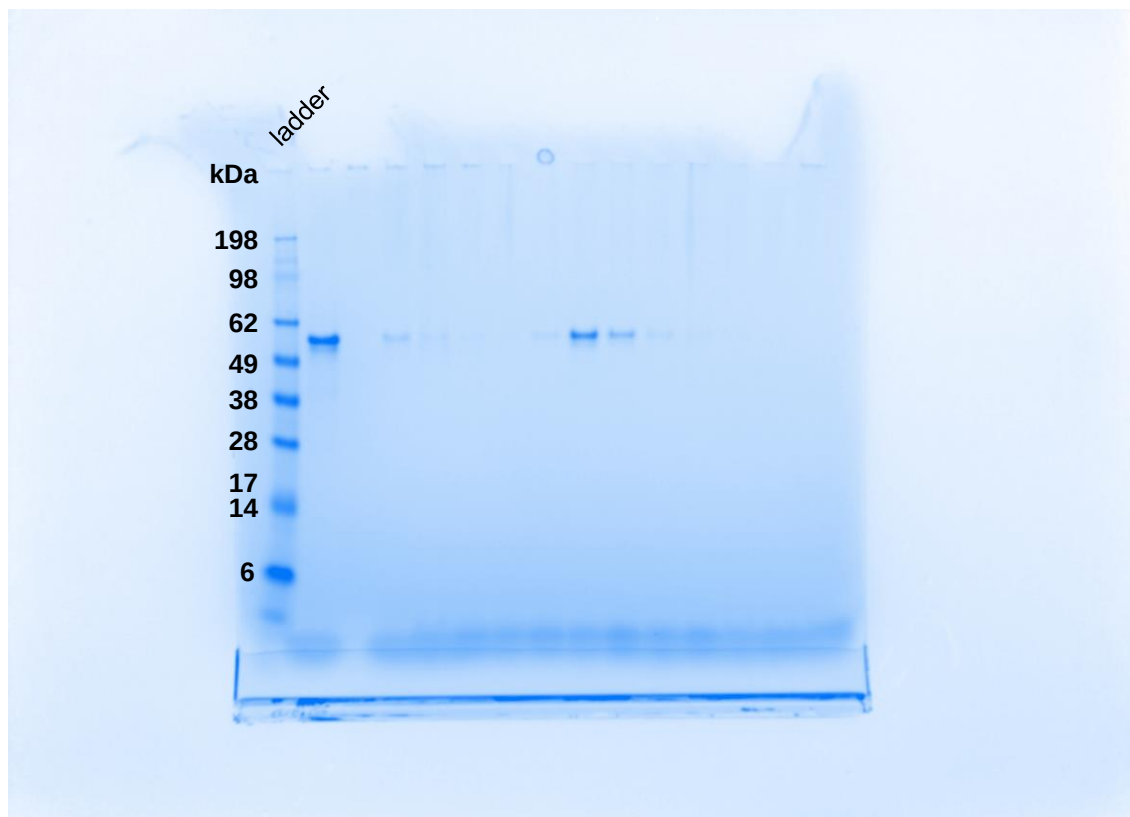


Third
(Diamide)

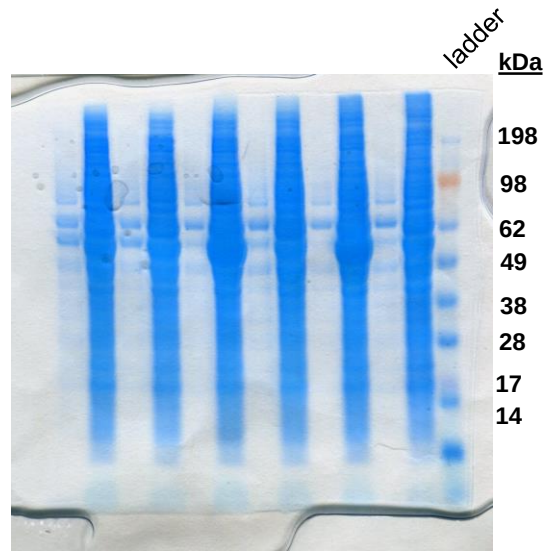


C

A

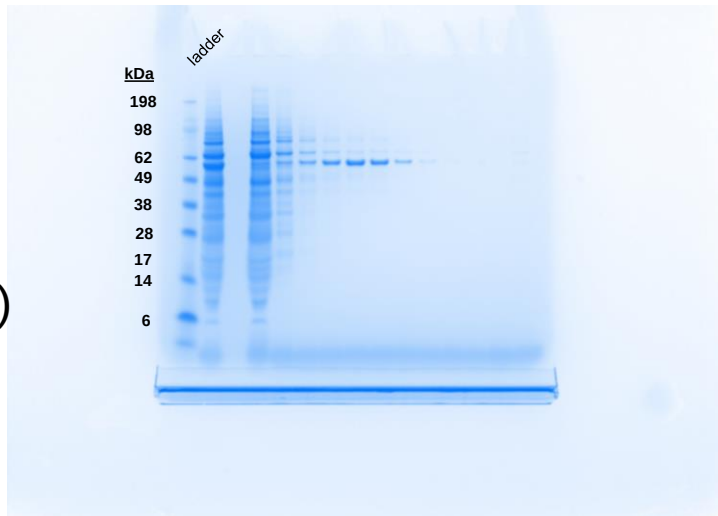


D

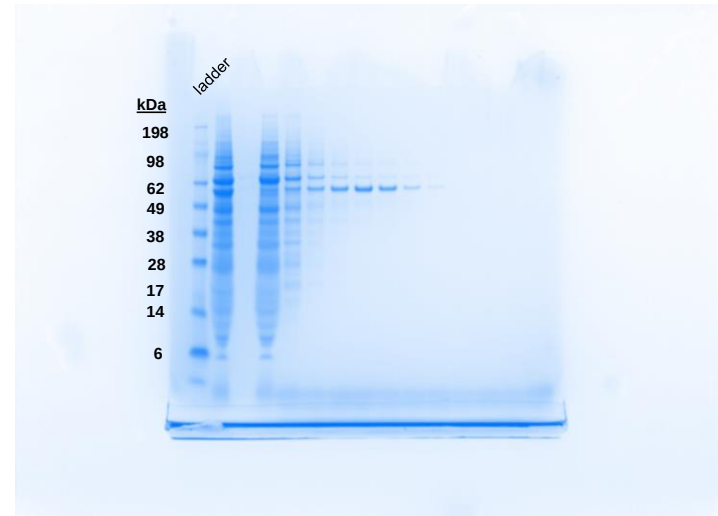


E

First (GII.3 D302A)



Second (GII.3 D302S)



Supplemental Table 1 – P Value Table

P values for the mouse immunogenicity studies in Figure 6 and Supplemental Figure 5.

Figure 6				
Panel	Assay	Week	Data Set	P Value
a	ELISA	2	0.1 ug	0.0079
a	ELISA	2	0.5 ug	0.0002
a	ELISA	2	2 ug	0.4089
a	ELISA	6	0.1 ug	0.0006
a	ELISA	6	0.5 ug	0.0541
a	ELISA	6	2 ug	0.2316
b	HBGA	2	0.1 ug	N/A
b	HBGA	2	0.5 ug	0.0002
b	HBGA	2	2 ug	0.0289
b	HBGA	6	0.1 ug	0.0020
b	HBGA	6	0.5 ug	0.4845
b	HBGA	6	2 ug	0.6126
Supplemental Figure 5				
Panel	Assay	Week	Data Set	P Value
a	ELISA	6	0.1 ug	0.0104
a	ELISA	6	0.5 ug	0.0140
a	ELISA	6	2 ug	0.6078
b	HBGA	6	0.1 ug	0.0044
b	HBGA	6	0.5 ug	N/A
b	HBGA	6	2 ug	N/A