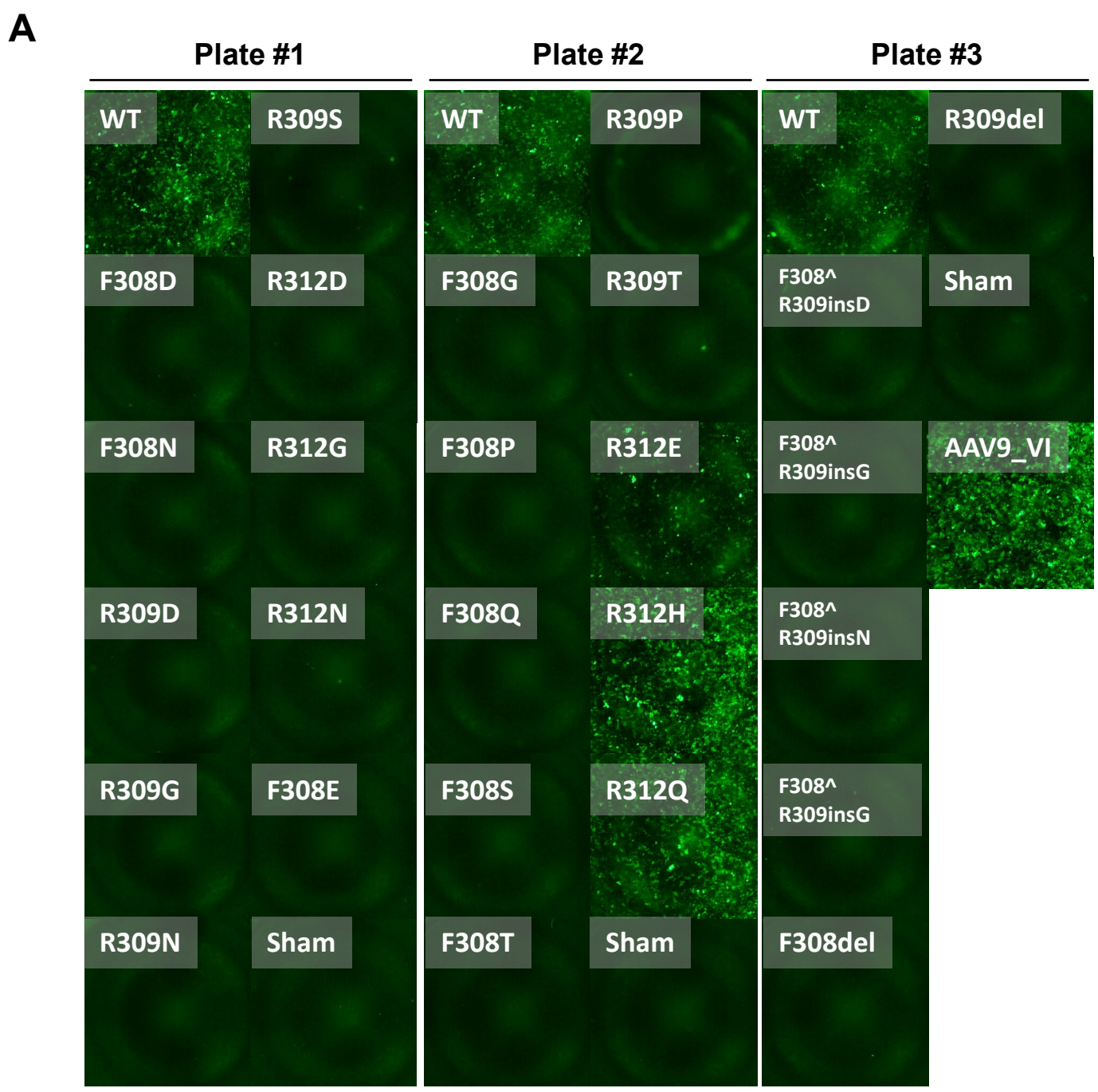
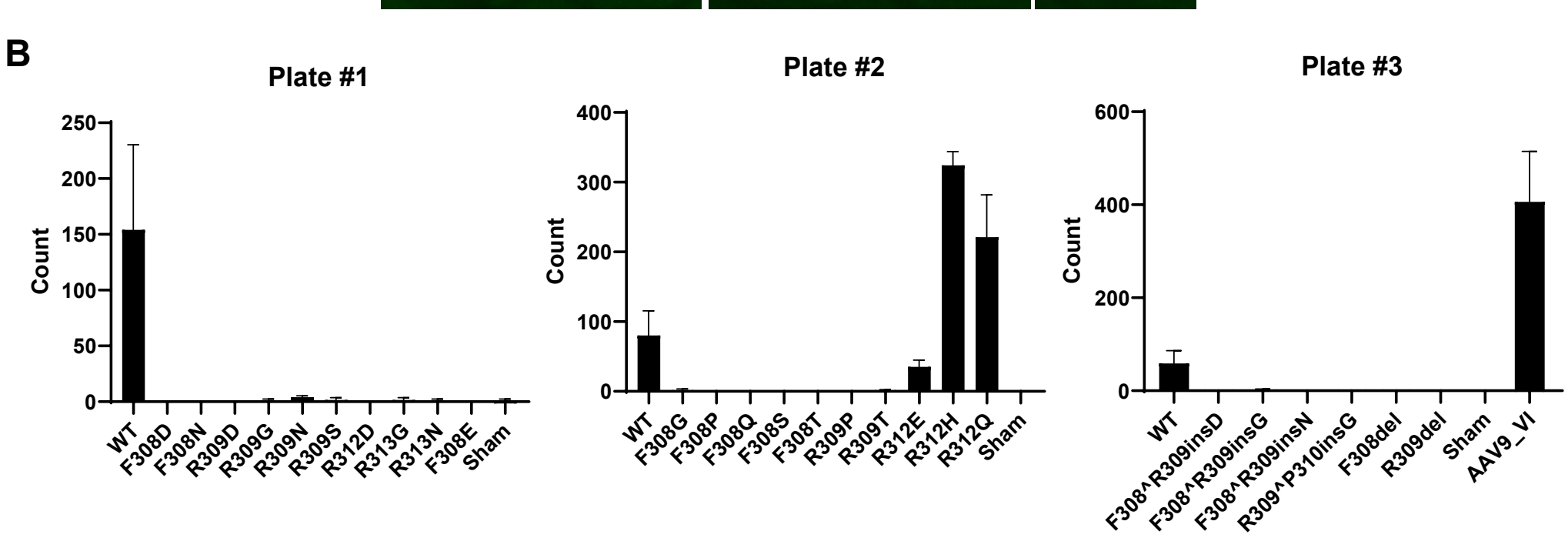
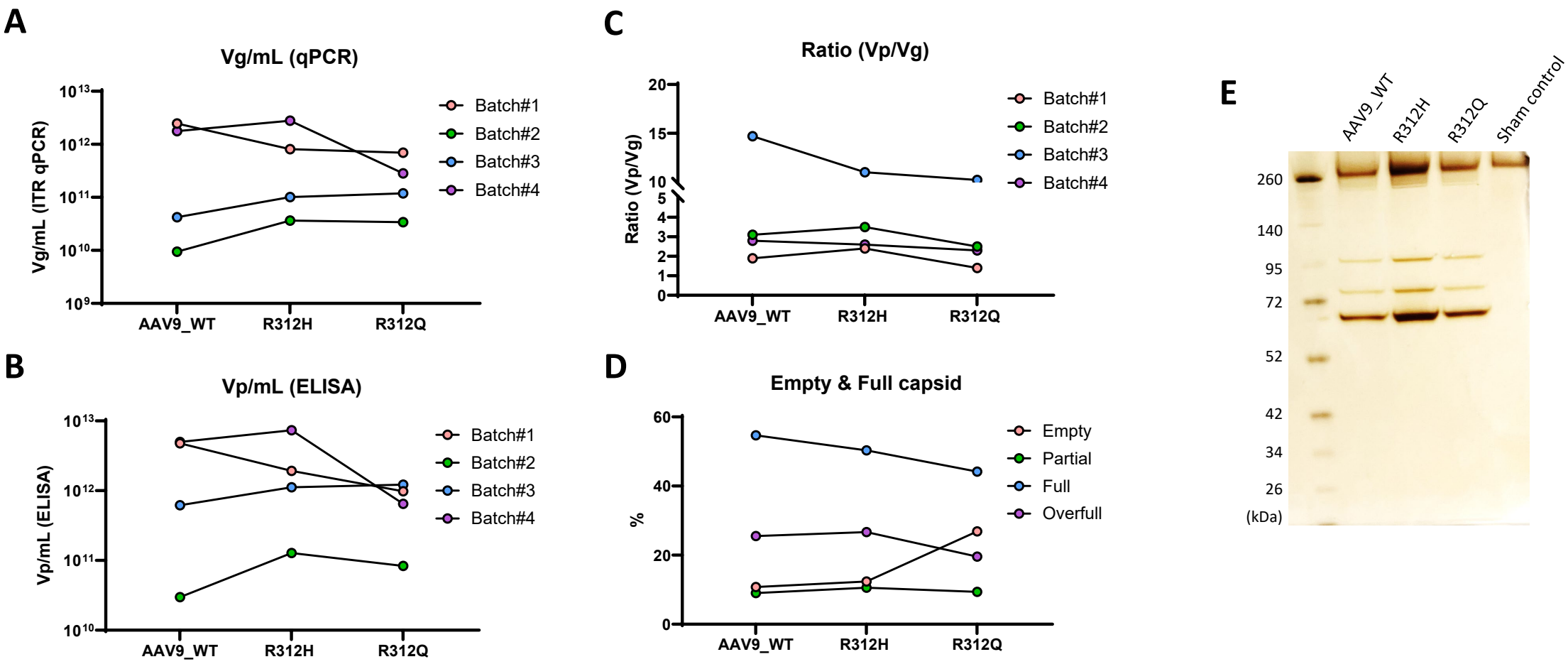
Supplementary figure 1





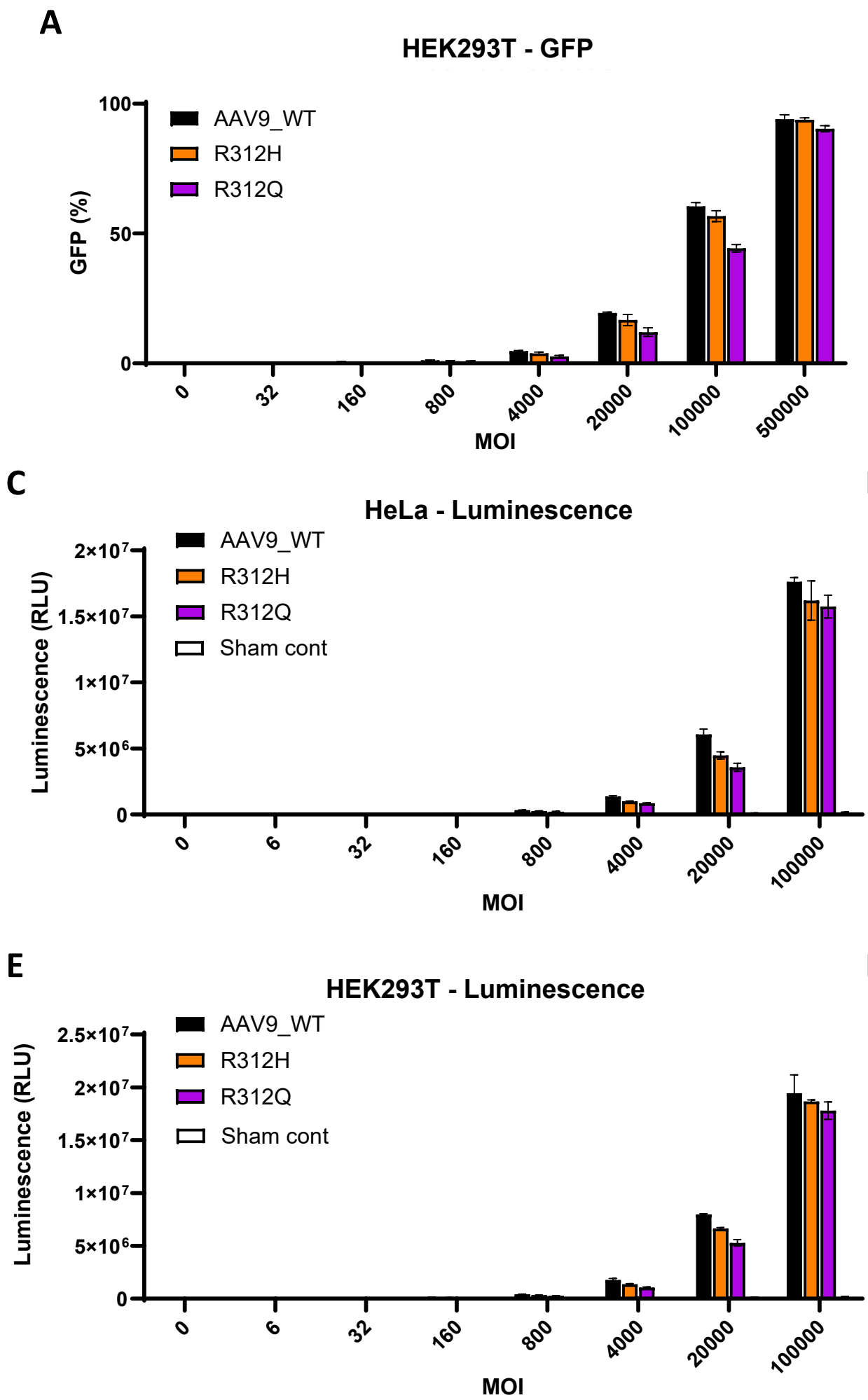
Supplementary Figure 1. Analysis of GFP-expressing HeLa cells using ELISpot reader. A total of 26 mutated AAV variants carrying the GFP transgene were isolated using the method described in Figure 4. Viral production cells (VPCs) were transfected in three 12-well plates, each including wild-type (WT) AAV9 and a sham control. The precipitated AAVs were directly applied to HeLa cells and incubated for 3 days. (A) GFP-expressing HeLa cells were imaged using an ELISpot reader. (B) GPF-expressing cells were then counted using ImmunoSpot software. Each sample was prepared in duplicate, with one replicate in each of two separate plates.

Supplementary figure 2



Supplementary Figure 2. Mutations in AAV9 capsid had no effect on the production yield or size of the vector. Four batches of WT and mutated AAVs were manufactured by triple transfection of HEK293 cells. (A-C) viral genome concentration (A), total viral particles (B), and ratio between viral genome and viral particle (C) were measured. (D) Empty, partial, full and overfull AAV in the representative batch were measured by charge-detection mass spectrometry (CDMS). (E) representative silver staining of WT and mutated AAVs.

Supplementary figure 3



Supplementary Figure 3. Comparison of the transduction efficiency of WT AAV9 and mutated AAVs with other cell line or other transgene (NanoLuc) (A, B) HEK293T cells were transduced with the AAV9, R312H (R26), and R312Q (R27) vectors at an indicated MOI. The percentage of GFP-positive cells was determined by flow cytometry. HeLa cells (C, D) and HEK293T cells (E, F) were transduced with the AAV9, R312H (R26), and R312Q (R27) vectors that express NanoLuc at an indicated MOI. Next day, bioluminescence intensities of the cells were determined by luminometer. (B, D, F) Four-parameter curve fit was calculated for each vector and area under the curve (AUC) were calculated. Each bar shows the mean ± SD. P values were determined by one way ANOVA with Tukey's multiple comparisons test. **p<0.01, ***p<0.001, ****p<0.0001