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Supplemental Information

GPR108 Is a Highly Conserved AAV Entry Factor

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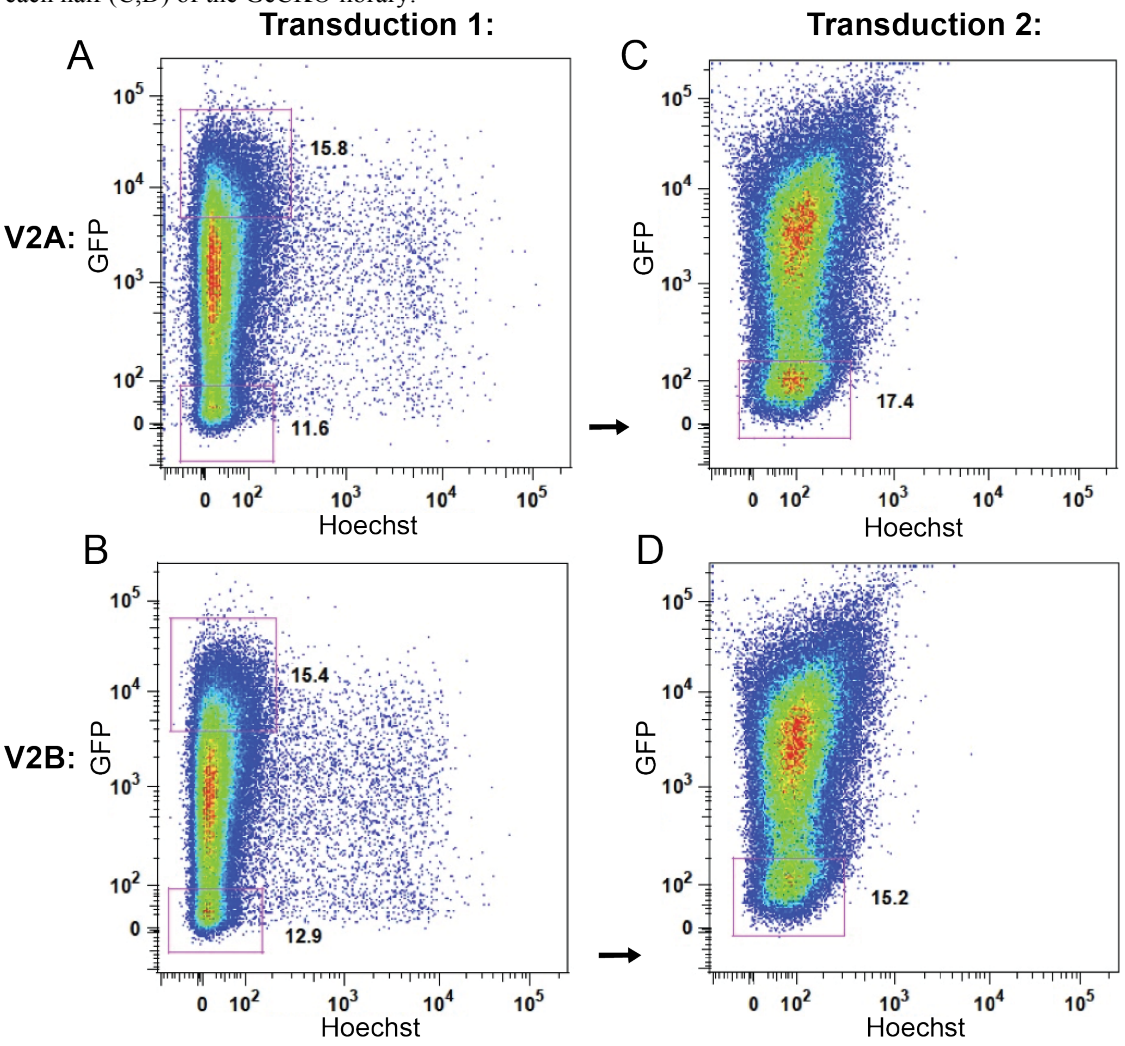
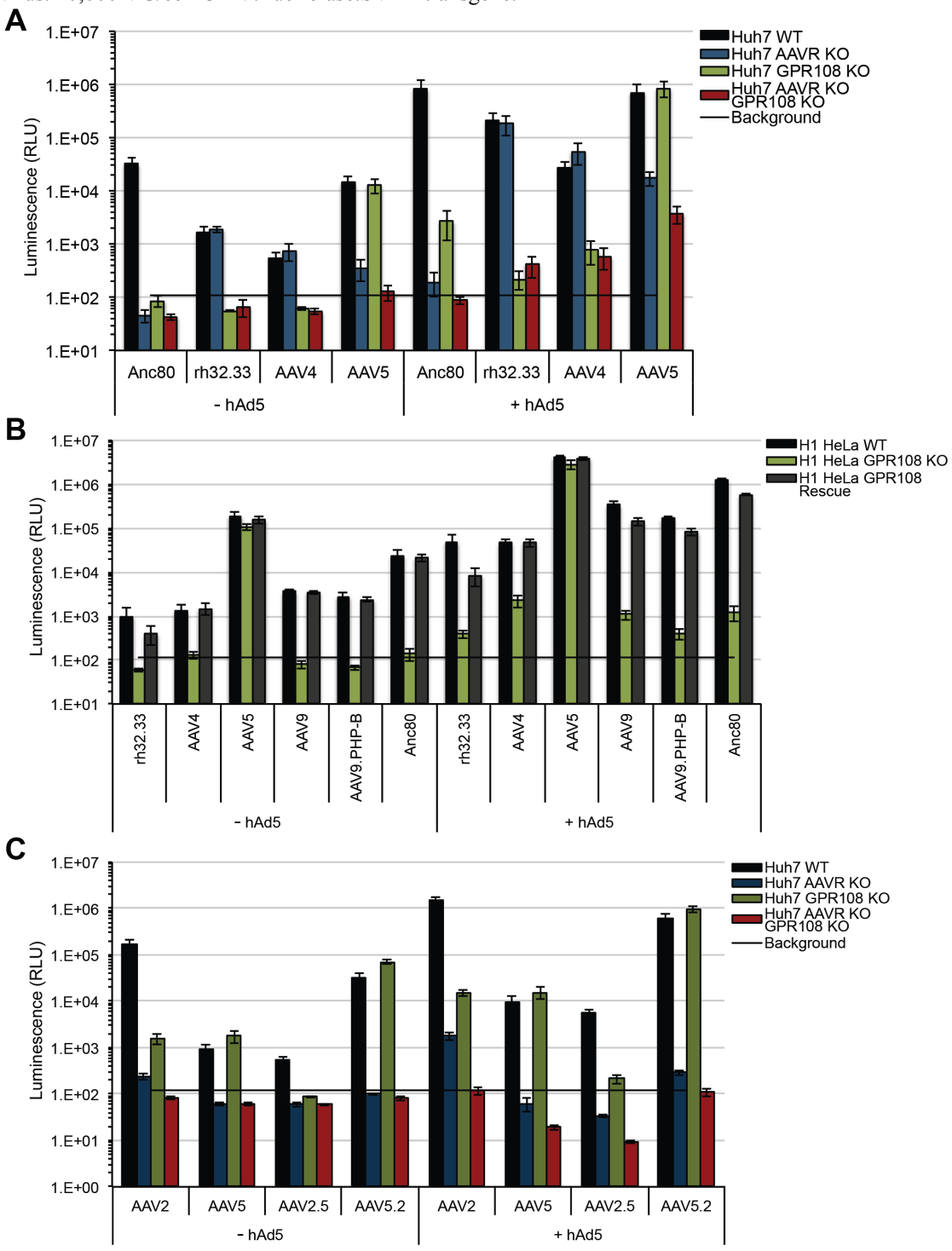
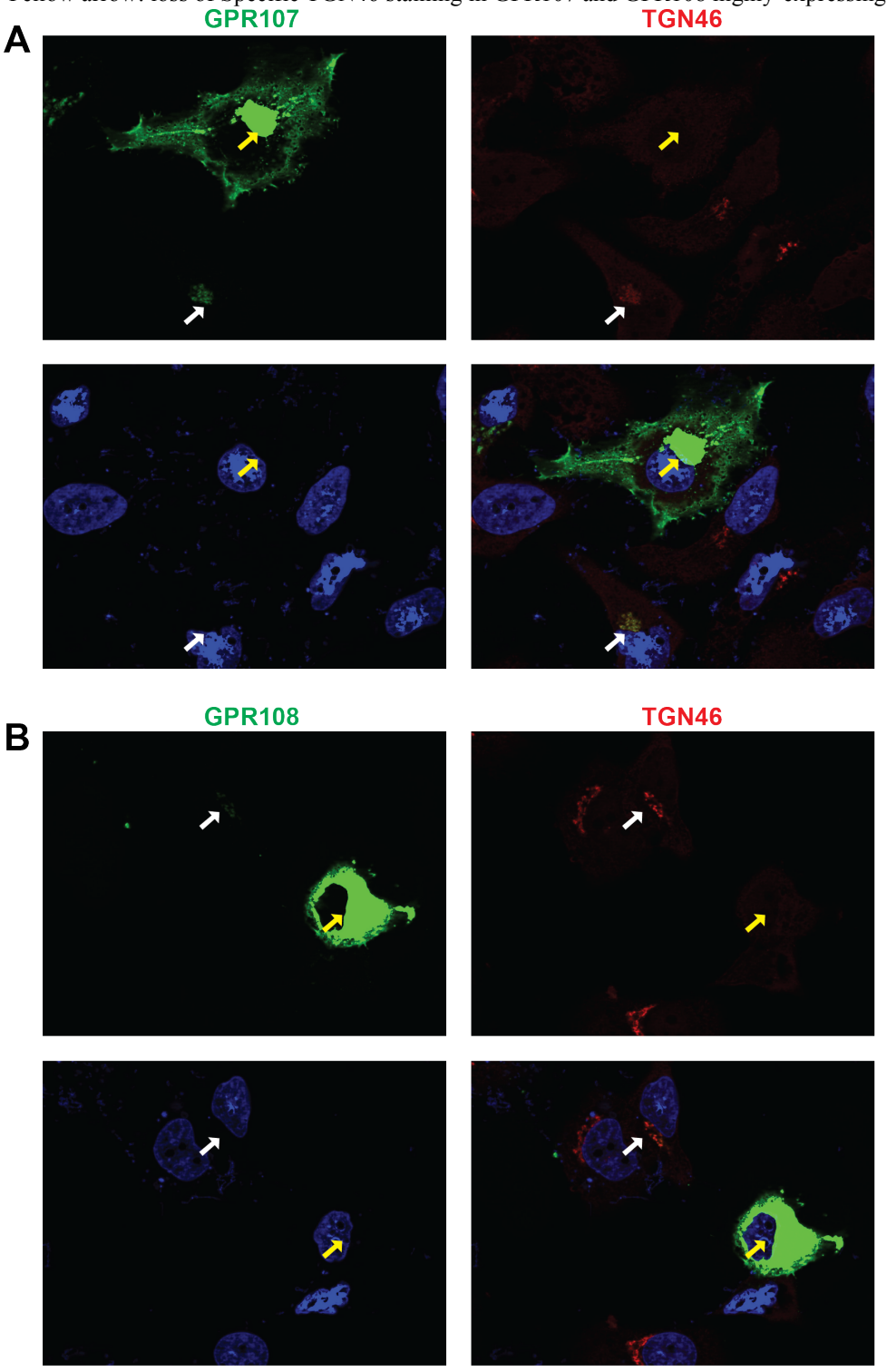
Supplemental Figure S1: FACS selection of rh32.33 entry screen. Gating strategy for FACS selection of GFP+ and GFP- cells in each of two halves [V2A and V2B, (A,B)] of the GeCKO library. Gating strategy for FACS selection of GFP- cells expanded and subjected to a second round of high MOI transduction for each half (C,D) of the GeCKO library.



Supplemental Figure S2: GPR108 usage is independent of helper virus co-infection. Transduction level of indicated serotypes in AAVR KO, GPR108 KO, or double KO cells relative to WT Huh7 cells (A,C), or H1 HeLa WT, GPR108 KO, or GPR108 rescue cells (B), in the presence or absence of helper virus. 10,000 VG/cell CMV.Luciferase.SVPA transgene.



Supplemental Figure S3: GPR107 or GPR108 overexpression causes disruption of intracellular membrane structures. Indirect immunofluorescence of flag-tagged GPR107 (A) or GPR108 (B) with anti-flag M2 primary antibody (green) or anti-TGN46 antibody as a trans-golgi network marker (red) and DAPI staining of cell nuclei (blue). White arrow: co-localization of GPR107 and GPR108 in low-expressing cells. Yellow arrow: loss of Specific TGN46 staining in GPR107 and GPR108 highly-expressing cells.

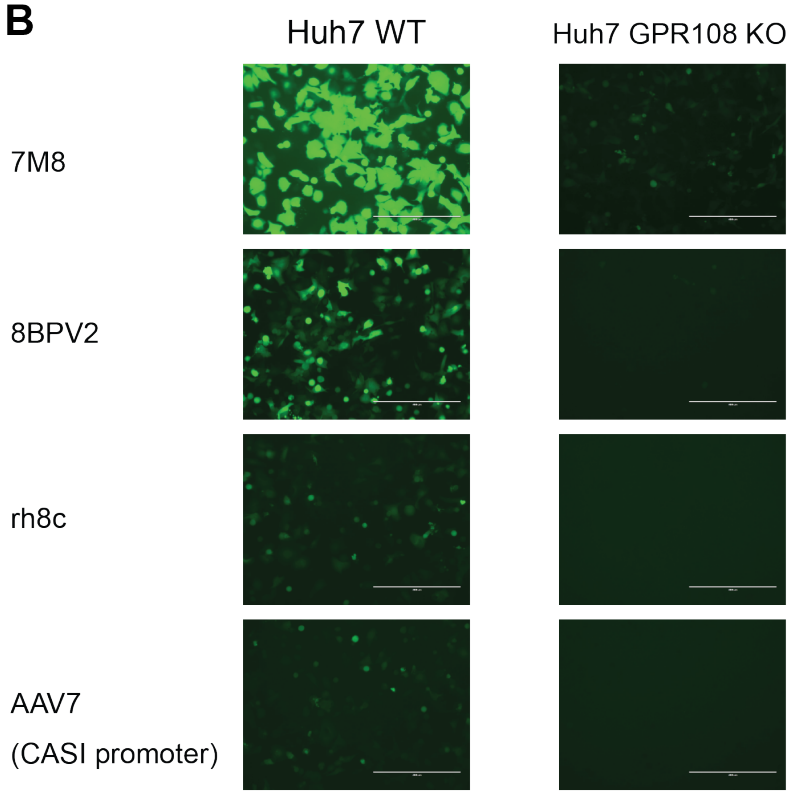


Supplemental Figure S4: Peptide insertion and glycan binding alteration does not alter GPR108 requirement. A) Table of tested peptide insertion mutants and parental capsid. B) Transduction of indicated AAV serotypes in WT or GPR108 KO Huh7 cells at 10,000 VG/cell with hAd5 helper virus (CMV.eGFP.WPRE transgene). C) Transduction of glycan-binding defective AAV2HSPG- and parental capsid AAV2 in WT or GPR108 KO Huh7 or H1 HeLa cells (CMV.Luciferase.SVPA transgene).

A

Parental	Peptide insertion
AAV2	7M8
AAV8	8BPV2
AAV9	PHP-B

B



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