Supplemental Materials

Figure S1. Genes enriched in the second round of screen of mCherry negative cells.

The x-axis shows all the genes enriched. The genes with an enrichment score of 3 are grouped by gene ontology analysis. Genes are indicated as circles with size corresponding to fold change of sgRNA reads in $gDNA^{Screen}$ and $gDNA^{Ctrl}$. The enrichment score [$-\log_{10}$] of each gene based on MAGeCK analysis is shown in the y-axis.

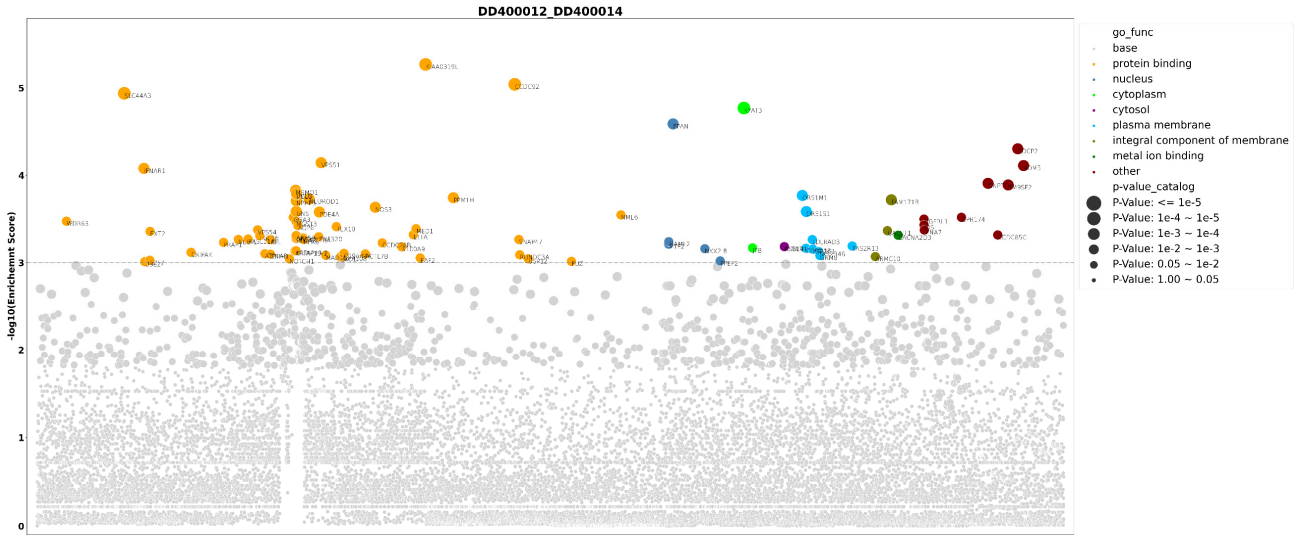

Figure S2. Silencing of 27 candidate genes using shRNA-expressing lentiviruses in HeLa cells.

HeLa cells seeded in 24-well plates were transduced with shRNA-expressing lentiviruses against the top 27 genes as indicated, respectively. After selection in puromycin, the cells were collected and lysed for Western blotting using antibodies against the proteins as indicated. β-actin was detected as a loading control.

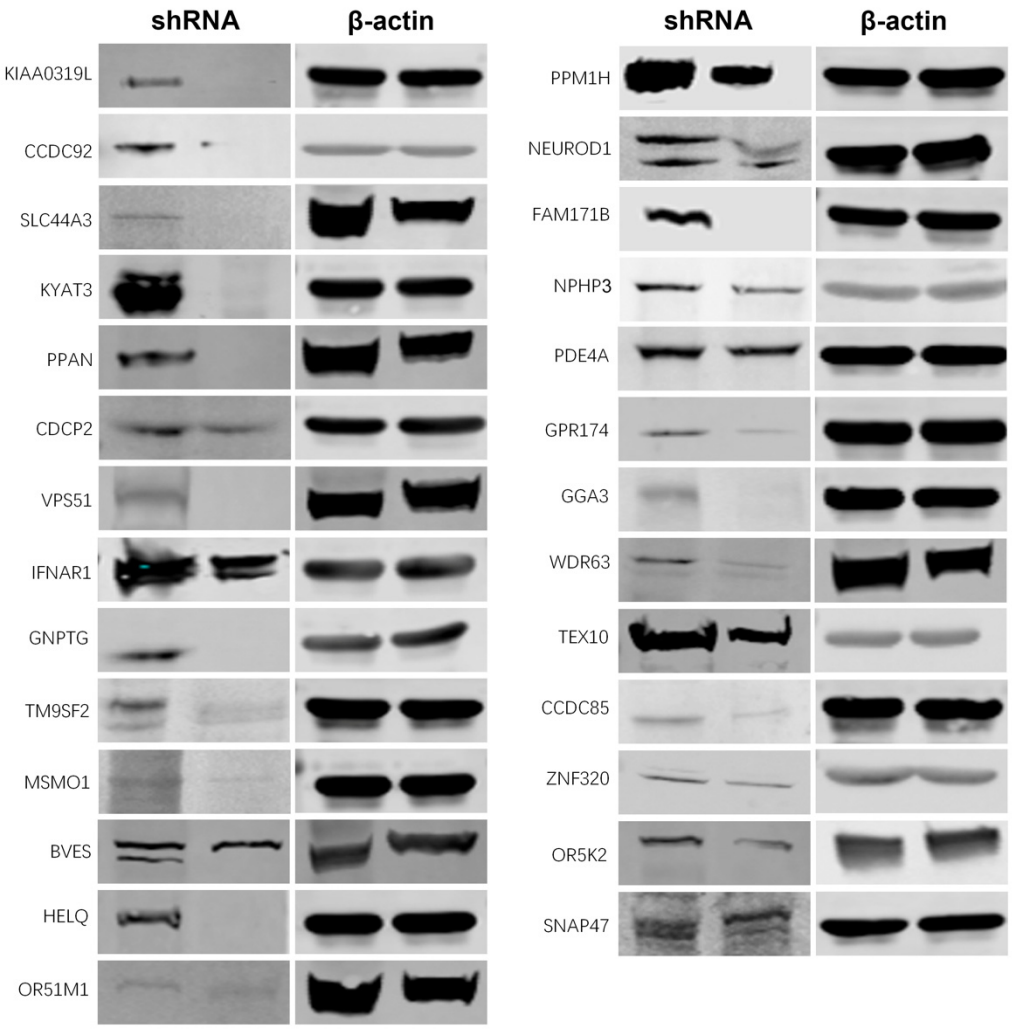

Figure S3. Fold changes of the hits of the selected genes in the gRNA library screen using mCherry negative cells transduced with rAAV2.5T.

This panel displays the individual gene fold-changes of 4 gRNAs per gene between the unselected and selected groups.

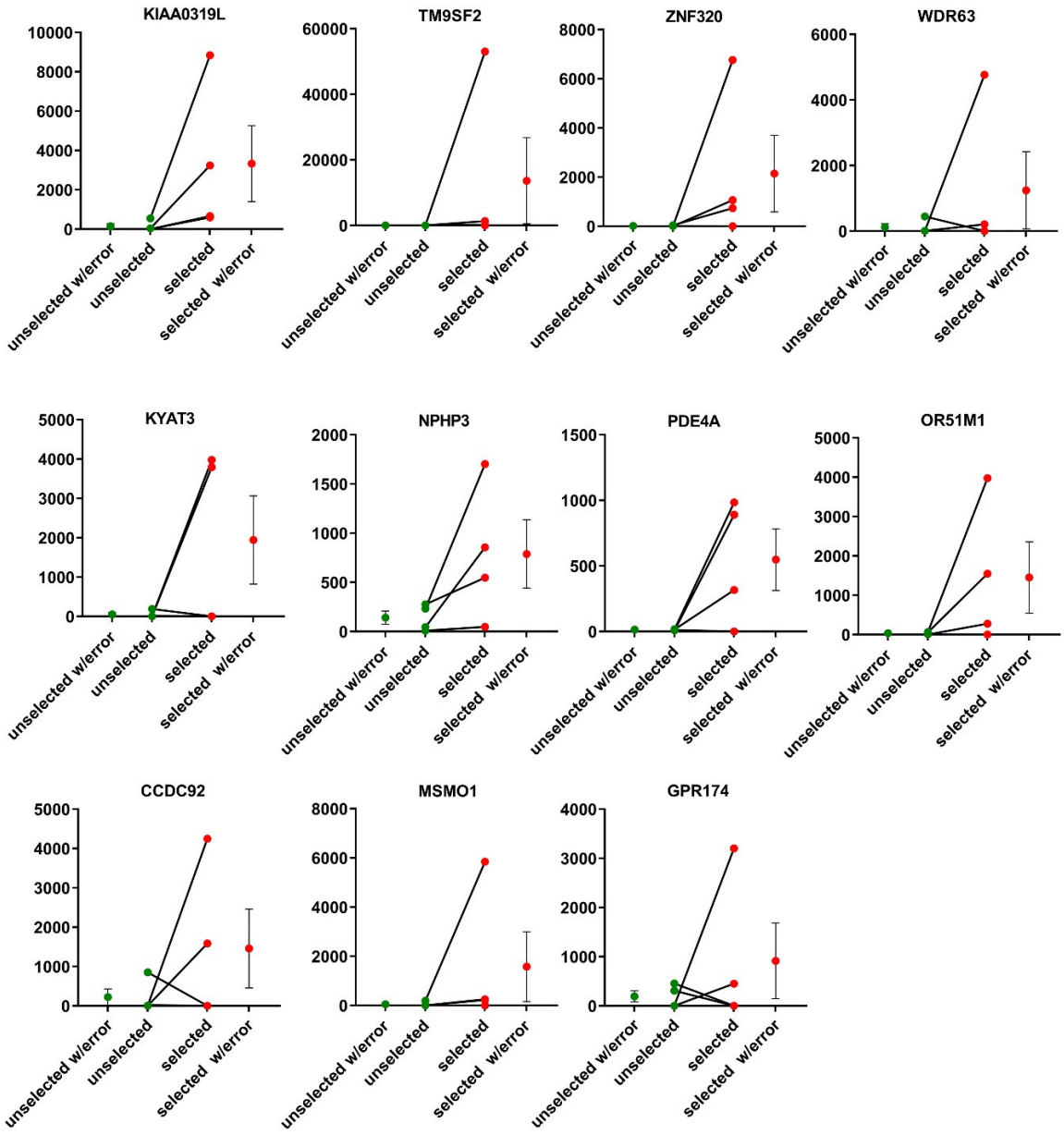

Figure S4. rAAV2.5T internalization assay of HeLa cells.

HeLa cells were incubated with rAAV2.5T at an MOI of 20,000 DRP/cell. After two hours at 37°C, the cells were used for vector internalization assay. The boundary of the box closest to zero indicates the 25th percentile, a black line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles.

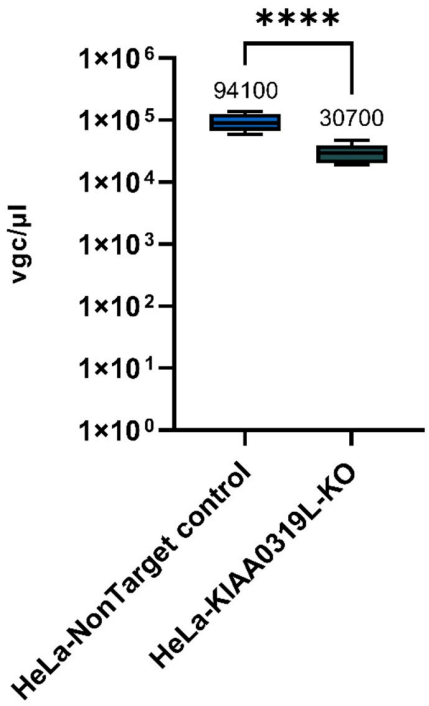

Figure S5. Co-staining of AAV2.5T capsid with WDR63 in cells of HAE-ALI cultures.

AAV2.5T was transduced into HAE-ALI cells at an MOI of 20,000. Three days after transduction, CuFi cells of the ALI culture were Accutase treated and cytospun onto slides for staining. The stained cells were subjected to imaging under a confocal microscope at a magnitude × 100 (CSU-W1 SoRa, Nikon). Green represents viral capsid, yellow represents WDR63, red represents mCherry expression, and blue represents DAPI staining.

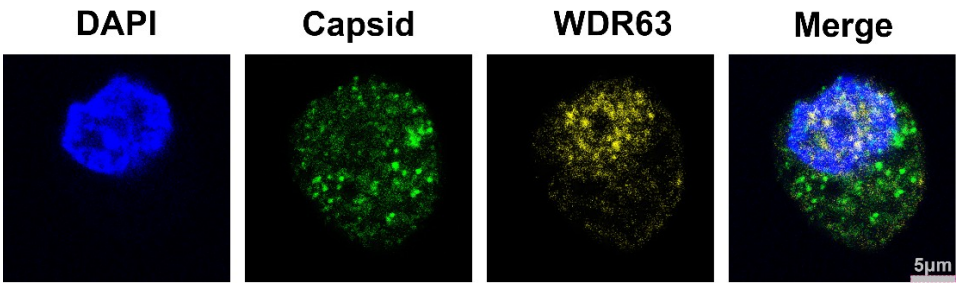

Figure S6. Knockout of *WDR63* causes a significant decrease in transduction efficiency of rAAV2 and rAAV5 in Dox-treated HAE-ALI cultures.

HAE-ALI cultures of non-target control (NT) or WRD63-knockout (KO) cells were transduced with rAAV2.5T, rAAV2(F5tg83gluc-CMVmCherry) and rAAV5(F5tg83gluc-CMVmCherry) at an MOI of 20K from the apical side. Dox was added with rAAV2.5T at 2.0 μM. After 16 hours post-transduction, both the apical and basolateral chambers were refreshed with culture media. Luciferase activity was measured at 3 days post-transduction. Data shown were means with an SD from three replicates.

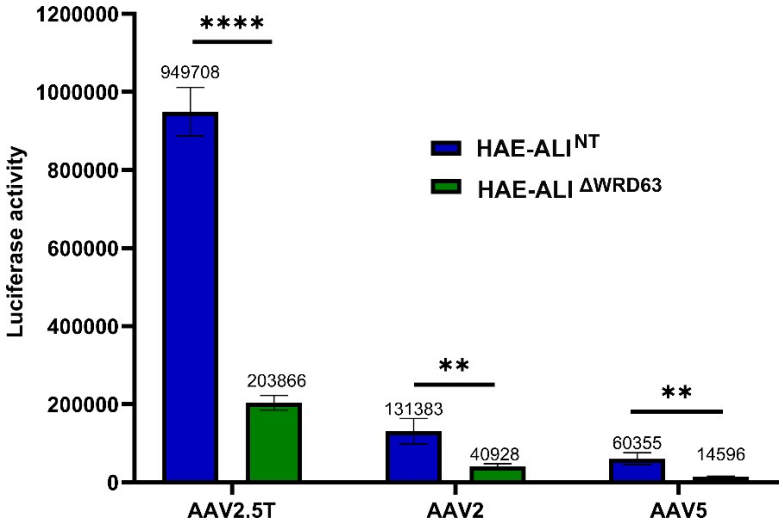

Table S1. A list of genes enriched in the second round of mCherry negative cell screening and ranked by the -$\log_{10}$ enrichment score.

NGS results of the sgRNA-targeted genes screened in the second-round screening ($gDNA^{Screen}$) were compared with the results from the background cells ($gDNA^{Ctrl}$) based on MAGeCK analysis. The analysis results are listed in **Supplemental Material 2**.

Table S2. List of shRNAs used in the study.

Gene	**Cat. no. of shRNA at MilliporeSigma**
Non-Target (NT) shRNA Control	SHC016V
KIAA0319L	TRCN0000123109
CCDC92	TRCN0000353704
SLC44A3	TRCN0000158746
KYAT3	TRCN0000150908
PPAN	TRCN0000128301
CDCP2	TRCN0000064638
VPS51	TRCN0000151062
IFNAR1	TRCN0000059013
GNPTG	TRCN0000036049
TM9SF2	TRCN0000059772
MSMO1	TRCN0000230198
BVES	TRCN0000153094
HELQ	TRCN0000051559
OR51M1	TRCN0000187514
PPM1H	TRCN0000052772
NEUROD1	TRCN0000019859
FAM171B	TRCN0000020096
NPHP3	TRCN0000118612
PDE4A	TRCN0000048812
GPR174	TRCN0000008261
GGA3	TRCN0000232888
WDR63	TRCN0000158457
TEX10	TRCN0000364593
CCDC85C	TRCN0000337094
ZNF320	TRCN0000152095
OR5K2	TRCN0000187322
SNAP47	TRCN0000167217

Table S3. gRNA sequences

Gene	**gRNA sequence (5' → 3')**
KIAA0319L	GAG GTG ACA CAA TAG CAA TG
TM9SF2	CTT GTT ACT TAT GTC CAT GG
ZNF320	GGA CAT CGT AGA GTT CAC AC
WDR63	TCC GGT TCA AGG AGA AAC AT
KYAT3	AAG GAC TTG ATA GTA ATG TG
NPHP3	TTA TTC ACT TAA CAT TAC CA
PDE4A	CAC AGT GCA CCA TGT TCC GG
OR51M1	GGC CTA ATT GTC ATC TTC CG
CCDC92	AGT GCT GGA GAA CAC CAT CA
MSMO1	AAG TTC CAG ATT GCA ACA TG
GPR174	TAT AAC CAT AGA ATA CCC AC

Table S4. The first antibodies used in this study.

Protein	Vendor	Cat. No.
KIAA0319L	Proteintech	21016-1-AP
CCDC92	Proteintech	27192-1-AP
SLC44A3	AB Clonal	A12820
KYAT3	Novus	87387
PPAN	AB Clonal	A7377
CDCP2	Novus	87438
VPS51	AB Clonal	A15651
IFNAR1	AB Clonal	A1715
GNPTG	Novus	88443
TM9SF2	Novus	95189
MSMO1	Novus	59450
BVES	AB Clonal	A0123
HELQ	AB Clonal	A12661
OR51M1	Biorbyt	ORB398077
PPM1H	Abcepta	AP9093
NEUROD1	Proteintech	12081-1-AP
FAM171B	Novus	93847
NPHP3	Proteintech	22026-1-AP
PDE4A	Abcepta	AP17181
GPR174	Abcepta	AP16459
GGA3	Novus	HD120C2913
WDR63	Novus	32639
TEX10	AB Clonal	A18118
CCDC85C	Novus	82622
ZNF320	Proteintech	24882
OR5K2	Novus	9805
SNAP47	Novus	56894
β-tubulin IV	MilliporeSigma	T7941
ZO-1	BD Bioscience	610966
Cytokeratin k5	ThermoFisher Invitrogen	MA5-12596
SCGB1A1	ThermoFisher Invitrogen	MAB4218
MUC5AC	Santa Cruz Biotechnology	sc-33667
AAV5 Capsid	ARP	608021
β-Actin	MilliporeSigma	A5441
Histon H3	Proteintech	17168-1-AP