Supplemental information

Supplementary Table 1. AAV variants in 51 'AAV Kit'

#	Capsid variant	Origin	Library type (if directed evolution)	Citation
1	AAV1	Human cell culture isolate	N/A	1
2	AAV2	Human cell culture isolate	N/A	2, 3
3	AAV3b	Human cell culture isolate	N/A	2
4	AAV4	Non-human primate cell culture isolate	N/A	4
5	AAV5	Human cancer isolate	N/A	5
6	AAV6	Human cell culture isolate	N/A	3
7	AAV7	Non-human primate isolate	N/A	6
8	AAV8	Non-human primate isolate	N/A	6
9	AAV9	Human isolate	N/A	7
10	AAV10	Non-human primate isolate	N/A	8
11	AAV11	Non-human primate isolate	N/A	8
12	AAV12	Non-human primate isolate	N/A	9
13	AAV13	Human cell culture isolate	N/A	10
14	AAV-rh10	Non-human primate isolate	N/A	11
15	AAV-h.Lvr6	Human liver isolate	N/A	12
16	AAV-2i8	Murine muscle rational design	N/A	13
17	AAV-Anc80	Ancestral reconstruction	N/A	14
18	AAV-DJ	HuH-7 cells directed evolution	Shuffled library	15
19	AAV-DJ8	AAV-DJ modification for murine tropism	Shuffled library + rational design	15
20	AAV-LK01	Human liver xenograft directed evolution	Shuffled library	16
21	AAV-LK02	Human liver xenograft directed evolution	Shuffled library	16
22	AAV-LK03	Human liver xenograft directed evolution	Shuffled library	16
23	AAV-LK19	Human liver xenograft directed evolution	Shuffled library	16
24	AAV-NP6	Human muscle xenograft directed evolution	Shuffled library	17
25	AAV-NP22	Human muscle xenograft directed evolution	Shuffled library	17
26	AAV-NP40	Human liver xenograft directed evolution	Shuffled library	18
27	AAV-NP59	Human liver xenograft directed evolution	Shuffled library	18
28	AAV-NP66	Human muscle xenograft directed evolution	Shuffled library	17
29	AAV-NP84	Human liver xenograft directed evolution	Shuffled library	18
30	AAV-NP94	Human muscle xenograft directed evolution	Shuffled library	17
31	AAV-KP1	Human islet directed evolution	Shuffled library	19
32	AAV-KP2	Human islet directed evolution	Shuffled library	19
33	AAV-KP3	Human islet directed evolution	Shuffled library	19
34	AAV-7m8	Murine retina directed evolution	Peptide insertion	20
35	AAV-PHP.eB	Murine CNS directed evolution	Peptide insertion	21
36	AAV2-Retro	Murine CNS directed evolution	Peptide insertion	22
37	AAV-HRP5	HuH-7 cells directed evolution	Shuffled library	23*
38	AAV-HRS1	HuH-7 cells directed evolution	Shuffled library	23*
39	AAV-HRS19	HuH-7 cells directed evolution	Shuffled library	23*
40	AAV-CD15	Human HSPC directed evolution	Shuffled library	23*
41	AAV-CD-SYD01	Human HSPC directed evolution	Shuffled library	23*
42	AAV-CD-SYD03	Human HSPC directed evolution	Shuffled library	23*
43	AAV-CD-SYD09	Human HSPC directed evolution	Shuffled library	23*
44	AAV-T33	Human T-cell directed evolution	Shuffled library	23*
45	AAV-R588I	Human liver xenograft replicative evolution	Shuffled library	12
46	AAV-N496D	Human liver xenograft directed evolution	Shuffled library	24
47	AAV-SYD04	Human liver xenograft directed evolution	Shuffled library	25
48	AAV-SYD11	Human liver xenograft directed evolution	Shuffled library	25
49	AAV-SYD12	Human liver xenograft directed evolution	Shuffled library	25
50	AAV2-RC01	Human liver xenograft directed evolution	Peptide insertion	26
51	AAV2-RC02	Human liver xenograft directed evolution	Peptide insertion	26

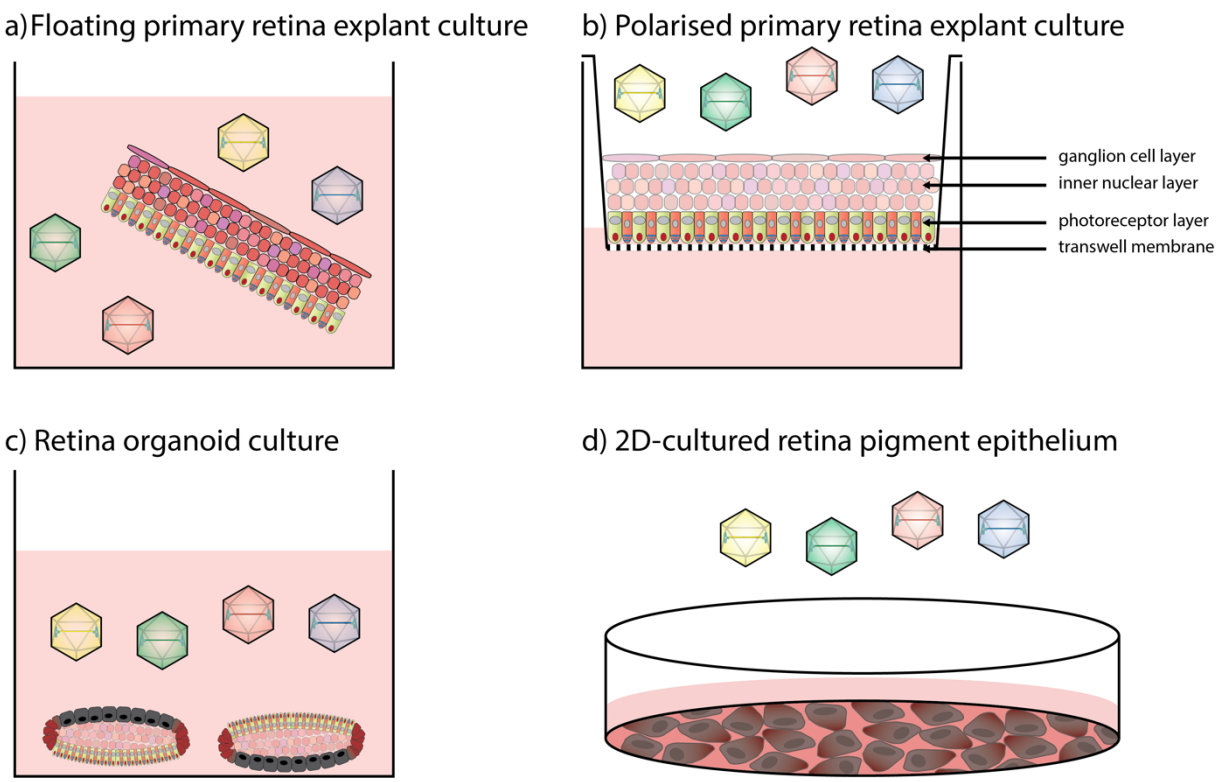
*variants 37-44 were previously published in a PhD Thesis.[23]

Supplementary Table 2. Oligonucleotides for peptide cloning

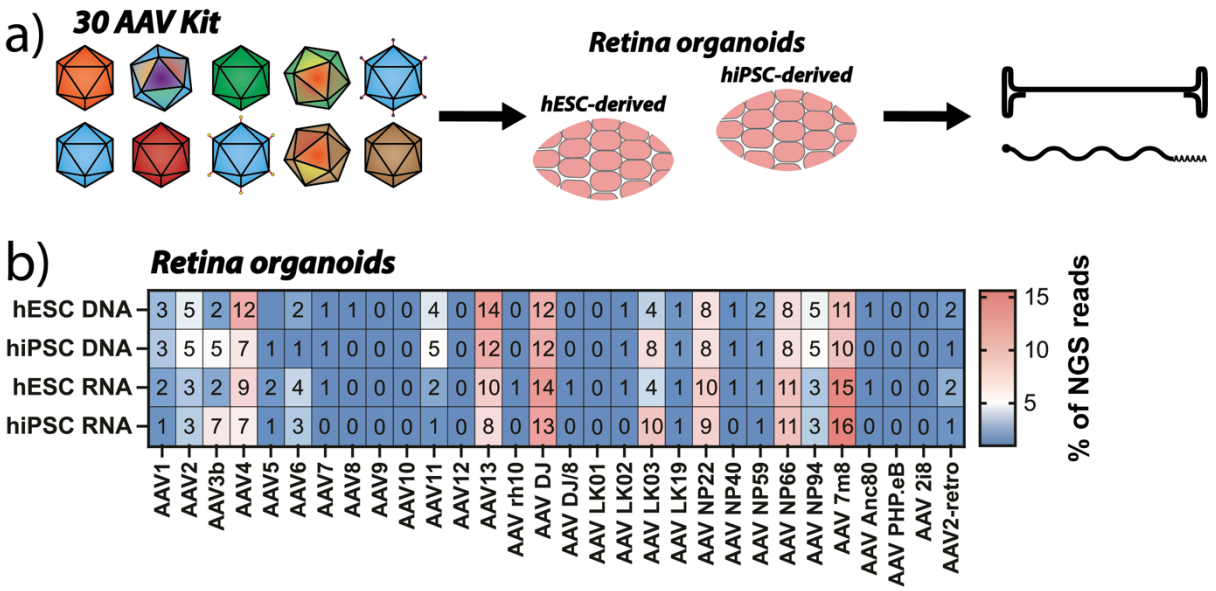
1.1_F	AGGCGGCGCACTCTGGCCCTGGAGGTTGGT
1.1_R	CGGCATCCGTCTGAGGCGGCCGCGACCGCA
1.2_F	CTGCGTAGTCCTCTGGCCCTGGAGGTTGGT
1.2_R	TTTCATCCGCCGGAGGCGGCCGCGACCGCA
1.3_F	CTGCTTAGGCCTCTGGCCCTGGAGGTTGGT
1.3_R	CCTACGCAGCCTAAGGCGGCCGCGACCGCA
1.4_F	ATAAGAAGGCCTCTGGCCCTGGAGGTTGGT
1.4_R	AGTCCTTCGAATCAGGCGGCCGCGACCGCA
1.5_F	CACAACACTACTCTGGCCCTGGAGGTTGGT
1.5_R	AGTTCGCGTTGTGAGGCGGCCGCGACCGCA
L1_F	AGGCTGCTGCCTCTGGCCCTGGAGGTTGGT
L1_R	CAGAATACTCGTCAGGCGGCCGCGACCGCA
L2_F	CTGAAGCGTACTCTGGCCCTGGAGGTTGGT
L2_R	CGGACGATGGCGAAGGCGGCCGCGACCGCA
L3_F	AGCCAGATGCCTCTGGCCCTGGAGGTTGGT
L3_R	GTGGCGCCGCCGCAGGCGGCCGCGACCGCA
L4_F	ACGCCAAGGCCTCTGGCCCTGGAGGTTGGT
L4_R	GAGTCGTCTCAGGAGGCGGCCGCGACCGCA
L5_F	CGTAGTCGTACTCTGGCCCTGGAGGTTGGT
L5_R	CGTGATATGCCTAAGGCGGCCGCGACCGCA
M1_F	CCTCTGCCGCCTCTGGCCCTGGAGGTTGGT
M1_R	ATTCCGGGGGGGGAGGCGGCCGCGACCGCA
M2_F	ATGATGATTACTCTGGCCCTGGAGGTTGGT
M2_R	ACGAATAATCCGAAGGCGGCCGCGACCGCA
M3_F	ACCATAATTCCTCTGGCCCTGGAGGTTGGT
M3_R	AGGCAGGATTCTCAGGCGGCCGCGACCGCA
M4_F	CCGAGGCAAACTCTGGCCCTGGAGGTTGGT
M4_R	CGGGATGCTCCTAAGGCGGCCGCGACCGCA
M5_F	CTTATCCAGACTCTGGCCCTGGAGGTTGGT
M5_R	AAGAATGCGACGAAGGCGGCCGCGACCGCA
H1_F	CTTATTCTTCCTCTGGCCCTGGAGGTTGGT
H1_R	GATACGCCGGTGAAGGCGGCCGCGACCGCA
H2_F	ATTCTGATTCCTCTGGCCCTGGAGGTTGGT
H2_R	GAGACTAAGAGGCAGGCGGCCGCGACCGCA
H3_F	AAGCTGCTTACTCTGGCCCTGGAGGTTGGT
H3_R	CCGACTAATAATAAGGCGGCCGCGACCGCA
H4_F	CGGATTCTGCCTCTGGCCCTGGAGGTTGGT
H4_R	AAGCTGGGTTCGGAGGCGGCCGCGACCGCA
H5_F	ATTAATCTTACTCTGGCCCTGGAGGTTGGT
H5_R	CCGAATGCTTCGAAGGCGGCCGCGACCGCA

Supplementary Table 3. Selected peptides

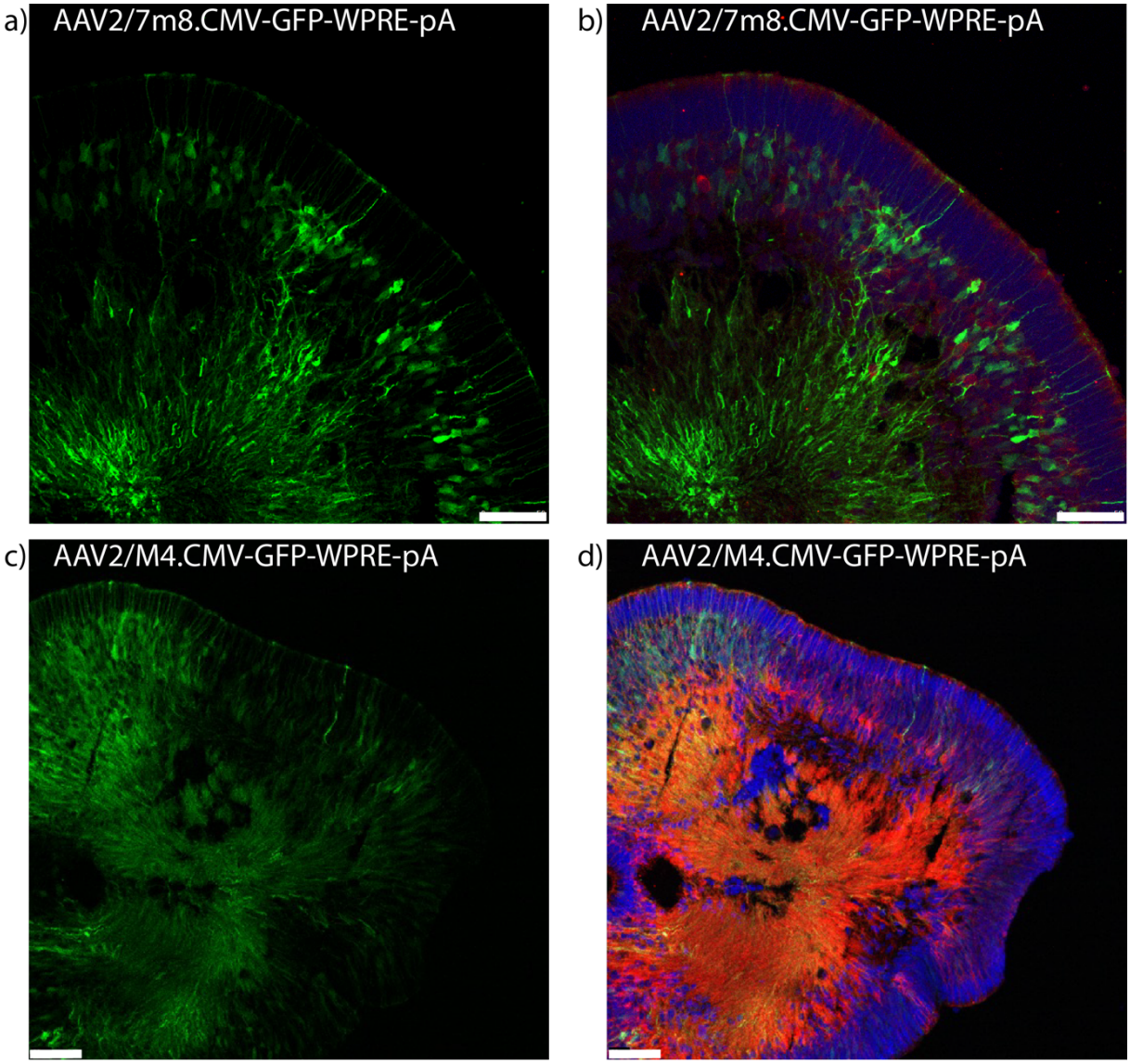
Variant name	Peptide	Counts	Percentage
Round 1			
1.1	SAPPRHPSE	25658	1.50%
1.2	RTTQFHPPE	19916	1.17%
1.3	RPKQPTQPK	13524	0.79%
1.4	RPSYSPSNQ	13341	0.78%
1.5	SSVVSSRCE	13163	0.77%
Round 2 – Unsorted; low dose			
L1	RKNKDTPVK	51022	2.21%
L2	RNQNETKRQ	41336	1.79%
L3	SKQLPTNNK	39001	1.69%
L4	RQNPKLGSE	33414	1.44%
L5	SKINPNASK	27632	1.19%
M4	*SLPRRDAPK*	*27304*	*1.18%*
Round 2 – CD73^{+}; medium dose			
L1	*RKNKDTPVK*	*89900*	*2.94%*
M1	RRQRIPGGE	86102	2.81%
M2	SNHHTNNPK	78538	2.56%
M3	RNYGRQDSQ	71595	2.34%
M4	SLPRRDAPK	69491	2.27%
M5	SLDKKNATK	69108	2.26%
L2	*RNQNETKRQ*	*60017*	*1.96%*
L3	*SKQLPTNNK*	*50421*	*1.65%*
M6	SSQRLPTTQ	49495	1.62%
M7	RPTKHLDRE	48752	1.59%
H6	*RRKAENQMK*	*45759*	*1.49%*
L5	*SKINPNASK*	*41970*	*1.37%*
Round 2 – CD73^{+}; high dose			
H1	RQQPQNTRQ	228671	10.29%
H2	STLQRTMAK	128948	5.80%
H3	RHLAVAPPQ	127910	5.75%
H4	RPWRESSQE	126922	5.71%
H5	STTTRDMPK	126428	5.69%
H6	RRKAENQMK	119396	5.37%
H7	RRGSDPVRK	118018	5.31%
H9	RKKNEETKK	113619	5.11%
H11	RRINMATGQ	111675	5.02%
L1	*RKNKDTPVK*	*107193*	*4.82%*



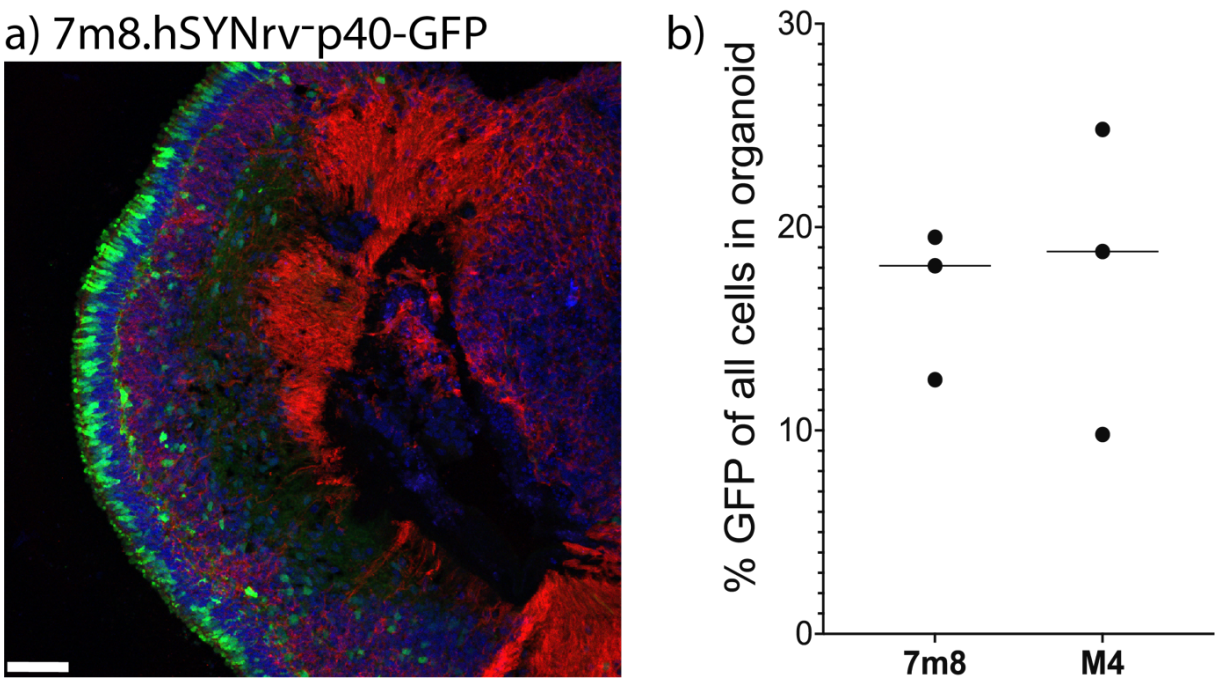
Supplementary Figure 1. Overview of retina models used in this study. a) Primary human retina holepunches submerged in culture media and referred to as "floating" explants in the presented study. Vectors were exposed to the explant from all sides. b) Polarised primary retina explants cultured on transwell inserts. The photoreceptor layer is in contact with culture media through the transwell membrane. Vectors contacted the tissue only from the ganglion cell layer. c) Submerged human induced pluripotent stem cell (hiPSC)-derived retina organoid cultures. AAVs could access organoids from all sides. d) 2D-cultured retina pigment epithelium derived from primary human tissue or differentiated from hiPSC.



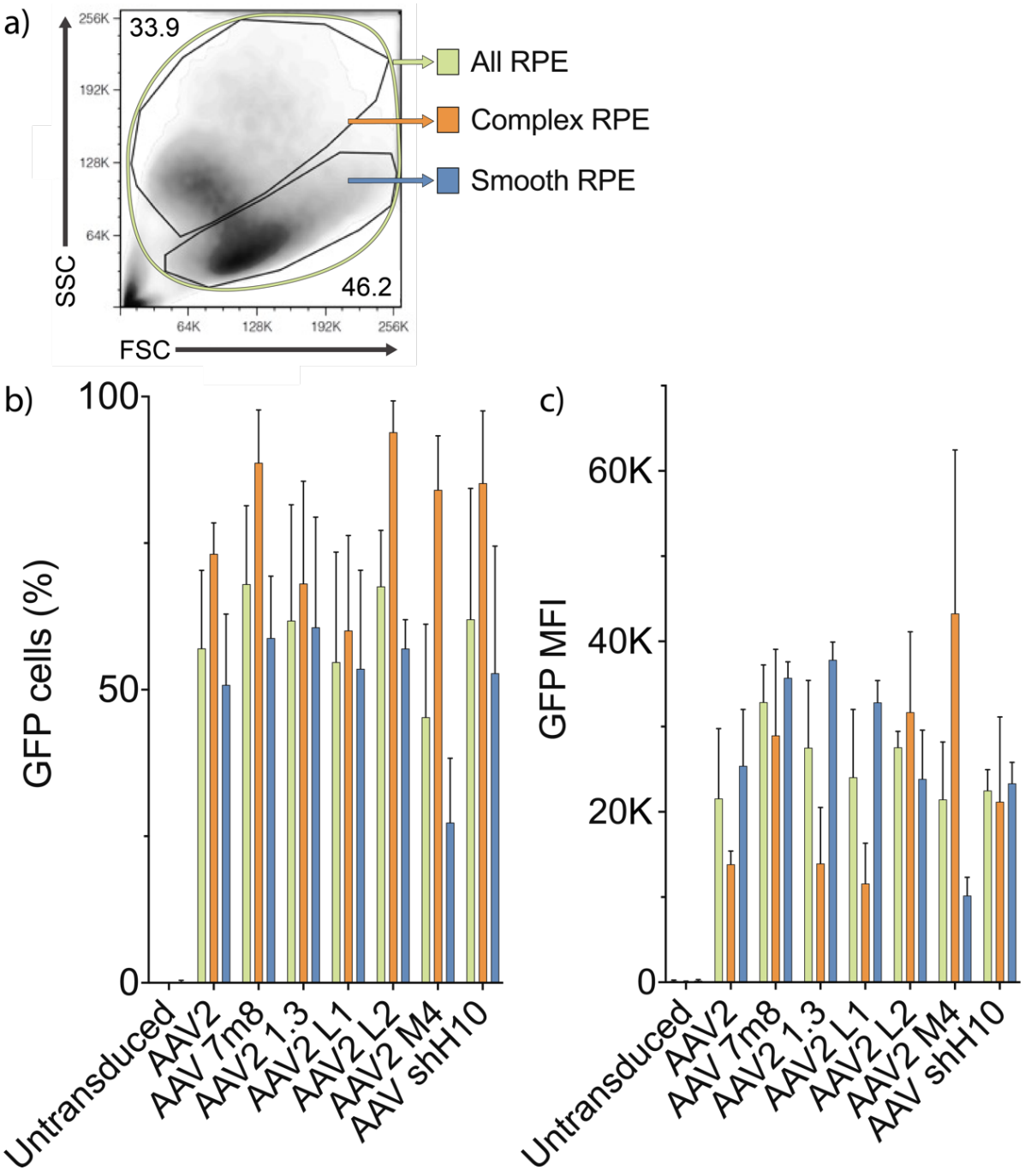
Supplementary Figure 2. Results from the 30 'AAV Kit' on retina organoids
(a) Schematic representation of the performed experiment. **(b)** Results of NGS-based quantification of AAV-delivered DNA and RNA/cDNA in retina organoids. Values are given as percentage of total reads. Number of replicates for each model: n=1.



Supplementary Figure 3. Detailed analysis of the transduction of AAV2-M4 in retinal organoids. Transduction of iPSC-derived retinal organoids with 5×10^{10} vector genomes using AAV-7m8 and AAV2-M4; n=1. (a-b) Representative confocal images showing AAV2-7m8 transduction with native CMV-driven eGFP expression [a], DAPI, and an overlay of eGFP (green), DAPI (blue) and cone arrestin (red) [b]. (c-d) Representative confocal images showing AAV2-M4 transduction with native CMV-driven eGFP expression [c], and an overlay of eGFP (green), DAPI (blue), and cellular retinaldehyde-binding protein (red) [d].



Supplementary Figure 4. Comparing the transduction of AAV-7m8 and AAV2-M4 using the hSYNrv-p40 promoter. (a) Representative microscopy image showing transduction of AAV-7m8 capsid delivering hSYNrv-p40-driven eGFP green: anti-GFP antibody-counterstained eGFP expression; red: cellular retinaldehyde-binding protein; blue: DAPI; n=1. (b) Flow cytometry showing eGFP expression of all DAPI-negative cells in three independently prepared retinal organoid cultures; n=3.



Supplementary Figure 5. Detailed analysis of the transduction of the top candidates in iPSC-derived retinal pigment epithelium. (a) Representative graph of cell populations within all DAPI-negative cells of iPSC-derived RPE. (b-c) eGFP positive cells [b] and mean fluorescent intensity [c] within the three cell populations defined in [a] shown for the indicated novel and benchmark AAV capsids; n=2. Green: all DAPI-negative cells; orange: granular (high SSC) cells; blue: smooth (low SSC) cells.

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