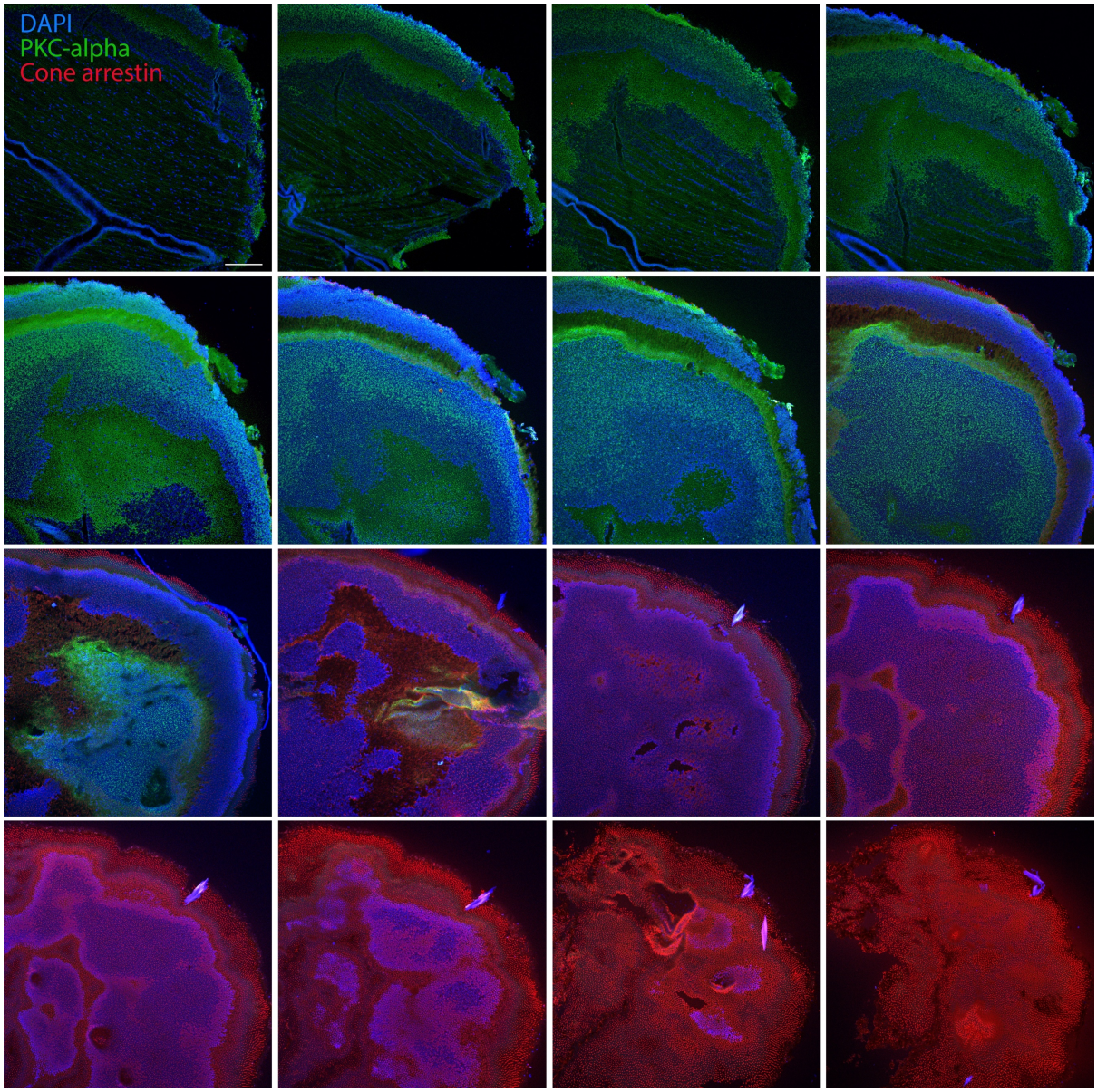
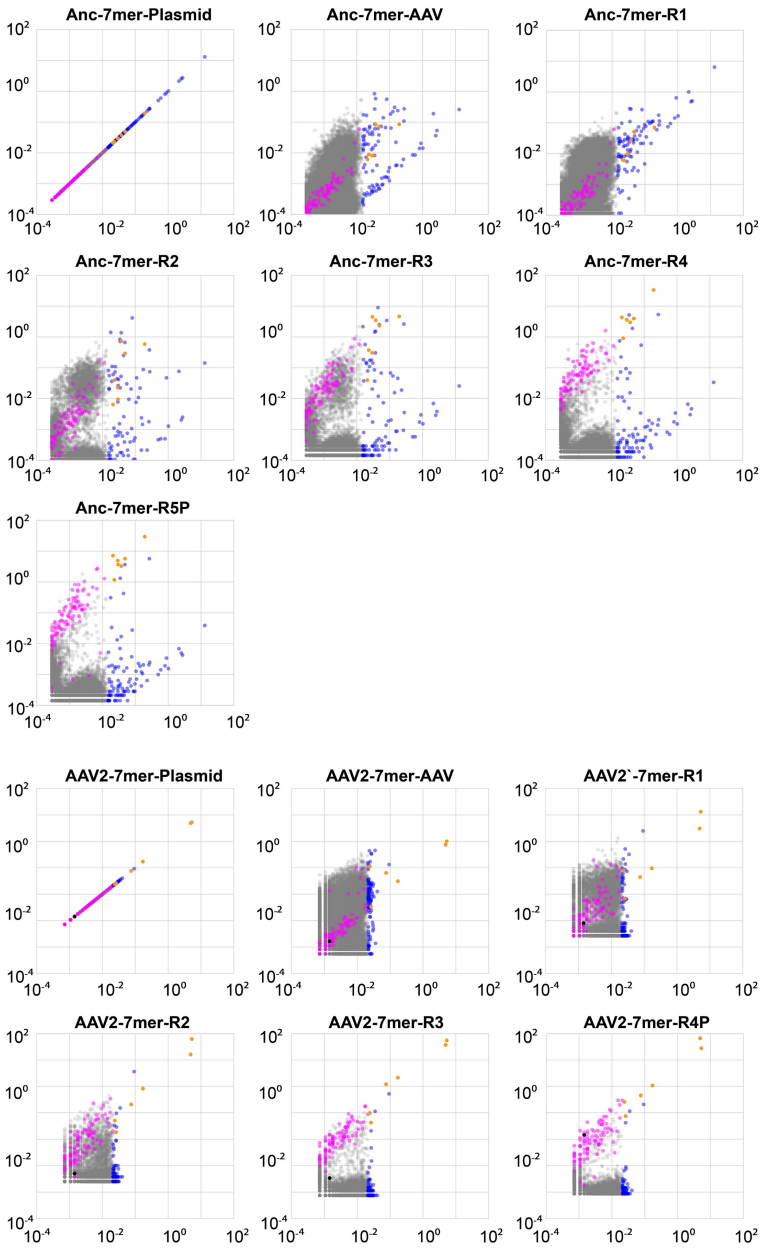
Supplementary Materials



Supplementary Figure 1. Transverse cryosectioning of mouse retinal punch illustrates the method used to isolate the outer retina. A punch of retina was flatmounted, embedded in OCT cutting medium, and flash frozen, then mounted in a cryostat and sectioned at 20 µm sections through retinal layers. Layers were then stained for PKC-alpha (a marker of bipolar cells in the inner nuclear layer) and cone arrestin (a marker of photoreceptors in the outer retina). Labeling shows successful isolation of outer retinal tissue from inner retinal cells, which was then used for amplification of libraries. RPE was peeled away prior to sectioning.



Supplementary Figure 2. Scatter plots track variants across rounds of selection. Scatterplots illustrate the behavior of individual variants over all rounds of selection for the ~Ancestral-7mer library and the 588-Loopswap library. Variants overrepresented in the original library are colored blue. Variants that had the greatest fold increase in representation in the final round of selection are shown in magenta. Variants that were overrepresented in the original library and increased significantly in representation over rounds of selection are colored orange. Black dots in AAV2-7mer scatter plots indicate the variant NHP#9.

Round	NHP ID	Age/Weight/Sex	Libraries injected	Amount Virus Injected	Notes
1	V002278	Approx. 7 years (age unknown at import) 5.98 kg/ Male	Loop Swap Ancestral-7mer	$2x10^{11}$ vg per library; 100 µl volume.	
2a	V002262	Approx. 7 years (age unknown at import) 6.35 kg/ Male	Recovered variants from round 1 AAV2-7mer	$2.5x10^{11}$ vg ONL $2.5x10^{11}$ vg RPE $5x10^{10}$ vg AAV2-7mer; 100 µl volume.	No variants were PCR amplified following injection from this round, no obvious immune response noted.
2b	V002148	Approx. 7 years (age unknown at import) 6.48 kg/ Male	Recovered variants from round 1 AAV2-7mer	$1.3x10^{11}$ vg ONL $1.3x10^{11}$ vg RPE $5x10^{10}$ vg AAV2-7mer; 100 µl volume.	Repeat of previous round
3	V002265	8 years 5 months 4.92 kg/ Male	Recovered variants from round 3	$4.3x10^{12}$ vg ONL $3.7x10^{12}$ vg RPE; 100 µl volume.	Error prone PCR conducted No adverse events
4	V002540	6 years 9 months 4.59 kg/ Male	Recovered variants from round 4	~$1x10^{12}$ vg per library; 100 µl volume.	No adverse events
5	V002861	6 years 6 months 6.60 kg/ Male	Recovered variants from round 5	$2.4x10^{12}$ vg ONL $6.3x10^{12}$ vg RPE; 100 µl volume.	No adverse events
GFP-barcode	V002361	9 years 5 months 6.00 kg/ Male	Barcoded individual variants	~$1x10^{10}$ vg each variant; 100 µl volume.	Both eyes injected with GFP-BC library. Hyphema in left eye resolved in 12 days
Variant validation	106	9 years 5 months 14.5 kg/ Male	7m8 and NHP9	~$1.5x10^{12}$ vg 7m8-pR1.7-GFP + $1.5x10^{12}$ vg 7m8-SNCG-tdTomato; 100 µl volume. OR ~$1.5x10^{12}$ vg NHP#9-pR1.7-GFP + $1.5x10^{12}$ vg NHP#9-SNCG-tdTomato; 100 µl volume.	No adverse events
Variant validation	735	17 years Male	NHP26 in one eye	~$5x10^{10}$ vg NHP#26-scCAG-GFP; 100 µl volume.	No adverse events

Supplemental Table 1. Summary of the rounds of selection performed in primates. The table indicates the age and weight of the primates injected, the virus and titer injected at each round, and notes on the rounds of selection completed. ONL refers to virus libraries recovered from ONL samples. RPE refers to virus libraries recovered from RPE samples, which were processed in parallel. Round 2b was a repeat of the 2nd round of selection, which did not result in PCR amplification of variants.

Primer	Sequence
SDM1	GACCTTAATCACAATCTTTTAAAACCCCGGCATGGCGGCT
SDM2	GGCTCGTGGACAAGTAAAGGGATTACCTCGGA
Neb Genomic_F	GTAAGGGTCTGCTCCATTGCCACTT
Neb Genomic_R	CTAAATCAAAAAAGAGTGAAAAGTTAGGAGG
IFA_F	TGGCTCGTGGACAAGGTAAGGGTCTGCTCCATTGC
IFA_R	CTCCGAGGTAATCCCCTAAATCAAAAAAGAGTGAAAAGTT
HindIII_F1	GACGTCAGACGCGGAAGCTTC
NotI_R1	GGTTTATTGATTAACAAGCGGCCG
AscI_R1	TGGCGGACTTTATAGGCGCG
SpeI_R1	GCCCAGTTCGAATAGCGAGT
LS588_Forward adapter	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**NNNNNGTTCTGTATCTACCAACCTCCA**
LS588_rev_index1	CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS453_Forward adapter	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**NNNNNATCGACCAGTACCTGTATTACT**
LS453_rev_index1	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
Anc_Forward adapter	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**NNNNNCCTGCAGTCGTCTAACACCGC**
Anc_rev_index1	CAAGCAGAAGACGGCATACGAGAT**CTCTAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
5_Forward adapter	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**NNNNNATGGCCACCAACAACCAGAGC**
5_rev_index1	CAAGCAGAAGACGGCATACGAGAT**ATCAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGAGGTTGTACGTGCCGGTCGC
4_Forward adapter	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**NNNNNACCTACCTGGCGGTGACCAGA**
4_rev_index1	CAAGCAGAAGACGGCATACGAGAT**TAGTTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTCCACGGTCGGCAGGTTGCTG
LS588_rev_index2	CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS588_rev_index3	CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS588_rev_index4	CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS588_rev_index5	CAAGCAGAAGACGGCATACGAGAT**CACTGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS588_rev_index6	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS588_rev_index7	CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS588_rev_index8	CAAGCAGAAGACGGCATACGAGAT**TCAAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGACCATGCCTGGAAGAACGCC
LS453_rev_index2	CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
LS453_rev_index3	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
LS453_rev_index4	CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
LS453_rev_index5	CAAGCAGAAGACGGCATACGAGAT**TTGACT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
LS453_rev_index6	CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
LS453_rev_index7	CAAGCAGAAGACGGCATACGAGAT**TGACAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
LS453_rev_index8	CAAGCAGAAGACGGCATACGAGAT**GGACGG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGTCCCGAATGTCACTCGCTC
Anc_rev_index2	CAAGCAGAAGACGGCATACGAGAT**GCGGAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
Anc_rev_index3	CAAGCAGAAGACGGCATACGAGAT**TTTCAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
Anc_rev_index4	CAAGCAGAAGACGGCATACGAGAT**GGCCAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
Anc_rev_index5	CAAGCAGAAGACGGCATACGAGAT**CGAAAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
Anc_rev_index6	CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
Anc_rev_index7	CAAGCAGAAGACGGCATACGAGAT**CCACTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
Anc_rev_index8	CAAGCAGAAGACGGCATACGAGAT**GCTACC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTAAGGCTCCCTGGCTGTTGAC
5_rev_index2	CAAGCAGAAGACGGCATACGAGAT**GCTCAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGAGGTTGTACGTGCCGGTCGC
5_rev_index3	CAAGCAGAAGACGGCATACGAGAT**AGGAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGAGGTTGTACGTGCCGGTCGC
5_rev_index4	CAAGCAGAAGACGGCATACGAGAT**CTTTTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGAGGTTGTACGTGCCGGTCGC
4_rev_index2	CAAGCAGAAGACGGCATACGAGAT**CCGGTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTCCACGGTCGGCAGGTTGCTG
4_rev_index3	CAAGCAGAAGACGGCATACGAGAT**ATCGTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTCCACGGTCGGCAGGTTGCTG
4_rev_index4	CAAGCAGAAGACGGCATACGAGAT**TGAGTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNTCCACGGTCGGCAGGTTGCTG
2-7mer Forward adapter	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**NNNNNTCTACCAACCTCCAGAGAGG**
rev_index1	CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index2	CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index3	CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index4	CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index5	CAAGCAGAAGACGGCATACGAGAT**CACTGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index6	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index7	CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index8	CAAGCAGAAGACGGCATACGAGAT**TCAAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index9	CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index10	CAAGCAGAAGACGGCATACGAGAT**AAGCTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index11	CAAGCAGAAGACGGCATACGAGAT**GTAGCC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
rev_index12	CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGTTGACATCTGCGGTAGCTG
F_adapter_GFPBC	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNGGCCATCAAGCTTATCGATACC
R_adapter_GFPBC	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNCTGATCAGCGAGCTCTAGTCG
NHP GAPD F	TGCACCACCAACTGCTTAGC
NHP GAPD R	GGCATGGACTGTGGTCATGAG
K9 GAPDH F	TGTCCCCACCCCCAATGTATC
K9 GAPDH R	CTCCGATGCCTGCTTCACTACCTT

Supplemental Table 2. Primers used in the study.