

1 **FIGURE LEGEND**

2 Supplementary Figure 1. IHC of subretinally injected rAAV2i8 and rAAV2
3 capsids. Both capsids showed the majority of transduction in the ONL and RPE,
4 with fewer transduced cells of the INL and RGC. Staining for rhodopsin indicated
5 that transduction occurred in rods for both rAAV2 and rAAV2i8 capsids (a).
6 Transduction of rod bipolar cells is shown by PKC-alpha co-localization
7 (arrowhead, b). Co-staining with glutamine synthetase indicate Muller glia are
8 transduced by rAAV2i8 (arrowhead, c). Scale bar = 50 μ m. RGC, retinal ganglion
9 cell layer; INL, inner nuclear layer; ONL, outer nuclear layer.

10

11 Supplementary Figure 2. Intravitreal delivery of high-titer rAAV2 capsids resulted
12 in transduction only in the presence of HS binding. Fundus images of eyes
13 injected with 2×10^9 vg rAAV2 or rAAV2-R585E vector were collected at 8 weeks
14 following delivery. Transduction with rAAV2-R58E is absent, but rAAV2 shows a
15 diffuse pattern that does not accumulate in any particular pattern.

16

17 Supplementary Figure 3. Subretinal transduction of rAAV2 capsid variants mixed
18 with vitreous to confirm vitreous did not inhibit transduction. Vector solution was
19 preincubated with equal volume of vitreous collected from mouse eyes for 1 hour
20 before being injected. Transduction of RPE was seen with both rAAV2 and
21 rAAV2-R585E, although the transduction of RGC is evident for rAAV2
22 (arrowhead).

23

24

25 Supplementary Figure 4. Staining of HS in the retinas of multiple animal models.
26 Retinas of mouse, rabbit, non-human primate, and human are shown. Each
27 animal retina has been stained with secondary antibody only (without antibody)
28 and with an antibody specific to HSPG (with antibody). Intense staining is seen at
29 the ILM in all animal species and is not present in the negative control. Scale bar
30 = 50 μm . RGC, retinal ganglion cell layer; INL, inner nuclear layer; ONL, outer
31 nuclear layer

32

33 Supplementary Figure 5. Analyses of HS-binding variants of rAAV6 eight weeks
34 after intravitreal delivery. Images show typical fundus and fluorescence of eyes
35 treated with rAAV6 capsid or rAAV6-K531E capsid at a low titer of 5×10^7 vg.
36 Punctate expression around the retinal vessels could only be seen in eyes
37 injected with rAAV6. Quantification of fundus images did not indicate
38 significance.

39

40 Supplementary Figure 6. *In vitro* competition assay using soluble heparin to
41 block the transduction of rAAV of HEK293 cells. Viruses were incubated with
42 increasing doses of soluble heparin and applied to cell culture at a multiplicity of
43 infection of 10,000 vg per cell. rAAV2 displayed a dose-dependent decrease in
44 transduction which was not observed with either rAAV1 or rAAV1-E531K. The
45 amount of transduction of rAAV1-E531K was lower than rAAV1 in all conditions.
46 Error bars shown as SEM.

47

48 Supplementary Figure 7. Analyses of HS-binding variants of rAAV8 eight weeks
49 after intravitreal delivery. Images show typical fundus and fluorescence of eyes
50 treated with rAAV8 capsid or rAAV8-E533K capsid at a titer of 1×10^8 vg. Hazy
51 expression could only be detected with HS-binding rAAV8-E533K capsid.
52 Quantification of fundus images show error bars as the SEM and trend toward
53 significance ($p < 0.055$).

54

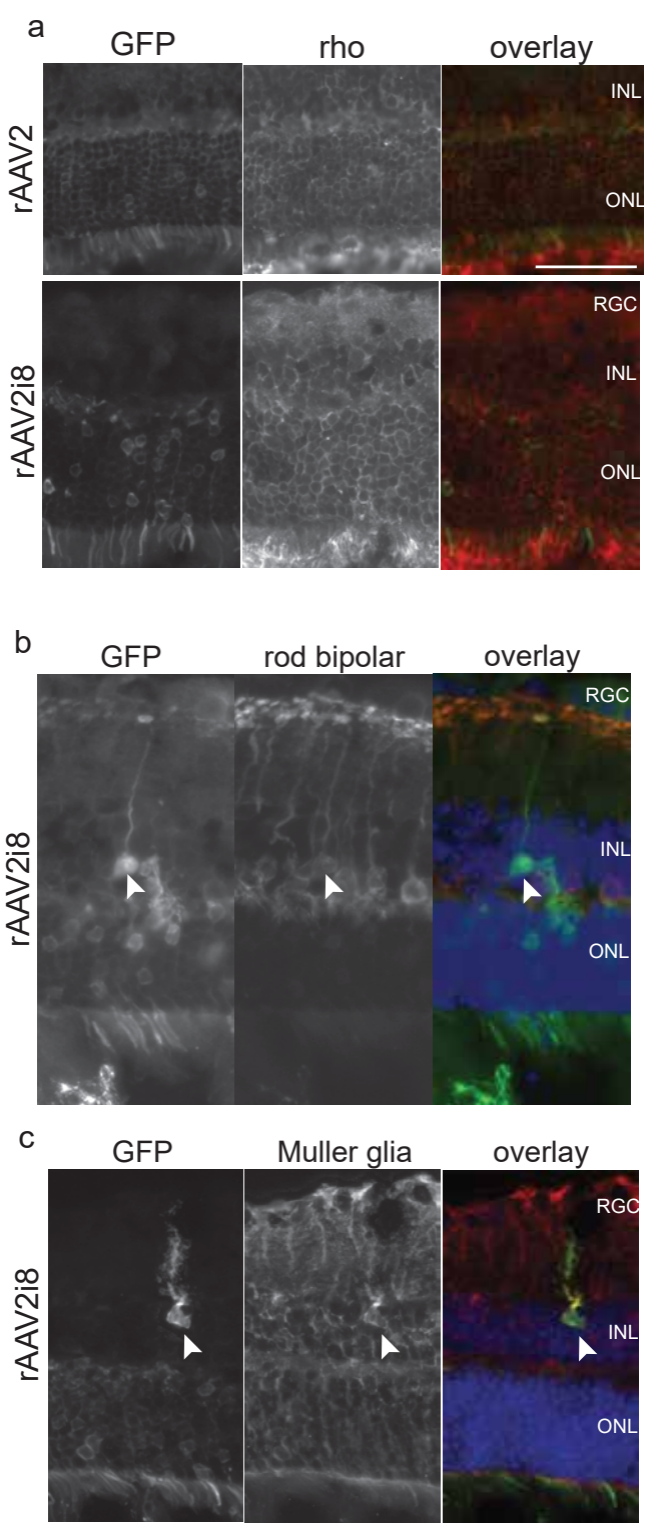
55 Supplementary Figure 8. FISH detection of retinas injected with AAV serotypes
56 and their HS-binding mutants three days after injection. The detection reaction
57 was carried out twice and image levels were adjusted equally to enhance weak
58 labeling. Transgenes found in the retina were more abundant with eyes injected
59 with the HS-binding motif compared to their natural serotypes. rAAV8-E533K
60 capsid could be seen accumulating at the ILM in addition to its presence in the
61 retina. PBS-injected retinas had minimal background signal. Scale bar = 20 μm .
62 RGC, retinal ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear
63 layer.

64

65 Supplementary Figure 9. The transduction of rAAV2.5 is due to the residue
66 mutation at position 265. The single amino acid mutant of rAAV2-265D was
67 tested for intravitreal delivery at 8 weeks post-injection. The transduction by this
68 single point mutant was similar to that of rAAV2.5, where transduction along
69 retinal vessels is prominent.

70

71 Supplementary Figure 10. Galactose binding alone on rAAV capsid does not
72 transduce the retina following IVit delivery. Fundus images of rAAV9 showed
73 hazy expression around the retina, while HS-deficient rAAV2i8G9 no longer
74 transduced the retina (a). The staining for galactose was carried out with
75 fluorescein-labeled lectin and showed the strongest expression in the ONL (b).
76 Scale bar = 20 μ m. RGC, retinal ganglion cell layer; INL, inner nuclear layer;
77 ONL, outer nuclear layer.

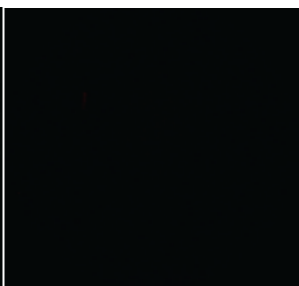
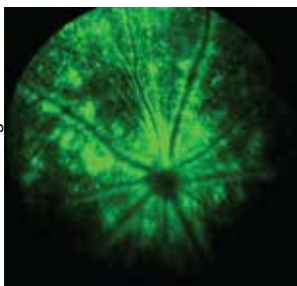


Supp. Fig. 1

AAV2

AAV2-R585E

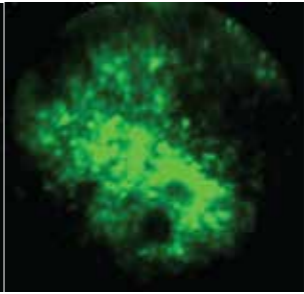
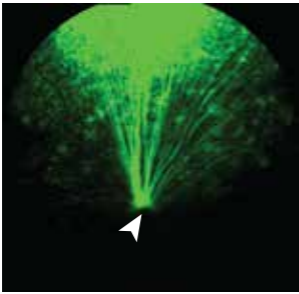
2E9 vg

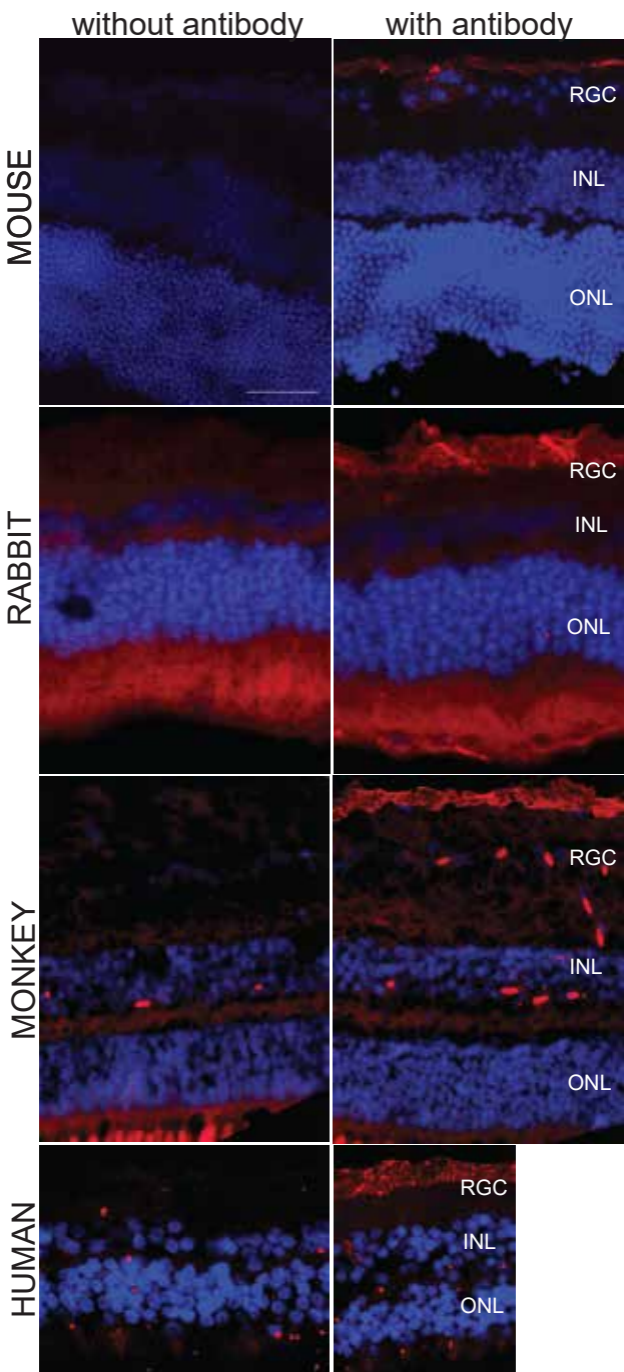


AAV2

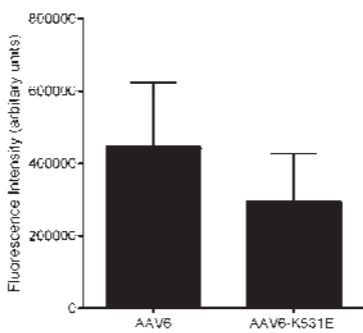
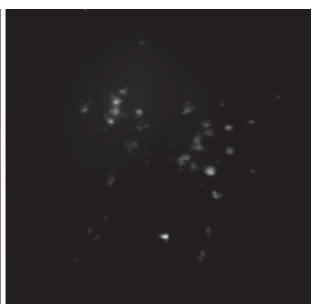
AAV2-R585E

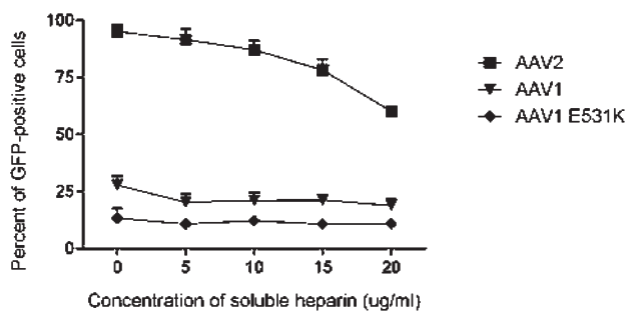
AAV mixed with vitreous





Supp. Fig. 4

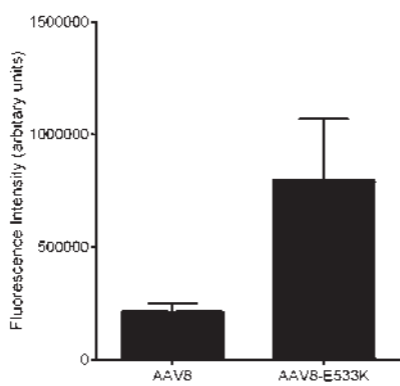




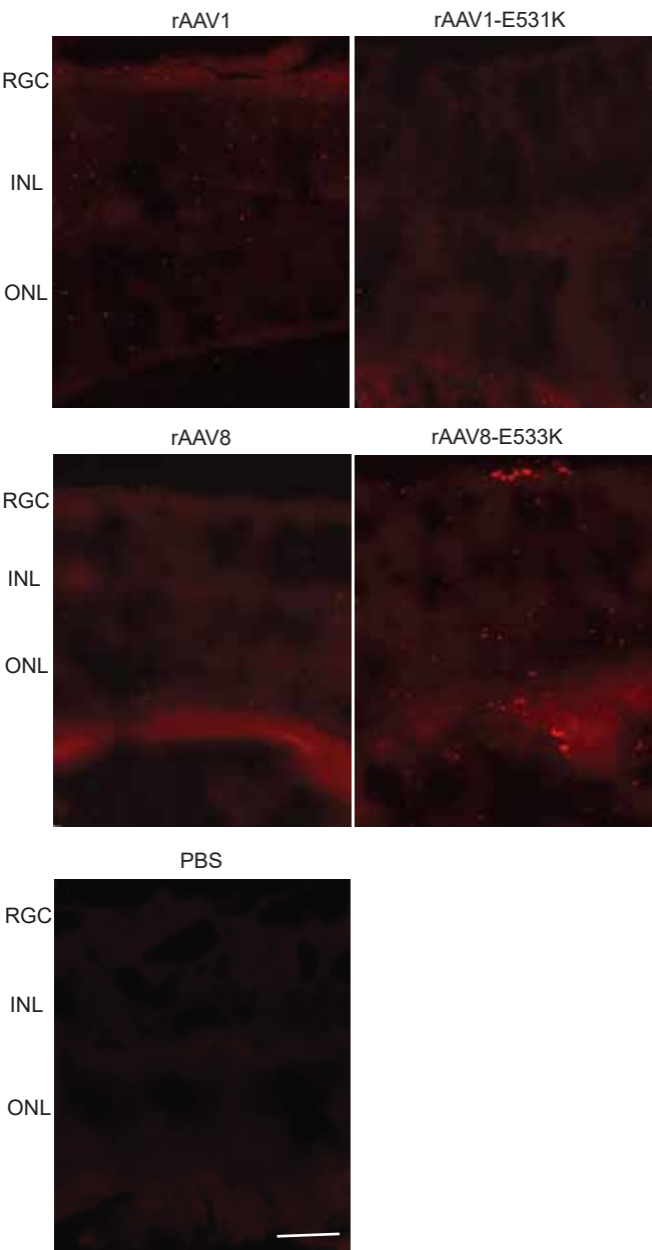
rAAV8



rAAV8-E533K

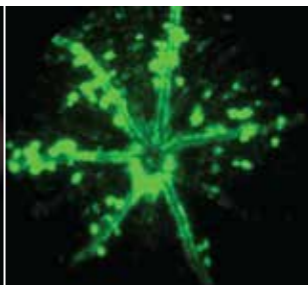
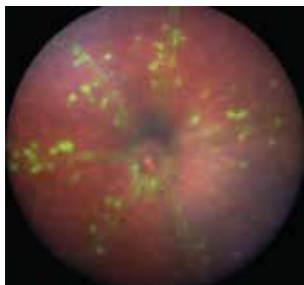


Supp. Fig. 7



Supp. Fig. 8

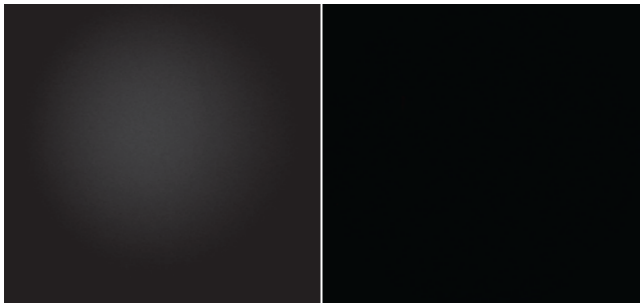
rAAV2-265D



a

rAAV9

rAAV2i8G9



b

No antibody

Galactose

