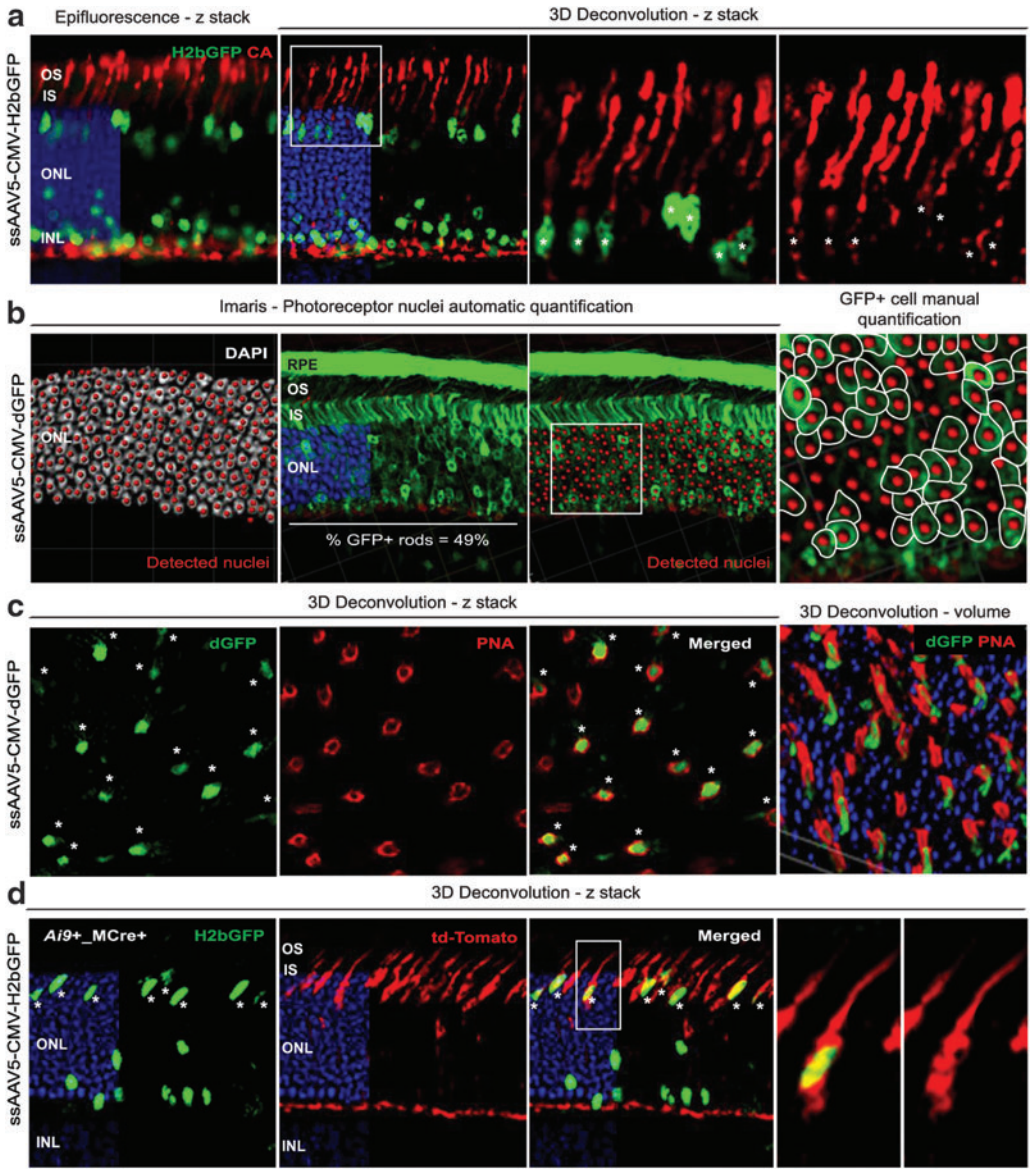
Supplementary Data



Supplementary Figure S1. Quantification of cone and rod transduction. **(a** and **b)** For each group of injected mice, quantification of cone and rod transduction was first performed on retinal sections after immunostaining with cone arrestin (CA; *red*). Images were acquired using epifluorescence microscopy with deconvolution for confocal-like image quality. Briefly, images were recorded at different focal plans (z-stacks) and 3D deconvolved in order to eliminate out-of-focus signals from separate objects that would have overlapped and could have been misinterpreted as colocalization. **(a)** Shown is a representative image of a mouse injected with AAV2/5-CMV-*H2bGFP* before and after 3D deconvolution. First panel shows one of the focal planes that are part of the z-stack series that is acquired by epifluorescence. Panels 2–4 show same focal plane after 3D deconvolution of the z-stack series. Panels 3–4 show higher magnification of the boxed area in panel 2, with panel 4 showing only red signal. Cone quantification was performed in one selected plane of the z-stack series for each image, based on the colocalization of the GFP signal (*green*) with the cone arrestin marker (*red*; usually at the level of cell nuclei: *asterisks* mark cones). **(b)** Shown are images from a mouse injected with AAV2/5-CMV-*dGFP* exemplifying the images obtained after processing the epifluorescence z-stack series with the Imaris software in order to determine rod transduction efficiency. Rod quantification was performed within the same focal plane of the deconvolved z-stack series by manually counting the number of total GFP^+ PRs (*green: white circles* in panel 4) and the number of GFP^+ cones (as described above) per image, and by determining through an automated counting algorithm the total number of PR nuclei in the ONL of the same image using the Imaris software (*red dots* in panels 1, 3, and 4). Rod transduction was then calculated as an average per eye and per group by determining the ratio of the number of total GFP^+ PRs – number of GFP^+ cones to the total number of PR nuclei per image (panel 1, *red dots*). Panel 4 shows higher magnification of the boxed area in panel 3. **(c** and **d)** Cone transduction was also quantified using two additional methods. **(c)** For the groups of mice injected with the AAV-dGFP or AAV-*eGFP* vectors, cone quantification was also performed on retinal flat mounts stained with PNA. Images were recorded at different focal plans (z-stacks), 3D deconvolved, and cone quantification was performed at the level of the cone segments, where GFP fluorescence (*green*) in cones was unambiguously surrounded by PNA staining (*red*). Shown is a representative focal plane of the deconvolved z-stack series at the level of the cone segment (panels 1–3) and a 3D reconstruction of the acquired series (panel 4). Images are from a mouse injected with AAV2/5-CMV-*dGFP*. **(d)** AAV-*H2bGFP* vectors were also evaluated in *Ai9*$^+$*_MCre*$^+$ mice, which express CRE-mediated td-Tomato (*red*) throughout the entire cone cell body. Shown is a representative focal plane of a z-stack series after 3D deconvolution from a mouse injected with AAV2/5-CMV-*H2bGFP*. The last two panels show a higher magnification of the boxed area in the third panel. *Asterisks* indicate GFP^+ cones. INL, inner nuclear layer; GCL, ganglion cell layer; ONL, outer nuclear layer; ss, single-stranded.