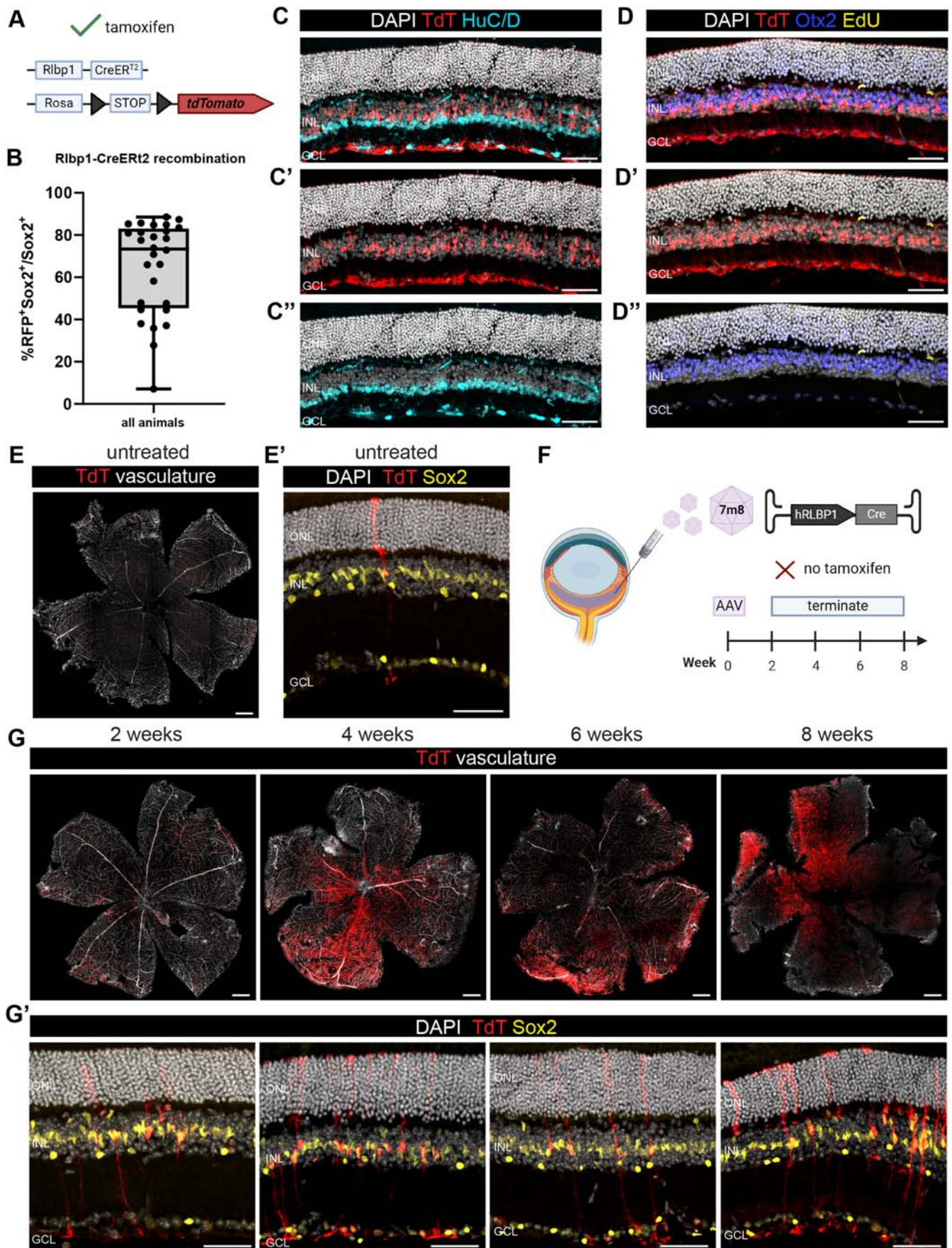


Expanded View Figures

Figure EV1. Assessment of transgenic lineage tracer mouse line and AAV kinetics in vivo.

(A) Schematic of tamoxifen-inducible transgenic mouse line for MG-specific expression, (B) bar plot of min to max quantification of recombination efficiency counted as percentage ratio of RFP⁺Sox2⁺ cells over all Sox2⁺ where each dot is a biological replicate (error bar: mean plus standard deviation), (C–C'') fluorescence images of Rbp1-CreERT2 x LSL-TdT retina cross-sections after tamoxifen showing DAPI-stained nuclei in white, TdT in red and HuC/D in cyan, (D–D'') fluorescence images of Rbp1-CreERT2 x LSL-TdT retina cross-sections after tamoxifen showing DAPI-stained nuclei in white, TdT in red, Otx2 in blue and EdU in yellow, (E) fluorescence image of an untreated retina flatmount with TdT in red and vasculature in white, (E') representative retina cross-section of untreated retina with TdT in red and Sox2 in yellow (F) schematic of experimental design, (G) fluorescence images of retina flatmounts for each timepoint with TdT in red and vasculature in white, (G') representative retina cross-sections from timepoints in (G) with DAPI-stained nuclei in white, TdT in red and Sox2 in yellow; scalebars for (C–C'', D–D'', G', E'): 50 μ m, for (E, G): 500 μ m, ONL outer nuclear layer, INL inner nuclear layer, GCL ganglion cell layer.



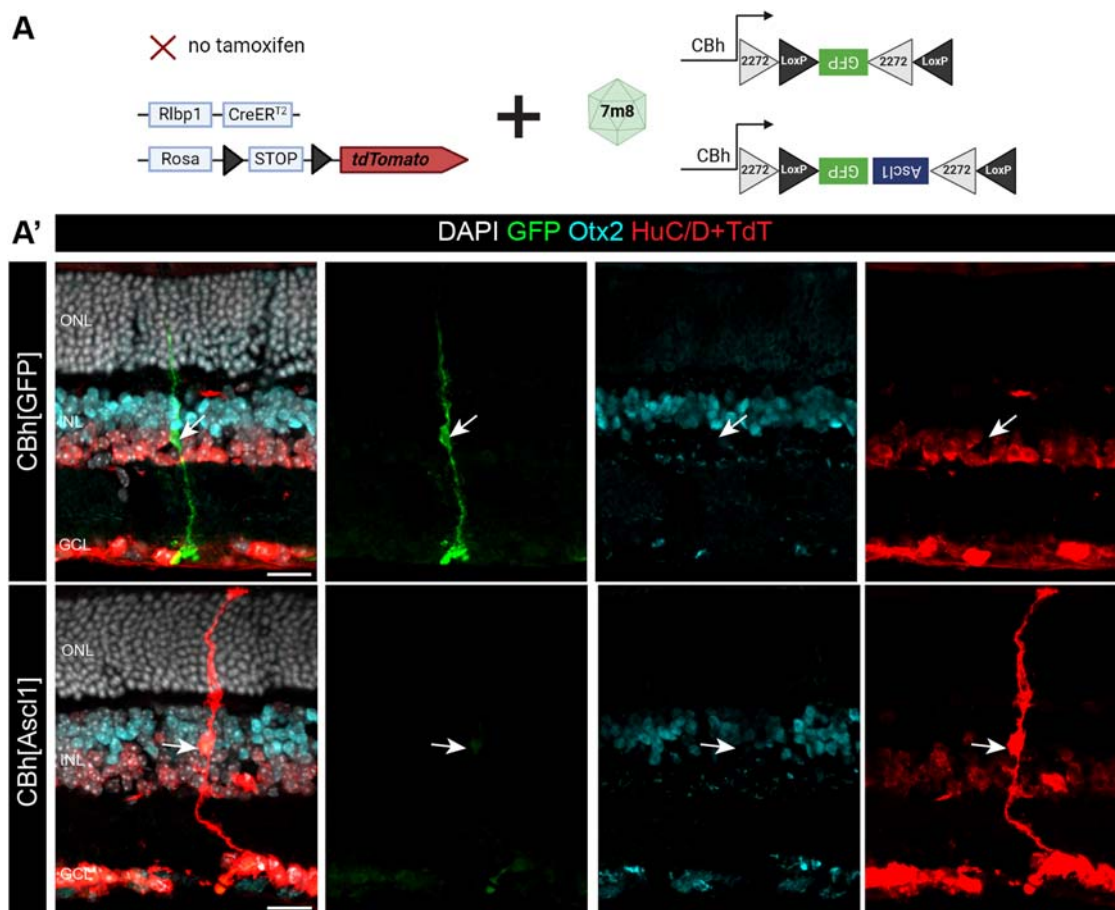
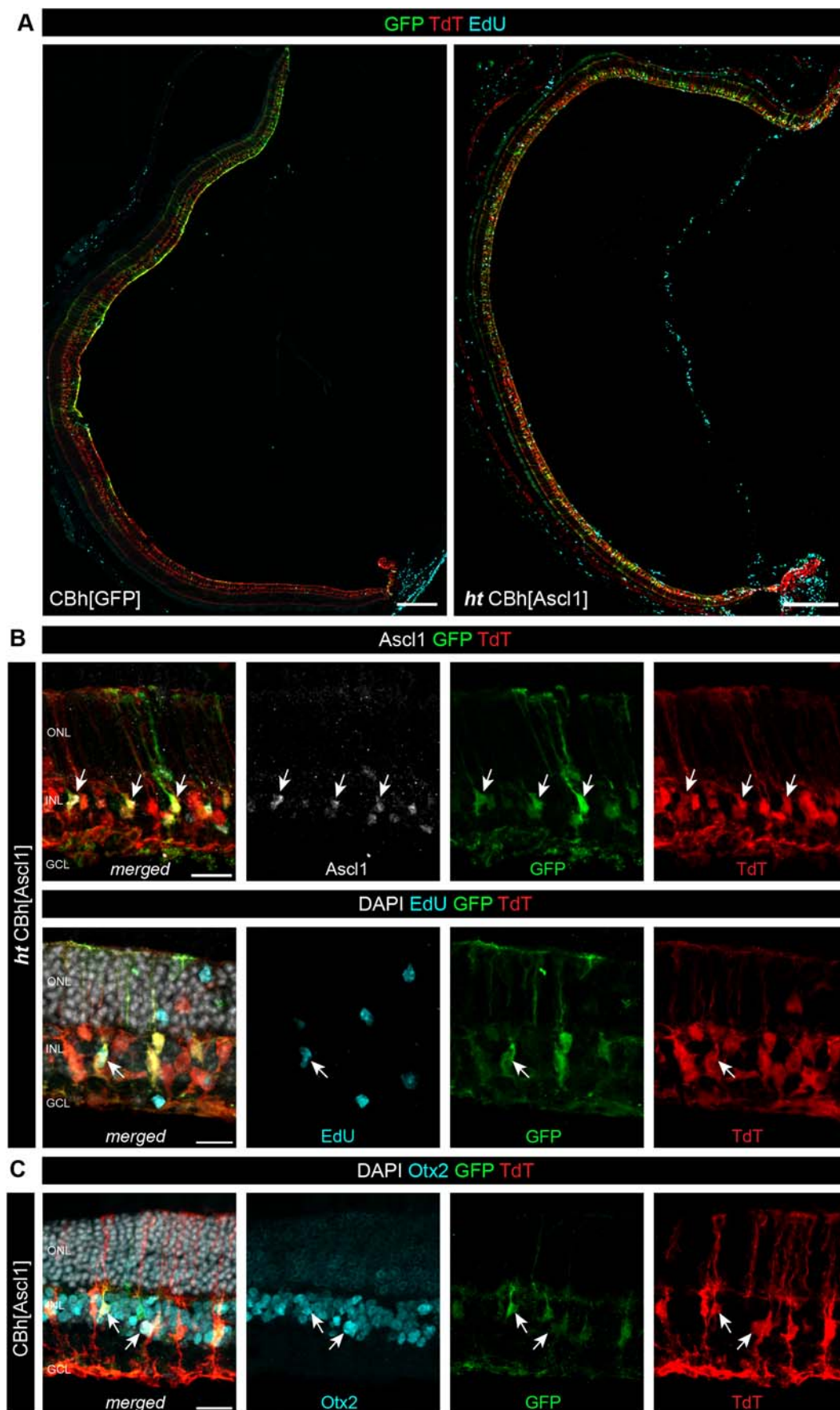


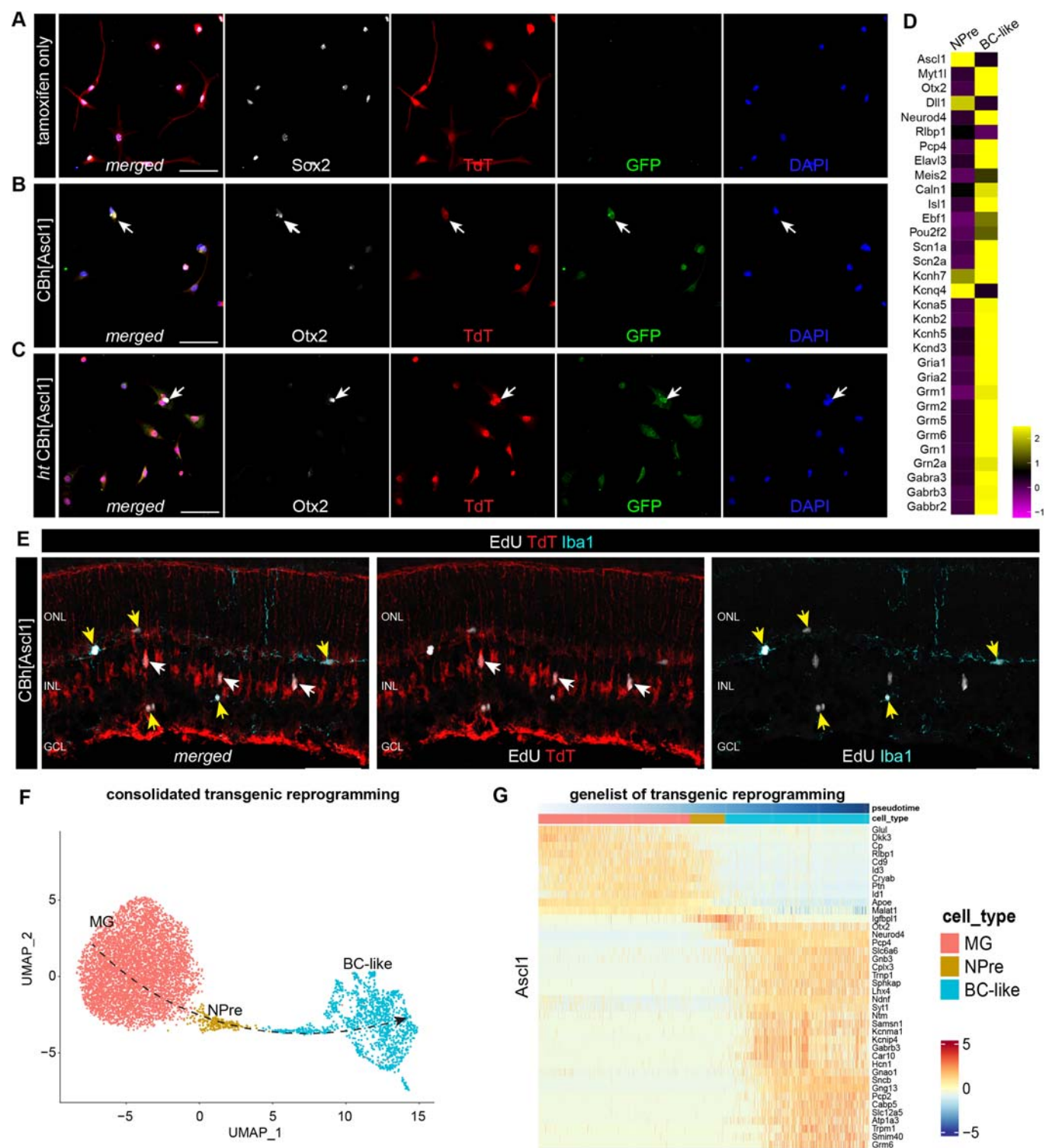
Figure EV2. Control experiments to assess Cre-dependent vector expression.

(A) Schematic of transgenic lineage tracer without tamoxifen administration and the FLEX vectors administered to assess traces of DNA recombination during AAV production, (A') fluorescence images of stained tissue post CBh-FLEX[GFP] and CBh-FLEX[Ascl1-GFP] injection, with white arrows indicating cells where vector or lineage tracer recombination occurs without tamoxifen in MG cells that are not Otx2 or HuC/D positive.



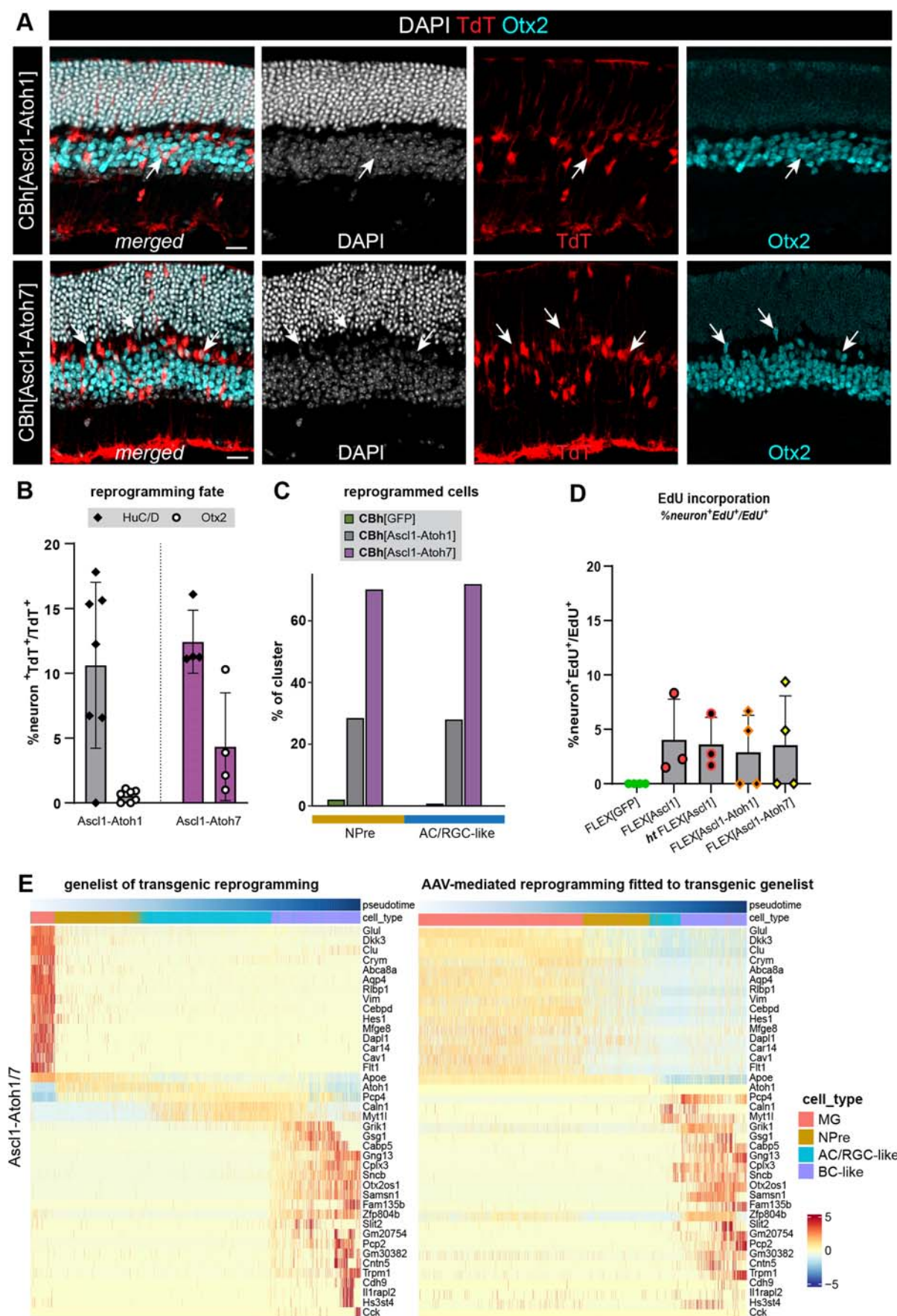
**Figure EV3. AAV transduction and validation of protein markers.**

(A) Fluorescence images of Rbp1-CreERT2 x LSL-TdT central retina cross-sections showing AAV-transduced cells in green, TdT in red and EdU proliferating cells in cyan for control and reprogramming vector conditions, (B) fluorescence images after reprogramming with ht AAV/CBh-FLEX[Ascl1], top row: transduced cells (white arrowhead) co-labeled with Ascl1 in white, GFP in green and TdT in red; bottom panel: transduced cells (white arrowhead) co-labeled with EdU in cyan, GFP in green and TdT in red, (C) fluorescence images after reprogramming with AAV/CBh-FLEX[Ascl1] showing transduced cells (white arrowhead) co-labeled with Otx2 in cyan, GFP in green and TdT in red; scale bar for (A): 200 μ m, (B, C): 20 μ m.



**Figure EV4. Transcriptional analysis of AAV-mediated reprogramming.**

(A–C) Fluorescence images of sorted lineage-traced cells on coverslips 24 h post FACS split by condition (white arrows indicate reprogrammed cells) showing merged and single channels of DAPI in blue, GFP vector reporter in green, TdT lineage tracer in red and glial marker Sox2 or bipolar marker Otx2 in white, (D) heatmap of average gene expression for selected neuronal genes in clusters NPre and MG-derived neurons following AAV-mediated reprogramming, (E) fluorescence images of retinal cross-section with proliferating lineage-traced MG (white arrows) and microglia (yellow arrows) with TdT in red, Iba1 in cyan and EdU in white, (F) UMAP of consolidated scRNA-seq data of Ascl1-mediated neurogenesis from transgenic animals (Glast-CreERT2 x LSL-tTA x tetO-Ascl1-GFP), (G) heatmap of top 40 differentially expressed genes across pseudotime trajectory from glial cell fate to neuronal cell fate based on scRNA-seq data from (F); scale bar: 50 μ m, ONL outer nuclear layer, INL inner nuclear layer, GCL ganglion cell layer.



◀ **Figure EV5. AAV-borne *Ascl1*-*Atoh1/7* expression induces neurogenesis that phenocopies transgenics.**

(A, A') Fluorescence images of reprogrammed cells (white arrows) per condition showing merged and single channels of DAPI in white, TdT in red and Otx2 in cyan, (B) bar plot of MG reprogramming to distinct neuronal fates (HuC/D or Otx2) after AAV-mediated *Ascl1*-*Atoh1* or *Ascl1*-*Atoh7* expression counted as a percentage ratio of neuron⁺TdT⁺ over all TdT⁺ cells with each dot being a biological replicate, (C) bar plot of the percentage that each vector treatment contributed to the formation of reprogrammed clusters NPre and MG-derived AC/RGC-like neurons where each column represents a color-coded sample. (D) Bar plot of Edu incorporation counted as a ratio of neuron⁺Edu⁺ over all Edu⁺ cells with each dot being a biological replicate, (E) heatmap of top 40 differentially expressed genes across pseudotime trajectory from glial cell fate to neuronal cell fate based on scRNA-seq data from transgenic experiments, followed by heatmap of gene expression in scRNA-seq data from AAV experiments that follows the genelist generated from consolidated transgenic data for *Ascl1*-*Atoh1/7* reprogramming; error bars for bar plots: mean plus standard deviation, statistical significance based on ordinary one-way ANOVA with Tukey's multiple comparisons test (significance $P < 0.05$, * = 0.05, ** = 0.01, *** = 0.001, **** = 0.0001, no significance detected); scale bar for (A-A'): 20 μ m.