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Supplemental Information

Preclinical Evaluation of a Cell-Based Gene Therapy Using the Sleeping Beauty Transposon System in Choroidal Neovascularization

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Figure S1

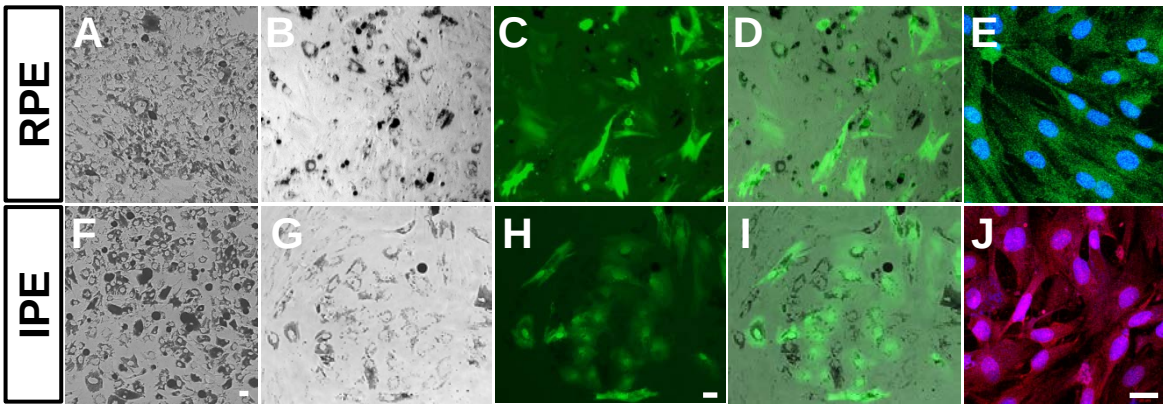

Figure S1. Phase contrast and fluorescence micrographs of rat primary RPE and IPE cells phenotype in culture. (A, F) Phase contrast micrograph of rat RPE cells and IPE cells respectively in culture showing the typical cobblestone morphology of pigment epithelial cells. (B-D and G-I) RPE and IPE cells after transfection with pFAR4-ITRs-CAGGS Venus plasmid. RPE and IPE preserve the morphology after transfection. B and G show bright field images; C and H images confirm fluorescent Venus expression; D and I images are a merged micrograph from bright field and fluorescent images in RPE and IPE cells, respectively. (E) rat RPE cells are positive for RPE65 (green) and (J) IPE cells are positive for cytokeratin 18 (CK18) (red). Nuclei are labeled with DAPI (blue). Scale bar: 20 μm. Abbreviations: RPE (retinal pigment epithelial cells), IPE (iris pigment epithelial cells).

Figure S2

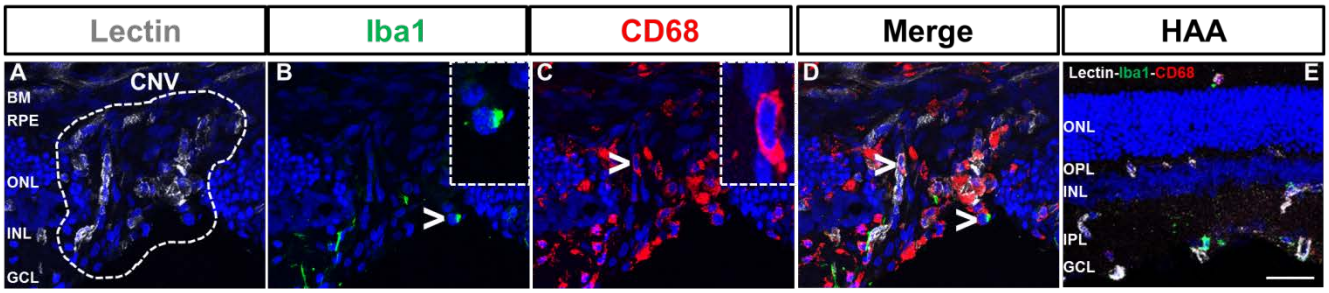

Figure S2. Confocal microscopic images displaying the expression of Iba1 and CD68 in rat CNV areas injected with transfected RPE and IPE cells. (A) New vessels positive to lectin marker (grey). (B) Microglial cell immunolabeled with Iba1 (green) in CNV area (arrow). (C) Macrophage cell immunolabeled with CD68 (red) in CNV area (arrow). (D) Merged images. (E) Healthy adjacent areas near to CNV areas immunostained with Iba1 and CD68.

Figure S3

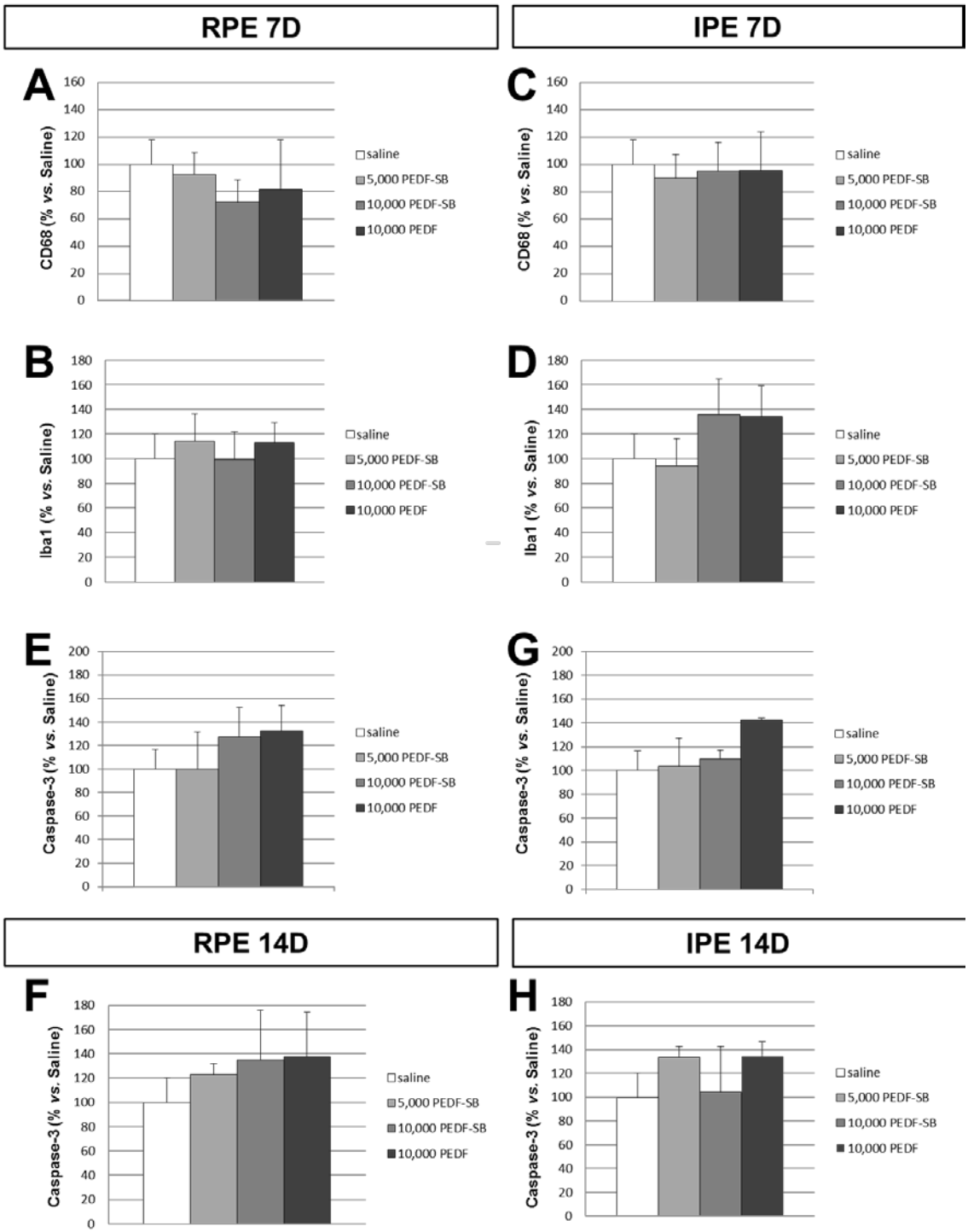

Figure S3. No changes in the analysis of the immunofluorescence intensity with CD68 and Iba1 at 7 days post-laser and caspase-3 immunoreactivity at 7 and 14 days post-laser in CNV areas in all groups studied. Fluorescence intensity measurement of CD68 (A and C), Iba1 (B and D) and caspase-3 (E-H) in rats injected with tRPE (A, B, E and F) and tIPE (C, D, G and H) (5,000 RPE-PEDF-SB, 10,000 PEDF-SB and 10,000-PEDF cells) vs. saline injection at 7 days postlaser (A-D, E and G) and 14 days post-laser (F and H). Data are presented as percentage mean ± SEM (n=4). Abbreviations: PEDF (pigment epithelium derived factor), tIPE (transfected iris pigment epithelial cells), tRPE (transfected retinal pigment epithelium cells).

Figure S4

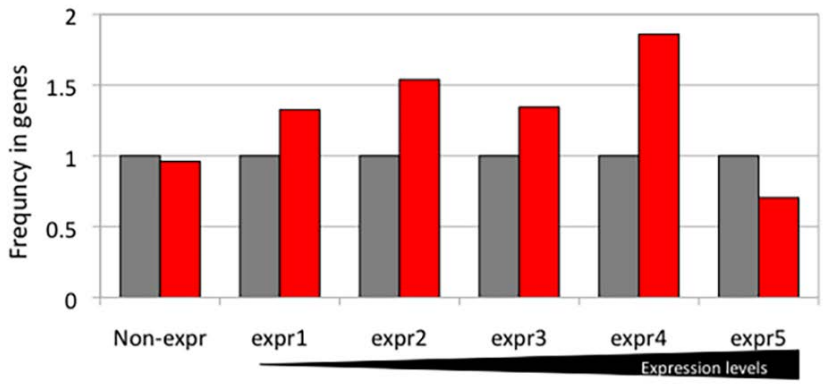

Figure S4. Correlation between integration rates and transcriptional activity of the insertion sites. The numbers of the x axis stand for groups of transcription units of increasing activity in rat retinal cells. Enrichment or depletion of *SB* insertions (in red) are shown in relation to random expected frequency (in gray), shown as 1.