

Supplemental Information

Long-Term PEDF Release in Rat Iris and Retinal

Epithelial Cells after *Sleeping Beauty*

Transposon-Mediated Gene Delivery

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Figure S1.

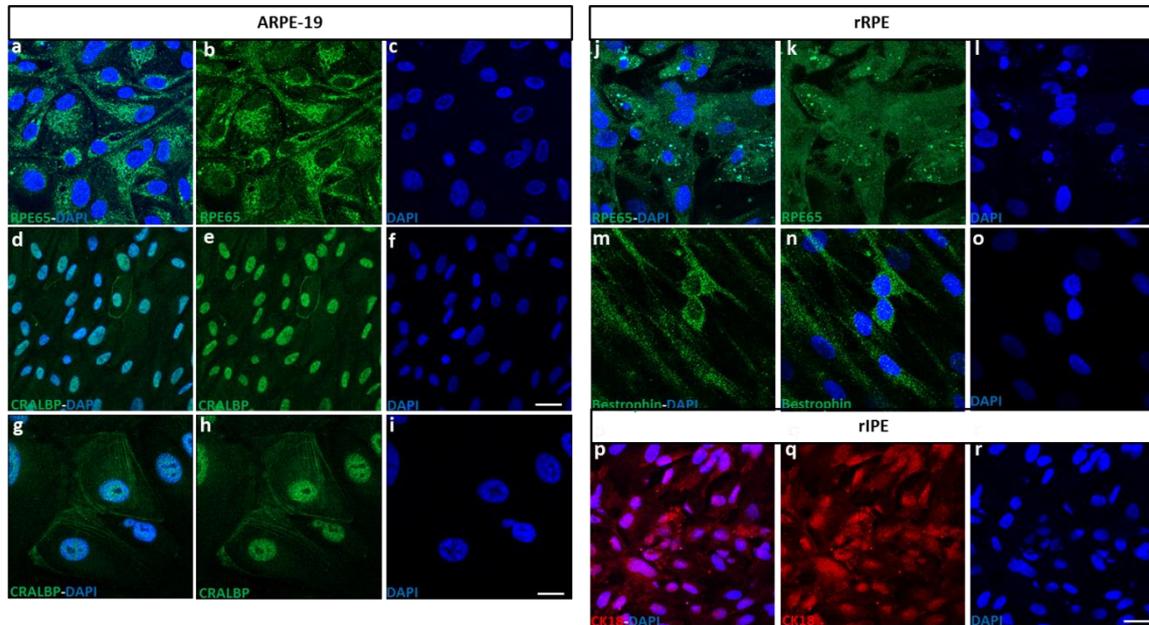


Figure S1. ARPE-19, RPE and IPE cells phenotype in culture. ARPE-19 cells were positive for RPE65 (a-c, green) and CRALBP (d-i, green), rat RPE cells were positive for RPE65 (j-l, green) and Bestrophin (m-o, green) and rat IPE cells were found to be positive for cytokeratin 18 (CK18) (p-r, red). Nuclei were stained with DAPI (blue). Scale bar (a-c and j-r), 20 μ m. Scale bar (d-f), 10 μ m. Scale bar (g-i), 40 μ m.

Figure S2

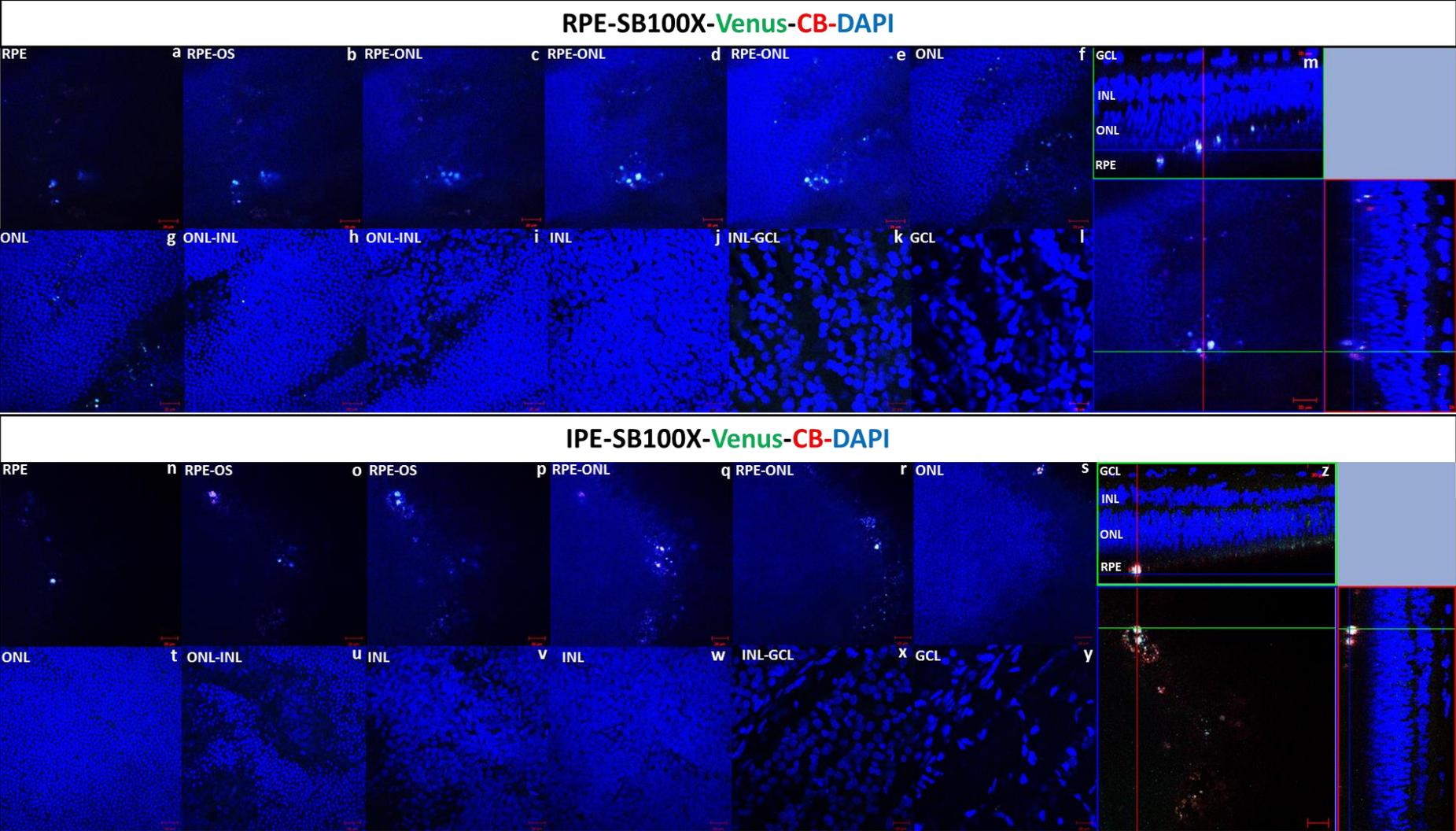


Figure S2. Fluorescence confocal images of the primary cells injected. Representative images of the rat RPE (a-m) and IPE (n-z) injected and transfected cells with pFAR4-SB100X-Venus (green) plasmid into the subretinal space. Stacks from RPE and GCL images that provide a wide 3D-view of the transfected cells. Figures m and z correspond to an orthogonal projection of RPE and IPE injected cells. Before the injection, cells were stained with an organelle and plasmatic membrane dye, CellBrite (red). The flat mounts obtained were counterstained with DAPI (blue). Abbreviations: CB (CellBrite), RPE (Retinal Pigment Epithelium), OS (Outer Segment), ONL (Outer Nuclear Layer), INL (Inner Nuclear Layer), GCL (Ganglion Cell Layer). Scale bar, 20 μ m.

Figure S3.

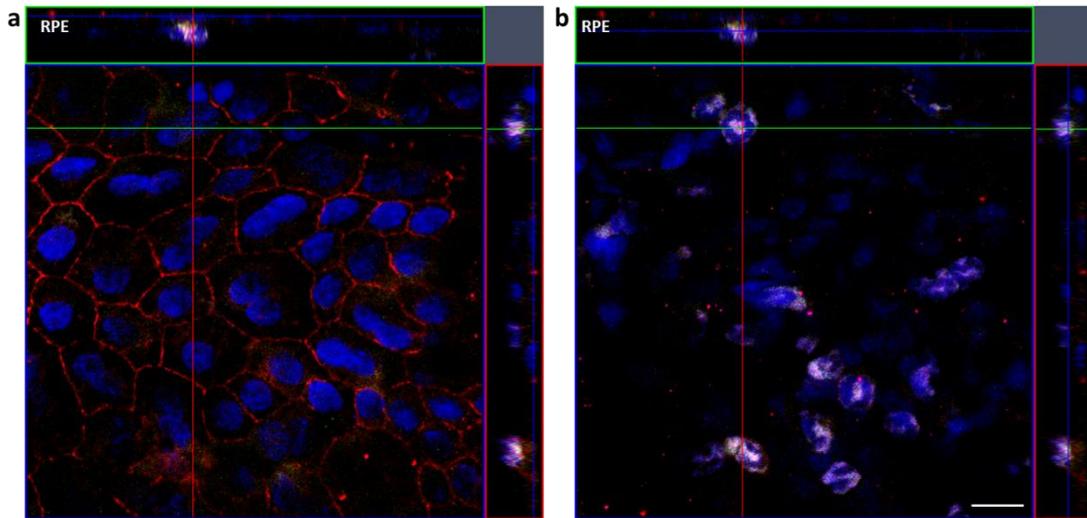


Figure S3. Localization of transplanted RPE transfected with the Venus gene. Both images correspond to the same sample (a) RPE layer with rat RPE transplanted cells transfected with pFAR4-SB100X-Venus (pink) and stained for ZO-1 (red) (b) the apical side of the RPE layer observed in a. Nuclei were stained for TOPRO-3 (blue). Scale bar, 20 μ m. RPE (Retinal Pigment Epithelium)

Figure S4.

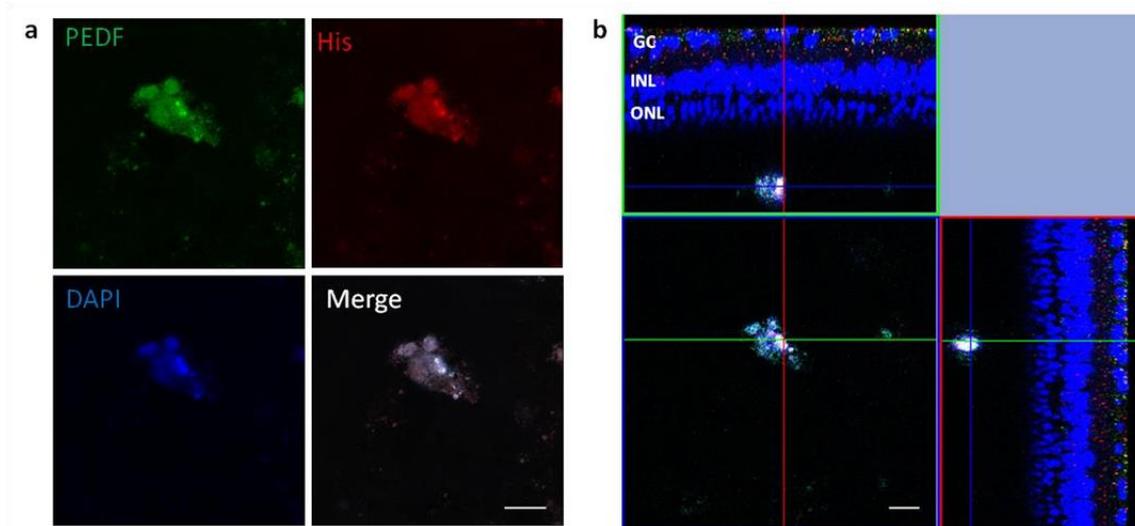


Figure S4. Localization of transplanted cells by PEDF and His double immunostaining. Anti-PEDF and anti-His immunofluorescence was performed in flat mounted retinas 15 days after subretinal transplantation of IPE cells transfected with pFAR4-ITRs CMV PEDF His and pFAR4-CMV SB100x SV40. **(a)** Colocalization of PEDF (green) and His staining (red); Nuclei are stained with DAPI (blue) and in white it is represented the merged image of the other three staining. **(b)** Z-orthogonal image of **a** showing the localization of the transplanted cells in the RPE layer. Scale bar: 20 μm . Abbreviations: GC: Ganglion cells; INL: Inner nuclear layer; ONL: Outer nuclear layer; RPE: Retinal pigment epithelium.

Figure S5.

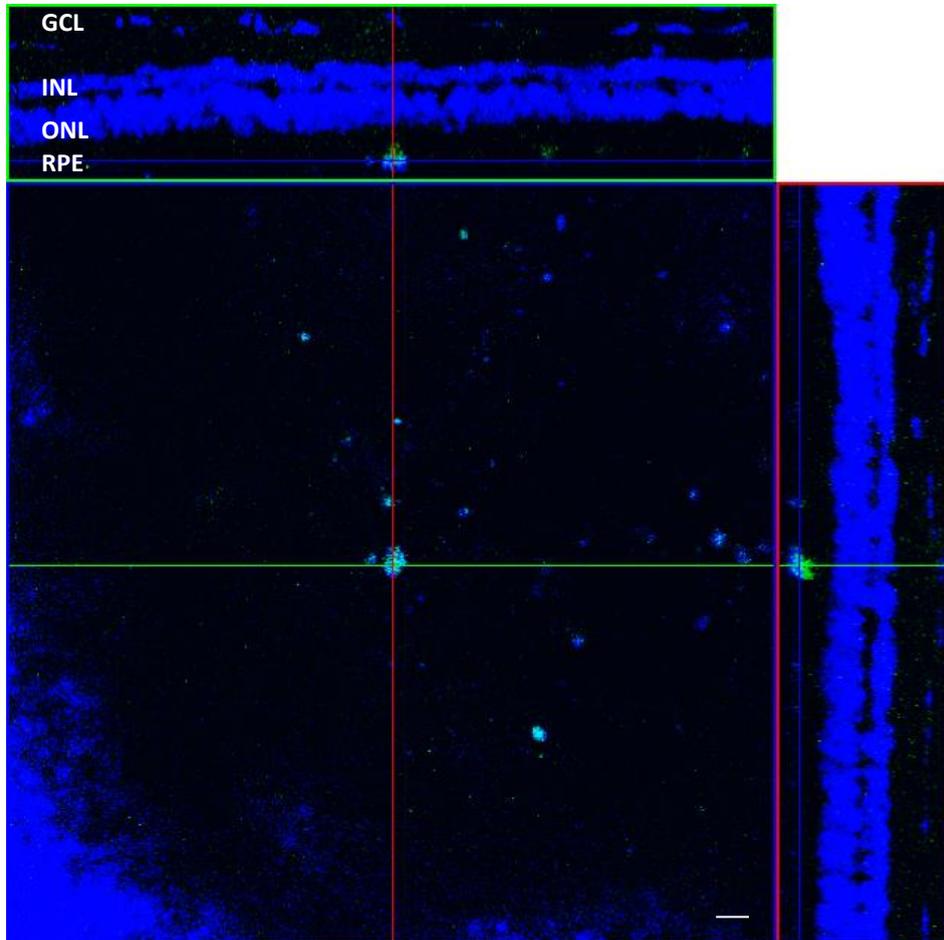


Figure S5. Localization of Venus positive RPE cells 4 months after transplantation. Cells are located above the RPE and producing Venus at least up to 4 months after transplantation. Nuclei were stained with TOPRO-3 (blue). Scale bar: 20 μ m. Abbreviations: GCL: Ganglion cell layer; INL: Inner nuclear layer; ONL: Outer nuclear layer; RPE: Retinal pigment epithelium.