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Supplemental information

Effect of degeneration stage on non-viral tissue transfection of *rd10* retina *ex vivo*

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Supplemental Information

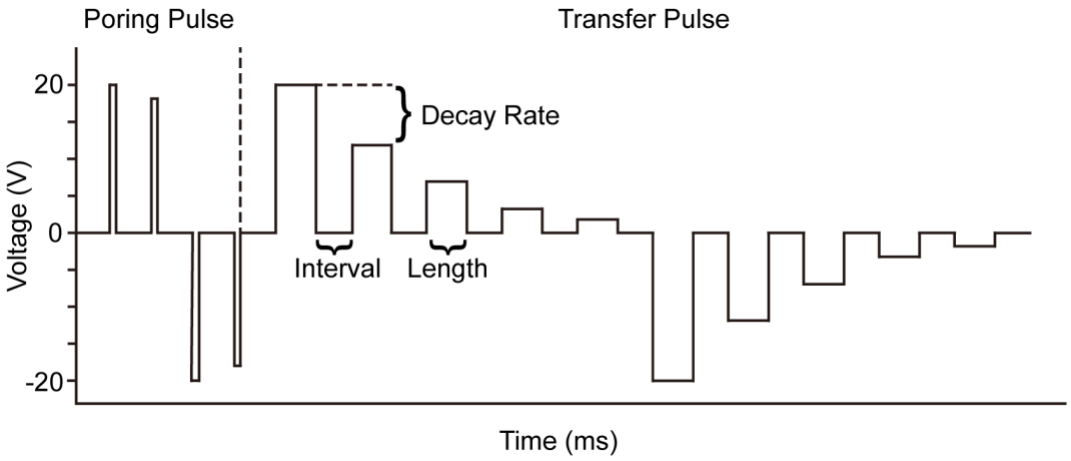

Figure S1. Diagram of output pulses using the NEPA21 Type II Electroporator. The diagram shows the following settings in an electroporation procedure: voltage, pulse length (duration of one transfer pulse), pulse interval (interval between two transfer pulses), number of pulses, decay rate (voltage decay rate of the transfer pulse), and polarity change.

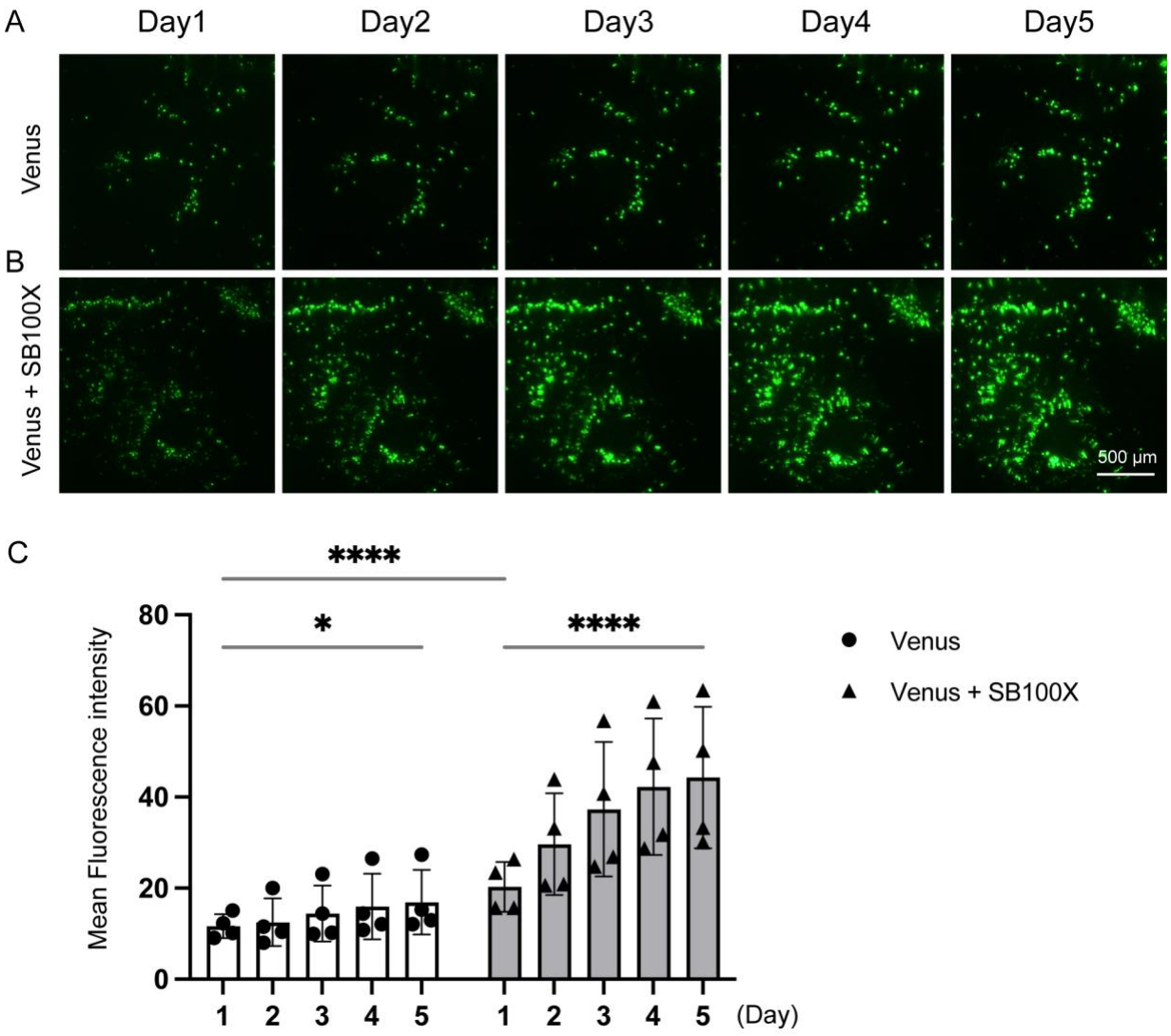

Figure S2. Effect of the *SB100X* transposase on transfection efficiency in P61 *rd10* retinas. Flat-mounted *rd10* retinas were electroporated with **(A)** the transposon plasmid pT2-CAGGS-*Venus* alone or **(B)** the transposon plasmid pT2-CAGGS-*Venus* and the *SB100X* transposase plasmid (ratio 16:1). Transfection efficiency was assessed from day 1 to day 5. Scale bar: 500 µm. **(C)** Quantitative analysis of transfection efficiency, determined by measuring mean fluorescence intensity, showed that co-administration of *SB100X* transposase significantly increased transgene expression compared to controls containing the pT2-CAGGS-*Venus* transposon alone. Furthermore, transgene expression increased over time in both groups (n=4, *p = 0.0409, ****p < 0.0001, using two-way ANOVA with Šídák's multiple comparisons test).

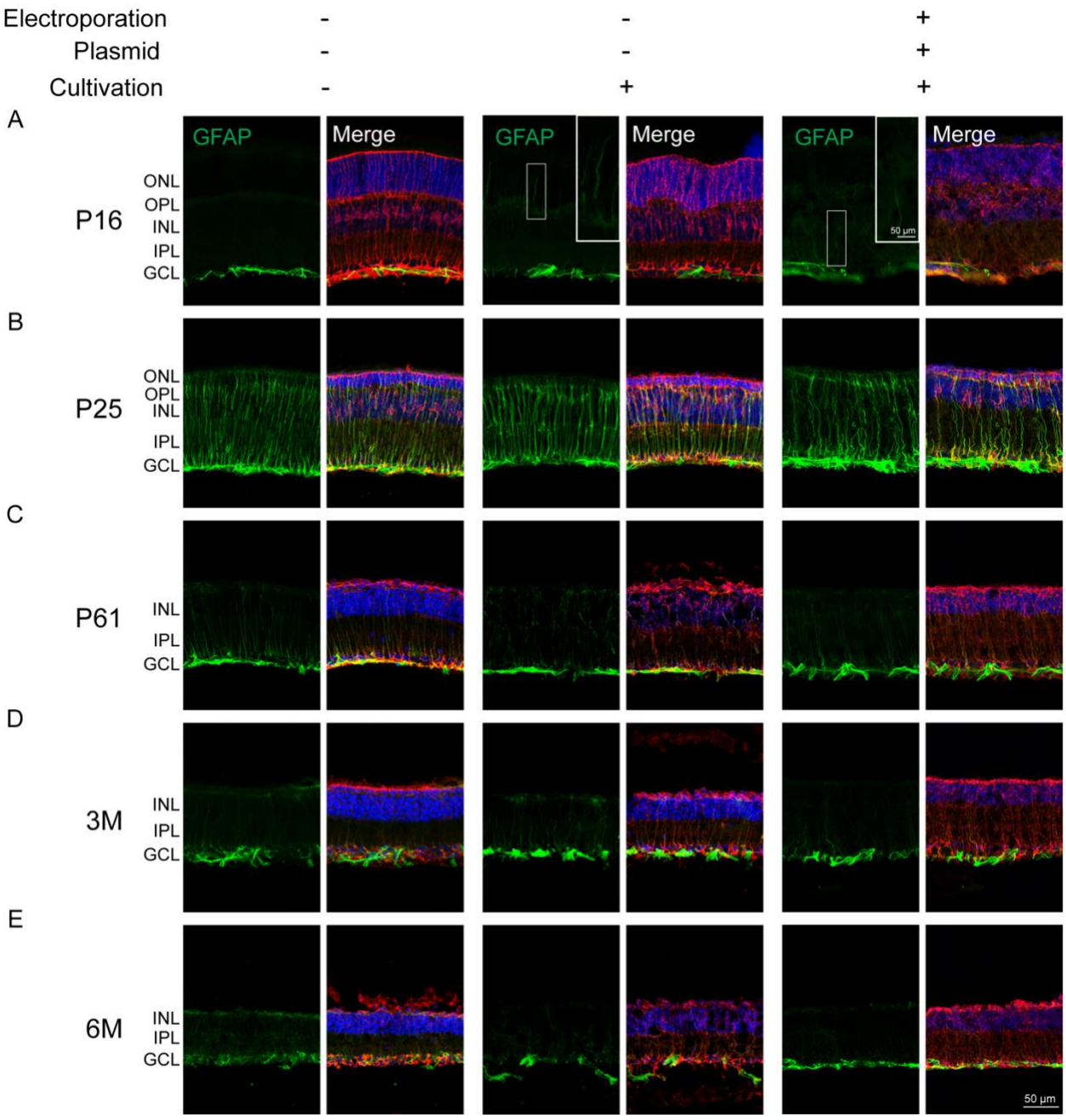

Figure S3. GFAP expression in *rd10* retinas of different ages after transfection and cultivation. The study included three groups, divided into non-transfected and non-cultured, cultured only, and retinas transfected with the pFAR4-CAGGS-*Venus* plasmid and subsequently cultivated. Anti-GFAP stained reactivated Müller cells green. Merged channels include anti-GFAP green, anti-GS labeled Müller cells red, and DAPI-labeled cell nuclei blue. **(A)** In P16 *rd10* retinas, GFAP expression was found in astrocytes in the GCL before transfection and cultivation. After transfection and/or cultivation, GFAP expression was additionally observed in Müller cells, with notable morphological damage, especially in the arrangement of Müller cells (indicated by white frames). **(B)** In P25 *rd10* retinas, high GFAP expression was present in all

Müller cells regardless of the transfection and cultivation procedures. **(C)** In P61 *rd10* retinas, GFAP expression remained positive in Müller cells before and after transfection and cultivation procedures. **(D, E)** In 3M and 6M *rd10* retinas, GFAP expression in Müller cells remained positive before and after transfection and cultivation. 3 retinas were included in each group. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar: 50 μm.