Supplemental information

Transplanted human induced pluripotent stem cells- derived retinal ganglion cells embed within mouse retinas and are electrophysiologically functional

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A) hiPSC-RPCs were analyzed by FACS on day 15 for ki67 and Chx10 markers. B) hiPSC-RGCs were analyzed by FACS on day 35 for RGC-specific markers such as Brn3, RBPMS, SNCG, and CD90. *Indicative of negligible/low presence of markers and we were in the process of finalizing antibodies to be used for analysis. Related to STAR Methods.

### Table S1. Flow-cytometric analysis of patient-derived iPSC for RPCs and RGCs.

| PennID for iPSC Line | Race              | Gender | Ki67  | Chx10 |
|----------------------|-------------------|--------|-------|-------|
| 1) Penn123i-SV20     | European American | Male   | 90%   | *     |
| 2) Penn087i-38-1     | European American | Male   | 100%  | 73.5% |
| 3) Penn019i-136-6    | European American | Male   | 77.5% | 80%   |
| 4) Penn024i-370-3    | African American  | Female | 95.5% | 82%   |

![](image.png)

| PennID for iPSC Line | Race              | Gender | Brn3  | RBPMS | SNCG  | CD90  |
|----------------------|-------------------|--------|-------|-------|-------|-------|
| 1) Penn123i-SV20     | European American | Male   | 71%   | 46%   | *     | *     |
| 2) Penn087i-38-1     | European American | Male   | 49.5% | 4%    | 25%   | 87%   |
| 3) Penn019i-136-6    | European American | Male   | 87%   | 22.5% | 93%   | 85%   |
| 4) Penn027i-40-2     | European American | Male   | 77.5% | 5%    | 6%    | 42%   |
| 5) Penn039i-63-1     | African American  | Female | 85%   | 2.3%  | 78%   | 58%   |
| 6) Penn059i-555-1    | African American  | Female | 83%   | 34%   | 84%   | 91%   |

Table S1. Flow-cytometric analysis of patient-derived iPSC for RPCs and RGCs. A) hiPSC-RPCs were analyzed by FACS on day 15 for ki67 and Chx10 markers. B) hiPSC-RGCs were analyzed by FACS on day 35 for RGC-specific markers such as Brn3, RBPMS, SNCG, and CD90. *Indicative of negligible/low presence of markers and we were in the process of finalizing antibodies to be used for analysis. Related to STAR Methods.
Table S2. **The number of eGFP+ donor hiPSC-RGCs detected per mouse retina.** Following intravitreal injections, SNCG-eGFP+ hiPSC-RGCs were quantified by Ilastik cell density counting software. *Indicative of hiPSC-RGCs detected in a mouse retina that was more than 0.1% (500 cells) of the total donor hiPSC-RGCs injected and hence considered as successful transplantation. Data is represented as 672 +/- 94 hiPSC-RGCs. Related to Figure 1.

| Mouse ID | PennID for iPSC Line | Gender | Number of eGFP+ hiPSC-RGCs Detected per Mouse Retina |
|----------|-----------------------|--------|-----------------------------------------------------|
| 1)       | Penn123i-SV20         | Male   | 1098*                                               |
| 2)       | Penn123i-SV20         | Male   | 1535*                                               |
| 3)       | Penn123i-SV20         | Male   | 816*                                                |
| 4)       | Penn087i-38-1         | Female | 702*                                                |
| 5)       | Penn087i-38-1         | Female | 403                                                 |
| 6)       | Penn087i-38-1         | Female | 787*                                                |
| 7)       | Penn087i-38-1         | Female | 375                                                 |
| 8)       | Penn087i-38-1         | Female | 753*                                                |
| 9)       | Penn087i-38-1         | Male   | 428                                                 |
| 10)      | Penn087i-38-1         | Male   | 1166*                                               |
| 11)      | Penn087i-38-1         | Male   | 282                                                 |
| 12)      | Penn087i-38-1         | Male   | 445                                                 |
| 13)      | Penn087i-38-1         | Male   | 230                                                 |
| 14)      | Penn059i-555-1        | Female | 537*                                                |
| 15)      | Penn059i-555-1        | Female | 525*                                                |

**Average 672**
Figure S1. Transplanted hiPSCs increase firing in response to depolarization-induced by light or current injection. Two-photon images obtained from the single optical slice acquired at the end of the patch-clamp recording session are shown on the left of the figure. EGFP fluorescence from hiPSCs is illustrated on panel A, red fluorescence from the CF 633 filled pipette and targeted cell on panel B, and combined EGFP and CF 633 fluorescence on panel C. The pipette can be seen on the right side of the cell on panels B and C. Targeted cell has a bright yellow color on panel C, the yellowish cell above it was stained with CF 633 during the preceding patching attempt. The scale bar is 50 µm. Traces from panels D and E show an increase in firing upon light stimulation similar to the corresponding traces from Figure 4, but with a much lower baseline firing. Firing rates were calculated using a 200 ms time bin. Traces on panel F illustrate membrane depolarization and action potential firing caused by an injection of depolarizing currents (two current steps of 30 and 40 pA, and the current ramp from 0 to 40 pA). For simplicity, current vs time traces on panel F (1st and 3rd graphs from the top) show command current, not the actual membrane current measured by the amplifier (the latter will have the same amplitude and time course but will also include electrical noise and spiking activity). The membrane voltage from panels E and F wasn’t corrected for LJP, correction can be done by subtracting 15 mV from the reported values. Related to Figure 4.
Figure S2. Example of hiPSC firing triggered by large and mostly spontaneous depolarizations. Two-photon images (single optical slice) on panels A, B, and C show EGFP, CF 633, and combined EGFP+CF 633 fluorescence respectively at the end of the patch-clamp recording. The scale bar is 50 µm. Graphs from panel D show firing (bottom trace, firing rate calculated using 200 ms time bin) triggered by two depolarizations, one aligned with the flash and a spontaneous one (middle trace). The trace at the top shows the time course of the stimulation, colored bars indicate light stimulation. Traces on panel E show membrane depolarization and action potential firing in response to current injections (two steps of 20 and 30 pA and a ramp from 0 to 40 pA). To correct for the LJP 15 mV should be subtracted from the reported membrane voltages. All traces were recorded in the current-clamp mode. Related to Figure 4.
Figure S3. Larger membrane depolarizations caused by repeated stimuli can lead to smaller increases in the firing rate. All traces were recorded in the current-clamp mode from the same cell as that presented in Figure 4. Panels A and B show depolarization and a related increase in the firing rate caused by light exposures. The flash from panel B was delivered 30 s after that from panel A. Traces on panel C were recorded following repeated stimulation with two flashes like that and including that illustrated on panel B, and two 2x brighter flashes. The traces at the top of each panel illustrate the time course and intensity of the stimulation (panel C trace shows command current, not the actual membrane current recorded by the amplifier). Stimulation events are also indicated by the colored (light stimulation) and gray (current injection) bars. The firing rates were calculated using a 200 ms time bin. For each stimulation time bins were aligned with stimulation onset (stimulation onset is between two-time bins). The membrane voltages were not corrected for the LJP, correction can be done by subtracting 15 mV from the reported values. Related to Figure 4.
Figure S4. Examples of light responses of two On-type wild type (WT) RGCs. The cellular membrane voltage was measured in the current-clamp mode, the upper traces and colored bars give the time course of the light stimulation, and flash intensities are indicated on the graphs. The firing rate traces were calculated using a 200 ms time bin. In general, hiPSC-RGCs generated responses similar to those produced by WT-RGCs. They may demonstrate elevated baseline firing (although some WT RGCs also showed elevated baseline firing which is illustrated by the cell 2 response) long after the flash recovery time. The detailed comparison of hiPSC-RGC and WT RGC light responses is beyond the scope of the current study and will be a topic of future investigation. Related to Figure 4.
Figure S5. Fundus image of a murine eye was taken following Pronase E injection in BAF and IR mode in the cSLO. 0.0001% of Pronase E was intravitreally injected into the vitreous cavity of C57BL/6 mice to degrade the ILM/NFL layer of the retina. The use of Pronase E A) caused cataract formation and B) induced inflammation as observed in the fundus image obtained in the BAF and IR modes of the cSLO. The scale bar is 200 µm. Related to STAR Methods.