

Biological and synthetic membranes for human induced pluripotent stem cell-based transplantation therapy

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Introduction

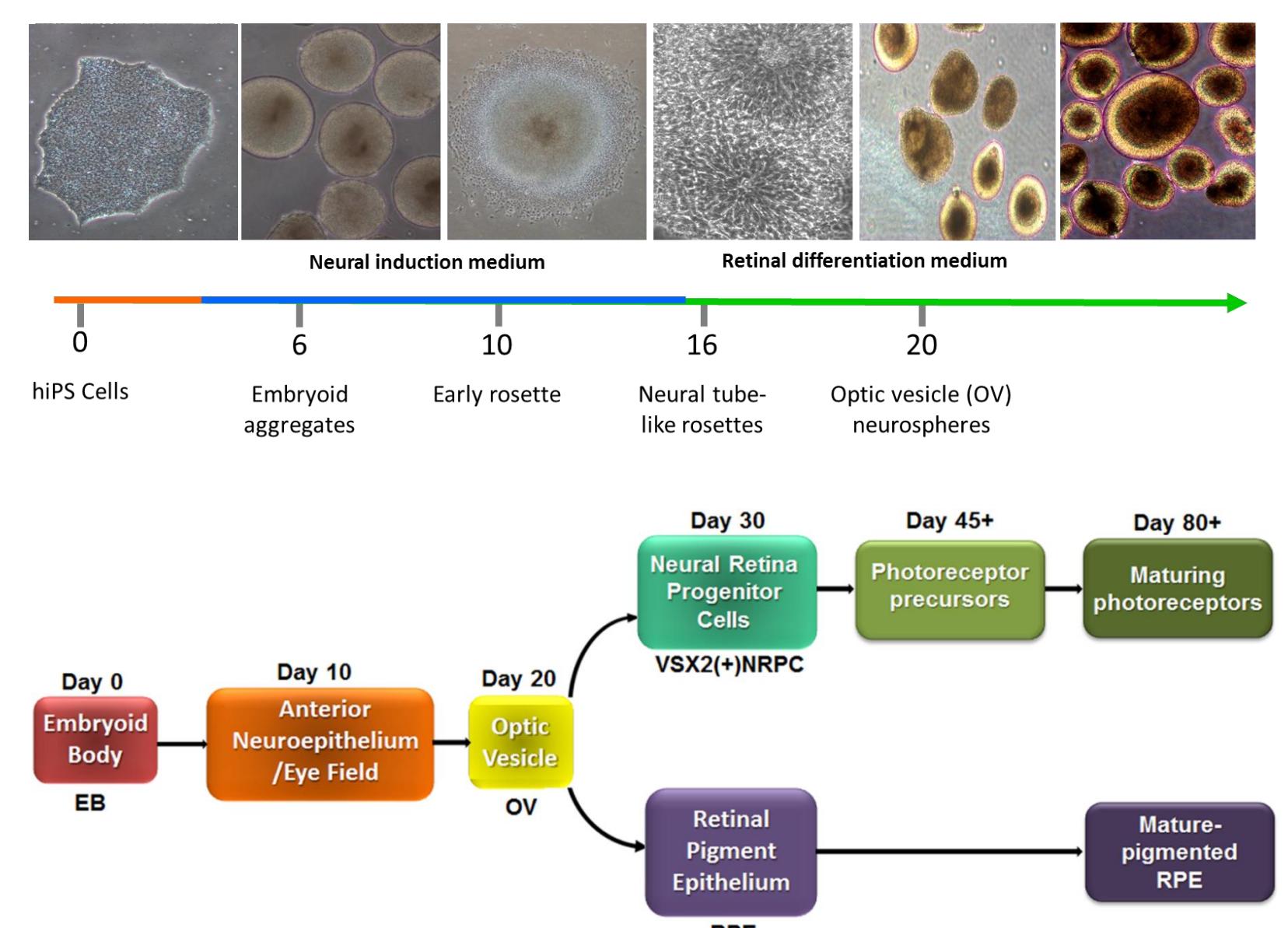
Scaffolds may improve transplantation therapy by providing an organized platform for cell delivery. In this study, we evaluated two membranes for human induced pluripotent stem cell (hiPSC)-based transplantation therapy.

SIS membrane (biological membrane): An acellular, porous membrane derived from porcine small intestine submucosa (SIS, Cook Biotech). SIS membrane is ~15µm thick and consists primarily of collagen, including types I, III, IV, V, and VI. While SIS membrane is already FDA approved for several applications outside of the eye, its use as a scaffold for intraocular transplantation has not been evaluated.

SU-8 membrane (synthetic membrane): An epoxy based viscous polymer that can be micro-patterned by photolithography. SU-8 membranes were generated at 8 µm thickness, with 5 µm pores.

Methods

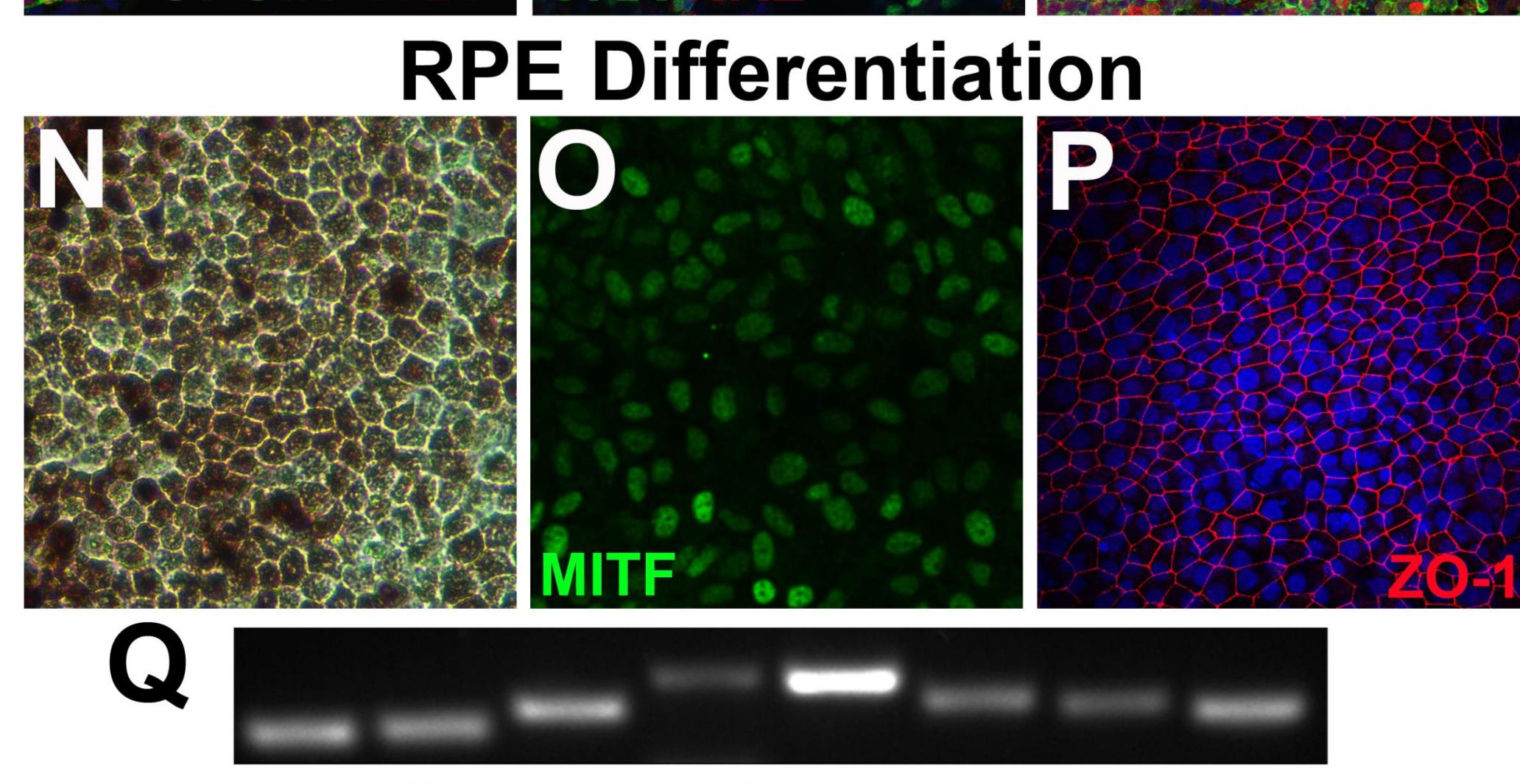
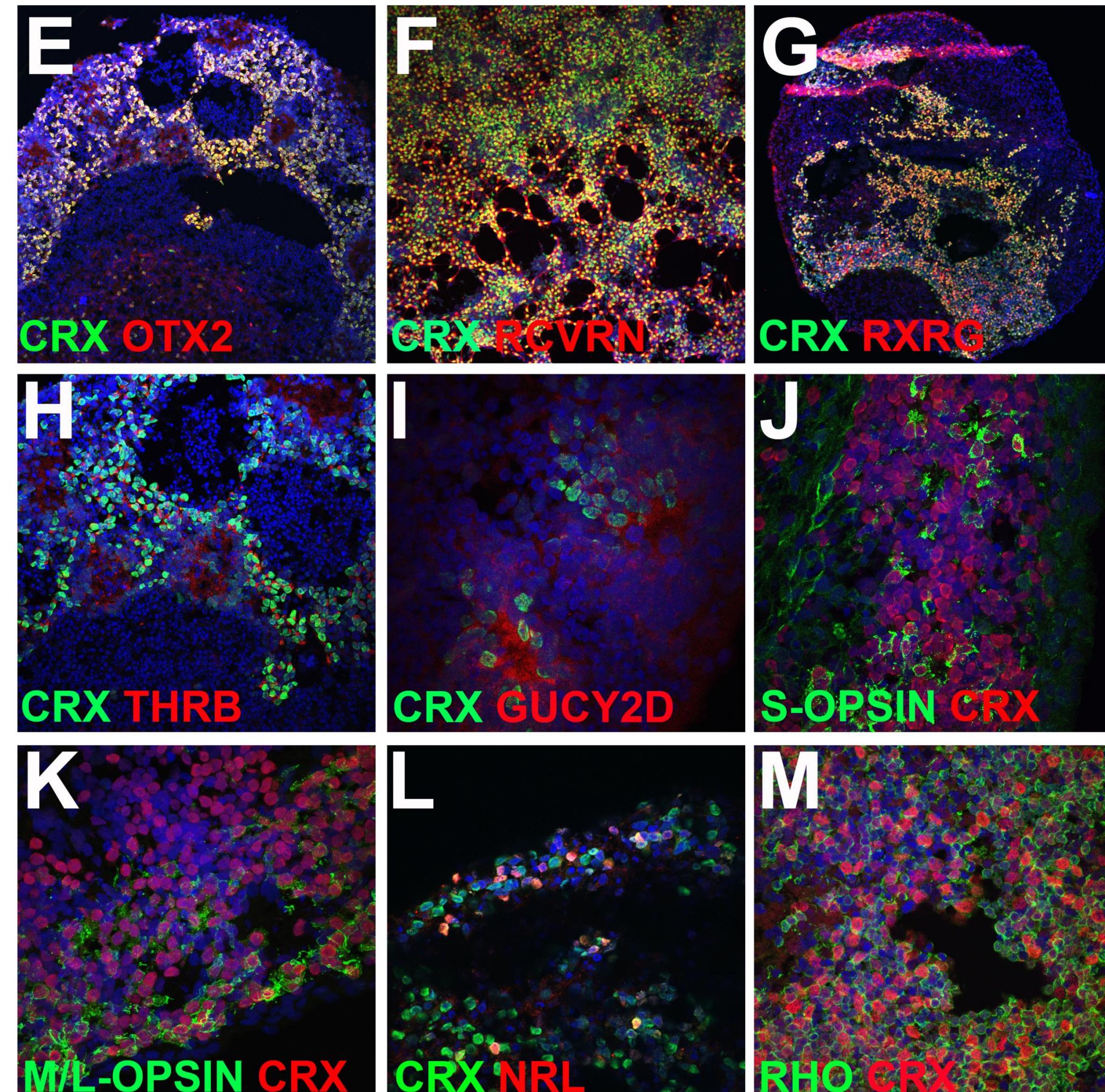
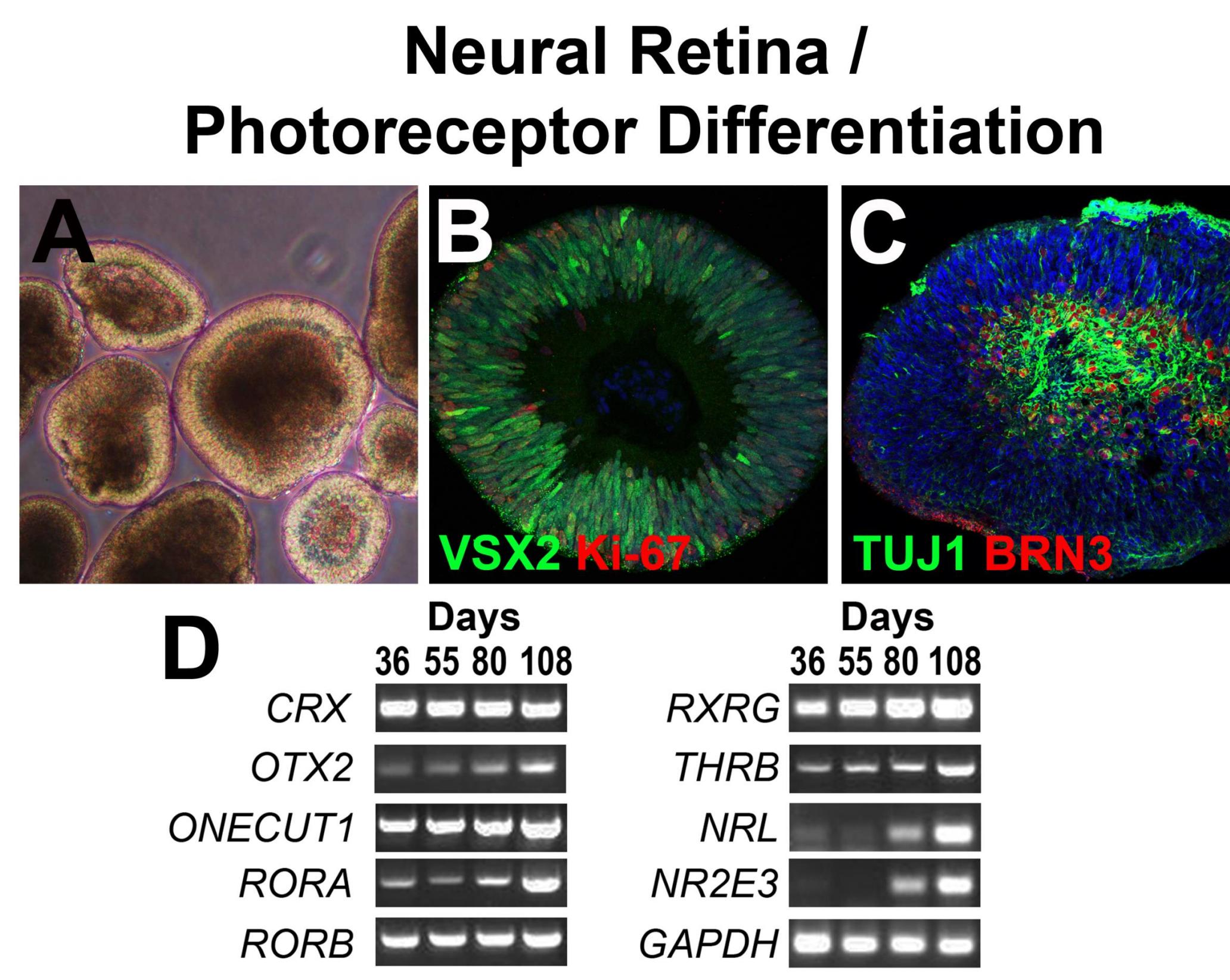
Differentiation of hiPSCs: Human induced pluripotent stem cells (hiPSCs) were differentiated to a neural retina cell fate as previously described (Meyer et al., *Stem Cells*, 2011). To generate neural retinal cells, optic vesicle-like structures (OVs) were isolated at day 20 using our established protocol, summarized below. OVs give rise to all neural retinal cell types, including a large percentage of photoreceptors.



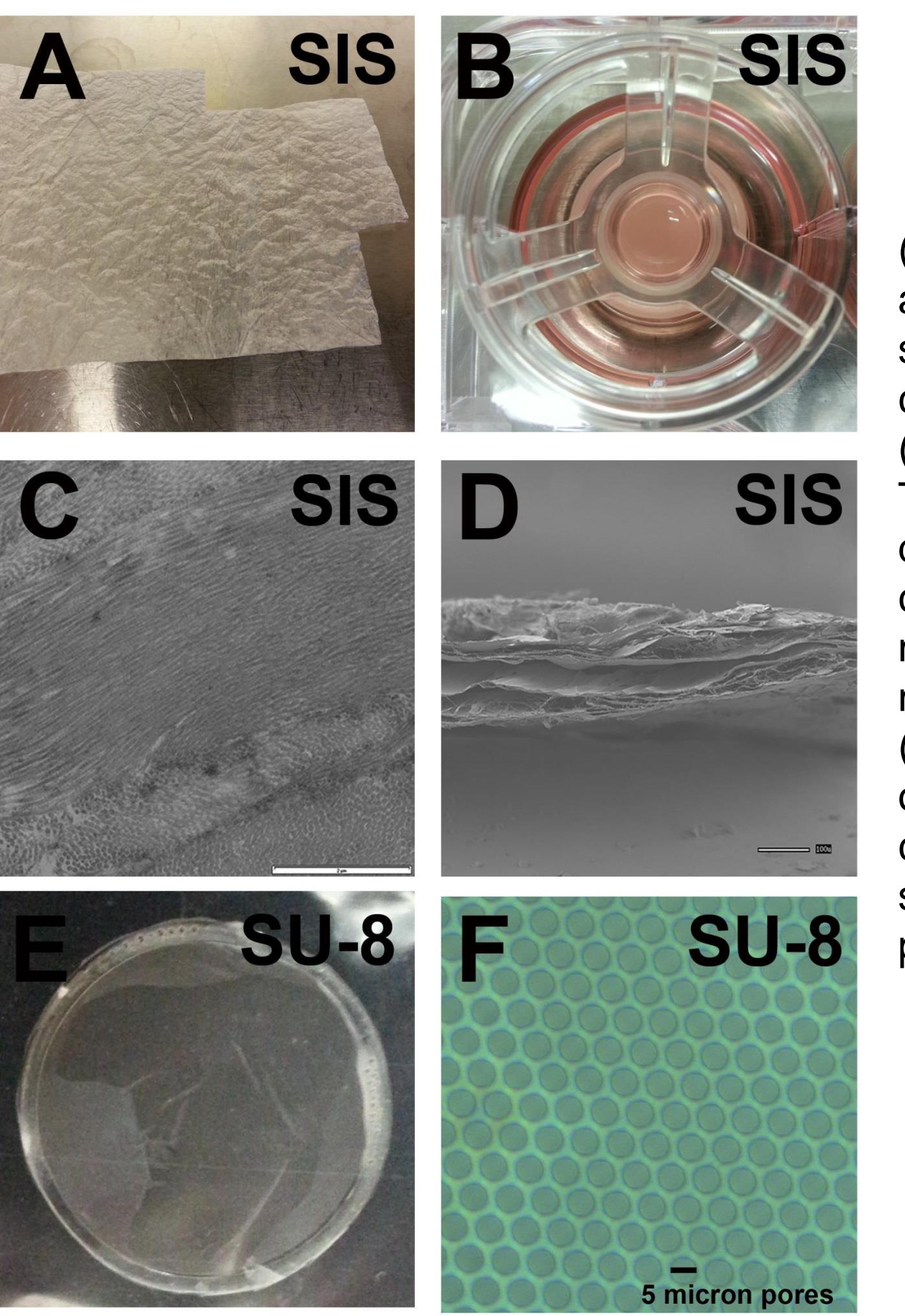
RPE were generated using a spontaneous differentiation method (Singh et al., *IOVS*, 2013). Differentiated RPE were microdissected and grown and expanded on laminin coated dishes. Following differentiation, hiPSC-RPE, or hiPSC-OVs containing neural retinal cells, were dissociated and plated onto laminin coated SIS or SU-8 membranes. Gene and protein expression were monitored with RT-PCR and immunocytochemistry (ICC). Cell growth, viability, and polarity were analyzed by standard light, confocal, and electron microscopy.

Subretinal transplantation of membranes into rat eyes: A patented human subretinal transplantation device (Eyevation) was modified for rat retina transplantation. Briefly, Long Evans, RCS rat, or immune compromised Foxn1-RNU rat eyes were proptosed and the overlying conjunctiva was removed. A 1-2mm incision was made in the superior sclera. The SIS membrane was loaded flat into the instrument tip by suction with a minimal amount of HBSS. The instrument tip was guided into the subretinal space and the SIS membrane was gently inserted by movement of the rounded mandrel. Rats were followed for 1-4 weeks post-transplantation.

1. Differentiation of hiPSCs toward photoreceptors and RPE

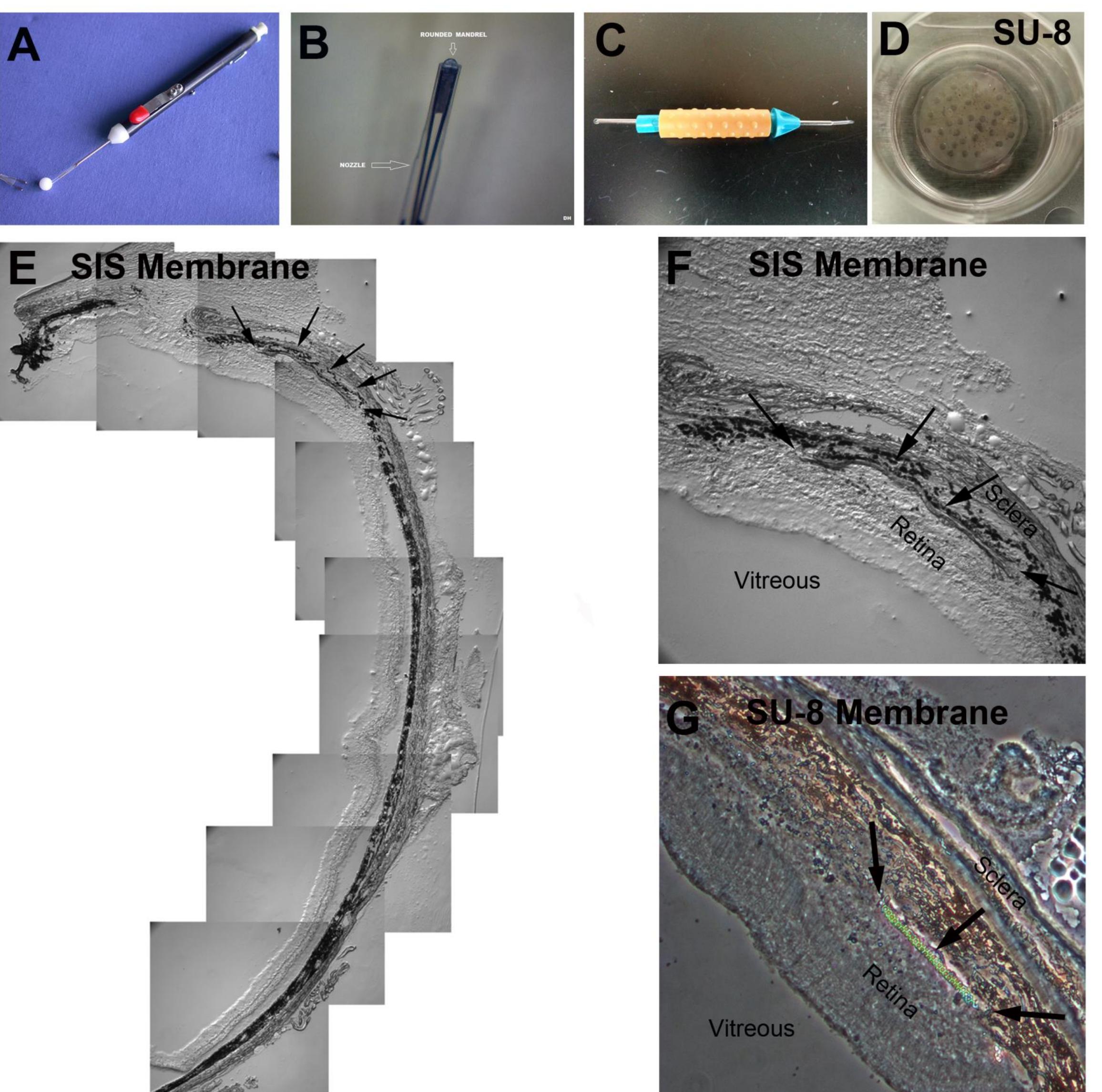


2. SIS and SU-8 Membranes



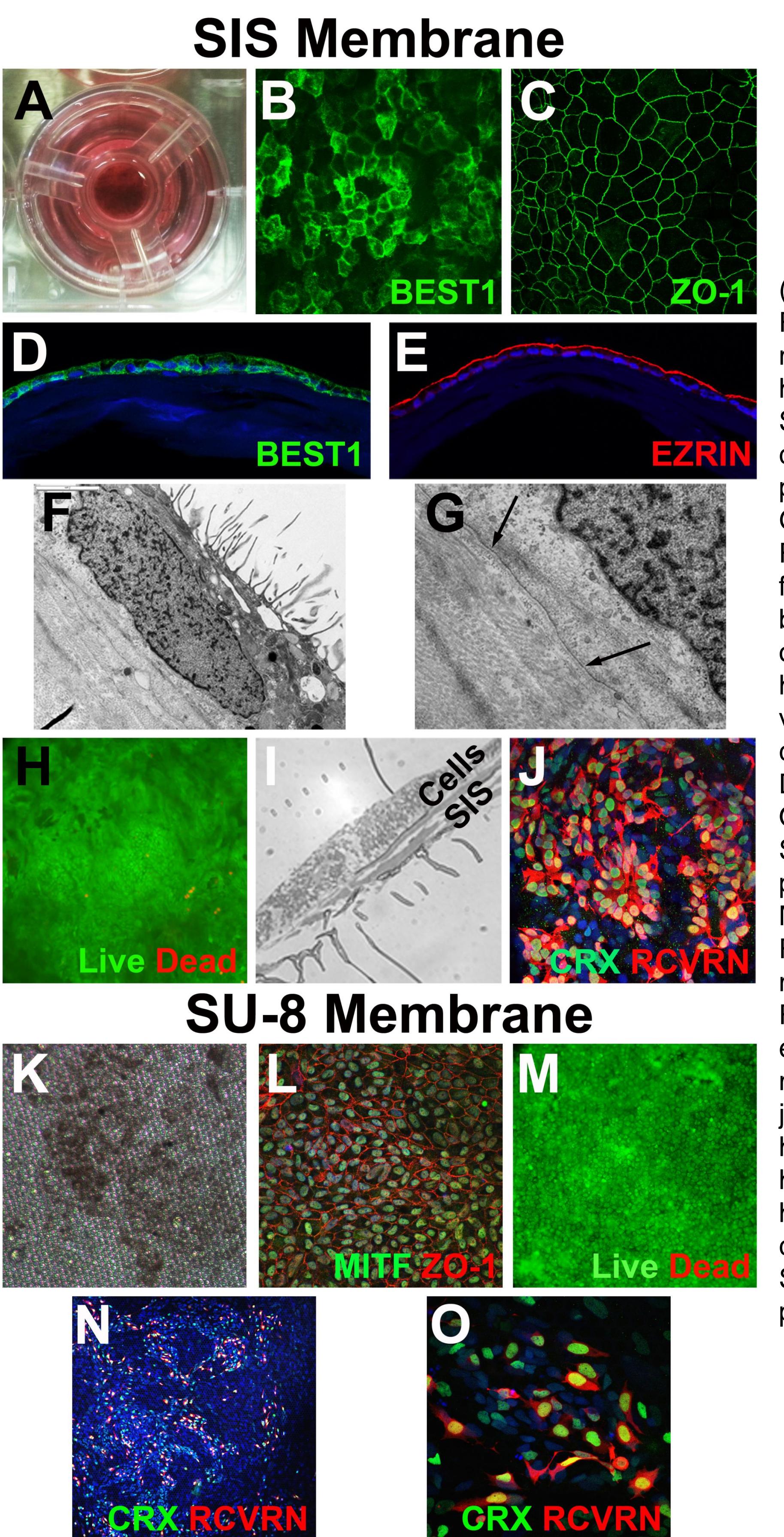
(A) SIS membranes are produced as sheets that can be cut to any shape. (B) SIS membranes are cultured using Snapwell inserts (Corning) to hold them in place. (C) Transmission electron micrograph demonstrates the porosity of collagen fibrils within the SIS membrane. (D) Scanning electron micrograph of SIS in cross-section. (E) SU-8 membranes have a thick outer ring to promote sinking in culture. (F) Micrograph of SU-8 shows micro-patterned rings with pores that are 5 microns in diameter.

4. Transplantation of membranes into the rat subretinal space



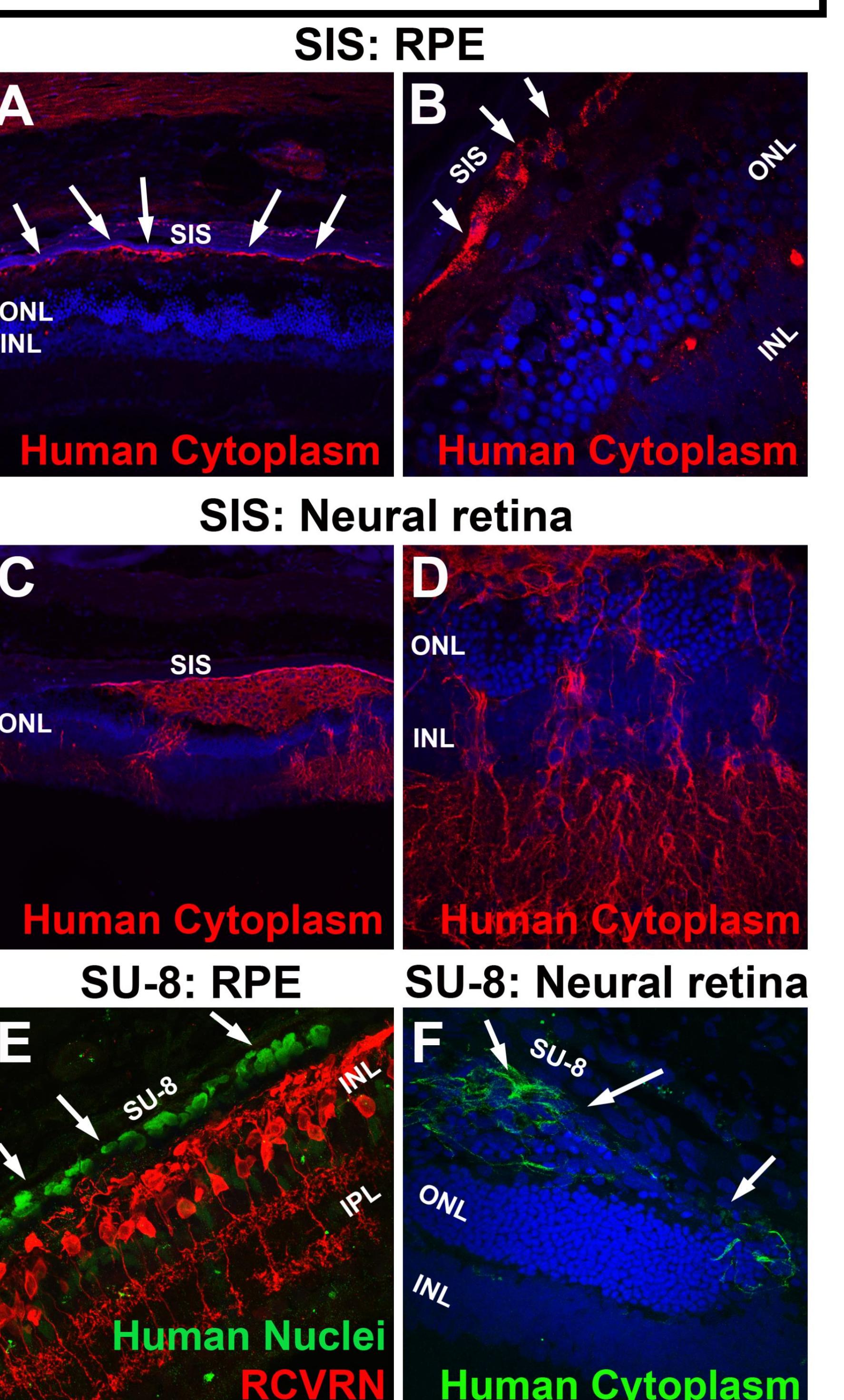
(A) Subretinal transplantation device allows delivery of membrane + retinal cell constructs. (B) Higher magnification image of the retractable mandrel used to deliver the membranes. (C) Trehpine instrument produces circular punches of membranes 1mm in diameter. (D) SU-8 with many circular punches removed for transplantation. (E) Montage demonstrating the successful insertion of SIS membrane into the rat subretinal space. (F) Higher magnification of the SIS membrane in the subretinal space. (G) Subretinal transplantation of the SU-8 membrane. (E-G) Arrows demarcate transplanted membranes.

3. Retinal cell types maintain structure and gene expression on membranes



(A) Pigmented hiPSC-RPE grown on SIS membrane. (B-E) hiPSC-RPE grown on SIS retain characteristic RPE protein expression. (F, G) TEM shows hiPSC-RPE microvilli and formation of a basement membrane on SIS (arrows). (H) hiPSC-RPE remain viable on SIS in culture. (I, J) Dissociated hiPSC-OVs form layers on SIS and include photoreceptors. (K) Micrograph of hiPSC-RPE grown on SU-8 membrane. (L) hiPSC-RPE maintain gene expression and form monolayers with tight junctions on SU-8. (M) hiPSC-RPE are healthy on SU-8. (N, O) hiPSC-neural retina cells form layers on SU-8 and include photoreceptors.

5. Transplanted cells on membranes survive for up to 1 month in vivo



(A, B) 1 week post-transplantation of SIS membrane plus hiPSC-RPE into the adult normal Long Evans rat retina. (C, D) 1 month post-transplant of SIS + hiPSC-neural retina cells into Long Evans retina. (E) 2 weeks post-transplant of SU-8 + hiPSC-RPE into the degenerate RCS rat retina. (F) 2 weeks post-transplant of SU-8 + hiPSC-neural retina cells into immune-compromised Foxn1-RNU rat retina.

Conclusions

- Retinal cell types derived from hiPSCs adhere to SIS and SU-8 membranes, proliferate, differentiate, and survive in culture for extended periods of time.
- hiPSC-derived RPE form monolayers on both membranes and retain characteristic RPE structure and gene expression.
- Dissociated hiPSC-derived OVs form layers on both membranes that include photoreceptor cells.
- Membranes can be transplanted into the rat subretinal space. Transplanted cells survive for at least 1 month in vivo.

Future Directions

- Examining methods to promote cell survival and integration in vivo.
- Development and transplantation of RPE + photoreceptor membrane constructs.
- Examining the long term consequences of membrane transplantation.

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