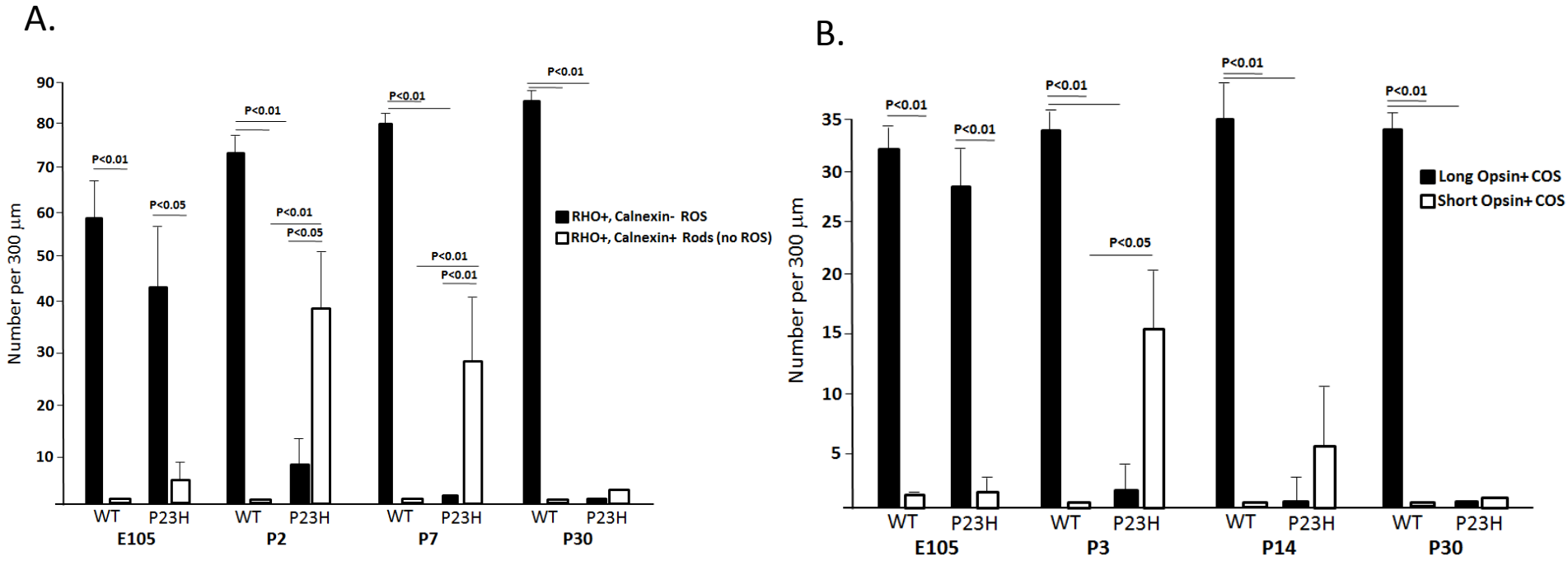

Fig. S1. Quantification of rod and cone OS in the visual streak of WT and P23H litter mates in Figs. 1 and 2. (B. A.. Rods with ROS that are RHO+ and calnexin- were counted and compared to rods where OS were lost and RHO expression colocalized with calnexin (RHO+, Calnexin+) at the indicated ages. Note diminished overall rod number at P30. n=3. B. These results demonstrate similar numbers of long cone OS (> 2 mm in length) in P23H and WT litter mates at E105. But there is rapid loss of cone OS (COS) length (Short indicates < 2mm in length) by P3, which progresses at P14, and then COS are essentially lost by P30. n=3.

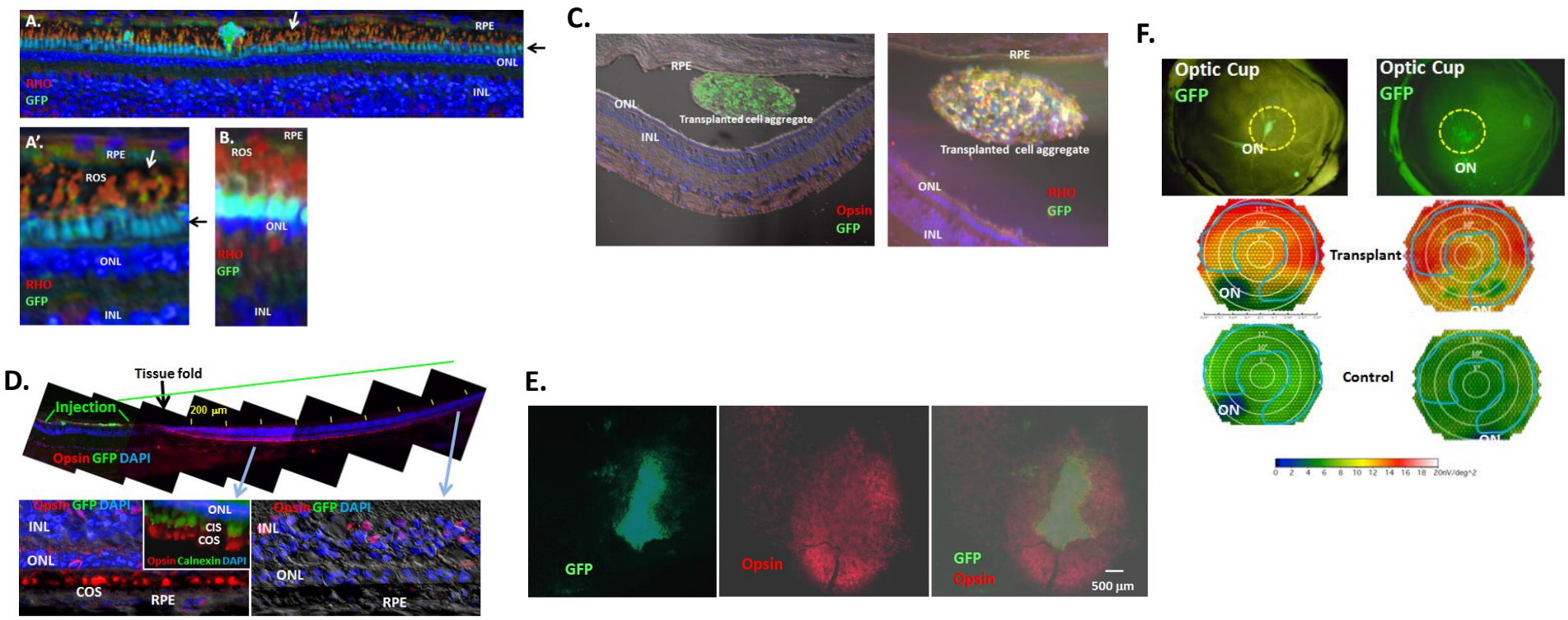

Fig. S2. Immunostaining and mfERG related to Fig. 3. A. High power view of immunostaining related to Fig. 3. The black arrows shows a monolayer of GFP+ transplanted cells apposed to the ONL and extending RHO+ OS to the RPE. A' is a higher power view. B. Higher power view of GFP+ transplanted cells integrated into the ONL and forming RHO+ OS. C. While transplant of 5 x 10^5 GFP+ cells led primarily to a monolayer of cells either integrated into the ONL or apposed to the ONL containing RHO+ OS, when 1 x 10^6 GFP+ cells were transplanted into the subretinal space, cells frequently formed aggregates. Although cells in these aggregates expressed RHO, they did not form OS directed toward the RPE. And, importantly, such aggregates failed to maintain opsin+ OS in endogenous cones. D. Low power view of a cell injection site in Fig. 3. E. Retinal flatmount in the region of GFP+ cell injection. Note induced cone opsin expression surrounding the GFP+ cells. F. Transplantation of GFP^+ rod precursors into the subretinal space of P23H pigs preserves photopic mfERG. Above, positions of injected GFP^+ rod precursors just superior to the optic nerve (ON) and the region of mfERG assessment (yellow circle) in the optic cup is shown. Below, representative mfERGs showing increased local photopic mfERG linked to preservation of COS in the region surrounding transplants are shown. n=5. Blue lines denote representative areas around injection sites used for hexagon quantification in Fig. 3.

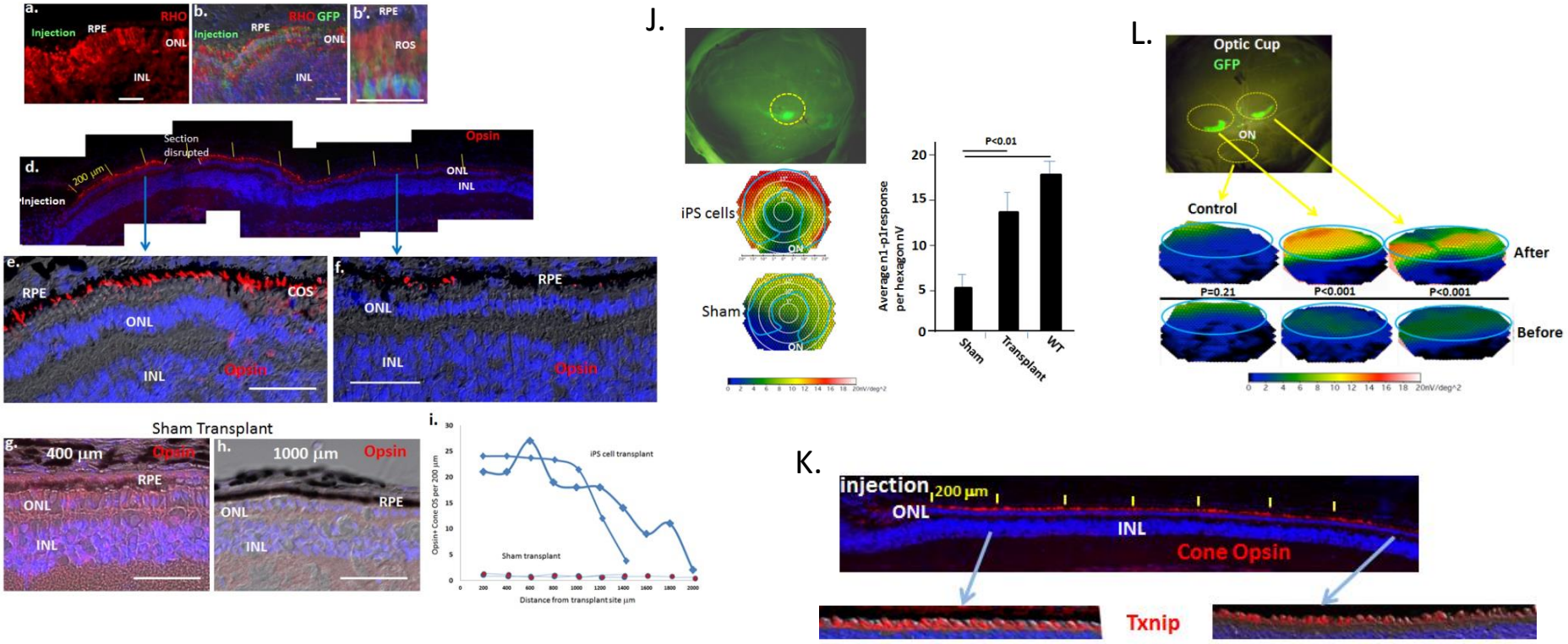

Fig. S3. Immunostaining and mfERG related to Figs. 3 and 4. GFP+ pig skin iPS cells were generated and differentiated as in Methods, and cells were injected subretinally below the visual streak at P14 as in Fig. 3. (A-F) Representative immunostaining is shown at P60. (A-C) GFP+ cells integrate into the ONL and form long RHO+ OS at the injection site. (D-F) Endogenous (GFP-) cone OS are maintained out to approximately 1500 µm from the injection site. (G-H) A control sham transplant has no effect on endogenous cone OS. (I) Quantification of the effect of transplanted iPS cells on cone OS at two injection sites. Bars are 50 µm. J. Photopic mfERG is increased in the region of transplanted iPS cells in Panels A-I. J. Above, optic cup showing the location of GFP+ transplanted differentiated iPS cells. Below, mfERG in the region of transplanted cells or sham control at P60. Blue lines show representative regions of hexagons counted. Right, quantification of mfERG responses. Error bars are standard deviations. K. Low power view showing opsin and Txnip expression with distance from the cell injection site in Fig. 4. L. Transplantation of GFP+ rod precursors restores cone mfERG in rodless P23H pigs. Above, optic cup showing representative GFP+ cell transplant sites at tow sites from Fig. 4. Regions of mfERG assessment are shown with yellow circles in the visual streak. ON, is optic nerve. Below, representative photopic mfERGs in regions of cell transplants. mfERGs are shown before and after cell transplant. An uninjected site is also shown. Blue lines denote representative areas around injection sites used for hexagon quantification in Fig. 4. See Fig. S4 for representative histological analysis of a vector through an injection site.

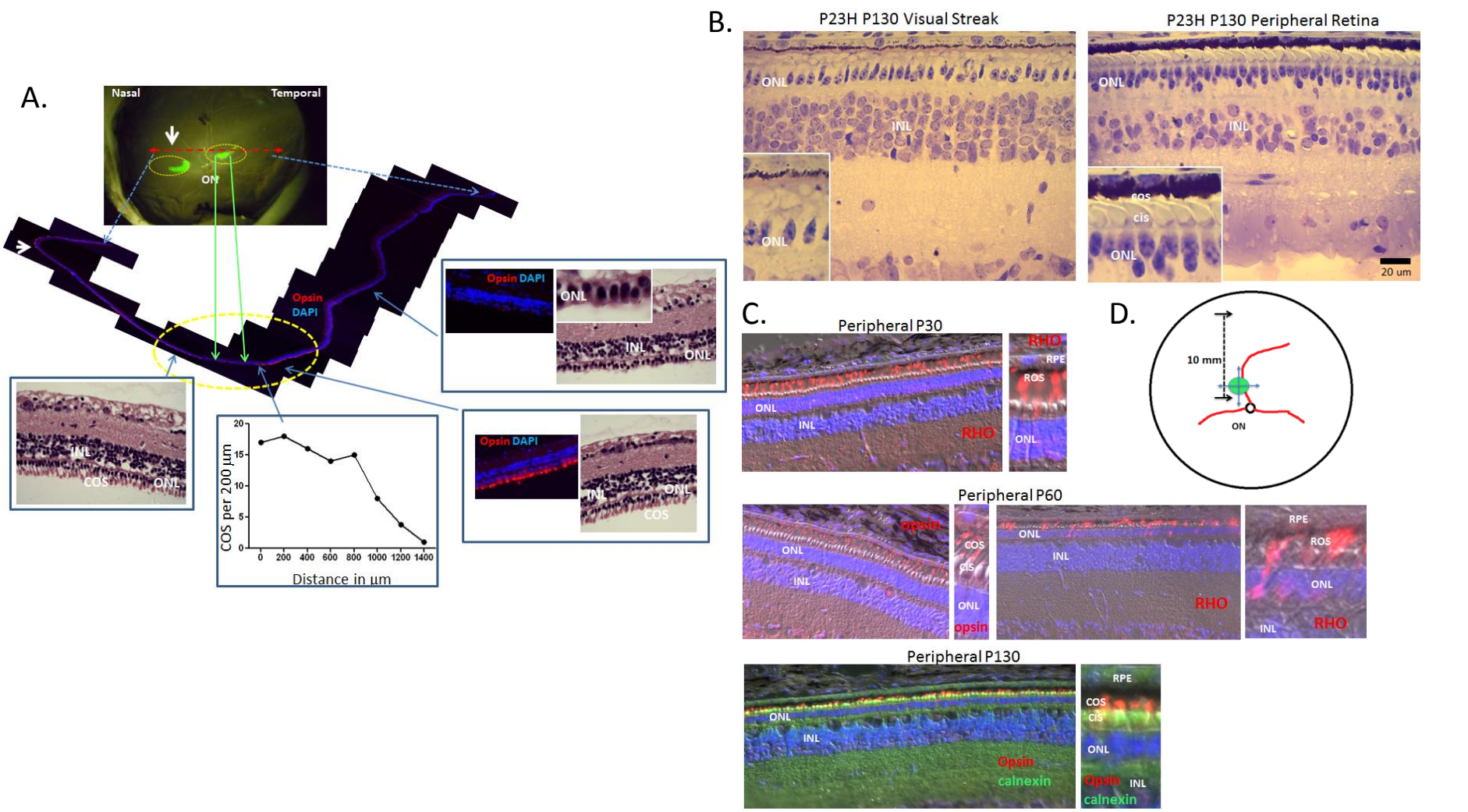

Fig. S4. Representative histologic vector analysis and histological and immunostaining analysis of the peripheral retina in P23H pigs relating to Figs. 3-4. A. Representative horizontal vector analysis (red arrow) from Figs. 4. Yellow circles show the areas of mfERG in Fig. S3L. Representative cone OS count is to the right of the arrow (temporally). White arrows show influence of the left transplant site. Green arrows show the cell transplant site. B-C. Rod and cone OS persist in the P23H peripheral retina. See Panel D below for position of peripheral sections. Note in contrast to the visual streak (Fig. 1), rod OS are maintained in the P23H retina at P30. By P60, rods and rod OS are diminishing in the peripheral retina, but opsin+ cone OS persist. By P130, rods are lost in the peripheral retina, but calnexin+ cone IS and opsin+ cone OS persist. D. Red lines indicate retinal vessels. "ON" is optic nerve. The green circle indicates sites of cell transplant. Blue lines show horizontal and vertical meridians of retinal sections. The central arrow indicates the position of the sections within the visual streak and the upper arrow the position in the peripheral retina in panels B-C. The diameter of the pig retina is 22 mm.

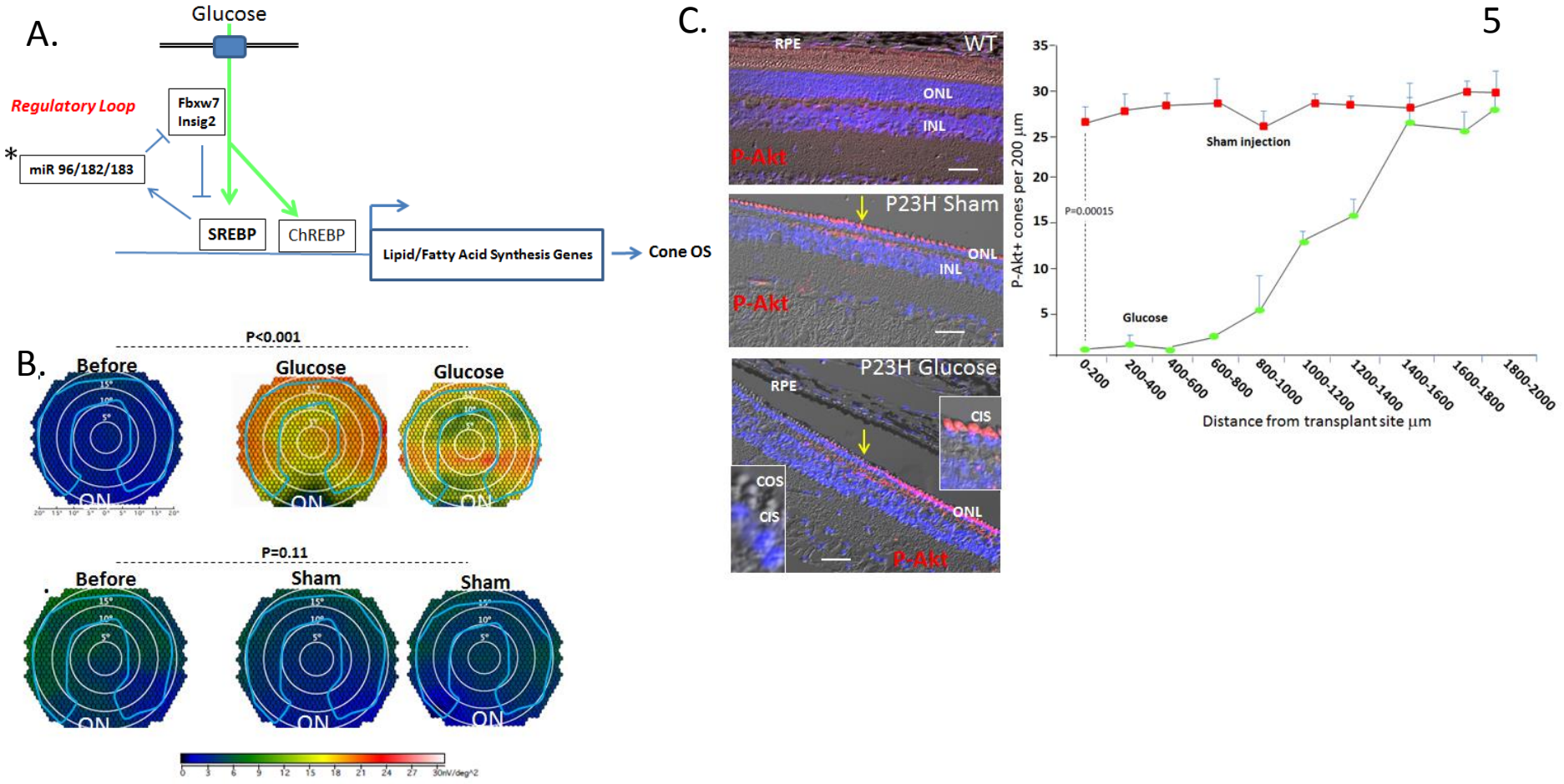

Fig. S5. A potential role for the miR 96/182/183 cluster in cone OS synthesis, and immunostaining and mfERG related to Fig. 5. A. Insulin/glucose induces SREBP, which in turn induces the miR 96/182/183 cluster. The individual miRs are then processed from a common transcript. Fbxw7 and Insig2 inhibit nuclear transport and cause turnover of SREBP. The miR 96/182/183 cluster targets Fbxw7 and Insig2 to increase SREBP activity in the liver. Mutation of miR 96/182/183 leads to loss of cone OS and function. B. Representative mfERGs from Fig. 5. ON, optic nerve. The blue lines indicate representative areas surrounding injection sites used for quantification of responses. C. Glucose injection blocks constitutive Akt activation (P-Akt) in P23H cones. Akt phosphorylated on serine 473 (P-Akt)+ cones were counted in multiple vectors from injection sites (Fig. 5) and these vectors were averaged, and then combined data for injection sites was averaged. The yellow arrows indicates a position 1000 mm from the injection site to the left. Quantification of P-Akt expression is shown on the right. Error bars are standard deviations. n=4. Bars are 40 µm.

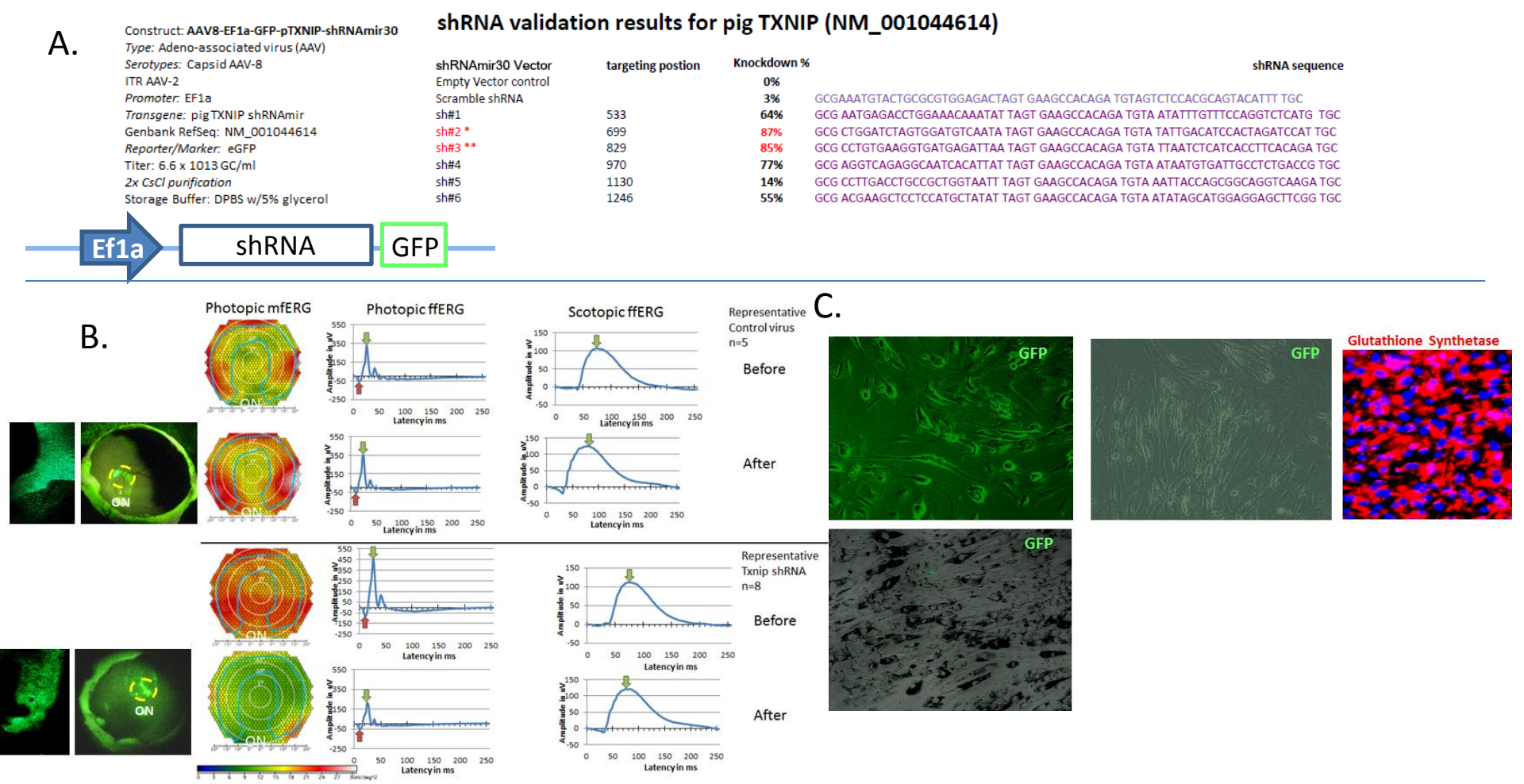

Fig. S6. AAV8 virus construction and analysis related to Fig. 6. A. Viruses were constructed by Vector Biolabs. The 1.2 Kb elongation factor-1 (EF-1a) promoter is not methylated and silenced like viral promoters such as CMV. For subretinal injection and cell infection, viruses were diluted 100-fold. Then, 30 ul consisting of approximately 2 x 10^{10} vector genomes were injected in vivo in Fig. 6. Primary cells cultures were infected with 2 x 10^{11} vector genomes per ml, and RNA for PCR and GFP fluorescence was analyzed 1 week later. B. Txnip shRNA AAV viruses inhibit photopic mfERG but do not affect scotopic ffERG. Representative ERGs are shown before injection and at the 4 week time point after injection when eyes were harvested (Fig. 6). "ON", is optic nerve. The blue lines denote representative hexagon areas surrounding the injection site that were counted in Fig. 6. Representative GFP expression in optic cups is shown on the left. Yellow circles show areas of mfERG analysis and RNA extraction (see Fig. 6J). Higher power views of injection sites are shown on the far left. C. Infection of pig primary cultures with AAV8 Txnip shRNA. Primary culture of pig neural retina from P14 (top right), pig Muller cells (top middle) and pig RPE (bottom left) are shown. Muller cells immunostained for the Muller marker glutathione synthetase (top right) show that the cultured cells are uniformly positive. Note little or no GFP expression from the virus in Muller or RPE cells. Cells were infected with 2 x10^{11} vector genomes per ml, and immunostaining was after one week. See Methods.

Primer name	Sequence	Tm ˚C	Amplicon (bp)
Ss TXNIP1 LP	ATCGCCCAAGCCAGCCAACT	62.5	150
Ss TXNIP1 RP	CAGACACCCGGCCATCAGGAAT	61.6	
Ss ACTB LP	GCCAACCGTGAGAAGATGAC	56	125
Ss ACTB RP	GAGTCCATCACGATGCCAGT	57.1	
Ss GAPDH LP	GTCGGTTGTGGATCTGACCT	56.8	118
Ss GAPDH RP	GTCCTCAGTGTAGCCCAGGA	58.4	

Table **S1**. Pig PCR primers related to Figs. 4 and 6.