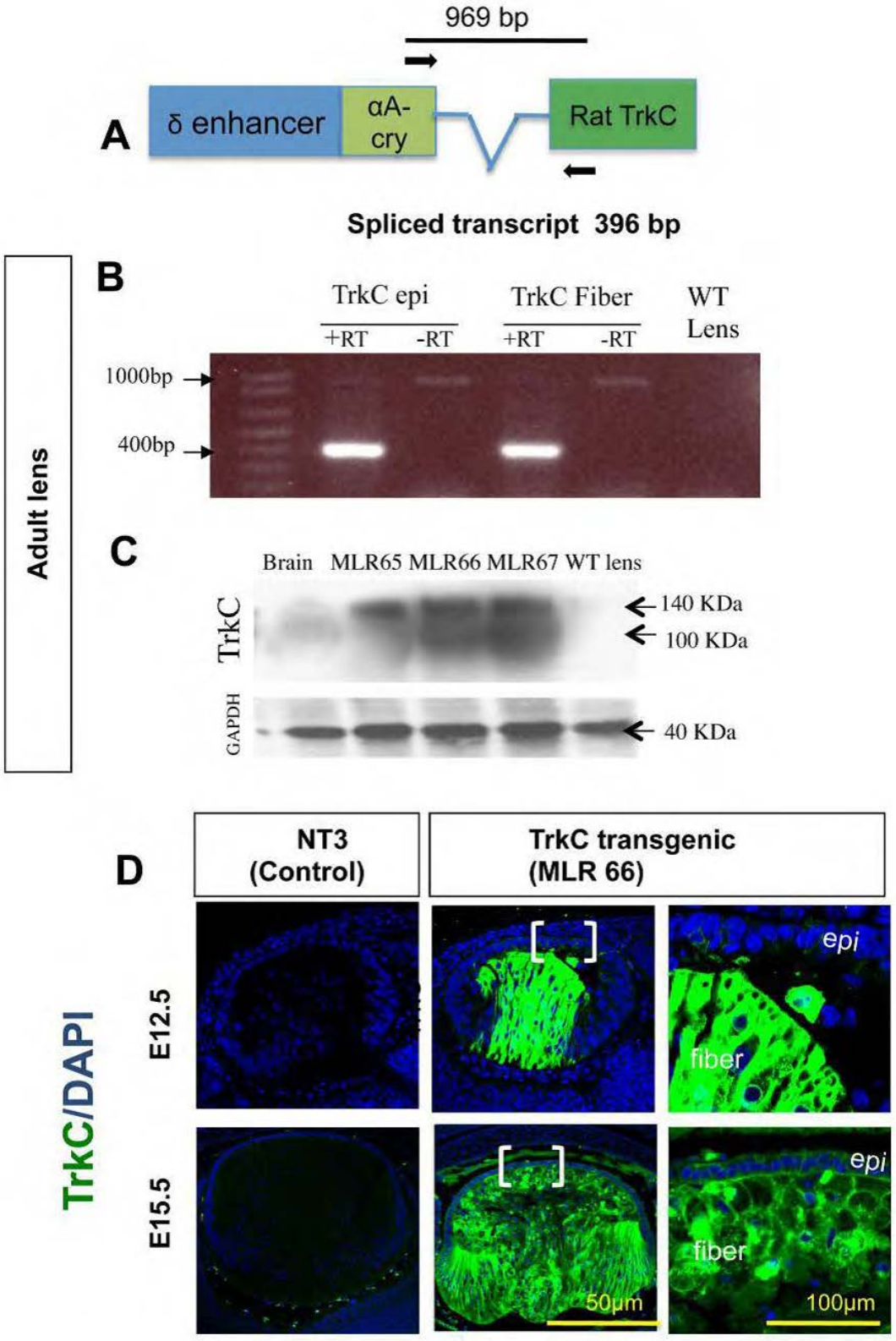

Fig. S1. Three different transgenic lines expressing TrkC in the lens epithelium and fiber cells were produced. (**A**) A transgenic construct was designed to express a rat TrkC cDNA under the regulatory control of a chimeric promoter consisting of the mouse αA-crystallin promoter (αA-cry) fused to the chicken δ-crystallin enhancer. The cDNA and promoter were separated by a rabbit β-globin intron. Location of PCR primers used for genotyping and RT-PCR are indicated by arrows. (**B**) RT-PCR of lens RNA amplified a 396 bp band specifically from reverse-transcribed RNA samples from TrkC transgenic lens epithelial and lens fiber cells. A trace 969 bp band could be amplified from transgenic RNA samples that had not been reverse transcribed, representing unspliced RNA or contaminating genomic DNA. (**C**) Western blots using antibodies to TrkC-detected protein bands of 140 kDa and 100 kDa in lens protein homogenates from all three TrkC transgenic lines but no such signal was detected in wild-type lens homogenates. The western blot was stripped and probed with Gapdh antibodies as a protein loading control. (**D**) Immunohistochemistry to detect TrkC in MLR66 transgenic lenses revealed expression of TrkC protein by E12.5 in the fiber cells as well as weakly in the epithelial cells. By E15.5, strong expression of TrkC protein was observed in both epithelial and fiber cells of the transgenic lens. For the TrkC transgenic lens histology, the right-most panels represent higher magnifications of the bracketed regions of the adjacent panels. epi, lens epithelium; fiber, lens fiber cells.

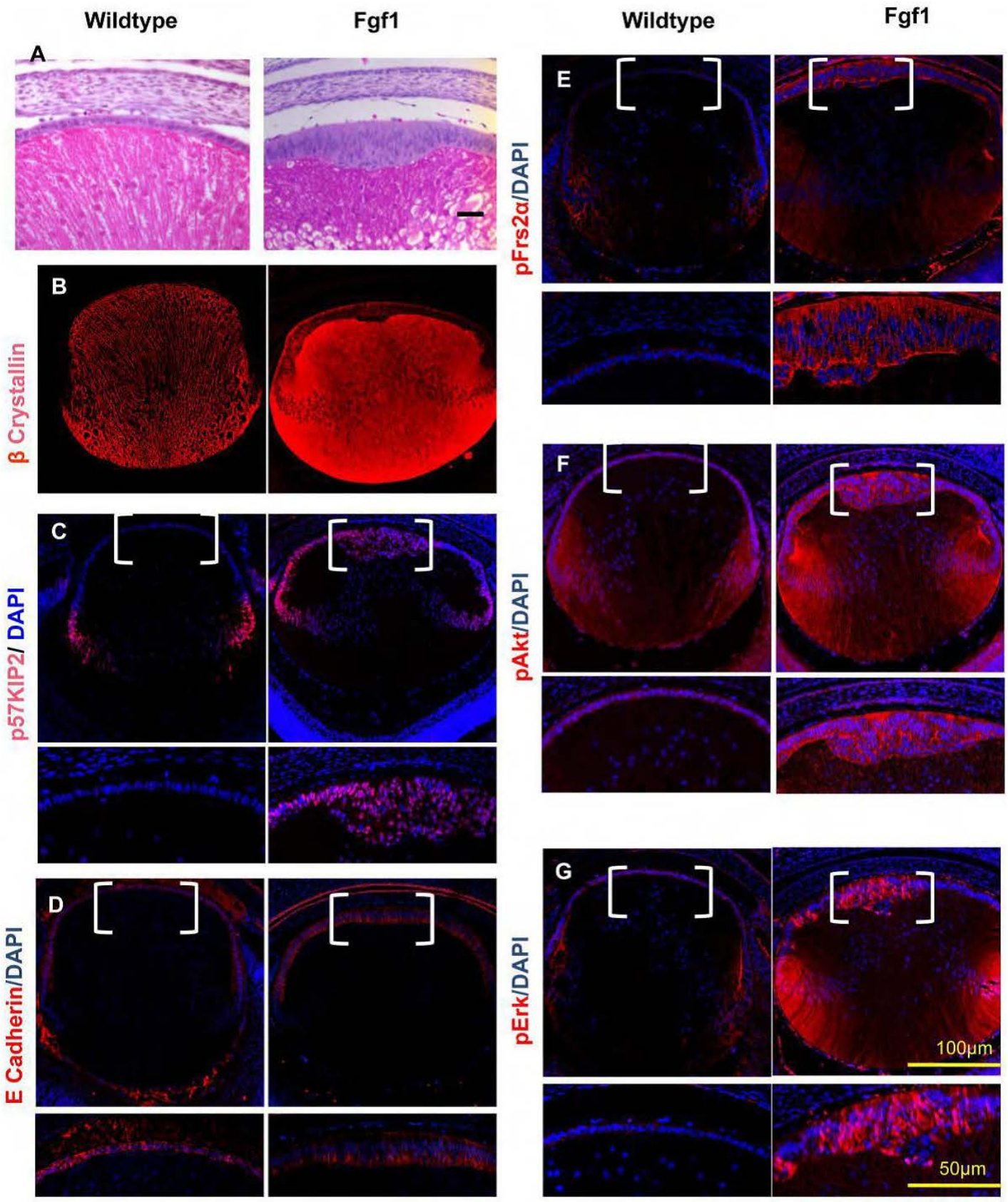

Fig. S2. Expression of Fgf1 in the lens induces premature fiber cell differentiation and activation of Frs2α, Erk and Akt. (**A-G**) Fgf1 transgenic lenses at E15.5 have elongated lens epithelial cells (A), which express β-crystallin (B) and $p57^{KIP2}$ (C), normal characteristics of lens fiber cells. E-cadherin expression in the transgenic lens epithelium is reduced and stochastic relative to the wild-type lens epithelium (compare left and right panels in D). Transgenic expression of Fgf1 also induces activation (phosphorylation) of Frs2α (E), Akt (F) and Erk (G) in the transgenic lens epithelium. The bracketed regions in C-G are shown at higher magnification below each bracketed panel.

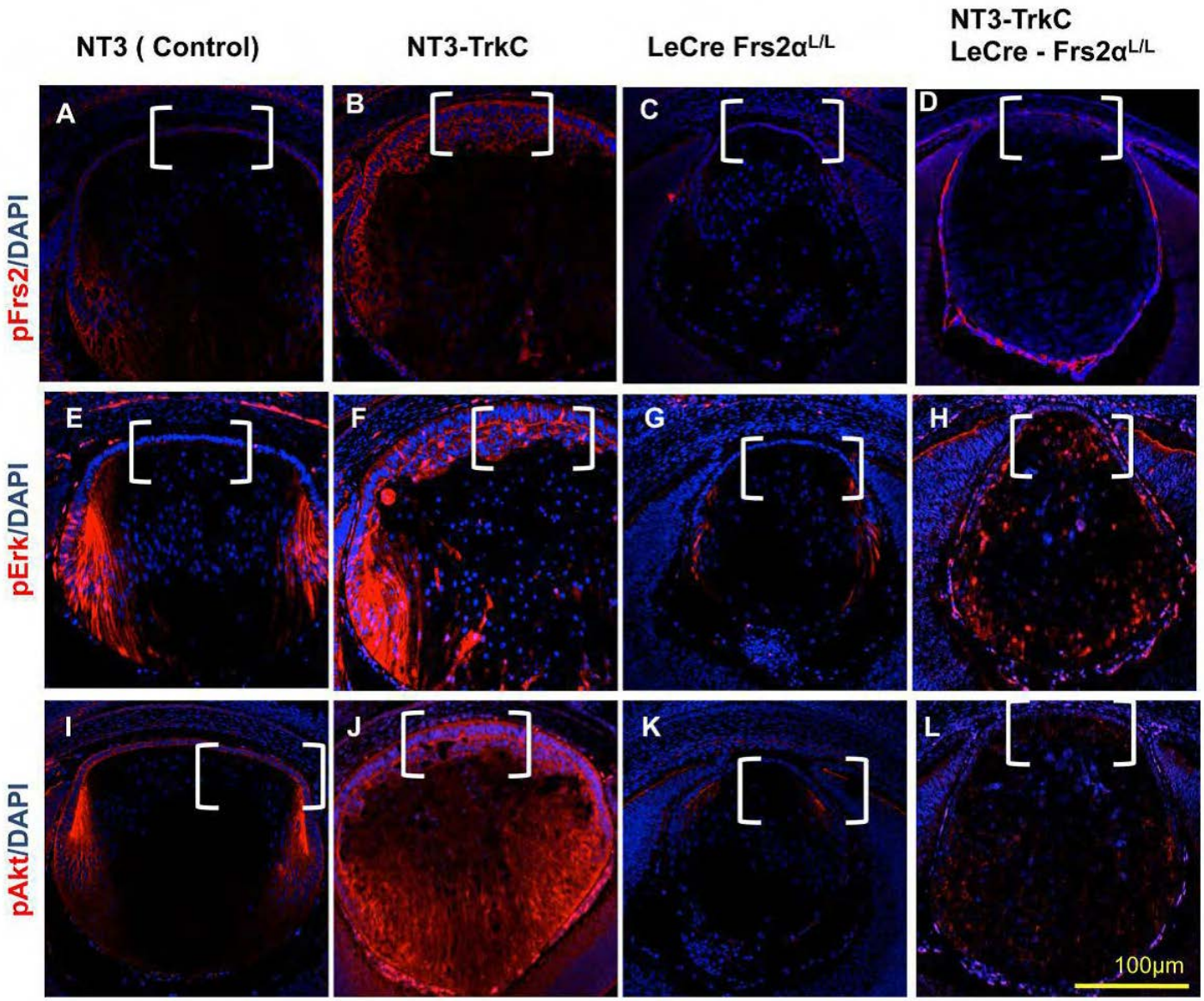

Fig. S3. Activated TrkC signaling induces Frs2α-dependent phosphorylation of Frs2α, Erk and Akt in the lens. (**A-L**) Lower magnification images for those seen in Fig. 8. Control (A,E,I), NT3-TrkC (B,F,J), Frs2α-deficient (C,G,K) and NT3-TrkC lenses lacking Frs2α (D,H,L) were analyzed at E15.5 to determine the activation (phosphorylation) state of Frs2α (A-D), Erk (E-H) and Akt (I-L) in the lens. The bracketed regions are shown in higher magnification in Fig. 8.

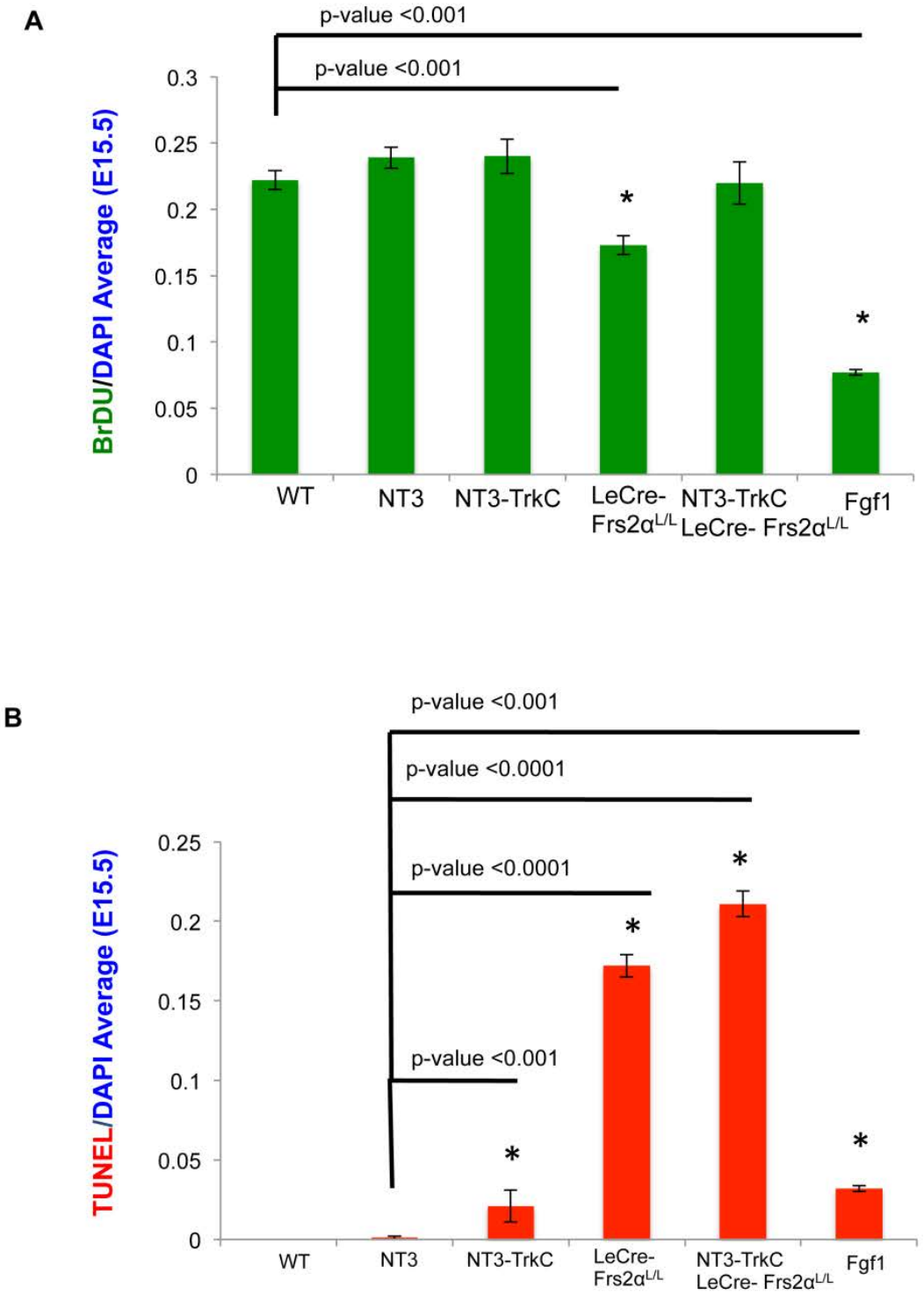

Fig. S4. Comparison of proliferation and apoptosis among the genetically modified lenses at E15.5. Proliferation rates were assayed by BrdU incorporation to determine the S-phase fraction of lens cells (**A**) and cell death was analyzed using the TUNEL assay (**B**) in E15.5 wild-type, NT3, NT3-TrkC, LeCre-Frs2αL/L, NT3-TrkC LeCre-Frs2αL/L and Fgf1 lenses. Cell proliferation was significantly decreased only in the LeCre-Frs2αL/L and Fgf1 lenses at E15.5 compared with wild type. Apoptosis was significantly increased in all the transgenics examined except NT3. Error bars represent s.e.m. Asterisks represent significant differences.

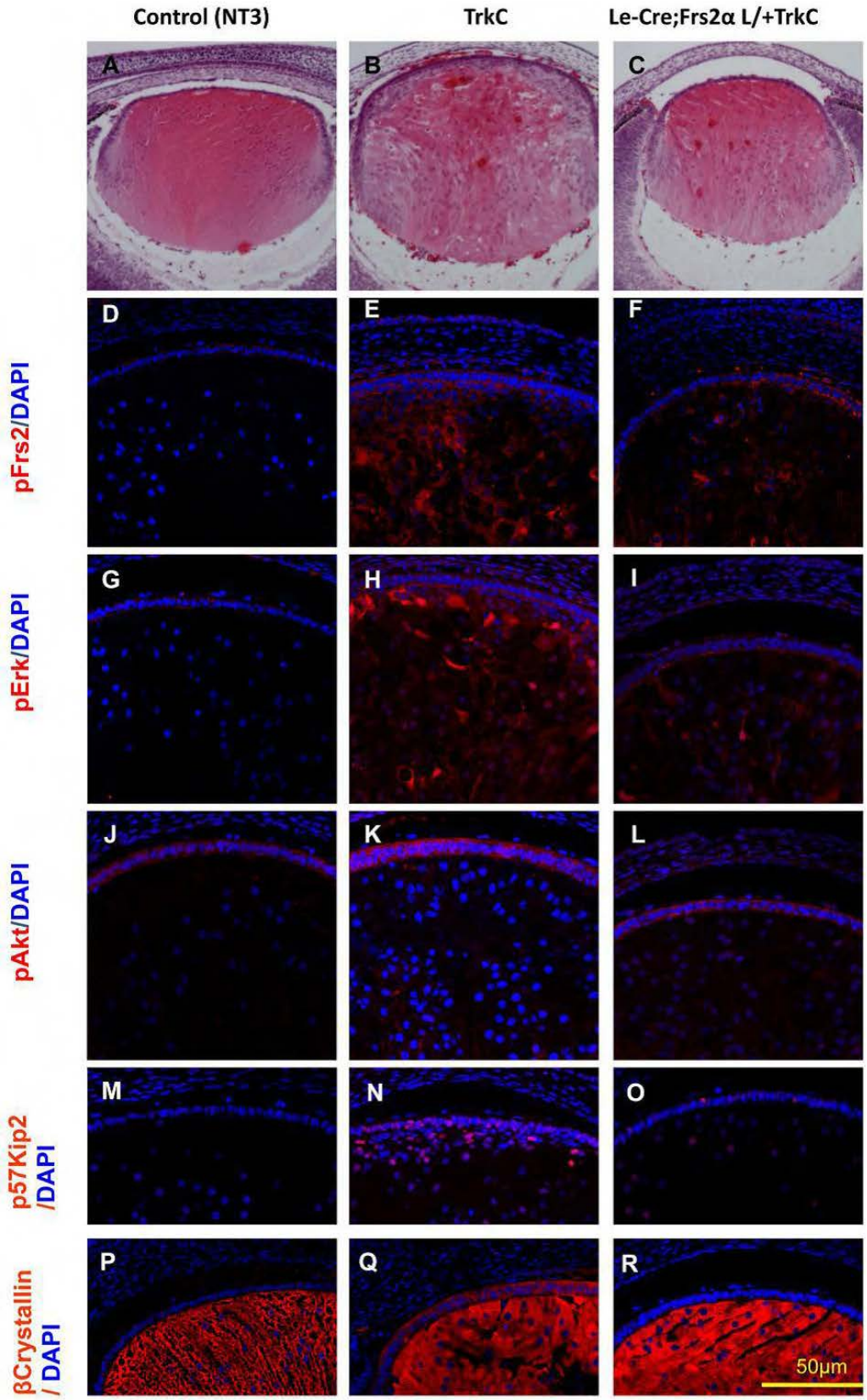

Fig. S5. TrkC alone can induce modest fiber cell differentiation and increased Frs2α, Erk and Akt activation that can be inhibited by the loss of a single allele of Frs2α. (**A-R**) E15.5 control NT3-transgenic embryos have normal lens morphology whereas the transgenic TrkC lenses exhibit slightly elongated lens epithelial cells, fiber cell dysmorphology and epithelial multilayering compared with control (compare A, B). Lens epithelial cells do not elongate in the TrkC transgenic lenses when one allele of Frs2α is deleted and appear phenotypically similar to control lens (compare A, C). Control (D,G,J), TrkC (E,H,K) and TrkC lenses lacking one allele of Frs2α (F,I,L) were analyzed at E15.5 to determine the activation (phosphorylation) state of Frs2α (D-F), Erk (G-I) and Akt (J-L) in the lens epithelium. Compared with control lenses, TrkC lenses had increased activation of Frs2α (compare D, E), Erk (compare G, H) and Akt (compare J, K) in the lens epithelium. However, this effect is Frs2α dependent, as the loss of one allele of Frs2α in the TrkC background results in expression levels of these proteins similar to that of control (compare D, F; G, I; J, L). Increased downstream signaling led to premature fiber cell differentiation in the TrkC lenses observed by the expression of fiber cell specific proteins $p57^{KIP2}$ (compare M, N) and β-crystallin (compare P, Q) in the epithelium; however, this effect is Frs2α dependent (compare M, O; P, R).

Table S1. Primers

Gene	Primers for PCR	Expected size (bp)
Frs2α cDNA	F: TAT CCC CGC TAC CCC TCG TT R: TGT TGC CCG GTC CAC TC	420
Frs2β cDNA	F: GCA CCT CGC CGA CCC TCC ACA A R: GGC CAC TCC ACC CTC GCT CCT CAG	410
TrkC cDNA	F: ACC CCC ACT TGC GTT AT R: ATG CGG AAG AGG GGG AGT TGG	230
P75-NTR cDNA	F: GGC ACC TCC AGA ACA AGA CCT C R: CCA CAA GGC CCA CAA CCA CAG C	156
GAPDH cDNA	F: AGG CCG GTG CTG AGT ATG TC R: TGC CTG CTT CAC CAC CTT CT	530
TrkA cDNA	F: ACG GTA ACA GCA CAT CAA GAG R: CGG AGG AAA CGG TTG AGG TC	610
ALK cDNA	F: AGC CTC CCA GCC CCG CCT TCT CT R: CAG CTC TGA TTC CCG TGG TTG TGC	234
Ret cDNA	F: CTG GCC TCC TCT ACC TCA ATC R: AGT GCC CAC CGA GTG CTC	412
Trk C transgene	F: ACC TGC CCT CCC ACC CTC TTC R: GGC TGG CTG CTT GCT CTA TCA CAC	401
Le-Cre transgene	F: CCT GTT TTG CAC GTT CAC CG R: ATG CTT CTG TCC GTT TGC CG	300
NT3 transgene	F: GCA TTC CAG CTG CTG ACG GT R: TGA CTG GCC TGG CTT CTT TAC A	900
Fgfr2 wild type (WT) and floxed	F: CTC CAC TGA TTA CAT CTA AAG AGC	WT: 300

allele for genotyping	R: GTC AAT TCT AAG CCA CTG TCT GCC	Floxed: 373
Frs2_ wild type (WT) and floxed	F: GAG TGT GCT GTG ATT GGA AGG CAG	WT: 224
allele for genotyping	R: GGCACGAGTGTCTGCAGACACATG	Floxed: 319

F, forward primer; R, reverse primer

Table S2. Antibodies

Primary antibody	Dilution	Source
Anti-Frs2α	1:100	Abcam, ab10425
Anti-BrdU	1:200	Abcam, ab6326
Anti-E-cadherin	1:100	Abcam, ab15148
Anti-Prox1	1:150	Abcam, ab37128
Anti-β-crystallins	1:200	Samuel Zigler, Johns Hopkins University School of Medicine, Baltimore, MD, USA
Anti-aquaporin0	1:100	Abcam, ab15077
Anti-pFrs2α	1:50	R&D Systems, AF5126
Anti-p44/48ERK1/2	1:100	Cell Signaling Technology, 9101
Anti-pAkt (Ser473)	1:100	Cell Signaling Technology, 9271
Anti-p57KIP2	1:100	Abcam, ab4058
Anti-TrkC	1:100	Cell Signaling Technology, 3376
Anti-GFP	1:100	Cell Signaling Technology, 2555