Supplementary Figures and Tables

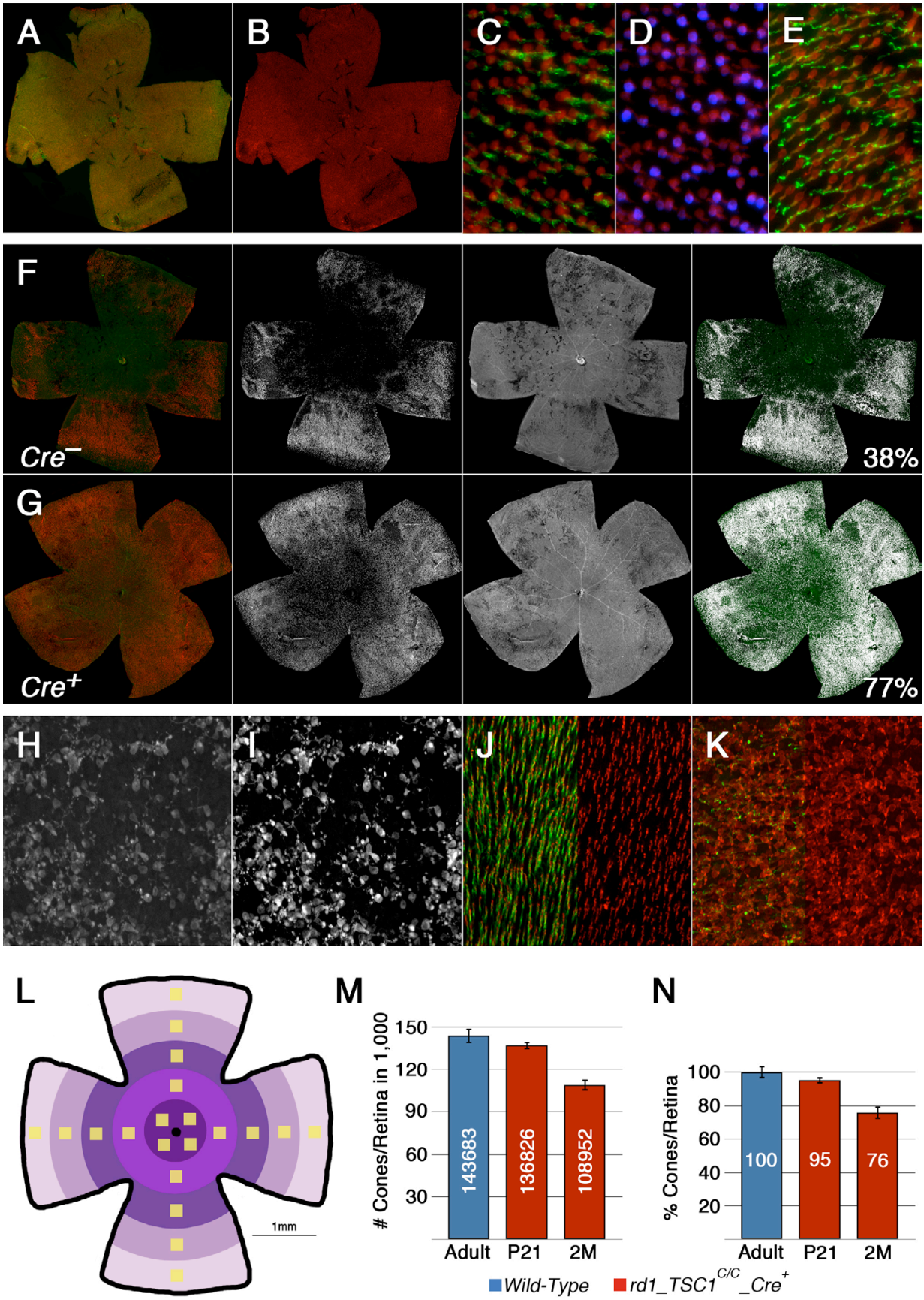

Figure S1

Cone survival analysis. (**A**, **B**) Tiled retinal flat mounts showing PNA (green signal) and the Ai9 Cre reporter expression (red signal) in combination (**A**) and Ai9 reporter only (**B**). The uniform distribution of the red signal

in (**B**) indicates that Cre recombinase is expressed and active across the entire retina. (**C-E**) Higher magnifications showing PNA and Cre reporter (**C**), Cre recombinase (blue signal) and Cre reporter (**D**), and cone arrestin (green signal) and Cre reporter (**E**), in cones. (**F**, **G**) Examples of tiled retinal flat mounts used for cone survival quantification at 2 months of age in *Cre* ± *TSC1*$^{C/C}$*_rd1* mice (compare to Figure 4A). First column, actual scan showing cone arrestin (red signal) and the FITC-PNA signal (green). Second and third columns show gray scale images of cone arrestin and PNA-FITC, respectively. Last column shows actual colocalization as seen after analysis (white signal) with Colocalizer Pro. Calibrated percentage of cone survival is indicated in panel. The percentage of cone survival in the *Cre*$^{-}$ animal (**F**: 38%) corresponds to 21% of colocalization between the red and green signal while the percentage of cone survival in the *Cre*$^{+}$ animal (**G**: 77%) corresponds to 43% of colocalization. (**H**) Example of the actual image resolution obtained by the tile scan used for the cone survival quantification. Shown is cone arrestin staining in gray scale. (**I**) Same image as in (**H**) after adjusting the upper and lower thresholds such that only cones are visible (same as second column in **F** and **G**). After this initial adjustment images were used to calculate colocalization in Colocalizer Pro. (**J**, **K**) Example of retinal flat mount stained for cone arrestin (red signal) and PNA (green signal) in a wild-type (**J**) and *rd1* mutant (**K**) background. In right half of each panel PNA has been removed to better visualize the cone arrestin staining. In wild-type less red signal is seen as cone arrestin is in the inner an outer segments. Therefore colocalization values of red versus green in *rd1* cannot be calibrated with colocalization values obtained in wild-type. (**L**) Cartoon of the 5 sectors used to count cones and location of squares in which cones were counted. (**M**) Average cone count per retina (n=3) for genotypes and ages indicated. (**N**) Percentage representation of cones per retina of values in (**M**) where wild-type is set at 100%. Percentage of cone survival obtained in the *rd1_Tsc1*^{cKO} at 2 months of age is similar between the counting method (75.8% ± 3.7%) and the colocalization method (77.6% ± 3.9%; see also Figure 4B). (**M, N**) Numbers in bars: actual value of graph rounded to the next number. Error bars: SD.

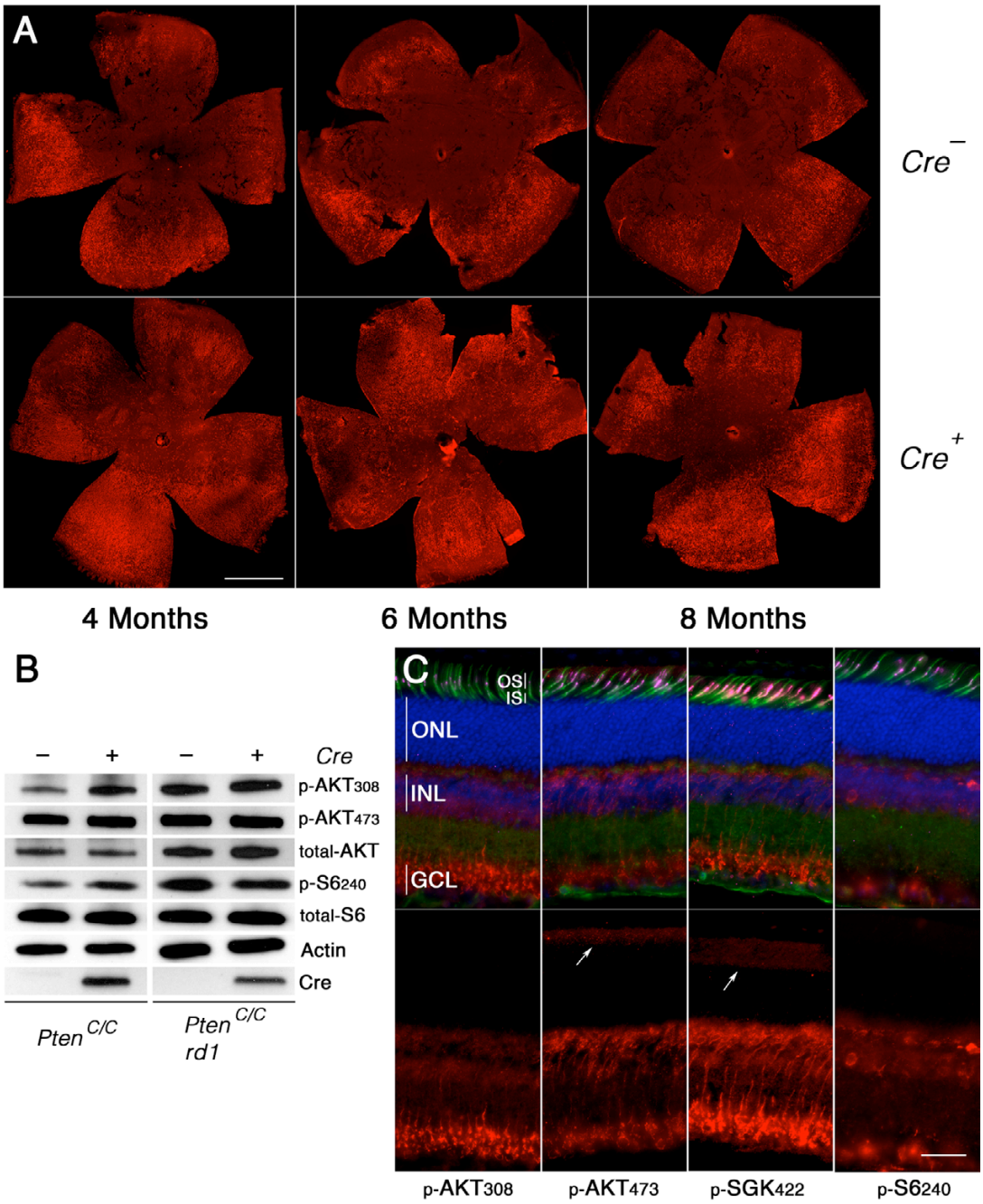

Figure S2

Loss of *Pten* promotes cone survival in *rd1* mice. (**A**) Representative retinal flat mounts at time points indicated from mice in a *rd1* mutant background harboring the *Pten*$^{c/c}$ allele (red signal: cone arrestin). Scale bar: 1mm. (**B**) Western blot analyses from whole retinal extracts at P21 from *Pten*$^{c/c}$ mice in a wild-type or *rd1* background (2nd and 4th columns contain extracts from *Cre*$^{+}$ animals). Proteins of interest are indicated on the right. (**C**) Immunofluorescence analysis at P21 on retinal cyrosections of *Pten*$^{c/c}$ mice for phosphorylation on sites of proteins indicated (red signal). Arrows show possible low level of expression or background noise of phospho (p)-AKT^{473} and p-SGK^{422} in PRs, while p-AKT^{308} and p-S6^{240} appear almost undetectable in PRs. In contrast, robust expression is seen in the inner nuclear layer (INL) and/or ganglion cell layer (GCL). This makes it difficult to detect phosphorylation changes of these proteins by western blot using whole retinal extracts as PTEN is lost only in cones. Additionally, after the loss of most rods retinal extracts contain mainly proteins of INL and GCL cells (green: PNA; magenta: short-wave opsin; blue: nuclear DAPI; IS: inner segment; ONL: outer nuclear layer; OS: outer segment). Scale bar: 50μm.

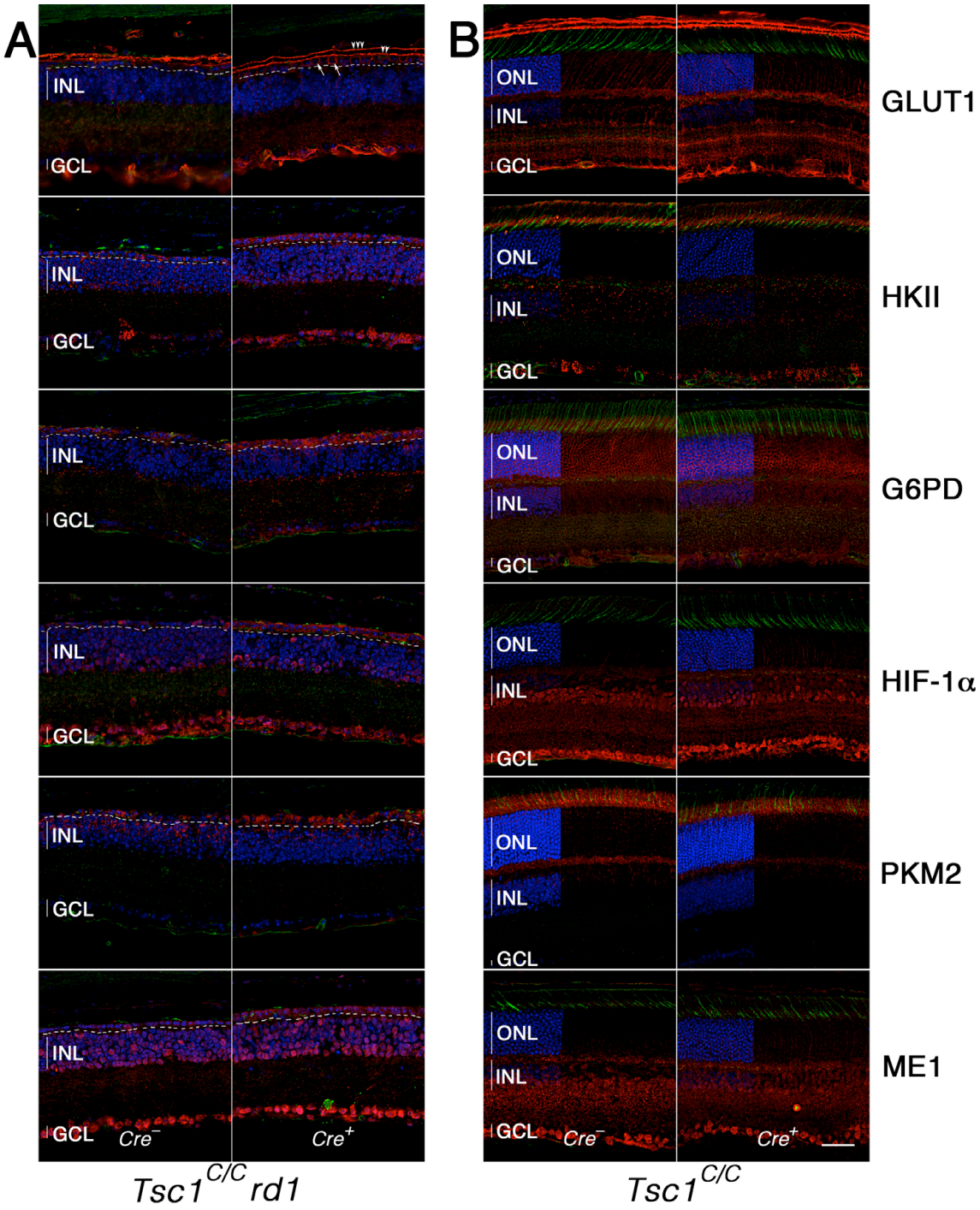

Figure S3

Loss of *Tsc1* upregulates metabolic genes only in diseased cones. Immunofluorescence analysis for proteins indicated (red signal) on retinal cryo-sections from *rd1* (**A**) and wild-type (**B**) mice harboring the *Tsc1*$^{c/c}$ allele at 2 months of age. Dotted lines (**A**) depict the border between the cone layer and the INL. In *rd1* mice all 6 proteins are expressed at higher levels in the cone layer upon loss of TSC1 (see also Figure 6A and Figure S4 for immunofluorescence on flat mounts at 2 months). In contrast, none of these proteins appear upregulated in a wild-type background upon loss of TSC1 (**B**). Arrows (**A**) show expression of glucose transporter-1 (GLUT1) on cone membrane, while double and triple arrowheads show expression on the apical and basal side of the retinal-pigmented epithelium (RPE) respectively (green: PNA; blue: nuclear DAPI; in (**B**) 3/5 of DAPI signal has been removed to better visualize the expression of the protein indicated to the right; HKII: hexokinase II; G6PD: glucose-6-phosphate dehydrogenase; HIF-1α: hypoxia inducible factor 1 alpha; PKM2: pyruvate kinase M2; ME1: malic enzyme 1). Scale bar: 50μm.

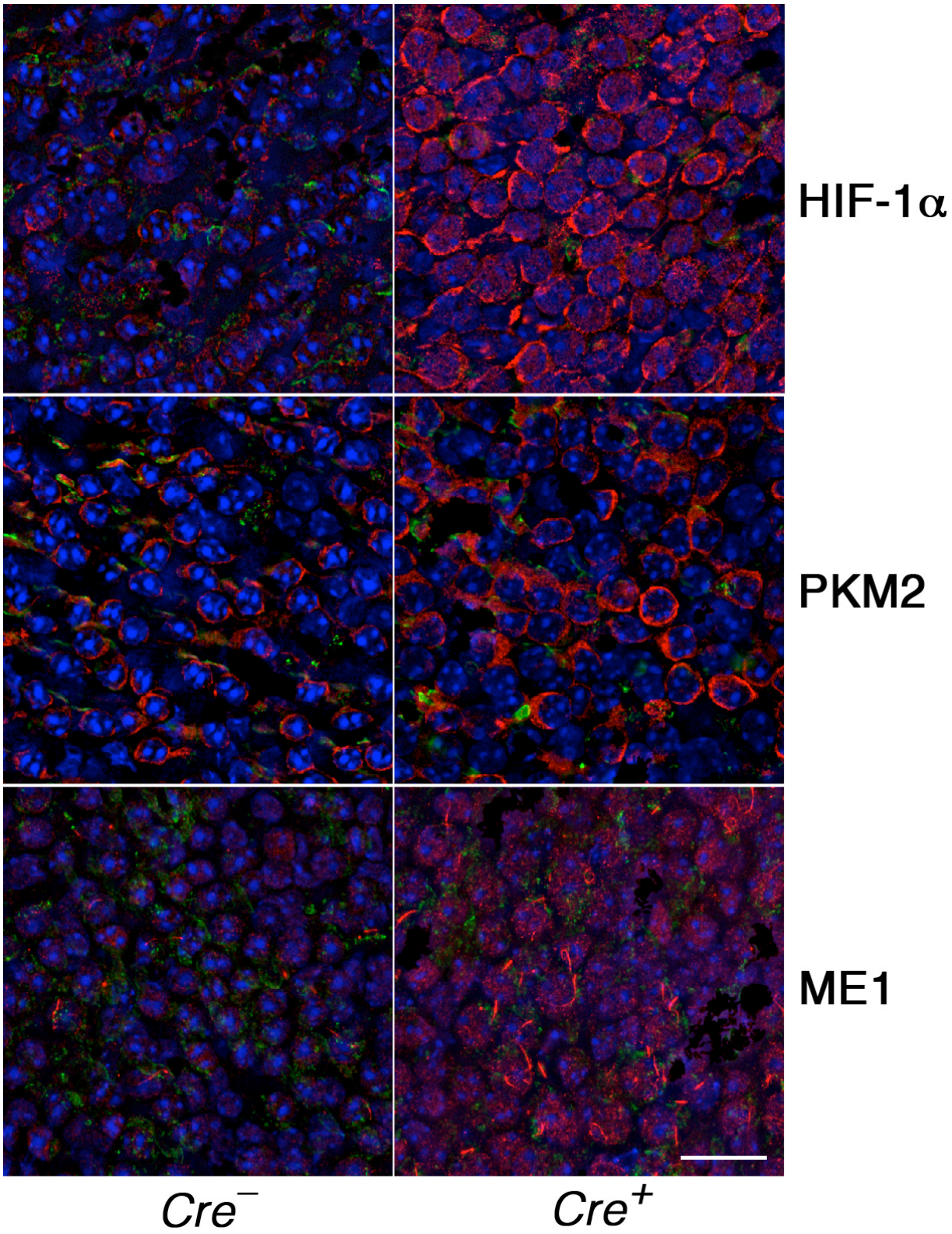

Figure S4

Loss of *Tsc1* upregulates HIF-1α, PKM2 and ME1 expression in cones of *rd1* mice. Immunofluorescence analyses on retinal flat mounts of *rd1* mutant mice harboring the *Tsc1*$^{c/c}$ allele at 2 months of age. Proteins of interest (red signal) are indicated to the right (green: PNA; blue: nuclear DAPI). Scale bar: 20μm.

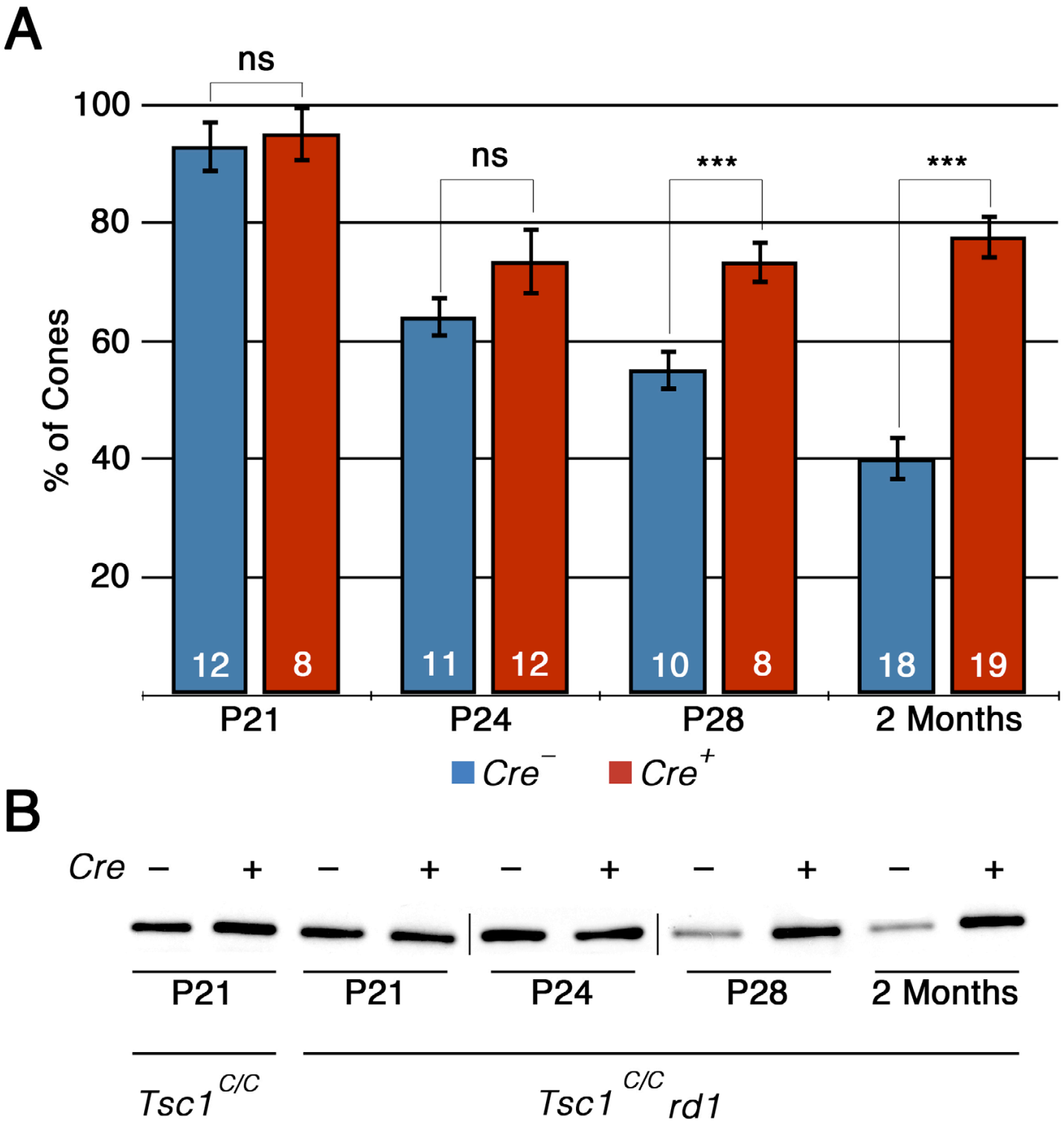

Figure S5

Early cone death kinetics. (**A**) Quantification of cone survival in *rd1* mice harboring the *Tsc1*$^{c/c}$ allele at time points indicated. Numbers in bars: numbers of retinas analyzed. P-values: ***<0.005; ns: not significant, calculated by t-Test. (**B**) Western blot for cone-arrestin from whole retinal extracts of *Tsc1*$^{c/c}$ mice in either a wild-type or *rd1* mutant background at time points indicated (Lanes separated by vertical lines of the time point P24 were run on a separate gel and inserted into the figure).

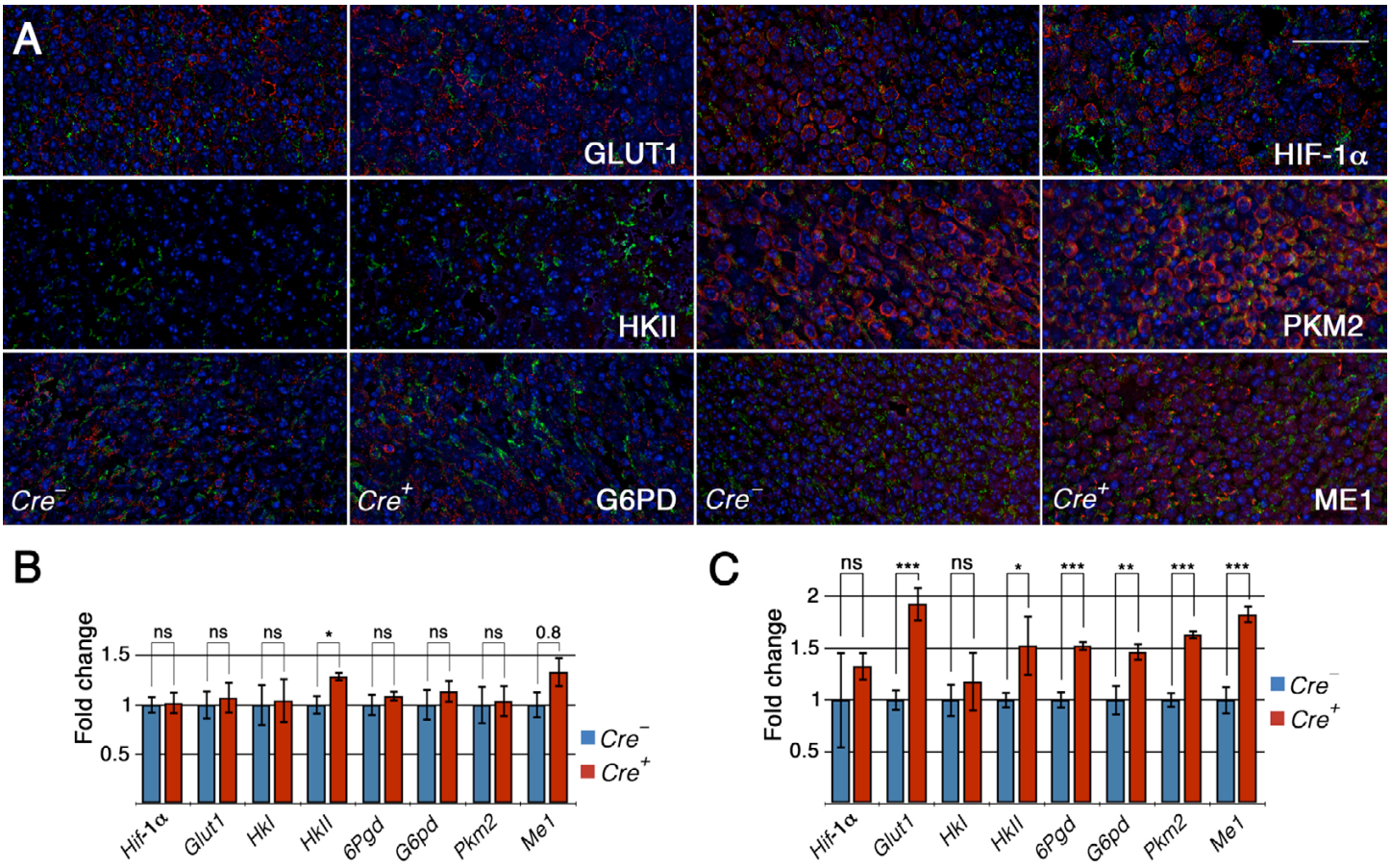

Figure S6

Increased expression of mTORC1 targets over time. (**A-C**) Immunofluorescence analysis on retinal flat mounts (**A**) and quantitative real-time polymerase chain reaction (qRT-PCR) (**B**, **C**) on *rd1* mutant mice harboring the *Tsc1*$^{c/c}$ allele. (**A**) Immunofluorescence analyses at P21 for proteins indicated (red signal). Apart from ME1, none of the other genes showed a clear increase in expression upon loss of TSC1 at P21 when compared to 2 months (Figure 6A and Figure S3A, S4) (green: PNA; blue: nuclear DAPI). Scale bar: 20μm. (**B**, **C**) qRT-PCR analysis for genes indicated on cDNA synthesized from retinal extracts at P21 (**B**) or P24 (**C**). Error bars: SD. P-values: ***<0.005; **<0.01; *<0.05; ns: not significant, calculated by t-Test. Data represents average of 3 biological samples run in duplicates with two animals per sample (HKI: hexokinase I; 6PGD: 6-phospho gluconate dehydrogenase).

Table S1:

Primers for qRT-PCR analysis

Gene	**Forward Primer (5'-3')**	**Reverse Primer (5'-3')**
Hif-1α	GATGACGGCGACATGGTTTAC	CTCACTGGGCCATTTCTGTGT
Glut1	TCAACACGGCCTTCACTG	CACGATGCTCAGATAGGACATC
HkI	GACAAAGCGGTTCAAAGCCAG	CGACTTCACACTGTTGGTCATCG
HkII	GGAACCCAGCTGTTTGACCA	CAGGGGAACGAGAAGGTGAAA
6pgd	AGACAGGCAGCCACTGAGTT	AAGTTCTGGGTTTCGCTCAA
G6pd	CCTACCATCTGGTGGCTGTT	TGGCTTTAAAGAAGGGCTCA
Pkm2	ATTGCCCGAGAGGCAGAGGC	ATCAAGGTACAGGCACTACACGCAT
Me1	AGAGGTGTTTGCCCATGAAC	GCTGGTCGGATTACTCAAAGC
β-actin	ACTGGGACGACATGGAGAAG	GGGGTGTTGAAGGTCTCAAA