Supporting Information

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Materials and Methods

Generation of Gpr48 Mutant Mice. To explore the biological function of Gpr48 *in vivo*, we generated Gpr48 homozygous mutant mice ($Gpr48^{-/-}$ mice) by microinjecting gene trap-mutated Gpr48 embryonic stem cells (ES) into blastocysts of C57BL/6 mice. Heterozygous mice were intercrossed to obtain homozygous mice. The genotypes of offspring of mutant mice were confirmed by PCR analysis (Fig. S1*b*). The mRNA levels of Gpr48 in the eyes of homozygous, heterozygous, and wild-type mice were detected by RT-PCR analysis. We demonstrated that Gpr48 is expressed in wild-type and heterozygous mice, but no expression of Gpr48 was detected in homozygous mutant mice by RT-PCR (Fig. 1*a*), confirming the successful deletion of Gpr48 in homozygous mutant. The $Gpr48^{-/-}$ mice are growth retarded in the embryo stages. Postnatal day 0 mice weighed ≈80% of their wild-type littermates. $Gpr48^{-/-}$ mice show a perinatal death phenotype, and ≈60% of newborn homozygous mice die around postnatal day 0 and day 1, which is consistent with previous reports (9).

Mouse Husbandry and PCR Genotyping. Mice were housed under a controlled humidity, temperature, and light regimen and fed *ad libitum.* Animal care was consistent with institutional and National Institutes of Health guidelines. Approximately 727 mice from heterozygous intercrosses were genotyped to perform the present study. PCR analysis was used for genotyping DNA obtained from tail samples. Genomic DNA was extracted by ethanol precipitation after proteinase K digestion in a tissue lysis buffer [50 mM Trish (pH 7.5), 50 mM EDTA (pH 8.0), 100 mM NaCl, 0.5 mM spermidine, 1% SDS, 5 mM DTT]. Three primers were used to distinguish the mutated from the wild-type allele (16). Two PCR products were expected: the wild-type PCR product A/B (805 bp) and the transgenic PCR product A/C (650 bp). Due to the insertion of the large 11.98-kb trap vector between primers A and B, the A/B PCR product could not be amplified in the transgenic allele under the present PCR conditions (Fig. S1*B*).

X-Gal Staining and Gpr48 Expression. To perform X-gal staining, embryo mouse or adult mouse eyes were dissected in 1× PBS and incubated with the β-glycosidase fixative buffer (0.2% glutaraldehyde, 1.5% formaldehyde, 2 mM MgCl2, 5 mM EGTA, 0.1 M sodium phosphate buffer, pH 8) for 30 min. Tissues were then washed three times for 30 min each in the washing buffer [0.1 M sodium phosphate buffer (pH 8), 2 mM $MgCl_2$, 5 mM EGTA, 0.01% (wt/vol) sodium deoxycholate, 0.02% (vol/vol) Nonidet P-40]. Subsequently, mouse tissues were incubated in the staining solution [washing buffer containing 5 mM K3Fe (CN), 5 mM K4Fe (CN) 6·6 H_2O]. The tissues were rinsed in PBS before being stored in 70% ethanol. Photography was performed with transillumination using a dissecting microscope (Leica). For section of the X-gal staining tissues, tissues were serially dehydrated and embedded in paraffin with Tissue-Tek (Sakura Finetek). The tissue was then sectioned at 10 μm thick and counterstained with eosin (Sigma-Aldrich).

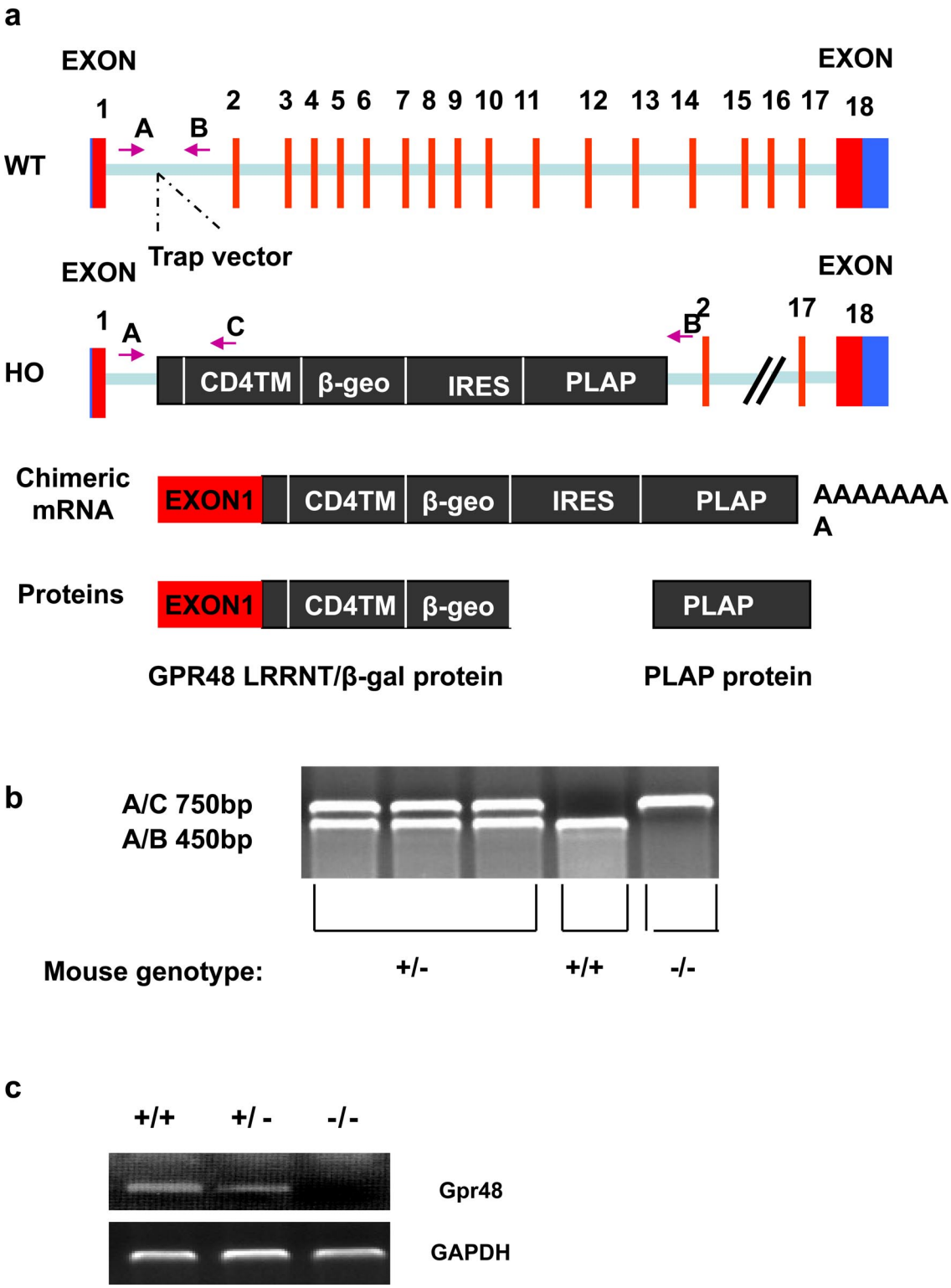

Fig. S1. Targeted disruption of the Gpr48 gene. (*a*) Schematic drawing of the Gpr48 gene-trapping strategy. The position of primers (A, B, and C) used in PCR genotyping are indicated by arrowheads. β-geo, a fusion protein between β-galactosidase and neomycin phosphotransferase; IRES, internal ribosomal entry site; PLAP, human placental alkaline phosphatase. (*b*) PCR genotyping of a Gpr48 fragment and the transgenic allele in wild-type (+/+), heterozygous (+/-), and homozygous mutant (-/-) mice. The size of PCR fragments is 805 and 650 bp for wild-type and insertional mutant alleles, respectively. (*c*) RT-PCR result of Gpr48 expression in E12.5 mouse embryos of different genotypes.

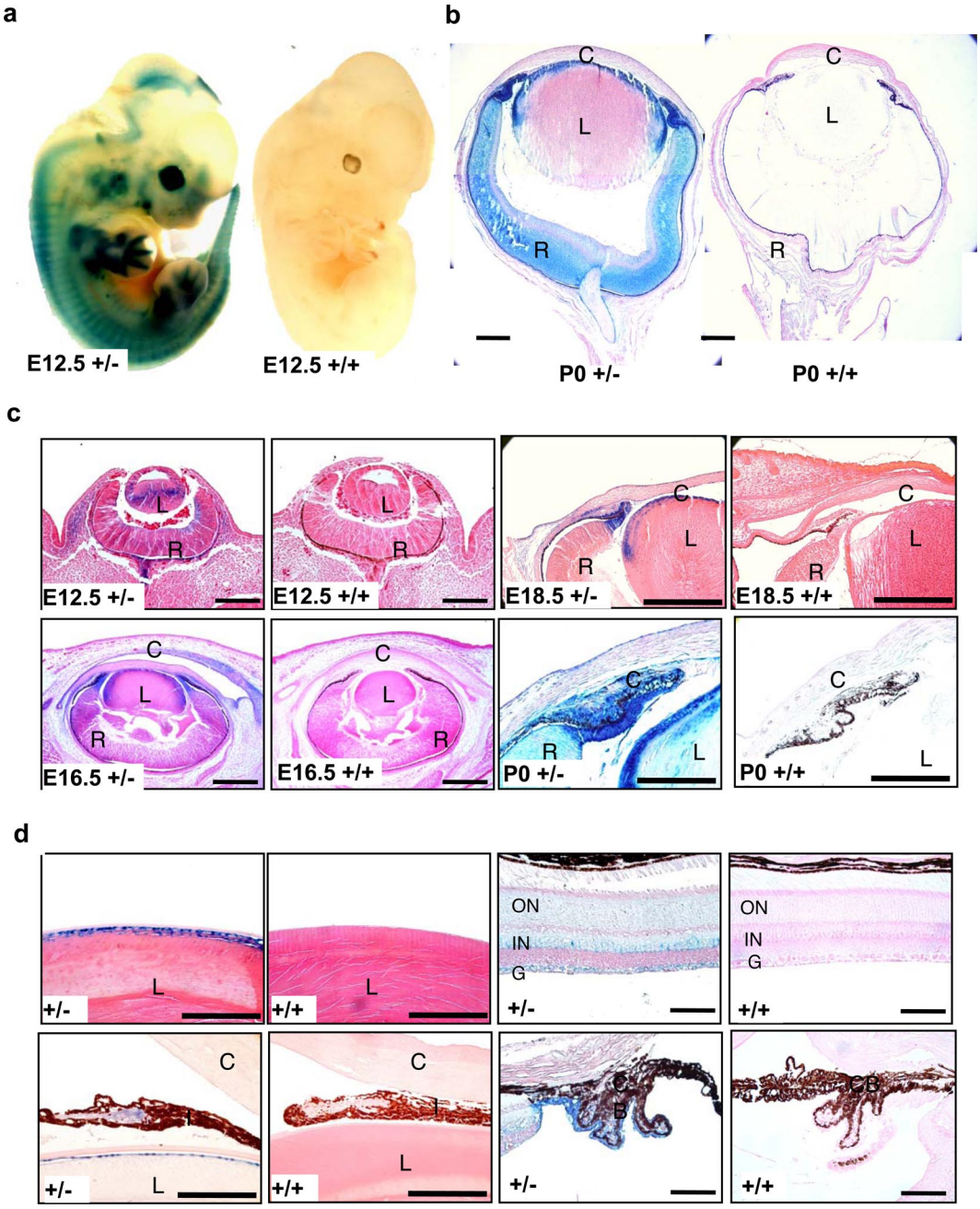

Fig. S2. Temporal and spatial expression of Gpr48 in mouse eyes. (*a*) Expression of Gpr48 at E12.5 days using β-galactosidase staining in Gpr48 heterozygous (+/-) and wild-type (+/+) mice. (*b*) Expression of Gpr48 at postnatal day 0 (P0) using β-galactosidase staining. (*c*) Temporal expression of Gpr48 during different stages of anterior segment development from E12.5 to P0. In the E12.5 embryo, mesenchymal cells expressing Gpr48 β-galactosidase are present around the eye between the surface ectoderm and the lens and inner layer cells of optical cup. At E16.5 and E18.5, the expression of Gpr48 β-galactosidase is very high at the tip of the optical cup and in the mesenchymal tissue around the tip of the optical cup. At postnatal day 0, Gpr48 β-galactosidase expression is strong in the ciliary body, iris stroma, corneal epithelial cell, keratocytes, and endoepithelial cells. Wild-type mice (+/+) with no β-galactosidase staining were used as controls. (*d*) Expression of Gpr48 in different tissues of adult mouse eyes. Gpr48 β-galactosidase staining was found in the lens epithelial cells, ganglion cells, inner nuclear cell layer of retina, iris stroma, and out layer cells of ciliary body. Wild-type mice with no β-galactosidase staining were used as controls. C, cornea; I, iris; R, retina; L, lens; CB, ciliary body; G, ganglion cells; IN, inner nuclear layer; ON, outer nuclear layer. [Scale bars: 170 μm (*b* and *c*) and 85 μm (*f*).]

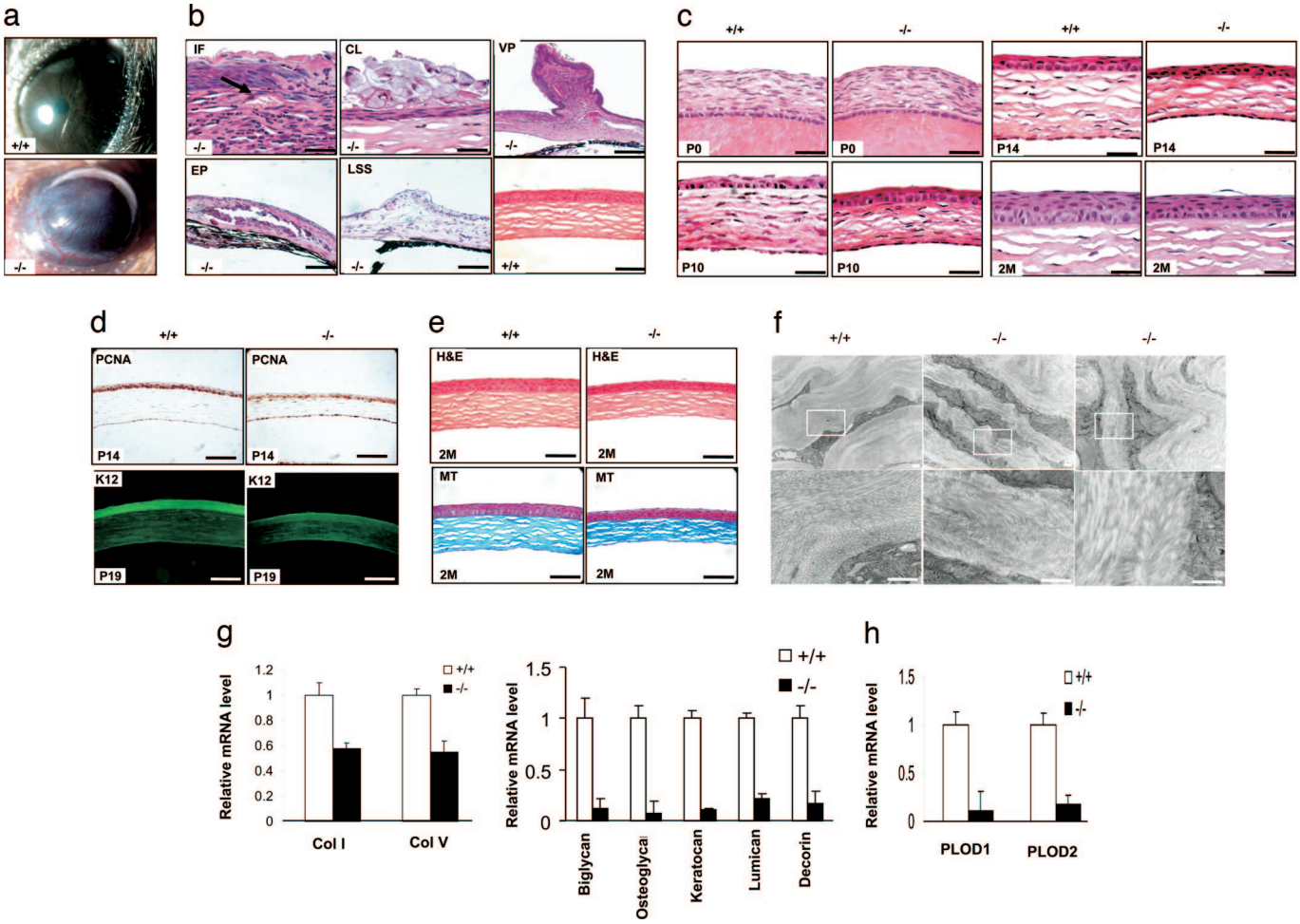

Fig. S3. Cornea dysgenesis in *Gpr48*$^{-/-}$ mice. (*a*) Cornea opacity associated with cornea neovascularization in *Gpr48*$^{-/-}$ mice. (*b*) Histological analyses of cornea in *Gpr48*$^{-/-}$ mice reveal different types of keratopathy in *Gpr48*$^{-/-}$ mice. IF, cornea inflammation and vascularization; CL, cytsic-like structure in front of the cornea epithelium; VP, cornea vascular pannus; EP, cornea epithelial plug; LSS, loss of cornea epithelium smoothness. Cornea in wild-type control mice have no defects (+/+). (*c*) Histological analysis of the cornea epithelium at different developmental stages in *Gpr48*$^{-/-}$ and wild-type mice. At P0, *Gpr48*$^{-/-}$ mice have similar cornea epithelium morphology compared with wild-type mice. At P10 and day 14 and in 2-month-old mice, the cornea epithelium is much thinner and more compact in *Gpr48*$^{-/-}$ compared with wild-type mouse corneas. (*d*) PCNAs staining and Keratin 12 staining are both decreased in the cornea epithelium of *Gpr48*$^{-/-}$ mice, indicating defects in proliferation and differentiation. (*e*) Thin cornea stroma in *Gpr48*$^{-/-}$ mice was found in both H&E and Masson's trichrome staining (blue). (*f*) Disturbed and disorganized fiber structures were found in the cornea of *Gpr48*$^{-/-}$ mice by electron microscope. (*g*) The expression levels of collagen I, collagen V, BGN, OGN, keraton, Den, and lumican were significantly decreased in *Gpr48*$^{-/-}$ mice in the P8 mouse eye. (*h*) The expression levels of PLOD1 and PLOD2, two key enzymes in collagen homeostasis, are also decreased in *Gpr48*$^{-/-}$ compared with wild-type mice. [Scale bars: 85 μm (*b* and *d*), 43 μm (*c*), and 500 nm (*f*).]

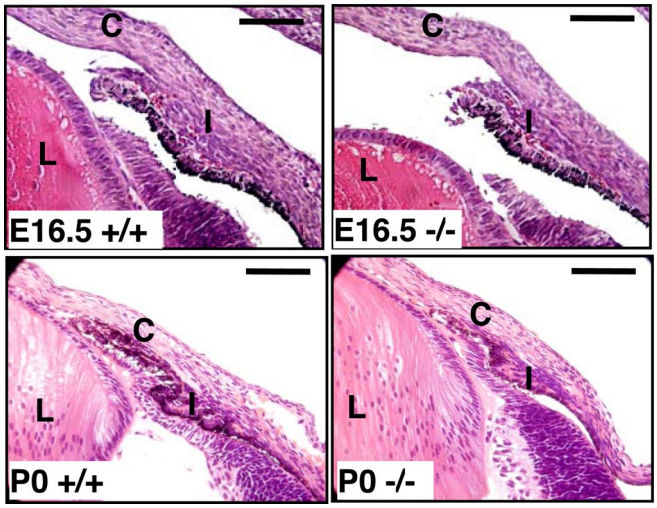

Fig. S4. Iris hypoplasia at postnatal day 0 Gpr48 null mutant mice. H&E staining of iris in the early stage of eye development. The irises show similar lengths between wild-type and Gpr48 null mutant mice at E16.5. At P0, the iris of Gpr48 mutant mice is significantly shorter and thinner than the wild type. (Scale bar: 43 μm.)