Supplementary Methods

Immunostainings and antibodies

Primary antibodies against the following proteins were used at the dilution indicated: anti-green fluorescent protein, conjugated to Alexa Fluor 488 (Invitrogen, Grand Island, NY, USA, 1/1,000, A21311); TGFβRII (C-16; Santa Cruz Biotechnology, Dallas, TX, USA, 1/500, sc-220); LM332 (a generous gift from Peter Marinkovich, 1/1,000); p63 (LabVision/NeoMarkers, Fremont, CA, USA, 1/50, MS-1081); keratin 10 (Covance, Princeton, NJ, USA, 1/500, PRB-159P); keratin 12 (a generous gift from Chia-Yang Liu) ; Pax6 (Covance, Princeton, NJ, USA, 1/50, PRB-278P); keratin 13 (Abcam, Cambridge, MA, USA, 1/100, ab58744); Muc5AC (Abcam, Cambridge, MA, USA, 1/200, ab3649); KLF5 (a generous gift from Jeff Whitsett, 1/100); SPDEF (a generous gift from Jeff Whitsett, 1/100). Secondary antibodies conjugated to Alexa Fluor 488 or 555 (Molecular Probes, Grand Island, NY, USA) were used at a dilution of 1/1,000. Sections were counterstained with hematoxylin (Ventana Medical Systems, Inc, Tuscon, AZ, USA), nuclear fast red (Sigma Chemical Co., St. Louis, MO, USA) or 4',6-diamidino-2-phenylindole (DAPI; Sigma Chemical Co., St. Louis, MO, USA, 1/5,000) to visualize cell nuclei. Fluorescence images were acquired with the AxioImager M1 fluorescent microscope (Zeiss, Oberkochen, Germany) and pictures were taken with the AxioCam MRm camera (Zeiss, Oberkochen, Germany). Images in different focal planes were combined using the Extended Focus Module within the Axiovision software suite (Zeiss, Oberkochen, Germany).

Real-time PCR

Primers used: GAPDH-F: CGTAGACAAAATGGTGAAGGTCGG; GAPDH-R: AAGCAGTTGGTGGTGCAGGATG; TGFβRII-F: GCAAGTTTTGCGATGTGAGA; TGFβRII-R: TCCGTGTTGTGGTTGATGTT. Taqman probes used: GAPDH:

Mm99999915_g1; 18S: 4352930E; SPDEF: Mm00600221_m1; Muc5AC: Mm01276725_g1, FoxA3: Mm00484714_m1; Gcnt3: Hs00191070_m1.

Chromatin immunoprecipitation

Primers used: Sites 1&2-F: ACAAGCCAGGAAGGCAGCTGTC; Sites 1&2-R: TCCAGAGCTGGTGTCAGCAATG (Sites 1&2 PCR product size: 131bp); Site3-F: AGGCCTCAGACTCACACTCAAG; Site 3-R: CGCCTGCTGTCAGAAGAGTCGT (Site 3 PCR product size: 193bp); Site 4-F: TAGCCATGTGGATGTCTAGC; Site 4-R: AGGCCAAGCCAGAAGACACT (Site 4 PCR product size: 138bp); Site 5-F: GAACGCAACAGATGTGTCCT; Site 5-R: TTCACTGCAGCCTTCCAACTCC (Site 5 PCR product size: 166bp).

Isolation of primary keratinocytes and cell culture

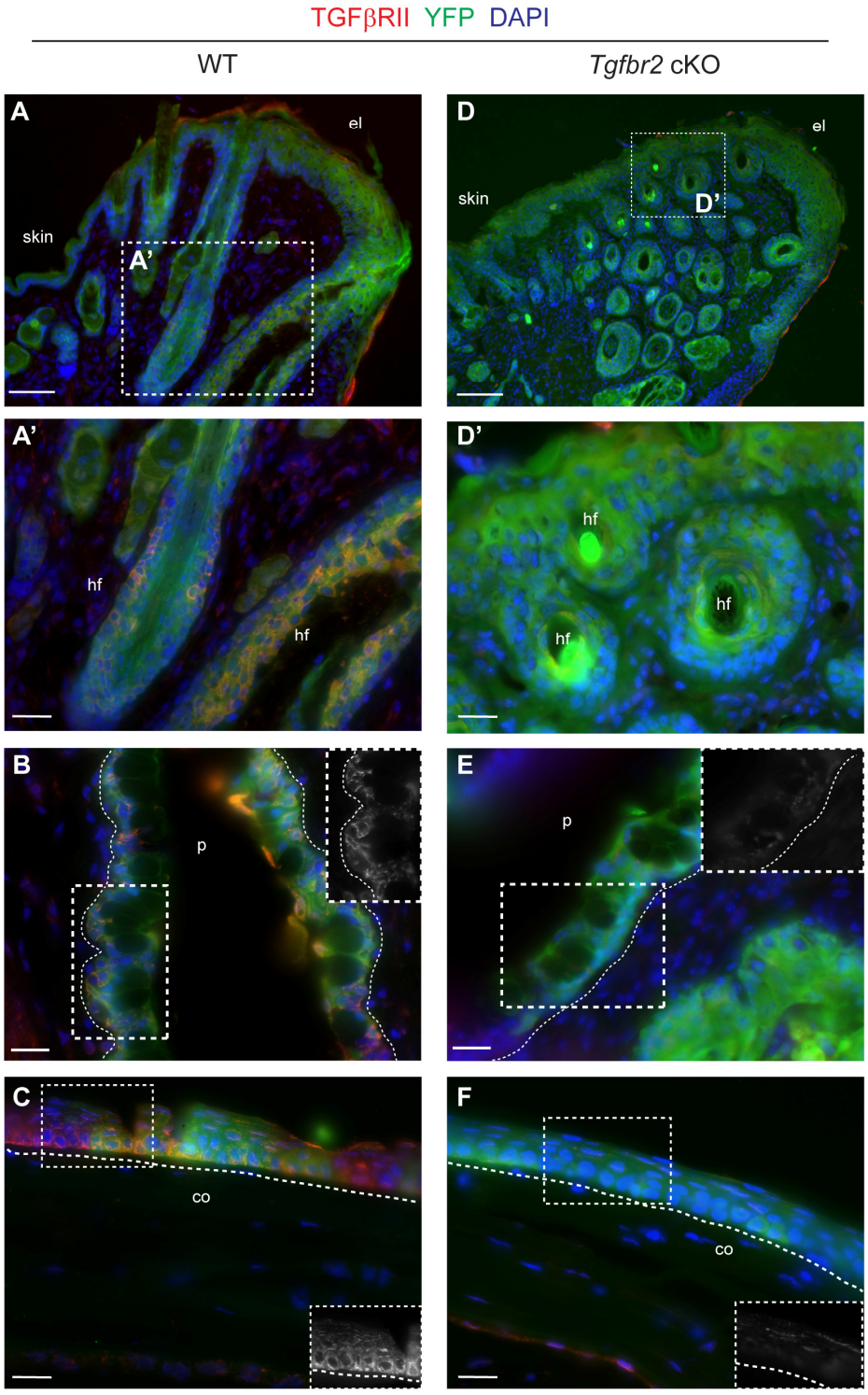
WT and *Tgfbr2* cKO keratinocytes were isolated from newborn C57BL/6 mice at postnatal day 1 by removing the backskin, dissociating epidermis from dermis by incubating in dispase (Gibco, Grand Island, NY, USA) overnight at 4ºC, extracting keratinocytes using 0.12% Trypsin-EDTA (Gibco, Grand Island, NY, USA) diluted in versene containing 0.1% glucose, and plating on a feeder layer of irradiated mouse embryonic fibroblasts (MEFs) in Epithelial cell culture media (E media) (Nowak and Fuchs, 2009) containing 0.3 mM calcium. MEFs were isolated from WT CD-1 mice at embryonic day 13.5 and cultured in DMEM (Gibco, Grand Island, NY, USA) containing 10% serum and 1% penicillin-streptomycin. After expanding MEFs, confluent plates were trypsinized with 0.05% Trypsin-EDTA (Gibco, Grand Island, NY, USA) and irradiated with 60 Gy by the CCHMC Comprehensive Cancer Core Facility. Irradiated MEFs were replated at 100% confluency in DMEM containing 10% serum and 1% penicillin-streptomycin one day before plating primary cells. WT and *Tgfbr2* cKO keratinocytes were passaged to a new feeder layer of irradiated MEFs in E media

containing 0.3 mM calcium once confluent. After the third passage, WT and *Tgfbr2* cKO keratinocytes were grown on plastic in E media containing 0.05 mM calcium.

Reference

Nowak, J. A. and Fuchs, E. (2009). Isolation and culture of epithelial stem cells. *Methods Mol Biol* **482**, 215-232.

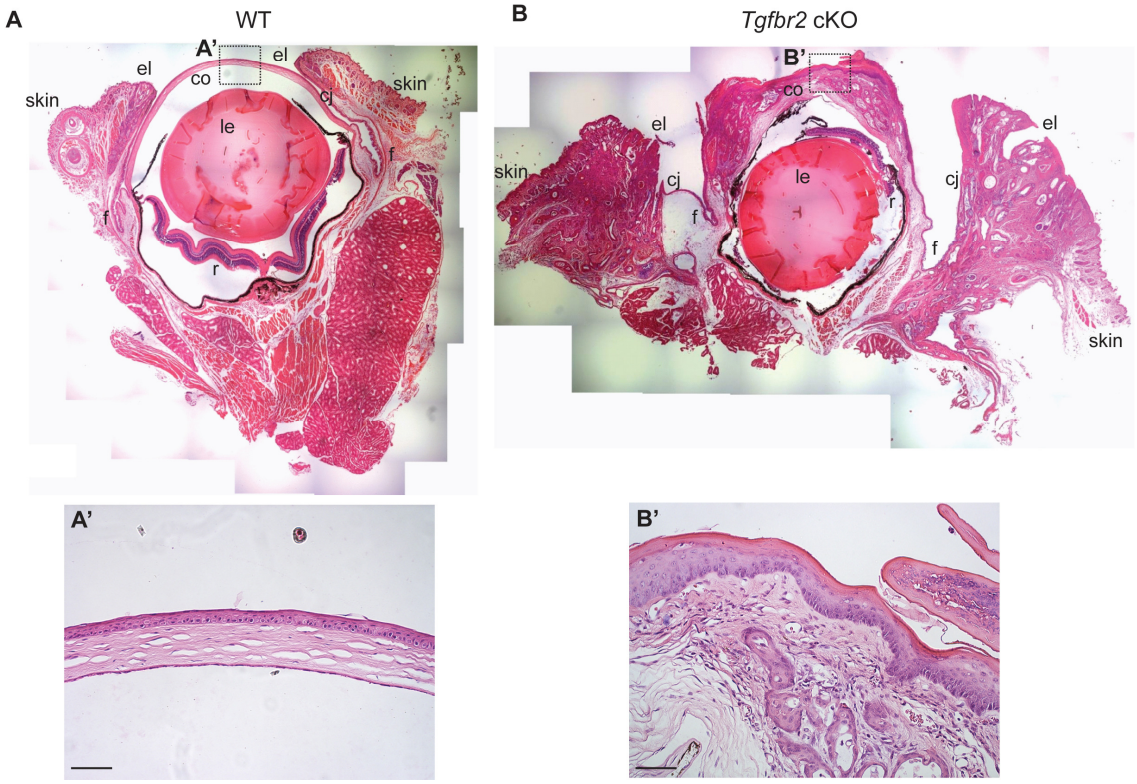
McCauley, et al, Supplemental Figure 1



Supplemental Figure 1 – The ocular surface epithelium is derived from K14-expressing cells and YFP expression reflects conditional deletion of *Tgfbr2* in ocular surface epithelium

(A-C) Immunofluorescence staining with antibodies against YFP and TGFβRII indicated that the eyelid epithelium **(A, A')**, conjunctival epithelium **(B),** and corneal epithelium **(C)** in WT mice was derived from keratin 14-expressing cells and normally expressed TGFβRII (red channel; black and white inset in **B** and **C**). **(D-F)** Immunofluorescence staining with antibodies against YFP and TGFβRII (red channel; black and white insets in **E** and **F**) confirmed efficient deletion of TGFβRII in the eyelid epithelium **(D, D')**, conjunctiva **(E)**, and cornea **(F)**. Dotted lines indicate the basal layer. Scale bars: 100µm **(D)**, 50µm **(A, C, E)**, 20µm **(A', C, D', F).** Abbreviations: el: eyelid; hf: hair follicle; p: palpebral conjunctiva; co: cornea.

McCauley et al, Supplemental Figure 2

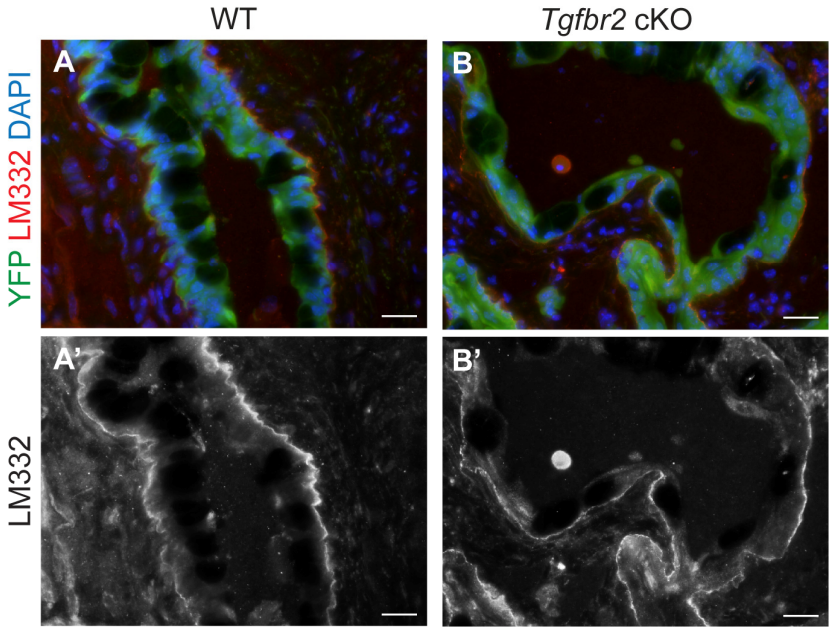


Supplemental Figure 2 – *Tgfbr2*-deficient mice develop ocular surface pathology: a macroscopic view

(A-B) Hematoxylin and eosin (H&E) staining revealed marked disorganization and aberrant structures in the adult *Tgfbr2* cKO eye **(B)** compared to comparable regions of an age-matched (11 months) WT eye **(A)**. The cornea of this *Tgfbr2* cKO mouse **(B'**, zoom on boxed region of **B)** displayed squamous epithelial hyperplasia with hyperkeratosis, parakeratosis and acute inflammation, compared to WT cornea **(A'**, zoom on boxed region of **A)**. Hyperplastic conjunctival epithelium formed cell nests

and epithelial-lined cystic structures that invaginated into the subconjunctival stroma, expanding the eyelid epithelium. The improper location of the retina in the *Tgfbr2* cKO section is a sectioning artifact. Abbreviations: el: eyelid; cj: conjunctiva; f: conjunctival fornix; co: cornea; le: lens; r: retina. Scale bars: 50µm.

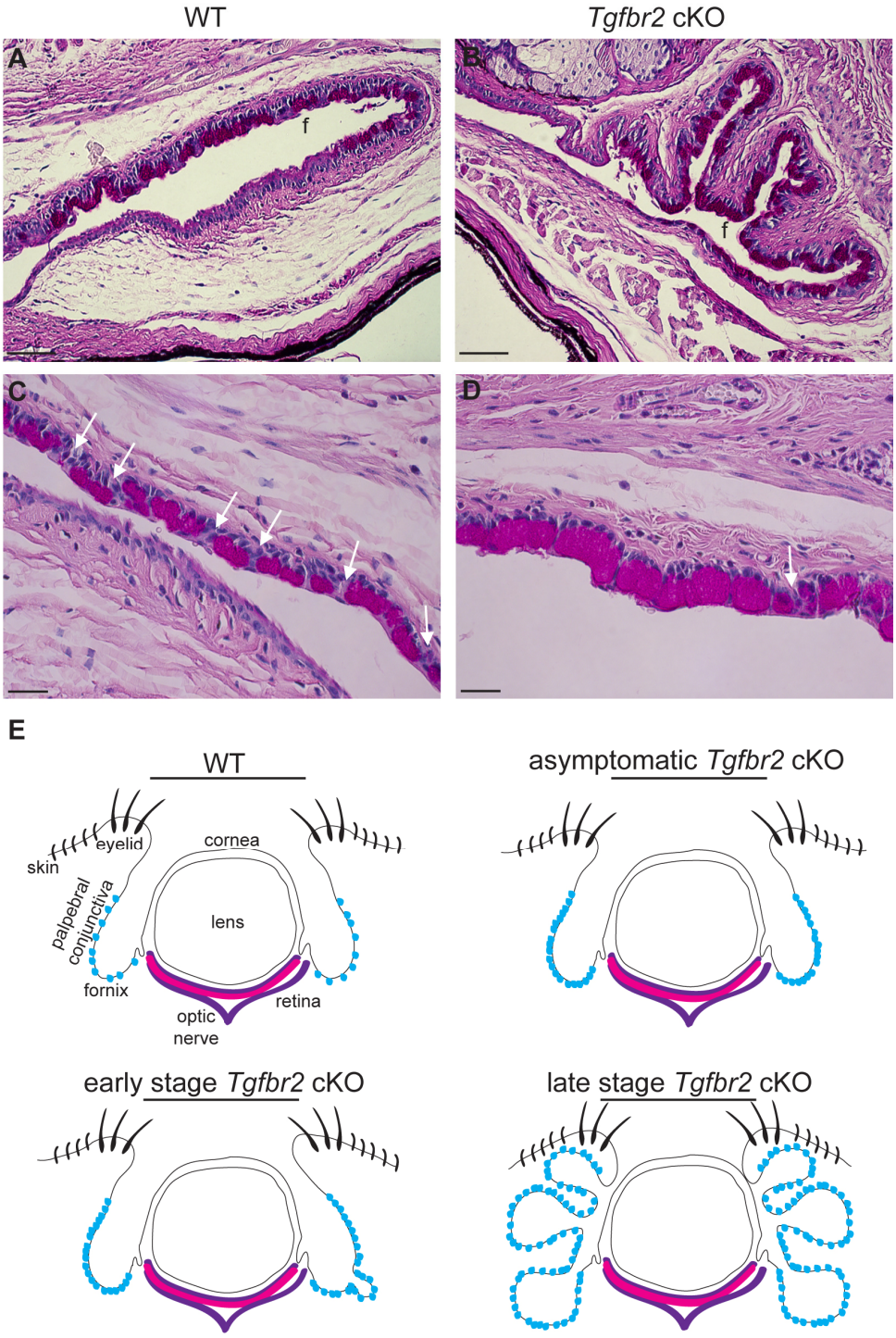
McCauley et al, Supplemental Figure 3



Supplemental Figure 3 - The hyperplastic *Tgfbr2* cKO conjunctival cell nests within the stroma maintain an intact basement membrane

Immunofluorescence staining with an antibody against LM332 (formerly called kalinin or laminin 5) (red channel in **A-B**; black and white in **A'-B'**) revealed a continuous basement membrane underlying the WT conjunctival epithelium (**A, A'**) as well as surrounding the expanded *Tgfbr2* cKO conjunctiva invaginating into the underlying stroma (**B, B'**). YFP marks stratified epithelial cells in green and DAPI counterstains nuclei in blue. Scale bars: 20µm.

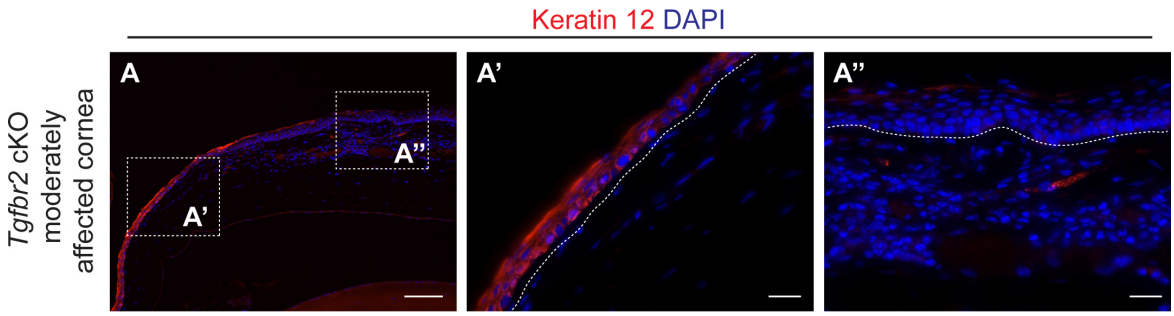
McCauley et al, Supplemental Figure 4



Supplemental Figure 4 – Asymptomatic *Tgfbr2*-deficient mice develop hyperplastic papillary structures at the conjunctival fornix

(A-D) In the absence of an overt ocular phenotype between 6 weeks and 5 months of age, combined PAS and H&E staining of slides from comparable regions demonstrated that as early as 6 weeks of age, the *Tgfbr2* cKO conjunctiva contained hyperplastic papillary structures **(A-B)**, and contained an expanded goblet cell compartment **(D)** compared to WT littermate control mice **(C)**. White arrows **(C-D)** denote stratified conjunctival epithelium interspersed between goblet cells. Scale bars: 50 µm **(A-B)**, 20 µm **(C-D)**. Abbreviations: f: fornix. **(E)** Schematic representation of a WT mouse eye and progression of the goblet cell expansion and beginning stages of conjunctival epithelial hyperplasia and invagination occurring upon loss of *Tgfbr2* in asymptomatic mice. As the phenotype progresses, continual goblet cell expansion and extensive epithelial invagination into the underlying stroma are observed in *Tgfbr2*-deficient aged mice. Goblet cells are represented in blue.

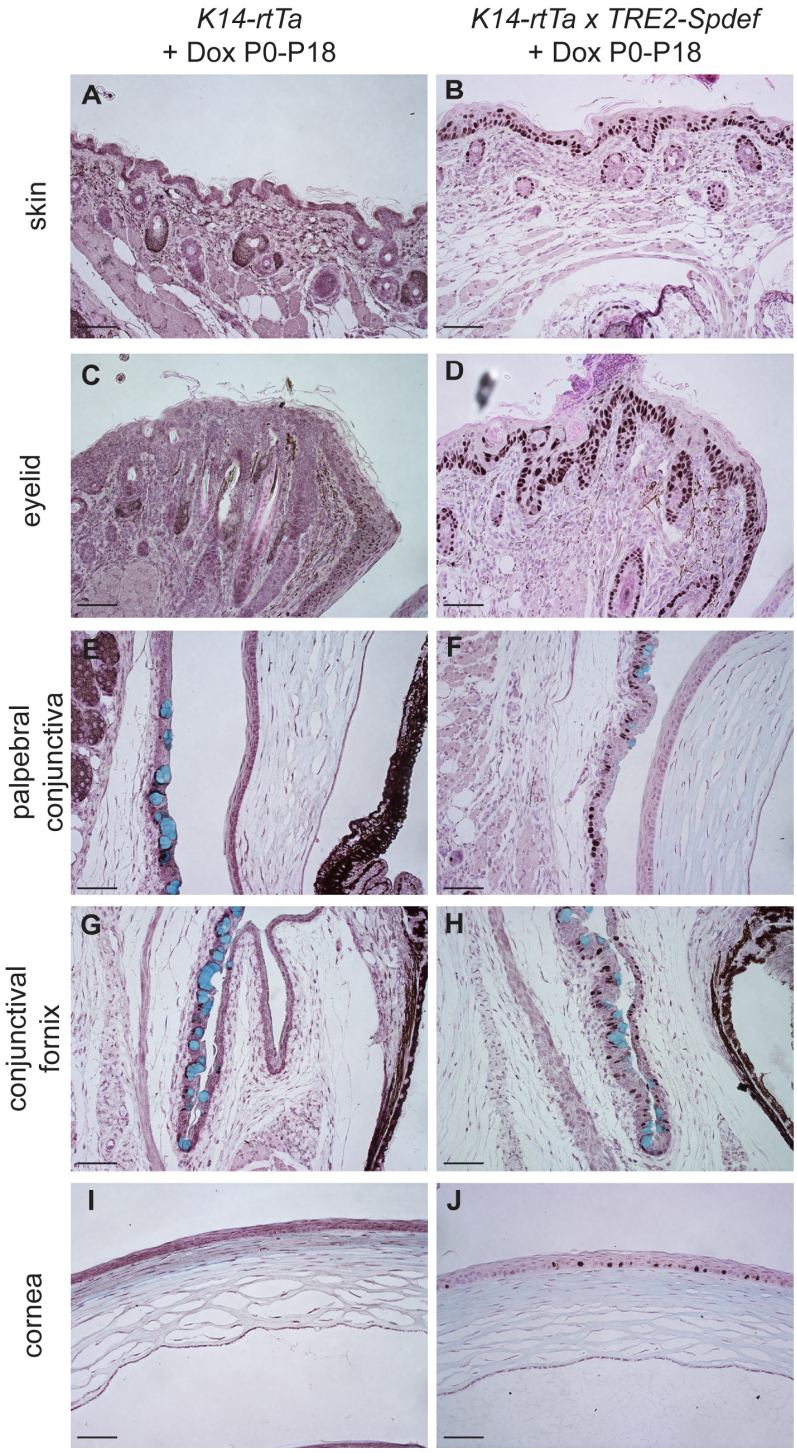
McCauley, et al, Supplemental Figure 5



Supplemental Figure 5 – Adult *Tgfbr2*-deficient corneal epithelium progressively loses keratin 12 expression in the central cornea

Corneal epithelium of adult *Tgfbr2* cKO mice which began to display a phenotype lost keratin 12 expression in the central cornea as it became hyperplastic **(A, A', A'').** Dotted lines indicate the basal layer. DAPI counterstains all nuclei in blue. Scale bars: 100µm **(A);** 20µm **(A', A'')**.

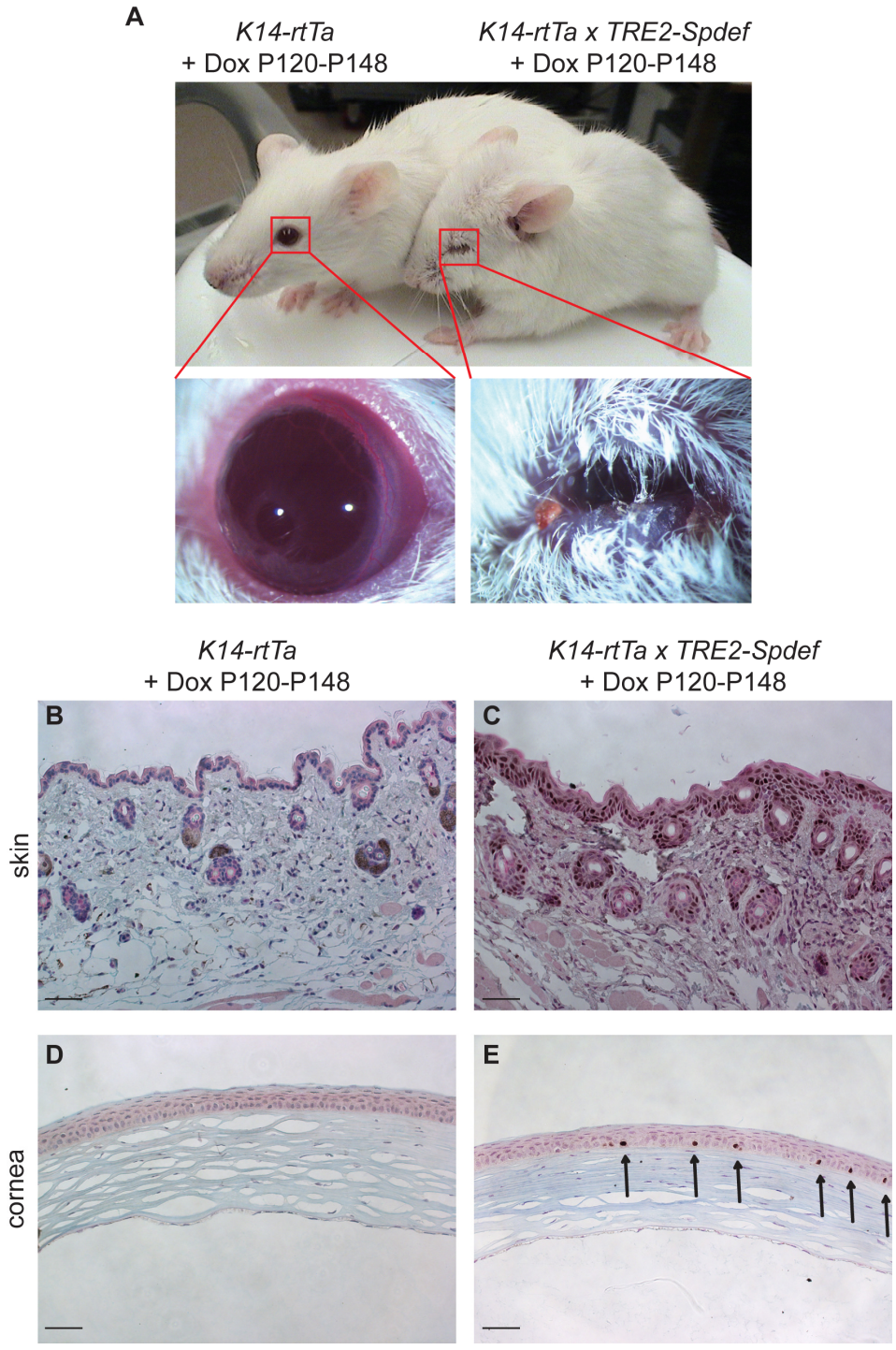
McCauley, et al, Supplemental Figure 6



Supplemental Figure 6 – Expression of SPDEF in keratin 14-positive ocular surface epithelia is not sufficient to drive goblet cell metaplasia by postnatal day 18

Immunohistochemistry staining using an antibody against SPDEF in postnatal day 18 mice expressing SPDEF in all keratin-14 derived epithelia *(K14-rtTA x TRE2-Spdef* with Dox administration since birth, see Fig. 9A for schematic) revealed strong nuclear expression (brown) in the ocular surface epithelia, including skin **(A-B),** eyelid **(C-D)**, palpebral conjunctiva **(E-F),** conjunctival fornix **(G-H)**, and cornea **(I-J)**, compared to WT mice (*K14-rtTA* alone), but no increase in goblet cell differentiation or goblet cell metaplasia. Goblet cells are marked by Alcian blue. Nuclear fast red counterstains all nuclei in red. Scale bars: 50µm.

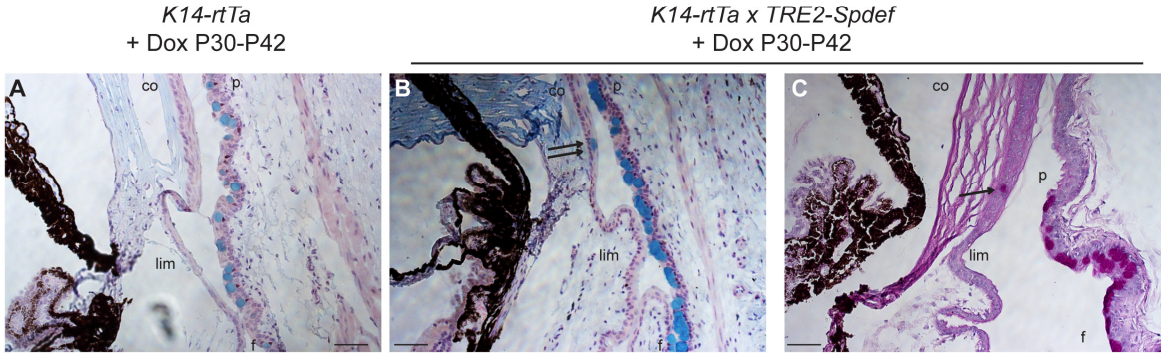
McCauley, et al, Supplemental Figure 7



Supplemental Figure 7 – Transgenic overexpression of SPDEF in keratin 14-derived cells results in an overt ocular phenotype

K14-rtTA x *TRE2-Spdef* mice and *K14-rtTA* control littermates were fed doxycycline to induce SPDEF expression in keratin 14-derived epithelium from P120 to P148. *K14-rtTA* x *TRE2-Spdef* mice developed progressive periorbital tissue expansion with narrowing of the palpebral fissure, compared to *K14-rtTA* control littermates **(A)**. **(B-E)** Immunohistochemistry with an antibody against SPDEF revealed abundant nuclear expression in the skin **(C)** and patchy expression in the cornea **(E;** arrows**)** of adult doxycycline-induced *K14-rtTA* x *TRE2-Spdef* mice, but no expression in *K14-rtTA* control skin **(B)** or corneal epithelium **(D)**. Sections were analyzed by PAS staining every 100µm throughout the entire eyes of adult doxycycline-induced *K14-rtTA* x *TRE2-Spdef* mice, with no goblet cells found in the skin or central cornea. Nuclear fast red counterstains all nuclei in red. Scale bars: 50µm.

McCauley, et al, Supplemental Figure 8



Supplemental Figure 8 - Transgenic overexpression of SPDEF from P30-P42 results in ectopic goblet cell formation in the peripheral cornea

K14-rtTA x *TRE2-Spdef* mice and *K14-rtTA* control littermates were fed doxycycline to induce SPDEF expression in keratin 14-derived epithelium from P30 to P42. *K14-rtTA* x *TRE2-Spdef* mice expressed SPDEF abundantly **(B)** and formed ectopic Alcian-blue and PAS-positive goblet cells in the peripheral cornea **(B-C,** arrows**)**, whereas *K14-rtTA* alone did not **(A)**. Scale bars: 50µm. Nuclei are counterstained with nuclear fast red **(A-B)** and hematoxylin **(C)**. Abbreviations: co: cornea; lim: limbus; f: conjunctival fornix; p: palpebral conjunctiva.