Supplemental Figures and Table for:

Epithelial stem cell homeostasis in Meibomian gland development, dysfunction, and dry eye disease

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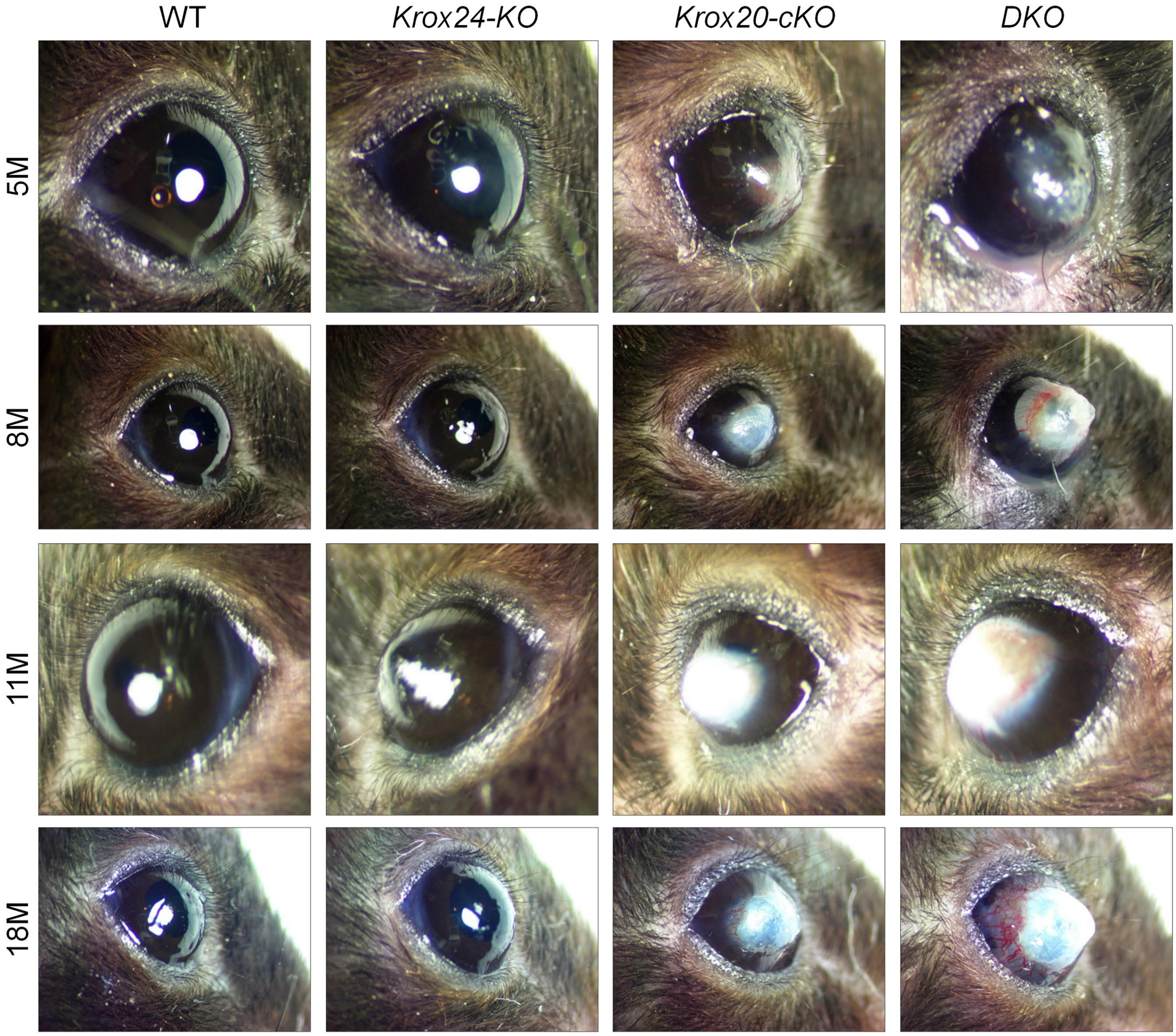
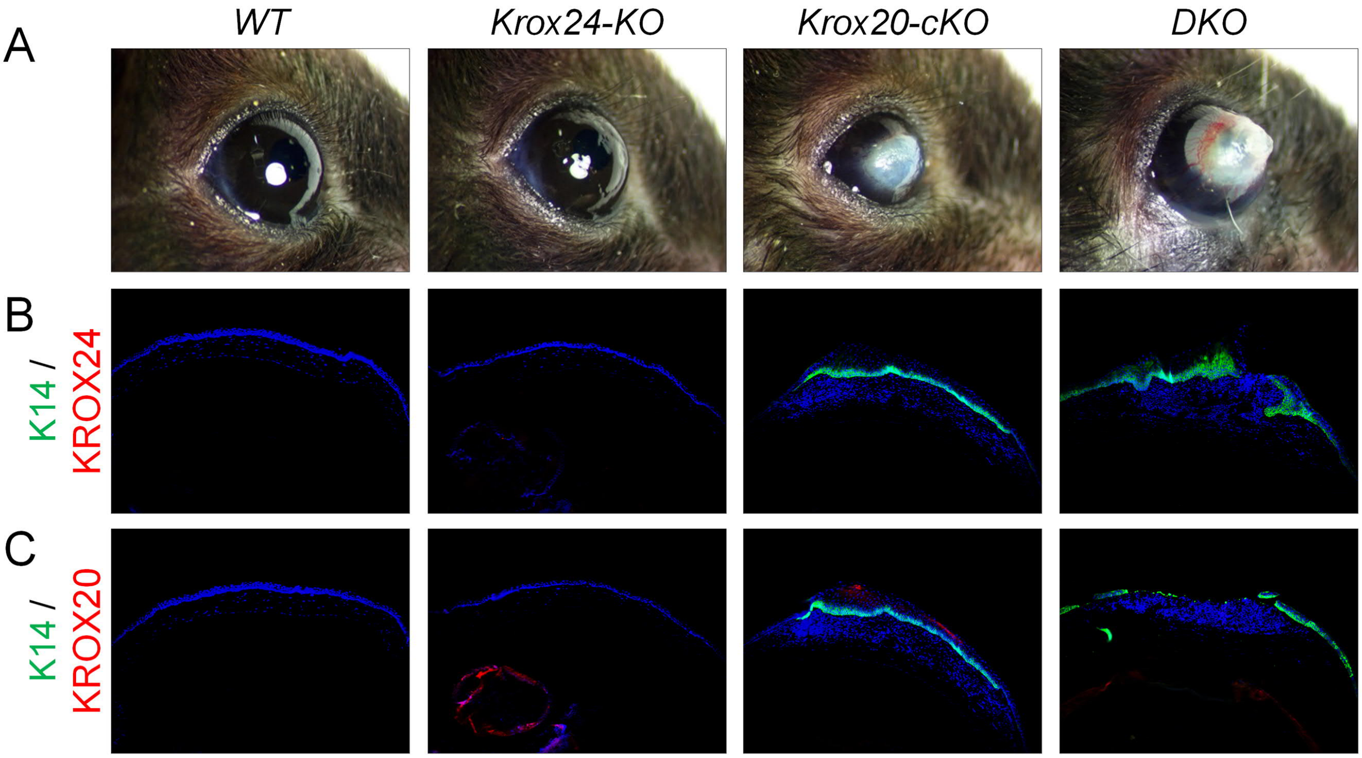
Conflict of interest: The authors have declared that no conflict of interest exists.

Supplemental Figure 1



Supplemental Figure 1. Loss of both *Krox20* and *Krox24* accelerates development and progression of corneal lesion. Gross images of the eye in wild type (*WT; Krox20*$^{f/f}$), *Krox24-KO(Krox24*$^{-/-}$*), Krox20-cKO (Krox20*$^{f/f}$*;K14-Cre),* and *DKO (Krox24*$^{-/-}$*; Krox20*$^{f/f}$*;K14-Cre)* mice at various ages (5, 8, 11, and 18 months), showing the progression of corneal lesion. Gross images shown for 8-month-old mice (8 M) are the same as in Figure 7 (panel **A**), Figure 8 (panel **A**), and Supplemental Figure 2 (panel **A**). n = 50 mice for each genotype. Among mice analyzed, 100% of *Krox20-cKO* and *DKO* mice developed corneal lesions. Representative images are shown. Scale bar represents 100 µm.

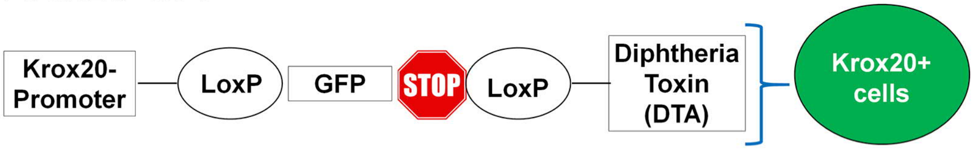
Supplemental Figure 2



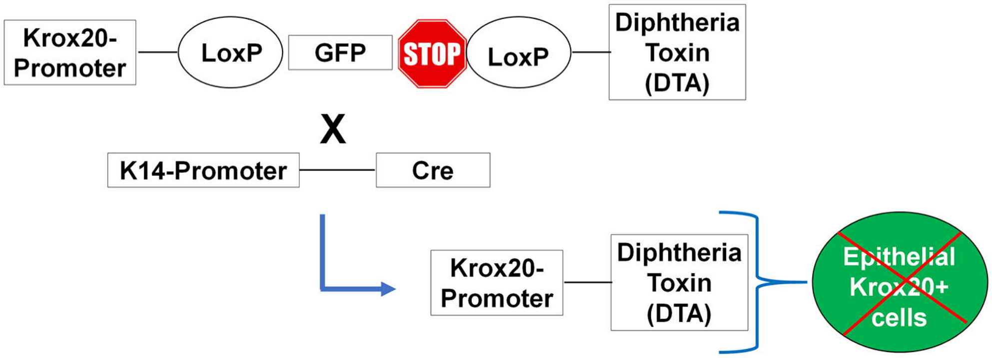
Supplemental Figure 2. Acceleration of phenotype severity is independent of KROX20 and KROX24 expression in the cornea. (A) Gross images of the eye in wild type (*WT; Krox20*$^{f/f}$), *Krox24-KO (Krox24*$^{-/-}$*), Krox20-cKO (Krox20*$^{f/f}$*;K14-Cre),* and *DKO (Krox24*$^{-/-}$*; Krox20*$^{f/f}$*;K14-Cre)* mice. Immunofluorescence staining for (**B**) K14 and KROX24, and (**C**) K14 and KROX20 in the indicated genotypes. n = 50 mice for each genotype. Among mice analyzed, 100% of *Krox20-cKO* and *DKO* mice developed corneal lesions. Representative images are shown. Gross images in (**A**) are the same as in Figure 7 (panel **A**), Figure 8 (panel **A**), and Supplemental Figure 1 (8M).

Supplemental Figure 3

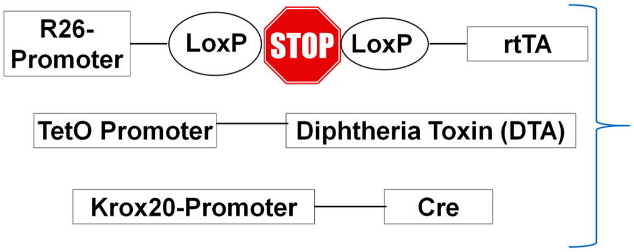
A *Krox20-GFP*



B *Krox20-DTA; K14-Cre*



C *R26-rtTA; tetO-DTA; Krox20-Cre*



Non-Krox20 lineage cells + Water --→ Alive
Krox20 lineage cells + Water --→ Alive
Non-Krox20 lineage cells + Doxy --→ Alive
Krox20 lineage cells + Doxy --→ Dead

Supplemental Figure 3. Schematic illustration of Krox20 cell depletion. (A) Illustration of *Krox20-GFP* (*Krox20-Flox-GFP-Flox-DTA*). In the absence of Cre expression, GFP serves as a reporter for *Krox20* expression. However, **(B)** when crossed with *K14-Cre* to generate *Krox20-DTA; K14-Cre*, the diphtheria toxin A (DTA) is expressed, resulting in the ablation of epithelial *Krox20*-expressing cells from K14 lineage. **(C)** Illustration of *R26-rtTA; tetO-DTA; Krox20-Cre+*. When Cre is expressed under the *Krox20* promoter, it results in the DTA-mediated death of *Krox20* lineage cells upon doxycycline (doxy) treatment.

Genotype	**Primer Sequence**	**Size**
Cre	**Fwd:** CAC CCT GTT ACG TAT AGC CG **Rev:** GAG TCA TCC TTA GCG CCG TA	Cre: 300bp
Beta-Actin	**Fwd:** CCT AGG CAC CAG GGT GTG AT **Rev:** TCA CGG TTG GCC TTA GGG TT	ActB: 239bp
GFP	**Fwd:** GAG CTG GAC GGC GAC GTA AAC **Rev:** CGT TGT GGC TGT TGT TAG TTG TAC	eGFP: 400bp
R26-TdTomato	**Fwd (Tom):** CTG TTC CTG TAC GGC ATG G **Rev (Tom):** GGC ATT AAA GCA GCG TAT CC **Fwd (WT):** AAG GGA GCT GCA GTG GAG TA **Rev (WT):** CCG AAA ATC TGT GGG AAG TC	WT: 300bp Tomato: 200bp
Krox20 fl/fl	**Fwd (WT):** GGG CTT GCA TTC TAC AGT GGT GGT C **Rev (Krox20-ΔFlox):** AGT TGA CAG CCC GAG TCC AGT GG **Fwd (WT; Krox20-ΔFlox):** GTG TCG CGC GTC AGC ATG CGT G **Rev (WT):** GGG AGC GAA GCT ACT CGG ATA CGG	Krox20-Flox: 195bp Krox20-ΔFlox: 210bp WT: 162bp
Egr1 -/-	**Fwd (WT):** AAC CGG CCC AGC AAG ACA CC **Fwd (KO):** CTC GTG CTT TAC GGT ATC GC **Rev (Common):** GGG CAC AGG GGA TGG GAA TG	WT: 414bp KO: 470bp
K14-Cre	**Fwd:** TTC CTC AGG AGT GTC TTC GC **Rev:** GTC CAT GTC CTT CCT GAA GC	K14-Cre: 494 bp
Krox20-Cre	**Fwd (WT):** CGC TTC CTC GTG CTT TAC GGT AT **Rev (WT):** TCA TCA GTC GGG TTA GAG CTG **Rev (Mut):** GGG CTG AGG AAG ACG ACT TTA	Krox20-Cre: 480bp WT: 312bp
TetO-DTA	**Fwd:** GGC GTG TAC GGT GGG AGG **Rev:** GGC ATT ATC CAC TTT TAG TGC	TetO-DTA: 420bp

Supplemental Table 1. Genotyping Primer Sequences