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Supplemental Information

Two-photon live imaging of single corneal stem cells reveals compartmentalized organization of the limbal niche

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Figure S1

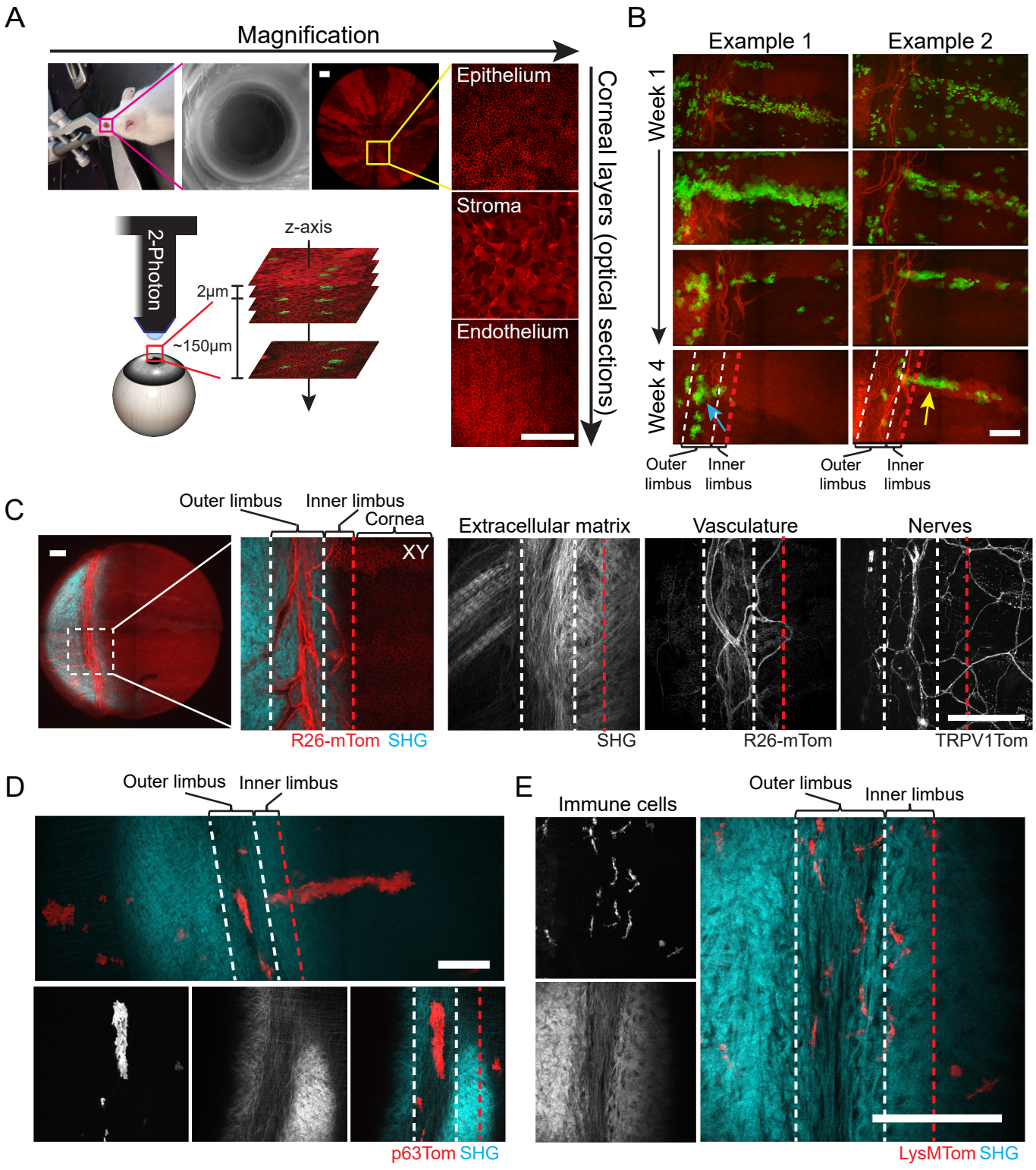

Figure S1. ***In vivo*** **2-photon imaging of the mouse eye.** (A) Representative images of the mouse eye acquired by 2-photon intravital imaging. (B) Expanded view of the limbus in p63^{CreERT2}; R26-mTmG mice that show the distinct organization and relative position of clones in the outer (cyan arrow) and inner (yellow arrow) limbus after one month of lineage tracing. (C) Histological characteristics of the limbus revealed by using *in vivo* reporters and 2-photon microscopy. Co-localization of circumferentially oriented collagen fibers (SHG), blood vessels (R26-mTom) and sensory nerves (TRPV1Tom) within the limbal niche. (D, E) Co-localization of inner limbal clones (p63Tom) and myeloid cells (LysMTom) with circumferentially oriented collagen fibers (SHG) in the limbal stroma. Scale bars: 200 µm.

Figure S2

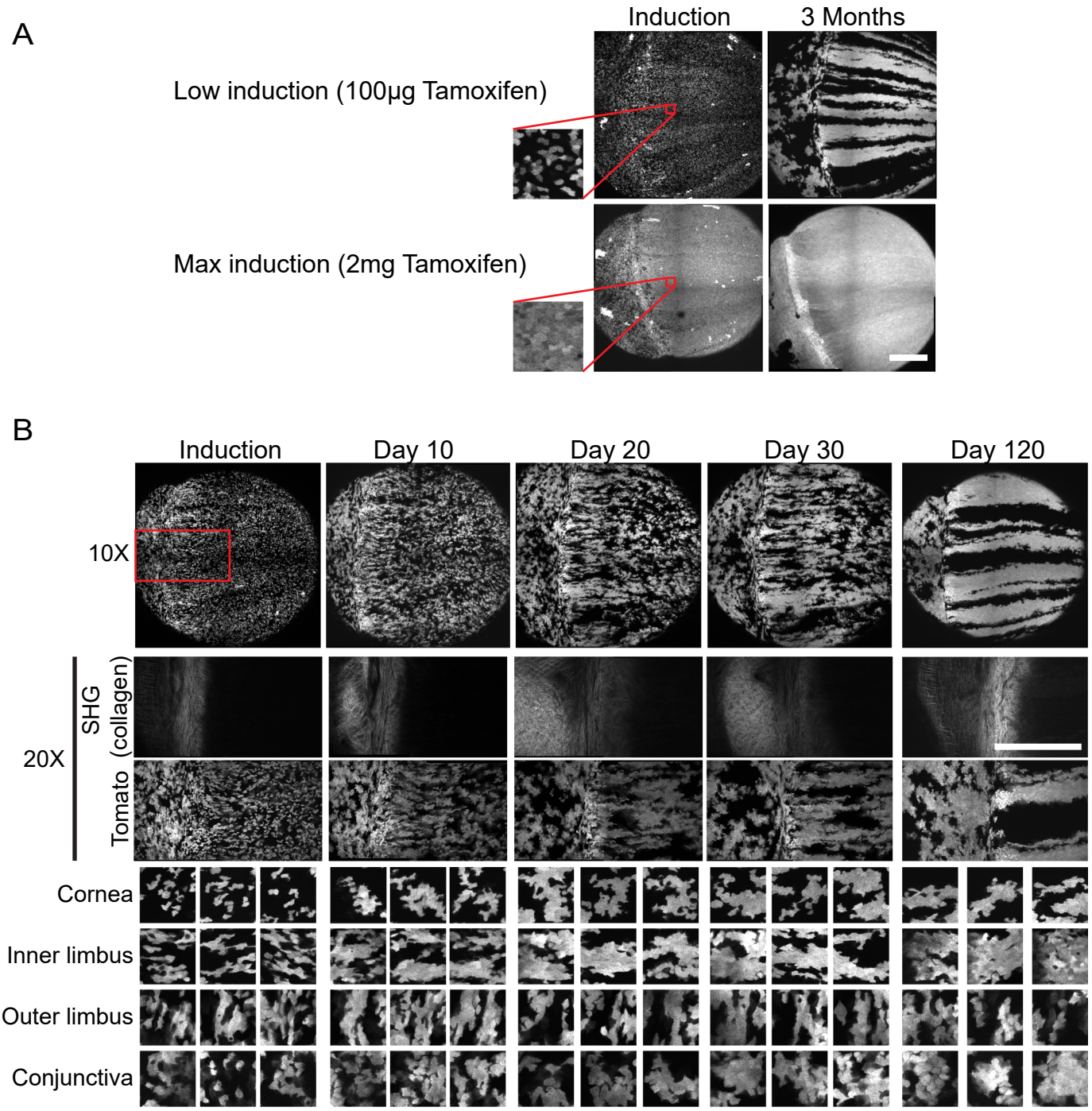

Figure S2. Live imaging of epithelial clonal dynamics. (A) Representative images of the eyes of p63^{CreERT2}; R26-tdTom mice after low or max Tamoxifen induction. (B) Images of the whole ocular surface epithelium aquired during a 120-day lineage tracing time course (top panel). Area outlined in red is shown in higher magnification (middle panels). Examples of distinct patterns of clone anisotropy (lower panels). See also Figure 1J. Scale bars: 1 mm (A), 500 µm (B).

Figure S3

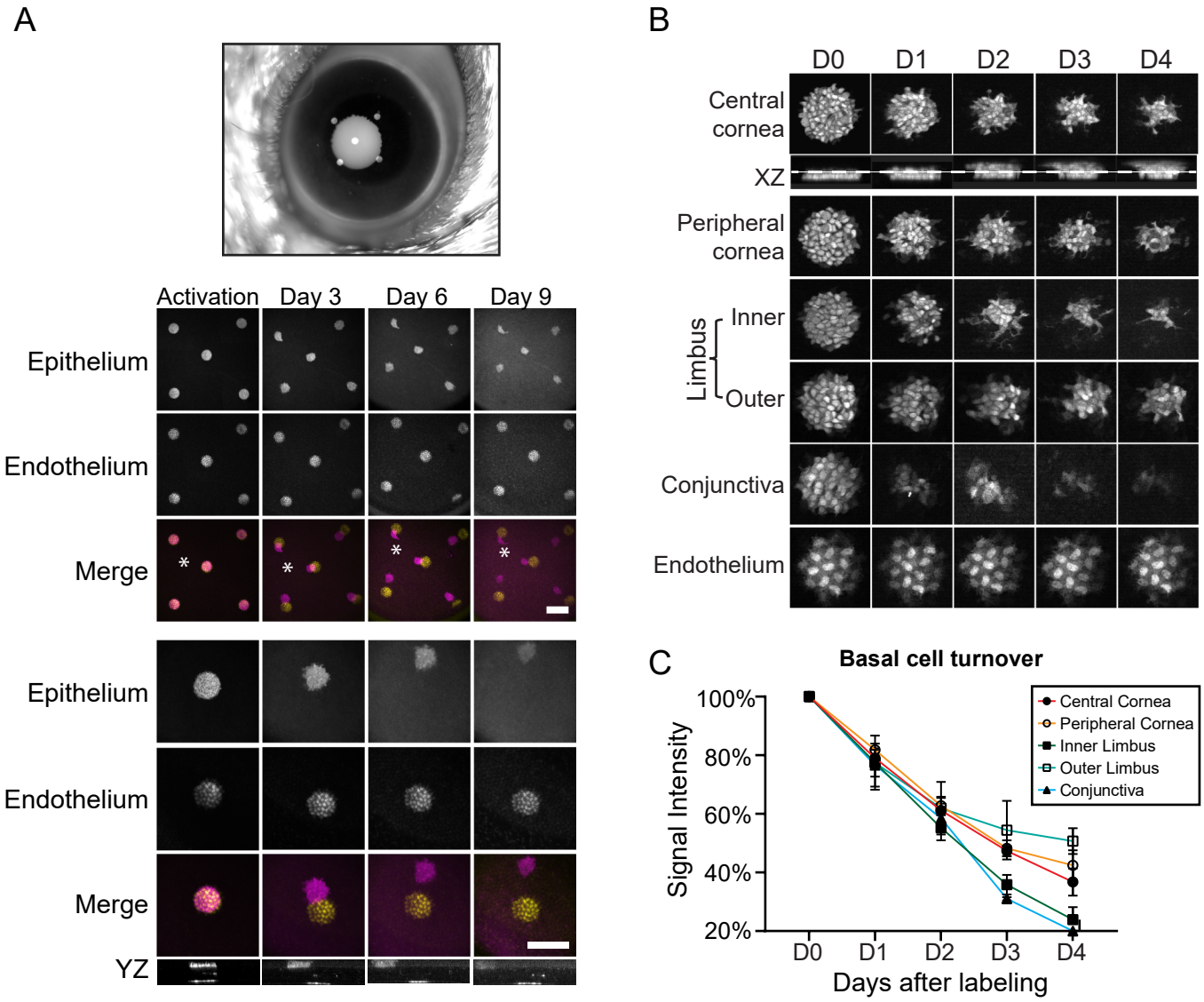

Figure S3. Direct capture of the centripetal movement of corneal progenitors. (A) Five groups of basal epithelial cells within the central cornea were unbiasedly photo-labeled using a globally expressed photo-activatable GFP reporter. Endothelial cells immediately below were used as a reference. Low magnification views of the central cornea where photo-labeled groups of cells can be seen during the time course (middle panels). The movement of labeled cells was visualized every three days over the course of nine days. Basal cells in the central cornea moved centripetally in unison towards the center of the eye (asterisk). One example of a group of labeled epithelial and endothelial cells (bottom panels). (B) Representative high magnification images of photo-labeled basal cells from the indicated epithelial compartments. The same groups of cells was re-imaged at daily time intervals. A group of quiescent endothelial cells (bottom row) were used as a control. The size and signal intensity of the labeled basal group is a function of their rate of proliferation and terminal differentiation, which is accompanied by their departure and transit towards the suprabasal epithelial layers. (C) Quantification of basal cellular turnover as a function of PAGFP signal decay. (n = 15 tracked groups of labeled cells from 3 mice, ****$p < 0.0001$, 2-way ANOVA). Scale bars: 200 μm.

Figure S4

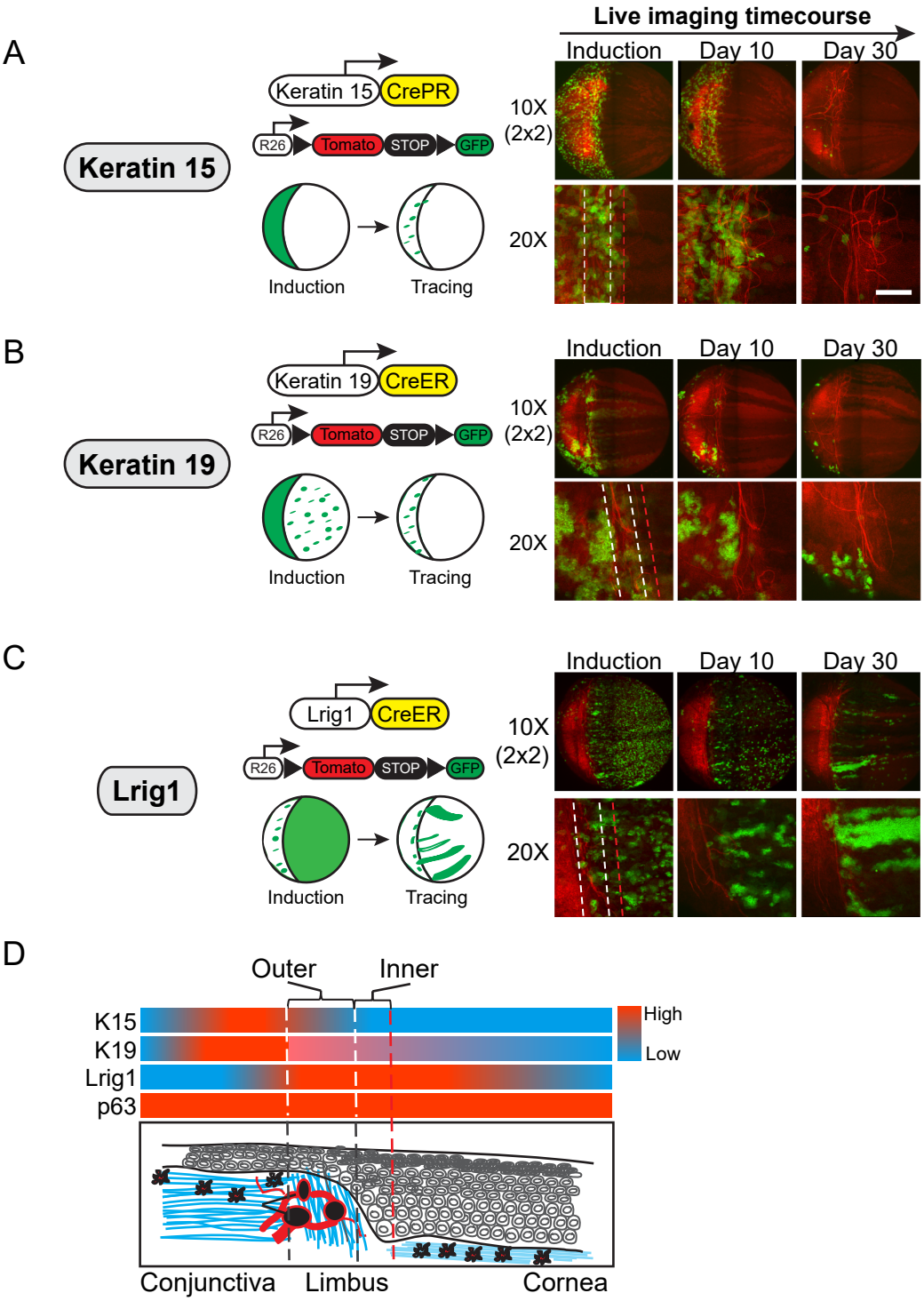

Figure S4. Validation of putative limbal stem cell markers. (A-C) Representative examples of lineage tracing time courses performed by longitudinal live imaging using the indicated genetic drivers. Low magnification views of the whole eye where traced labeled cells can be seen in green (GFP) (upper right panels). High magnification views of the same areas of the limbus at the indicated time points (lower right panels). (D) Diagram summarizes the specificity of each validated marker for stem and progenitor cell populations in the ocular surface epithelium. Dotted lines indicate the margins of the outer (white) and inner (red) limbus. Scale bar: 200 μm.

Figure S5

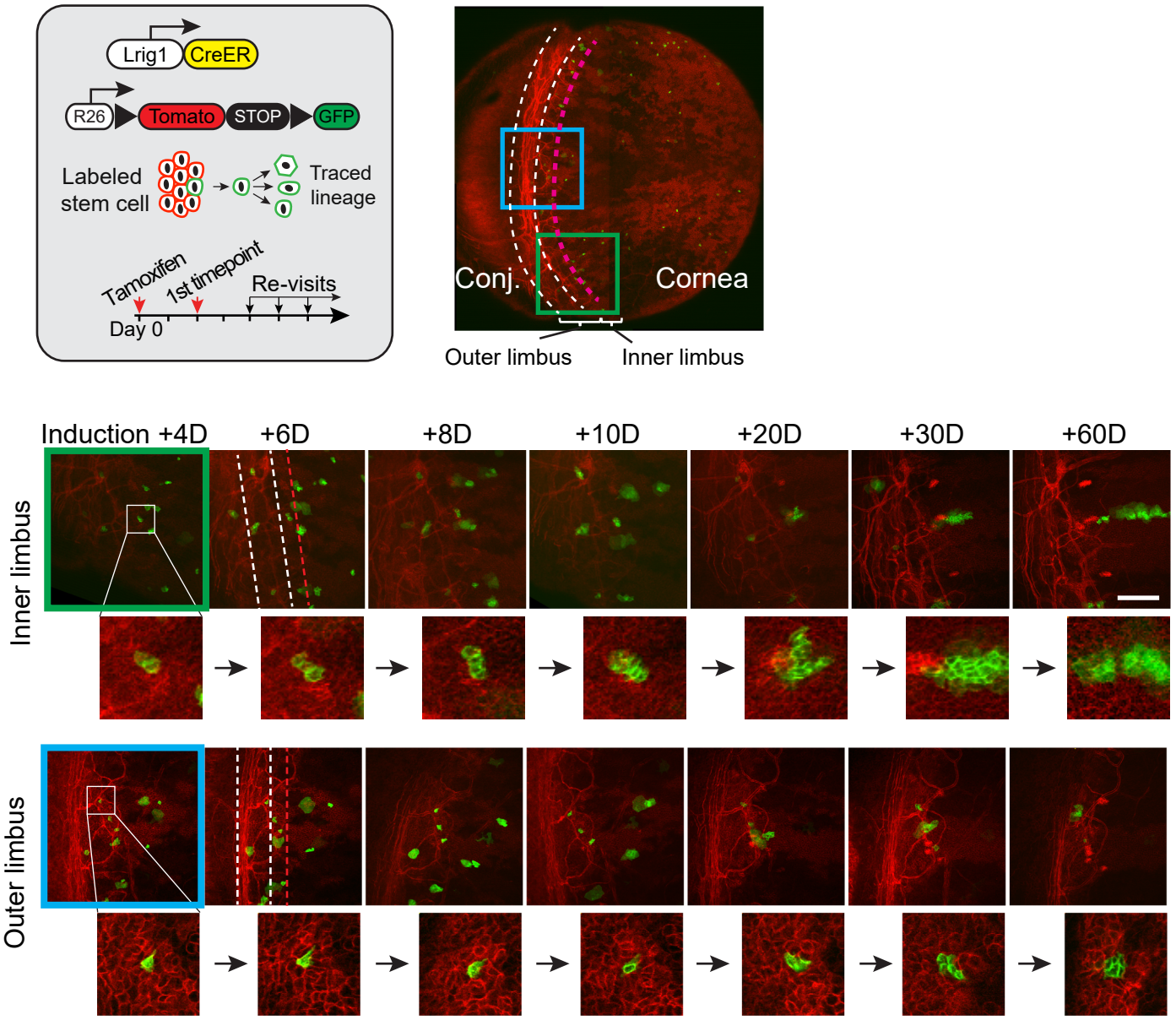

Figure S5. Lrig1 marks single limbal stem and progenitor cells. Experimental strategy and representative examples of capturing the activity of single limbal cells by longitudinal live imaging over a two-month period. A low dose of Tamoxifen marks single Lrig1+ cells in the limbus. The location of each founding cell and its progeny is accurately recognized across different time points due to their spatial separation and distinctive histological features of their limbal environment where they reside. (Top panels) An example of a stem cell captured in the inner limbus. This cell undergoes rounds of symmetric cell divisions and its progeny expand centripetally away from the limbus and towards the cornea. (Bottom panels) An example of a stem cell marked in the outer limbus. This cell is slow cycling and its progeny remain in the same location. Dotted lines indicate the margins of the outer (white) and inner (red) limbus. Scale bar: 200 μm.

Figure S6

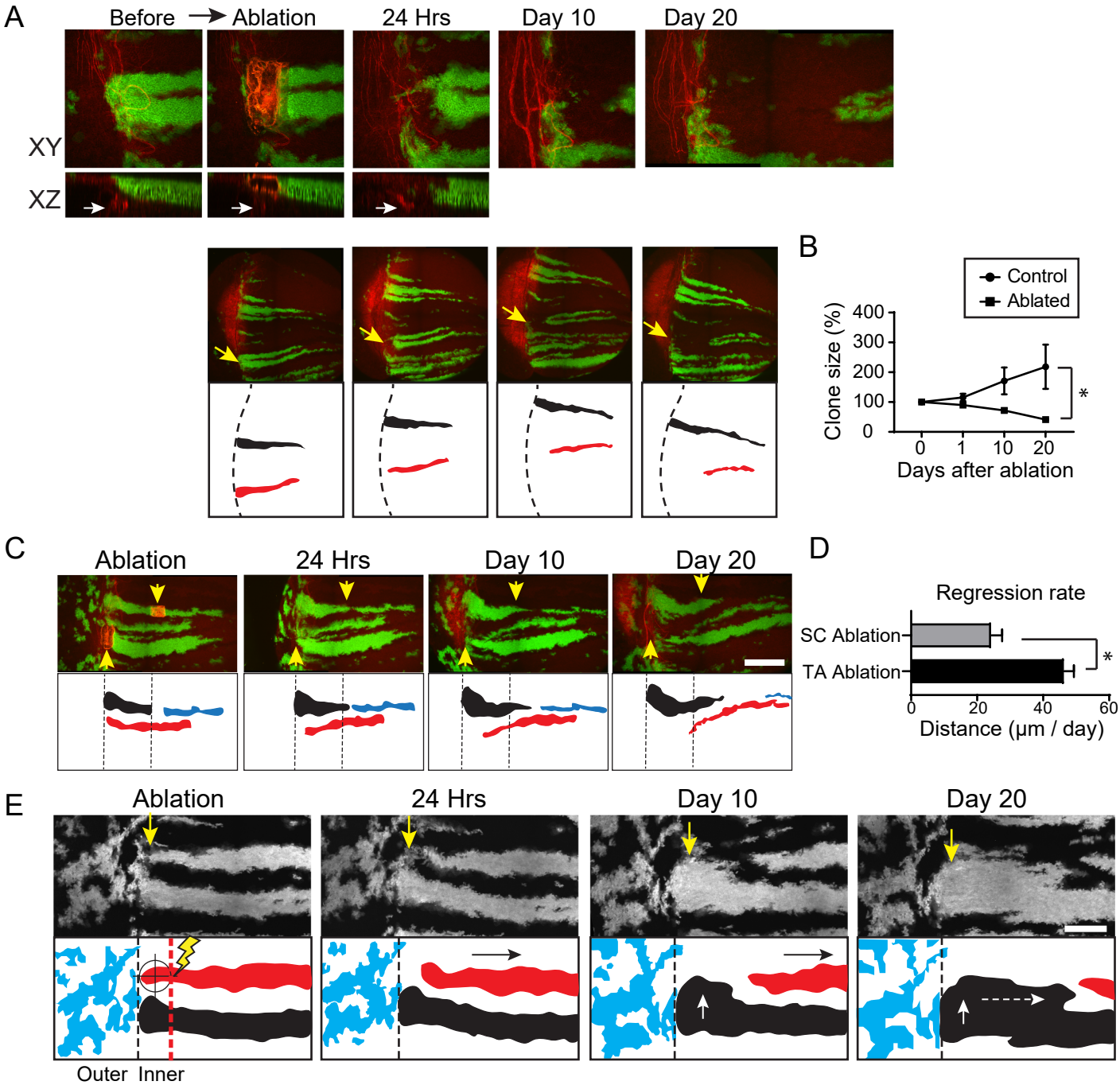

Figure S6. 2-photon ablation and lineage tracing of limbal cells. (A) Time course series following ablation of cells in the inner limbus. Bottom panel shows traces of individual corneal lineages with intact (black) or ablated (red) inner limbal stem cells. White arrows point to the underlying limbal niche that is not affected by the ablation cells in the epithelium. Yellow arrows indicate the site of ablation and the dotted lines mark the inner limbal border. (B) Quantification of the size of corneal clones with intact or ablated inner limbal cells (n = 6 clones tracked in 2 mice; p = 0.018, 2-way ANOVA). (C) Traces indicate segments of a corneal lineage upstream (black) or downstream (blue) of the of the ablated cells. A separate lineage (red) with ablated stem cells in the inner limbus is also shown for comparison. Arrows indicate the site of ablations. (D) Quantification of the centripetal regression rate of corneal lineages downstream of the stem and progenitor cell ablation sites (n = 8 ablated clones tracked in 3 mice; p = 0.0119, unpaired t-test). (E) Representative views of the limbal area taken at the indicated timepoints after stem cell ablation. A corneal lineage (red trace) regresses after ablation of its associated stem cells. The expansion of inner stem cells from a proximal lineage (black trace) compensates for the cell loss and resumes the centripetal regeneration of that segment of the cornea. Yellow arrows indicate the site of ablation. Black arrows indicate the regression of the ablated clone. White arrows indicate the early lateral expansion and late centripetal growth of a neighboring clone. Scale bars: 200 μm.

Figure S7

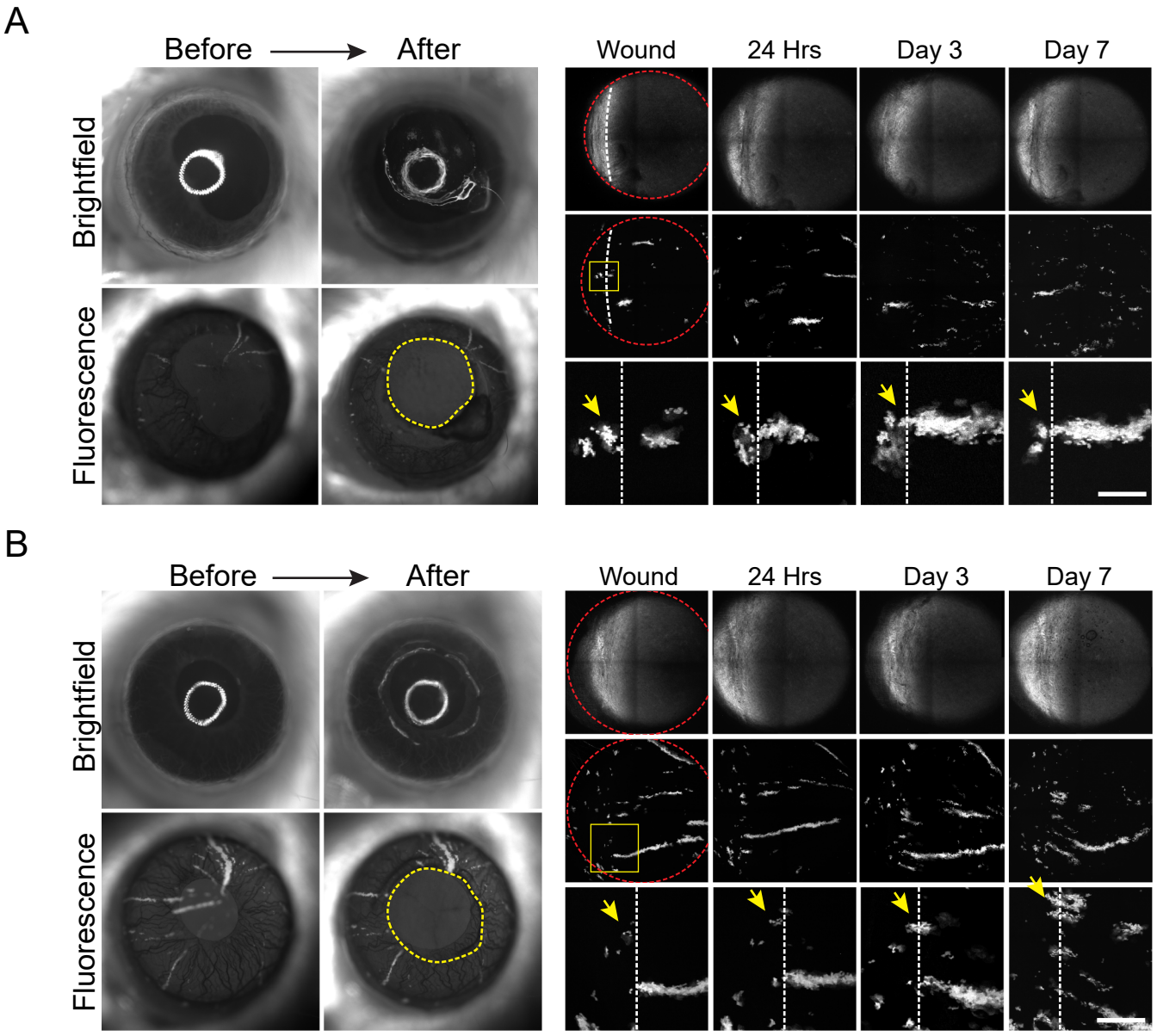

Figure S7. Stem cells in the outer limbus contribute to corneal wound healing. (A, B) Representative time series from two independent experiments showing cells traced by live imaging before and after an epithelial debridement wound in the central cornea. Yellow arrows point to the clones in the outer limbus that expand into the cornea after wounding. The yellow dotted line indicates the area of epithelial debridement. The white dotted line indicates the margin of the inner limbus. Scale bars: 200 μm.