Supporting Information

Walker et al. 10.1073/pnas.0910382107

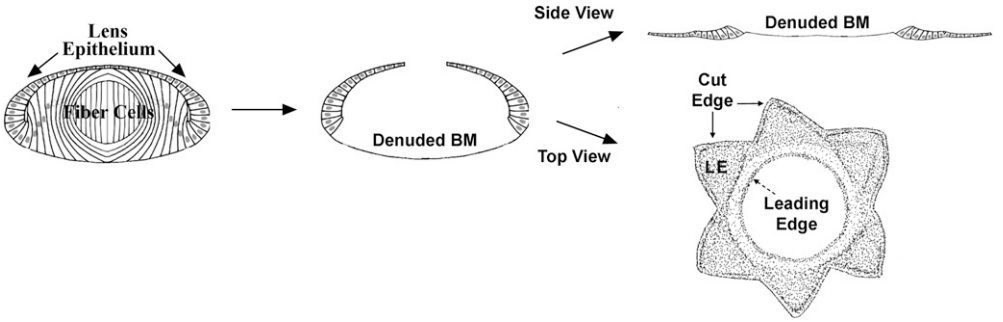

Fig. S1. Ex vivo mock cataract surgery explant cultures. Model of mock cataract surgery as performed to prepare ex vivo wounded epithelial explants. The fiber cell mass was removed from the lens capsule [a basement membrane (BM)] creating a wounded leading edge in the lens epithelium (LE) where it had abutted the fiber cells. Cuts made in the anterior capsule to flatten the explant created a wounded cut edge.

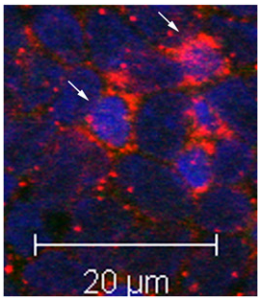

Fig. S2. G8-positive cells in newborn rat epithelial explants. Explants prepared from newborn rat lenses (gift from Peggy Zelenka and Senthil Saravanamuthu) were immunostained with mAb to the G8 antigen and a rhodamine-tagged secondary antibody (red) and the nuclear stain TO-PRO-3 (blue). $G8^{pos}$ cells (arrows) were found to be innate to the lens epithelium of the newborn rat.

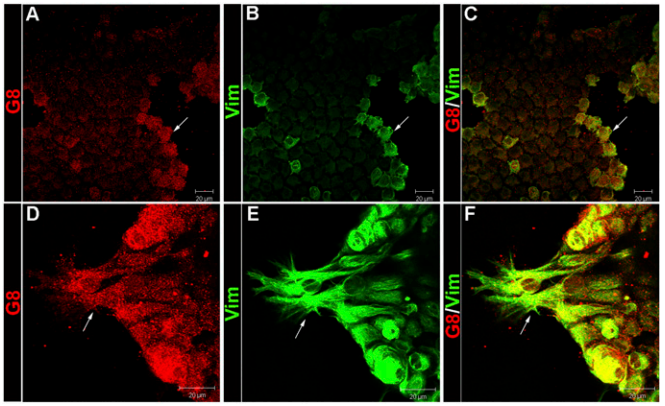

Fig. S3. $G8^{pos}$/$vimentin^{pos}$ cells rapidly respond to injury of an adult epithelium by migrating to wound edges. Adult rat lens epithelial explants were prepared following mock cataract surgery and fixed either immediately following injury (*A*–*C*) or at 24 h postinjury (*D* and *E*). These lens explants were coimmunostained with antibodies to both the G8 antigen (red) and vimentin (green). $G8^{pos}$/$vimentin^{pos}$ cells quickly migrated to the wounded edges (arrow, *A*–*C*) and were a prominent feature of the wound edges at 24 h postinjury (arrow, *D*–*F*). These studies demonstrate that the phenomenon of $G8^{pos}$/$vimentin^{pos}$ cells as rapid responders to injury is a feature of adult epithelium.

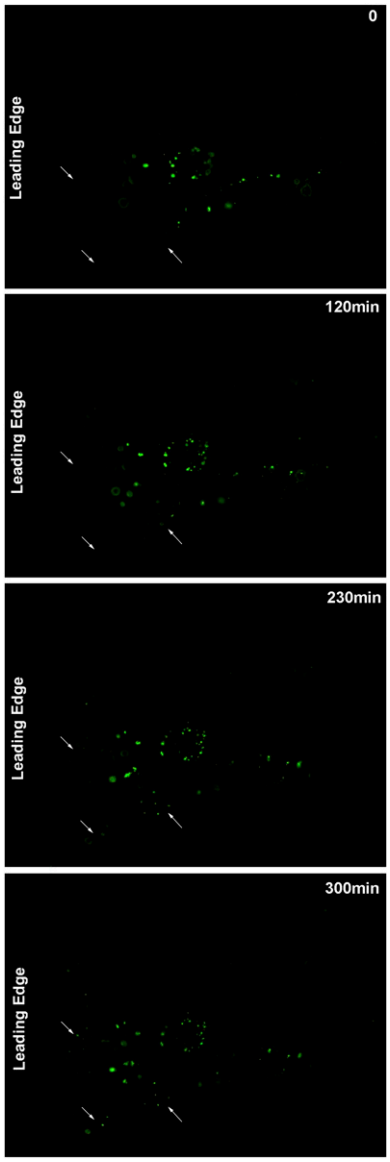

Fig. S4. Time-lapse imaging of G8^{pos} cells migrating to the leading edge. Immediately after wounding of the lens epithelium, G8 cells were labeled with mAb to G8 and a fluorescent secondary antibody, and images were acquired of the ex vivo explants at 10 min intervals over a 5-h time period. Individual frames are presented representing time 0 (first frame), 120 min, 230 min, and 300 min (last frame). Arrows denote areas at or near the leading edge where G8^{pos} cells appear.

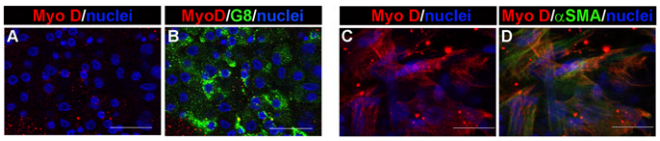

Fig. S5. Temporal expression of MyoD protein after wounding. The expression of MyoD protein by G8^{pos} cells in response to injury was determined by confocal analysis at 1 h after wounding (*A* and *B*), when G8^{pos} cells had emerged from their niches, and 6 d after injury (*C* and *D*), when G8^{pos} cells had differentiated into myofibroblasts. Samples were immunostained for MyoD (red) and G8 (green) (*A* and *B*) or MyoD (red) and αSMA (green) (*C* and *D*). Nuclei were stained with TO-PRO-3 (blue). *B* is an overlay of MyoD and G8, and *D* is an overlay of MyoD and αSMA. Punctate staining for MyoD in *A* and *B* is nonspecific. Although G8^{pos} cells that respond to injury express message for MyoD, MyoD protein expression was not detected until G8^{pos} cells differentiated into myofibroblasts.

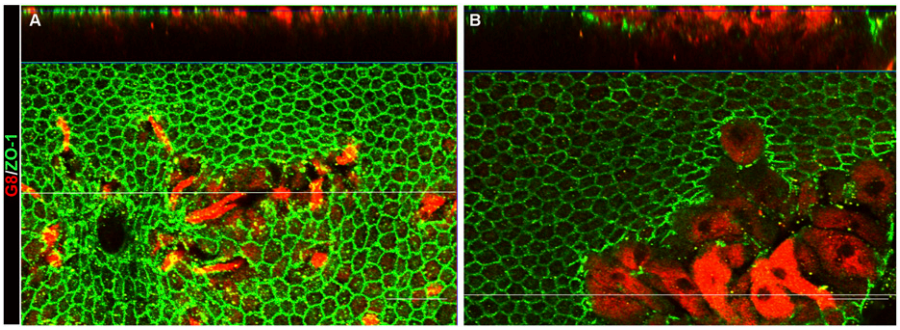

Fig. S6. Localization of the epithelial cell marker Z0-1 restricted to the epithelium in wounded lens explants. Ex vivo explants were fixed at 1 h after injury inflicted by mock cataract surgery and double-stained for the G8 antigen (red) and the epithelial cell junctional protein ZO-1 (green). $G8^{pos}$ cells were observed as they emerged from their niches (*A*) and migrated toward the leading edge (*B*). ZO-1 formed cell–cell junctions in lens epithelial cells that were polarized to the lens cells' apical surfaces (seen in orthogonal section; *A Upper* and *B Upper*), whereas the G8 cells lack ZO-1 junctions, confirming the mesenchymal phenotype of the G8 cells. In addition, the orthogonal sections clearly show the positioning of $G8^{pos}$ cells along the apical surfaces of the lens epithelium.

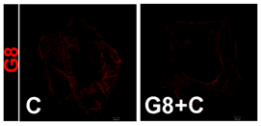

Fig. S7. Efficacy of ablation approach. Epithelial explants were exposed to G8+C or C alone at culture day 1, cultured another 5 d, and immunostained with antibody to the G8 antigen (red). This ablation protocol effectively ablates most of the $G8^{pos}$ cell population that is associated with the lens epithelium.

Movie S1. Time-lapse imaging of $G8^{pos}$ cells migrating to the leading edge. Immediately after wounding of the lens epithelium, G8 cells were labeled with mAb to G8 and a fluorescent secondary antibody and the images acquired of the ex vivo explants at 10-min intervals over a 5-h time period.

Movie S1