Figure S1  Very little heterozygous tissue remained in mosaic discs generated with either en-GAL4, UAS-FLP (A) or ey-FLP (B-C).
(A-B) A posterior compartment of a mosaic wing disc generated with en-GAL4, UAS-FLP (A) and an eye-antennal mosaic disc generated with ey-FLP (B). sec5<sup>55</sup> clones are GFP-positive (green). Sister clones are RFP-positive (red). Heterozygous cells are yellow and were largely absent. Larvae were maintained at 18° to prevent ablation of homozygous sec5<sup>55</sup> tissue and were dissected at the wandering stage (C) An adult eye following sec5<sup>55</sup>-ablation. Wild-type tissue lacks pigmentation (white) and comprised the majority of the adult eye. Residual heterozygous cells or non-ablated sec5<sup>55</sup> cells are pigmented (orange) and remained only in the posterior portion of the adult eye.
Figure S2  (A) Dying sec52 cells were cleared by a combination of basal extrusion and engulfment. Discs were dissected 12 hours AUS. Anti-cleaved Caspase3 (aC3, blue) marks dying cells, sec52 cells are GFP-negative (green), Phalloidin (Actin, red) outlines cells. Arrows show engulfed GFP-negative, cleaved Caspase3-positive apoptotic bodies within GFP-positive neighboring cells. Asterisk shows basally extruded GFP-negative apoptotic bodies. A single z-slice is shown. Apical is up; basal is down. (B-B’) sec52 ablation did not delay the larval to pupal transition. (B) Developmental timing of pupariation for sec52 and control animals. Shifted 30°C ablated experiments shown as solid lines; 18°C non-ablated experiments shown as dashed lines (B’). Calculated average time (dAEL) to 50% pupariation from data in (B). sec52  30°C 9.8 +/- 0.2, control 30°C 9.6 +/- 0.04, sec52  18°C 10.5 +/- 0.02, control 18°C 10.2 +/- 0.1. (C) The anterior compartment of ablated discs was also reduced in size at 12 hours AUS. The posterior compartment data (Figure 1, H) was shown for comparison.
Figure S3  Localized proliferation was not observed by EdU incorporation in proliferating cells. EdU (white) labels proliferating cells, anti-cleaved Caspase3 (aC3, magenta) marks dying cells. White lines mark the approximate A-P boundary as assessed by GFP. Posterior is to the right. Scale bar is 100μm. (A-A’) Discs dissected from ablated (A) and control (A’) animals 6 hr AUS. (B-B’) Discs dissected from ablated (B) and control (B’) animals 12 hr AUS.
Figure S4  A bias in sequencing quality scores or base composition did not explain the disproportionate number of mutations found at early read positions. (A) The average sequencing quality as a function of read position is shown. Data shown represents the average of the mean quality scores from all sequencing runs. (B) The base composition at each read position was calculated by averaging the mean % A, T, C and G at each position from each sequencing run. No base bias was evident across the read length. (C) The percentage of predicted mutations at each sequencing read position (Figure 6, E) is shown for comparison.
Figure S5  Developmental timing of pupariation of ablated (30°C) or non-ablated (18°C) Bu^n\textsuperscript{ASG3}, Rnrl\textsuperscript{A4BS} and Gmd\textsuperscript{B3-1} animals as compared to control. All animals were crossed to the sec5\textsuperscript{St} wing disc ablation system. One set of animals were shifted to 30°C at 7.5 dAEL, shown in red. A second set was maintained at 18°C, shown in dark grey. The calculated average time (dAEL) to 50% pupariation is shown. Error bars show one STDEV. Dashed lines mark the average time to 50% pupariation for the parental control. Developmental timing was relatively unaffected at 18°C in Gmd\textsuperscript{B3-1} and Rnrl\textsuperscript{A4BS} animals, suggesting that the adult phenotype was not due to accelerated development. Ablated Rnrl\textsuperscript{A4BS} animals showed a slight delay in pupariation, suggesting that the inability of these animals to compensate might result in a developmental delay. Animals carrying the bu^n\textsuperscript{ASG3} were delayed under both conditions, indicating that these animals were at a slightly earlier development stage at the time of temperature shift. The delayed development of these animals could account for the reduced size of the wing discs (Figure 7, E), as these animals would be “younger” at the time of dissection; however, the resulting adult wings were also reduced in size when compared both to control ablated wings (Figure 7, A) and to bu^n\textsuperscript{ASG3} non-ablated 18°C control wings (data not shown), suggesting that loss of bu^n prevents full recovery of the disc after sec5\textsuperscript{St} ablation.
File S1

Supporting Materials and Methods

Drosophila Stocks

\(w; P(\text{mw}, \text{ubi-GFP.nls}), \text{sec5}\), \(\text{FRT40A, en-GAL4, UAS-FLP/CyO}\)

\(y, w, \text{ey-FLP}; P(\text{mw}, \text{ubi-GFP.nls}), \text{sec5}, \text{FRT40A/SM6-TM6b}\)

\(w; P(\text{mw}, \text{ubi-RFP.nls}), \text{FRT40A}\)

\(w; \text{FRT40A}\)

Immunohistochemistry

Antibodies used for supplemental data were rabbit anti-cleaved Caspase3 (1:100, Cell Signaling); mouse anti-GFP (1:500, Roche). Phalloidin was used to stain the actin cytoskeleton and outline cells (1:200, Sigma). Click-iT EdU labeling kit (Invitrogen) was used to mark cells in S-phase. Incorporation and detection was performed according to manufacturer’s instructions. A 10 minute incubation in 10\(\mu\)M EdU was used. Images of eye and wing imaginal discs shown in Supplemental Figure 1 were captured on a Zeiss Axio Imager M1 and processed using Adobe Photoshop.

Developmental timing

Eggs were collected on agar grape juice plates at room temperature for 4-6 hours. After 48 hours at 18\(^\circ\)C, 55 L1 larvae per a vial were transferred to standard fly food supplemented with fresh yeast paste. Vials were either maintained at 18\(^\circ\)C or shifted to 30\(^\circ\)C 7.5 dAEL for 48 hours, after which they were returned to 18\(^\circ\)C. Pupae were counted every 10-14 hours until all animals had pupariated. Data are presented as the fraction of larvae pupariated or the time to 50% pupariated. Data points represent the average of at least 4 vials. Error bars are one STDEV.