Supplemental Data

Intrinsic Tumor Suppression and Epithelial Maintenance by Endocytic Activation of Eiger/TNF Signaling in *Drosophila*

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GENOTYPES

Figure 1
yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B (A-B'), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (C-D'), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP, egr^{1}/egr^{1}; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (E-F'), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP, egr^{1}/UAS-Eiger^{+W}, egr^{1}; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (G-H'), *UAS-mGFP, hsFLP1.22/+* or Y; *Tub-Gal4, FRT82B, Tub-Gal80/FRT82B* (I-K'), *UAS-mGFP, hsFLP1.22/+* or Y; *Tub-Gal4, FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (L-N'), *UAS-mGFP, hsFLP1.22/+* or Y; *egr^{1}/egr^{1}; Tub-Gal4, FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (O-Q'), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (R-U), and *yw, eyFLP1/UAS-Bsk^{DN}; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (V-Y).

Figure 2
UAS-mGFP, hsFLP1.22/+ or Y; *UAS-Eiger^{+W}/+; Tub-Gal4, FRT82B, Tub-Gal80/FRT82B* (A-C, A'-C'), *UAS-mGFP, hsFLP1.22/+* or Y; *UAS-Eiger^{+W}/+; Tub-Gal4, FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (D-F, D'-F'), *yw, eyFLP1/UAS-Bsk^{DN}; Act>y+>Gal4, UAS-myrRFP, G454/UAS-Eiger^{W}, UAS-GFP-Rab5; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (G-J), *yw, eyFLP1/UAS-Bsk^{DN}; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (K-N), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/UAS-p35; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (O-R), and *y,w eyFLP1/+ or Y; Tub-Gal80, FRT40A/FRT40A; Act>y^{+}>Gal4, UAS-GFP/UAS-Hep^{CA}* (S-V).

Figure 3
UAS-mGFP, hsFLP1.22/+ or Y; *Tub-Gal4, FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (A-C, G-I) and *UAS-mGFP, hsFLP1.22/+* or Y*; egr^{1}/egr^{1}; Tub-Gal4, FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (D-F, J-L).

Figure 4
yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/UAS-p35; FRT82B, Tub-Gal80/FRT82B, scrib^{1} (A-B'''), *ey-Gal4, UAS-FLP/+; FRT82B, GMR-hid, CL3R/FRT82B* (C, C', E, E'), *ey-Gal4, UAS-FLP/+; FRT82B, GMR-hid, CL3R/FRT82B, scrib^{1}* (D, D', F, F'), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B* (G-H'), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/UAS-Eiger^{W}; FRT82B, Tub-Gal80/FRT82B* (I-J'), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (K-L'), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/ UAS-Eiger^{W}; FRT82B, Tub-Gal80/FRT82B, scrib^{1}* (M-N'), *GMR-Gal4/+* (O), *GMR-Gal4/UAS-Eiger^{W}* (P), *GMR-Gal4/+; UAS-Rab5/+* (Q), *GMR-Gal4/UAS-Eiger^{W}; UAS-Rab5/+* (R), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/UAS-Rab5^{DN}, FRT82B* (S), and *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/UAS-Rab5^{DN}, FRT82B, scrib^{1}* (T-X).

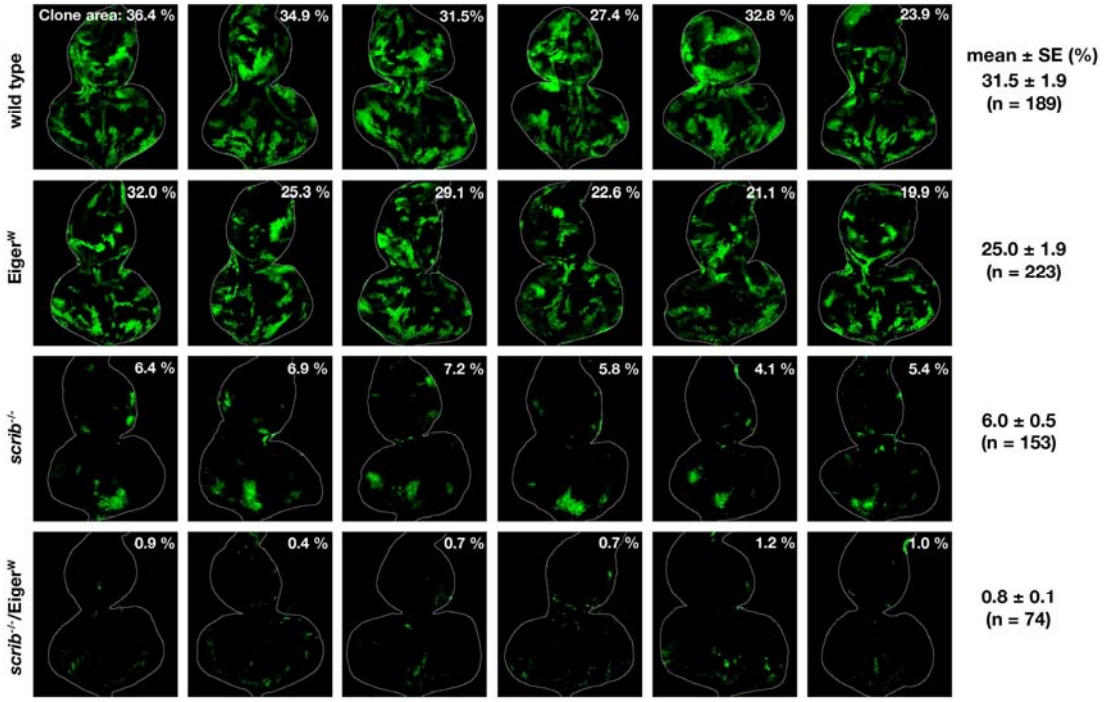

Figure S1. *scrib* Clones Are Hyper-Sensitive to Eiger Signaling
GFP-labeled wild-type, Eiger^{+W}, *scrib*$^{-/-}$, or *scrib*$^{-/-}$/Eiger^{+W} clones were generated in eye-antennal discs and the sizes of these clones were quantified using the ImageJ program. Clone area was determined by the percentage of the size of the clones/total size of the eye-antennal disc. Five discs were analyzed in each experiment (n, number of clones analyzed), and the data were collected as mean ± SE (%).
Genotypes are as follows: *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B* (wild type), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/UAS-Eiger*^{W}*; FRT82B, Tub-Gal80/FRT82B* (Eiger^{+W}), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B, scrib*^{1} (*scrib*$^{-/-}$), and *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP/UAS-Eiger*^{W}*; FRT82B, Tub-Gal80/FRT82B, scrib*^{1} (*scrib*$^{-/-}$/Eiger^{+W}).

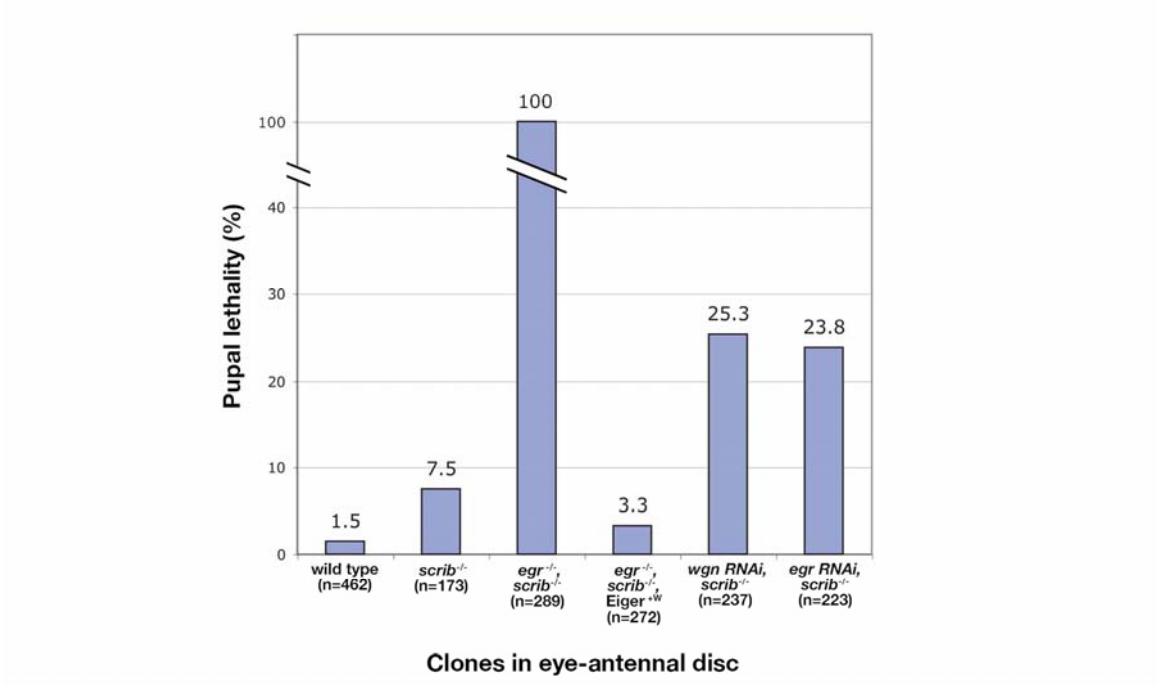

Figure S2. Pupal Lethality Caused by Clones Induced in Eye-Antennal Discs

Clones of wild-type, *scrib*$^{-/-}$, *egr*$^{-/-}$/*scrib*$^{-/-}$, *egr*$^{-/-}$/*scrib*$^{-/-}$/Eiger^{+W}, *scrib*$^{-/-}$/*wengen* RNAi, or *scrib*$^{-/-}$/*eiger* RNAi cells were induced in eye-antennal discs, and pupal lethality of these animals was scored (n, number of pupae analyzed).
Genotypes are as follows: *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B* (wild type), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/FRT82B, scrib*^{1} (*scrib*$^{-/-}$), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP, egr*^{1}/*egr*^{1}*; FRT82B, Tub-Gal80/FRT82B, scrib*^{1} (*egr*$^{-/-}$, *scrib*$^{-/-}$), *yw, eyFLP1/+* or Y; *Act>y+>Gal4, UAS-GFP, egr*^{1}/*UAS-Eiger*$^{+W}$, *egr*^{1}*; FRT82B, Tub-Gal80/FRT82B, scrib*^{1} (*egr*$^{-/-}$, *scrib*$^{-/-}$/*Eiger*$^{+W}$), *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/UAS-wengen-IR; FRT82B, Tub-Gal80/FRT82B, scrib*^{1} (*wgn* RNAi/*scrib*$^{-/-}$), and *yw, eyFLP1/+ or Y; Act>y+>Gal4, UAS-GFP/+; FRT82B, Tub-Gal80/UAS-eiger-IR*$^{\#3}$*, FRT82B, scrib*^{1} (*egr* RNAi/*scrib*$^{-/-}$).

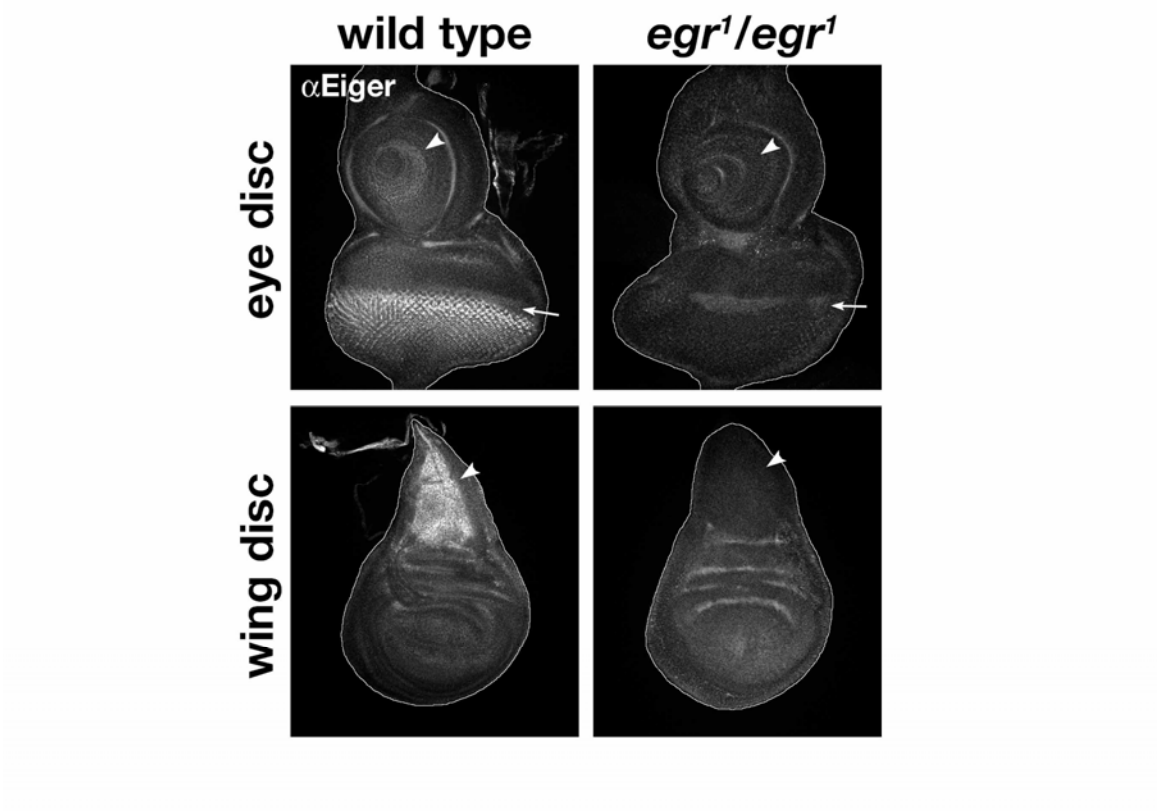

Figure S3. Validation of the Anti-Eiger Antibody
The anti-Eiger R1 antisera was raised in rabbits against the extracellular domain of Eiger (except for the TNF homology domain) fused with GST protein. The antisera was then absorbed with tissues from *eiger* homozygous mutant larvae. Imaginal discs from wild type or *eiger* homozygous mutant larvae were stained with the anti-Eiger antibody (1: 250). The signals of Eiger staining in eye disc cells (posterior to the morphogenetic furrow; arrow), antennal disc cells (arrowhead), and myoblasts in wing discs (arrowhead) were completely disappeared in *eiger* homozugous mutants.