**Supplemental Figure Legends**

**Figure S1. Related to Figure 1**

**Ablation and gene expression using DUAL Control**

(**A**) A schematic of the domains used for ablation and genetic manipulation in most DUAL Control experiments. The ablation domain coincides with an enhancer of the *salm* gene, which patterns the distal wing pouch. The domain used for genetic manipulation utilizes an enhancer of the *dve* gene, which drives *UAS*-based constructs in the entire wing pouch.

(**B**) A wing imaginal disc bearing *DCNA* with *lexAop-GFP* to mark the ablation domain (green) and *UAS-RFP* to mark the pouch domain (red), as diagrammed in the schematic in (**A**). DAPI, blue.

(**C**-**F**) Wing discs bearing combinations of the transgenes that comprise DUAL Control, showing that heat shock induced ablation only occurs when all three genes (*hsp65, lexAop-GluRI, salm-LexADBD*) are present, as indicated by the presence of Nub (red) in the *salm* domain. DAPI, blue. Genes omitted: (**D**) *lexAop-GluR1* (**E**) *hs-p65* (**F**) *salm-LexADBD.*

**Figure S2. Related to Figure 2**

**Quantification of cell death in GluRI ablated discs**

(**A**-**B**) Wing discs bearing a *lexAop-GFP* transgene and ablated with *DCGluR1* (**A**) and *DChepCA* (**B**) and stained for PI (yellow). DAPI, blue. PI staining in the notum is due to physical manipulation of the wing discs and acts as an internal control.

(**C**) Quantification of the PI signal. The *lexAop-GFP* was used to outline the ablated tissue for quantification, as seen in (A) and (B). n = 10 discs for each treatment.

(**D**-**E**) Wing imaginal discs bearing a *DRWNT-GFP ; UAS-RFP* transgene, ablated with *DCGluR1* (**D**) and *DChepCA* (**E**), and stained for DCP1, gray. DAPI, blue. Solid arrowheads in (D) highlight GFP negative, DCP1 positive cells. Open arrowheads in (E) indicate a lack of GFP negative, DCP1 positive cells in *DChepCA* ablated discs.

(**F**). Quantification of DCP1 cells at the wound edge (GFP+) vs. the lateral wing pouch (GFP-). ns: not significant. P\*\*\*\* < 0.0001. Data analyzed with a Kruskal-Wallis test and a Tukey’s multiple comparisons test. n = 10 discs for each treatment.

**Figure S3. Related to Figure 3**

**Blocking apoptosis with P35 leads to wing overgrowth phenotypes**

(**A**) A wing disc ablated with *DCrpr>>p35* and labeled with TUNEL (red). DAPI, blue. The lack of signal demonstrates the ability of P35 to inhibit cell death.

(**B**) Adult wings showing the overgrown phenotype accompanied by blistering and ectopic vein tissue observed following *DC­GluR1>>p35* ablation.

(**C**) Quantification of overgrown wing phenotype frequency following ablation with *DCrpr>>p35* (n = 89), *DChepCA>>p35* (n = 30)*, DCGluR1>>p35* (n = 43)*,* and *DCGluR1 DRWnt-GAL80 >>p35* (104). No overgrown wings are observed in *DCrpr>>p35*.

(**D-F**) Wing imaginal discs stained for Wg (red)and DCP1 (gray) following ablation with *DChepCA* (**D**), *DChepCA>>p35* (**E**) or *DCGluR1>>p35*(**F**). Ectopic Wg overlaps with the diffuse DCP1 signal in *DChepCA>>p35* discs, while in *DCGluR1>>p35* discs ectopic Wg is only observed at the wound edge (arrowhead) and not in the diffuse DCP1 signal in the rest of the pouch.

(**G-H**) Wing imaginal discs ablated with *DCGluR1>>wgRNAi* (**G**) *DCGluR1>>p35; wgRNAi* (**H**) and stained for Wg (red) and DCP1 (gray).

(**I**) Quantification of overgrown wing phenotype frequency following ablation with *DCGluR1* (n = 43)*, DCGluR1>>wgRNAi* (n = 62)*,* and *DCGluR1>>p35; wgRNAi* (n = 46).

(**J**) Schematic of wing discs bearing *DCGluR1>>GFP* with and without *DRWNT-GAL80*, demonstrating the ability of *DRWNT-GAL80* to limit *UAS-GFP* expression to the lateral pouch.

(**K**) A wing disc ablated by *DCGluR1>>GFP , DRWnt-GAL80* and stained for DCP1 (gray). DAPI, blue. The GFP signal overlaps with the NiA cells but not the wound edge apoptosis.

(**L**) A wing disc ablated by *DCGluR1>>p35 , DRWnt-GAL80* and stained for DCP1 (gray). DAPI, blue. The wound edge DCP1 is still strong and punctate while the NiA cells are no longer visible.

**Figure S4. Related to Figure 5.**

**Manipulation of JNK pathway elements in *DCGluRI* ablated discs.**

(**A**) A wing disc ablated with *DCGluR1* in a *hepr75* hemizygous background and stained for DCP1 (gray) and Mmp1 (red). DAPI, blue. Wound edge apoptosis and NiA cells are still observed in this background, although are overall reduced. As in other panels, the dashed line outlines the ablated *salm* domain, indicated by the change in DAPI appearance.

(**B**) A wing disc ablated with *DCGluR1>>bskRNAi* andstained for DCP1 (gray) and Mmp1 (red), DAPI, blue. Wound edge apoptotic cells are reduced following *bsk* knockdown, while NiA cells remain unaffected.

(**C**) A wing disc ablated with *DCGluR1>>egrRNAi* stained for DCP1 (gray) and Mmp1 (red). DAPI, blue. Both wound edge apoptotic cells and NiA cells remain unaffected by *egr* knockdown.

(**D**) A wing disc ablated with *DCGluR1>>grndRNAi* andstained for DCP1 (gray) and Mmp1 (red). DAPI, blue. As in (**C**), both wound edge apoptotic cells and NiA cells remain unaffected following *grnd* knockdown.

(**E**) A wing disc ablated with *DCGluR1>>rprRNAi* andstained for DCP1 (gray) and Mmp1 (red). DAPI, blue. Knockdown of *rpr* leads to a mild reduction of both wound edge apoptotic cells and NiA cells (open arrowheads).

(**F**) A wing disc ablated with *DCGluR1* in a *hid1* heterozygousbackground,andstained for DCP1 (gray) and Mmp1 (red), DAPI, blue. Both wound edge apoptotic cells and NiA cells are unaffected in this background.

(**G**) A wing imaginal disc ablated with *DCGluR1>>miRHG* andstained for DCP1 (gray) and Nub (red). DAPI, blue. Tissue loss indicated by loss of Nub (arrowhead) is still observed following ablation with *DCGluR1* despite inhibition of apoptosis by knockdown of *rpr, hid,* and *grim.*

(**H**) Schematic of the *egr-Gal4 ; UAS-GFP* reporter showing activity in the adult muscle precursor cells (AMPs) just below the notum.

(**I-J**) Wing imaginal discs bearing an *egr* reporter (*egr-GAL4; UAS-GFP*, green) in an undamaged heat shocked disc (**I**) and following ablation with a version of *DCGluR1* that lacks the *DVE>>GAL4* (**J**). DAPI, blue. Reporter activity is not observed in the pouch with or without ablation

**Figure S5. Related to Figure 6.**

**Quantification of proliferation with NiA manipulation.**

(**A-B)** Wing imaginal discs bearing the *CycE-GFP* reporter (green) at 18 hr (**A**) and 24 hr (**B**) of recovery following ablation by *DCGluR1* and stained for DCP1 (gray). DAPI, blue. The open arrowhead in (A) indicates a lack of reporter activity in the *salm* domain, while the arrowhead in (B) highlights stronger reporter activity at 24 hr.

(**C**-**D**) Wing imaginal discs after 18 hr (**C**) and 24 hr (**D**) of recovery following *DCGluR1* ablation, and stained for DCP1 (gray), PH3 (magenta), and DAPI. The open arrowhead in (C) indicates a lack of PH3 in the *salm* domain, while the arrowhead in (D) highlights increased PH3 at 24 hr.

(**E**) Quantification of PH3+ cells in *DCNA* disc and in *DCGluR1* ablated discs, both in the presence and absence of *miRHG* and after 18 hr and 24 hr after the heat shock/recovery. P\*\* < 0.005. Data was analyzed via a Kruskal-Wallis test and a Tukey’s multiple comparisons test. n = 10 discs for each treatment.

(**F**-**G**) Wing discs bearing the *PCNA-GFP* reporter at 18 hr (**F**) and 24 hr (**G**) of recovery following *DCGluR1* ablation, and stained for DCP1 (gray) and Mmp1 (red). DAPI, blue.

(**H**) Quantification of the mean fluorescence intensity of the *PCNA-GFP* signal at 18 hr and 24 hr of recovery in the presence and absence of *miRHG*, following *DCGluR1* ablation*.* ns. P\* < 0.0445. Data was analyzed via a Kruskal-Wallis test and a Tukey’s multiple comparisons test. n = 10 discs for each treatment.