

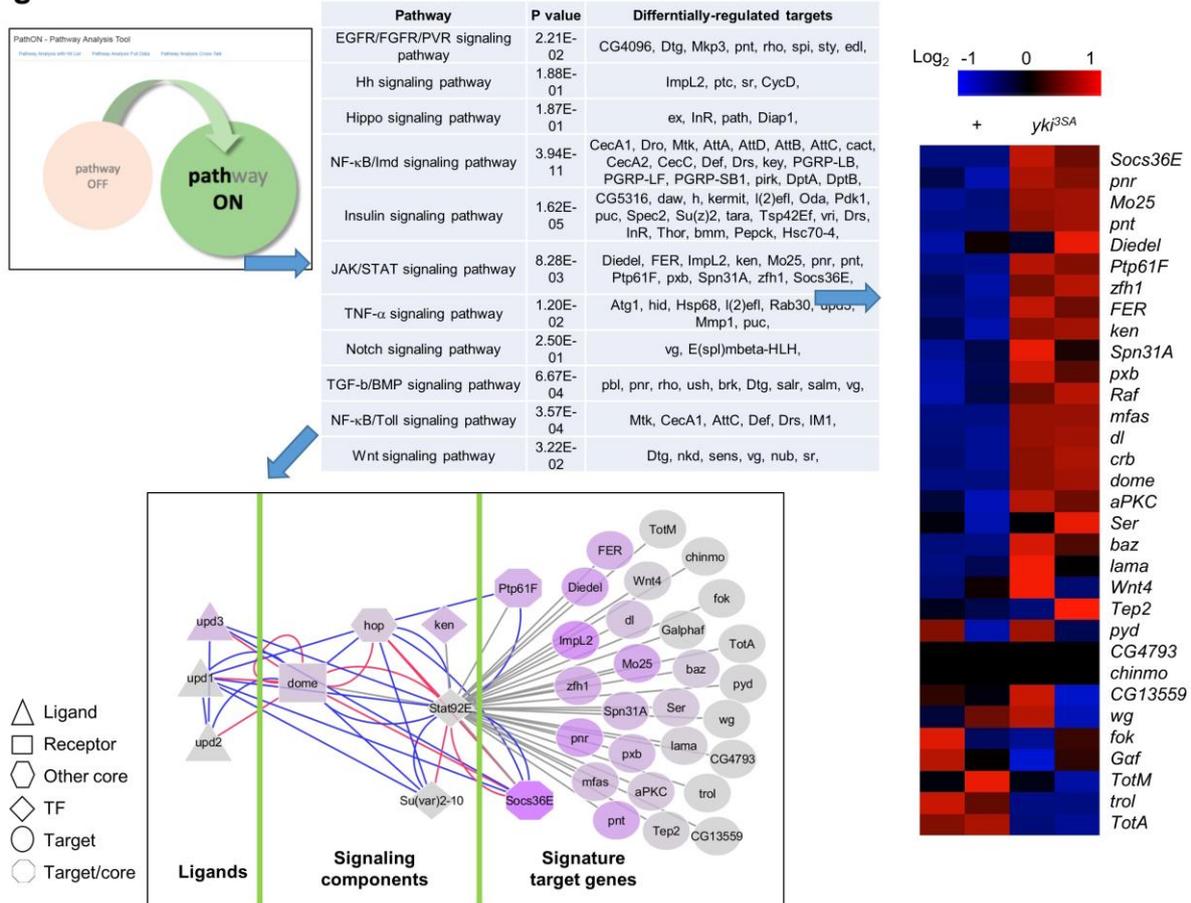
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**Supplemental information**

**Coordination of tumor growth  
and host wasting by tumor-derived Upd3**

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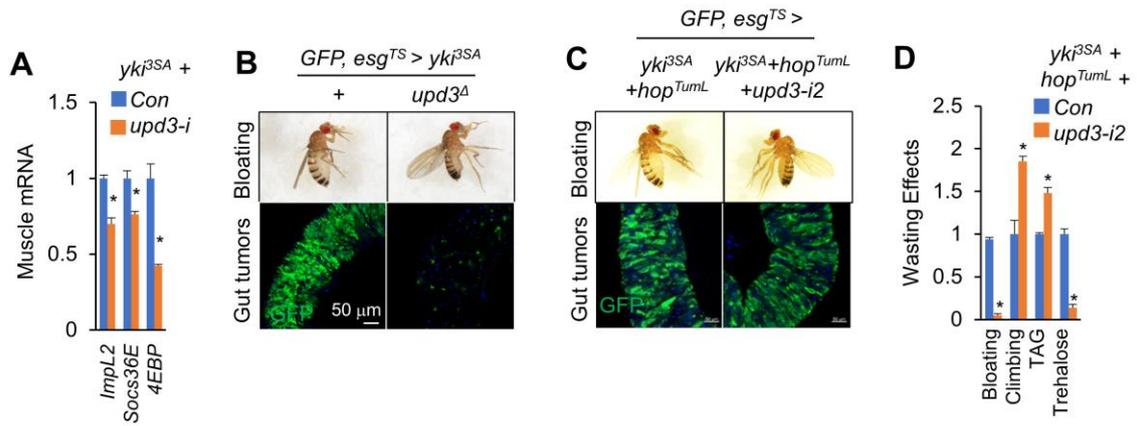
**Fig. S1**



**Supplemental Figure S1. Using PathOn to analyze pathway activation. Related to Figure 1.**

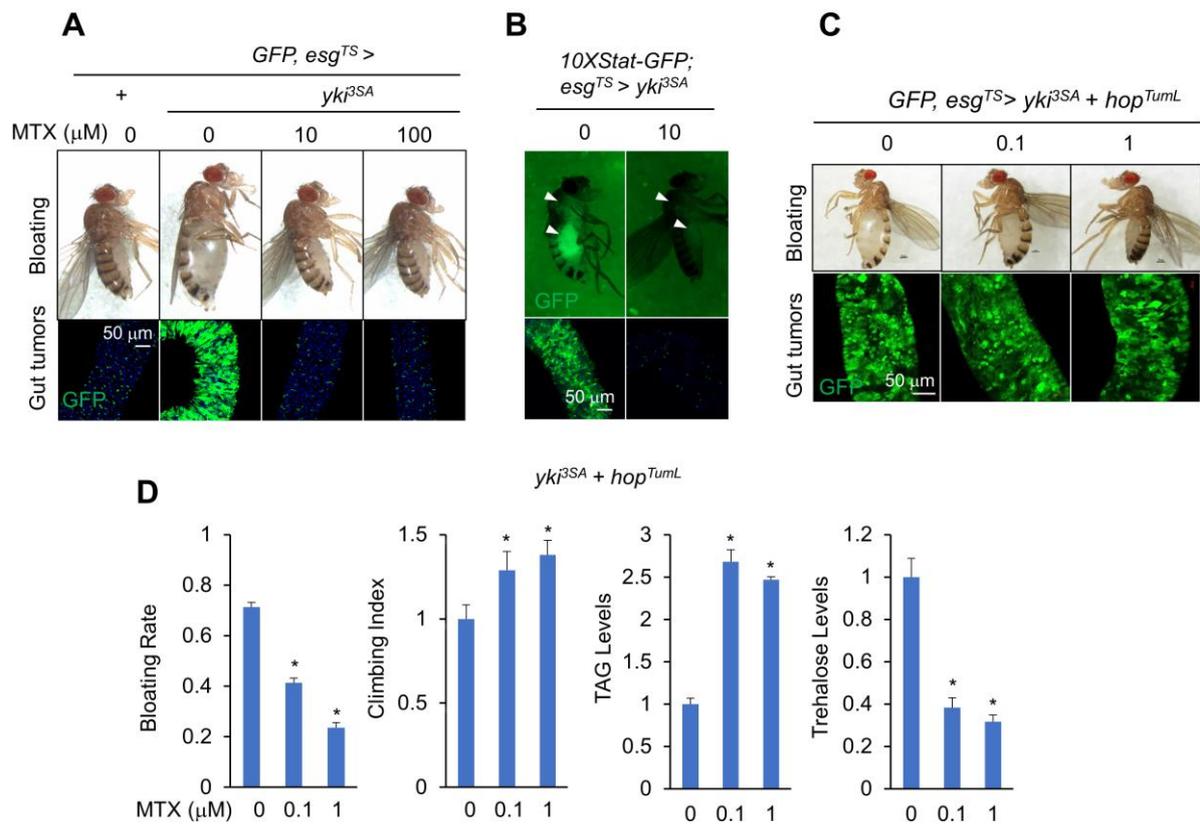
PathOn is a web-based analysis tool useful to predict the ON/OFF status of 14 major signaling pathways by analyzing the enrichment of pathway signaling components or signature target genes based on expression profiles provided by the user. The RNA-seq dataset obtained from the muscle of *yki*-tumor bearing flies, as well as a dataset from control flies, were uploaded and analyzed by PathON. Enrichment *p*-values of signature target genes were calculated using a hypergeometric test. PathOn predicts that the Jak/Stat signaling pathway is activated. A heatmap illustration and a network illustration are also provided by PathOn to visualize the transcriptional changes in mutant vs control samples.

**Fig. S2**



**Supplemental Figure S2. *upd3* knockdown using another RNAi line in *yki<sup>3SA</sup>+hop<sup>TumL</sup>* tumors alleviates host wasting. Related to Figures 1 and 2. (A)** Gene expression levels in the muscle were analyzed with qPCR at day 8 (n=3, 10 flies/replicate). **(B)** Bloating phenotypes (up) and gut tumors (bottom, scale bar, 50 μm) of flies bearing *yki<sup>3SA</sup>* tumors with or without systemic *upd3* mutation at day 8. **(C-D)** Wasting effects of flies bearing *yki<sup>3SA</sup>+hop<sup>TumL</sup>* tumors with or without *upd3* RNAi (HM05061) at day 6: Bloating phenotype (up) and gut tumors (bottom, scale bar, 50 μm) **(C)**, bloating rates (n=4, 20 flies/replicate), climbing rates (n=15), TAG and trehalose storages (n=3, 5 flies/replicate) **(D)**. Data are presented as mean ± SEM. \**p* < 0.05.

**Fig. S3**

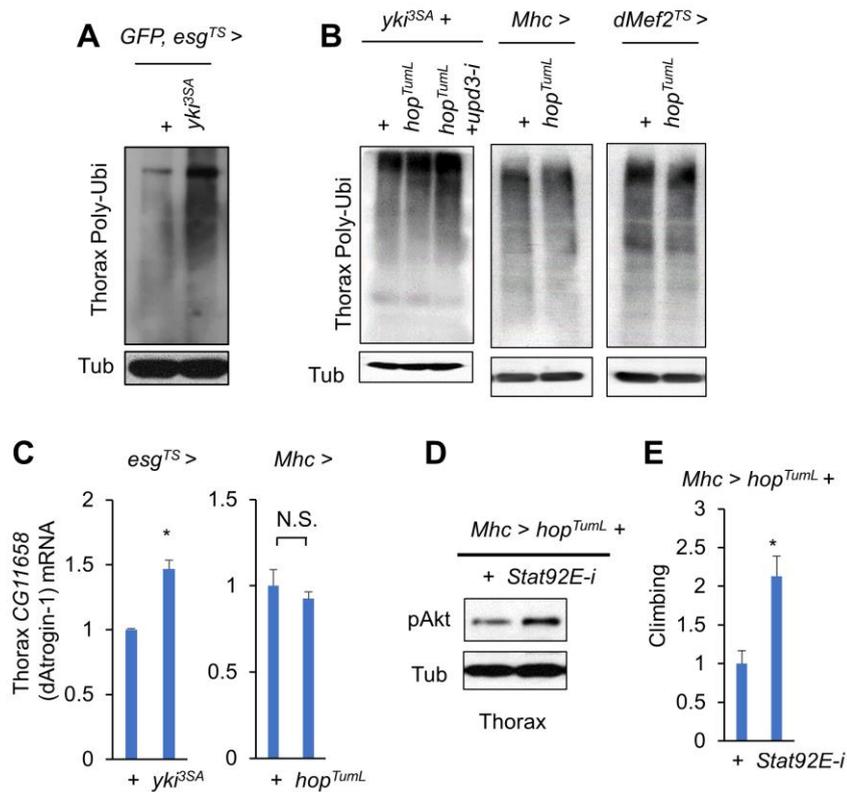


**Supplemental Figure S3. Pharmaceutical Jak/Stat inhibition alleviates host wasting. Related to Figure 2. (A-C)**

Bloating phenotypes (**A**, **C**, up) and gut tumors (**A**, **C**, bottom, scale bar, 50  $\mu$ m) and 10XStat-GFP signals in the whole body and midgut (**B**, scale bar, 50  $\mu$ m) under MTX treatment simultaneously with tumor induction at day 6. (**D**)

Wasting effects such as bloating rates (n=4, 20 flies/replicate), climbing rates (n=15), and TAG and Trehalose storages (n=3, 5 flies/replicate) at day 6. Data are presented as mean  $\pm$  SEM. \*  $p < 0.05$ .

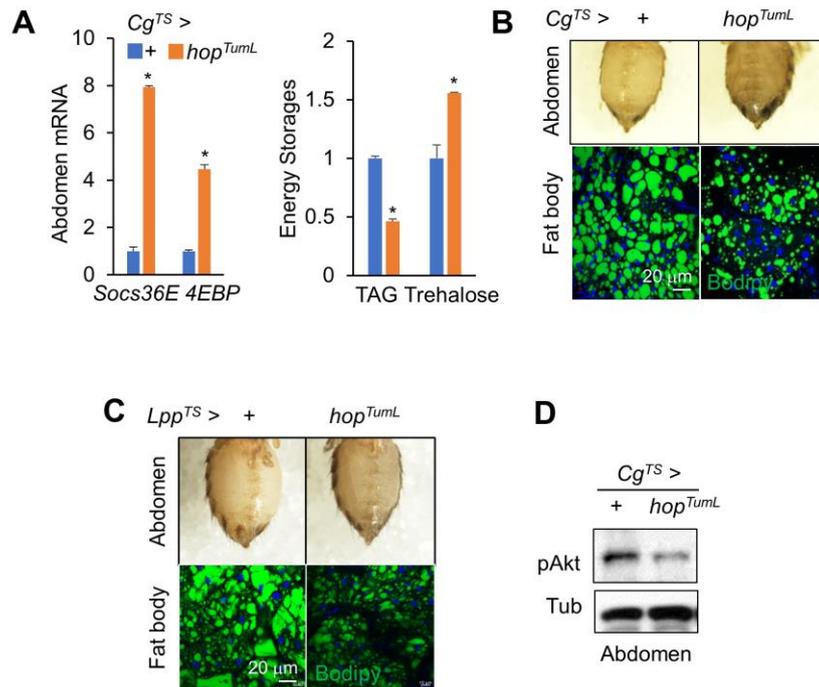
**Fig. S4**



**Supplemental Figure S4. Jak/Stat signaling does not regulate proteasome-associated protein degradation.**

**Related to Figures 3 and 4.** (A-B) Polyubiquitin proteins in adult thoracic muscles (A, day 8) (B, left, day 6; middle, day 4; right, day 4). (C) mRNA levels of *CG11658* in the thoracic muscles of indicated genotypes (left, day 8; right, day 4) (n=3, 5 flies/replicate). (D-E) Thoracic muscle p-Akt levels (D) and fly climbing rates (E, n=15) of adult flies of indicated genotypes at day 4. Data are presented as mean ± SEM. \**p* < 0.05.

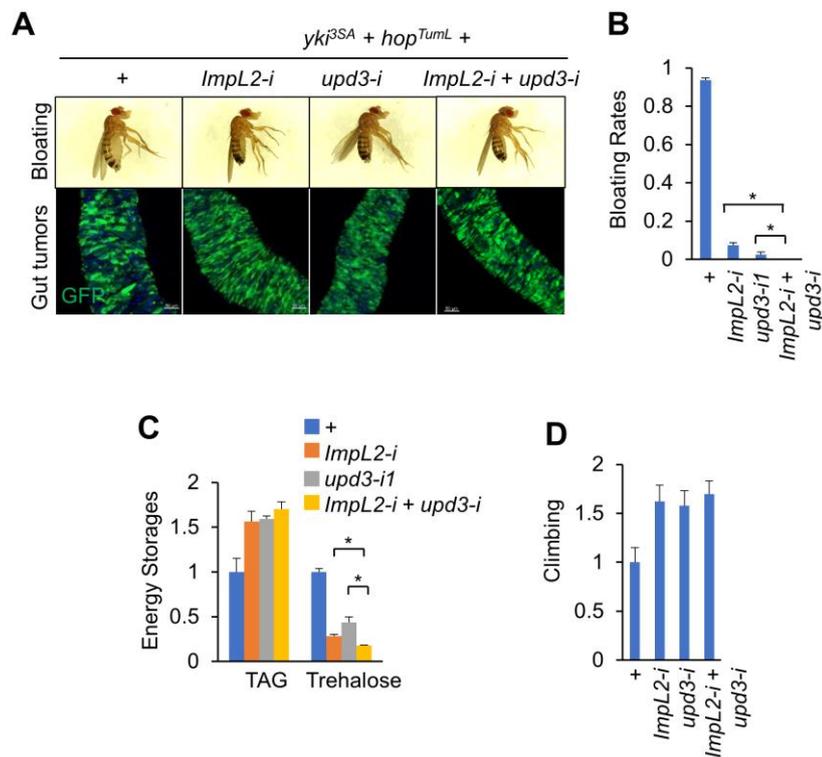
**Fig. S5**



**Supplemental Figure S5. Fat body Jak/Stat signaling regulates lipid loss and hyperglycemia. Related to Figure 6.**

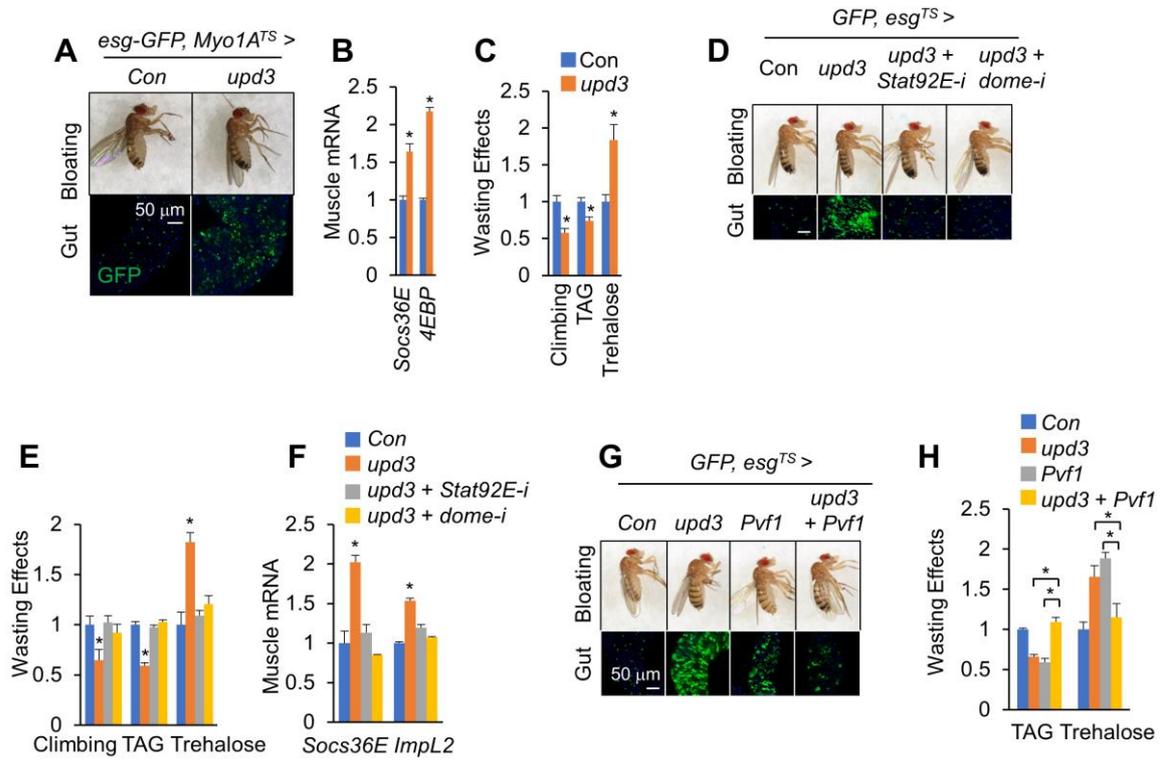
Gene expression in abdomens containing fat bodies (**A**, left, n=3, 5 flies/replicate), TAG and Trehalose storages (**A**, right, n=3, 5 flies/replicate), abdomen appearance (**B-C**, up), Bodipy-labeled lipid droplets in the abdominal fat body (**B-C**, bottom, scale bar, 20  $\mu$ m), as well as abdomen insulin signaling as indicated by p-Akt (**D**), in flies with activation of Jak/Stat signaling in the fat body at day 4. Data are presented as mean  $\pm$  SEM. \* $p < 0.05$ .

**Fig. S6**



**Supplemental Figure S6. Double knockdown of *ImpL2* and *Upd3* in  $yki^{3SA}+hop^{TumL}$  gut tumors further alleviates host wasting. Related to Figure 2. (A-D) Wasting effects of flies bearing  $yki^{3SA}+hop^{TumL}$  tumors with *ImpL2* RNAi (NIG 15009R-3) and/or *upd3* RNAi (HMS00646) at day 6: (A) Bloating phenotypes (up) and gut tumors (bottom, scale bar, 50  $\mu$ m), (B) bloating rates (n=4, 20 flies/replicate), (C) TAG and trehalose levels (n=3, 5 flies/replicate), (D) climbing rates (n=15). Data are presented as mean  $\pm$  SEM. \* $p < 0.05$ .**

**Fig. S7**



**Supplemental Figure S7. Gut-derived Upd3 results in host Jak/Stat activation and organ wasting. Related to Figure 7.** Fly appearances (A, D, G, up), gut tumors (A, D, G, bottom, scale bar, 50  $\mu$ m), thoracic muscle gene expression (B, F, n=3, 5 flies/replicate), and wasting effects such as climbing (n=15), TAG and Trehalose storages (C, E, H, n=3, 5 flies/replicate) of adult flies with indicated genotypes at day 8. *Stat92E* RNAi (HMS00035, III) and *dome* RNAi (v19717) were used. Data are presented as mean  $\pm$  SEM. \**p* < 0.05.

**Supplemental Table S3. Genotypes in this study. Related to each Figure.**

<b>Figure 1</b>	
<b>A-C,</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i>
<b>E-F</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i>
<b>D</b>	<i>10XStat-GFP; esg-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i> <i>10XStat-GFP; esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-yki<sup>3SA</sup></i>
<b>G</b>	<i>upd3-LacZ; esg-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i> <i>upd3-LacZ; esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-yki<sup>3SA</sup></i>
<b>H</b>	<i>hs-Flp, FRT19A, upd3-LacZ; tub-Gal4, tub-GAL80 &gt; UAS-GFP</i> <i>hs-Flp, FRT19A, upd3-LacZ; tub-Gal4, tub-GAL80 &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i>
<b>I</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-upd3-i1</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-upd3-i2</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-dome-i</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop-i</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-Stat92E-i</i>
<b>Figure 2</b>	
<b>B-E</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-upd3-i1</i>
<b>Figure 3</b>	
<b>A-C</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-upd3-i1</i>
<b>D-F</b>	<i>Mhc-GAL4 &gt; +</i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup></i>
<b>Figure 4</b>	
<b>A-B</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-upd3-i1</i>
<b>C-G</b>	<i>Mhc-GAL4 &gt; +</i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup></i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup>, UAS-InR<sup>AC</sup></i> <i>dMef2-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i> <i>dMef2-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-hop<sup>TumL</sup></i>
<b>Figure 5</b>	
<b>E</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i>

	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i>
<b>F</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-upd3-i1</i>
<b>G</b>	<i>Mhc-GAL4 &gt; +</i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup></i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup>, UAS-Stat92E-i</i>

<b>Figure 6</b>	
<b>A-C</b>	<i>Mhc-GAL4 &gt; +</i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup></i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup>, UAS-Impl2-i</i>
<b>D-F</b>	<i>Cg-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i> <i>Cg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-hop<sup>TumL</sup></i> <i>Cg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-hop<sup>TumL</sup>, UAS-Impl2-i</i>

<b>Figure 7</b>	
<b>A-D</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-upd3</i>

<b>Figure S2</b>	
<b>A</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-upd3-i1</i>
<b>B</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i> <i>upd3<sup>A</sup>; esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i>
<b>C-D</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-upd3-i2</i>

<b>Figure S3</b>	
<b>A</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i>
<b>B</b>	<i>10XStat-GFP; esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-yki<sup>3SA</sup></i>
<b>C-D</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i>

<b>Figure S4</b>	
<b>A</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i>
<b>B-E</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-upd3-i1</i> <i>Mhc-GAL4 &gt; +</i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup></i> <i>Mhc-GAL4 &gt; UAS-hop<sup>TumL</sup>, UAS-Stat92E-i</i> <i>dMef2-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i>

	<i>dMef2-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-hop<sup>TumL</sup></i>
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<b>Figure S5</b>	
<b>A, B,</b>	<i>Cg-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i>
<b>D</b>	<i>Cg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-hop<sup>TumL</sup></i>
<b>C</b>	<i>Lpp-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i> <i>Lpp-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-hop<sup>TumL</sup></i>

<b>Figure S6</b>	
<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup></i>	
<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-Impl2-i</i>	
<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-upd3-i1</i>	
<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-yki<sup>3SA</sup>, UAS-hop<sup>TumL</sup>, UAS-Impl2-i, UAS-upd3-i1</i>	

<b>Figure S7</b>	
<b>A-C</b>	<i>esg-GFP; Myo1A-GAL4, tub-GAL80<sup>TS</sup> &gt; +</i> <i>esg-GFP; Myo1A-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-upd3</i>
<b>D-F</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-upd3</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-upd3, UAS-Stat92E-i</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-upd3, UAS-dome-i</i>
<b>G-H</b>	<i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-upd3</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-Pvf1</i> <i>esg-GAL4, tub-GAL80<sup>TS</sup> &gt; UAS-GFP, UAS-upd3, UAS-Pvf1</i>

**Supplemental Table S4: Oligonucleotide information. Related to STAR Methods.**

Oligonucleotides
Upd3 forward: CTGGTCACTGATCTTACTCGCC
Upd3 reverse: GGATTGGTGGGATTGATGGGA
Pvf1 forward: CTGTCCGTGTCCGCTGAG
Pvf1 reverse: CTCGCCGGACACATCGTAG
Impl2 forward: AAGAGCCGTGGACCTGGTA
Impl2 reverse: TTGGTGAACCTTGAGCCAGTCG
Socs36E forward: GAGATCCTCACAGAGGCCACT
Socs36E reverse: GCGAAACTTTCCACCTGACC
4EBP forward: CTCCTGGAGGCACCAAACCTTATC
4EBP reverse: TTCCCCTCAGCAAGCAACTG
InR forward: ACAAATGTAAACCTTGCAAATCC
InR reverse: GCAGGAAGCCCTCGATGA
RpL32 forward: GCTAAGCTGTGCGACAAATG
RpL32 reverse: GTTCGATCCGTAACCGATGT
Stat92E-RB forward: TAGTCCAGTGTGGTGGGAATTCGCCACCATGCCGCTAAATCCCTACAACATGAAC
Stat92E-RB reverse: ATCATGTCTGGATCCCTCGAGTCAAAAGTTCTCAAAGTTTGTAAATCGTATCGAAGTCC
hop <sup>TumL</sup> forward: TAGTCCAGTGTGGTGGGAATTCGC CACCATGGCCCTGGCCAACGGG
hop <sup>TumL</sup> reverse: ATCATGTCTGGATCCCTCGA GTCACTCGGCATCCGTCGGCT
Impl2-BS1 forward: TACGCGTGCTAGCCCGGGCTCGAGG GAAACTCCAGCCGAAGTTCTAGAAC
Impl2-BS1 reverse: CTTACTTAGATCGCAGATCTCGAG CGAAATGCAGATCGGATTAACCCA
Impl2-BS2 forward: TACGCGTGCTAGCCCGGGCTCGAGGAGCAGGTGTTTTCCCGCTAACAC
Impl2-BS2- reverse: CTTACTTAGATCGCAGATCTCGAG GCGCTATTAATGCGCTGTAATGC
Impl2-BS3 forward: TACGCGTGCTAGCCCGGGCTCGA GGGACGTGCTATCCGATTGCGATA
Impl2-BS3- reverse: CTTACTTAGATCGCAGATCTCGAG CGTCGGCATTGTCATCGAAGTTT
Impl2-BS4 forward: TACGCGTGCTAGCCCGGGCTCGA GCACCGTCAACGGGCTTTAAGTTC
Impl2-BS4- reverse: CTTACTTAGATCGCAGATCTCGA GATCGCTGGCGAATGTTGCATC
Impl2-BS2-mut-#1 forward: TGCTAGCCC GGCTCGAG
Impl2-BS2-mut-#1- reverse: CTCAAAGTGAAAACCTTGAATTAAGTCTAG
Impl2-BS2-mut-#2 forward: CTAGACTTTAATTCAAGGTTTTCACTTTGAG
Impl2-BS2-mut-#2 reverse: CTGGAAAACCTCGAATGCCTCAC
Impl2-BS2-mut-#3 forward: GTGAGGCATTGAGGTTTTCCAG
Impl2-BS2-mut-#3 reverse: AAGCTTACTTAGATCGCAGATCTCGAG
BS2-amplicon forward: TCGCTGAGCAATACTAAACACCTGC
BS2-amplicon reverse: GTGGAACCGAAAACGTGATACCC
BS3-amplicon forward: GGGCGAGAGCCGGAGTCACA
BS3-amplicon reverse: GCGCTGTAATGCGACGCTCTGG
BS4-amplicon forward: GGTGATTGCCGCCGAACGCAG
BS4-amplicon reverse: TGGCGAATGTTGCATCAGACGGT
Negative control- amplicon forward: CACGGCGGCGGTGCATTCTC
Negative control- amplicon reverse: CAGCGCTTGCAGAGACCGGC