# Supporting Information

## Yan and Perrimon 10.1073/pnas.1515891112

### SI Methods

**Fly Strains.** The following stocks were used in this study: $w^{1118}$ (used as wild-type, WT), *MTD-Gal4*, *ap-Gal4*, *nub-Gal4*, *dome-Gal4, UAS-2xEYFP* (43), *Sxl* shRNA (HMS00609), *nito* shRNA (HMS00166), *nito* shRNA (HMS02013), *nito* shRNA (HMJ02081), *nito* dsRNA (VDRC 20942), *nito* dsRNA (VDRC 114704). Experiments presented in Figs. 1, 2, and 4 and Fig. S3 were done using *nito* shRNA (HMS00166).

To generate a null *nito* mutation, we used the homozygous viable P-element insertion $nito^{HP25329}$ located in the 5′UTR of the *nito* gene. After mobilization of the HP25329 P-element, we screened for homozygous lethal lines and recovered a null allele, $nito^{1}$, that deletes 1,357 bp and is lethal over *Df(2R)Exel6055* (43F1 to 44A4) that uncovers the *nito* locus. The Nito antibody, raised against amino acids 479–500, cannot detect any Nito antigens in $nito^{1}$ mutant clones (Fig. 3 *H* and *H′*).

To generate mutant clones, $nito^{1}$ was recombined with $FRT^{G13}$ and crossed to *y w hsflp; ubiGFP* $FRT^{G13}$ flies. The progeny were heat-shocked at 37 °C for 1 h twice at first- and second-instar larval stage.

**Antibody Stainings in Discs and Ovaries.** Larval wing discs and female ovaries were stained as described (19). Briefly, tissues were dissected in PBS and fixed in 4% formaldehyde in PBST (PBS + 0.1% Triton X-100). After blocking in 1% normal donkey serum in PBST for 1 h, the samples were incubated with the primary antibody in the same solution at 4 °C overnight. After three washes in PBST, samples were incubated with the secondary antibody for 2 h at room temperature, washed in PBST three times, and subsequently mounted in Vectashield. All images were taken on a Zeiss LSM 780 microscope.

The following antibodies were used: mouse anti-α-Spectrin (1:10) (3A9, DSHB), rabbit anti-Vasa (1:250) (Santa Cruz Biotechnology), mouse anti-Sxl (1:10) (M18, DSHB), rabbit anti-Nito (1:500), rabbit anti-phospho-Histone H3 (1:1,000) (Millipore), rabbit anti-GFP (1:1,000) (Molecular Probes), mouse anti-GFP (1:200) (Molecular Probes), Alexa 488- or 555- conjugated secondary antibodies (1:1,000) (Molecular Probes) and DAPI (1:1,000) (Molecular Probes).

Nito antibodies were generated in rabbits against a peptide containing amino acids 479–500 (KSSKPPYDESALEYRRPEYDPY) and affinity-purified at YenZym Antibodies. Polyclonal antisera were raised in two rabbits, YZ3137 and YZ3138, and gave similar staining patterns. All of the experiments described in the paper were performed with antiserum from YZ3137.

Adult legs and wings were mounted in a 1:1 (vol/vol) mixture of Permount (Fisher Scientific) and xylene. The genitalia images were taken in stacks and rendered with HeliconFocus software.

**Coimmunoprecipitation.** To generate the GFP-Nito plasmid, a *nito* full-length cDNA (GH11110) was cloned into the *Drosophila* Gateway vector pAGW. HA-Sxl and GFP-Sxl were constructed following PCR of *Sxl* (the MS3 isoform) from *UAS-Sxl* flies (44) and cloned into pAHW and pAGW, respectively. GFP was cloned into pAWM as a control.

*Drosophila* S2 cells were maintained at 25 °C in Schneider's medium supplemented with 10% FBS. One microgram of total DNA was transfected into S2 cells in a single well of six-well plates with Effectene (QIAGEN). After 48 h, cells were lysed in IP lysis buffer (Pierce) with Halt Protease Inhibitor (Thermo Scientific). Lysates were incubated with anti-GFP nanobody agarose beads (Allele Biotechnology) or anti-HA agarose (Sigma) for 2 h at 4 °C. The beads were washed 3–4 times with 1 mL lysis buffer. Protein complexes were eluted and detected by Western blotting using anti-GFP antibody (A6455, Molecular Probes) or anti-HA antibody (3F10, Roche). For RNase treatment experiment, 100 μL of RNase A (10 mg/mL, Thermo Scientific) and 5 μL of RNase T1 (1,000 U/μL, Thermo Scientific) were added to 1 mL of lysate and incubated for 30 min at 30 °C, then overnight at 4 °C with beads (12, 17).

**RT-PCR.** Total RNA was extracted from dissected wing discs or ovaries using TRIzol (Invitrogen), digested with DNase I (Qiagen) and purified using the RNeasy Mini kit (Qiagen). cDNA was generated from 1 μg of purified RNA using the iScript cDNA Synthesis lit (Bio-Rad). For nonreal time PCR, TaKaRa Taq polymerase was used. For qPCR, iQ SYBR Green Supermix (Bio-Rad) was used and reactions were measured in a CFX96 Real-Time PCR detection system (Bio-Rad). qPCR results for *nito* expression in male and female wing discs (Fig. 3*B*) were normalized to the reference gene *αTubulin84B*. *Sxl* primers used in Fig. 4*G* are described in ref. 12. *nito* primers in Fig. 3*B* and *αTubulin84B* primers are listed below.

| | |
|---|---|
| nito_PP17280_f | GGCTACAAGGTACTTTGCGTC |
| nito_PP17280_r | TACTCGCGGTACAGTGTCTCC |
| Sxl-f | GTGGTTATCCCCCATATGGC |
| Sxl-r | GATGGCAGAGAATGGGAC |
| αTubulin84B-f | CAACCAGATGGTCAAGTGCG |
| αTubulin84B-r | ACGTCCTTGGGCACAACATC |

**RNA Immunoprecipitation (RIP).** RIP experiments were performed following previous protocols (45, 46). One microgram of DNA was transfected into S2 cells in 60-mm plates with Effectene (QIAGEN). After 48 h, cells were lysed in IP lysis buffer (Pierce) with Halt Protease Inhibitor (Thermo Scientific) and RNasin plus (40 U/mL, Promega). After centrifugation, 10% of the supernatant were saved as the input and the rest were incubated with anti-GFP nanobody agarose beads (Allele Biotechnology) for 2 h at 4 °C. The beads were washed 3–4 times with 1 mL of lysis buffer. To elute RNA from the RNA/protein complexes, the beads were treated with proteinase K solution for 10 min at 55 °C. Total RNA from the input and the beads were extracted by using RNeasy Micro kit (QIAGEN). cDNA was synthesized using SuperScript III First-Strand Synthesis System (Life Technologies) with four of the eluted RNA and random hexamers. TaKaRa Taq polymerase was used for two rounds of PCR amplification with primers and conditions described in ref. 12.

| | |
|---|---|
| Sxl-intron | GAGGGTCAGTCTAAGTTATATTCG |
| Sxl-r | GATGGCAGAGAATGGGAC |

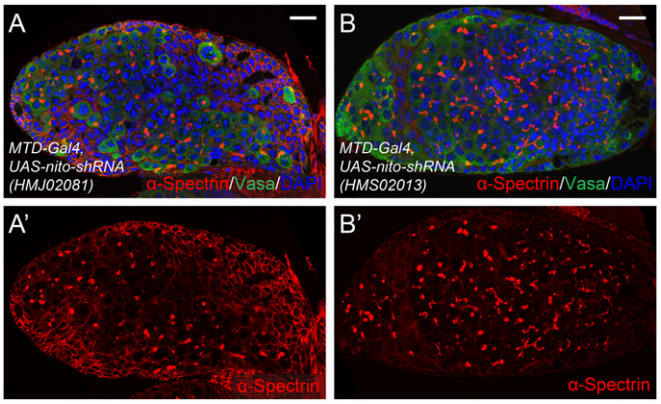


**Fig. S1.** Two independent *nito* shRNAs result in similar stem-cell-tumor in the germ-line. (*A*–*B*′) Egg chambers expressing shRNAs targeting *nito* (HMJ02081) or *nito* (HMS02013) using *MTD-Gal4* stained for α-Spectrin, Vasa and DAPI. (Scale bars: 20 μm.)

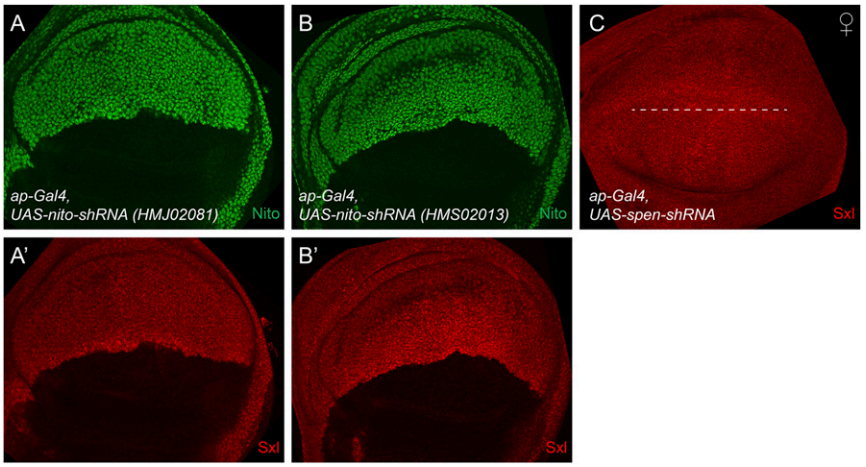


**Fig. S2.** *nito*, but not *spen*, regulates Sxl levels in wing discs. (*A*–*B*′) Expression of *nito* shRNA (HMJ02081 or HMS02013) in the dorsal half of the wing disk using *ap-Gal4* leads to a strong reduction of both Nito (*A* and *B*) and Sxl (*A*′ and *B*′) stainings. (*C*) Expression of *spen* shRNA in the dorsal half of the disk (below the dashed line) by *ap-Gal4* does not affect Sxl protein levels. *spen* shRNA generates embryonic lethality with cuticle and head defects when expressed using *MTD-Gal4* (47), which resembles the phenotype of the *spen* mutant, indicating that the shRNA is functional.

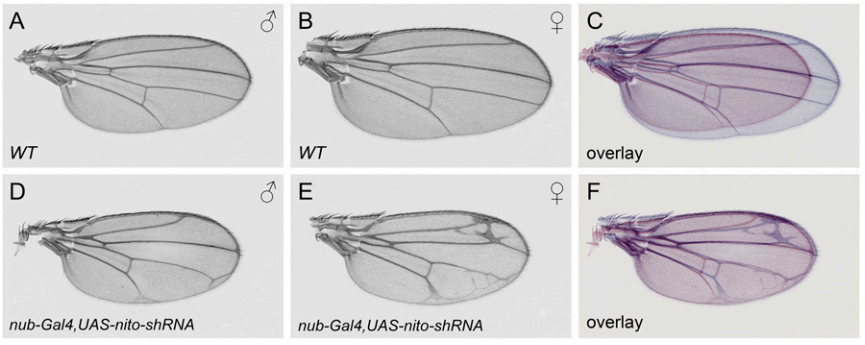


**Fig. S3.** Nito shRNA affects wing growth more strongly in females than in males. (*A*) WT male wing. (*B*) WT female wing. (*C*) Overlay of the images in *A* (red) and *B* (blue) shows that a WT female wing is about 30% larger than a WT male wing. Male (*D*) and female (*E*) wings in which *nito* shRNA was expressed using the *nub-Gal4* driver. (*F*) Overlay of *D* and *E* showing that both male and female wings reach about the same size upon *nito* knockdown.

**Dataset S1. Screen results of 316 RNAi lines targeting 247 splicing genes using *MTD-Gal4*, *dome-Gal4* and *nub-Gal4***

Dataset S1