Figure S1:

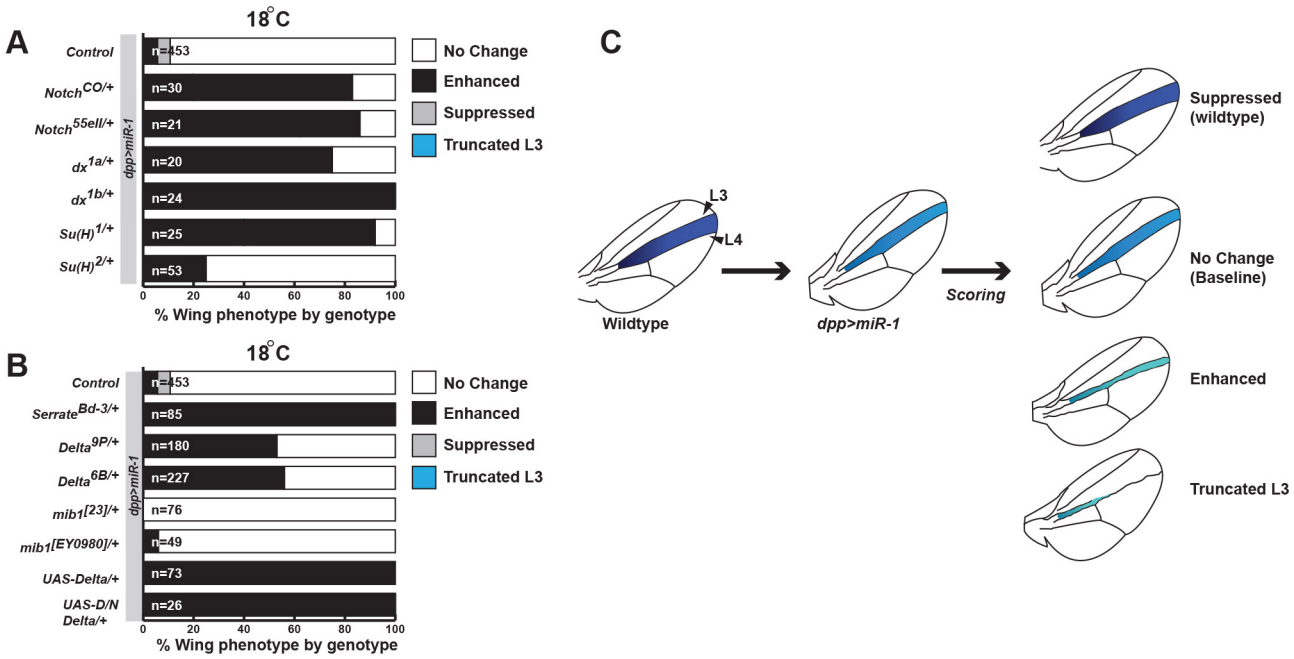


Fig. S1. Positive Effectors of Notch Signaling Do Not Mediate the Truncation of L3. (A) Percentage of progeny displaying "no change," "enhanced," "suppressed," or "truncated L3" by genotype when crossed to the *dpp>dmiR-1* line at 18°C. At this temperature, L3 is formed normally in the control *dpp>miR-1* line and loss of the L3-L4 intervein distance predominates. Note that no animals display the L3 truncation phenotype. n=number of progeny scored. (B) As in (A), with emphasis on the mutants of Notch ligands Delta and serrate and the regulator of Delta, mindbomb1. Control, *dpp>dmiR-1;* D/N Delta, dominant-negative Delta; dx, deltex; mib1, mindbomb1; Su(H), Suppressor of Hairless. (C) Schematic of the scoring system based on changes in the L3-L4 intervein distance observed with the *dpp>dmiR-1* fly line.

Figure S2:

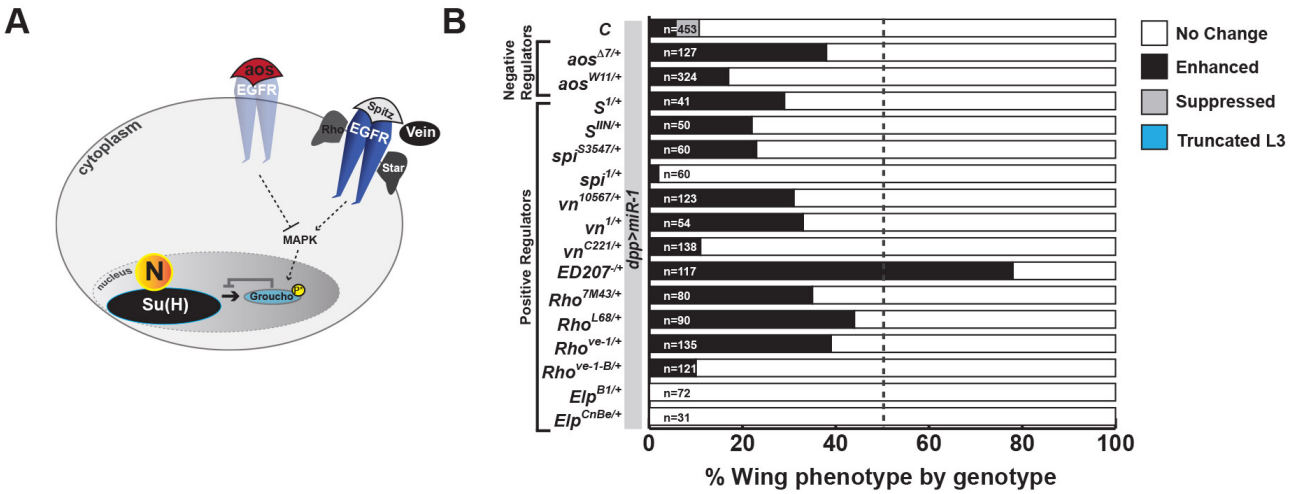


Fig. S2. Phosphorylation of Groucho does not Result in L3 truncation. (A) Schematic of Epidermal growth factor Receptor (EFGR) signaling as it pertains to the regulation of the Notch (N) repressor Groucho. (B) Genetic interaction studies. Graph shows the percentage of affected offspring for the noted crosses (performed at 18°C) for each of the possible alterations in L3-L4 intervein distance as depicted in. The loss of L3 does not occur for any of the stated crosses indicating that phosphorylation of Groucho does not play a significant role in the truncation of L3. n=number of flies scored. *C: control, aos:argos, S:star, Spi:spitz, vn: vein, Elp: elipse* (EGFR gain of function allele)

Figure S3:

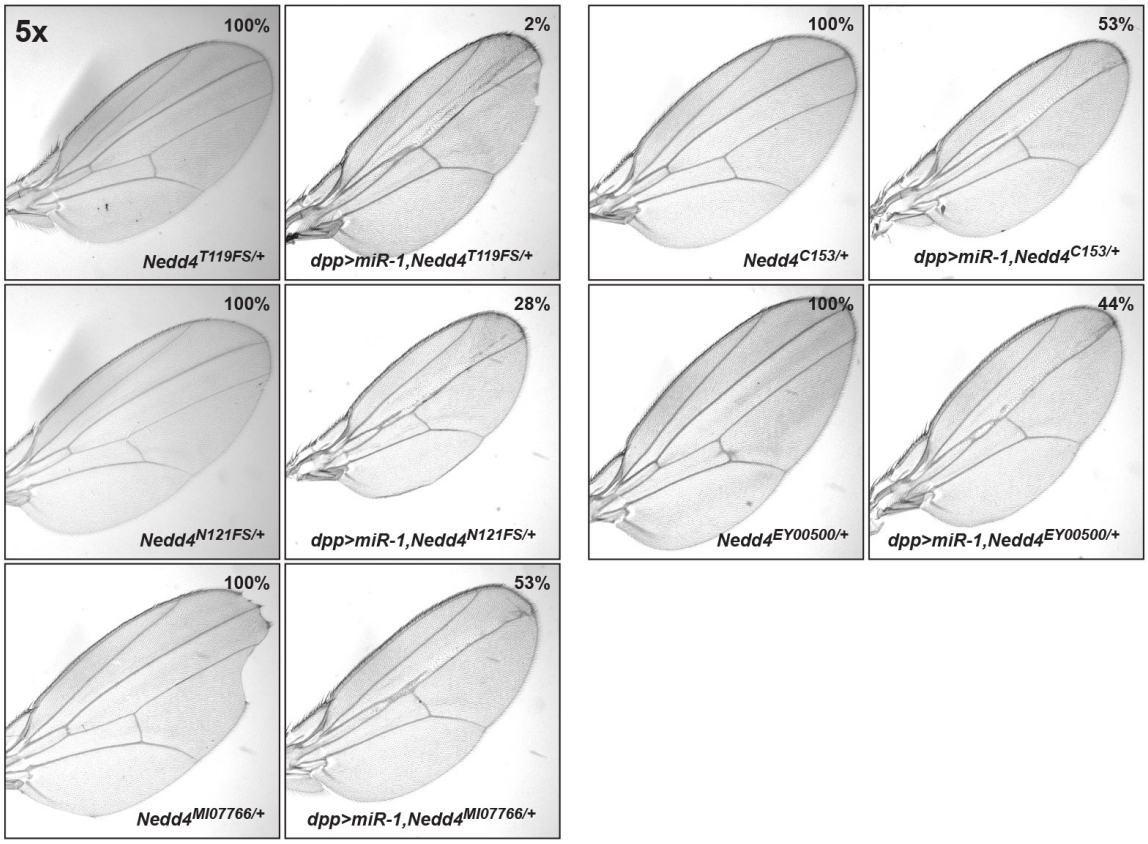
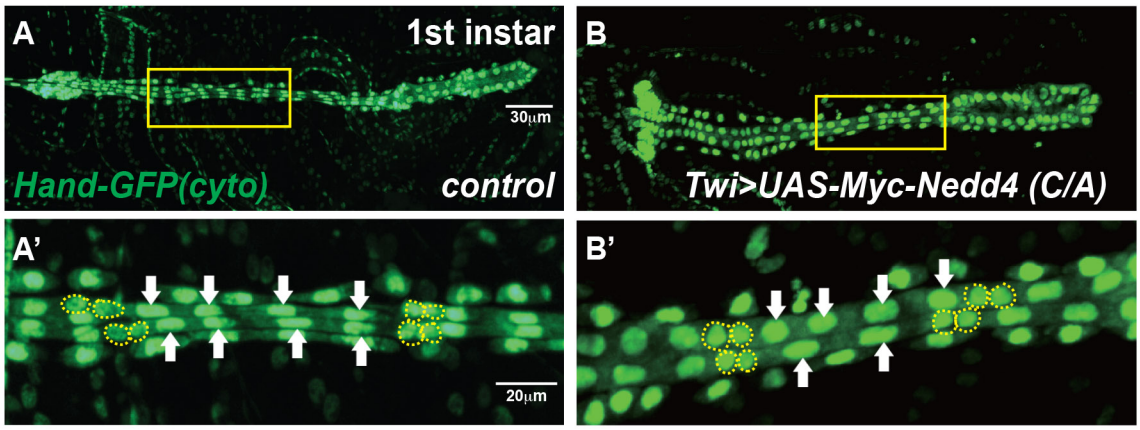


Fig. S3. *dNedd4* Genetically Interacts with *dmiR-1*, resulting in a Truncated L3. Representative wing morphology of control (parental) or *dpp>dmiR-1,dNedd4*$^{(-/+)}$ mutant progeny with L3 truncation were quantified as in Figure 2C. Genotypes are noted. Penetrance for each genotype is shown at top right.

Figure S4:



C

	X Hand-Gal4/Cyo		
	Curly wing (with CyO)	Straight wing (with Hand-GAL4)	Lethality (%)
Control (n=228)	115	113	1.7
UAS-Myc-Nedd4 (n=168)	90	78	13
UAS-Myc-Nedd4 (C/A) (n=211)	113	98	13
UAS-Nedd4-RNAi (n=159)	82	77	6.1

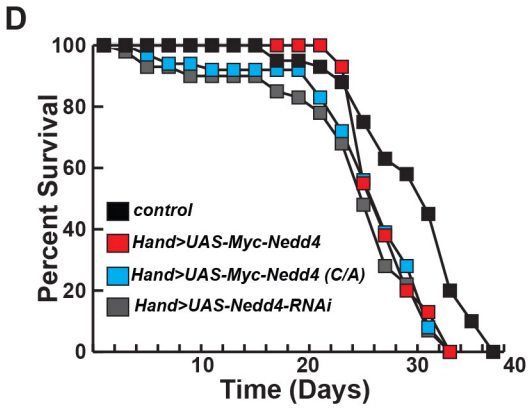


Fig. S4 Misexpression of dNedd4 Produces Abnormal Numbers of Cardioblasts and Mildly Decreases Fitness and Lifespan. (A–B) First-instar larvae marked by 20X magnification of first-instar larvae with genotypes as noted at bottom right. Anterior is to the left. (A'–B') 20X magnification of the area heighted by the yellow box noted in (A–B), with ostial cells outlined in dashed lines and cardioblasts marked by white arrows. Normally, four cardioblasts exist between each set of four ostial cells (A), while in *twist>UAS-Myc-dNedd4(C/A)* mutant larvae, the localization and/or number of these cardioblasts is abnormal (n=6 per phenotype). (C) Rates of eclosion by genotype at 29°C to enhance the temperature-sensitive UAS-transgene expression, with misexpression of dNedd4 mildly increasing lethality. (D) Adult male flies maintained at 29°C in vials containing approximately 15 animals. 60 flies were assayed per genotype, with misexpression of dNedd4 modestly decreasing life span.