Kavaler et al., https://doi.org/10.1083/jcb.201706101

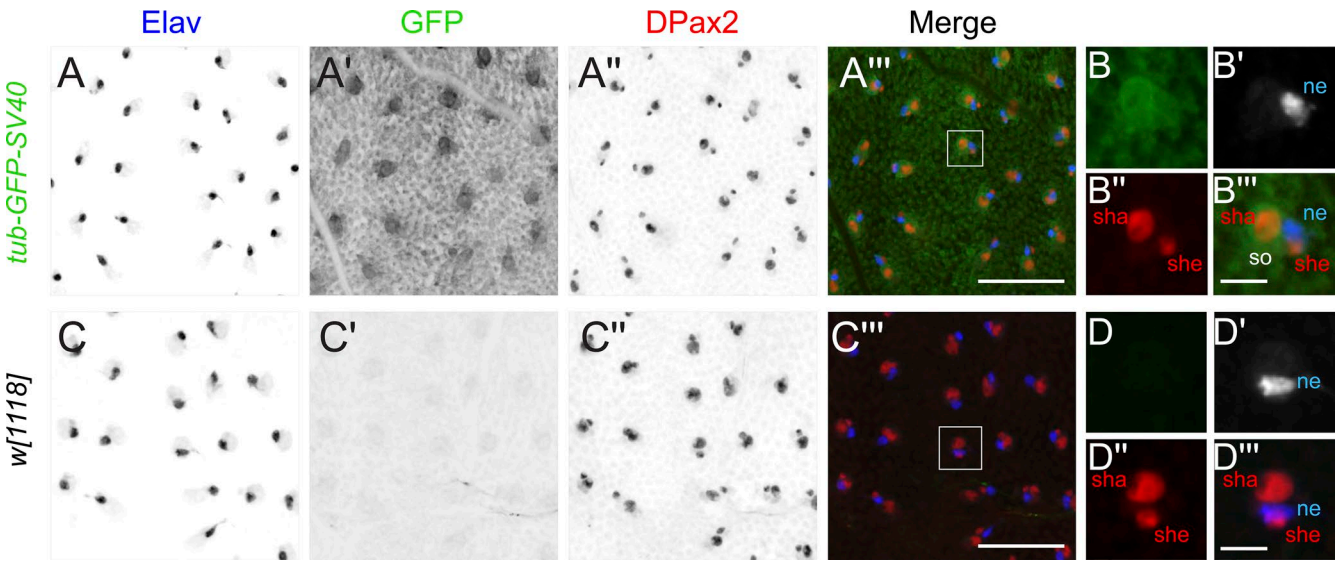

Figure S1. **Analysis of a *tub-GFP-SV40* sensor in the pupal notum.** These experiments were to control whether the neuronal pattern of the *tub-GFP-miR-279* sensor was specific to this miRNA sensor. Here, a *tub-GFP-SV40 3′UTR* sensor is shown to have broad expression in the ∼30 h pupal notum. An apparent sensory organ–related up-regulated GFP pattern is observed (A′), particularly in the larger external cells that include the Dpax2+ shaft cell. This is seen more clearly in the close-up view (B). This may reflect preferential accumulation of GFP in these polyploid external sensory organ cells. The staining is specific, as compared with relative absence of signal in parallel staining of *w[1118]* notum lacking a GFP transgene (C and D). These tests show that a control unregulated sensor does not induce neuron-specific up-regulation as seen with *tub-GFP-miR-279* sensor (Fig. 1). Bars: (A and C) 50 µM; (B and D) 10 µM.

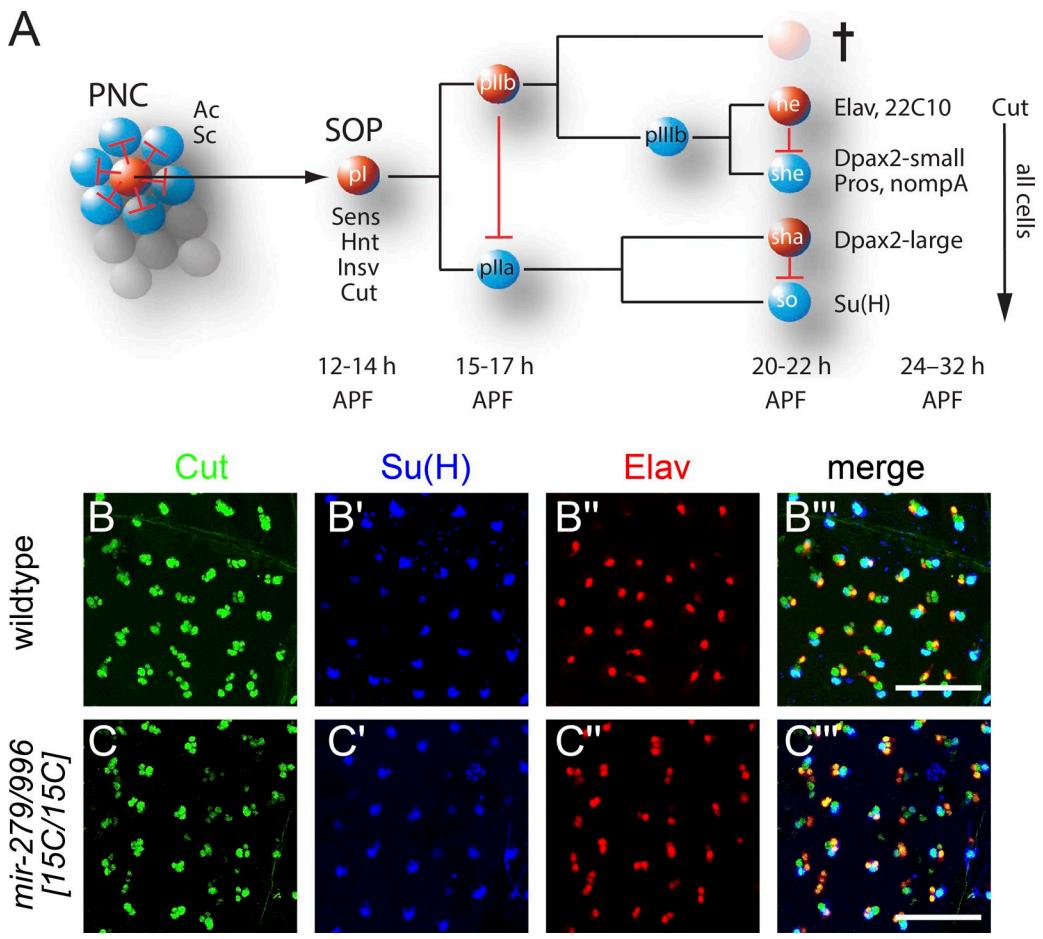

Figure S2. ***mir-279/996* null mutants have specific defects in internal cell fates. (A)** Schematic of the PNS mechanosensory lineage and key markers. **(B and C)** Staining of ∼32 h APF nota with the pan-lineage marker Cut, the socket cell marker Su(H), and the neuronal marker Elav. Compared with control wild-type tissue (B), *mir-279/996[15C/15C]* deletion mutants have normal total numbers of lineage cells and socket cells but a high frequency of double neuron sensory clusters (C). Bars, 50 µM.

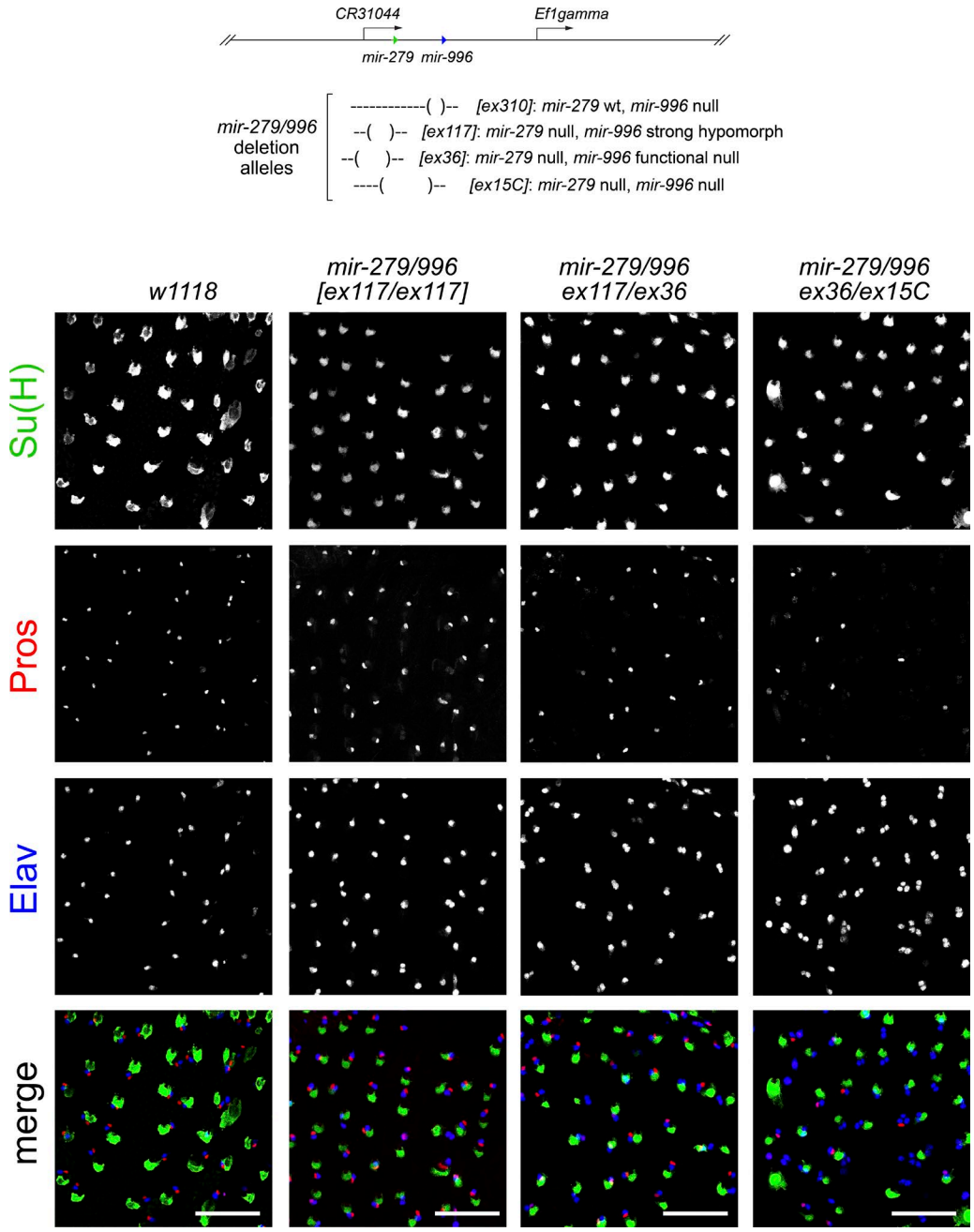

Figure S3. **Analysis of phenotypic series of mir-279/996 alleles reveals a graded response in the severity of the ectopic neuron defects.** Top: Summary of *mir-279/996* alleles and their relative phenotypic strength. Bottom: Staining of ~32 h APF nota with the socket cell marker Su(H), the sheath cell marker Pros, and the neuronal marker Elav. Among the allelic combinations tested here, the *ex117* homozygote has the weakest effect in terms of sheath cell loss/extra neuron phenotypes, *ex117/ex36* is intermediate, and *ex36/15C* is strongest. Bars, 50 μM.

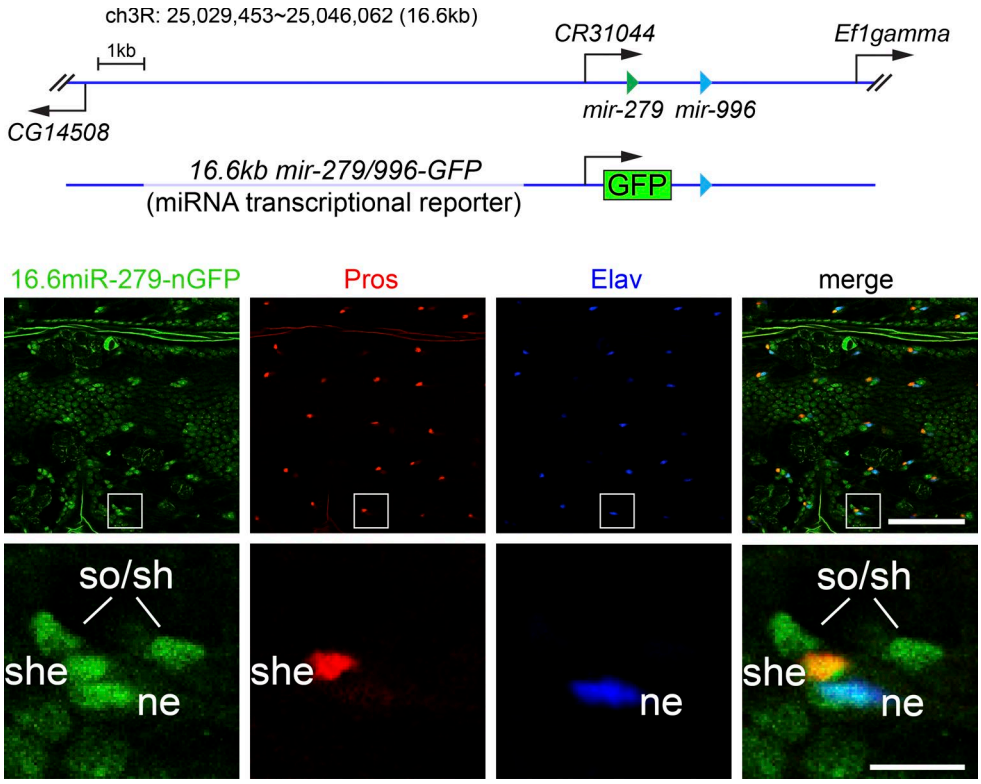

Figure S4. **Analysis of a 16.6-kb *mir-279/996*-GFP transcriptional reporter in the notum.** This reporter exhibits general epidermal expression as well as elevated expression in sensory organ cells. Note that it is elevated in the Elav+ neuron, even though functional sensor assays indicate that miR-279/996 activity is low in Elav+ neurons (Fig. 1). Bars: (top) 50 µM; (bottom) 10 µM.

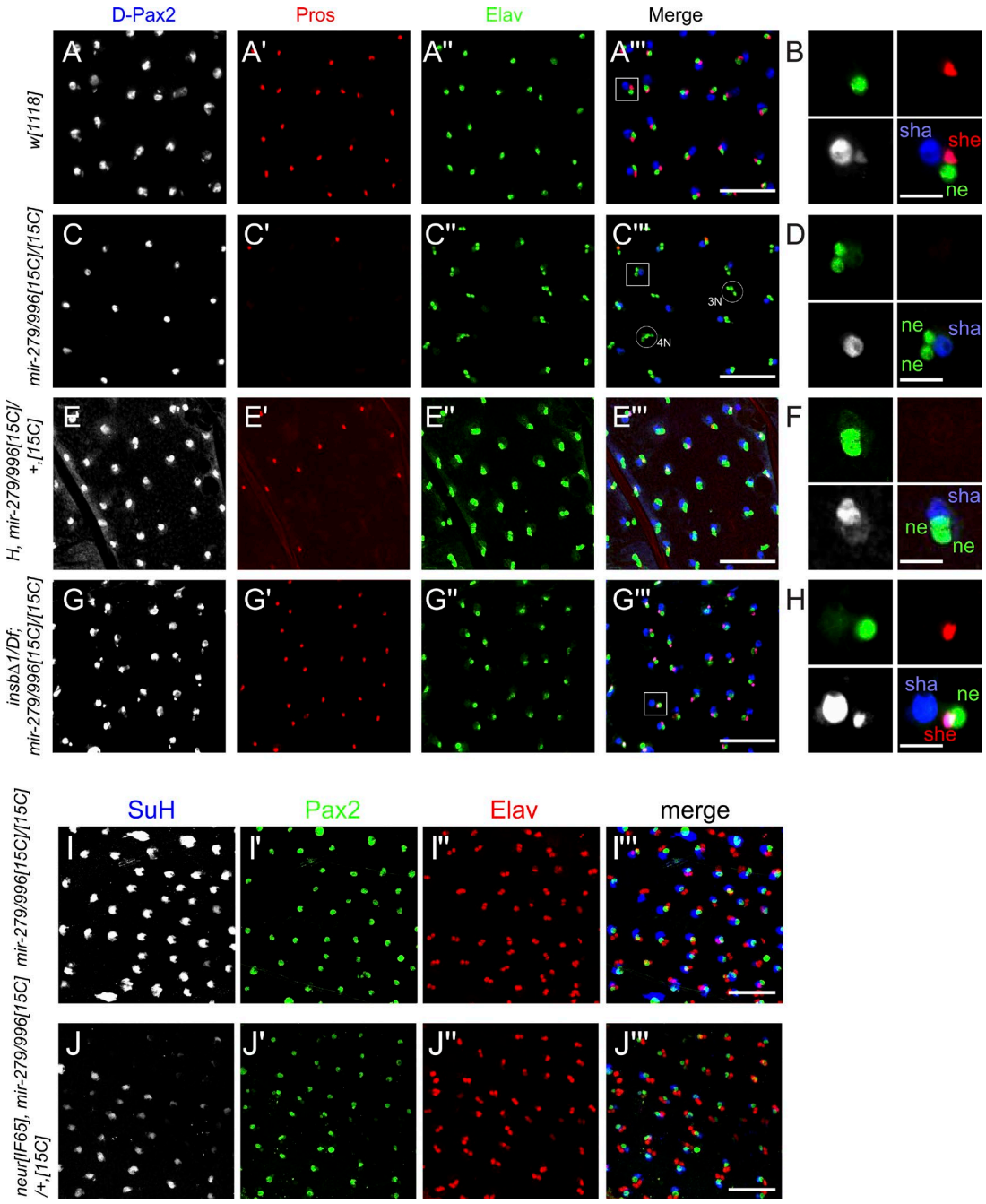

Figure S5. **Genetic interactions of *mir-279/996* and Notch pathway mutants. (A–H)** Shown are ~32 h APF nota stained for the indicated lineage markers: DPax2 stains the larger nuclei of shaft cells and the smaller nuclei of sheath cells, Pros is specific to sheath cells, and Elav accumulates in neuronal nuclei. Close-ups of individual sensory organ clusters are shown at right (B, D, F, and H). As described in Figs. 1 and 2, compared with wild-type nota (A and B), deletion of *mir-279/996* causes high-frequency conversion of sheath cells into neurons (C and D). Because this defect resembles a loss of Notch signaling and miRNAs are thought to act as repressors, this implies deregulation of a negative component of the Notch pathway. **(E and F)** Heterozygosity of *H*, the major known Notch pathway repressor and a haploinsufficient factor for normal PNS development, does not modify the *mir-279/996* sheath-to-neuron conversion phenotype. **(G and H)** Hemizygosity for *insb*, a neural-specific but nonessential Notch pathway repressor, fully rescues the *mir-279/996* PNS defect. **(I and J)** Shown are >28 h APF nota stained for the indicated lineage markers: Su(H) stains the socket cell nuclei, DPax2 stains the larger nuclei of shaft cells and the smaller nuclei of sheath cells, and Elav accumulates in neuronal nuclei. **(I)** The typical phenotype of *mir-279/996[15C/15C]* null mutants, with a high frequency of double Elav sensory organ clusters lacking a DPax2+ sheath cell, is not modified in animals lacking one copy of the core Notch pathway component and miR-279/996 target *neuralized* (J). Bars: (left) 50 µM; (right, magnified individual sensory organs) 10 µM.