

Figure S1. Bouton numbers for type I and II NMJs on m1 to m3.

(A) A representative image of the nerve endings at m1, m2 and m3. Type II endings were labeled by ANF-GFP driven by Tdc2-GAL4. TRITC-conjugated HRP outlined the neuronal membrane of both type I (Ib+Is) and II terminals. Muscles are numbered from the most distal (m1) to the most proximal (m4) to the soma in the CNS. Scale bar, 100 μm . (B) Quantification of the number of type I (Ib+Is) and II boutons on m1, m2, and m3. The data is displayed from the most distal m1 to the most proximal m3. Numbers on the bars indicate how many neurons were analyzed for each muscle. Data were derived from 14 animals.

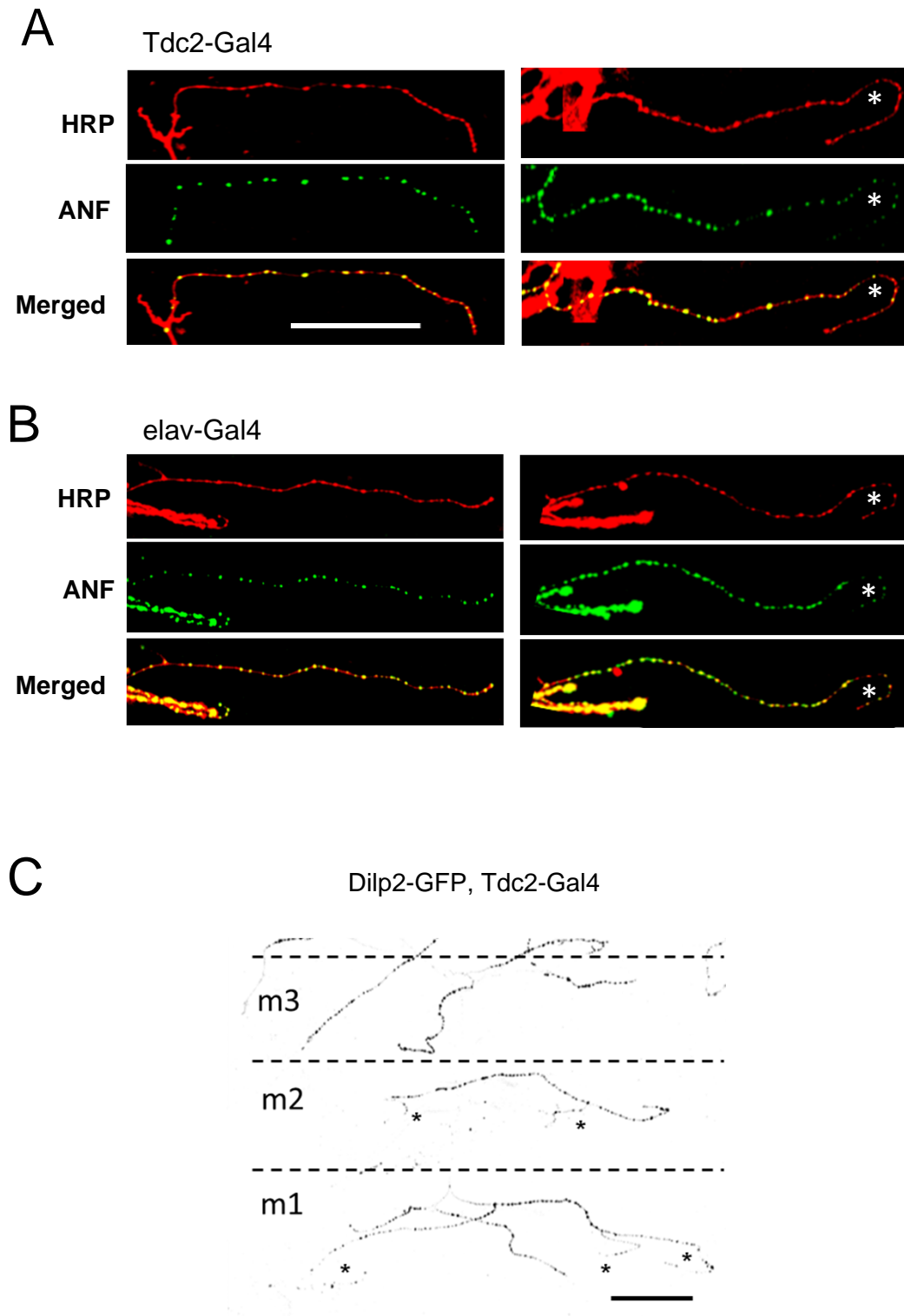


Figure S2. Attenuated distal neuropeptide stores. ANF-GFP expression was driven by Tdc2-Gal4 (A) or elav-Gal4 (B) and neuronal membranes were labeled by TRITC- HRP immunofluorescence. The left panels show examples with DCVs widely distributed among in type II boutons. The right panels show examples with distal boutons with weaker DCV/neuropeptide signals (indicated with asterisks). Bar, 50 μm . (A) left from m2, while other images from m1. (C) Inverted gray scale image of Dilp2-GFP in type II neurons innervating of m1, m2 and m3. In this image, a strong neuropeptide signal is black, while a dimmer signal is gray and no signal is white. Asterisks indicate distal regions in which the neuropeptide signal becomes dimmer compared to proximal boutons. Note that such regions are more prevalent in the distal innervation (e.g., on m1 compared to m3). Bar, 50 μm .

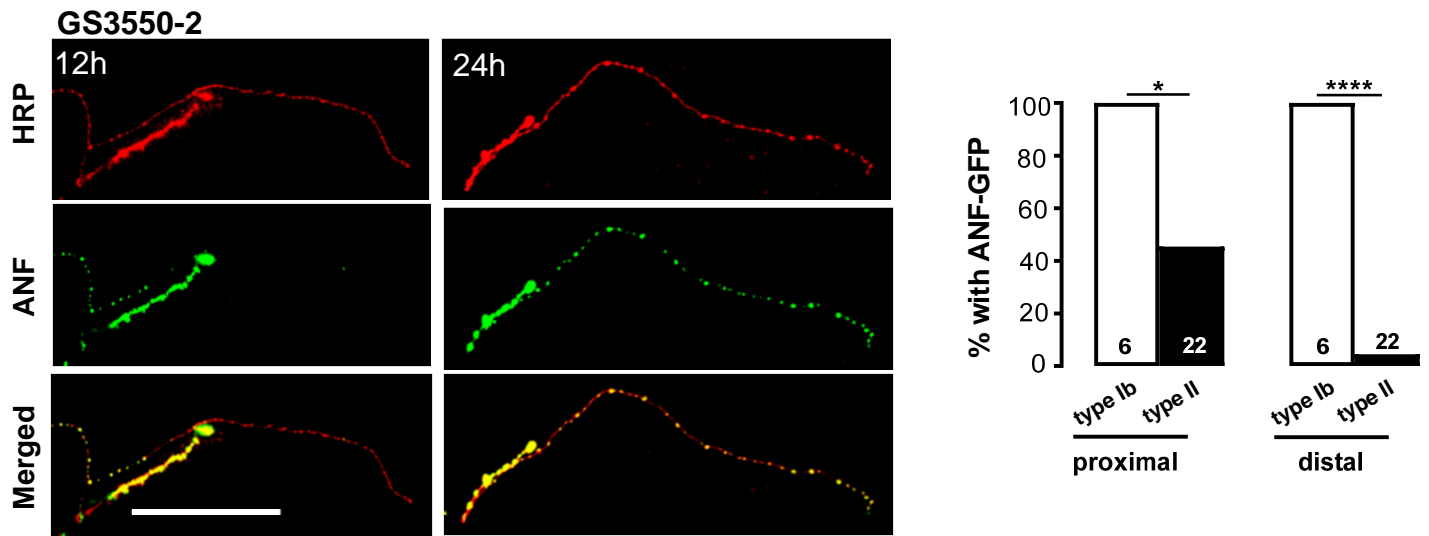


Figure S3. Differential appearance of neuropeptides in type Ib and II branches upon GeneSwitch induction in the GS3550-2 line. Left panels: ANF-GFP fluorescence in type Ib and II boutons on m1 after RU486 induction for 12h and 24h. TRITC-conjugated HRP stains the neuronal membrane. Scale bar, 50 μ m. Right: Quantification of the percentage of type Ib and type II NMJs with ANF-GFP after 12h RU486 treatment. N on each bar represents the number of type I or II endings examined in the experiment. Fisher's Exact Test was performed to calculate the p-values. * $p < 0.05$; **** $p < 0.0001$. Data derived from 6 animals for type Ib boutons and 16 animals for type II boutons.