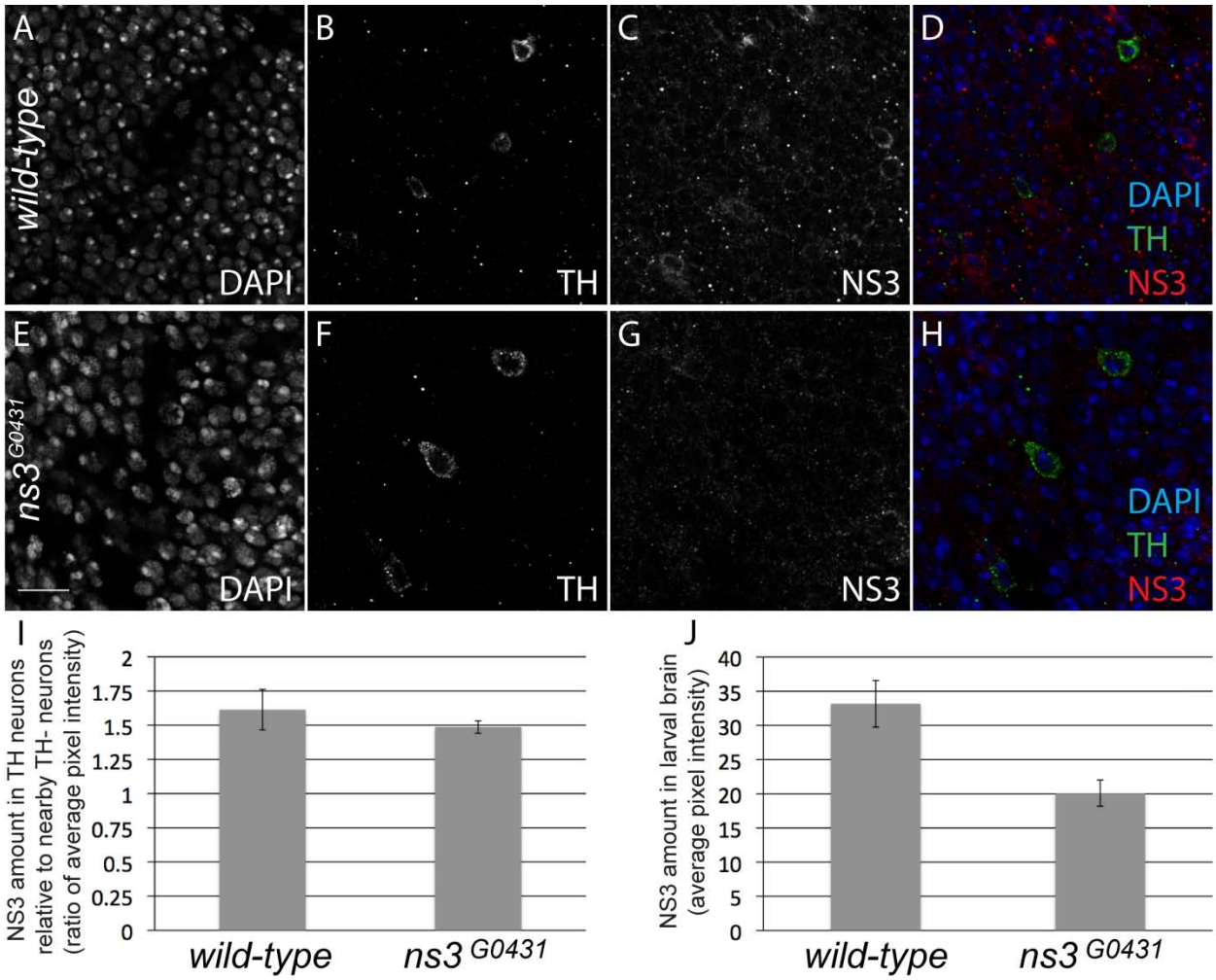

Figure S3 NS3 levels in wild-type and *ns3*^{G0431} mutant larval DA neurons. A-H. The CNS was dissected from wild-type and *ns3*^{G0431} first-instar larvae and stained with anti-tyrosine hydroxylase (TH) and anti-NS3 antibodies. DA neurons were identified by positive staining for TH. Levels of DA neuron NS3 were quantified by manually drawing four lines in the cytoplasm of TH-positive cells, measuring the average pixel intensity of NS3 staining, and averaging the four replicates. These cytoplasmic NS3 values were normalized by dividing by the average pixel intensities of NS3 in surrounding TH-negative cells. TH-negative levels of NS3 were measured by manually drawing five 225 x 225 pixel boxes within TH-negative regions, measuring average pixel intensities of these regions, then taking the mean of all five measurements. **I.** The ratios of TH-positive versus TH-negative NS3 levels, as described above, are plotted for wild-type and *ns3*^{G0431} DA neurons (*N* = 10 DA neurons of each genotype). The levels of NS3 were not changed in DA neurons of *ns3*^{G0431} mutants versus wild-type. **J.** The levels of NS3 in wild-type and *ns3*^{G0431} mutant DA neurons not normalized to surrounding regions. These values served as the normalizing denominator for the data plotted in I. NS3 levels in *ns3*^{G0431} mutants were lower compared to wild-type. However, this trend is not DA neuron-specific and instead represents a general decrease in NS3 levels throughout the CNS. When the DA levels of NS3 were normalized to surrounding regions -- shown in panel I -- there was no decrease in DA neuron NS3 of *ns3*^{G0431} mutants. Thus, NS3 levels throughout the CNS were uniformly decreased throughout the CNS of *ns3*^{G0431} mutants relative to wild-type.