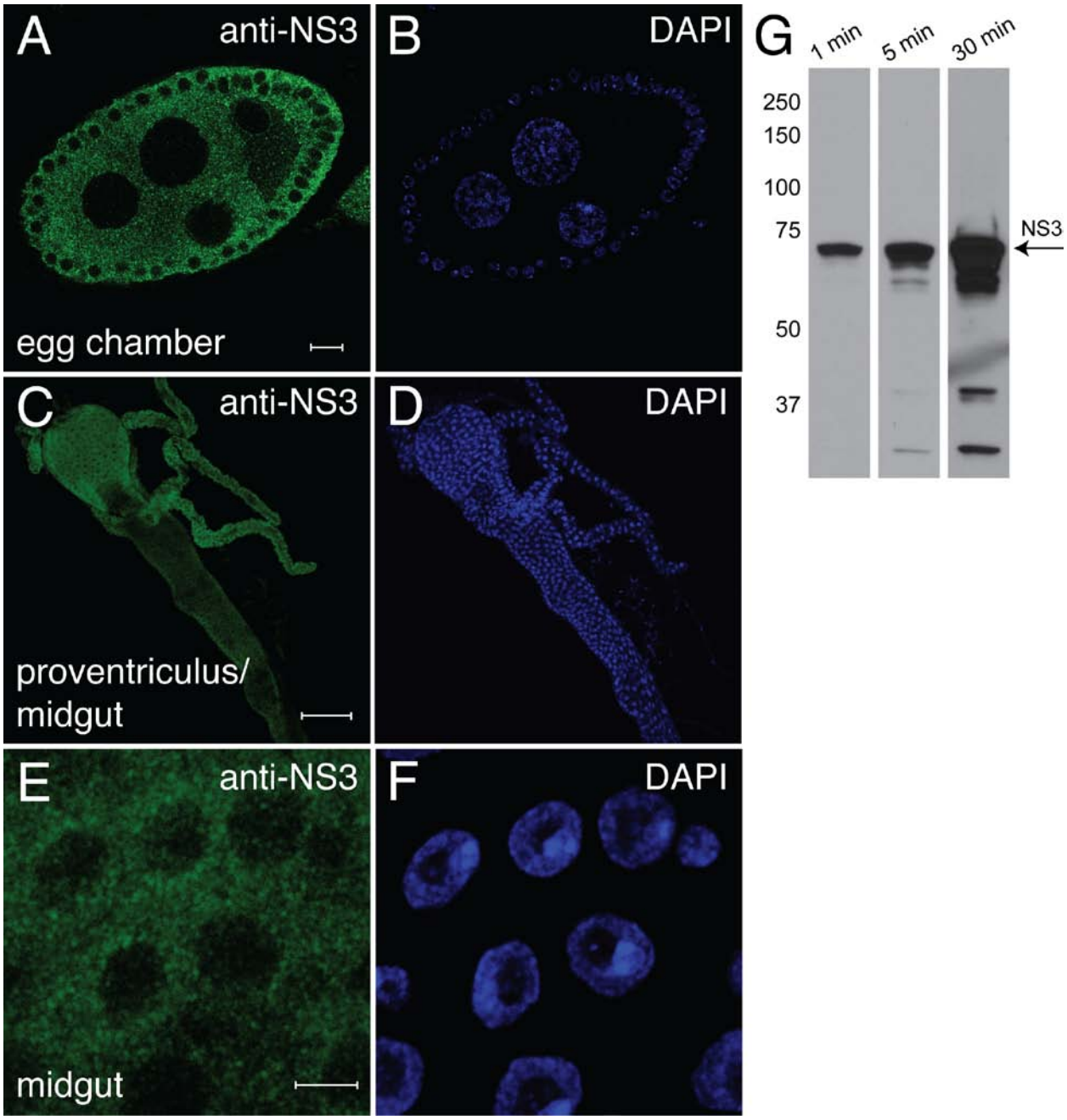

Figure S1 NS3 localization in the ovary/egg chamber, proventriculus, and midgut. A, B. NS3 staining in an egg chamber (stage 8) from a wild-type female demonstrating cytoplasmic enrichment and localization to the periphery of the germinal vesicle. Image captured with a 40X objective at 2X optical zoom. Scale bar in A represents 10µm. **C, D**. NS3 staining in the proventriculus and midgut of a second-instar wild-type larva. NS3 protein is more abundant in the proventriculus than the midgut, yet cytoplasmic in both tissues. Image captured with a 20X objective; the scale bar in E indicates 100µm. **E, F.** NS3 protein in the midgut illustrating cytoplasmic localization and perinuclear enrichment. Image captured with a 63X objective and 8X optical zoom. Scale bar in E indicates 5µm. **G.** Western analysis of embryonic protein extract with the NS3 antibody. The 1 min film exposure reveals that the antibody primarily recognizes NS3 (70kDa) and other minor epitopes become apparent upon longer exposures (5 and 30 min).

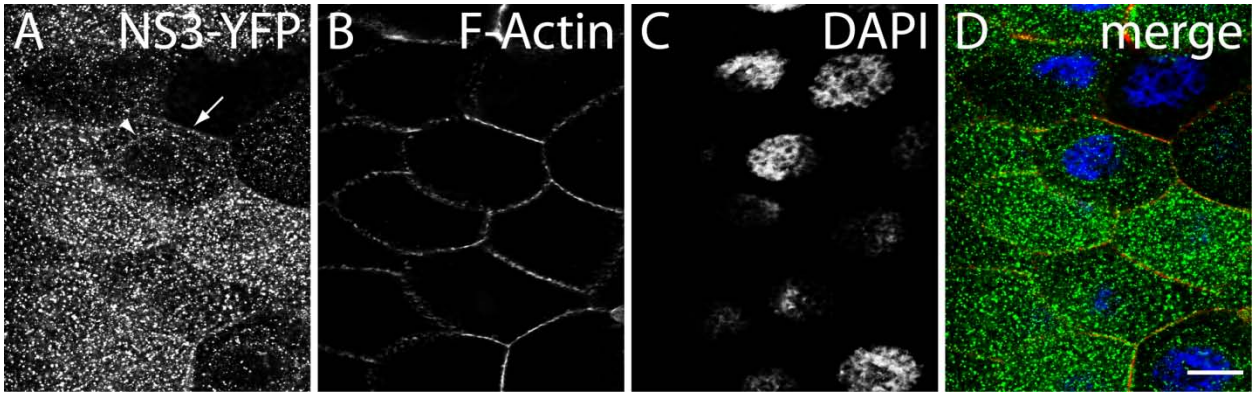

Figure S2 NS3-YFP localizes to cytoplasmic puncta and is enriched proximal to the nuclear envelope and cellular membrane of salivary glands. A-D. NS3-YFP was expressed in salivary glands via *c061-gal4,* a driver that is active at variable levels in cells of the salivary glands. (**A**) NS3-YFP exists within cytoplasmic puncta and is enriched at the nuclear periphery (arrow head) and proximal to the cellular membrane (arrow). (**B**) F-Actin was detected via AlexaFluor conjugated phalloidin to visualize cell exteriors. (**C**) Dapi staining to visualize DNA within the nucleus. (**D**) Merged image of NS3-YFP (green), F-Actin (red), and Dapi (blue) staining. Note the co-localization of NS3-YFP and F-Actin at the cell membrane. Scale bar = 50 µm.

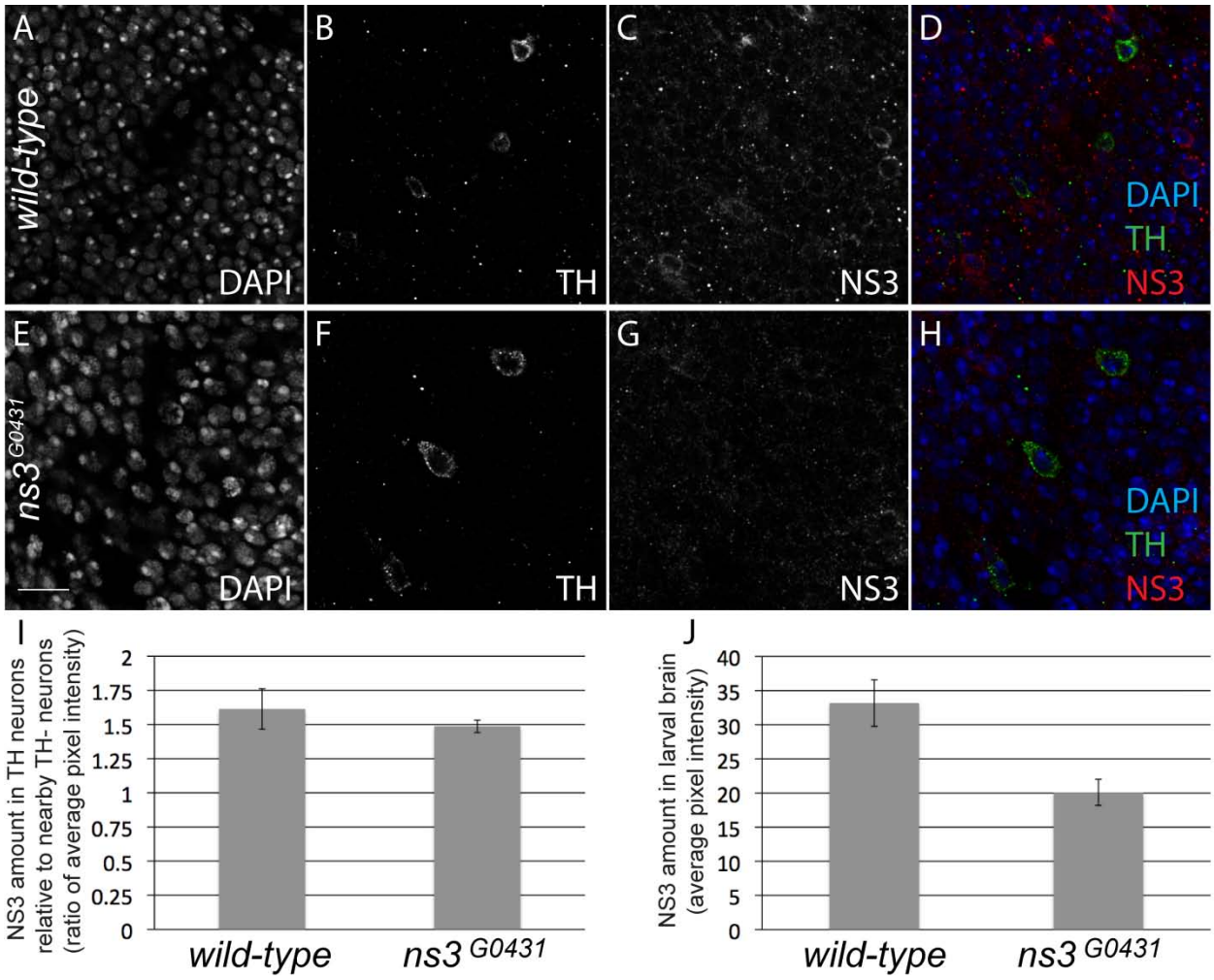

Figure S3 NS3 levels in wild-type and *ns3*^{G0431} mutant larval DA neurons. A-H. The CNS was dissected from wild-type and *ns3*^{G0431} first-instar larvae and stained with anti-tyrosine hydroxylase (TH) and anti-NS3 antibodies. DA neurons were identified by positive staining for TH. Levels of DA neuron 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.