



Figure S5: *Wolbachia* distribution in somatic tissues in N7NO:wNo flies (+W). Negative controls that do not contain *Wolbachia* (-W) are included for each tissue (A, C, E, G, I, K). Flies that contain *Wolbachia* are shown in (B, D, F, H, J, L), punctate red staining indicates *Wolbachia* presence in tissues. Diffuse red staining of increased intensity compared to that observed in the negative control images indicates *Wolbachia* presence in the tissue above or below the plane of focus. Nucleic acid is stained blue using DAPI. Images were processed by fluorescent *in situ* hybridization, using two rhodamine-labeled probes to target conserved regions of the *Wolbachia* 16S rRNA gene. Images shown are representative of at least five biological replicates, except for panel J where 40% of individuals contained *Wolbachia* infected tissues, and H where 20% of individuals contained punctuate staining indicative of *Wolbachia*. Here, images are representative of the tissues where *Wolbachia* was not detected. Note that no *Wolbachia* was detected in either the fat body or muscle sections of any N7NO-wNo individual, as shown in B and D. Each paired tissue set (ie, A and B; C and D; E and F etc) were taken under the same magnification and exposure. Due to the size difference in organs of interest, magnification differs across tissues. Auto-fluorescence varies with respect to tissue type, so exposure varies between, but not within, tissue sets. All images have been cropped and resized but otherwise remain unaltered. Malpighian tubules (Malp. tubules) and thoracic ganglia (Thoracic g.) have been abbreviated in the panel labels.