



Figure S3: *Wolbachia* distribution in somatic tissues in DSR:wRi flies (+W). *Wolbachia*-free negative controls (-W) are included for each tissue (A, C, E, G, I, K, M). Flies that contain *Wolbachia* are shown in (B, D, F, H, J, L, N), 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 D, where 20% of individuals contained *Wolbachia* within the fat body. Here, panel D contains an image representative of the fat body of DSR-wRi flies where *Wolbachia* was not detected. Each paired tissue set (ie, A and B; C and D; E and F etc) were taken under the same magnification and exposure. 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.