

Supplementary Material

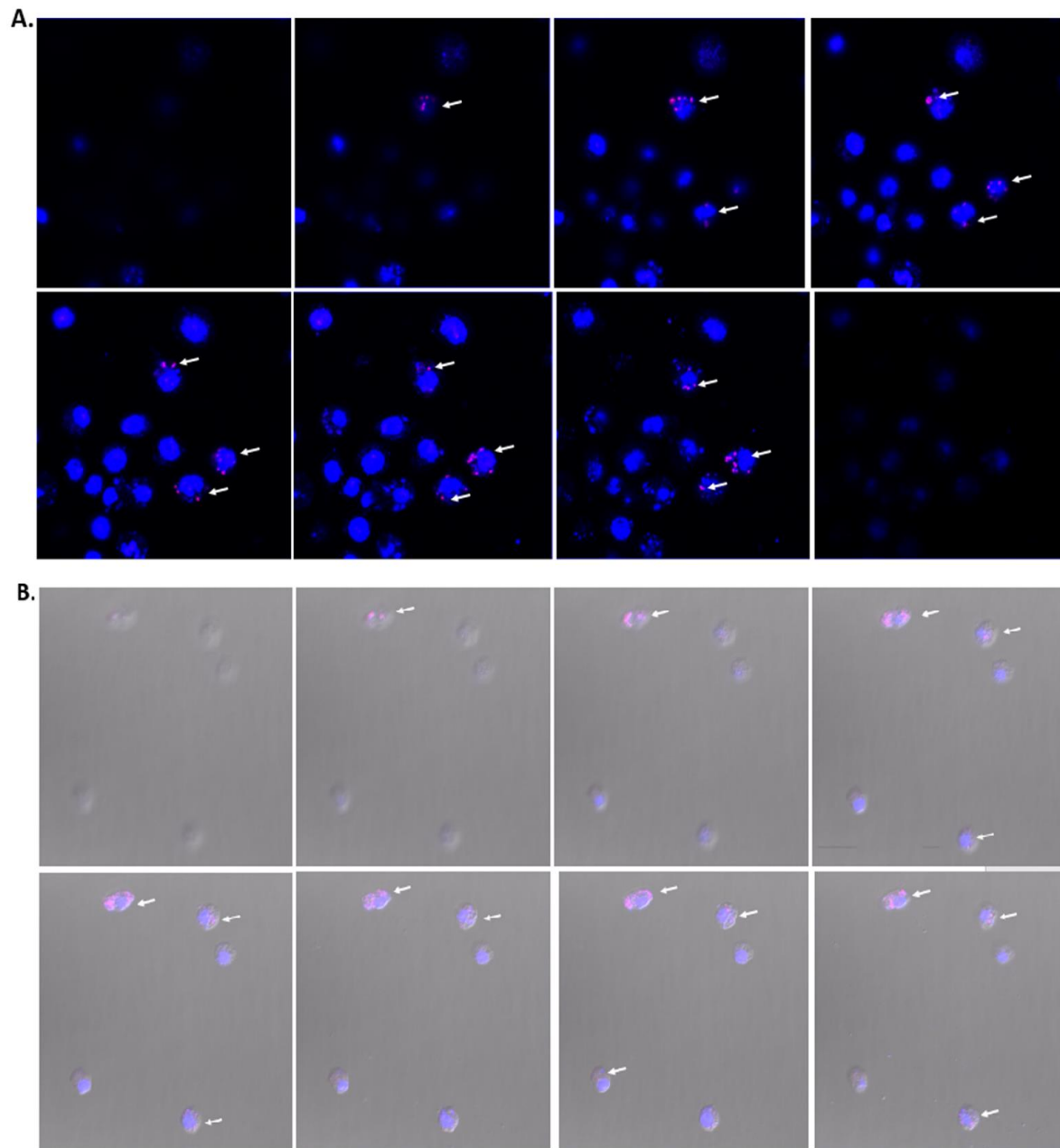


Figure S1. Representative Z-stack of *Drosophila* S2 cells after infection with *Wolbachia* in a transwell chamber. Uninfected *Drosophila* S2 cells were seeded beneath *Wolbachia*-infected JW18 cells in a transwell insert. After co-culture for 1 day, new *Wolbachia* infections in previously uninfected S2 cells were visualized by **(A)** FISH only or **(B)** FISH with DIC using a Leica

SP5 confocal microscope. Sections were taken at 1.5 μm increments. Red=*Wolbachia* (arrows), blue= nuclei stained with DAPI.

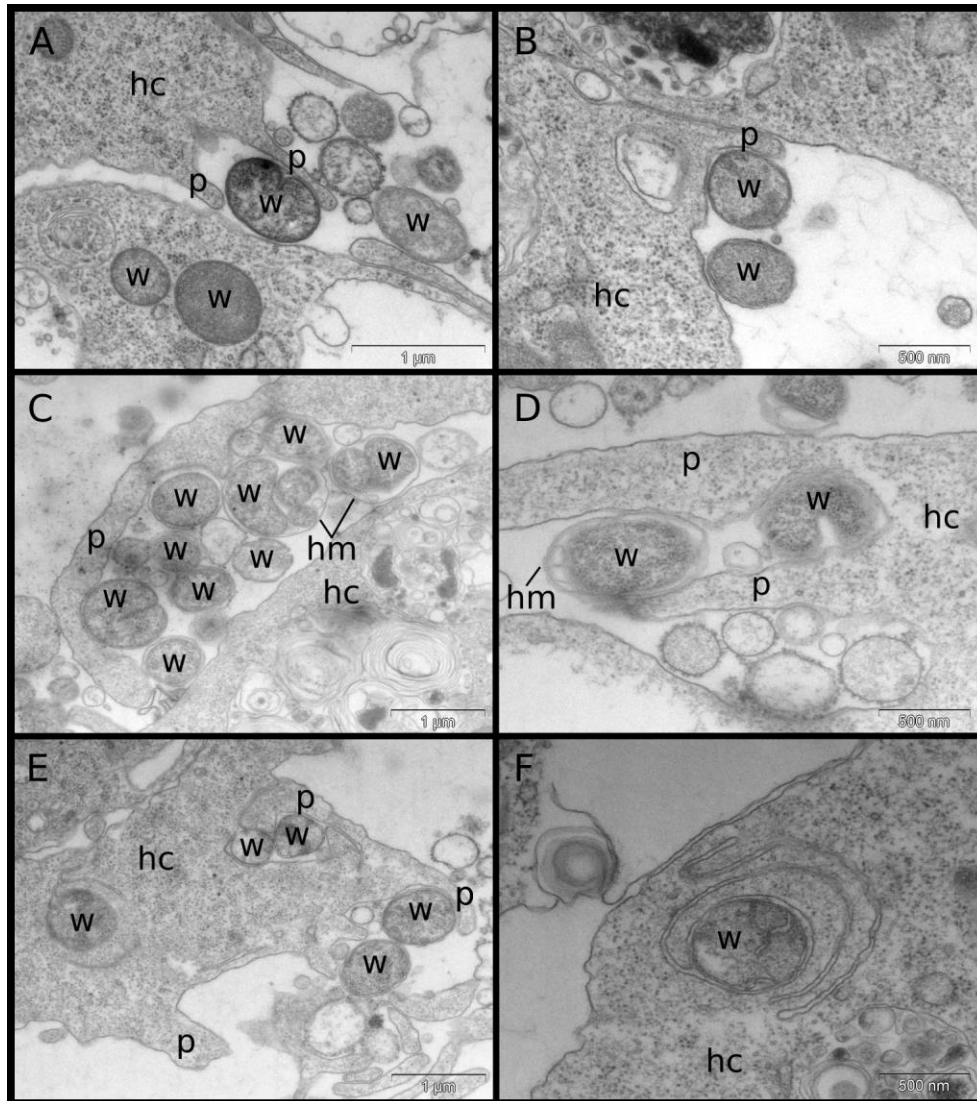


Figure S2. Transmission electron micrographs of LDW/JW18 cells exposed to *Wolbachia* from cell lysates or infected JW18 cells showing putative uptake events. A-E) Pseudopodia-like extensions of the host cell surround *Wolbachia* free in the culture media. E-F) Membrane structure suggests recent internalization for some *Wolbachia* cells. Arrows, host-derived membrane; hc, host cell; hm, host membrane; p, pseudopodia; w, *Wolbachia*.

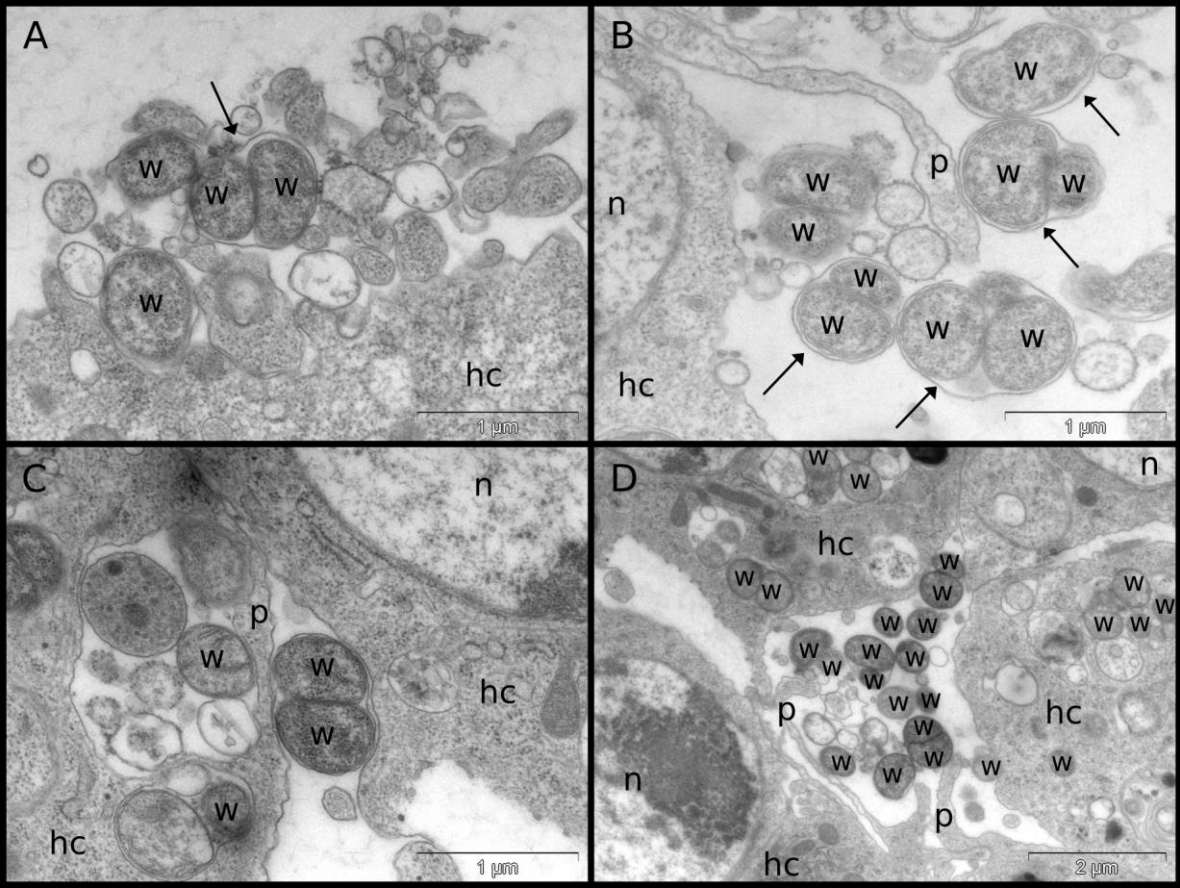


Figure S3. Transmission electron micrographs of LDW/JW18 cells exposed to *Wolbachia* from cell lysates or infected JW18 cells showing the different forms of extracellular *Wolbachia*. A, B) *Wolbachia* occurred free in the culture medium and contained within a host-derived membrane (arrows). A-D) Many *Wolbachia* appear to have recently divided, occurring as paired daughter cells. Arrows, host-derived membrane; hc, host cell; hm, host membrane; n, nucleus; p, pseudopodia; w, *Wolbachia*.

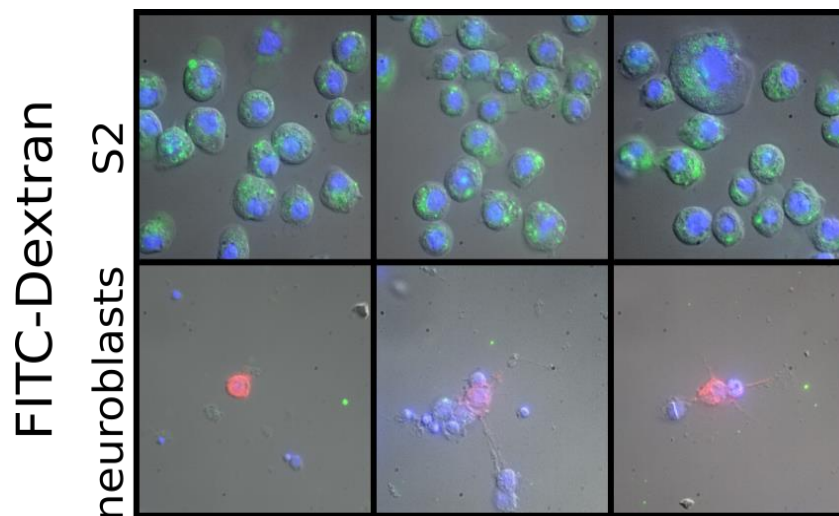


Figure S4. S2 cells uptake FITC-labelled 40K MW dextran and neuroblasts do not. Merged DIC and fluorescent channels. FITC dextran, green. DAPI, blue. CD-ChRFP under GAL4 expression in neuroblasts, red.

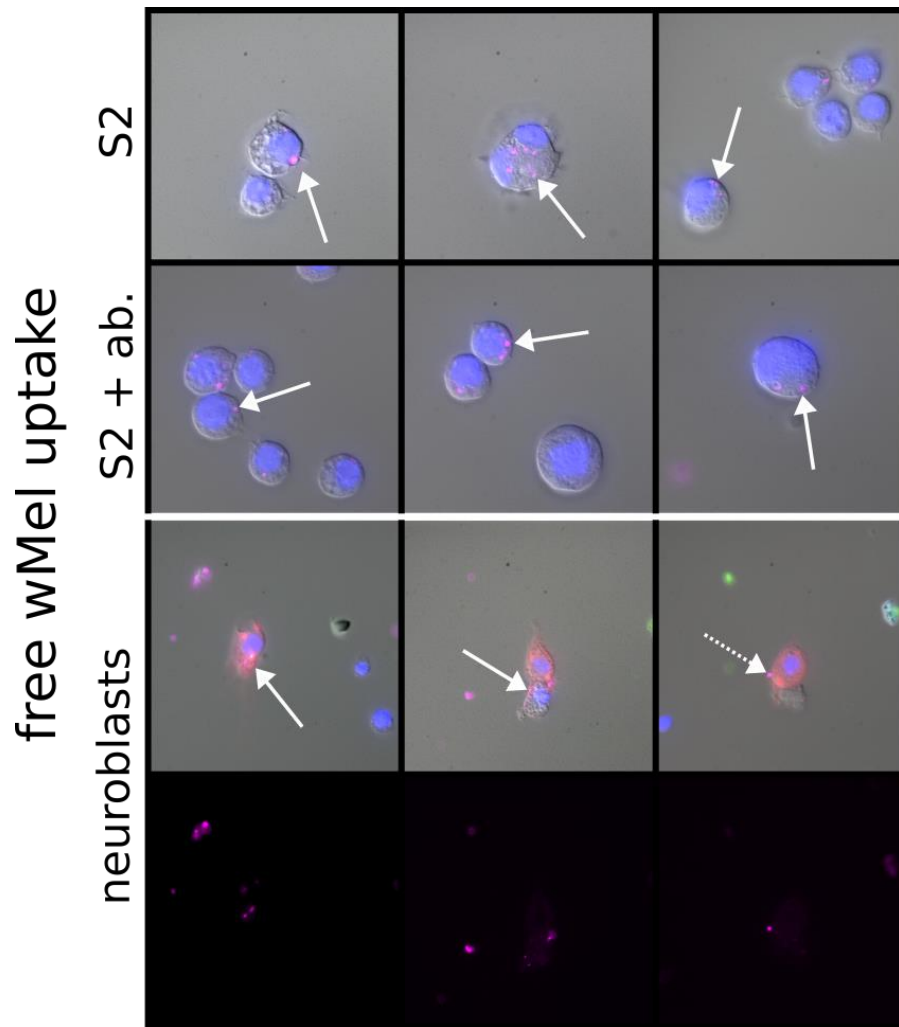


Figure S5. S2 cells maintained with and without pen-strep antibiotics readily uptake Wolbachia in 1 day, whereas neuroblasts do so at a low level. DAPI, blue. wMel W2 16S rRNA probe, magenta. CD-ChRFP under GAL4 expression in neuroblasts, red. Cell debris from extracting wMel from JW18 cells expressing Jupiter-GFP, green. Solid arrows indicate intracellular Wolbachia. Dashed arrow indicates extracellular Wolbachia. Bottom two rows are merged DIC and fluorescence channels and the far-red channel (magenta) showing the wMel probe signal, respectively.