Figure S1. Latrunculin A treatment disrupts axonal branching in HSN, **Related to Figure 2** (A) Myristolated GFP highlights the morphology of HSNL neuron and yellow arrowheads point to collateral axonal branches that extend from the synaptic region in untreated animals. (B) DMSO treated animals does not affect HSN branch formation. (C) In animals injected with Latrunculin A, there is an increase in number of animals that fail to form any branches. Scale bars represent 10 μm. (D) Graph quantifies the percentage of animals that elaborate zero, one or two branches. Statistics for each mutant was compared against the wildtype values (*$p<0.05$ with $n>35$, Fisher's exact test).

Figure S2. General F-actin levels along the entire HSN neuron are unaffected in *wve-1* an *gex-3* mutants, Related to Figure 3 (A) GFP::moesinABD labels the entire HSN neuron with no significant enrichment at presynaptic sites as compared to cytoplasmic mCherry. The fluorescence of GFP::moesin is unaffected in (B) *wve-1* (C) or *gex-3* mutants when compared to cytoplasmic mCherry. Scale bars represent 10 μm.

Figure S3. Rac GTPases function redundantly to assemble an Arp2/3 mediated actin network at synapses, Related to Figure 3 (A–D) Loss of each individual racs does not have strong effects on the recruitment of F-actin to synapses as labeled by GFP::utCH when compared to wildtype. However, double mutants (E) *mig-2;rac-2* and (F) *ced-10;rac-2* show a strong loss of F-actin from the HSN synaptic region. Scale bars represent 10 μm. (G) GFP::utCH fluorescence is quantified in the graph where each bar represents the average fluorescence value and error bars are ± S.E.M. (*$p<0.05$ and ***$p<0.001$ with $n>20$, Two-tailed Student's t-test).

Figure S4. SYG-1 localization and HSN axon outgrowth is unaffected in *wve-1* or *gex-3* mutants, Related to Figure 4 (A) GFP::utCH labels synaptic F- actin network which localizes to the HSN synaptic region with SYG-1::mCherry in the L4 stage. In both (B) *wve-1* and (C) *gex-3* mutants, F-actin recruitment is reduced but SYG-1::mCherry remains unaffected and localizes to the synaptic region. Scale bars represent 10 μm. (D) In a L3 worm, GFP::utCH labels the anterior stretch of the outgrowing axon all the way into the axonal tip. White dashed box highlights the developing synaptic region around the vulva which is also labeled by GFP::utCH. (E) In *wve-1* mutants,

GFP::utCH still labels the outgrowing axon tip, but fails to be recruited to the synaptic region. Scale bars represent 10 μm.

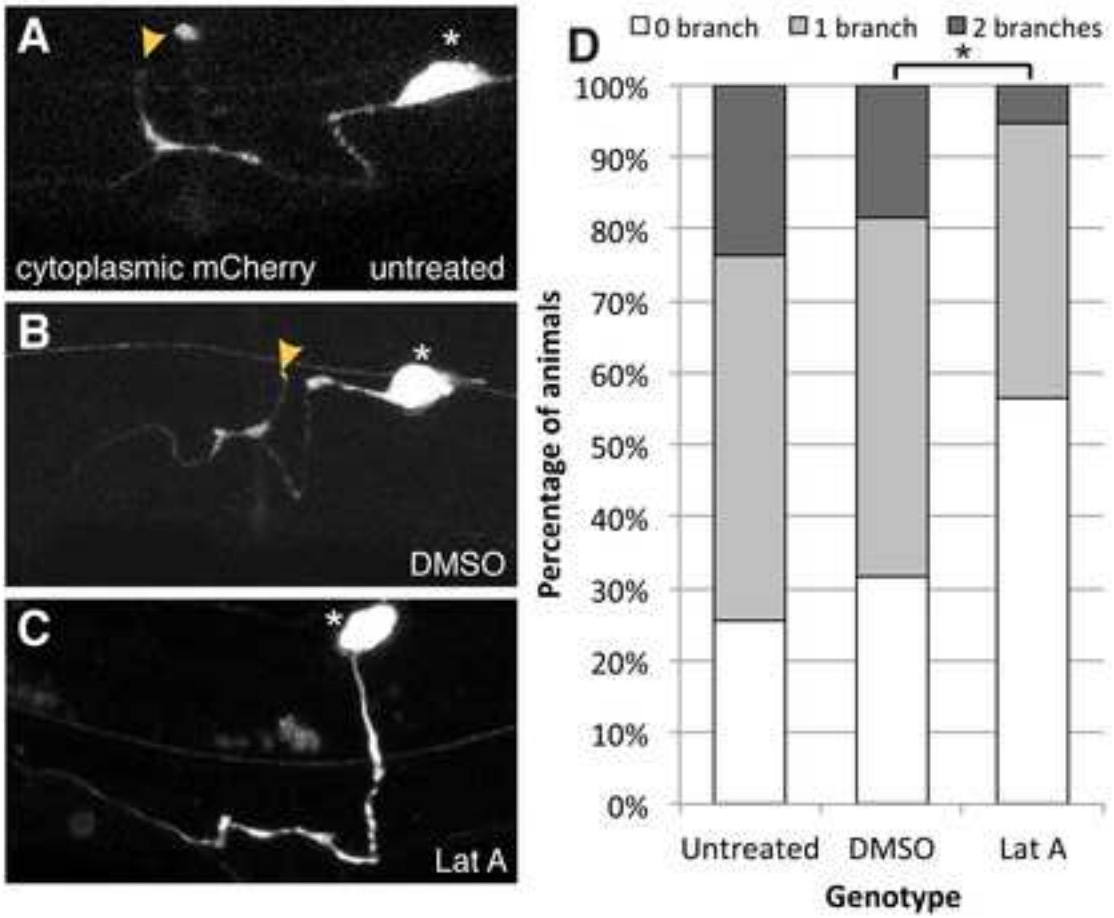
Figure S5. wve-1/WAVE complex is required for presynapse assembly and axonal branch formation in VC4/5 neurons, Related to Figure 4 (A-D) Ventral views (A) of the morphology and axonal branches of VC4 and VC5 neurons in wild type (B), *wve-1* (C) and *gex-3* (D) animals. Most *wve-1* and *gex-3* mutants show reduced number of axonal branches. Arrowheads point to axonal branches. * indicates the cell body. Arrow points to vulval slit. Scale bar represent 10 □m. (E-H) Lateral view (E) of the morphology and axonal branches of VC4 and VC5 neurons in wild type (F), *wve-1* (G) and *gex-3* (H) animals. Anterior (A), Posterior (P), Left (L), Right (R), Dorsal (D), Ventral (V). (I-K) Synapses of VC4 and VC5 neurons are labeled by synaptobrevin(SNB-1)::GFP in wild type (I), *wve-1* (J) and *gex-3* (K) animals. *wve-1* and *gex-3* mutants show significant loss of SNB-1::GFP fluorescence. Dashed boxes indicate the synaptic regions. (L) Quantification of the relative fluorescence of SNB-1::GFP in wild type, *wve-1* and *gex-3* animals. Error bars are +/- S.E.M. (*** $p<0.001$, $n>20$, Two-tail Student's t-test). (M) Quantification of the percentage of animals with zero, one or two branches on each side. (*** $p<0.001$, $n>80$, Fisher's exact test). *wve-1* and *gex-3* mutants have less SNB-1::GFP fluorescence signal and reduced number of axon branches as compared to wild type animals.

Supplementary Figure 6

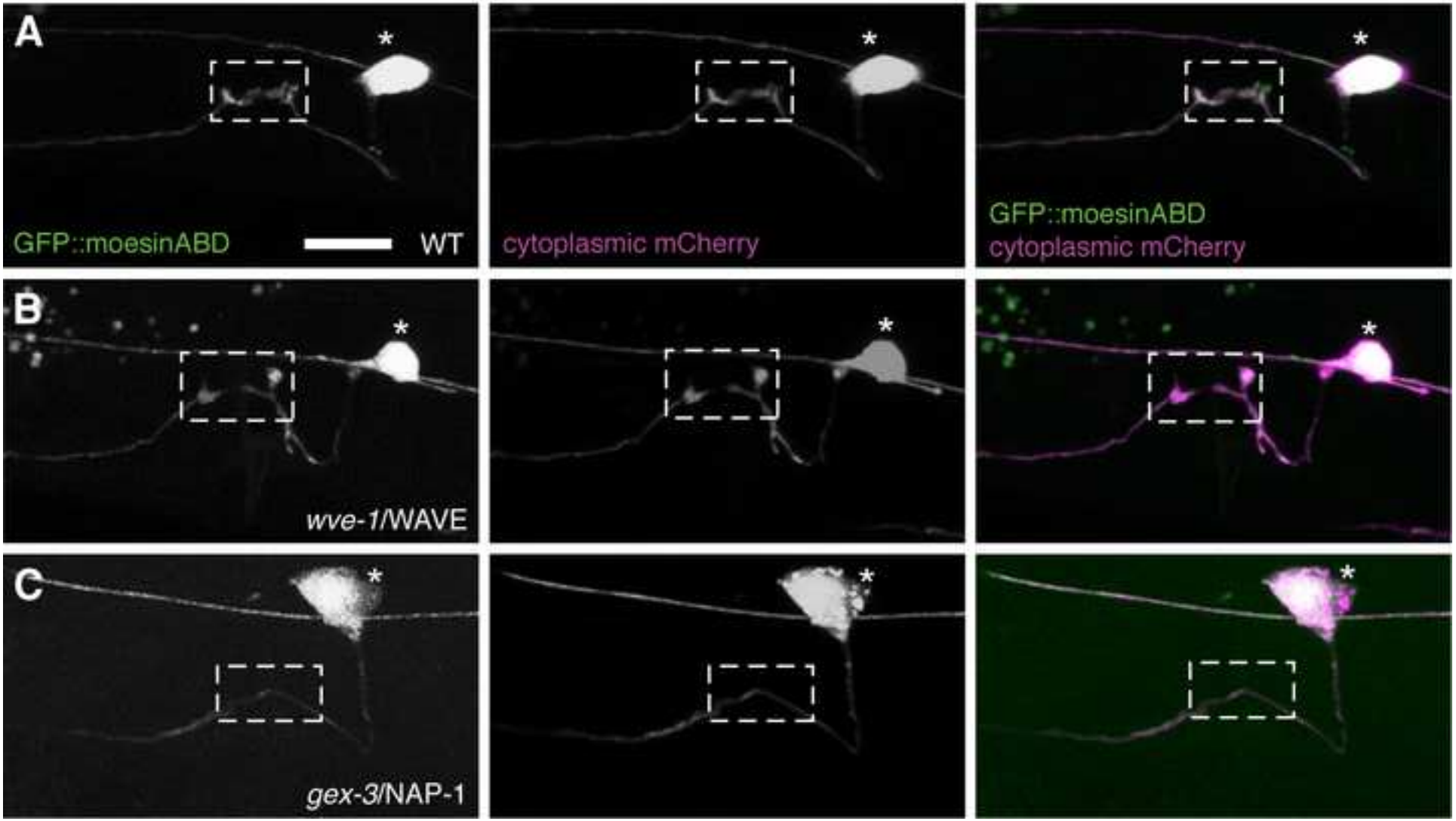
Figure S6. *Drosophila* WRC binds to the WIRS motif in SYG-1, Related to Figure 5 and Figure 6 (A) Pull-down using immobilized di-MBP-tagged *Drosophila* WRC complex as bait (wild type 2MBP-dWRC, or containing R106A/G110W mutations in the Abi2 subunit (AW), which impair binding to WIPS motifs). This is an SDS-PAGE gel stained by coomassie blue with the eluted proteins. GST-tagged *C. elegans* SYG-1 cytoplasmic tail (GST-ceSYG-1 CT) is pulled down by MBP-dWRC (band is highlighted by black arrow). Making the AW mutation in hWRC interface that interferes with WIRS binding decreases this binding. Mutating the WIRS sequence in GST-ceSYG-1 CT (2 Ala) also decreases the ability for dWRC to pull down GST-ceSYG-1 CT. Competitors

were chemically synthesized 15 amino acid WIRS peptides (WT for wildtype and 2A for the mutant peptide) and only the wildtype peptide was able to compete for binding in the pull-down. *In vivo* expression of mutated SYG-1 constructs are properly recruited and localized to the HSN synaptic region. (B–D) Localization of SYG-1Δcyto and SYG-1(2A) to the synaptic region resemble full-length SYG-1.

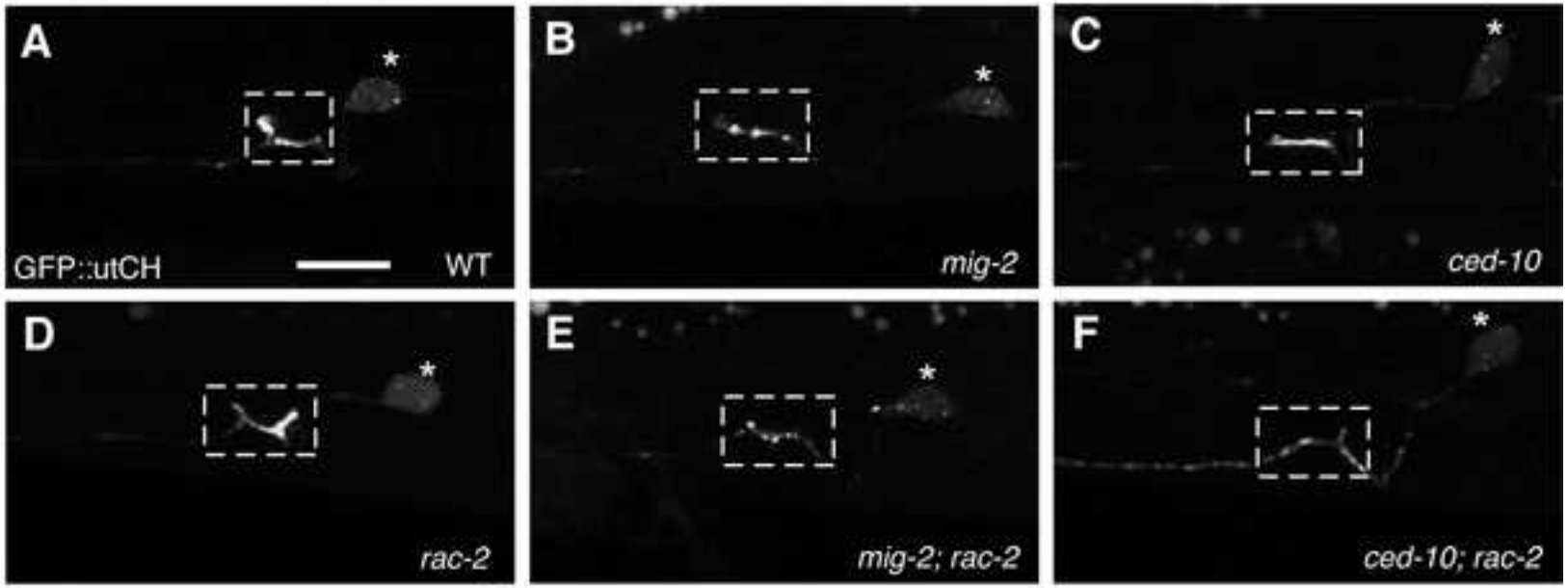
Supplementary Figure 1, related to Figure 2

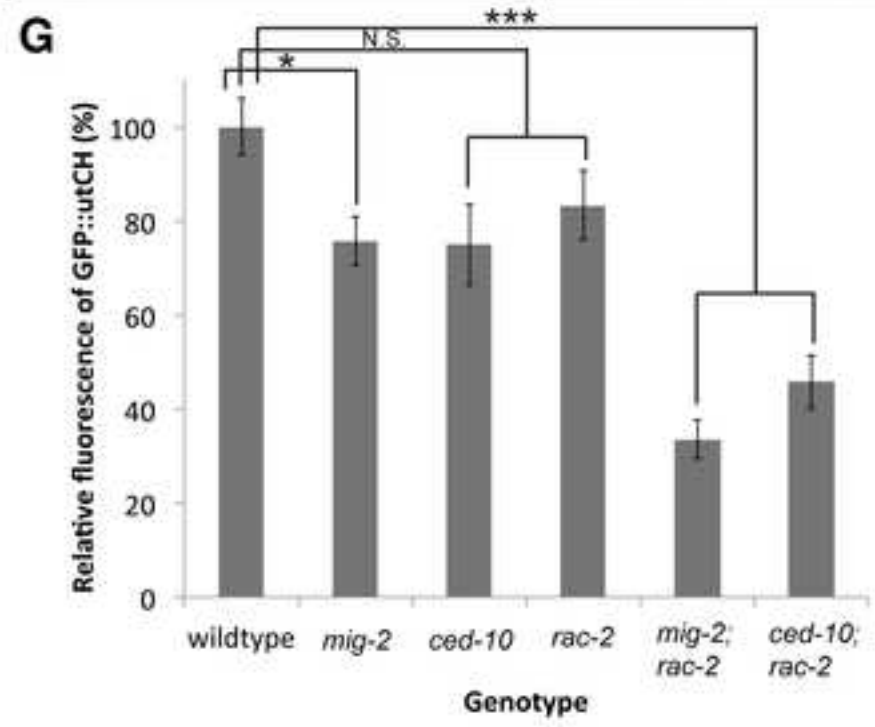


Supplementary Figure 2

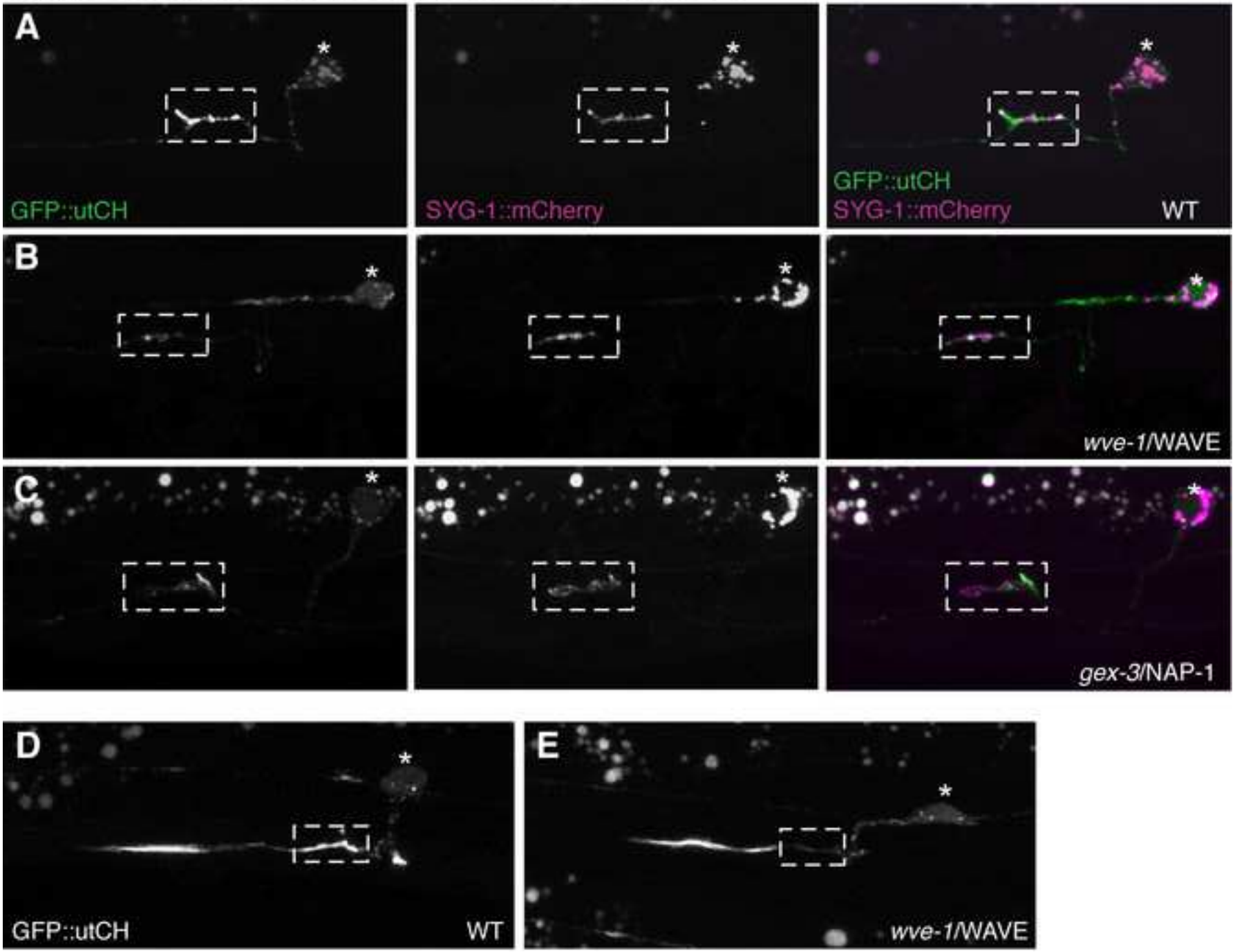


Supplementary Figure 3



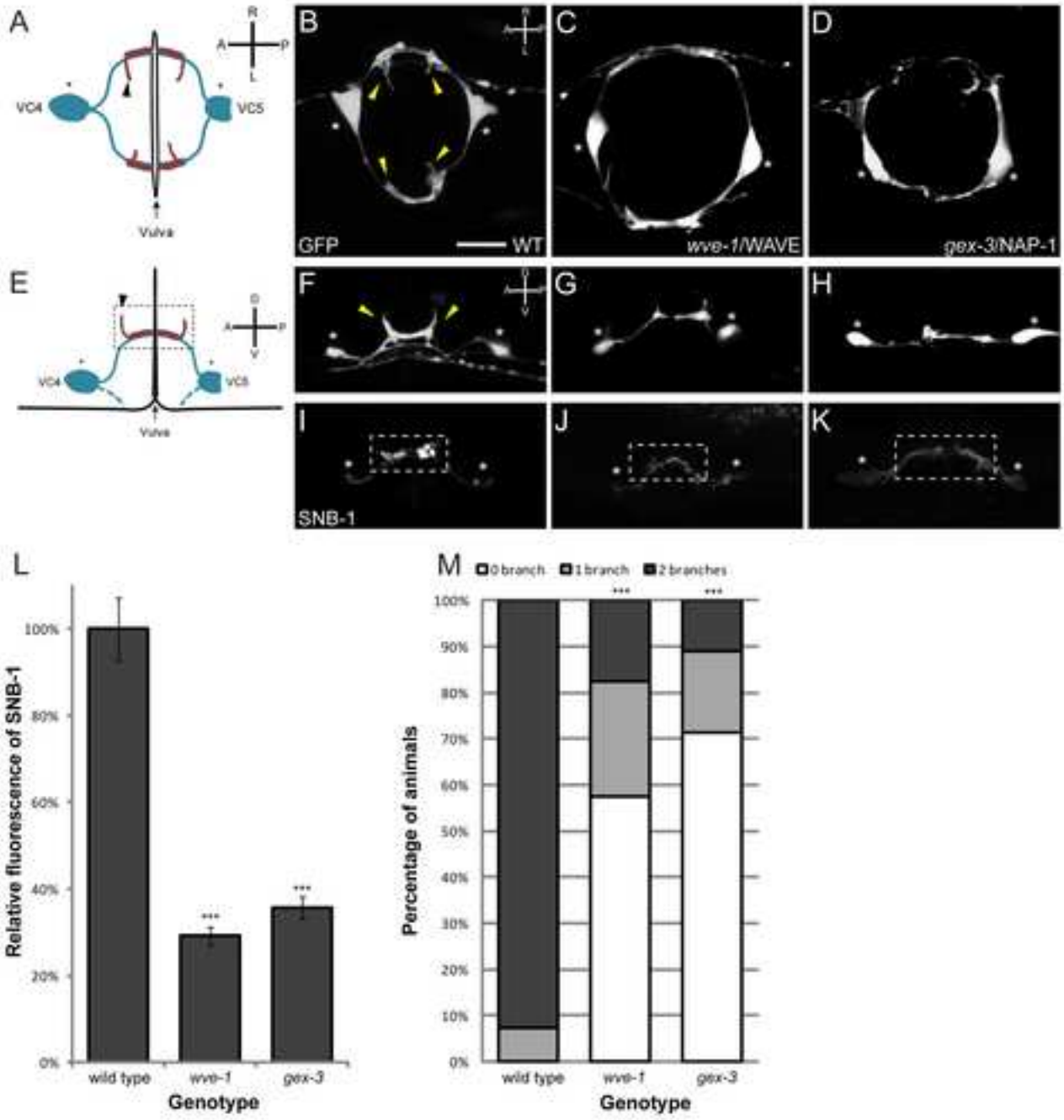


Supplementary Figure 4, related to Figure 4



Supplemental Figure

Supplementary Figure 5, related to Figure 4



Supplementary Figure 6, related to Figure 5 and Figure 6