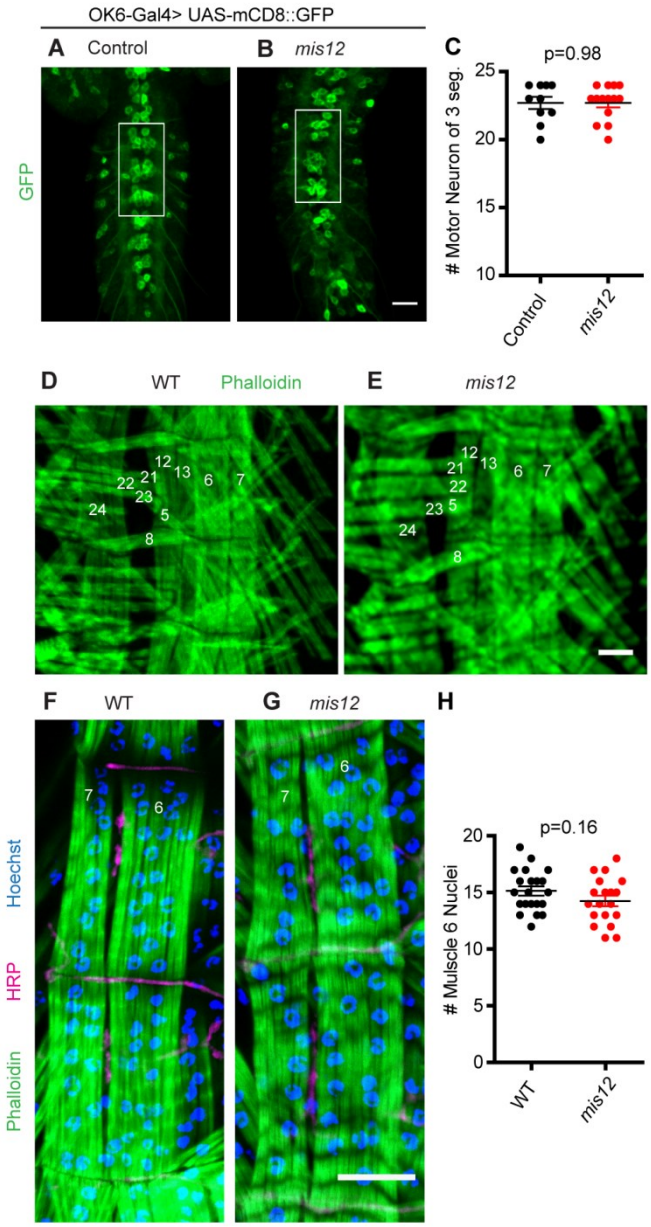

Figure S1. Muscles and motor neurons differentiate normally in *mis12* mutants, related to Figure 1

(A-C) Motor neurons were labeled and counted by using *OK6-GAL4* to drive *UAS-mCD8::GFP* in either *mis12*^{f03756}*/+* (control, **A**) or *mis12*^{f03756}*/mis12*^{f03756} (**B**) embryos. The number of neurons in the three indicated segments were counted (**C**). It was not always possible to discern all 8 motor neurons in each segment and therefore some embryos were scored at fewer than the predicted 24 motor neurons; the counts were nonetheless equivalent

between the genotypes. (D,E) Muscles were stained with rhodamine-phalloidin (green). In each hemisegment of 21-h AEL embryos of *mis12*^{f03756} homozygotes, the normal pattern of muscle fibers was present. Muscles are numbered according the nomenclature of Crossley (1978) to facilitate the comparison. **(F, G)** Rhodamine-phalloidin stained muscles 6 and 7 in two adjacent segments of control and *mis12*^{f03756} homozygous embryos. Nuclei were stained with Hoechst dye (blue) and innervating axons with anti-HRP (Magenta). **(H)** Quantification of nuclei in muscle 6; unpaired t test with Welch's correction. Scale bar 20µm.

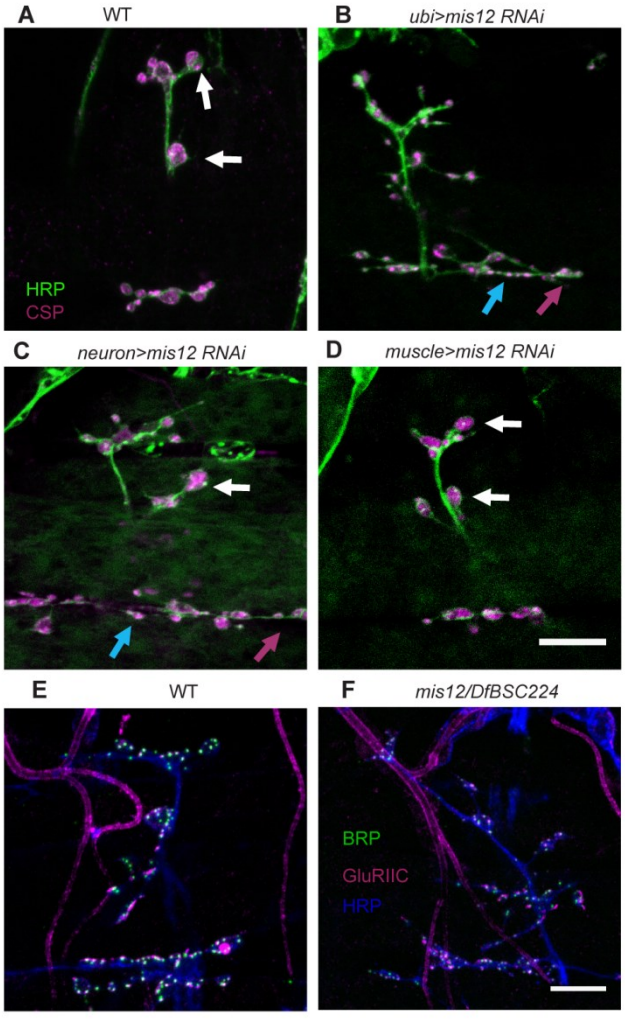

Figure S2. Knockdown of *mis12* ubiquitously or selectively in neurons causes NMJ growth defects and further characterization of *mis12* synapses, related to Figure 1

(A-D) Embryonic neuromuscular junctions on muscles 6, 7, 12, and 13 stained with anti-HRP

and anti-CSP. RNAi against *mis12* expressed either ubiquitously (**B**) or in neurons (**C**), but not in muscle (**D**), gave rise to overly long neurites (magenta arrows) with many small clusters of vesicles (blue arrows) in place of the normal large boutons (white arrows) as quantified in Figure 1. **(E, F)** Immunolocalization of the presynaptic active zone marker Brp (green) and postsynaptic receptor GluRIIC (magenta), co-stained with anti-HRP (blue) as a marker of neuronal membranes. Neuromuscular junctions on muscles 6, 7, 12, and 13 are shown for wild type (**A**) and *mis12*^{f03756}*/DfBSC224* embryos (**B**). Scale bar, 10 µm.

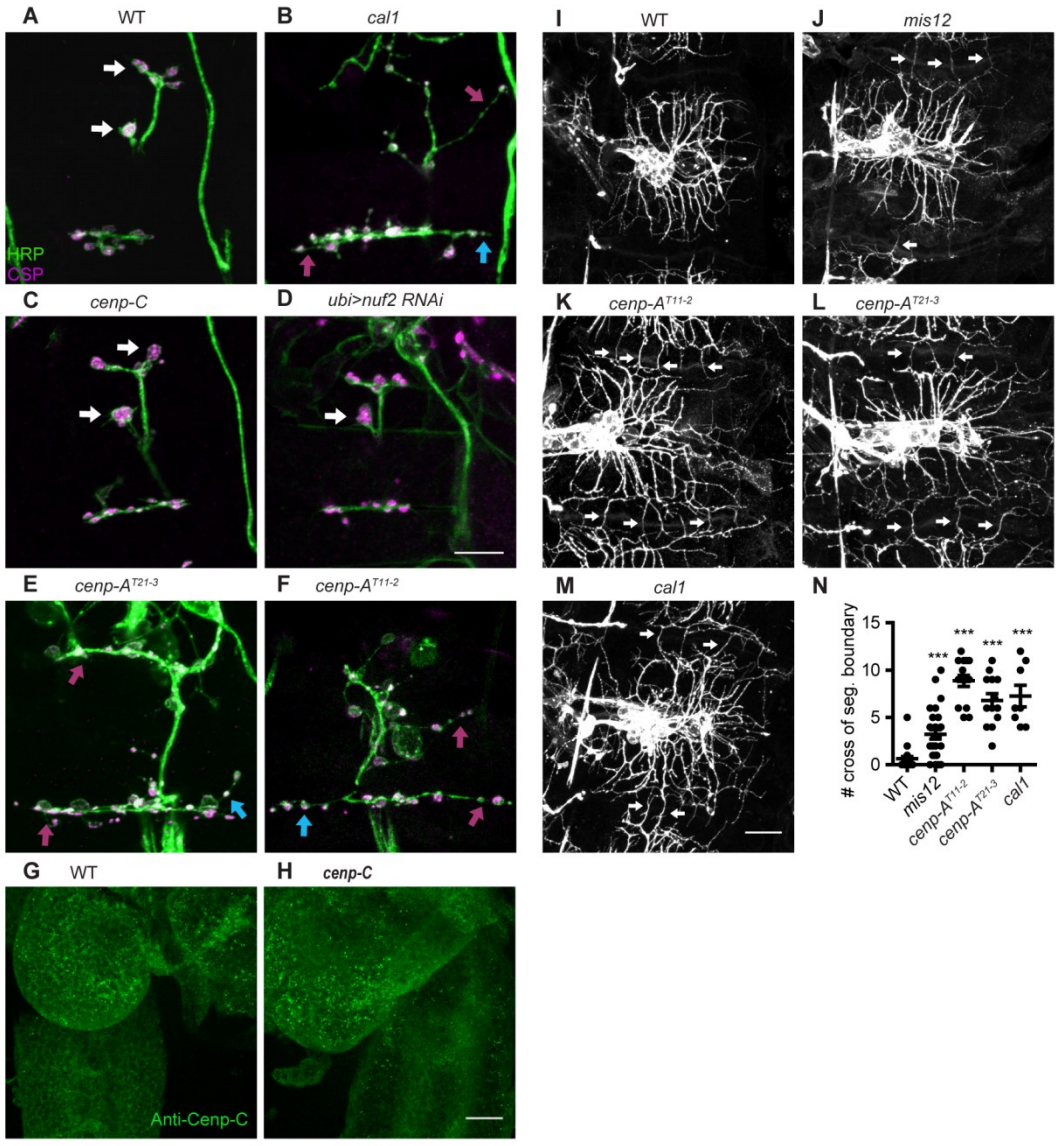

Figure S3. Kinetochore phenotypes at the NMJ and in sensory neuron dendrites, related to

Figure 2

(A-F) anti-HRP and CSP staining of junctions on muscles 6,7,12, and 13, as in Figure 2, for Wild-type (**A**), *cal1*^{c03646} (**B**) , *cenp-C*^{prl41} (**C**), and ubiquitous RNAi knockdown of *nuf2* (**D**), and *Cenp-A* (*cid*$^{T21-3}$ and *cid*$^{T11-2}$) (**E**,**F**). White arrows mark normal boutons. *Cenp-A* and cal1 mutant embryos had overly long neurites (magenta arrows) and many small boutons (blue arrows), but these phenotypes were not present in *Cenp-C* mutants or the *nuf2* knockdown, genotypes that also retained the large boutons that characterize wildtype synapses. Quantification appears in Figure 2. **(G,H)** Cenp-C staining of embryonic brain indicates that extensive maternally contributed Cenp-C protein perdures into late stages of embryonic development (21 h AEL) in *cenp-C*^{prl41} and likely offers an explanation for the lack of a synaptic phenotype in that genotype. **(i-M)** anti-HRP staining of dorsal sensory neuron clusters for Wild-type (**I**), *mis12*^{f03756} (**J**), *Cenp-A* (*cid*$^{11-2}$) (**K**), *cenp-A* (*cid*$^{T21-3}$) (**L**) and *cal1*^{c03646} (**M**). White arrows indicate dendrites that cross the segment boundary. (**N**) Quantification of the number of dendrites that crossed the segment boundary in the indicated genotypes. Each genotype was compared with WT using one-way ANOVA with Dunnett's multiple comparisons test. ***p<0.001. Error bars, mean ± s.e.m. Scale bar, 10 μm (A-F and I-M) and 20 um (G, H).

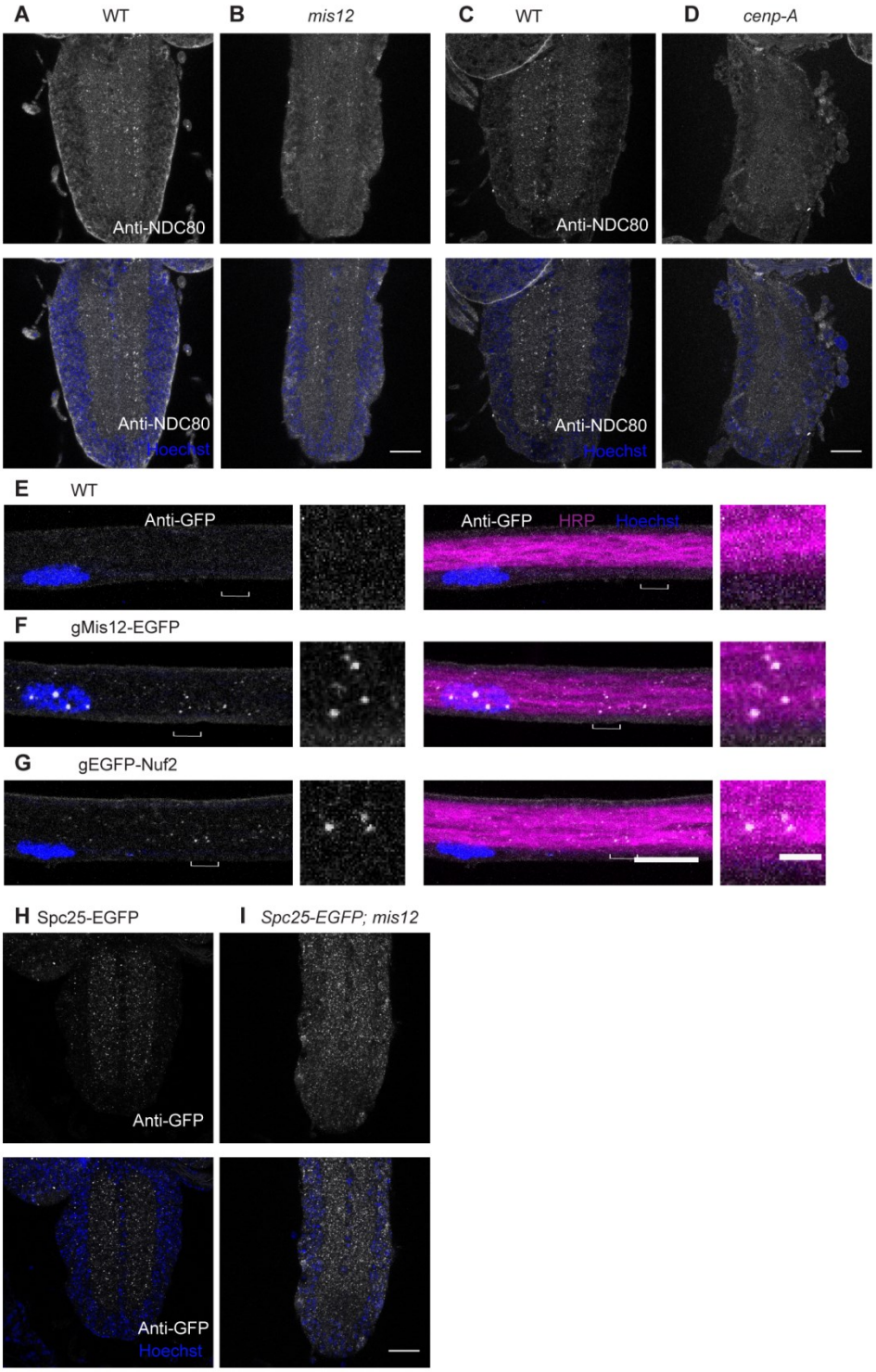

Figure S4. Kinetochore protein localization in VNC and axons, related to Figure 3

(A-D) Ventral nerve cords stained with anti-Ndc80 (white) and Hoechst dye (blue). In wildtype embryos Ndc80 puncta are seen in the synaptic neuropil. This signal is reduced in

embryos mutant for *mis12* **(B)** or *cenp-A/cid* **(D)**. Scale bar, 20 µm. **(E-G)** In axons of third instar larvae, Mis12-EGFP **(F)** and EGFP-Nuf2 **(G)** formed sparse puncta (white) in axonal cytoplasm. The Mis12 signal was not as bright as those present, and presumably centromeric, in nearby Hoechst-stained (blue) glial nuclei. Axons are labelled with anti-HRP (magenta). Right panels show enlargements of the axonal regions marked by brackets. Scale bar, 10 µm, 2 µm in right-hand panels. **(H,I)** EGFP tagged Spc25was expressed under control of its endogenous promoter. In the neuropil of the embryonic ventral nerve cord Spc25-EGFP formed bright puncta of GFP immunoreactivity (white) in a region devoid of nuclei (Hoechst dye, blue). In a *mis12* mutant background, the GFP-tagged spc25 was more abundant and broadly distributed, including the retention of spc25 in the cell bodies. Scale bar, 20 µm.