Figure Legends

Figure 1. Model showing *C. elegans* UNC-45 structure domains and interacting proteins. The large oval encloses the solved crystal structure of *C. elegans* UNC-45 (PDBID 4I2Z). The UNC-45 UCS domain binds to striated muscle myosin S1 (chicken skeletal muscle myosin, PDBID 2MYS with light chains excluded). The TPR domain binds to Hsp90 (*S. cerevisiae* Hsp90 dimer in the ATP bound closed conformation, PDBID 2CG9). UNC-45 also binds to Hsp70 (*E. coli* DnaK, PDBID 4B9Q) and citrate synthase (porcine heart CS, PDBID 1CTS), as well as GATA4, progesterone receptor (directly or indirectly), Apobec2 and other myosins. Additional unknown proteins may serve as UNC-45 co-chaperones or targets. Known protein structures are modeled in PyMOL (PyMOL Molecular Graphics System, Version 1.6.0.0 Schrödinger LLC).

Figure 2. Model of UNC-45 function in assembling and protecting sarcomeric myosin. During normal development, myosin maturation involves chaperones such as TriC/CCT and Hsp70 (Srikakulam and Winkelmann, 1999; Srikakulam and Winkelmann, 2004). Data from zebrafish studies suggest myosin then forms a complex with UNC-45 and Hsp90 in the cytosol (Etard et al., 2008). The complex remains stable until the end of myofibrillogenesis. Once myosin successfully incorporates into thick filaments, UNC-45 and Hsp90 may dissociate from myosin and move to the Z-disk for storage. Upon stress, UNC-45 and Hsp90 translocate from the Z-disk to the A-band, potentially protecting myosin from denaturation. Since both *Drosophila* and *C. elegans* UNC-45 display chaperone function *in vitro* (Barral et al., 2002; Melkani et al., 2010), it is possible that UNC-45 could protect myosin independently of Hsp90. Upon cessation of stress, UNC-45 and Hsp90 may return to the Z-disk until needed. In cases where UNC-45 needs to be cleared, this is accomplished by the ubiquitin/proteasome protein degradation system. UNC-45 associates with various enzymes such as ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2 (Let-70), and ubiquitin ligases E3/E4 (CHN-1, UFD-2) (Hoppe et al., 2004). Eventually, UNC-45, CHN-1 and UFD-2 form a complex with an AAA ATPase (p97/VCP/CDC-48) (Janiesch et al., 2007) before transiting to the 26S proteasome for degradation.

Pink area represents a single sarcomere. Z, Z-disk; M, M-line. Blue-green actin thin filaments project from the Z-disks, and two myosin thick filaments are pictured across the M-line. Tric/CCT (Bovine, PDBID 4A0O). Hsp70 (*E. coli* DnaK in the ATP-bound open conformation, PDBID 4B9Q). Hsp90 (*S. cerevisiae* Hsp90 dimer in the ATP bound closed conformation, PDBID 2CG9). UNC-45 (*C. elegans* UNC-45, PDBID 4I2Z). Myosin S1 (chicken skeletal muscle myosin, PDBID 2MYS with light chains excluded). UFD-2 (Yeast UFD-2, PDBID 2QIZ). p97/VCP (VCP-like AAA ATPase from *Mycobacterium tuberculosis*, PDBID 3FP9). 26S proteasome (yeast proteasome, PDBID 4CR2). Known protein structures are modeled in PyMOL (PyMOL Molecular Graphics System, Version 1.6.0.0 Schrödinger LLC).

Figure 3. Conserved requirement of UNC-45 for worm, fly and vertebrate skeletal muscle structure and function. (A) Accumulation of myosin-containing thick filaments is compromised in a heat-sensitive *unc-45* UCS domain mutant of *C. elegans*. Electron micrographs of region II (posterior to the pharynx) cross sections from strain CB286 nematodes grown at (a) 15° or (b) 25°C. Scale bar, 0.5 µm. Figure reprinted, with permission, from Barral et al. (1998) [©1998 Rockefeller University Press. Journal of Cell Biology. 143:1215-1225.] **(B)** Ultrastructural defects associated with body-wall muscle of 22-hour-old *Drosophila unc-45* embryos. Electron micrograph of *yw* control line (a, b). *T-33* embryo (*unc-45* null mutant) exhibits reduced level of thick filaments and a loss of the thick-thin filament lattice spacing (c, d). Transverse (a, c) and longitudinal (b, d) orientations are shown. Scale bars: 0.25 µm (a, c); 1.0 µm (b, d). Figure reprinted, with permission, from Lee et al. (2011b). **(C)** Zebrafish with morpholino (MO)-injected knockdown of *unc-45b* display lack of trunk muscle striation. Compared to control MO-injected embryos (a, c), *unc-45b* MO-injected organisms (b, d) display lack of striation. Immunofluorescence images with phalloidin staining of control MO-injected embryos (a) *unc-45b* MO-injected embryos (b) as well as transmission electron micrographs of control MO-injected (c) and *unc-45b* MO-injected (d) embryos at 48 hpf are shown. Figure reprinted, with permission, from Wohlgemuth et al. (2007). **(D)** Missense mutant *unc-45* (*dicky ticker*) results in loss of myosin staining in *Xenopus* embryonic muscle. Immunofluorescence micrographs of wild-type (left panels) and

mutant embryos (right panels) stained with MyHC and α-actinin antibodies. As shown in subpanels a and c, A-bands and Z-disks are well-organized in wild-type somites at stage 43. Muscle myosin staining is greatly reduced in the mutant (b). However, α-actinin-containing sarcomeres are observed (d). Phalloidin-stained thin filaments are present but disorganized in the mutant. Figure reprinted, with permission, from Geach and Zimmerman (2010).

Figure 4. Cardiac defects associated with UNC-45 mutations. (A) Immunofluorescence micrographs of 1-week-old *Drosophila* hearts (third segment) from (a) control and (b) *unc-45* knockdown adults were probed with antibody against muscle myosin. Control cardiac tubes show typical spiral myofibrillar arrangements within the cardiomyocytes (arrow). Myofibrillar organization is completely disrupted in the conical chamber and third segment of *unc-45* knockdown cardiac tubes (indicated by *) with loss of most myosin-containing myofibrils and significant dilation. Scale bars: 75 μm. Figure reprinted, with permission, from Melkani et al. (2011). **(B)** Electron micrographs of sections through cardiac muscle cells from 5-day-old (a) wild-type and (b) *unc-45 steif* mutant zebrafish embryos. Arrows show Z-disks of sarcomeres, The mutant lacks proper intercalated disks and myofibrils. Scale bar, 0.5 μm. Figure reprinted, with permission, from Etard et al. (2007). **(C)** Control (a, c, e) and *unc-45b* mutant (b, d, f) mouse embryonic cardiac tissue. The mutation results in the blockage of embryonic cardiogenesis at the stage of right heart formation. Frontal, left lateral and right lateral views at E9.5 are shown from top to bottom in the control and mutant hearts. Control hearts (UNC-45b$^{C57/C57}$, a, c, e) show right and left ventricles (RV, LV), left atrium (LA) and the outflow tract (OFT). In contrast, UNC-45b gene-trap mutant hearts (UNC-45b$^{gt/gt}$, b, d, f) display a single ventricle (V), one atrium (A) and the OFT. Scale bar, 200 μm (a-f). Figure reprinted, with permission, from Chen et al. (2012).

Figure 5. Structural similarities and differences among the three UCS domain proteins, *Drosophila* UNC-45 (PDBID 3NOW, pink), *C. elegans* UNC-45 (PDBID 4I2Z, blue), *S. cerevisiae* She4p (PDBID 3OPB, green). (**A**) Putative myosin binding groove. Here, only the UCS domain is shown with helix 3 of armadillo repeat layers 14-20 colored white to highlight the groove. **(B)** Putative myosin interacting loop. By

aligning the crystal structures using the overall protein conformations, the positions of the proposed myosin interacting loops can be compared. *Drosophila* UNC-45 (in gray) was used as the reference to show relative position of the loops to the UCS domain. The gaps in the loops were filled in manually for better visualization. In addition to the loops, the helices where the loops originate and terminate are also represented in color. **(C)** The overall bent shape. The angle between the central and the UCS domains was measured using PyMOL. Since the *Drosophila* and the *C. elegans* UNC-45 proteins were very similar, the small difference in the bend angle is probably not significant; however, the yeast She4 protein has a drastically larger bend angle. Since all three proteins are composed of layers of armadillo repeats, the difference in the bend angle could suggest flexibility between the central and the UCS domains. Protein structures are modeled in PyMOL (PyMOL Molecular Graphics System, Version 1.6.0.0 Schrödinger LLC).

Figure 6. UNC-45 protein sequence alignment based on the three-dimensional crystal structures. Crystal structures of *C. elegans* UNC-45 (PDBID 4I2Z) and the yeast UCS protein She4p (PDBID 3OPB) were aligned to the *Drosophila* UNC-45 crystal structure (PDBID 3NOW) using the "align to selection" function in PyMOL (PyMOL Molecular Graphics System, Version 1.6.0.0 Schrödinger, LLC). Chain B of the two molecules in the She4 protein structure was utilized for this comparison, because of its higher degree of completeness. Due to the bend in the structures, the N-terminal regions (amino acids 138-439 for *Drosophila*, 130-452 for *C. elegans*, and 31-323 for yeast) and the C-terminal regions (amino acids 440-923 for *Drosophila*, 453-930 for *C. elegans*, and 324-771 for yeast) were aligned separately. After the three-dimensional structural alignment, the α-helices (*Drosophila* in pink, *C. elegans* in blue, and yeast in green) were compared and amino acids occupying comparable positions were aligned manually. Each armadillo repeat layer, designated according to Lee et al. (2011a), is indicated above the helices with the corresponding H1, H2, or H3 labeled. Lower case letters indicate deletions, mutations, or variations from the PubMed sequences.

Figure 7. Mutations from the various UCS domain containing proteins mapped onto the *Drosophila* UNC-45 structure (PDBID 3NOW). Mutations from *S. pombe*, *C.*

elegans, zebrafish, *Xenopus*, mouse and human were mapped onto the *Drosophila* UNC-45 crystal structure using the protein alignment in Figure 6. A majority of the mutations is located in the UCS domain at the bottom of the figure, which underscores the importance of this domain to UNC-45 function. Residue numbers correspond to those of the mutant organisms. Protein structure modeled in PyMOL (PyMOL Molecular Graphics System, Version 1.6.0.0 Schrödinger LLC).

Figure 8. UNC-45 filament structure and its putative interactions with myosin S1 and Hsp90. Based upon its crystal structure configuration, *C. elegans* UNC-45 (cUNC-45) forms filaments as a result of interactions between TPR domains and central/UCS neck regions of adjacent molecules (Gazda et al., 2013). The UCS domains of these filaments are proposed to interact with the periodically-spaced S1 heads of myosin thick filaments to facilitate their folding (Gazda et al., 2013). The region of muscle myosin II that may interact with UNC-45 is colored in red, based upon the ability of the homologous region of myosin V to interact directly with the She4p UCS protein *in vitro* (Shi and Blobel, 2010). The C-terminal peptide (MEEVD) of Hsp90 interacts with the TPR domain of cUNC-45, which lies behind the central domain as depicted (Gazda et al., 2013). The C-terminus of one Hsp90 subunit of the homodimer is colored red to show the area of possible interaction with cUNC-45. It is hypothesized that the UNC-45 filament serves as a scaffold to present Hsp90 chaperone activity to the UCS-bound unfolded myosin S1 domains (Gazda et al., 2013). In this diagram, a stylized thick filament displays folded myosin heads that have detached from cUNC-45. The Hsp90 molecules have detached from cUNC-45 as well. cUNC-45 (PDBID 4I2Z), myosin II (PDBID 2MYS), hsp90 (PDBID 2CG9) are modeled in PyMOL. Protein structures are modeled in PyMOL (PyMOL Molecular Graphics System, Version 1.6.0.0 Schrödinger LLC).