A Drosophila model for Duchenne muscular dystrophy

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APPENDIX

Color figures

Chapter 2: Figure 3

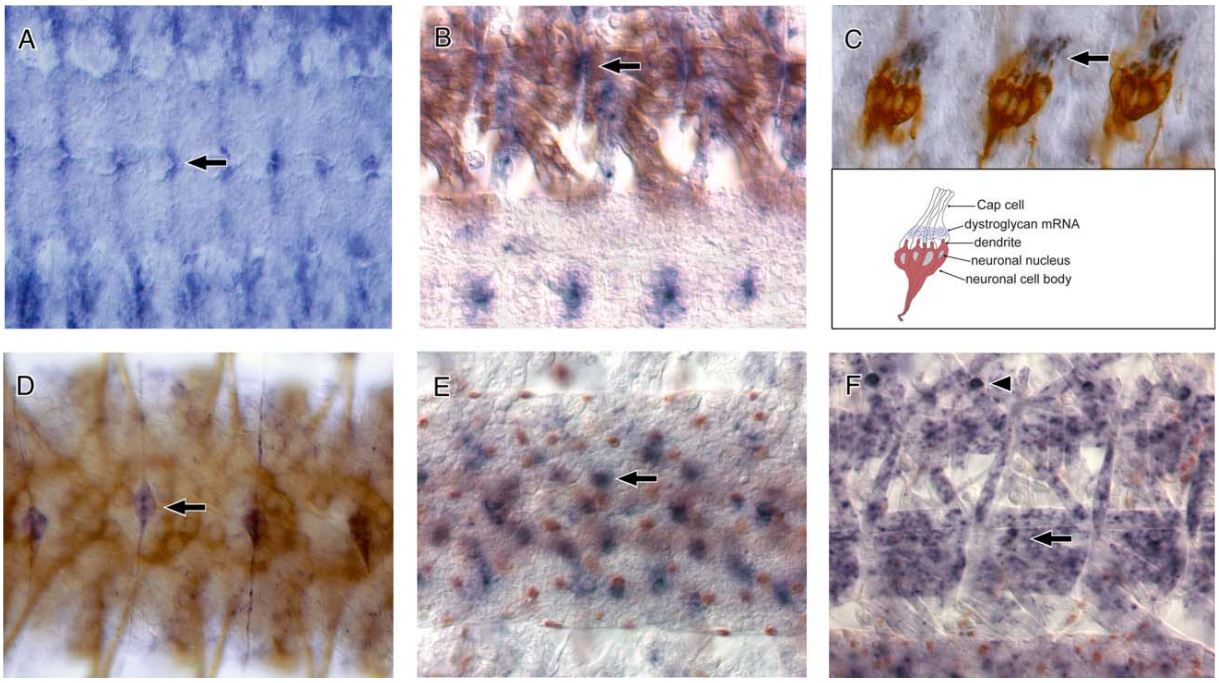

Figure 3: RNA expression of a subset of the *Drosophila* DGC orthologues in the embryonic mesoderm and derived musculature and in the nervous system. The RNA expression patterns of a subset of the *Drosophila* DGC orthologs expressed in the embryonic mesoderm and derived musculature and the nervous system are shown in dissected ventral cords and embryonic body walls: (**A**) The large *dystrophin* isoforms dmDLP1/dmDLP2 are detected in the mesectodermal cells underlying the ventral nerve cord (arrow). (**B**) At a later stage, the large *dystrophin* isoforms are expressed at the muscle attachment sites (arrow). The *dystrophin* RNA (blue) is shown relative to the muscle fibres that are labeled by the anti-muscle myosin antibody (brown). (**C**) *dystroglycan*-like RNA in blue is expressed in the accessory or cap cells (arrow) of the chordotonal sensory organs. A schematic drawing of a chordotonal organ (Campos-Ortega and Hartenstein, 1997) is shown, illustrating the relative location of the *dystroglycan*-like expression domain. *dystrobrevin*-like RNA is also expressed in the cap cells (data not shown). (**D**) *dystroglycan*-like is expressed in the dorsal-median cells (arrow). Neuronal cell bodies and axonal and dendritic processes in (**C** and **D**) are labeled by anti-22C10 (brown). (**E**) *scgdelta* RNA (blue, arrow) is expressed by subsets of ventral cord neurons, likely the larval neuroblasts, and is not present in the glial cells, here labeled by the anti-Repo antibody (brown). (F) *scgdelta* is expressed in most muscle fibres (arrow) and in a unidentified triangular-shaped cell, likely a muscle (arrowhead). The *scgdelta* mRNA expression is shown in blue, while the glial cells are labeled by the Repo antibody in brown. All panels represent dissected embryonic body walls with associated ventral cords: (**A**) stage 13, (**B**) stage 15, (**C**) stage 16, (**D**) stage 14, (**E**, **F**) stage 16. Anterior is to the left.

Chapter 3: Figure 2

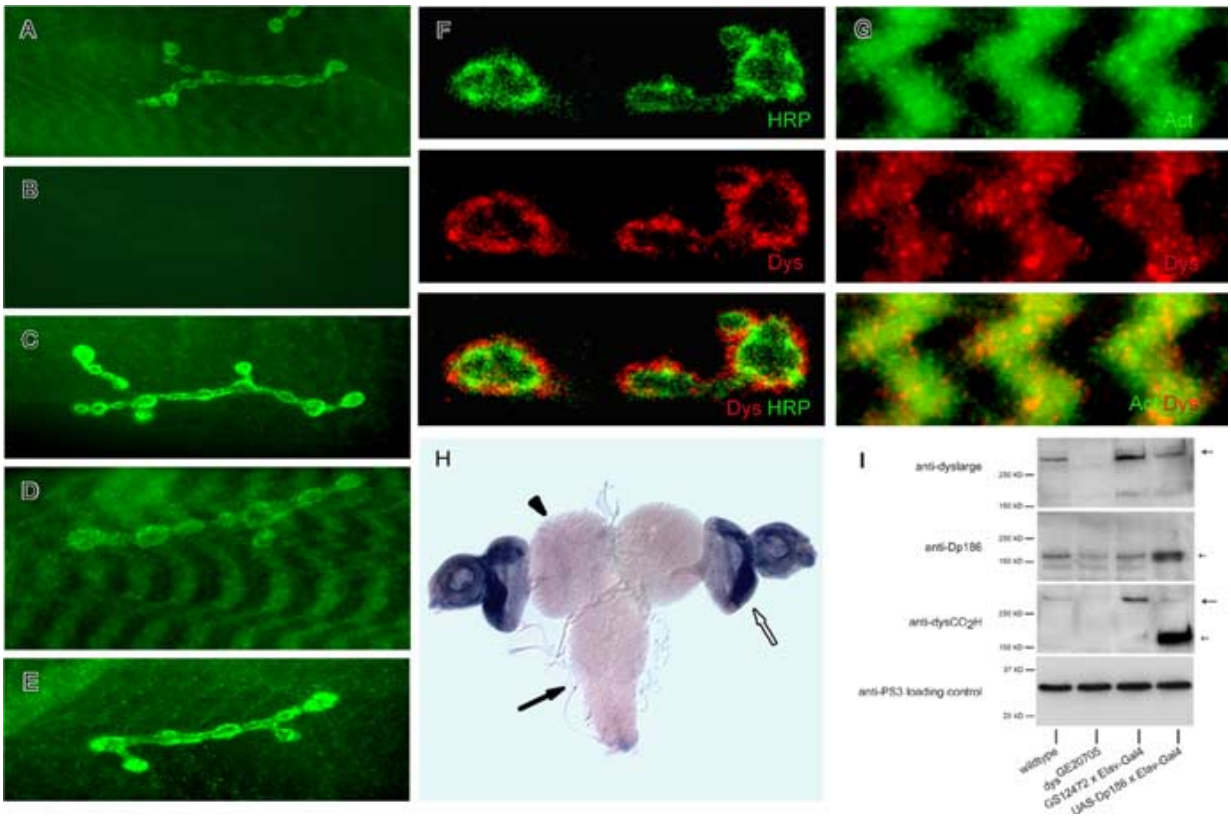

Figure 2. The large Dystrophin isoforms are localized postsynaptically. Third instar larval body walls were stained with anti-DysCO_2H, which recognizes all Dystrophin isoforms (**A**–**C**), anti-Dyslarge, which recognizes only the large isoforms (**D**, **E**), double labeled with anti-HRP and anti-DysCO_2H(**F**), or double labeled with anti-actin and anti-DysCO_2H(***G***). **A**, Dystrophin protein is expressed at the wild type third instar larval NMJ at synaptic and extrasynaptic sites. **B**, Dystrophin protein is severely reduced in the $dys^{GE20705}$ mutant. **C**, The DLP2 isoform protein accumulates highly at the NMJ after overexpression in the muscle (G14-Gal4/+; GS12472/+). **D**, Wild type Dystrophin protein is recognized at the NMJ by the large-isoform-specific antibody anti-Dyslarge. **E**, Overexpressed DLP2 (G14-Gal4/+; GS12472/+) can also be visualized using anti-Dyslarge antisera. **F**, Double labeling of a wild type larval body wall with anti-HRP, staining presynaptic boutons (green), and anti-DysCO_2H (red) reveals that the Dystrophin protein is postsynaptically localized at the NMJ. **G**, Double labeling of a wild type larval body wall with anti-actin (green) and anti-DysCO_2H (red) reveals that the Dystrophin colocalizes with actin at the NMJ extrasynaptic sites of expression. **H**, RNA *in situ* hybridization of wild type larval neuropile (filled arrow), brain (arrowhead), and associated eye-antennal discs (open arrow) with an exon 4 antisense probe that labels all large *dystrophin* isoform mRNAs reveals no apparent expression of large *dystrophin* isoform mRNAs in the neuropile or brain. **I**, Western blot analysis of embryo extracts prepared from wild type, $dys^{GE20705}$ Elav-Gal4/GS12472 (overproducing DLP2), and Elav-Gal4/UAS-Dp186 (overproducing Dp186) using the indicated antibodies. The large Dystrophin isoforms (anti-Dyslarge panel) are absent from the mutant $dys^{GE20705}$ and overexpressed in the Elav-Gal4/GS12472 embryos. Dp186 is expressed at decreased levels in $dys^{GE20705}$ and is overexpressed in Elav-Gal4/UAS-Dp186 (anti-Dp186 panel). The pan-Dystrophin antibody (anti-DysCO_2H panel) confirms the overexpression of both the long isoforms and Dp186 in the overproducing embryos. The arrows indicate the large Dystrophin isoforms, and the short arrows indicate the Dp186 isoform. All lanes were loaded equally, as confirmed by the anti-ribosomal subunit PS3 antibody (anti-PS3 panel), except the last lane of the Dp186 blot, in which 5% of the protein was loaded to permit comparison of the extracts in a single exposure.

Chapter 4: Figure 2

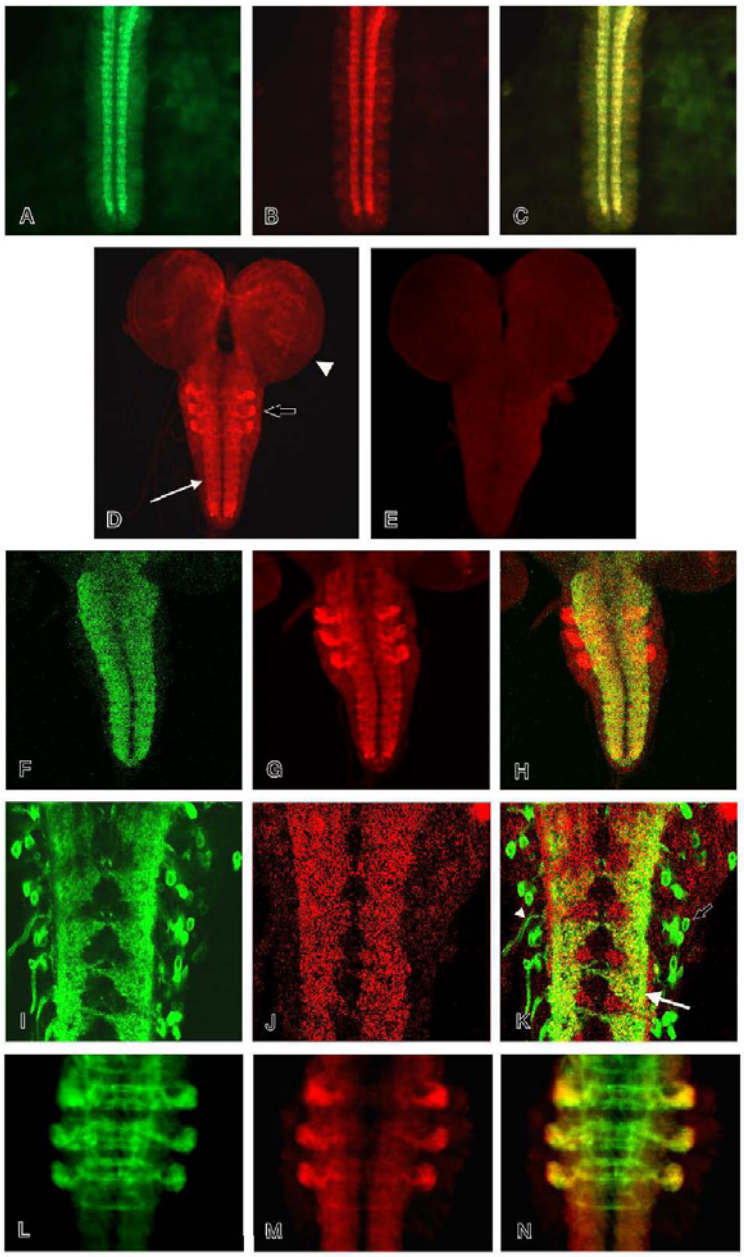

Figure 2. Dp186 protein is expressed in synapse-rich regions of the embryonic and larval neuropiles, likely in motoneuronal dendrites. A dissected stage 16 embryo ventral nerve cord (**A-C**), entire third instar larval central nervous systems (**D, E**) or only the neuropile (**F-H**) or a portion thereof (**I-N**) are shown. (**A**) The synapse-rich embryonic neuropile as detected by mAb nc82 which recognizes the presynaptic Bruchpilot protein. (**B**) antiDp186 staining of the ventral nerve cord. (**C**) The merge of the channels displayed in A and B. (**D**) Dp186 is expressed in the neuropile (arrow), the brain lobes (arrowhead) and in three lateral clusters, likely the thoracic neuromeres (open arrow, see also L-N) in third instar larvae. (**E**) Dp186 expression cannot be detected in the mutant $dys^{Dp186\ 166.3}$.(**F-H**) The presynaptic Synapsin protein (**F**) and Dp186 (**G**) are expressed throughout the larval neuropile. The merge of the channels shown in F and G is shown (**H**). (**I-K**) mCD8-GFP protein driven by OK6-Gal4 (**I**) colocalizes with Dp186 protein (**J**) in motoneuron dendrites (arrow), but not in cell bodies (open arrow), or axons (arrowhead)(**K**). (**L-N**) Alexa Fluor 488-conjugated phalloidin (**L**), which binds to F-actin, overlaps with Dp186 (**M**) at three lateral clusters, likely the thoracic neuromeres (**N**). Anterior is up in all preparations.

Chapter 6: Figure 6

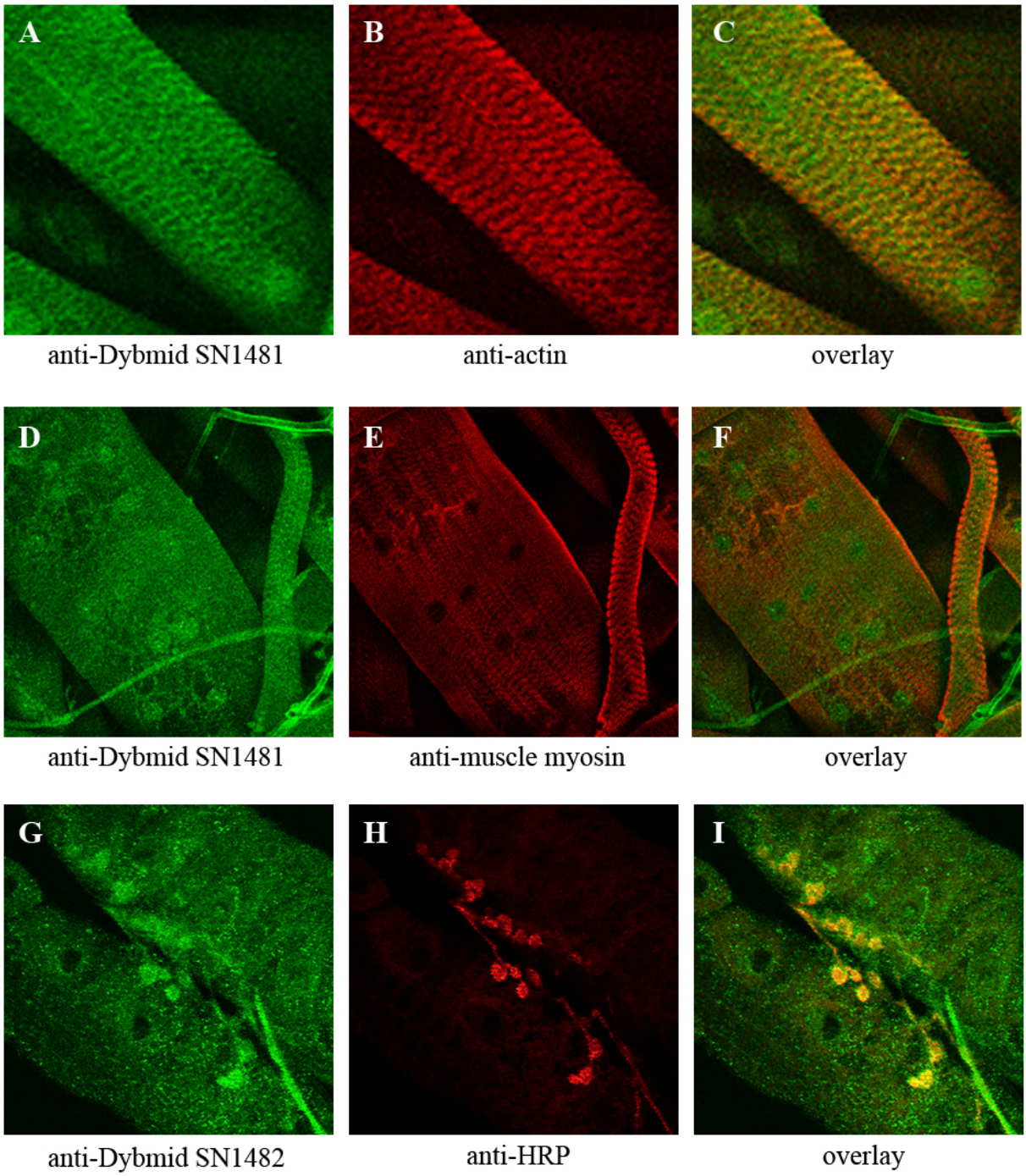

Figure 6: Dystrobrevin localizes with actin to the sarcomeric I-band and is expressed at the synapse, but does not colocalize with muscle myosin. Representative photographs of double labelings of wild type third instar larval body walls with anti-Dybmid SN1481 and anti-actin (**A-C**), anti-Dybmid SN1481 and anti-muscle myosin (**D-F**), and anti-Dybmid SN1482 and anti-HRP (**G-I**).