Supplemental figure 1: DP71 dystrophin associates with ankyrin-B through its membrane-binding domain (AA1-949) and not the ZU5, UPA or death domains (AA 966-1535). GST-DP71, GST-Dyn4 (6-his) and GST(6-his) control protein were expressed in bacteria and bound to Ni– sepharose beads (GE Healthcare) in column buffer (20mM Na Phosphate pH 7.4, 0.3M NaBr, 20mM imidazole, 0.5mM DTT, 1mM sodium azide), eluted with 0.3M imidazole, then immobilized onto glutathione sepharose beads. Beads were washed and equilibrated in binding assay buffer (20mM HEPES pH7.4, 60mM NaCl, 0.5mM DTT, 1mM sodium azide) with the additions of 0.1mM EGTA, and 20µM zinc sulfate. 220 kDa full length ankyrin B (6-his tag) was expressed in Sf21 cells and purified by Ni-sepharose followed by gel filtration on a superose-6 column. Ankyrin-B (966-1535) (6-his tag) containing ZU5, UPA, and Death domains, and Ankyrin-B membrane-binding domain (6-his tag) were expressed as pMAL fusion proteins in bacteria and isolated by NiNTA–sepharose chromatography. Ankyrins (2µM final) were incubated with the above protein glutathione-sepharose beads in a final volume of 50µl overnight on a shaker platform at 4°C. Samples were layered over 20% glycerol gradients in 0.4 ml tubes and centrifuged at 5000xg for 15min. Tubes were frozen on dry ice, and tips containing the pelleted beads and bound protein cut off into 5XPAGE gel-loading buffer. SDS gels were run and duplicates were either stained for protein with Pierce GelCode Blue stain or immunoblots performed and labeled with an anti-histidine antibody followed by iodinated protein A/G.

Supplemental figure 2: Asparagine 331 in dynactin-4 is required for interaction with ankyrin-B. A schematic representation of the series of dynactin-4 truncation-mutants tested for interaction with ankyrin-B. In Blue and marked with (+), are the truncation mutants that positively interacted with ankyrin-B in the yeast two-hybrid assay (see methods). In black and marked with (-), are the truncation mutants and point mutation N331A (in red asterisk) that failed to interact with ankyrin-B in the yeast two-hybrid assay. Numbers represent the nucleotide boundaries of the dynactin-4 fragments tested within the ORF, where the full-length dynactin-4 is 1-1380.