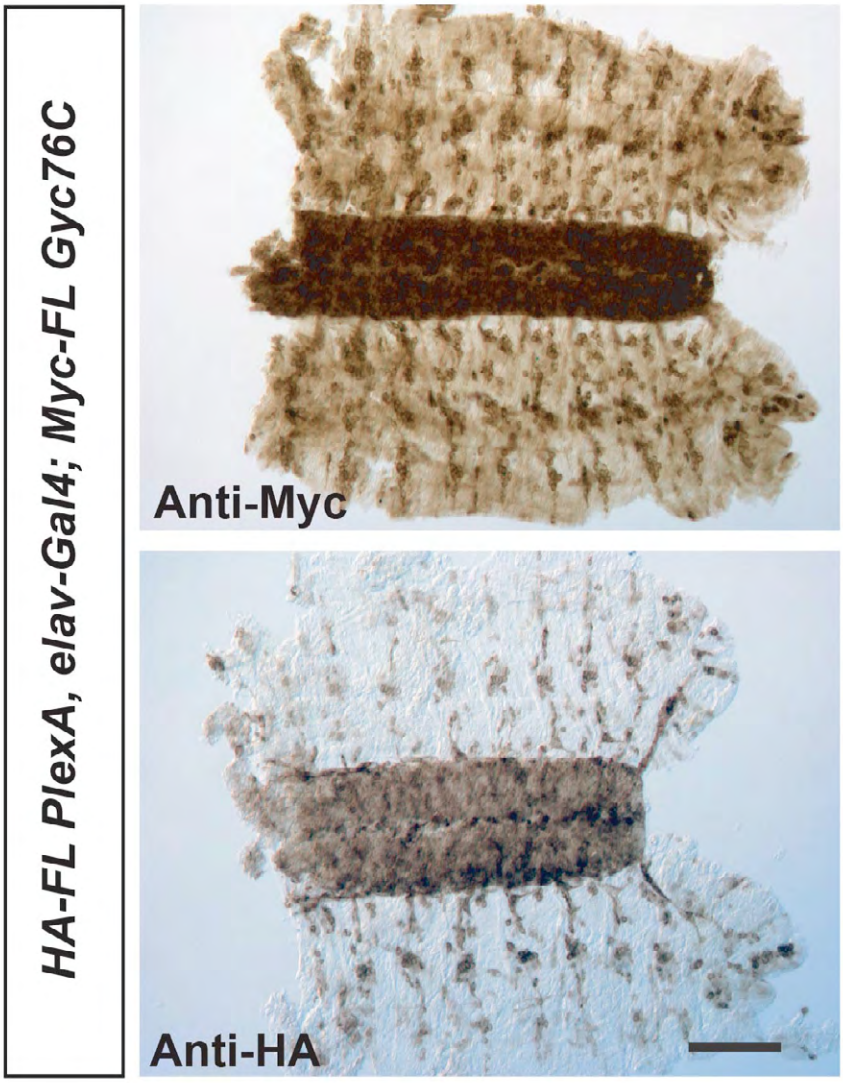

Fig. S1. Immunohistochemistry of *UAS:HA-FL PlexA, elav-Gal4; UAS:Myc-FL Gyc76C* embryos. Filleted stage 14-15 *UAS:HA-FL PlexA, elav-Gal4; UAS:Myc-FL Gyc76C Drosophila* embryos stained with anti-Myc (top panel) or anti- HA (bottom panel) reveal the neuronal expression of each transgene. Scale bar, 20 μm.

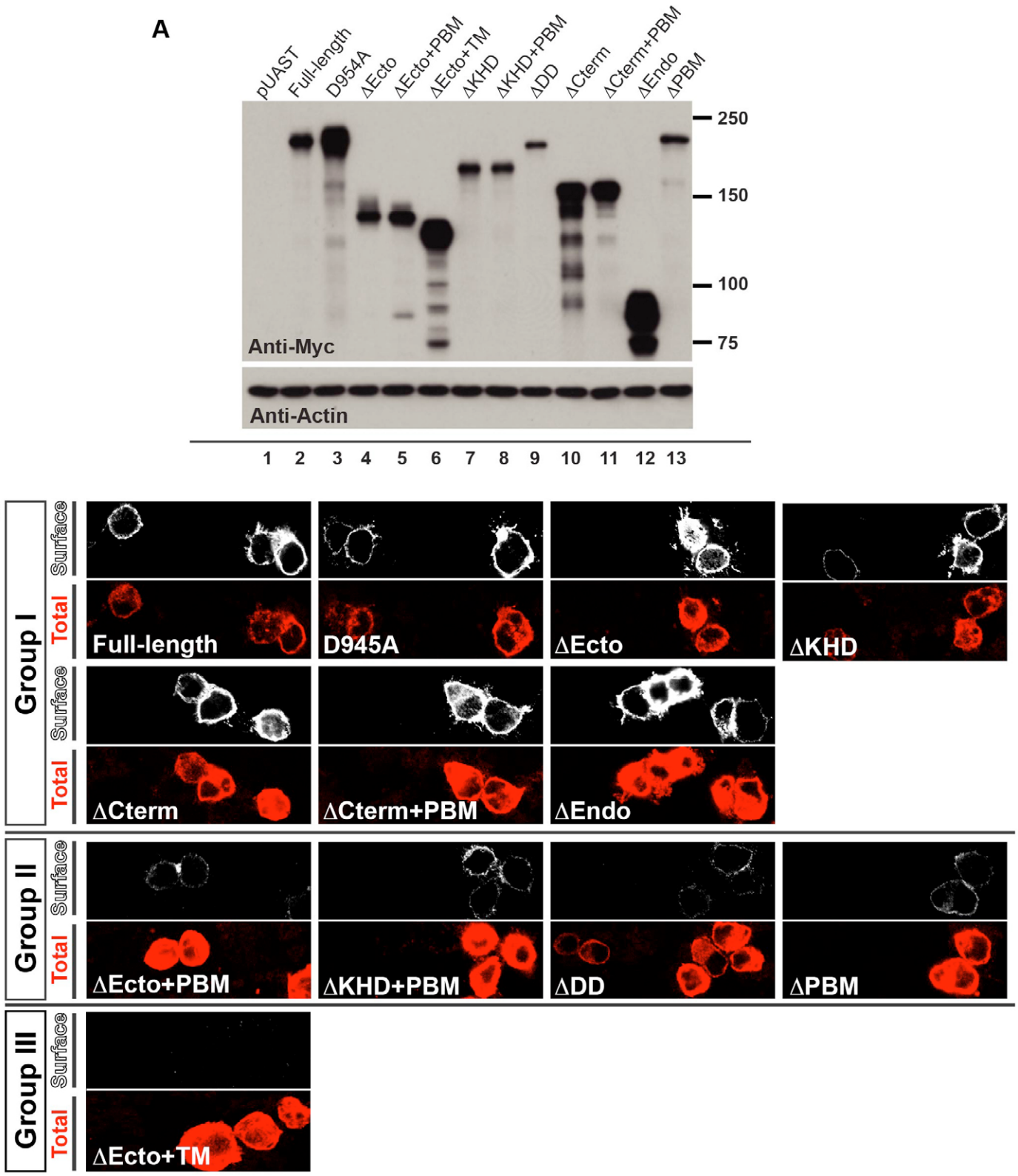

Fig S2. Western blot analysis and live cell-surface immunostaining reveal protein expression level and protein localization of Gyc76C constructs expressed in S2R+ cells under the control of *Actin-Gal4*. (**A**) Western analyses of lysates from *Drosophila* S2R+ cells used in cGMP assays. Cells were transfected with *Myc-FL Gyc76C*, or all *Myc-Gyc76C* deletion constructs described in the text, and then immunoblotted with anti-Myc (top panel). The control for protein loading was provided by immunoblotting for anti-actin (bottom panel). (**B**) Live cell-surface immunostaining using anti-Myc (9E10) was performed to label surface proteins (in white) of all Myc-Gyc76C constructs expressed in S2R+ cells prior to fixation and permeablization. The total protein produced by each Myc-Gyc76C construct (in red; pseudo color) was observed by then labeling with rabbit anti-Myc (71D10) following fixation and permeablization. The Gyc76C constructs in Group I (FL, ΔEcto, ΔKHD, ΔCterm, ΔCterm+PBM, and ΔEndo) show strong cell surface localization; those in Group II (ΔEcto+PBM, ΔKHD+PBM, ΔDD, and ΔPBM) show weak cell surface localization are; and (ΔEcto+TM) in Group III shows no cell surface protein localization.

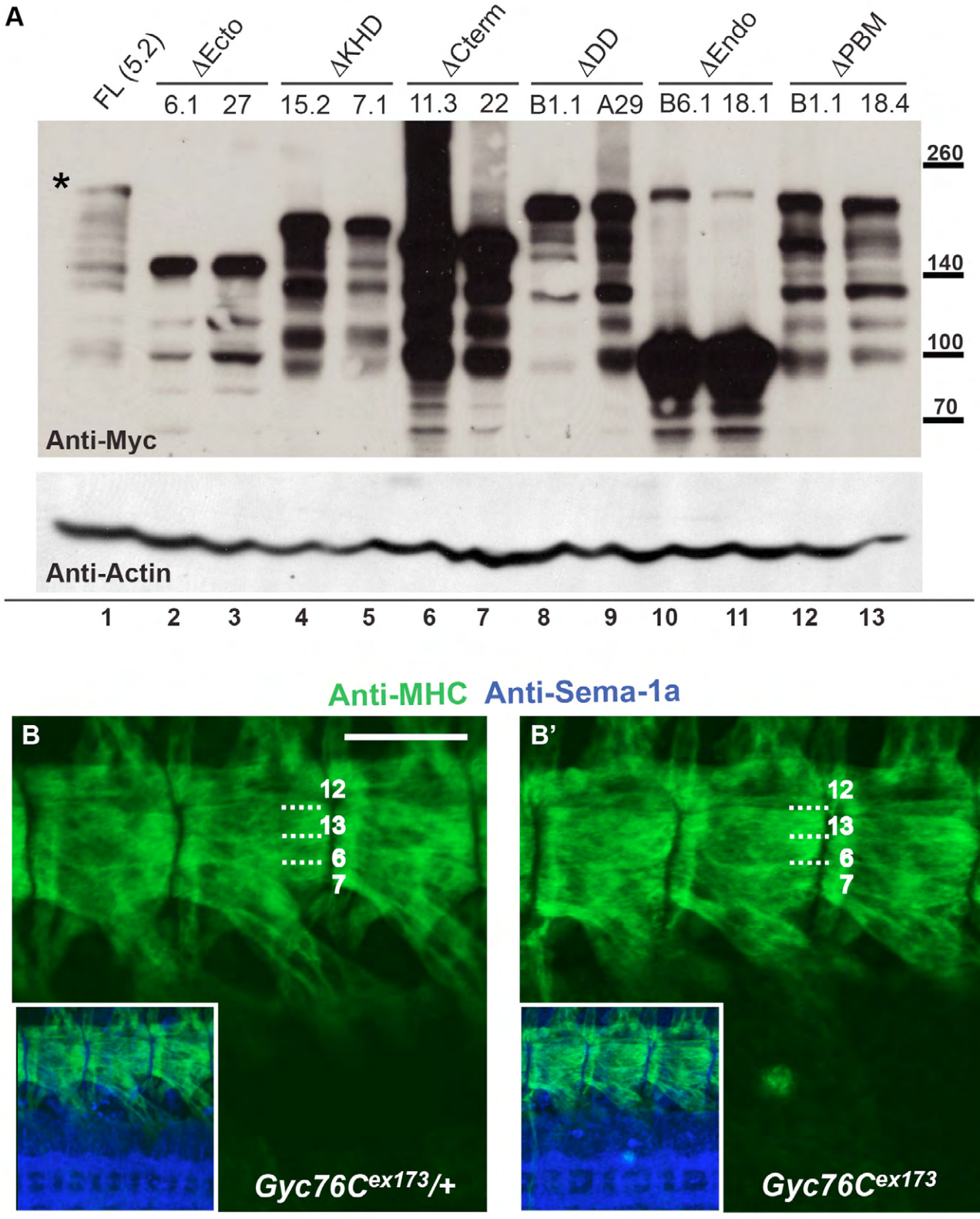

Fig. S3. Western blot analysis of Gyc76C transgene expression levels driven by *elav-Gal4*, and overall muscle development and organization of *Gyc76C* homozygous mutant embryos. (**A**) Equal amounts of embryonic lysates (1 embryo/4ul of Laemmli buffer) obtained from each transgenic line were subjected to Western analysis and blotting with anti-Myc (top panel). The loading control is provided by immunoblotting using anti-actin (bottom panel). (**B**) and **B′)** Stage 16 embryos stained with anti-MHC and anti-Sema-1a (blue) to reveal overall ventro-lateral muscle field organization (green). *Gyc76C* heterozygous and *Gyc76C* homozygous embryos show no apparent differences in muscle development or organization. Scale bar, 10 μm.

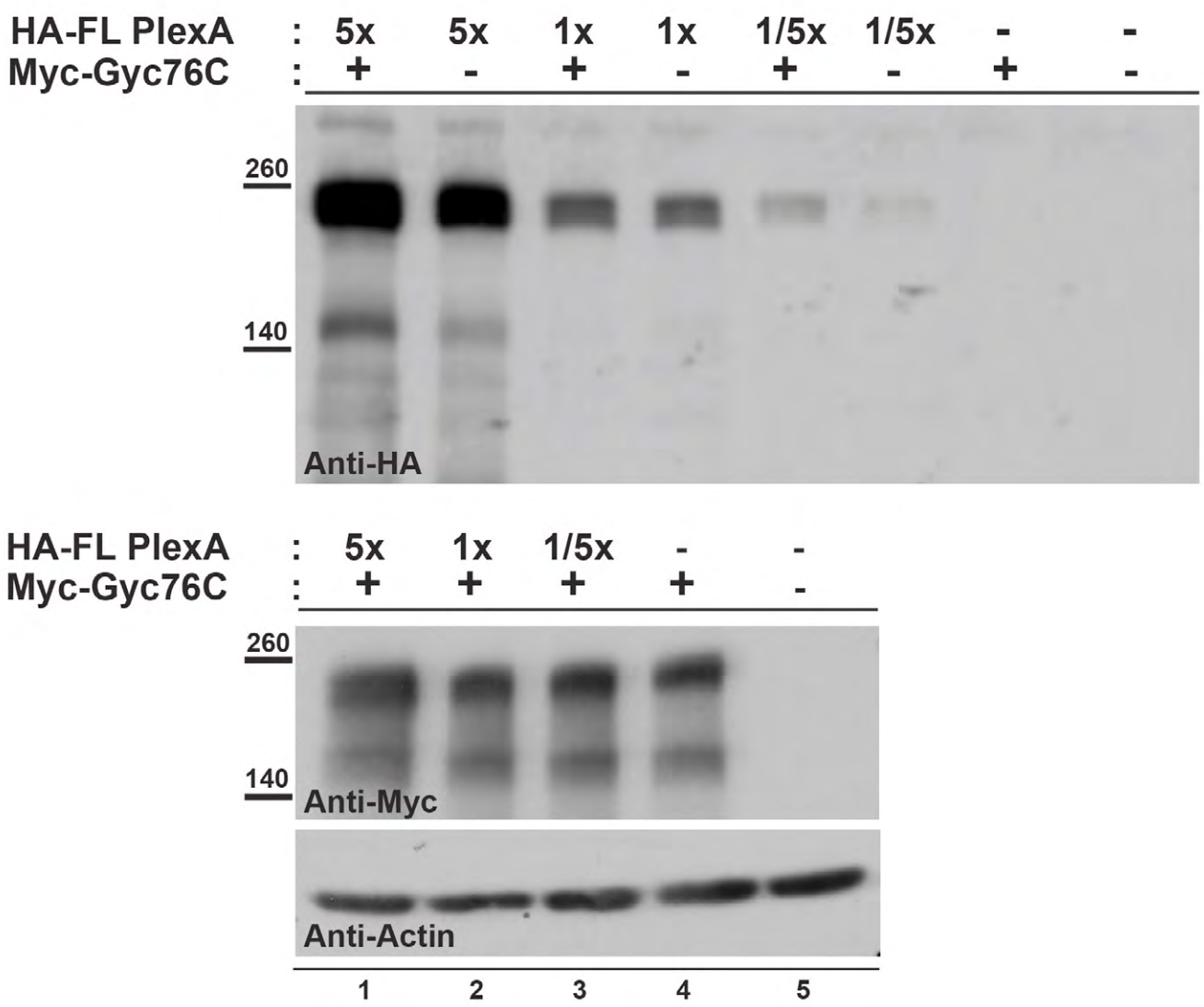

Fig. S4. Representative Western blots for PlexA: Gyc76C *in vitro* co-expression experiments used for GC assays. Lysates from Myc-FL Gyc76C-expressing DmBG2 cells with, or without, transfection using different amounts of HA-FL PlexA cDNA. Anti-HA and anti-Myc were used to detect HA-FL PlexA (top panel) and Myc-FL Gyc76C (middle panel), respectively. Different concentrations of HA-FL-PlexA did not influence the expression levels of Myc-FL Gy76C (middle panel, lanes 1-4). The loading control was performed by immunoblotting using anti-actin (bottom panel).

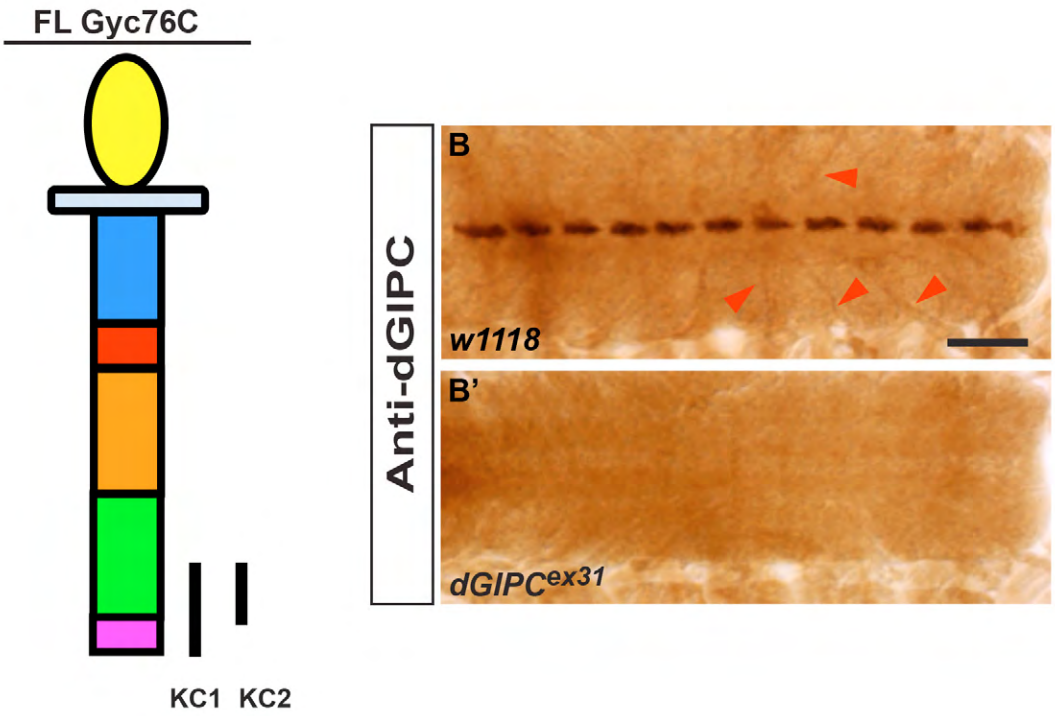

Fig. S5. Gyc76C baits for yeast-two-hybrid and endogenous dGIPC protein expression in the developing ventral nerve cord of *Drosophila* embryos. (**A**) Schematic diagram showing the protein landscape of Gyc76C that includes the PCR-amplified Gyc76C KC1 (aa 1451-1525) and KC2 (aa 1451-1522) fragments (black lines) used for constructing "baits" for yeast-two-hybrid assay. (**B**) and **B′)** Filleted preparations of wild-type and *dGIPC*^{ex31} stage 14 embryos stained with a mouse monoclonal antibody directed against dGIPC. (C) dGIPC protein is strongly expressed in midline glia but is also detected in other regions, including ventral nerve roots (red arrowheads). (C′) dGIPC staining, including at ventral nerve roots, is absent in *dGIPC*^{ex31} null mutants. Scale bar, 10 μm.