Kittelmann et al., http://www.jcb.org/cgi/content/full/jcb.201302022/DC1

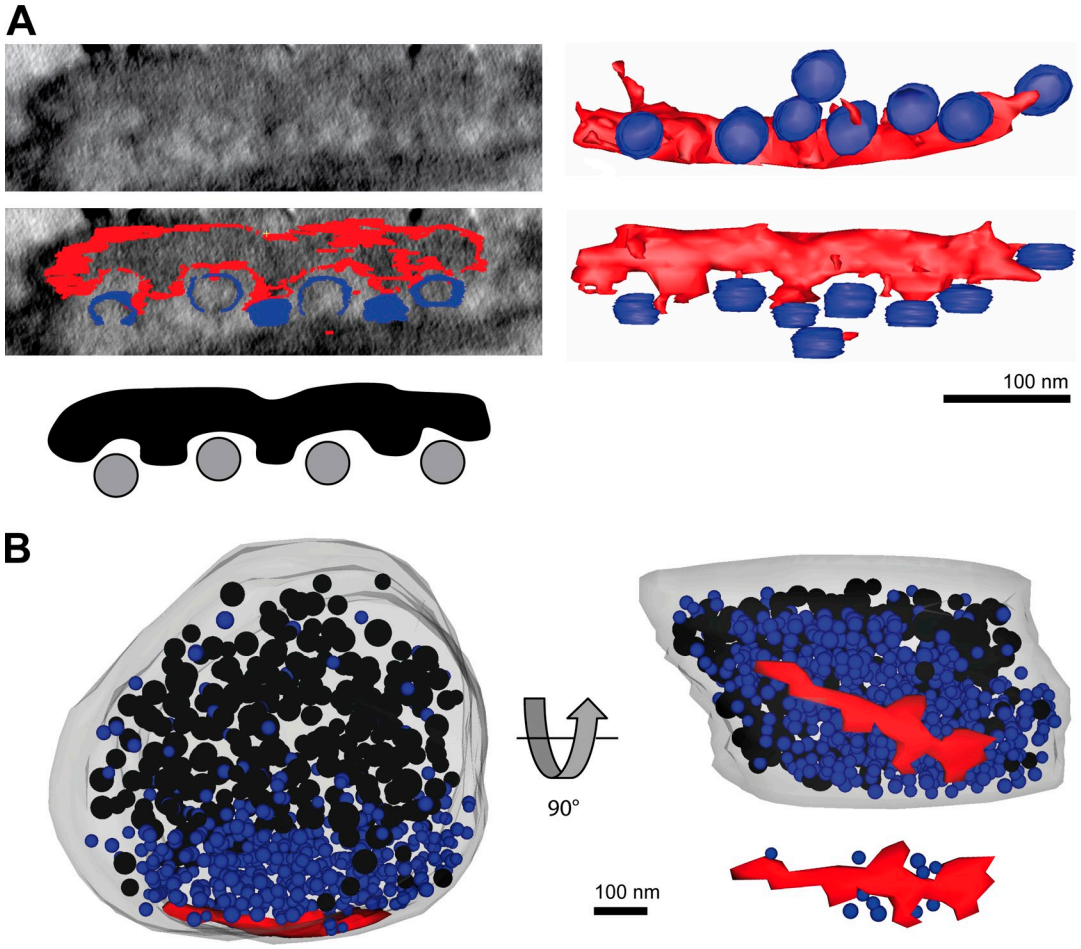

Figure S1. **3D reconstructions of a neuron–neuron DPs.** (A, left) Projection from a wild-type ventral cord neuron–neuron synapse HPF EM tomographic reconstruction. Borders of the DP (red) and SVs (blue) selected during volume segmentation are shown in the projection. Schematic representation shows the DP structure and SVs. (right) 3D view of this neuron–neuron AZ DP, which clearly shows the branched structure of the DP and localization of SVs in the bays close to the presynaptic membrane. (B) 3D reconstructions from serial 50-nm sections of a wild-type nerve ring neuron–neuron synapse. Axon membrane (gray), DP (red), SVs (blue), and DCVs (black) were traced. The synaptic terminal is shown in cross section view and 90° tilted to allow a top view onto the AZ DP. Branching of the DP is clearly visible. SVs located within the bays formed between branches.

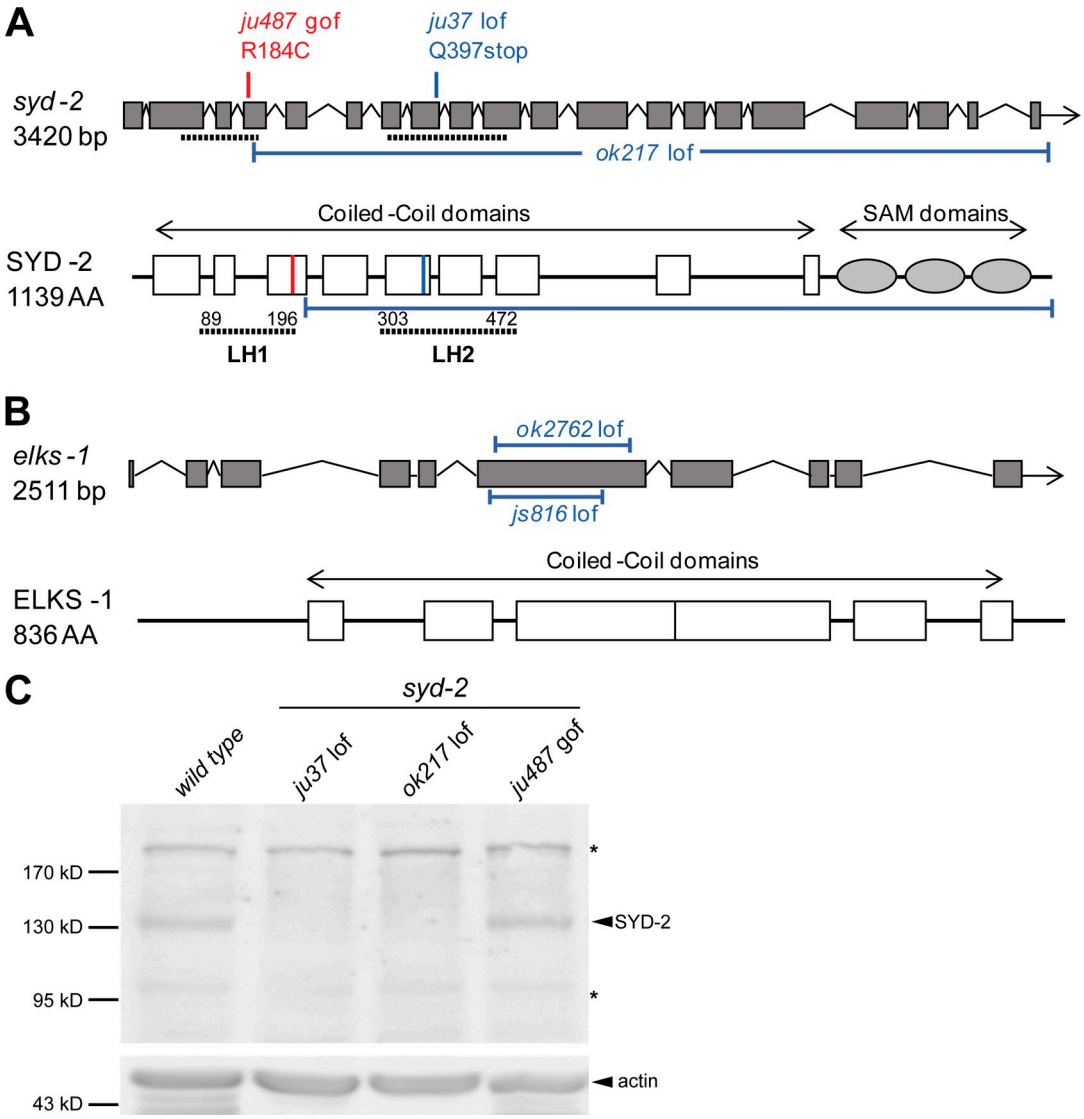

Figure S2. **Gene and protein structure of *syd-2* and *elks-1*.** Exons are shown as gray boxes, and introns are indicated with connecting lines. Mutations are shown in blue for loss-of-function (lof) and in red for gain-of-function (gof) mutations. (A) The SYD-2 protein is composed of N-terminal coiled-coil domains (white boxes) and three C-terminal sterile α motif domains (gray ellipses; adapted from Taru and Jin, 2011). Two Liprin homology domains (LH1 and LH2) have been identified within the coiled-coil domains and are indicated by dotted lines (Serra-Pagès et al., 1998; Taru and Jin, 2011). (B) The ELKS-1 protein mainly consists of coiled-coil domains (white boxes). Coiled-coil predictions were adapted from Wagh et al. (2006; HUSAR [Heidelberg Unix Sequence Analysis Resources] sequence analysis package). (C) Western blot analyses of SYD-2 protein levels were performed with total worm lysates from wild-type, *syd-2(ju37)* and *syd-2(ok217)* lof, and *syd-2(ju487)* gof worms. Polyclonal antibody against the N terminus of *C. elegans* SYD-2 recognizes full-length SYD-2 protein (130 kD) in lysate from wild-type and *syd-2* gof mutant animals. The amount of SYD-2 protein in *syd-2* gof mutants is similar to wild-type worms. No full-length SYD-2 protein was detected in *syd-2* lof mutants. Actin was used as a loading control. Asterisks mark nonspecific bands.

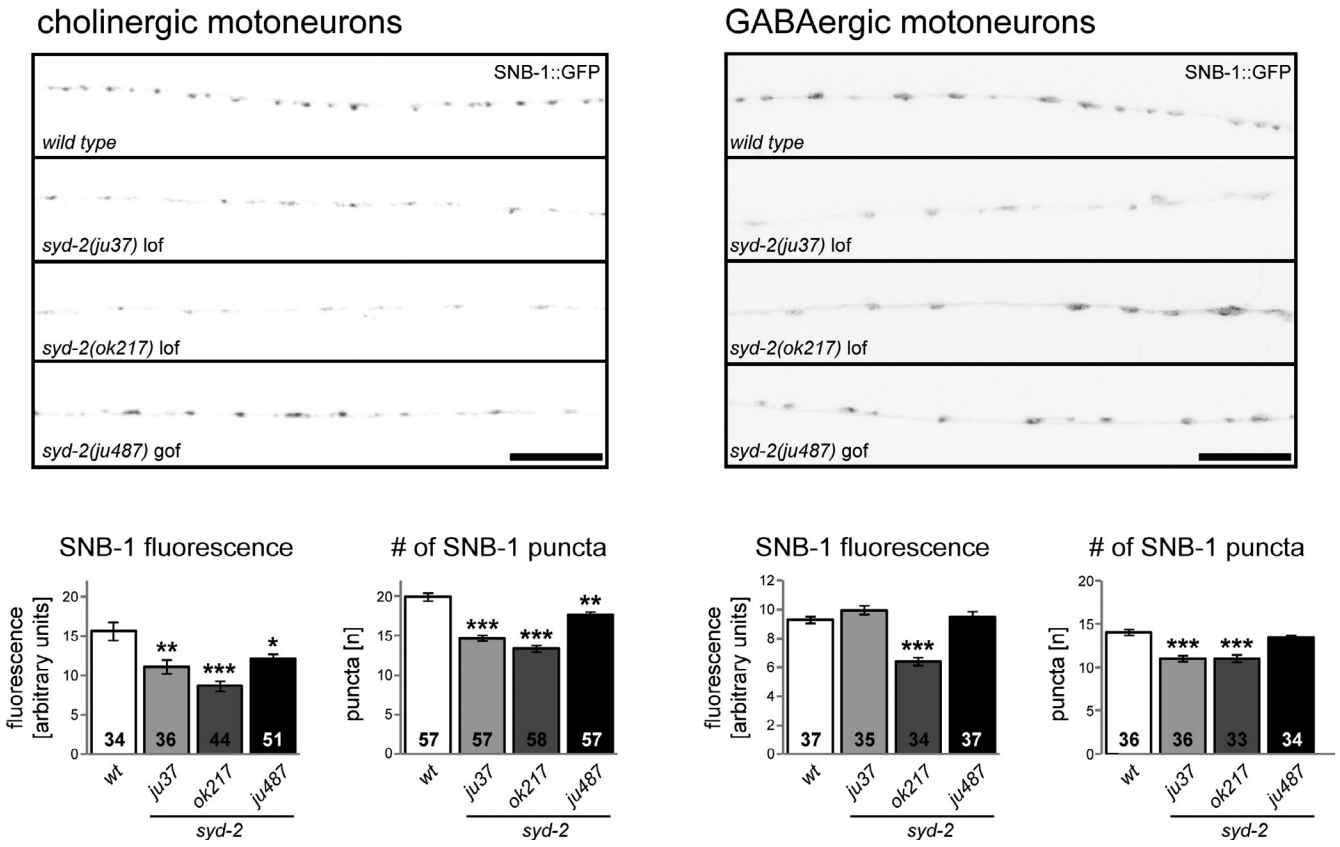

Figure S3. ***syd-2* mutant animals show altered SV clustering at NMJs.** Fluorescence analysis of marker protein SNB-1 in the different *syd-2* mutant strains (see Materials and methods) revealed defects in the axonal distribution of the SV protein SNB-1 in cholinergic DA and DB and GABAergic DD motoneurons. Quantifications of the total axonal SNB-1–GFP fluorescence as well as the number of synaptic puncta within 70 µm of posterior dorsal cord in wild-type and *syd-2* mutants are shown on the bottom. SNB-1 fluorescence as well as the number of synaptic SNB-1 puncta is reduced in *syd-2(ju37)* and *syd-2(ok217)* lof mutants. Bars, 10 µm. Statistics: analysis of variance and Dunnett's post test (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$). Error bars represent SEMs. Bars without asterisks fail to reach significance.

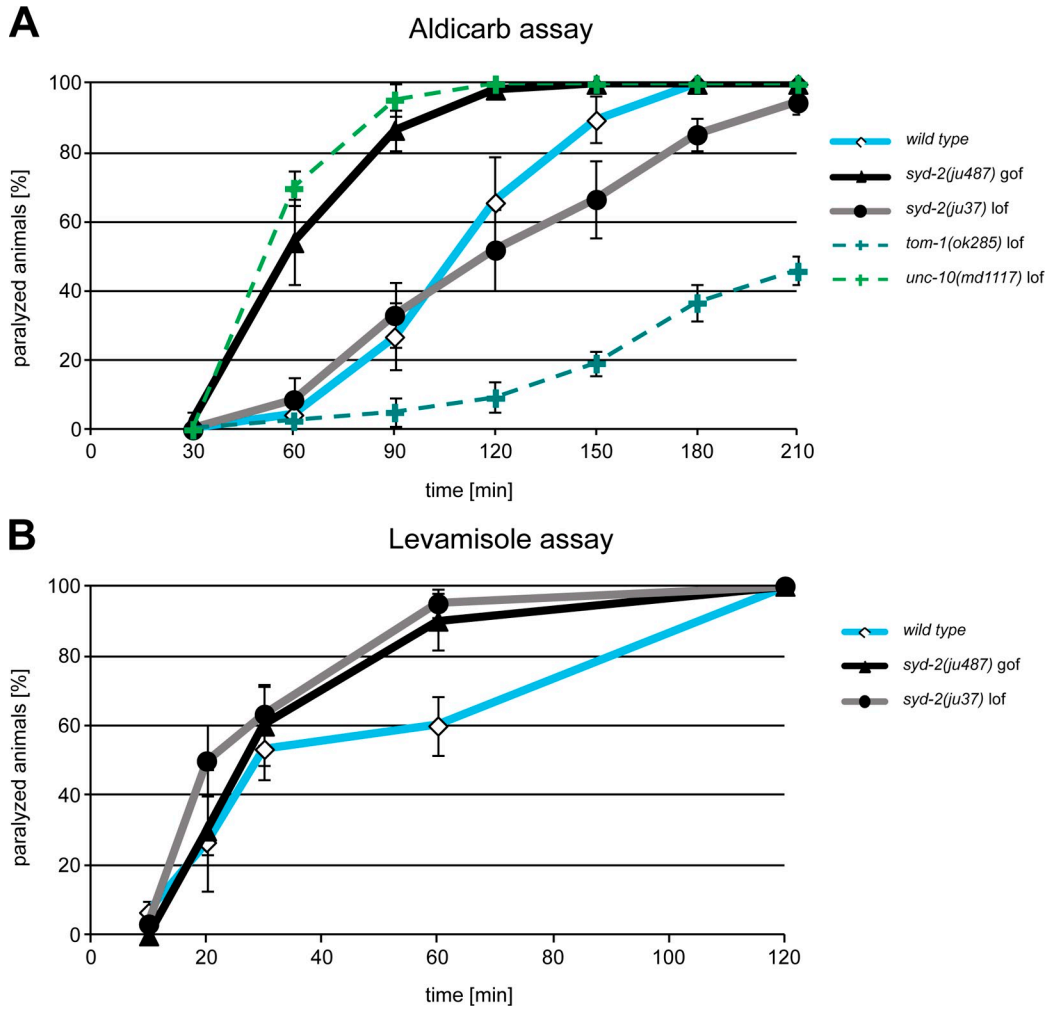

Figure S4. **Aldicarb and levamisole assays.** (A) Worms were scored for paralysis over time on 1.5-mM aldicarb plates. As markers, *tom-1* and *unc-10* mutants were used for strong aldicarb sensitivity and resistance, respectively (Mahoney et al., 2006). Four independent repeat experiments were averaged for each time point. The *syd-2(ju37)* lof mutants show moderate resistance to aldicarb, whereas *syd-2(ju487)* gof mutants are hypersensitive compared with wild type. (B) 10 worms were scored for paralysis over time on 0.2-mM levamisole plates. Three independent repeat experiments were averaged for each time point. *Syd-2(ju37)* lof as well as *syd-2(ju487)* gof mutants show similar levels of levamisole sensitivity as wild-type animals. Error bars indicate the SEMs.

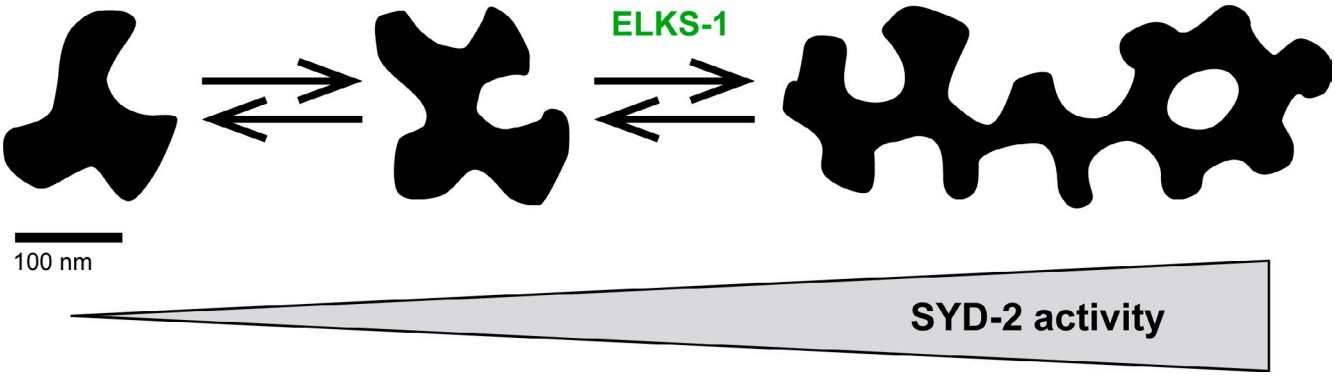

Figure S5. **DP size is regulated by SYD-2 activity.** According to our morphological analysis, we suggest that the level of SYD-2 activity determines the size of presynaptic DPs. Increased SYD-2 activity in *syd-2* gof mutants leads to the elongation of DPs, whereas the loss of SYD-2 in *syd-2* lof mutants results in the reduction of DP size. Other molecules are likely to be involved in the regulation of DPs size. Elongation of DPs through increased SYD-2 activity depends on the presynaptic protein ELKS-1.

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