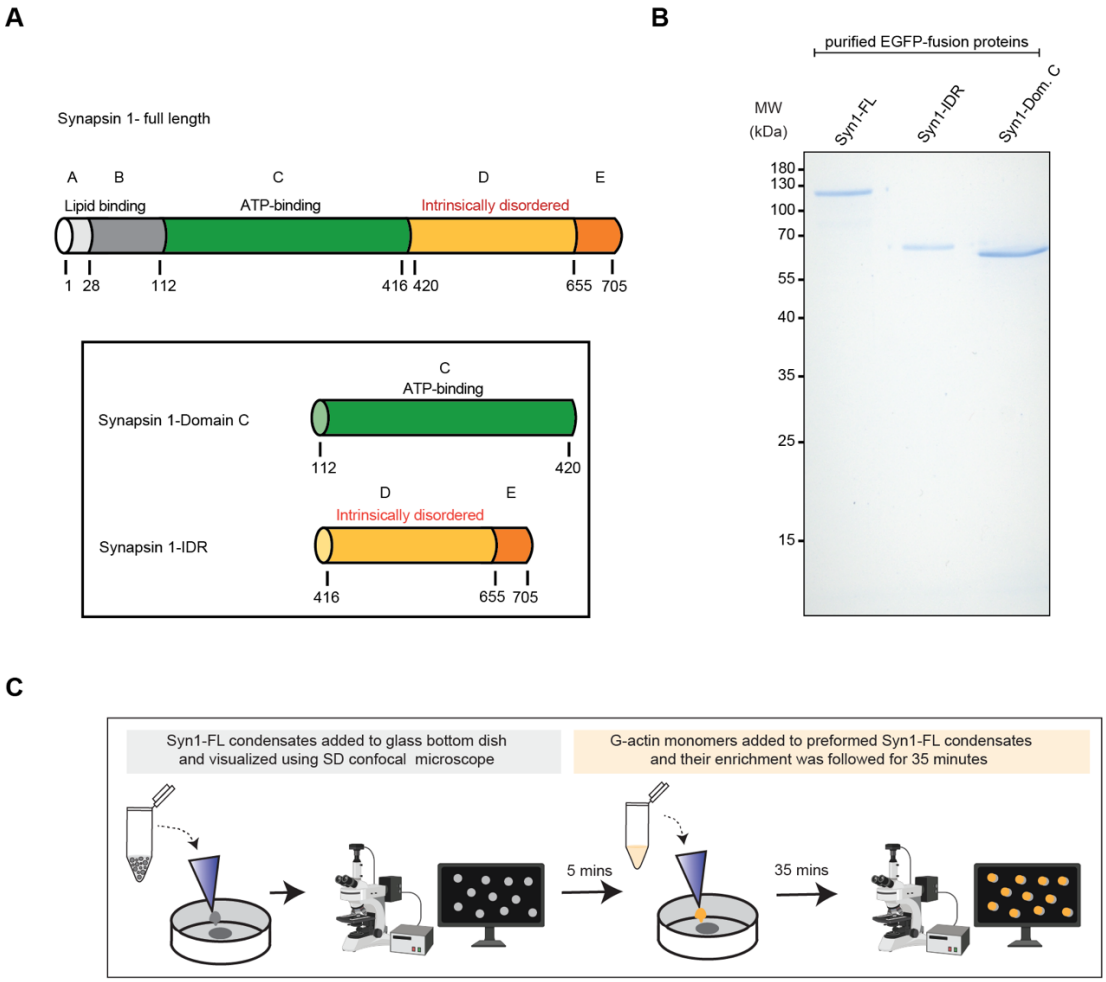
Supplementary Figure with Figure Legends

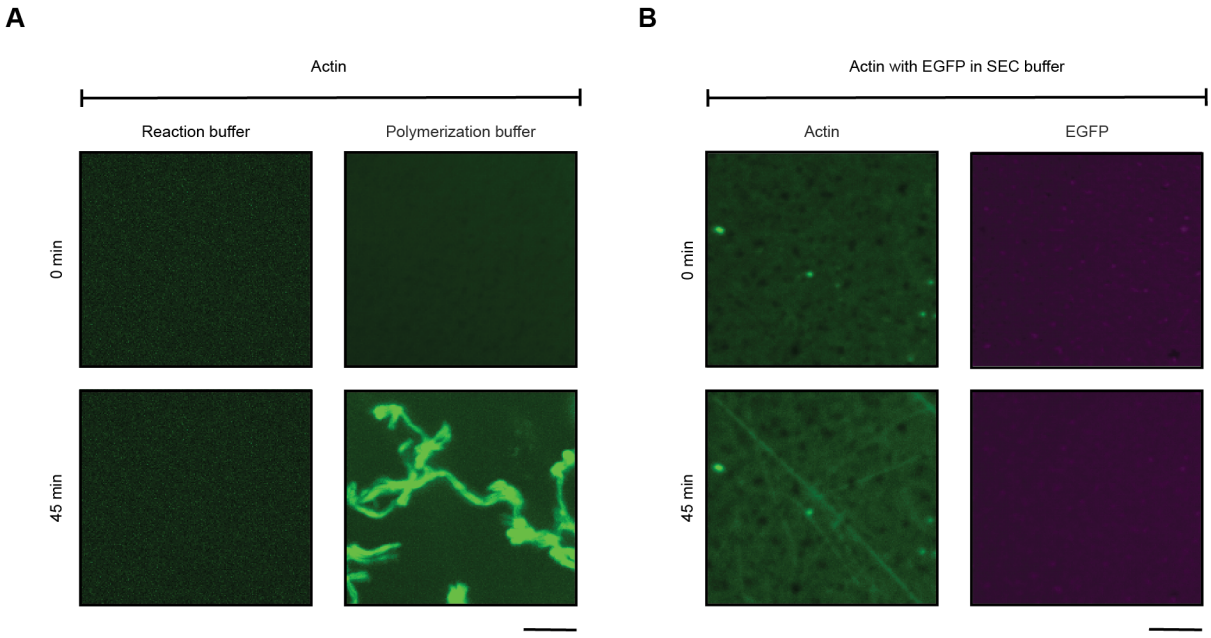


Supplementary figure 1: Recombinant proteins used for in-vitro reconstitutions.

A. Domain organization of Synapsin 1.

B. SDS-PAGE gel of the proteins – EGFP-Syn1-FL (102.235 kDa), EGFP-Syn1-IDR (57.621 kDa) and EGFP-Syn1-domain C (63.116 kDa) employed for in-vitro reconstitutions in the present study.

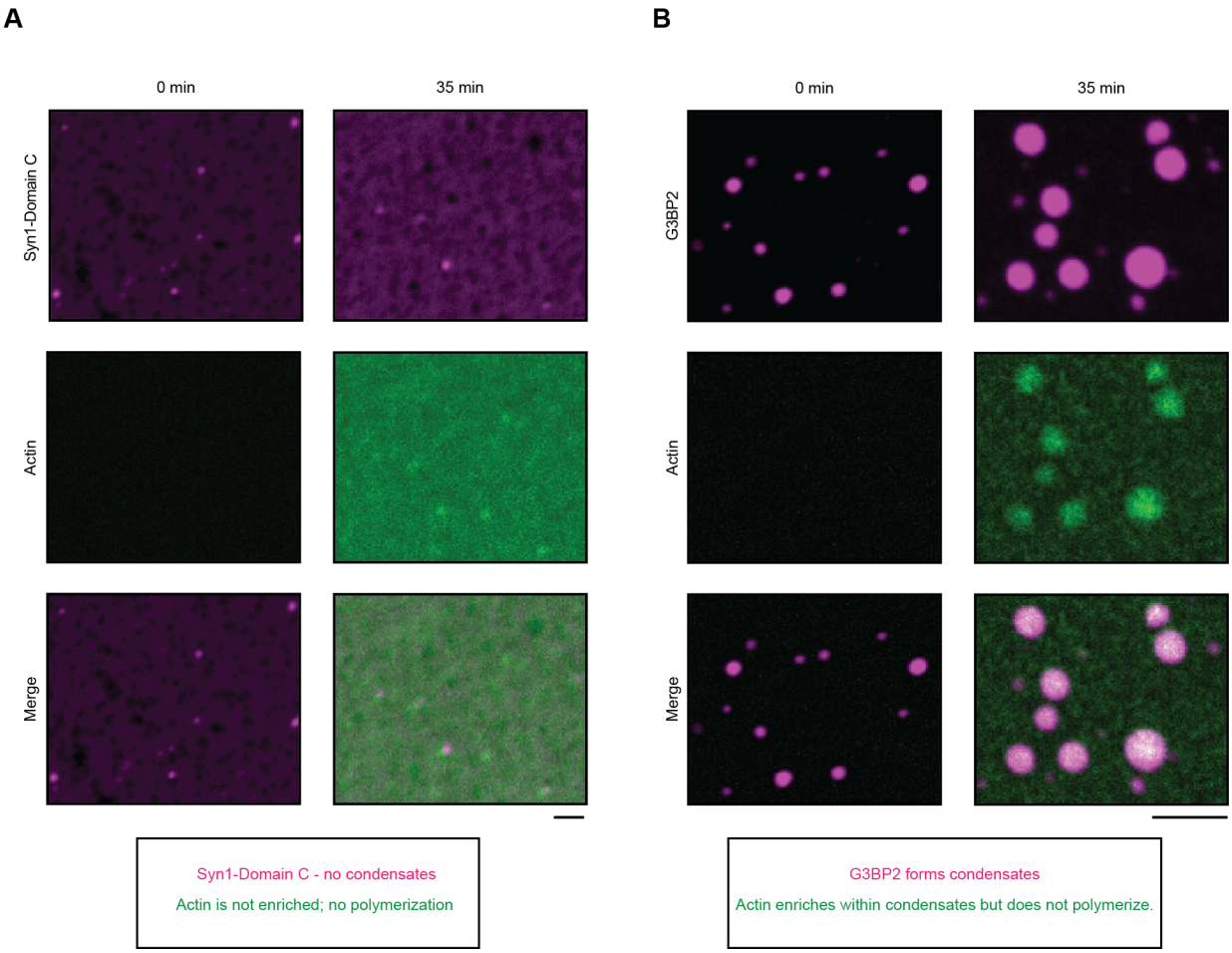
C. Schematic illustration of Syn1-actin reconstitution assay. Actin polymerization from Syn1 phases was examined by first forming 6 µM EGFP-Syn1-FL condensates with 3% (w/v) PEG 8000 on a glass bottom dish. After 5 minutes, when EGFP-Syn1-FL condensates became 3-4 µm in size, ATTO647 labelled G-actin monomers were added from top into these pre-formed EGFP-Syn1-FL condensates such that the final concentration of actin and EGFP-Syn1-FL in the final reaction mix was 4 and 4 µM respectively.



Supplementary figure 2: Positive and negative controls for actin polymerization.

A. Representative images for actin polymerization in reaction buffer and commercial polymerization buffer (FluMaXx) at t = 0 and t = 45 minutes. Images were acquired using spinning-disk confocal microscope at 488 nm (control for the background signal; represented in magenta) and 647 nm for actin (represented in green). Scale bar, 5 µm.

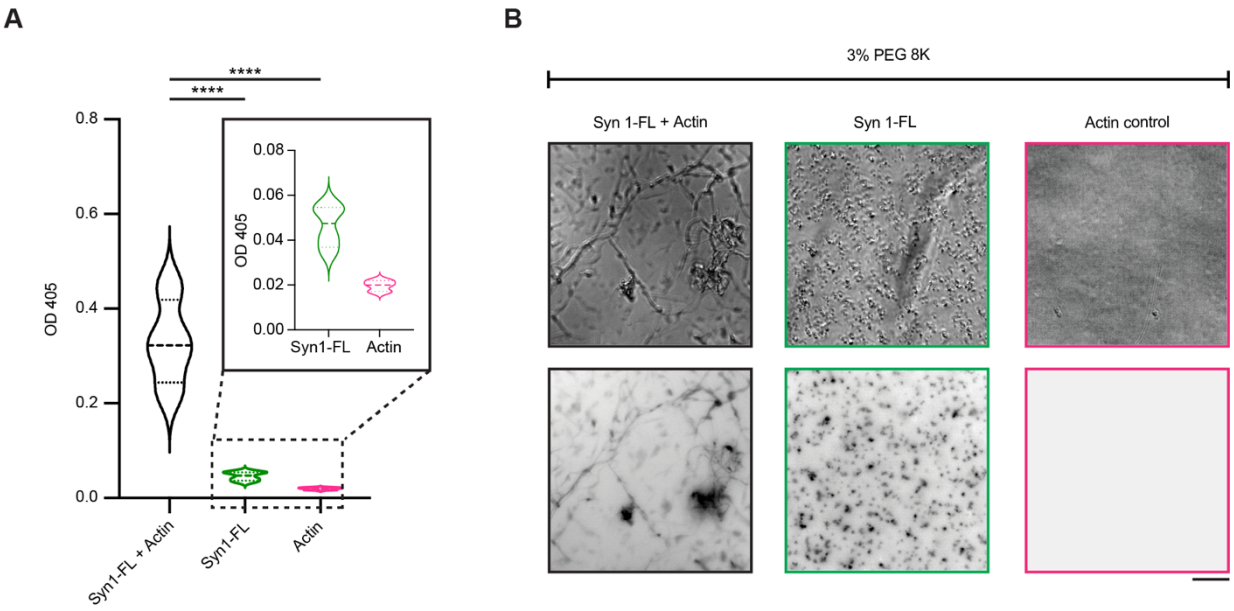
B. Representative images for actin polymerization in presence of EGFP protein, 3% PEG 8K in reaction buffer (buffer used for final elution of the protein from the size-exclusion column, SEC buffer) at t = 0 and t = 45 minutes at 488 nm and 647 nm for EGFP and actin, respectively. Images were acquired using spinning-disk confocal microscope at 488 nm (control for the background signal) and 647 nm for actin. Scale bar, 5 µm.



Supplementary figure 3: In-vitro reconstitution of EGFP-Syn1-Domain C with actin.

A. Representative confocal images from the in-vitro reconstitution of EGFP-Syn1-Domain C (4 µM, 3% (w/v) PEG 8000) with ATTO-647 labelled G-actin monomers (4 µM) at t = 0 (left) and t = 35 minutes (right). Image acquisition for EGFP-Syn1-Domain C and ATTO647 G-actin was carried out at excitation wavelengths 488 nm and 647 nm respectively. Scale bar: 5 µm

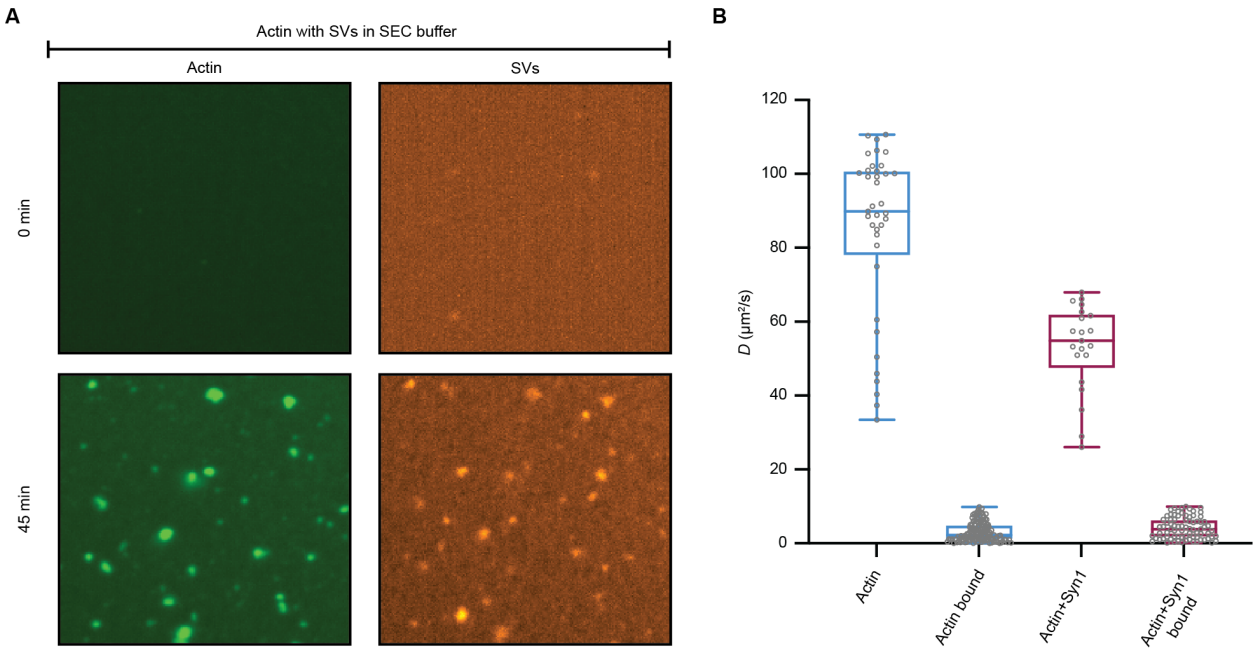
B. Representative confocal images from the in-vitro reconstitution of EGFP-G3BP2 (4 µM, 3% PEG 8,000) with ATTO-647 labeled G-actin monomers (4 µM) at t = 0 (left) and t = 35 minutes (right). Images were acquired at excitation wavelengths 488 nm and 647 nm for EGFP-G3BP2 and ATTO647 G-actin, respectively. Scale bar: 5 µm.



Supplementary figure 4: Turbidity measurements for in-vitro reconstitutions of synapsin 1 with actin.

A. Quantification of the turbidimetry assay. Plot comparing the turbidity measurements for EGFP-Syn1-FL with actin, EGFP-Syn1-FL alone and actin alone in presence of 3% (w/v) PEG 8000. Actin polymerization was assessed as increase in optical density after 48 h of incubation period Actin used for the assay was Mg^{2+} exchanged and supplemented with 0.5 mM ATP. Data shown here is quantified from 4 independent experiments (N = 4). ****p<0,0001; one-way ANOVA test.

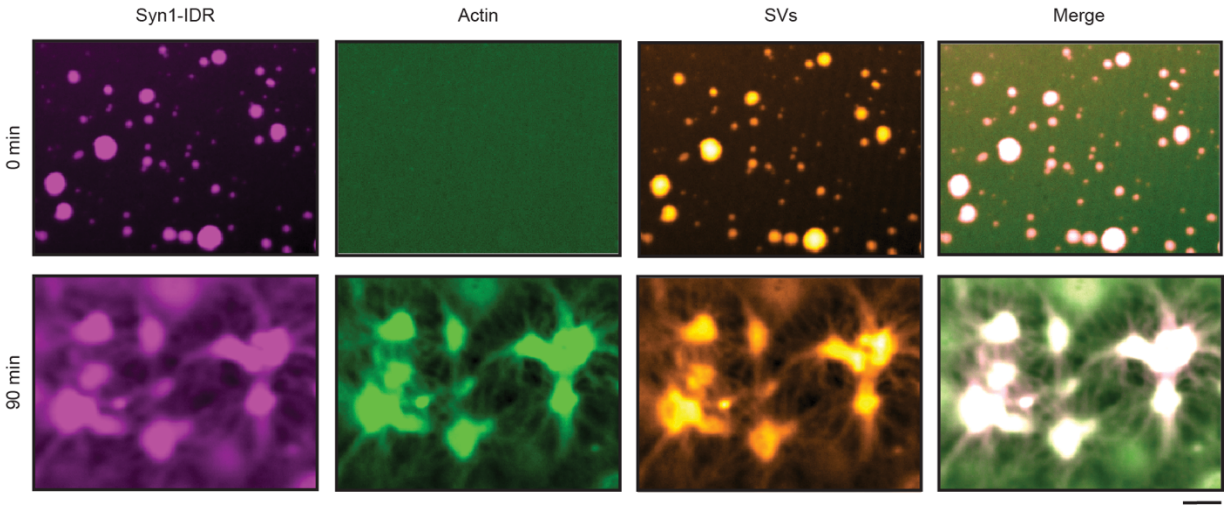
B. Top – representative brightfield images of reaction mixes after turbidimetry assay from a. Bottom – epifluorescence images of the same regions at 488 nm excitation wavelength. Scalebar, 50 µm.



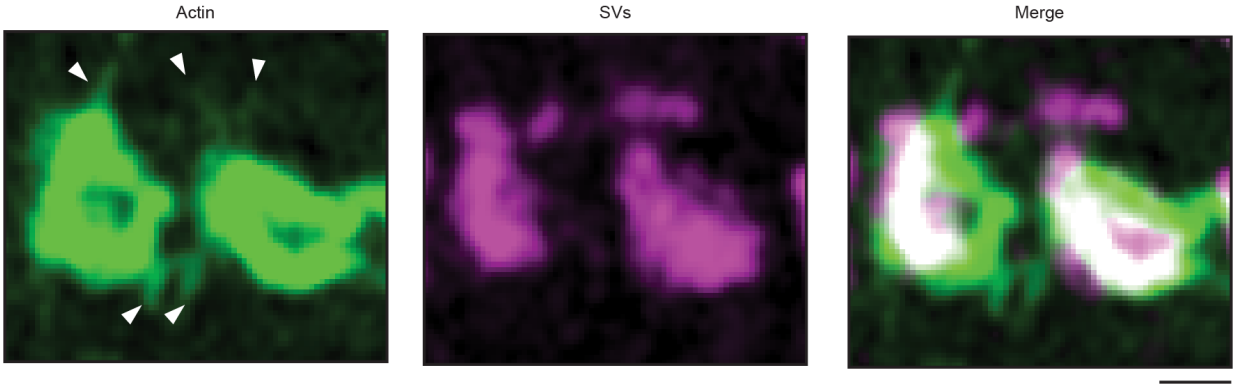
Supplementary figure 5: Actin reconstitution with native SVs in reaction buffer.

A. Representative images for actin reconstitution in presence of natively purified 3 nM SVs labelled with FM4-64 (1.65 µM final concentration), 3% PEG 8K in reaction buffer at t = 0 and t = 45 minutes. Images were acquired using a spinning-disk confocal microscope at 561 nm and 647 nm for SVs and actin, respectively. Scale bar, 5 µm.

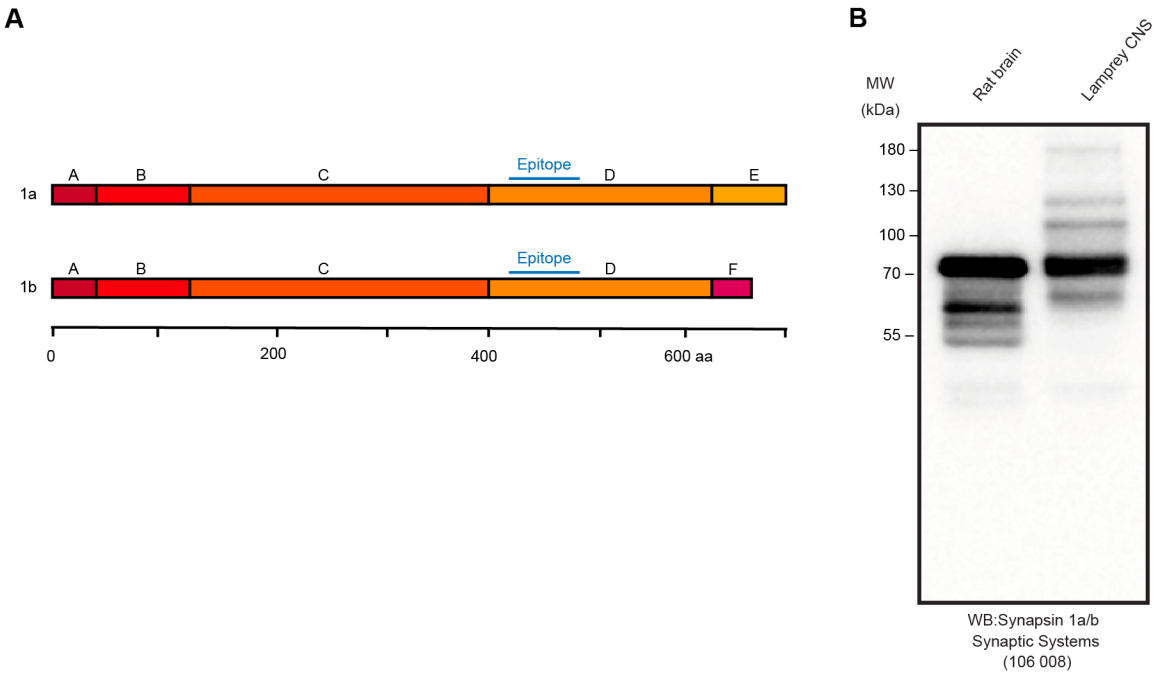
B. Diffusion coefficients of actin bound to SVs immobilized on a functional surface as described in Perego et al., 2020. Blue boxes, diffusion of actin alone; magenta boxes, diffusion of actin in the presence of EGFP-Synapsin 1.



Supplementary figure 6: Reconstitution of actin with synapsin 1 IDR-SV phases and actin asters. Representative confocal images of the reconstituted EGFP-Syn1-IDR [4 µM, 3% (w/v) PEG 8000] and SVs (3 nM, labelled with FM4-64, 1.65 µM) condensates after adding ATTO-647 labelled G-actin monomers (4 µM) at t = 0 (top) and 90 minutes (bottom). Excited wavelengths were 488 nm for EGFP-Synapsin 1 IDR, 560 nm for SVs labelled with FM4-64 and 647 for ATTO647 G-actin. Scale bar: 5 µm.



Supplementary figure 7: Actin-asters visualized in lamprey synapses. Immuno-fluorescent images showing "actin-asters" at lamprey synapses. Alexa Fluor 488-Phalloidin and SV2 were used here for labelling actin and anti-SV2 antibody, respectively. Images were acquired using a Zeiss CellDiscoverer7 with LSM900 Airyscan2 (plan apochromat 50x/1.2 objective). Scale bar, 1 µm.

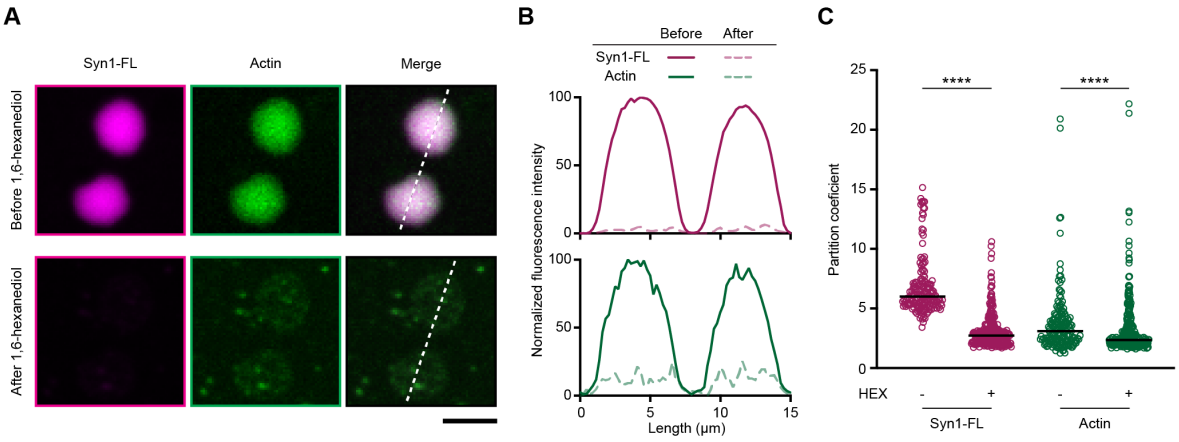


Supplementary figure 8: Characterization of antibodies against endogenous lamprey synapsin 1.

A. Scheme indicating the region of antibody binding (epitope) within the domains of synapsin 1 isoforms *a* and *b.*

B. Western Blot indicating the successful recognition of synapsin 1 in both rat and lamprey CNS lysates (rabbit anti- synapsin 1 a/b antibody; Synaptic Systems 106 008).

C.



Supplementary figure 9: 1,6-Hexanediol disperses EGFP-synapsin 1:actin condensates lacking an apparent actin polymerization.

A. Representative confocal images from the in vitro reconstituted EGFP-synapsin 1:actin:GUV assemblies before and after 1,6-hexanediol treatment. Yellow line represents the line used for plotting the intensity profile. Scale bar, 5 µm.

B. Fluorescence intensity profiles of EGFP-synapsin 1 and actin along the yellow line from a. Solid lines, fluorescence intensity before and dashed lines, after treatment with 1,6-hexanediol.

C. Quantification of synapsin 1 and actin partitioning in EGFP-synapsin 1:actin:GUV assemblies before and after 1,6-hexanediol treatment. Data from three independent reconstitutions, >1500 condensates analyzed for each condition. **** $p<0.0001$; Mann-Whitney nonparametric test.

Supplementary Table 1. Primer sequences for plasmid construction inserted into DNA sequences of 6xHis-EGFP-*Bgl*II…*Sac*I-SV40 expression cassette encoding for human Syn1-Dom.C and Syn1-IDR inserts.

#DML0022+0023 (A206K)	**FW:** CACCCAGTCC**AAG**CTGAGCAAAGACCCC **RV:** CTTTGCTCAG**CTT**GGACTGGGTGCTCAGG