

Fig. S1 Syntenin-a and -b in zebrafish, developmental expressions and effectiveness of morpholinos. (A) Sequence alignment of the two zebrafish Syntenins with human (h) Syntenin. Both proteins have similar domain organizations as the Syntenins found in other organisms, namely an N-terminal region, two PDZ domains in tandem and a C-terminal domain. Syntenin-a and Syntenin-b are 61% identical and, respectively, 69% and 61% similar to human Syntenin. The PDZ tandem, mediating most of the Syntenin interactions described so far, is highly conserved compared to the N-terminal region and the C-terminal domain. Underlined residues in the PDZ1 domain correspond to residues mutated for the PIP₂-interaction studies, Ala substituting for Lys120 and His-Glu-Gln replacing the residues 172Ser-Asp-Lys174. Underlined residues in the PDZ2 domain correspond to residues mutated for the syndecan-interaction studies, Ala substituting for Lys204 and Ser-His-Glu replacing the residues 251Lys-Asp-Thr253. (B,C) WISH illustrating the spatio-temporal expression of *syntenin-a* (B) and *syntenin-b* (C) transcripts at different stages of embryonic development, as indicated. Images correspond to lateral views except for the 2-cell stages which are animal pole views and the 100% epiboly stages which are dorsal views. The specificity of the reaction is illustrated for 72 hours post fertilization (hpf) embryos, showing no staining when a sense probe is used. Note that by 24 hpf, both signals became more restricted to the nervous systems. By 72 hpf, the Syntenin transcripts distributed in different brain areas. (D) Syntenin-a morpholinos, MOsynta and MOsyntabis, designed to inhibit the translation of *syntenin-a* mRNA are effective, as tested by in vitro transcription-translation assays, while their mismatch derivatives (mMO) have no effect on this translation.

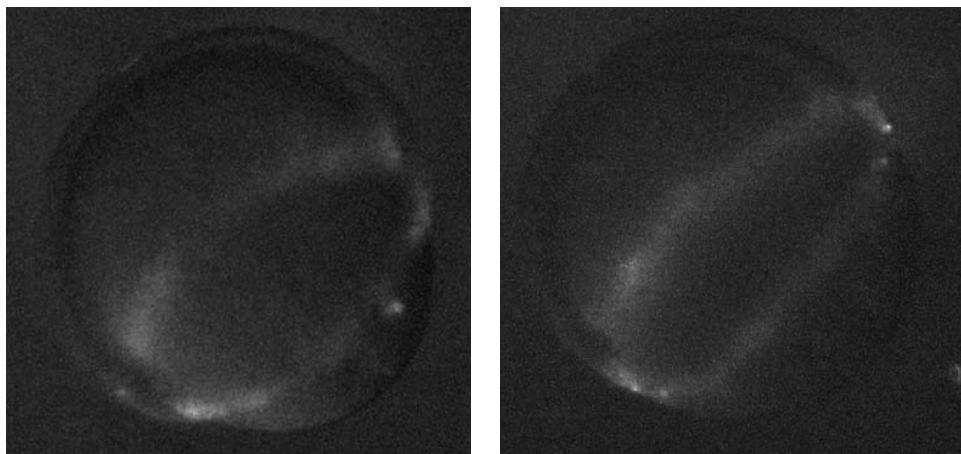


Fig. S2. The process of YSL endocytosis is not disrupted in Syntenin-a morphants. Views of embryos at 6.7 hpf incubated with Rhodamine-dextran showing the endocytic ring in control (left) and in 6 ng syntaMO-injected (right) embryos. To label endocytic vesicles, embryos at shield stage were placed in a 10 mg/ml solution of Rhodamine-labeled dextran (10.000 MW, Molecular probes). After 30 min of loading, embryos were washed and analysed immediately. Microscopic analyses were performed with a Leica Fluo Combi stereomicroscope connected to a Leica DC300 F camera.

atg aag ctc ccg tgc tgg ata acg ctg ctg ctg cag acc gca ctg gcc cag cac agc aaa tctcaa cca agt gat gat gag agc tct
M K L P C W I T L L L Q T A L A Q H S K S Q P S D D E S S
gga gat gag cct tac gat gat gaa gac ttc tac tct gga tcc ggc tct ggc tat cca gat ata aaa gtt aga ccc tct tca gtg ggc gtg
G D E P Y D D E D F Y S G S G S G Y P D I K V R P S S V G V
gtt ttc act aca gag gag ccc ctc cca ctc tcc acc acc cag gcc aca ggc cct gcc ccc tcg gcc tct cct gca gca gag cca agc agt
V F T T E E P L P L S T T Q A T G P A P S A S P A A E P S S
ccg cct cca ccc gag gta gag cgg ggt ttg ggt caa gat gtc gac agc aaa cta gaa att gag aaa aag tat gaa gag tat gac
P P P E V E R E S G L G Q D V D S K L E I E K K Y E E Y D
gag gag gaa gaa att cgg cca acg agg aag gag cag gaa cct gat act gag aaa gta caa gag cga gag caa gat cgg tcg aag gct aca
E E E I R P T R K E Q E P D T E K V Q E R E Q D R S K A T
gtt gcg cca cga ctg act gac gtt cct ata gtc ttt ttg ggc tcc tct act gtt ggt gga gcc aca gag acc act act gac ctc gag gat
V A P R L T D V P I V F L G S S T V G G A T E T T D L E D
ctc ggc ggc aga gaa gaa aca gat gaa gat ctg tat att act aaa gaa act atc gtt tta gat cca tcc agc gag aca gat atg ata
L G G R E E T D E D L Y I T K E T I V L D P S S E T D M I
acc gat gag atc aca acc acc gag ttt ata ccc acc att cca tcc acc act get aaa ccc acc agg cca cgt cct att ctg acc act
T D E I T T E F I P T T I P S T T A K P T R P R P I L T T
cca agc ccc acc gct gtc cgc ccc agg caa cca cag acc acg ccc agc aga gcc gct ccc acc gag agc agc act cgt tca gtg atg acc
P S P T A V R P R Q P Q T T P S R A A P T E S S T R S V M T
aca aca cag acg caa gtc cca gat gaa act gta aat aat gaa gtc gca ggg ccc ggt cca agt gga gat ttc gaa atc cgc gag aat gag
T T Q T Q V P D E T V N N E V A G P G P S G D F E I R E N E
gtc cgc cag aac aat gat ctg ggg cga ggc cga gcg gtc cca gga gag ccc gat ctg acc gga aac aca gta gtt gat gct gga agt tca
V R Q N N D L G R G R A V P G E P D L T G N T V D A A G S S
gct gca cag ctt cca cag aag aac atc ctc gag agg aag gag gtt ttg ata ggc gtc atc gtc gga ggt gta gtc ggc gct ctc ttc gcc
A A Q L P Q K N I L E R K E V L I A V I V G G V V G A L F A
gcg ttc ctg gta atg tta ctc gtc tac cgg atg aaa aag aaa gac gaa ggc agc tac aca ttg gag gag cct aaa caa gcc acc gtt acc
A F L V M L L V Y R M K K K D E G S Y T L E E P K Q A T V T
tac cag aaa cca gac aag cag gag gaa ttc tac gcc taa
Y Q K P D K Q E E F Y A *

Fig. S3 cDNA and protein sequence of zebrafish syndecan-3. Regions corresponding to putative attachment sites for heparan sulfate chains and to predicted transmembrane and cytoplasmic domains are underlined.

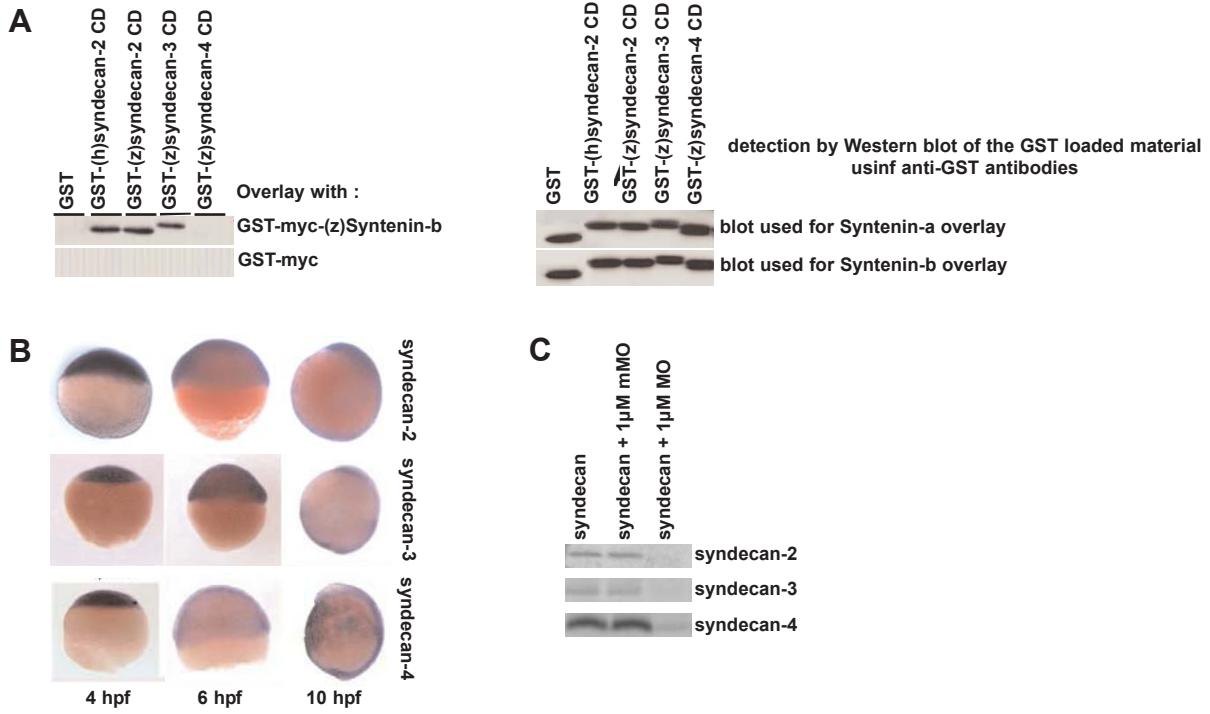


Fig. S4 (A) (Left) Ligand overlay illustrating the binding of GST-myc-Syntenin-b (upper panel) to various syndecan cytoplasmic domains and the absence of binding of GST-myc (lower panel). (Right) Control experiment by Western blot with anti-GST antibodies showing that similar amounts, of GST and GST-syndecan cytoplasmic domains were loaded for the overlays with GST-Syntenin-a (upper panel) or GST-Syntenin-b (lower panel). **(B)** WISH experiment showing the broad spatio-temporal expression of the different zebrafish syndecans at sphere (4 hpf), shield (6 hpf) and bud (10 hpf) stages. **(C)** In vitro transcription-translation experiment showing the activity of the MOs for the different syndecans and the ineffectiveness of their mismatch controls (mMO).