

Figure S1. Agrin-induced assembly of presynaptic specializations requires newly synthesized proteins in cultured neurons.

(A) Representative images showing the inhibition of agrin bead-induced presynaptic differentiation in CHX-treated cultured neurons.

(B-C) Quantitative analyses showing the effects of CHX treatment on the percentage of agrin bead-neurite contacts with presynaptic markers (B) and their intensities (C).

Scale bar = 10 μ m. Asterisks indicate the bead-neurite contact sites. Arrows indicate the localization of presynaptic markers, while arrowheads indicate the absence of these markers, at the bead-neurite contacts. Data are means \pm S.E.M.. Numbers indicated in the bar regions represent the total numbers of bead-neurite contacts measured from 3 independent experiments. * p < 0.05, ** p < 0.01, and *** p < 0.001 (unpaired Student's t-test).

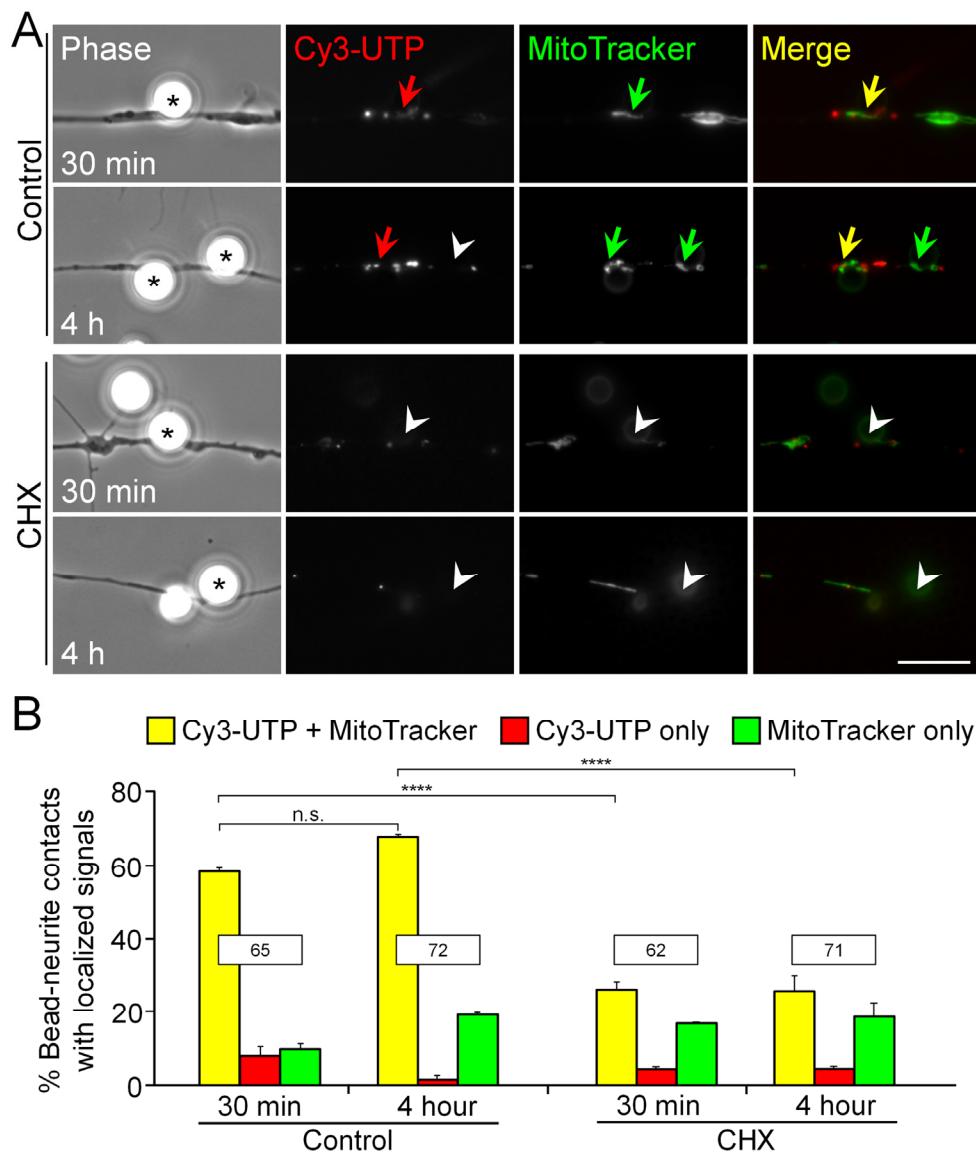


Figure S2. Agrin-induced Cy3-UTP localization and mitochondrial clustering are temporally coupled events via CHX-sensitive mechanisms.

(A) Representative images showing that both Cy3-UTP-labelled RNP granules and mitochondrial clusters were detected as early as 30-minute agrin bead stimulation, and they were significantly inhibited in CHX-treated neurons.

(B) Quantitative analyses showing the effects of CHX treatment on the localization of RNP granules and mitochondrial clusters induced by agrin beads for 30-minute or 4-hour.

Scale bar = 10 μ m. Asterisks indicate the bead-neurite contact sites. Arrows indicate the localization of Cy3-UTP granules and mitochondrial clusters, while arrowheads indicate the absence of these markers, at the bead-neurite contacts. Data are means \pm S.E.M.. Numbers indicated in the chart represent the total numbers of bead-neurite contacts measured from 3 independent experiments. n.s. = non-significant, **** p < 0.0001 (two-way ANOVA with Sidak's multiple comparison test).

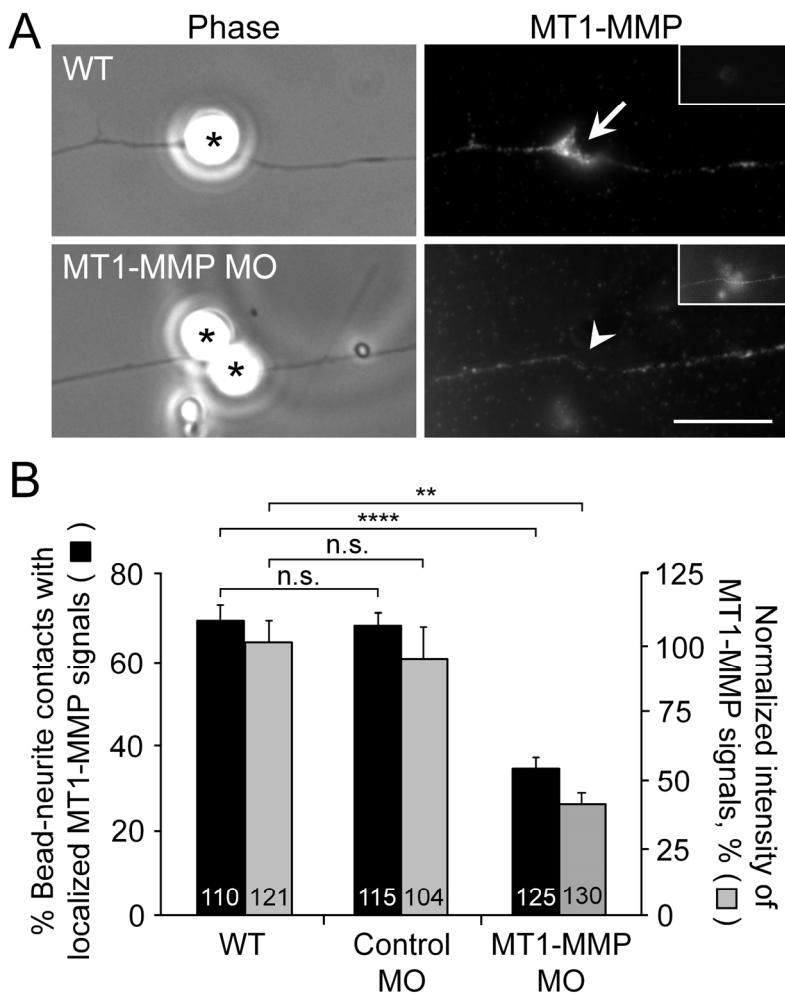


Figure S3. MT1-MMP antisense morpholino oligonucleotides inhibit agrin-induced localization of endogenous MT1-MMP proteins.

(A) Representative images showing the reduction of localized MT1-MMP immunostaining signals in MT1-MMP antisense morpholino oligonucleotide (MO)-expressing neurons, compared with that in wild-type (WT) or control MO-expressing neurons. Insets show the fluorescent dextran signals as a cell-lineage tracker.

(B) Quantitative analyses showing the reduced percentage of agrin bead-neurite contacts with localized endogenous MT1-MMP signals and their intensities in cultured neurons expressing MT1-MMP MO.

Scale bar = 10 μ m. Asterisks indicate the bead-neurite contact sites. An arrow indicates the localization of endogenous MT1-MMP proteins, while an arrowhead indicates the absence of MT1-MMP localization, at the bead-neurite contacts. Data are means \pm S.E.M.. Numbers indicated in the bar regions represent the total numbers of bead-neurite contacts measured from 3 independent experiments. n.s. = non-significant, ** p < 0.01, and *** p < 0.0001 (one-way ANOVA with Dunnett's multiple comparison test).

Probe ID	Sequence	Probe ID	Sequence
MT1-MMP_1	ccgagtatattgctgtgac	MT1-MMP_25	gcaattgtgtcaaagttgcc
MT1-MMP_2	gttattcacagtgcata	MT1-MMP_26	gaatacaaaacatctcccc
MT1-MMP_3	ttatccactgatctgttc	MT1-MMP_27	tccatccattacacgtttat
MT1-MMP_4	ctgcagagcaagaatagggt	MT1-MMP_28	gaattgccaaataggcatag
MT1-MMP_5	atcctgtgcgactgagtaaa	MT1-MMP_29	ttaatggagctggaaagacc
MT1-MMP_6	aaacaggcaaattcaggctg	MT1-MMP_30	acaatttgcacatccttcg
MT1-MMP_7	tctgggctgaatttggagg	MT1-MMP_31	atcaaacaccccagtgcatt
MT1-MMP_8	ggcagatattccatactgttg	MT1-MMP_32	gataccctggctctaaaaca
MT1-MMP_9	catagaacttctgcata	MT1-MMP_33	agcatcaattctgcgttag
MT1-MMP_10	actgtcaaacttccatgtca	MT1-MMP_34	ttccatttggcatccaatat
MT1-MMP_11	cttcatttcattgtcttcgt	MT1-MMP_35	taatacttggfaccctgaa
MT1-MMP_12	caaatttgtcaggactcca	MT1-MMP_36	ctctcatctctcgtaaac
MT1-MMP_13	aaatgtgatgtccttgtct	MT1-MMP_37	ttgacaggttgggtattc
MT1-MMP_14	cgcctcataggtagaatact	MT1-MMP_38	ctttgatggagctggatg
MT1-MMP_15	tcacactctccaaacttta	MT1-MMP_39	gctgctgatttgtgaacttc
MT1-MMP_16	gcatgatgtcagcatgtta	MT1-MMP_40	cgaccaaaaacggatttcggg
MT1-MMP_17	ccatcaaaaggagtgtc	MT1-MMP_41	caggaactacaatggctgca
MT1-MMP_18	tatgcatgtgcaaaaagcc	MT1-MMP_42	ttgcctgaagagcacaacag
MT1-MMP_19	ggttctgcagagtcaaagt	MT1-MMP_43	ccagtatagaatccttcg
MT1-MMP_20	ccaccaggaacagatcatta	MT1-MMP_44	agttaggtgttcacacttt
MT1-MMP_21	aatgttccaaaccgagagca	MT1-MMP_45	tggagaggcagtaattcagg
MT1-MMP_22	gagccataattgcagatgga	MT1-MMP_46	attgcattageacttctc
MT1-MMP_23	aatttagtgtgtccatccac	MT1-MMP_47	ctggcagtgaaggggttaaa
MT1-MMP_24	ggatcatgtgggttatcatc	MT1-MMP_48	ttgagagaacaggccataat

Table S1. A set of 48 unique sequences of Quasar 670-conjugated Stellaris RNA FISH probes against *Xenopus* MT1-MMP mRNA.