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Supplemental information

Molecular and functional architecture of striatal dopamine release sites

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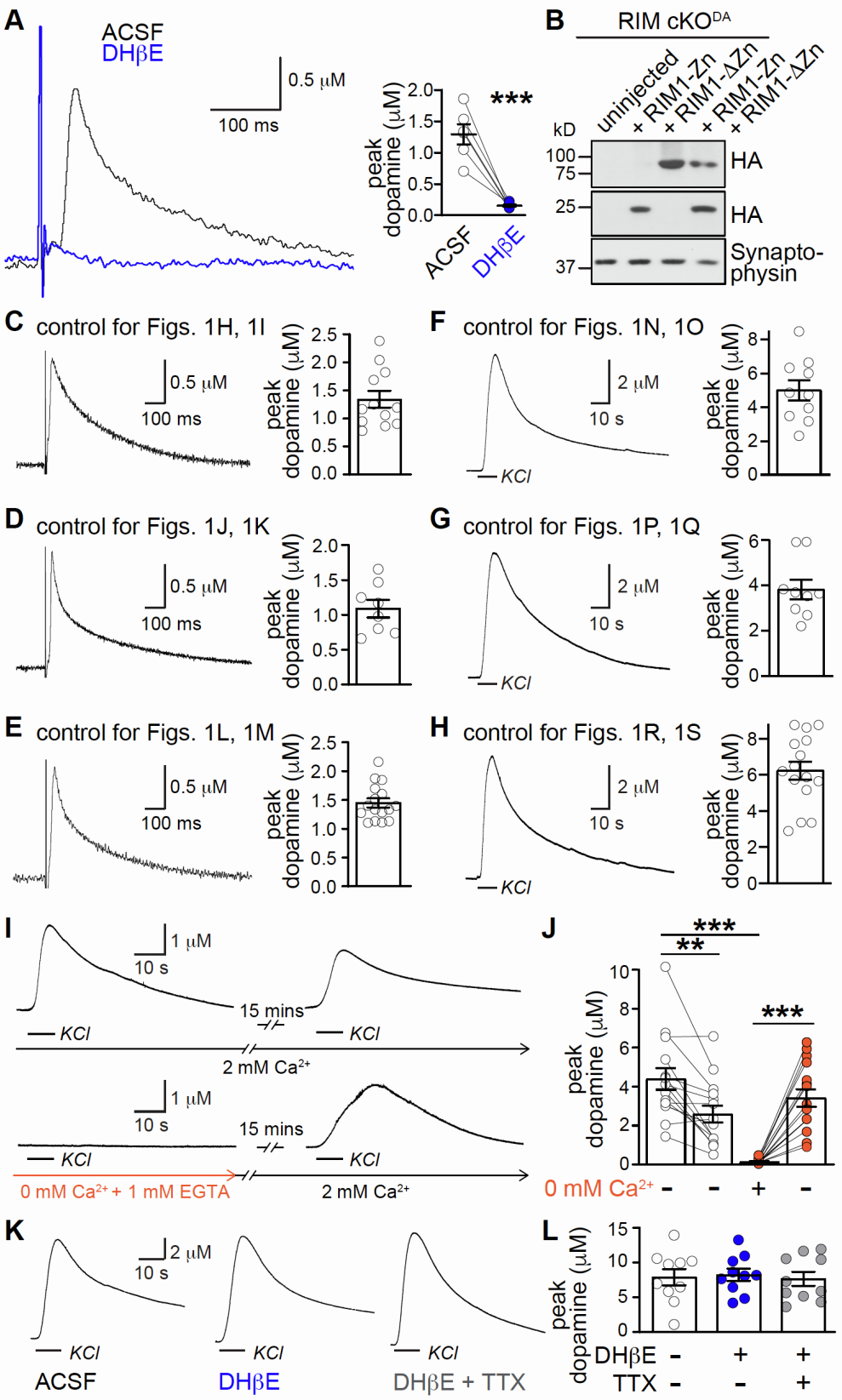

Figure S1. Additional data for RIM rescue experiments and for KCl-triggered release,

related to Fig. 1

(A) Sample traces (single sweeps) and quantification of dopamine release evoked by a 90 µA electrical stimulus in wild type slices before and after application of DHβE, 6 slices/3 mice.
(B) Western blot of striatal lysates from RIM cKO^{DA} mice injected with cre-dependent AAV viruses into the midbrain to assess expression of RIM1-Zn, RIM1-ΔZn, or RIM1-Zn + RIM1-ΔZn. HA antibodies were used to assess expression of rescue constructs, and synaptophysin antibodies were used to blot for loading on separate gels (with 6.5 times less material).
(C-E) Sample traces (single sweeps) and quantification of dopamine release in the dorsolateral striatum evoked by a 90 µA electrical stimulus in brain slices of unrelated control mice. For rescue experiments shown in Figs. 1 and 8, stable amperometric recordings were established with unrelated control slices first (using the same carbon fiber) on each day before proceeding with assessment of rescue, C: 12/4; D: 8/3; E: 16/4.
(F-H) Same as A-C but for a local 100 mM puff of KCl, F: 10/4; G: 9/3; H: 15/4.
(I-J) Sample traces (I) and quantification of peak dopamine amplitudes (J) evoked by 2 consecutive 100 mM KCl-puffs at an interval of 15 min. Slices were incubated either in 2 mM Ca^{2+} throughout the experiment or in 0 mM Ca^{2+} + 1 mM EGTA containing ACSF for the first pulse and then in 2 mM Ca^{2+} for the second pulse, 2/2 mM Ca^{2+} 15/4; 0/2 mM Ca^{2+} 15/4.
(K-L) Sample traces (K) and quantification of peak dopamine (L) evoked by a 100 mM KCl puff in slices of control mice in ACSF, DHβE, or DHβE + TTX, 10/4 each.
Data are mean ± SEM, ** $p < 0.01$, *** $p < 0.001$ as assessed by paired t-test in A, repeated measures one-way ANOVA followed Sidak's multiple comparisons tests in J and one-way ANOVA followed Sidak's multiple comparisons tests in L. For numerical values of means and errors, exact p-values and the number of observations used for statistics for this and all following supplementary figures, see corresponding Tables S1-S8.

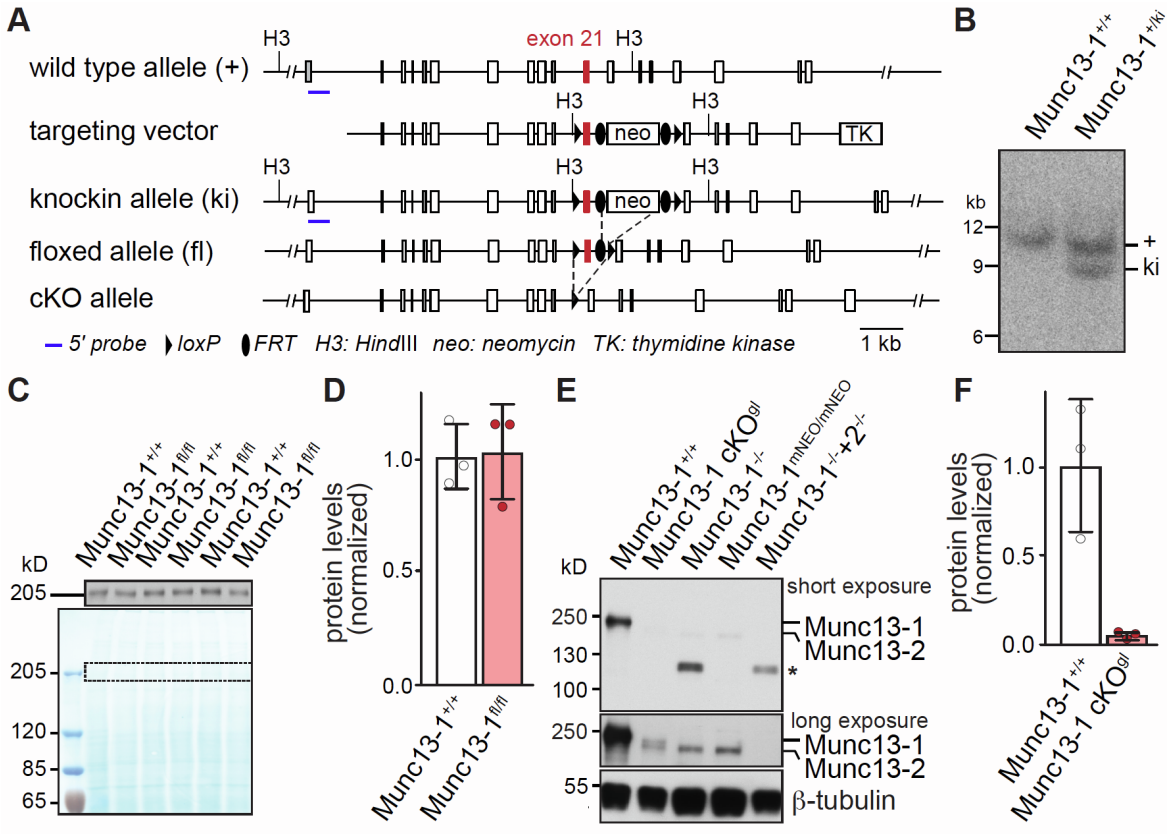

Figure S2. Generation of Munc13-1 floxed mice, related to Fig. 3

(A) Schematic of the Munc13-1 wild type allele, the targeting vector, the knock-in allele (ki) after homologous recombination, the floxed allele after flp recombinase-mediated excision of the neomycin resistance cassette (neo), and the Munc13-1 cKO allele after cre-mediated recombination. The targeted exon 21 is shown in red. A 5' probe (blue bar) was used for Southern blot in B.

(B) Southern blotting after *Hind*III digest of genomic DNA purified from a positive embryonic stem (ES) cell clone produced 9.8 kb Munc13-1 wild type and 8.4 kb Munc13-1 ki band.

(C, D) Western blot (C) and estimation of Munc13-1 levels (D) in P0 whole brain homogenates from wild type (Munc13-1$^{+/+}$) and homozygote floxed (Munc13-1$^{fl/fl}$) littermate mice, 5 µg of protein were loaded per lane of a 3-8% gradient Tris-Acetate gel and bands were detected by chemiluminescence. The scanned Western blot (top) and a protein stain of the nitrocellulose membrane (bottom, dotted rectangle indicates the region represented in the upper panel in C),

and estimated Munc13-1 protein levels normalized to total measured protein are shown in D, 3 mice each.

(E) Western blot analysis of Munc13-1 levels from E18 or P0 whole brain tissue homogenates from different Munc13-1 knockout mouse lines. Lane 1, wild type (Munc13-$1^{+/+}$); lane 2, homozygote germline recombined allele of the new Munc13-1 floxed (Munc13-1 cKO^{gl}) mice; lane 3, Munc13-1 constitutive knockout (Munc13-$1^{-/-}$) published in (Augustin et al., 1999); lane 4, Munc13-1 constitutive knockout (Munc13-$1^{mNeo/mNeo}$) published in (Rhee et al., 2002); lane 5, Munc13-1/Munc13-2 double knockout (Munc13-$1^{-/-}$+$2^{-/-}$) published in (Varoqueaux et al., 2002). Munc13-1 cKO^{gl} exhibit a severe reduction of Munc13-1 protein levels comparable to that in well-characterized constitutive Munc13-1 knockout lines. Note that the Munc13-$1^{-/-}$ (Augustin et al., 1999) mice express a truncated Munc13-1 protein product (*) that is detected by antibodies that bind N-terminal epitopes as discussed in Fig. 1 of (Man et al., 2015). A long exposure (middle) reveals that the anti-Munc13 antibody detects a protein band with slightly lower molecular weight in Munc13-1 knockout samples, which corresponds to ubMunc13-2 (Munc13-2) and is absent in the Munc13-$1^{-/-}$+$2^{-/-}$ sample. Munc13-1 cKO^{gl} animals express very low levels of a Munc13-1 protein product, likely corresponding to a protein lacking both exon 21 and 22, for which transcripts could be detected in brains of Munc13-1 cKO^{gl} mice using reverse transcriptase PCR (not shown, see methods).

(F) Estimation of Munc13-1 levels in P0 whole brain homogenates from Munc13-$1^{+/+}$ and littermate Munc13-1 cKO^{gl} mice indicates that the residual Munc13-1 protein is reduced to at most a few % of Munc13-$1^{+/+}$ levels, 3 mice each.

Estimated protein levels in D and F are mean ± SEM.

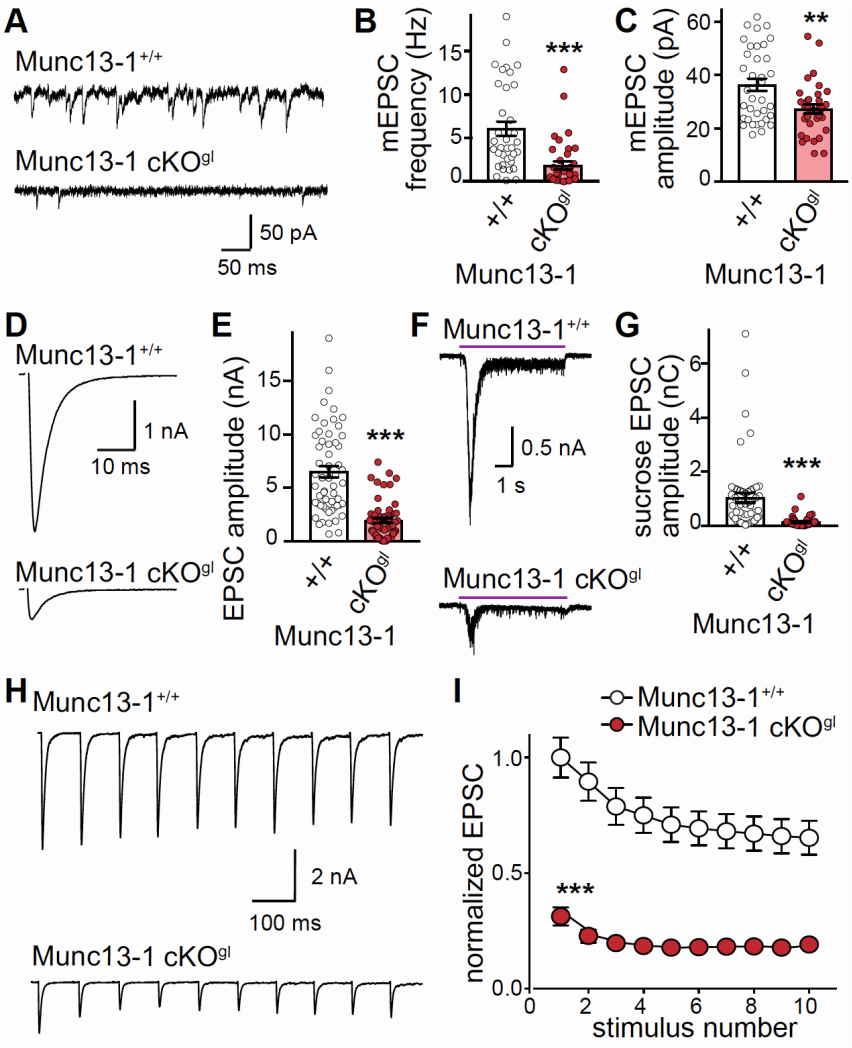

Figure S3. Munc13-1 deletion strongly impairs neurotransmitter release in autaptic hippocampal cultures, related to Fig. 3

(A-C) Sample traces (A) and quantification of miniature EPSC (mEPSC) frequency (B) and amplitude (C) recorded in presence of tetrodotoxin (TTX). B: Munc13-1$^{+/+}$, 36 neurons/3 independent cultures; Munc13-1 cKO^{gl} (homozygote germline recombined mice generated from the new Munc13-1 floxed mice), 39/3; C: Munc13-1$^{+/+}$ 36/3; Munc13-1 cKO^{gl} 33/3.

(D, E) Sample traces (D) and quantification of average EPSC amplitudes (E) evoked by a 2-ms depolarization to 0 mV, Munc13-1$^{+/+}$ 57/3; Munc13-1 cKO^{gl} 56/3.

(F, G) Sample traces (F) and quantification of EPSCs (G) evoked by application of 500 mM sucrose, Munc13-1$^{+/+}$ 54/3; Munc13-1 cKO^{gl} 53/3.

(H, I) Sample traces (H) and quantification (I) of EPSCs triggered by 10 stimuli at 10 Hz. Quantification in I is shown normalized to the mean of the first EPSC of Munc13-1$^{+/+}$, Munc13-1$^{+/+}$ 40/3; Munc13-1 cKO^{gl} 44/3.

Data are mean ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as assessed by Mann-Whitney tests in B, C, E, G; two-way ANOVA (*** $p < 0.001$ for genotype and stimulus number, n.s. for interaction) followed by Sidak's multiple comparisons tests in I (*** $p < 0.001$ for all stimuli).

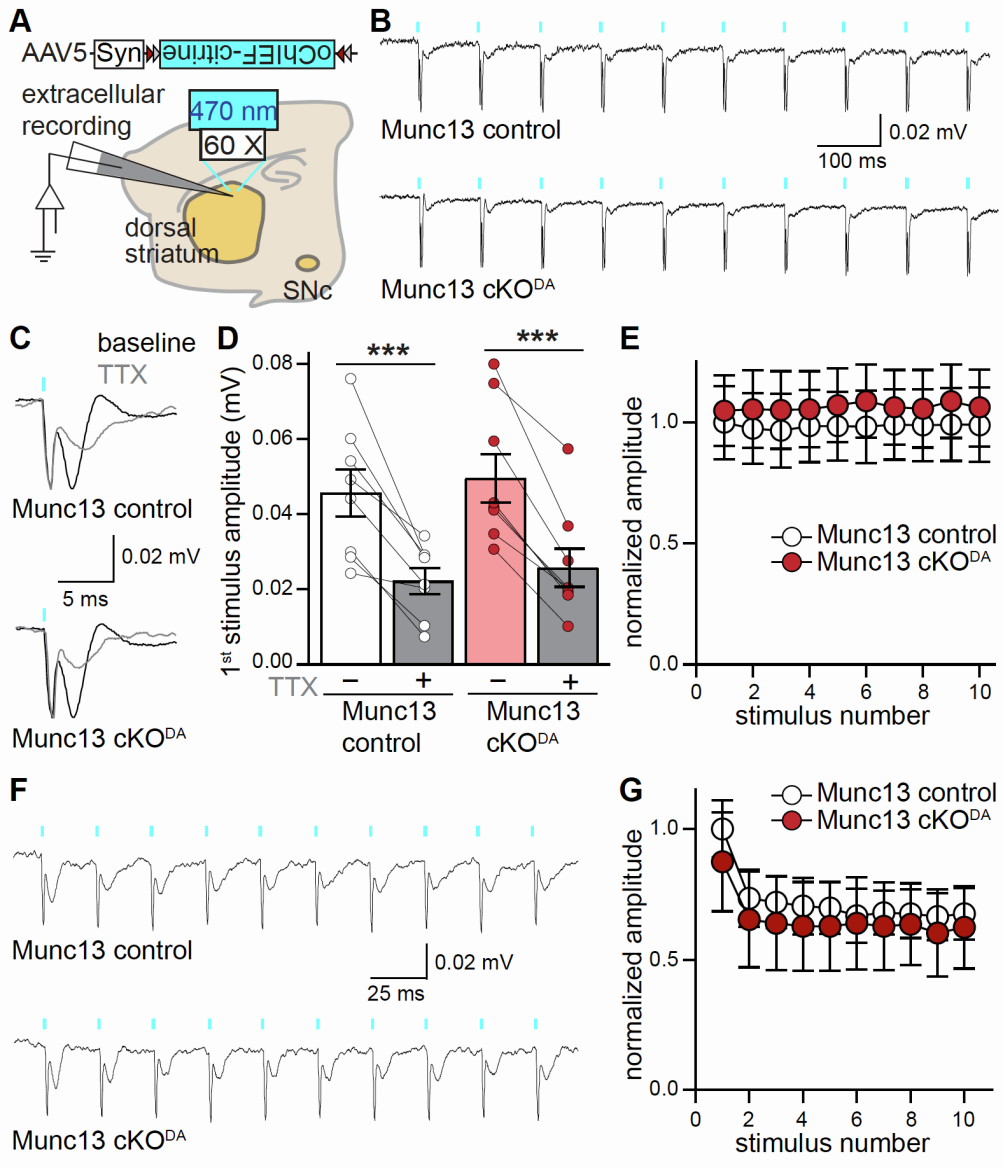

Figure S4. Dopamine axons of Munc13 cKO^{DA} mice fire action potentials, related to Fig. 3

(A) Schematic outlining cre-dependent expression of oChIEF for dopamine neuron activation and recording of extracellular field potentials in dorsolateral striatum.

(B-E) Sample traces (B, C, average of 100 sweeps) and quantification (D, E) of extracellular potentials evoked by ten optogenetic stimuli at 10 Hz. In C, the extracellular potential for the first stimulus of a 10-Hz train before (black) and after 1 µM TTX (grey) is magnified (the full train in B and the first response in C are from different slices). Quantification of extracellular potential amplitudes evoked by the 1st stimulus of a 10 Hz train before and after TTX is shown in D, and

the extent of TTX inhibition is similar between Munc13 control and cKO^{DA}. The normalized extracellular potential amplitudes for 10 Hz after normalization to the mean first response amplitude of Munc13 control are shown in E, 8 slices/6 mice each.

(F, G) Same as B and E but for the first 10 stimuli of a 40 Hz train. The amplitudes are decreased after the first stimulus because dopamine axons do not reliably follow optogenetic activation at frequencies above 20 Hz (Liu et al., 2018), 6/5 each.

Data are mean ± SEM, *** $p < 0.001$ as assessed by repeated measures one-way ANOVA followed by Sidak's multiple comparisons tests in D; two-way ANOVA (n.s. for genotype, stimulus number and interaction) in E; two-way ANOVA (*** $p < 0.001$ for stimulus number, n.s. for genotype and interaction) in G.

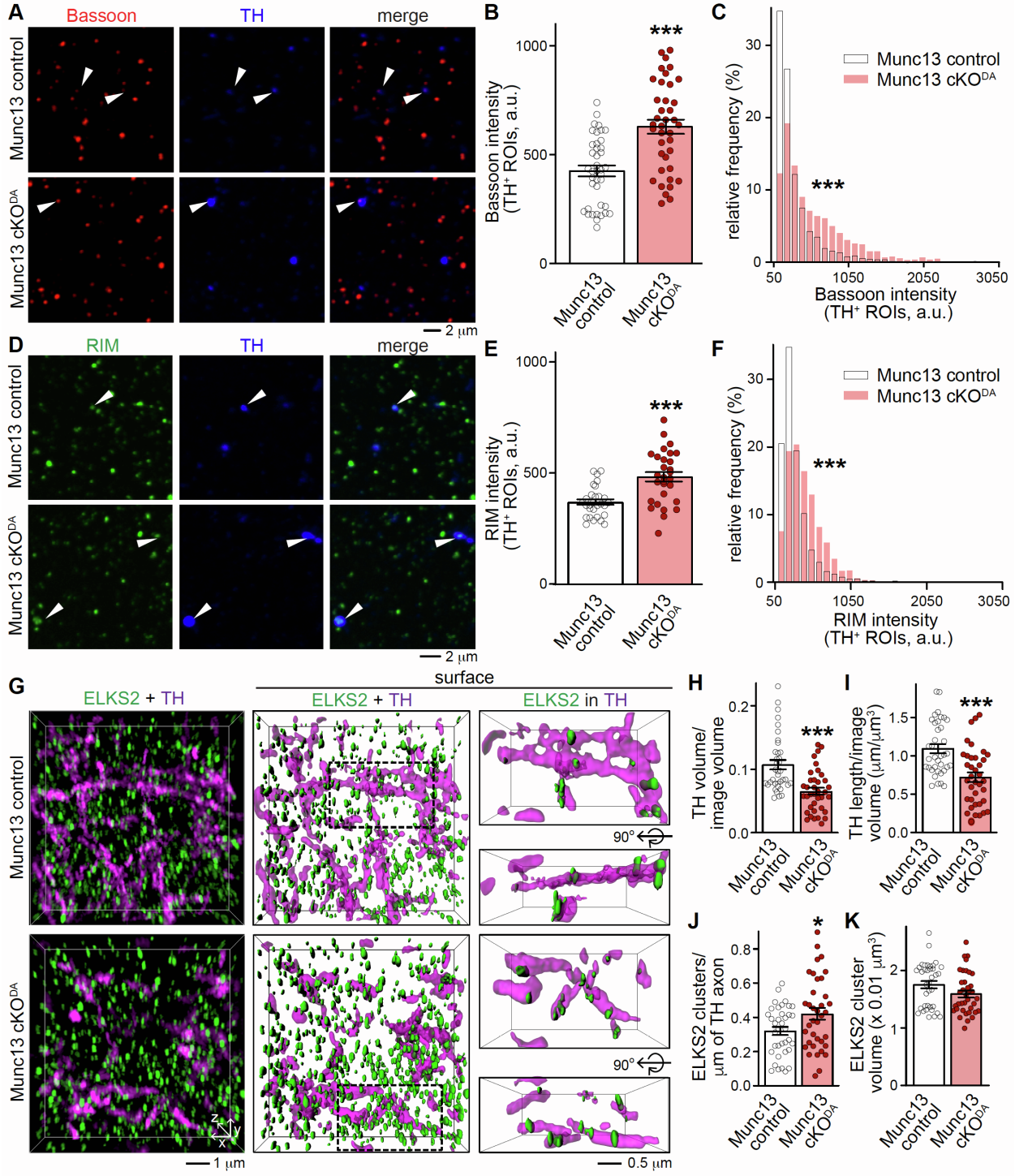

Figure S5. Dopamine axon and release site structure after Munc13 ablation, related to Fig. 5

(A-C) Sample confocal images (A) and quantification (B, C) of striatal synaptosomes stained for the active zone marker Bassoon and the dopamine axon marker TH. Quantification of Bassoon

intensity within TH^+ ROIs pooled by images (B) and frequency distribution histogram for all Bassoon intensities within TH^+ ROIs (C) are shown, B: Munc13 control 40 images/4 mice; Munc13 cKO^{DA} 39/4; C: Munc13 control 3035 ROIs/40 images/4 mice, Munc13 cKO^{DA} 1745/39/4.

(D-F) Sample confocal images (D) and quantification (E, F) of striatal synaptosomes stained for the active zone scaffold RIM (green) and the dopamine axon marker TH (blue). Quantification of RIM intensity within TH^+ ROIs pooled by images (E) and frequency distribution histogram for RIM intensities within TH^+ ROIs (F) are shown, E: 29 images/3 mice each; F: Munc13 control 4766 ROIs/29 images/3 mice, Munc13 cKO^{DA} 1639/29/3.

(G) Sample 3D-SIM images of dorsolateral striatum stained for ELKS2 and TH. Volume rendered images (left, 10 x 10 x 2 μm^3) with all ELKS2; surface rendering of the same images with all ELKS2 (middle) and magnified views (right, 5 x 3 x 2 μm^3, frontal view and rotated by +90° along the x axis) of only ELKS2 within TH (> 40% volume overlap) are shown.

(H-K) Quantification of the experiment shown in G displaying TH volume per image (H), TH axon length per image volume (I), density (J) and volume (K) of ELKS2 clusters (J) localized within TH axons (> 40% volume overlap), 37 images/3 mice each.

Data are mean ± SEM, * $p < 0.05$, *** $p < 0.001$ as assessed by unpaired t-tests in B, E, H, I, J, K and Kolmogorov-Smirnov tests in C, F.

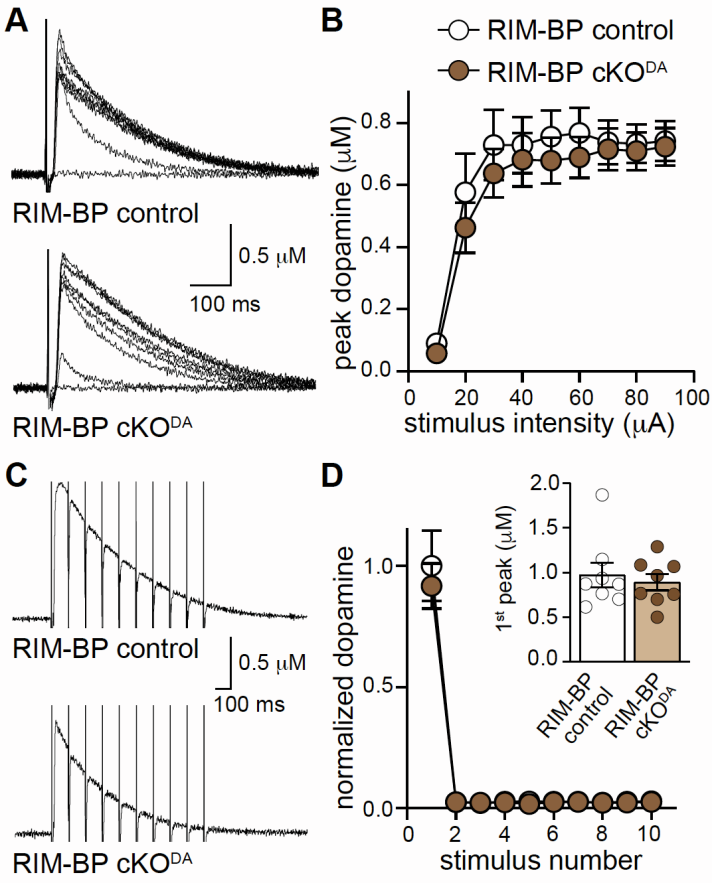

Figure S6. Ablation of RIM-BP1 and RIM-BP2 does not detectably impair electrically evoked dopamine release, related to Fig. 6

(A, B) Sample traces (A, single sweeps) and quantification of peak amplitudes (B) of dopamine release evoked by electrical stimulation (10-90 µA single electrical pulses at increasing stimulation intensities), 16 slices/5 mice each.

(C, D) Sample traces (C, average of 4 sweeps) and quantification of peak amplitudes (D) of dopamine release normalized to the first peak amplitude of RIM-BP control in response to 10 stimuli at 10 Hz train, inset in D shows the peak dopamine amplitude for 1st stimulus, 8/4 each. Data are mean ± SEM, *** $p < 0.001$ as assessed by two-way ANOVA (*** for stimulus, n.s. for genotype and interaction) followed by Sidak's multiple comparisons tests in B, D and Mann-Whitney test for inset in D.

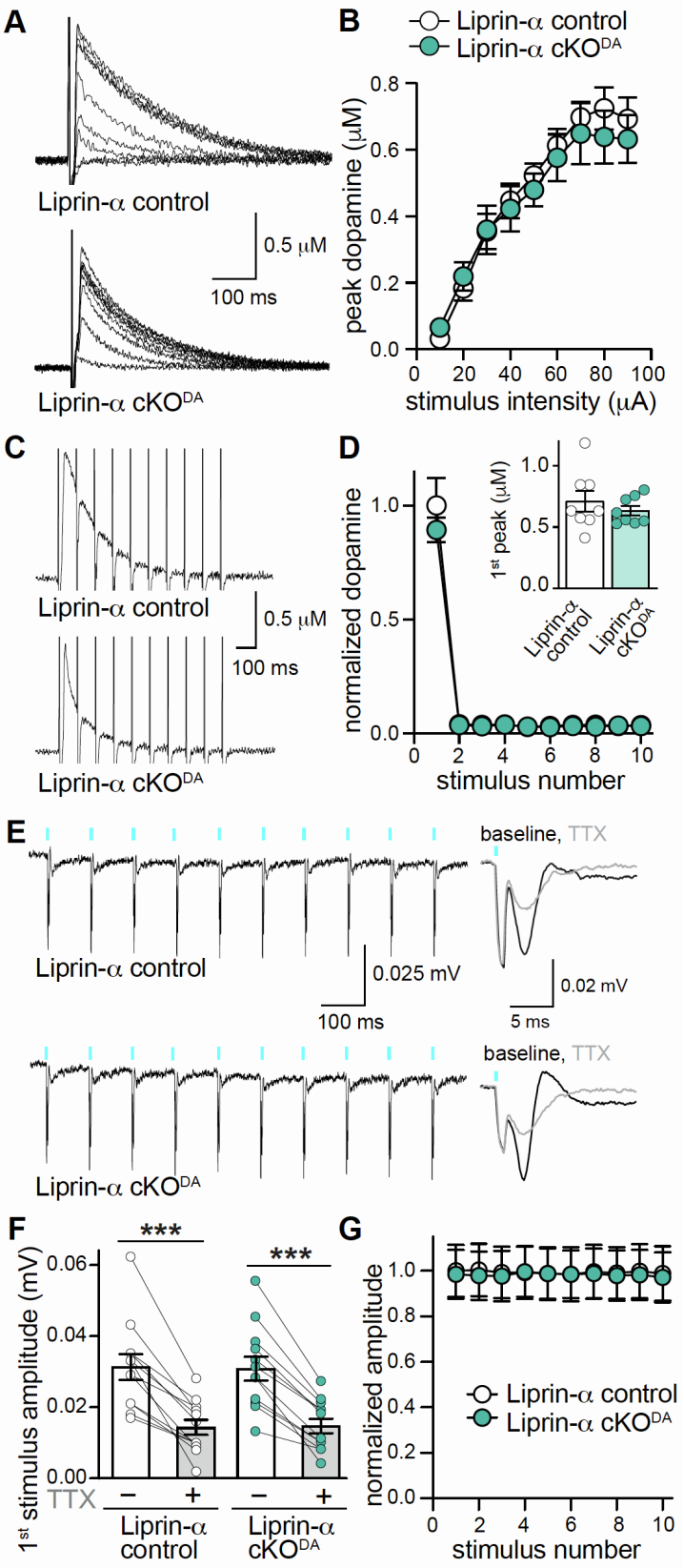

Figure S7. Additional electrophysiological characterization of Liprin-α cKO^{DA} mice,

related to Fig. 7

(A, B) Sample traces (A, single sweeps) and quantification of peak amplitudes (B) of dopamine release evoked by electrical stimulation (10-90 µA single electrical pulses at increasing stimulation intensities), 12 slices/8 mice each.

(C, D) Sample traces (C, average of 4 sweeps) and quantification of peak amplitudes (D) of dopamine release normalized to the first peak amplitude of Liprin-α control in response to 10 electrical stimuli at 10 Hz train, inset in D shows the peak dopamine amplitude for the 1st stimulus, 8/3 each.

(E-G) Sample traces (E, average of 100 sweeps) and quantification (F, G) of extracellular potentials evoked by 10 stimuli at 10 Hz of optogenetic stimulation. In E (inset, right), the extracellular potential for the first stimulus of a 10-Hz train before (black) and after 1 µM TTX (grey) is magnified, the full train (left) and inset (right) are from different slices. Quantification of extracellular potential amplitudes evoked by the 1st stimulus of a 10 Hz train before and after TTX is shown in F, and the extent of TTX inhibition is similar between Liprin-α control and cKO^{DA}. The normalized extracellular potential amplitudes for 10 Hz after normalization to the mean first response amplitude of Liprin-α control are shown in G, 12/3 each.

Data are mean ± SEM, ** $p < 0.01$, *** $p < 0.001$ as assessed by two-way ANOVA (*** for stimulus intensity/stimulus number, n.s. for genotype, interaction) followed by Sidak's multiple comparisons tests in B, D; $p > 0.05$ unpaired t-test for inset in D; or repeated measures one-way ANOVA in F followed by Sidak's multiple comparisons tests for specific pairs; and two-way ANOVA (n.s. for genotype, stimulus and interaction) in G.

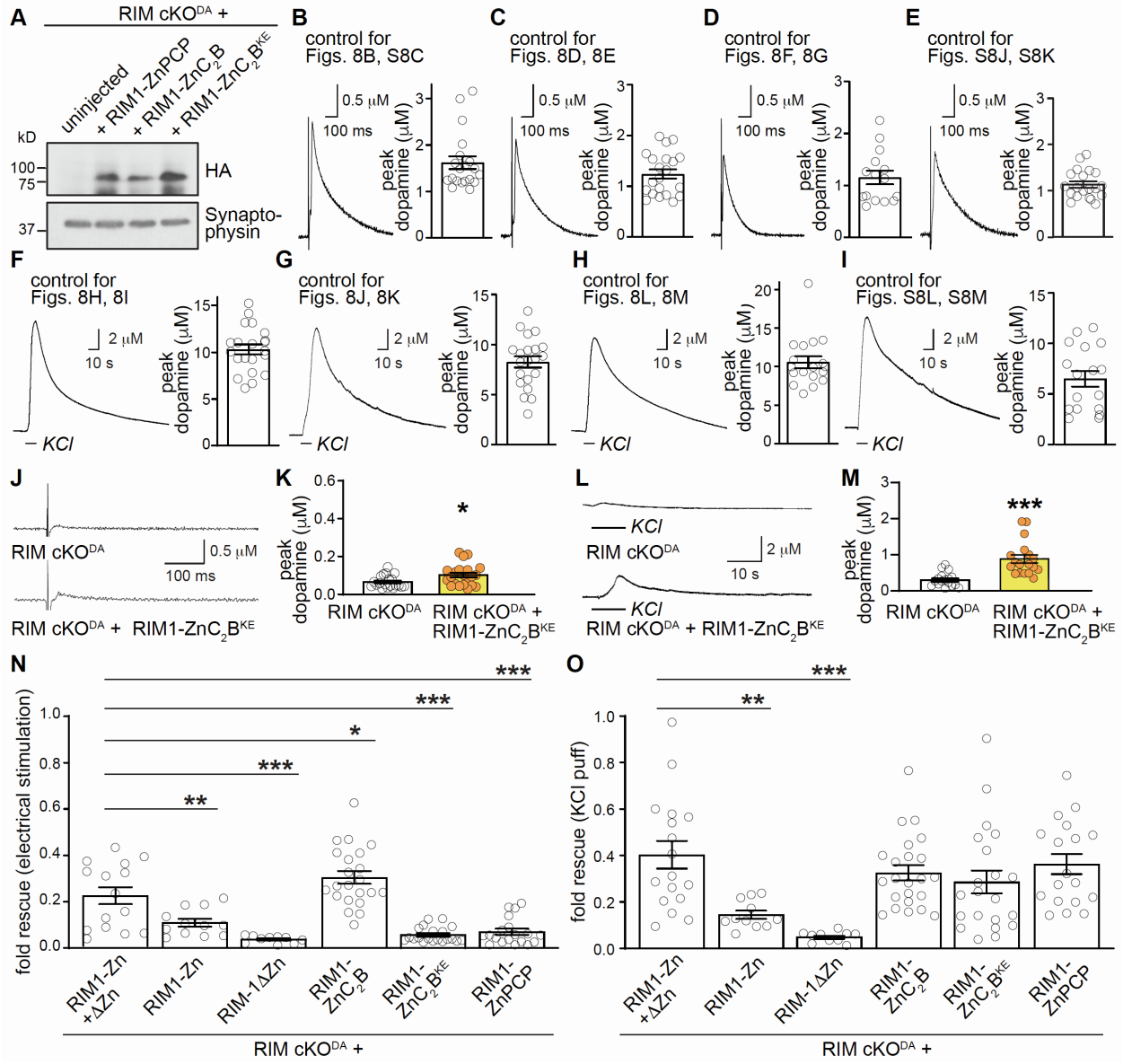

Figure S8. Control recordings and expression analyses for rescue experiments and comparison of rescue efficacies, related to Fig. 8

(A) Western blot of striatal lysates from RIM cKO^{DA} mice injected with cre-dependent AAV viruses into the midbrain to assess expression of RIM1-ZnPCP, RIM1-ZnC_2B, and RIM1-ZnC_2B^{KE}. HA antibodies were used to assess expression of rescue constructs, and synaptophysin antibodies were used to blot for loading on separate gels (with 6.5 times less material).

(B-E) Sample traces (single sweeps) and quantification of dopamine release in the dorsolateral

striatum evoked by a 90 µA electrical stimulus in brain slices of unrelated control mice recorded on the same days as the experiment shown in Figs. 8 and S8, B: 20 slices/4 mice; C: 21/5; D: 15/4; E: 20/4.

(F-I) Same as B-E but for a local 100 mM KCl-puff, F: 21/4; G: 21/5; H: 18/4; I: 17/4.

(J, K) Sample traces (single sweeps, J) and quantification of peak dopamine (K) evoked by a 90 µA electrical stimulus in brain slices, RIM cKO^{DA} 20/4; RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} 21/4.

(L, M) Sample traces (L) and quantification (M) of peak dopamine evoked by a local 100 mM KCl-puff, RIM cKO^{DA} 17/4; RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} 18/4.

(N) Rescue of peak dopamine evoked by a 90 µA electrical stimulus for each rescue experiment in Figs. 1, S1, 8, and S8, normalized to the average of the unrelated control mice used for all rescue conditions, RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn 15/4; RIM cKO^{DA} + RIM1-Zn 12/4; RIM cKO^{DA} + RIM1-ΔZn 10/3; RIM cKO^{DA} + RIM1-ZnC$_2$B 22/5; RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} 20/5; RIM cKO^{DA} + RIM1-ZnPCP 19/4; control 72/20 (used for normalization). Rescue data from the RIM cKO^{DA} + RIM1- ZnC$_2$B vs. RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} experiment (Figs. 8D, 8E) was used for the summary plot.

(O) Same as N but for rescue estimated by KCl evoked dopamine release, RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn 17/4; RIM cKO^{DA} + RIM1-Zn 11/4; RIM cKO^{DA} + RIM1-ΔZn 10/3; RIM cKO^{DA} + RIM1-ZnC$_2$B 23/5; RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} 21/5; RIM cKO^{DA} + RIM1-ZnPCP 18/4; control 73/20 (used for normalization). Rescue data from the RIM cKO^{DA} + RIM1- ZnC$_2$B vs. RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} experiment (Figs. 8J, 8K) was used for the summary plot.

Data are mean ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as assessed by Mann-Whitney tests in K, M and one-way ANOVA followed by Dunnett's multiple comparisons tests in N and O, where all comparisons were made to RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn.

Table S1. Means, number of observations and statistical testing, related to Figs. 1 and S1

For each dataset, mean ± SEM are shown in the corresponding figure. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size**	**Statistical test** **p-value**
1D Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM control = 1.50 ± 0.13 (n = **17 slices**, 3 mice) RIM cKO^{DA} = 0.07 ± 0.01 (n = **17 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
1F Peak dopamine (µM) evoked by a KCl puff	RIM control = 6.45 ± 0.67 (n = **20 slices**, 3 mice) RIM cKO^{DA} = 0.40 ± 0.05 (n = **20 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
1I Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM cKO^{DA} = 0.09 ± 0.01 (n = **12 slices**, 4 mice) RIM cKO^{DA} + RIM1-Zn = 0.14 ± 0.02 (n = **12 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p = 0.07
1K Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM cKO^{DA} = 0.053 ± 0.009 (n = **9 slices**, 3 mice) RIM cKO^{DA} + RIM1-ΔZn = 0.050 ± 0.006 (n = **10 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p = 0.8
1M Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM cKO^{DA} = 0.07 ± 0.01 (n = **15 slices**, 4 mice) RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn = 0.28 ± 0.05 (n = **15 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p = 0.0003
1O Peak dopamine (µM) evoked by a KCl puff	RIM cKO^{DA} = 0.19 ± 0.04 (n = **11 slices**, 4 mice) RIM cKO^{DA} + RIM1-Zn = 1.09 ± 0.13 (n = **11 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
1Q Peak dopamine (µM) evoked by a KCl puff	RIM cKO^{DA} = 0.25 ± 0.06 (n = **10 slices**, 3 mice) RIM cKO^{DA} + RIM1-ΔZn = 0.38 ± 0.05 (n = **10**	Mann-Whitney test p = 0.08

	slices, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	
1S Peak dopamine (μM) evoked by a KCl puff	RIM cKO^{DA} = 0.17 ± 0.03 (n = **16 slices**, 4 mice) RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn = 2.98 ± 0.44 (n = **17 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
S1A Peak dopamine (μM) evoked by a 90 μA electrical stimulus before and after DHβE	ACSF = 1.29 ± 0.16 (n = **6 slices**, 3 mice) DHβE = 0.14 ± 0.02 (n = **6 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Paired t-test p = 0.001
S1C Peak dopamine (μM) evoked by a 90 μA electrical stimulus	Control = 1.34 ± 0.15 (n = 12 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S1D Peak dopamine (μM) evoked by a 90 μA electrical stimulus	Control = 1.09 ± 0.13 (n = 8 slices, 3 mice) Each circle in the figure represents a slice recording.	not applicable
S1E Peak dopamine (μM) evoked by a 90 μA electrical stimulus	Control = 1.45 ± 0.08 (n = 16 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S1F Peak dopamine (μM) evoked by a KCl puff	Control = 5.00 ± 0.60 (n = 10 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S1G Peak dopamine (μM) evoked by a KCl puff	Control = 3.83 ± 0.43 (n = 9 slices, 3 mice) Each circle in the figure represents a slice recording.	not applicable
S1H Peak dopamine (μM) evoked by a KCl puff	Control = 6.23 ± 0.51 (n = 15 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S1J Peak dopamine (μM) evoked by a KCl puff	(a) KCl evoked 1st response in ACSF with 2 mM Ca^{2+} = 4.46 ± 0.56 (b) KCl evoked 2nd response in ACSF with 2 mM Ca^{2+} after 15 min = 2.64 ± 0.44 (c) KCl evoked 1st response in ACSF with 0 mM Ca^{2+} + 1 mM EGTA = 0.14 ± 0.03 (d) KCl evoked 2nd response after switching (c) to ACSF with 2 mM Ca^{2+} = 3.47 ± 0.46 Each circle in the figure represents a slice recording that has been used for sample size in statistics and sample size was **15 slices** from 4 mice each.	Repeated measures one-way ANOVA (p < 0.0001, F = 32.05) followed by Sidak's multiple comparisons tests for specific pairs: a vs. b (p = 0.0029); a vs. c (p < 0.0001); c vs. d (p < 0.0001); and b vs. d (p = 0.1).
S1L Peak dopamine (μM) evoked by a KCl puff	ACSF = 7.98 ± 1.17 (n = **10 slices**, 4 mice) DHβE = 8.31 ± 0.87 (n = **10 slices**, 4 mice) DHβE + TTX = 7.75 ± 1.01 (n = **10 slices**, 4 mice)	One-way ANOVA (p = 0.9, F = 0.07609) followed by Sidak's multiple comparisons tests for specific pairs:

	Each circle in the figure represents a slice recording that has been used for sample size in statistics.	ACSF vs. DHβE ($p = 0.9$) and ACSF vs. DHβE + TTX ($p = 0.9$).

Table S2. **Means, medians, number of observations and statistical testing, related to Fig. 2**

For each dataset, mean ± SEM are shown in the corresponding figure. For image data, median values are reported here in addition to mean ± SEM. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size** **Median (for imaging data only)**	**Statistical test** **p-value**
2C Munc13-1 clusters/image volume (#/μm^3)	Munc13-1-EYFP = 3.598 ± 0.033, median: 3.611 (n = **88 images**, 3 mice) wild type = 0.017 ± 0.001, median: 0.016 (n = **86 images**, 3 mice) The distribution of values for each image is shown as violin plots with superimposed mean ± SEM for Munc13-1-EYFP. All values for wild type were very close to zero and are shown as scatter plots, where each circle represents an image that has been used for sample size in statistics.	Unpaired t-test $p < 0.0001$
2D Munc13-1 volume/image volume ($\mu m^3/\mu m^3$)	Munc13-1-EYFP = 0.047 ± 0.001, median: 0.047 (n = **88 images**, 3 mice) wild type = 0.00022 ± 0.00002, median: 0.0002 (n = **86 images**, 3 mice) The distribution of values for each image is shown as violin plots with superimposed mean ± SEM for Munc13-1-EYFP. All values for wild type were very close to zero and are shown as scatter plots, where each circle represents an image that has been used for sample size in statistics.	Unpaired t-test $p < 0.0001$
2E TH volume/image volume ($\mu m^3/\mu m^3$)	Munc13-1-EYFP = 0.078 ± 0.002, median: 0.077 (n = **88 images**, 3 mice) wild type = 0.079 ± 0.002, median: 0.077 (n = **86 images**, 3 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test $p = 0.5$
2F TH length/image volume ($\mu m/\mu m^3$)	Munc13-1-EYFP = 1.02 ± 0.03, median: 0.98 (n = **88 images**, 3 mice) wild type = 0.98 ± 0.02, median: 0.94 (n = **86 images**, 3 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test $p = 0.2$
2G Munc13-1 clusters/µm of TH axon	Munc13-1-EYFP = 0.448 ± 0.009, median: 0.445 (n = **88 images**, 3 mice) wild type = 0.003 ± 0.001, median: 0.002 (n = **86 images**, 3 mice) The distribution of values for each image is shown as violin plots with superimposed mean ± SEM for Munc13-1-EYFP. All values for wild type were very close to zero and are shown as scatter plots, where each circle represents an	Unpaired t-test $p < 0.0001$

	image that has been used for sample size in statistics.	
2H Munc13-1 clusters/µm of TH axon	Munc13-1-EYFP actual = 0.448 ± 0.009, median: 0.445 (n = **88 images**, 3 mice) Munc13-1-EYFP shuffled = 0.270 ± 0.003, median: 0.272 (n = **88 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Paired t-test $p < 0.0001$
2I Munc13-1 cluster volume (x 0.01 μm^3)	Munc13-1-EYFP actual = 1.17 ± 0.01, median: 1.18 (n = **88 images**, 3 mice) Munc13-1-EYFP shuffled = 1.08 ± 0.01, median: 1.08 (n = **88 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Paired t-test $p < 0.0001$

Table S3. Means, number of observations and statistical testing, related to Figs. 3 and S2-S4

For each dataset, mean ± SEM are shown in the corresponding figure. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size**	**Statistical test** **p-value**
3D Peak dopamine (µm) evoked by the 1st stimulus of a 10 Hz train of optogenetic stimulation	Munc13 control = 0.482 ± 0.033 (n = **5 slices**, 3 mice) Munc13 control + TTX = 0.008 ± 0.002 (n = **5 slices**, 3 mice) Munc13 cKO^{DA} = 0.076 ± 0.008 (n = **5 slices**, 3 mice) Munc13 cKO^{DA} + TTX = 0.012 ± 0.002 (n = **5 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Repeated measures one-way ANOVA ($p < 0.0001$, $F = 193.7$) followed by Sidak's multiple comparisons tests for specific pairs: Munc13 control vs. Munc13 control + TTX ($p = 0.0006$); Munc13 control vs. Munc13 cKO^{DA} ($p = 0.0006$); Munc13 cKO^{DA} vs. Munc13 cKO^{DA} + TTX ($p = 0.0062$); and Munc13 control + TTX vs. Munc13 cKO^{DA} + TTX ($p = 0.7$).
3E Peak dopamine evoked by a 10 Hz train of optogenetic stimulation normalized to the 1st peak amplitude of Munc13 control (mean ± SEM for stimulus 2, 6 and 10 are listed here)	Munc13 control stimulus 2: 0.36 ± 0.05; stimulus 6: 0.09 ± 0.02 and stimulus 10: 0.06 ± 0.01. Munc13 cKO^{DA} stimulus 2: 0.11 ± 0.02; stimulus 6: 0.06 ± 0.01 and stimulus 10: 0.06 ± 0.01. Each circle in the figure represents the mean ± SEM from all recorded slices; Munc13 control = **6 slices**, 3 mice and Munc13 cKO^{DA} = **6 slices**, 3 mice.	Two-way ANOVA ($p < 0.0001$ for genotype $F = 445.4$; $p < 0.0001$ for stimulus number $F = 101.5$ and $p < 0.0001$ for interaction $F = 112.1$) followed by Sidak's multiple comparisons tests between Munc13 control vs. Munc13 cKO^{DA} for each stimulus: stimulus 1-4 ($p < 0.0001$); stimulus 5 ($p = 0.0128$); and stimulus 6-10 ($p > 0.9$).
3G Peak dopamine (µM) evoked by a KCl puff	Munc13 control = 3.55 ± 0.53 (n = **7 slices**, 3 mice) Munc13 cKO^{DA} = 0.09 ± 0.01 (n = **7 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.0006$
3H Total dopamine (x 100 µM x s) evoked by a KCl puff	Munc13 control = 1.012 ± 0.205 (n = **7 slices**, 3 mice) Munc13 cKO^{DA} = 0.021 ± 0.004 (n = **7 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.0006$
S2D Normalized protein level	Munc13-1$^{+/+}$ = 1.00 ± 0.08 (n = 3 mice) Munc13-1$^{fl/fl}$ = 1.02 ± 0.12 (n = 3 mice)	no statistics used
S2F	Munc13-1$^{+/+}$ = 1.00 ± 0.21 (n = 3 mice)	no statistics used

Normalized protein level	Munc13-1 cKO^{gl} = 0.05 ± 0.01 (n = 3 mice)	
S3B mEPSC frequency (Hz)	Munc13-1$^{+/+}$ = 6.04 ± 0.84 (n = **36 neurons**, 3 independent cultures) Munc13-1 cKO^{gl} = 1.79 ± 0.44 (n = **39 neurons**, 3 independent cultures) Each circle in the figure represents a whole cell recording from a neuron that has been used for sample size in statistics.	Mann-Whitney test ($p < 0.0001$)
S3C mEPSC amplitude (pA)	Munc13-1$^{+/+}$ = 36.35 ± 2.30 (n = **36 neurons**, 3 independent cultures) Munc13-1 cKO^{gl} = 27.27 ± 1.76 (n = **33 neurons**, 3 independent cultures) Each circle in the figure represents a whole cell recording from a neuron that has been used for sample size in statistics.	Mann-Whitney test ($p = 0.0176$)
S3E EPSC amplitude (nA)	Munc13-1$^{+/+}$ = 6.50 ± 0.54 (n = **57 neurons**, 3 independent cultures) Munc13-1 cKO^{gl} = 1.89 ± 0.25 (n = **56 neurons**, 3 independent cultures) Each circle in the figure represents a whole cell recording from a neuron that has been used for sample size in statistics.	Mann-Whitney test ($p < 0.0001$)
S3G sucrose EPSC amplitude (nC)	Munc13-1$^{+/+}$ = 1.04 ± 0.18 (n = **54 neurons**, 3 independent cultures) Munc13-1 cKO^{gl} = 0.14 ± 0.25 (n = **53 neurons**, 3 independent cultures) Each circle in the figure represents a whole cell recording from a neuron that has been used for sample size in statistics.	Mann-Whitney test ($p < 0.0001$)
S3I EPSC triggered by 10 stimuli at 10 Hz normalized to the mean of the 1st EPSC of Munc13-1$^{+/+}$ (mean ± SEM for stimulus 1, 2, 6 and 10 are listed here)	Munc13-1$^{+/+}$ stimulus 1: 1.00 ± 0.09; stimulus 2: 0.90 ± 0.08; stimulus 6: 0.70 ± 0.07 and stimulus 10: 0.65 ± 0.07. Munc13-1 cKO^{gl} stimulus 1: 0.31 ± 0.04; stimulus 2: 0.23 ± 0.03; stimulus 6: 0.18 ± 0.02 and stimulus 10: 0.19 ± 0.02. Each circle in the figure represents the mean ± SEM from all neuronal recordings that has been used for sample size in statistics; Munc13-1$^{+/+}$ = **40 neurons**, 3 independent cultures and Munc13-1 cKO^{gl} = **44 neurons**, 3 independent cultures.	Two-way ANOVA ($p < 0.0001$, $F = 496.4$ for genotype, $p < 0.0001$, $F = 3.901$ for stimulus number and $p = 0.4$, $F = 1.013$ for interaction) followed by Sidak's multiple comparisons tests between Munc13-1$^{+/+}$ vs. Munc13-1 cKO^{gl} for each stimulus: stimulus 1-10 ($p < 0.0001$).
S4D Amplitude (mV) of the extracellular potential evoked by the 1st stimulus of a 10 Hz train of optogenetic stimulation	Munc13 control = 0.046 ± 0.006 (n = **8 slices**, 6 mice) Munc13 control + TTX = 0.022 ± 0.003 (n = **8 slices**, 6 mice) Munc13 cKO^{DA} = 0.050 ± 0.006 (n = **8 slices**, 6 mice) Munc13 cKO^{DA} + TTX = 0.026 ± 0.005 (n = **8 slices**, 6 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Repeated measures one-way ANOVA ($p < 0.0001$, $F = 16.38$) followed by Sidak's multiple comparisons tests for specific pairs: Munc13 control vs. Munc13 control + TTX ($p = 0.0003$); Munc13 control vs. Munc13 cKO^{DA} ($p = 0.8$); Munc13 cKO^{DA} vs. Munc13 cKO^{DA} + TTX ($p = 0.0003$); and

		Munc13 control + TTX vs. Munc13 cKO^{DA} + TTX (p = 0.9).
S4E Amplitudes of the extracellular potentials evoked by a 10 Hz train of optogenetic stimulation normalized to the 1st peak amplitude of Munc13 control (mean ± SEM for stimulus 2, 6 and 10 are listed here)	Munc13 control stimulus 2: 0.98 ± 0.15; stimulus 6: 0.98 ± 0.15 and stimulus 10: 0.99 ± 0.15. Munc13 cKO^{DA} stimulus 2: 1.06 ± 0.16; stimulus 6: 1.09 ± 0.15 and stimulus 10: 1.06 ± 0.16. Each circle in the figure represents the mean ± SEM from all recorded slices; Munc13 control = **8 slices**, 6 mice and Munc13 cKO^{DA} = **8 slices**, 6 mice.	Two-way ANOVA (p = 0.7, F = 0.1554 for genotype; p = 0.0646, F = 1.923 for stimulus number and p = 0.4, F = 0.9617 for interaction).
S4G Amplitudes of the extracellular potentials evoked by a 40 Hz train of optogenetic stimulation normalized to the 1st peak amplitude of Munc13 control (mean ± SEM for stimulus 1, 2, 6 and 10 are listed here)	Munc13 control stimulus 1: 1.00 ± 0.11; stimulus 2: 0.73 ± 0.11; stimulus 6: 0.67 ± 0.10 and stimulus 10: 0.68 ± 0.10. Munc13 cKO^{DA} stimulus 1: 0.88 ± 0.19; stimulus 2: 0.65 ± 0.18; stimulus 6: 0.64 ± 0.18 and stimulus 10: 0.62 ± 0.16. Each circle in the figure represents the mean ± SEM from all recorded slices; Munc13 control = **6 slices**, 5 mice and Munc13 cKO^{DA} = **6 slices**, 5 mice.	Two-way ANOVA (p = 0.7, F = 0.1394 for genotype; p < 0.0001, F = 74.06 for stimulus number and p = 0.3, F = 1.187 for interaction).

Table S4. **Means, number of observations and statistical testing, related to Fig. 4**

For each dataset, mean ± SEM are shown in the corresponding figure. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size**	**Statistical test** **p-value**
4B Peak dopamine (µm) evoked by single electrical pulses at increasing stimulation intensities of 10-90 µA (mean ± SEM for 10 µA, 50 µA and 90 µA are listed here)	Munc13 control 10 µA: 0.06 ± 0.02; 50 µA: 0.65 ± 0.07 and 90 µA: 0.78 ± 0.14. Munc13 cKO^{DA} 10 µA: 0.02 ± 0.01; 50 µA: 0.08 ± 0.02 and 90 µA: 0.07 ± 0.01. Each circle in the figure represents the mean ± SEM from all recorded slices; Munc13 control = **10 slices**, 5 mice and Munc13 cKO^{DA} = **10 slices**, 5 mice.	Two-way ANOVA ($p < 0.0001$, $F = 92.43$ for genotype; $p < 0.0001$, $F = 13.28$ for stimulus intensity and $p < 0.0001$, $F = 10.00$ for interaction) followed by Sidak's multiple comparisons tests between Munc13 control vs. Munc13 cKO^{DA} for each stimulus intensity: 10 µA ($p > 0.9$); 20 µA ($p = 0.0119$); and 30-90 µA ($p < 0.0001$).
4D Peak dopamine evoked by a 10 Hz train of electrical stimulation at 90 µA normalized to the 1st peak amplitude of Munc13 control (mean ± SEM for stimulus 2, 6 and 10 are listed here)	Munc13 control stimulus 2: 0.028 ± 0.004; stimulus 6: 0.027 ± 0.003 and stimulus 10: 0.029 ± 0.005. Munc13 cKO^{DA} stimulus 2: 0.023 ± 0.005; stimulus 6: 0.026 ± 0.005 and stimulus 10: 0.029 ± 0.005 Each circle in the figure represents the mean ± SEM from all recorded slices; Munc13 control = **8 slices**, 4 mice and Munc13 cKO^{DA} = **8 slices**, 4 mice.	Two-way ANOVA ($p < 0.0001$, $F = 206.8$ for genotype; $p < 0.0001$, $F = 506.4$ for stimulus number and $p < 0.0001$, $F = 216.8$ for interaction) followed by Sidak's multiple comparisons tests between Munc13 control vs. Munc13 cKO^{DA} for each stimulus: stimulus 1 ($p < 0.0001$); and stimulus 2-10 ($p > 0.9$).
4D inset Peak dopamine (µm) evoked by 1st stimulus of a 10 Hz train of electrical stimulation	Munc13 control = 0.86 ± 0.04 (n = **8 slices**, 4 mice) Munc13 cKO^{DA} = 0.04 ± 0.02 (n = **8 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics; sample size as in Fig. 4D.	Mann-Whitney test $p = 0.0002$
4E In vivo extracellular dopamine measured in microdialysis, normalized to the average dopamine values of the 76th – 120th min of Munc13 control (mean ± SEM for 90th, 165th and 240th min are listed here)	Munc13 control 90th min: 1.00 ± 0.07; 165th min: 0.37 ± 0.05 and 240th min: 0.27 ± 0.04 Munc13 cKO^{DA} 90th min: 0.36 ± 0.08; 165th min: 0.25 ± 0.06 and 240th min: 0.22 ± 0.05 Each circle in the figure represents the mean ± SEM of all experimental mice that was used for sample size in statistics; Munc13 control = **7 mice** and Munc13 cKO^{DA} = **7 mice**.	Two-way ANOVA ($p = 0.0004$, $F = 50.18$ for genotype; $p < 0.0001$, $F = 158.60$ for time points and $p < 0.0001$, $F = 49.00$ for interaction) followed by Sidak's multiple comparisons tests between Munc13 control vs. Munc13 cKO^{DA} for each time point: 90-150th min ($p < 0.0001$); 165-195th min ($p < 0.01$); and 210-240th min ($p > 0.6$).

Table S5. **Means, medians, number of observations and statistical testing, related to Figs. 5 and S5**

For each dataset, mean ± SEM are shown in the corresponding figure, except for Fig. 5J (mean ± SD). For image data, median values are reported in the tables in addition to mean ± SEM. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size** **Median (for imaging data only)**	**Statistical test** **p-value**
5B TH intensity (a.u.) in TH^+ ROIs	Munc13 control = 1402 ± 102, median: 1374 (n = **30 images**, 3 mice) Munc13 cKO^{DA} = 1715 ± 79, median: 1723 (n = **30 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Unpaired t-test p = 0.018
5C Synaptophysin intensity (a.u.) in TH^+ ROIs	Munc13 control = 400 ± 22, median: 373 (n = **30 images**, 3 mice) Munc13 cKO^{DA} = 348 ± 13, median: 338 (n = **30 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Unpaired t-test p = 0.043
5D Bassoon intensity (a.u.) in synaptophysin$^+$TH$^-$ (syp$^+$TH$^-$) and synaptophysin$^+$TH$^+$ (syp$^+$TH$^+$) ROIs	syp$^+$TH$^-$ of Munc13 control = 480 ± 23, median: 433 (n = **30 images**, 3 mice) syp$^+$TH$^-$ of Munc13 cKO^{DA} = 493 ± 21, median: 495 (n = **30 images**, 3 mice) syp$^+$TH$^+$ of Munc13 control = 511 ± 29, median: 528 (n = **29 images**, 3 mice) syp$^+$TH$^+$ of Munc13 cKO^{DA} = 613 ± 39, median: 617 (n = **29 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	One-way ANOVA (p = 0.0055, F = 4.428) followed by Sidak's multiple comparisons tests for specific pairs: syp$^+$TH$^-$ of Munc13 control vs. Munc13 cKO^{DA} (p = 0.9); and syp$^+$TH$^+$ of Munc13 control vs. Munc13 cKO^{DA} (p = 0.0291).
5E Frequency histogram of Bassoon intensity (a.u.) in syp$^+$TH$^+$ ROIs	syp$^+$TH$^+$ of Munc13 control (n = **221 syp$^+$TH$^+$ ROIs**, 29 images, 3 mice) syp$^+$TH$^+$ of Munc13 cKO^{DA} (n = **168 syp$^+$TH$^+$ ROIs**, 29 images, 3 mice)	Kolmogorov-Smirnov test p < 0.0001
5G TH volume/image volume ($\mu m^3/\mu m^3$)	Munc13 control = 0.073 ± 0.002, median: 0.07 (n = **163 images**, 4 mice) Munc13 cKO^{DA} = 0.063 ± 0.002, median: 0.06 (n = **165 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test p < 0.0001
5H TH length/image volume ($\mu m/\mu m^3$)	Munc13 control = 0.99 ± 0.02, median: 0.92 (n = **163 images**, 4 mice) Munc13 cKO^{DA} = 0.68 ± 0.02, median: 0.62 (n = **165 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test p < 0.0001
5J Fraction of the TH surface area at increasing distances	Munc13 control 0.2 µm: 0.196 ± 0.007, median: 0.197; 0.4 µm: 0.022 ± 0.004, median: 0.021; 0.6 µm: 0.0020 ± 0.0004, median: 0.002	Two-way ANOVA (p < 0.0001, F = 68.16 for genotype; p < 0.0001, F = 22330 for distance

from the medial axis of the TH axon (mean ± SD and median for 0.2 µm, 0.4 µm, and 0.6 µm are listed here)	Munc13 cKO^{DA} 0.2 µm: 0.162 ± 0.014, median: 0.164; 0.4 µm: 0.043 ± 0.008, median: 0.043; 0.6 µm: 0.006 ± 0.002, median: 0.006 Sample size was **160 images** from 4 mice each for Munc13 control and Munc13 cKO^{DA}.	and $p < 0.0001$, $F = 506.1$ for interaction).
5K Bassoon clusters/µm of TH axon	Munc13 control = 0.172 ± 0.003, median: 0.18 (n = **163 images**, 4 mice) Munc13 cKO^{DA} = 0.254 ± 0.007, median: 0.26 (n = **165 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test $p < 0.0001$
5L Bassoon cluster volume (x 0.01 µm^3)	Munc13 control = 1.25 ± 0.01, median: 1.26 (n = **163 images**, 4 mice) Munc13 cKO^{DA} = 1.54 ± 0.02, median: 1.59 (n = **165 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test $p < 0.0001$
S5B Bassoon intensity (a.u.) in TH$^+$ ROIs	Munc13 control = 426 ± 25, median: 429 (n = **40 images**, 4 mice) Munc13 cKO^{DA} = 625 ± 33, median: 633 (n = **39 images**, 4 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Unpaired t-test $p < 0.0001$
S5C Frequency histogram of Bassoon intensity (a.u.) in TH$^+$ ROIs	Munc13 control (n = **3035 TH$^+$ ROIs**, 40 images, 4 mice) Munc13 cKO^{DA} (n = **1745 TH$^+$ ROIs**, 39 images, 4 mice)	Kolmogorov-Smirnov test $p < 0.0001$
S5E RIM intensity (a.u.) in TH$^+$ ROIs	Munc13 control = 371 ± 12, median: 363 (n = **29 images**, 3 mice) Munc13 cKO^{DA} = 485 ± 21, median: 495 (n = **29 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Unpaired t-test $p < 0.0001$
S5F Frequency histogram of RIM intensity (a.u.) in TH$^+$ ROIs	Munc13 control (n = **4766 TH$^+$ ROIs**, 29 images, 3 mice) Munc13 cKO^{DA} (n = **1639 TH$^+$ ROIs**, 29 images, 3 mice)	Kolmogorov-Smirnov test $p < 0.0001$
S5H TH volume/image volume (µm^3/µm^3)	Munc13 control = 0.11 ± 0.01, median: 0.1 (n = **37 images**, 3 mice) Munc13 cKO^{DA} = 0.07 ± 0.01, median: 0.06 (n = **37 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Unpaired t-test $p < 0.0001$
S5I TH length/image volume (µm/µm^3)	Munc13 control = 1.10 ± 0.06, median: 1.0 (n = **37 images**, 3 mice) Munc13 cKO^{DA} = 0.72 ± 0.06, median: 0.68 (n = **37 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Unpaired t-test $p < 0.0001$
S5J ELKS2 clusters/µm of TH axon	Munc13 control = 0.32 ± 0.02, median: 0.34 (n = **37 images**, 3 mice) Munc13 cKO^{DA} = 0.42 ± 0.04, median: 0.41 (n =	Unpaired t-test $p = 0.021$

	37 images, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	
S5K ELKS2 cluster volume (x 0.01 μm^3)	Munc13 control = 1.76 ± 0.06, median: 1.88 (n = **37 images**, 3 mice) Munc13 cKO^{DA} = 1.60 ± 0.06, median: 1.51 (n = **37 images**, 3 mice) Each circle in the figure represents an image that has been used for sample size in statistics.	Unpaired t-test p = 0.083

Table S6. **Means, number of observations and statistical testing, related to Figs. 6 and S6**

For each dataset, mean ± SEM are shown in the corresponding figure. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size**	**Statistical test** **p-value**
6C Peak dopamine evoked by a 10 Hz train of optogenetic stimulation normalized to the 1st peak amplitude of RIM-BP control (mean ± SEM for stimulus 2, 6 and 10 are listed here)	RIM-BP control stimulus 2: 0.28 ± 0.05; stimulus 6: 0.07 ± 0.01 and stimulus 10: 0.06 ± 0.01. RIM-BP cKO^{DA} stimulus 2: 0.24 ± 0.05; stimulus 6: 0.06 ± 0.01 and stimulus 10: 0.06 ± 0.01. Each circle in the figure represents the mean ± SEM from all recorded slices; RIM-BP control = **8 slices**, 5 mice and RIM-BP cKO^{DA} = **8 slices**, 5 mice.	Two-way ANOVA ($p = 0.6$, $F = 0.2854$ for genotype; $p < 0.0001$, $F = 106.7$ for stimulus number and $p = 0.9$, $F = 0.349$ for interaction) followed by Sidak's multiple comparisons tests between RIM-BP control vs. RIM-BP cKO^{DA} for each stimulus: stimulus 1-10 ($p > 0.8$).
6C inset 1 Peak dopamine (µm) evoked by the 1st stimulus of a 10 Hz train of optogenetic stimulation	RIM-BP control = 0.60 ± 0.07 (n = **8 slices**, 5 mice) RIM-BP cKO^{DA} = 0.57 ± 0.06 (n = **8 slices**, 5 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics; sample size as in Fig. 6C.	Mann-Whitney test $p = 0.7$
6C inset 2 Paired pulse ratio (PPR) of the 2nd to the 1st response in a 10 Hz train of optogenetic stimulation	RIM-BP control = 0.26 ± 0.03 (n = **8 slices**, 5 mice) RIM-BP cKO^{DA} = 0.23 ± 0.03 (n = **8 slices**, 5 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics; sample size as in Fig. 6C.	Mann-Whitney t-test $p = 0.6$
6D 20-80% rise time (ms) of the response evoked by the 1st stimulus of a 10 Hz train of optogenetic stimulation	RIM-BP control = 1.97 ± 0.24 (n = **8 slices**, 5 mice) RIM-BP cKO^{DA} = 2.29 ± 0.25 (n = **8 slices**, 5 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.3$
6F Peak dopamine (µM) evoked by a KCl puff	RIM-BP control = 5.99 ± 0.88 (n = **8 slices**, 3 mice) RIM-BP cKO^{DA} = 5.02 ± 0.39 (n = **8 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.2$
6G Total dopamine (x 100 µM x s) evoked by a KCl puff	RIM-BP control = 1.40 ± 0.17 (n = **8 slices**, 3 mice) RIM-BP cKO^{DA} = 1.12 ± 0.06 (n = **8 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.1$

S6B Peak dopamine (µm) evoked by single electrical pulses at increasing stimulation intensities of 10-90 µA (mean ± SEM for 10 µA, 50 µA and 90 µA are listed here)	RIM-BP control 10 µA: 0.09 ± 0.02; 50 µA: 0.76 ± 0.09 and 90 µA: 0.74 ± 0.07. RIM-BP cKO^{DA} 10 µA: 0.06 ± 0.02; 50 µA: 0.68 ± 0.07 and 90 µA: 0.72 ± 0.06. Each circle in the figure represents the mean ± SEM from all recorded slices; RIM-BP control = **16 slices**, 5 mice and RIM-BP cKO^{DA} = **16 slices**, 5 mice.	Two-way ANOVA ($p = 0.3$, $F = 0.9502$ for genotype; $p < 0.0001$, $F = 49.17$ for stimulus intensity and $p = 0.9$, $F = 0.3909$ for interaction) followed by Sidak's multiple comparisons tests between RIM-BP control vs. RIM-BP cKO^{DA} for each stimulus intensity: 10-90 µA ($p > 0.3$).
S6D Peak dopamine evoked by a 10 Hz train of electrical stimulation at 90 µA normalized to the 1st peak amplitude of RIM-BP control (mean ± SEM for stimulus 2, 6 and 10 are listed here)	RIM-BP control stimulus 2: 0.025 ± 0.004; stimulus 6: 0.028 ± 0.004 and stimulus 10: 0.029 ± 0.003. RIM-BP cKO^{DA} stimulus 2: 0.026 ± 0.003; stimulus 6: 0.026 ± 0.004 and stimulus 10: 0.027 ± 0.004. Each circle in the figure represents the mean ± SEM from all recorded slices; RIM-BP control = **8 slices**, 4 mice and RIM-BP cKO^{DA} = **8 slices**, 4 mice.	Two-way ANOVA ($p = 0.6$, $F = 0.2839$ for genotype; $p < 0.0001$, $F = 170.5$ for stimulus number and $p = 0.9$, $F = 0.1706$ for interaction) followed by Sidak's multiple comparisons tests between RIM-BP control vs. RIM-BP cKO^{DA} for each stimulus: stimulus 1-10 ($p > 0.8$).
S6D inset Peak dopamine (µm) evoked by 1st stimulus of a 10 Hz train of electrical stimulation	RIM-BP control = 0.97 ± 0.14 (n = **8 slices**, 4 mice) RIM-BP cKO^{DA} = 0.89 ± 0.09 (n = **8 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics; sample size as in Fig. S6D.	Mann-Whitney test $p = 0.9$

Table S7. **Means, medians, number of observations and statistical testing, related to Figs. 7 and S7**

For each dataset, mean ± SEM are shown in the corresponding figure, except for Fig. 7E (mean ± SD). For image data, median values are reported in the tables in addition to mean ± SEM. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size** **Median (for imaging data only)**	**Statistical test** **p-value**
7C TH volume/image volume ($\mu m^3/\mu m^3$)	Liprin-α control = 0.056 ± 0.003, median: 0.05 (n = **84 images**, 4 mice) Liprin-α cKO^{DA} = 0.051 ± 0.003, median: 0.043 (n = **85 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test p = 0.2
7D TH length/image volume ($\mu m/\mu m^3$)	Liprin-α control = 0.69 ± 0.03, median: 0.62 (n = **84 images**, 4 mice) Liprin-α cKO^{DA} = 0.63 ± 0.03, median: 0.55 (n = **85 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test p = 0.2
7E Fraction of the TH surface area at increasing distances from the medial axis of the TH axon (mean ± SD and medians for 0.2 µm, 0.4 µm, and 0.6 µm are listed here)	Liprin-α control 0.2 µm: 0.187 ± 0.014, median: 0.19; 0.4 µm: 0.024 ± 0.009, median: 0.024 and 0.6 µm: 0.002 ± 0.001, median: 0.002. Liprin-α cKO^{DA} 0.2 µm: 0.193 ± 0.014, median: 0.195; 0.4 µm: 0.028 ± 0.007, median: 0.028 and 0.6 µm: 0.003 ± 0.001, median: 0.002. Sample size: Liprin-α control = **84 images**, 4 mice and Liprin-α cKO^{DA} = **85 images**, 4 mice.	Two-way ANOVA (p = 0.9, F = 0.013 for genotype; p < 0.0001, F = 4244 for distance and p < 0.0001, F = 18.55 for interaction)
7F Bassoon clusters/µm of TH axon	Liprin-α control = 0.45 ± 0.01, median: 0.48 (n = **84 images**, 4 mice) Liprin-α cKO^{DA} = 0.50 ± 0.01, median: 0.51 (n = **85 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test p = 0.0005
7G Bassoon cluster volume (x 0.01 μm^3)	Liprin-α control = 1.47 ± 0.02, median: 1.5 (n = **84 images**, 4 mice) Liprin-α cKO^{DA} = 1.49 ± 0.02, median: 1.49 (n = **85 images**, 4 mice) The distribution of images in the figure is shown as violin plots with superimposed mean ± SEM.	Unpaired t-test p = 0.5
7I Peak dopamine evoked by a 10 Hz train of optogenetic stimulation normalized to the 1st peak amplitude of Liprin-α control (mean ± SEM for stimulus 2, 6 and 10 are listed here)	Liprin-α control stimulus 2: 0.32 ± 0.03; stimulus 6: 0.11 ± 0.01 and stimulus 10: 0.09 ± 0.01. Liprin-α cKO^{DA} stimulus 2: 0.18 ± 0.02; stimulus 6: 0.06 ± 0.01 and stimulus 10: 0.05 ± 0.01. Each circle in the figure represents the mean ± SEM from all recorded slices; Liprin-α control = **11 slices**, 4 mice and Liprin-α cKO^{DA} = **11 slices**, 4 mice.	Two-way ANOVA (p = 0.0008, F = 22.05 for genotype; p < 0.0001, F = 226.5 for stimulus number and p < 0.0001, F = 17.21 for interaction) followed by Sidak's multiple comparisons tests between Liprin-α control vs. Liprin-α cKO^{DA} for stimulus number: stimulus 1-2 (p <

		0.0001); and stimulus 3-10 ($p > 0.06$).
7I inset 1 Peak dopamine (µm) evoked by the 1st stimulus of a 10 Hz train of optogenetic stimulation	Liprin-α control = 0.56 ± 0.05 (n = **11 slices**, 4 mice) Liprin-α cKO^{DA} = 0.32 ± 0.03 (n = **11 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics, sample size as in Fig. 7I.	Mann-Whitney test $p = 0.0003$
7I inset 2 Paired pulse ratio (PPR) of the 2nd to the 1st response in a 10 Hz train of optogenetic stimulation	Liprin-α control = 0.32 ± 0.01 (n = **11 slices**, 4 mice) Liprin-α cKO^{DA} = 0.33 ± 0.02 (n = **11 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics, sample size as in Fig. 7I.	Mann-Whitney t-test $p = 0.3$
7J 20-80% rise time (ms) of the response evoked by the 1st stimulus of a 10 Hz train of optogenetic stimulation	Liprin-α control = 2.16 ± 0.12 (n = **11 slices**, 4 mice) Liprin-α cKO^{DA} = 2.32 ± 0.10 (n = **11 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.3$
7L Peak dopamine (µM) evoked by a KCl puff	Liprin-α control = 4.50 ± 0.50 (n = **12 slices**, 8 mice) Liprin-α cKO^{DA} = 2.82 ± 0.36 (n = **12 slices**, 8 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.01$
7M Total dopamine (x 100 µM x s) evoked by a KCl puff	Liprin-α control = 1.24 ± 0.13 (n = **12 slices**, 8 mice) Liprin-α cKO^{DA} = 0.72 ± 0.09 (n = **12 slices**, 8 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test $p = 0.002$
S7B Peak dopamine (µm) evoked by single electrical pulses at increasing stimulation intensities of 10-90 µA (mean ± SEM for 10 µA, 50 µA and 90 µA are listed here)	Liprin-α control 10 µA: 0.03 ± 0.01; 50 µA: 0.52 ± 0.04 and 90 µA: 0.69 ± 0.07. Liprin-α cKO^{DA} 10 µA: 0.07 ± 0.02; 50 µA: 0.48 ± 0.05 and 90 µA: 0.63 ± 0.07. Each circle in the figure represents the mean ± SEM from all recorded slices; Liprin-α control = **12 slices**, 8 mice and Liprin-α cKO^{DA} = **12 slices**, 8 mice.	Two-way ANOVA ($p = 0.6$, $F = 0.1755$ for genotype; $p < 0.0001$, $F = 61.02$ for stimulus intensity and $p = 0.8$, $F = 0.4974$ for interaction) followed by Sidak's multiple comparisons tests between Liprin-α control vs. Liprin-α cKO^{DA} for each stimulus intensity: 10-90 µA ($p > 0.7$).
S7D Peak dopamine evoked by a 10 Hz train of electrical stimulation at 90 µA normalized to the 1st peak amplitude of Liprin-α control	Liprin-α control stimulus 2: 0.041 ± 0.006; stimulus 6: 0.033 ± 0.006 and stimulus 10: 0.037 ± 0.004. Liprin-α cKO^{DA} stimulus 2: 0.037 ± 0.004; stimulus 6: 0.028 ± 0.005 and stimulus 10: 0.033 ± 0.004.	Two-way ANOVA ($p = 0.2$, $F = 1.493$ for genotype; $p < 0.0001$, $F = 178.9$ for stimulus number and $p = 0.7$, $F = 0.6439$ for interaction) followed by Sidak's multiple comparisons tests

(mean ± SEM for stimulus 2, 6 and 10 are listed here)	Each circle in the figure represents the mean ± SEM from all recorded slices; Liprin-α control = **8 slices**, 3 mice and Liprin-α cKO^{DA} = **8 slices**, 3 mice.	between Liprin-α control vs. Liprin-α cKO^{DA} for each stimulus: stimulus 1-10 ($p > 0.1$).
S7D inset Peak dopamine (µm) evoked by the 1st stimulus of a 10 Hz train of electrical stimulation	Liprin-α control = 0.71 ± 0.09 (n = **8 slices**, 3 mice) Liprin-α cKO^{DA} = 0.63 ± 0.04 (n = **8 slices**, 3 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics; sample size as in Fig. S7D.	Unpaired t-test $p = 0.4$
S7F Amplitude (mV) of the extracellular potential evoked by the 1st stimulus of a 10 Hz train of optogenetic stimulation	Liprin-α control = 0.031 ± 0.004 (n = **12 slices**, 3 mice) Liprin-α control + TTX = 0.014 ± 0.002 (n = **12 slices**, 3 mice) Liprin-α cKO^{DA} = 0.031 ± 0.003 (n = **12 slices**, 3 mice) Liprin-α cKO^{DA} + TTX = 0.015 ± 0.002 (n = **12 slices**, 3 mice). Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Repeated measures one-way ANOVA ($p < 0.0001$, $F = 11.06$) followed by Sidak's multiple comparisons tests for specific pairs: Liprin-α control vs. Liprin-α control + TTX ($p = 0.0005$); Liprin-α control vs. Liprin-α cKO^{DA} ($p > 0.9$); Liprin-α cKO^{DA} vs. Liprin-α cKO^{DA} + TTX ($p = 0.0011$); and Liprin-α control + TTX vs. Liprin-α cKO^{DA} + TTX ($p > 0.9$).
S7G Amplitudes of the extracellular potentials evoked by a 10 Hz train of optogenetic stimulation normalized to the 1st peak amplitude of Liprin-α control (mean ± SEM for stimulus 2, 6 and 10 are listed here)	Liprin-α control stimulus 2: 1.00 ± 0.12; stimulus 6: 0.99 ± 0.12 and stimulus 10: 0.99 ± 0.12. Liprin-α cKO^{DA} stimulus 2: 0.98 ± 0.11; stimulus 6: 0.99 ± 0.10 and stimulus 10: 0.97 ± 0.11. Each circle in the figure represents the mean ± SEM from all recorded slices; Liprin-α control = **12 slices**, 3 mice and Liprin-α cKO^{DA} = **12 slices**, 3 mice.	Two-way ANOVA ($p = 0.9$, $F = 0.006742$ for genotype; $p = 0.7$, $F = 0.6628$ for stimulus number and $p = 0.8$, $F = 0.5567$ for interaction).

Table S8. **Means, number of observations and statistical testing, related to Figs. 8 and S8**

For each dataset, mean ± SEM are shown in the corresponding figure. The sample size that has been used for statistical testing is shown in bold for each data set.

Figure **Parameter (units)**	**Mean ± SEM and sample size**	**Statistical test** **p-value**
8C Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM cKO^{DA} = 0.08 ± 0.01 (n = **16 slices**, 4 mice) RIM cKO^{DA} + RIM1-ZnC$_2$B = 0.42 ± 0.03 (n = **16 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
8E Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM cKO^{DA} + RIM1-ZnC$_2$B = 0.39 ± 0.04 (n = **22 slices**, 5 mice) RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} = 0.08 ± 0.01 (n = **20 slices**, 5 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
8G Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM cKO^{DA} = 0.05 ± 0.01 (n = **19 slices**, 4 mice) RIM cKO^{DA} + RIM1-ZnPCP = 0.09 ± 0.02 (n = **19 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p = 0.042
8I Peak dopamine (µM) evoked by a KCl puff	RIM cKO^{DA} = 0.38 ± 0.07 (n = **18 slices**, 4 mice) RIM cKO^{DA} + RIM1-ZnC$_2$B = 1.40 ± 0.14 (n = **18 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
8K Peak dopamine (µM) evoked by a KCl puff	RIM cKO^{DA} + RIM1-ZnC$_2$B = 2.42 ± 0.24 (n = **23 slices**, 5 mice) RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} = 2.13 ± 0.37 (n = **21 slices**, 5 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p = 0.1
8M Peak dopamine (µM) evoked by a KCl puff	RIM cKO^{DA} = 0.70 ± 0.11 (n = **18 slices**, 4 mice) RIM cKO^{DA} + RIM1-ZnPCP = 2.70 ± 0.32 (n = **18 slices**, 4 mice) Each circle in the figure represents a slice recording that has been used for sample size in statistics.	Mann-Whitney test p < 0.0001
S8B	Control = 1.62 ± 0.14 (n = 20 slices, 4 mice)	not applicable

Peak dopamine (µM) evoked by a 90 µA electrical stimulus	Each circle in the figure represents a slice recording.	
S8C Peak dopamine (µM) evoked by a 90 µA electrical stimulus	Control = 1.24 ± 0.09 (n = 21 slices, 5 mice) Each circle in the figure represents a slice recording.	not applicable
S8D Peak dopamine (µM) evoked by a 90 µA electrical stimulus	Control = 1.16 ± 0.13 (n = 15 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S8E Peak dopamine (µM) evoked by a 90 µA electrical stimulus	Control = 1.15 ± 0.07 (n = 20 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S8F Peak dopamine (µM) evoked by a KCl puff	Control = 10.30 ± 0.53 (n = 21 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S8G Peak dopamine (µM) evoked by a KCl puff	Control = 8.28 ± 0.57 (n = 21 slices, 5 mice) Each circle in the figure represents a slice recording.	not applicable
S8H Peak dopamine (µM) evoked by a KCl puff	Control = 10.58 ± 0.77 (n = 18 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S8I Peak dopamine (µM) evoked by a KCl puff	Control = 6.51 ± 0.78 (n = 17 slices, 4 mice) Each circle in the figure represents a slice recording.	not applicable
S8K Peak dopamine (µM) evoked by a 90 µA electrical stimulus	RIM cKO^{DA} = 0.07 ± 0.01 (n = **20 slices**, 4 mice) RIM cKO^{DA} + RIM1-ZnC_2B^{KE} = 0.11 ± 0.01 (n = **21 slices**, 4 mice) Each circle in the figure represents a slice recording.	Mann-Whitney test p = 0.04
S8M Peak dopamine (µM) evoked by a KCl puff	RIM cKO^{DA} = 0.31 ± 0.05 (n = **17 slices**, 4 mice) RIM cKO^{DA} + RIM1-ZnC_2B^{KE} = 0.89 ± 0.11 (n = **18 slices**, 4 mice) Each circle in the figure represents a slice recording.	Mann-Whitney test p < 0.0001

S8N
Rescue evoked by a 90 µA electrical stimulus normalized to the average of all controls
	(a) RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn = 0.218 ± 0.037 (n = **15 slices**, 4 mice)
	(b) RIM cKO^{DA} + RIM1-Zn = 0.111 ± 0.017 (n = **12 slices**, 4 mice)
	(c) RIM cKO^{DA} + RIM1-ΔZn = 0.039 ± 0.005 (n = **10 slices**, 3 mice)
	(d) RIM cKO^{DA} + RIM1-ZnC_2B = 0.304 ± 0.027 (n = **22 slices**, 5 mice)
	(e) RIM cKO^{DA} + RIM1-ZnC_2B^{KE} = 0.060 ± 0.007 (n = **20 slices**, 5 mice)
	(f) RIM cKO^{DA} + RIM1-ZnPCP = 0.072 ± 0.013 (n = **19 slices**, 4 mice)
	Each circle in the figure represents a slice recording. The sample size for control slices that has been used for normalization is **72 slices**, 20 mice.
		One-way ANOVA (p < 0.0001, F = 24.76) followed by Dunnett's multiple comparisons tests where all conditions were compared to RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn (a):
		a vs. b (p = 0.0128);
		a vs. c (p < 0.0001);
		a vs. d (p = 0.0230);
		a vs. e (p < 0.0001); and
		a vs. f (p < 0.0001).

S8O Rescue evoked by a KCl puff	(a) RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn = 0.401 ± 0.060 (n = **17 slices**, 4 mice)	One-way ANOVA (p < 0.0001, F = 6.731) followed by

normalized to the average of all controls	(b) RIM cKO^{DA} + RIM1-Zn = 0.147 ± 0.018 (n = **11 slices**, 4 mice)
	(c) RIM cKO^{DA} + RIM1-ΔZn = 0.051 ± 0.007 (n = **10 slices**, 3 mice)
	(d) RIM cKO^{DA} + RIM1-ZnC$_2$B = 0.326 ± 0.033 (n = **23 slices**, 5 mice)
	(e) RIM cKO^{DA} + RIM1-ZnC$_2$B^{KE} = 0.287 ± 0.050 (n = **21 slices**, 5 mice)
	(f) RIM cKO^{DA} + RIM1-ZnPCP = 0.364 ± 0.043 (n = **18 slices**, 4 mice)
	Each circle in the figure represents a slice recording. The sample size for control slices that has been used for normalization is **73 slices**, 20 mice.
		Dunnett's multiple comparisons tests where all conditions were compared to RIM cKO^{DA} + RIM1-Zn + RIM1-ΔZn (a):
		a vs. b ($p = 0.0026$);
		a vs. c ($p < 0.0001$);
		a vs. d ($p = 0.5$);
		a vs. e ($p = 0.2$); and
		a vs. f ($p = 0.9$).