Supplementary Information

Tomosyns attenuate SNARE assembly and synaptic depression by binding to VAMP2-containing template complexes

Marieke Meijer^{1, 5}, Miriam Öttl^{2, 5}, Jie Yang^{3, 5}, Aygul Subkhangulova², Avinash Kumar³, Zicheng Feng³, Torben W. van Voorst², Alexander J. Groffen¹, Jan R.T. van Weering¹, Yongli Zhang^{3, 4, 6, *} and Matthijs Verhage^{1, 2, 6, *}

 ¹ Department of Human Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam University Medical Center, 1081HV Amsterdam, The Netherlands
 ² Department of Functional Genomics, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, 1081HV Amsterdam, The Netherlands
 ³ Department of Cell Biology, Yale School of Medicine, New Haven, CT 06511, USA
 ⁴ Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA
 ⁵ These authors contributed equally: Marieke Meijer, Miriam Öttl, Jie Yang
 ⁶ These authors jointly supervised this work: Yongli Zhang, Matthijs Verhage

*Correspondence: <u>m.meijer@vu.nl</u> (M.M.), <u>jie.yang.jy546@yale.edu</u> (J.Y.), <u>Yongli.zhang@yale.edu</u> (Y.Z), <u>m.verhage@vu.nl</u> (M.V.)

Supplementary Fig. 1



Supplementary Figure 1. A novel conditional KO mouse for both tomosyn paralogs. a Structures of the tomosyn-1 and tomosyn-2 genes. Zoom-ins to early exons show floxed regions around exon 2 of tomosyn-1 and exon 3 of tomosyn-2. b Example Western blot showing loss of tomosyn-1 (STXBP5) and levels of synaptic SNAREs in cDKO high-density neuronal cultures. Numbers on the left indicate approximate molecular weight of the detected bands in kDa. c Quantification of synaptic SNAREs expression from Western blots exemplified in **b**. cDKO levels were normalized to control levels in the corresponding culture after normalizing to actin. N = 4 independent cultures. Open circles denote the average of 3 technical replicates per culture. Bars show means of biological replicates ± SD. Data were analyzed using one sample t-test. **p<.01. d Example images of autaptic hippocampal neurons immunostained for MAP2 as a dendrite marker, synaptophysin-1 as a synapse marker, and tomosyn-1 (scale bar = 50 μ m). Dashed boxes in MAP2 images correspond to dendrite zoom-ins on the right. Dendrite zoom-ins show synaptic localization of tomosyn-1 and loss of expression in cDKO neurons (scale bar = 10 μ m). **e-h** Quantifications from images shown in **d. e** Loss of tomosyn expression in synapses was confirmed by measuring the intensity of the tomosyn-1 signal within synaptophysin-1 puncta in cDKO (n = 30/2) and control (n = 31/2) neurons; ***p<.0001. **f** Total dendritic length was determined by tracing of the MAP2 signal. Control n = 62/4, cDKO n = 57/4; p=0.295. g Sholl plot showing similar dendritic complexity of both genotypes. h Synapse density was determined by the number of synaptophysin-1-positive puncta within one μ m dendrite. Control n = 62/4, cDKO n = 57/4; p=0.2035. i-k Morphological analysis from immunostainings at day in vitro (DIV) 7 and 15 shows similar morphology of both groups throughout development. DIV7: control n = 13/1, cDKO n = 14/1; DIV15: control n = 19/1, cDKO n = 18/1. i Total neurite length derived from MAP2 tracing; p=0.7628 (DIV7: WT vs cDKO), ***p=0.0002 (WT: DIV7 vs DIV15), p=0.3685 (DIV15: WT vs cDKO), ***p=0.0005 (cDKO: DIV7 vs DIV15). j Sholl plot of dendrites. k Synapse density was determined from synaptophysin-1positive puncta within one µm dendrite; p=0.8865 (DIV7: WT vs cDKO), ***p<0.0001 (WT: DIV7 vs DIV15), p=0.2869 (DIV15: WT vs cDKO), ***p<0.0001 (cDKO: DIV7 vs DIV15). I Signal intensity of tomosyn-1 in synaptophysin-positive puncta confirms loss of tomosyn at both DIV7 and DIV15; ***p<0.0001 (DIV7: WT vs cDKO), p=0.1895 (WT: DIV7 vs DIV15), ***p<0.0001 (DIV15: WT vs cDKO), *p*=0.0215 (cDKO: DIV7 vs DIV15).

N = cells/independent cultures, unless stated otherwise. In **e**, **f**, **h**, **I**, **k** and **I**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. In **g** and **j**, data are presented as mean \pm SEM. A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. When applicable, p-value thresholds (*<0.05; **<0.01;***<0.001) were adjusted with a Bonferroni correction (α /number of tests). Abbreviations: n.s. (not significant); DIV (day in vitro). Source data are provided as a Source Data file.



Supplementary Figure 2. Normal EPSC kinetics and more quantifications of release probability and short-term plasticity. a-c Analysis of the kinetics of single EPSCs. Control n = 44/6, cDKO n = 49/6. a 20 – 80% rise time; p=0.7124. b 100 - 50% decay time; p=0.0602. c EPSC width at half maximum; p=0.0907. d Paired-pulse ratios at different inter-pulse intervals. Same data as in Fig. 1 g. 20 ms IPI: control n = 45/6, cDKO n = 47/6; ***p<0.0001. 50 ms IPI: control n = 37/6, cDKO n = 42/6; ***p<0.0001. 100 ms IPI: control n = 39/6, cDKO n = 44/6; ***p<0.0001. 200 ms IPI: control n = 47/6, cDKO n = 47/6; ***p<0.0001. e Same analysis as in Fig. 1 h-k but for a 5 Hz train of 5 pulses. The rundown of the absolute (left) and normalized (center) amplitude were plotted and the ratio of the fifth over the first amplitude was calculated (right) to quantify short-term plasticity. Control n = 39/6, cDKO n = 42/6; ***p<0.0001. f Same as in e but for a 20 Hz train. Control n = 39/6, cDKO n = 42/6; ***p<0.0001.

N = cells/independent cultures. In **a-f**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. In left and center plots in **e** and **f**, data are presented as mean ± SEM. A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. Abbreviations: n.s. (not significant). Source data are provided as a Source Data file.



Supplemental Figure 3. Tomosyn cDKO neurons in micro-networks enhances the release probability of spontaneous and evoked release. a-c Analysis of spontaneous vesicle release in micro-networks of hippocampal neurons. Control n = 21/4, cDKO n = 19/4. a Example traces of miniature EPSCs (mEPSCs). b The frequency of mEPSCs; ***p<0.0001. c mEPSC amplitude; p=0.2802. d-g Analysis of evoked synaptic transmission in micro-networks of hippocampal neurons. Control n = 23/5, cDKO n = 23/5. d Example traces of EPSCs evoked by local field stimulation (5 pulses at 5 Hz). e Amplitudes of EPSCs. f Amplitudes of EPSCs normalized to the first pulse. g STP quantified by the ratio of the fifth pulse over the first pulse; ***p<0.0001.

N = cells/independent cultures. In **b**, **c** and **g**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. In **e** and **f**, data are presented as mean \pm SEM. A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. Abbreviations: n.s. (not significant). Source data are provided as a Source Data file.



Supplementary Fig. 4. **Tomosyns increase the calcium affinity of the release machinery. a-b** Autaptic hippocampal neurons were stimulated with paired pulses of 50 ms intervals at different extracellular calcium concentrations. Control n = 9 - 13/4, cDKO n = 10 - 14/4. **a** Example traces. **b** The paired-pulse ratio (PPR) was calculated by dividing the amplitude of the second pulse by the amplitude of the first pulse. 1mM: control n = 13/4, cDKO n = 14/4, **p=0.0052; 1.5mM: control n = 9/4, cDKO n = 14/4, **p=0.0086; 2mM: control n = 13/4, cDKO n = 14/4, **p=0.0002; 4mM: control n = 9/4, cDKO n = 11/4, **p=0.0028; 8mM: control n = 9/4, cDKO n = 10/4, *p=0.0288. **c** Concentration-response curves of the 1st EPSC amplitudes from data in a-b, normalized to responses in standard 2 mM [Ca²⁺]. **d** A Hill function was fitted to the data shown in **c** from which the dissociation constant (Kd) for calcium (left) and the Hill coefficient (right) were calculated. Control n = 9/4, cDKO n = 11/4; *p=0.0193 (Kd); p=0.7792 (Hill coefficient).

N = cells/independent cultures. In **b** and **d**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. In **c**, data are presented as mean \pm SEM. A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. Abbreviations: n.s. (not significant). Source data are provided as a Source Data file.



Supplementary Figure 5. Fitting of an energy barrier model yields similar results as manual analysis of sucrose traces. a Illustration of the minimal vesicle state model. Vesicles first start out in a depot pool of vesicles from which they transition to a primed state at a priming rate k1. De-priming occurs at a rate of k-1. Primed vesicles will fuse with a rate k2. b RRP size as estimated from the fitted data. Control n = 16/6, cDKO n = 18/6. c Pves calculated as the ratio of the charge released by a single EPSC to the fitted RRP. Control n = 15/6, cDKO n = 15/6. d The fraction of the RRP released by the submaximal sucrose concentration as calculated from the fitted data. Control n = 10/6, cDKO n = 15/6; ***p<0.0001. e Priming and f de-priming rates during the 500 mM sucrose application, derived from the fitting. Control n = 16/6, cDKO n = 16/6. g Neurons were stimulated with 80 action potentials at 40 Hz (see Fig. 2g). The cumulative charge released during the train was plotted and a line was drawn through the last 20 pulses to back-extrapolate to the y-intercept, which marks the estimate for the RRP (readily releasable pool) size. h RRP size as calculated by back-extrapolation shown in g. Control n = 33/6, cDKO n = 38/6; p=0.1090. i The refilling rate of vesicles during the pulse is estimated from the slope of the back-extrapolation line shown in g. Control n = 33/6, cDKO n = 38/6; p=0.1419. j The amplitude of recovery pulse R2 (see Fig. 2 g) was divided by the first amplitude of the train. Control n = 31/6, cDKO n = 35/6; p=0.1933. k The absolute amplitude of recovery pulse R2. Control n = 31/6, cDKO n = 35/6; *p=0.0316.

N = cells/independent cultures. In **b-f** and **h-k**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. In **g**, data are presented as mean \pm SEM (shaded area). A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. Source data are provided as a Source Data file.



Supplementary Figure 6. Loss of tomosyns does not increase vesicle docking.

a-e High-pressure freeze electron microscopy was performed on micro-networks of control (n = 132 synapses / 4 sapphires), cDKO (n = 89 synapses/ 4 sapphires), and cDKO neurons expressing tomosyn-1m (+WT; n = 128 synapses/ 4 sapphires). **a** Example images. Edges of the active zone region on the presynaptic membrane are indicated by arrowheads. Scale bar = 100 nm. **b** No significant difference between control and cDKO synapses is detected, but the number of docked vesicles is reduced in + WT synapses compared to control. *p*=0.0424 (control vs cDKO); **p*=0.0153 (control vs +WT), *p*=0.804 (cDKO vs +WT). **c** The total number of synaptic vesicles (*p*=0.3097) and **d** the length of the active zone (*p*=0.2044) in the cross section is normal in all groups. **e** The distribution of vesicles proximal to the active zone is normal in all groups.

A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. Abbreviations: n.s. (not significant). Source data are provided as a Source Data file.



Supplementary Figure 7. **The tomosyn-VAMP2 hybrid is properly targeted to synapses. a-d** Morphological analysis of neurons infected with wild-type tomosyn or hybrid tomosyn (see Fig. 3). Control n = 35/3, cDKO n = 30/3, + WT n = 33/3, + Hybrid n = 35/3. **a** Example images of autaptic hippocampal neurons immunostained for MAP2, synaptophysin-1, and tomosyn-1. Scale bar = 50 µm. **b** Total dendrite length as derived from the MAP2 mask; *p*=0.0622 (control vs +WT); *p*=0.0826 (cDKO vs +WT), *p*=0.0354 (+hybrid vs +WT). **c** Synapse density as derived from the synaptophysin-1-positive puncta within the MAP2 mask; *p*=0.2991 (all groups). **d** The ratio of the tomosyn-1 signal intensity in synapses to the signal in the soma was calculated to demonstrate synaptic targeting; *p*=0.2934 (control vs +WT); ****p*<0.0001 (cDKO vs +WT), *p*=0.3730 (+hybrid vs +WT). **e** Absolute EPSC amplitudes. cDKO n = 17/4, + WT n = 21/4, + Hybrid n = 21/4; ***p*=0.00137 (cDKO vs +WT), ***p*=0.00147 (+hybrid vs +WT). **f** EPSC amplitude measured 90 seconds after STP protocol (5 pulses at 10Hz) normalized to initial EPSC amplitude (1st pulse 10Hz train). cDKO n = 14/4, + WT n = 20/4, + Hybrid n = 18/4.

N = cells/independent cultures. In **b-f**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. For post-hoc comparison to + WT group, p-value thresholds (*<0.05; **<0.01;***<0.001) were adjusted with a Bonferroni correction (α /number of tests). Grey asterisks show comparison to + WT group. Abbreviations: n.s. (not significant). Source data are provided as a Source Data file.



Supplementary Figure 8. The probability density functions (PDFs) of the time-dependent extensions at constant forces revealed an intermediate state induced by the tomosyn SNARE motif (state 7*). The PDFs can be well fitted by a sum of two Gaussian functions in the absence of the tomosyn SNARE motif and three Gaussian functions when present. The three peaks represent the template complex (state 7), the tomosyn-bound template complex (state 7*), and the Munc18-1-bound open syntaxin (Fig. 5 c).



Supplementary Figure 9. The tomosyn SNARE motif does not affect the folding of Munc18-1-bound closed syntaxin-1. a Schematic diagrams of Munc18-1-bound closed syntaxin (i) and unfolded SNARE motif (ii). Note that the syntaxin-1 molecule was pulled from its C-terminus and I187C to which a DNA handle was attached. b Force-extension curves obtained by pulling Munc18-1 bound syntaxin-1 in the absence or presence of 2 μ M tomosyn SNARE motif in the solution. c Extension-time trajectories at constant forces showing reversible unfolding and refolding of the Munc18-1-bound syntaxin.

Abbreviations: N-terminal regulatory domain (NRD).



Supplementary Figure 10. Reduced lifetimes of the tomosyn-bound template complex state due to tomosyn truncation. a Closed-up views of the extension trajectories (black) containing the tomosyn-bound template complex state shown in Fig. 6a. b Histograms of the dwell time of the tomosyn-bound template complex. The whole trajectories span 40 s-90 s and were first analyzed by three-state hidden-Markov modeling. Then, the idealized state transitions (red traces in a) were derived by the Viterbi algorithm. Finally, the states corresponding to the tomosyn-bound template complex state (state 7*) were extracted and their dwell times were calculated for the histogram distributions. The distributions were fit with a single exponential function (red curves) to derive the average lifetimes (τ) indicated.



Supplementary Figure 11. SNARE-truncating constructs support neuronal morphology. a-d Morphological analysis of neurons infected with different mutant constructs (see Fig. 5). Control n = 26 – 27/3, cDKO n = 27/3, + WT n = 29/3, + FA n = 33 – 34/3, + ΔPB n = 30 – 31/3, + ΔSNARE n = 36 – 38/3, + WDonly n = 34/3. a Example images of autaptic hippocampal neurons immunostained for MAP2, synaptophysin-1, and tomosyn-1. Dashed boxes correspond to dendrite zoom-ins in Fig. 6 f. Scale bar = 50 μ m. **b** Somatic intensity of tomosyn-1 normalized to controls. Control n = 26/3, cDKO n = 27/3, + WT n = 29/3, + FA n = 33/3, + ΔPB n = 30/3, + ΔSNARE n = 36/3, + WDonly n = 34/3. Post hoc comparisons against +WT: p=0.1271 (control); ***p<0.0001 (cDKO), p=0.00927 (+FA), *p=0.00492 (+ΔPB), p=0.009623 (+ΔSNARE), p=0.1967 (+WDonly). c Total dendrite length as derived from the MAP2 mask. Control n = 27/3, cDKO n = 27/3, + WT n = 29/3, + FA n = 34/3, + ΔPB n = 31/3, + ΔSNARE n = 38/3, + WDonly n = 34/3. Post hoc comparisons against +WT: p=0.0567 (control); p=0.2442 (cDKO), p=0.6569 (+FA), p=0.1724 (+ Δ PB), p=0.2624 (+ Δ SNARE), p=0.2592 (+WDonly). **d** Synapse density as derived from the synaptophysin-1-positive puncta within the MAP2 mask. Control n = 27/3, cDKO n = 27/3, + WT n = 29/3, + FA n = 34/3, + ΔPB n = 31/3, + ΔSNARE n = 38/3, + WDonly n = 34/3; p=0.7383 (all groups). **e** Absolute EPSC amplitudes. Control n = 21/6, cDKO n = 25/8, +WT n = 27/6, +FA n = 18/5, $+\Delta PB$ n = 13/4, $+\Delta SNARE$ n = 8/4, +WDonly n = 10/3; p=0.06294 (all groups). f EPSC amplitude measured 90 seconds after STP protocol (5 pulses at 10Hz) normalized to initial EPSC amplitude (1st pulse 10Hz train). Control n = 21/6, cDKO n = 23/8, +WT n = 26/6, +FA n = 16/5, $+\Delta$ PB n = 12/4, $+\Delta$ SNARE n = 6/3, +WDonly n = 10/3; *p*=0.06294 (all groups).

N = cells/independent cultures. In **b-f**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. For post-hoc comparison, p-value thresholds (*<0.05; **<0.01;***<0.001) were adjusted with a Bonferroni correction (α /number of tests). Abbreviations: n.s. (not significant). Source data are provided as a Source Data file.

Supplementary Fig. 12



Supplementary Figure 12. **The partial SNARE-hybrid constructs support neuronal morphology. a-d** Morphological analysis of neurons infected with different mutant constructs (see Fig. 7). **a** Example images of autaptic hippocampal neurons immunostained for MAP2, synaptophysin-1, and tomosyn-1. Scale bar = 50 μ m. Control n = 22/4, cDKO n = 23/4, + WT n = 24/4, + Core n = 23/4, + LR n = 25/4. **b** Total dendrite length as derived from the MAP2 mask; *p*=0.0553 (all groups). **c** Synapse density as derived from the synaptophysin-1-positive puncta within the MAP2 mask; *p*=0.4820 (all groups). **d** The ratio of the tomosyn-1 signal intensity in synapses to the signal in the soma was calculated to demonstrate synaptic targeting; *p*=0.013 (all groups). Post hoc comparisons against +WT: *p*=0.1836 (control); ***p*=0.00025 (cDKO), *p*=0.6171 (+Core), *p*=0.03678 (+LR). **e** Absolute EPSC amplitudes cDKO. n = 20/4, + WT n = 22/4, + Core n = 24/4, + LR n = 23/4; *p*=0.0054. Post hoc comparisons against +WT: **p*=0.0051 (cDKO), *p*=0.9399 (+Core), *p*=0.3096 (+LR). **f** EPSC amplitude measured 90 seconds after STP protocol (5 pulses at 10Hz) normalized to initial EPSC amplitude (1st pulse 10Hz train). cDKO n = 19/4, + WT n = 22/4, + Core n = 22/4, + LR n = 22/4.

N = cells/independent cultures. In **b-f**, boxplots display median (center), upper and lower quartiles (box bounds) and whiskers to the last datapoint within 1.5x interquartile range. A one-way ANOVA tested the significance of adding experimental group as a predictor, see Supplementary Table 1. For post-hoc comparison, p-value thresholds (*<0.05; **<0.01;***<0.001) were adjusted with a Bonferroni correction (α /number of tests). Abbreviations: n.s. (not significant). Source data are provided as a Source Data file.

Control Control <t< th=""><th>PARAMETER</th><th>GROUP</th><th>(N/n)</th><th>MEAN±SD</th><th>OUTLI</th><th>ANOVA BETWEEN MODELS</th><th>POSTHOC ANOVA ON SUBSETS</th></t<>	PARAMETER	GROUP	(N/n)	MEAN±SD	OUTLI	ANOVA BETWEEN MODELS	POSTHOC ANOVA ON SUBSETS
Sequence intermation Construction Proper printConstruction Proper prin					SUPP	LEMENTAL FIGURE 1	
$ \begin{aligned} \begin{array}{c} \text{Dath Section 1} \\ \text{Support at } \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Support at } \\ \text{Support at } \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Support at } \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Support at } \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \{Dath Section 2} \\ \begin{array}{c} \text{Dath Section 2} \\ \\\ \\\ \e& \text{Dath Section 2} \\ \begin{array}{c} Dath Section 2$	Synaptic tom-1 intensity (a u)	Control cDKO	2/31 2/30	1399 ± 334 424 ± 129	0	F(1,58) = 231.1, p<0.0001 ***	
dbC 4DC 4DC 4DC 4DC 4DC Nergenging Como 4.2 4DL 4	Dendrite length (mm)	Control	4/62	1764 ± 944	0	F(1,114) = 1.107, p =0.295	
convertional product theoremconvertional product theoremconvertional product theoremconvertional product theoremconvertional product theoremconvertional product theoremconvertional product theoremconvertional product theoremconvertional product theoremconvertional product 	Symoneoe/ um	cDKO Control	4/57	1605 ± 889	0	E(1 112) = 1.626 = 0.2025	
Nach Legender, Noch Legender, Martin Grouper, Martin Grouper, Martin 	Synapses/ µm	cDKO	4/02	0.27 ± 0.1 0.28 ± 0.09	0	r(1,113) = 1.030, p=0.2033	
Nome BU30Nome BU30Nome BU30Nome 	Neurite length (mm),	Control	1/13	1048 ± 569	0	F(3,60) = 11.54, p<0.0001 ***	WT-DIV7 vs cDKO-DIV7: $F(1,25) = 0.0931$, $p = 0.7628$
PDY5OKO	Neurite length (mm),	Control	1/19	2432 ± 1065	0		WT-DIV15 vs cDKO-DIV15: F(1,35) = 0.8302, p= 0.3685
Spectra (DNP) Control Hole Hole <td>DIV15</td> <td>cDKO</td> <td>1/18</td> <td>2136 ± 899</td> <td>0</td> <td></td> <td>cDKO-DIV7 vs cDKO-DIV15: F(1,30) = 14.96, p= 0.0005**</td>	DIV15	cDKO	1/18	2136 ± 899	0		cDKO-DIV7 vs cDKO-DIV15: F(1,30) = 14.96, p= 0.0005**
Space-Space Space-Space Space-Space	Synapses/µm, DIV7	cDKO	1/13 1/14	0.11 ± 0.05 0.11 ± 0.05	0	F(3,59) = 55.81, p < 0.0001 ****	WT-DIV/ vs cDKO-DIV/:F(1,25) = 0.0208, p=0.8865 WT-DIV7 vs WT-DIV15: F(1,29) = 67.93, p<0.0001 ***
Jackson Pars, Prof. John Pars, Pro	Synapses/µm, DIV15	Control	1/19	0.33 ± 0.14	1		WT-DIV15 vs cDKO-DIV15: F(1,34) = 1.17, p= 0.2869
Latery Tea, DVI Long Teal of the second seco	Intensity Tom, DIV7	cDKO Control	1/18	0.32 ± 0.07 1399 ± 406	0	F(3.58) = 101.7, p<0.0001 ***	cDKO-DIV7 vs cDKO-DIV15: F(1,30) = 100.9, p<0.0001 *** WT-DIV7 vs cDKO-DIV7: F(1,24) = 55.84, p<0.0001 ***
Index Provide Action (2000)103<		cDKO	1/14	624 ± 352	1		WT-DIV7 vs WT-DIV15: $F(1,29) = 1.806$, $p = 0.1895$
Interform <	Intensity Tom, DIV15	control	1/19	1499 ± 268 412 ± 161	0		WT-DIV15 vs cDKO-DIV15: $F(1,34) = 381$, p<0.0001 *** cDKO-DIV7 vs cDKO-DIV15: $F(1,29) = 5.912$, p= 0.0215
mPPC (require) mPC (map) mPC (map)Conset mPC (map) mPC (map)Conset mPC (map) mPC (map)Conset mPC (map) mPC (map) mPC (map)Conset mPC (map) mPC (map) mPC (map)Conset mPC (map) mPC (map) mPC (map) mPC (map)Conset mPC (map) mPC (map) 					FIGURE 1 +	- SUPPLEMENTAL FIGURE 2	
$ \begin{aligned} & \text{def} \\ \text{and price angle Ads} & Control & 647 & 11.164 + 538 & 1 & 1 & 11.064 + 0.1002 \\ & \text{def} \\ \\ & \text{def} \\ & \text{def} \\ \\ \\ & \text{def} \\ \\ & \text{def} \\ \\ \\ & de$	mEPSC frequency	Control cDKO	6/47 6/44	6.54 ± 6.54 27 11 + 13 87	1	F(1,83) = 111.7, p<0.0001 ***	
1700 0.44 0.934 + 6.8 0 1 1285 Campido L. 0 0.000 13.1 + 14 0 10.1 + 14.5, pri0.000 *** 1285 Campido L. 0 0.000 13.1 + 14 1 1 10.0 + 14.5, pri0.000 *** 1285 Campido L. 0 0.000 0.000 13.1 + 14 1 1 10.0 + 14.5, pri0.000 *** 1285 Campido L. 0 0.000 0.000 0.000 1.0 + 0.000 1.0 + 0.000 1285 Campido L. 0 0.000 0.000 0.000 1.0 + 0.000 1.0 + 0.000 1285 Campido L. 0 0.000 0.000 0.000 1.0 + 0.000 1.0 + 0.000 1285 Campido L. 0 0.000 0.000 0.000 1.0 + 0.000 1.0 + 0.000 1285 Campido L. 0 0.000 0.000 0.000 1.0 + 0.000 1.0 + 0.000 1285 Campido L. 0 0.000 0.000 0.000 0.000 1.0 + 0.000 1285 Campido L. 0 0.000 0.000 0.000 1.0 + 0.000 1.0 + 0.000 1285 Campido L. 0 0.000 0.000	mEPSC amplitude	Control	6/47	31.16 ± 5.85	1	F(1,83) = 3.075, p=0.0832	
Cale and part of the set of	$FPSC$ amplitude $(n\Lambda)$	cDKO Control	6/44	32.84 ± 5.68	0	F(1.91) = 16.44 n = 0.0001 ***	
BixB	Er be unprivate (mr)	cDKO	6/49	12.2 ± 5.3	0	1(1,91) 10.11, p 0.0001	
Instrument of and the second of the	EPSC charge (pC)	Control cDKO	6/50 6/49	124 ± 145 194 + 121	1	F(1,90) = 14.63, p=0.0002 ***	
char EPC decryment BCPC decryment 	EPSC rise time (ms)	Control	6/44	1.68 ± 0.64	2	F(1,83) = 0.137, p=0.7124	
Lab Constr PAP 2 Sol PAP 2 Sol PAP 2 Sol PRN: Indivation Constr PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PRN: Indivation Constr PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PRN: Indivation Constr PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PARD FULS ELEVENCE Constr PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PARD FULS ELEVENCE Constr PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAR 2 Sol PAP 2 Sol PAR 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol PAP 2 Sol	EBSC doogy time (mg)	cDKO Control	6/49	0.64 ± 0.47	1	E(1.85) = 2.627 n=0.0602	
FTSC lands DATE6489.86.12% 6401FLADFLADPALED PLASE KATUS5406400.31.030PALED PLASE KATUS5506470.34.031FLAD200 m IP5004400.34.030FLAD1500 m IP5006400.34.030FLAD1600 m IP5006400.34.030FLAD1600 m IP5006400.34.030FLAD7.05.07.57.ph.0001***500 m IP500 m IP500 m IP500 m IP500 m IP500 m IP1FLAD500 m IP500 m IP6400.92.050FLADFLAD500 m IP6470.93.051FLADFLADFLAD500 m IP6470.93.051FLADFLADFLAD500 m IP6470.93.051FLADFLADFLAD500 m IP6470.93.051FLADFLADFLAD500 m IP6470.93.051FLADFLADFLAD6167 mall AL (AD0.83.050FLADFLADFLAD6167 mall AL (AD0.84.050FLADFLAD<	EFSC decay time (ms)	cDKO	6/49	0.92 ± 2.81 7.67 ± 2.56	0	r(1,85) = 5.027, p=0.0002	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	EPSC half-width (ms)	Control	6/44	9.46 ± 2.96 10.22 ± 3.06	1	F(1,83) = 2.929, p=0.0907	
Dam BP Connal 643 694 0.33 1 P(DA) = 12.5 p.0.0001*** 30 m BP Connal 647 0.1 = 0.23 1 P(D) = 27.5 p.0.0001*** 30 m BP Connal 643 1.1 ± 0.41 1 P(D) = 27.5 p.0.0001*** 00 m BP Connal 647 0.51 0.19 0 P(D) = 27.5 p.0.0001*** 30 m BP Connal 647 0.51 0.19 0 P(D) = 0.5 p.0.0001*** 30 m BP Connal 647 0.51 0.19 0 P(D) = 0.5 p.0.0001*** 310 m BP Connal 647 0.51 0.15 0.10 P(D) = 0.5 p.0.0001*** 310 m BP Connal 647 0.51 0.5 0.15 p.0.0001*** P(D) = 0.5 p.0.0001*** 310 m BP Connal 640 0.41 0.16 0 P(D) = 0.5 p.0.0001*** 310 m BP Connal 641 0.41 0.16 0 P(D) = 0.5 p.0.0001*** 118 P(D) Connal 531 532 P(D) = 0.5 p.0.0001*** P(D) = 0.5 p.0.0001*** 10 BP Connal 532 532 1	PAIRED PULSE RATIO (#	#2/#1)	0/49	10.22 ± 5.00	0		
90 mtH 100 mtH 11 mtJ 1 F(170 - 27.0, p-0.001***) 100 mtH 642 0.3 + 0.3 0 P(7.0 - 22.7, p-0.001***) 20 mtH 644 0.5 ± 0.19 0 P(7.0 - 22.7, p-0.001***) 20 mtH 647 0.5 ± 0.19 0 P(7.0 - 22.7, p-0.001***) 20 mtH 647 0.5 ± 0.19 0 P(7.0 - 22.7, p-0.001***) 20 mtH Control 647 0.5 ± 0.13 0 P(7.0 - 27.0, p-0.001***) 21 ferrin Control 647 0.5 ± 0.13 0 P(7.0 - 27.0, p-0.001***) 21 ferrin Control 647 0.3 ± 0.31 1 P(7.0 - 25.16, p-0.001***) 21 ferrin Control 642 0.3 ± 0.37 1 P(7.0 - 25.16, p-0.001***) 11 fER frequency Control 531 632.6 0 P(7.0 - 25.16, p-0.001***) 11 fER frequency Control 531 632.6 0 P(7.0 - 15.16, p-0.001***) 11 fER frequency Control 532 0.62 ± 0.7 0 P(7.0 - 15.16, p-0.005**) 11 fER frequency </td <td>20 ms IPI</td> <td>Control</td> <td>6/45</td> <td>0.95 ± 0.35</td> <td>1</td> <td>F(1,83) = 17.25, p<0.0001 ***</td> <td></td>	20 ms IPI	Control	6/45	0.95 ± 0.35	1	F(1,83) = 17.25, p<0.0001 ***	
Internal ProbabilityNote of the second	50 ms IPI	Control	6/37	0.71 ± 0.23 1.11 ± 0.41	1	F(1,70) = 27.70, p<0.0001***	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	100 101	cDKO	6/42	0.74 ± 0.20	1	F(1.5() 22.55 - 0.0001***	
200 mI 0K00.93 m.03 m.03 m.031 0 0 0 0 0 0 0 0 	100 ms IPI	cDKO	6/39	1.04 ± 0.33 0.75 ± 0.17	0	$F(1, 6) = 22.77, p < 0.0001^{***}$	
IDENT ISAND 4 IDENT 1	200 ms IPI	Control	6/47	0.95 ± 0.19	1	F(1,86) = 37.86, p<0.0001***	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	SHORT-TERM PLASTICI	CDKO TY (#5/#1)	6/4/	0.74 ± 0.14	0		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	5 Hz train	Control	6/47	0.90 ± 0.36	1	F(1,86) = 69.15, p<0.0001***	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	10 Hz train	cDKO Control	6/47 6/39	0.50 ± 0.15 0.85 ± 0.43		F(1.75) = 32.54, p<0.0001***	
20 HE ruin Control 643 0.38 + 0.30 1 F(1,70) = 35. 6, p=0.0001*** mEPSC megnets Control 642 0.38 + 0.20 1 mEPSC megnets Control 421 5.33 + 6.77 1 F(1,74) = 51.65, r=0.0001*** mEPSC megnitod Control 421 5.35 + 6.77 1 F(1,73) = 1.21, p=0.3002 EPSC amplitod Control 522 8.73 + 4.25 0 F(1,40) = 51.21, p=0.3002 EPSC amplitod Control 522 8.73 + 4.25 0 F(1,40) = 51.21, p=0.3002 EPSC amplitod Control 522 0.62 + 0.17 1 F(1,30) = 50.001*** after STP (9.67)*1 Control 522 0.40 + 0.10 0 F(1,21) = -0.601*** after STP (9.67)*1 Control 522 0.40 + 0.10 0 F(1,13) = 5.75 + 0.54 after STP (9.67)*1 Control 522 0.40 + 0.10 0 F(1,13) = 5.70, p=0.0000*** after STP (9.67)*1 Strand (20*) Control 413 0.57 + 0.50 F(1,15) = 0.57, p=0.0000***		cDKO	6/44	0.44 ± 0.16	0		
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	20 Hz train	Control cDKO	6/39 6/42	0.74 ± 0.37 0.38 ± 0.20	1	F(1,70) = 35.16, p<0.0001***	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $					SUPP	LEMENTAL FIGURE 3	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	mEPSC frequency	Control cDKO	4/21 4/21	5.35 ± 6.77 37.39 ± 19.02	1	F(1,34) = 51.05, p<0.0001 ***	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	mEPSC amplitude	Control	4/21	20.55 ± 8.83	1	F(1,33) = 1.21, p=0.2802	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	EPSC amplitude	cDKO Control	4/21	24.41 ± 15.39 8 73 + 4 25	0	F(1 40) = 2 19 n = 0 1463	
SH2 Control 5/23 0.62 ± 0.17 1 F(1.39) = 30.60, p<0.0001*** PR $5/3$ 0.49 ± 0.10 0 SUPPLEMENTAL FIGURE 4 PR<($e^{2/4}$), 50 ms IP $-$ SUPPLEMENTAL FIGURE 4 0 InM (Ca^{-1}/e Control 4/13 1.57 ± 0.54 0 F(1.22) = 9.620, p= 0.0552** InM (Ca^{-1}/e Control 4/14 1.12 ± 0.14 0 F(1.22) = 9.620, p= 0.0052** InM (Ca^{-1}/e Control 4/13 0.85 ± 0.06 0 F(1.18) = 8.703, p= 0.002*** InM (Ca^{-1}/e Control 4/13 0.98 ± 0.20 0 F(1.22) = 2.034, p= 0.002*** InM (Ca^{-1}/e Control 4/10 0.54 ± 0.07 0 F(1.15) = 12.79, p=0.0028** InM (Ca^{-1}/e Control 4/9 0.63 ± 0.1 0 F(1.14) = 5.932, p=0.028** InM (Ca^{-1}/e Control 4/9 1.61 ± 0.35 0 F(1.15) = 6.85, p= 0.019* $Int Ca^{-1}/e$ Control 4/9 1.61 ± 0.35 0 F(1.15) = 0.082, p=0.7792	Er be umphtade	cDKO	5/23	10.82 ± 5.67	0	1(1,40) 2.19, p 0.1405	
$\begin{tabular}{ c c c c c c c } \hline Control & 4/13 & 1.57 \pm 0.54 & 0 & F(1.22) = 9.620, p=0.0052^{**} \\ COKO & 4/14 & 1.12 \pm 0.14 & 0 & F(1.22) = 9.620, p=0.0052^{**} \\ COKO & 4/14 & 0.85 \pm 0.16 & 0 & F(1.18) = 8.703, p=0.0086^{**} & COKO & 4/14 & 0.74 \pm 0.14 & 0 & F(1.18) = 8.703, p=0.0086^{**} & COKO & 4/14 & 0.72 \pm 0.08 & 0 & F(1.22) = 2.034, p=0.0002^{***} & COKO & 4/14 & 0.72 \pm 0.08 & 0 & F(1.22) = 2.034, p=0.0002^{***} & COKO & 4/14 & 0.72 \pm 0.08 & 0 & F(1.22) = 2.034, p=0.0002^{***} & COKO & 4/14 & 0.74 \pm 0.14 & 0 & F(1.15) = 12.79, p=0.0028^{**} & COKO & 4/10 & 0.51 \pm 0.08 & 0 & F(1.14) = 5.932, p=0.0288^{**} & COKO & 4/10 & 0.51 \pm 0.08 & 0 & F(1.14) = 5.932, p=0.0288^{**} & COKO & 4/10 & 0.51 \pm 0.08 & 0 & F(1.14) = 5.932, p=0.0288^{**} & COKO & 4/11 & 1.57 \pm 0.2 & 0 & F(1.15) = 6.865, p=0.0193^{**} & COKO & 4/11 & 1.27 \pm 0.2 & 0 & F(1.15) = 6.865, p=0.0193^{**} & COKO & 4/11 & 1.27 \pm 0.2 & 0 & F(1.15) = 6.865, p=0.0193^{**} & COKO & 4/11 & 1.27 \pm 0.2 & 0 & F(1.15) = 0.082, p=0.7792 & COKO & 4/11 & 1.27 \pm 0.2 & 0 & F(1.15) = 0.082, p=0.7792 & COKO & 4/11 & 1.287 \pm 0.45 & 0 & F(1.43) = 1.778, p=0.1894 & COKO & 4/11 & 2.87 \pm 0.45 & 0 & F(1.43) = 1.778, p=0.1894 & COKO & 6/26 & 2.15 \pm 1.59 & 0 & F(1.43) = 0.013^{**} & COKO & 6/26 & 2.15 \pm 1.59 & 0 & F(1.43) = 0.000^{***} & COKO & 6/26 & 2.15 \pm 1.59 & 0 & F(1.43) = 0.032, p=0.013^{**} & COKO & 6/26 & 0.25 & 0.07 \pm 0.06 & 0 & F(1.42) = 0.626, p=0.013^{**} & COKO & 6/26 & 0.55 & 0.07 \pm 0.06 & 0 & F(1.43) = 0.000^{***} & COKO & 6/26 & 0.55 & 0.07 \pm 0.06 & 0 & F(1.43) = 0.000^{***} & COKO & 6/26 & 0.05 \pm 0.07 \pm 0.06 & 0 & F(1.43) = 0.034, p=0.000^{***} & COKO & 6/26 & 0.05 \pm 0.07 \pm 0.06 & 0 & F(1.43) = 0.000^{***} & COKO & 6/26 & 0.05 \pm 0.07 \pm 0.06 & 0 & F(1.23) = 0.230, p=0.6347 & COKO & 6/18 & 1.33 \pm 0.91 & 0 & F(1.23) = 0.230, p=0.6347 & COKO & 6/18 & 1.33 \pm 0.91 & 0 & F(1.23) = 0.230, p=0.6347 & COKO & 6/18 & 1.33 \pm 0.91 & 0 & F(1.23) = 0.230, p=0.6347 & COKO & 6/18 & 1.33 \pm 0.91 & 0 & F(1.23) = 0.230, p=0.6347 & COKO & 6/18 & 1.33 \pm 0.91 & 0 & F(1.23) = 0$	5Hz STP (#5/#1)	Control cDKO	5/23	0.62 ± 0.17 0.40 ± 0.10	1	F(1,39) = 30.60, p<0.0001 ***	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		CDRO	5/25	0.40 ± 0.10	SUPP	LEMENTAL FIGURE 4	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	PPR (#2/#1), 50 ms IPI		4/12	1.57 + 0.54			
$I.5mM [Ca^{2+}]e$ Control49 1.18 ± 0.3 0 $F(1,18) = 8.703, p=0.0086^{**}$ $2mM [Ca^{2+}]e$ Control4/13 0.88 ± 0.20 0 $F(1,22) = 20.34, p=0.0002^{***}$ $aMM [Ca^{2+}]e$ Control4/14 0.72 ± 0.08 0 $aMM [Ca^{2+}]e$ Control4/19 0.72 ± 0.08 0 $bMM [Ca^{2+}]e$ Control4/9 0.56 ± 0.07 0 $bMM [Ca^{2+}]e$ Control4/9 0.65 ± 0.1 0 $bMM [Ca^{2+}]e$ Control4/10 0.51 ± 0.08 0 $bMT [Ca^{2+}]e$ Control4/19 1.61 ± 0.35 0 $bMT [Ca^{2+}]e$ Control4/19 1.61 ± 0.35 0 $bMT [Ca^{2+}]e$ Control4/11 1.27 ± 0.2 0 $bMT [Ca^{2+}]e$ Control4/11 1.27 ± 0.2 0 $bH1$ [coefficient (c)Control6/25 2.45 ± 1.26 0 $cDKO$ 4/11 1.27 ± 0.2 0 $cDKO$ 6/25 2.45 ± 1.26 0 $cDKO$ 6/25 0.45 ± 0.21 0 $cDKO$ 6/25 0.45 ± 0.21 0 $cDKO$ 6/26 0.05 ± 0.04 0 $cDKO$ 6/26 0.85 ± 0.24 0 $cDKO$ 6/26 </td <td>Imm [Ca-]e</td> <td>cDKO</td> <td>4/13</td> <td>1.57 ± 0.54 1.12 ± 0.14</td> <td>0</td> <td>$F(1,22) = 9.620, p = 0.0052^{++}$</td> <td></td>	Imm [Ca-]e	cDKO	4/13	1.57 ± 0.54 1.12 ± 0.14	0	$F(1,22) = 9.620, p = 0.0052^{++}$	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	1.5mM [Ca ²⁺]e	Control	4/9	1.18 ± 0.33	0	F(1,18) = 8.703, p=0.0086**	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	2mM [Ca ²⁺]e	Control	4/14 4/13	0.85 ± 0.16 0.98 ± 0.20	0	F(1,22) = 20.34, p= 0.0002***	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	4 14 50 2+1	cDKO	4/14	0.72 ± 0.08	0	E(115) 12 70 0 0020**	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	4mM [Ca ⁺⁺]e	cDKO	4/9 4/11	0.74 ± 0.14 0.56 ± 0.07	0	$F(1,15) = 12.79, p=0.0028^{**}$	
Normalized EPSC amp, (to 2mM)Control4/100.51 \pm 0.080F(1,15) = 0.82, p=0.7192KD Ca ^{2*} (mM)Control4/91.61 \pm 0.350F(1,15) = 0.82, p=0.7192Hill coefficient (c)Control4/93.02 \pm 0.570F(1,15) = 0.082, p=0.7792CDKO4/112.87 \pm 0.450FIGURE 2 + SUPPLEMENTAL FIGURE 5RRP size (nC) - manualControl6/252.45 \pm 1.260Pves (EPSC/RRP) -Control6/240.04 \pm 0.030F(1,43) = 1.778, p=0.1894Control6/250.07 \pm 0.060Pves (EPSC/RRP) -Control6/250.07 \pm 0.060Pves (EPSC/RRP) -Control6/260F(1,43) = 42.13, p<0.0001***	$8mM [Ca^{2+}]e$	Control	4/9	0.63 ± 0.1	0	F(1,14) = 5.932, p=0.0288*	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Normalized EPSC amp. (to	2mM)	4/10	0.51 ± 0.08	0		
Hill coefficient (c) $CDKO$ $4/11$ 1.27 ± 0.2 0 $F(1,15) = 0.082, p=0.7792$ Hill coefficient (c) $COntrol$ $4/9$ 3.02 ± 0.57 0 $F(1,15) = 0.082, p=0.7792$ FIGURE 2 + SUPPLEMENTAL FIGURE 5RRP size (nC) - manual $Control$ $6/25$ 2.45 ± 1.26 0 $F(1,43) = 1.778, p=0.1894$ OVER (C) - manual $Control$ $6/26$ 2.15 ± 1.59 0 $F(1,42) = 6.626, p=0.0137*$ manual $Control$ $6/25$ 0.04 ± 0.03 0 $F(1,43) = 42.13, p<0.0001***$ $250/500mM - manual$ $Control$ $6/25$ 0.45 ± 0.21 0 $F(1,43) = 42.13, p<0.0001***$ $cDKO$ $6/25$ 0.45 ± 0.21 0 $F(1,43) = 42.13, p<0.0001***$ $cDKO$ $6/26$ 0.85 ± 0.24 0 0 $F(1,23) = 0.230, p= 0.6347$ $initial$ $cDKO$ $6/16$ 1.99 ± 1.02 0 $F(1,27) = 0.687, p=0.4144$ $cDKO$ $6/16$ 1.99 ± 1.02 0 $F(1,23) = 2.080, p=0.1627$ $reg (EPSC/RRP) Control$ $6/15$ 0.05 ± 0.04 0 $F(1,23) = 2.080, p=0.1627$ $rited$ $COntrol$ $6/15$ 0.10 ± 0.06 0 $F(1,19) = 129.3, p<0.0001***$	KD Ca ²⁺ (mM)	Control	4/9	1.61 ± 0.35	0	F(1,15) = 6.865, p= 0.0193*	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Hill coefficient (c)	cDKO Control	4/11 4/9	1.27 ± 0.2 3.02 ± 0.57	0	F(1.15) = 0.082, p=0.7792	
FIGURE 2 + SUPPLEMENTAL FIGURE 5 RRP size (nC) - manual cDKO 6/25 (cDKO 2.45 ± 1.26 (cDKO 0 (cDKO F(1,43) = 1.778, p=0.1894 Pves (EPSC/RRP) - manual Control 6/24 (cDKO 0.04 ± 0.03 (cDKO 0 (cDKO F(1,42) = 6.626, p=0.0137* 250/500mM - manual Control 6/25 (cDKO 0.45 ± 0.21 (cDKO 0 (cDKO F(1,43) = 42.13, p<0.0001*** Refilled pool (% of initial) Control 6/19 (cDKO 0.45 ± 0.21 (cDKO 0 (cDKO F(1,32) = 0.230, p= 0.6347 Refilled pool (% of initial) Control 6/16 (cDKO 1.99 ± 1.02 (cDKO 0 (cDKO F(1,23) = 0.080, p=0.1637 Refilled pool (% of initial) Control 6/15 (c18 1.33 ± 0.91 (cDKO 0 (c18 F(1,23) = 2.080, p=0.4144 Pves (EPSC/RRP) - fitted Control 6/15 (c15 0.05 ± 0.04 (c18 0 (c18 ± 0.10 ± 0.06 F(1,23) = 2.080, p=0.1627 fitted Control 6/15 0.10 ± 0.06 0 F(1,19) = 129.3, p<0.0001***		cDKO	4/11	2.87 ± 0.45	0		
InternationControl </td <td>RRP size (nC) - manual</td> <td>Control</td> <td>6/25</td> <td>2 45 + 1 26</td> <td>FIGURE 2 +</td> <td>- SUPPLEMENTAL FIGURE 5 F(1 43) = 1 778 p=0 1894</td> <td></td>	RRP size (nC) - manual	Control	6/25	2 45 + 1 26	FIGURE 2 +	- SUPPLEMENTAL FIGURE 5 F(1 43) = 1 778 p=0 1894	
Pves (EPSC/RRP) - manual Control cDKO $6/24$ 0.04 ± 0.03 0 $F(1,42) = 6.626, p=0.0137^*$ 250/500mM - manual Control cDKO $6/25$ 0.07 ± 0.06 0 $F(1,42) = 6.626, p=0.0137^*$ 250/500mM - manual Control cDKO $6/26$ 0.45 ± 0.21 0 $F(1,43) = 42.13, p<0.001^{***}$ Refilled pool (% of initial) Control $6/19$ 0.79 ± 0.07 0 $F(1,32) = 0.230, p= 0.6347$ initial) cDKO $6/20$ 0.80 ± 0.09 0 RRP size (nC) - fitted cDKO $6/16$ 1.99 ± 1.02 0 $F(1,27) = 0.687, p=0.4144$ Pves (EPSC/RRP) - fitted Control $6/15$ 0.05 ± 0.04 0 $F(1,23) = 2.080, p=0.1627$ fitted cDKO $6/15$ 0.10 ± 0.06 0 $F(1,19) = 129.3, p<0.0001^{***}$		cDKO	6/26	2.15 ± 1.59	Ő	(.,,	
250/500mM - manual Control 6/25 0.45 ± 0.21 0 F(1,43) = 42.13, p<0.0001*** cDKO 6/26 0.85 ± 0.24 0 6 <td>Pves (EPSC/RRP) - manual</td> <td>Control cDKO</td> <td>6/24 6/25</td> <td>0.04 ± 0.03 0.07 ± 0.06</td> <td>0</td> <td>F(1,42) = 6.626, p=0.0137*</td> <td></td>	Pves (EPSC/RRP) - manual	Control cDKO	6/24 6/25	0.04 ± 0.03 0.07 ± 0.06	0	F(1,42) = 6.626, p=0.0137*	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	250/500mM - manual	Control	6/25	0.45 ± 0.21	0	F(1,43) = 42.13, p<0.0001***	
Initial port (vol) Collor (vol) <thcollor (<="" td=""><td>Refilled pool (% of</td><td>cDKO Control</td><td>6/26</td><td>0.85 ± 0.24 0.79 ± 0.07</td><td>0</td><td>F(1,32) = 0.230 $p = 0.6247$</td><td></td></thcollor>	Refilled pool (% of	cDKO Control	6/26	0.85 ± 0.24 0.79 ± 0.07	0	F(1,32) = 0.230 $p = 0.6247$	
RRP size (nC) - fitted Control cDKO $6/16$ 1.99 ± 1.02 0 $F(1,27) = 0.687, p=0.4144$ Pves (EPSC/RRP) - fitted Control cDKO $6/15$ 0.05 ± 0.04 0 $F(1,23) = 2.080, p=0.1627$ Gitted cDKO $6/15$ 0.10 ± 0.06 0 $F(1,23) = 2.080, p=0.1627$ 250/500mM - fitted Control $6/10$ 0.55 ± 0.09 0 $F(1,19) = 129.3, p<0.0001***$	initial)	cDKO	6/20	0.80 ± 0.09	0	1(1,52) 0.250, p= 0.0547	
Pres (EPSC/RRP) - Control 6/15 0.05 ± 0.04 0 F(1,23) = 2.080, p=0.1627 fitted cDKO 6/15 0.10 ± 0.06 0 6/15 10.10 ± 0.06 0 250/500mM - fitted Control 6/10 0.55 ± 0.09 0 F(1,19) = 129.3, p<0.0001***	RRP size (nC) - fitted	Control cDKO	6/16	1.99 ± 1.02 1 33 + 0.91	0	F(1,27) = 0.687, p=0.4144	
fitted cDKO $6/15$ 0.10 ± 0.06 0 250/500mM - fitted Control $6/10$ 0.55 ± 0.09 0 $F(1,19) = 129.3, p < 0.0001^{***}$	Pves (EPSC/RRP) -	Control	6/15	0.05 ± 0.04	0	F(1,23) = 2.080, p=0.1627	
2.507.500.0001 - Fitted Control 0/10 0.53 \pm 0.09 0 F(1,19) = 129.3, P<0.0001	fitted	cDKO Control	6/15	0.10 ± 0.06	0	$E(1.10) = 120.2 = -0.0001 \pm \pm \pm$	
	250/500mm - mued		0/10	0.55 ± 0.09	0	$1(1,17) = 127.3, p > 0.0001^{+++}$	1

	cDKO	6/15	0.92 ± 0.06	0		
K _{2,max} (s ⁻¹)						1
0mM HS	cDKO	6/13	0.0001 ± 0.00008 0.0013 ± 0.0010	0	F(1,14) = 6.366, p = 0.02436*	
250mM HS	Control	6/12	0.43 ± 0.81	0	F(1,22) = 21.34, p=0.0001***	
	cDKO	6/17	0.80 ± 0.42	1		
500mM HS	Control	6/16	3.02 ± 3.53	0	F(1,26) = 41.96, p < 0.0001 ***	
AE (PT)	CDKU	6/18	5.13 ± 1.19	0		
0mM HS	Control	6/13	0.00 ± 0.98	0	F(1,14) = 21.03, p= 0.0004***	
	cDKO	6/7	-2.43 ± 0.68	0		
250mM HS	Control	6/12	-7.84 ± 0.85	0	$F(1,23) = 21.36, p = 0.0001^{***}$	
500mM HS	Control	6/17	-8.95 ± 0.44 -10.1 ± 0.63	0	F(1, 26) = 48, 63, n < 0,0001 ***	
2000000 110	cDKO	6/18	-10.9 ± 0.24	1	(1,20) 10100, p 1010001	
Priming rate K ₁ (s ⁻¹)	Control	6/16	0.17 ± 0.20	1	F(1,24) = 3.768, p=0.06409	
	cDKO	6/16	0.07 ± 0.14	0		
Unpriming rate K-1(s ⁻¹)	cDKO	6/16	0.07 ± 0.07 0.04 ± 0.06	0	F(1,24) = 3.959, p=0.05814	
		0.10	SYNA	PTIC RECOV	I / RY AFTER TRAIN STIMULATION	
40Hz train						
Norm. EPSC – R1	cDKO	6/33	1.00 ± 0.31 0.59 ± 0.13	0	$F(1,64) = 53.49, p < 0.0001^{***}$	
Norm. EPSC – R2	Control	6/31	0.95 ± 0.21	0	F(1,59) = 1.732, p=0.1933	
	cDKO	6/35	0.90 ± 0.14	0		
1st EPSC (nA)	Control	6/33	6.95 ± 4.85	1	F(1,62) = 7.586, p<0.007711**	
$R1 EPSC(n\Delta)$	cDKO Control	6/38	9.51 ± 5.08 6.38 ± 4.42		F(1,62) = 0.485 p=0.4889	
KI LI SC (IIA)	cDKO	6/38	5.57 ± 2.94	1	1 (1,02) = 0.405, p=0.4009	
R2 EPSC (nA)	Control	6/31	6.36 ± 4.89	1	F(1,57) = 4.858, p=0.03157*	
DDD : (0)	cDKO	6/35	8.14 ± 4.54	1		
RRP size (nC)	Control	6/33	0.78 ± 0.51 0.61 ± 0.46	0	F(1,63) = 2.642, p=0.109	
Refilling rate (nC/s)	Control	6/33	1.05 ± 1.00	1	F(1,61) = 2.214, p=0.1419	
<i>o ()</i>	cDKO	6/38	0.83 ± 0.61	2		
			FI	TTED PARA	METERS RECOVERY 500mM HS	1
$K_{2,max}(S^{-1})$	cDKO	6/12	2.06 ± 1.31 2.52 + 1.10	0	F(1,16) = 0.738, p = 0.403	
$\Delta E_a(RT)$	Control	6/12	-9.83 ± 0.64	0	F(1,16) = 0.691, p=0.4182	
	cDKO	6/11	-10.03 ± 0.72	0		
			0.44 - 0.50	SUPF	PLEMENTAL FIGURE 6	
Micro-network Docked SVs (um)	cDKO	4/132 4/89	8.11 ± 3.78 6.57 ± 3.45	2	L ratio $(2,339) = 7.153$, p=0.2044	Control-cDKO: p=0.0424 Control-WT: p=0.0153*
	+WT	4/128	6.87 ± 4.57	2		<i>cDKO-WT</i> : p=0.804
Micro-network	Control	4/132	623 ± 271	2	L ratio (2,335) = 3.176, p=0.2044	
AZ length (µm)	cDKO	4/89	580 ± 308	4		
Micro-network	Control	4/128	154 ± 96	4	L ratio $(2.335) = 2.344$ n=0.3097	
Total SV number	cDKO	4/89	134 ± 92	3		
	+WT	4/128	143 ± 99	2		
Micro-network	Control	4/132	0.42 ± 1.04	2	L ratio (2, 339) = 3.118, p=0.2103	
outside AZ	+WT	4/89	0.20 ± 0.03 0.16 ± 0.57	2		
				FIGURE 3 +	+ SUPPLEMENTAL FIGURE 7	
EPSC amp. (nA)	cDKO	4/17	9.73 ± 5.33	0	F(2,52) = 7.508, p=0.001366**	<i>vsWT</i> : F(1,32) = 12.30, p=0.001366**
	+WT	4/21	5.03 ± 6.13	1		
STP (5th/1st)	+Hybrid	4/21	10.75 ± 7.33 0.35 ± 0.15	0	$F(2,53) = 15.08 \ p < 0.0001 ***$	v_{SWT} : F(1,36) = 11.87, p=0.001468** v_{SWT} : F(1,33) = 25.50, p<0.0001***
511 (571.)	+WT	4/21	0.93 ± 0.47	0	1(2,55) = 15.06, p < 0.0001	<i>vsw</i> 1. 1 (1,55) – 25.50, p < 0.0001
	+Hybrid	4/21	0.54 ± 0.27	0		<i>vsWT</i> : F(1,37) = 10.76, p=0.002262**
EPSC recovery	cDKO	4/14	0.51 ± 0.10	0	F(2,46) = 28.71, p < 0.0001 ***	<i>vsWT</i> : F(1,29) = 36.20, p<0.0001***
	+WI +Hybrid	4/20	1.48 ± 0.67 0.65 ± 0.23	0		$v \in WT$: E(1,33) = 30.10 m < 0.0001***
Norm. synaptic tom-1	Control	3/35	1.00 ± 0.26	0	F(3,125) = 50.52, p<0.0001***	vsWT: F(1,63) = 0.749, p=0.3902
	cDKO	3/30	0.35 ± 0.16	0		<i>vsWT:</i> F(1,58) = 50.52, p<0.0001***
	+WT +hybrid	3/33	1.12 ± 0.48 1.69 ± 0.88			$vsWT \cdot F(1.62) = 15.89 n = 0.0001707***$
Synaptic / somatic	Control	3/35	0.66 ± 0.40	0	F(3,124) = 22.94, p<0.0001***	v_{sWT} : F(1,63) = 1.123, p=0.2934
tomosyn	cDKO	3/30	1.04 ± 0.44	0	(., , <u>.</u> , , <u>r</u> , ., <u>r</u>	<i>vsWT</i> : F(1,58) = 45.35, p<0.0001***
	+WT	3/33	0.60 ± 0.25	1		
Total dandrita langth	+hybrid Control	3/35	0.65 ± 0.27	1	E(2, 127) = 6,000, n=0,000,7***	vsWT: F(1,63) = 0.805, p=0.373
(mm)	cDKO	3/30	1.72 ± 0.69	0	1 (3,127) = 0.007, p=0.0007 · · ·	v_{sWT} : F(1,59) = 3.118, p= 0.0826
	+WT	3/33	2.05 ± 0.87	0		
	+hybrid	3/35	2.45 ± 1.17	0		<i>vsWT</i> : F(1,64) = 4.617, p= 0.03544
Synapses/µm dendrite	cDKO	3/35 3/30	0.28 ± 0.09 0.26 ± 0.08	0	F(3,127) = 1.237, p = 0.2991	
	+WT	3/33	0.30 ± 0.09	0		
	+hybrid	3/35	0.30 ± 0.10	0		
EPSC amp (nA)	Control	6/21	7.23 ± 4 90	FIGURE 7 +	SUPPLEMENTAL FIGURE 11 F(6,106) = 2.069 p=0.06294	
	cDKO	8/25	9.89 ± 6.77	1	-(0,100) 2.000, p 0.002)4	
	+WT	6/27	4.69 ± 4.22	1		
	+FA	5/18	8.81 ± 6.61	0		
	$+\Delta PB$ + $\Delta SNARE$	4/15	9.20 ± 5.08 7.08 ± 5.46	0		
	+WDonly	3/10	8.07 ± 4.83	0		
STP (5 th /1 st)	Control	6/21	0.78 ± 0.41	0	F(6,108) = 13.21, p<0.0001 ***	<i>vsKO</i> : F(1,37) = 19.35, p=0.00011***
	cDKO +WT	8/25	0.44 ± 0.16 1.25 ± 0.72			$v_{\rm E}KO$: $F(1/43) = 26.30 \text{ pc}(0.0001***)$
	+FA	5/18	0.50 ± 0.14	0		v_{sKO} : F(1,34) = 1.102, p=0.3013
	$+\Delta PB$	4/13	0.37 ± 0.08	0		<i>vsKO</i> : F(1,29) = 0.024, p= 0.8781

I	+ASNARE	4/8	0.44 ± 0.20	0		$v_s KO$: F(1.24) = 0.078, p= 0.7821
	+WDonly	3/10	0.41 ± 0.16	0		<i>vsKO</i> : F(1,26) = 1.331, p=0.2592
EPSC recovery	Control	6/21	0.94 ± 0.36	0	F(6,96) = 16.18, p<0.0001 ***	<i>vsKO</i> : F(1,34) = 30.28, p=0.00011***
	cDKO	8/22	0.53 ± 0.16	0		
	+WT	6/25	1.49 ± 0.79	1		<i>vsKO</i> : F(1,37) = 41.17, p<0.0001***
	+FA	5/16	0.69 ± 0.24	0		<i>vsKO</i> : F(1,29) = 4.793, p=0.03677
	$+\Delta PB$	4/12	0.52 ± 0.15	0		<i>vsKO</i> : F(1,25) = 0.524, p=0.4757
	+ΔSNARE	3/6	0.56 ± 0.15	0		<i>vsKO</i> : F(1,19) = 0.173, p=0.682
	+WDonly	2/9	0.52 ± 0.14	0		<i>vsKO</i> : F(1,22) = 0.036, p=0.8506
Norm. synaptic tom-1	Control	3/27	1.00 ± 0.29	1	F(6,206) = 25.82, p < 0.0001 ***	<i>vsWT:</i> F(1,51) = 1.909, p=0.1731; <i>vsKO:</i> F(1,49) = 285, p<0.0001***
levels	CDKO	3/27	0.22 ± 0.05	0		w V O E(1.52) = 48.47 + <0.0001 * * *
	+W1 +EA	3/29	1.15 ± 0.85 0.56 ± 0.40	0		v_{SKO} : $F(1,52) = 48.47$, $p < 0.0001^{+++}$ v_{SKO} : $F(1,52) = 19.22$, $p < 0.0001^{+++}$, v_{SKO} : $F(1,56) = 33.43$, $p < 0.0001^{+++}$
	+APB	3/31	1.48 ± 1.03	0		$v_{SWT} = f(1,56) = 4.163$ n=0.04605: $v_{SKO} = f(1,56) = 53.45$, p<0.0001 v_SWT: F(1.56) = 4.163 n=0.04605: $v_{SKO} = f(1.54) = 53.70$ n<0.0001***
	+ASNARE	3/38	0.42 ± 0.21	1		v_{SWT} : F(1.61) = 29.97. p<0.0001***: v_{SKO} : F(1.59) = 51.26. p<0.0001***
	+WDonly	3/34	0.64 ± 0.30	1		<i>vsWT</i> : F(1,58) = 13.40, p= 0.00054**; <i>vsKO</i> : F(1,56) = 89.86, p<0.0001***
Norm. somatic tom-1	Control	3/26	1.00 ± 0.18	1	F(6,205) = 34.03, p<0.0001 ***	vsWT: F(1,50) = 2.408, p=0.1271
levels	cDKO	3/27	0.26 ± 0.11	0		vsWT: F(1,52) = 78.04, p<0.0001***
	+WT	3/29	0.90 ± 0.41	0		
	+FA	3/33	0.66 ± 0.38	0		<i>vsWT</i> : F(1,58) = 7.25, p=0.009266
	$+\Delta PB$	3/30	1.09 ± 0.22	0		<i>vsWT</i> : F(1,55) = 8.59, p= 0.004917*
	+ΔSNARE	3/36	0.68 ± 0.25	0		<i>vsWT</i> : F(1,61) = 7.147, p=0.009623
	+WDonly	3/34	0.96 ± 0.27	0		<i>vsWT</i> : F(1,59) = 1.705, p= 0.1967
Total dendritic length	Control	3/27	1.00 ± 0.45	0	F(6,210) = 2.871, p= 0.0104*	<i>vsWT</i> : F(1,52) = 3.798, p= 0.05672
(mm)	cDKO	3/27	1.11 ± 0.62	0		vsWT: F(1,52) = 1.387, p= 0.2442
	+WT	3/29	1.29 ± 0.79	0		WT F(1.59) = 0.100 = 0.0500
		2/21	1.45 ± 0.89 1.58 ± 0.02			v_{SW1} : F(1,58) = 0.199, p= 0.0509 v_{SW1} : F(1,56) = 1.011, p= 0.1724
	+ASNARE	3/31	1.38 ± 0.93 1.42 ± 0.66	0		$v_{SW}T$: $F(1,50) = 1.911$, $p = 0.1724$ $v_{CW}T$: $F(1,63) = 1.279$, $p = 0.2624$
	+WDonly	3/34	1.42 ± 0.00 1 44 + 0.75	0		$v_{SWT} = \Gamma(1, 59) = 1.279$, p= 0.2592
Synapses/um dendrite	Control	3/27	0.26 ± 0.07	0	F(6,211) = 0.590 p= 0.7383	10// 11 ((,0)) 112/0, p 0120/2
o ynapoes pin dename	cDKO	3/27	0.25 ± 0.08	0	r(0,211) 0.550, p 0.7505	
	+WT	3/29	0.26 ± 0.07	0		
	+FA	3/34	0.24 ± 0.09	0		
	$+\Delta PB$	3/31	0.23 ± 0.06	0		
	+ΔSNARE	3/38	0.25 ± 0.06	0		
	+WDonly	3/34	0.24 ± 0.07	0		
TRAG (1)	DVO	4/80		FIGURE 8	+ SUPPLEMENTAL FIGURE 12	
EPSC amp. (nA)	cDKO	4/20	11.30 ± 5.99	0	$F(3,81) = 4.542, p=0.005406^{**}$	vsWT: F(1,37) = 8.863, p=0.005108*
	+W1	4/22	0.78 ± 4.89	0		wWT: E(1.40) = 0.006 m=0.0200
	+L R	4/23	7.95 + 5.59	0		$v_{SW1} \cdot r(1,40) = 0.000, p=0.3099$ $v_{SW1} \cdot r(1,40) = 1.059, p=0.3096$
STP (5 th /1 st)	cDKO	4/20	0.35 ± 0.09	0	F(3.81) = 28.33, p < 0.0001 ***	$v_{SWT} = F(1, 37) = 52.42, p < 0.0001 ***$
(- · · ·)	+WT	4/22	0.97 ± 0.44	0	((,,,,)),, F	······································
	+Core	4/24	0.56 ± 0.17	0		<i>vsWT</i> : F(1,41) = 34.32, p<0.0001***
	+LR	4/23	0.58 ± 0.41	1		vsWT: F(1,39) = 29.40, p<0.0001***
EPSC recovery	cDKO	4/19	0.54 ± 0.11	0	F(3,77) = 15.46, p<0.0001***	<i>vsWT</i> : F(1,35) = 36.88, p<0.0001***
	+WT	4/22	1.52 ± 0.98	1		
	+Core	4/22	1.00 ± 0.62	0		<i>vsWT</i> : F(1,38) = 9.200, p=0.004347*
	+LR	4/22	0.79 ± 0.33	0		<i>vsWT</i> : F(1,38) = 21.07, p<0.0001***
Norm. synaptic tom-1	Control					
levels		4/22	1.00 ± 0.28	0	F(4,109) = 16.67, p < 0.0001 ***	<i>vsWT</i> : F(1,41) = 0.099, p=0.7549
	cDKO	4/22 4/23	1.00 ± 0.28 0.30 ± 0.12	0	F(4,109) = 16.67, p<0.0001***	vsWT: F(1,41) = 0.099, p=0.7549 vsWT: F(1,42) = 57.04, p<0.0001***
	cDKO +WT	4/22 4/23 4/24	$1.00 \pm 0.28 \\ 0.30 \pm 0.12 \\ 1.04 \pm 0.52 \\ 0.01 \pm 0.58$	0 0 0 0	F(4,109) = 16.67, p<0.0001***	vsWT: F(1,41) = 0.099, p=0.7549 vsWT: F(1,42) = 57.04, p<0.0001***
	cDKO +WT +Core	4/22 4/23 4/24 4/23 4/25	$1.00 \pm 0.28 \\ 0.30 \pm 0.12 \\ 1.04 \pm 0.52 \\ 0.91 \pm 0.58 \\ 1.21 \pm 0.52$	0 0 0 0	F(4,109) = 16.67, p<0.0001***	vsWT: F(1,41) = 0.099, p=0.7549 vsWT: F(1,42) = 57.04, p<0.0001*** vsWT: F(1,42) = 0.936, p=0.3389 vsWT: F(1,42) = 0.922, r=0.2110
Synantic / somatic	cDKO +WT +Core +LR	4/22 4/23 4/24 4/23 4/25	$1.00 \pm 0.28 \\ 0.30 \pm 0.12 \\ 1.04 \pm 0.52 \\ 0.91 \pm 0.58 \\ 1.21 \pm 0.53 \\ 0.83 \pm 0.40 $	0 0 0 0 0	F(4,109) = 16.67, p<0.0001***	vsWT: F(1,41) = 0.099, p=0.7549 vsWT: F(1,42) = 57.04, p<0.0001*** vsWT: F(1,42) = 0.936, p=0.3389 vsWT: F(1,42) = 0.923, p=0.3419 vsWT: F(1,42) = 0.331, p=0.3366
Synaptic / somatic	cDKO +WT +Core +LR Control cDKO	4/22 4/23 4/24 4/23 4/25 4/22 4/23	1.00 ± 0.28 0.30 ± 0.12 1.04 ± 0.52 0.91 ± 0.53 0.83 ± 0.40 0.98 ± 0.37	0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013*	vsWT: F(1,41) = 0.099, p=0.7549 $vsWT: F(1,42) = 57.04, p<0.0001***$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 1.3 a = 0.0002531**$
Synaptic / somatic tomosyn	cDKO +WT +Core +LR Control cDKO +WT	4/22 4/23 4/24 4/23 4/25 4/22 4/22 4/23 4/24	$\begin{array}{c} 1.00 \pm 0.28 \\ 0.30 \pm 0.12 \\ 1.04 \pm 0.52 \\ 0.91 \pm 0.58 \\ 1.21 \pm 0.53 \\ 0.83 \pm 0.40 \\ 0.98 \pm 0.37 \\ 0.74 \pm 0.28 \end{array}$		F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013*	vsWT: F(1,41) = 0.099, p=0.7549 vsWT: F(1,42) = 57.04, p<0.0001*** vsWT: F(1,42) = 0.936, p=0.3389 vsWT: F(1,44) = 0.923, p=0.3419 vsWT: F(1,40) = 1.831, p=0.1836 vsWT: F(1,40) = 16.13, p=0.0002531**
Synaptic / somatic tomosyn	cDKO +WT +Core +LR Control cDKO +WT +Core	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23	$\begin{array}{c} 1.00\pm 0.28\\ 0.30\pm 0.12\\ 1.04\pm 0.52\\ 0.91\pm 0.58\\ 1.21\pm 0.53\\ \hline 0.83\pm 0.40\\ 0.98\pm 0.37\\ 0.74\pm 0.28\\ 0.74\pm 0.33\\ \end{array}$	0 0 0 0 0 0 1 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013*	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$
Synaptic / somatic tomosyn	cDKO +WT +Core +LR Control cDKO +WT +Core +LR	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24	$\begin{array}{c} 1.00\pm0.28\\ 0.30\pm0.12\\ 1.04\pm0.52\\ 0.91\pm0.58\\ 1.21\pm0.53\\ \hline 0.83\pm0.40\\ 0.98\pm0.37\\ 0.74\pm0.28\\ 0.74\pm0.28\\ 0.74\pm0.23\\ 0.82\pm0.27\\ \hline \end{array}$	0 0 0 0 0 0 1 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013*	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,42) = 4.653, p=0.03678$
Synaptic / somatic tomosyn Total dendritic length	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24 4/23 4/25 4/22	$\begin{array}{c} 1.00\pm0.28\\ 0.30\pm0.12\\ 1.04\pm0.52\\ 0.91\pm0.58\\ 1.21\pm0.53\\ \hline\\ 0.98\pm0.37\\ 0.74\pm0.28\\ 0.74\pm0.28\\ 0.74\pm0.33\\ 0.82\pm0.27\\ \hline\\ 1.72\pm0.53\\ \end{array}$	0 0 0 0 0 1 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,42) = 4.653, p=0.03678$ $vsWT: F(1,41) = 0.430, p=0.5159$
Synaptic / somatic tomosyn Total dendritic length (mm)	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24 4/23 4/25 4/22 4/23	$\begin{array}{c} 1.00\pm0.28\\ 0.30\pm0.12\\ 1.04\pm0.52\\ 0.91\pm0.58\\ 1.21\pm0.53\\ 0.83\pm0.40\\ 0.98\pm0.37\\ 0.74\pm0.28\\ 0.74\pm0.33\\ 0.82\pm0.27\\ 1.72\pm0.53\\ 1.56\pm0.57\\ \end{array}$	0 0 0 0 0 1 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,42) = 4.653, p=0.03678$ $vsWT: F(1,42) = 0.430, p=0.5159$ $vsWT: F(1,42) = 2.220, p=0.1437$
Synaptic / somatic tomosyn Total dendritic length (mm)	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24 4/22 4/23 4/24	$\begin{array}{c} 1.00\pm0.28\\ 0.30\pm0.12\\ 1.04\pm0.52\\ 0.91\pm0.58\\ 1.21\pm0.53\\ \hline\\ 0.83\pm0.40\\ 0.98\pm0.37\\ 0.74\pm0.28\\ 0.74\pm0.28\\ 0.74\pm0.33\\ 0.82\pm0.27\\ \hline\\ 1.72\pm0.53\\ 1.56\pm0.57\\ \hline\\ 1.86\pm0.85\\ \hline\end{array}$	0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,42) = 4.653, p=0.03678$ $vsWT: F(1,41) = 0.430, p=0.5159$ $vsWT: F(1,42) = 2.220, p = 0.1437$
Synaptic / somatic tomosyn Total dendritic length (mm)	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT +Core	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23	$\begin{array}{c} 1.00\pm0.28\\ 0.30\pm0.12\\ 1.04\pm0.52\\ 0.91\pm0.58\\ 1.21\pm0.53\\ 0.83\pm0.40\\ 0.98\pm0.37\\ 0.74\pm0.28\\ 0.74\pm0.28\\ 0.74\pm0.33\\ 0.82\pm0.27\\ 1.72\pm0.53\\ 1.56\pm0.57\\ 1.86\pm0.85\\ 2.12\pm1.05\\ \end{array}$	0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,42) = 4.653, p=-0.03678$ $vsWT: F(1,42) = 4.263, p=0.1437$ $vsWT: F(1,42) = 1.493, p=0.2286$
Synaptic / somatic tomosyn Total dendritic length (mm)	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT +Core +LR	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24 4/23 4/22 4/23 4/24 4/23 4/25	$\begin{array}{c} 1.00\pm0.28\\ 0.30\pm0.12\\ 1.04\pm0.52\\ 0.91\pm0.58\\ 1.21\pm0.53\\ 0.83\pm0.40\\ 0.98\pm0.37\\ 0.74\pm0.28\\ 0.74\pm0.28\\ 0.74\pm0.33\\ 0.82\pm0.27\\ 1.72\pm0.53\\ 1.56\pm0.57\\ 1.86\pm0.85\\ 2.12\pm1.05\\ 2.11\pm0.91\\ \end{array}$	0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,41) = 0.254, p=0.03678$ $vsWT: F(1,41) = 0.430, p=0.5159$ $vsWT: F(1,42) = 4.653, p=0.01437$ $vsWT: F(1,42) = 1.493, p= 0.2286$ $vsWT: F(1,44) = 0.911, p= 0.3451$
Synaptic / somatic tomosyn Total dendritic length (mm) Synapses/µm dendrite	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT +Core +LR Cortrol cDKO	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/25 4/22	$\begin{array}{c} 1.00 \pm 0.28 \\ 0.30 \pm 0.12 \\ 1.04 \pm 0.52 \\ 0.91 \pm 0.58 \\ 1.21 \pm 0.53 \\ \hline 0.83 \pm 0.40 \\ 0.98 \pm 0.37 \\ 0.74 \pm 0.28 \\ 0.74 \pm 0.28 \\ 0.74 \pm 0.33 \\ \hline 0.82 \pm 0.27 \\ \hline 1.72 \pm 0.53 \\ 1.56 \pm 0.57 \\ 1.86 \pm 0.85 \\ 2.12 \pm 1.05 \\ 2.11 \pm 0.91 \\ \hline 0.24 \pm 0.07 \end{array}$	0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553 F(4,109) = 0.874, p= 0.482	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,41) = 0.430, p=0.5159$ $vsWT: F(1,42) = 1.493, p=0.2286$ $vsWT: F(1,42) = 1.493, p=0.2286$ $vsWT: F(1,44) = 0.911, p= 0.3451$
Synaptic / somatic tomosyn Total dendritic length (mm) Synapses/µm dendrite	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/25 4/22 4/23	$\begin{array}{c} 1.00 \pm 0.28 \\ 0.30 \pm 0.12 \\ 1.04 \pm 0.52 \\ 0.91 \pm 0.58 \\ 1.21 \pm 0.53 \\ \hline 0.83 \pm 0.40 \\ 0.98 \pm 0.37 \\ 0.74 \pm 0.28 \\ 0.74 \pm 0.33 \\ 0.82 \pm 0.27 \\ \hline 1.72 \pm 0.53 \\ 1.56 \pm 0.57 \\ 1.86 \pm 0.85 \\ 2.12 \pm 1.05 \\ 2.11 \pm 0.91 \\ \hline 0.24 \pm 0.07 \\ 0.23 \pm 0.10 \end{array}$	0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553 F(4,109) = 0.874, p= 0.482	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,41) = 0.430, p=0.5159$ $vsWT: F(1,42) = 1.493, p=0.2286$ $vsWT: F(1,44) = 0.911, p= 0.3451$
Synaptic / somatic tomosyn Total dendritic length (mm) Synapses/µm dendrite	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24	$\begin{array}{c} 1.00\pm0.28\\ 0.30\pm0.12\\ 1.04\pm0.52\\ 0.91\pm0.58\\ 1.21\pm0.53\\ \hline 0.83\pm0.40\\ 0.98\pm0.37\\ 0.74\pm0.28\\ 0.74\pm0.28\\ 0.74\pm0.33\\ 0.82\pm0.27\\ \hline 1.72\pm0.53\\ 1.56\pm0.57\\ 1.86\pm0.85\\ 2.12\pm1.05\\ 2.11\pm0.91\\ 0.24\pm0.07\\ 0.23\pm0.10\\ 0.21\pm0.07\\ \hline 0.21$	0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553 F(4,109) = 0.874, p= 0.482	$vsWT: F(1,41) = 0.099, p=0.7549$ $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ $vsWT: F(1,42) = 0.936, p=0.3389$ $vsWT: F(1,44) = 0.923, p=0.3419$ $vsWT: F(1,40) = 1.831, p=0.1836$ $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ $vsWT: F(1,41) = 0.254, p=0.6171$ $vsWT: F(1,42) = 4.653, p=0.03678$ $vsWT: F(1,42) = 2.220, p= 0.1437$ $vsWT: F(1,42) = 1.493, p= 0.2286$ $vsWT: F(1,44) = 0.911, p= 0.3451$
Synaptic / somatic tomosyn Total dendritic length (mm) Synapses/µm dendrite	cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT +Core +LR Control cDKO +WT +Core +LR	4/22 4/23 4/24 4/23 4/25 4/22 4/23 4/24 4/23 4/24 4/23 4/24 4/23 4/22 4/23 4/22 4/23 4/24 4/23 4/24 4/23 4/24	$\begin{array}{c} 1.00 \pm 0.28 \\ 0.30 \pm 0.12 \\ 1.04 \pm 0.52 \\ 0.91 \pm 0.58 \\ 1.21 \pm 0.53 \\ \hline 0.83 \pm 0.40 \\ 0.98 \pm 0.37 \\ 0.74 \pm 0.28 \\ 0.74 \pm 0.28 \\ 0.74 \pm 0.33 \\ 0.82 \pm 0.27 \\ \hline 1.72 \pm 0.53 \\ 1.56 \pm 0.57 \\ 1.86 \pm 0.85 \\ 2.12 \pm 1.05 \\ 2.11 \pm 0.91 \\ 0.24 \pm 0.07 \\ 0.23 \pm 0.10 \\ 0.21 \pm 0.07 \\ 0.22 \pm 0.07 \\ 0.22 \pm 0.07 \\ 0.22 \pm 0.07 \\ 0.22 \pm 0.07 \\ 0.24 $	0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0	F(4,109) = 16.67, p<0.0001*** F(4,106) = 3.332, p=0.013* F(4,109) = 2.389, p=0.0553 F(4,109) = 0.874, p= 0.482	vsWT: F(1,41) = 0.099, p=0.7549 $vsWT: F(1,42) = 57.04, p<0.0001^{***}$ vsWT: F(1,42) = 0.936, p=0.3389 vsWT: F(1,44) = 0.923, p=0.3419 vsWT: F(1,40) = 1.831, p=0.1836 $vsWT: F(1,40) = 16.13, p=0.0002531^{**}$ vsWT: F(1,41) = 0.254, p=0.6171 vsWT: F(1,42) = 4.653, p=0.03678 vsWT: F(1,42) = 2.220, p=0.1437 vsWT: F(1,42) = 1.493, p=0.2286 vsWT: F(1,44) = 0.911, p= 0.3451

Supplementary table 1. **Overview of statistical analyses per figure.** The results of the one-way ANOVA to test whether the more complex model (including information on the experimental group observations belong to) is significantly better at capturing the data than the simpler model. Results are reported as F(between groups df, within groups df) = [F-value], p = [p-value]. P-value thresholds = *<0.05; **<0.01;***<0.001. For post-hoc tests, p-value thresholds were adjusted with a Bonferroni correction (α /number of tests). N = independent cultures, n = neurons.