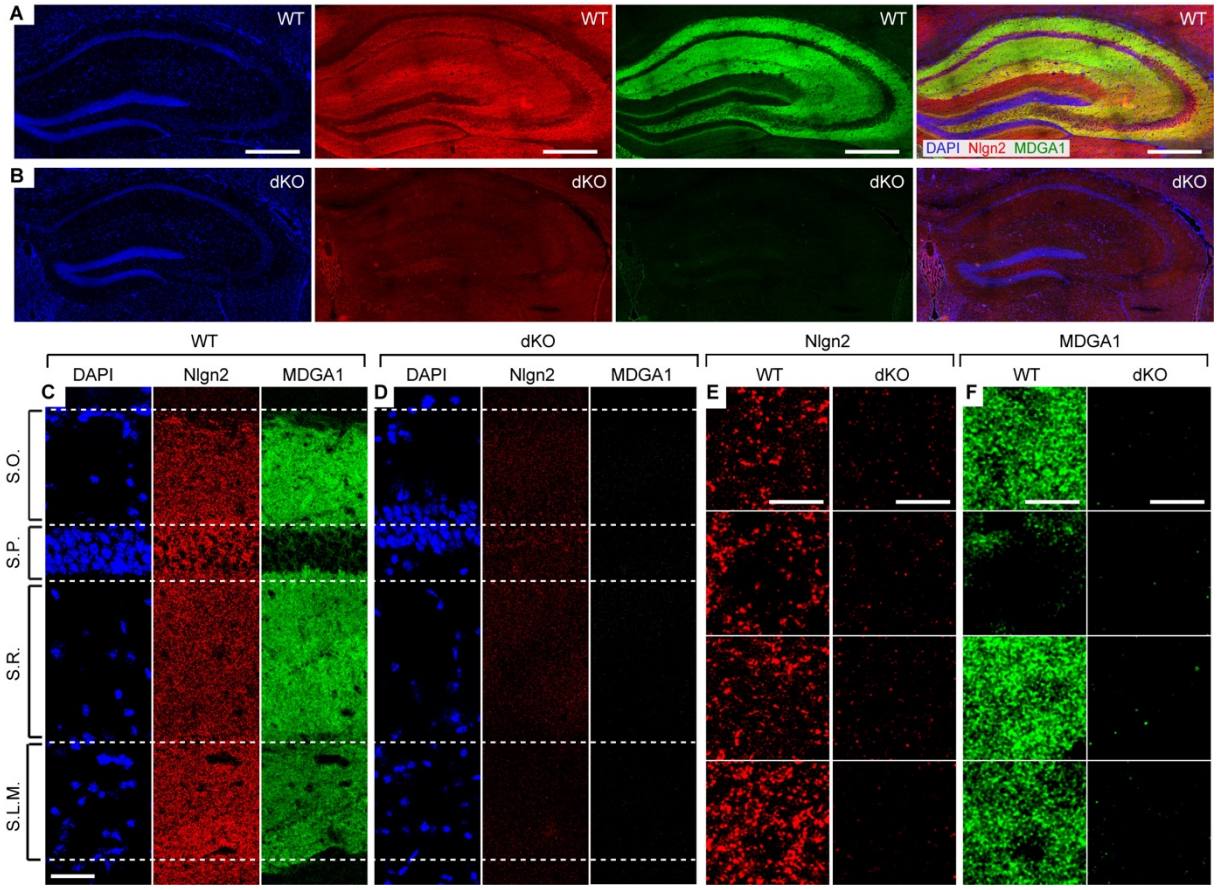
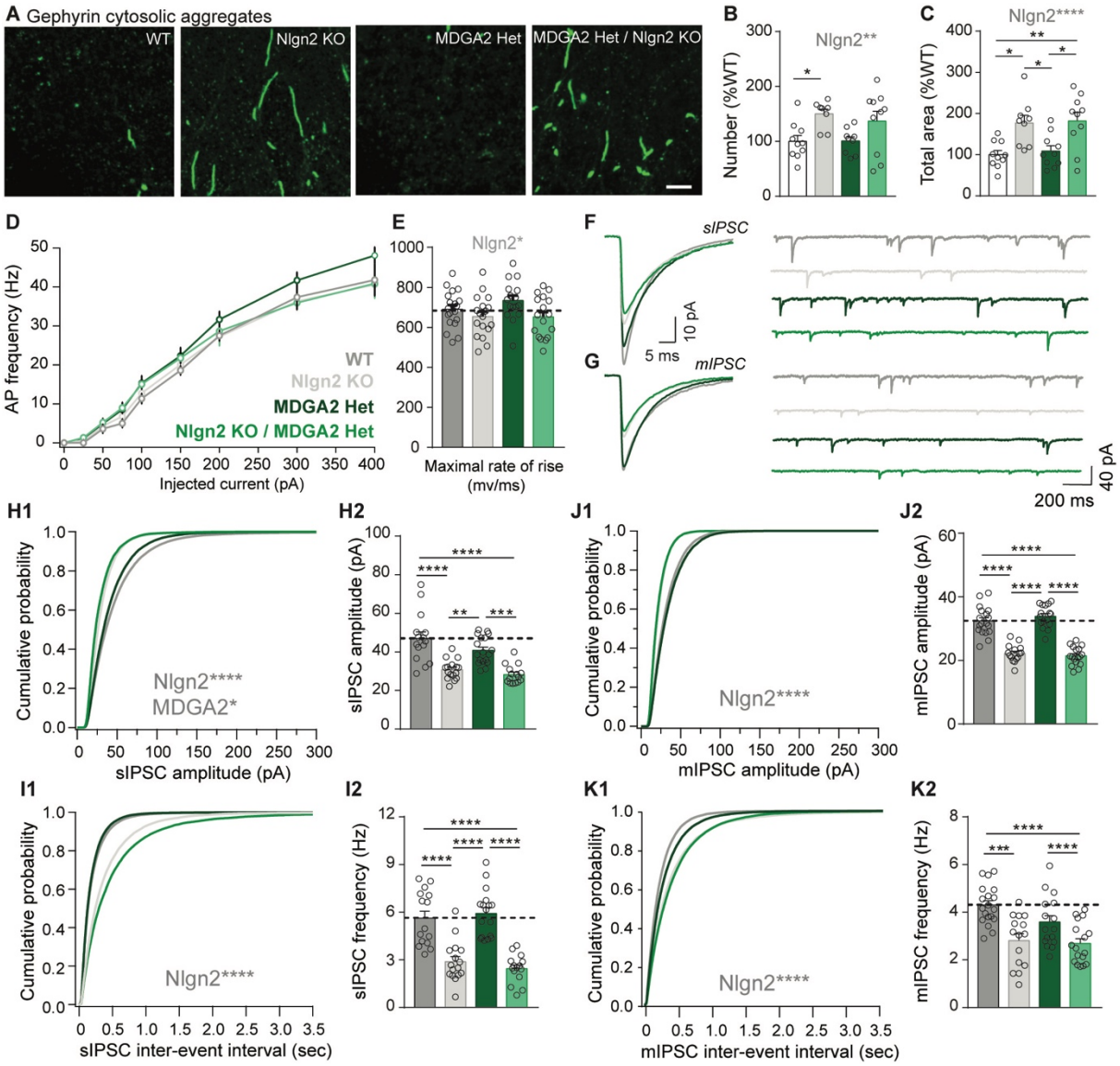
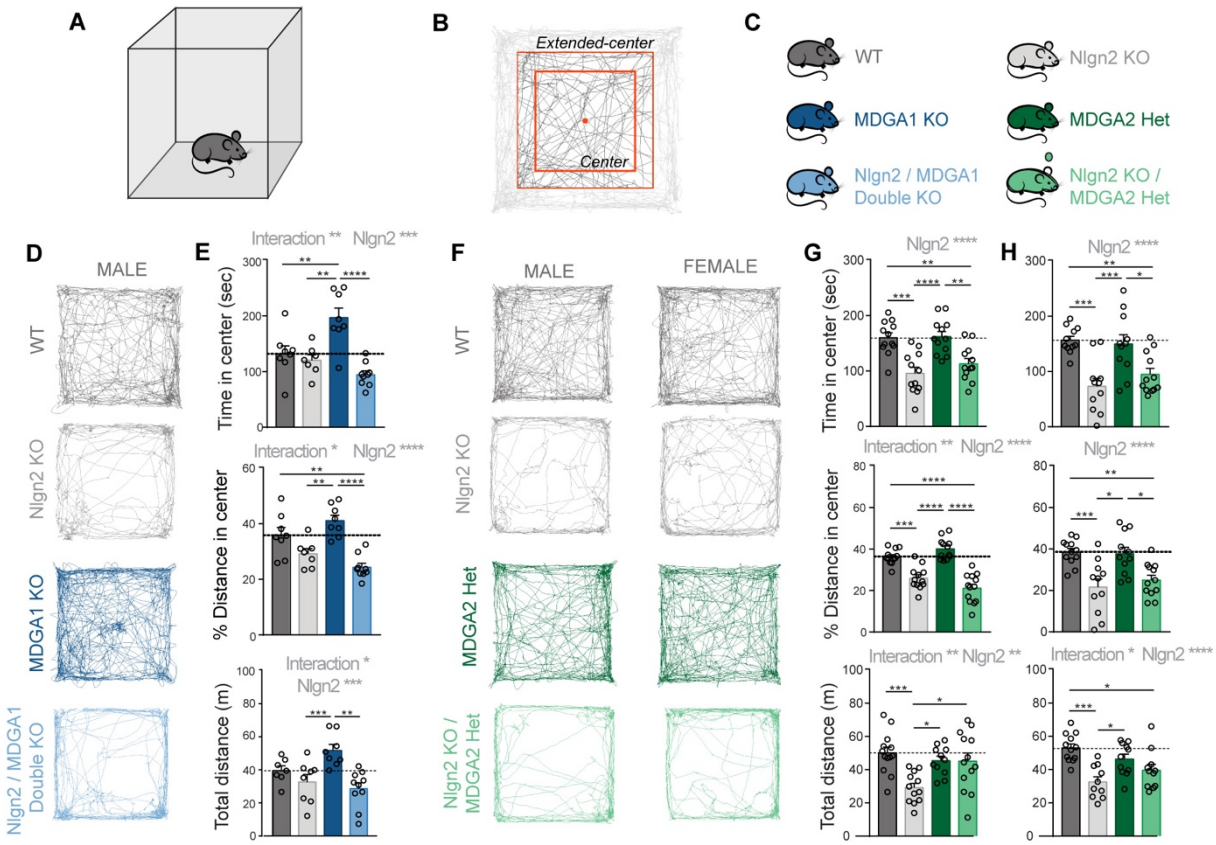


Supplementary Figure 1. Validation of the specificity of antibodies against MDGA1 and Nlgn2. (A-B) Photomicrographs showing an overview of the hippocampus in WT (A) versus Nlgn2-MDGA1 dKO mice (B) labelled with DAPI (blue), and antibodies against Nlgn2 (red) and MDGA1 (green). Scale bar 500 µm. (C-D) Photomicrographs showing an overview of area CA1 labelled with DAPI, and with antibodies against Nlgn2 and MDGA1 in WT (C) versus Nlgn2 / MDGA1 dKO mice (D). Scale bar 50 µm. (E-F) High magnification photomicrographs showing Nlgn2 and MDGA1 labeling within different hippocampal layers in WT (E) versus Nlgn2 / MDGA1 dKO mice (F), scale bar 5 µm.



Supplementary Figure 2. Heterozygous MDGA2 deletion shows no effects on the formation of gephyrin aggregates and on GABAergic transmission in CA1 pyramidal cells. (A) High magnification photomicrographs of gephyrin aggregates in WT, Nlgn2 KO, MDGA2 Het and Nlgn2 KO / MDGA2 Het mice. Scale bar 5 µm. (B-C) Quantification of the number (B) and the total area (C) of gephyrin aggregates, expressed as percentage of WT. Statistically significant ANOVA comparisons are marked in gray at the top of panels and listed in Supplementary Table 3. For all other ANOVA comparisons, F<1. Post-hoc analysis (Tukey's comparison test): * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Error bars represent SEM, and each circle represents an experimental animal (n = 8-10). (D) Frequency of action potentials (APs) in response to steps of injected current. (E) Quantification of the maximal rate of AP rise in WT, Nlgn2 KO, MDGA2 Het, Nlgn2 KO / MDGA2 Het mice. (F) Representative average sIPSCs and sIPSC recording traces from all four genotypes analysed. (G) Representative average mIPSCs and mIPSC recording traces from all four genotypes analysed. (H-I) Average cumulative distribution and bar-graphs showing the quantification of sIPSC amplitude (H) and sIPSC inter-event frequency (I). (J-K) Average cumulative distribution and bar-graphs showing the quantification of mIPSC amplitude (J) and mIPSC inter-event frequency (K). Statistically significant ANOVA comparisons are marked in gray at the top of panels and listed in Supplemental Table 3. For all other ANOVA comparisons, F<1. Post-hoc analysis (Tukey's comparison test): * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. In bar graphs, each circle represents a single cell (n = 14-19 cells for APs and rate of rise; 16-18 cells for sIPSC recordings; 15-19 cells for mIPSC recordings; each from four animals per genotype).



Supplementary Figure 3. Heterozygous MDGA2 deletion does not alter anxiety-related avoidance behavior in Nlgn2 KO mice. (A-C) Schematics representing the OF arena (A), the center (B), and the genotypes analyzed (C). (D) Representative tracks of OF exploration in MDGA1 male mice. (E) OF scores of MDGA1 male mice: Time spent in the anxiogenic region (top) of the OF arena, distance traveled in the center of the OF expressed as percentage of total distance traveled (center), total distance travelled in the OF (bottom). (F) Representative tracks of OF exploration in MDGA2 mice. (G) OF scores of MDGA2 male mice: Time spent in the anxiogenic region (top) of the OF arena, distance traveled in the center of the OF expressed as percentage of total distance traveled (center), total distance travelled in the OF (bottom). (H) OF scores of MDGA2 female mice: Time spent in the anxiogenic region (top) of the OF arena, distance traveled in the center of the OF expressed as percentage of total distance traveled (center), total distance travelled in the OF (bottom). Statistically significant ANOVA comparisons are marked in gray at the top of panels and listed in Supplementary Table 3. For all other ANOVA comparisons, F<1. Post-hoc analysis (Tukey's comparison test): * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Error bars represent SEM, and each circle represents an experimental animal (n = 7-9 for male MDGA1 set, n = 11-13 for female MDGA2 set, n = 10-12 for male MDGA2 set).

Supplementary Table 1. Analysis of the number and size of gephyrin, $GABA_AR\gamma2$ and VIAAT puncta in layers S.O. and S.L.M. of hippocampal area CA1 in WT, Nlgn2 KO, MDGA1 KO and Nlgn2 / MDGA1 dKO mice (all data expressed as %WT).

		WT		Nlgn2 KO		MDGA1 KO		Nlgn2 / MDGA1 dKO		Main source of variation	
		n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	F-value	p-value
Stratum Oriens (S.O.)	Gephyrin (number)	8	100.00 ± 6.37	8	78.96 ± 6.96	8	86.56 ± 5.67	8	76.63 ± 9.63	Nlgn2: $F_{(1,28)} = 4.484$	0.043
	Gephyrin (size)	8	100.00 ± 3.21	8	90.26 ± 3.28	7	94.27 ± 2.51	7	89.39 ± 3.27	Nlgn2: $F_{(1,28)} = 4.227$	0.049
	$GABA_AR\gamma2$ (number)	8	100.00 ± 5.35	8	90.33 ± 6.30	9	93.14 ± 7.77	9	82.92 ± 6.54	/	/
	$GABA_AR\gamma2$ (size)	9	100.00 ± 3.91	8	81.46 ± 4.09	9	84.87 ± 3.48	9	81.99 ± 3.31	Nlgn2: $F_{(1,31)} = 8.38$ Interaction $F_{(1,31)} = 4.48$	Nlgn2: 0.007 Interaction 0.042
	VIAAT (number)	8	100.00 ± 15.71	8	101.19 ± 12.14	7	81.78 ± 12.35	7	74.33 ± 14.10	/	/
	VIAAT (size)	8	100.00 ± 2.44	8	98.99 ± 3.21	8	89.01 ± 3.09	8	87.57 ± 1.92	MDGA1: $F_{(1,28)} = 18.4$	<0.001
Stratum lacunosum moleculare (SLM)	Gephyrin (number)	7	100.00 ± 7.90	7	98.50 ± 8.66	7	109.89 ± 4.36	7	93.18 ± 2.79	/	/
	Gephyrin (size)	8	100.00 ± 3.83	8	95.64 ± 2.30	7	97.97 ± 1.76	7	88.85 ± 3.58	/	/
	$GABA_AR\gamma2$ (number)	8	100.00 ± 6.04	8	94.59 ± 4.05	8	107.69 ± 3.97	9	102.60 ± 4.38		
	$GABA_AR\gamma2$ (size)	8	100.00 ± 4.27	9	85.47 ± 4.1	8	84.19 ± 2.74	9	83.23 ± 3.39	Nlgn2: $F_{(1,30)} = 4.38$ MDGA1: $F_{(1,30)} = 5.94$	Nlgn2: 0.045 MDGA1: 0.021
	VIAAT (number)	6	100.00 ± 26.44	6	106.59 ± 22.35	4	117.51 ± 23.79	4	120.34 ± 17.85	/	/
	VIAAT (size)	7	100.00 ± 3.32	6	100.82 ± 5.50	4	84.13 ± 3.68	4	95.16 ± 3.82	MDGA1: $F_{(1,19)} = 7.09$	MDGA1: 0.015

Supplementary Table 2 (Part 1). Analysis of the number and size of gephyrin, $GABA_AR\gamma2$ and VIAAT puncta in layers S.O., S.P., S.R. and S.L.M. of hippocampal area CA1 in WT, Nlgn2 KO, MDGA2 Het and Nlgn2 KO / MDGA2 Het mice (all data expressed as %WT).

		WT		Nlgn2 KO		MDGA2 Het		Nlgn2 KO / MDGA2 Het		Main source of variation	
		n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	F-value	p-value
Stratum Oriens (S.O.)	Gephyrin (number)	11	100.00 ± 8.92	10	86.87 ± 6.49	11	104.31 ± 8.61	9	78.32 ± 6.99	Nlgn2: $F_{(1,37)} = 5.951$	Nlgn2: 0.020
	Gephyrin (size)	10	100.00 ± 3.03	10	91.35 ± 2.43	10	96.68 ± 2.77	10	94.13 ± 2.28	Nlgn2: $F_{(1,37)} = 4.527$	Nlgn2: 0,040
	$GABA_AR\gamma2$ (number)	12	100.00 ± 7.65	11	87.60 ± 8.94	12	117.91 ± 13.8	12	60.32 ± 8.05	Interaction: $F_{(1,43)} = 5.130$ Nlgn2: $F_{(1,43)} = 12.31$	Interaction: 0.029 Nlgn2: 0.001
	$GABA_AR\gamma2$ (size)	10	100.00 ± 2.55	10	86.17 ± 3.14	12	93.84 ± 5.02	11	76.82 ± 2.17	Nlgn2: $F_{(1,39)} = 18.65$ MDGA2: $F_{(1,39)} = 4.71$	Nlgn2: <0.001 MDGA2: 0.036
	VIAAT (number)	7	100.00 ± 17.70	7	84.99 ± 10.96	6	8297 ± 18.43	6	58.58 ± 9.60	/	/
	VIAAT (size)	7	100.00 ± 5.32	7	99.28 ± 3.09	6	89.99 ± 2.47	7	93.22 ± 6.13	/	/
Stratum Pyramidale (S.P.)	Gephyrin (number)	9	100.00 ± 10.72	9	88.69 ± 6.88	9	79.64 ± 7.10	9	70.07 ± 8.67	MDGA2: $F_{(1,32)} = 5.281$	MDGA2: 0.028
	Gephyrin (size)	9	100.00 ± 2.40	9	90.28 ± 3.64	9	92.68 ± 3.08	8	89.18 ± 1.27	Nlgn2: $F_{(1,31)} = 5.515$	Nlgn2: 0.025
	$GABA_AR\gamma2$ (number)	9	100.00 ± 3.98	10	81.33 ± 7.56	10	84.78 ± 8.66	9	59.50 ± 5.25	Nlgn2: $F_{(1,34)} = 10.36$ MDGA2: $F_{(1,34)} = 7.359$	Nlgn2: 0.003 MDGA2: 0.010
	$GABA_AR\gamma2$ (size)	10	100.00 ± 5.39	10	88.88 ± 4.56	10	87.27 ± 3.88	10	70.77 ± 3.43	Nlgn2: $F_{(1,36)} = 10.00$ MDGA2: $F_{(1,36)} = 12.34$	Nlgn2: 0.003 MDGA2: 0.001
	VIAAT (number)	8	100.00 ± 19.34	8	91.39 ± 10.93	8	71.34 ± 11.78	7	86.27 ± 12.51	/	/
	VIAAT (size)	8	100.00 ± 9.21	8	98.07 ± 2.99	7	109.34 ± 3.65	7	103.67 ± 4.69	/	/
Stratum Radiatum (S.R.)	Gephyrin (number)	11	100.00 ± 10.69	9	85.62 ± 8.19	11	94.89 ± 9.10	8	92.60 ± 3.56	/	/
	Gephyrin (size)	10	100.00 ± 3.27	9	95.68 ± 1.80	9	94.87 ± 2.51	10	93.62 ± 2.30	/	/
	$GABA_AR\gamma2$ (number)	11	100.00 ± 8.02	11	75.02 ± 9.24	12	90.91 ± 9.06	12	55.97 ± 8.86	Nlgn2: $F_{(1\ 42)} = 11.48$	Nlgn2: 0.002
	$GABA_AR\gamma2$ (size)	10	100.00 ± 3.37	10	83.94 ± 2.98	11	99.99 ± 5.22	11	78.23 ± 3.04	Nlgn2: $F_{(1,38)} = 24.41$	Nlgn2: <0.001
	VIAAT (number)	7	100.00 ± 25.15	7	81.17 ± 13.19	7	68.68 ± 11.52	5	90.43 ± 7.13	/	/
	VIAAT (size)	7	100.00 ±7.12	7	101.76 ± 2.85	7	92.45 ± 4.57	7	99.49 ± 4.95	/	/

Supplementary Table 2 (Part 2). Analysis of the number and size of gephyrin, $GABA_AR\gamma2$ and VIAAT puncta in layers S.O., S.P., S.R. and S.L.M. of hippocampal area CA1 in WT, Nlgn2 KO, MDGA2 Het and Nlgn2 KO / MDGA2 Het mice (all data expressed as %WT).

		WT		Nlgn2 KO		MDGA2 Het		Nlgn2 KO / MDGA2 Het		Main source of variation	
		n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	F-value	p-value
Stratum lacunosum moleculare (S.L.M.)	Gephyrin (number)	10	100 .00 ±11.18	9	99.21 ± 3.61	11	88.76 ± 5.43	8	108.41 ± 6.41	/	/
	Gephyrin (size)	11	100.00 ± 3.60	9	94.38 ± 1.26	11	93.48 ± 2.56	10	92.63 ± 1.90	/	/
	$GABA_AR\gamma2$ (number)	9	100.00 ± 8.93	7	85.92 ± 3.43	10	82.21 ± 9.79	9	71.60 ± 9.97	/	/
	$GABA_AR\gamma2$ (size)	10	100.00 ± 4.8	9	84.29 ± 2.72	9	88.32 ± 3.23	9	77.98 ±1.69	Nlgn2: $F_{(1,33)} = 14.50$ MDGA2: $F_{(1,33)} = 6.914$	Nlgn2: <0.001 MDGA2: 0.013
	VIAAT (number)	5	100.00 ± 20.59	6	49.13 ± 11.20	8	30.52 ± 8.52	7	32.86 ± 6.18	Interaction: $F_{(1,22)} = 5.483$ Nlgn2: $F_{(1,22)} = 4.549$ MDGA2: $F_{(1,22)} = 14.22$	Interaction: 0.029 Nlgn2: 0.044 MDGA2: 0.001
	VIAAT (size)	7	100.00 ± 7.76	6	101.08 ± 10.61	8	87.57 ± 7.34	7	99.64 ± 3.74	/	/

Supplementary Table 3. Two-way ANOVA comparisons for Supplementary Figures 2-3.

Figure	Nlgn2 x MDGA2 interaction		Main effect of Nlgn2		Main effect of MDGA2	
	F-value	p-value	F-value	p-value	F-value	p-value
S2 B	$F_{(1,35)} < 1$	0.913	$F_{(1,35)} = 21.46$	<0.0001	$F_{(1,5)} < 1$	0.662
S2 C	$F_{(1,33)} < 1$	0.582	$F_{(1,33)} = 11.73$	0.0017	$F_{(1,33)} < 1$	0.621
S2 F	$F_{(1,66)} < 1$	0.338	$F_{(1,66)} = 6.247$	0.0149	$F_{(1,66)} < 1$	0.382
S2 I	$F_{(1,59)} = 1.095$	0.300	$F_{(1,59)} = 78.98$	<0.0001	$F_{(1,59)} < 1$	0.843
S2 J	$F_{(1,60)} < 1$	0.359	$F_{(1,60)} = 51.10$	<0.0001	$F_{(1,60)} = 4.661$	0.035
S2 K	$F_{(1,62)} = 1.655$	0.203	$F_{(1,62)} = 171.6$	<0.0001	$F_{(1,62)} < 1$	0.679
S2 L	$F_{(1,63)} = 1.590$	0.212	$F_{(1,63)} = 26.12$	<0.0001	$F_{(1,63)} = 3.204$	0.078
S3G ***Time in centre***	$F_{(1,44)} < 1$	0.777	$F_{(1,44)} = 11.61$	0.0014	$F_{(1,44)} = 1.727$	0.196
S3G ***%Distance in centre***	$F_{(1,44)} = 5.679$	0.022	$F_{(1,44)} = 56.75$	<0.0001	$F_{(1,44)} < 1$	0.401
S3G ***Total distance***	$F_{(1\ 45)} = 7.953$	0.007	$F_{(1,45)} = 8.532$	0.0054	$F_{(1,45)} = 2.383$	0.130
S3H ***Time in centre***	$F_{(1,41)} = 1.350$	0.252	$F_{(1,41)} = 13.21$	0.0008	$F_{(1,41)} < 1$	0.707
S3H ***%Distance in centre***	$F_{(1,41)} < 1$	0.961	$F_{(1,41)} = 20.41$	<0.0001	$F_{(1,41)} < 1$	0.822
S3H ***Total distance***	$F_{(1,40)} = 5.186$	0.028	$F_{(1,40)} = 20.65$	<0.0001	$F_{(1,40)} < 1$	0.959
Figure	**Nlgn2 x MDGA1 interaction**		**Main effect of Nlgn2**		**Main effect of MDGA1**	
	F-value	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
S3E ***Time in centre***	$F_{(1,27)} = 2.473$	0.128	$F_{(1,27)} = 21.55$	<0.001	$F_{(1,27)} = 3.264$	0.082
S3E ***%Distance in centre***	$F_{(1,29)} = 2.814$	0.104	$F_{(1,29)} = 28.64$	<0.001	$F_{(1,29)} < 1$	0.832
S3E ***Total distance***	$F_{(1,29)} = 4.665$	0.039	$F_{(1,29)} = 16.04$	<0.001	$F_{(1,29)} = 1.198$	0.283

Supplementary Table 4. Passive and AP properties of CA1 pyramidal cells in WT, Nlgn2 KO, MDGA2 Het and Nlgn2 KO / MDGA2 Het mice.

	WT		Nlgn2 KO		MDGA2 Het		Nlgn2 KO / MDGA2 Het		Main source of variation	
	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	F-value	p-value
Membrane resistance (MOhm)	41	92.07 ± 4.41	35	97.43 ± 3.06	36	99.59 ± 5.26	35	104.19 ± 4.64	\	\
Membrane capacitance, proximal compartments (pF)	41	45.67 ± 1.94	35	45.48 ± 2.03	36	40.53 ± 1.39	35	42.65 ± 2.34	MDGA2: $F_{(1,143)} = 4.14$	0.044
Membrane capacitance, distal compartments (pF)	41	116.42 ± 4.95	35	106.48 ± 3.75	36	107.6 ± 5.55	35	109.56 ± 4.85	\	\
Resting membrane potential (mV)	20	-58.83 ± 1.19	19	-60.39 ± 1.46	17	-56.99 ± 1.75	17	-55.92 ± 1.85	MDGA2: $F_{(1,69)} = 4.47$	0.038
AP threshold (mV)	19	-45.56 ± 1.03	18	-45.50 ± 0.77	17	-45.58 ± 0.77	17	-45.65 ± 0.55	\	\
AP amplitude (mV)	19	117.16 ± 0.98	18	119.14 ± 1.47	17	120.34 ± 1.11	17	116.89 ± 1.46	Interaction: $F_{(1,67)} = 4.60$	0.036
AP Maximal rate of rise (mv/ms)	19	690.66 ± 21.03	18	654.42 ± 24.01	16	734.23 ± 24.52	17	652.39 ± 24.99	Nlgn2: $F_{(1,66)} = 6.25$	0.015