

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	<input type="text" value="No custom software used"/>
Data analysis	<input type="text" value="No custom software used"/>

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	No human research participants involved
Reporting on race, ethnicity, or other socially relevant groupings	No human research participants involved
Population characteristics	No human research participants involved
Recruitment	No human research participants involved
Ethics oversight	No human research participants involved

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	This information is provided in the figure legends and / or the section on Statistical Analysis in the Methods section.
Data exclusions	This information is provided in the figure legends and / or the section on Statistical Analysis in the Methods section.
Replication	This information is provided in the section on Experimental Subjects in the Methods section.
Randomization	This information is provided in the section on Experimental Subjects in the Methods section.
Blinding	This information is provided in the section on Experimental Subjects in the Methods section.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Not applicable
Research sample	Not applicable
Sampling strategy	Not applicable
Data collection	Not applicable
Timing	Not applicable
Data exclusions	Not applicable
Non-participation	Not applicable
Randomization	Not applicable

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Not applicable
Research sample	Not applicable
Sampling strategy	Not applicable
Data collection	Not applicable
Timing and spatial scale	Not applicable
Data exclusions	Not applicable
Reproducibility	Not applicable
Randomization	Not applicable
Blinding	Not applicable

Did the study involve field work? ☐ Yes ☒ No

Field work, collection and transport

Field conditions	Not applicable
Location	Not applicable
Access & import/export	Not applicable
Disturbance	Not applicable

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	This information is available in the section on Immunohistochemistry in the Methods section.
Validation	All antibodies were validated, either here or previously, using either knockout tissue (Nlgn2, MDGA1) or a no primary antibody control.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Not applicable.
Authentication	Not applicable.
Mycoplasma contamination	Not applicable.
Commonly misidentified lines (See ICLAC register)	Not applicable.

Palaeontology and Archaeology

Specimen provenance	Not applicable.
Specimen deposition	Not applicable.
Dating methods	Not applicable.
<input type="checkbox"/> Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	Not applicable.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	This information is available in the section on Experimental Subjects in the Methods section.
Wild animals	Not applicable.
Reporting on sex	This information is available in the section on Experimental Subjects in the Methods section.
Field-collected samples	Not applicable.
Ethics oversight	This information is available in the section on Experimental Subjects in the Methods section.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	Not applicable.
Study protocol	Not applicable.
Data collection	Not applicable.
Outcomes	Not applicable.

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | | |
|-------------------------------------|---|
| No | Yes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Public health |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> National security |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Crops and/or livestock |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Ecosystems |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Any other significant area |

Experiments of concern

Does the work involve any of these experiments of concern:

- | | |
|-------------------------------------|--|
| No | Yes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Demonstrate how to render a vaccine ineffective |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Enhance the virulence of a pathogen or render a nonpathogen virulent |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Increase transmissibility of a pathogen |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Alter the host range of a pathogen |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Enable evasion of diagnostic/detection modalities |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Enable the weaponization of a biological agent or toxin |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Any other potentially harmful combination of experiments and agents |

Plants

Seed stocks	<input type="text" value="Not applicable."/>
Novel plant genotypes	<input type="text" value="Not applicable."/>
Authentication	<input type="text" value="Not applicable."/>

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <small>May remain private before publication.</small>	<input type="text" value="Not applicable."/>
Files in database submission	<input type="text" value="Not applicable."/>
Genome browser session <small>(e.g. UCSC)</small>	<input type="text" value="Not applicable."/>

Methodology

Replicates	<input type="text" value="Not applicable."/>
Sequencing depth	<input type="text" value="Not applicable."/>
Antibodies	<input type="text" value="Not applicable."/>
Peak calling parameters	<input type="text" value="Not applicable."/>
Data quality	<input type="text" value="Not applicable."/>
Software	<input type="text" value="Not applicable."/>

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

- Sample preparation
- Instrument
- Software
- Cell population abundance
- Gating strategy

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

- Design type
- Design specifications
- Behavioral performance measures

Acquisition

- Imaging type(s)
- Field strength
- Sequence & imaging parameters
- Area of acquisition
- Diffusion MRI ☐ Used ☐ Not used

Preprocessing

- Preprocessing software
- Normalization
- Normalization template
- Noise and artifact removal
- Volume censoring

Statistical modeling & inference

- Model type and settings
- Effect(s) tested
- Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Not applicable.

(See [Eklund et al. 2016](#))

Correction

Not applicable.

Models & analysis

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Functional and/or effective connectivity
<input type="checkbox"/>	<input checked="" type="checkbox"/> Graph analysis
<input checked="" type="checkbox"/>	<input type="checkbox"/> Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Not applicable.

Graph analysis

All relevant parameters are reported in the statistical analysis section or the figure legends.

Multivariate modeling and predictive analysis

Not applicable.

Description of Additional Supplementary Files

1

2

3 **File name:** Supplementary Data 1

4 **Description:** Source data for Figures 1-6

5

6 **File name:** Supplementary Data 2

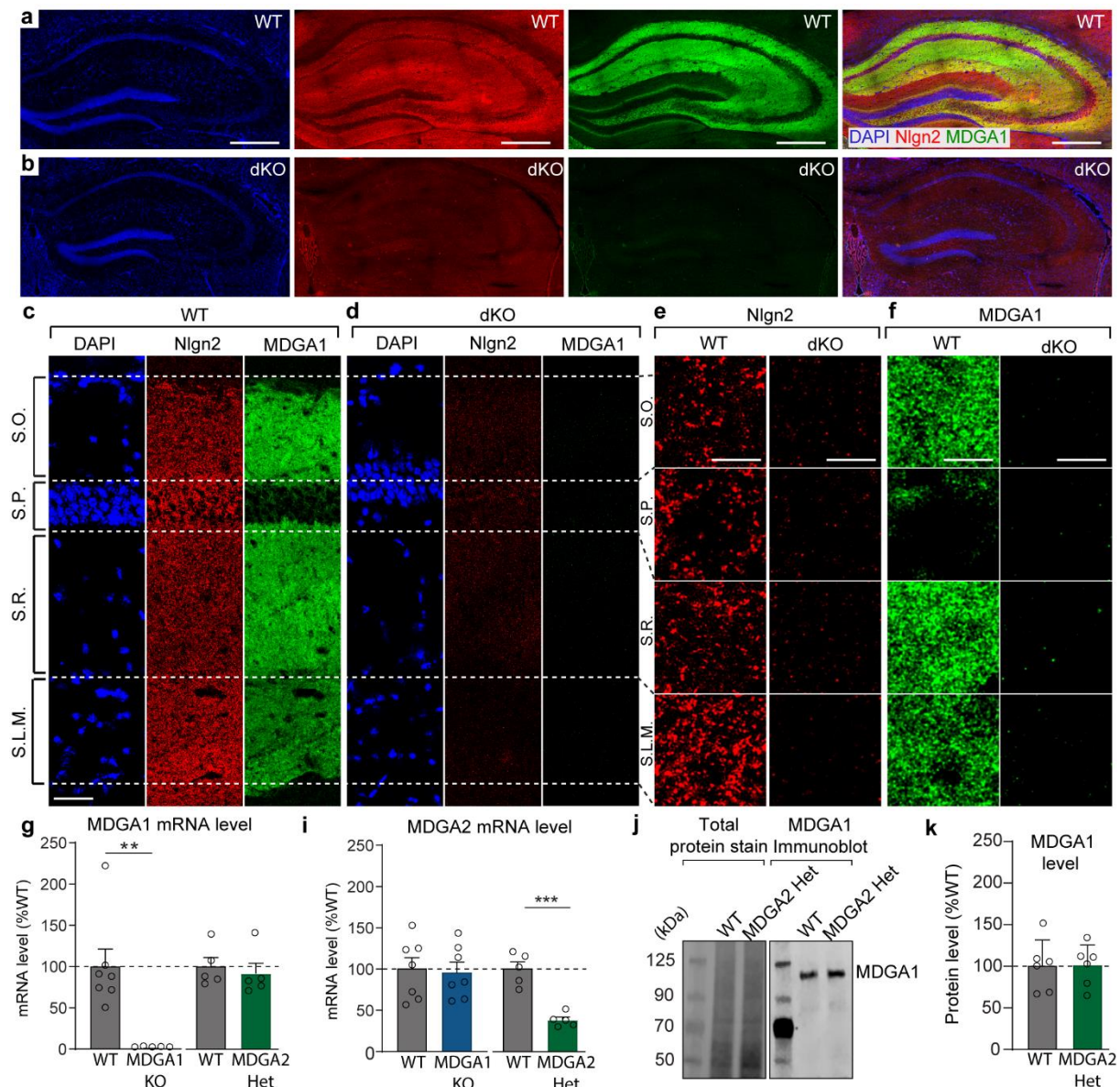
7 **Description:** Source data for Supplementary Figures 1-5 and Supplementary Tables 1-6

8

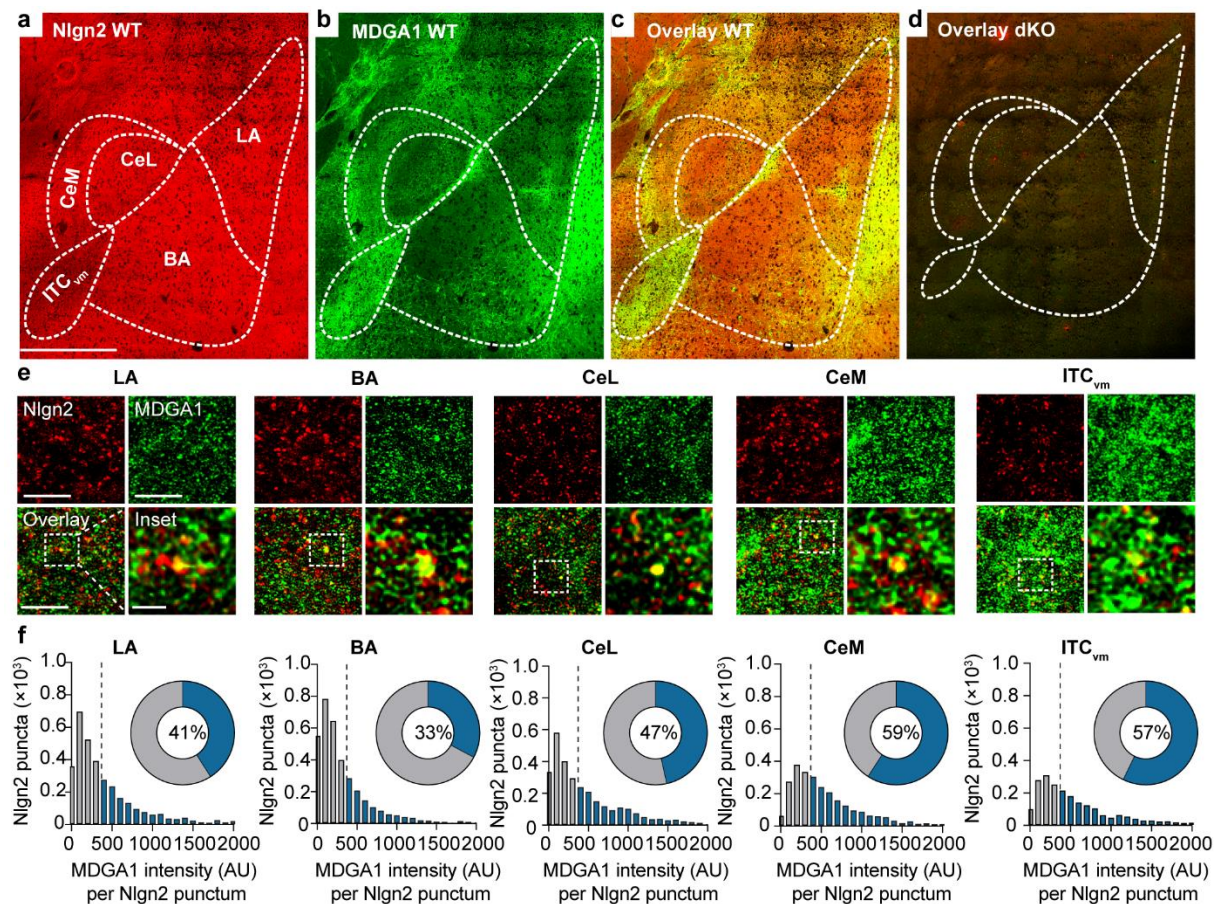
SUPPLEMENTARY INFORMATION

Functional Neuroligin-2-MDGA1 interactions differentially regulate synaptic GABA_ARs and cytosolic gephyrin aggregation

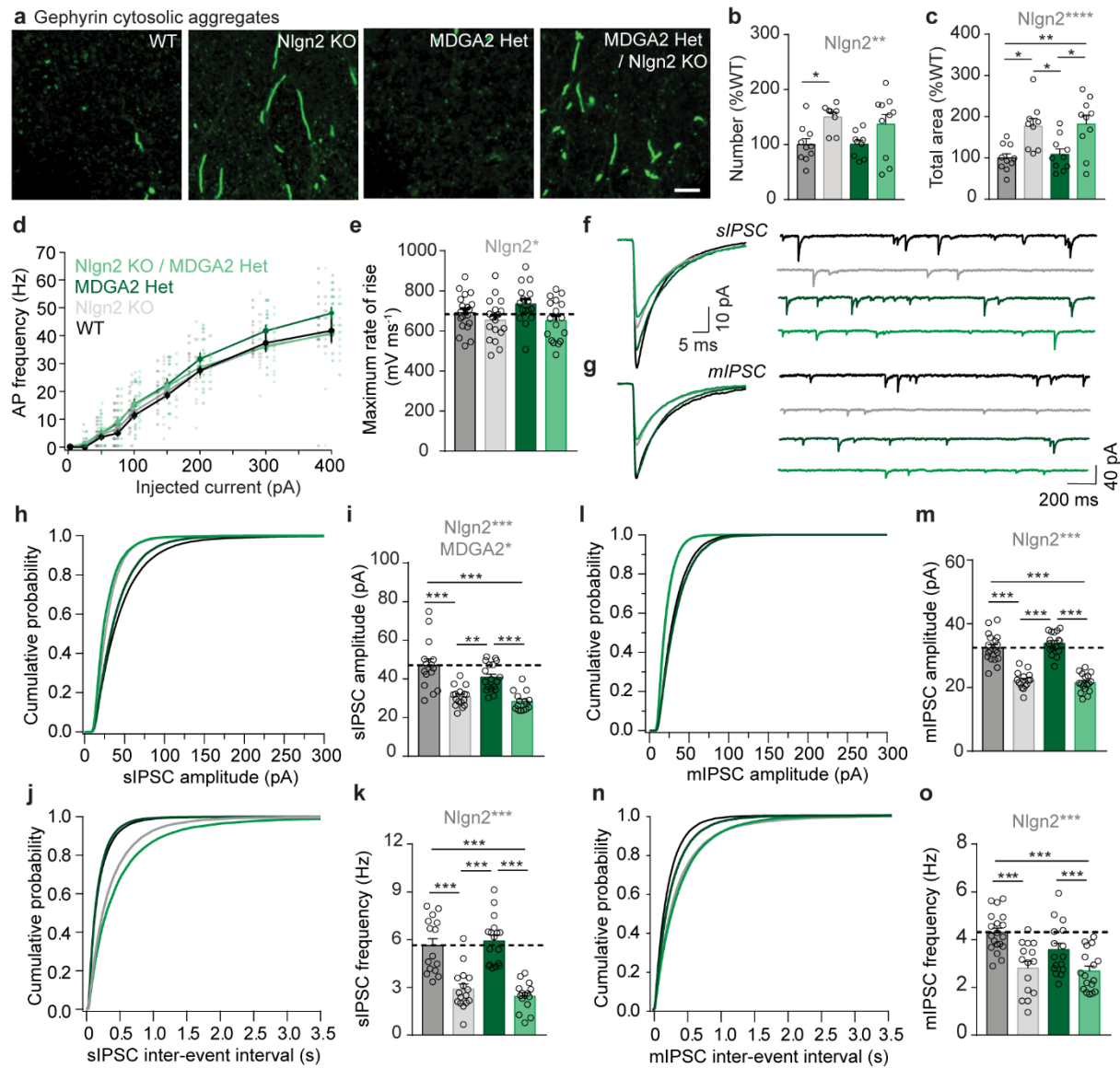
Tommaso Zeppillo[#], Heba Ali[#], Sowbarnika Ravichandran[§], Tamara C. Ritter[§], Sally Wenger, Francisco J. López-Murcia, Erinn Gideons, Janetti Signorelli, Michael J. Schmeisser, Jens Wiltfang, JeongSeop Rhee, Nils Brose, Holger Taschenberger and Dilja Krueger-Burg



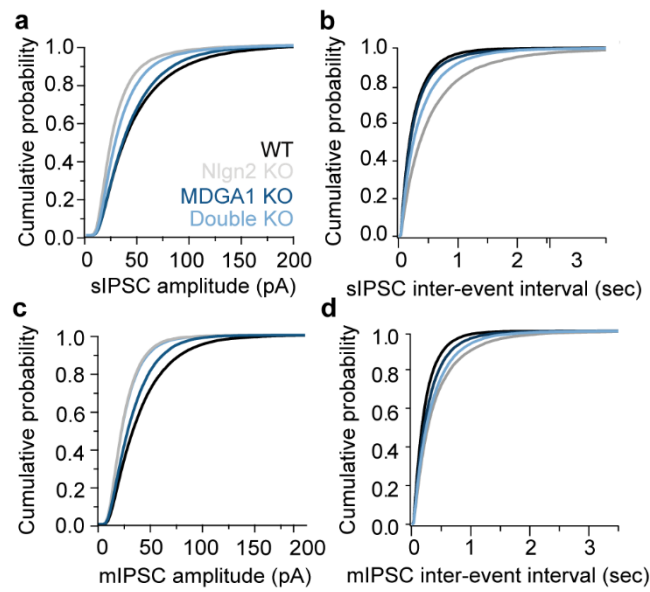
Supplementary Fig. 1: Validation of the specificity of antibodies against MDGA1 and Nlgn2, and of the mouse models used in the study. (a-b) Photomicrographs showing an overview of the hippocampus in WT (a) versus Nlgn2-MDGA1 dKO mice (b) labelled with DAPI (blue), and antibodies against Nlgn2 (red) and MDGA1 (green). Scale bar 500 μ m. **(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 μ m. **(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 μ m. **(g)** Bar graph showing MDGA1 mRNA level in WT, MDGA1 KO and MDGA1 Het mice, relative values normalized by the expression of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as percentage of the WT mice. **(i)** Bar graph showing the MDGA2 mRNA level in WT, MDGA1 KO and MDGA1 Het mice. **(j)** Western blot membrane showing the total protein stain and the result of the immunoblot against MDGA1 in WT and MDGA2 Het mice. **(k)** Bars graph showing the MDGA1 protein level between WT and MDGA2 Het mice normalized by the average sample value of all lanes on the same blot, and expressed as a percentage of the WT mice. Statistically significant unpaired t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars represent SEM, and each circle represents an experimental animal ($n = 5-7$), details listed in supplementary table 1.



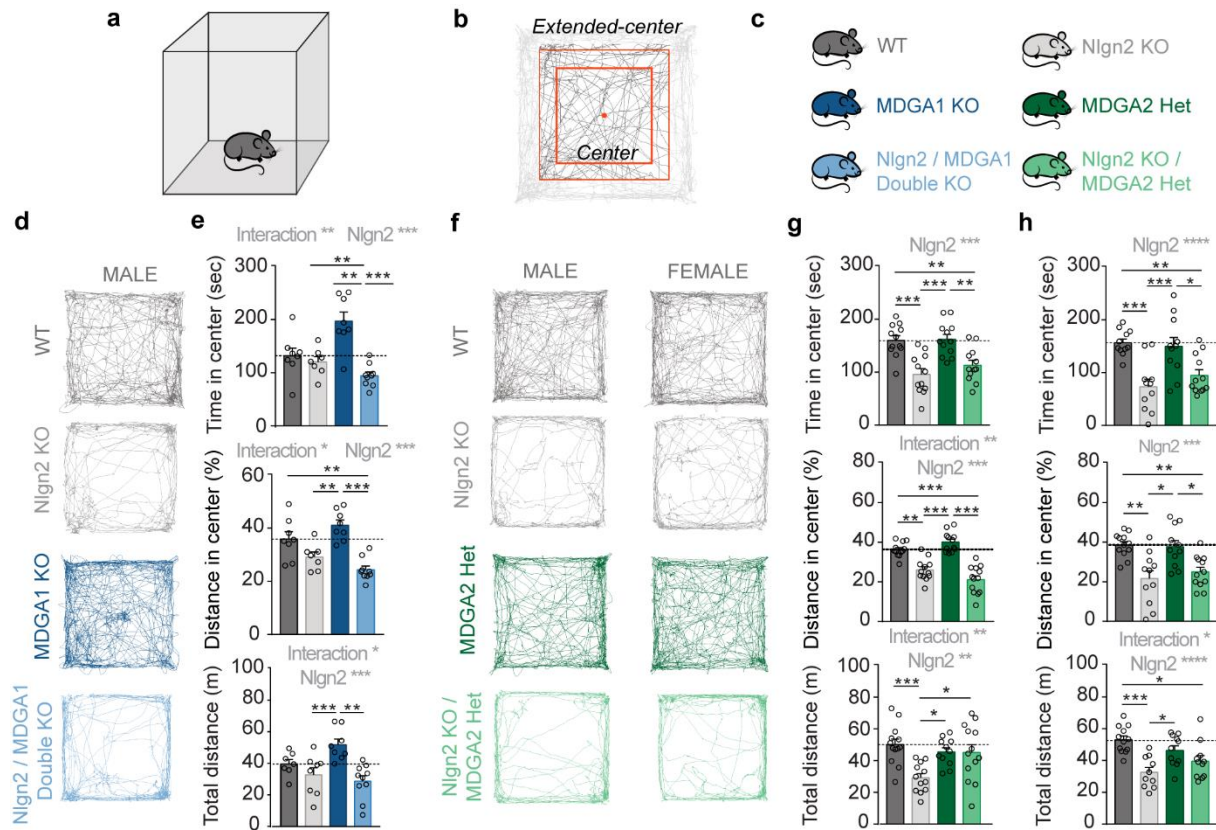
Supplementary Fig. 2. Colocalization of Nlgn2 and MDGA1 in the amygdala of WT mice. (a-d) Photomicrographs showing the amygdala in WT (a-c) and dKO (d) mice, labeled with antibodies against Nlgn2 (a), MDGA1 (b), or an overlay of both antibodies (c, d). (e) High magnification photomicrographs showing representative images of Nlgn2 (red) and MDGA1 (green) staining, and their colocalization (overlay) in subregions of the amygdala. LA, lateral amygdala; BA basal, amygdala; CeL, centrolateral amygdala; CeM, centromedial amygdala; ITC_{vm}, ventromedial intercalated cluster. (f) Histograms showing the frequency distribution of MDGA1 fluorescence (intensity in arbitrary units) within Nlgn2-labeled puncta in subregions of the amygdala (LA, BA, CeL, CeM, ITC_{vm}). Bars in blue represent Nlgn2-labeled puncta with above-threshold MDGA1 fluorescence intensity (see Methods section for threshold determination). Doughnut chart insets display the percentage of Nlgn2-labelled puncta with an above-threshold MDGA1 fluorescence intensity (in blue, percentage in the center of the doughnut chart).



Supplementary Fig. 3: Heterozygous MDGA2 deletion does not affect the formation of gephyrin aggregates nor GABAergic transmission in CA1 pyramidal cells. (a) High magnification photomicrographs of gephyrin aggregates in the hippocampal CA1 area of WT, Nlgn2 KO, MDGA2 Het and Nlgn2 KO / MDGA2 Het mice. Scale bar 5 μ m. (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): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars represent SEM, and each circle represents an experimental animal ($n = 8-10$). (d) Frequency of action potentials (APs) in response to depolarizing current steps. (e) Quantification of the maximal rate of AP rise in CA1 pyramidal neurons of WT, Nlgn2 KO, MDGA2 Het, Nlgn2 KO / MDGA2 Het mice. (f) Representative average sIPSC waveforms (left) obtained from individual sIPSCs (right) recorded in the four genotypes. (g) Representative average mIPSC waveforms (right) obtained from individual mIPSCs (right) recorded in the four genotypes. (h-k) Average cumulative distributions of sIPSC amplitudes (h) and sIPSC inter-event intervals (j) shown together with the respective mean values for sIPSC amplitudes (i) and sIPSC frequencies (k) for all genotypes. (l-o) Average cumulative distributions of mIPSC amplitudes (m) and mIPSC inter-event intervals (n) shown together with the respective mean values for mIPSC amplitudes (i) and mIPSC frequencies (o) for all genotypes. Statistically significant ANOVA comparisons are marked in gray at the top of panels and listed in Supplementary Table 8. For all other ANOVA comparisons, $F < 1$. Post-hoc analysis (Tukey's comparison test): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars represent SEM, and 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; four animals per genotype).



Supplementary Fig. 4: Loss of MDGA1 expression perturbs spontaneous GABAergic transmission in CA1 pyramidal neurons. (a-b) Average cumulative amplitude distributions and average waveforms of sIPSCs amplitude (a) and frequency (b) for all genotypes (WT, Nlgn2 KO, MDGA1 KO, and Nlgn2-MDGA1 double KO). **(c-d)**. Average cumulative distributions of mIPSCs amplitude (c) and frequency (d) for all analyzed genotypes.



Supplementary Fig. 5. Heterozygous MDGA2 deletion does not influence abnormal 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 8. For all other ANOVA comparisons, $F < 1$. Post-hoc analysis (Tukey's comparison test): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 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. Summary of MDGA1 and MDGA2 mRNA and protein levels. MDGA1 and MDGA2 mRNA levels in hippocampal tissue of WT, MDGA1 KO and MDGA2 Het mice (unpaired t-test) were normalized by the expression of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, and expressed as percentage of the expression in WT mice. MDGA1 protein level between WT and MDGA2 Het mice were normalized by the average sample value of all lanes on the same blot, and expressed as a percentage of WT mice. (Avg.Cq represent the cycle number at which the sample's reaction curve intersects the threshold line).

	WT		MDGA1 KO		p-value
	n	Mean ± SEM	n	Mean ± SEM	
MDGA1 mRNA level (S1g)	7	100.0 ± 21.3	5	0.6 ± 0.2	<0.001
MDGA2 mRNA level (S1i)	7	100.0 ± 11.0	7	91.6 ± 12.7	0.631
Avg.Cq MDGA1	7	31.2 ± 0.3	5	38.6 ± 0.4	<0.001
Avg.Cq MDGA2	7	30.6 ± 0.2	7	30.9 ± 0.3	0.515
Avg.Cq GAPDH	7	23.2 ± 0.3	5	23.1 ± 0.3	0.849
	WT		MDGA2 Het		p-value
	n	Mean ± SEM	n	Mean ± SEM	
MDGA1 mRNA level (S1i)	5	100.0 ± 11.0	5	91.6 ± 12.7	0.631
MDGA2 mRNA level (S1i)	5	100.0 ± 8.6	5	39.6 ± 3.8	<0.001
Avg.Cq MDGA1	5	31.6 ± 0.2	5	31.4 ± 0.2	0.516
Avg.Cq MDGA2	5	30.1 ± 0.2	5	31.1 ± 0.1	0.003
Avg.Cq GAPDH	5	23.2 ± 0.1	5	22.88 ± 0.17	0.166
MDGA1 Protein expression (Immunoblotting) – S1j-k	5	100.0 ± 12.7	5	100.4 ± 10.1	0.983

Supplementary Table 2. Analysis of the number and size of gephyrin, GABA_AR γ 2 and VIAAT puncta in layers S.O., S.P., S.R. and S.L.M. of hippocampal area CA1 in WT, Nlgn2 KO, MDGA1 KO and Nlgn2 / MDGA1 dKO mice (all data expressed as percentage of WT).

		WT		Nlgn2 KO		MDGA1 KO		Nlgn2 / MDGA1 dKO		Main source of variation	
		n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	F-value	p-value
Stratum oriens (S.O.)	Gephyrin (number)	8	100.0 \pm 6.4	8	79.0 \pm 7.0	8	86.6 \pm 5.7	8	76.6 \pm 9.6	Nlgn2: $F_{(1,28)} = 4.48$	0.04
	Gephyrin (size)	8	100.0 \pm 3.2	8	90.3 \pm 3.3	7	94.2 \pm 2.2	7	91.3 \pm 3.4	Nlgn2: $F_{(1,28)} = 4.23$	0.05
	GABA _A R γ 2 (number)	8	100.0 \pm 5.3	8	90.3 \pm 6.3	9	93.1 \pm 7.8	9	3.0 \pm 6.5	/	/
	GABA _A R γ 2 (size)	9	100.0 \pm 3.9	8	81.5 \pm 4.1	9	84.9 \pm 3.5	9	82.0 \pm 3.3	Nlgn2: $F_{(1,31)} = 8.38$ Interaction $F_{(1,31)} = 4.48$	Nlgn2: 0.01 Interaction 0.04
	VIAAT (number)	8	100.0 \pm 15.7	8	101.2 \pm 12.1	8	87.1 \pm 11.9	8	79.7 \pm 14.1	/	/
	VIAAT (size)	8	100.0 \pm 2.4	8	99.0 \pm 3.2	8	89.0 \pm 3.1	8	87.6 \pm 1.9	MDGA1: $F_{(1,28)} = 18.40$	<0.001
Stratum lacunosum moleculare (SLM)	Gephyrin (number)	7	100.0 \pm 7.9	7	98.5 \pm 8.7	7	109.9 \pm 4.4	6	93.2 \pm 2.8	/	/
	Gephyrin (size)	8	100.0 \pm 3.8	8	95.6 \pm 2.3	7	98.0 \pm 1.8	8	91.9 \pm 4.3	/	/
	GABA _A R γ 2 (number)	8	100.0 \pm 6.0	8	94.6 \pm 4.1	8	107.7 \pm 4.0	9	102.6 \pm 4.4		
	GABA _A R γ 2 (size)	8	100.0 \pm 4.3	9	85.5 \pm 4.1	8	84.2 \pm 2.7	9	83.2 \pm 3.4	Nlgn2: $F_{(1,30)} = 4.38$ MDGA1: $F_{(1,30)} = 5.94$	Nlgn2: 0.05 MDGA1: 0.02
	VIAAT (number)	6	100.0 \pm 26.4	6	10.6 \pm 22.3	4	117.5 \pm 23.8	4	120.3 \pm 17.9	/	/
	VIAAT (size)	7	100.0 \pm 3.3	6	100.8 \pm 5.5	5	85.2 \pm 3.0	5	94.0 \pm 3.2	MDGA1: $F_{(1,19)} = 7.10$	MDGA1: 0.02

Supplementary Table 3 (Part 1). Analysis of the number and size of gephyrin, GABA_AR γ 2 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 percentage of WT)

		WT		Nlgn2 KO		MDGA2 Het		Nlgn2 KO / MDGA2 Het		Main source of variation	
		n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	F-value	p-value
Stratum oriens (S.O.)	Gephyrin (number)	11	100.0 \pm 8.9	10	86.9 \pm 6.5	11	104.3 \pm 8.6	9	78.3 \pm 7.0	Nlgn2: F _(1,37) = 6.0	Nlgn2: 0.02
	Gephyrin (size)	10	100.0 \pm 3.0	10	91.4 \pm 2.4	10	96.7 \pm 2.8	11	94.1 \pm 2.3	Nlgn2: F _(1,37) = 4.5	Nlgn2: 0.04
	GABA _A R γ 2 (number)	12	100.0 \pm 7.7	11	87.6 \pm 8.9	12	117.9 \pm 13.8	12	60.3 \pm 8.1	Interaction: F _(1,43) = 5.1 Nlgn2: F _(1,43) = 12.3	Interaction: 0.03 Nlgn2: 0.001
	GABA _A R γ 2 (size)	10	100.00 \pm 2.5	10	86.2 \pm 3.1	12	93.8 \pm 5.0	11	76.8 \pm 2.2	Nlgn2: F _(1,39) = 18.65 MDGA2: F _(1,39) = 4.7	Nlgn2: <0.001 MDGA2: 0.04
	VIAAT (number)	7	100.0 \pm 17.7	7	85.0 \pm 11.0	6	83.0 \pm 18.4	6	58.6 \pm 9.6	/	/
	VIAAT (size)	7	100.0 \pm 5.3	7	99.3 \pm 3.1	6	90.0 \pm 2.5	7	93.2 \pm 6.1	/	/
Stratum pyramidale (S.P.)	Gephyrin (number)	9	100.0 \pm 10.7	9	88.7 \pm 6.9	9	79.6 \pm 7.1	9	70.1 \pm 8.7	MDGA2: F _(1,32) = 5.3	MDGA2: 0.03
	Gephyrin (size)	9	100.0 \pm 2.4	9	90.3 \pm 3.6	9	92.7 \pm 3.1	8	89.2 \pm 1.3	Nlgn2: F _(1,31) = 5.5	Nlgn2: 0.03
	GABA _A R γ 2 (number)	9	100.0 \pm 4.0	10	81.3 \pm 7.6	10	84.8 \pm 8.7	9	59.5 \pm 5.2	Nlgn2: F _(1,34) = 10.4 MDGA2: F _(1,34) = 7.4	Nlgn2: 0.003 MDGA2: 0.01
	GABA _A R γ 2 (size)	10	100.0 \pm 5.4	10	88.9 \pm 4.6	10	87.3 \pm 3.9	10	70.8 \pm 3.4	Nlgn2: F _(1,36) = 10.0 MDGA2: F _(1,36) = 12.3	Nlgn2: 0.003 MDGA2: 0.001
	VIAAT (number)	8	100.0 \pm 19.3	8	91.4 \pm 10.9	8	71.3 \pm 11.8	7	86.3 \pm 12.5	/	/
	VIAAT (size)	8	100.0 \pm 9.2	8	98.1 \pm 3.0	7	109.3 \pm 3.7	7	103.7 \pm 4.7	/	/
Stratum radiatum (S.R.)	Gephyrin (number)	11	100.0 \pm 10.7	9	85.6 \pm 8.2	11	94.9 \pm 9.1	8	92.6 \pm 3.6	/	/
	Gephyrin (size)	10	100.0 \pm 3.3	9	95.7 \pm 1.8	9	94.9 \pm 2.5	10	93.6 \pm 2.3	/	/
	GABA _A R γ 2 (number)	11	100.0 \pm 8.0	11	75.0 \pm 9.2	12	90.9 \pm 9.1	12	56.0 \pm 8.7	Nlgn2: F _(1,42) = 11.48	Nlgn2: 0.002
	GABA _A R γ 2 (size)	10	100.0 \pm 3.4	10	83.9 \pm 3.0	11	100.0 \pm 5.2	11	78.2 \pm 3.0	Nlgn2: F _(1,38) = 24.41	Nlgn2: <0.001
	VIAAT (number)	7	100.0 \pm 25.2	7	81.2 \pm 13.2	7	68.7 \pm 11.5	5	90.4 \pm 7.1	/	/
	VIAAT (size)	7	100.0 \pm 7.1	7	101.76 \pm 2.8	7	92.5 \pm 4.6	7	99.5 \pm 5.0	/	/

Supplementary Table 3 (Part 2). Analysis of the number and size of gephyrin, GABA_AR γ 2 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 percentage of WT).

		WT		Nlgn2 KO		MDGA2 Het		Nlgn2 KO / MDGA2 Het		Main source of variation	
		n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	F-value	p-value
Stratum lacunosum moleculare (S.L.M.)	Gephyrin (number)	10	100.0 \pm 11.2	9	99.2 \pm 3.6	11	88.8 \pm 5.4	8	108.4 \pm 6.4	/	/
	Gephyrin (size)	11	100.0 \pm 3.6	9	94.4 \pm 1.3	11	93.5 \pm 2.6	10	92.6 \pm 1.9	/	/
	GABA _A R γ 2 (number)	9	100.0 \pm 8.9	7	85.9 \pm 3.4	10	82.2 \pm 9.8	9	71.6 \pm 10.0	/	/
	GABA _A R γ 2 (size)	10	100.0 \pm 4.8	9	84.3 \pm 2.7	9	88.3 \pm 3.2	9	78.0 \pm 1.7	Nlgn2: F _(1,33) = 14.5 MDGA2: F _(1,33) = 6.9	Nlgn2: <0.001 MDGA2: 0.01
	VIAAT (number)	5	100.0 \pm 20.6	6	49.1 \pm 11.2	8	30.5 \pm 8.5	7	32.9 \pm 6.2	Interaction: F _(1,22) = 5.5 Nlgn2: F _(1,22) = 4.6 MDGA2: F _(1,22) = 14.2	Interaction: 0.03 Nlgn2: 0.04 MDGA2: 0.001
	VIAAT (size)	7	100.0 \pm 7.8	6	101.1 \pm 10.6	8	87.6 \pm 7.3	7	99.6 \pm 3.7	/	/

Supplementary Table 4. Passive and AP properties of CA1 pyramidal cells in WT, Nlgn2 KO, MDGA1 KO, Nlgn2 KO / MDGA1 double KO, and MDGA2 Het and Nlgn2 KO / MDGA2 Het mice.

	WT		Nlgn2 KO		MDGA1 KO		Nlgn2 / MDGA1 dKO		Main source of variation	
	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	F-value	p-value
Membrane resistance ($M\Omega$)	37	100.5 \pm 4.5	34	95.0 \pm 5.6	41	114.6 \pm 7.4	36	100.9 \pm 7.7	\	\
Membrane capacitance, proximal compartments (pF)	37	42.9 \pm 2.4	34	45.1 \pm 2.7	41	39.5 \pm 1.5	36	46.9 \pm 1.8	Nlgn2 KO: $F_{(1,144)} = 5.1$	0.03
Membrane capacitance, distal compartments (pF)	37	122.7 \pm 4.8	34	113.2 \pm 6.0	41	111.2 \pm 3.4	36	126.6 \pm 5.1	Interaction: $F_{(1,144)} = 6.6$	0.01
Resting membrane potential (mV)	21	-58.3 \pm 1.7	16	-57.2 \pm 2.2	23	-55.4 \pm 1.3	18	-59.9 \pm 1.5	\	\
AP threshold (mV)	20	-44.4 \pm 0.8	15	-43.7 \pm 0.7	24	-45.4 \pm 0.7	18	-44.7 \pm 0.7	\	\
AP amplitude (mV)	20	117.9 \pm 1.2	15	117.8 \pm 1.5	24	120.8 \pm 1.8	19	120.9 \pm 1.4	\	\
AP maximum rate of rise (mV/ms)	21	582.4 \pm 17.5	16	591.9 \pm 34.9	19	648.6 \pm 20.2	24	733.8 \pm 21.5	Nlgn2 KO: $F_{(1,73)} = 5.3$ Interaction: $F_{(1,73)} = 3.55$	Nlgn2 KO: 0.02 Interaction: 0.004

	WT		Nlgn2 KO		MDGA2 Het		Nlgn2 KO / MDGA2 Het		Main source of variation	
	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	F-value	p-value
Membrane resistance ($M\Omega$)	41	92.1 \pm 4.4	35	97.4 \pm 3.1	36	99.6 \pm 5.3	35	104.2 \pm 4.6	\	\
Membrane capacitance, proximal compartments (pF)	41	45.7 \pm 1.9	35	45.5 \pm 2.0	36	40.5 \pm 1.4	35	42.7 \pm 2.3	MDGA2: $F_{(1,143)} = 4.1$	0.04
Membrane capacitance, distal compartments (pF)	41	116.4 \pm 5.0	35	106.5 \pm 3.8	36	107.6 \pm 5.6	35	109.6 \pm 4.9	\	\
Resting membrane potential (mV)	20	-58.8 \pm 1.2	19	-60.4 \pm 1.5	17	-57.0 \pm 1.8	17	-55.9 \pm 1.9	MDGA2: $F_{(1,69)} = 4.5$	0.04
AP threshold (mV)	19	-45.6 \pm 1.0	18	-45.5 \pm 0.8	17	-45.6 \pm 0.8	17	-45.7 \pm 0.6	\	\
AP amplitude (mV)	19	117.2 \pm 1.0	18	119.1 \pm 1.5	17	120.3 \pm 1.1	17	116.9 \pm 1.5	Interaction: $F_{(1,67)} = 4.6$	0.04
AP Maximum rate of rise (mV/ms)	19	690.7 \pm 21.0	18	654.4 \pm 24.0	16	734.2 \pm 24.5	17	652.4 \pm 25.0	Nlgn2: $F_{(1,66)} = 6.3$	0.02

Supplementary Table 5. Analysis of the number and size of PSD95 and vGluT1 puncta in layers S.O., S.P., S.R. and S.L.M. of hippocampal area CA1 in WT and MDGA1 KO mice (all data expressed as percentage of WT).

		WT		Mdga1 KO		p-value
		n	Mean ± SEM	n	Mean ± SEM	
Stratum Oriens (S.O.)	PSD95 (number)	6	100.0 ± 7.9	6	82.5 ± 7.9	0.15
	PSD95 (size)	6	100.0 ± 2.9	6	100.1 ± 2.9	0.99
	vGluT1 (number)	6	100.0 ± 11.6	6	123.7 ± 11.6	0.18
	vGluT1 (size)	6	100.0 ± 4.2	6	115.3 ± 4.2	0.03
Stratum Pyramidale (S.P.)	PSD95 (number)	6	100.0 ± 11.0	6	70.0 ± 11.0	0.08
	PSD95 (size)	6	100.0 ± 3.3	6	99.7 ± 3.3	0.94
	vGluT1 (number)	6	100.0 ± 21.8	6	160.8 ± 21.8	0.08
	vGluT1 (size)	6	100.0 ± 5.5	6	103.2 ± 5.5	0.68
Stratum Radiatum (S.R.)	PSD95 (number)	6	100.0 ± 15.2	6	100.4 ± 15.2	0.99
	PSD95 (size)	6	100.0 ± 5.3	6	105.7 ± 5.3	0.47
	vGluT1 (number)	6	100.0 ± 15.6	6	123.3 ± 15.6	0.31
	vGluT1 (size)	6	100.0 ± 9.8	6	127.8 ± 9.8	0.07
Stratum Lacunosum moleculare (S.L.M.)	PSD95 (number)	6	100.0 ± 9.3	6	89.2 ± 9.3	0.43
	PSD95 (size)	6	100.0 ± 2.9	6	93.8 ± 2.9	0.17
	vGluT1 (number)	6	100.0 ± 12.2	6	85.0 ± 12.2	0.41
	vGluT1 (size)	6	100.0 ± 4.5	6	93.2 ± 4.5	0.31

Supplementary Table 6. Comparison of: mean amplitudes and mean frequencies of spontaneous mEPSCs, and passive properties of CA1 pyramidal cells in WT and MDGA1 KO mice (unpaired t-test).

	WT		MDGA1 KO		p-value
	n	Mean ± SEM	n	Mean ± SEM	
mEPSC frequency	23	0.12 ± 0.01	22	0.13 ± 0.01	0.07
mEPSC amplitude	23	9.4 ± 0.2	22	9.7 ± 0.3	0.52
Membrane resistance (MOhm)	23	164.8 ± 6.4	23	149.8 ± 6.0	0.1
Membrane capacitance, proximal compartments (pF)	23	34.3 ± 2.5	23	27.2 ± 0.8	0.02
Membrane capacitance, distal compartments (pF)	23	135.8 ± 5.9	23	117.4 ± 4.6	0.01

Supplementary Table 7. Two-way ANOVA comparisons for Supplementary Fig. 3/5.

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
S3b	$F_{(1,35)} < 1$	0.9	$F_{(1,35)} = 21.5$	<0.001	$F_{(1,5)} < 1$	0.7
S3c	$F_{(1,33)} < 1$	0.6	$F_{(1,33)} = 11.7$	0.002	$F_{(1,33)} < 1$	0.6
S3e	$F_{(1,66)} < 1$	0.3	$F_{(1,66)} = 6.3$	0.02	$F_{(1,66)} < 1$	0.4
S3i	$F_{(1,59)} = 1.1$	0.3	$F_{(1,59)} = 78.98$	<0.001	$F_{(1,59)} < 1$	0.8
S3m	$F_{(1,60)} < 1$	0.4	$F_{(1,60)} = 51.1$	<0.001	$F_{(1,60)} = 4.7$	0.04
S3k	$F_{(1,62)} = 1.66$	0.2	$F_{(1,62)} = 171.6$	<0.001	$F_{(1,62)} < 1$	0.7
S3o	$F_{(1,63)} = 1.59$	0.2	$F_{(1,63)} = 26.1$	<0.001	$F_{(1,63)} = 3.2$	0.1
S5g Time in center	$F_{(1,44)} < 1$	0.8	$F_{(1,44)} = 11.6$	0.001	$F_{(1,44)} = 1.8$	0.2
S5g Center distance	$F_{(1,44)} = 5.68$	0.02	$F_{(1,44)} = 56.8$	<0.001	$F_{(1,44)} < 1$	0.4
S5g Total distance	$F_{(1,45)} = 7.95$	0.01	$F_{(1,45)} = 8.5$	0.01	$F_{(1,45)} = 2.3$	0.1
S5h Time in center	$F_{(1,41)} = 1.4$	0.3	$F_{(1,41)} = 13.2$	<0.001	$F_{(1,41)} < 1$	0.7
S5h Center distance	$F_{(1,41)} < 1$	1.0	$F_{(1,41)} = 20.4$	<0.001	$F_{(1,41)} < 1$	0.8
S5h Total distance	$F_{(1,40)} = 5.19$	0.03	$F_{(1,40)} = 20.7$	<0.001	$F_{(1,40)} < 1$	1.0
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
S5e Time in center	$F_{(1,27)} = 2.47$	0.13	$F_{(1,27)} = 21.6$	<0.001	$F_{(1,27)} = 3.3$	0.1
S5e Center distance	$F_{(1,29)} = 2.8$	0.1	$F_{(1,29)} = 28.6$	<0.001	$F_{(1,29)} < 1$	0.8
S5e Total distance	$F_{(1,29)} = 4.67$	0.04	$F_{(1,29)} = 16.0$	<0.001	$F_{(1,29)} = 1.2$	0.3