NMDARs drive the expression of neuropsychiatric disorder risk genes within GABAergic interneuron subtypes in the juvenile brain

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Supplementary Figure Legends

FIGURE1, SUPP1 | Schematic overview and quality control for scRNAseq

A, Breeding strategy, **B_i, C_i, D_i, E_i**, Representative FACS gates to sequentially isolate live: dead cells using DAPI: DRAQ5 staining, singlet-gating and TdT^+-reporter gating to obtain reporter-positive MGE-derived interneurons from frontal neocortex and hippocampus. **B_{ii}, C_{ii}, D_{ii}, E_{ii}**, Barcode Rank Plots for cells from WT and NULL mice, demonstrating separation of cell-associated barcodes and those associated with empty partitions. UMI, unique molecular identifier; MR, Mean Reads; MG, Median Genes. **B_{iii}, C_{iii}, D_{iii}, E_{iii}**, Distributions of the total number of genes, percentage of mitochondrial genes and UMIs per cell in control mice. **B_{iv}, C_{iv}, D_{iv}, E_{iv},** Pearson correlation coefficient of the distributions of the total number of genes and the UMI

FIGURE1, SUPP2 | Biological replicates

Representative UMAP plots of the 3 biological replicates from neocortex and hippocampus indicates **A,** similar clustering, and **B,** similar expression profiles of *Nkx2.1*-derived, *Gad1*-expressing MGE-derived interneurons and *Nkx2.1* derived, *Olig1*-expressing oligodendrocytes.

FIGURE1, SUPP3 | Select marker gene expression across the subtypes of merged cortical and hippocampal MGE-*Grin1*$^{wt/wt}$

A_i, UMAP representation of cardinal MGE markers genes in the cortical and hippocampal merged dataset. **A_{ii},** UMAP representation colored by region, highlighting the region-specific enrichments of MGE subsets. **A_{iii},** Pie chart indicating the percentages of cells recovered across the interneuron subtypes from neocortex and the hippocampus. **B,** Violin plot showing the distribution of expression levels of well-known representative cell-type-enriched marker genes across the 11 MGE subtypes.

FIGURE1, SUPP4 | MGE-derived interneuron subtype differences between neocortex and hippocampus

Volcano plot representing the −log10 False Discovery Rate (FDR) versus log2 fold change (FC) between **A,** TH-expressing MGE subsets and the remaining MGE subset SST, PVALB and NGFC; **B,** Differential expression of the cardinal MGE classes between neocortex and hippocampus, at a fold change ≥0.5 and FDR <10e-5.

FIGURE1, SUPP5 | MGE-derived interneuron subtype annotation based on marker expression

A, Table indicating the subtype-defining marker genes observed in the present study and their descriptions in the previous scRNAseq datasets (*indicates the genes expressed in the cortex-exclusive PVALB.2 subcluster). Representative UMAP plots of MGE subtype-enriched genes in **B,** SST subclusters, **C,** NGFC subclusters, and **D,** PVALB subclusters.

FIGURE2, SUPP1 | Validation of MGE subtype abundances subsequent to *Grin1*-ablation by immunostaining

A, Boxplots indicating the cell counts of MGE-derived interneurons expressing **(A_i)** Ai14/tdTomato, **(A_{ii})** PV, SST immunostaining from P30 somatosensory neocortex of *Grin1*$^{wt/wt}$ and *Grin1*$^{fl/fl}$. **B,** Boxplots indicating the cell counts of hippocampal MGE-derived interneurons expressing **(B_i)** Ai14/tdTomato, and **(B_{ii})** PV, SST immunostaining from P30 *Grin1*$^{wt/wt}$ and *Grin1*$^{fl/fl}$.

FIGURE2, SUPP2 | scRNAseq differential recoveries of MGE-derived interneuron subtypes

A_i, Number of cells recovered across cardinal subtypes SST, PV and NGFC. **A_{ii},** Number of cells recovered within the subtypes of PV / SST/ NGFC. **B,** UMAP representation colored by cardinal MGE-derived interneuron subtypes SST, PVALB and NGFC, highlighting the differential enrichments of cells **C,** Representative UMAP plots indicating the granularity among PV/SST/NGFC subtypes between both brain regions and both genotypes.

FIGURE2, SUPP3 | Select marker gene expression across the subtypes of merged MGE-derived interneurons from *Grin1*$^{wt/wt}$ and *Grin1*$^{fl/fl}$

A, Violin plot showing the distribution of expression levels of well-known representative cell-type-enriched marker genes across the MGE subtypes. **B$_i$,** −log10 False Discovery Rate (FDR) versus log2 fold change (FC) between *Pthlh*-PVALB.2 and *Pthlh*-PVALB.3 at a fold change ≥0.5 and FDR <10e-3. **B$_{ii}$,** Dot plots representing the normalized expressions of NGFC marker genes mis expressed in *Pthlh*-PVALB.3 upon *Grin1*-ablation.

FIGURE2, SUPP4 | Expression of control genes in the MGE-derived interneuron subtypes subsequent to *Grin1*-ablation

A, Split-violin plot from both genotypes indicating the expression of *Grin1* in the MGE-derived interneurons, pyramidal neurons and non-neurons. **B,** Representative UMAP plots of cardinal MGE markers genes.

FIGURE4, SUPP1 | Validation of Nkx2.1-expressing interneuron in P25-30 mouse cortex and hippocampus; anti-Nkx2-1 and anti-PV/SST co-immunostaining

A$_{i}$, Representative saggital section from P25-30 MGE-*Grin1*$^{wt/wt}$-mice immunostained with anti-Nkx2-1 (cyan), and counter-stained with DAPI (blue). While arrow heads in the cortex indicate Nkx2-1 (+) cells in deep and in mid-cortical layers. **A$_{ii}$**, Boxplots indicate the normalized density of cortical vs hippocampal Ai14(+): Nkx2-1(+) double-positive cell counts normalized to total Ai14(+) counts. Representative immunostaining of P25-30 MGE-*Grin1*$^{wt/wt}$ brains from **B**, somatosensory cortex or **C$_{i}$**,hippocampus using anti-Nkx2-1 (cyan), DAPI (blue), PV/SST (green) and endogenous Ai14 reporter (red) expression. **C$_{ii}$**, Indicates rare representative example of rare PV(+): Nkx2-1(+) double-positive cells from MGE-*Grin1*$^{fl/fl}$ hippocampus. (0 cells from WT, 4 cells from null tissue; n = 3 brains (4 sections) from each genotype for immunostaining); Error bars reflect s.e.m from individual sections; two-tailed unpaired t-test, for statistical analysis.

FIGURE5, SUPP1 | Differential gene expression in the MGE-derived interneuron subtypes subsequent to *Grin1*-ablation

A_i, Bar plot denoting the number of genes up/downregulated in the cortical and hippocampal MGE clusters. Volcano plot representing the −log10 False Discovery Rate (FDR) versus log2 fold change (FC) between **B,** hippocampal and **C,** cortical MGE cardinal clusters upon *Grin1*-loss, at a fold change ≥0.2 and FDR <10e-6.

FIGURE5, SUPP2 | Molecular pathways differentially expressed in MGE-derived interneuron subtypes subsequent to *Grin1*-ablation

A, Hierarchical clustering tree summarizing the correlation among significant pathways enriched among the DEGs. Pathways with many shared genes are clustered together. Bigger dots indicate more significant P-values. **B,** Bar plot showing the classification of molecular functions of the DEGs, across the MGE subtypes. Total number of DEGs in the particular molecular class indicated in parentheses.

FIGURE6, SUPP1 | Differential expression of intracellular signaling cascades across subtypes upon *Grin1*-ablation

Heatmap of log2 FC of significant DEGs in cortical and hippocampal MGE cardinal subtypes, showing a subset of **A,** genes regulating intracellular Ca^{2+} homeostasis and Ca^{2+} binding proteins; **B,** notable second messengers; **C,** Ca^{2+} dependent / activated kinases and phosphatases.and, **D,** cellular energetics and mitochondrial function. **E,** Breeding strategy to obtain MGE-derived interneuron-specific expression of Ribotag (*Rpl22-*^{HA}) transgene in the background of *Grin1*$^{fl/fl}$ alleles.

FIGURE7, SUPP1 | Differential expression of fundamental interneuron marker genes across subtypes upon *Grin1*-ablation

Differential expression of cardinal interneuron marker genes that are broadly expressed across interneurons by **A_i,** scRNAseq approach, and cross-validated using **A_{ii},** the Ribotag-seq approach.

FIGURE8, SUPP1 | Aberrant NMDAR-signaling result in misexpression of regulators of membrane excitability that are high-risk Sz genes

A, Schema representing the field of hippocampal pyramidal cell innervated by the interneurons. Heatmap of log2 FC of significant DEGs in cortical and hippocampal MGE cardinal subtypes, showing a subset of **B_i,** Neurotransmitter release machinery. **B_{ii},** Postsynaptic GABA / Glutamate receptor complexes, Kcn-, Scn- ion channel complexes and other miscellaneous regulators of excitability.

List of Supplementary Tables

Table1A | List of differentially expressed genes in MGE-interneuron subtypes after *Grin1*-ablation.

Table1B | scRNAseq cell number recoveries across different clusters from both *Grin1*$^{wt/wt}$ *and Grin1*$^{fl/fl}$.

Table1C | scRNAseq cluster marker genes from both *Grin1*$^{wt/wt}$ *and Grin1*$^{fl/fl}$.

Table2 | Intracellular signaling pathways differentially expressed after *Grin1*-ablation.

Table3 | Differentially enriched disease ontology mapping after *Grin1*-ablation.

Table4A | Differentially expressed genes mapped to high-ranked SZDB and SFARI databases.

Table4B | All schizophrenia risk genes catalogued in SZDB database.

Table4C | All autism risk genes catalogued in SFARI database.

Table4D| Protein-protein interaction statistics for disease-annotated genes