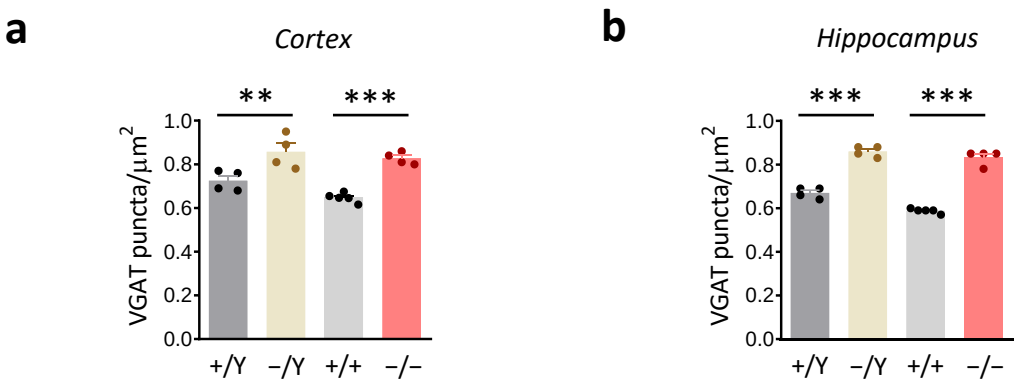
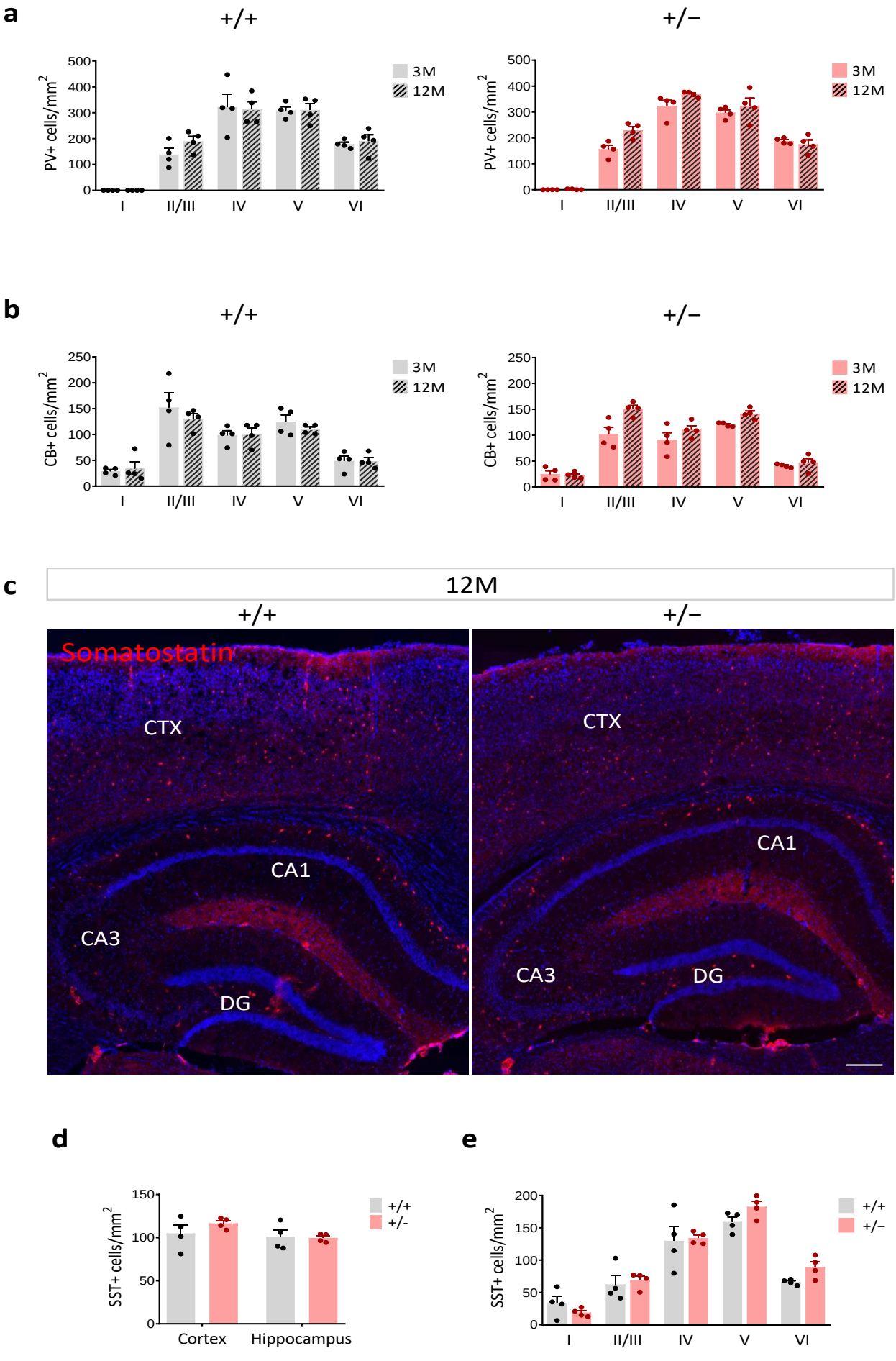
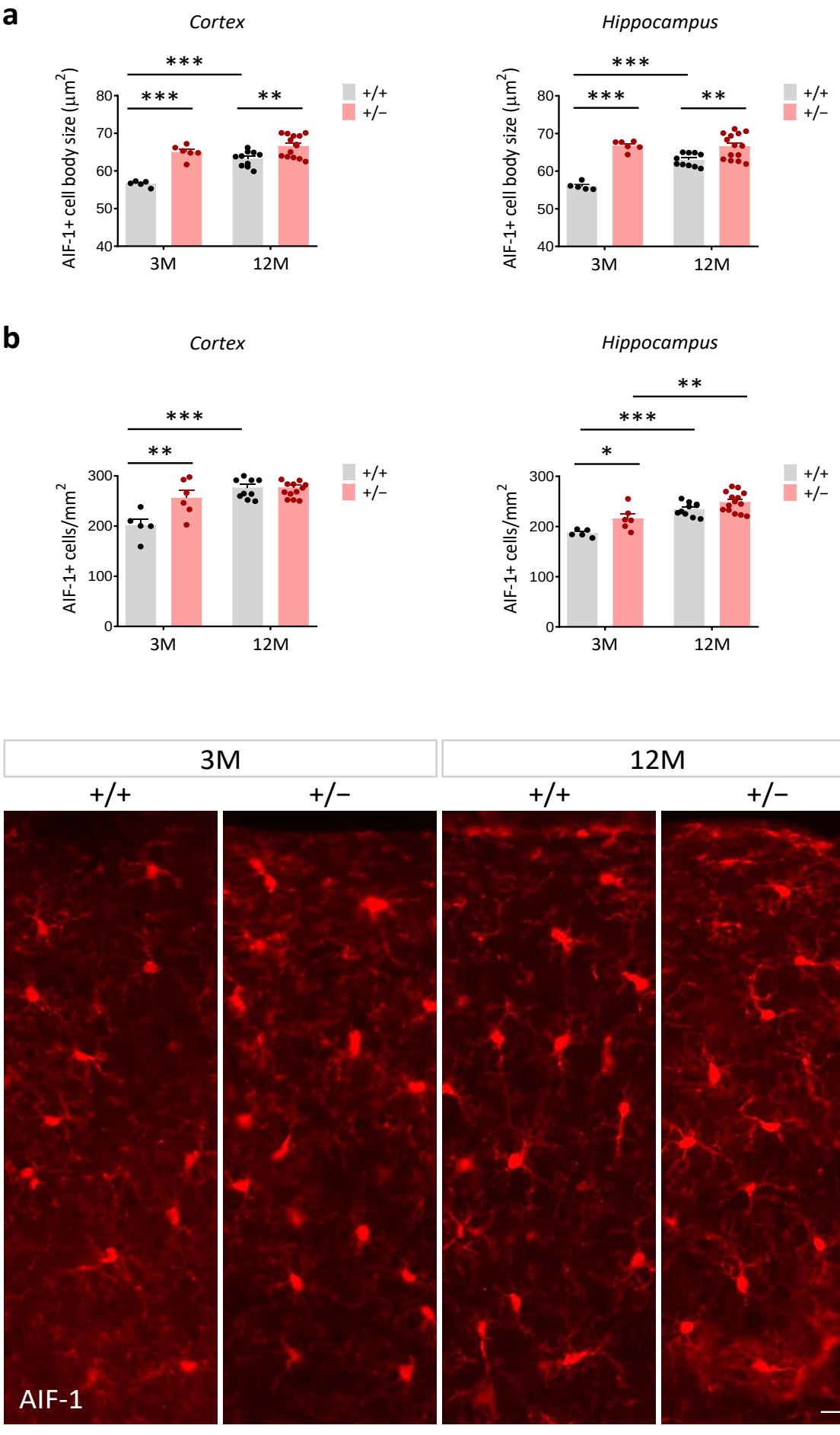


Supplementary Figure 1. Density of VGAT-immunoreactive synaptic puncta in the somatosensory cortex and hippocampus of middle-aged *Cdkl5* knockout mice.

(**a**,**b**) Density of vesicular GABA transporter (VGAT)-immunoreactive puncta in layers II-III of the somatosensory cortex (**a**) and in the stratum oriens of the CA1 hippocampal field (**b**) of 12-month-old *Cdkl5* wild-type (+/Y, *n* = 4) and hemizygous (−/Y, *n* = 4) male mice, and *Cdkl5* wild-type (+/+, *n* = 5) and homozygous (−/−, *n* = 4) female mice. Data are presented as mean ± SEM. Statistical significance was assessed by two-way ANOVA followed by Sidak's post hoc test. ** $p < 0.01$, *** $p < 0.001$.

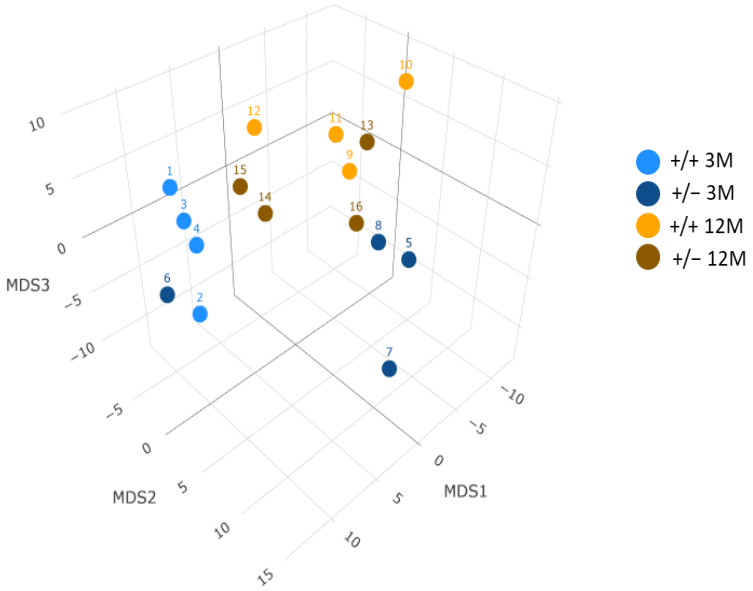

a
+/+
+/−
PV+ cells/mm2
500
400
300
200
100
0
I
II/III
IV
V
VI
3M
12M
b
CB+ cells/mm2
250
200
150
100
50
c
12M
Somatostatin
CTX
CA1
CA3
DG
d
SST+ cells/mm2
150
Cortex
Hippocampus
+/+
+/-
e
+/−

Supplementary Figure 2. Layer-specific distribution of inhibitory interneurons in the somatosensory cortex of young and middle-aged heterozygous *Cdkl5* +/– female mice. (**a**,**b**) Density of parvalbumin (PV)-positive interneurons (**a**) and calbindin (CB)-positive interneurons (**b**) across somatosensory cortical layers in 3-month-old (3M) and 12-month-old (12M) wild-type (+/+, left histograms) and *Cdkl5* heterozygous (+/−, right histograms) female mice (3M: *Cdkl5* +/+ *n* = 4; *Cdkl5* +/− *n* = 4; 12M: *Cdkl5* +/+ *n* = 4; *Cdkl5* +/− *n* = 4). (**c**) Representative fluorescence images of brain sections immunostained for somatostatin (SST) from one 12-month-old (12M) wild-type (+/+) and *Cdkl5* heterozygous (+/−) female mouse. Scale bar = 200 µm. (**d**) Density of SST-positive interneurons in the somatosensory cortex and hippocampus of 12-month-old wild-type (+/+, *n* = 4) and *Cdkl5* heterozygous (+/−, *n* = 4) female mice. (**e**) Density of SST-positive interneurons across somatosensory cortical layers in 12-month-old wild-type (+/+, *n* = 4) and *Cdkl5* heterozygous (+/−, *n* = 4) female mice. Data in (**a**), (**b**), (**d**), and (**e**) are presented as mean ± SEM. Statistical significance was assessed by two-way repeated measures ANOVA followed by Sidak's post hoc test. Abbreviations: CTX, cortex; CA1, hippocampal CA1 field; CA3, hippocampal CA3 field; DG, dentate gyrus; I-VI, cortical layers.


a
Cortex
Hippocampus
AIF-1+ cell body size (µm²)
***
**
+/+
+/−
3M
12M
b
AIF-1+ cells/mm²
*
c
3M
12M
+/+
+/−
AIF-1

Supplementary Figure 3. Microglial cell body size and density in the somatosensory cortex and hippocampus of young and middle-aged heterozygous *Cdkl5* +/– female mice. (**a**,**b**) Mean microglial cell body size (**a**) and cell density (**b**) in the somatosensory cortex (left histograms) and hippocampus (right histograms) of 3-month-old (3M) and 12-month-old (12M) wild-type (+/+) and heterozygous *Cdkl5* (+/−) female mice (3M: *Cdkl5* +/+ $n = 5$; *Cdkl5* +/− $n = 6$; 12M: *Cdkl5* +/+ $n = 10$; *Cdkl5* +/− $n = 14$). (**c**) Representative fluorescence images of brain sections immunostained for AIF-1 in the somatosensory cortex from one mouse per experimental group. Scale bar = 20 µm. Data in (**a**) and (**b**) are presented as mean ± SEM. Statistical significance was assessed by two-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

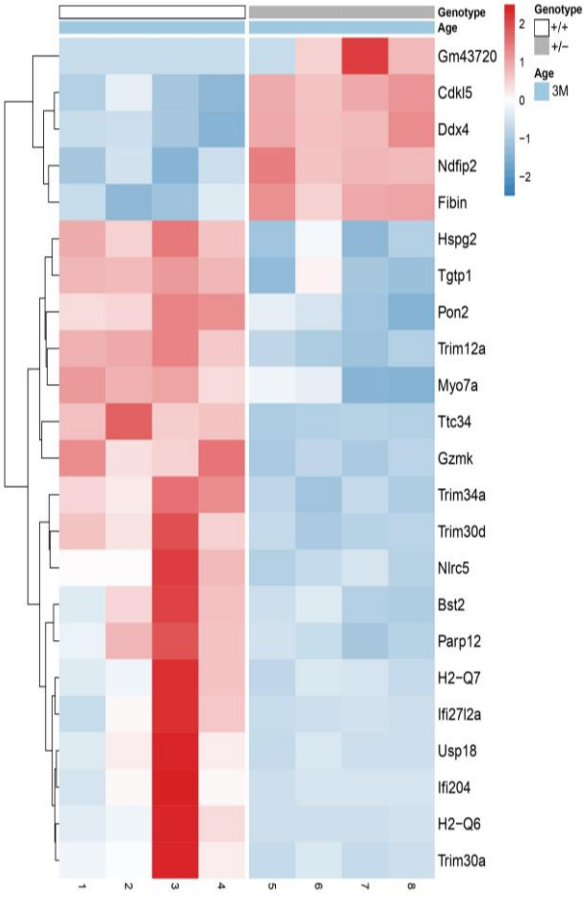
a



b

3M

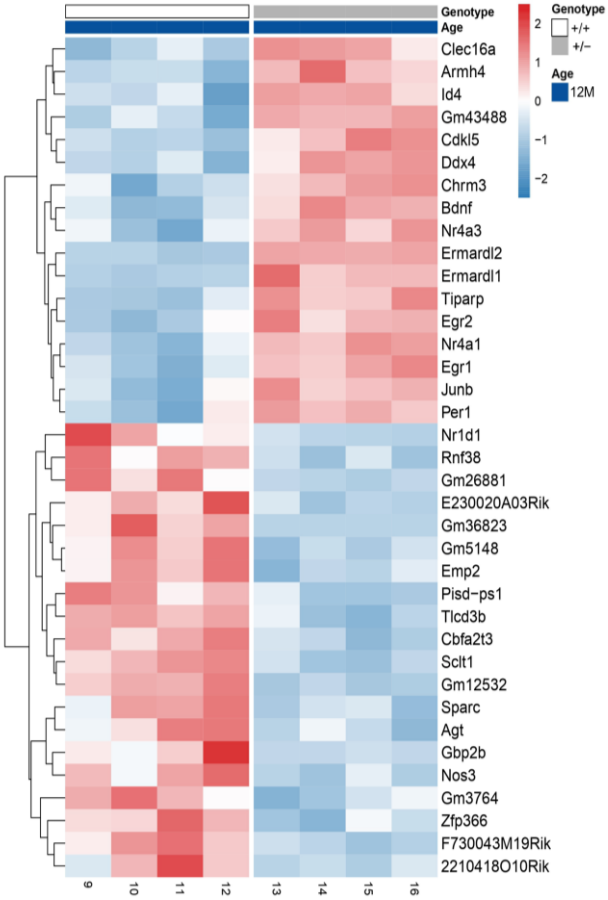
(*Cdkl5* +/− vs. *Cdkl5* +/+)



c

12M

(*Cdkl5* +/− vs. *Cdkl5* +/+)



Supplementary Figure 4. Quality control and transcriptome analysis of hippocampal gene expression in young and middle-aged heterozygous *Cdkl5* +/– female mice. (**a**) Multidimensional scaling (MDS) plot showing the overall transcriptomic similarity among hippocampal samples of 3-month-old (3M) and 12-month-old (12M) wild-type (+/+) and heterozygous *Cdkl5* (+/−) female mice. Each point represents one animal: *Cdkl5* +/+ 3M (light blue), *Cdkl5* +/− 3M (dark blue), *Cdkl5* +/+ 12M (yellow), *Cdkl5* +/− 12M (brown). Distances between points reflect the leading $\log_2$ fold change between pairs of samples across the most variable genes. (**b**,**c**) Heatmaps showing normalized expression levels of genes significantly differentially expressed between *Cdkl5* +/− and wild-type mice at 3 months (3M, **b**) and 12 months (12M, **c**) of age (p-adj < 0.05, DESeq2). Each column represents an individual animal; each row represents a gene. Red indicates higher expression; blue indicates lower expression. Samples are grouped by genotype.