Supplemental Figure 1. Npas4-RNAi has no adverse effect on the overall health of neurons. (a) Npas4-RNAi does not induce apoptosis. Cultured cerebellum granule neurons were co-transfected with GFP and Npas4-RNAi or control-RNAi at 5 DIV. Apoptosis was induced at 6 DIV by replacing serum-containing media with serum-free media containing 5 mM KCl (low KCl) or 60 mM KCl (high KCl) for 24 hours. Cells were fixed and stained with Hoechst to visualize cell nuclei. Apoptotic cells were identified based on their fragmented or condensed nuclei and the percentage of transfected cells undergoing apoptosis was determined. Data are displayed as mean ± SEM from 3 independent experiments. (b) Npas4-RNAi does not inhibit dendritic growth by Sholl analysis. Hippocampal neurons were co-transfected with GFP and vector control, Npas4-RNAi or control-RNAi constructs at 6 DIV, fixed at 25–26 DIV, and confocal z-stacks acquired. The number of dendrites crossing concentric circles of increasing radii centered on the cell body was counted. Two independent experiments are shown. n = 20, 21, and 21 for vector control, Npas4-RNAi and control-RNAi, respectively, in the left panel. n = 49, 39, and 51 for vector control, Npas4-RNAi and control-RNAi, respectively, in the right panel. Data are displayed as mean ± SEM. * p < 0.05 by t-test.
Supplemental Figure 2. Construction and validation of the Npas4<sup>−/−</sup> mouse. (a) Schemes used to generate the Npas4<sup>−/−</sup> mouse. (b) Southern blot showing the successful removal of the Npas4 allele in mouse ES cells. Genomic DNA was digested with AluI and hybridized with the 5′ probe shown in (a). (c) Semi-quantitative PCR shows that Npas4 mRNA is absent in Npas4<sup>−/−</sup> mice. Dissociated cortical neurons from Npas4<sup>+/+</sup> and Npas4<sup>−/−</sup> littermates were cultured and stimulated with KCl (50 mM, 7 DIV). RNA samples were collected at the indicated times. (d) Western blots showing that Npas4 protein is absent in Npas4<sup>−/−</sup> mice. Experiments were conducted as described in (c) except that samples were collected 2 hours after stimulation.
Supplemental Figure 3. Npas4 knockout mice have similar mIPSCs to wildtype littermates. (a) Cumulative distributions of mIPSC interevent intervals (left) and amplitudes (right) recorded from acute hippocampal slices prepared from wildtype (thick line) and Npas4<sup>-/-</sup> (thin line) mice (P15-18). (b) mean ± SEM from (a). mIPSC interevent intervals are 339.5 ±16.0 and 311.6 ± 11.0 ms and amplitudes are 29.2 ± 0.7 and 30.6 ± 0.7 pA for wildtype and Npas4<sup>-/-</sup> mice, respectively.
Supplemental Figure 4. Construction and validation of the Npas4^flx/flx mouse. (a) Schemes used to generate the Npas4^flx/flx mouse. (b) Representative images showing that Npas4 expression is abolished in neurons transfected with a Cre recombinase construct (bottom) in organotypic hippocampal slices prepared from Npas4^flx/flx mice.
Supplemental Figure 5. Expression of Cre recombinase has no effect on GABAergic synapses in wildtype hippocampal slices. (a) Cumulative distributions of mIPSC interevent intervals (left) and amplitudes (right) recorded from wildtype mouse neurons transfected with control construct (thin line) or Cre recombinase (thick line). (b) Summary data from (a) are shown as mean ± SEM. mIPSC interevent intervals are 750.1 ± 35.5 and 732.9 ± 29.1 ms and amplitudes are 26.6 ± 0.6 and 25.0 ± 0.6 pA for control and Cre, respectively.
**Supplemental Figure 6. Validation of the Npas-minigene construct.** (a) Npas4-minigene is induced by neuronal activity. Npas4−/− mouse neurons were transfected with Npas4-minigene (6 DIV) and stimulated with NMDA (20 mM, 14 DIV) for 2 hours. (b) Npas4-minigene drives expression of an Npas4-responsive luciferase reporter. Rat hippocampal neurons were transfected (5 DIV) with the control plasmid, Npas4-RNAi, or Npas4-RNAi and an RNAi-resistant Npas4-minigene together with either Npas4-Luc (left) or MEF2-Luc (right). Cultures were subsequently stimulated with KCl (55 mM, 7 DIV) for 7 hours and luciferase activity measured. Data were compiled from 3 independent experiments, each conducted in triplicate.
Supplemental Figure 7. Lentivirus expressing Npas4-RNAi knocks down the expression of Npas4. Mouse hippocampal neurons were infected with lentivirus (3 DIV) and stimulated with KCl (8 DIV) for 2 hours. GFP is used to demonstrate comparable levels of infection between neurons infected with Npas4-RNAi and control virus.
### Supplemental List

List of candidate genes whose expression levels are affected by Npas4-RNAi (U = Up-regulated; D = Down-regulated; N = No change)

<table>
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<tr>
<th>Gene Symbol</th>
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### Category 2: Nucleotide Binding

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### Category 3: Ion Channels

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### Category 4: Membrane Proteins/Synaptic Proteins

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Category 5: Kinases/Phosphatases

- Pkp1
- Paraneoplastic antigen MA2
- Cathepsin D

Category 6: Protein Signaling

- Pp1
- Pp2

Category 7: Calcium Signaling

- Ppp1
- Ppp2

Category 8: Ubiquitination

- Ppi1
- Ppi2

Category 9: Cytoskeleton Trafficking/Intracellular Signaling

- Pkm2
- Pki

Category 10: Catalytic Activity/Metabolic

- Pnp1
- Pnp2

Category 11: Other

- Pk
- Ppi
Uncharacterized

Nanog
N-acetylneuraminic acid synthase (sialic acid synthase)
Aryl hydrocarbon receptor
Aryl hydrocarbon receptor interacting protein
Arsenyi RNA synthase
Ulk1
Ulk1
Ulk1
Ulk1
Ulk1
Ulk1
Ulk1
Ulk1
Ulk1
Down syndrome critical region gene 3
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N
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**SUPPLEMENTARY INFORMATION**

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