Table of Contents:

- Appendix Figure S1
 Phenotype of Satb2^{flx/flx} and Satb2^{NesCre}cKO mouse brain
- 2. Appendix Figure S2 Coomassie Brilliant Blue Staining of purified GST-hybrid proteins
- Appendix Figure S3
 AP bursting increases nuclear phospho-MSK1 immunoreactivity in both Satb2^{flx/flx}
 and Satb2^{NesCre} hippocampal neurons



Appendix Figure S1.: Phenotype of Satb2^{*fix/fix*} and Satb2^{NesCre}cKO mouse brain

- A Nissl-staining of coronal brain sections from P15 Satb2 ^{fix/fix} (left panel) and Satb2^{NesCre} knockout mice (right panel). Satb2^{NesCre} mice (Cera et al., 2019) lack corpus callosum as has been described for Satb2-germ line knockout mutants (Alcamo et al., 2008; Britanova et al., 2008). Scale bar: 200 μm.
- B Immunoblotting analysis for Satb2 in DIV7 primary hippocampal cultures derived from Satb2^{NesCre} or Satb2^{loxP/loxP} mice demonstrates absence of detectable Satb2 immunoreactivity in cultures from knockout animals compared to cultures from floxed littermate control mice.



Appendix Figure S2.: Coomassie Brilliant Blue Staining of purified GST-hybrid proteins

Coomassie Brilliant Blue (CCB)-stained gel of protein fractions obtained after affinity purification of GST-hybrid proteins is shown. Asterisks indicate the GST-SATB2 and GST-LEMD2⁽⁴¹³⁻⁵⁰³⁾ hybrid proteins.



Appendix Figure S3.: AP bursting increases nuclear phospho-MSK1 immunoreactivity in both Satb2flx/flx and Satb2-defficient hippocampal neurons

Immunocytochemical analysis of the phosphorylation of MSK1 at threonine 581 following Bicinduced AP bursting for 1 h in primary hippocampal cultures derived from neonatal Satb2^{flx/flx} mice and Satb2^{flx/flx}::Nes-Cre mice. The mean nuclear phospho-MSK1 immunoreactivity was measured as absolute 8 bit gray levels. DAPI staining was used to identify nuclei. Representative images **(A)** and the quantitative analysis **(B)** are shown. AP bursting caused a significant increase in the nuclear phospho MSK1 immunoreactivity in both Satb2^{flx/flx} and Satb2^{flx/flx}::Nes-Cre cultures, *n* = 3 independent primary cultures, two-way ANOVA; main effect of treatment, $F_{1,8} = 46.48$, p = 0.0001; main effect of genotype, $F_{1,8} = 0.003$, p = 0.95, not significant interaction, $F_{1,8} = 0.15$, p = 0.7081; untreated vs. Bic-treated Satb2^{flx/flx} cultures p = 0.0081; untreated vs. Bic-treated Satb2^{flx/flx} cultures, untreated); 461 (Satb2^{flx/flx} cultures, Bic-treated); 520 (Satb2^{flx/flx}::Nes-Cre cultures, untreated), 492 (Satb2^{flx/flx}::Nes-Cre , Bic-treated). Data are presented as mean ± SEM, **p < 0.01, Scale bar, 10 µm.