

Expanded View Figures

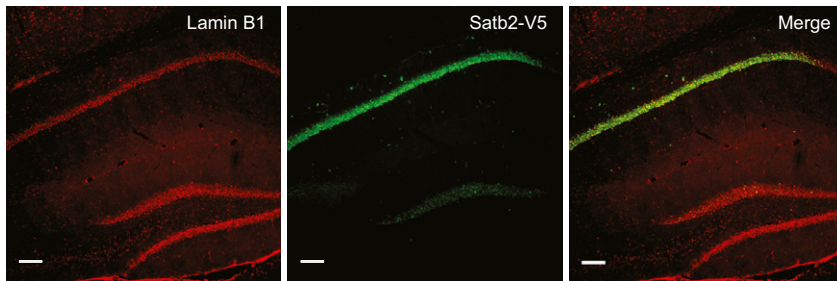


Figure EV1. Re-introduction of V5-tagged SATB2 into the adult dorsal hippocampus of *Satb2* conditional mutants.

rAAV8-hSyn-*Satb2*-V5 virus was injected into the dorsal hippocampus of *Satb2*^{CamKCre} knockout mice. Lamin B2 staining (left panel) was used to mark cell nuclei in hippocampus. V5 immunoreactivity was used to detect expression of SATB2-V5 in hippocampus (middle panel). Overlay of both pictures (right panel) demonstrates re-expression of SATB2 in CA1-neuronal nuclei in SATB2-deficient mice. Representative images are shown. Scale bars: 100 μ m.

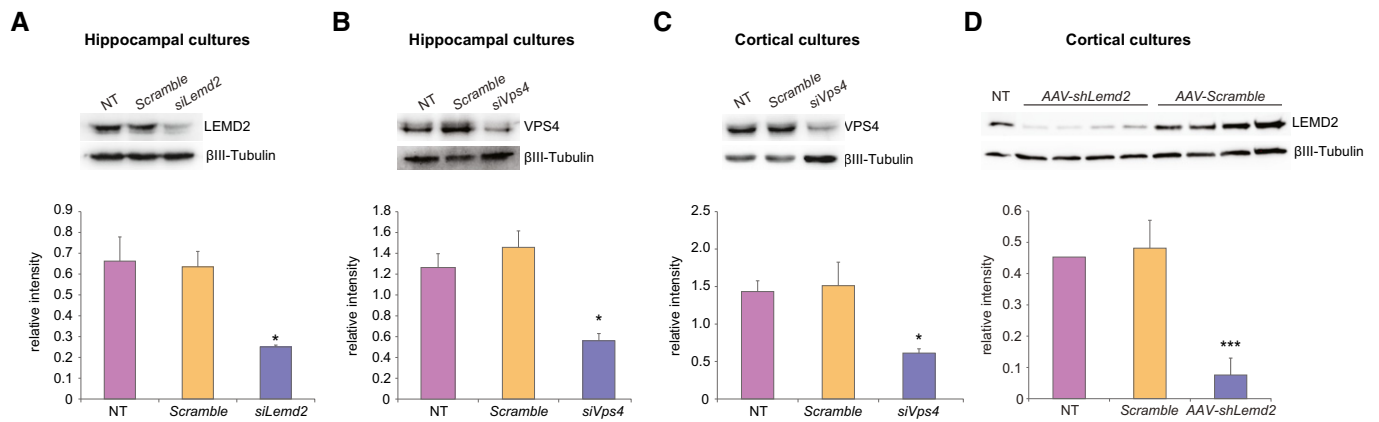


Figure EV2. Gene silencing of *Lemd2*, *Vps4a* and *Vps4b* in primary neuronal cultures.

- A** Gene silencing of *Lemd2* in hippocampal cultures by siRNA. Upper panel: Representative Western blot for LEMD2 protein in primary hippocampal neurons, NT (non-transfected), *Scramble* (transfected with control siRNA), *siLemd2* (transfected with siRNA against *Lemd2*). Lower panel: Western blot quantification of LEMD2 in hippocampal cultures, $n = 4$ independent primary cultures. One-way Welch's ANOVA followed by Tukey *post hoc* test, $F_{2,9} = 16.783$, $P = 0.011$, NT vs *siLemd2* $P = 0.013$, *Scramble* vs *siLemd2* $P = 0.019$. Data are presented as mean \pm SEM, * $P < 0.05$ compared to NT.
- B** Gene silencing of *Vps4* in hippocampal cultures by siRNA. Upper panel: Representative Western blot for VPS4 protein level in primary hippocampal neurons. NT (non-transfected), *Scramble* (transfected with control siRNA), *siVps4* (transfected with siRNAs against *Vps4a* and *Vps4b*). Lower panel: Western blot quantification of VPS4 protein level in primary hippocampal cultures, $n = 3$ independent experiments, one-way Welch's ANOVA followed by Tukey *post hoc* test, $F_{2,6} = 14.226$, $P = 0.027$, NT vs *siVps4* $P = 0.063$, *Scramble* vs *siLemd2* $P = 0.045$. Data are presented as mean \pm SEM, * $P < 0.05$ compared to *scramble*.
- C** Gene silencing of *Vps4* in cortical cultures by siRNA. Upper panel: Representative Western blot for VPS4 protein level in primary cortical neurons. NT (non-transfected), *scramble* (transfected with control siRNA), *Vps4* (transfected with siRNA against *Vps4a* and *Vps4b*). Lower panel: Western blot quantification of VPS4 protein level in primary cortical cultures, $n = 3$ independent experiments, one-way ANOVA followed by Tukey *post hoc* test, $F_{2,8} = 9.423$, $P = 0.014$, NT vs *siVps4* $P = 0.041$, *scramble* vs *siVps4* $P = 0.015$. Data are presented as mean \pm SEM, * $P < 0.05$ compared to NT.
- D** Gene silencing of *Lemd2* in cortical cultures by shRNA. Upper panel: Western blot for LEMD2 in primary cortical neurons, NT (non-transfected), *Scramble* (transfected with control shRNA), AAV-*shLemd2* (transfected with shRNA against *Lemd2*). Lower panel: Western blot quantification of LEMD2 protein level, $n = 4$ independent experiments, unpaired *t*-test, $t(6) = 5.98$, $P = 0.000979$. Data are presented as mean \pm SEM, *** $P < 0.001$ compared to *Scramble*.

Source data are available online for this figure.

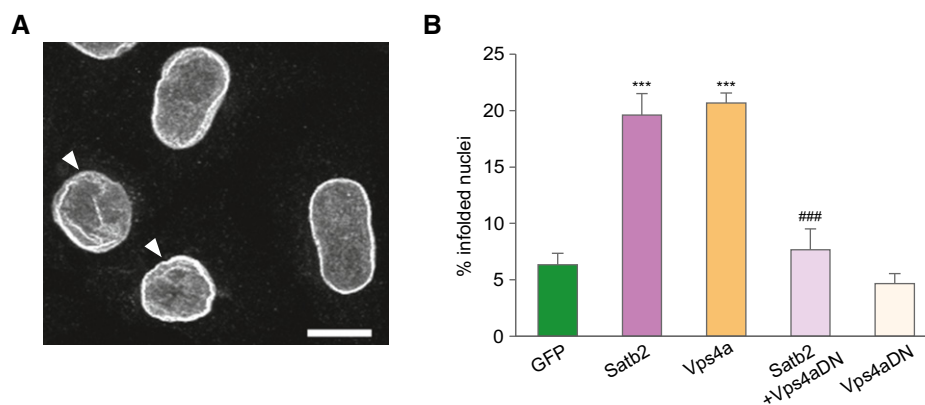


Figure EV3. 5 ESCRT-III/VPS4 complex is required for SATB2-triggered nuclear infolding formation in HeLa cells.

A Confocal image (z-axis projection of confocal image stack) of HeLa cell nuclei immunostained for Lamin B2. Arrowheads indicate infolded nuclei. Scale bar: 10 μ m.

B Percentage of infolded nuclei in HeLa cells after ectopic expression of *Satb2*, *Vps4a*-GFP, a dominant-negative *Vps4a* mutant fused to GFP (*Vps4aDN*-GFP), and a combination of *Satb2* and *Vps4aDN*-GFP. The number of infolded nuclei is significantly increased in both *Satb2*- and *Vps4a*-transfected cells compared with GFP-transfected cells. Expression of the dominant-negative *Vps4a* mutant abolished the increase in the number of infolded nuclei induced by SATB2, $n = 3$ –5 independent experiments, ANOVA followed by Tukey *post hoc* test, $F_{4,14} = 25.4$, GFP vs *Satb2*, $P < 0.0001$, GFP vs *Vps4a*, $P < 0.0001$, *Satb2* vs *Satb2* + *Vps4aDN*, $P = 0.0006$. Number of analyzed nuclei: 521 (GFP), 586 (*Satb2*), 302 (*Vps4a*), 345 (*Satb2*/*Vps4aDN*), 317 (*Vps4aDN*). Data are presented as mean \pm SEM, *** $P < 0.0001$ compared to GFP; ### $P < 0.001$ compared to *Satb2*.

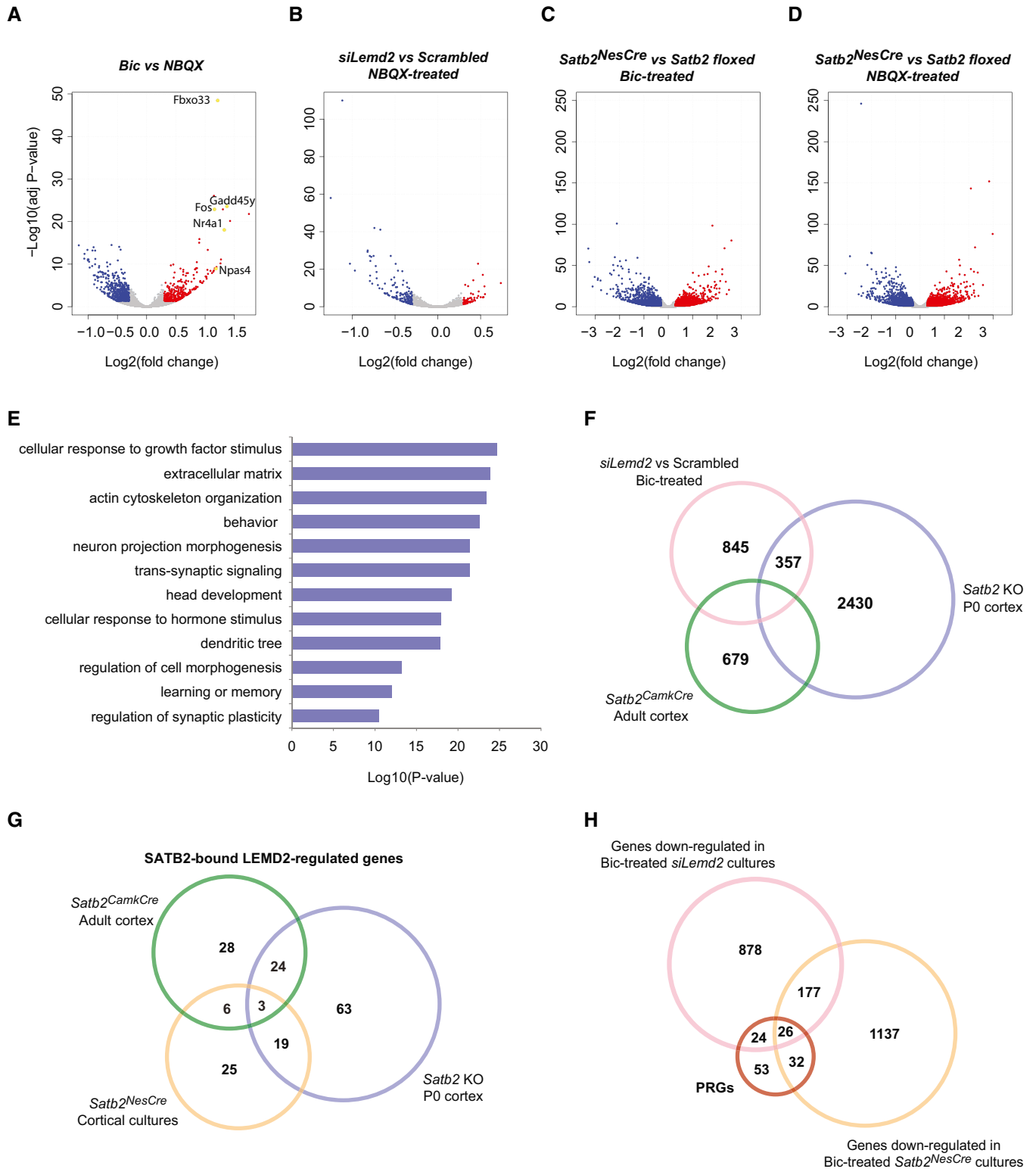


Figure EV4.

Figure EV4. LEMD2 and SATB2 coregulate neuronal gene transcription.

- A “Volcano plot” of statistical significance against fold change (FC) between silenced (NBQX-treated) and active (Bic-treated) primary cortical cultures from *Satb2* floxed mice. The differentially expressed genes (adjusted P -value < 0.05 , 0.3 FC cut-off) are indicated in red (up-regulated) and in blue (down-regulated), $n = 5$ – 7 independent primary cultures. Examples of some IEGs strongly up-regulated upon Bic treatment are marked and highlighted in yellow.
- B–D “Volcano plots” illustrating the differential gene expression between NBQX-treated *siLemd2*- vs scrambled siRNA-transfected cortical cultures (B), Bic-treated *Satb2^{NesCre}* vs *Satb2* floxed cultures (C), and NBQX-treated *Satb2^{NesCre}* vs *Satb2* floxed cultures (D). The differentially expressed genes (adjusted P -value < 0.05 , 0.3 FC cut-off) are indicated in red (up-regulated) and in blue (down-regulated), $n = 3$ – 7 independent primary cultures.
- E GO enrichment analysis of differentially expressed genes between *siLemd2*- and scrambled siRNA-transfected Bic-treated cultures.
- F Venn diagram illustrating the overlap between the differentially expressed genes (adjusted P -value < 0.05 , 0.3 FC cut-off) in *siLemd2*- vs scrambled siRNA-transfected cultures, SATB2-deficient vs wild-type P0 cortices (Fischer’s exact test, P -value $< 1E-15$, OR = 2.93), and *Satb2^{CamkCre}* vs *Satb2* floxed adult cortices (Fischer’s exact test, P -value $< 1E-15$, OR = 3.54).
- G Venn diagram of SATB2-bound LEMD2-regulated genes specific to or shared by the differentially expressed genes in *Satb2^{NesCre}* cortical neurons (Fischer’s exact test, P -value $< 1E-15$, OR = 3.059), P0 cortex (Fischer’s exact test, P -value $< 1E-15$, OR = 2.627), and adult cortex (Fischer’s exact test, P -value $< 1E-15$, OR = 2.962).
- H Venn diagram showing the overlap between rapid and delayed PRG (Tyssowski *et al*, 2018) and the genes down-regulated in Bic-stimulated *Satb2^{NesCre}* CKO (Fischer’s exact test, P -value $< 1E-15$, OR = 9.99) and *siLemd2*-silenced cultures (Fischer’s exact test, P -value $< 1E-15$, OR = 10.17).