Supplementary Data-Lee et al.

Supplementary Materials and Methods

ES Cell Culture & ShRNA Experiment

CBP $^{+/+}$ and CBP$^{-/-}$ embryonic stem (ES) cells were cultured on γ-irradiated (30 Gy) mouse embryo fibroblast feeder layers in ES growth medium (R1 media) containing DMEM (Gibco, Rockville, MD) supplemented with nonessential amino acids (Gibco), 2 mM L-glutamine (Gibco), 0.1 mM β-mercaptoethanol (Gibco), leukemia inhibitory factor (LIF; 1,000 units/ml; Chemicon, Temecula, CA), 100 units/ml penicillin and 100 μg/ml streptomycin (Gibco), and 20% fetal bovine serum (Atlanta Biological, Atlanta, GA) at 37°C in 5% CO_2 to proliferate and maintain their undifferentiated status. Subconfluent ES cell cultures from 100-mm dishes were treated with trypsin and transferred to nonadherent Petri dishes and cultured in ES differentiation medium (EB media) containing DMEM supplemented with nonessential amino acids, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum to promote embryoid body (EB) formation. After 3 additional days of EB culture, 1 μM of all-*trans* retinoic acid (RA; Sigma, St. Louis, MO) was added to the medium and EBs were cultured for 3 additional days. The day when RA was added to the culture was designated as day 0. EBs (2 x 10^5 cells/ml) were then transferred to poly-D-lysine (Sigma)-coated 8-chamber slides in ES differentiation Neurobasal medium (Gibco) containing 1 μM of all-*trans* retinoic acid (RA; Sigma, St. Louis, MO), 5 ng/ml of basic fibroblast growth factor (bFGF), N-2 supplement (Gibco) and 0.5 mM glutamine. Half of the medium in differentiating EB culture was replaced with fresh medium every 2 days until the shRNA experiment begin (Supplementary Reference #1, 2).

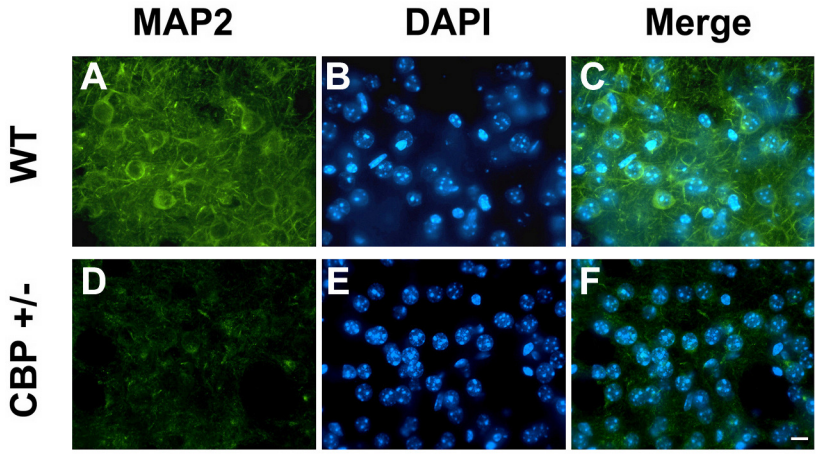
SureSilencing shRNA plasmids for ESET/SETDB1 with GFP marker (KH17319G) as well as shRNA control plasmids with GFP marker were purchased from SuperArray Bioscience Corp. (Frederick, MD). One microgram (per milliliter) of plasmids were transiently transfected using DMRIE-C transfection reagent (Invitrogen Life Tech., Carlsbad, CA) in neuronally differentiated CBP null ($^{-/-}$) ES cells for 24hr. Cells were fixed with 2% PFA and stained with ESET and TMH-H3 (K9) antibodies. GFP positive cells indicated the expression of shRNA plasmids. Transfection efficiency of shRNA-GFP was ~20%. The levels of ESET knock down and TMH-H3 (K9) by ESET shRNA and control shRNA were determined by Spinning Disk Confocal Microscopy. The quantification of immunoreactivity was analyzed using IP Lab software (BD Biosciences, Rockville, MD) and AQI-X-COMBO-CWF (Media cybernetics Inc. Bethesda, MD).

Supplementary Reference:

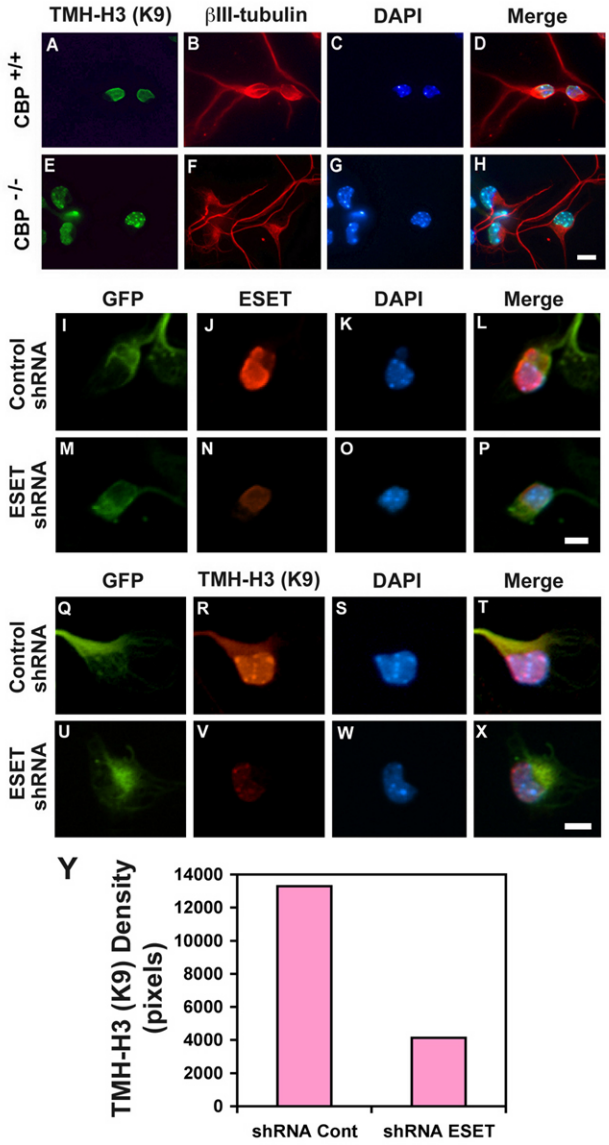
1. Bain G, Kitchens D, Yao M, Huettner JE, Gottlieb DI. (1995) Embryonic stem cells express neuronal properties in vitro. *Dev Biol.* **168**, 342-357.

2. Mujtaba T, Piper DR, Kalyani A, Groves AK, Lucero MT, Rao MS. (1999) Lineage-restricted neural precursors can be isolated from both the mouse neural tube and cultured ES cells. *Dev Biol.* ***214***, 113-127.

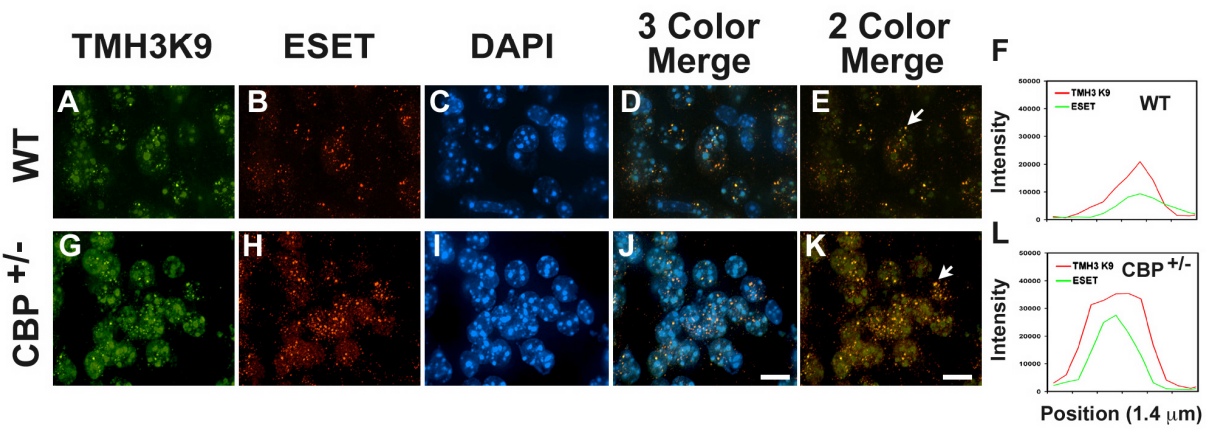
Supplemental Figures and Legends



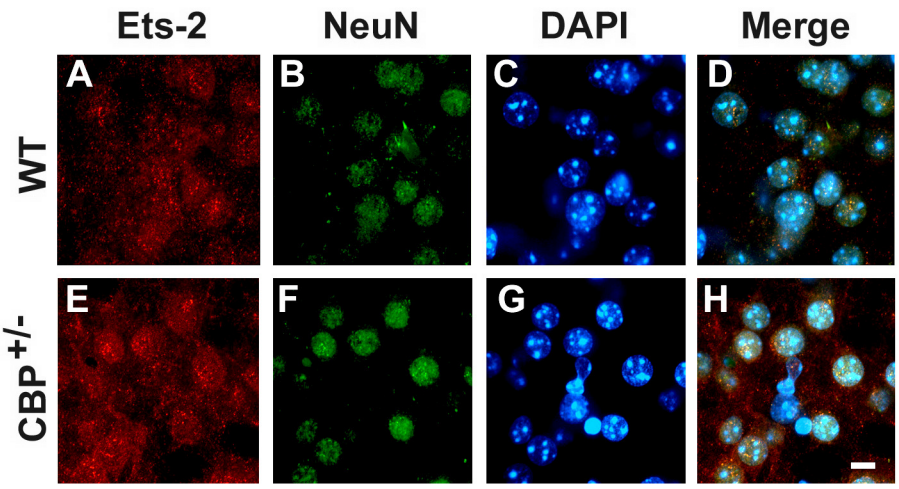
Supplementary Figure 1. CBP deficiency ($CBP^{+/-}$) results in the reduction of MAP2 immunoreactivity in striatal neurons (D-F) compared to wild type (WT) ($CBP^{+/+}$) mice (A-C) at 6 months of age. MAP2 staining shows the marked alteration of dendritic arborization in CBP deficiency mice. MAP2, microtubule associated protein 2. Scale bar, 10 μm.



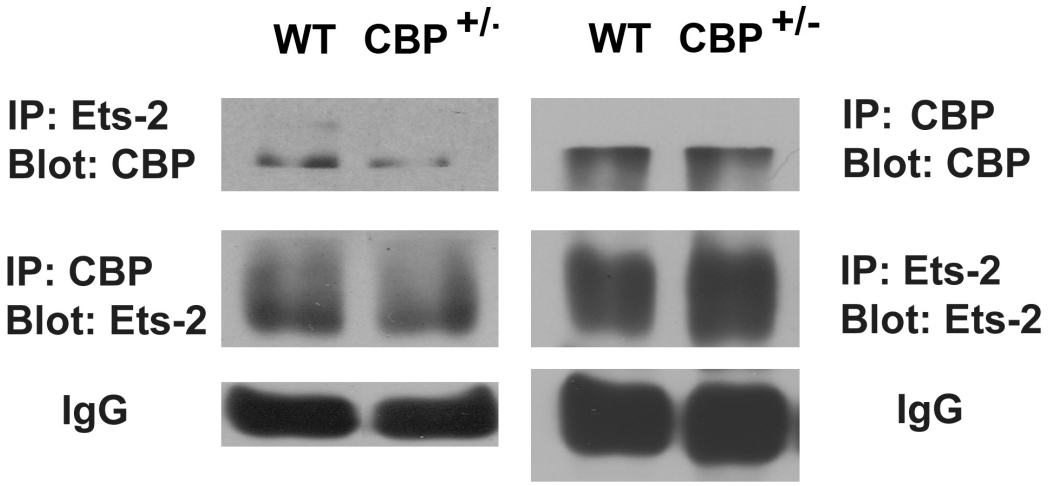
Supplementary Figure 2. Neuronally differentiated CBP null ($^{-/-}$) embryonic stem (ES) cells show the increased level of TMH-H3 (K9) in the nucleus. $CBP^{+/+}$ (A-D) and $CBP^{-/-}$ ES cells (E-H) were differentiated *in vitro*. TMH-H3 (K9) (green, A and E), βIII-tubulin (red, B and F), DAPI (blue, C and G), and overlay images (D and H). Condensed and punctate structures of heterochromatin were colocalized with TMH-H3 (K9) in differentiated CBP null ES cells. **ESET knock down by ESET shRNA reduces the endogenous level of ESET (M-P) and TMH-H3 (K9) (U-X) compared to control shRNA in neuronally differentiated CBP null ($^{-/-}$) ES cells.** SureSilencing shRNA plasmids for ESET/SETDB1 with GFP marker (KH17319G) (SuperArray Bioscience Corp., Frederick, MD) were transiently transfected for 24hr. Cells were fixed with 2% PFA and stained with ESET and TMH-H3 (K9) antibodies. GFP positive cells indicate the expression of shRNA plasmids. Transfection efficiency of shRNA-GFP was ~20%. Scale bars: 10 μm. **Panel Y** represents the quantification of TMH-H3 (K9) level in GFP-positive neurons.



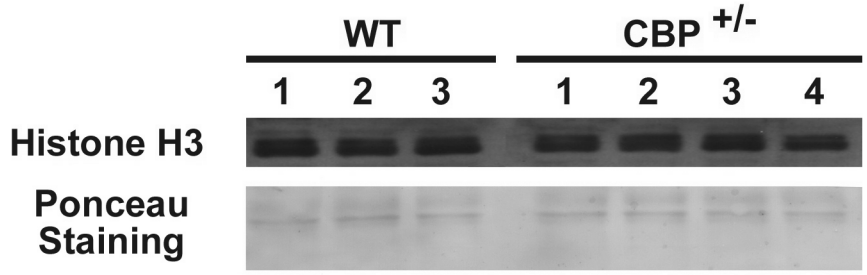
Supplementary Figure 3. Confocal microscopic analysis confirms the colocalization of ESET and TMH-H3(K9) in the striatal neuron of wild type (WT) ($CBP^{+/+}$) and $CBP^{+/-}$ mouse. Colocalization of ESET and TMH-H3 (K9) was elevated in striatal neurons of $CBP^{+/}$ mice. White arrows indicate spots where colocalization intensity of ESET (green) and TMH-H3(K9) (red) were determined by the line measurement (AQI-X-COMBO-CWF, Media cybernetics Inc. Bethesda, MD). Scale bar, 10 μm.



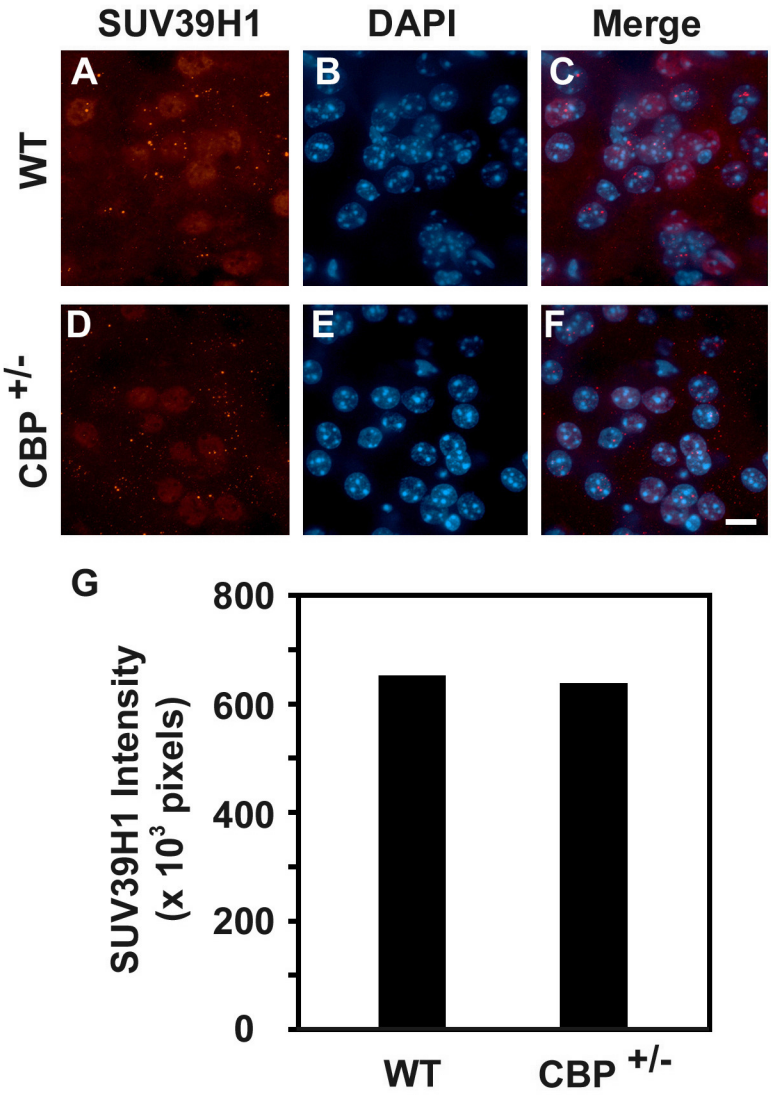
Supplementary Figure 4. Ets-2 immunoreactivity is increased in the nucleus of striatal neurons in $CBP^{+/-}$ mice compared to wild type (WT) ($CBP^{+/+}$) mice. Ets-2 is shown as condensed and punctate structures in the nucleus of $CBP^{+/-}$ mice. NeuN, neuronal nuclear marker. Scale bar, 10 μm.



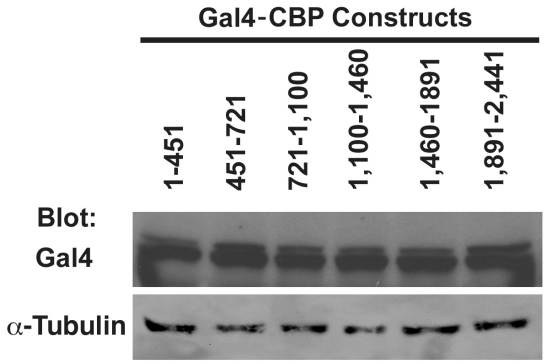
Supplementary Figure 5. Co-immunoprecipitation analysis of CBP and Ets-2 from striatal neuronal lysates of wild type (WT) ($CBP^{+/+}$) and $CBP^{+/-}$ mouse. Striatal tissue lysates (500 µg) were incubated with 5 µg of CBP or Ets-2 antibody for 12 hours at 4ºC. Samples were immunoprecipitaed with protein-Sepharose A and run on the SDS-PAGE. The IgG blot confirms that equal amount of samples were loaded on the gel. The data indicates the physical interaction between CBP and Ets-2 in neurons (left panel). The blots of IP were reprobed with anti-Ets-2 and anti-CBP antibody (right panel), respectively.



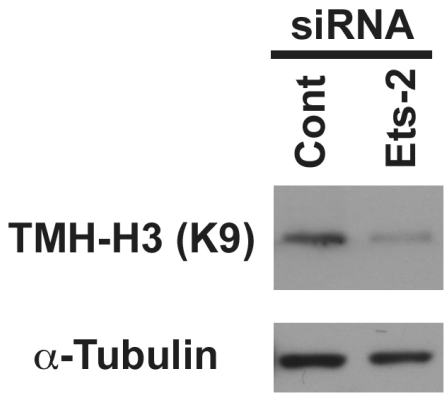
Supplementary Figure 6. Western blot analysis for the total histone H3 in wild type (WT) ($CBP^{+/+}$) and $CBP^{+/-}$ mice. There was no change in the level of total histone H3 protein between wild type ($CBP^{+/+}$) and $CBP^{+/-}$ mice at 6 months of age. Ponceau staining demonstrates equal protein loading for Western blots.



Supplementary Figure 7. CBP deficiency ($CBP^{+/-}$) does not alter the level of SUV39H1 immunoreactivity in striatal neurons (D-F) compared to wild type (WT) ($CBP^{+/+}$) mice (A-C). SUV39H1 was stained in the nucleus of striatal neurons. Scale bar, 10 μm.



Supplementary Figure 8. Western blot analysis for the expression of Gal4-CBP deletion constructs. Gal-4 fused CBP deletion constructs (1-451, 451-721, 721-1100, 1100-1460, 1460-1891, and 1891-2441) were transiently transfected in SH-SY5Y cells for 24hr. Quantified cell lysates were run on the 12 % SDS-PAGE. The blot was developed with Gal4 antibody. α-Tubulin staining demonstrates equal protein loading for Western blots.



Supplementary Figure 9. Ets-2 knock down by Ets-2 siRNA reduces the level of TMH-H3 (K9) compared to control (cont) siRNA in neuronally differentiated CBP null ($^{-/-}$) ES cells. α-Tubulin staining demonstrates equal protein loading for Western blots.