

results are displayed in the same manner as in figure 5. Data as shown were from 2-3 month old wildtype control and DKO mouse forebrains. Data are presented as means±s.e.m. Unpaired student t-test. *indicates P<0.05, **indicates P<0.01.

Supplementary Figure 1 Characterization of cre-recombinase induced gene deletion in DKO mouse brain. **a**, Immunohistochemistry of cre-recombinase staining in cortex (CTX) and hippocampal dentate gyrus (DG) of adult DKO mouse brain showed cre only expressed in NeuN positive mature neurons. Scale bar at bottom right, 100um, applied to all panels. **b**, Southern blot analysis showed functional *Dnmt1* 2lox allele in control (con) mouse brain of 1 mo, 3 mo, and 4 mo. The deletion happened in the presence of cre-recombinase and led to 1lox recombination null allele as identified in the DKO mutant brain.

Supplementary Figure 2 Morphology analysis and subgranule zone (SGZ) cell proliferation assay in DKO mouse brain. **a**, Representative dorsal view of one pair of brains from 3 mo control and DKO. Scale bar at bottom right, 3 mm, applied to both panels. **b**, BrdU labeling cell proliferation assay of dentate gyrus subgranule zone showed similar level of cell proliferation between DKO and control mice. Four pairs of 3 month old mice were used. Data are presented as means±s.e.m. P>0.05

Supplementary Figure 3 Normal basal synaptic transmission in *Dnmt* mutant mice and normal long term plasticity in single *Dnmt* KO mouse brain. **a**, Input-output curves from the DKO, *Dnmt1* SKO as well as wildtype control groups were essentially identical as

shown by plotting the synaptic responses against presynaptic fiber volley amplitudes. **b**, LTP of adult *Dnmt1* SKO was indistinguishable from control mice ($P>0.05$). fEPSP slopes in control (round mark) versus SKO mice (triangle mark) were recorded 30 min before till 180 min after tetanic stimulation (100Hz, 1sec). Scale bar, 5 msec/1mV. **c, d**, No abnormalities of LTD was observed in adult *Dnmt1* SKO (**c**) and *Dnmt3a* SKO (**d**) mice ($P>0.05$). fEPSP slopes were recorded 12 min before till 60 min after stimulation (1Hz, 15min). Scale bar, 10 msec/0.5mV. Representative recordings are shown in the insets (**b, c, d**). 28 slices from 9 Con and 11 slices from 4 *Dnmt1* SKO were used in **b**. 10 slices from 5 Con and 6 slices from 3 *Dnmt1* SKO were recorded in **c**. 15 slices from 8 Con and 7 slices from 3 *Dnmt3a* SKO were recorded in **d**. Slice numbers were used for statistical analysis. Data are presented as means \pm s.e.m. $P>0.05$

Supplementary Figure 4 Normal memory formation of *Dnmt1* and *Dnmt3a* SKO mice in Morris water maze test. **a, d**, Escape latency time to find the hidden platform plotted versus training day. Both *Dnmt1* SKO (**a**) and *Dnmt3a* SKO (**d**) improved similarly as littermate control mice to find the hidden platform (*Dnmt1* SKO: genotype $F(1, 253)=0.00$, $P=0.944$; day $F(11, 253)=17.46$, $P<0.0001$; genotype X day $F(11, 253)=0.27$, $P=0.991$. *Dnmt3a* SKO: genotype $F(1, 154)=0.37$, $P=0.555$; day $F(11, 154)=9.47$, $P<0.0001$; genotype X day $F(11, 154)=0.23$, $P=0.995$). **b, e**, Percentage time spent in target quadrant during three individual probe trials. *Dnmt1* SKO (**b**) and *Dnmt3a* SKO (**e**) performed as well as the control mice in the target quadrant. **c, g**, The swimming speeds between the groups of *Dnmt1* SKO (**b**) or *Dnmt3a* SKO (**e**) with control mice were indistinguishable from each other. 17 control mice and 8 *Dnmt1* SKO were used in

figure a-c, 10 control mice and 6 Dnmt3a SKO were used in figure d-f. Data are presented as means±s.e.m. $P>0.05$

Supplementary Figure 5 Contextual fear conditioning test in Dnmt single knockouts.

a, Contextual memory consolidation was normal in Dnmt1 SKO mice when tested immediately (for 3 min) and 24h later in a conditioning chamber. **b**, Contextual memory consolidation was normal in Dnmt3a SKO mice presentation. 6 mice for Dnmt1 SKO group, 8 mice for Dnmt3a SKO group and 21 control mice were used. Data are presented as means±s.e.m. $P>0.05$

Supplementary Figure 6 DKO mouse brain has normal expression of *Reelin*, *PP1β* and *PP1γ*. Real time PCR analysis showed no significant expression change of *Reelin*, *PP1β* or *PP1γ* in DKO hippocampi as compared with control ($P>0.05$). 7 control and 5 DKO samples were used for this experiment. Data are presented as means±s.e.m. $P>0.05$

Supplementary Figure 7 Dnmt single knockout mouse brain did not show significant gene expression change. **a**, Real time PCR analysis showed similar gene expression between Dnmt1 SKO and control ($P>0.05$). **b**, Real time PCR analysis showed similar gene expression between Dnmt3a SKO and control ($P>0.05$). 3-4 pairs of samples were used. Data are presented as means±s.e.m.

Supplementary Figure 8 Stat1 promoter methylation analysis in SKO and proximal region. **a**, No DNA methylation level change was found in Dnmt SKO. The region of interest was shown in Figure 5b. **b**, Bisulfite sequencing of *Stat1* proximal promoter region (-400bp to -750bp) showed no methylation change within DKO as compared with control samples. Data as shown were from 3 pairs of genomic DNAs of 2-3 month old wildtype and DKO mouse forebrains. Schematic gene promoter structure is shown on top with arrow pointing out transcription starting site (+1). 8 CpG site that marked with a vertical slash from -400bp to -750bp were analysed. The results are displayed in the same manner as in Figure 5. Data are presented as means±s.e.m. $P>0.05$.

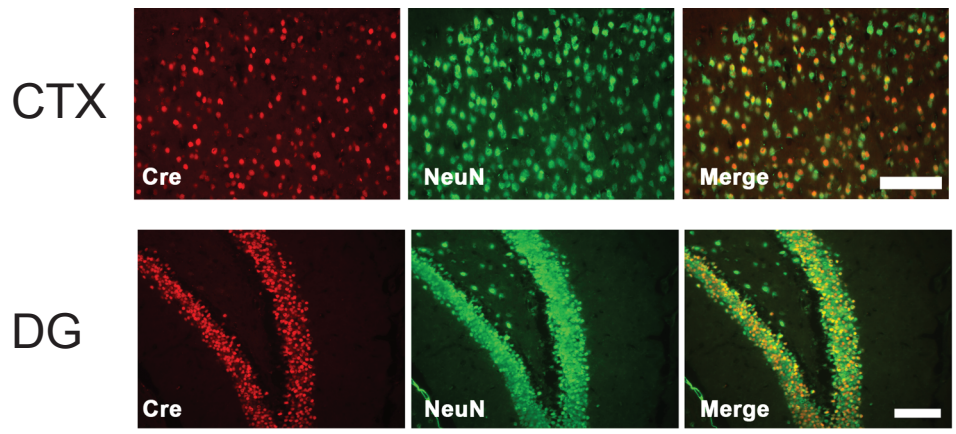
Supplementary Figure 9 Purity test of FACS sorted NeuN positive and negative nuclei subpopulations. **a**, Representative microscopy picture showed mixed nuclei population before sorting. NeuN positive and negative nuclei populations were separated after FACS sorting. DNA dye Hoechst was used to label all the nuclei. White arrows were used to point out the NeuN positive nuclei or Hoechst positive nuclei in left or right column respectively. **b-e**, Post FACS sorting confirmed the purity of both positive (**d&e**) and negative (**b&c**) populations from either DKO (**c&e**) or Control (**b&d**) samples.

Supplementary Figure 10 Quantitative analysis of 5-methylcytosine and 5-hydroxymethylcytosine using LC-MS/MS. **a**, No significant difference was found between Dnmt1 or Dnmt3a SKO with control mice. 5-methylcytosine (5mdC) and 5-hydroxymethylcytosine (5hmdC) contents are expressed as the percentage in the total cytosine pool. **b**, Significantly less 5-hydroxymethylcytosine was found in DKO mouse

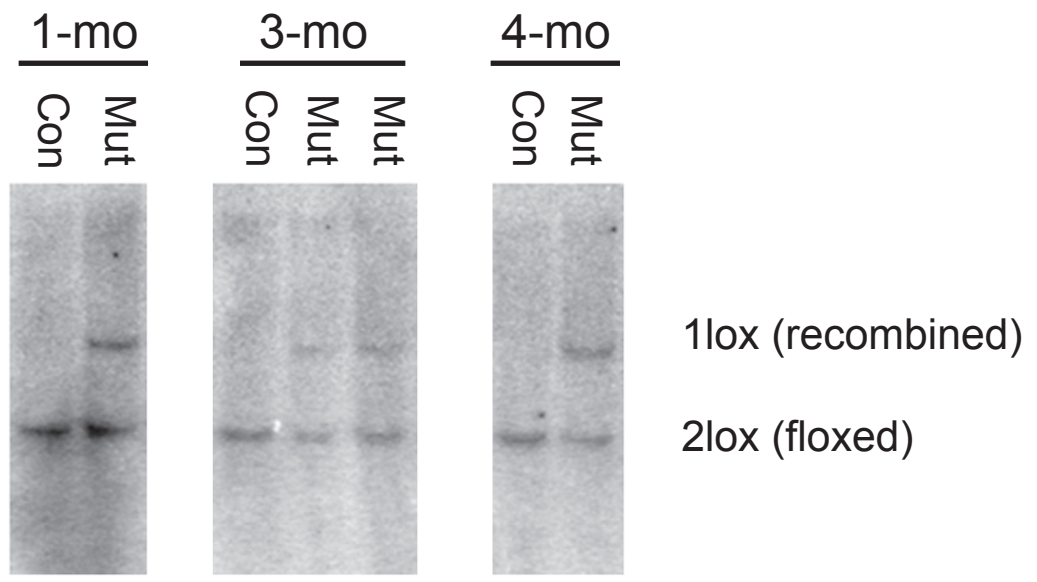
brain. Data are from replicates of 3 separate experiments for Dnmt1 or Dnmt3a SKO and 4 for DKO 3 month old forebrain DNAs. Data are presented as means \pm s.e.m. * P < 0.05

Supplemental Fig1 (Fan et al.)

a

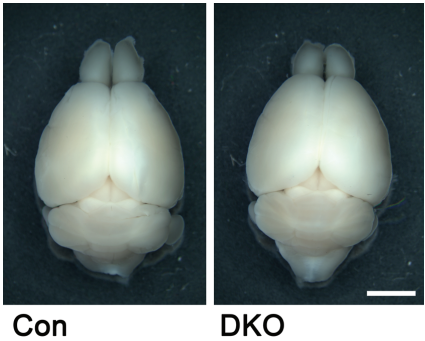


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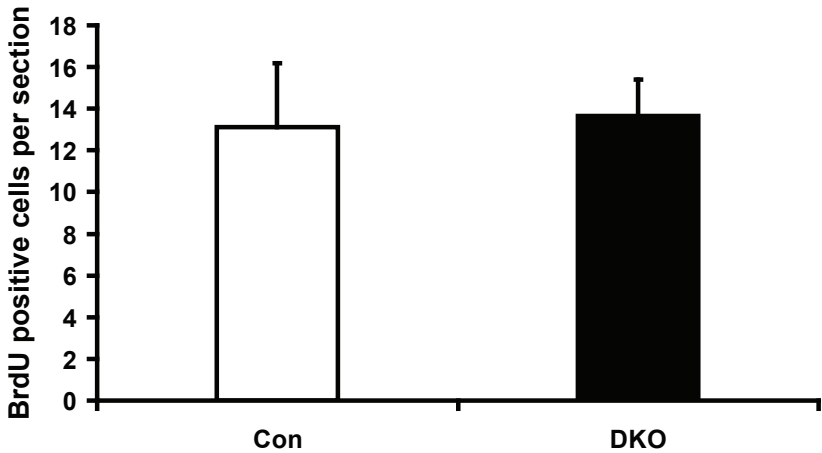


Supplemental Fig2 (Fan et al.)

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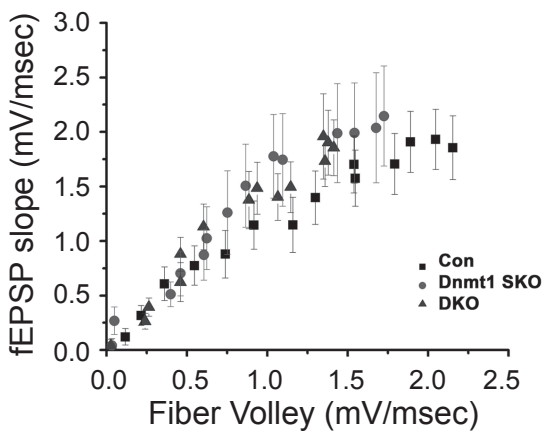


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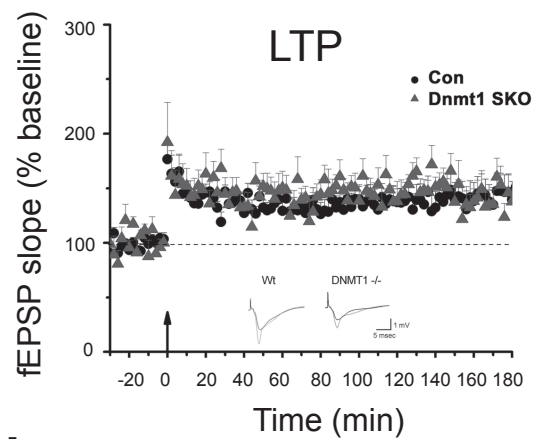


Supplemental Fig3 (Fan et al.)

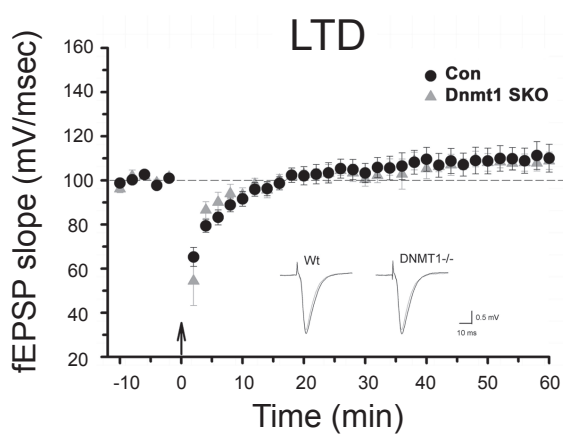
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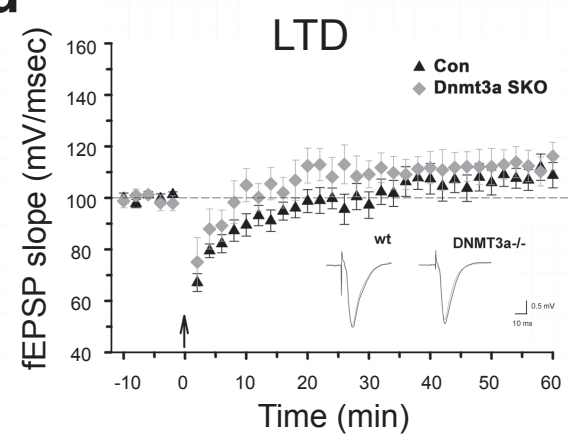
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c

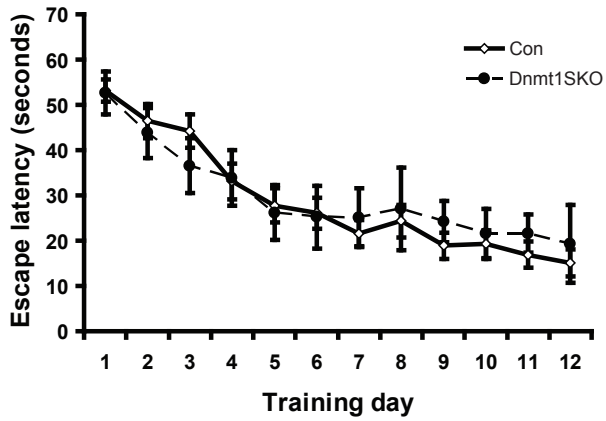


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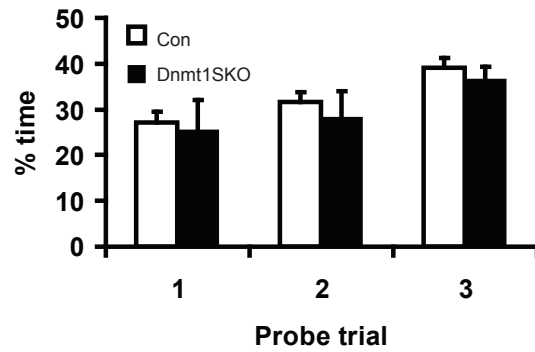


Supplemental Fig4 (Fan et al.)

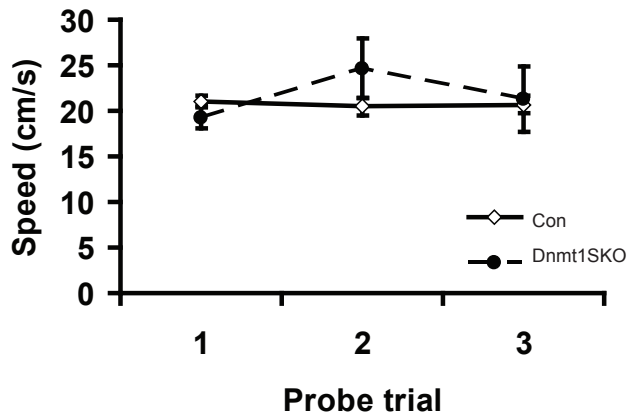
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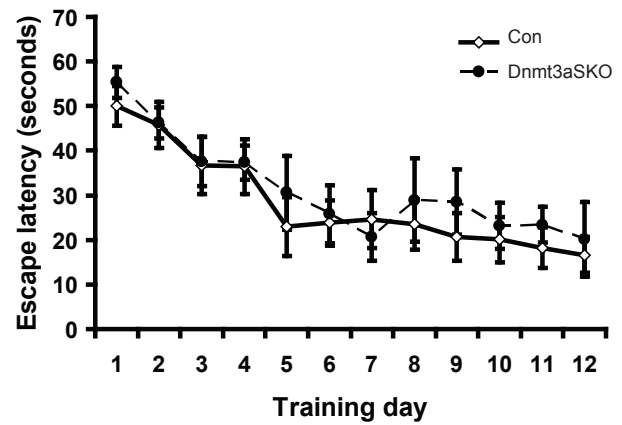
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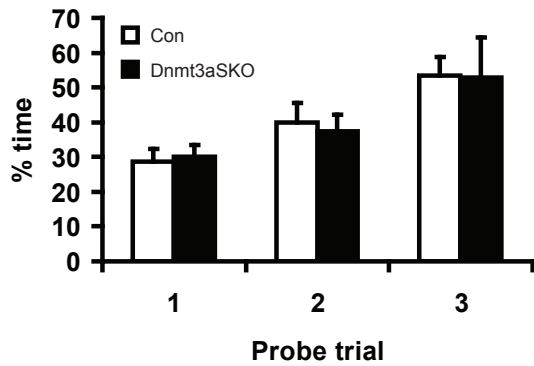
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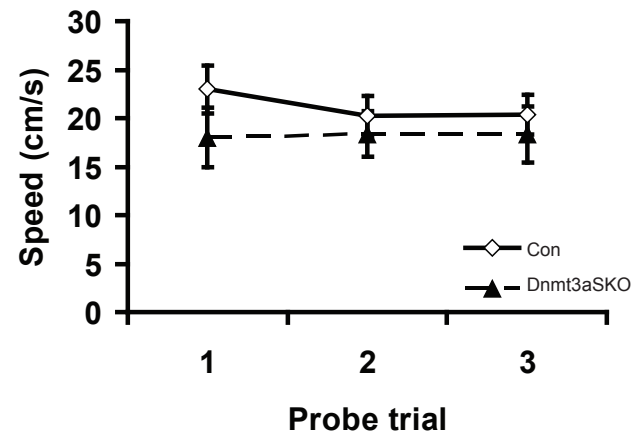
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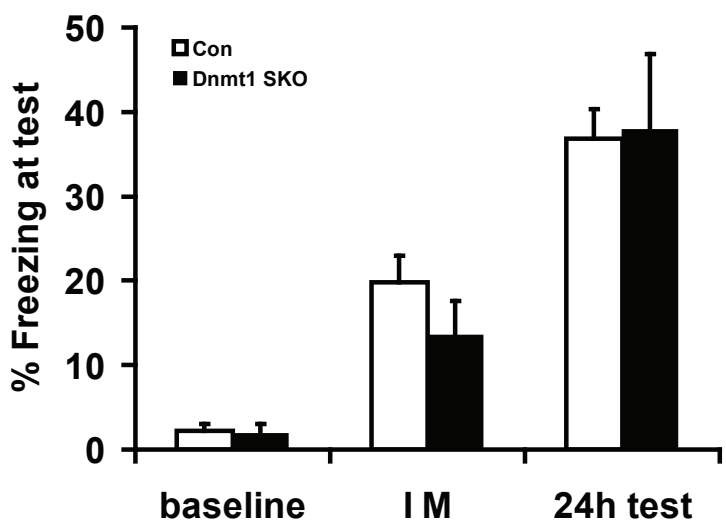
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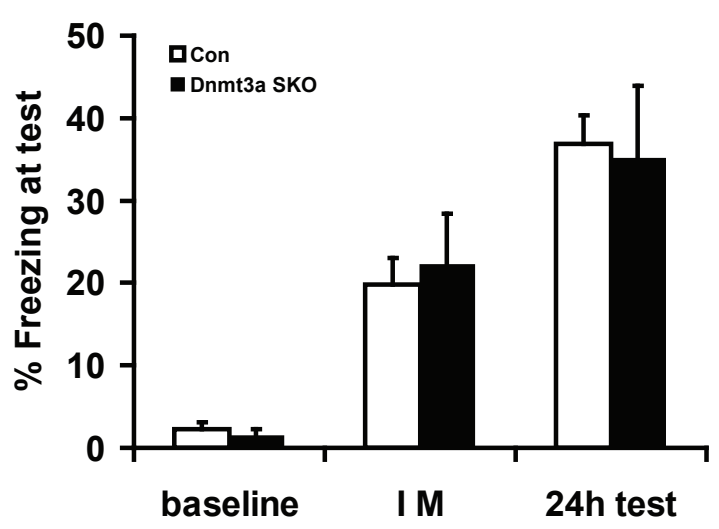
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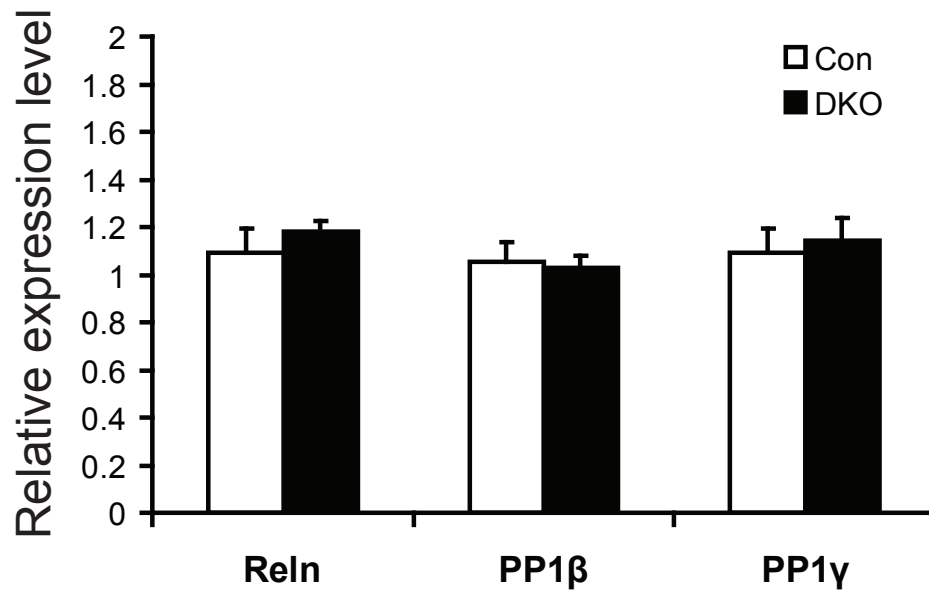
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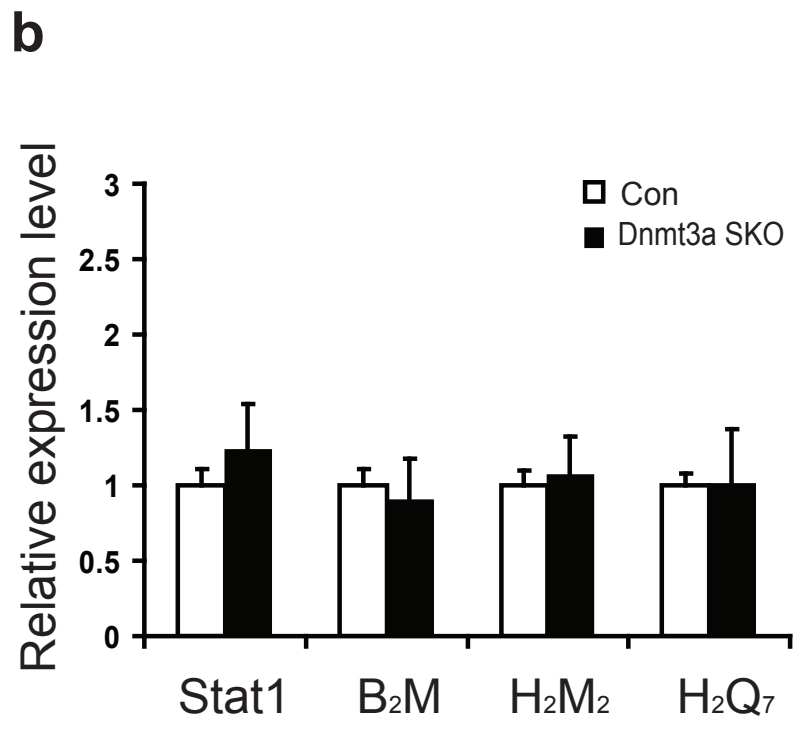
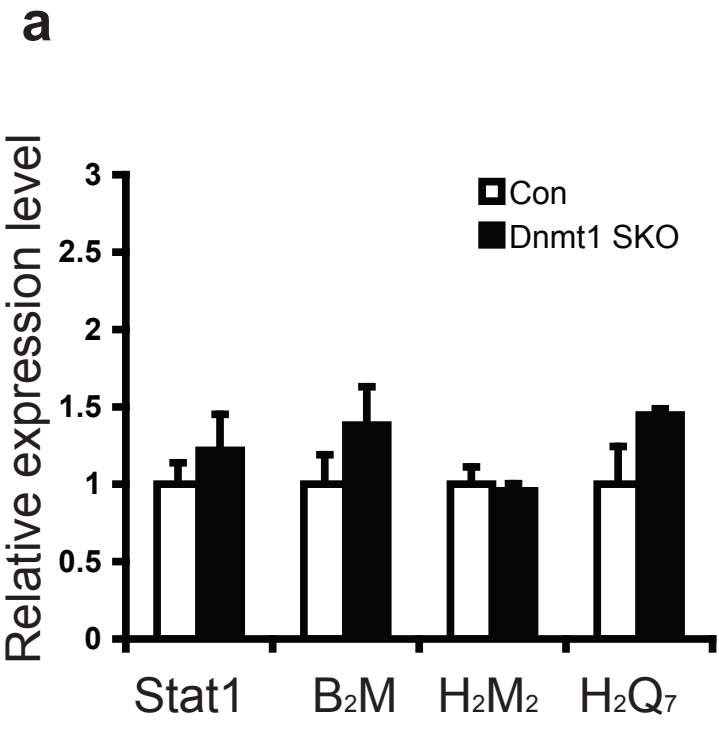


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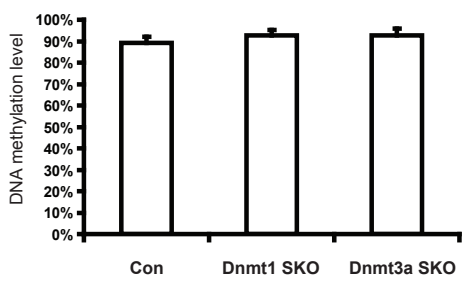
Supplemental Fig6 (Fan et al.)



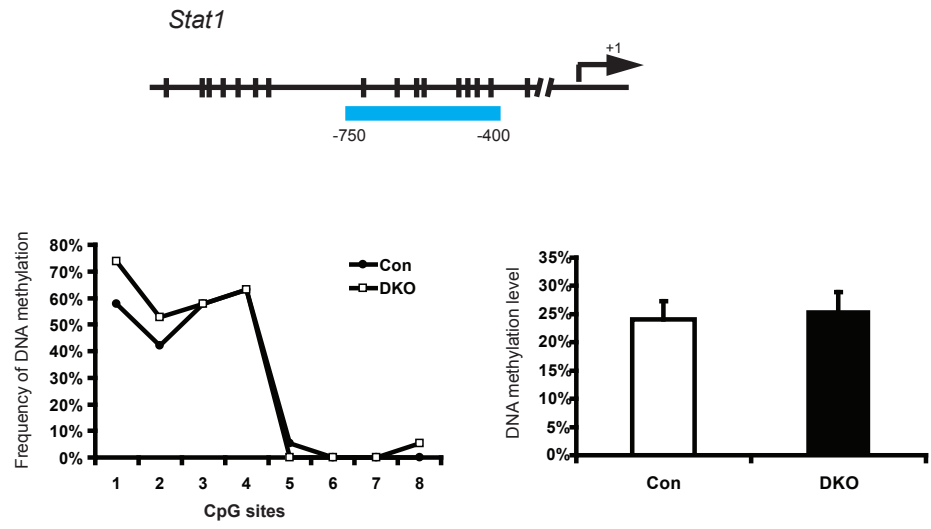


Supplemental Fig8 (Fan et al.)

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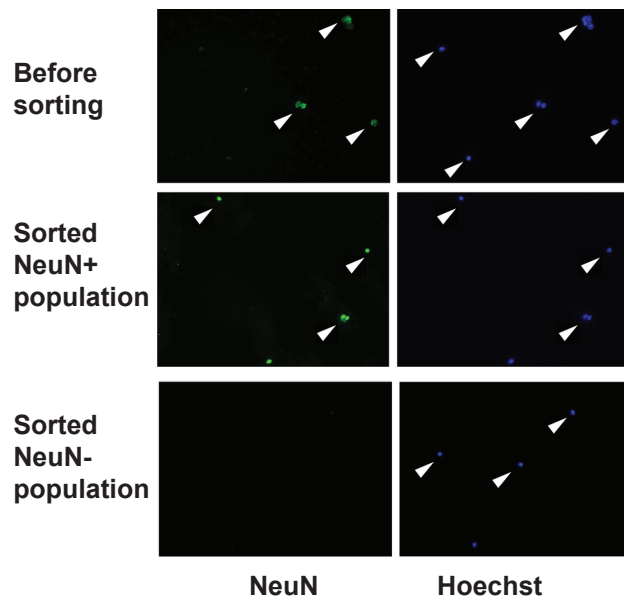


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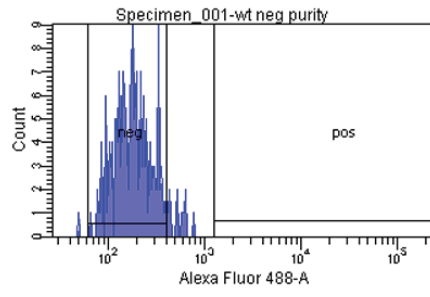


Supplemental Fig9 (Fan et al.)

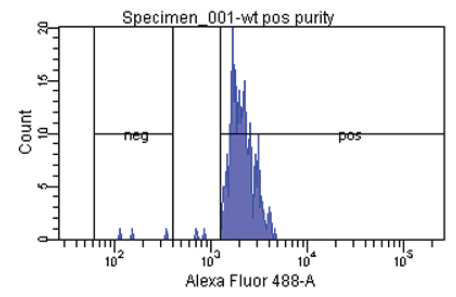
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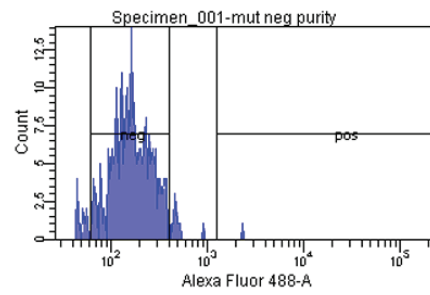
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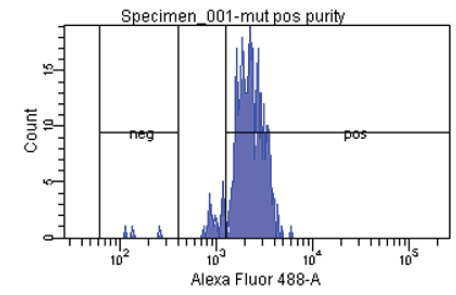
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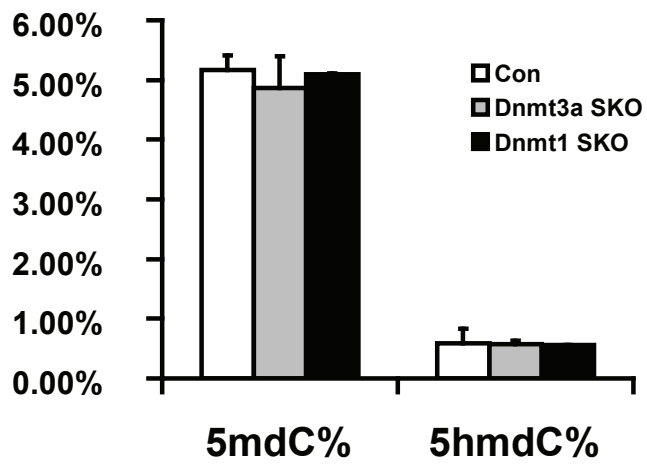
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