Tet1-mediated DNA demethylation regulates neuronal cell death induced by oxidative stress

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Supplemental Figure 1. Expression of Klotho is regulated by DNA methylation in mouse cortical neurons.

(a,b) Expression of Klotho in cortical neurons is regulated by DNA methylation. Cortical neurons were treated with 5-aza-2-dC (0-10 μM) for 12 h and collected for analyzing mRNA levels of Klotho and BDNF. Quantitative PCR were performed. All data points are normalized to untreated samples.

All data were collected from 3 independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001; one-way ANOVA with Tukey's test.

(c) Mouse cortical neurons were treated with 5-aza-2-dC (0-1 μM) for 12 h and collected for measuring Klotho protein level.

Supplemental Figure 2. DNA demthylation inhibits neuronal cell death induced by staurosporine in mouse cortical neuron.

(a) Mouse cortical neuron transfected with GFP vector or klotho shRNA and with or without pretreated with 5-aza-2-dC, a Dnmt inhibitor, for 6h and treated with

staurosporine (500nM 10-12h) to induce the apoptotic cell death. Yellow arrowhead stands for the healthy cells and red arrowhead indicates apoptotic cells. Chromatin condensation was monitored by Hoechst staining. Scale bar=50μm.

(b) Quantitation of (a). (ANOVA followed by Tukey's test, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, n=4). Error bar represents s.e.m. Each bar represents at least 600 cells of each condition.

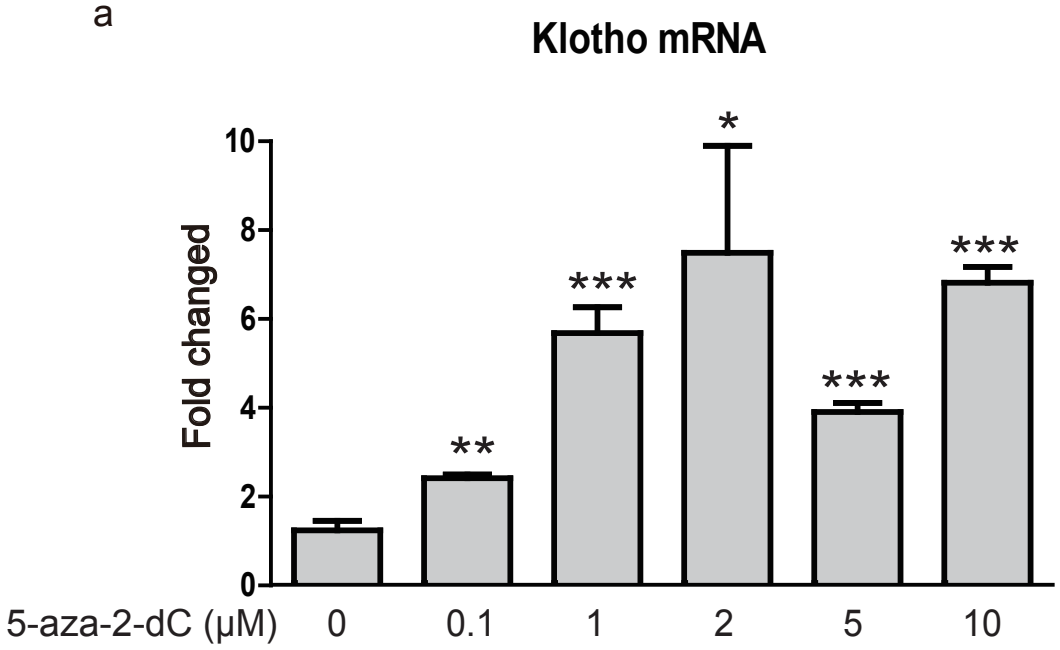
Supplemental Figure 3. Tet1 is critical for neuronal cell death induced by staurosporine in mouse cortical neuron via regulating Klotho.

(a) Mouse cortical neuron transfected single with GFP vector and Tet1 shRNA or together with GFP/Tet1CD and Tet1CD/shKL and treated with staurosporine to induce the apoptotic cell death. Yellow arrowhead stands for the healthy cells and red arrowhead indicates apoptotic cells. Chromatin condensation was monitored by Hoechst staining. Scale bar=50μm.

(b) Quantitation of (a). (ANOVA followed by Tukey's test, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, n=4). Error bar represents s.e.m. Each bar represents at least 600 cells of each condition.

Supplemental Figure 1. Xin et al.

a



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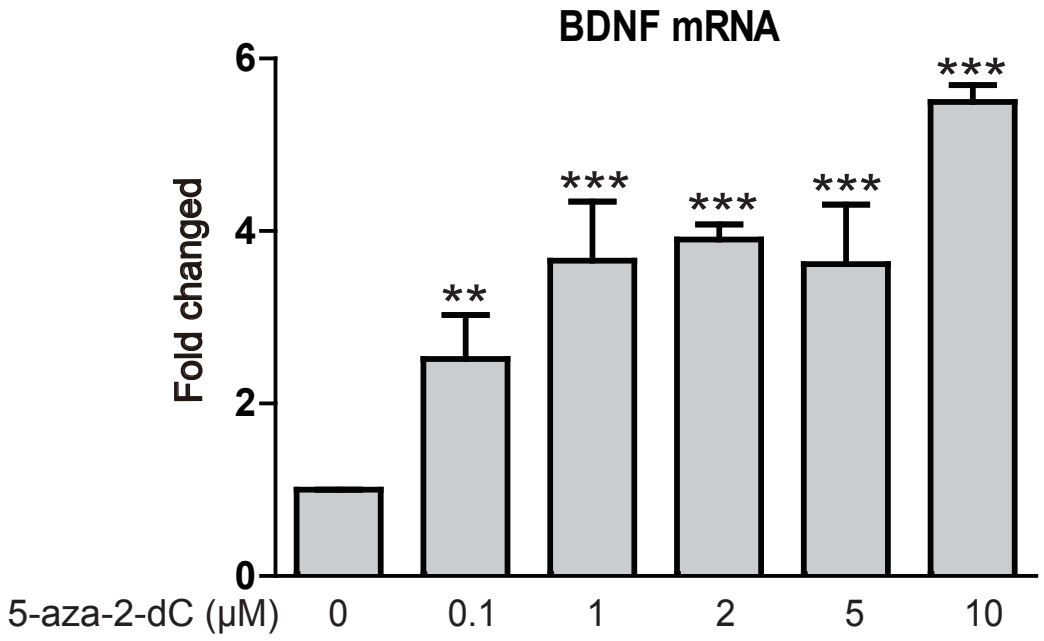

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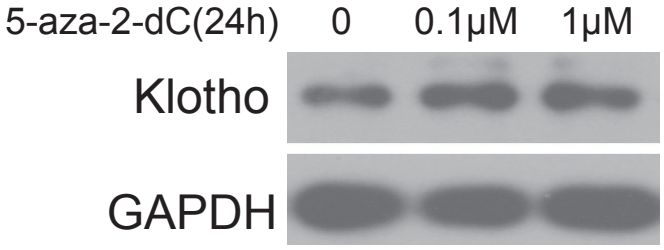
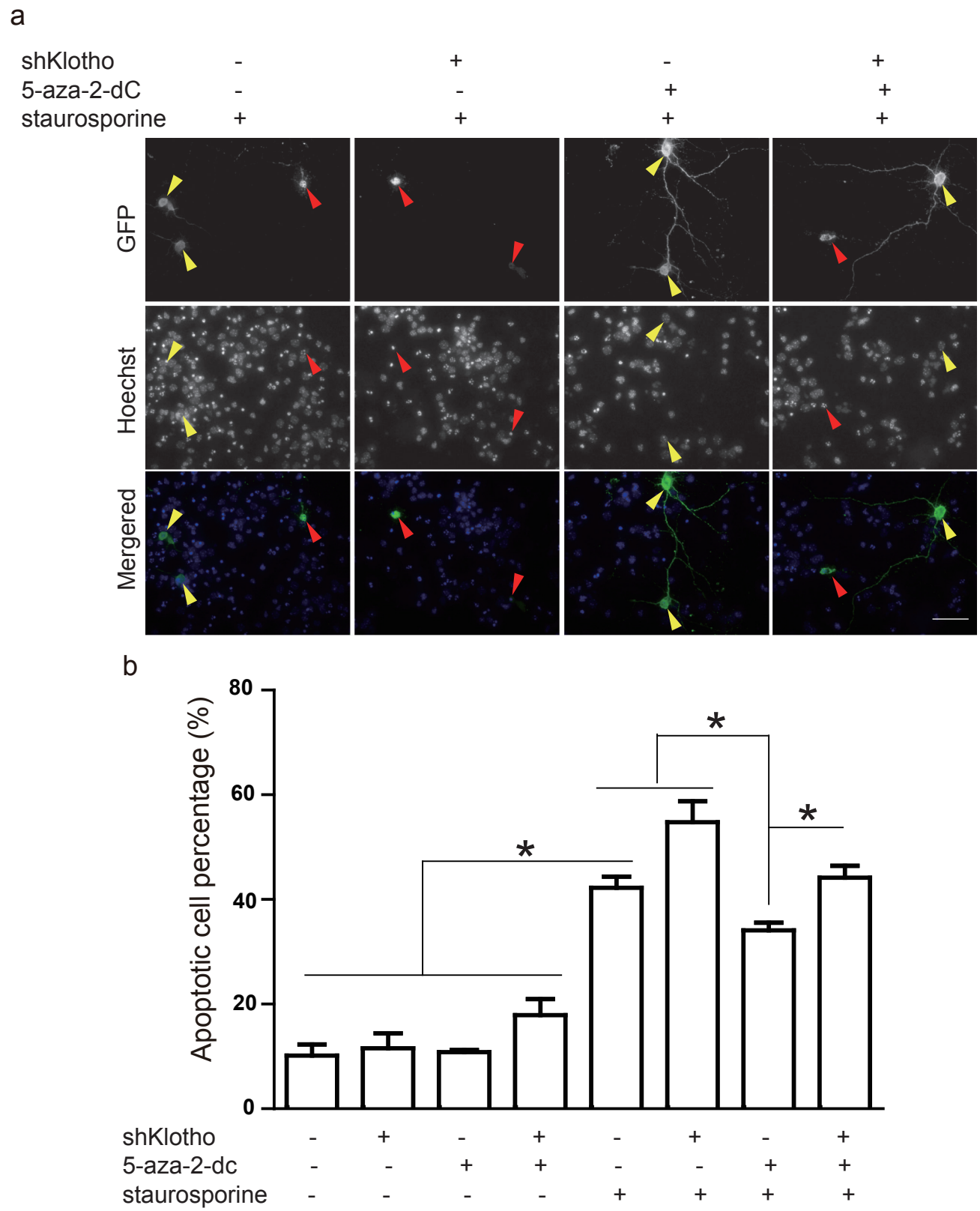


Supplemental Figure 2. Xin et al.



Supplemental Figure 3. Xin et al.