

## Supplementary Data

### Materials and Methods

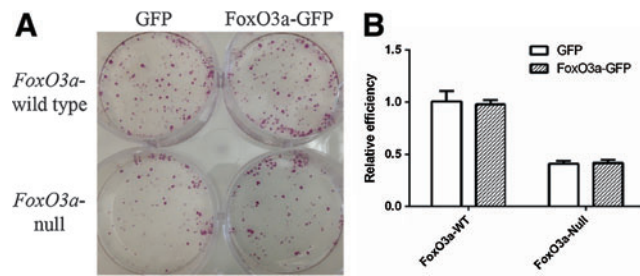
#### *Determination of reactive oxidative stress production*

Reactive oxidative stress levels were detected by staining the *FoxO3a*-wild type and -null mouse embryonic fibroblasts (MEFs) with dihydroethidium (DHE) (Molecular Probes) as previously described [1]. Cells were loaded with 5  $\mu$ M DHE for 15 min at 37°C with 5% CO<sub>2</sub> in phosphate buffered saline (PBS) and then washed with PBS. The cells were returned to MEF media for a 30-min recovery period and then fixed with

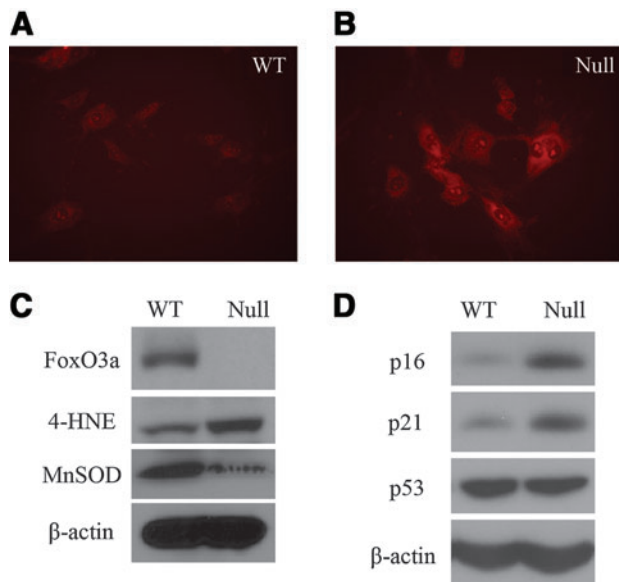
4% paraformaldehyde for 7 min at room temperature. The pictures of *FoxO3a*-wild type and -null MEFs were taken under fluorescence microscope.

### Reference

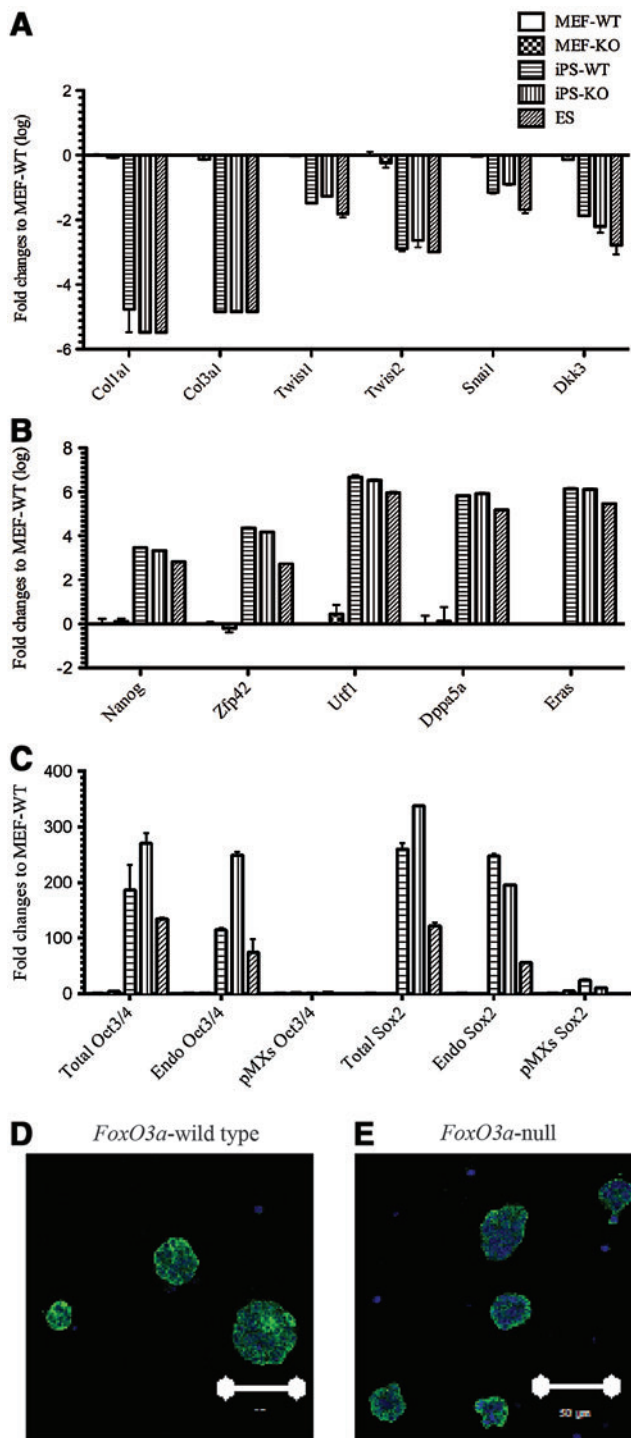
1. Tian C, L Sun, B Jia, K Ma, N Curthoys, J Ding and J Zheng. (2012). Mitochondrial glutaminase release contributes to glutamate-mediated neurotoxicity during human immunodeficiency virus-1 infection. *J Neuroimmune Pharmacol* 7:619–628.



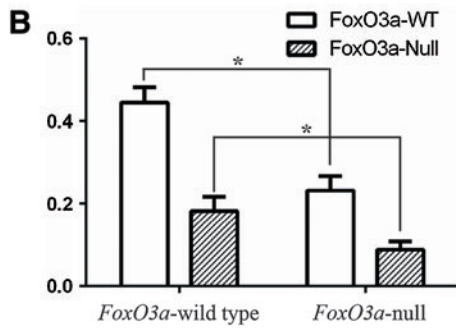
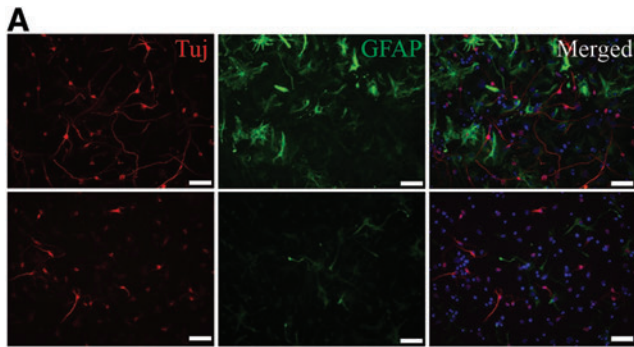
**SUPPLEMENTARY FIG. S1.** Overexpression of GFP or forkhead class O3a (FoxO3a)-GFP together with Yamanaka factors in *FoxO3a*-wild type and -null mouse embryonic fibroblasts (MEFs). **(A)** Overview of alkaline phosphatase (AP) staining in the *FoxO3a*-wild type and -null MEFs after the overexpression of GFP or FoxO3a-GFP together with Yamanaka factors for 12 days. **(B)** The number of AP+ colonies was normalized to the *FoxO3a*-wild type MEFs transduced with retroviruses encoding GFP together with Yamanaka factors. GFP, green fluorescent protein; MEFs, mouse embryonic fibroblasts.



**SUPPLEMENTARY FIG. S2.** Reactive oxidative stress (ROS) detection in the *FoxO3a*-wild type and -null MEFs. The ROS levels in *FoxO3a*-wild type (**A**) and -null (**B**) MEFs were detected by dihydroethidium staining. The expression levels of FoxO3a, 4-HNE, and MnSOD (**C**) and the expression levels of p16, p21, and p53 (**D**) in the *FoxO3a*-wild type and -null MEFs were determined by western blotting analysis with  $\beta$ -actin as the internal control. 4-HNE, hydroxynonenal.



**SUPPLEMENTARY FIG. S3.** Marker genes expression in *FoxO3a*-wild type and -null induced pluripotent stem (iPS) cells. The expression of fibroblast-specific genes (**A**) and iPS cell-specific genes (**B**) in MEFs, iPS cells, and embryonic stem (ES) cells were analyzed by SYBR-Green based quantitative RT-PCR with GAPDH as the internal control. The total, transgenic, and endogenous levels of Oct3/4 and Sox2 (**C**) were analyzed through SYBR-Green based quantitative RT-PCR with specific primer pairs (Supplementary Table S1). Immunofluorescence staining of Nanog (green) in *FoxO3a*-wild type (**D**) and -null (**E**) iPS cells and nuclear staining with DAPI (blue). Scale bar = 50  $\mu$ m. RT-PCR, real-time polymerase chain reaction.



**SUPPLEMENTARY FIG. S4.** Neurons and astrocytes differentiation of *FoxO3a*-wild type and -null iPS cell-derived neural stem cells (NSCs). **(A)** Immunofluorescence staining of neuron marker-Tuj (*red*) and astrocyte marker-GFAP (*green*) in *FoxO3a*-wild type and -null iPS cell-derived NSCs, and nuclear staining with DAPI (*blue*). **(B)** Quantification of the proportion Tuj+ / DAPI and GFAP+ / DAPI in *FoxO3a*-wild type and -null NSCs derived from iPS cells. Scale bar = 50  $\mu$ m (\* $p < 0.05$ ).

SUPPLEMENTARY TABLE S1. PRIMER SEQUENCES FOR SYBR-GREEN BASED QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION

<i>Genes</i>	<i>Primers-Forward</i>	<i>Primers-Reverse</i>
<i>GAPDH</i>	AAGGGCTCATGACCACAGTC	GGATGACCTTGCCCACAG
<i>Dkk3</i>	CAGCTCTCAACTACCCTCAGG	ACCTCAGAGGACGTTTTAGCA
<i>Twist1</i>	GAGGTCTTGCCAATCAGCCA	CCAGTTTGATCCCAGCGTTT
<i>Twist2</i>	CAGCAAGATCCAGACGCTCAA	GGTTGTCCAGGTGCCGAAAG
<i>Snai1</i>	GTCTGCACGACCTGTGGAAA	AGCCAGACTCTTGGTGCTTG
<i>Col1a1</i>	CTGACGCATGGCCAAGAAGA	ATACCTCGGGTTTCCACGTC
<i>Col3a1</i>	GAGGAATGGGTGGCTATCCG	TTGCGTCCATCAAAGCCTCT
<i>Nanog</i>	TCTTCCTGGTCCCCACAGTTT	GCAAGAATAGTTCTCGGGATGAA
<i>Zfp42</i>	CCCTCGACAGACTGACCCTAA	TCGGGGCTAATCTCACTTTCAT
<i>Ulf1</i>	TGTCCCGGTGACTACGTCT	CCCAGAAGTAGCTCCGTCTCT
<i>Dppa5a</i>	ATGATGGTGACCCTCGTGAC	ACCTCGATAAGTTCTTCGGGAG
<i>Eras</i>	TGCCTACAAAGTCTAGCATCTTG	CTTTTACCAACACCACTTGCAC
<i>Total Oct3/4</i>	GGCTTCAGACTTCGCCTTC	AACCTGAGGTCCACAGTATGC
<i>Total Sox2</i>	CCTCCGGGACATGATCAGCATG	GCAGTGTGCCGTTAATGGCCGTG
<i>Endo Oct3/4</i>	CCTCTGTTCCCGTCACTGCTCTG	ATGAGTGACAGACAGGCCAG
<i>Endo Sox2</i>	CCTCCGGGACATGATCAGCATG	CGGCATCACGGTTTTTGCCT
<i>pMXs Oct3/4</i>	CCTCTGTTCCCGTCACTGCTCTG	TTTATCGTCGACCACTGTGCTGG
<i>pMXs Sox2</i>	CCTCCGGGACATGATCAGCATG	TTTATCGTCGACCACTGTGCTGG