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Supplemental Information

Alternative Routes to Induced Pluripotent Stem Cells Revealed by Reprogramming of the Neural Lineage

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Supplementary Figures

Supplementary Figure 1:

A) Immunofluorescence images of Nanog colonies from reprogramming NSC with E-cadherin at d6 and d12. Scale bars are 50μm.

B) Cell counts on day 1 of reprogramming following plating of $1x10^5$ cells on day 0.

C) Relative expression of exogenous Oct4, Sox2, Klf4 and c-Myc in NSC, Astrocyte and MEF. Error bars are standard deviation of two technical replicates from one representative experiment. d6-Dox samples were exposed to doxycycline from d0-3 with no doxycycline from d3-6. O; Oct4, S; Sox2, K; Klf4, M; c-Myc.

D) Immunofluorescence images of Nanog colonies from reprogramming Astrocytes with E-cadherin and/or SSEA1. Scale bars are 50μm.

E) Counts of Nanog, E-cadherin (E-cad) and Nanog+/E-cadherin+ (N+E+) colonies from NSC reprogramming cultures treated with mock treated or exposed to siRNA to E-cadherin from days 7-11 and fixed on day 11. Error bar represents standard deviation of two independent experiments. There is a decrease in the number of Nanog colonies suggesting that a threshold of E-cadherin may be required for expression of Nanog in some cases.

F) Western blot analysis for Oct4. NSCs were exposed to dox alone or in combination with DMSO or SGC0946 for 1 day before dox was removed. Samples were harvested at d1, d2, d3 and d4.

Supplementary Figure 2:

A) Left Panel – Scheme of experiment for samples labeled “Fix” in B and C. Dox was added to reprogramming cells at d0 and remained until cultures were fixed on days indicated. Right Panel – Scheme of experiment for samples labeled “-Dox” in B and C. Dox was added to reprogramming cells at d0 and removed at the days indicated. Cells remained in culture until d14.

B) Day 14 counts for Nanog+/E-cadherin+ colonies obtained from reprogramming astrocyte cultures upon dox withdrawal on indicated days. Multiple biological replicates are stacked.

C) As in B but for MEFs.

D) Nanog colony counts from NSC, Astrocyte and MEF reprogramming cultures derived from mice with two different reprogramming alleles incorporated into the Col1a locus (JSS)- please see below.

E) Western blot analysis of Oct4, Sox2, Klf4 and c-Myc in two different genotypes of MEFs: JSS- Contains the reprogramming factors in two different configurations on each allele, OSKM and OKSM and heterozygous for the rtta allele; SSRW- homozygous for the OKSM allele and heterozygous for the rtta allele. Samples were harvested at d1 or d3 after doxycycline treatment.

F) Immunostaining of Sox2 from a NSC isolation in Brightfield, DAPI (Blue) and Sox2 (green).

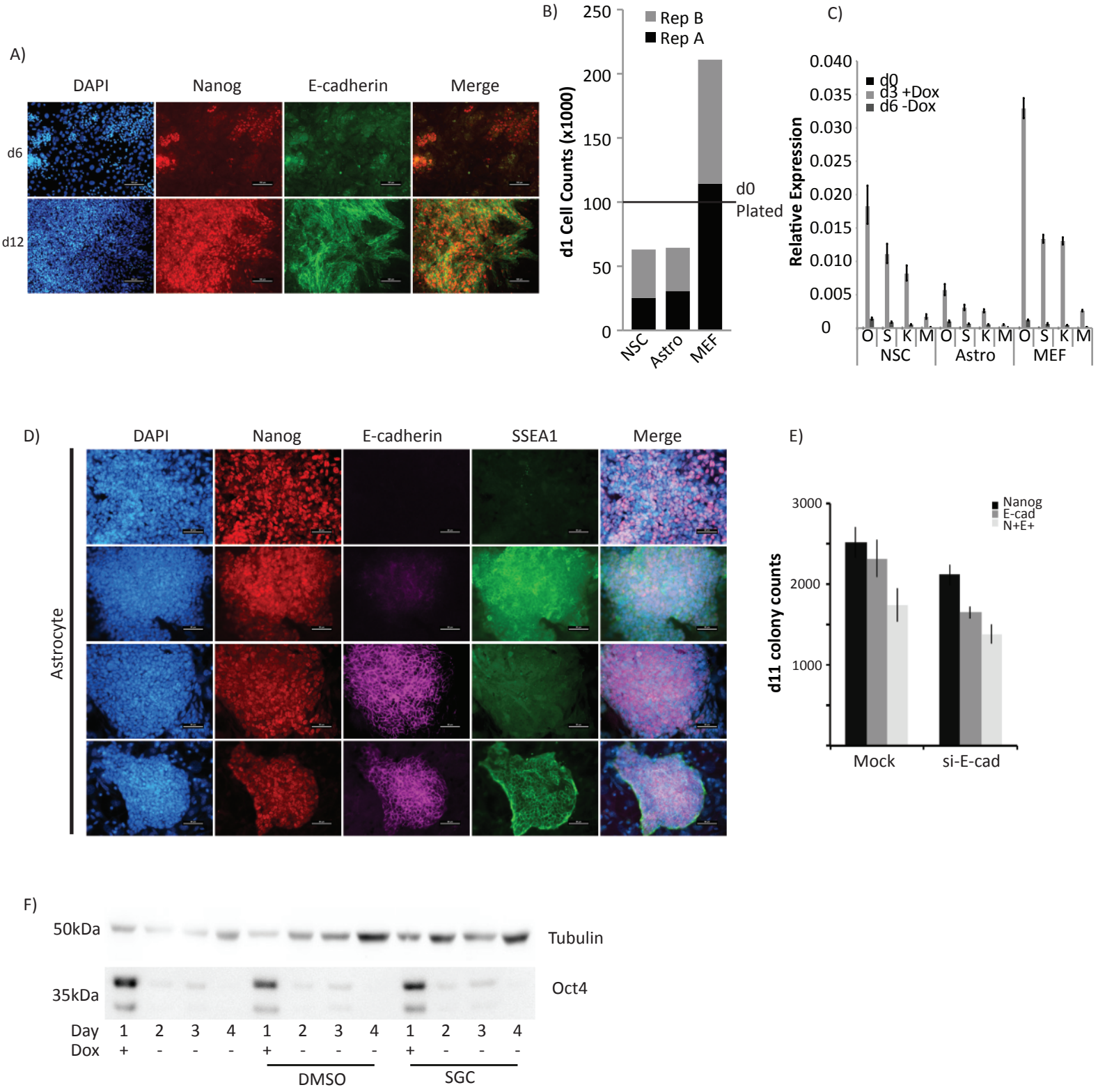
Supplementary Table 1

This table contains all primary data for each replicate reprogramming experiment as well as percent of each population- Nanog+ E-cadherin+, Nanog+ E-cadherin-, along with statistical significance of difference in populations.

Supplementary Table 2

This table contains the reads from the RNA-Seq of populations of N+E- and N+E- on day 7.

Supp Figure 1



Supp Figure 2