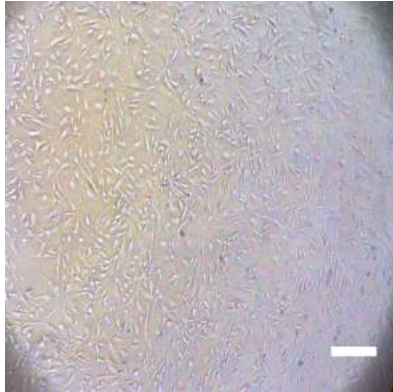


**Permissiveness to form pluripotent stem cells may be an evolutionarily  
derived characteristic in *Mus musculus***

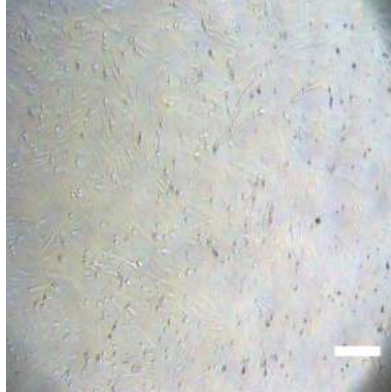
Tiffany A. Garbutt, Thomas I. Konneker, Kranti Konganti, Andrew E. Hillhouse, Francis  
Swift-Haire, Alexis Jones, Drake Phelps, David L. Aylor, David W. Threadgill

## Supplemental Information

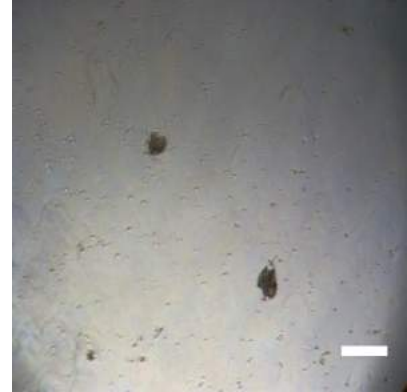
NOD/LtJ



A. day before virus

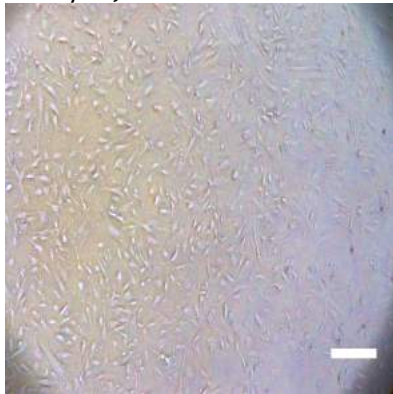


B. 5 days after virus

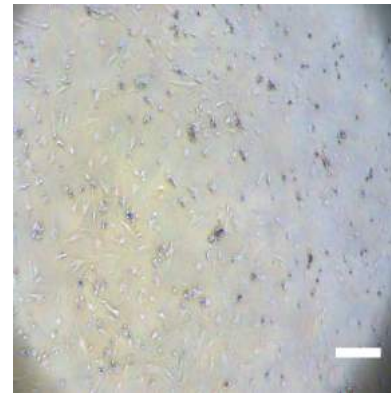


C. 21 days after virus

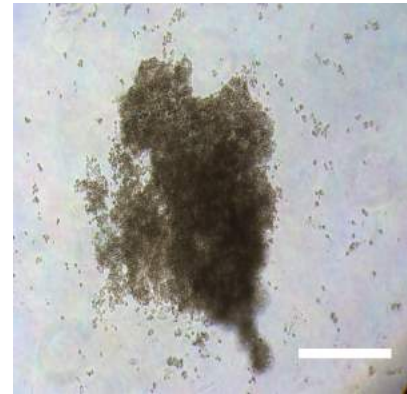
WSB/EiJ



D. day before the virus



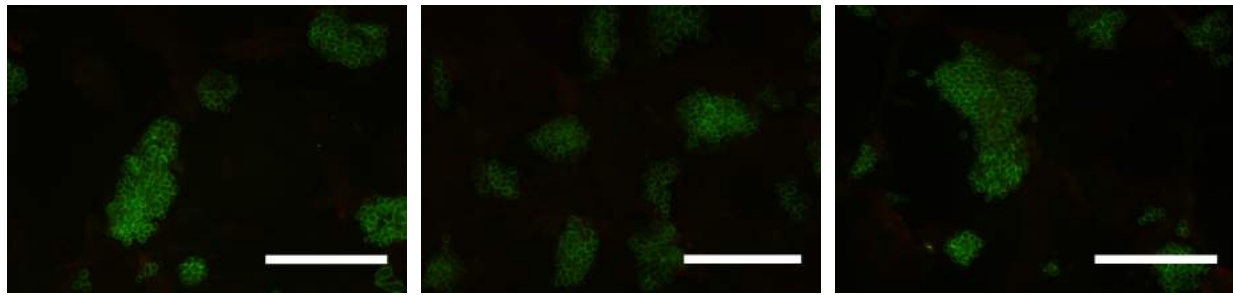
E. 5 days after virus



F. 18 days after virus

### **Figure 1: Serum free conditions during reprogramming leads to cell death**

(A,B,D,E) Approximately five days after the addition of 2i in serum free conditions, cell death became obvious in both backgrounds. (C,F) By 18 – 21 days after the addition of 2i in serum free conditions all the cells were dead and detached from the plate. Some floating clumps were selected and placed on MEFs but did not expand. Scale bars are 400  $\mu\text{m}$ .

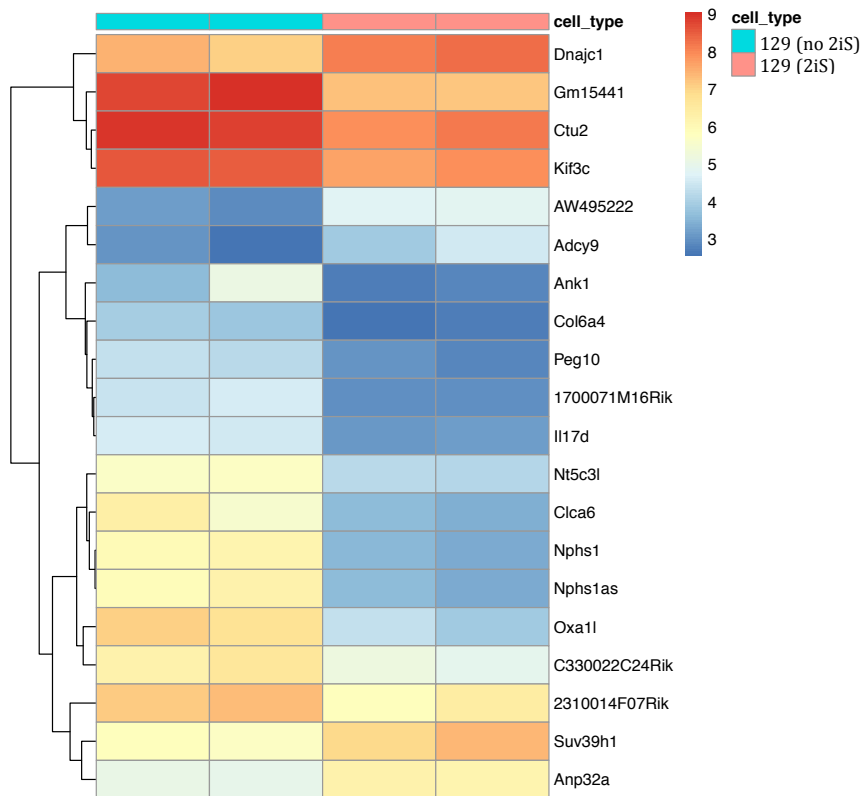


A. NOD/LtJ iPSC.2iS Male

B. NOD/LtJ iPSC.2iS Female

C. WSB/EiJ iPSC.2iS Male

**Figure 2: NOD male and female iPSC grown in 2iS stain homogenously for PECAM1**  
The cells were stained for the ESC marker PECAM1 (green) and the EpiSC maker CD40 (red). There are no differences in cell surface staining between male and female NOD/ShiLtJ iPSC. Scale Bars are 200  $\mu$ m.



**Figure 3: Difference between 129S1/SvImJ iPSC maintained in 2iS media versus ESC/LIF media**

129S1/SvImJ male iPSC were derived and maintained in standard ESC/LIF media for 16 passages and then transferred and maintained in 2iS media for eight passages. Their transcriptional profiles were compared, revealing 39 differentially expressed genes, 20 of which are depicted here with varying degrees of expression. Cells maintained in 2iS media had reduced expression of *Ank1*, *Col18A1*, *Nphs1*, and *Il17D*, genes associated with cell death. Lower expression of *Kif3C*, *Basp1*, and *Nphs1* in 2iS maintained cells compared to ESC/LIF maintained cells, is associated with reduced cytoskeleton organization.