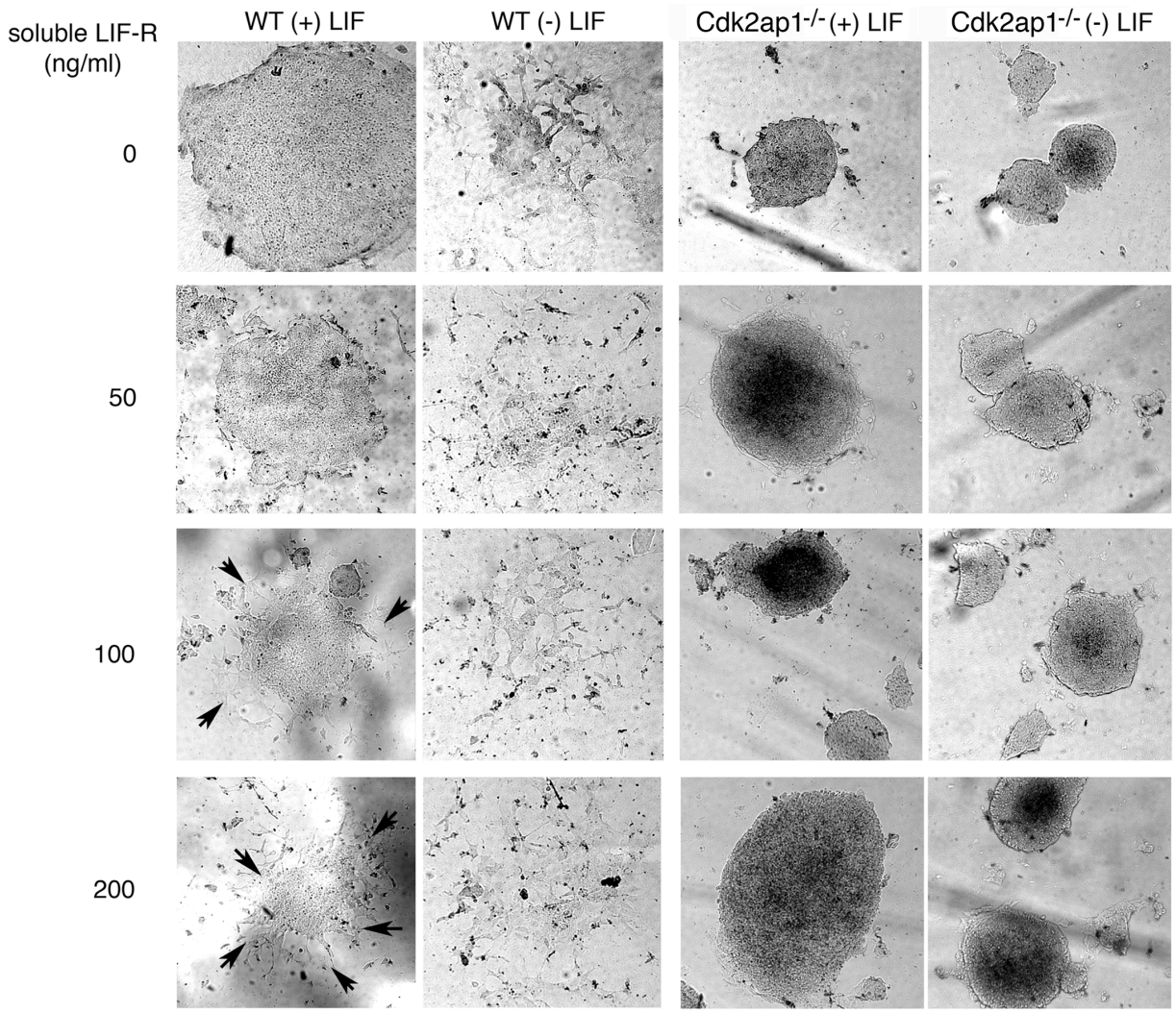
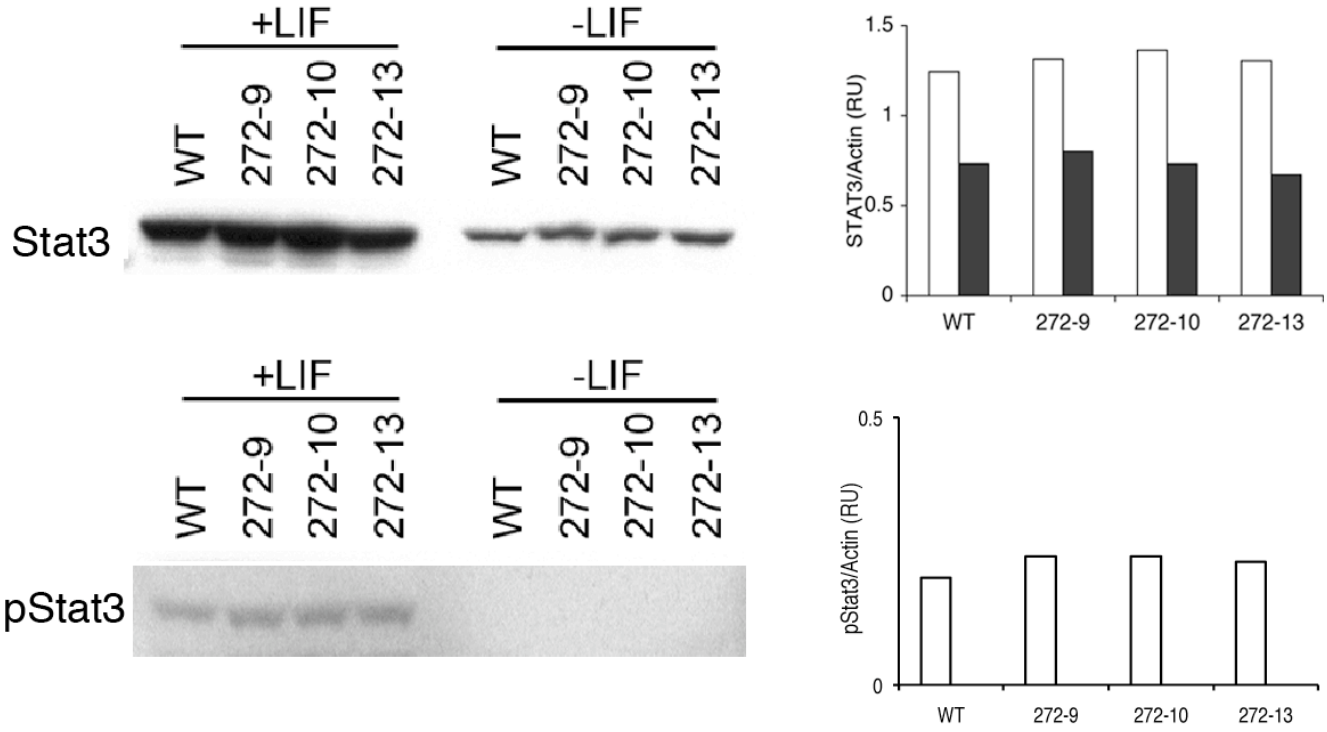
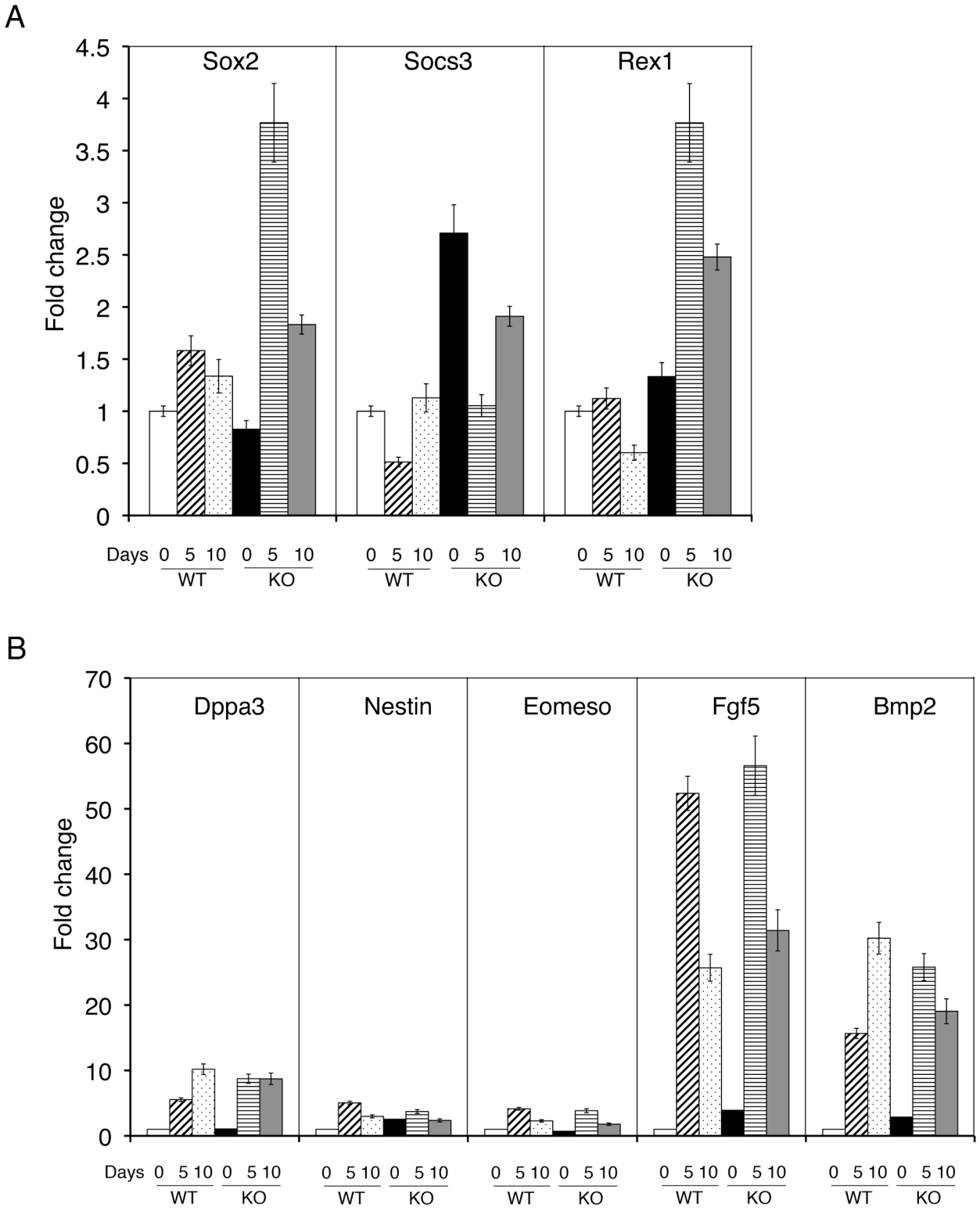
SUPPLEMENTAL INFORMATION



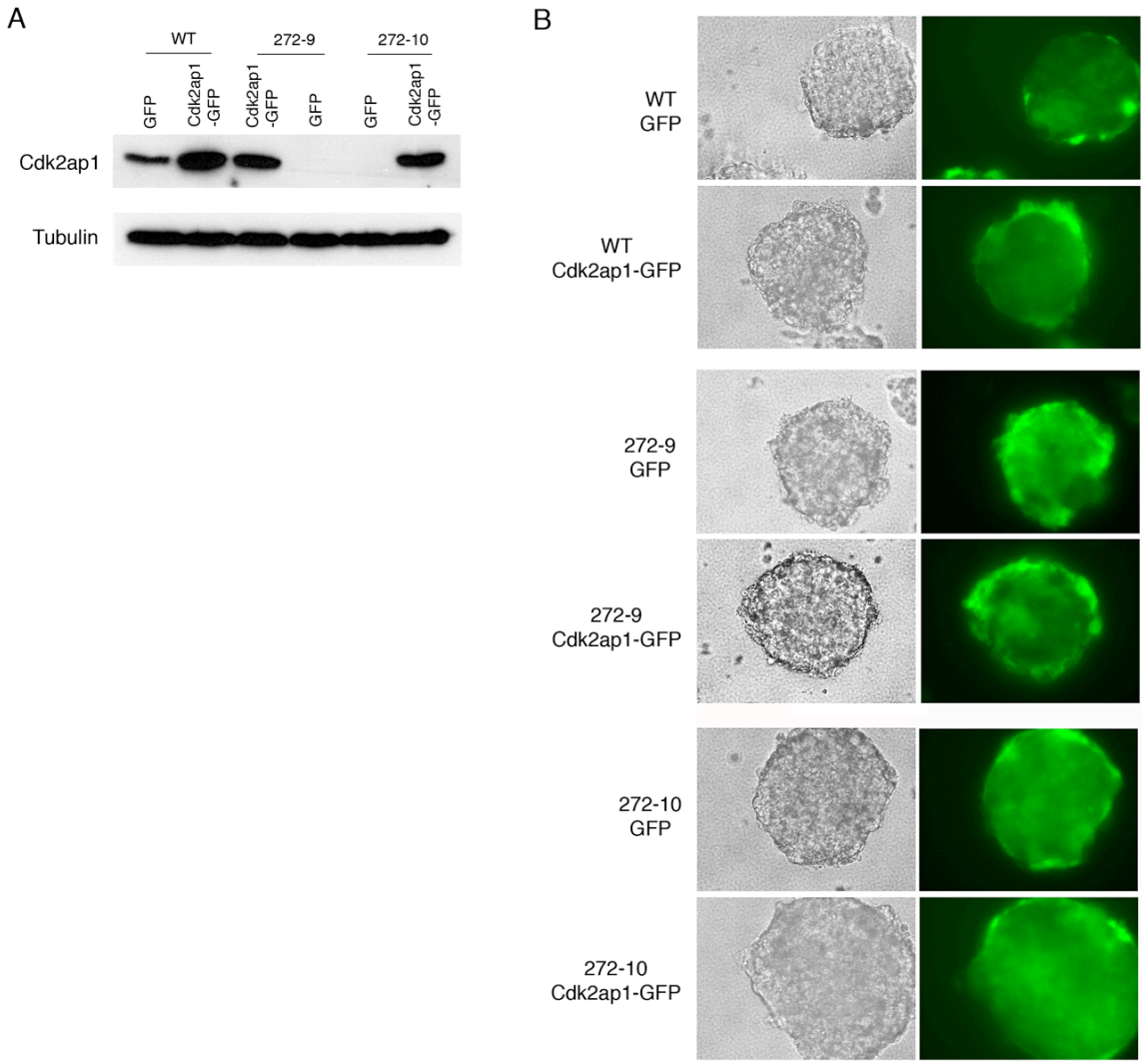
S1. *Cdk2ap1*$^{-/-}$ mESCs showed resistance to soluble LIF-receptor. To test if compromised differentiation of *Cdk2ap1*$^{-/-}$ mES cells was due to the production of LIF in differentiating cells, we treated WT (*Cdk2ap1*$^{+/+}$) and *Cdk2ap1*$^{-/-}$ mESCs with soluble LIF receptor. Cells were seeded on gelatin coated chamber slides and grown in the presence or absence of LIF supplemented with various concentrations of soluble LIF receptor alpha (0, 50, 100, and 200 ng/ml) (Sigma-Aldrich, St. Louis, MO). After 10 days in culture, the chambers were disassembled and the cells were stained for alkaline phosphatase activity (Cell BioLabs, San Diego, CA). Photomicrograph was taken and processed by using SPOT software. While *Cdk2ap1*$^{+/+}$ cells showed differentiation upon LIF withdrawal or after the addition of soluble LIF receptor, *Cdk2ap1*$^{-/-}$ cells did not show differentiation. The arrow is used to indicate the cells with differentiated morphology where needed.



S2. Intact LIF mediated signaling pathway in *Cdk2ap1*$^{-/-}$ mES cells.
The signaling molecules involved in LIF mediated pathway were examined to see if there is any defect due to the knockout of *Cdk2ap1* in mES cells. No significant alterations were found, implicating that the compromised differentiation in *Cdk2ap1*$^{-/-}$ cells is independent of this known mechanism and suggesting that Cdk2ap1 is working downstream of these molecules. WT and *Cdk2ap1*$^{-/-}$ (272-9, 272-10, 272-13) mES cells were grown in the presence or absence of LIF and the level of Stat3 and pStat3 was examined by Western analysis. Densitometry scanning was done by using UN-SCAN-IT gel software V4.3 (Silk Scientific Corporation, Orem, UT) and results were normalized against the level of β-actin.

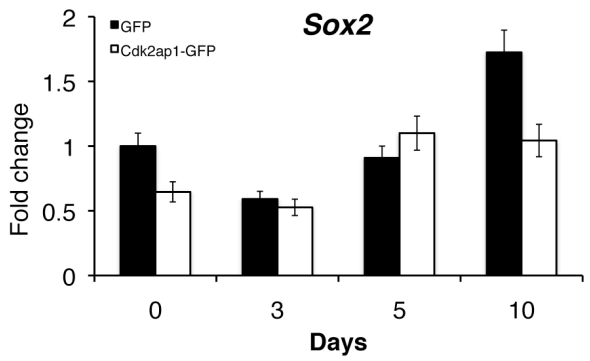


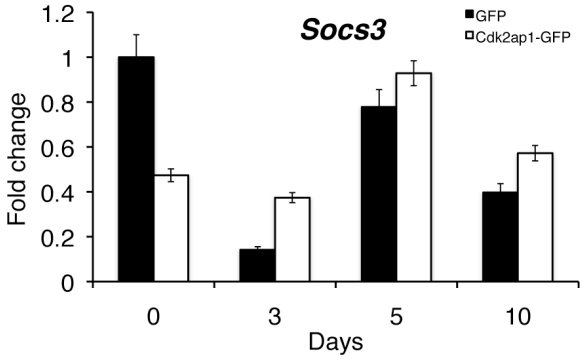
S3. Molecular changes in *Cdk2ap1*$^{-/-}$ mESC embryoid bodies. Gene expression profiles of (**A**) additional stem cell genes (*Sox2*: $p<0.2$, *Socs3*: $p<0.01$, and *Rex1*: $p<0.02$) and (**B**) differentiation-related genes (*Dppa3*: $p<0.04$, *Nestin*: $p<0.03$, *Eomeso*: $p<0.01$, Fgf5: $p<0.01$, Bmp2: $p<0.01$) in differentiating *Cdk2ap1*$^{-/-}$ mESC embryoid bodies are shown in support of Fig. 2. Statistical p value was determined by Student's t-test by comparing values from two different genotypes for each differentiation time point. The error bar represents Standard error.

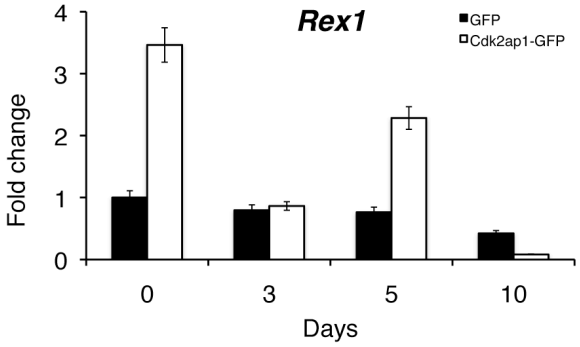


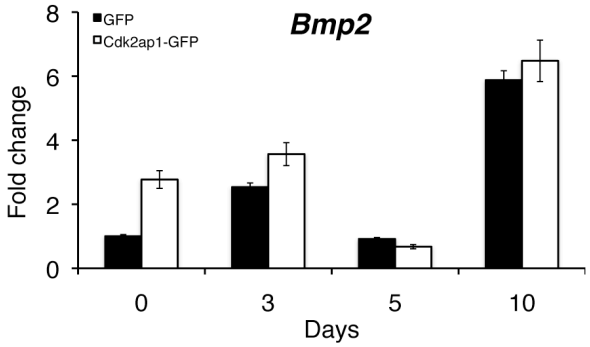
S4. Ectopic restoration of *Cdk2ap1* in *Cdk2ap1*$^{-/-}$ mESCs. To examine the effect of restoring *Cdk2ap1* expression in *Cdk2ap1*$^{-/-}$ mESCs, stable clones were generated by transducing *WT* (*Cdk2ap1*$^{+/+}$) or *Cdk2ap1*$^{-/-}$ mESCs with lentiviral *Cdk2ap1* construct (p*HAGE-Cdk2ap1-IRES-GFP-Puro*). Resulting transduced cells were selected by culturing in the ESC growth medium supplemented with 1μg/ml puromycin for 2 weeks. Control clones were generated by transducing *Cdk2ap1*$^{-/-}$ mESCs with lentiviral p*HAGE-IRES-GFP-Puro*. **A**. The expression of Cdk2ap1 was monitored by Western analysis. **B**. The expression of GFP was monitored by fluorescence microscopy on embryoid bodies from selected clones.

S5-1

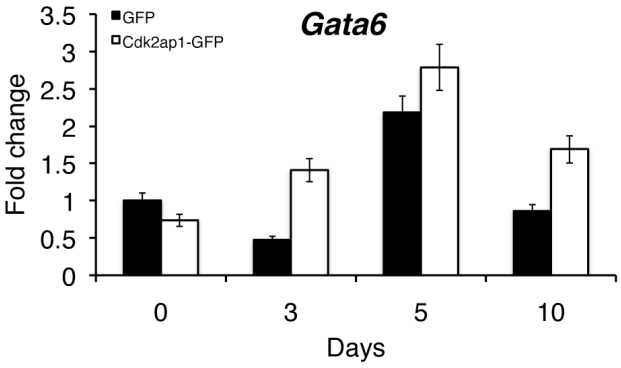

Sox2
GFP
Cdk2ap1-GFP
Fold change
Days
0
3
5
10

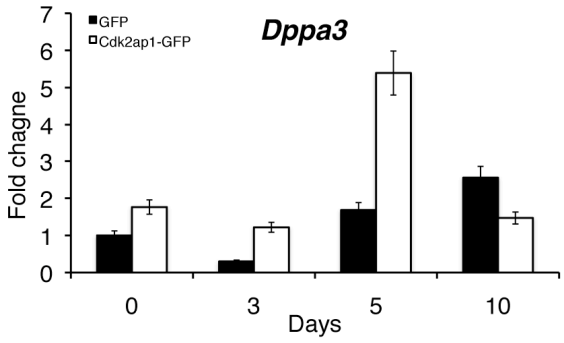

Socs3
GFP
Cdk2ap1-GFP
Fold change
Days

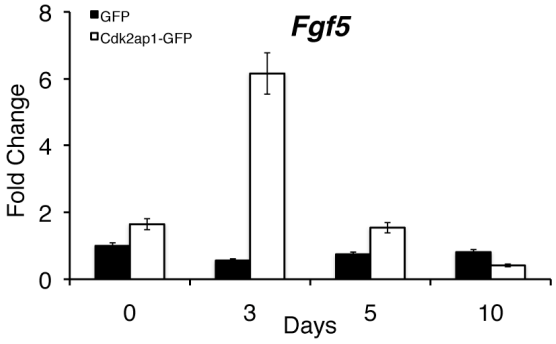

Rex1
GFP
Cdk2ap1-GFP
Fold change
Days

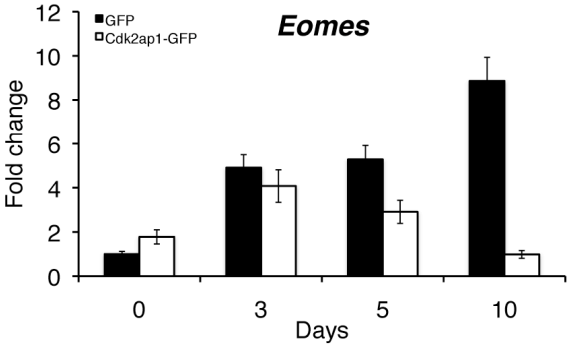

Bmp2
GFP
Cdk2ap1-GFP
Fold change
Days

S5-2

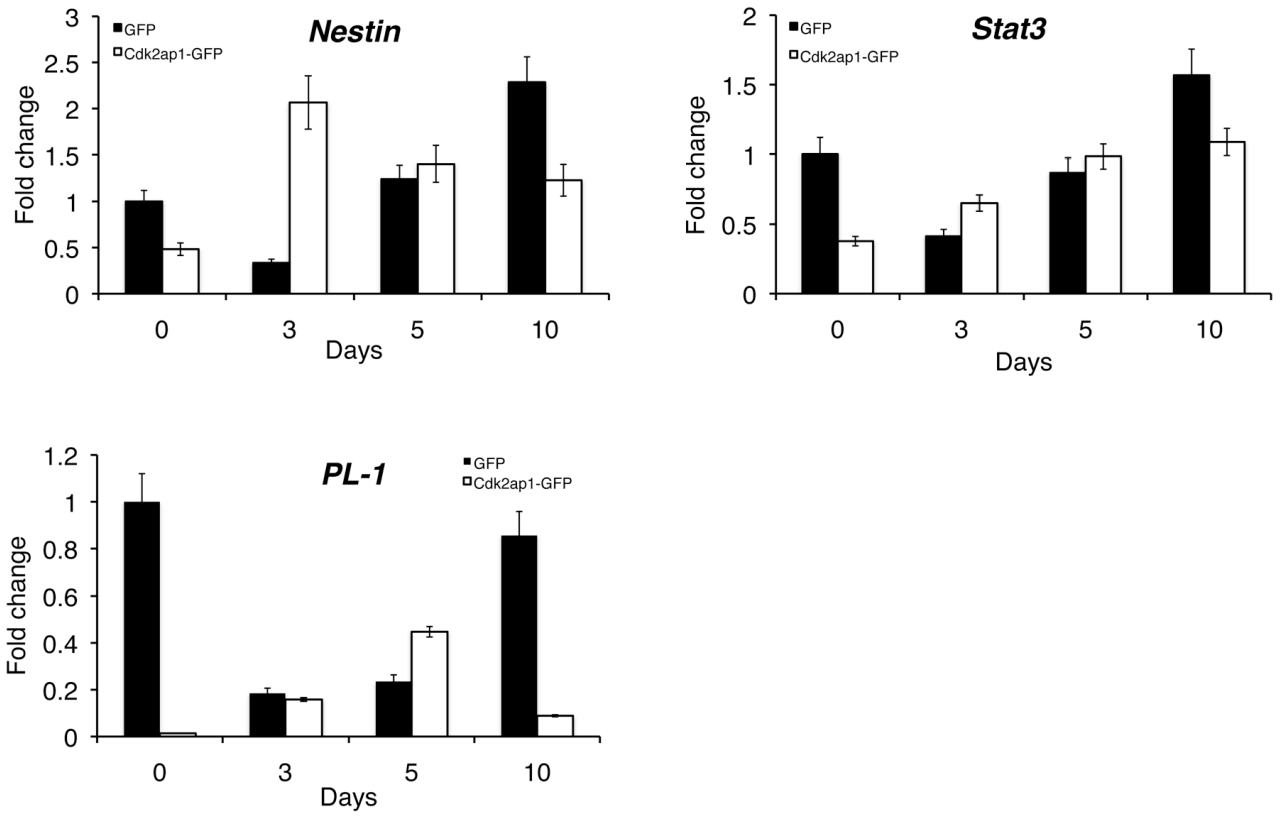

Gata6
GFP
Cdk2ap1-GFP
Fold change
Days


Dppa3
GFP
Cdk2ap1-GFP
Fold chagne
Days

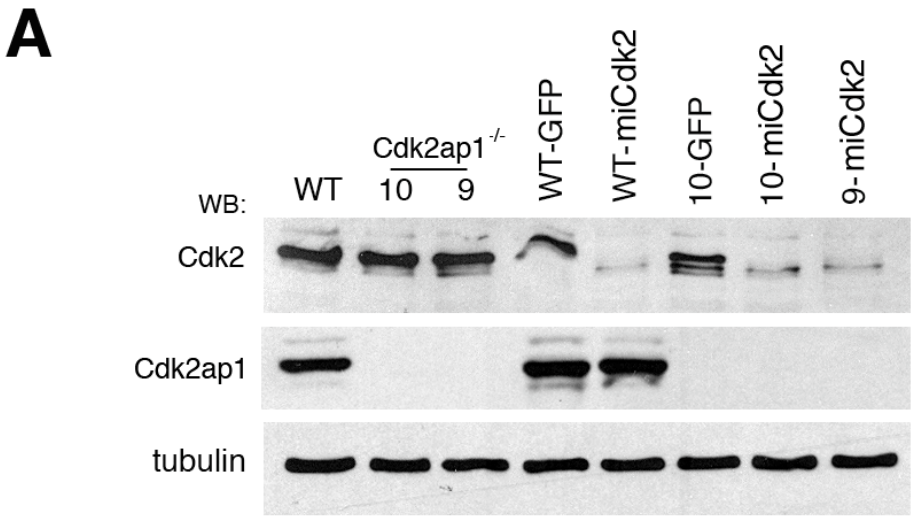

Fgf5
GFP
Cdk2ap1-GFP
Fold Change
Days

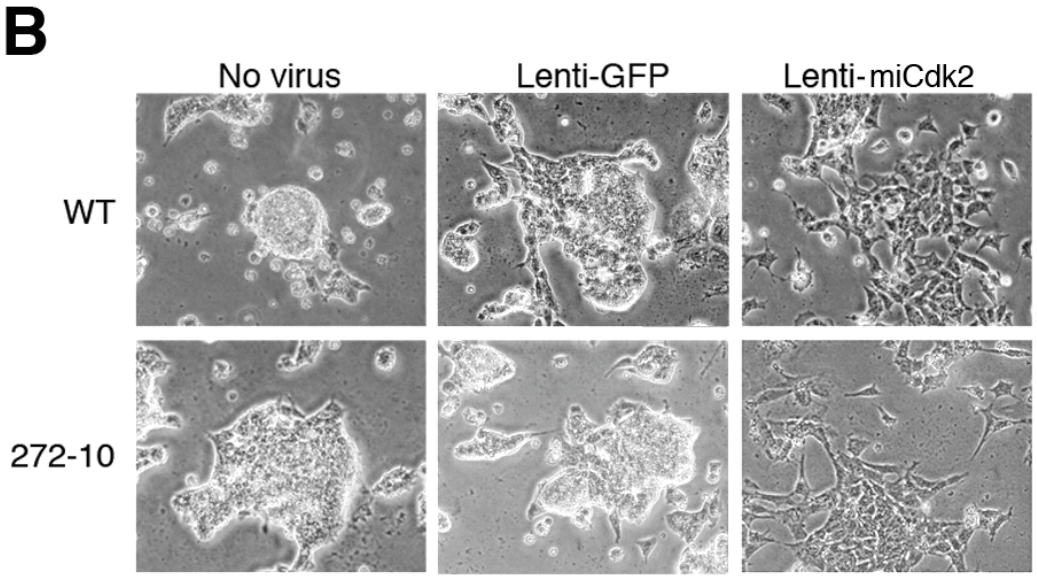

Eomes
GFP
Cdk2ap1-GFP
Fold change
Days

S5-3



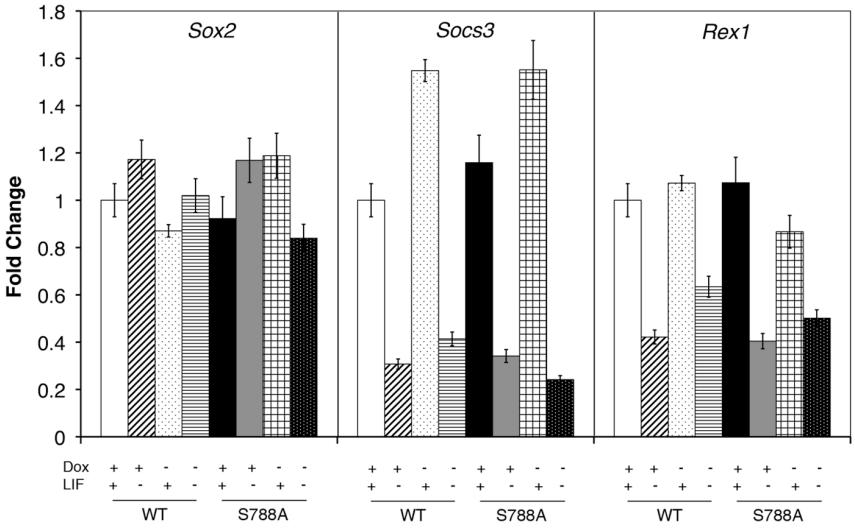
S5 (S5-1, S5-2, and S5-3). The effect of the restored *Cdk2ap1* on the gene expression profiles in *Cdk2ap1*$^{-/-}$ mESCs. Molecular effect of the restoration of *Cdk2ap1* in *Cdk2ap1*$^{-/-}$ mESC embryoid bodies was determined as described in Fig. 2. Supplemental data on additional stem cell genes (*Sox2*: $p<0.001$, *Socs3*: $p<0.04$, *Rex*1: $p<0.01$) and differentiation-related genes (*Bmp2*: $p<0.07$, *Gata6*: $p<0.01$, *Dppa3*: $p<0.03$, *Fgf5*: $p<0.02$, *Eomeso*: $p<0.005$, *Nestin*: $p<0.02$, and *Pl-1*: $p<0.02$) are shown. The error bar represents Standard error. The statistical p value was determined by comparing values against day 0.



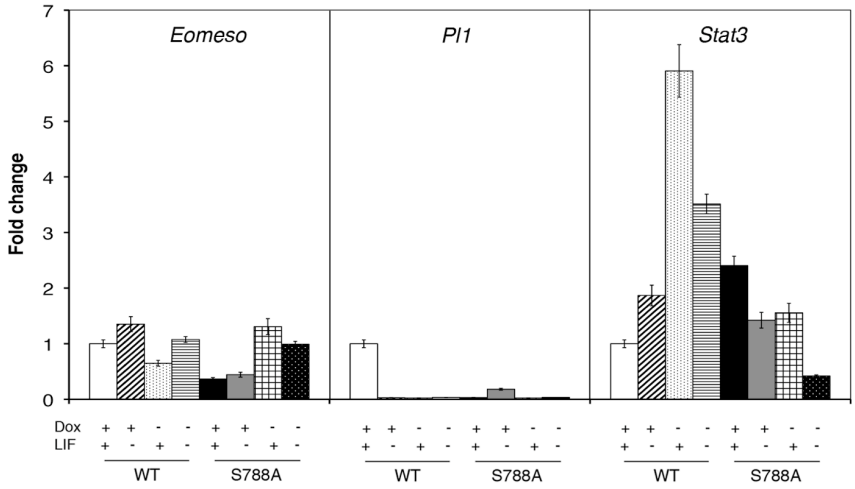


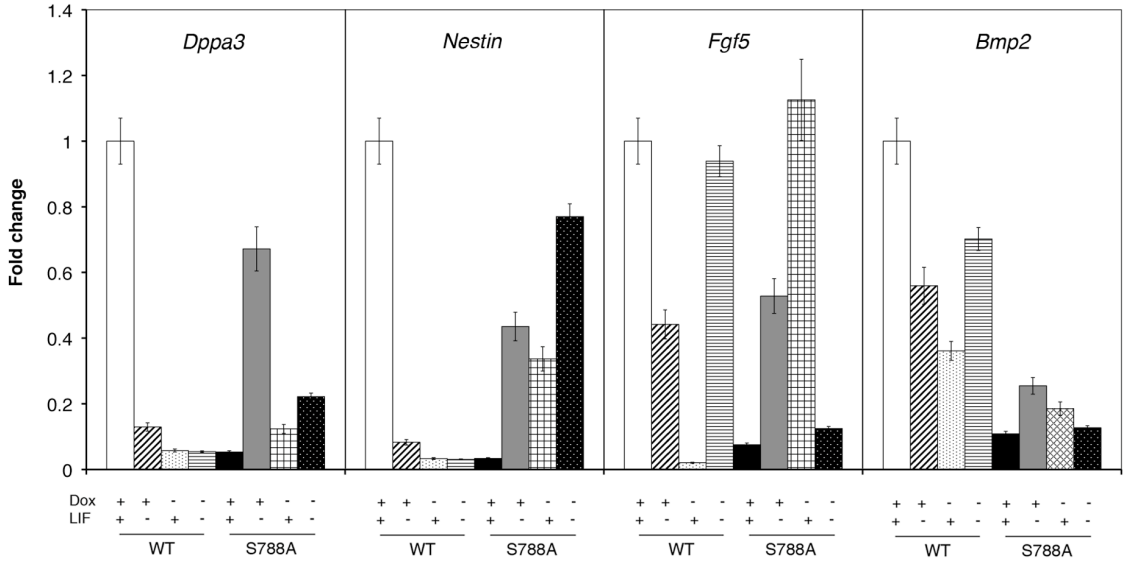
S6. Down-regulation of *Cdk2* resulted in spontaneous differentiation of *Cdk2ap1*$^{-/-}$ mESCs. The significance of Cdk2 in compromised differentiation of *Cdk2ap1*$^{-/-}$ mES cells was examined by knocking down *Cdk2* with lentiviral *Cdk2* microRNA. *Cdk2ap1*$^{+/+}$ (WT) and *Cdk2ap1*$^{-/-}$ (clone 9 and 10) cells were transduced with lentiviral *Cdk2* microRNA and selected by puromycin resistance. **A**. The expression of Cdk2 was monitored by Western analysis. Cells transduced with *Cdk2* microRNA (9-miCdk2 and 10-miCdk2) showed drastic decrease in Cdk2 level compared to no virus (*Cdk2ap1*$^{-/-}$ 9 and 10) and control GFP virus transduced cells (9-GFP and 10-GFP). *Cdk2ap1*$^{+/+}$ cells were also used for comparison (WT, WT-GFP, and WT-miCdk2). The same blot was probed with anti-Cdk2ap1 and also anti-tubulin antibody. **B**. Morphological examination showed differentiation of both *Cdk2ap1*$^{+/+}$ and *Cdk2ap1*$^{-/-}$ mES cells after transduction with *Cdk2* microRNA (lenti-miCdk2) compared to cells transduced with no virus or control virus (lenti-GFP).

A


Sox2
Socs3
Rex1
Fold Change
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Dox
LIF
WT
S788A

B


Eomeso
Pl1
Stat3
Fold change
7
6
5
4
3
2
1
0
Dox
LIF
WT
S788A


Dppa3
Nestin
Fgf5
Bmp2
Fold change
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Dox
LIF
WT
S788A

S7. The molecular significance of the inducible pRb S788A mutant expression in *Cdk2ap1*$^{-/-}$ mESCs. The effect of ectopic expression of pRb S788A mutant in *Cdk2ap1*$^{-/-}$ mESCs was determined as shown in Fig. 5. Expression profiles of additional stem cell genes (*Sox2*: $p<0.01$, *Socs3*: $p<0.05$, *Rex1*: $p<0.4$) and differentiation-related genes (*Eomeso*: $p<0.1$, *Pl-1*: $p<0.4$, *Stat3*: $p<0.1$, *Dppa3*: $p<0.5$, *Nestin*: $p<0.5$, *Fgf5*: $p<0.4$, and *Bmp2*: $p<0.2$) are presented. Statistical p values were determined by Student t-test by comparing values against the samples treated +Dox+LIF. . The error bar represents Standard error.