

## SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1: Cdk2ap1 and Mbd3 interact via amino acids 8-15 in the N-terminal of Cdk2ap1. Western blot analysis for expression showed high levels of endogenous Cdk2ap1 and very low levels of MBD3 proteins showed low levels of endogenous Mbd3 (Suppl. Fig. 1A - lanes 1 and 2 of  $\alpha$ -Mbd3 panel). Immunoprecipitation with anti-Cdk2ap1 antibody showed positive interaction of myc-Mbd3 with Cdk2ap1<sup>WT</sup> and not Cdk2ap1 <sup>$\Delta$</sup>  (Suppl. Fig. 1B). This was reproduced in a reciprocal CoIP using the anti-myc antibody (Suppl. Fig. 1C). As seen in Suppl. Fig. 1D, endogenous Mbd3 was also successfully immunoprecipitated from the wild-type EB using the  $\alpha$ -Cdk2ap1 antibody, further validating the intracellular interaction the two endogenous proteins. Panels 1 and 2 were exposures of the western blot membranes for the indicated times. Differences in migration are possibly due to the high levels of IgG present in the IP lanes. In Suppl. Fig. 1D, panel 2, the two forms of Cdk2ap1, namely the monomer (12kD) and the dimer (24kD) can be seen in the second lane. \* indicates IgG light chain (Suppl. Fig. 1B and 1D) and unknown band (Suppl. Fig. 1C). Similar results were obtained from 293T cell lysates (Suppl. Fig. 1E). Exposure time for detection of Cdk2ap1 in Suppl. Fig. 1E is 15 seconds, and that for Mbd3 is 10 minutes.

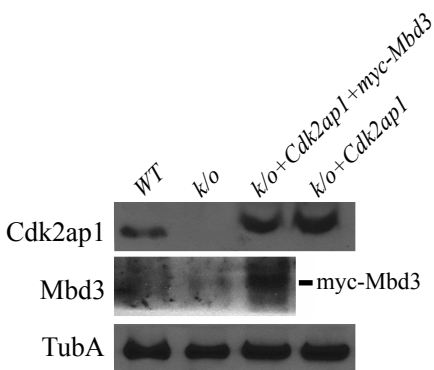
## MATERIALS AND METHODS

Bisulfite Sequencing: Bisulfite sequencing was performed using the EZ DNA Methylation Kit (Zymo Research) according to the manufacturer's protocol. 1 $\mu$ g of genomic DNA isolated from the undifferentiated mESC and EB was used as a template in the CT conversion reaction, followed by column purification and PCR amplification using previously published primer sets (1). The PCR product was further subcloned into pCR2.1 plasmid and transformed into DH5 $\alpha$  cells. Plasmids from individual colonies were isolated and sequenced to identify differentially methylated CpG islands.

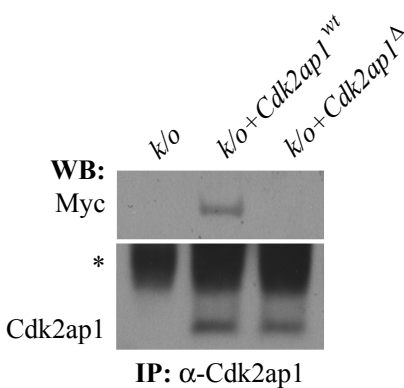
1. Gu, P., Le Menuet, D., Chung, A. C., and Cooney, A. J. (2006) *Molecular and cellular biology* **26**, 9471-9483

# Supplemental Figure 1

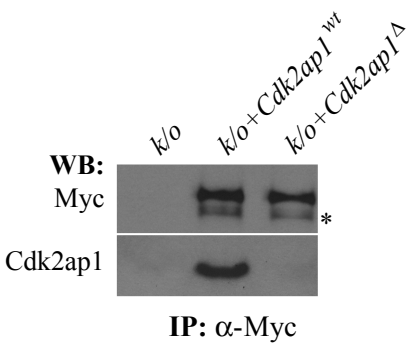
**A**



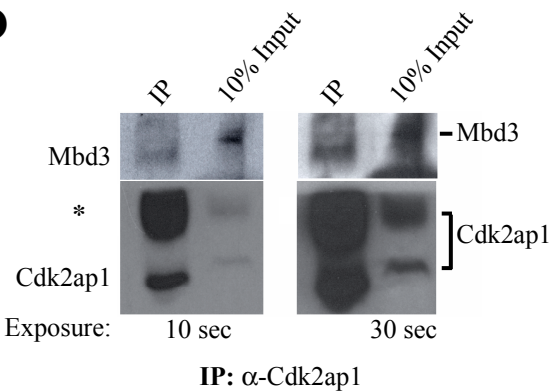
**B**



**C**



**D**



**E**

