

Supplementary Materials for

Nodal Signaling Recruits the Histone Demethylase Jmjd3 to Counteract Polycomb-Mediated Repression at Target Genes

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Table S1. Primers used for RT-PCR analysis.

Table S2. Primers used for ChIP analysis.

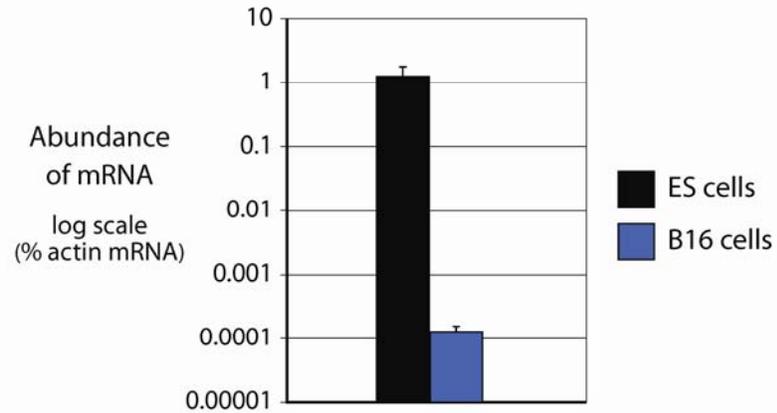


Fig. S1. Quantification of the abundance of *Nodal* mRNA in WT ES cells and in B16 melanoma cells. ES cells are represented by black bars and B16 cells by blue bars. Data are presented in log scale. In ES cells, the abundance of *Nodal* mRNA was on average 1.26% of that of actin; SEM = 0.53; n = 2 experiments. In B16 cells analyzed in parallel, the abundance of *Nodal* mRNA was on average 0.00013% of that of actin; SEM= 0.00003; n = 4 experiments.

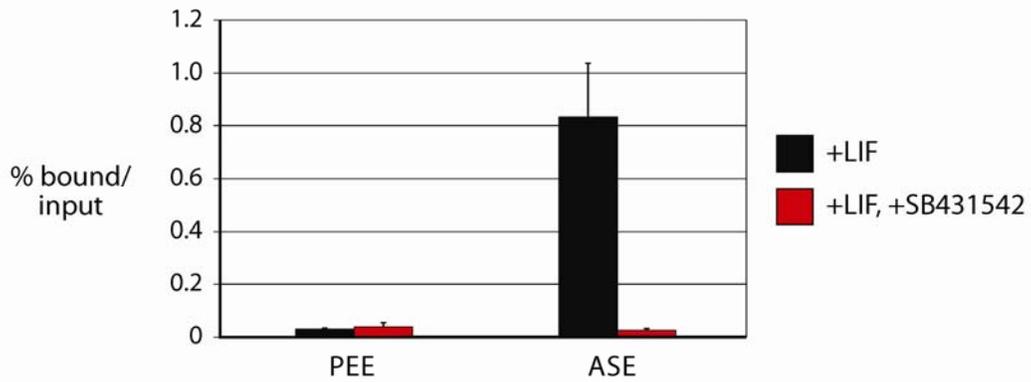


Fig. S2. ChIP analysis of Smads2/3 at the PEE and ASE regions of the *Nodal* locus. WT ES cells were grown in LIF (black bars). The binding of Smads2/3 occurs only in the ASE region. After treatment with SB431542 for 48 hours (red bars), binding was completely lost. Error bars represent the SEM; n = 3 experiments.

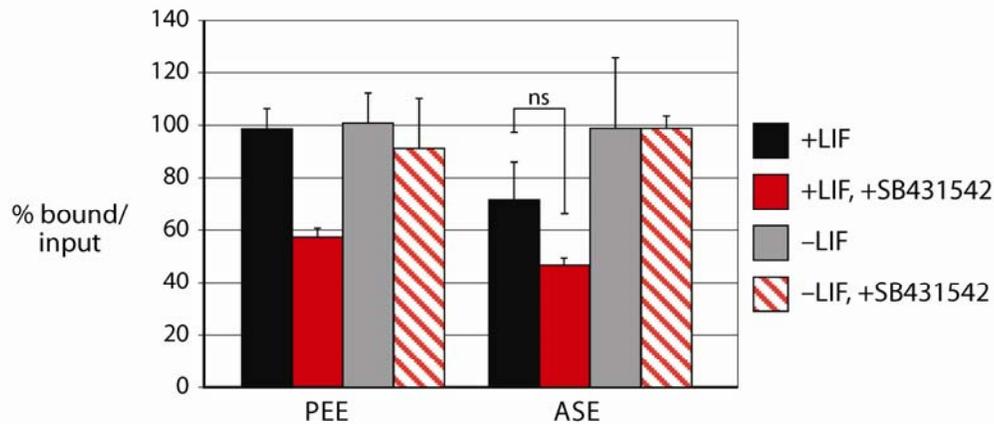
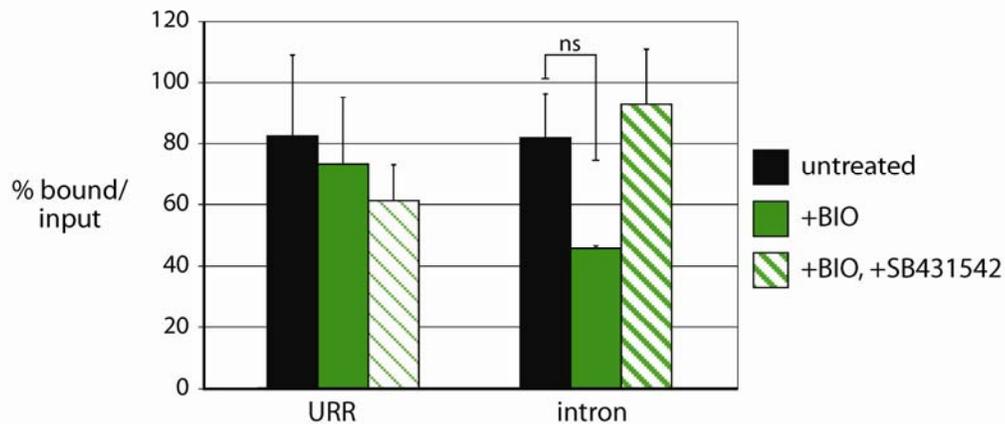
A**B**

Fig. S3. The abundance of total histone H3 at the *Nodal* and *Brachyury* loci. **(A)** ChIP analysis of histone H3 was performed on untreated WT E14 ES cells (black bars), 96 hours withdrawal of LIF (gray bars), and after treatment with SB431542 (5 μ M) for 96 hours in the presence or absence of LIF (red filled bars and red striped bars, respectively), corresponding to the conditions described in Figs. 1 and 2 for the *Nodal* locus. Some reduction in the abundance of H3 in the *Nodal* locus was observed in cells treated with SB431542 in the presence of LIF. **(B)** ChIP analysis of total H3 was performed on WT ES cells left untreated (black bars), treated with BIO for 48 hours (green bars), or treated with BIO in combination with SB431542, corresponding to conditions described in Figs. 3 and 4 for the *Brachyury* locus. There were no substantial changes within the URR under any condition, and there was only a small reduction in total amount of histone H3 upon BIO treatment in the intronic region. Error bars represent the SEM; n = 3 experiments.

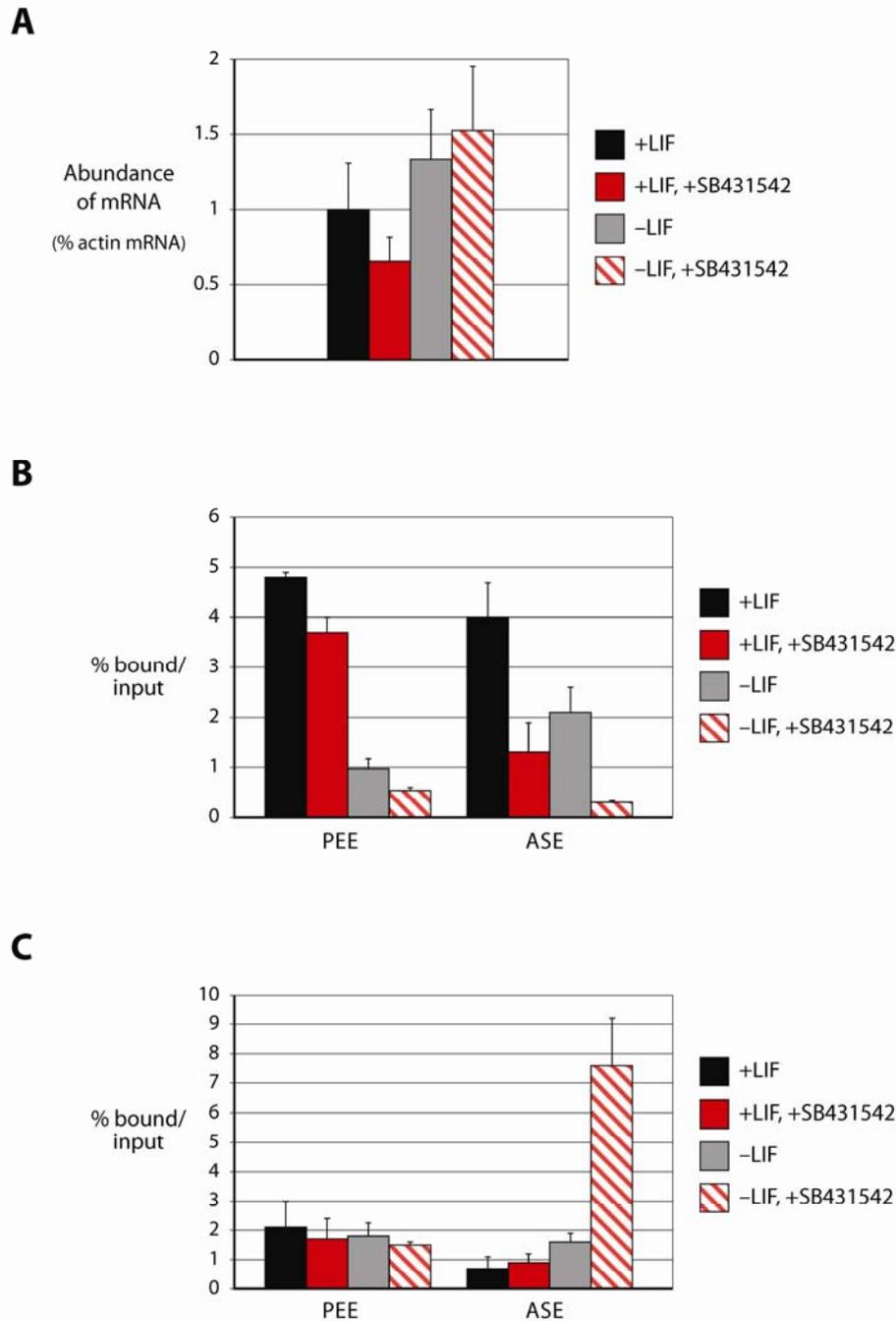


Fig. S4. Changes in *Nodal* expression and chromatin status after 24 hours of signaling inhibition. (A) Quantification of the abundance of *Nodal* mRNA in WT ES cells. ChIP analysis of the amounts of Jmjd3 (B) and H3K27me3 (C) at the *Nodal* locus after 24 hours of the indicated treatments. Whereas there was only a limited effect on the expression of *Nodal*, the abundance of Jmjd3 at the ASE was already reduced at this early time point of SB431542 treatment both in the presence and absence of LIF (red filled bars and red striped bars, respectively). The abundance of Jmjd3 in the absence of LIF and SB431542 was very low; this amount was apparently low enough to enable the accumulation of H3K27me3.

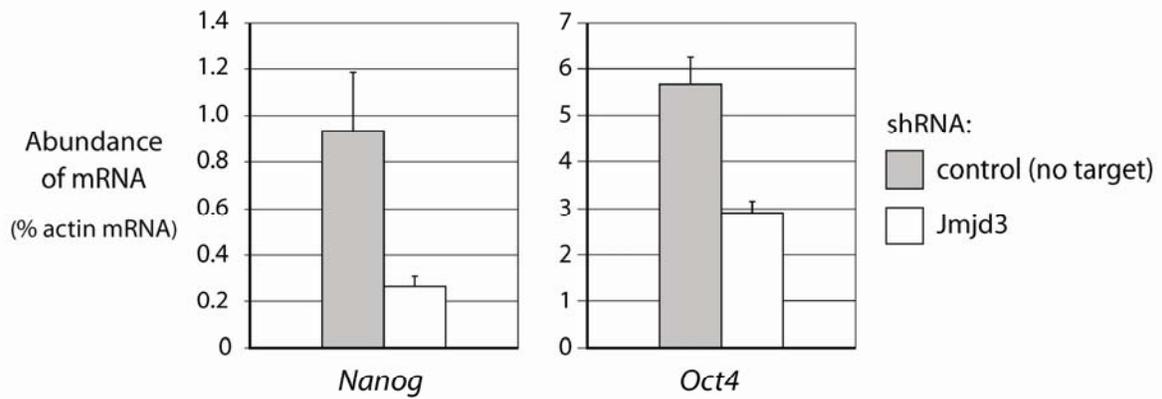


Fig. S5. Expression of *Nanog* and *Oct4* after knockdown of *Jmjd3*. The extent of expression of *Nanog* and *Oct4* after treatment with control shRNA (gray bars) or *Jmjd3*-specific shRNA (white bars) was measured with the same samples as those in Fig. 3C. These data are consistent with previous reports of *Jmjd3* being necessary for maintaining pluripotency and may explain why knockdown cells could not be maintained.

Table S1. Primers used for RT-PCR analysis.

Gene name	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>Nodal</i>	GGGGCTCCTGGATCATCTAC	AAGCATGCTCAGTGGCTTGG
<i>Oct4</i>	GGCGTTCTCTTTGGAAAGGTGTTT	CTCGAACCACATCCTTCTCT
<i>Nanog</i>	CTCTTCAAGGCAGCCCTGAT	CCATTGCTAGTCTTCAACCAC
<i>Brachyury</i>	AACTTTCCTCCATGTGCTGAGAC	TGACTTCCCAACACAAAAAGCT
<i>β-actin</i>	CCATCCTGCGTCTGGACCTG	GTAACAGTCCGCCTAGAAGC

Table S2. Primers used for ChIP analysis.

Gene name	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>Nodal</i> PEE	TGATGTGAGTGGGGAATGC	TGGGTGACTGAACGAAGGTC
<i>Nodal</i> ASE	CCACATCTTCTAATCCGGTCTG	ACCCTTCAAGAGAGGGTCAC
<i>Brachyury</i> URR	CGGCCAGTCTGATATGGCCGCGCA	CCCGCAAGGCGCGACAAGAG TAA
<i>Brachyury</i> intron	GCCATAGGTGAGCATTGGTT	GGTGGCAACTTGGAGTGAGT