Neri et al. Supplementary Information

Supplementary Figure 1.
(A) Flow cytometry cell cycle analysis of 3T3-MycER cells starved and arrested in G1/G0 for 36 hours in 0.2% FCS medium and then treated for indicated time with 10% FCS and with or without OHT and with OHT and CHX. ChIP analysis at the transcription start site of
(B) ChIP analisys at the TSS of Suz12, Ezh2, and Eed in mouse 3T3-MycER fibroblasts arrested by serum starvation and then treated for 2, 6, or 12 hours with FCS with or without OHT using the E2F1 antibody. Statistical analysis was performed with respect to non induced cells. * p-value < 0.01.
(C) Western blot analysis of mouse NIH-3T3 fibroblasts expressing exogenous Myc-ER protein after OHT treatment. Cells arrested by serum starvation were treated with 10% FCS with or without OHT.
(D) ChIP analisys of Suz12, Hoxa9 and Nucleolin in mouse 3T3-MycER fibroblasts using the IgG, TIP60 and MOF antibodies. Statistical analysis was performed with respect to IgG. * p-value < 0.01.
(E) ChIP analisys of Suz12, Ezh2, and Eed in mouse ESC sh GFP, sh c-Myc, sh N-Myc or dKD #1 using IgG, c-Myc or N-Myc antibodies as indicated. Statistical analysis was performed with respect to sh GFP samples. * p-value < 0.01.

Supplementary Figure 2.
(A) Western blot analysis of c-Myc and N-Myc levels in control mouse ESC E14 or ESC silenced with the indicated constructs.
(B) Flow cytometry 2-dimensional cell cycle analysis in control mouse ESC E14 or ESC silenced with the indicated constructs.
(C) Mean time of cell replication in double c-Myc/N-Myc knockdown (dKD#2) ESC. The data are the mean of three independent experiments. * p-value < 0.01.
(D) Growth of control ESC cells sh GFP (dark blue line), dKD #1 (yellow line), dKD #2 (light blue line) and without LIF (pink line).
(E) Flow cytometry viability assay performed using LIVE/DEAD® Cell Viability Assays from Invitrogen on sh GFP, dKD #1 and dKD #2 ESC. Live cells percentages are indicated. The mean±SD of 3 experiments is shown.
(F) Flow cytometry cell trace analysis using dye dilution. Red lines indicate the control (sh GFP) stem cell proliferative tracing at the times indicated. The blue lines indicate dKD #2 stem cell proliferative tracing.
(G) Phosphatase Alcaline (AP) staining of ESC colonies. The cells were plated as single cells to measure the capacity to form AP+ colonies in control cells (sh GFP) or in double knockdown (dKD #2). Two different magnifications are shown (scale bar: top panels 50μm, bottom panels 200μm).
(H) Quantification of AP+ colonies in control cells (shGFP) and in double silenced cells (dKD #2). * p-value < 0.01.

Supplementary Figure 3.
(A) Cell size analysis. In red control sh GFP, in blue ESC cultured without LIF (left), dKD #1 (middle), or dKD #2 (right). The size increase of dKD #1 or #2 cells correlates with that observed in mouse stem cells cultured in the absence of LIF for 72 hours. Flow cytometry analysis of cell size was performed using the FSC (forward scattering cell).
(B) Immunofluorescence of SSEA1 (top panels) and Oct3/4 (bottom panels) expression in control shGFP cells (first and third column), dKD #1 cells (second column) and dKD #2 cells (fourth column). DNA staining with ToPro shows cells nuclei.
(C) Flow cytometry analysis of Oct3/4 (top panels), SSEA1 (central panels) and E-Cadherin (bottom panels) expression in control sh GFP cells (first and third column), dKD #1 cells (second column) and dKD #2 cells (fourth column).
(D) Flow cytometry analysis of SSEA1 (top panels) and E-Cadherin (bottom panels) expression in mouse embryonic stem cells cultured in the absence of LIF for 0 (left panels), 2 (central panels) and 4 (right panels) days.

Supplementary Figure 4.
(A) Western blot analysis of c-Myc, N-Myc, and PRC2 protein levels in total cell extracts from

ESC grown in presence of LIF, in the absence of LIF, in the absence of LIF expressing either activated Myc-ER, or ectopic PCR2. β-actin was used as a control loading.
(B) Western blot analysis of Myc-ER (using anti-ERα antibody) in cytoplasmatic or nuclear extracts in ESC grown as above. β-actin and histone H3 were used as a control loading.
(C) H3K27 (top panel) and H3K4 (bottom panel) trimethylation quantification by colorimetric quantification assay (see materials and methods) in mouse ESC grown as indicated. Data are presented as mean±SD of three independent experiments * p-value < 0.01.
(D) H3K4 methyltransferase activity assay in ESC grown as in A. Data are presented as the mean±SD of three independent experiments.
(E) ChIP analysis of the promoters regions of the indicated bivalent genes in ESC grown as in A using the H3K4me3 antibodies. Purified rabbit IgG were used as negative control. Data are presented as the mean±SD of three independent experiments. Statistical analysis was performed with respect to non treated ESC. * p-value < 0.01.
(F) Cell growth of the control sh GFP, dKD #1 and dKD #1 + PRC2 ESC cultured as in Figure 6.
(G) Mean time of cell replication in control sh GFP, dKD #1 and dKD #1 + PRC2 mESC. The data are the mean of three independent experiments.

Supplementary Figure 5.
(A) Western blot analysis of c-Myc, N-Myc, and PRC2 core proteins in ES cells cultured with or without LIF for 3 days, and of control sh GFP or dKD #1 or dKD #2 cells. β-Actin antibody was used as loading control.
(B) Western blot analysis of control cells (sh GFP) or dKD (dKD #2) ESC. H3 antibody was used as loading control.
(C) H3K27 (left panel) and H3K4 (right panel) trimethylation quantification in control (sh GFP) or dKD (dKD #2) ESC by colorimetric quantification assay (see materials and methods). Data are presented as mean±SD of three independent experiments. * p-value < 0.01.