
A. Schematic representation of the Eed gene locus, targeting vector (above) and targeted allele. Exons (black boxes), loxP sites (triangles), WD40 domains encoded by exons 3 to 5, the position of probes (open bars), and GC-rich region (gray crescent) are indicated. B. Bars represent the relative colony forming efficiency of indicated ES cell lines (clone 36 was set to 1). Error bars represent the standard deviation. C. Proliferation and self-renewal is not dependent on Eed. Cumulative cell numbers of indicated ES cell lines cultured in the presence of LIF for 3 days. To correct for the colony formation defect in Eed<sup>−/−</sup> ES cells 36<sup>Eed−/−</sup> and ΔSX cells were seeded in an appropriate ratio compared to clone 36 cells. D. Normal proliferation of differentiated Eed deficient cells under adherent cell culture conditions. The graph shows cumulative cell numbers for clone 36, 36<sup>Eed−/−</sup>, 36<sup>EedTG</sup>, ΔSX and ΔSX<sup>Eed−/−</sup> ES cells differentiated with all-trans-retinoic acid for 9 days. E. Impaired embryoid body formation of Eed deficient ES cells. Representative images of embryoid bodies derived from clone 36 and 36<sup>Eed−/−</sup> ES cells after one week. F. Expression of the EGFP-Eed fusion protein in 36<sup>EedTG</sup> cells causes restoration of PRC2 function. Immunofluorescence / RNA FISH showing recruitment of the EGFP-Eed fusion protein (green) in 36<sup>EedTG</sup> cells by Xist restoring efficient tri-methylation of histone H3K27 (red). Below, combined immunofluorescence / RNA FISH showing Mph1 recruitment by Xist in 36<sup>EedTG</sup> cells. DNA was stained with DAPI (blue).
Supplementary Figure 1