Pasini et al. Supplementary information

Supplementary Materials

Embryo harvesting

Successful mating was controlled by the presence of a vaginal plug referred to as 0.5 dpc. Pregnant females were sacrificed by cervical dislocation. Whole 8.5 dpc embryos or entire 7.5 and 8.5 dpc deciduae were extracted from pregnant females. 8.5 dpc embryos were photographed and analyzed as indicated. Deciduae were fixed for 3-4 hours at 4°C in 4% Para-Formaldehyde/PBS and dehydrated at 4°C as follows: 1h wash in PBS, 2h wash in 50% EtOH in PBS, O/N incubation in 70% EtOH, 2h incubation in 100% EtOH. Deciduae were stored in 100% EtOH at –20°C.

The deciduae were paraffin-embedded as follows: 2h wash at RT in Xylene/EtOH 1:1, 2h wash at RT in 100% Xylene, 2h wash at 60°C in Xylene/Paraffin 1:1, O/N incubation at 60°C in 100% Paraffin and subsequently embedded in 100% Paraffin.

Histological analysis

Embryo sections (4μm) were stained with Hematoxylin and Eosin. Suz12 IHC staining were performed on consecutive sections as follows: paraffin-embedded sections were incubated in 0.25 mM EDTA pH 8.0 at 95°C for 50 minutes and subsequently at RT for 20 minutes for antigen retrieval. Then, they were incubated in blocking solution (1x TBS, 2% BSA, 2% goat serum- 0.02% Tween20) at RT for 60 minute, and finally incubated for 2 hours at RT in a humid chamber with primary antibodies diluted in blocking solution: anti-Suz12 1:100, anti BrdU 1:50 anti-Phospho (Ser10) Histone H3 (1:1000), anti-di/tri Methyl Histone H3K27 (1:50). Staining was visualized using anti rabbit or mouse Envision™ kit (DAKO) according to the manufacture's instructions. Sections were counterstained in haematoxylin. TUNEL assays were performed using Apoptag® Peroxidase Plus Kit, Chemicon (Cat N# S7101) following the manufacture's suggestions.

Percentage of both BrdU and P-H3 (Ser15) positive cells in both WT and KO 7.5 and 8.5 dpc embryos was determined as follows: Positive cells were counted independently on two or more sections per embryo. Two different KO and WT embryos at each day of development were counted. Separate counts were averaged and the results were plotted. SD were determined and presented in the figures. The total number of cells counted is indicated in each figure legend.