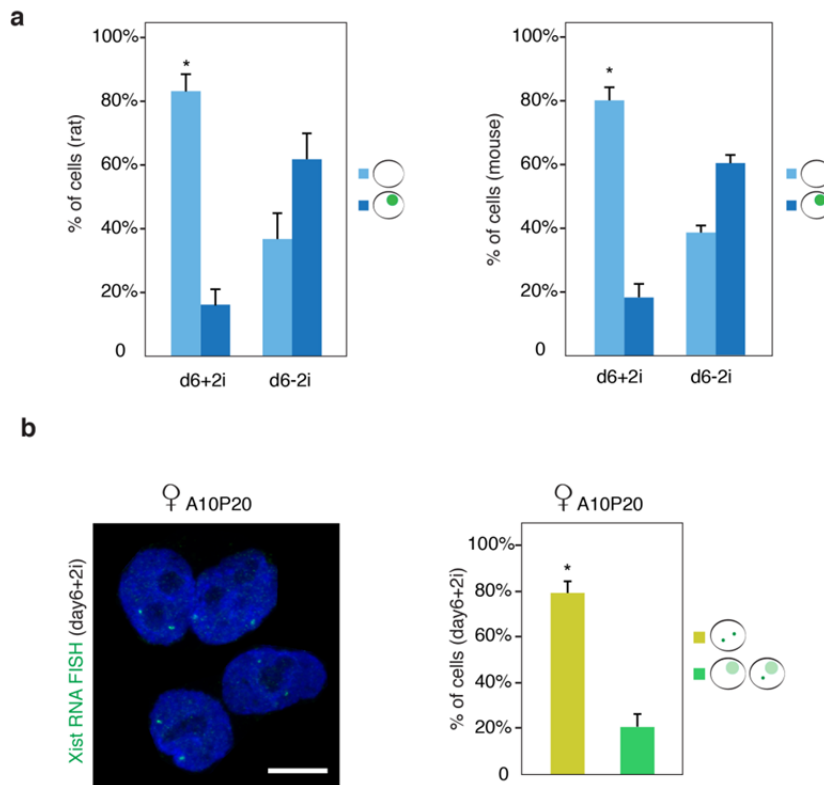


**Supplemental Information to:**

**A novel approach to differentiate rat embryonic stem cells *in vitro*  
reveals a role for RNF12 in activation of X chromosome inactivation**

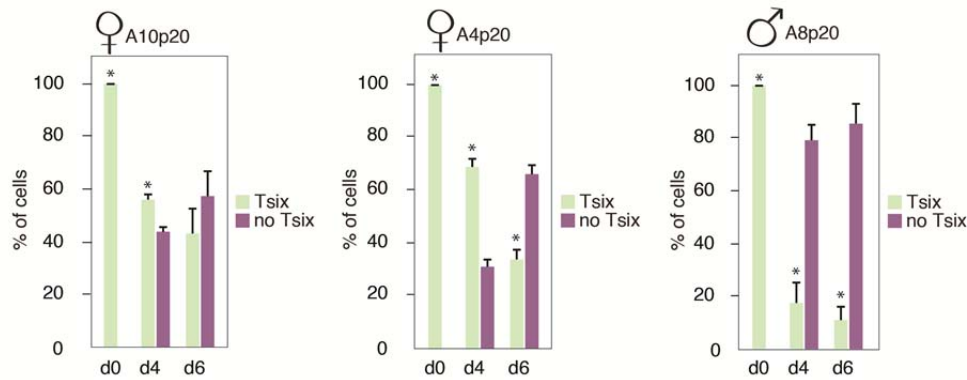
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**Sup. Figure 1. Delayed XCI in the presence of MEK and GSK3β inhibitors.**

(a) Quantification of H3K27me3 immunofluorescence analysis in female clone A10p20 (left) and in mouse ES cells (F12 strain, right) at day 6 of neural differentiation in the presence (+2i) or absence (-2i) of MEK and GSK3β inhibitors. Error bars indicate standard deviation of two experiments. Asterisks indicate significant differences with the following p-values: p-value (rat\_d6+2i)<sub>cloud</sub> VS no cloud=0.008 and p-value (mouse\_d6+2i)<sub>cloud</sub> VS no cloud=0.006.

(b) Left: Representative image of Xist RNA FISH analysis performed at day 6 of neuronal differentiation of female clone A10p20 in the presence of MEK and GSK3β inhibitors. Xist is shown in FITC (green) and DNA is stained with DAPI (blue). Scale bar represents 10 μm. Right: Quantification of cells showing pinpoints or clouds at day 6 of neuronal differentiation in the presence of MEK and GSK3β inhibitors. Error bars represent standard deviation between two experiments. Asterisk shows significant difference with p-value<sub>pinpoints</sub> VS clouds=0.008.



**Sup. Figure 2. Quantification of Tsix pinpoints during neuronal differentiation.**

Quantification of relative number of cells with *Tsix* RNA signals (pinpoint) and without (no pinpoint) in A10p20 (left graph), A4p20 (middle graph) female and A8P20 (right graph) male rESCs at day 0, 4 and 6 neuronal differentiation. More than 100 nuclei were counted for each time point and for each experiment. Error bars indicate standard deviation between two experiments. Asterisks indicate significant difference between the two groups (signal (*Tsix*) versus no signal (no *Tsix*)) within each differentiation time point. P-values are provided: p-value<sub>A10-d0 Tsix VS no Tsix</sub><0.001, p-value<sub>A10-d2 Tsix VS no Tsix</sub>=0.037, p-value<sub>A4-d0 Tsix VS no Tsix</sub><0.001, p-value<sub>A10-d2 Tsix VS no Tsix</sub>=0.006, p-value<sub>A10-d6 Tsix VS no Tsix</sub>=0.01, p-value<sub>A8-d0 Tsix VS no Tsix</sub><0.001, p-value<sub>A8-d2 Tsix VS no Tsix</sub>=0.009, p-value<sub>A8-d6 Tsix VS no Tsix</sub>=0.01.

