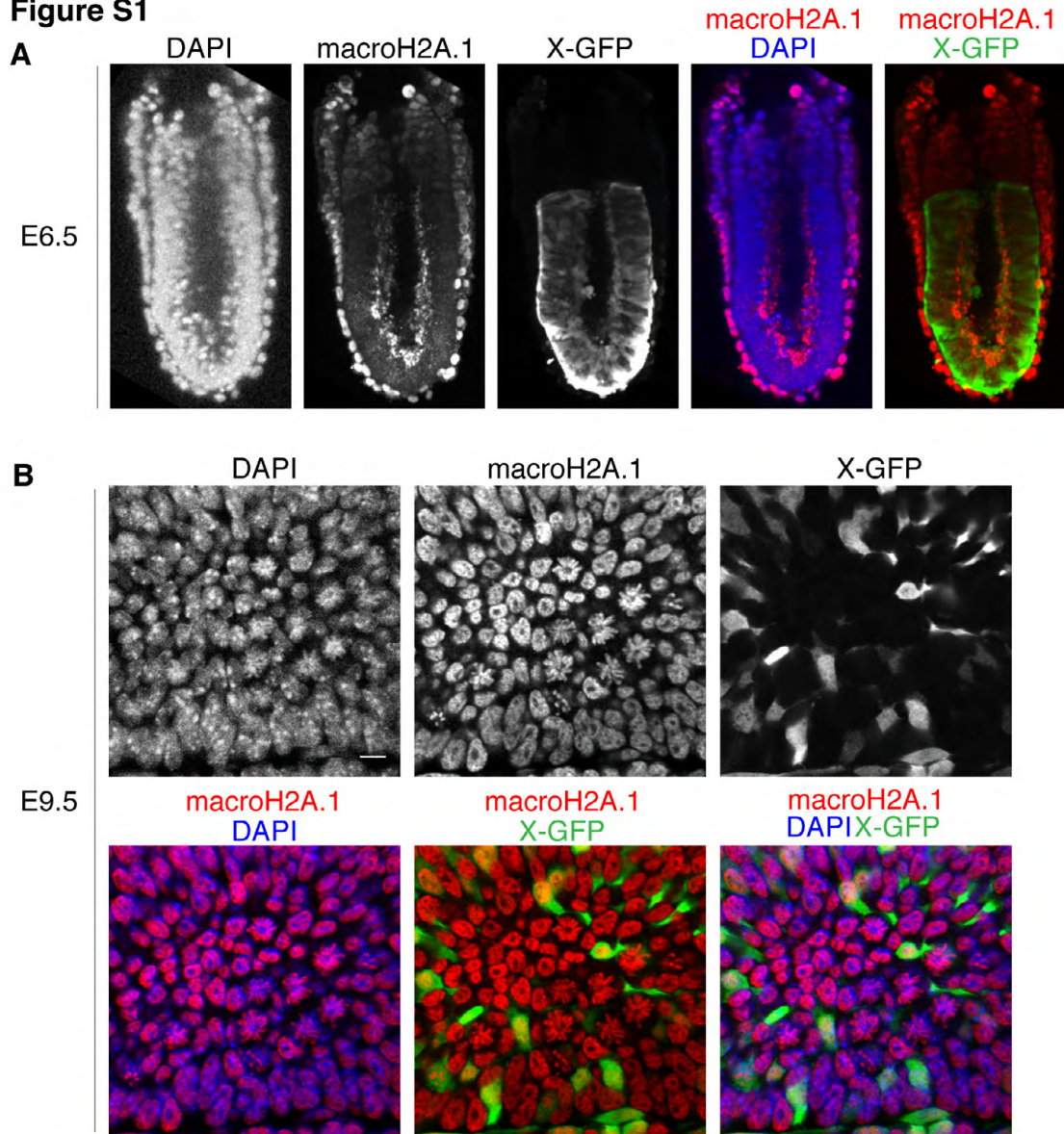
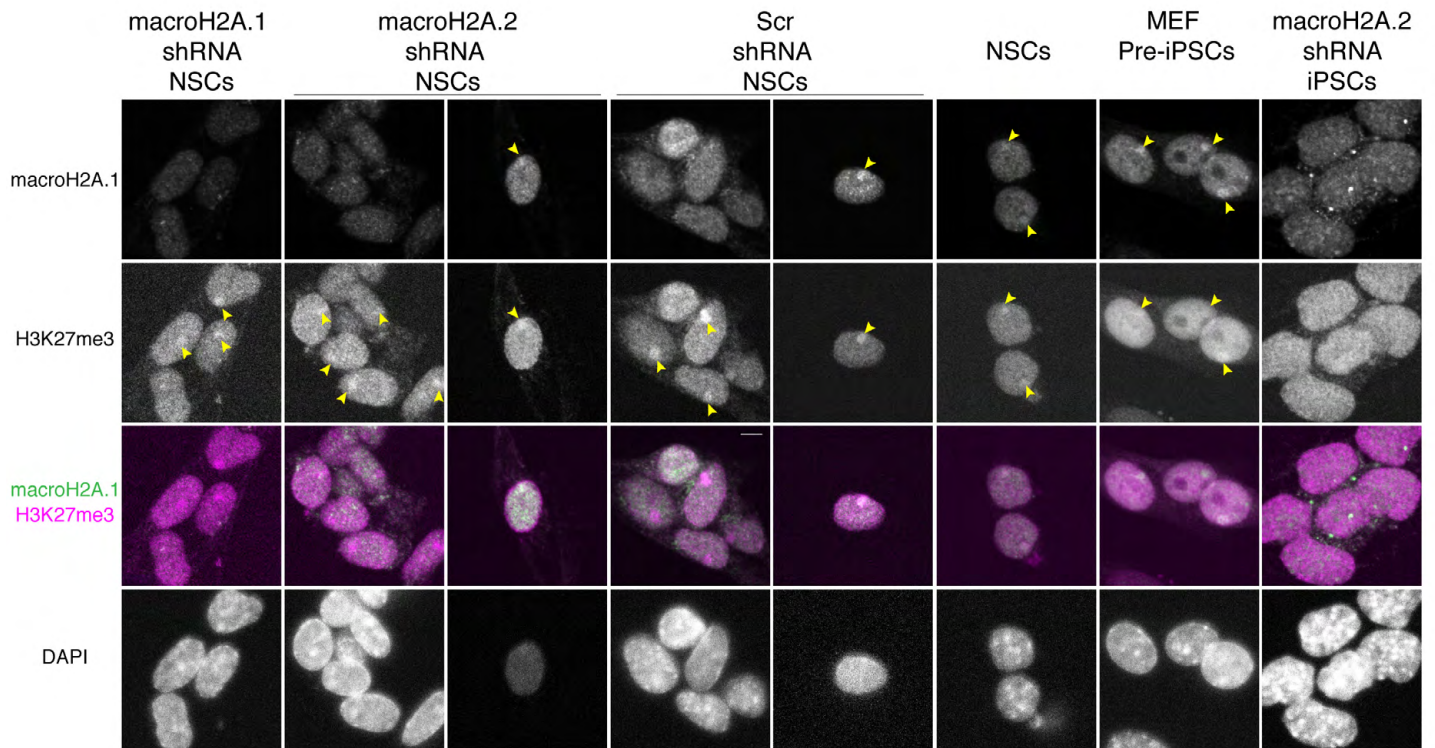


**Figure S1**

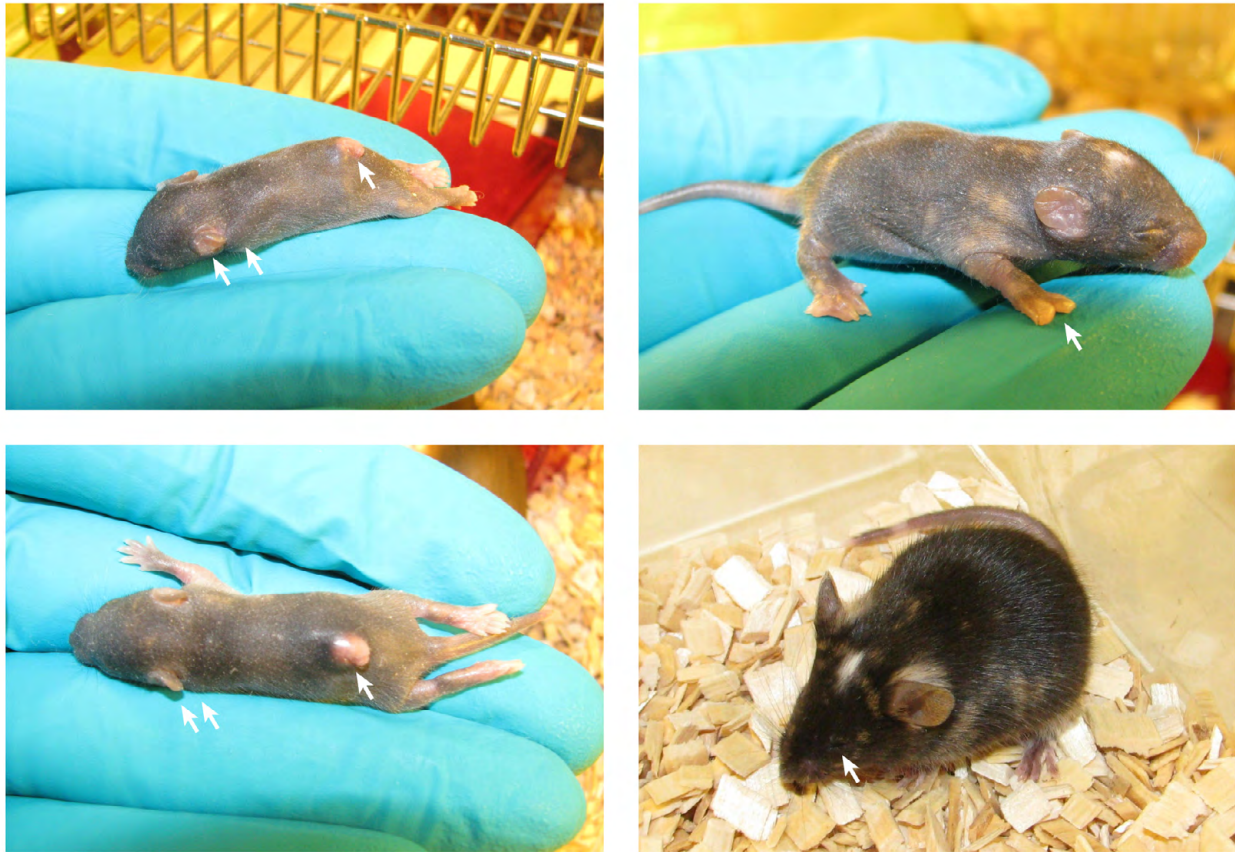


**Fig. S1. (A)** Example of E6.5 female *X-GFP* mouse conceptus wholemount immunofluorescence against macroH2A.1 (red) and GFP (green). MacroH2A.1 is highly expressed in the visceral endoderm and to some extent in the extra embryonic ectoderm but is not detected in the epiblast, precursor of all somatic lineages (mosaic *X-GFP* expression due to random X chromosome inactivation). DAPI is in blue. Images are projected confocal Z-sections. **(B)** E9.5 *X-GFP* mouse embryo immunofluorescence against macroH2A.1 (red) and GFP (green) showing cells of a somite. Nuclear macroH2A.1 is detected in all cells. DAPI is in blue. Images are projected confocal Z-sections. Scale bar = 5  $\mu$ m.



**Fig. S2.** Immunofluorescence analysis of macroH2A.1 and H3K27me3 in NSCs, pre-iPSCs and iPSCs. Yellow arrowheads indicate the inactive X chromosome. MacroH2A.1 is in green and H3K27me3 in magenta in merge panels. Scale bar = 5  $\mu$ m.

**Figure S3**  
macroH2A.1+.2 shRNA iPSCs chimeras



**Fig. S3.** Examples of developmental defects seen in macroH2A.1 + .2 shRNA iPSCs chimeras.

**Table S1**

<b>Applied Biosystems Custom Taqman probes</b>	
<b>Gene name</b>	<b>Probe</b>
<i>Oct4</i> DE	AATCTGCTATTGAGGAAGC
<i>Oct4</i> PE	AAGCAGGGTATCTCC
<i>Oct4</i> PP	TGTCCGGTGACCCAAG
<i>Sox2</i> RR1	AGCAATGCTGAGAAAT
<i>Sox2</i> PP	ACAGGCGTGCGCCGT
<i>Sox2</i> RR2	AGACTCTAAAAGAATTTCCCG
<i>G3PDH</i> PP	AGCCCACACGCTTG
<i>C-Jun</i> Promoter	CCAATGGGAAAGCC
<i>B-globin</i> PP	CGTAGAGCCACACCCT
<i>Thy1</i> PP	TTCCCTGGAGACCTGT
<b>Primers</b>	
<b>Gene name</b>	<b>Sequence</b>
<i>Oct4</i> DE-F	CCTCTCGTCCTAGCCCTTCCT
<i>Oct4</i> DE-R	GAAGCCGCCAAGTTCACAA
<i>Oct4</i> PE-F	CCGGAGTCCCTGGAGGAA
<i>Oct4</i> PE-R	CTCCTCAAAGACAGAGCCTCAGA
<i>Oct4</i> PP-F	TCCGAGCAACTGGTTTGTGA
<i>Oct4</i> PP-R	TTTCAACCTTCAAGGTCCTCTCA
<i>Sox2</i> RR1-F	GGTGGTCGTCAAACCTCTGCTAAT
<i>Sox2</i> RR1-R	CCTCCTCTCCTAATCTCCTTATGGA
<i>Sox2</i> PP-F	TGGTGCTGTTTACCCACTTCCT
<i>Sox2</i> PP-R	CGCCCCCGTTTTTCAG
<i>Sox2</i> RR2-F	CAGGTTCCCCTCTAATTAATGCA
<i>Sox2</i> RR2-R	CATTACCACGTGAATAATCCTATATGC
<i>G3PDH</i> PP-F	TCCCCTCCCCCTATCAGTTC
<i>G3PDH</i> PP-R	GACCCGCCTCATTTTTGAAA
<i>B-globin</i> PP-F	TGTCATCACCGAAGCCTGATT
<i>B-globin</i> PP-R	TGTGAGCAGATTGGCCCTTAC
<i>C-Jun</i> Promoter-F	CCGCCCCCTGAGAAC
<i>C-Jun</i> Promoter-R	CAATCCCTAAAATAGCCCATGAT

<i>Thyl</i> PP-F	GGCTGCTTCTGATTATTTAGTTTGTC
<i>Thyl</i> PP-R	ACCCACCATACGCCCTTATG

**Table S2**

<b>Applied Biosystems Taqman probe/assays</b>	
<b>Gene name</b>	<b>Probe ID</b>
GAPDH	4352339E
Nanog	Mm02384862_g1
Rex1	Mm03053975_g1
Klf4	Mm00516104_m1
Oct4	Mm00658129_gH
T / Brachyury	Mm01318252_m1
Gata4	Mm00484689_m1
<b>Primers and custom probes</b>	
<b>Gene name</b>	<b>Sequence</b>
Retroviral Oct4-F	TGGTACGGGAAATCACAAGTTTGTA
Retroviral Oct4-R	GGTGAGAAGGCGAAGTCTGAAG
Retroviral Oct4-probe	FAM-CACCTTCCCCATGGCTG-MGB
Retroviral Klf4-F	TGGTACGGGAAATCACAAGTTTGTA
Retroviral Klf4-R	GAGCAGAGCGTCGCTGA
Retroviral Klf4-probe	FAM-CCCCTTACCATGGCTG-MGB
Endogenous Oct4-F	TTCCACCAGGCCCCC
Endogenous Oct4-R	GGTGAGAAGGCGAAGTCTGAAG
Endogenous Oct4-probe	FAM-CCCACCTTCCCCATGGCT-MGB
<b>Primers used with SYBR green</b>	
<b>Gene name</b>	<b>Primer sequence</b>
GAPDH-F	CCCACTAACATCAAATGGGG
GAPDH-R	CCTTCCACAATGCCAAAGTT
mH2A1-F	CACCATGTGAGCCGCGGGGAAG (Kapoor et al., 2010)
mH2A1-R	GTTGGCGTCCAGCTTGGCCA (Kapoor et al., 2010)
mH2A2-F	GGACCAAAGGCAAGTCAGAG (Kapoor et al., 2010)
mH2A2-R	TCCGAGGTGGAATTTGATGT (Kapoor et al., 2010)