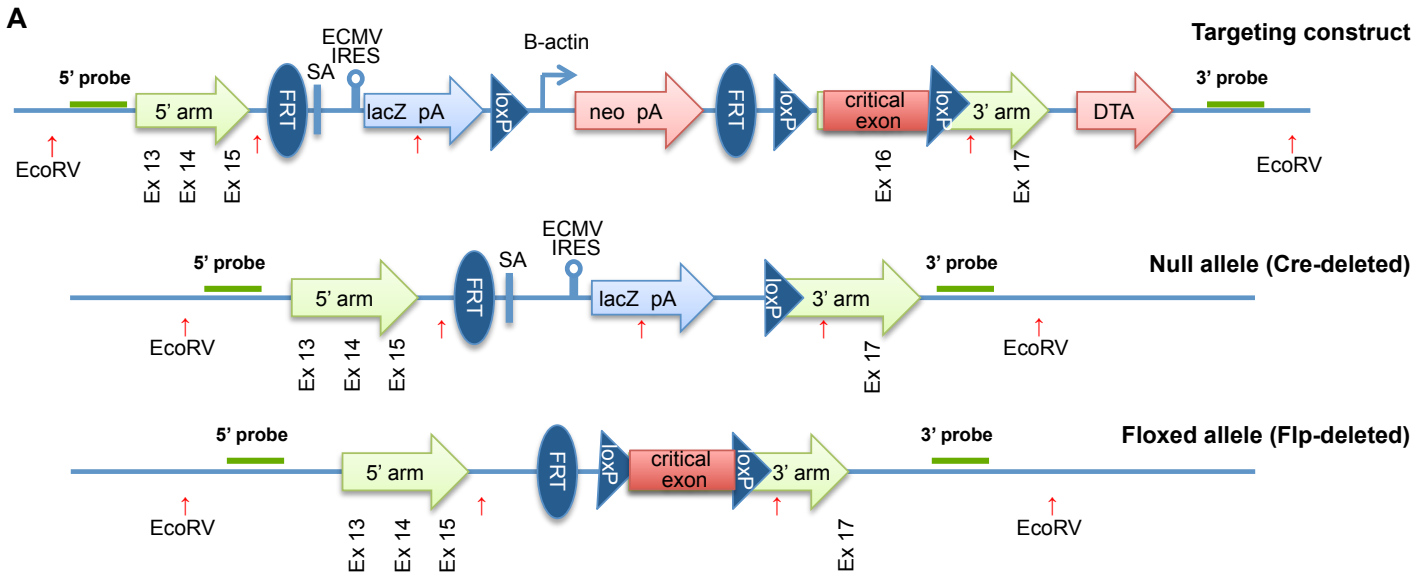
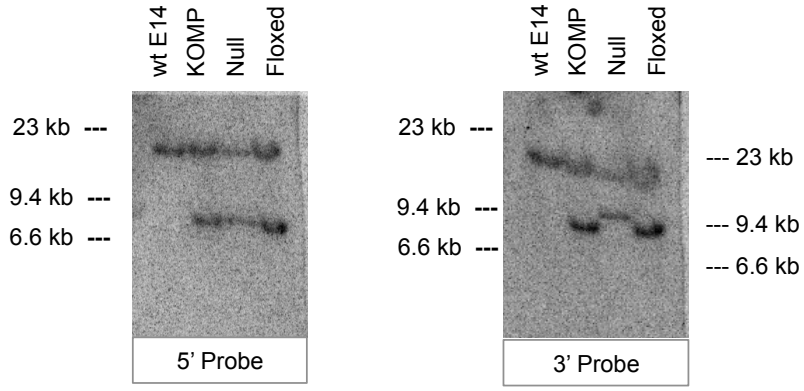
## Supplementary Figures

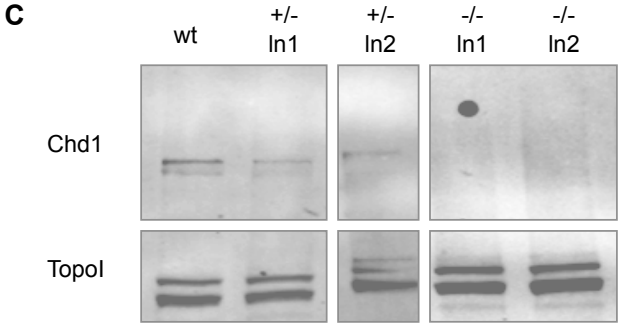



**B**

**Expected size of DNA fragment**

| Sample | 5' probe | 3' probe |
|---|---|---|
| wt E14 | 15.6 kb | 15.6 kb |
| KOMP | 7.5 kb | 8.3 kb |
| Null | 7.5 kb | 9.8 kb |
| Floxed | 7.5 kb | 8.3 kb |



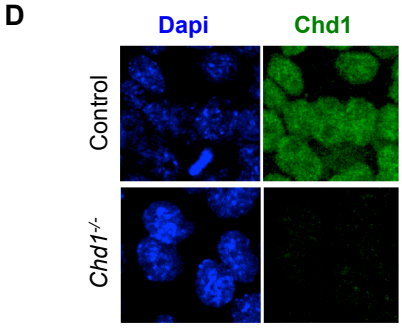



**Fig. S1.** Generation and validation of Chd1 null and conditional alleles. (A) Schematic diagram showing the targeting vector used to generate *Chd1*$^{-}$ and *Chd1*$^{flox}$ alleles in ES cells. (B) Southern blotting was conducted on genomic DNA isolated from ES cell clones and digested with EcoRV ( ↑ ). A 5' probe was designed against the genomic region upstream of the targeted insertion, while another 3' probe was designed against the region downstream of the insertion. Bands of the expected sizes were obtained in the clones before and after Cre (null) or Flp (flox) recombination. Note the 9.8kb band visible for the 3' probe only upon generation of the null allele. (C) Western blot showed loss of Chd1 protein in two independent mutant cell lines (ln1-/- and ln2-/-) when compared to wild type (wt) and 2 heterozygous (ln1+/- and ln2+/-) ES cell lines. All protein samples were run on the same blot. (D) Chd1 was undetectable by IF in *Chd1*$^{-/-}$ ES cells.

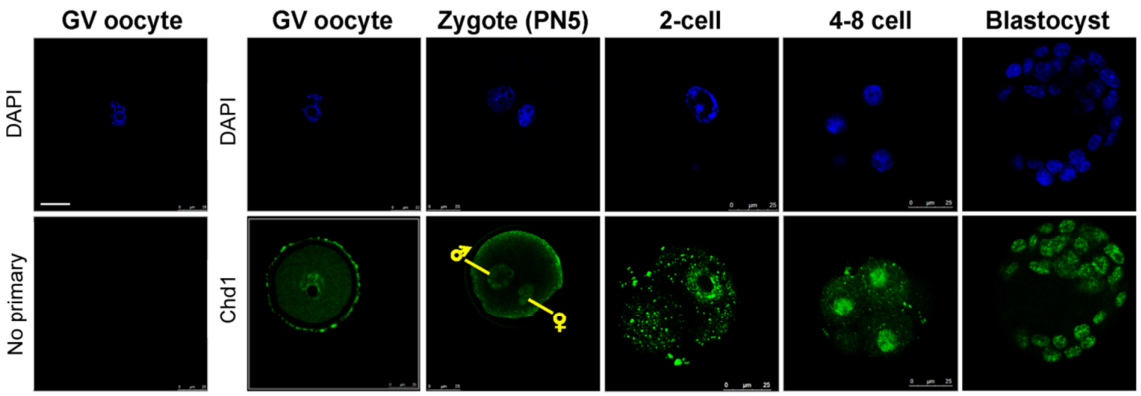
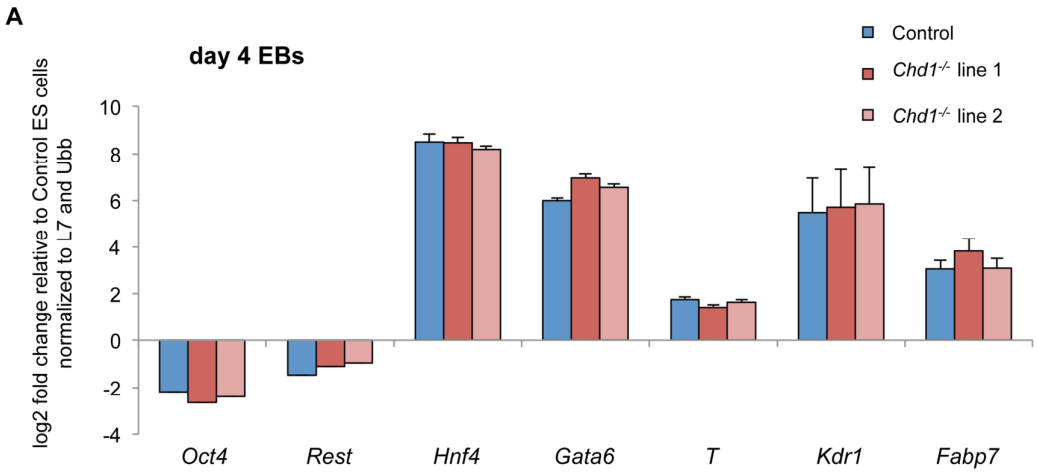
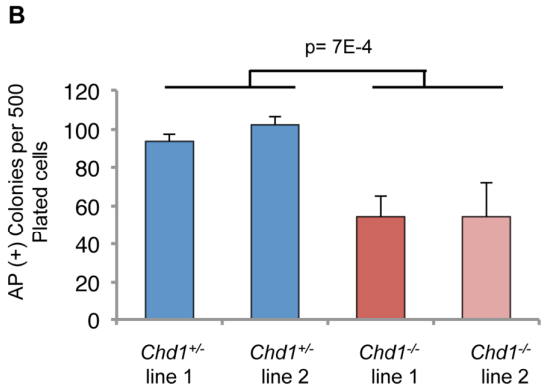



**Fig. S2.** Chd1 is expressed during pre-implantation stages. Analysis of protein expression from unfertilized oocyte to blastocyst stage by immunofluorescence.




C

| | Control | *Chd1*$^{-/-}$ |
|---|---|---|
| **Viability %** | 98.58 (±0.863) | 97.89 (±1.157) |
| **G0/G1** | 7.9 | 11.4 |
| **S** | 67.3 | 64.6 |
| **G2/M** | 12.2 | 10.3 |

**Fig. S3.** *Chd1*$^{-/-}$ cells are viable, but have a self-renewal defect. (A) Gene expression analysis at day 4 of EB differentiation showing that Chd1 mutant ES cells are able to induce differentiation genes at comparable levels to control cells. Note that regular qRT-PCR normalization for housekeeping genes was performed in this case due to the difficulty in performing cell number normalization from EBs. (B) Self-renewal capacity is impaired in absence of Chd1, as assessed in 2 different mutant and control ES cell lines using a colony formation assay in FBS/LIF. Error bars represent standard deviation of 2 replicate wells. (C) *Chd1*$^{-/-}$ ES cells have similar viability and cell cycle patterns as control ES cells. Data are averages from two independent *Chd1*$^{-/-}$ and control heterozygous cell lines.

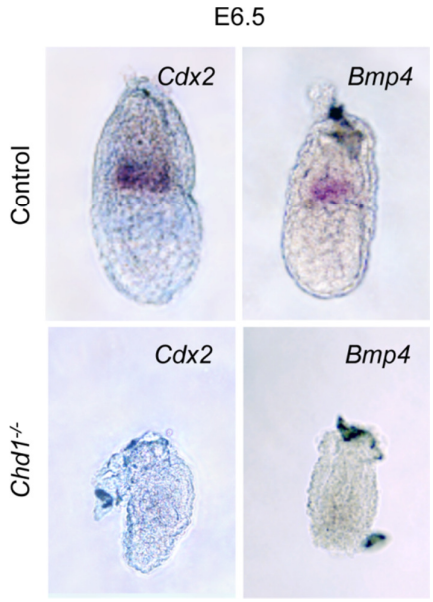



**Fig. S4.** Analyses of extra-embryonic ectoderm gene expression in Chd1 mutants. Expression of the extra-embryonic ectoderm markers *Cdx2* and *Bmp4* is lost at E6.5 in *Chd1*$^{-/-}$ embryos (bottom), compared with littermate controls (top).

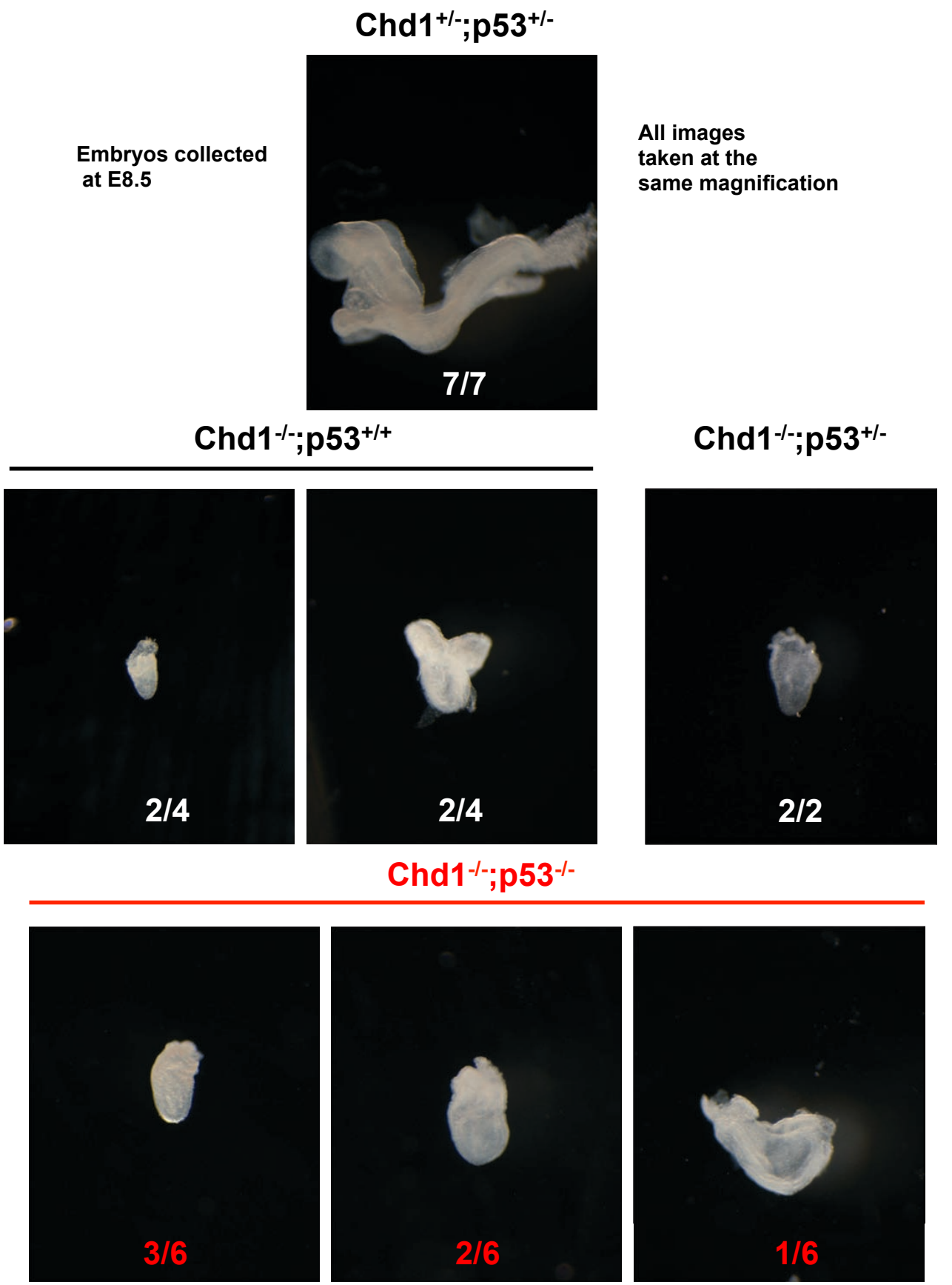

Chd1+/-;p53+/-
Embryos collected at E8.5
All images taken at the same magnification
7/7
Chd1-/-;p53+/+
Chd1-/-;p53+/-
2/4
2/4
2/2
Chd1-/-;p53-/-
3/6
2/6
1/6

**Fig. S5**. *Chd1*$^{-/-}$;*p53*$^{-/-}$ embryos arrest at E7.0-8.0. Embryos from *Chd1*$^{+/-}$;*p53*$^{+/-}$ intercrosses were recovered at E8.5. *Chd1*$^{-/-}$;*p53*$^{-/-}$ embryos are severely delayed and arrested when recovered at this stage, with a range of severity (bottom images).

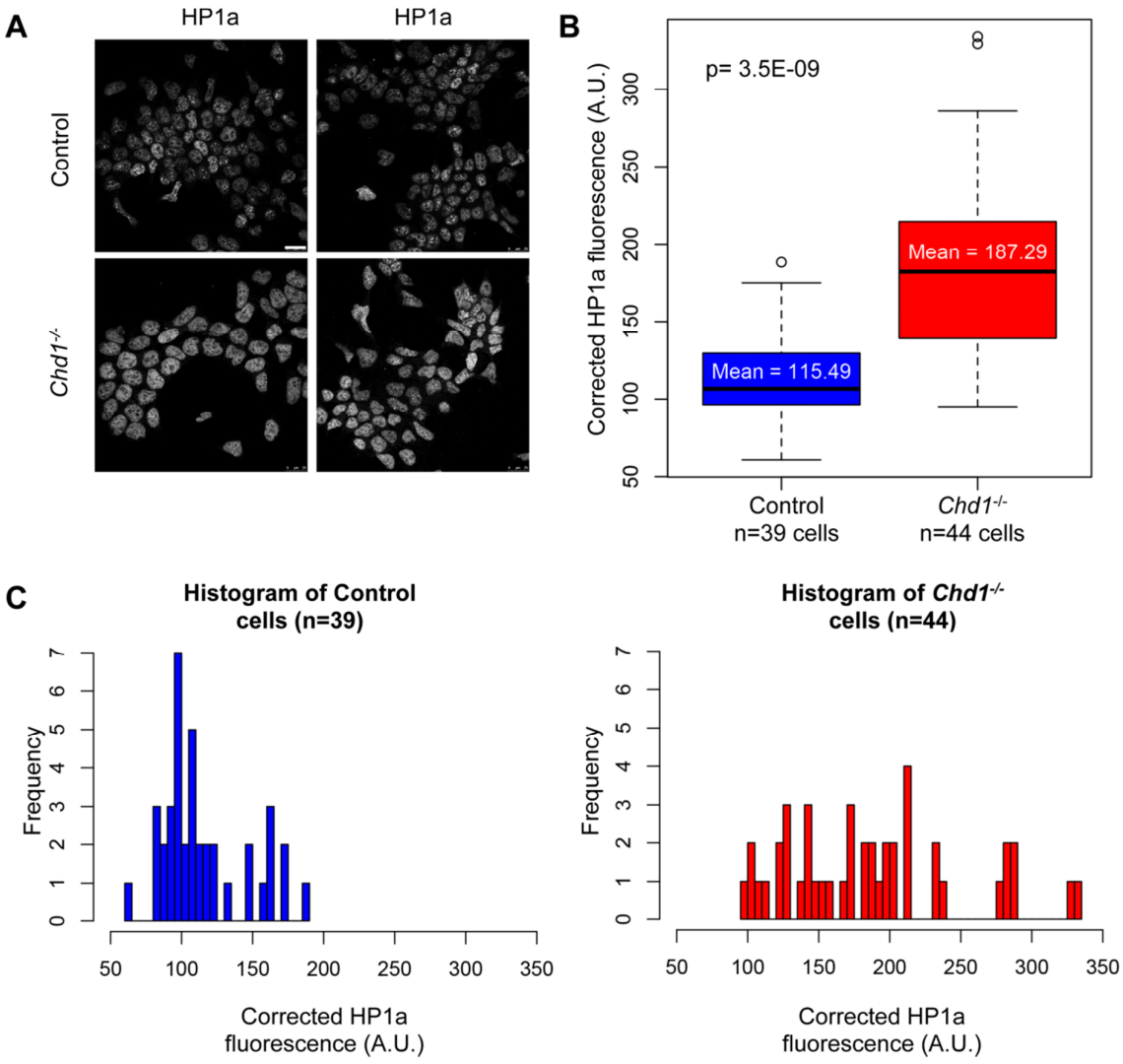



**Fig. S6**. Increased levels of Hp1a in *Chd1*$^{-/-}$ ES cells. (A) Hp1a immunofluorescence in control and *Chd1*$^{-/-}$ cells. (B) Box-plot showing an increase of corrected Hp1a fluorescence in *Chd1*$^{-/-}$ cells, Welch paired t-test p-value=3.5E-09. (C) Histogram representing the distribution of corrected Hp1a fluorescence.

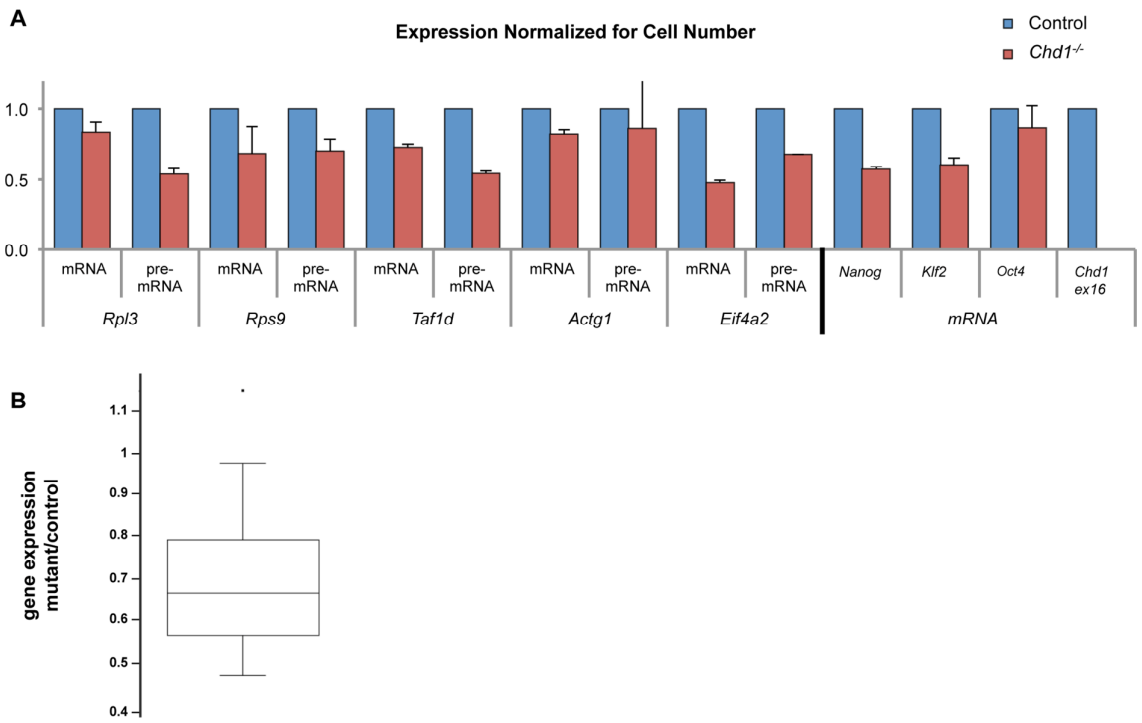



**Fig. S7.** Reduced transcriptional output in *Chd1*$^{-/-}$ ES cells. (A) *Chd1*$^{-/-}$ ES cells show a decreased transcriptional output when assessed by cell number-normalized qRT-PCR for the indicated genes. Mature messenger RNA (mRNA) represents a primer set in two consecutive exons that only detects post-splicing mRNA. Pre-mRNA represents a primer set amplifying an exon-intron boundary that only detects pre-splicing mRNA. Data are representative of two independently derived ES cell lines. Expression level in mutant ES cells is plotted as an average of the percentage of the level in the control cell line ± standard deviation from 2 cell number-normalized replicates. (B) Box-plot showing the statistical analysis for the cumulative reduction in gene expression in mutant cells relative to controls, using the data shown in (A). *Chd1*$^{-/-}$ ES cells show a median reduction in gene expression of 33.8%, paired t-test p-value=2.1E-09.

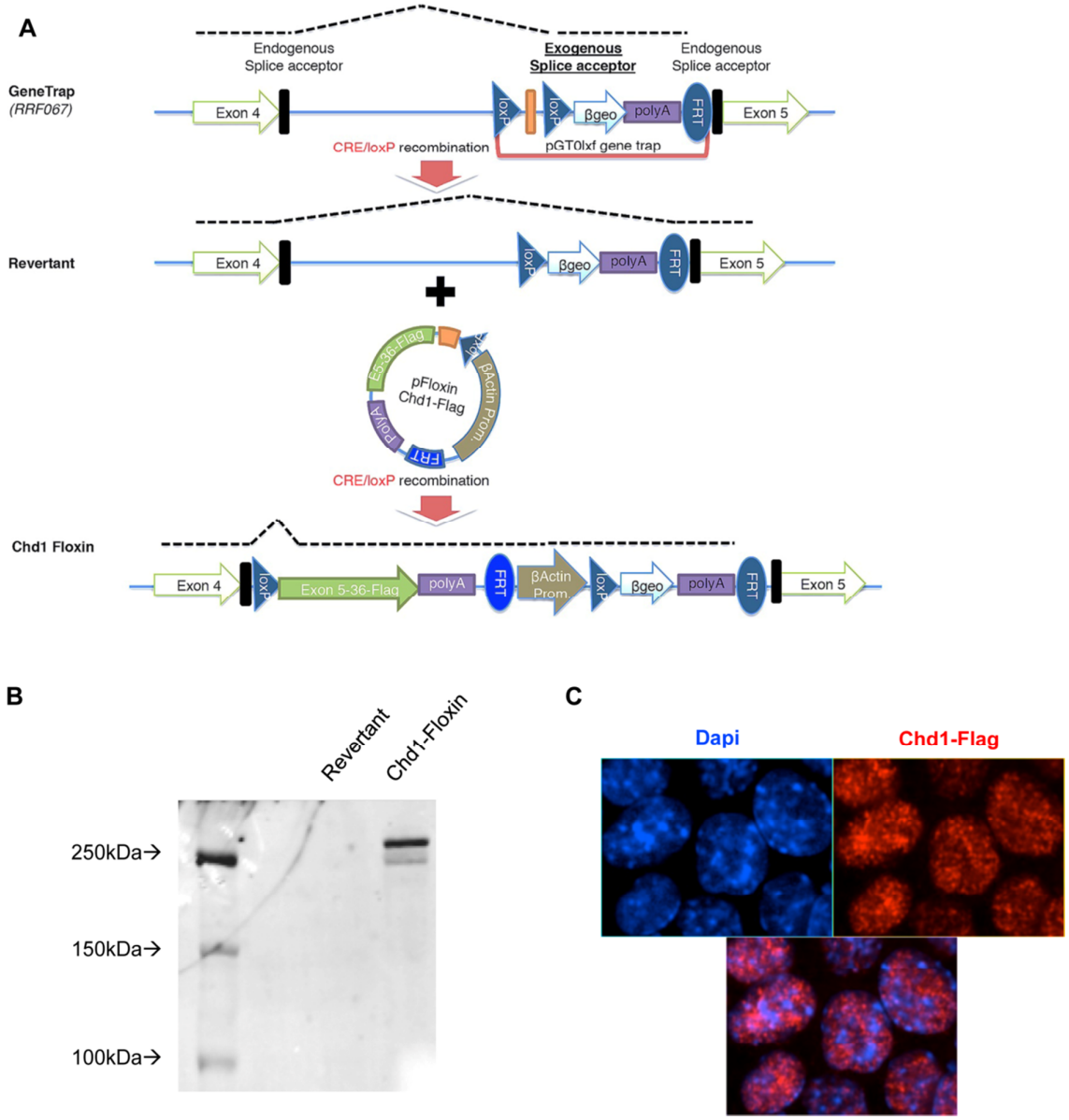



**Fig. S8.** Generation of Chd1-flag knock-in (KI) ES cells. (A) Diagram illustrating the generation of Flag-tagged Chd1 KI ES cell line. (B) Validation of the Chd1-Flag KI ES cell line by Western blotting for the Flag epitope, showing a band at the expected size for Chd1. (C) IF for Flag in the Chd1-Flag KI ES cells shows that DAPI-dense heterochromatin and Chd1 do not overlap.

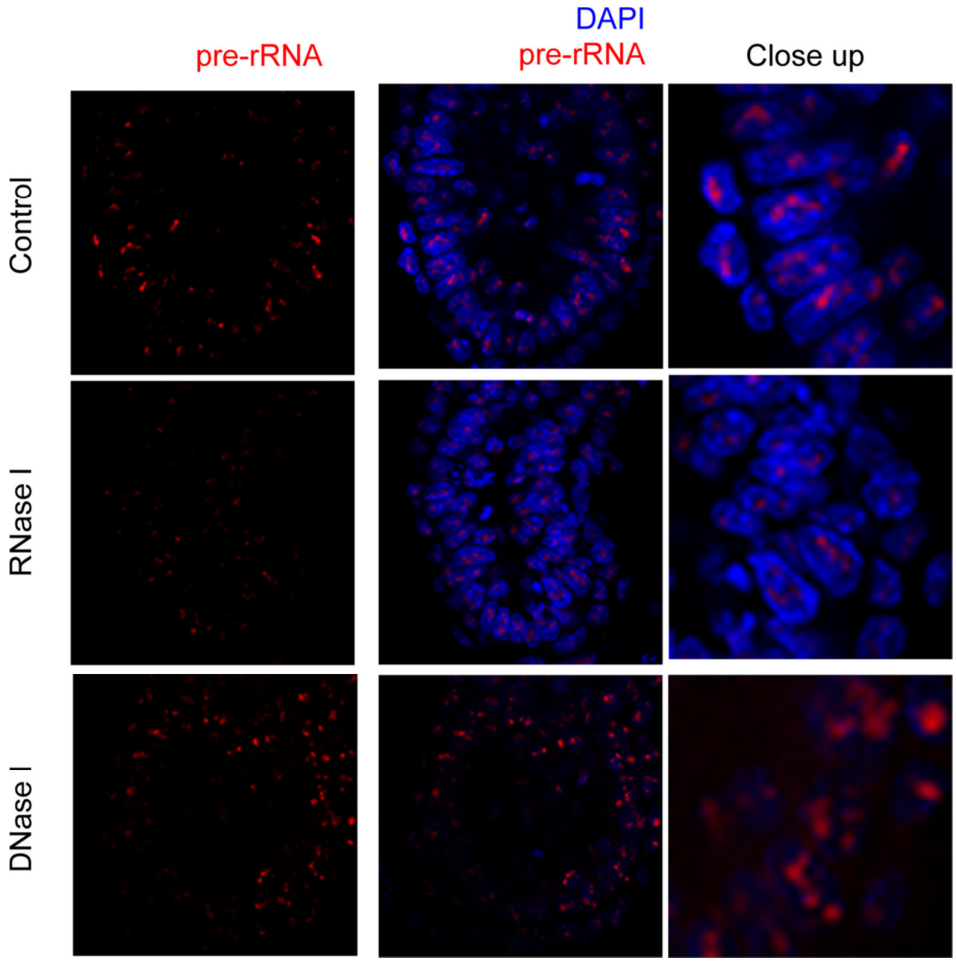



**Fig. S9**. Pre-rRNA FISH signal is sensitive to RNase I but not DNase I treatment. Levels of pre-RNA FISH signal are reduced in E.5.5 control embryos after RNase I treatment but not DNase treatment.

**Table S1. ChIP Primers**

| oligo name | sequence | cited from |
|---|---|---|
| intergenic_Ch8-F | AAGGGGCCTCTGCTTAAAAA | Boyer et al., Nature 2006 |
| intergenic_Ch8-R | AGAGCTCCATGGCAGGTAGA | |
| Rpl3TSSchipF2 | GCCATCCCTACCAGACTGAC | |
| Rpl3TSSchipR2 | GAATGGGCTGTTTTGTGCTT | |
| Rpl3GBchipF | CTGGGGGCACATCTCATAGT | |
| Rpl3GBchipR | AGATAGGTCCCCTGCCTCTG | |
| Rps9TSSchipF2 | GGTTGTGGAACAATCCCATT | |
| Rps9TSSchipR2 | CTCGGCTCTCAGAGAAATCC | |
| Rps9GBchipF | TCAAGCAGCTGGACAGGTAA | |
| Rps9GBchipR | TGACTCCAATCTCCCTCCAG | |
| Klf2TSSchipF2 | ACCGGGTGCAGATCTTGAG | |
| Klf2TSSchipR2 | CTACCCTGCACCCCAAAGT | |
| Klf2GBchipF | TGTGAAGAATGTTTTCAAAATGG | |
| Klf2GBchipR | GGACCCGAGGGAAATAAGTC | |
| Gapdh ChIP-F1 | AGGCTCAAGGGCTTTTAAGG | |
| Gapdh ChIP-R1 | ATCCTGTAGGCCAGGTGATG | |
| rDNA: | | |
| enhancer_f | TACTTCTGAGGCCGAGAGGA | |
| enhancer_r | GATCCAAAGCTCCAGCTGAC | |
| promoter_f | CCTTTGAGGTCCGGTTCTTT | |
| promoter_r | TCCAGGTCCAATAGGAACAGAT | |
| 5'ETS_f | ACTGACACGCTGTCCTTTCC | |
| 5'ETS_r | CGACAGACCCAAGCCAGTA | |
| 18S_f | GTGGAGCGATTTGTCTGGTT | |
| 18S_r | CGCTGAGCCAGTCAGTGTAG | |
| 5.8S_f | GACTCTTAGCGGTGGATCACTC | |
| 5.8S_r | GACGCTCAGACAGGCGTAG | |
| ITS_f | GTGTCGTTCCCGTGTTTTTC | |
| ITS_r | ATCGGTATTTCGGGTGTGAG | |
| 28S_f | AAATGTGGCGTACGGAAGAC | |
| 28S_r | CGTGCCGGTATTTAGCCTTA | |
| 3'ETS_f | CGTCTTCTCCTCCGTCTCC | |
| 3'ETS_r | GATCCCACCGTCGGTCAC | |
| IGS1_f | TCTTCCGAAGGTGCAGAGTT | |
| IGS1_r | TCCTCCTCCTCCTCCTCTTC | |
| IGS2_f | CTTCCCAAATGCTGGGATTA | |

| | |
|---|---|
| IGS2_r | AAGGCAGCTAGGGCTACACA |
| IGS3_f | CTTCCCAAATGCTGGGATTA |
| IGS3_r | ACAAGGCAGCTAGGGCTACA |
| IGS4_f | CCATCTCGTGGGCTTATGTT |
| IGS4_r | AGGCAGAGATGGGAGGATTT |

**Table S2. RT Primers**

| oligo name | sequence |
|---|---|
| Oct4RTf | AGCCGACAACAATGAGAACC |
| Oct4RTr | TGGTCTCCAGACTCCACCTC |
| Rpl3RTf | GATGAGTGTAAAAGGCGCTTC |
| Rpl3RTr | CTTGGTGAAAGCCTTCTTCTT |
| Rpl3RTintronR | TTCTAAGGGAACCCAAGAGC |
| Rps9RTf | CGTCTCGACCAGGAGCTAAA |
| Rps9RTr | CTTGACCCTCCAAACCTCAC |
| Rps9RTintronR | GATCCGCAATTCGAAATTCTAC |
| Taf1dRTf | TGGATGATGATGGTTCACTTTC |
| Taf1dRTr | GCCTGAGGATTTGTTGCTTC |
| Taf1dRTintronR | AACCCCATAAATTGCCCTTC |
| Actg1RTf | CCTGAACCCCAAAGCTAACA |
| Actg1RTr | ACATGGCTGGGGTATTGAAG |
| Actg1RTintronR | GTCCCGGCTCAAGCATAC |
| Eif4a2RTf | GAATTCCGATCAGGGTCAAG |
| Eif4a2RTr | CACTTGTTGCACGTCAATCC |
| Eif4a2RTinitronR | AAAATACACAGGAGCAGACTCAC |
| prerDNAF | TGTCGTTGTCACACCTGTCC |
| prerDNAR | AAATAAGGTGGCCCTCAACC |
| Gapdh-F | GGGGTCGTTGATGGCAACA |
| Gapdh-R | AGGTCGGTGTGAACGGATTTG |
| NanogRT-F | TTGCTTACAAGGGTCTGCTACT |
| NanogRT-R | ACTGGTAGAAGAATCAGGGCT |
| Chd1ex15/16-F | CAAGGAGCTTGAGCCATTTC |
| Chd1ex15/16-R | TGGTGGTTTAATGAGGTAGCAA |