Maternal SMCHD1 regulates *Hox* gene expression and patterning in the mouse embryo

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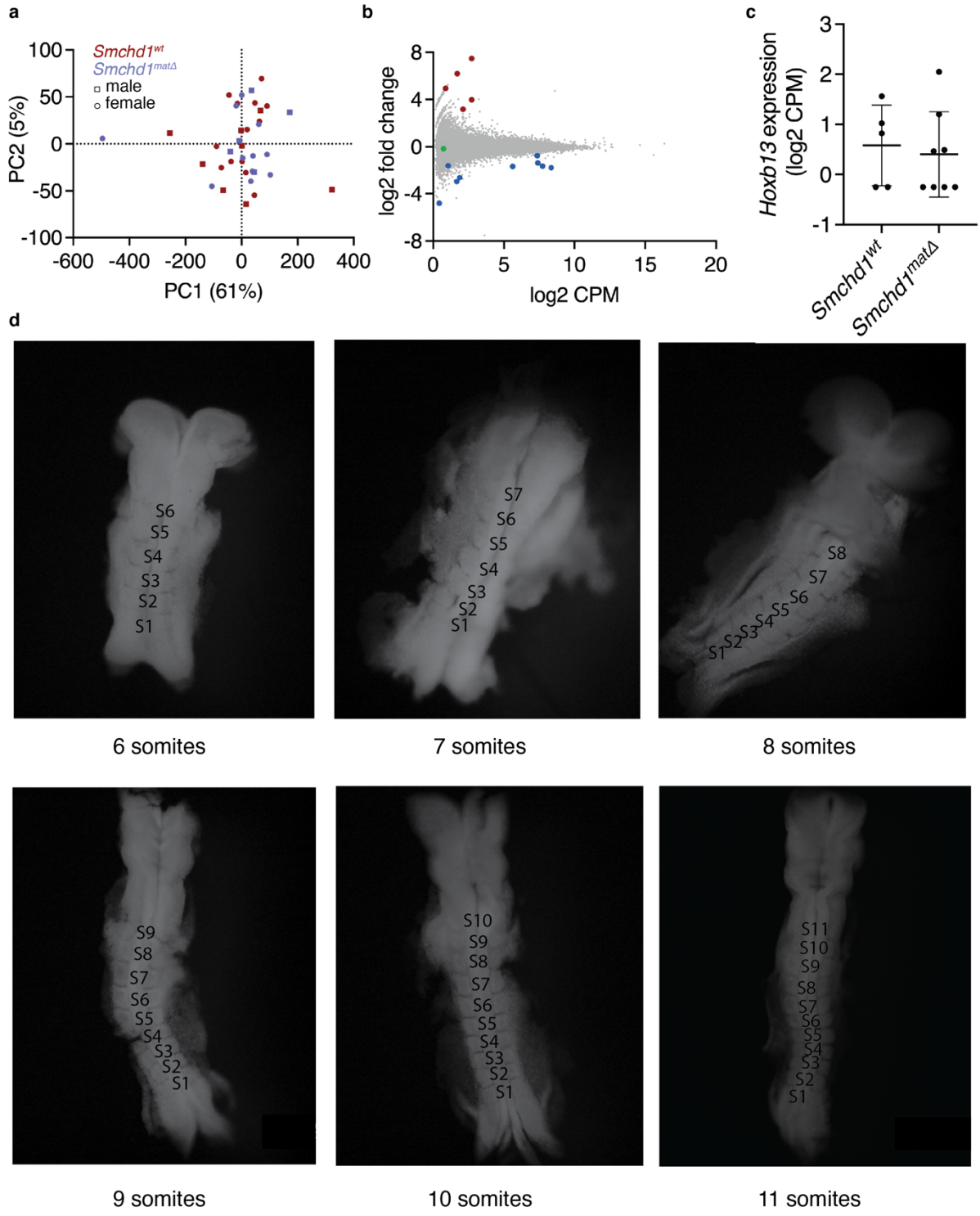
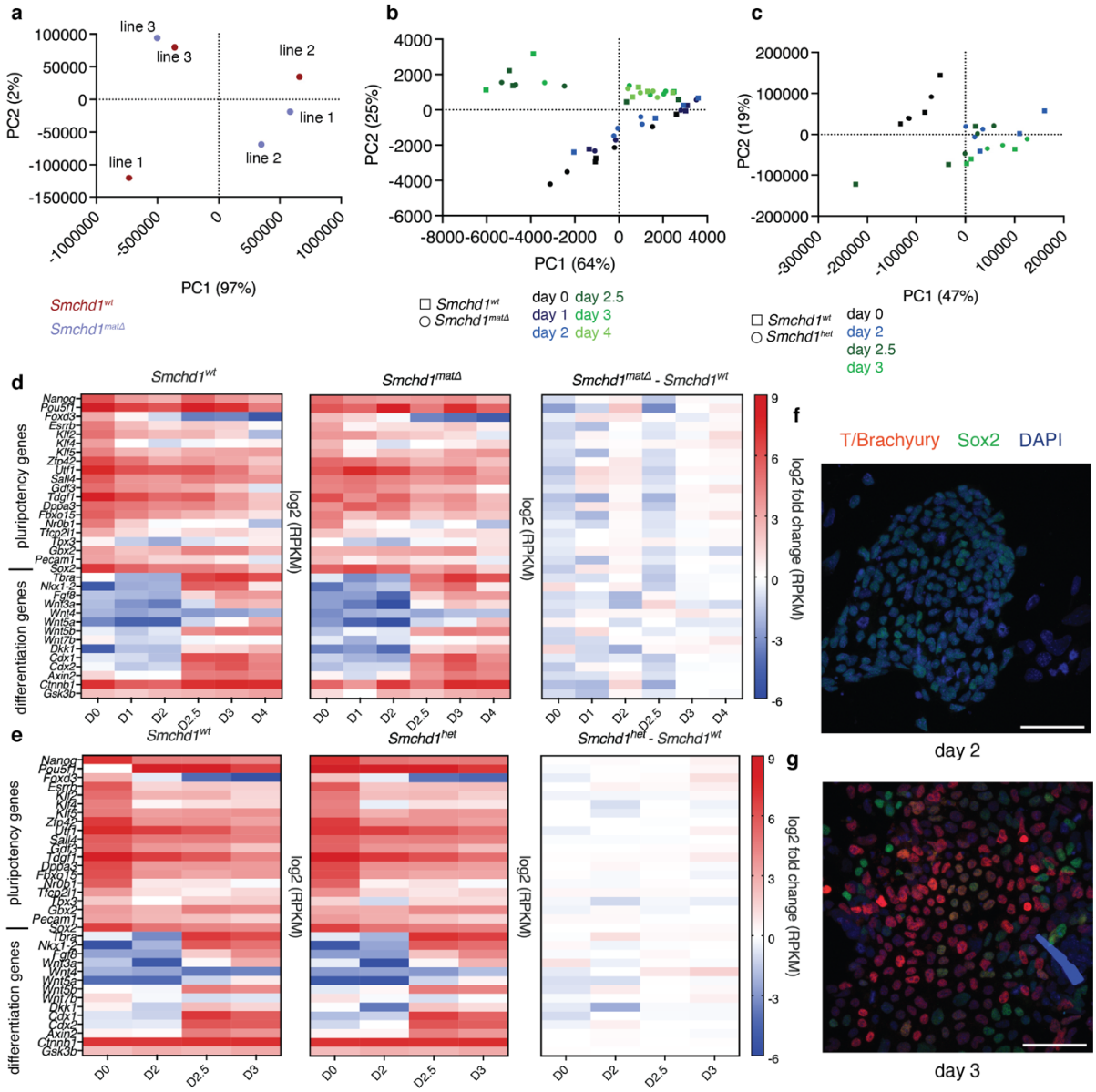


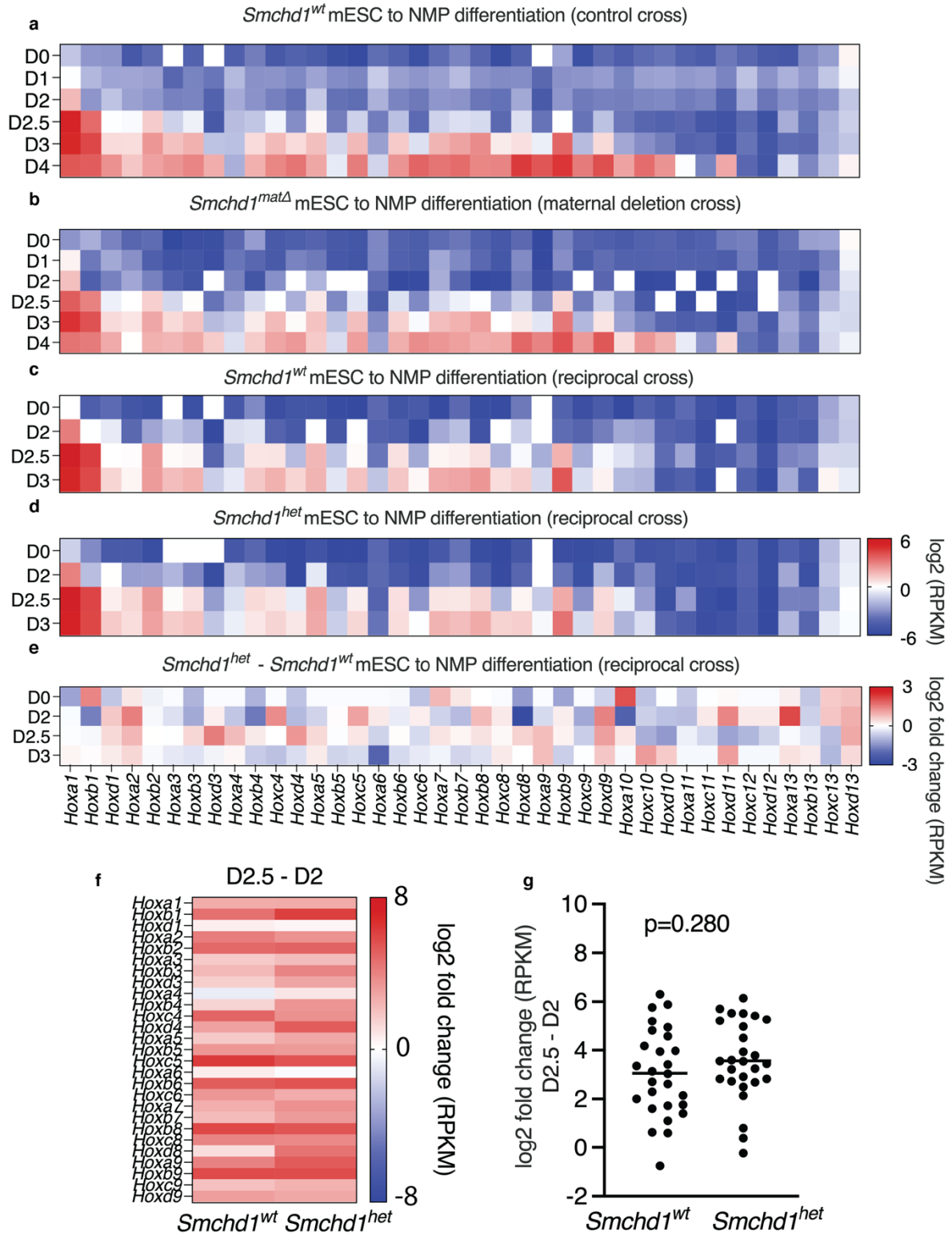
Supplementary Figure 1. RNA-seq expression analysis in embryos. a. Principal Component analysis (PCA) of all RNA-seq samples from E8.0-8.5 tailbud. Red squares represent control *Smchd1*^{wt} males, red circles represent *Smchd1*^{wt} females, purple squares represent *Smchd1*^{matΔ} males, purple circles represent *Smchd1*^{matΔ} females. **b**. MA plot of differential expression in *Smchd1*^{matΔ} males compared with *Smchd1*^{wt} E2.75 embryos, from data published in [7]. The x-axis shows log counts per million (CPM), while the y-axis is the log2 fold change between the *Smchd1*^{matΔ} and control embryos. Significantly

differentially expressed genes are shown in red (upregulated) and blue (downregulated). *Hoxb13* is the only detectable *Hox* gene and is shown in green. Source data are provided in Supplementary Data 1. **c**. *Hoxb13* expression in logCPM in E2.75 male embryos from (b), mean plotted with SD (n=5 and 8 individual embryos for $Smchd1^{wt}$ and $Smchd1^{mat\Delta}$ respectively). **d.** Representative dorsal-view images of E8.0-8.5 embryos at each somite stage after tailbud dissection. Note the ventral view of the 8 somite embryo is shown.



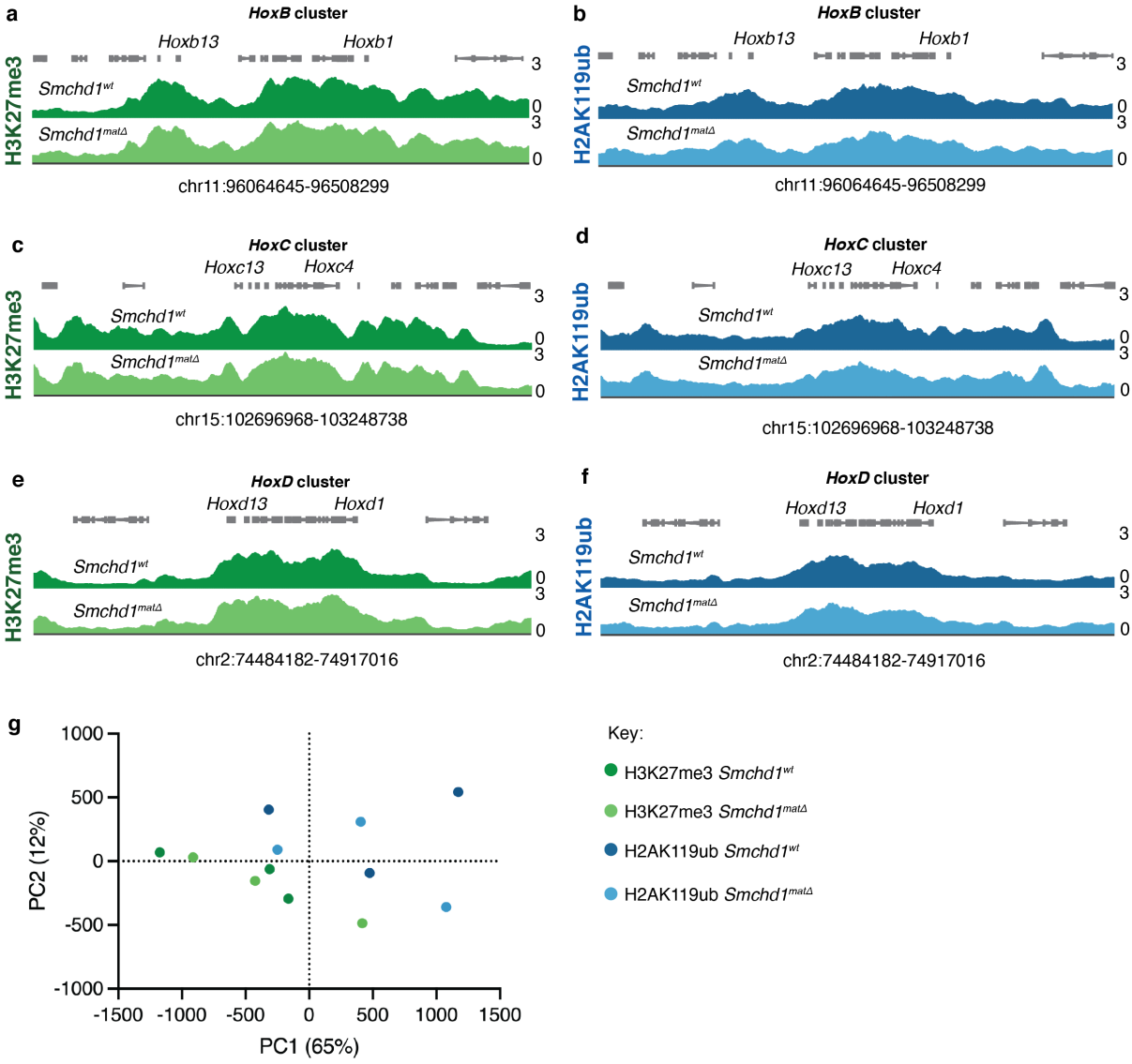
Supplementary Figure 2. Analysis of expression of differentiation factors for differentiating *Smchd1*^{wt}, *Smchd1*^{matΔ} and *Smchd1*^{het} male mESC. a. PCA plot for RNA-seq performed on *Smchd1*^{wt} and *Smchd1*^{matΔ} male mESC maintained in 2i+LIF medium. n=3 independent mESC lines per genotype. **b**. PCA plot for RNA-seq time course from day 0 to day 4 of differentiation as shown in Figure 3a. n=4 from two technical replicates of two mESC lines derived from separate blastocysts for all days except *Smchd1*^{wt} day 1 and *Smchd1*^{matΔ} days 0 and 2.5 where n=3 from technical replicates of two independent mESC lines. **c**. As in (b) but for *Smchd1*^{wt} and *Smchd1*^{het} male mESC generated from the reciprocal of the maternal deletion cross. n=3 independent mESC lines per genotype. **d.** Heatmaps of average RPKM of differentiation and pluripotency factor gene expression, in *Smchd1*^{wt} and *Smchd1*^{matΔ} samples, and the average log2 fold change between these two genotypes. Differentiation and pluripotency factor list compiled from [40, 55, 56]. Source data is provided in Supplementary Data 3. **e.** As in (d) but for for

Smchd1^{wt} and *Smchd1*^{het} male mESC generated from the reciprocal of the maternal deletion cross as shown in Figure 1c. Source data are provided in Supplementary Data 4. **f-g**. Immunofluorescence (IF) images of Sox2 and T/Brachyury at days 2 (f) and 3 (g) of differentiation, representative of IF experiments from n=2 of the differentiation experiments from independent mESC lines, each for *Smchd1*^{wt} and *Smchd1*$^{mat\Delta}$. Scale bar is 50 μm.

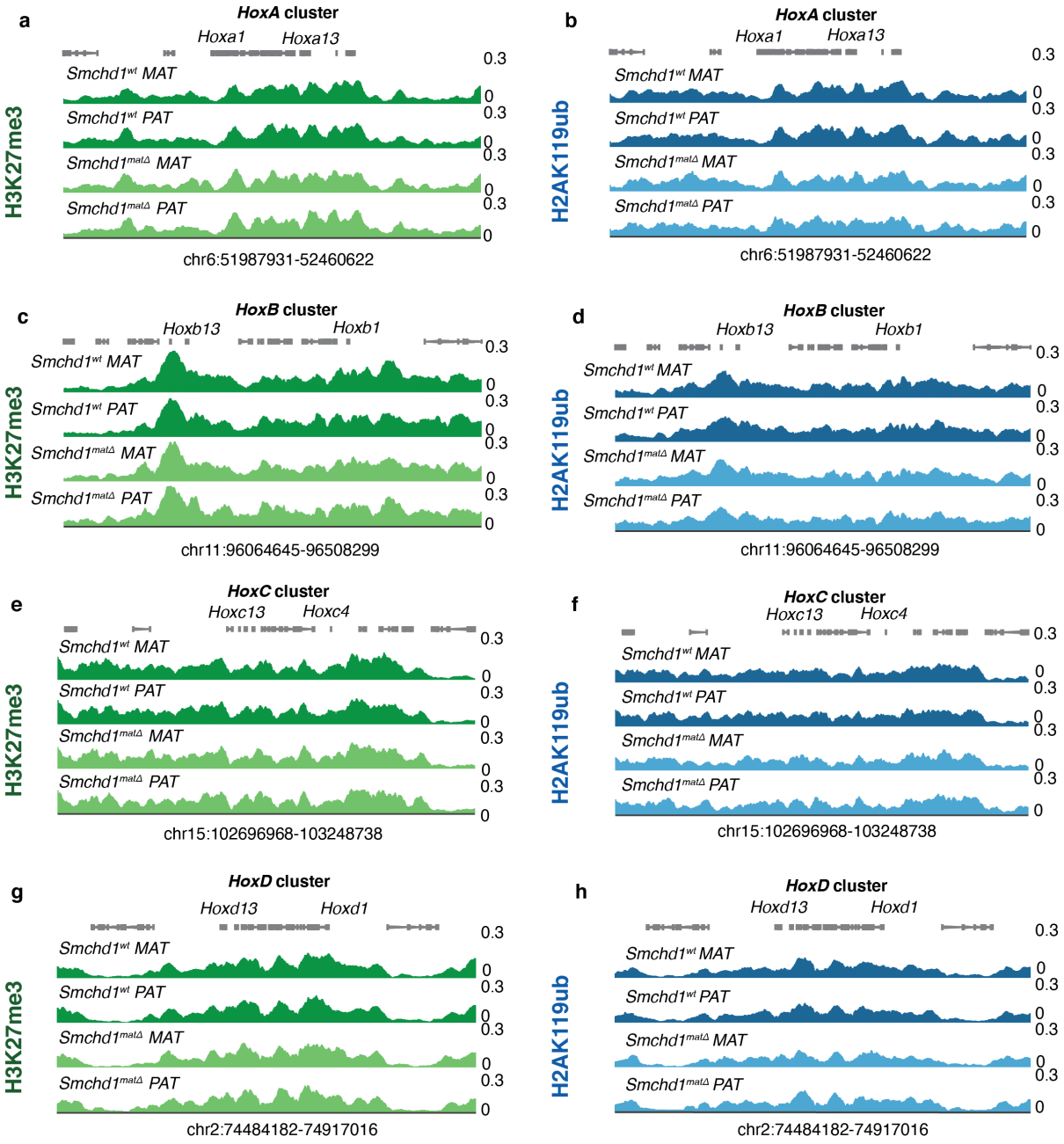


Supplementary Figure 3. Expression analysis for differentiating *Smchd1*^{wt}, *Smchd1*^{matΔ} and *Smchd1*^{het} male mESC. a,b. Heatmaps of average log2 RPKM values over all Hox genes during the RNA-seq time course from day 0 to day 4 of differentiation as shown in Figure 3a. n=4 from two

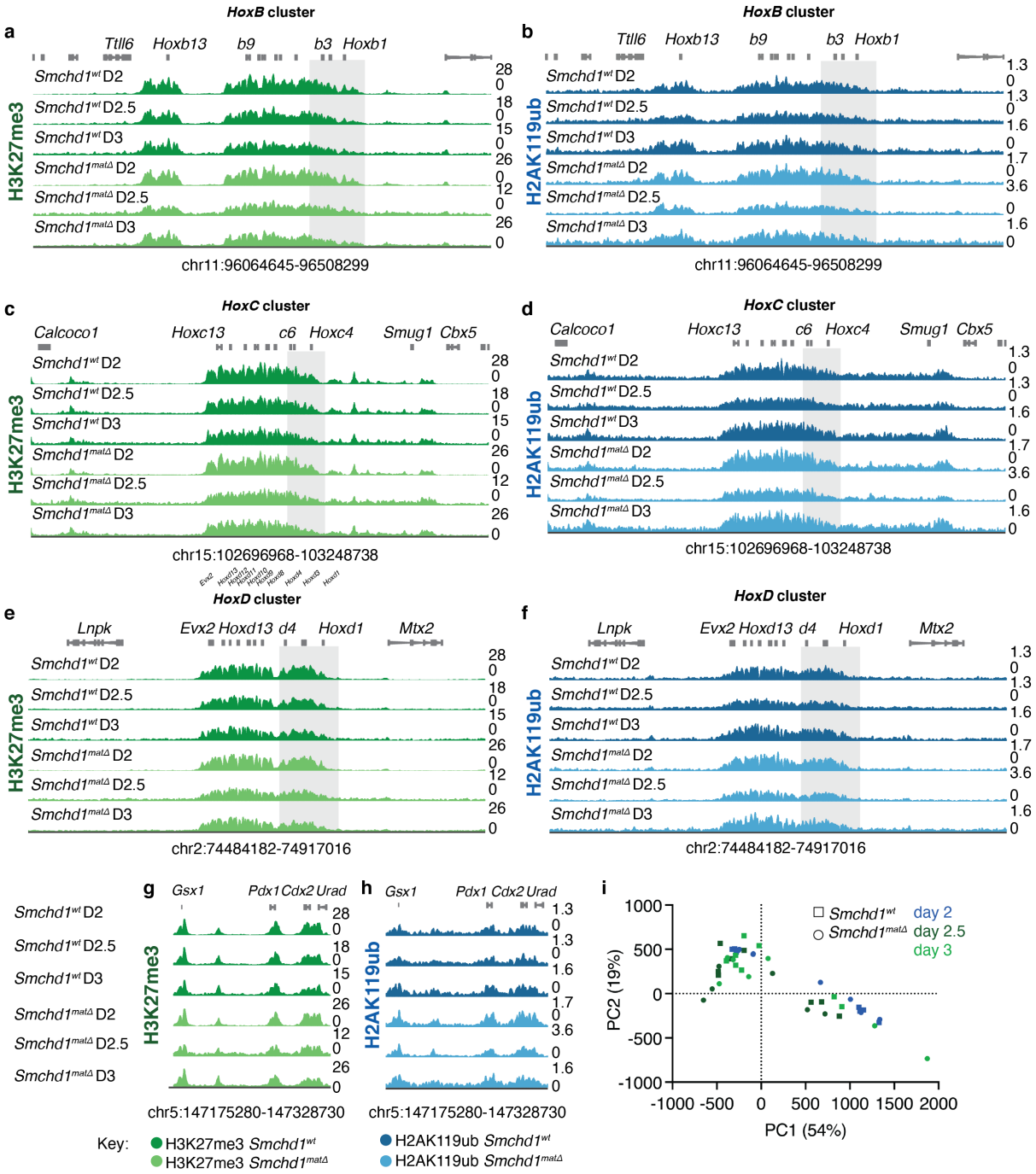
technical replicates of two mESC lines derived from separate blastocysts for all days except *Smchd1*^{wt} day 1 and *Smchd1*$^{mat\Delta}$ days 0 and 2.5 where n=3 from technical replicates of two independent mESC lines, shown for *Smchd1*^{wt} (a) and *Smchd1*$^{mat\Delta}$ (b) mESCs generated from the control and maternal deletion crosses as shown in Figure 1a-b respectively. **c, d.** As in (a) but for *Smchd1*^{wt} (c) and *Smchd1*^{het} (d) mESCs generated from the reciprocal of the maternal deletion cross as shown in Figure 1c. n=3 independent mESC lines per genotype, sampled at days 0, 2, 2.5 and 3 of differentiation. **e.** Heatmaps of the average log2 fold change of Hox genes between *Smchd1*^{wt} (c) and *Smchd1*^{het} samples. **f.** Heatmap of the average log2 fold change of *Hox* gene expression between the day 2.5 and day 2 samples, for *Smchd1*^{wt} and *Smchd1*^{het} samples. **g**. The log2 fold change for the *Hox1-9* genes between day 2.5 and day 2 of differentiation, for the average of the replicates for each genotype (Student's t-test, two-tailed, equal variance). Source data are provided in Supplementary Data 3 and 4.



Supplementary Figure 4. H3K27me3 and H2AK119ub coverage over Hox clusters in 2i + LIF mESCs. a, c, e. H3K27me3 CUT&RUN in *Smchd1*^{wt} and *Smchd1*^{matΔ} ESCs cultured in 2i+LIF medium over the *HoxB* (a), *HoxC* (c) *and HoxD* (e) clusters as marked. n=3 independent mESC lines per genotype, the average of which is shown. Genes are shown in grey above the CUT&RUN enrichment tracks and genome coordinates are shown below. Green indicates H3K27me3, dark green for *Smchd1*^{wt} and light green for *Smchd1*^{matΔ}. Y-axis is FPM. **b,d,f.** as in (a, c, e) but for H2AK119ub. Blue represents H2AK119ub, dark blue for *Smchd1*^{wt} and light blue for *Smchd1*^{matΔ}. **g.** PCA plot for H3K27me3 and H2AK119ub CUT&RUN in *Smchd1*^{wt} and *Smchd1*^{matΔ} mESCs after quantification of 10kb probes sliding by 10kb windows genome-wide, corrected for total read count (FPM). Source data are provided in Supplementary Data 5.

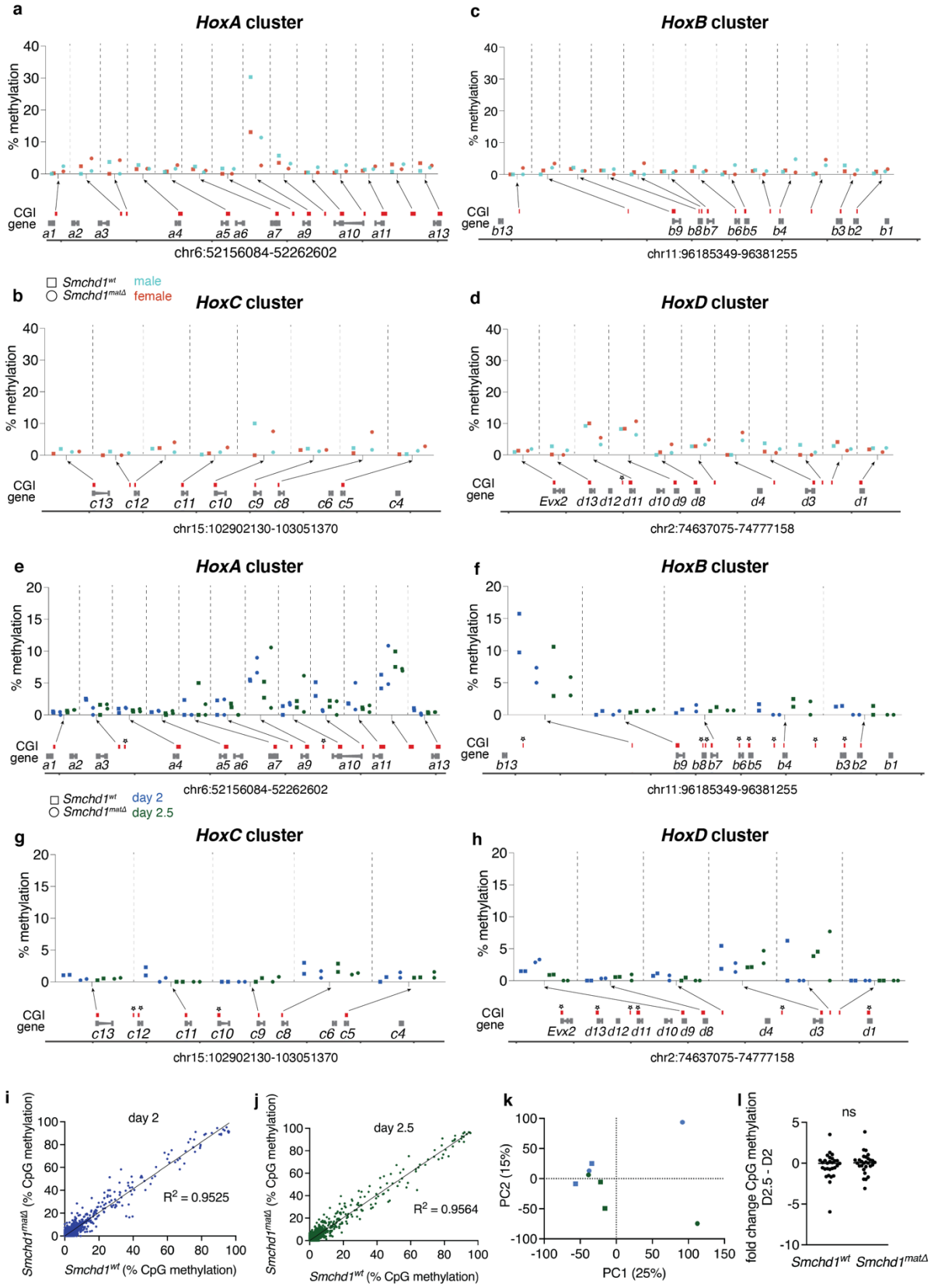


Supplementary Figure 5. H3K27me3 and H2AK119ub over the maternal and paternal allele of each *Hox* cluster. a, c, e, g. CUT&RUN analysis of H3K27me3 over each *Hox* cluster in *Smchd1*^{wt} and *Smchd1*$^{mat\Delta}$ ESCs cultured in 2i+LIF medium, where the reads have been split based on SNPs between the C57BL/6 maternal (MAT) and Cast paternal (PAT) genomes. n=3 independent mESC lines per genotype, the average of which is shown. Genes are shown in grey above the CUT&RUN enrichment tracks, genome coordinates are shown below. Green indicates H3K27me3, dark green for *Smchd1*^{wt} and light green for *Smchd1*$^{mat\Delta}$. Note the read counts are significantly lower than for Figure 4 as reads without an informative SNP are removed. Y-axis is FPM. **b, d, f, h**. as in a. but for H2AK119ub. Blue represents H2AK119ub, dark blue for *Smchd1*^{wt} and light blue for *Smchd1*$^{mat\Delta}$. Source data are provided in Supplementary Data 5.



Supplementary Figure 6. H3K27me3 and H2AK119ub coverage over Hox clusters in differentiating mESCs. a, c, e H3K27me3 CUT&RUN in *Smchd1*^{wt} and *Smchd1*^{matΔ} ESCs collected at days 2, 2.5 and 3 of differentiation over the *HoxB* (a), *HoxC* (c) *and HoxD* (e) clusters as marked. n=4 from two technical replicates of two mESC lines derived from separate blastocysts for all days except *Smchd1*^{matΔ} days 2.5 and 3 where n=3 from technical replicates of two independent mESC lines, the average for which is shown. Genes are shown in grey above the CUT&RUN enrichment tracks and genome coordinates are shown below. Green indicates H3K27me3, dark green for *Smchd1*^{wt} and light green for *Smchd1*^{matΔ}. **b,d,f.** as in (a, c, e) but for H2AK119ub. n=4 from two technical replicates of two mESC lines derived from separate blastocysts for all days except *Smchd1*^{wt} day 2 and *Smchd1*^{matΔ}

day 2.5 where n=3 from technical replicates of two independent mESC lines, the average for which is shown. Blue represents H2AK119ub, dark blue for *Smchd1*^{wt} and light blue for *Smchd1*$^{mat\Delta}$. **g.** as in (a) but for genome browser tracks showing *Pdx1* and *Cdx2* for H3K27me3 CUT&RUN as example tracks of a genomic region outside *Hox*. **h.** as in (g) but for H2AK119ub. **i.** PCA plot for H3K27me3 and H2AK119ub CUT&RUN in *Smchd1*^{wt} and *Smchd1*$^{mat\Delta}$ samples collected at days 2, 2.5 and 3 during differentiation, after quantification of 10kb probes sliding by 10kb windows genome-wide, corrected for total read count (FPM). Source data are provided in Supplementary Data 6.



Supplementary Figure 7. DNA methylation of CpG islands is unchanged in *Smchd1* maternal null E2.75 embryos and differentiating mESCs. a-d. % DNA methylation levels from whole genome bisulfite sequencing data of *Smchd1*^{wt} (circles) and *Smchd1*^{matΔ} (squares) E2.75 embryos at CpG islands over Hoxa (a), Hoxb (b), Hoxc (c) and Hoxd (d) clusters from [7, 45]. Each datapoint displays the complied

reads of n=6 *Smchd1*^{wt} females, n=5 *Smchd1*^{wt} males, n=4 *Smchd1*^{matΔ} females and n=8 *Smchd1*^{matΔ} male E2.75 embryos deleted with MMTV-Cre. Male embryos are shown in light blue, females in red. Genomic coordinates, genes in grey and CpG islands in red are shown below the % methylation graphs. Stars indicate no coverage over that CpG island. **e-h.** As in a-d but for reduced representation bisulfite sequencing data of *Smchd1*^{wt} (circles) and *Smchd1*^{matΔ} (squares) differentiating mESCs at days 2 (blue) and 2.5 (green) of differentiation. n=2 biological replicates per genotype and timepoint. **i,** Scatterplot showing overall low % methylation over CpG islands genome-wide in *Smchd1*^{wt} and *Smchd1*^{matΔ} samples at day 2 (blue) of differentiation. The Pearson coefficient indicates very high correlation between the two genotypes (R^2 = 0.9525). **j.** as in (i) but for day 2.5 (green) of differentiation (R^2 = 0.9564). **k.** PCA plot for *Smchd1*^{wt} (circles) and *Smchd1*^{matΔ} (squares) bismark files at day 2 (blue) and 2.5 (green) of differentiation. **l.** Fold change in % DNA methylation at CpG islands surrounding *Hox1-9* genes between day 2 and day 2.5 each for *Smchd1*^{wt} and *Smchd1*^{matΔ} samples. Source data are provided in Supplementary Data 7.