Supplementary Figure Legends

Figure S1: Expression pattern of *Hotair* in developing embryos, and KO phenotype characterization, Relate to Figure 1.

(A) Whole mount *in situ* hybridization of *Hotair* in E11.5(a), E12.5(b), E13.5(c) embryos(scale bar:1mm).

(B) H&E staining and *in situ* hybridization of *Hotair* in E11.5 forelimb(a-f) and E15.5 wrist sections(g-l). H&E staining(a, d, g, j), *in situ* hybridization of *Hotair* antisense probe(b, e, h, k) and sense control(c, f, i, l) are shown. AER(apical ectodermal ridge), ZPA(zone of polarizing activity) are marked presenting the proximal-distal(PD) and anterior-posterior(AP) axes in the E11.5 limb bud. (Scale bar: 200μm for B-j/k/l; 100 μm for others).

(C) Genotype analysis of Hotair WT, heterozygous(Het) and KO genomic DNAs with a primer set for the wild type allele(329bp) and KO allele(566bp).

(D) Alizarin Red-Alcian Blue staining of the whole skeleton shows "L6→S1" homeotic transformation of the lumbar vertebraes in KO animals. Note missing lumbar 6 in KO.

Figure S2: Gene expression analysis in *Hotair* KO mouse, Related to Figure 2.

(A) Expression comparison of *Hotair* and adjacent *Hoxc11* between TTFs and E11.5 posterior half of embryos. The Ct values of each gene from qRT-PCR with 30ng total RNA were shown.

(B) Expression of *Hoxa11* and *Hoxa1* in TTFs and E11.5 posterior half of embryos.

(C) Single cell qPCR analysis of *Hotair* expression in TTF and MEF cells. Individual cells are sorted and lysed to perform specific target amplification (STA) for 18 cycles. The preamplified cDNAs are used as template for qPCR analysis. The Ct values of each individual cells are presented, and classified as expression high or low with threshold of 30 (left panel). Pie charts illustrating the percentage of *Hotair* positive(Ct<30) and negative cells(Ct>30) cells from TTF and MEF populations. Note here the Ct values of single cells are not comparable with those generated from conventional qPCR.

(D) Enriched Gene Ontology (GO) terms in the up-regulated gene set from *Hotair* KO cells ($p<0.05$, FDR<0.05 for each).

(E) RNA-seq analysis of the gene expression from HoxA, B, D, Dlk1-Dio3 and H19-Igf2 loci in Hotair WT and KO cells. x-axis is genomic coordinate; y-axis is the normalized RNA-seq signals of each genes. Selectively de-repressed imprinted genes in KO are highlighted with arrows.

(F) Intracellular staining and FACS analysis of Dlk1 and Igf2 expression level in WT and KO fibroblast cells($n>3$). The Geometric Mean Fluorescence Intensity (GMFI) showing the relative expression level in the cells (left). Mean ± s.d. is shown. **$p < 0.01$ relative to WT determined by student's t-test.

(G) Immuno-fluorescence staining of Dlk1 in WT and KO TTF cells.

Figure S3: Whole mount *in situ* hybridization of *Hoxc11* on E12.5 developing embryos, Related to Figure 3.

Staining of the whole embryos (a, b) and forelimbs(c, d) are shown. (Scale bar: 1mm for a, b; 400μm for c, d)

Figure S4: Epigenetic analysis of genes affected by *Hotair* deletion, Related to Figure 4.

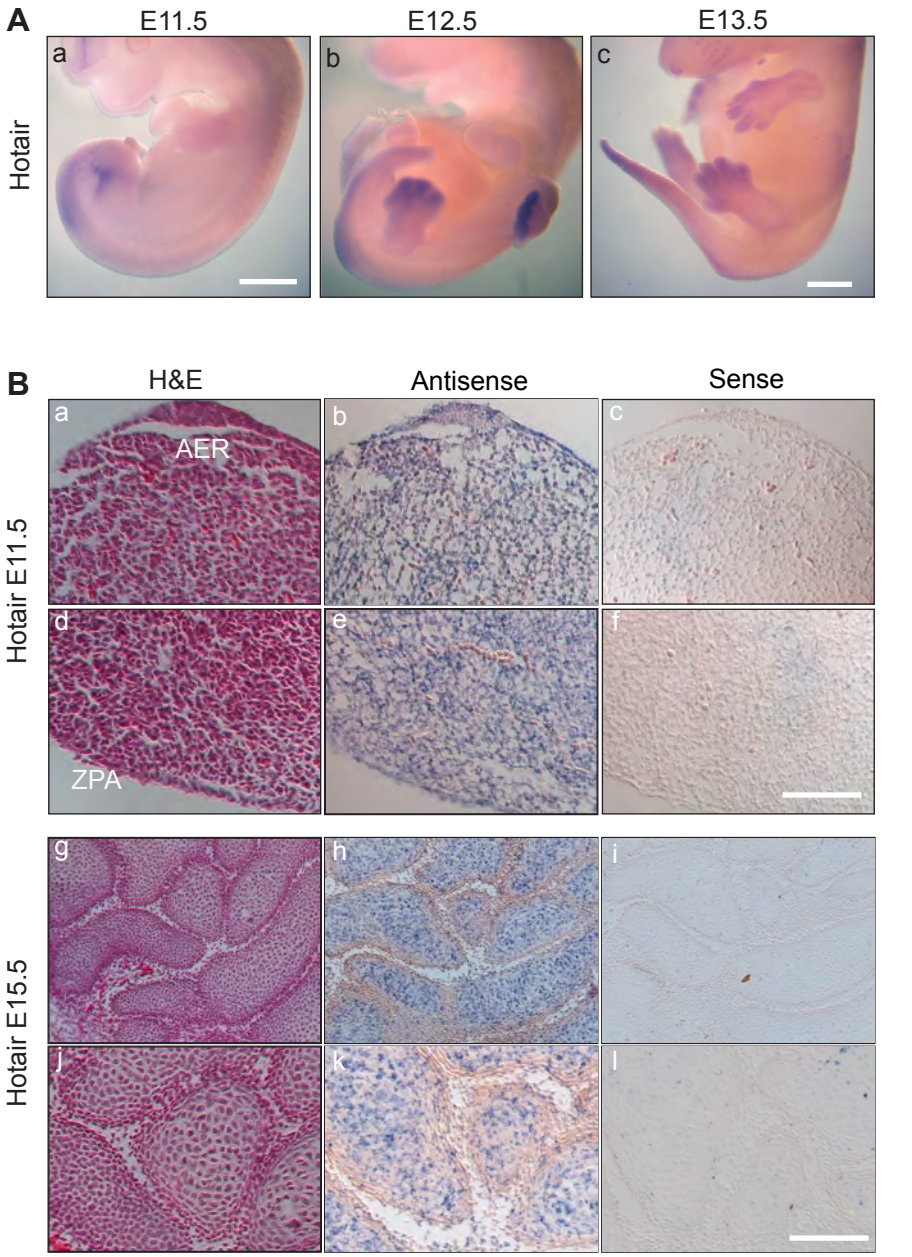
(A) ChIP-qPCR shows reduced binding of PRC2 complex on *HoxD*(D1, D3, D11) and imprinted genes(*Dlk1, Plag1, Dcn, H19*) in KO cells. ChIP is performed with PRC2 antibodies (combined antibodies of EZH2 and Suz12) along with IgG as a negative control. Mean ± s.d. is shown; *$p<0.05$ relative to control(WT) by student's t-test.

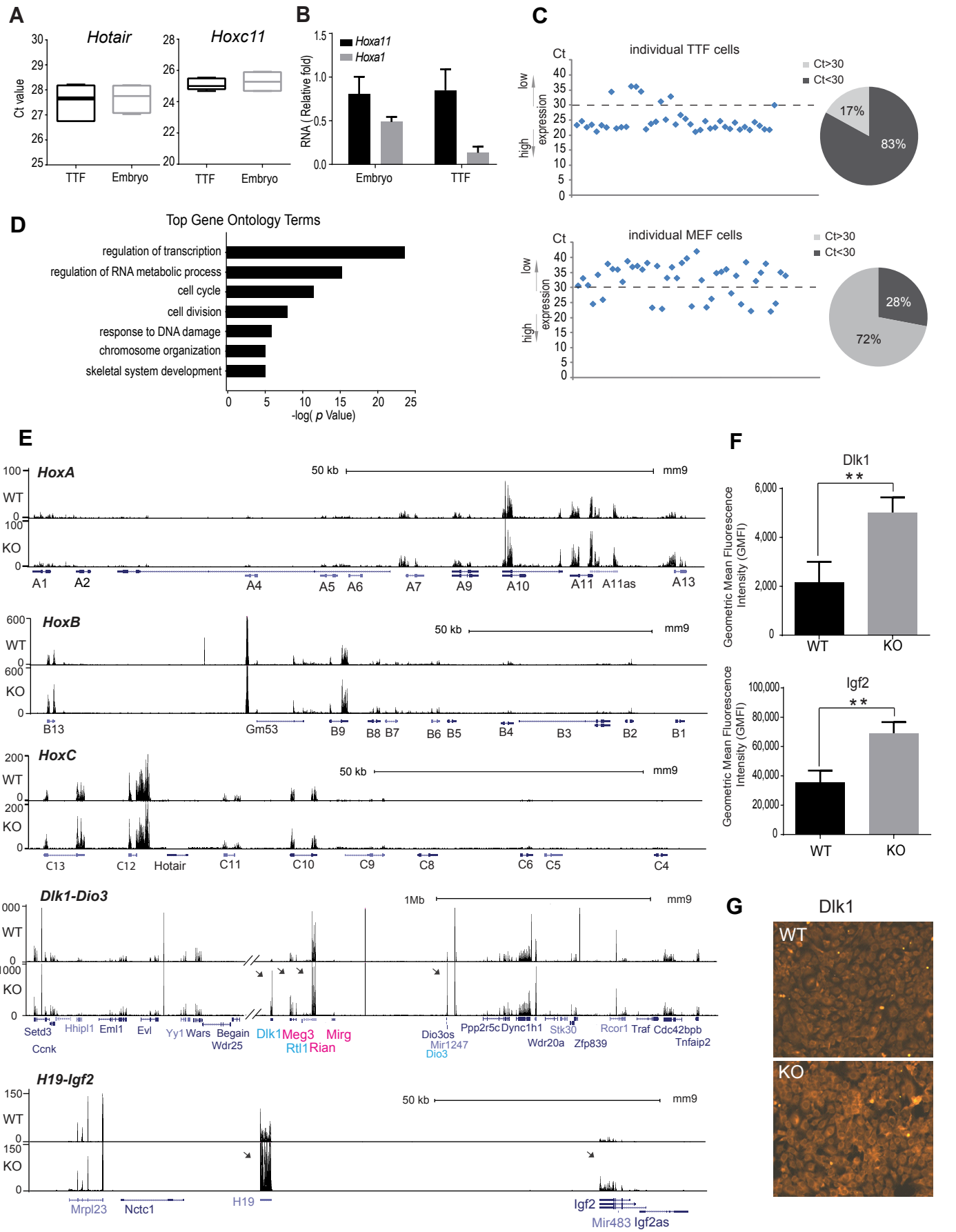
(B) DNA methylation analysis of the imprinted region at *Dlk1-Gtl2* locus. *Upper*, A schematic diagram of *Dlk1-Gtl2* locus containing the intergenic differentially methylated region(IG-DMR). *Lower*, bisulfite sequencing of DNA from IG-DMR in *Hotair* WT and KO cells. Black and white squares represent methylated and unmethylated CpG islands along this region. Individual alleles from independently sequenced clones are presented by a row.

(C, D) Gene expression analysis of *HoxCΔ* RNA-seq data (Schoderet and Duboule, 2011)

(C) Significant de-repression of other *Hox* loci in *HoxCΔ* RNA-seq data. We noticed that multiple *Hox* genes are among the most up-regulated genes by rank in *HoxCΔ* compared to wild type (i.e. in the top 5 percentile), despite modest fold of up-regulation (~1.5- to 2-fold). To estimate the significance of this observation, we compared the average ranking of the observed coordinate up-regulation of *Hox* genes versus that of 10,000 randomized 10-gene data sets where the gene labels are permuted. The number of instances in the observed data versus permutated data allowed us to estimate a *p*-value of obtaining the observation by chance alone. *HoxB* and *HoxD* loci are significant with $p<0.05$ whereas *HoxA* just misses the cutoff.

(D) Cumulative counts of RNA-seq reads from each of the Hox loci in WT vs. *HoxCΔ.* Y-axis shows the sum of Reads Per Kilobase per Million mapped reads (RPKM). *HoxCΔ* removes all *HoxC* genes except *Hoxc4*, which in the altered genomic context is now expressed as much as the sum of all *HoxC*-derived transcripts in wild type. Rather than simply reducing the dosage of *HoxC* transcripts, *HoxCΔ* actually increases the total dosage of all *Hox* transcripts, including from *HoxA*, *HoxB*, and *HoxD*.


A
E11.5
E12.5
E13.5
Hotair
a
b
c
B
H&E
Antisense
Sense
Hotair E11.5
AER
ZPA
Hotair E15.5
C
WT
Het
KO
WT allele
(392bp)
KO allele
(566bp)
D
WT
KO
L1
L6
L1
L5


A
Hotair
Hoxc11
Ct value
TTF
Embryo
B
Hoxa11
Hoxa1
RNA (Relative fold)
C
individual TTF cells
individual MEF cells
Ct>30
Ct<30
low expression
high
17%
83%
28%
72%
D
Top Gene Ontology Terms
regulation of transcription
regulation of RNA metabolic process
cell cycle
cell division
response to DNA damage
chromosome organization
skeletal system development
-log(p Value)
E
HoxA
HoxB
HoxC
Dlk1-Dio3
H19-Igf2
WT
KO
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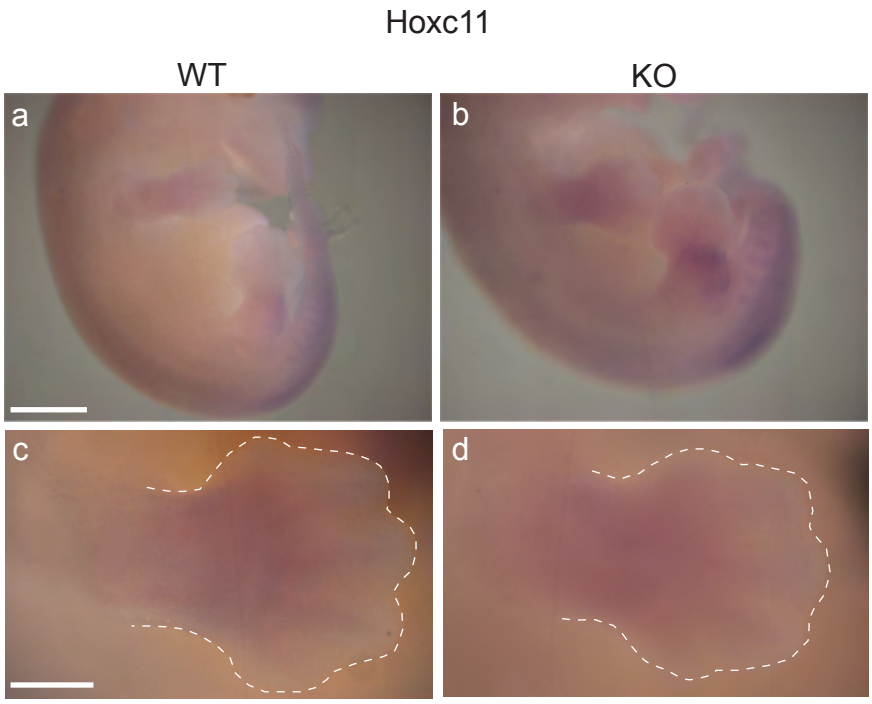
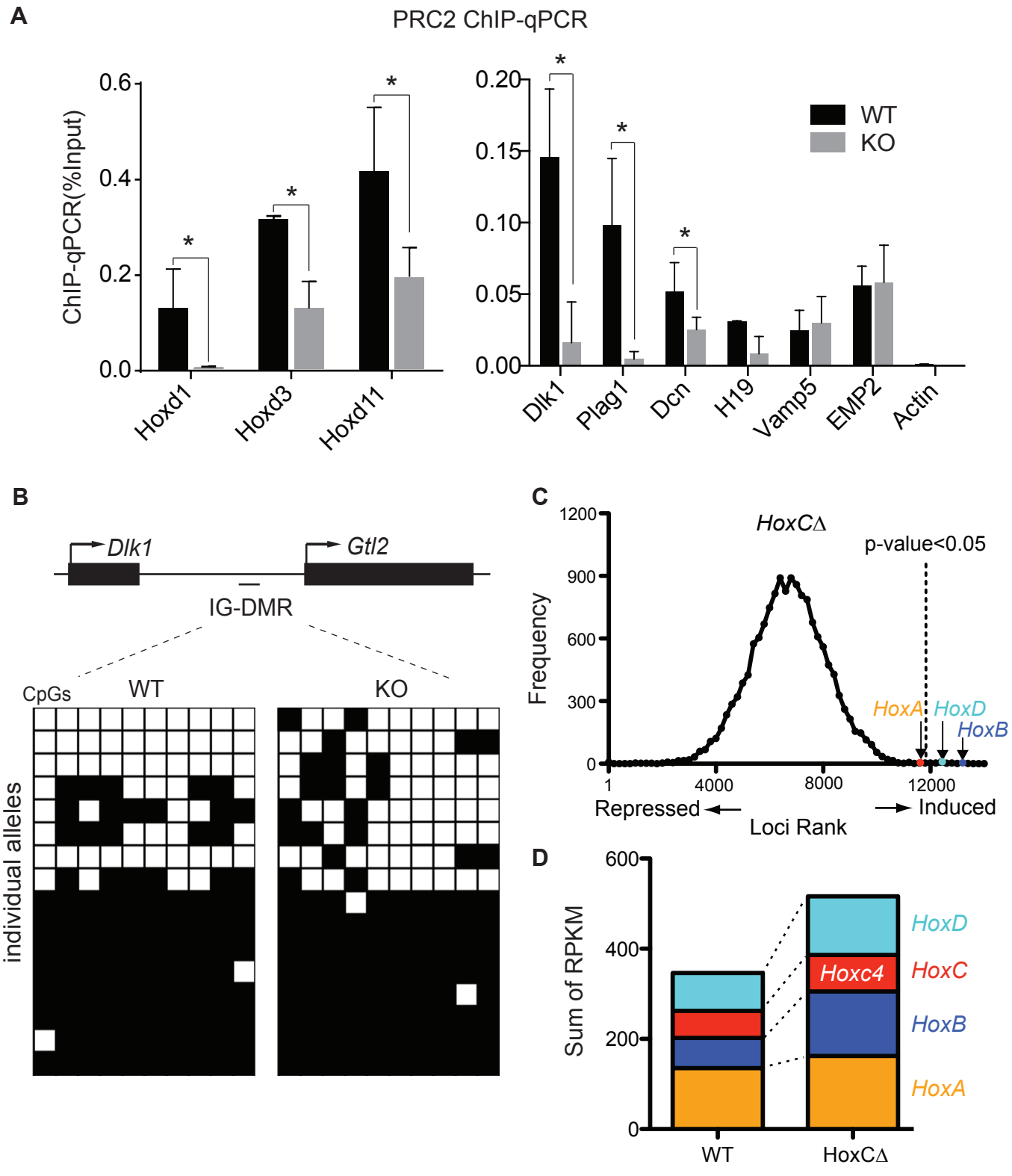
1Mb
mm9
F
Dlk1
Igf2
Geometric Mean Fluorescence Intensity (GMFI)
G
Dlk1
WT
KO


Hoxc11
WT
KO
a
b
c
d


A
PRC2 ChIP-qPCR
ChIP-qPCR(%Input)
0.6
0.4
0.2
0.0
Hoxd1
Hoxd3
Hoxd11
0.20
0.15
0.10
0.05
0.00
Dlk1
Plag1
Dcn
H19
Vamp5
EMP2
Actin
WT
KO
B
Dlk1
Gtl2
IG-DMR
CpGs
WT
KO
individual alleles
C
HoxCΔ
p-value<0.05
Frequency
1200
900
600
300
0
HoxA
HoxD
HoxB
1
4000
8000
12000
Repressed
Induced
Loci Rank
D
Sum of RPKM
600
400
200
0
HoxD
Hoxc4
HoxC
HoxB
HoxA
WT
HoxCΔ