

Supplementary Material

Table S1. PCR primers used for ChIP analysis

Primer set	Forward primer	Reverse primer
C4	GAG CTG GCC CTT GGG AAT ATG	GCC AGG AGT CAG TGC CCT GAC
C5	CAA CAT ATC AAG ATG CGT TTC	TTG ATT AAG GGT GAT TTG TGA C
C6	CAA GGC CCT GCT CAT AGA CCT TC	CAA TCG CAA GCC AGA TGT CAA G
C8	CCT CAG AGA ACT CAT CTT TTG G	CTA GCT AAT GGA CAA GGC CAG C
C9A	GCA CTC CTT CCA AAG GCT CAG	CAA ACA TCA GGG CGA CCC GCG
C9	CTC CAC TTC TTC AGG CTG GCA AAG	TCT GCC CCT GGT CAG CTC AGT TTC
C10	ATC TTT GAT GGC ACT TAG GGG CGT	AAC ATG GGT GTC TAG ACC ACA GCG
C11A	CGG TGT GCG TCT GTG AAC	GGG AGA AGC AAC CAC CAG AG
C11B	CTC TGG CGA ATC TTC CTG TAA CTC	GCC TTC TGC CTG CAC ATT GAC TTC
C11C	CCC CAG CTT GTC ATA AGG AG	TCA ATT GCA GAT TTG TAT TCG TTT
C11D	GGC AGG AGA AGA GAA CGA TG	GTT CCA GGC CGT AGG AGA C
C12A	GGC TCA TCC GTC TCC TTA AAC	CAA AAA TAT CCG CAC ATA CAC AC
C12B	TCT CCT GAA TCC TGG GTT TG	GGT TGA GAG ACA CGG GAC TG
C12C	GGT CAA GAT TTG GTT CCA GAA CCG	TGG CGT TGT AAG CGG GAC TAT GTC
C13A	GCA CTG TAA AGA GGA CTA TGG GTG	CTT CGG AGG AAT GGA GGA ATG GAG
C13B	CCA TAC ACC ACC TTC TCC TCA TTG	CTC TAG GCC TGA GTC CTT ACC TAC
GAPDH	CTG CAG TAC TGT GGG GAG GT	CAA AGG CGG AGT TAC CAG AG

Table S2. 3C PCR primers for gene desert region control.

Primer	Gene desert region primer
Desert 1	CAT GTG AAG CAC AGA CTG ATA C
Desert 2	CTG GCT GTG TAT GCA AGA AGA G
Desert 3	CAT GGT CGG TCT TCA TCC AGA AG
Desert 4	CTC AGG TTT GGT TAA AGA GCT GC
Desert 5	GAG CTT TCA AGA GTG GGA TAT C
Desert 6	CAC TTG GTG TGT GTG AGA CAC
Desert 7	GCA GGA AAG CCT TCC TAA TGT C

Table S3. Quantitation of chromatin immunoprecipitation (ChIP) products by semi-qPCR (Fig. 2).

*H3K4me3 and H3K27me3 relative to histone H3.

Primer	H3K4me3/H3			H3K27me3/H3		
	Brain	Trunk-anterior	Trunk-posterior	Brain	Trunk-anterior	Trunk-posterior
C4	0.000	0.753±0.01	1.494±0.11	2.187±0.23	0.631±0.00	0.618±0.01
C5	0.000	1.238±0.06	1.816±0.17	1.526±0.05	0.838±0.01	0.934±0.05
C6	0.000	1.456±0.32	1.824±0.17	4.348±1.16	2.695±0.81	1.250±0.34
C8	0.000	0.938±0.03	4.529±0.46	2.034±0.18	0.878±0.02	1.969±0.15
C9A	0.000	0.365±0.04	1.097±0.01	1.074±0.04	0.247±0.05	0.109±0.07
C9A	0.000	0.164±0.08	2.328±0.18	1.424±0.11	0.350±0.09	0.659±0.06
C10	0.000	0.000	0.492±0.07	0.472±0.06	0.037±0.05	0.000
C11D	0.000	0.000	0.727±0.04	0.848±0.02	0.396±0.06	0.547±0.04
C11C	0.000	0.000	0.959±0.03	1.319±0.06	0.360±0.09	0.939±0.06
C11B	0.000	0.000	0.584±0.02	0.838±0.02	0.344±0.05	0.337±0.06
C11A	0.000	0.000	0.023±0.03	0.610±0.05	0.229±0.06	0.258±0.08
C12C	0.000	0.000	0.714±0.02	1.661±0.07	0.865±0.02	0.743±0.06
C12B	0.000	0.000	0.267±0.07	0.679±0.04	0.190±0.06	0.119±0.08
C12A	0.000	0.000	0.206±0.10	0.911±0.04	0.246±0.04	0.388±0.04
C13B	0.000	0.000	0.358±0.05	0.974±0.03	0.138±0.07	0.184±0.08
C13A	0.000	0.000	0.283±0.05	1.273±0.11	0.171±0.07	0.221±0.07

A



B

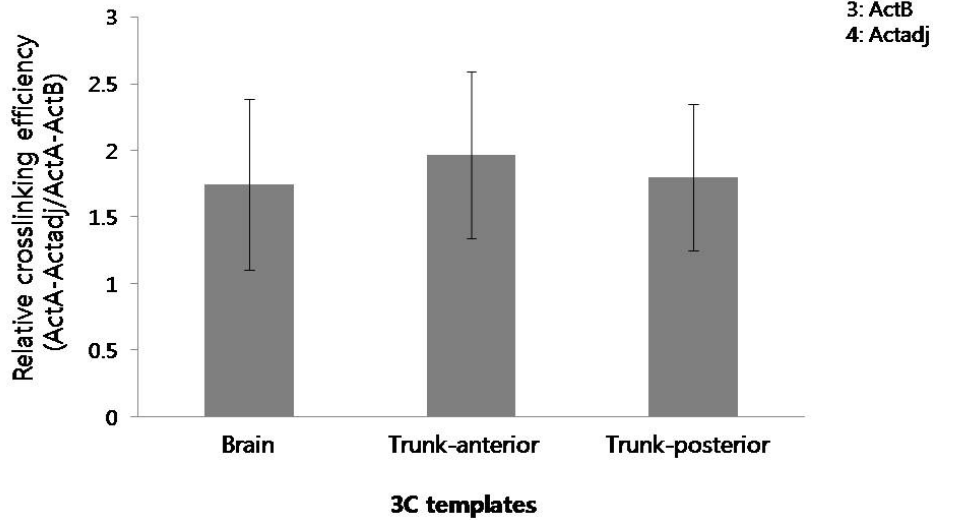


Fig. S1. The crosslinking efficiency control of 3C templates. (A) A schematic presentation of the beta-actin locus. The black arrows indicate 3C PCR primers of 1, 2 and 4 that were used to amplify the ligation products resulting from the crosslinking of the adjacent *Hind*III fragments. The 2, 3 primers were used to normalize the 3C results from different 3C templates. (B) The 3C PCR results were normalized using the genomic quantity signal (2 and 3 primers). Relative crosslinking efficiency was characterized to allow for comparison of the crosslinking signal in brain, trunk-anterior and trunk-posterior templates.

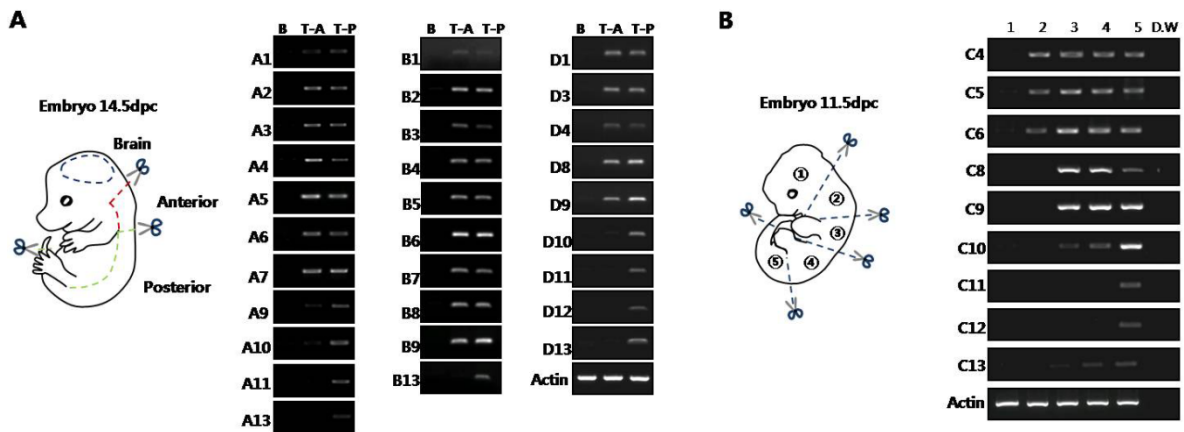


Fig. S2. Expression pattern of *Hox* cluster genes in E11.5 and E14.5 mouse embryos along the anteroposterior axis. (A) RT-PCR quantification of the expression of *Hoxc* cluster genes in five parts of E14.5 mouse embryos. Each lane refers to the five embryonic parts (see left panel for details). (B) RT-PCR analysis of *Hoxa*, *-b*, and *-d* cluster genes in the brain, and trunk-anterior and trunk-posterior regions of E11.5 mouse embryos. Genes located in the 5' end of each cluster tend to be expressed more in the posterior regions. RT-PCR was performed with the same materials that were used for the analysis of the *Hoxc* gene expression shown in Fig. 1. β -actin was used as a positive control for RNA integrity and cDNA synthesis. The data are representative of one of the three replicate experiments. B, brain; T-A, trunk-anterior; T-P, trunk-posterior.

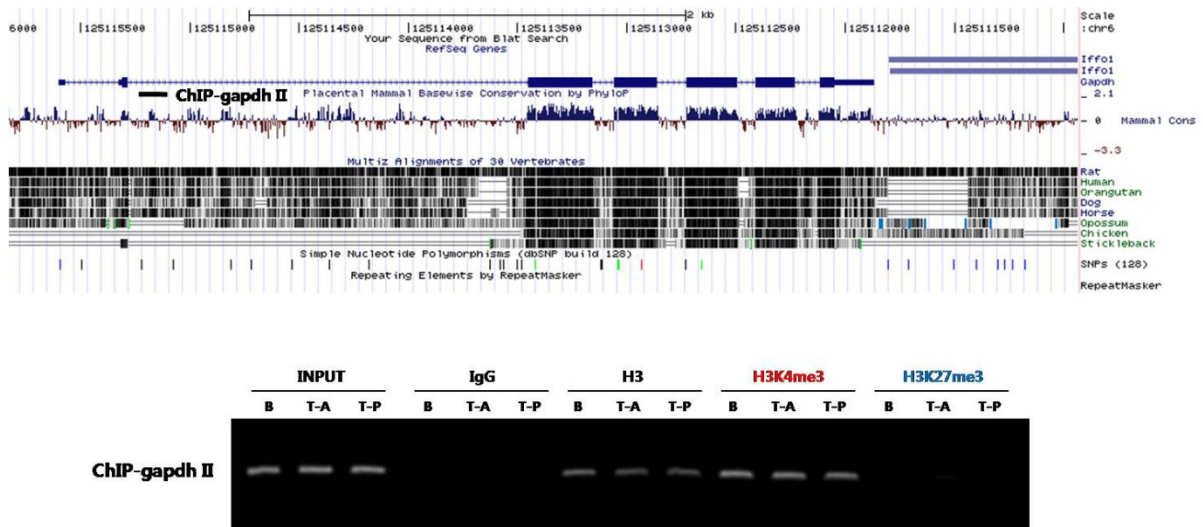


Fig. S3. The schematic diagram shows the *GAPDH* genomic locus and the position of the primer used as a control in ChIP-PCR. The PCR primers of the *GAPDH* gene was used as a positive control for the active chromatin mark H3K4me3 and a negative control for the inactive chromatin mark H3K27me3. ChIP assays were performed with the indicated antibodies on crosslinked chromatin samples from the E14.5 brain and anterior and posterior trunk tissues. Immunoprecipitated and input DNAs were amplified by PCR using region-specific primers. The data show the results of one of the three independent experiments giving comparable results. B, brain; T-A, trunk-anterior; T-P, trunk-posterior.