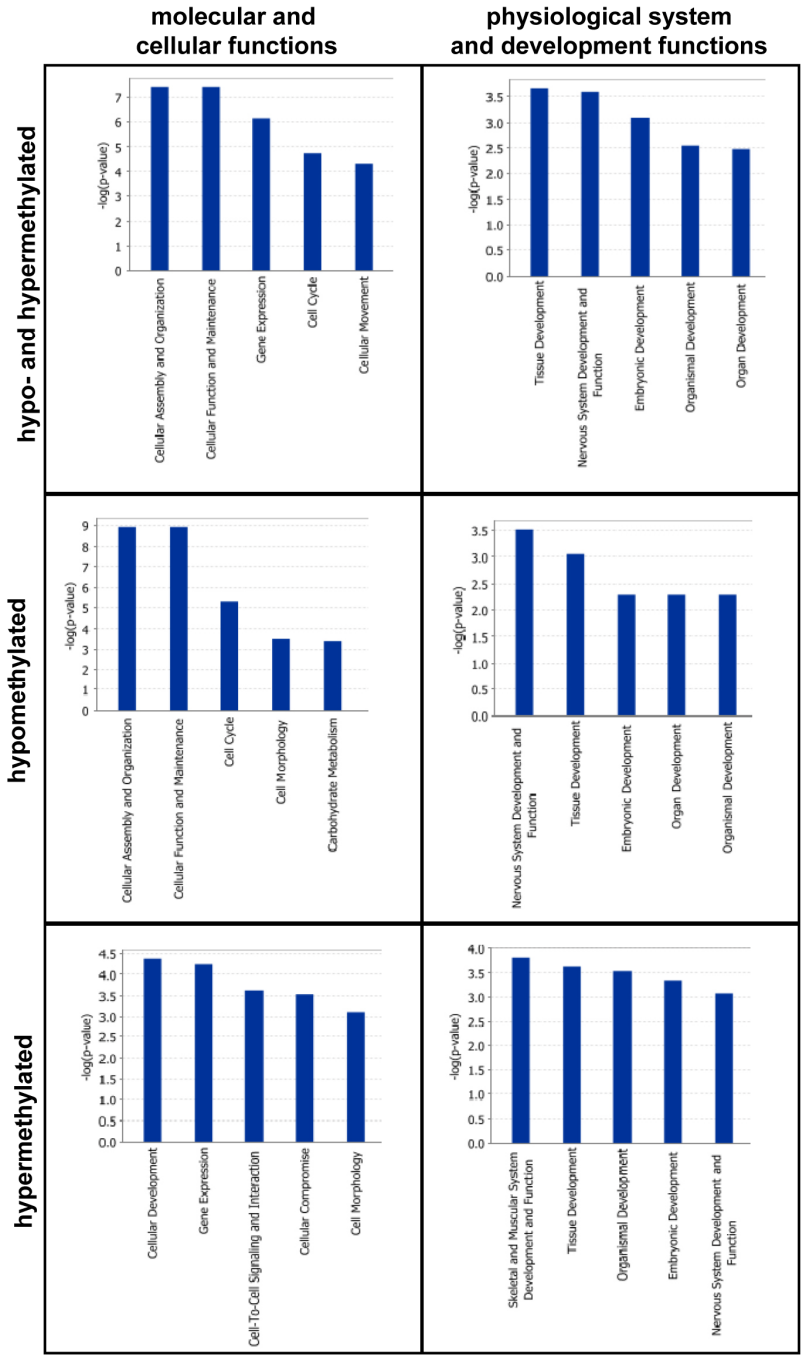
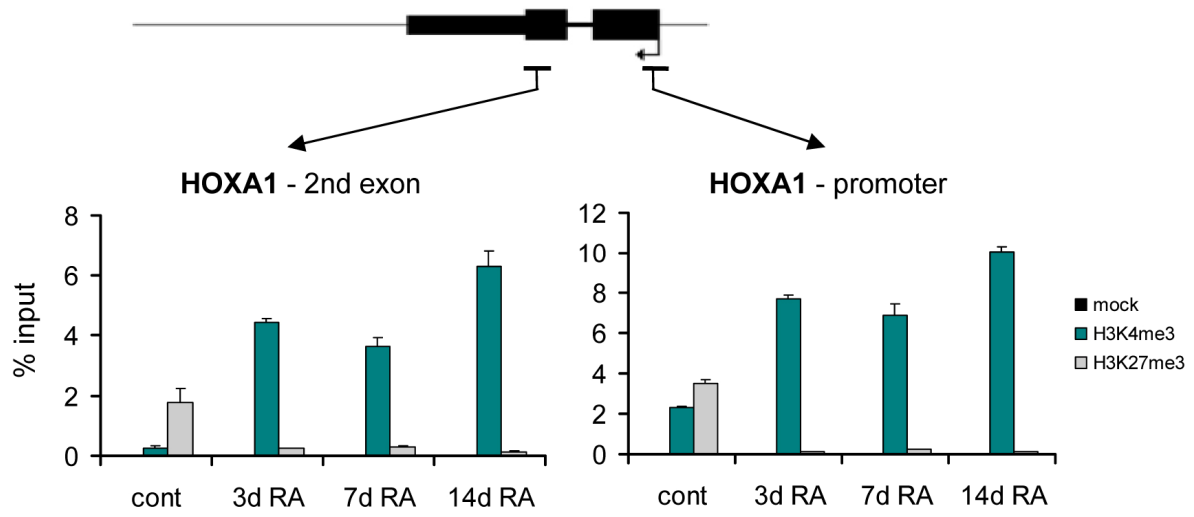
Supplementary information for Bocker et al. "Hydroxylation of 5-methylcytosine by TET2 maintains the active state of the mammalian HOXA cluster".

Supplementary Figure S1



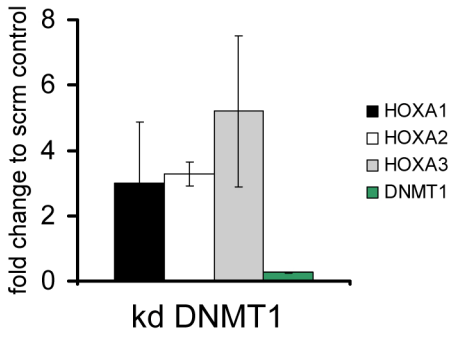
Ingenuity Pathway Analysis of differentially methylated genes of NT2 cells after 14 days of RA treatment. The bar diagrams show the five most enriched molecular and cellular functions (right) and physiological functions (left) found correlated with differentially methylated genes.

Supplementary Figure S2



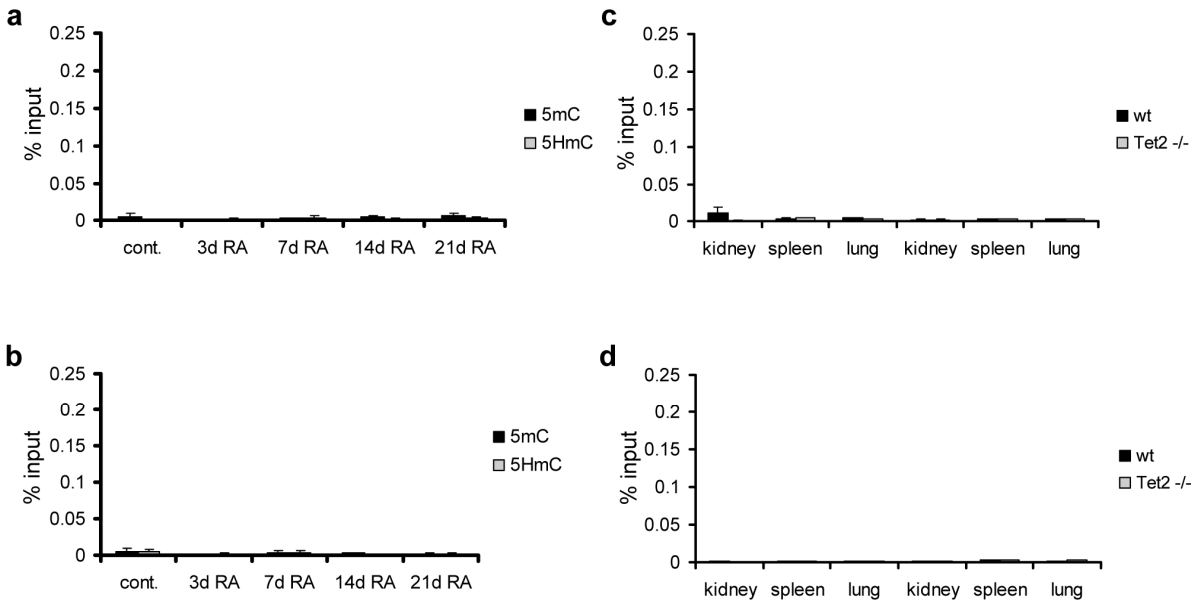
Histone modifications in the *HOXA1* region before and after RA treatment. Chromatin immunoprecipitation (ChIP) analysis of the *HOXA1* gene in control (cont.) NT2 cells and cells treated for 3 days, 7 days and 14 days with RA. ChIP was repeated at least three times with chromatin from biological replicates using ChIP-grade antibodies specific for H3K27me3 and H3K4me3. Immunoprecipitated DNA was analyzed by real time PCR using two primer pairs specific for the *HOXA1* promoter region and the second exon. The sketch above the diagrams shows the positions of the primers. The primer pair in the second exon covers a potential NANOG/OCT4 binding site[37]. Enrichments are shown as percentage of the total input. Standard deviations are indicated by error bars.

Supplementary Figure S3



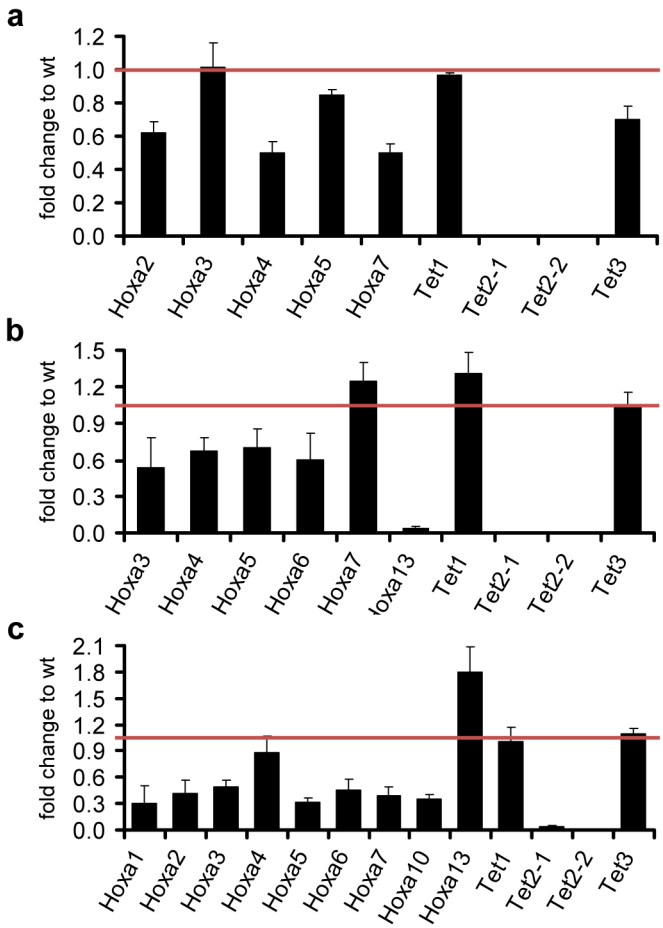
The effect of DNMT1 depletion on *HOXA* gene expression. Quantitative RT-PCR expression analysis of *HOXA1, HOXA2*, *HOXA3* and *DNMT1* after 3 days of DNMT1 knock down with a specific siRNA pool in NT2 cells. The diagrams shows fold differences compared to the scrambled control (non-targeting pool). Quantitative RT-PCR measurements are from two independent experiments and were internally normalised to the corresponding *lamin-b* and *β-actin* expression levels. The range is indicated by error bars.

Supplementary Figure S4



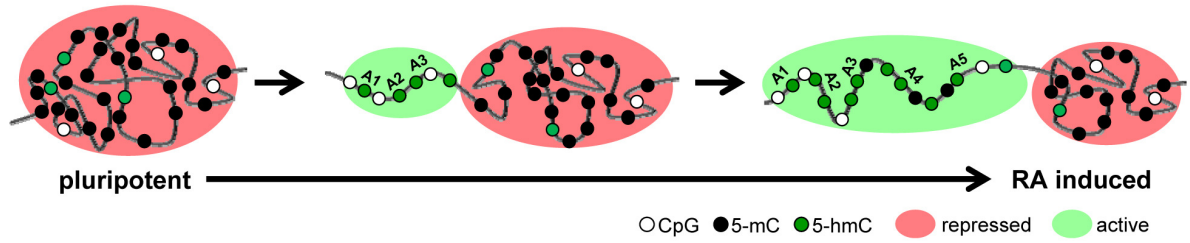
Examples of negative control experiments for gene specific MeDIP experiments. DNA immunoprecipitated with antibodies specific for 5mC or 5hmC using genomic DNA from NT2 cells or mouse tissues was amplified using primer pairs specific for the human unmethylated control regions[52] *UEB2B* (**a**) and *HIST1H3B* (**b**) or mouse unmethylated control regions *Gapdh* (**c**) and *β-actin* (**d**). No significant enrichments were found. Diagrams show the results of at least three independent experiments. Standard deviations are indicated by error bars.

Supplementary Figure S5



Expression of *Hoxa* genes is reduced in mouse *Tet2*$^{-/-}$ tissues. qRT-PCR expression analysis of *Hoxa* and *Tet* genes in three more tissues from the *Tet2*$^{-/-}$ mouse. Diagrams show the expression as fold change relative to the wild type (=1). For *Tet2*, two different primer pairs were used[39]. (**a**) spinal chord, (**b**) uterus and (**c**) ovary. Standard deviations of three replicates are indicated by error bars

Supplementary Figure S6



A model describing the role of DNA methylation during the activation of the HOXA cluster. The inactive cluster in pluripotent cells is part of an inactive higher order structure that is incompatible with transcription. The cluster is mainly marked by 5mC on the DNA level. During RA-induced differentiation HOXA genes move progressively into an active compartment, which is accompanied by 5mC-5hmC conversion.

Supplementary Table S1. HiSeq reads.

	Reads #	sample	reads per sample	mapped reads
MeDIP-pool	92.393 x 10^6	Control-1	45.246 x 10^6	31.513 x 10^6 (70%)
		14d RA-1	47.147 x 10^6	31.690 x 10^6 (68%)
hMeDIP-pool	38.373 x 10^6	Control-2	10.839 x 10^6	6.461 x 10^6 (68%)
		14d RA-2	27.534 x 10^6	20.606 x 10^6 (75%)

Supplementary Table S2. RT-PCR primer pairs.

gene	forward primer	reverse primer
human HOXA1	GCCGTACTCTCCAACTTTC	CTCGCCTCAATACATTCACC
human HOXA2	TGCAGCATCTGAATTACTAAAAACA	CCAAATAAAAGAAGGCAAAACC
human HOXA3	TGCTTTGTGTTTTGTCGAGACTC	CAACCCTACCCCTGCCAAC
human HOXA4	ATGAAGAAGATCCATGTCAGC	CAGACAAACAGAGCGTGTGG
human HOXA5	TGAAGTGGAACTCCTTCTCCAGC	CGCAAGCTGCACATAAGTCATG
human HOXA6	TGGGCTGCGTGGAATTGATGAGC	GATGCAGCGCATGAACTCCTGCG
human OCT4	CTGACAACAATGAAAATCTTCAG	GTTACAGAACCACACTCGGAC
human β-actin	GATCAAGATCATTGCTCCTCCTG	CTAGAAGCATTTGCGGTGGAC
human lamin-b	CTGGAAATGTTTGCATCGAAGA	GCCTCCCATTGGTTGATCC
human DNMT1	Hs_DNMT1_1_SG QuantiTect Primer Assay (Qiagen)	
human DNMT3a	Rn_Dnmt3a_2_SG QuantiTect Primer Assay (Qiagen)	
human DNMT3b	Hs_DNMT3B_1_SG QuantiTect Primer Assay (Qiagen)	
human TET1	TCTGTTGTTGTGCCTCTGGA	GCCTTTAAAACTTTGGGCTTC
human TET2	AAAGATGAAGGTCCTTTTTATACCC	TTTACCCTTCTGTCCAAACCTT
human TET3	CCATTGCAAAGTGGGTGA	CGCACCAGGCAGAGTAGC
mouse Hoxa1[1]	CCTTGGCAGTGGCGACTCT	GCGCAGGATTGGAAAGTTGT
mouse Hoxa2[1]	TCGCTGAGTGCCTGACATCT	AAAGCGTCGAGGTCTTGATTG
mouse Hoxa3[1]	CAATGGGTTCGCTTACAATGC	AGGCAGGTCGATGGTACTCAAC
mouse Hoxa4[1]	CCGGAGAATGAAGTGGAAGAAA	GCCGAGGCAGTGTTGGAA
mouse Hoxa5[1]	TAGTTCCGTGAGCGAACAATTC	GCTGAGATCCATGCCATTGTAG
mouse Hoxa6[1]	CCTATTTTGTGAATCCCACTTTC	CAGCTGGCCCAAGAAGGA
mouse Hoxa7[1]	ACGCGCTTTTTAGCAAATATACG	GGGTGCAAAGGAGCAAGAAG
mouse Hoxa9[1]	CCGAACACCCCGACTTCA	TTCCACGAGGCACCAAACA
mouse Hoxa10[1]	CACAGGCCACTTCGTGTTCTT	TTGTCCGCAGCATCGTAGAG
mouse Hoxa11[1]	AGATTTCTCCAGCCTCCCTTCTT	TGGAGGAGTAGGAGTATGTCATTGG
mouse Hoxa13[1]	CCTCCCCACCTCTGGAAGTC	TAAGGCACGCGCTTCTTTCT
mouse Tet1[2]	CTGAGCCTGTTCCTCGATGTGG	AGGTGAGAAGTAGATGAGGCTGATG
mouse Tet2-1[2]	GTCAACAGGACATGATCCAGGAG	CCTGTTCCATCAGGCTTGCT
mouse Tet2-2[2]	AGCCTGATGGAACAGGACAG	AACGGGCTTCCATTCTGGAG
mouse Tet3[2]	GGAGTTGGCTGGAGTCACCAC	CCACCGCATTGCCACTGTAC
mouse β-actin[2]	TGAACCCTAAGGCCAACCGTGAAA	CAGGATGGCGTGAGGGAGAGCATAG
mouse GPDH	CATGGCCTTCCGTGTTCCTA	TGCTTCACCACCTTCTTGATGT

Primer sequences published in:

[1] Reference 56

[2] Reference 39

Supplementary Table S3. Primer pairs for 454 bisulfite sequencing.

Each 454 primer consists of a 454 adaptor (forward GCCTCCCTCGCGCCATCAG, reverse GCCTTGCCAGCCCGCTCAG), a sample specific multiplex identifier (CACA for untreated NT2 cells and CTCT for 21d RA-induced NT2 cells as well as a gene specific sequence (see below).

amplicon name		primer
hoxa1-down	forward	TAAGAATTTAGAGGGTGAAGGTTG
	reverse	TCAACTATCCAAAACTTAAAAAAAA
hoxa1-1stex	forward	TGGATTATAATTTGAGTGGGAGTAG
	reverse	AAAATACCCCATACTTAACAATAAC
hoxa2-2ndex	forward	GTTTAGGTTGGGTTTTTAGGAAAG
	reverse	TTATCTCTCAATCAAATCCAACAAC
hoxa3-6	forward	ATGGGTTTTATATAGTTGTTTTTTA
	reverse	ACCAATCTACTAAACCTCACTAAAC
hoxa3-5-1	forward	GGTTATTGTGGGTGAGTTGAGTAG
	reverse	ATATCAAACCCCTATCAAAATATAC
hoxa3-3	forward	GTAAGAATTTGTTAGGGGAAGGG
	reverse	CTCCAAAACCAATCCTTTAAATTTA
hoxa3-3-3	forward	TTAAGAGGATAAATTAAGTTAGAATTTGAA
	reverse	CAAAACAAAAACAAATAAAAACCAC
hoxa3-2	forward	TTGATTAGAATTTTTTTTAATAGGT
	reverse	ATACCCTAATAACTCCCTAATATTC
hoxa4-2ndex	forward	GTTTTGAGTTTGTGTTTTTTTTGGT
	reverse	AATAATATCCAATTACTCTTCCCCAC
hoxa5-6-2	forward	AGTTGAGAGGTAAGTGGAGTTTTTT
	reverse	AAAAAAAATCTAAAAACTAAAACCC
hoxa6-1	forward	GGGGTTTTTTTGTTTGTTATTGTT
	reverse	CCTTCTTAAACCAACTACCCCTCTACC
hoxa7	forward	GGTGAGAGAAGATTTGGGTA
	reverse	CACCCCCAAATTTACACCAA
hoxa9	forward	ATTATTGTTTAATTTTATGTGAGGG
	reverse	AAAAACTAACCCAAAATCCC
hoxa10	forward	GTAGAGGTAGTAGGAGTTTTTTTT
	reverse	CATATTTTACACAAAAAATATCAACCAAA

Supplementary Table S4. ChIP and MeDIP qPCR-primer.

amplicon name	amplicon position (hg19 Feb 2009)	forward primer reverse primer
HOXA-down	Chr7:27127898-27128122	CAAGAACTCAGAGGGTGAAG CGAGTCGCTCGGGCAGC
HOXA1-second exon	Chr7:27133310-27133475	TGGGAAAGACTTAGCACAGGAAGC TGGGCAAACTCTCTAAACTGGTACAA
HOXA1-promoter	Chr7:27135417-27135585	ACTGGAAAGTTGTAATCCTATG AGAAAGTTGGCACAGTCACG
HOXA1 -upstream_RARE	Chr7:27136210-27136464	CATAGAACCGTGGTGGAGG CGAGCTCTGAGTTTCCAGG
HOXA2-promoter	Chr7:27142260-27142538	TTCACTGATTACAGCCGTATG CTCGCCCCCACGTACTCC
HOXA3-2nd_CGI	Chr7:27161528-27161808	TTTCAAATAGGAAAAGGAAACGC GTGGGCTGCTCTCTTTGTC
HOXA4-second exon	Chr7:27168958-27169218	GGATCGCATCTTGGTGTTGG CTTCTATTTCGGGGTTCTGG
HOXA5-promoter	Chr7:27182940-27183304	GGAGAGTGCGTGGACGTG CATCATAAATTGTGCAAGGGTG
HOAX5-6	Chr7:27184407-27184732	AGAGGCAAGTGGAGTCTCC CCAGAACAGGAAGGAAGCTC
HOXA6-promoter	Chr7:27187228-27187495	GAGACTCGACGCCCCGTAC GGTGCGATTTGCTGCTGTC
UEB2B*	Chr5:133706782-133706958	CTCAGGGGTGGATTGTTGAC TGTGGATTCAAAGACCACGA
HIST1H3B*	Chr6:26032431-26032716	CTGTGCCTGGTTGCAGATTA CCCACACTTCTTATGCGACA
mHoxa2-promoter	Chr6:52114601-52114889	GCAGAAGCAATCATGTGACAGC GGCTATTGATAAAACCAATCTCTCG
mHoxa4-first exont	Chr6:52141145-52141397	GCGACCCGAGCAGTCCC ACGCCCGCTGCCCTCC
mHoxa5-first exon	Chr6:52153936-52154206	CTACAATGGCATGGATCTCAGC GCTGATGTGGGTGCTGCC
mHoxa7-promoter	Chr6:52168388-52168649	GTCTGAAGCCCAGCAAGGTAAG GCAAGCCTTCCACCCTGTG
mGpdh-promoter*	Chr6:125115488-125115620	CTCTGCTCCTCCCTGTTCC TCCCTAGACCCGTACAGTGC
mBeta-actin-promoter*	Chr5:143668015-143668193	AGCCAACTTTACGCCTAGCGT TCTCAAGATGGACCTAATACGGC

* Primer sequences published in Reference 52.

Supplementary Reference

56.Lebert-Ghali, C.E. *et al.* HoxA cluster is haploinsufficient for activity of hematopoietic stem and progenitor cells. *Exp. Hematol.* **38**, 1074-1086 (2011).