

Guiding the HBO1 complex function through the JADE subunit

In the format provided by the
authors and unedited

Table of content

Supplementary Figure 1. HEK293T cells transiently expressing FLAG-tagged JADE1, JADE2 or JADE3 and Xpress-tagged HBO1, ING5, and MEAF6 were subjected to fanChIP analysis.

Supplementary Figure 2. A schematic of reconstitution of NCPs.

Supplementary Figure 3. In vitro HAT assays with purified HBO1-JADE1-ING4/5-MEAF6 and HBO1-JADE1S complexes.

Supplementary Figure 4. Acetylation of H4 and H3 tails by the indicated HBO1 complexes at an active H3K4me3-enriched gene promoter *in vivo*.

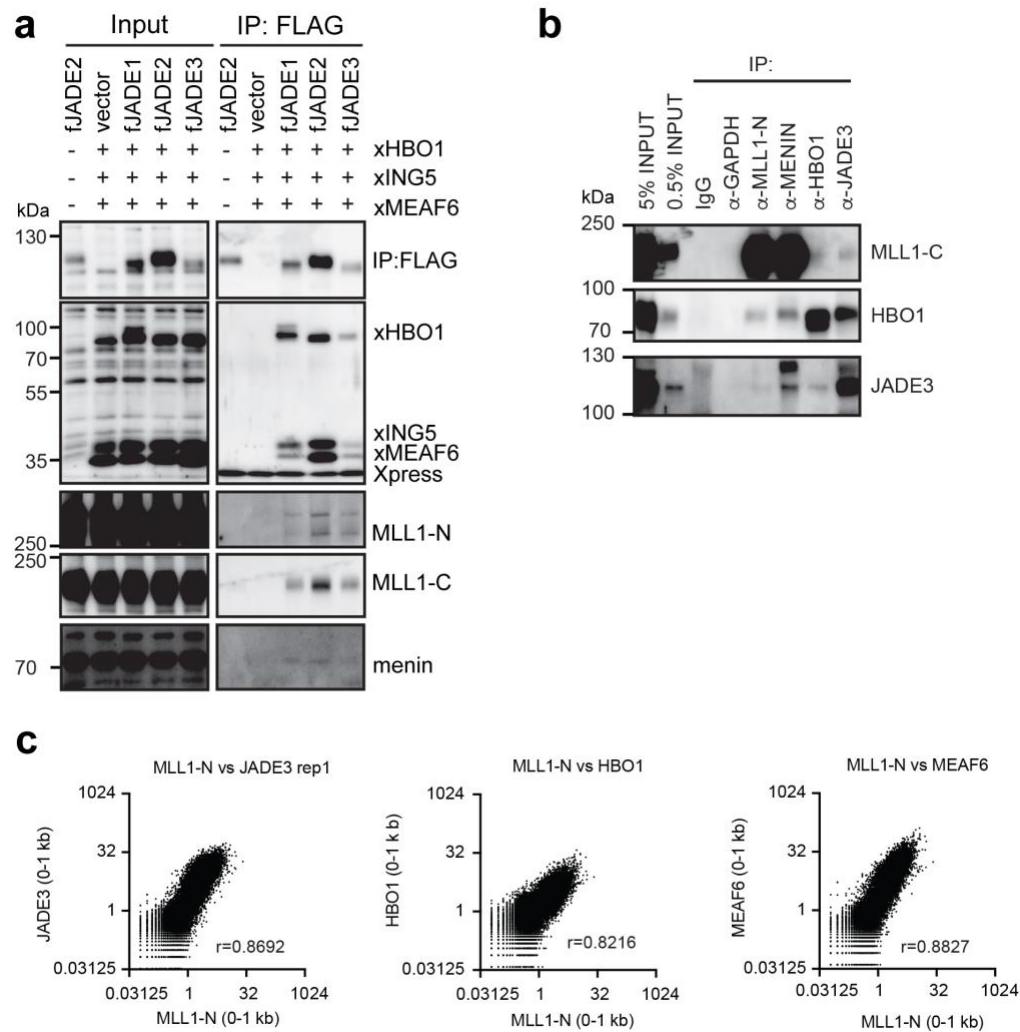
Supplementary Figure 5. Subcellular localization of JADE3/HBO1 complex components.

Supplementary Figure 6. Protein expression of NUP98-JADE fusions.

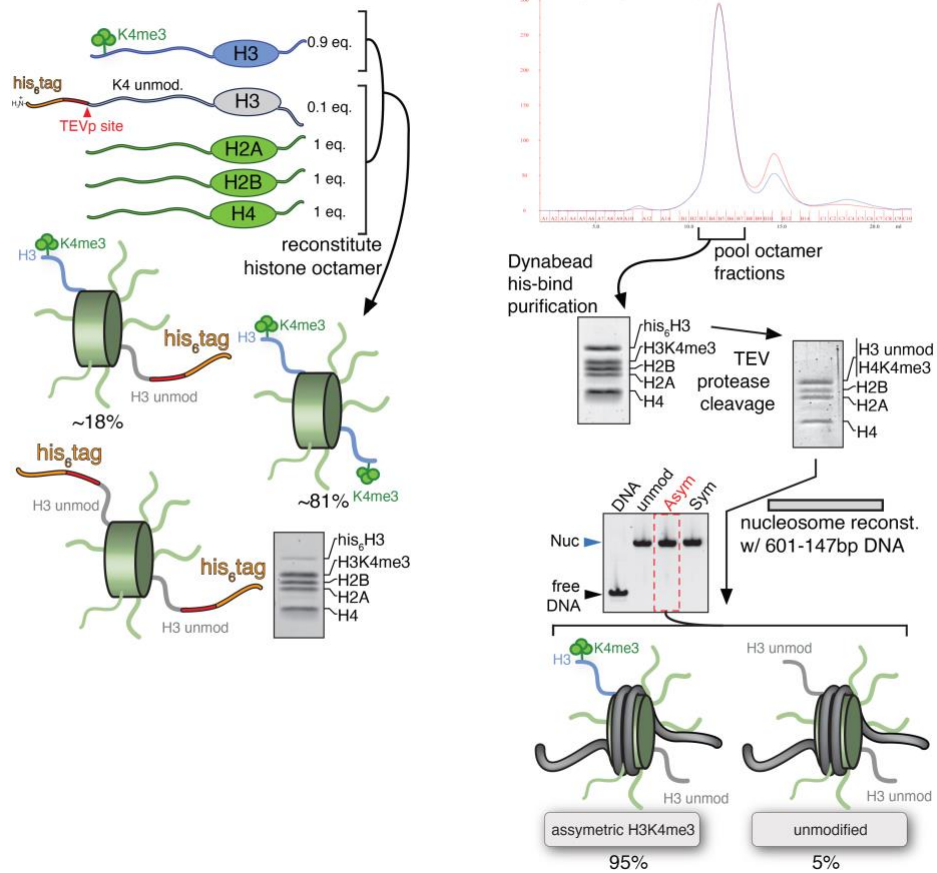
Supplementary Table 1. Antibodies and other reagents.

Supplementary Table 2. Custom qPCR probe/primer sequences.

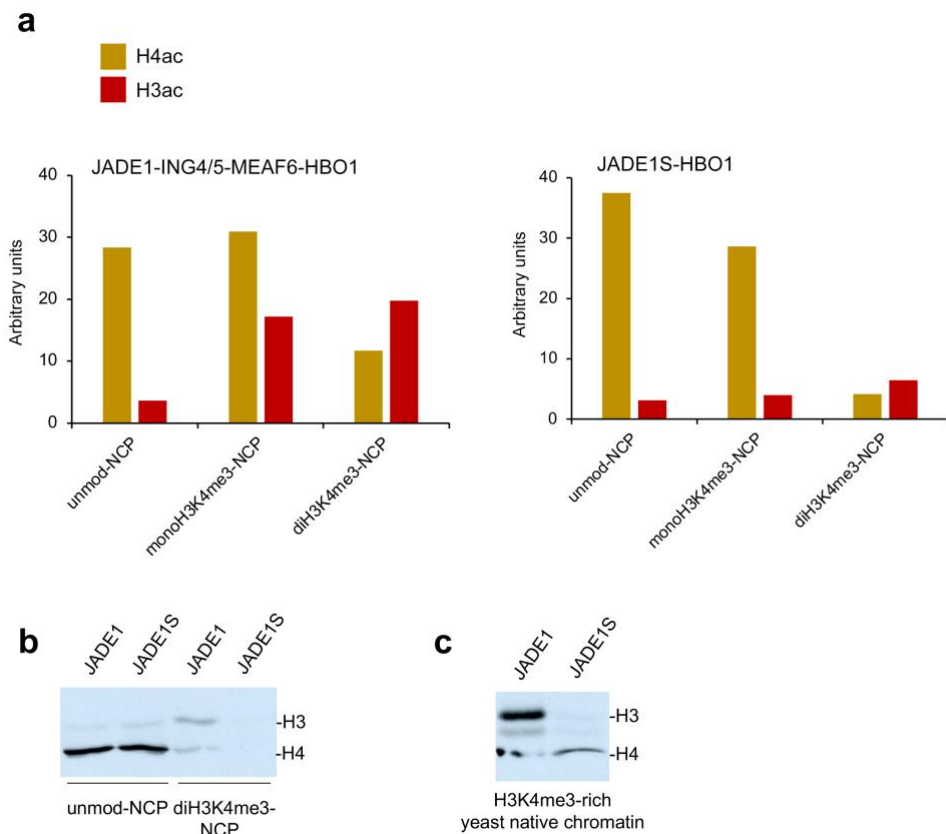
Supplementary Table 3. Accession numbers of the NGS data.



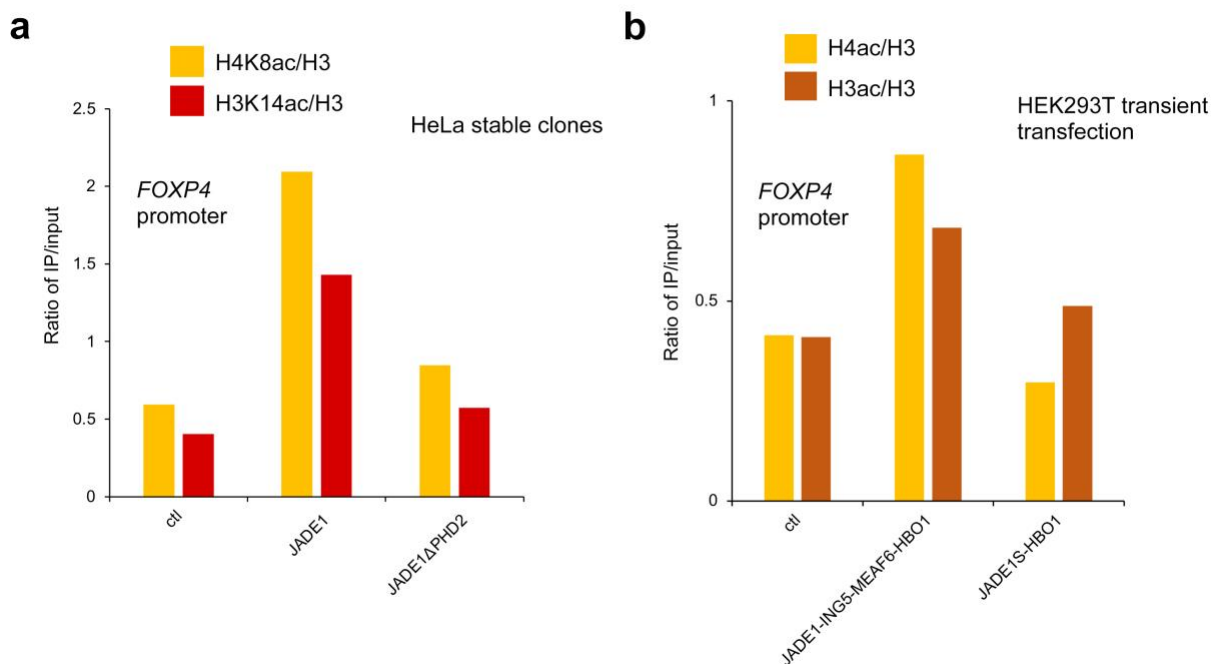
Supplementary Figure 1. (a) HEK293T cells transiently expressing FLAG-tagged JADE1, JADE2 or JADE3 and Xpress-tagged HBO1, ING5, and MEAF6 were subjected to fanChIP analysis using anti-FLAG antibody. The exogenously expressed proteins and endogenous MLL1 and MENIN were detected by indicated antibodies. Note that although components of the JADE/HBO1 complex associate with MLL1 on chromatin, they may not be direct components of a soluble MLL1 complex, as fanChIP assays capture protein-chromatin complexes. (b) HEK293T cells were subjected to fanChIP analysis using specific antibodies to endogenous proteins. Interactions of the MLL1 complex and HBO1 complex components were confirmed at endogenous level. (c) The ChIP-seq tags were clustered into a 1 kb bin (0 to +1 kb from the TSS) and are presented as XY scatter plots with the Spearman's rank correlation coefficient (r). Related to Figure 1.



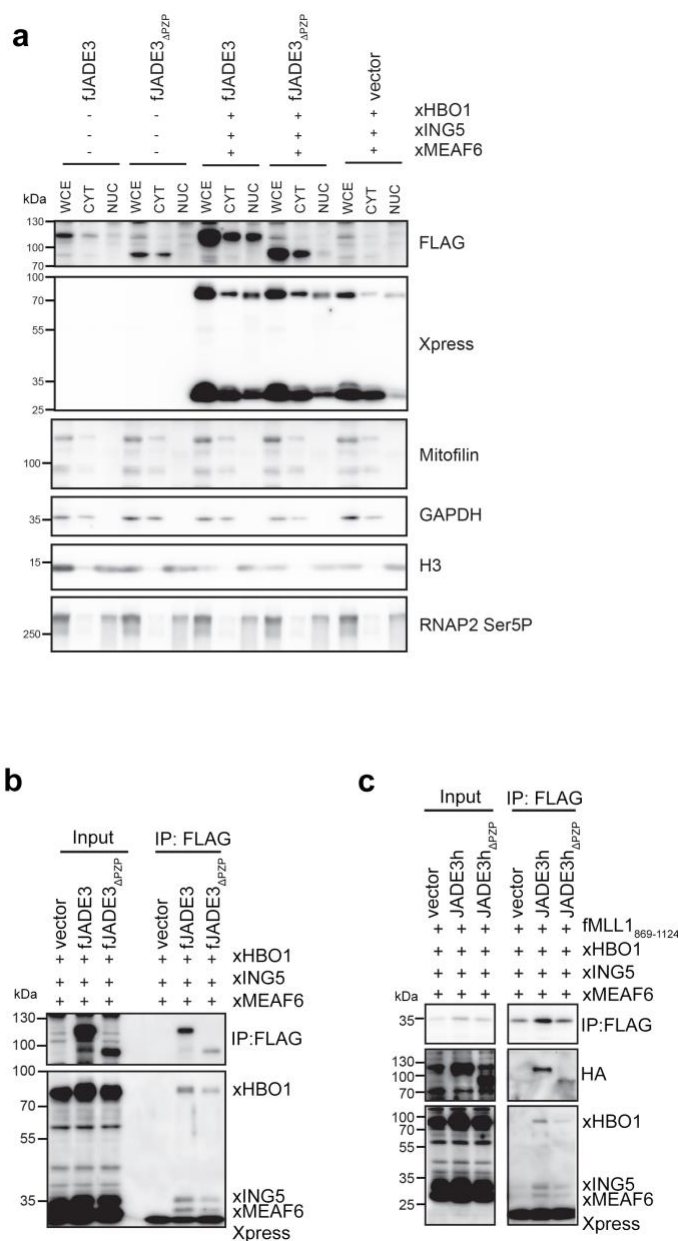
Supplementary Figure 2. A schematic of reconstitution of NCPs. Related to Figure 2.



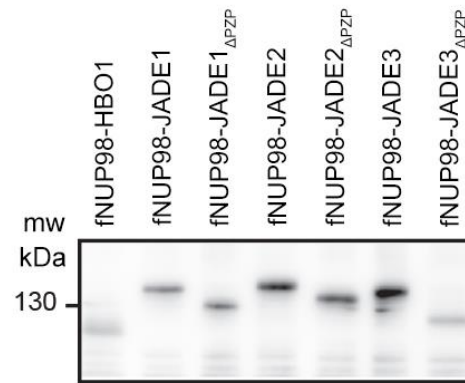
Supplementary Figure 3. In vitro HAT assays with purified HBO1-JADE1-ING4/5-MEAF6 and HBO1-JADE1S complexes. (a) quantification of the radioactive HAT assay shown in Figure 2d using the ImageJ software. Signal intensity of H3 and H4 bands were measured on unmodified, asymmetrically methylated, and symmetrically methylated nucleosomes. (b) The radioactive HAT assay (fluorogram) on unmodified or symmetrically methylated nucleosomes. (c) The radioactive HAT assay using purified yeast native chromatin (oligonucleosomes). Related to Figure 2.



Supplementary Figure 4. Acetylation of H4 and H3 tails by the indicated HBO1 complexes at an active H3K4me3-enriched gene promoter *in vivo*. (a) ChIP-qPCR assays in HeLa cells stably expressing JADE1 or JADE1ΔPHD2 compared to non-transfected cells. H4K8ac, H3K14ac and H3 levels were measured in three different cell lines at the transcriptionally active *FOXP4* promoter. Values are presented as ratio of IP/input normalized on H3 signals to correct for nucleosome occupancy. n=2 independent samples. (b) ChIP-qPCR assays as in (a) but in HEK293T cells transiently transfected with all components of JADE1 or JADE1S complexes. Related to Figure 2.



Supplementary Figure 5. (a) Subcellular localization of JADE3/HBO1 complex components. HEK293T cells transiently expressing FLAG-tagged JADE3 proteins and Xpress-tagged HBO1, MEAF6 and ING5 were separated into the cytosolic fraction (CYT) and the nuclear fraction (NUC). Whole cell extracts (WCE) were also analyzed for comparison. The distribution of the exogenous proteins was visualized using anti-FLAG and Xpress antibodies. Representative endogenous proteins were detected using indicated antibodies to ensure successful fractionation. (b) HEK293T cells transiently expressing FLAG-tagged JADE3 proteins and Xpress-tagged HBO1, ING5, and MEAF6 were subjected to fanChIP analysis using anti-FLAG antibody. The co-precipitates were visualized using indicated antibodies. (c) HEK293T cells transiently expressing FLAG-tagged MLL1 fragment (869-1124aa), HA-tagged JADE3 proteins, and Xpress-tagged HBO1, ING5, and MEAF6 were subjected to fanChIP analysis using anti-FLAG antibody. The co-precipitates were visualized using indicated antibodies. Related to Figure 6.



Supplementary Figure 6. Protein expression of NUP98-JADE fusions. The whole cell extracts of the virus packaging cells were subjected to WB analysis using anti-FLAG antibody. Related to Figure 7.

Supplementary Table 1. Antibodies and other reagents. Related to Figures 1, 6 and 7.

Reagent/Resource	Source	Identifier
Antibodies		
FLAG [WB 1000:1]	Sigma-Aldrich	Cat#F7425; RRID: AB_439687
FLAG (M2) [ChIP: 1 µg/ 400 µL]	Sigma-Aldrich	Cat#:F3165 RRID:AB_259529
Xpress [WB 1000:1]	Santa Cruz Biotechnology	Cat#sc-499; RRID: AB_675764
HA (3F10) [WB 1000:1]	Roche	Cat#11867423001; RRID: AB_390918
JADE3 [ChIP: 1 µg/400 µL]	Abcam	Cat#129495
MLL(N) [ChIP: 1 µg/400 µL]	Cell Signaling Technology	Cat# 14689 RRID: AB_2688009
RNAP2 Ser5-P [ChIP: 1 µg/400 µL] [WB 1000:1]	Millipore	Cat#05-623; RRID: AB_309852
RNAP2 non-P [ChIP: 1 µg/400 µL]	Abcam	Cat#ab817; RRID: AB_306327
HBO1 [ChIP: 1 µg/400 µL]	Abcam	Cat#:70183 RRID:AB_1269226
ING4 [ChIP: 1 µg/400 µL]	Abcam	Cat#:108621 RRID:AB_10860023
MEAF6[ChIP: 1 µg/400 µL]	STJ	Cat#116836
Histone H3 [WB 1000:1]	Cell Signaling Technology	Cat#4499
Mitofilin [WB 1000:1]	Abcam	Cat# AB_137057
GAPDH [WB 1000:1]	Cell Signaling Technology	Cat#1118
MENIN [WB 1000:1]	Santa Cruz Biotechnology	Cat#sc-8200
MLL1(C) [WB 1000:1]	Cell Signaling Technology	Cat#14197
Histone H3K4me3[ChIP: 1 µg/400 µL]	Active motif	Cat#39159
HRP-conjugated anti-rabbit IgG(H+L) [WB 5000:1]	Bethyl Laboratories	Cat#A120-201P
HRP-conjugated anti-mouse IgG(H+L) [WB 5000:1]	Bethyl Laboratories	Cat#A90-516P
HRP-conjugated anti-rat IgG(H+L) [WB 5000:1]	Bethyl Laboratories	Cat#A110-305P
Chemicals, peptides, and recombinant proteins		
MNase	Sigma-Aldrich	Cat#N3755-200U
Protease inhibitor cocktail	Roche	Cat#11873580001
Protein-G Magnetic beads	Thermo Scientific	Cat#1004D
mGM-CSF	Fisher Peprotech	Cat#500-P65

mIL3	Peprotech	Cat#213-13
mSCF	Peprotech	Cat#250-03
Critical commercial assays		
RNeasy Mini Kit	Qiagen	Cat#74106
SuperScript™ III First-Strand cDNA Synthesis System	Thermo Scientific	Fisher Cat#18080051
SureSelect Strand Specific RNA Library Prep Kit	Agilent Technologies	Cat#G9691A
TruSeq ChIP Sample Prep Kit SetB	Illumina	Cat#IP-202-1024
FLAG M2 antibody-conjugated beads		Cat#M8823; RRID: AB_2637089
c-Kit magnetic beads	Sigma-Aldrich	Cat#130-091-224
ECL Prime Western Blotting Detection Reagent	Miltenyi Biotec	Cat# 12316992
Lipofectamine 2000 transfection reagent	GE Healthcare	Cat#11668027
MycoAlert Mycoplasma Detection Kit	Thermo Fisher	Cat#LT07-218
Lonza		
Experimental models: Cell lines		
Human: HEK293T	ATCC	Cat# CRL-3216, RRID:CVCL_0063
Human: PLAT-E	Laboratory of Toshio Kitamura	N/A
Experimental models: Organisms/strains		
Mouse: C57BL/6JJcl	CLEA Japan, Inc.	C57BL/6JJcl
Oligonucleotides		
Taqman probe for murine <i>Gapdh</i>	Life Technologies	Mm99999915_g1
Taqman probe for murine <i>Hoxa9</i>	Life Technologies	Mm00439364_m1
Custom qPCR probe/primer sequences (see Supplementary Table 3)		
Recombinant DNA		
pCMV5	Laboratory of Michael Cleary	N/A
pMD2.G		Addgene #12259 Plasmid
pMDLg/pRRE		Addgene #12251 Plasmid
pMSCV	Clontech	634401
pRSV-rev		Addgene #12253 Plasmid
pCDH-MSCV-MCS-EF1-Puro	System Biosciences	Cat#CD710B-1
Reagent/Resource	Source	Identifier
Antibodies		
FLAG [WB 1000:1]	Sigma-Aldrich	Cat#F7425; RRID: AB_439687
FLAG (M2) [ChIP: 1 µg/ 400 µL]	Sigma-Aldrich	Cat#:F3165 RRID:AB_259529

Xpress [WB 1000:1]	Santa Cruz	Cat#sc-499; RRID: AB_675764
HA (3F10) [WB 1000:1]	Biotechnology Roche	Cat#11867423001; RRID: AB_390918
JADE3[ChIP: 1 µg/400 µL]	Abcam	Cat#129495
MLL(N) [ChIP: 1 µg/400 µL]	Cell Signaling Technology	Cat# 14689 RRID: AB_2688009
RNAP2 Ser5-P [ChIP: 1 µg/400 µL] [WB 1000:1]	Millipore	Cat#05-623; RRID: AB_309852
RNAP2 non-P [ChIP: 1 µg/400 µL]	Abcam	Cat#ab817; RRID: AB_306327
HBO1 [ChIP: 1 µg/400 µL]	Abcam	Cat#:70183 RRID:AB_1269226
ING4 [ChIP: 1 µg/400 µL]	Abcam	Cat#:108621 RRID:AB_10860023
MEAF6[ChIP: 1 µg/400 µL]	STJ	Cat#116836
Histone H3 [WB 1000:1]	Cell Signaling Technology	Cat#4499
Histone H3K4me3[ChIP: 1 µg/400 µL]	Active motif	Cat#39159
HRP-conjugated anti-rabbit IgG(H+L) [WB 5000:1]	Bethyl Laboratories	Cat#A120-201P
HRP-conjugated anti-mouse IgG(H+L) [WB 5000:1]	Bethyl Laboratories	Cat#A90-516P
HRP-conjugated anti-rat IgG(H+L) [WB 5000:1]	Bethyl Laboratories	Cat#A110-305P
Chemicals, peptides, and recombinant proteins		
MNase	Sigma-Aldrich	Cat#N3755-200U
Protease inhibitor cocktail	Roche	Cat#11873580001
Protein-G Magnetic beads	Thermo Scientific	Cat#1004D
mGM-CSF	Peprotech	Cat#500-P65
mIL3	Peprotech	Cat#213-13
mSCF	Peprotech	Cat#250-03
Critical commercial assays		
RNeasy Mini Kit	Qiagen	Cat#74106
SuperScript™ III First-Strand cDNA Synthesis System	Thermo Scientific	Cat#18080051
SureSelect Strand Specific RNA Library Prep Kit	Agilent Technologies	Cat#G9691A
TruSeq ChIP Sample Prep Kit SetB	Illumina	Cat#IP-202-1024
FLAG M2 antibody-conjugated beads		Cat#M8823; RRID: AB_2637089
c-Kit magnetic beads	Sigma-Aldrich	Cat#130-091-224
ECL Prime Western Blotting Detection Reagent	Miltenyi Biotec	Cat# 12316992
Lipofectamine 2000 transfection reagent	GE Healthcare	Cat#11668027
MycoAlert Mycoplasma Detection Kit	Thermo Fisher	Cat#LT07-218
	Lonza	
Experimental models: Cell lines		

Human: HEK293T	ATCC	Cat# CRL-3216, RRID:CVCL_0063
Human: PLAT-E	Laboratory of Toshio Kitamura	N/A

Experimental models: Organisms/strains

Mouse: C57BL/6JJcl	CLEA Japan, Inc.	C57BL/6JJcl
--------------------	------------------	-------------

Oligonucleotides

Taqman probe for murine <i>Gapdh</i>	Life Technologies	Mm99999915_g1
Taqman probe for murine <i>Hoxa9</i>	Life Technologies	Mm00439364_m1
Custom qPCR probe/primer sequences (see Supplementary Table 3)		

Recombinant DNA

pCMV5	Laboratory of Michael Cleary	N/A	
pMD2.G		Addgene #12259	Plasmid
pMDLg/pRRE		Addgene #12251	Plasmid
pMSCV	Clontech	634401	
pRSV-rev		Addgene #12253	Plasmid
pCDH-MSCV-MCS-EF1-Puro	System Biosciences	Cat#CD710B-1	

Supplementary Table 2. Custom qPCR probe/primer sequences. Related to Figure 7.

Target name	Forward	Reverse	Reporter
CD4- pre-TSS	TGTCCGAGCAAGG GATGATATTG	CCAAGTCACTCTGC ACTACCA	ACTGCCACCATGCC AAT
CD4- post-TSS	GGACTCACTGTCCC TCCTGAA	CTTCAAGGCCATGA GGTCTCA	TCAGCCTTTCCGCC CTC
HOXA7 pre-TSS	GCCTTCCCCGTCTG GAT	ACTCTGCCCAAGTC TTCTCTCA	CAGGCCGGACTTA GAC
HOXA7 TSS	GACGCCTACGGCA ACCT	GCCTTTGGCGAGGT CACT	CCCTGCGCCTCCTA C
HOXA9 pre-TSS	TGGCTGCTTTTTTAT GGCTTCAATT	CCGCGTGCGAGTG C	CCCCTCACATAAAA TT
HOXA9 post-TSS	AGTGGCGGCGTAA ATCCT	TGATCACGTCTGTG GCTTATTTGAA	CCC GCAGCCTCATC
MYC pre-TSS	GCGGTATCTGCTGC TTTGG	GCATTATGTATGCA CAGCTATCTGGAT	CTGGGTGGAAGGT ATCC
MYC post-TSS	GGGTAGGCGCAGG CA	GGTTTTTCCAAGTC AACGATTCCA	ATGTGTCCGATTCT CC

Supplementary Table 3. Accession numbers of the NGS data. Related to Figures 1 and 7.

Sample name		DRA accession number	Sample ID	GEA accession number
293T-fanChIP-INPUT(21T0226-IN)		DRA012473	SAMD00393839	E-GEAD-446
293T-fanChIP-JADE3 (21T0226-PHF16)	rep1	DRA012473	SAMD00393842	E-GEAD-446
293T-fanChIP-JADE3 (20T0108-PHF16)	rep2	DRA010819	SAMD00247197	E-GEAD-401
293T-fanChIP-MLL (21T0226-MLL)		DRA012473	SAMD00393840	E-GEAD-446
293T-fanChIP-ING4 (21T0226-ING4)		DRA012473	SAMD00393843	E-GEAD-446
293T-fanChIP-HBO1(21T0226-HBO1)		DRA012473	SAMD00393841	E-GEAD-446
293T-fanChIP-H3K4me3 (15T0713_H3K4me3)		DRA004872	SAMD00055702	E-GEAD-320
293T-fanChIP-RNAP2 (21T0226-RNAP2 non-P)	non-P	DRA012473	SAMD00393846	E-GEAD-446
293T-fanChIP-RNAP2 P(21T0226-RNAP2 Ser5-P)	Ser5-P	DRA012473	SAMD00393847	E-GEAD-446
293T-fanChIP-MEAF6 (21T0226-MEAF6)		DRA012473	SAMD00393844	E-GEAD-446
293T-fanChIP-IN(22T0810 _IN)		DRA016087	SAMD00594505	E-GEAD-604
293T-fanChIP-pCMV5-FLAG(22T0831_pCMV5_M2)		DRA016087	SAMD00594508	E-GEAD-604