Inventory of Supplemental Figures:

Figure S1: Relates to Figures 1 and 4

Figure S2: Relates to Figure 2

Figure S3: Relates to Figure 4

Table S1: Fosmids for FISH analysis, relates to Figure 3, Asteriks indicate fosmids previously used in Eskeland et al, 2010a. Names are Ensembl(r 45) (http://jun2007.archive.ensembl.org/Mus_musculus/index.html).

	Region	name	Ensembl name	start	end
Alpha globin	I19r*	WI1-2837A17	G135P60495H4	32056569	32100774
	Hbq1*	WI1-2903N21	G135P603718B2	32196269	32241313
Sox2	Sox2Up	WI1-1766B8	G135P601568H9	34503694	34543065
	Sox2Down	WI1-1185J11	G135P603742D7	34686049	34722693
Nanog	NanogUp	WI1-2049I9	G135P604583D8	122574082	122610708
	NanogDown	WI1-196C18	G135P62993G4	122916571	122959014

Table S2: Coordinates for enhancer regions and primer pairs used for putative enhancer amplification, relates to Figure 6

NAME	MM9	FORWARD PRIMER 5'-3' (WITH KPN1 SITE)	REVERSE PRIMER5'-3' (WITH ECOR V SITE)
Nanog	chr6:122652450-122654050	CAGGGTACCCTGTAATTCTCTCCAAT	GACGATATCCAAGGCTGGCTGCT
AE1	chr3:63075951-63077049	CAGGGTACCGTGAGTTTCACATGATA	CAGGATATCGGTAAATTGAAGCAGT
AE2	chr10:110976751-110977649	CAGGGTACCTGGATATTTCTCTCCA	CAGGATATCGGCCTCTTACAACAT
AE3	chr5:75478951-75480049	CAGGGTACCGAAAGGAAGGAGAA	CAGGATATCGAAAAGAGAGAGGAACGGA
AE4	chr11:115205551-115206449	CAGGGTACCCTTCAGTGCAGGACCT	CAGGATATCGCTTCTGCCCAGACACT
In AE	chr5:101180951-101182649	CAGGGTACCCACTCCCCTGTGACTGTACAAGA	CAGGATATCGTCCCCTGTGATCTAGGGAA
In AE	chr18:75639551-75640449	CAGGGTACCCTGGACAGATAACTGCT	CAGGATATCAGCCAGAGCAATGAGGAGA

Supplementary Figure Legends:

Figure S1: ***H4K16ac antibody specificity and replication of ESC data in separate cell line***

A) Binding specificity of H4K16ac antibody (Millipore 07-329), calculated from the intensity of antibody-peptide interactions (y-axis) to the top list of histone modification on the MODified histone peptide array, arranged in decreasing specificity (x-axis).

B) Normalised (average reads per million/RPM) H4K16ac (solid line) or input (dotted line) ChIP-seq tag counts around (+/- 5kb) the transcription start site (TSS) and transcription end site (TES) of genes separated into quartiles according to expression in OS25 ESC (from high to low (Q1 to Q4).

C) H4K16ac in 46c ESC and OS25 ESC, and native ESC input (RPM/bp) in 200bp sliding windows with a 20bp step, across the active *Actb* and *Calm1* loci. Exons are shown as boxes below the graph and the direction of transcription is indicated.

D) Heatmaps of H4K16ac and input ChIP-seq data (RPM) in OS25 ESC (left) or 46c ESC (right) at H3K4me1+/H3K27ac+ (top), and H3K4me1+/H3K27ac- (bottom) marked enhancers. Data are ranked by sum of hits in the H4K16ac (46c) dataset; 10kb window around enhancer midpoint, in 500bp windows. Intensity is determined by RPM.

E) H4K16ac in 46c ESC and OS25 ESC, and native ESC input (RPM/bp) in 200bp sliding windows with a 20bp step across; (top) a putative active *Klf4* enhancer, (middle) a genetically defined enhancer active in ES cells (*Igll1-Vpreb1* loci), and (bottom) a putative active *Pou5f1* enhancer.

Figure S2: ***Gain and loss of H4K16ac during retinoic acid differentiation***

A) Normalised (average reads per million/RPM) H4K16ac (solid line) or input (dotted line) ChIP-seq tag counts around (+/- 5kb) the transcription start site (TSS) and transcription end site (TES) of genes separated into quartiles according to expression in OS25 ESC (left, UD)

or OS25s after 3 days differentiation induced with retinoic acid (right, D3) (from high to low (Q1 to Q4).

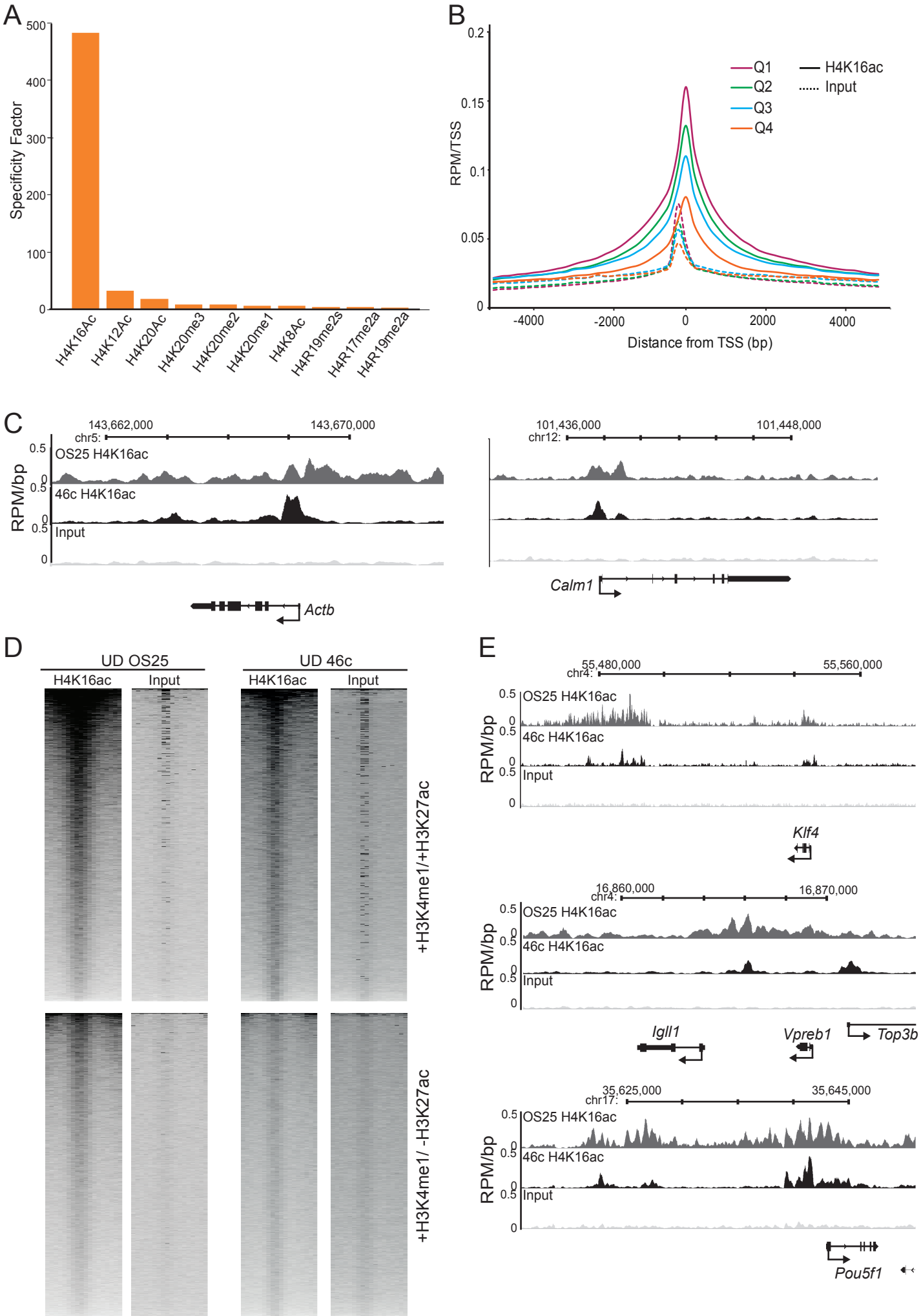
B) RPM H4K16ac ChIP-seq tag counts around (+/- 5kb) the TSS of genes differentially up regulated in either OS25 ES cells (ESC Up genes, red line) or OS25 D3 differentiated cells (D3 Up, green line) in undifferentiated OS25s (left) or D3 differentiated cells (right).

C) Log2 H4K16ac/input at the *Hoxb* locus established by hybridization to a custom microarray of ChIP DNA from undifferentiated (top) and retinoic acid differentiated (bottom) OS25 ESC. Chromosome position and RefSeq gene annotations are used from July 2007 (mm9) mouse genome build (UCSC).

D) Genomic distribution of H4K16ac and H3K27ac peaks in ESCs and NPCs, and $0.5x10^6$ peaks randomly distributed throughout the genome. Left, percentage of histone modification peaks found across each category of genomic sequence, relative to mm9 RefSeq genes. Right, schematic detailing the categorisation (peaks classified as distal intergenic do not fall into any of the previous categories.)

Figure S3: ***Regulatory landscape around pluripotency associated genes***

H4K16ac/Kat8/EP300/H3K27ac/H3K4me1/H3K4me3 around 8 pluripotency associated genes (Takahashi et al, 2007) including core pluripotency genes *Sox2*, *Pou5f1* and *Nanog*. ChIP data are shown as RPM per base pair (bp) in 200bp sliding windows with a 20bp step. Enhancer regions are indicated by purple shaded boxes.


A
Specificity Factor
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400
300
200
100
0
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H4K12Ac
H4K20Ac
H4K20me3
H4K20me2
H4K20me1
H4K8Ac
H4R19me2s
H4R17me2a
H4R19me2a
B
RPM/TSS
0.2
0.15
0.1
0.05
0
Q1
Q2
Q3
Q4
H4K16ac
Input
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-2000
0
2000
4000
Distance from TSS (bp)
C
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143,670,000
chr5:
OS25 H4K16ac
46c H4K16ac
Input
RPM/bp
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0
Actb
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101,448,000
chr12:
Calm1
D
UD OS25
UD 46c
H4K16ac
Input
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+H3K4me1/ -H3K27ac
-5
+5
Distance from Enhancer midpoint (kb)
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55,560,000
chr4:
OS25 H4K16ac
46c H4K16ac
Input
RPM/bp
Klf4
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Igll1
Vpreb1
Top3b
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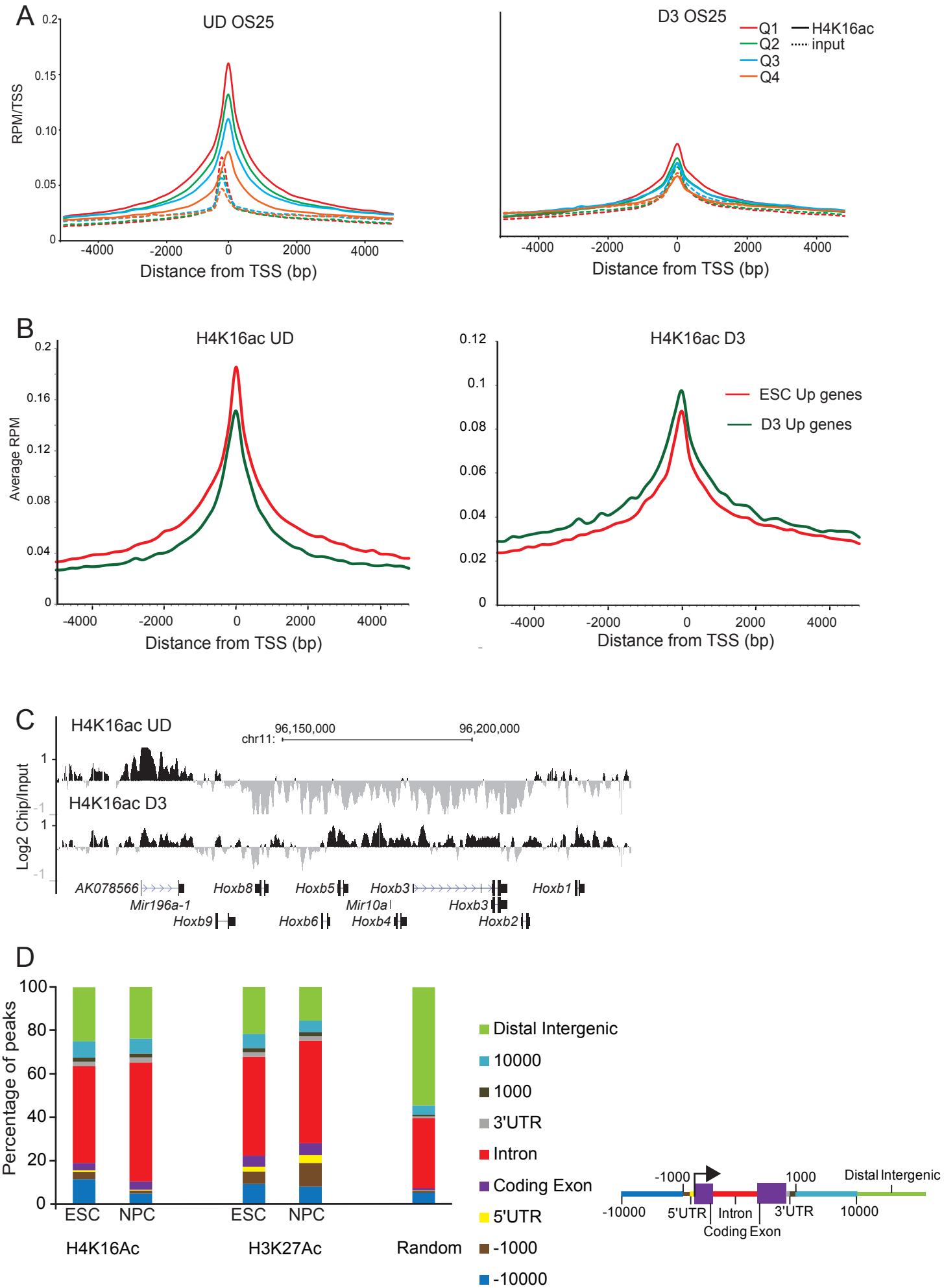
35,645,000
chr17:
Pou5f1

Figure S1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UD OS25
RPM/TSS
Distance from TSS (bp)
D3 OS25
Q1
Q2
Q3
Q4
H4K16ac
input
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Average RPM
H4K16ac D3
ESC Up genes
D3 Up genes
C
H4K16ac UD
chr11:
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96,200,000
H4K16ac D3
Log2 Chip/Input
AK078566
Hoxb8
Hoxb5
Hoxb3
Hoxb1
Mir196a-1
Mir10a
Hoxb3
Hoxb9
Hoxb6
Hoxb4
Hoxb2
D
Percentage of peaks
ESC
NPC
ESC
NPC
H4K16Ac
H3K27Ac
Random
Distal Intergenic
10000
1000
3'UTR
Intron
Coding Exon
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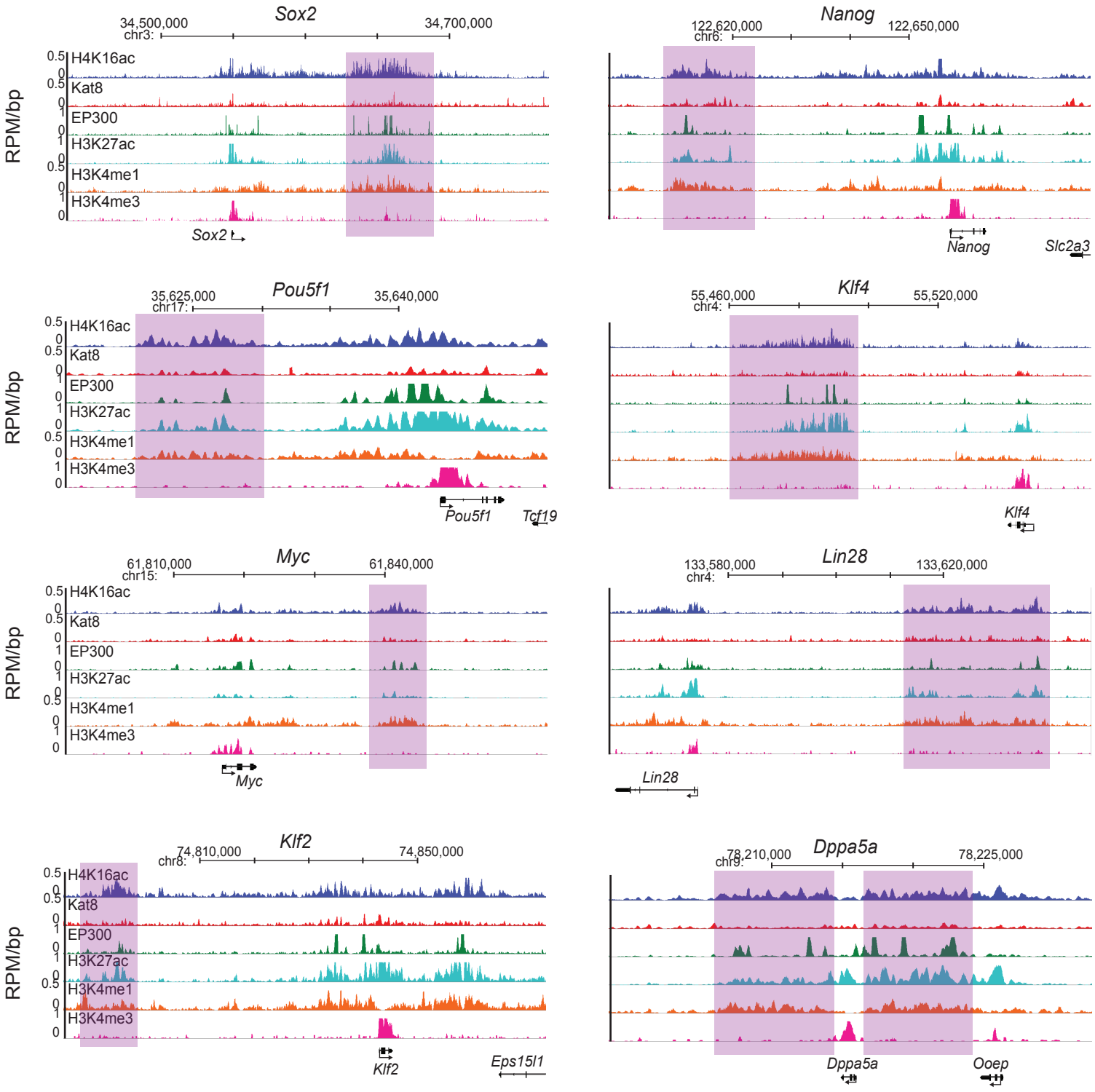
-10000
-1000
1000
Distal Intergenic
-10000
5'UTR
Intron
3'UTR
10000
Coding Exon

Figure S2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chr3:
34,700,000
RPM/bp
H4K16ac
Kat8
EP300
H3K27ac
H3K4me1
H3K4me3
Sox2
Nanog
122,620,000
chr6:
122,650,000
Nanog
Slc2a3
Pou5f1
35,625,000
chr17:
35,640,000
Pou5f1
Tcf19
Klf4
55,460,000
chr4:
55,520,000
Klf4
Myc
61,810,000
chr15:
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Myc
Lin28
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chr4:
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Lin28
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Klf2
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chr9:
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Dppa5a
Ooep

Figure S3