SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURE LEGENDS

Suppl. Fig 1. ***The murine Jmjd3 locus.*** H3K4me3 (from ChIP-Seq data) in untreated macrophages and the conserved NF-kB binding sites are shown.

Suppl. Fig 2. ***Validation of ChIP-Seq data. a***) Validation of ChIP-sequencing data at a representative set of genes with different tag counts. Data are expressed as fold enrichment relative to the average of the Q-PCR signals obtained in four Jmjd3-negative regions. ***b***) Specificity of the Jmjd3 ChIP. Signals were abrogated in Jmjd3$^{-/-}$ macrophages. Data are expressed as % of the signal detected in wt cells.

Suppl. Fig 3. ***Jmjd3 ChIP-Seq profiles at a representative panel of genes.***

Suppl. Fig 4. ***Rapid increase in H3K4me3 levels at the TSSs of a subset of inducible genes in macrophages.*** Representative ChIP-Seq profiles are shown.

Suppl. Fig 5. ***Binding of Jmjd3 to H3K4me3-positive genes***. Overlays of Jmjd3 and H3K4me3 signals in LPS-treated macrophages are shown.

Suppl. Fig. 6. ***ChIP-Seq profiles at the Upp1 and Nos2 genes***.

Suppl. Fig. 7. ***RNA Pol_II profiles at selected inducible genes***.

Suppl. Fig. 8. ***Association of Jmjd3 with an H3K27me3-negative TSS at a gene with multiple TSSs***. ChIP-Seq profiles (left) and validation by ChIP-QPCR (right) are shown. Error bars: s.e.m. from a triplicate experiment.

Suppl. Fig. 9. ***H3K27me3 levels at validated Jmjd3 target genes.***

Suppl. Fig. 10. ***H3K4me3 levels at representative target genes whose expression is impaired upon Jmjd3 depletion.***

SUPPLEMENTARY TABLE LEGENDS

Suppl. Table 1. ***Jmjd3 target genes.*** The table shows the genomic location and intensities (tag count) of the Jmjd3 peaks from the ChIP-Seq experiment (2h after LPS+IFNγ treatment).

Suppl. Table 2. ***H3K4me3 peaks in untreated macrophages.*** The table shows the peaks of H3K4me3 in untreated macrophages. Intensities are shown both as a 'maximum overlap count' and as a 'total number of tags' within each peak.

Suppl. Table 3. ***H3K4me3 peaks in LPS-stimulated macrophages.***

Suppl. Table 4. ***H3K4me3 peaks detected in both untreated and LPS-stimulated macrophages and their association with Jmjd3 peaks.***

Suppl. Table 5. ***H3K27me3 peaks in unstimulated macrophages.*** The table also shows the intensity change for each peak as compared to the library obtained in LPS-stimulated macrophages.

Suppl. Table 6. ***H3K27me3 peaks in LPS-stimulated macrophages.***

Suppl. Table 7. ***BLOC analysis of H3K27me3 ChIP-Seq data.*** The table lists the broad regions of H3K27me3 enrichment identified using the program described in Pauler et al (2009). See the supplementary discussion for details.

Suppl. Table 8. ***H3K27me3 peaks associated with Jmjd3 peaks.***

Suppl. Table 9. Microarray data in wt *vs.* Jmjd3 $^{-/-}$ macrophages stimulated with LPS+IFNγ for 4h. Data were obtained from a biological triplicate and are shown using a threshold of $\pm$ 1.4 fold change (FC). Genes that are bound by Jmjd3 are also indicated.

Suppl. Table 10. ***List of the ChIP-Seq libraries generated and relative tag counts.***

Suppl. Table 11. ***Q-ChIP primers used in this study.***

Suppl. Table 12. ***Q-RT-PCR primers and primers for nascent transcripts used in this study.***

Supplementary discussion.

BLOC analysis of the H3K27me3 libraries.

The H3K27me3 library at 0 hr has 9,372,377 uniquely mapped tags while the 4 hr library has 13,241,000 uniquely mapped tags. The 4 hr library was downsampled to 9.3 million tags.

Then the libraries were processed as described in the paper (Pauler et al. 2009 Genomes Res.) to create a "high density map". The high density map divides the chromosome into 25bp long windows. In each 25bp window, the fragment density is calculated by counting the tags as follows: tags within 200bp of the window and oriented towards it are counted as 1, and tags within 200bp -300 bp of the window and oriented towards it are counted as 0.25. All 25bp window which finally have no fragment density are assigned the value -1.

The BLOCs program (available at http://genauwiki.imp.ac.at) was run on this high-density map. The program defines a BLOC start when 20 windows (out of 20-23 consecutive windows) show a fragment density >0 and BLOC end when 6 windows (out of 6-8 consecutive windows) show a fragment density <0. The program also requires a "median_cutoff" parameter. Only those BLOCs are reported which by the program where the median number of tags in the windows within the BLOC is greater than the "median_cutoff". Different values of median_cutoff were tried, and finally a cutoff of 3 was selected.

Based on this analysis, the number of BLOCs reported in the two libraries is given below:

BLOCs discovered in H3K27me3 library at 0 hr: 2966
BLOCs discovered in H3K27me3 library at 4 hr: 4601

The average size of BLOCs is 21.2 kb at 0 hr and 27.8 kb at 4 hr

At 0 hr, there are 59,684 peaks of H3K27me3. Out of these, 27,947 peaks (46.8%) lie within the 2966 BLOCs reported in the 0hr library

At 0 hr, there are 89,093 peaks of H3K27me3. Out of these, 52,852 peaks (59.3%) lie within the 4601 BLOCs reported in the 4hr library

We found that a number of BLOCs reported by the program in the two libraries do not overlap. This may be a problem with the robustness of the program. Therefore we resorted to taking a union of all the BLOCs reported in both libraries, considering the overlapping blocs as a single bloc.

Total BLOCs (union of the two sets): 5733

Considering the union set of 5733 BLOCs,
out of 59,684 peaks of H3K27 at 0 hr, 43,181 peaks (72.3%) lie within the BLOCs, and
out of 89,093 peaks of H3K27 at 4 hr, 59,330 peaks (66.6%) lie within the BLOCs

What happens to peaks within and outside the BLOCs?

At 0hr, 43,181 out of 59,684 H3K27me3 peaks lie within blocs and 16,503 peaks lie outside blocs

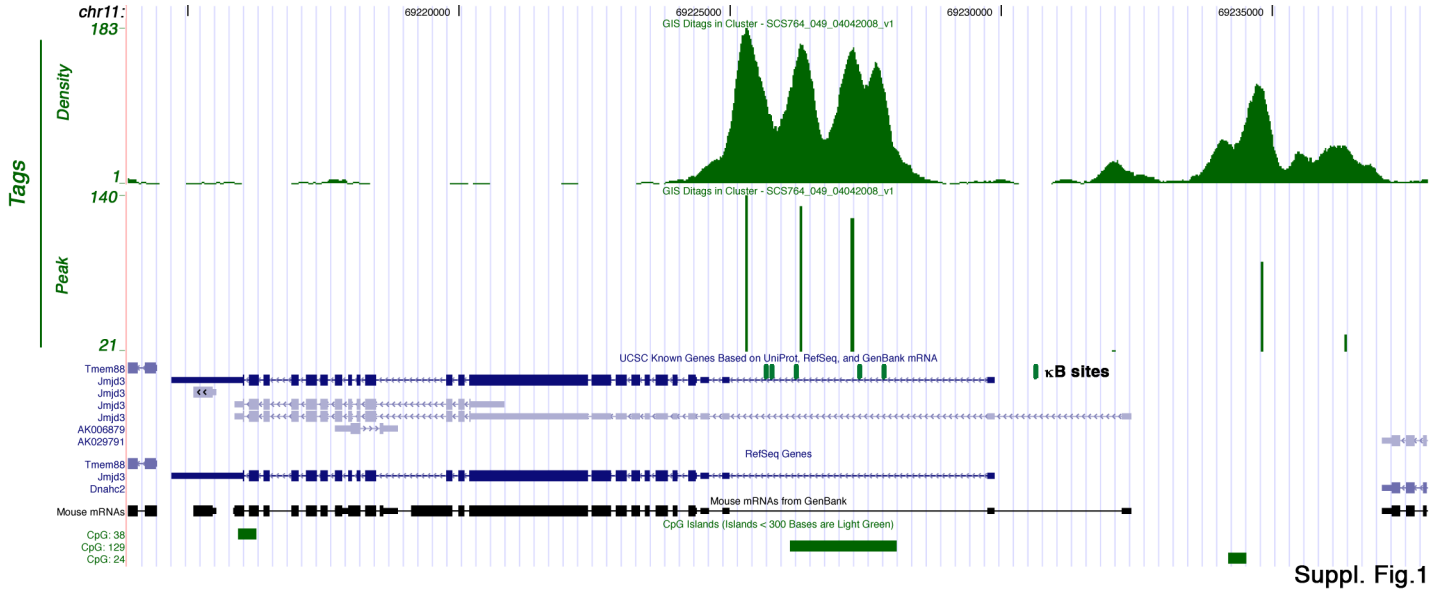
	peaks in BLOCS	peaks outside BLOCs
increase 2 fold from 0hr to 4hr	3.87%	2.20%
reduce 2 fold from 0hr to 4hr	0.11%	0.31%

For each BLOC in this union set, we calculated the total tag count in the region of the BLOC (start to end) in both libraries. Then we took the ratio of the total tag counts in 4 hr vs. the 0 hr library. The results are as follows:

BLOCs that show more than 2 fold increase in total tag counts from 0hr to 4hr: 113 out of 5733

BLOCs that show more than 2 fold decrease in total tag counts from 0hr to 4hr: 3 out of 5733

BLOCs that are similar based on 2-fold cutoff = 5633 out of 5733 (98%)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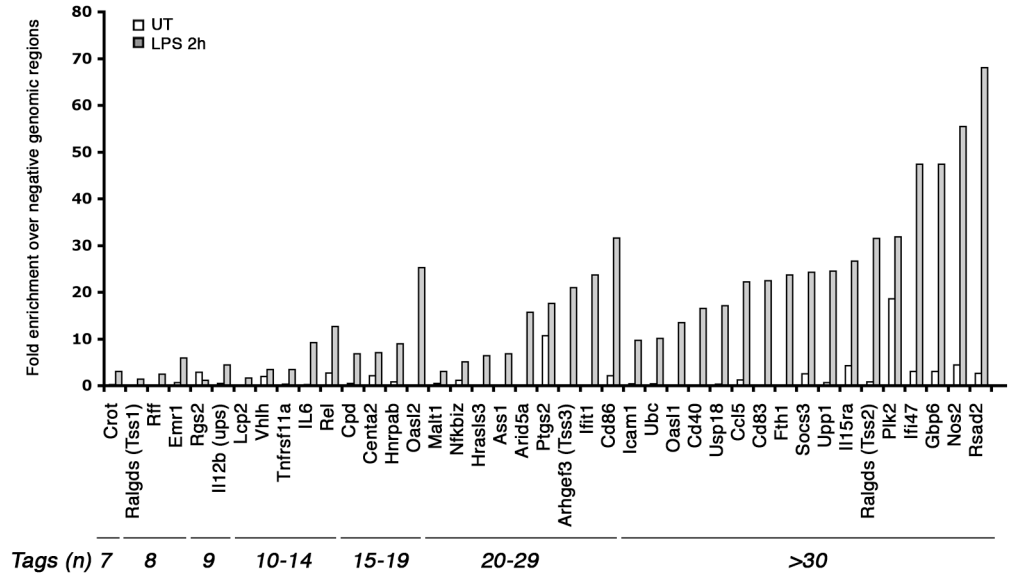
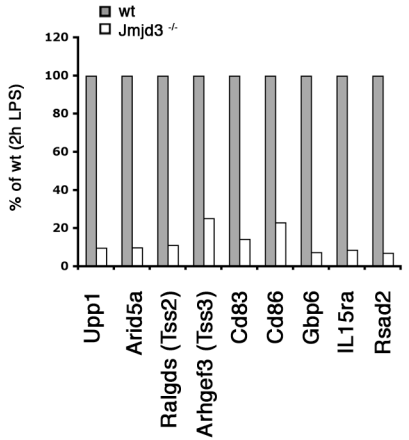
69220000
69225000
69230000
69235000
Tags
Density
183
1
GIS Ditags in Cluster - SCS764_049_04042008_v1
GIS Ditags in Cluster - SCS764_049_04042008_v1
140
Peak
21
UCSC Known Genes Based on UniProt, RefSeq, and GenBank mRNA
κB sites
Tmem88
Jmjd3
Jmjd3
Jmjd3
Jmjd3
AK006879
AK029791
RefSeq Genes
Tmem88
Jmjd3
Dnahc2
Mouse mRNAs
Mouse mRNAs from GenBank
CpG Islands (Islands < 300 Bases are Light Green)
CpG: 38
CpG: 129
CpG: 24

Suppl. Fig.1

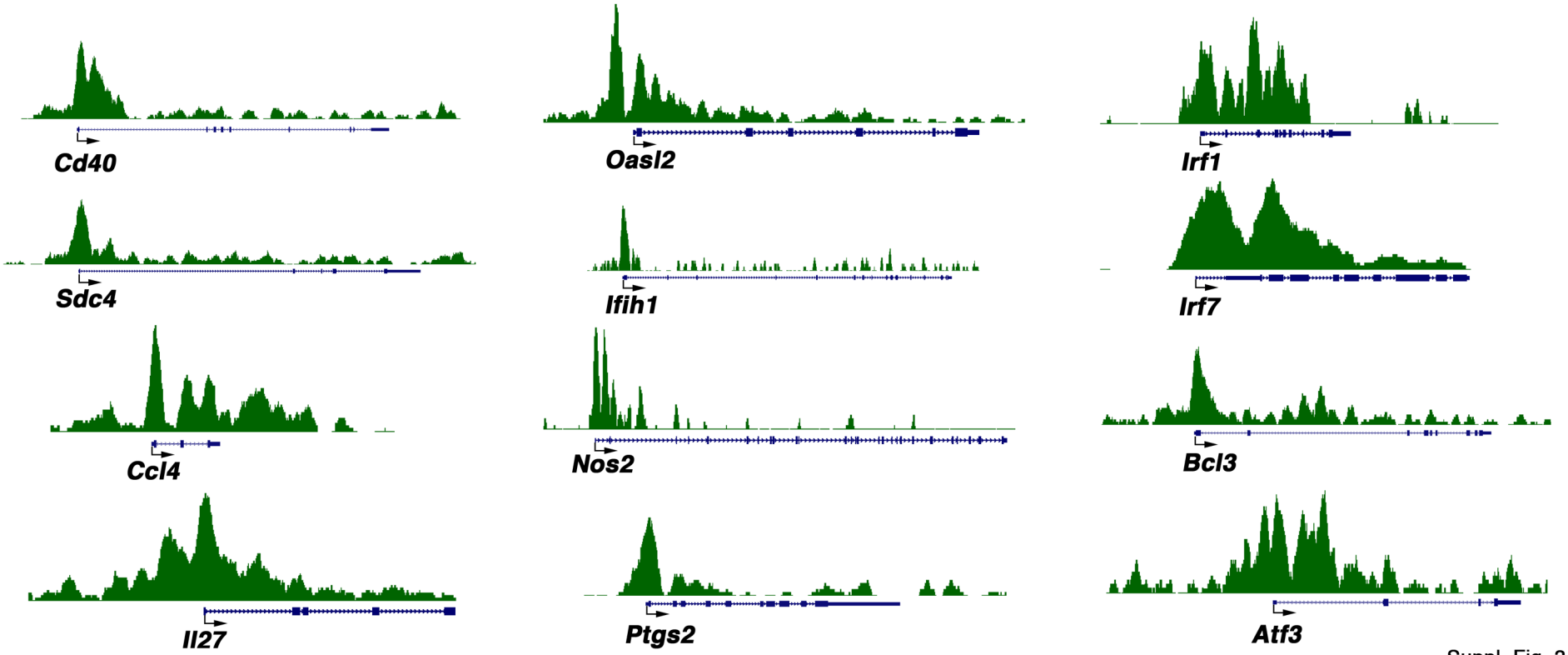
a.



b.



Suppl. Fig. 2


Cd40
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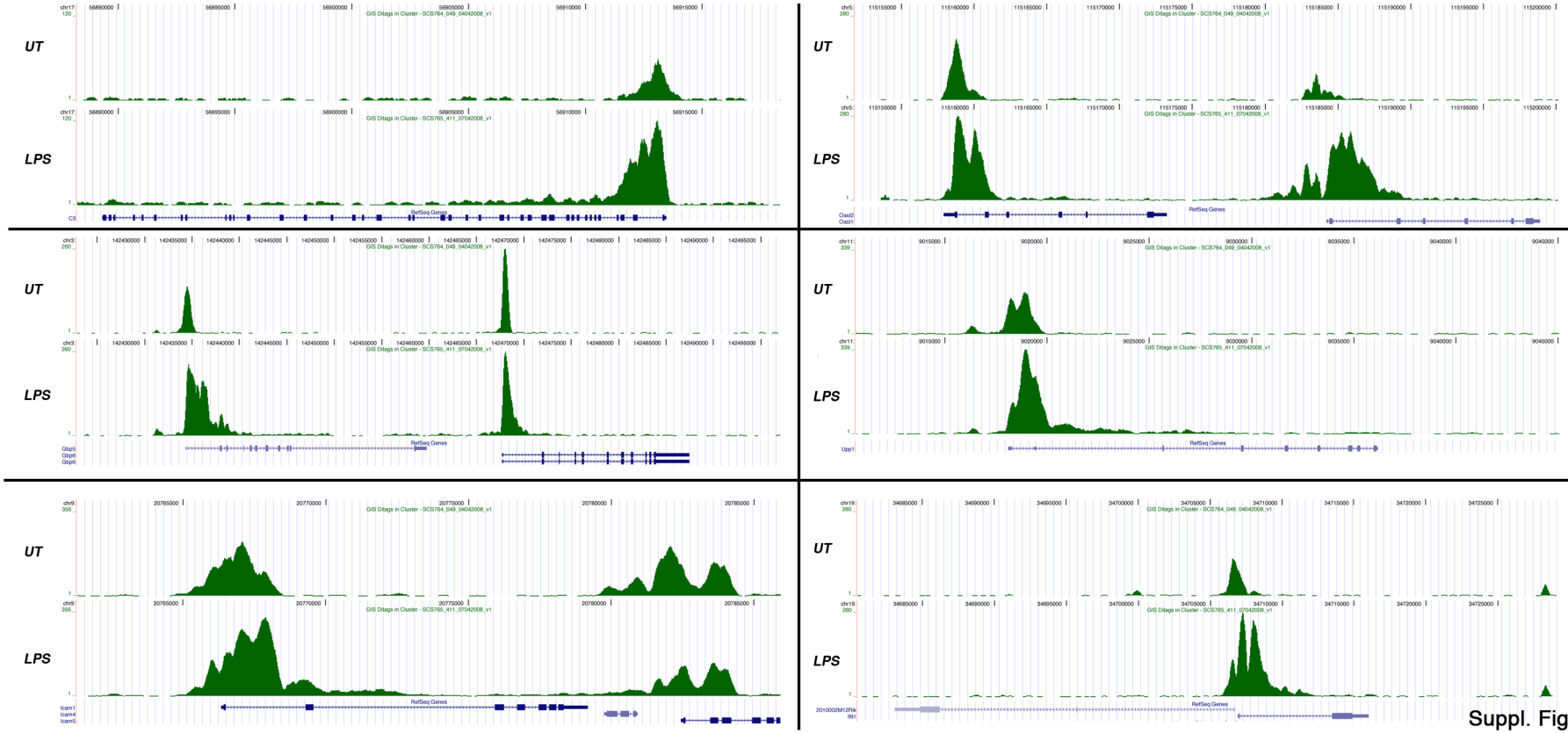
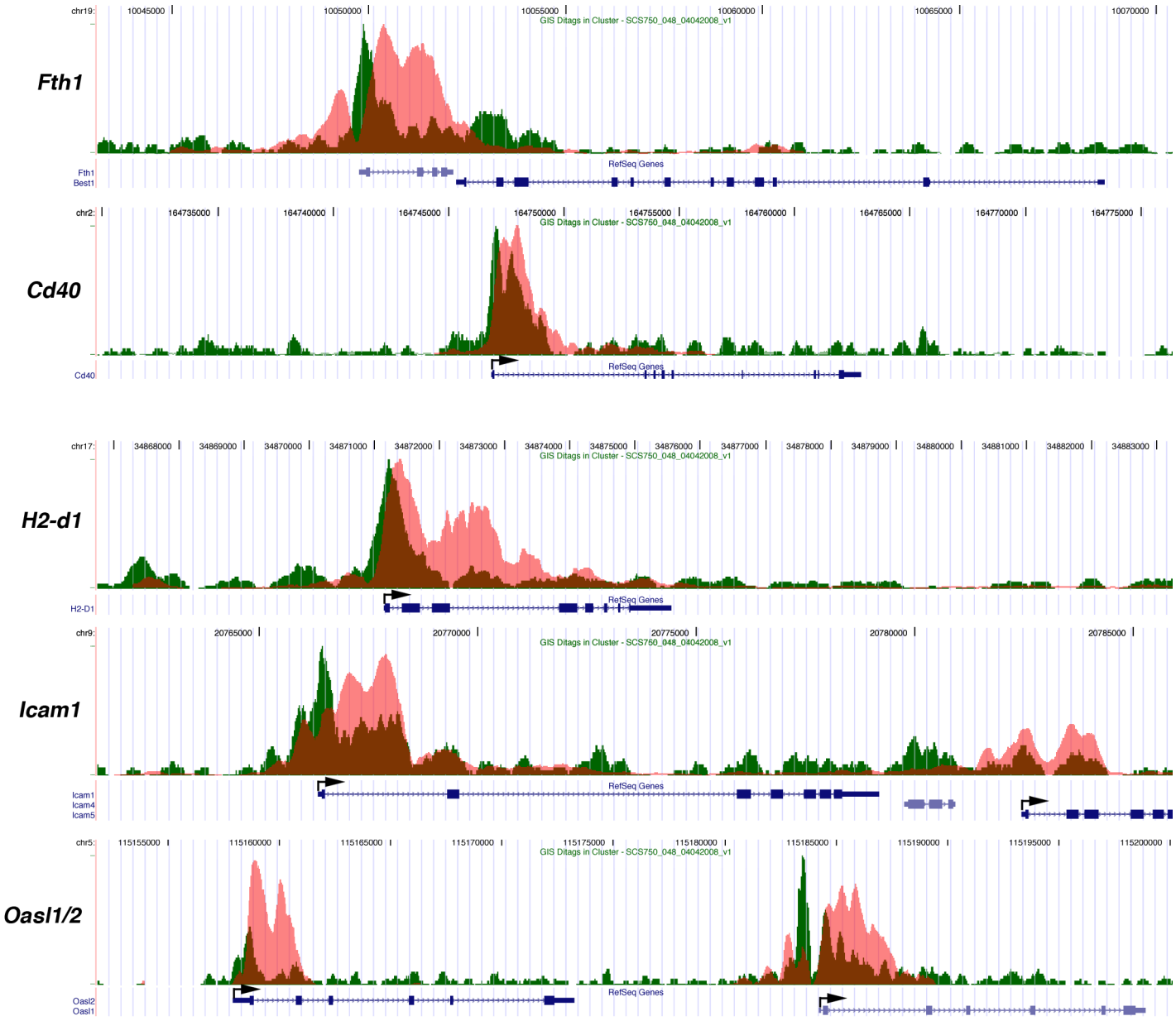
Irf1
Sdc4
Ifih1
Irf7
Ccl4
Nos2
Bcl3
Il27
Ptgs2
Atf3

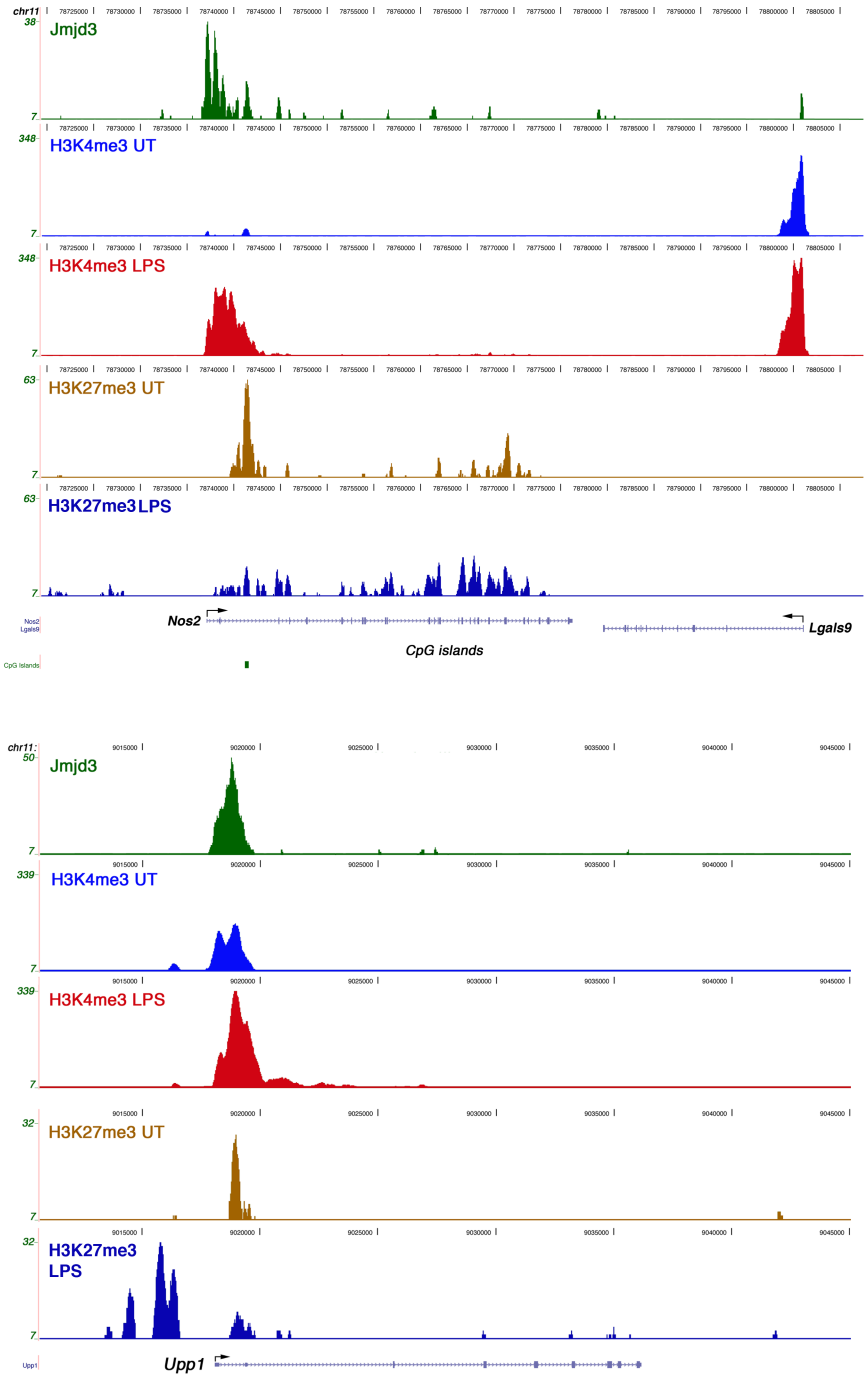
Suppl. Fig. 3



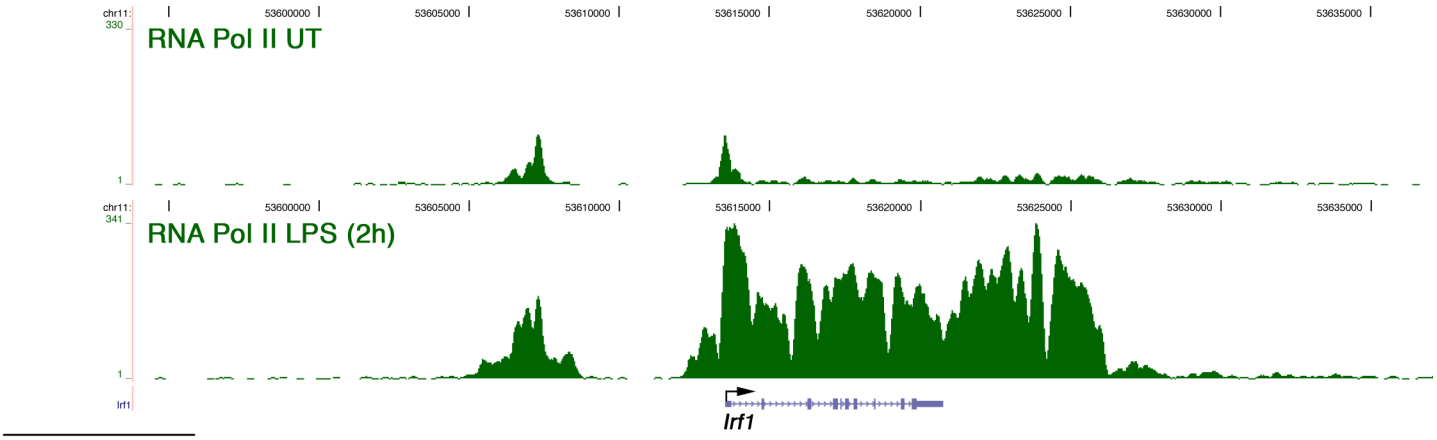
Suppl. Fig. 4


Fth1
chr19:
10045000
10050000
10055000
10060000
10065000
10070000
GIS Ditags in Cluster - SCS750_048_04042008_v1
RefSeq Genes
Fth1
Best1
Cd40
chr2:
164735000
164740000
164745000
164750000
164755000
164760000
164765000
164770000
164775000
GIS Ditags in Cluster - SCS750_048_04042008_v1
RefSeq Genes
Cd40
H2-d1
chr17:
34868000
34869000
34870000
34871000
34872000
34873000
34874000
34875000
34876000
34877000
34878000
34879000
34880000
34881000
34882000
34883000
GIS Ditags in Cluster - SCS750_048_04042008_v1
RefSeq Genes
H2-D1
Icam1
chr9:
20765000
20770000
20775000
20780000
20785000
GIS Ditags in Cluster - SCS750_048_04042008_v1
RefSeq Genes
Icam1
Icam4
Icam5
Oasl1/2
chr5:
115155000
115160000
115165000
115170000
115175000
115180000
115185000
115190000
115195000
115200000
GIS Ditags in Cluster - SCS750_048_04042008_v1
RefSeq Genes
Oasl2
Oasl1
H3K4me3 (+LPS)
Jmjd3

Suppl. Fig. 5


chr11
Jmjd3
H3K4me3 UT
H3K4me3 LPS
H3K27me3 UT
H3K27me3 LPS
Nos2
Lgals9
CpG islands
chr11:
Jmjd3
H3K4me3 UT
H3K4me3 LPS
H3K27me3 UT
H3K27me3
LPS
Upp1

Suppl. Fig. 6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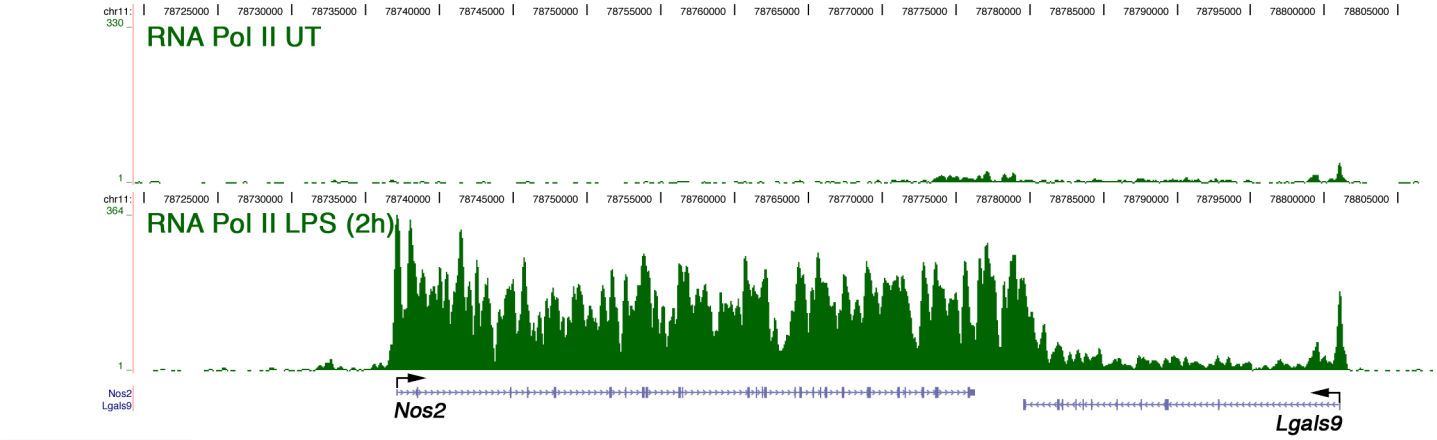
330
RNA Pol II UT
53600000
53605000
53610000
53615000
53620000
53625000
53630000
53635000
1
chr11:
341
RNA Pol II LPS (2h)
1
Irf1
Irf1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330
RNA Pol II UT
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78780000
78785000
78790000
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1
chr11:
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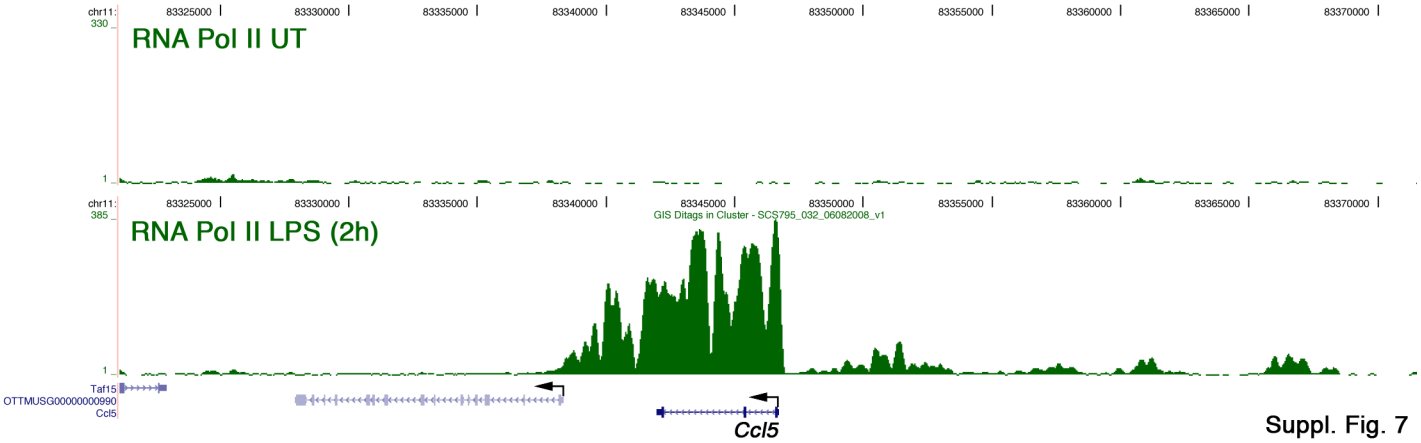
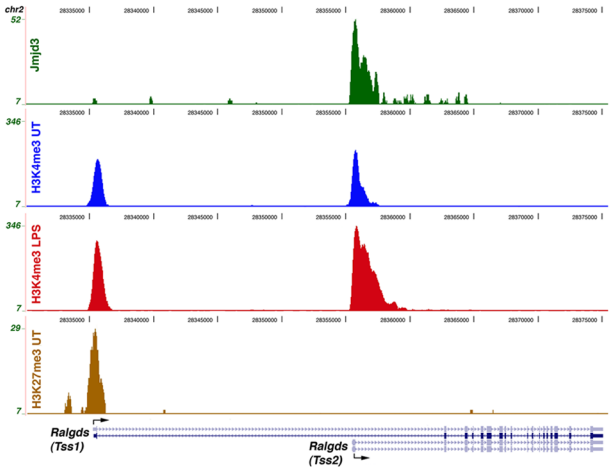
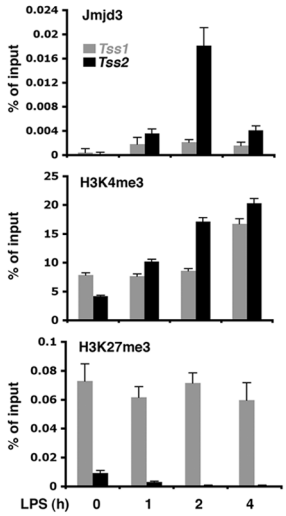
RNA Pol II LPS (2h)
1
Nos2
Lgals9
Nos2
Lgals9

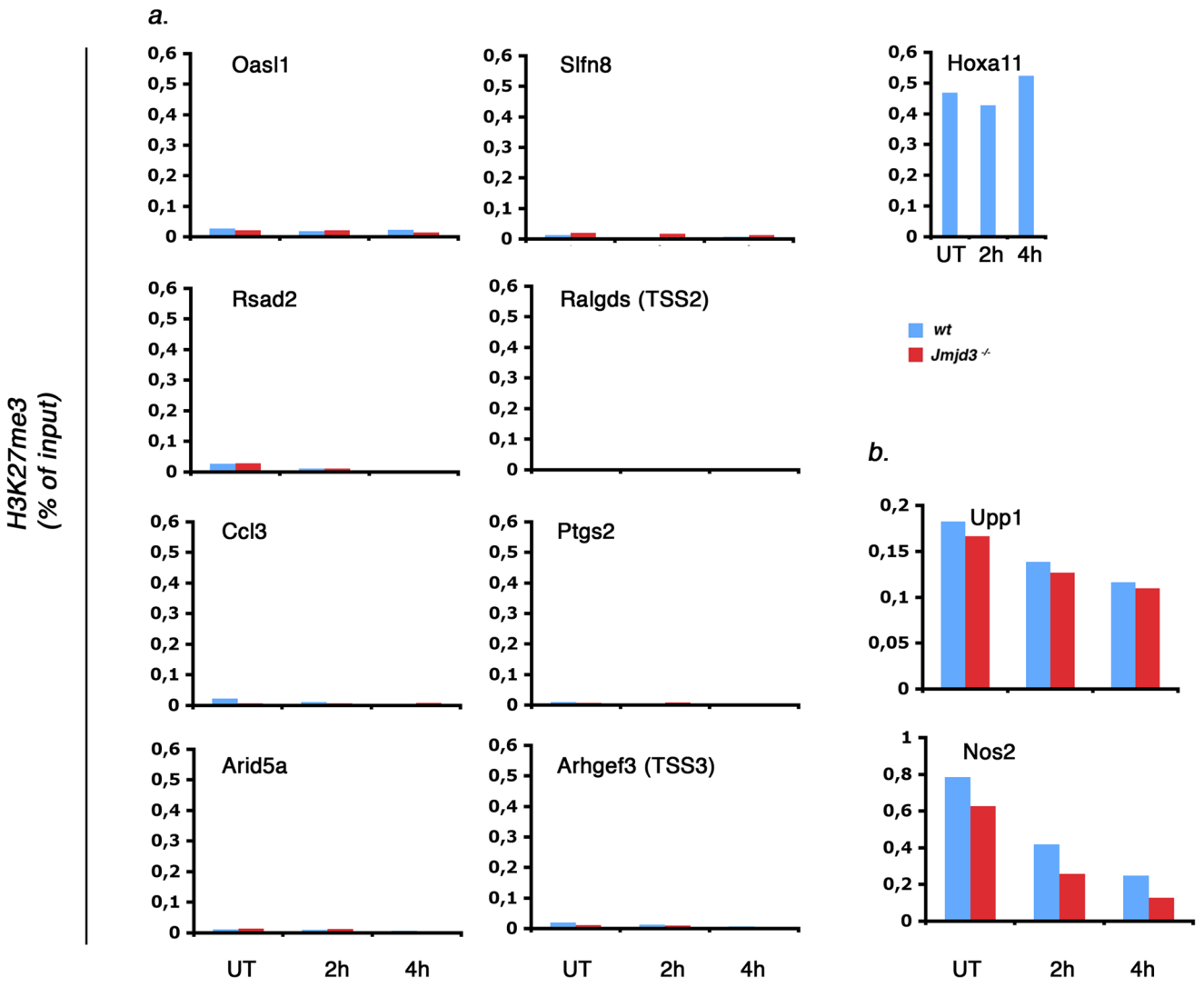

chr11:
330
RNA Pol II UT
83325000
83330000
83335000
83340000
83345000
83350000
83355000
83360000
83365000
83370000
1
chr11:
385
RNA Pol II LPS (2h)
GIS Ditags in Cluster - SCS795_032_06082008_v1
1
Taf15
OTTMUSG00000000990
Ccl5
Ccl5

Suppl. Fig. 7

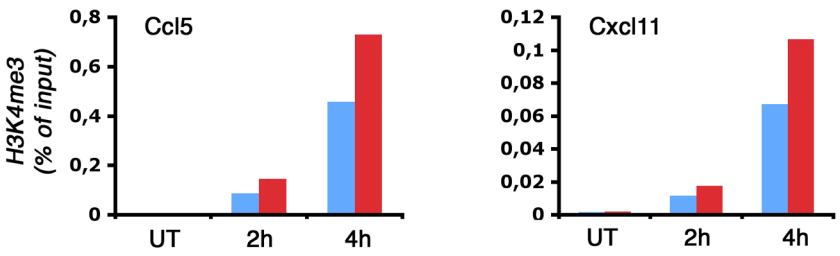

chr2
52
Jmjd3
7
346
H3K4me3 UT
7
346
H3K4me3 LPS
7
29
H3K27me3 UT
7
Ralgds
(Tss1)
Ralgds
(Tss2)


Jmjd3
Tss1
Tss2
% of input
H3K4me3
% of input
H3K27me3
% of input
LPS (h) 0 1 2 4

Suppl. Fig. 8


a.
b.
H3K27me3
(% of input)
Oasl1
Slfn8
Hoxa11
Rsad2
Ralgds (TSS2)
Ccl3
Ptgs2
Arid5a
Arhgef3 (TSS3)
Upp1
Nos2
UT
2h
4h
wt
Jmjd3 -/-

Suppl. Fig. 9


H3K4me3
(% of input)
Ccl5
0,8
0,6
0,4
0,2
0
UT
2h
4h
Cxcl11
0,12
0,1
0,08
0,06
0,04
0,02
0
UT
2h
4h

Suppl. Fig. 10