Figure S1

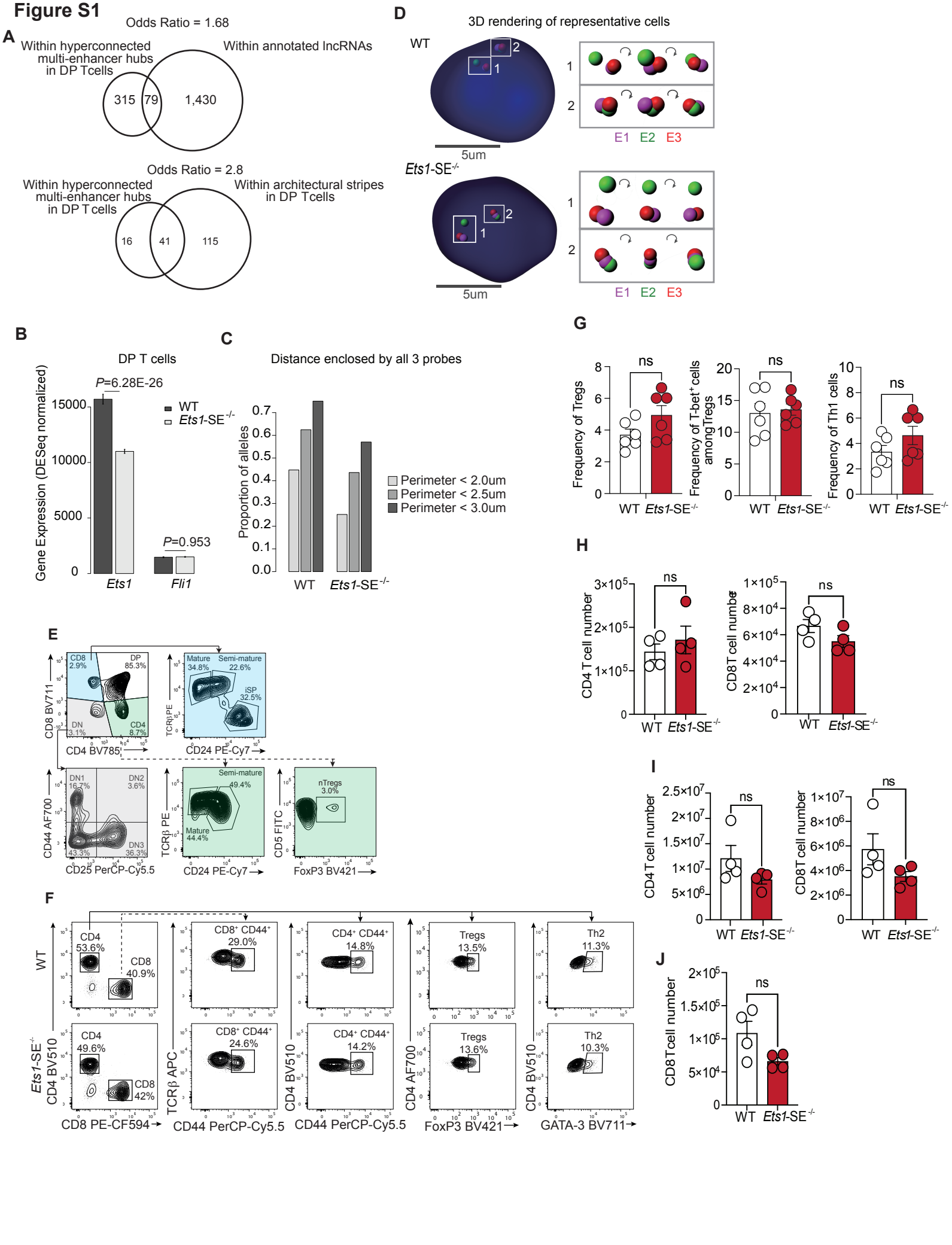

A
Odds Ratio = 1.68
Within hyperconnected multi-enhancer hubs in DP Tcells
Within annotated lncRNAs
315
79
1,430
Odds Ratio = 2.8
Within hyperconnected multi-enhancer hubs in DP T cells
Within architectural stripes in DP Tcells
16
41
115
D
3D rendering of representative cells
WT
Ets1-SE-/-
5um
E1 E2 E3
B
DP T cells
P=6.28E-26
P=0.953
Gene Expression (DESeq normalized)
WT
Ets1-SE-/-
Ets1
Fli1
C
Distance enclosed by all 3 probes
Proportion of alleles
Perimeter < 2.0um
Perimeter < 2.5um
Perimeter < 3.0um
G
Frequency of Tregs
Frequency of T-bet+ cells among Tregs
Frequency of Th1 cells
ns
H
CD4 T cell number
CD8 T cell number
E
CD8 BV711
CD4 BV785
CD24 PE-Cy7
TCRβ PE
CD44 AF700
CD25 PerCP-Cy5.5
CD5 FITC
FoxP3 BV421
I
J
F
CD8 PE-CF594
CD44 PerCP-Cy5.5
TCRβ APC
CD4 BV510
CD4 AF700
GATA-3 BV711

Figure S2

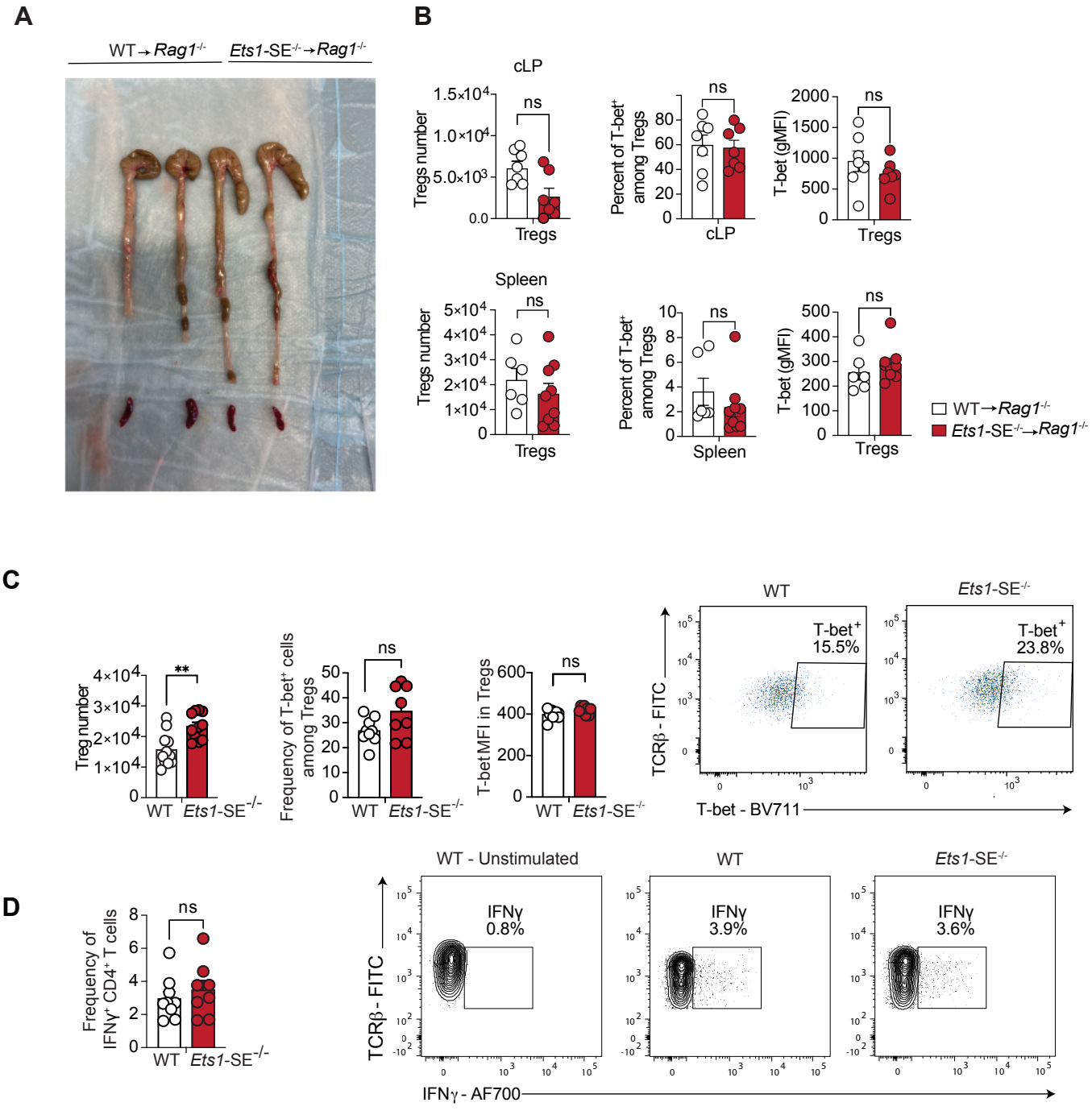
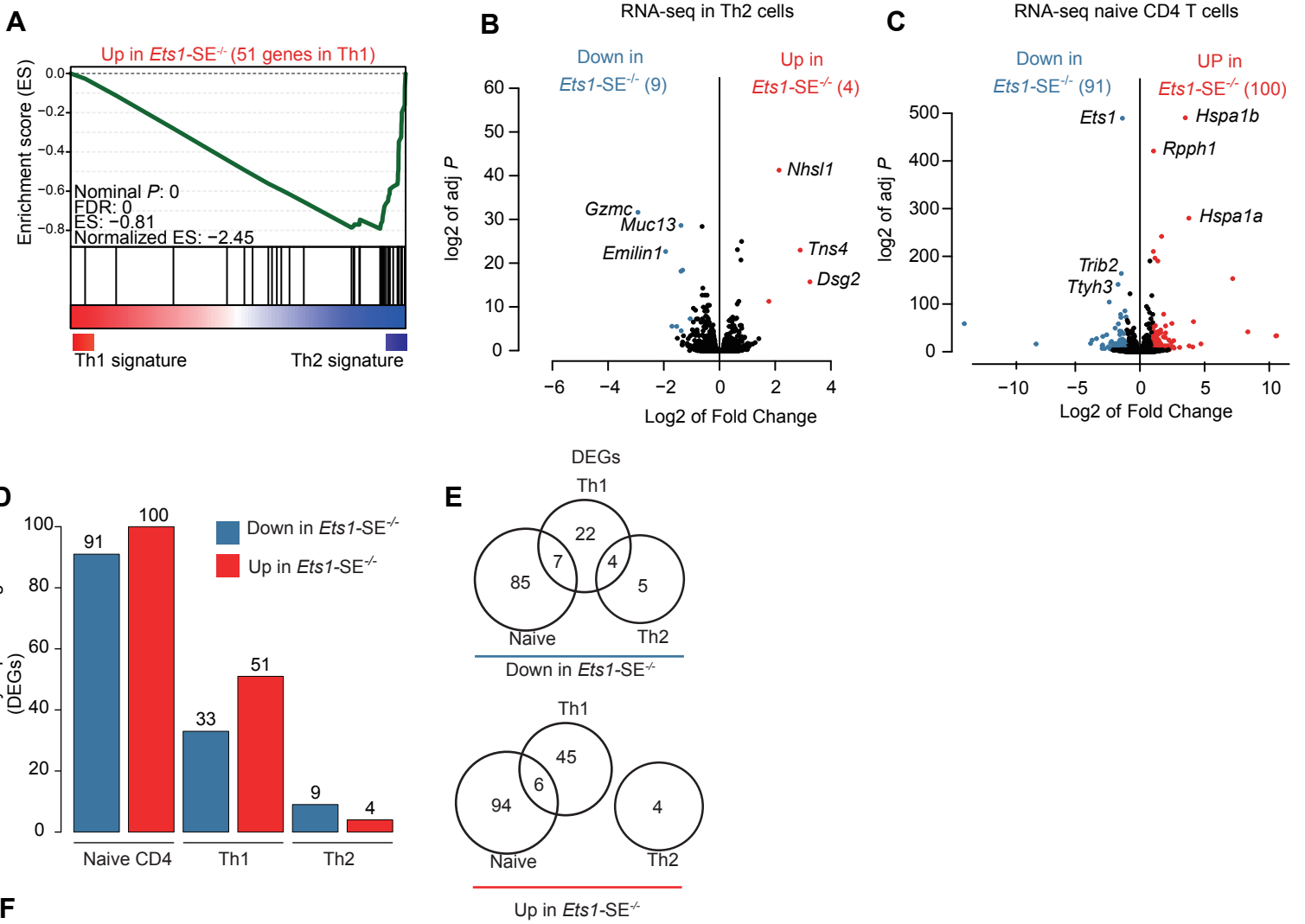
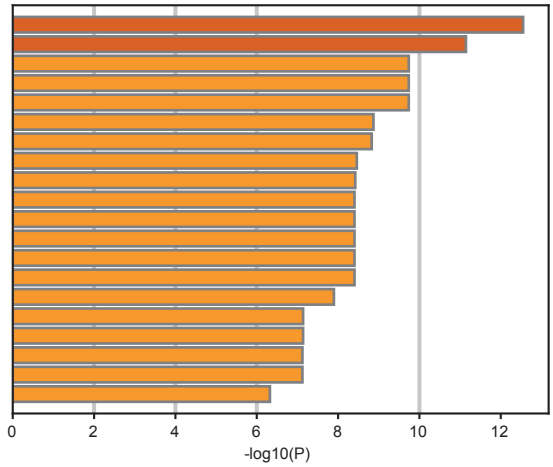

A
WT→Rag1-/-
Ets1-SE-/-→Rag1-/-
B
cLP
Tregs number
ns
Tregs
Percent of T-bet+ among Tregs
cLP
T-bet (gMFI)
Spleen
Spleen
WT→Rag1-/-
Ets1-SE-/-→Rag1-/-
C
Treg number
**
WT
Ets1-SE-/-
Frequency of T-bet+ cells among Tregs
T-bet MFI in Tregs
WT
Ets1-SE-/-
T-bet+ 15.5%
T-bet+ 23.8%
TCRβ - FITC
T-bet - BV711
D
Frequency of IFNγ+ CD4+ T cells
WT - Unstimulated
WT
Ets1-SE-/-
IFNγ 0.8%
IFNγ 3.9%
IFNγ 3.6%
TCRβ - FITC
IFNγ - AF700

Figure S3



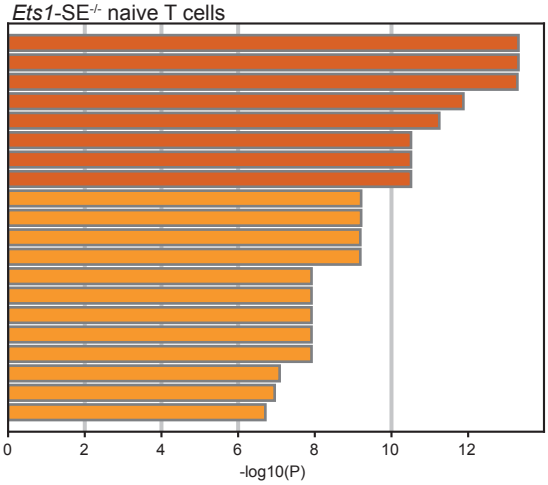
F

Genes downregulated in *Ets1*-SE$^{-/-}$ naive T cells



M4332: GSE20366 TREG VS NAIVE CD4 TCELL DEC205 CONVERSION UP
M7159: GSE15735 2H VS 12H HDAC INHIBITOR TREATED CD4 TCELL UP
M7289: GSE18893 TCONV VS TREG 24H CULTURE DN
M7427: GSE16451 CTRL VS WEST EQUINE ENC VIRUS IMMATURE NEURON CELL LINE UP
M5072: GSE32423 MEMORY VS NAIVE CD8 TCELL UP
M2925: GSE13522 WT VS IFNAR KO SKIN DN
M8900: GSE37301 PRO BCELL VS RAG2 KO NK CELL DN
M5385: GSE3982 MEMORY CD4 TCELL VS BCELL DN
M5737: GSE7852 LN VS THYMUS TREG UP
M5727: GSE7852 TREG VS TCONV LN UP
M5035: GSE30083 SP2 VS SP4 THYMOCYTE DN
M9263: GSE40068 CXCR5NEG BCL6NEG CD4 TCELL VS CXCR5POS BCL6NEG TFH DN
M9262: GSE40068 CXCR5POS BCL6POS TFH VS CXCR5NEG BCL6NEG CD4 TCELL DN
M2932: GSE13522 WT VS IFNAR KO SKIN UP
M8125: GSE19941 LPS VS LPS AND IL10 STIM IL10 KO MACROPHAGE DN
M6577: GSE5455 HEALTHY VS TUMOR BEARING MOUSE SPLEEN MONOCYTE 24H INCUBATION UP
M6576: GSE5455 HEALTHY VS TUMOR BEARING MOUSE SPLEEN MONOCYTE DN
M6550: GSE5142 CTRL VS HTERT TRANSDUCED CD8 TCELL EARLY PASSAGE CLONE DN
M9207: GSE40666 WT VS STAT1 KO CD8 TCELL DN
M380: GSE7219 UNSTIM VS LPS AND ANTI CD40 STIM DC UP

Genes upregulated in *Ets1*-SE$^{-/-}$ naive T cells



M7420: GSE16450 CTRL VS IFNA 6H STIM MATURE NEURON CELL LINE UP
M9269: GSE39916 B CELL SPLEEN VS PLASMA CELL BONE MARROW UP
M2933: GSE13522 WT VS IFNAR KO SKING T CRUZI Y STRAIN INF UP
M9262: GSE40068 CXCR5POS BCL6POS TFH VS CXCR5NEG BCL6NEG CD4 TCELL DN
M6316: GSE2935 UV INACTIVATED VS LIVE SENDAI VIRUS INF MACROPHAGE UP
M7430: GSE16450 IMMATURE VS MATURE NEURON CELL LINE 12H IFNA STIM UP
M5034: GSE30083 SP2 VS SP4 THYMOCYTE UP
M5690: GSE7460 TREG VS TCONV ACT UP
M7159: GSE15735 2H VS 12H HDAC INHIBITOR TREATED CD4 TCELL UP
M4575: GSE24142 DN2 VS DN3 THYMOCYTE FETAL DN
M5676: GSE7460 TCONV VS TREG THYMUS DN
M9765: GSE43863 TH1 VS LY6C INT CXCR5POS EFFECTOR CD4 TCELL DN
M9285: GSE39110 UNTREATED VS IL2 TREATED CD8 TCELL DAY6 POST IMMUNIZATION DN
M9201: GSE40443 INDUCED VS TOTAL TREG DN
M5030: GSE30083 SP1 VS SP4 THYMOCYTE DN
M9284: GSE39110 UNTREATED VS IL2 TREATED CD8 TCELL DAY6 POST IMMUNIZATION UP
M6567: GSE5679 PPARG LIGAND ROSIGLITAZONE VS ROSIGLITAZONE AND RARA AGONIST AM580 TREATED DC DN
M7010: GSE15330 LYMPHOID MULTIPOTENT VS MEGAKARYOCYTE ERYTHROID PROGENITOR IKAROS KO DN
M377: GSE7219 WT VS NIK NFKB2 KO DC UP
M6576: GSE5455 HEALTHY VS TUMOR BEARING MOUSE SPLEEN MONOCYTE DN

Figure S4

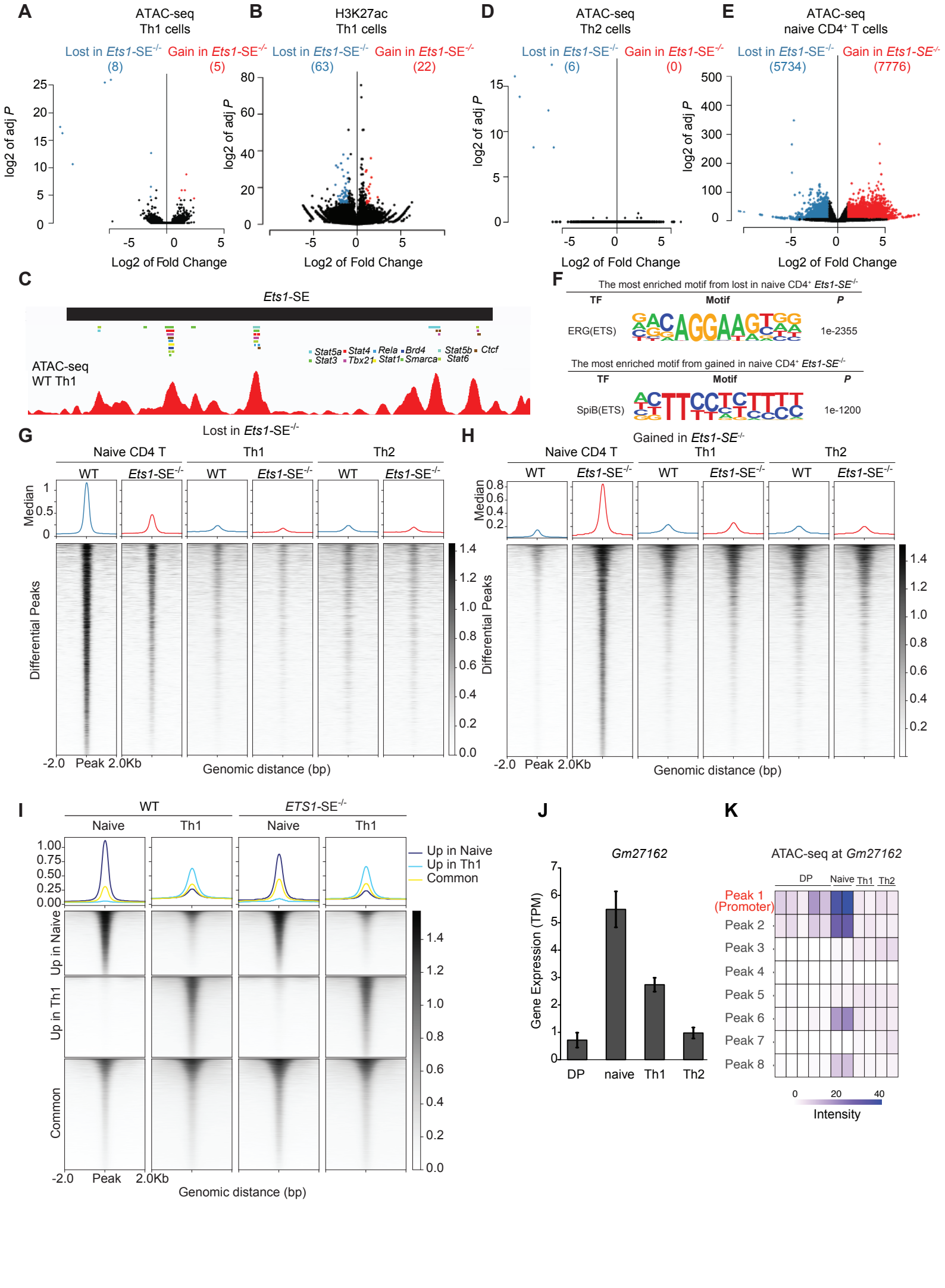

A
ATAC-seq
Th1 cells
Lost in Ets1-SE-/- (8)
Gain in Ets1-SE-/- (5)
log2 of adj P
Log2 of Fold Change
B
H3K27ac
Th1 cells
Lost in Ets1-SE-/- (63)
Gain in Ets1-SE-/- (22)
log2 of adj P
Log2 of Fold Change
D
ATAC-seq
Th2 cells
Lost in Ets1-SE-/- (6)
Gain in Ets1-SE-/- (0)
log2 of adj P
Log2 of Fold Change
E
ATAC-seq
naive CD4+ T cells
Lost in Ets1-SE-/- (5734)
Gain in Ets1-SE-/- (7776)
log2 of adj P
Log2 of Fold Change
C
Ets1-SE
ATAC-seq
WT Th1
Stat5a Stat4 Rela Brd4 Stat5b Ctcf
Stat3 Tbx21 Stat1 Smarca Stat6
F
The most enriched motif from lost in naive CD4+ Ets1-SE-/-
TF
Motif
P
ERG(ETS)
1e-2355
The most enriched motif from gained in naive CD4+ Ets1-SE-/-
TF
Motif
P
SpiB(ETS)
1e-1200
G
Lost in Ets1-SE-/-
Naive CD4 T
Th1
Th2
WT
Ets1-SE-/-
Median
Differential Peaks
-2.0
Peak
2.0Kb
Genomic distance (bp)
H
Gained in Ets1-SE-/-
Naive CD4 T
Th1
Th2
WT
Ets1-SE-/-
Median
Differential Peaks
-2.0
Peak
2.0Kb
Genomic distance (bp)
I
WT
ETS1-SE-/-
Naive
Th1
Up in Naive
Up in Th1
Common
-2.0
Peak
2.0Kb
Genomic distance (bp)
J
Gm27162
Gene Expression (TPM)
DP
naive
Th1
Th2
K
ATAC-seq at Gm27162
DP
Naive
Th1
Th2
Peak 1 (Promoter)
Peak 2
Peak 3
Peak 4
Peak 5
Peak 6
Peak 7
Peak 8
Intensity

Figure S5

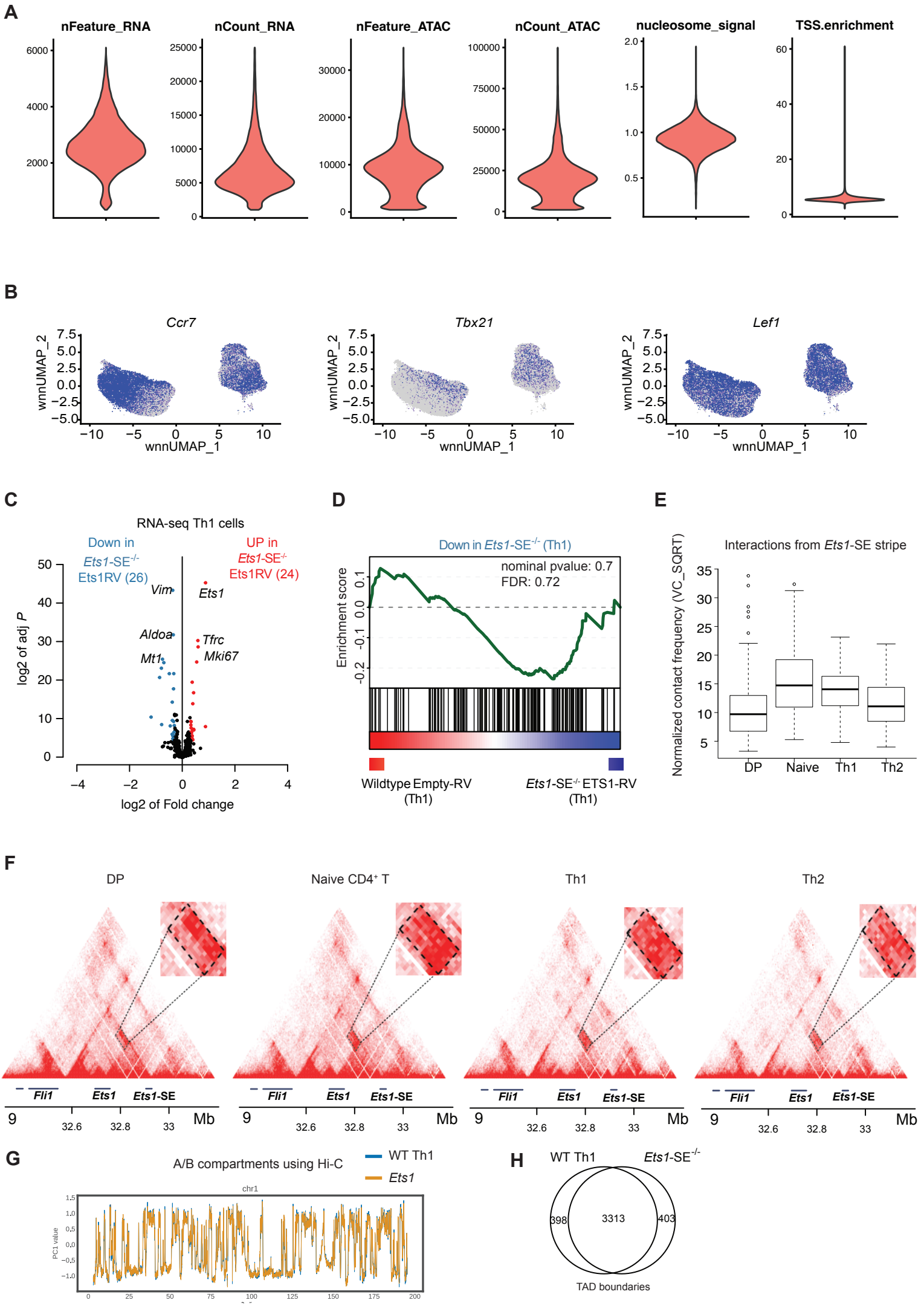
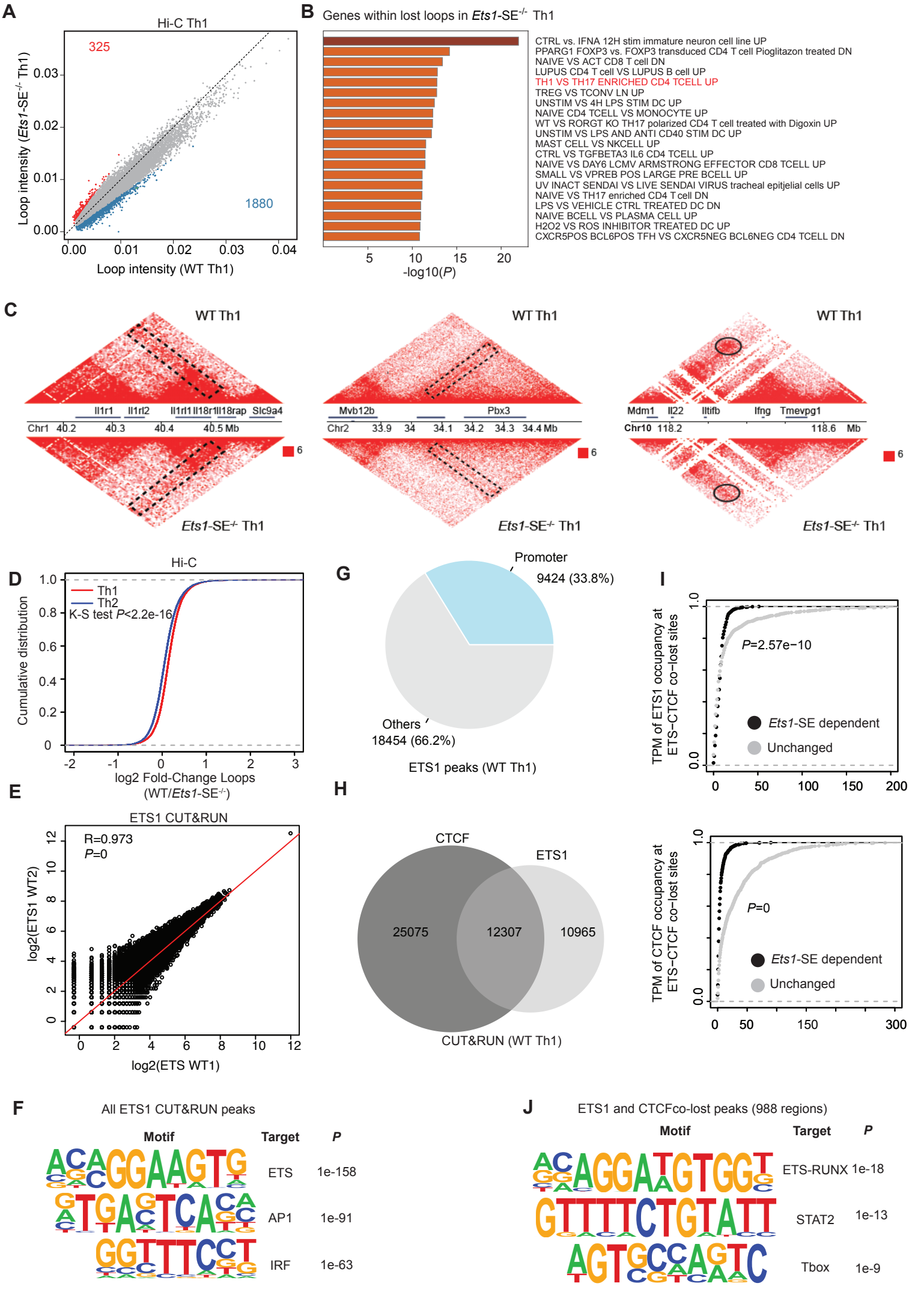

A
nFeature_RNA
nCount_RNA
nFeature_ATAC
nCount_ATAC
nucleosome_signal
TSS.enrichment
B
Ccr7
Tbx21
Lef1
wnnUMAP_2
wnnUMAP_1
C
RNA-seq Th1 cells
Down in Ets1-SE-/- Ets1RV (26)
UP in Ets1-SE-/- Ets1RV (24)
Vim
Ets1
Aldoa
Tfrc
Mki67
Mt1
log2 of adj P
log2 of Fold change
D
Down in Ets1-SE-/- (Th1)
nominal pvalue: 0.7
FDR: 0.72
Enrichment score
Wildtype Empty-RV (Th1)
Ets1-SE-/- ETS1-RV (Th1)
E
Interactions from Ets1-SE stripe
Normalized contact frequency (VC_SQRT)
DP
Naive
Th1
Th2
F
DP
Naive CD4+ T
Th1
Th2
Fli1
Ets1
Ets1-SE
Mb
G
A/B compartments using Hi-C
WT Th1
Ets1
chr1
PC1 value
H
WT Th1
Ets1-SE-/-
398
3313
403
TAD boundaries

Figure S6


A
Hi-C Th1
325
1880
Loop intensity (Ets1-SE-/- Th1)
Loop intensity (WT Th1)
B
Genes within lost loops in Ets1-SE-/- Th1
CTRL vs. IFNA 12H stim immature neuron cell line UP
PPARG1 FOXP3 vs. FOXP3 transduced CD4 T cell Pioglitazon treated DN
NAIVE VS ACT CD8 T cell DN
LUPUS CD4 T cell VS LUPUS B cell UP
TH1 VS TH17 ENRICHED CD4 TCELL UP
TREG VS TCONV LN UP
UNSTIM VS 4H LPS STIM DC UP
NAIVE CD4 TCELL VS MONOCYTE UP
WT VS RORGT KO TH17 polarized CD4 T cell treated with Digoxin UP
UNSTIM VS LPS AND ANTI CD40 STIM DC UP
MAST CELL VS NKCELL UP
CTRL VS TGFBETA3 IL6 CD4 TCELL UP
NAIVE VS DAY6 LCMV ARMSTRONG EFFECTOR CD8 TCELL UP
SMALL VS VPREB POS LARGE PRE BCELL UP
UV INACT SENDAI VS LIVE SENDAI VIRUS tracheal epitjelial cells UP
NAIVE VS TH17 enriched CD4 T cell DN
LPS VS VEHICLE CTRL TREATED DC DN
NAIVE BCELL VS PLASMA CELL UP
H2O2 VS ROS INHIBITOR TREATED DC UP
CXCR5POS BCL6POS TFH VS CXCR5NEG BCL6NEG CD4 TCELL DN
-log10(P)
C
WT Th1
Il1r1 Il1rl2 Il1rl1 Il18r1 Il18rap Slc9a4
Chr1 40.2 40.3 40.4 40.5 Mb
Mvb12b Pbx3
Chr2 33.9 34 34.1 34.2 34.3 34.4 Mb
Mdm1 Il22 Iltifb Ifng Tmevpg1
Chr10 118.2 118.6 Mb
Ets1-SE-/- Th1
D
Hi-C
Th1
Th2
K-S test P<2.2e-16
Cumulative distribution
log2 Fold-Change Loops (WT/Ets1-SE-/-)
E
ETS1 CUT&RUN
R=0.973
P=0
log2(ETS1 WT2)
log2(ETS WT1)
G
Promoter
9424 (33.8%)
Others
18454 (66.2%)
ETS1 peaks (WT Th1)
H
CTCF
ETS1
25075
12307
10965
CUT&RUN (WT Th1)
I
TPM of ETS1 occupancy at ETS−CTCF co−lost sites
P=2.57e−10
Ets1-SE dependent
Unchanged
TPM of CTCF occupancy at ETS−CTCF co−lost sites
P=0
F
All ETS1 CUT&RUN peaks
Motif
Target
P
ETS 1e-158
AP1 1e-91
IRF 1e-63
J
ETS1 and CTCFco-lost peaks (988 regions)
Motif
Target
P
ETS-RUNX 1e-18
STAT2 1e-13
Tbox 1e-9