SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Table S1: 1,25(OH)$_2$D$_3$-modulated FAIRE-peaks. At 8,979 genomic regions a treatment of THP-1 cells with 1,25(OH)$_2$D$_3$ significantly ($p < 0.05$) modulated chromatin accessibility at least at one time point. Absolute values of chromatin accessibility as measured in triplicate FAIRE-seq experiments and FC in reference to time point 0 h are indicated. Genomic regions that are modulated in average more than 2-fold (see **Fig. 1C**) are shaded in beige, regions with six or more FAIRE peaks in blue and the overlap of both categories in pink. Of note, the chromosomal region chr1:39,956,000-44,264,000 is amplified in THP-1 cells and shaded in grey. SOM categorization of FAIRE time course profiles by absolute values (groups I to IX, see **Fig. S3B**) and FC (groups A to I, see **Fig. 2D**) is marked. Furthermore, the overlap of the summits (+/-100 bp) of the FAIRE peaks with VDR and CTCF motifs (both at HOMER score 7) is indicated as well as the overlap (maximal distance 250 bp) with TSS regions and VDR (11,29) and CTCF ChIP-seq peaks found in THP-1 cells (see **Fig. 3A**).

Table S2: CTCF peaks. THP-1 cells were treated for 24 h with 1,25(OH)$_2$D$_3$ (1,25D) or solvent (EtOH) and chromatin was extracted for CTCF ChIP-seq analysis. The position of 71,993 peaks is indicated as well as their strength (fold enrichment) in the presence and absence of ligand and the resulting FC. In addition, the overlap of the CTCF loci with TSS regions, DR3-type sequences (HOMER score 7), VDR (found in absence or presence of 1,25(OH)$_2$D$_3$ (11)) and ligand-dependent FAIRE sites is shown.

Table S3: 1,25(OH)$_2$D$_3$ target genes. In triplicate independent experiments THP-1 cells were stimulated for 2.5, 4 and 24 h with vehicle (EtOH) or 100 nM 1,25(OH)$_2$D$_3$ (1,25D). From the 9,220 not expressed genes (grey, average expression < 0.3) at basal level (EtOH), 38 were expressed above the threshold after 1,25(OH)$_2$D$_3$ treatment. Including the latter, 46 genes

(marked in red) were significantly ($p < 0.001$) regulated by $1,25(OH)_2D_3$ after 2.5 h, 289 (green) after 4 h and 1,204 (blue) after 24 h.

Table S4: Canonical pathways, biofunctions and diseases associated with $1,25(OH)_2D_3$ target genes. Canonical pathways, biofunctions and diseases were analyzed through the use of IPA (Qiagen, www.qiagen.com/ingenuity), in order to rank concerning the involvement of $1,25(OH)_2D_3$ target genes at 2.5, 4 and 24 h after onset of stimulation (see **Table S3**) by significance of enrichment.

Table S5: $1,25(OH)_2D_3$ target genes predicted by epigenome data. Loci of 165 accessible chromatin regions overlapping with a TSS of a gene significantly modulated by $1,25(OH)_2D_3$ at 24 h are listed. The classification of the genes (see **Fig. S6A**) is indicated.

Table S6: Dicer substrate siRNA oligonucleotides (IDT, Leuven, Belgium)**.**

Gene	IDT catalog no.	Oligo nucleotide sequences (5'-3')
CTCF	HSC.RNAI.N006565.12.1	GCAUUUGAACCUUGUAUAAUUAACT AGUUAAUUAUACAAGGUUCAAAUGCUG
CTCF	HSC.RNAI.N006565.12.2	GGAGCCUGCCGUAGAAAUUGAACCT AGGUUCAAUUUCUACGGCAGGCUCCUC
CTCF	HSC.RNAI.N006565.12.3	AGCAAAUCAUGGAAUGUUCUGAGTC GACUCAGAACAUUCCAUGAUUUGCUAA
NC1	DS NC1	CGUUAAUCGCGUAUAAUACGCGUAT AUACGCGUAUUAUACGCGAUUAACGAC

Table S7: Reverse transcription qPCR primers.

Gene	Fragment size (bp)	Annealing temperature (°C)	Primer sequences (5'-3')
ALOX5[2]	246	60	TGGCGCGGTGGATTCATAC CAGGGGAACTCGATGTAGTCC
ASAP2[2]	210	60	AATAAGCGGAGCGGAAATTGC GTTTCAATGGAAGGTTTGAGGC
B2M[1,2]	246	60	GGCTATCCAGCGTACTCCAAA CGGCAGGCATACTCATCTTTTT
CAMP[2]	135	60	CAGCAGTCACCAGAGGATTGT CAGCAGGGCAAATCTCTTGTTA
CD14	142	60	ACGCCAGAACCTTGTGAGC GCATGGATCTCCACCTCTACTG
CTCF[2]	77	60	ATGTGCGATTACGCCAGTGTA TGAAACGGACGCTCTCCAGTA
CYP24A1	70	60	CAAACCGTGGAAGGCCTATC AGTCTTCCCCTTCCAGGATCA
ELL[2]	226	60	GCAGCATACAGGACAAGATCAC GCACTCGCCAAGTTGATGG
FANCE[2]	186	60	AAGCCTCTTTCTTGGACGGAT TCCATCTTCACAAGGCAACAC
FBP1[2]	102	60	AAACACGCCATCATAGTGGAAC TCCAACGGACACAAGGCAATC
GAPDH[1,2]	113	60	CATGAGAAGTATGACAACAGCCTAG TCCTTCCACGATACCAAAGT
G0S2[5]	102	64	GCCACTAAGGTCATTCCCGCCT CCTTGCGCTTCTGGGCCATCAT
HBEGF[2]	175	60	CAAGGAGGAGCACGGGAAAAG CCCATGACACCTCTCTCCA
HPRTI[1,3]	94	60	TGACACTGGCAAAACAATGCA GGTCCTTTTCACCAGCAAGCT
HTT[2]	175	64	AGCTCACCGCTGCTAAGGA ACATCCGATCTCGATTCAGAGTC
IL8	168	60	CAGAGACAGCAGAGCACAC AGCACTCCTTGGCAAAACTG
MPC1[2]	47	60	AGTCTCCAGAGATTATCAGTGGG GCAACAGAGGGCAAATGTCAT
MYC	147	60	CCAGCAGCGACTCTGAGG GGACCAGTGGGCTGTGAG
NFKBIA	227	60	GCAAAATCCTGACCTGGTGT GCTCGTCCTCTGTGAACTCC
NOD2[4]	83	60	TCTCTCGCAGAAGGACTGAA TCTGCCCCTAGGTAGGTGAT
PPARGC1B[6]	113	64	TGAGCAGACCTTGACAGTGGAG GACTATGCTTGATGTCTGGTTTGA
THBD[2]	107	60	GACCTTCCTCAATGCCAGTCA CGTCGCCGTTCAGTAGCAA

TMEM37[6]	147	64	TGGAGTTCCTCATGGTGTCCCA GTGTGACTTGGTTCCTGAGGAG
ZFP36[4]	104	60	CTCATGGCCAACCGTTACAC GACTCAGTCCCTCCATGGTC

[1] reference gene
[2] sequences obtained from PrimerBank (http://pga.mgh.harvard.edu/primerbank)
[3] see Vandesompele, *Genome Biol* **3**, R34 (2002)
[4] designed with Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi)
[5] designed with Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast)
[6] OriGene

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: FAIRE-seq analysis of a 1,25(OH)$_2$D$_3$ time course in THP-1 cells. In triplicate independent experiments THP-1 cells were stimulated with 100 nM 1,25(OH)$_2$D$_3$ (1,25D) for 0, 2, 24 and 48 h and chromatin was isolated for FAIRE-seq analysis. A pie chart indicates the number and percentage of FAIRE peaks that showed at no, at one, at two and at three time points a significant ($p < 0.001$) ligand effect (**A**). Of note, the total number of genomic regions showing a significant (FDR < 0.1%) FAIRE signal at least at one of the four time points was 80,586 but only at 62,231 regions the FAIRE signal was significant (FDR < 0.1%) at all time points. A stacked bar chart illustrates the absolute number of FAIRE peaks being modulated by 1,25(OH)$_2$D$_3$ treatment at 2, 24 and 48 h and the respective percentages of genomic regions displaying chromatin opening (**B**). The IGV browser was used to visualize representative examples of genomic loci (+/-2 kb of the peak summit) with 1,25(OH)$_2$D$_3$-induced chromatin opening that in parallel overlap with a TSS region (**C**) or display 1,25(OH)$_2$D$_3$-induced chromatin closing (**D**). The peak tracks display data from the triplicate FAIRE-seq time course experiment (grey for input lanes and turquoise for 1,25(OH)$_2$D$_3$ treatments for the indicated time periods) and a VDR ChIP-seq experiment (red, (29)). The presence of a DR3-type sequence below the summits (+/-100 bp) of the FAIRE peaks is indicated. Gene structures are shown in blue.

Figure S2: 1,25(OH)$_2$D$_3$-modulated FAIRE peaks. Histogram plots are shown that use 20 evenly sized bins, in order to display the distribution (FC, in log scale) of the 3,323 genomic regions after 2 h stimulation of THP-1 cells with 1,25(OH)$_2$D$_3$, the 4,586 regions after 24 h and the 2,399 regions after 48 h. Red vertical lines indicate the respective average changes in chromatin accessibility due to ligand treatment.

Figure S3: Overlap of ligand-modulated FAIRE peaks with TSS regions. Stacked column graphs indicate the overlap of all 62,231 regions of open chromatin with TSS regions (**A**),

which is in all cases approximately 20%. The remaining 80% non-TSS regions may be enhancers and silencers. For all 8,979 significantly $1,25(OH)_2D_3$-responsive genomic regions the time course of the absolute change of their chromatin accessibility was analyzed in a 3x3 SOM lattice (**B**). Each of the nine resulting groups (I to IX, marked in **Table S1**) is represented by a graph displaying the average chromatin accessibility over time. Three groups (III, VI and VII) overlap with 2.5-times more TSS regions than the reference (see **A**) or 2.5-times less (groups V and VIII).

Figure S4: Motifs below FAIRE peak summits. The genomic sequence below the summits (+/-100 bp) of the 8,979 ligand-responsive FAIRE peaks was screened by HOMER (score 7) for enriched transcription factor binding sites (**A**). The sequence logos of the 10 most significantly enriched motifs are listed. Public ENCODE ChIP-seq data from K562 cells were re-analyzed at harmonized settings. Venn diagrams were used to display the overlap of the 98,515 CTCF peaks in K562 cells (blue) with all 71,993 CTCF sites found in THP-1 cells (red) (left), the 46,920 CTCF sites in presence of solvent (EtOH) (center) and the 21,690 common CTCF sites (right, **B**). Due to variant peak width the percentages of overlap differ between K562 and THP-1 cells. The overlap of K562 CTCF peaks with binding sites for the transcription factors ETS1, GABPA, NR2F2 and NR4A1 in the same cell lines is shown (**C**). Both absolute numbers as well as percentages are indicated.

Figure S5: Impact of CTCF silencing. THP-1 cells were transfected with a mixture of three DsiRNA oligonucleotides directed against CTCF and after 24 h they were stimulated with solvent (EtOH) or 100 nM $1,25(OH)_2D_3$ for 24 h. qPCR was performed in order to determine changes in the expression of sixteen $1,25(OH)_2D_3$ target genes. Columns show the average of three independent experiments, each performed in triplicate individual transfections, and bars indicate standard deviations. Two-tailed Student's t-tests were performed to determine the significance of i) the mRNA changes by the CTCF knock-down (black) in reference to control DsiRNA-transfected cells, ii) the modulation of the ligand effect (blue) in reference to

control DsiRNA-transfected cells and iii) the induction by $1,25(OH)_2D_3$ (red) in reference to solvent-treated cells (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S6: Details of RNA-seq analysis. In triplicate independent experiments THP-1 cells were treated for 2.5, 4 and 24 h with vehicle (EtOH) or 100 nM $1,25(OH)_2D_3$ and RNA-seq analysis was performed. A pie chart illustrates the number of genes that were i) not expressed in THP-1 cells at basal level, ii) expressed but not regulated by $1,25(OH)_2D_3$ and iii) expressed and regulated by $1,25(OH)_2D_3$ at one or more time points (**A**, see **Table S2**). Venn diagrams demonstrate the overlap of genes being significantly ($p < 0.001$) up-regulated (**B**) or down-regulated (**C**) by $1,25(OH)_2D_3$ at the respective time points.

Figure S7: Integration of epigenomic and transcriptomic data. K-means analysis ($k = 6$) of the FC (in log scale) of gene expression after 24 h stimulation with $1,25(OH)_2D_3$ for 165 genes overlapping with at least one of the ligand-modulated 7,979 FAIRE peaks (**A**). Relevance (obtained by random forests) of the top 11 attributes for inferring the discrete state of the FC (in log scale) at 24 h for the corresponding (overlapping or closest) gene for all 8,979 FAIRE peaks (**B**) or the selected 165 $1,25(OH)_2D_3$ target genes (**C**) is indicated. Average prediction (test) error of the discrete state of the FC (in log scale) at 24 h for the corresponding genes for i) all 8,979 FAIRE peaks (**D**), ii) the selected 165 $1,25(OH)_2D_3$ target genes for epigenomic and transcriptomic attributes or iii) as a function of the number of attributes (**F**) is shown.

Fig. S1

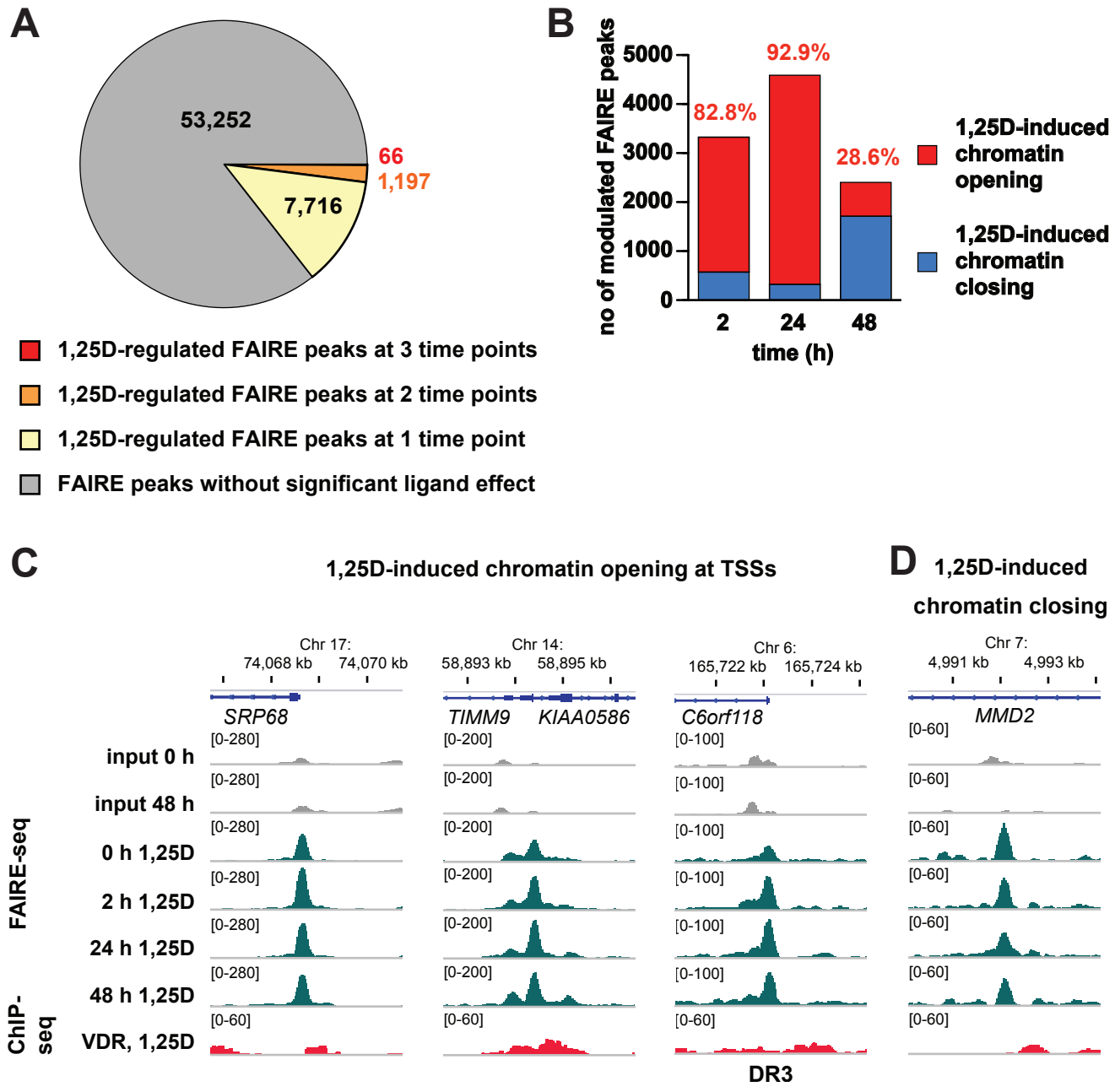
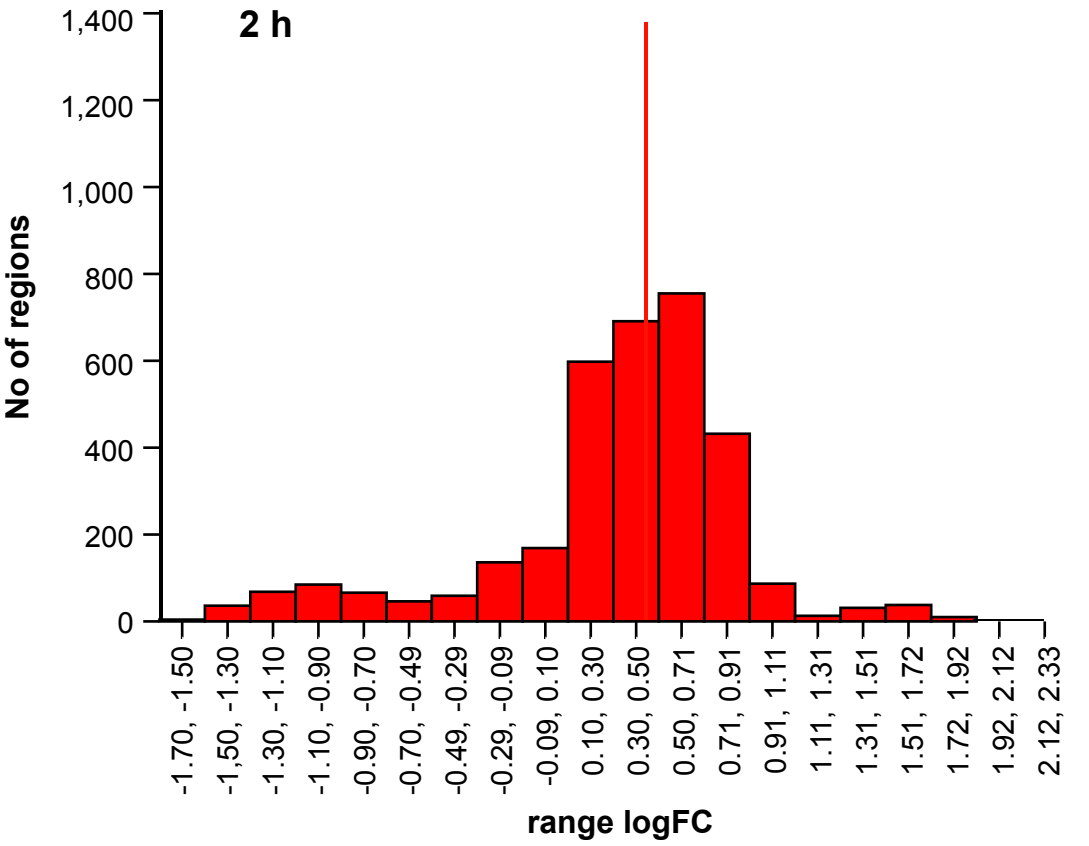
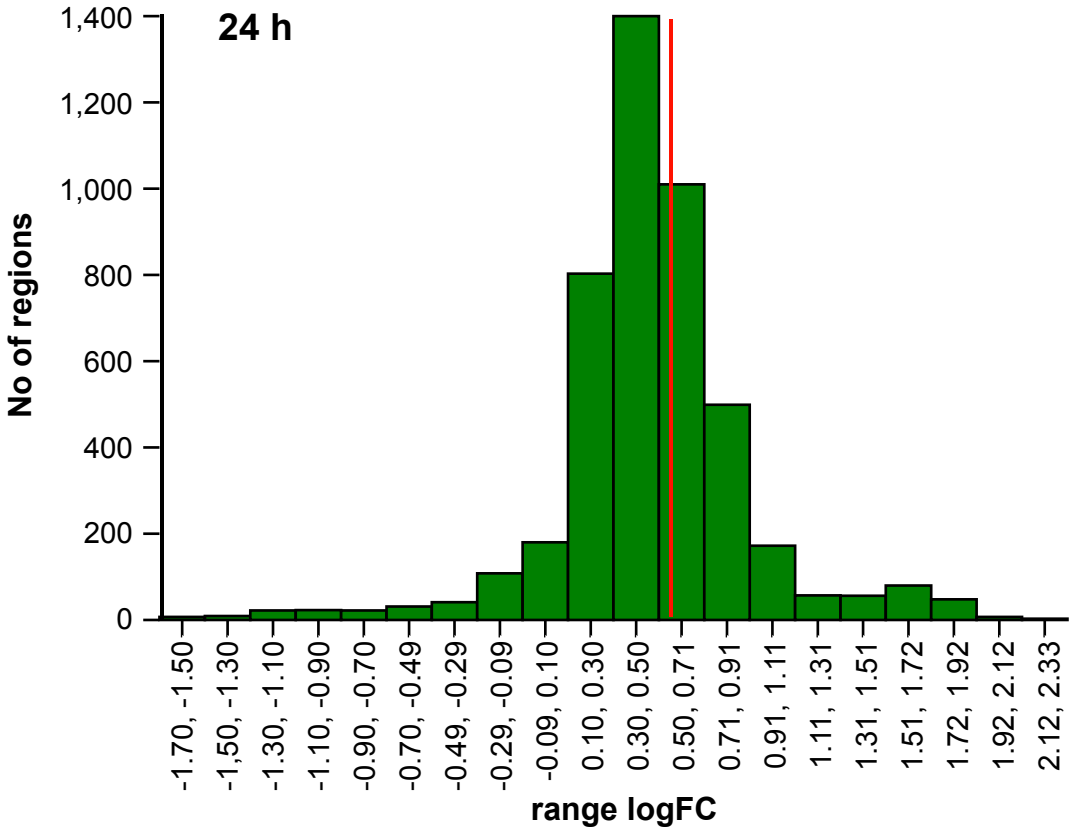

A
53,252
66
1,197
7,716
1,25D-regulated FAIRE peaks at 3 time points
1,25D-regulated FAIRE peaks at 2 time points
1,25D-regulated FAIRE peaks at 1 time point
FAIRE peaks without significant ligand effect
B
no of modulated FAIRE peaks
5000
4000
3000
2000
1000
0
82.8%
92.9%
28.6%
2
24
48
time (h)
1,25D-induced chromatin opening
1,25D-induced chromatin closing
C
1,25D-induced chromatin opening at TSSs
Chr 17:
74,068 kb
74,070 kb
SRP68
Chr 14:
58,893 kb
58,895 kb
TIMM9
KIAA0586
Chr 6:
165,722 kb
165,724 kb
C6orf118
FAIRE-seq
ChIP-seq
input 0 h
input 48 h
0 h 1,25D
2 h 1,25D
24 h 1,25D
48 h 1,25D
VDR, 1,25D
[0-280]
[0-200]
[0-100]
[0-60]
DR3
D
1,25D-induced chromatin closing
Chr 7:
4,991 kb
4,993 kb
MMD2

Fig. S2


2 h
No of regions
1,400
1,200
1,000
800
600
400
200
0
-1.70, -1.50
-1,50, -1.30
-1.30, -1.10
-1.10, -0.90
-0.90, -0.70
-0.70, -0.49
-0.49, -0.29
-0.29, -0.09
-0.09, 0.10
0.10, 0.30
0.30, 0.50
0.50, 0.71
0.71, 0.91
0.91, 1.11
1.11, 1.31
1.31, 1.51
1.51, 1.72
1.72, 1.92
1.92, 2.12
2.12, 2.33
range logFC


24 h
No of regions
1,400
1,200
1,000
800
600
400
200
0
-1.70, -1.50
-1,50, -1.30
-1.30, -1.10
-1.10, -0.90
-0.90, -0.70
-0.70, -0.49
-0.49, -0.29
-0.29, -0.09
-0.09, 0.10
0.10, 0.30
0.30, 0.50
0.50, 0.71
0.71, 0.91
0.91, 1.11
1.11, 1.31
1.31, 1.51
1.51, 1.72
1.72, 1.92
1.92, 2.12
2.12, 2.33
range logFC

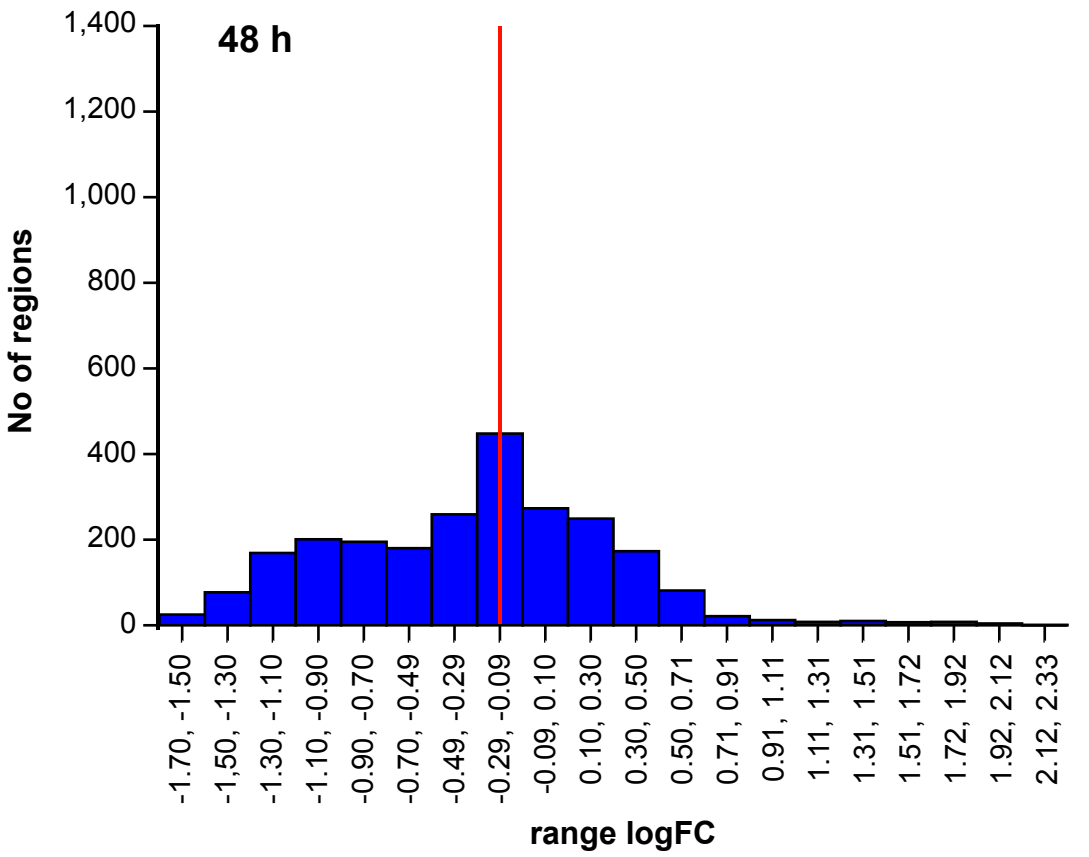
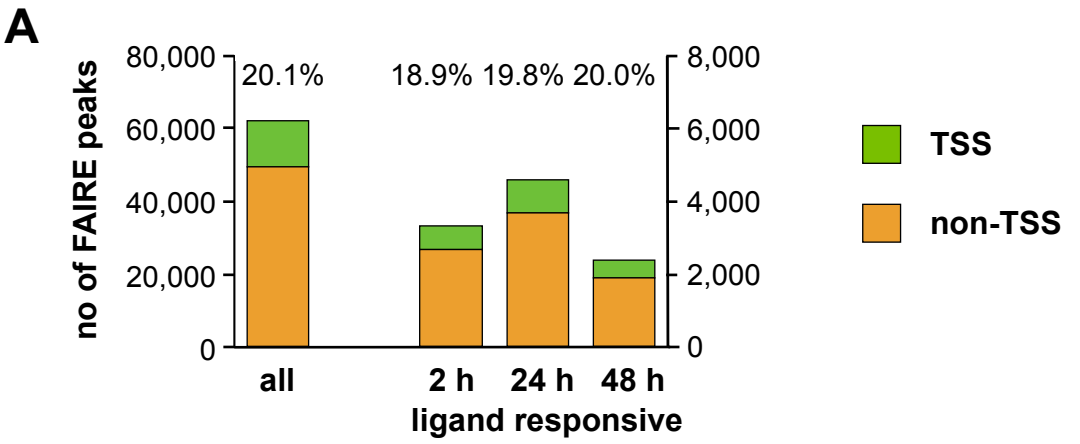

48 h
No of regions
1,400
1,200
1,000
800
600
400
200
0
-1.70, -1.50
-1,50, -1.30
-1.30, -1.10
-1.10, -0.90
-0.90, -0.70
-0.70, -0.49
-0.49, -0.29
-0.29, -0.09
-0.09, 0.10
0.10, 0.30
0.30, 0.50
0.50, 0.71
0.71, 0.91
0.91, 1.11
1.11, 1.31
1.31, 1.51
1.51, 1.72
1.72, 1.92
1.92, 2.12
2.12, 2.33
range logFC

Fig. S3


A
no of FAIRE peaks
80,000
60,000
40,000
20,000
0
8,000
6,000
4,000
2,000
0
20.1%
18.9%
19.8%
20.0%
all
2 h
24 h
48 h
ligand responsive
TSS
non-TSS

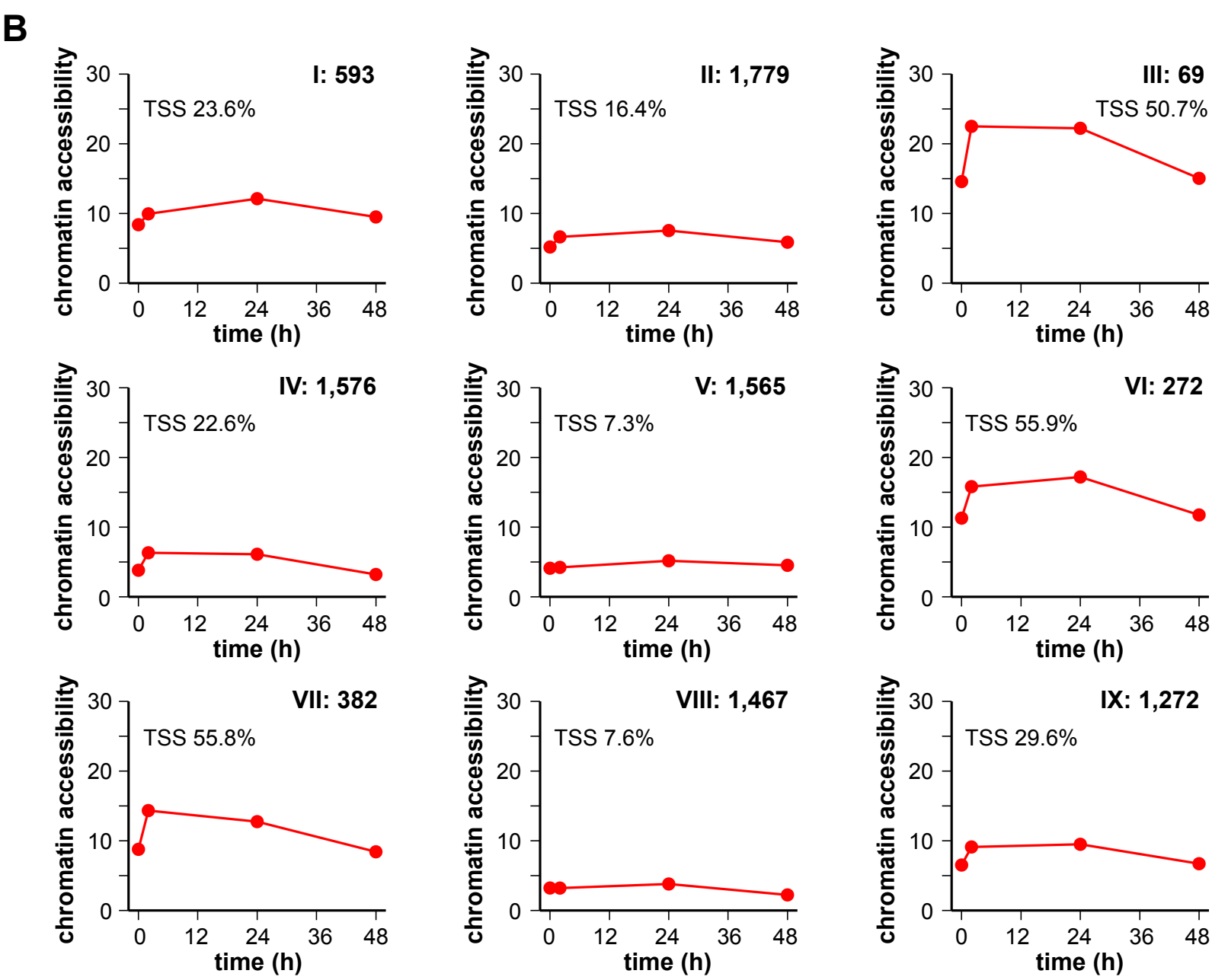
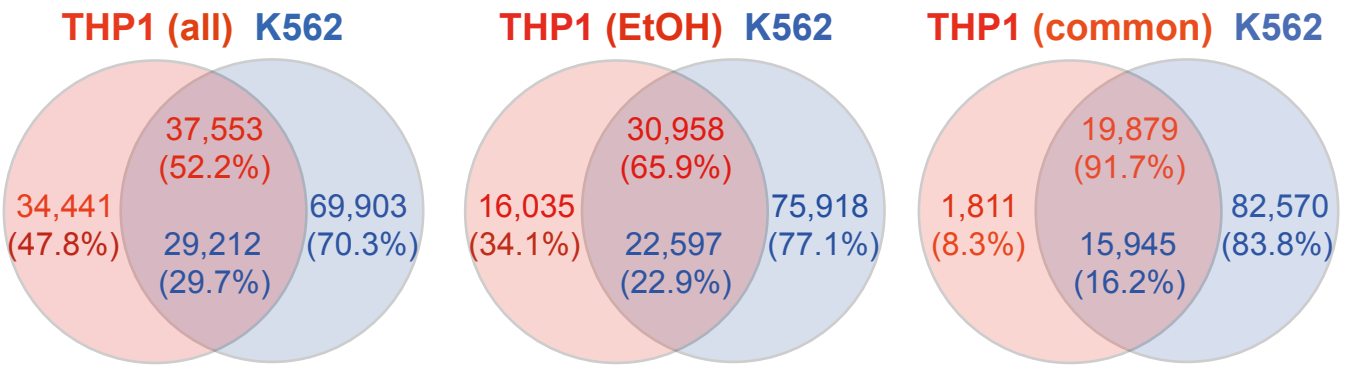

B
chromatin accessibility
time (h)
I: 593
TSS 23.6%
II: 1,779
TSS 16.4%
III: 69
TSS 50.7%
IV: 1,576
TSS 22.6%
V: 1,565
TSS 7.3%
VI: 272
TSS 55.9%
VII: 382
TSS 55.8%
VIII: 1,467
TSS 7.6%
IX: 1,272
TSS 29.6%

Fig. S4

A Transcription factor binding motif below ligand-modulated FAIRE peaks

Sequence logo	Transcription factor name	p value enrichment
	CTCF	1e-417
	BORIS	1e-338
	PU.1	1e-320
	ELF5	1e-256
	ETS1	1e-228
	ETV1	1e-193
	Fli1	1e-173
	GABPA	1e-168
	ERG	1e-166
	Ets-distal	1e-116

B CTCF binding site overlap between THP-1 and K562 cells



C Transcription factor binding overlap (ENCODE data from K562 cells)

	CTCF	ETS1	GABPA	NR2F2	NR4A1
CTCF	98,515 (100%)	9,962 (10.1%)	7,633 (7.7%)	14,915 (15.1%)	135 (0.1%)
ETS1	12,033 (33.1%)	36,341 (100%)	18,745 (51.6%)	24,350 (67.0%)	1,038 (2.9%)
GABPA	8,066 (27.8%)	16,236 (56.0%)	28,995 (100%)	21,062 (72.6%)	397 (1.4%)
NR2F2	16,774 (28.3%)	24,047 (40.5%)	23,229 (39.2%)	59,306 (100%)	995 (1.7%)
NR4A1	156 (12.5%)	747 (59.8%)	380 (30.4%)	765 (61.2%)	1,249 (100%)

Fig. S5

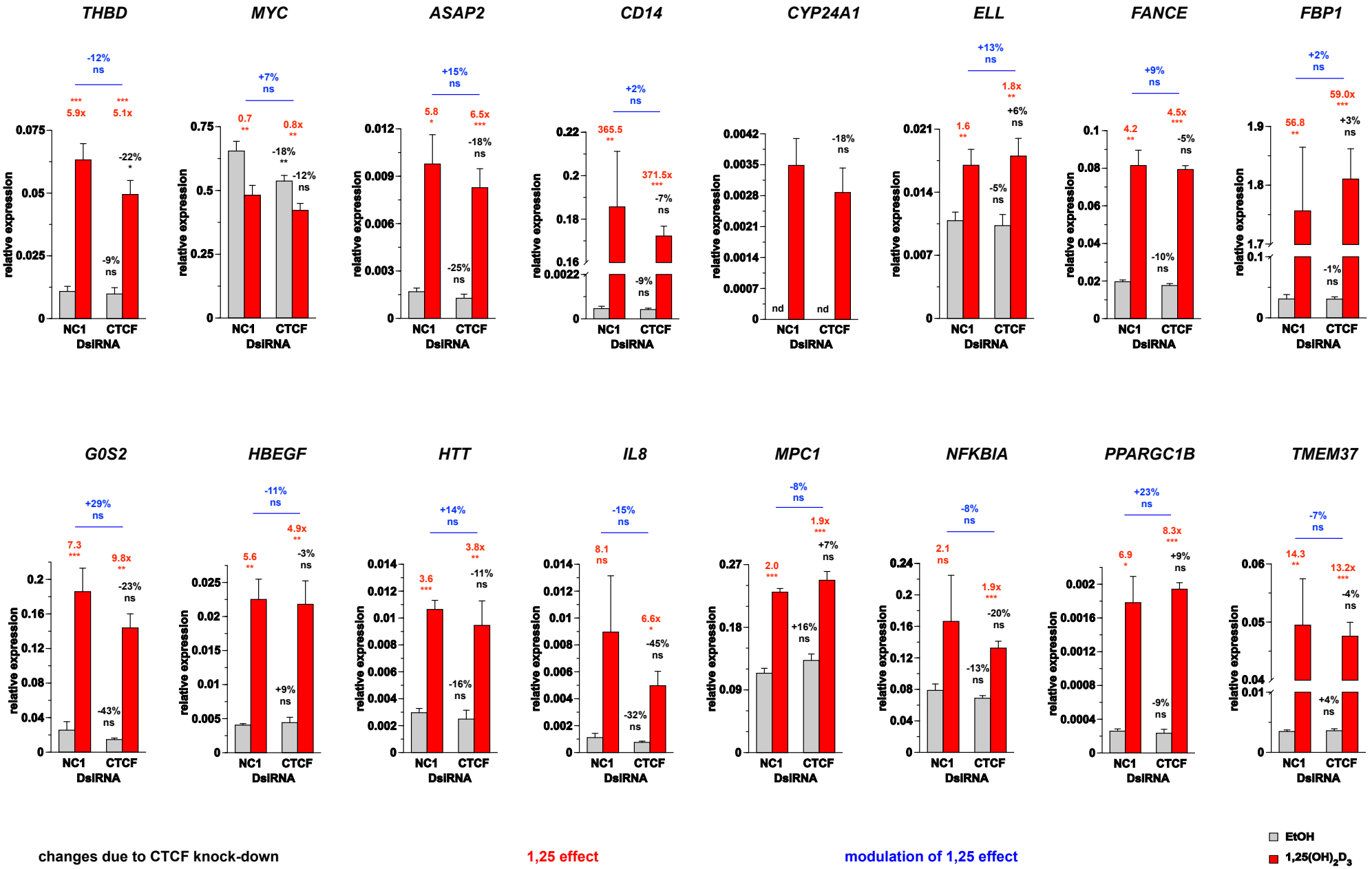

THBD
MYC
ASAP2
CD14
CYP24A1
ELL
FANCE
FBP1
G0S2
HBEGF
HTT
IL8
MPC1
NFKBIA
PPARGC1B
TMEM37
relative expression
NC1
CTCF
DsiRNA
changes due to CTCF knock-down
1,25 effect
modulation of 1,25 effect
EtOH
1,25(OH)2D3

Fig. S6

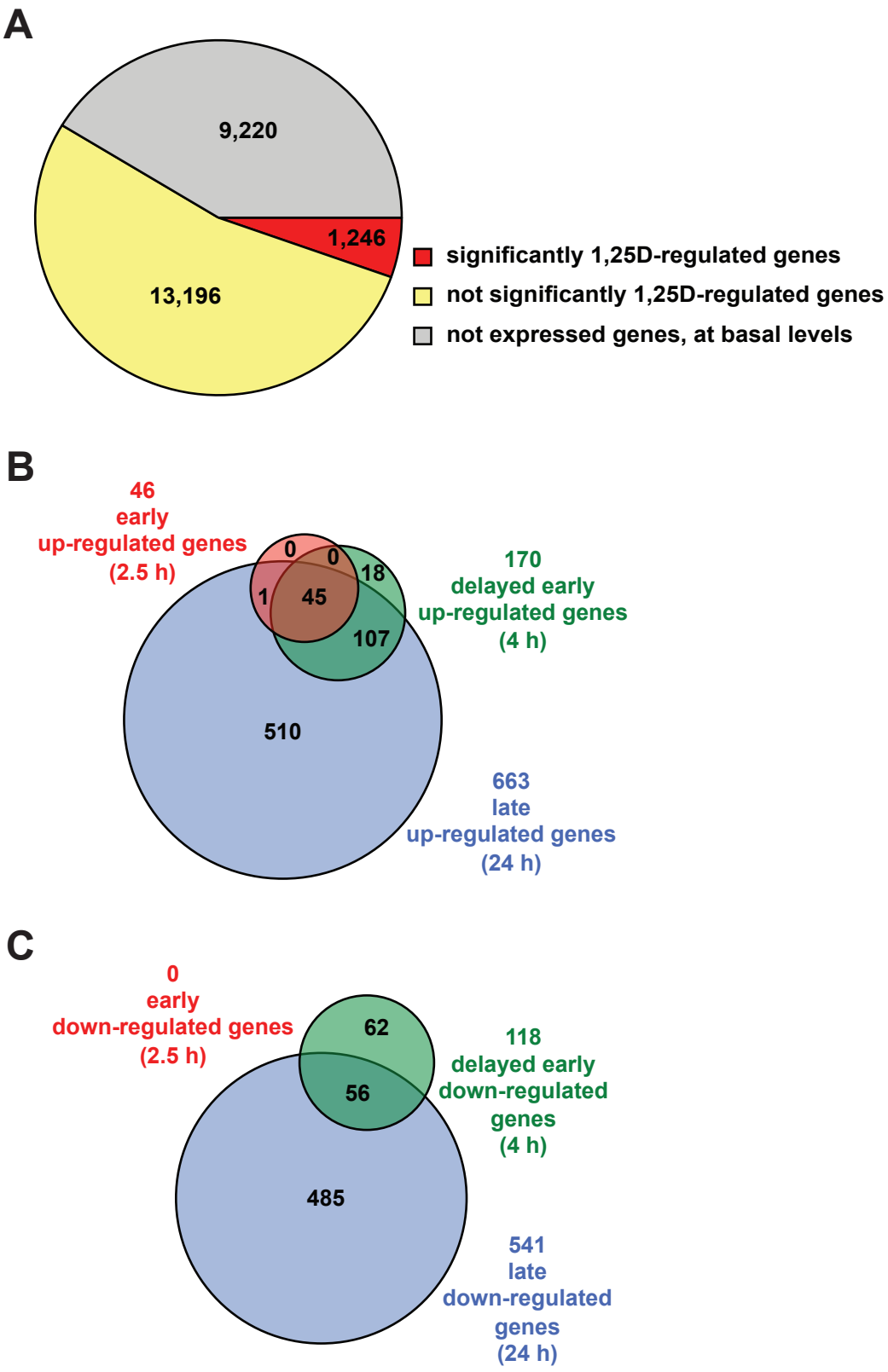
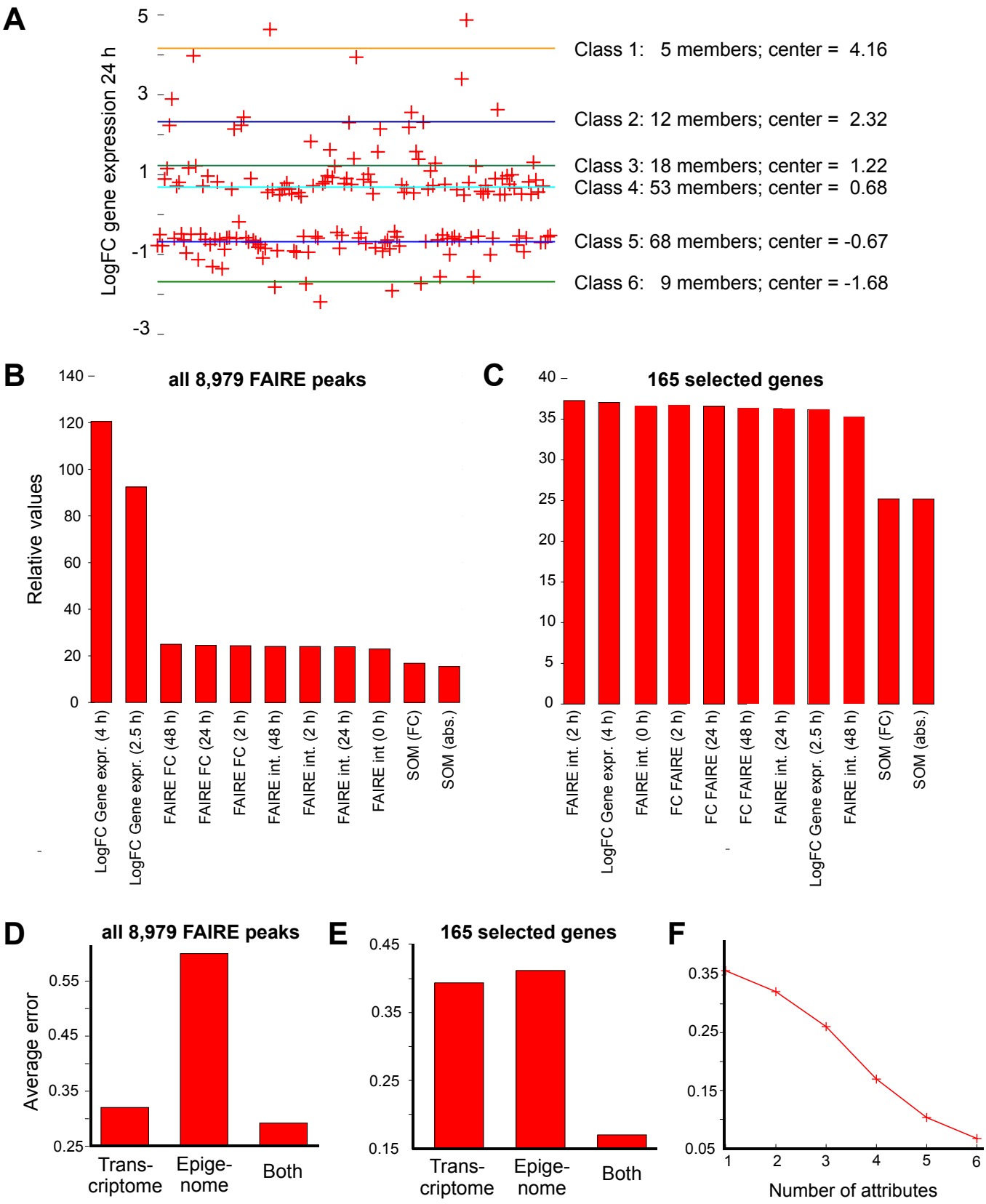

A
9,220
1,246
13,196
significantly 1,25D-regulated genes
not significantly 1,25D-regulated genes
not expressed genes, at basal levels
B
46
early
up-regulated genes
(2.5 h)
0
0
18
1
45
107
510
170
delayed early
up-regulated genes
(4 h)
663
late
up-regulated genes
(24 h)
C
0
early
down-regulated genes
(2.5 h)
62
56
485
118
delayed early
down-regulated
genes
(4 h)
541
late
down-regulated
genes
(24 h)

Fig. S7


A
LogFC gene expression 24 h
5
3
1
-1
-3
Class 1: 5 members; center = 4.16
Class 2: 12 members; center = 2.32
Class 3: 18 members; center = 1.22
Class 4: 53 members; center = 0.68
Class 5: 68 members; center = -0.67
Class 6: 9 members; center = -1.68
B
all 8,979 FAIRE peaks
Relative values
140
120
100
80
60
40
20
0
LogFC Gene expr. (4 h)
LogFC Gene expr. (2.5 h)
FAIRE FC (48 h)
FAIRE FC (24 h)
FAIRE FC (2 h)
FAIRE int. (48 h)
FAIRE int. (2 h)
FAIRE int. (24 h)
FAIRE int (0 h)
SOM (FC)
SOM (abs.)
C
165 selected genes
40
35
30
25
20
15
10
5
0
FAIRE int. (2 h)
LogFC Gene expr. (4 h)
FAIRE int. (0 h)
FC FAIRE (2 h)
FC FAIRE (24 h)
FC FAIRE (48 h)
FAIRE int. (24 h)
LogFC Gene expr. (2.5 h)
FAIRE int. (48 h)
SOM (FC)
SOM (abs.)
D
all 8,979 FAIRE peaks
Average error
0.55
0.45
0.35
0.25
Trans-criptome
Epige-nome
Both
E
165 selected genes
0.45
0.35
0.25
0.15
Trans-criptome
Epige-nome
Both
F
0.35
0.25
0.15
0.05
1
2
3
4
5
6
Number of attributes