SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Table S1: qPCR primers.

Gene	Fragment size (bp)	Annealing temperature (°C)	Primer sequences (5'-3')
B2M	246	60	GGCTATCCAGCGTACTCCAAA
			CGGCAGGCATACTCATCTTTTT
CAMP	135	60	CAGCAGTCACCAGAGGATTGT
			CAGCAGGGCAAATCTCTTGTTA
CD93	234	60	CCTCCCCAAGTGGTCTGAG
			TGAGTCTCGTCCTTGTCACCT
CDKN2D	202	60	CTTCCAATCCATCTGGCAGT
			CTCTTGCTGGAGAGGGTGAC
DUSP10	183	60	GCGGCAGTACTTTGAAGAGG
			ATTGGTCGTTTGCCTTTGAC
GAPDH	113	60	CATGAGAAGTATGACAACAGCCT
			AGTCCTTCCACGATACCAAAGT
GZF1	212	60	TGTCTGTGATGAATGTGGTGCAA
			GTGAATTCCGCTGGGCAAAAG
EP300	217	60	GCAGCATATGCTCCCAAATC
			AGCTACCAGTCCAGGATG
HBEGF	175	60	CAAGGAGGAGCACGGGAAAAG
			CCCATGACACCTCTCTCCA
HPRTI	94	60	TGACACTGGCAAAACAATGCA
			GGTCCTTTTCACCAGCAAGCT
LOC200261	140	60	TCTCATCAGTAACCCAGGAGG
			CCCACCAAAAGGGACACCAT
NAPB	225	60	GCCACTTCATAGTAGACGAGTTG
			TGGACTTTTTGATGCGAAGCA
NFKBIA	227	60	GCAAAATCCTGACCTGGTGT
			GCTCGTCCTCTGTGAACTCC
NXT1	91	60	CTTCCAGCGAGTTCCAAATCA
			CAGATGACAACAAGGACCGTG
SSTR4	174	64	CGTGGTCGTCTTTGTGCTCT
			AAGAATCGGCGGAAGTTGT
THBD	107	60	GACCTTCCTCAATGCCAGTCA
			CGTCGCCGTTCAGTAGCAA
VDR	332	60	AGATGACCCTTCTGTGACCC
			AGCTTCTTCAGTCCCACCTG

Table S2: Genomic primers.

Genomic region	Fragment size (bp)	Annealing temperature (°C)	Primer sequences (5'-3')
CAMP	347	65	AGCAAGTCCTGTGAAGCAATAGC
TSS			CAGGAGGCGGTAGAGGTTAGCATC
CAMP	224	65	ACAACCTTTCCTAAGACTTGGCTTG
VDR site			CCCAACCTAGAGACTCACTCTTGC
DUSP10	217	65	AGCTTCGGATAAACCCTCCTTAAA
TSS			TTGATCTCCAGCAGCAACATAGTTA
DUSP10	250	65	GTCAACCCTTTGAGCCGGAGTTAAG
VDR site			GGAGATCATGAAACCCATTGTCCAG
NFKBIA	235	65	AAATCCCCAGCCAGCGTTTATAG
TSS			GTCCAGTAGCCGCTCCTTCTTC
NXT1	225	65	CTACCGCCTCTTATAAGCCACACAT
TSS			CTCCTCCTTCTTGGAGGCTTACTCT
NXT1	153	65	TGAGGTCAAGGGTCCATTCTGATGGC
VDR site			ACTTCCTGACCCAAGCATGAATCCCCT
PSMA6	100	65	GTTGGGAATTTTCCATCTTGCTCT
VDR site			CTCGCTGACCTGCAGGAAACTC
THBD	140	65	CACCAGGCACTTCCTTCCTTTTC
TSS			GTCCCAGCCCAGACACTTCTTG

Table S3: Microarray results. Genes that had been also studied by qPCR are indicated in red.

SUPLEMENTARY FIGURE LEGENDS

Figure S1: Basal mRNA expression of the genes within the *THBD* locus. qPCR was used to determine the basal expression of the seven genes within the *THBD* locus (Fig. 1) in relation to the reference genes *B2M*, *GAPDH* and *HPRT1* in untreated THP-1 cells. A representative of at least three independent experiments, each performed in triplicate individual cell treatments, is shown. The data points represent the means of three cell treatments and the bars indicate standard deviations.

Figure S2: Effects of 1α,25(OH)$_2$D$_3$ on mRNA expression of reference genes. qPCR was performed to determine the relative changes of mRNA expression of the VDR target genes *DUSP10*, *NFKBIA*, *CAMP* and *HBEGF* and the negative control genes *CDKN2D*, *VDR* and *EP300* in relation to the reference genes *B2M*, *GAPDH* and *HPRT1* in THP-1 cells in response to incubation with 100 nM 1α,25(OH)$_2$D$_3$ for the indicated times. The columns represent the means of three cell treatments and the bars indicate standard deviations. Two-tailed Student's t-tests were performed to determine the significance of the mRNA induction by 1α,25(OH)$_2$D$_3$ in reference to solvent-treated cells (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S3: Confirming VDR target genes by siRNA-mediated knock-down. THP-1 cells were transfected with 200 pmol of a mixture of three siRNA oligonucleotides directed against VDR and after 43 h incubated with solvent (EtOH) or 100 nM 1α,25(OH)$_2$D$_3$ for 5 h. qPCR was performed to determine the reduction of the respective mRNA levels normalized by the three reference genes *B2M*, *GAPDH* and *HPRT1*. A representative of at least three independent experiments, each performed in triplicate individual cell treatments, is shown. The columns represent the means of three cell treatments and the bars indicate standard deviations. Two-tailed Student's t-tests were performed to determine the significance of the mRNA reduction by the VDR knock-down and the induction by 1α,25(OH)$_2$D$_3$ (red stars) in

reference to control siRNA-transfected cells and solvent-treated cells, respectively (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S4: Effects of HDAC inhibition on mRNA expression of reference genes. qPCR was performed to determine the relative changes of mRNA expression of the VDR target genes *DUSP10*, *NFKBIA*, *CAMP* and *HBEGF* and the negative control genes *CDKN2D*, *VDR* and *EP300* in relation to the reference genes *B2M*, *GAPDH* and *HPRT1* in THP-1 cells in response to incubation with 300 nM TsA for the indicated times. The columns represent the means of three cell treatments and the bars indicate standard deviations. Two-tailed Student's t-tests were performed to determine the significance of the mRNA induction by TsA in reference to solvent-treated cells (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S5: TsA dose response. qPCR was performed to determine the relative changes of mRNA expression of 12 selected genes normalized by the three reference genes *B2M*, *GAPDH* and *HPRT1* in THP-1 cells in response to incubation with the indicated concentrations of TsA for 90 min. The columns represent the means of three cell treatments and the bars indicate standard deviations. Two-tailed Student's t-tests were performed to determine the significance of the mRNA induction by TsA in reference to solvent-treated cells (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S6: Microarray analysis of TsA-treated THP-1 cells. The Venn diagram indicates the number of genes that responded after 90 and 150 min to a treatment with 300 nM TsA. For the sake of clarity, the sizes and overlaps of the circles are only approximate.

Figure S7: Early time course expression profiling of six representative genes. qPCR was performed to determine the relative changes of mRNA expression of selected genes normalized by the three reference genes *B2M*, *GAPDH* and *HPRT1* in THP-1 cells in

response to incubation with 100 nM 1α,25$(OH)_2D_3$ and 300 nM TsA, alone or in combination, over a time period of 150 min. A representative of at least three independent experiments, each performed in triplicate individual cell treatments, is shown. The data points represent the means of three cell treatments and the bars indicate standard deviations. Two-tailed Student's t-tests were performed to determine the significance of the mRNA induction by the stimuli in reference to solvent-treated cells (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S8: Microarray analysis of 1α,25$(OH)_2D_3$- and TsA-treated THP-1 cells. The Venn diagrams indicate the number of genes regulated in THP-1 cells that were treated for 150 min with 100 nM 1α,25$(OH)_2D_3$ and 300 nM TsA, alone and in combination. For the sake of clarity, the sizes and overlaps of the circles are only approximate.

Fig. S1

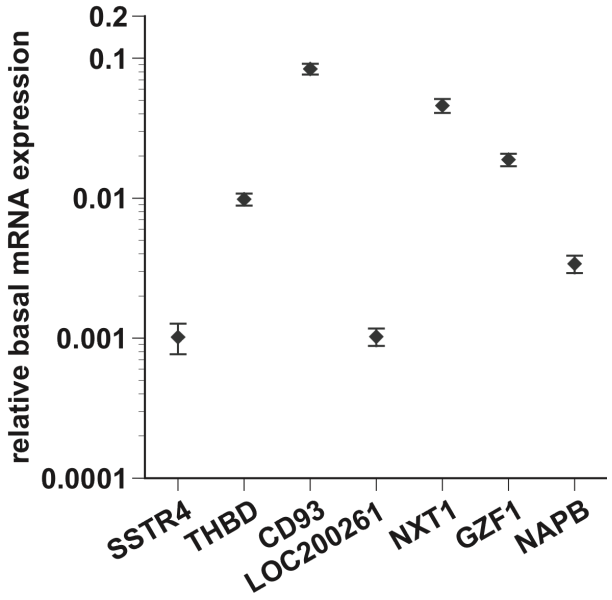

relative basal mRNA expression
0.2
0.1
0.01
0.001
0.0001
SSTR4
THBD
CD93
LOC200261
NXT1
GZF1
NAPB

Fig. S2

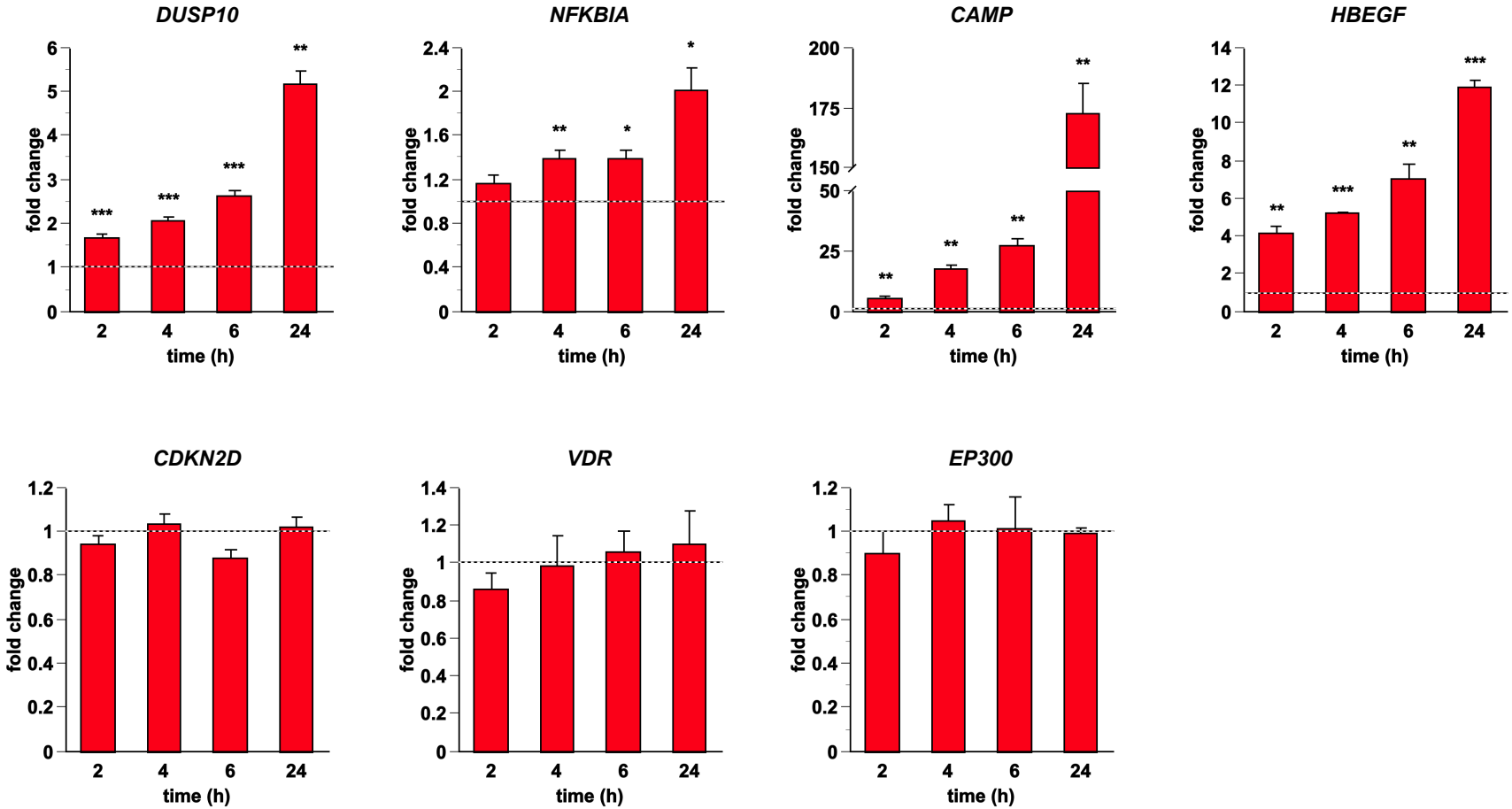
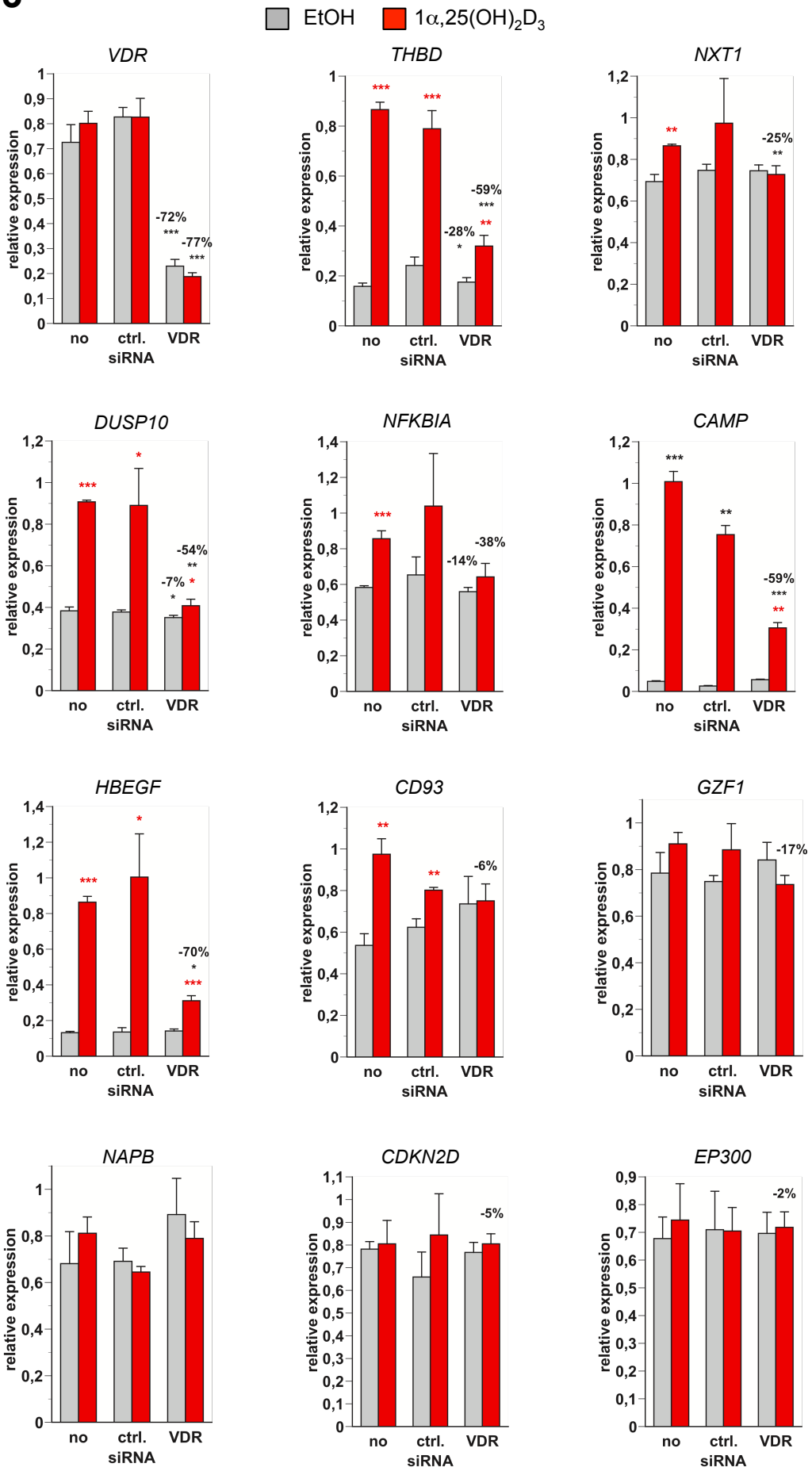

DUSP10
NFKBIA
CAMP
HBEGF
CDKN2D
VDR
EP300
fold change
time (h)
2
4
6
24

Fig. S3



red stars = significance of ligand treatment effect (vs solvent), black stars = significance of knock-down

Fig. S4

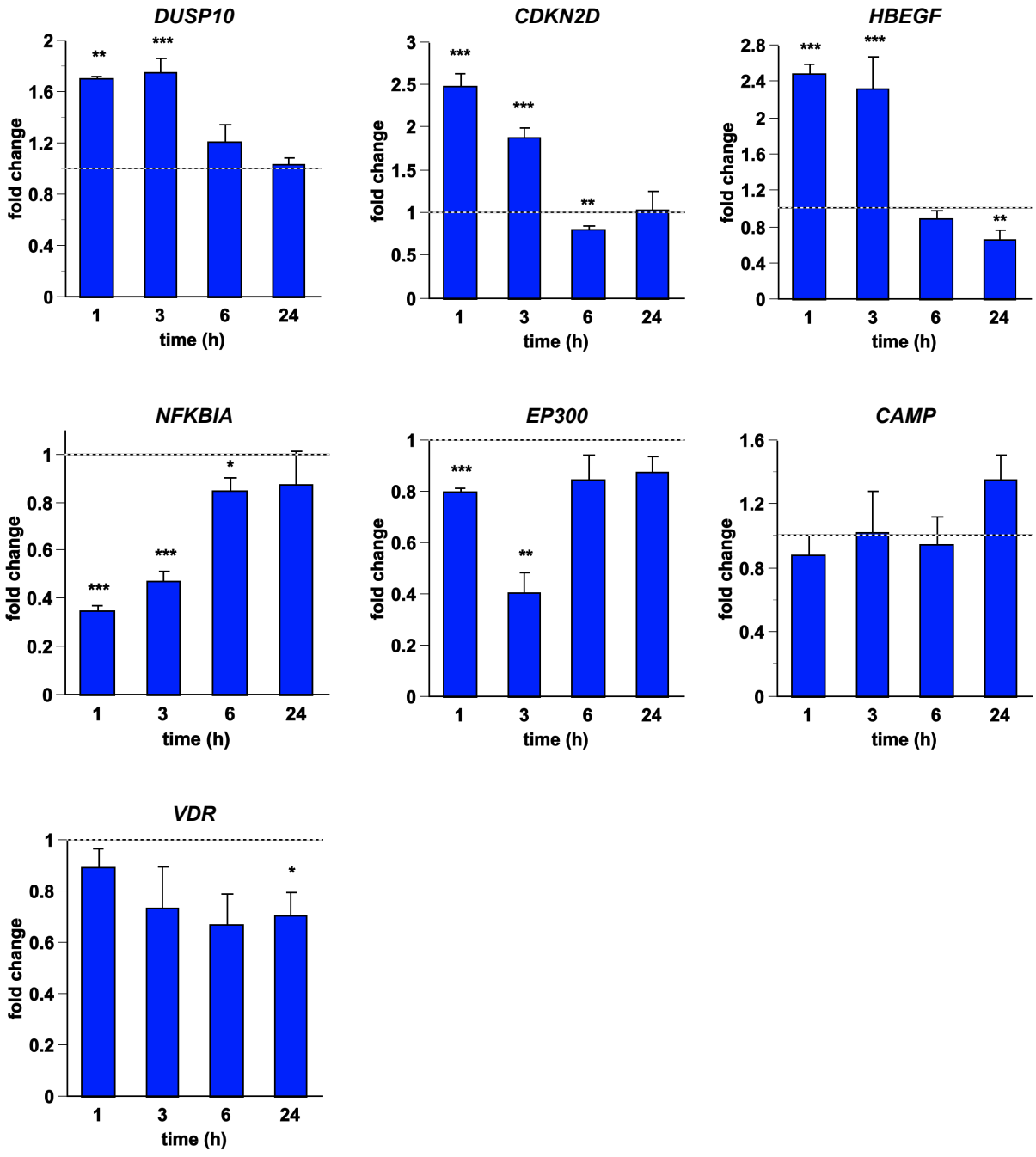

DUSP10
CDKN2D
HBEGF
NFKBIA
EP300
CAMP
VDR
fold change
time (h)
1
3
6
24

Fig. S5

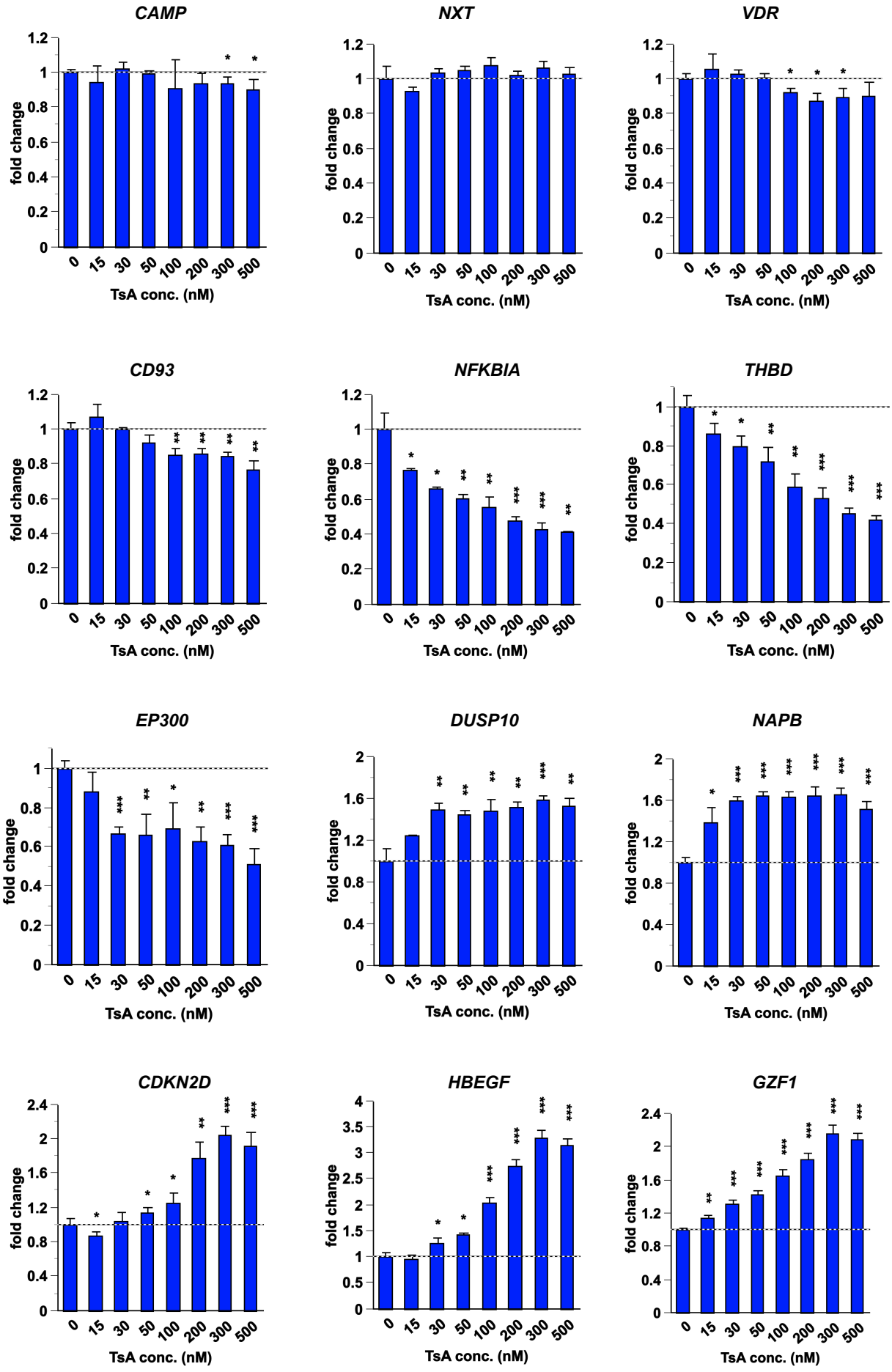

CAMP
NXT
VDR
CD93
NFKBIA
THBD
EP300
DUSP10
NAPB
CDKN2D
HBEGF
GZF1
fold change
TsA conc. (nM)
0
15
30
50
100
200
300
500

Fig. S6

Fig. S7

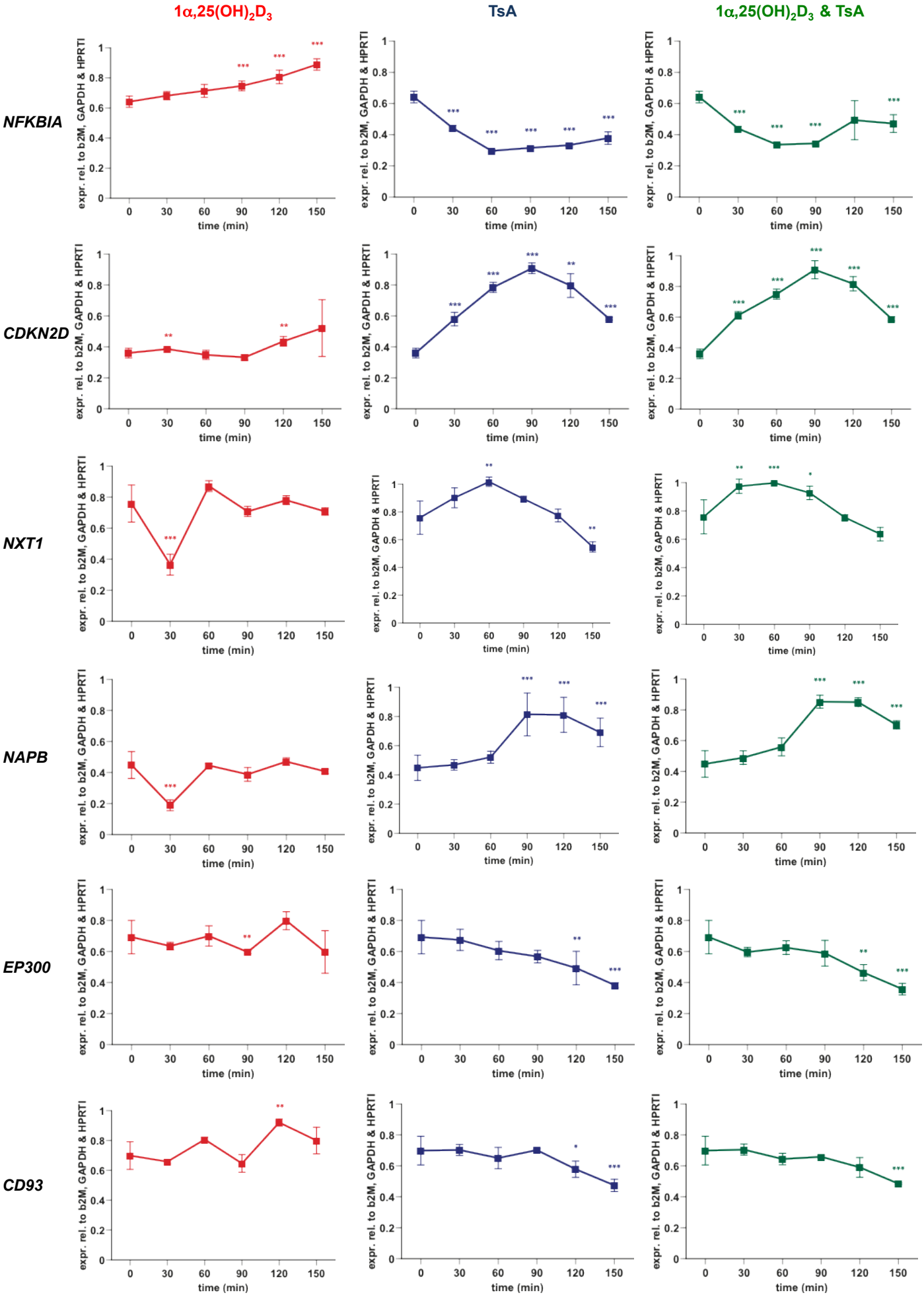

1α,25(OH)2D3
TsA
1α,25(OH)2D3 & TsA
NFKBIA
CDKN2D
NXT1
NAPB
EP300
CD93
expr. rel. to b2M, GAPDH & HPRTI
time (min)

Fig. S8