SUPPLEMENTARY TABLES

Table S1: Quantitative real-time PCR primers. Sequence and product size of primer pairs used for gene-specific quantitative real-time PCR.

Gene	Primer pairs (5' - 3')	Product size (bp)	Annealing temperature (°C)
RPLP0	AGATGCAGCAGATCCGCAT GTGGTGATACCTAAAGCCTG	318	58-62
IGFBP3	AAGTTGACTACGAGTCTCAG ACGGCAGGGACCATATTC	83	58
CYP24A1	CAAACCGTGGAAGGCCTATC AGTCTTCCCCTTCCAGGATCA	70	62
HDAC1	GATCTGCTCCTCTGACAAAC GACTTCTTTCTTCTCCTCTG	159	60
HDAC2	CAGTGGAGATGAAGATGGAG TTTCACCACTGTTGTCCTTG	241	60
HDAC3	CTTCATCCAGATGTCAGCAC TCCACATCGCTTTCCTTGTC	268	60
HDAC4	GCATGTGTTTCTGCCTTGCTG GTTCTCGCAAGTCTGAGCCT	191	60
HDAC5	ATGCTGTTGAAGGACATCTG TCTGGATCTCGATGACTTTC	272	60
HDAC6	GCAAGGGATGGATCTGAACC CTAGGCTGTGAACCAACATC	201	60
HDAC7	CTCACTGTCAGCCCCAGAG TGTCACGCAGGACCACTG	249	62
HDAC8	ACCAGATCTGTGAAAGTGTAC AACTAGACCACATGCTTCAG	414	60
HDAC9	GCAGATCCACATGAACAAACTG GATCTGAGCATCTTCATCACTG	240	60
HDAC10	ATTGAAAGAACAAATGCG CTCTGGGCTCCGTGGGAC	367	62
HDAC11	GGACGACAAGCGTGTGTACATC AGCGGTGTGTCTGAGTTCTGTG	443	60

Table S2: ChIP primers. Location (relative to TSS) and sequence of the PCR primer pairs used to detect two genomic regions of the human *IGFBP3* promoter.

VDRE	Location	Primer sequence (5' - 3')
RE1/2	-419 to -293	CGCTGTATGCCAGTTTCC TCACCCCAGTCACTCCTG
RE3	-3401 to -3256	CTCCCACATTGTTTAAGACTC GTAGGCAGTGTGACAGCAG

Table S3: Sequences of hydrolysis probes used for the quantification of ChIP products. The probes carry at their 5' a 6-FAM group and at their 3' a BHQ-1 group.

Probe target	Location	Probe sequence (5' - 3')
RE1/2	-389 to -369	TCGCCGCAGGGAGACCTCAC
RE3	-3376 to -3351	TCAAATGCCACCACCTCTCAGAAGT

Table S4: siRNA oligonucleotides. Location (relative to the TSS) and sequence of siRNA oligonucleotides used for gene-specific silencing.

Target gene	Location	Sequence of + oligonucleotide (5' - 3')
control	-	UGCGCUACGAUCGACGAUG
HDAC4	1117	CGAGCACAUCAAGCAACAA
	1521	CAGCUUCUGAACCGAAUCU
	3279	GCGUGAGCAAGAUCCUCAU
HDAC6	174	GUGUCACUUCGAAGCGAAA
	1771	CCGUGAGAGUUCCAACUUU
	2621	GGACCCUCCAGUUCUAAGU

SUPPLEMENTARY FIGURES

Fig. S1: Induction of *CYP24* transcription by 1α,25(OH)$_2$D$_3$ and Gemini in MCF-10A cells. Real-time quantitative PCR was performed in order to measure the time-dependent mRNA expression of the *CYP24* gene in MCF-10A cells after treatment with 10 nM 1α,25(OH)$_2$D$_3$ (A) or 10 nM Gemini (B). The data were normalized to the expression of the housekeeping gene *RPLP0* and fold inductions were calculated in reference to vehicle control. Data points indicate the means of at least three independent cell treatments and the bars represent standard deviations. A two-tailed Student's t-test was performed to determine the significance of the stimulation in reference to vehicle-treated control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Fig. S2: Induction of *IGFBP3* transcription by 1α,25(OH)$_2$D$_3$ and Gemini in RWPE-1 cells. Real-time quantitative PCR was performed in order to measure the time-dependent mRNA expression of the *IGFBP3* gene in MCF-10A cells after treatment with 10 nM 1α,25(OH)$_2$D$_3$ (A) or 10 nM Gemini (B). The data were normalized to the expression of the housekeeping gene *RPLP0* and fold inductions were calculated in reference to vehicle control. Data points indicate the means of at least three independent cell treatments and the bars represent standard deviations. A two-tailed Student's t-test was performed to determine the significance of the stimulation in reference to vehicle-treated control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Fig. S3: Basal expression of *HDAC* genes in MCF-10A cells. Real-time quantitative PCR was used to determine the basal expression of *IGFBP3* and the 11 *HDAC* genes in relation to the housekeeping gene *RPLP0* in non-treated MCF-10A cells. Data points represent the mean +/- standard deviation of at least three experiments.

Fig. S4: *In silico* screening for VDREs. Each 10 kB genomic DNA (thin black line) upstream of human genes *IGFBP3*, *HDAC4* and *HDAC6* were screened *in silico* for VDREs (red boxes) as described in (8). Repetitive sequence is indicated in blue. All VDRE are of DR3-type, their sequences are indicated below.

Fig. S5: No response of eight *HDAC* gene family members to 1α,25(OH)$_2$D$_3$. Real-time quantitative PCR was performed in order to measure the time-dependent mRNA expression of the genes *HDAC1* (A), *HDAC2* (B), *HDAC3* (C), *HDAC5* (D), *HDAC7* (E), *HDAC8* (F), *HDAC10* (G) and *HDAC11* (H) in MCF-10A cells after treatment with 10 nM 1α,25(OH)$_2$D$_3$. The data were normalized to the expression of the housekeeping gene *RPLP0* and fold inductions were calculated in reference to vehicle control. Data points indicate the means of at least three independent cell treatments and the bars represent standard deviations. A two-tailed Student's t-test was performed to determine any significance of the stimulation in reference to vehicle-treated control (* $p < 0.05$).

Fig. S6: No response of eight *HDAC* gene family members to Gemini. Real-time quantitative PCR was performed in order to measure the time-dependent mRNA expression of the genes *HDAC1* (A), *HDAC2* (B), *HDAC3* (C), *HDAC5* (D), *HDAC7* (E), *HDAC8* (F), *HDAC10* (G) and *HDAC11* (H) in MCF-10A cells after treatment with 10 nM Gemini. The data were normalized to the expression of the housekeeping gene *RPLP0* and fold inductions were calculated in reference to vehicle control. Data points indicate the means of at least three independent cell treatments and the bars represent standard deviations. A two-tailed Student's t-test was performed to determine any significance of the stimulation in reference to vehicle-treated control (* $p < 0.05$; ** $p < 0.01$).

Fig. S7: siRNA controls. MCF-10A cells were transfected for 24 h with 200 pmol of siRNAs against *HDAC4* and *HDAC6*. Real-time quantitative PCR was used to determine the reduction of the respective mRNA levels normalized by the control gene *RPLP0* (A). Bars represent the mean +/- standard deviation of at least three independent experiments. Western blotting in reference to β-actin expression was performed in order to prove the silencing effects also on the protein level (B). Representative gels are shown and their quantification is indicated below (standard deviations in brackets).

Fig. S8: Sonication efficiency. Sonicated reverse-crosslinked and purified chromatin used for ChIP experiments of this study was analyzed by agarose gel electrophoresis. A representative gel is shown. The experiments relate to ChIP assays with antibodies against VDR (1), AcH4 (2) and HDACs 4 and 6 (3) shown in Figs. 2 and 3.

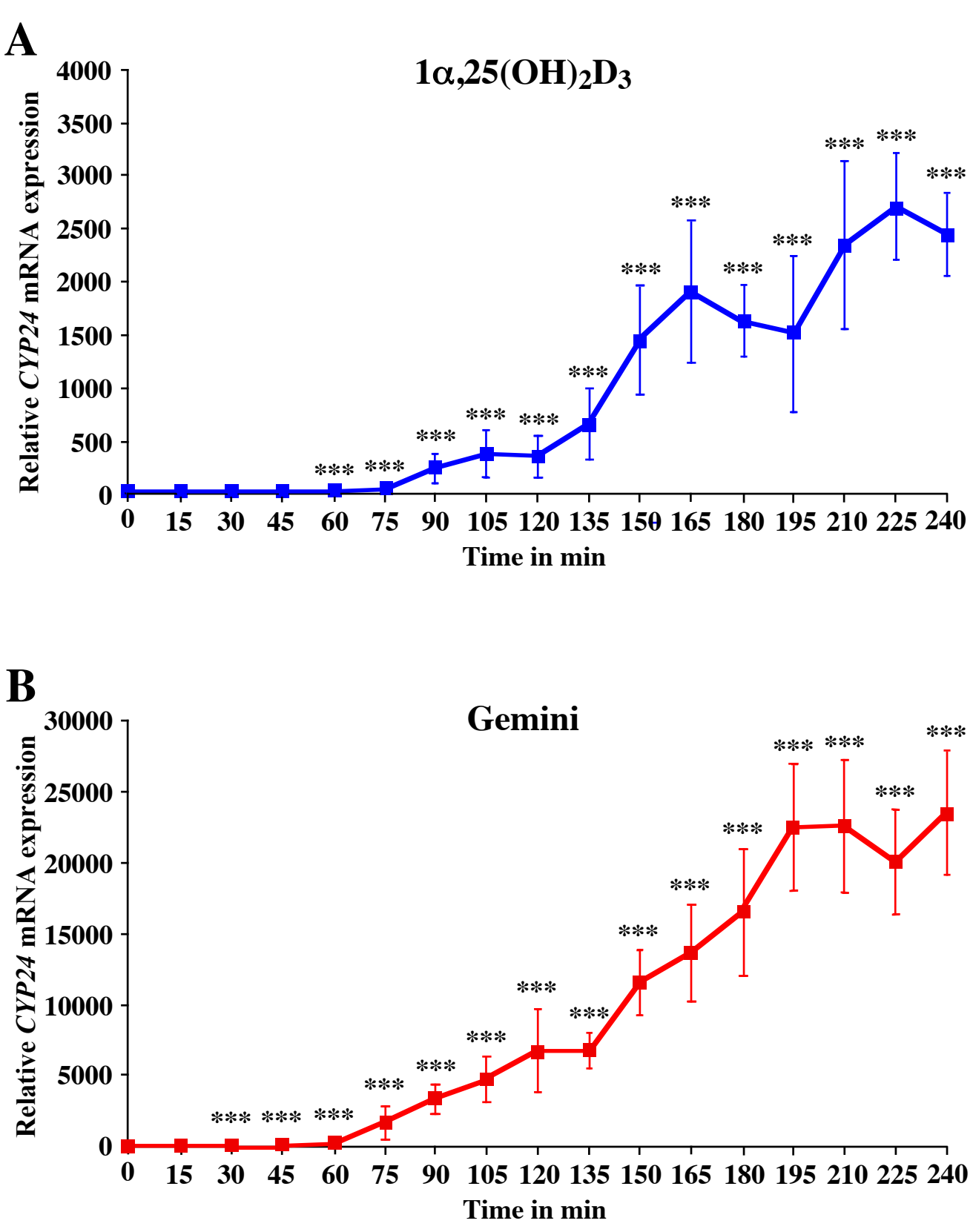

quantitative PCR
A
1α,25(OH)2D3
Relative CYP24 mRNA expression
4000
3500
3000
2500
2000
1500
1000
500
0
0 15 30 45 60 75 90 105 120 135 150 165 180 195 210 225 240
Time in min
B
Gemini
Relative CYP24 mRNA expression
30000
25000
20000
15000
10000
5000
0
0 15 30 45 60 75 90 105 120 135 150 165 180 195 210 225 240
Time in min

Fig. S1

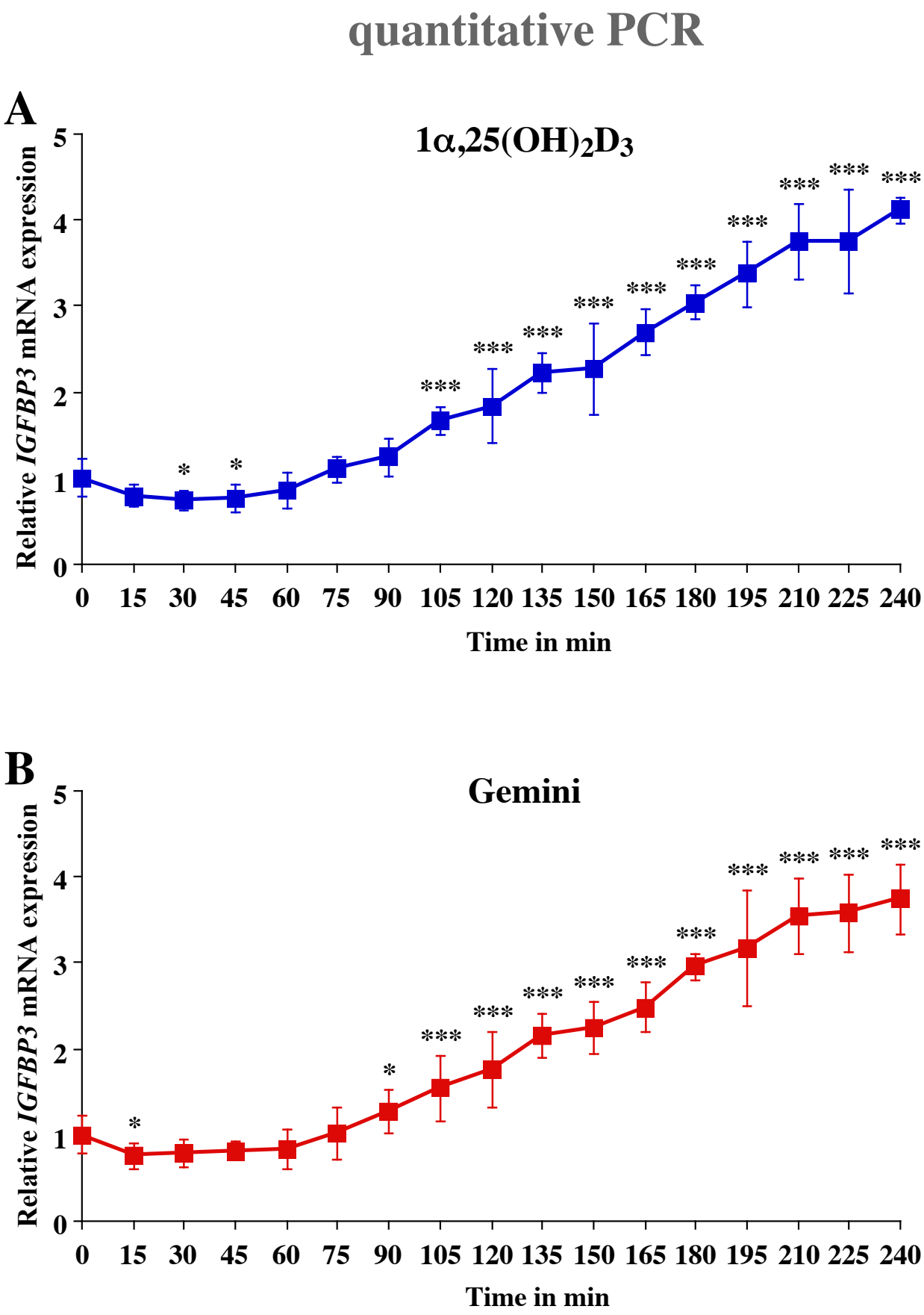

quantitative PCR
A
1α,25(OH)2D3
Relative IGFBP3 mRNA expression
Time in min
B
Gemini
Relative IGFBP3 mRNA expression
Time in min

Fig. S2

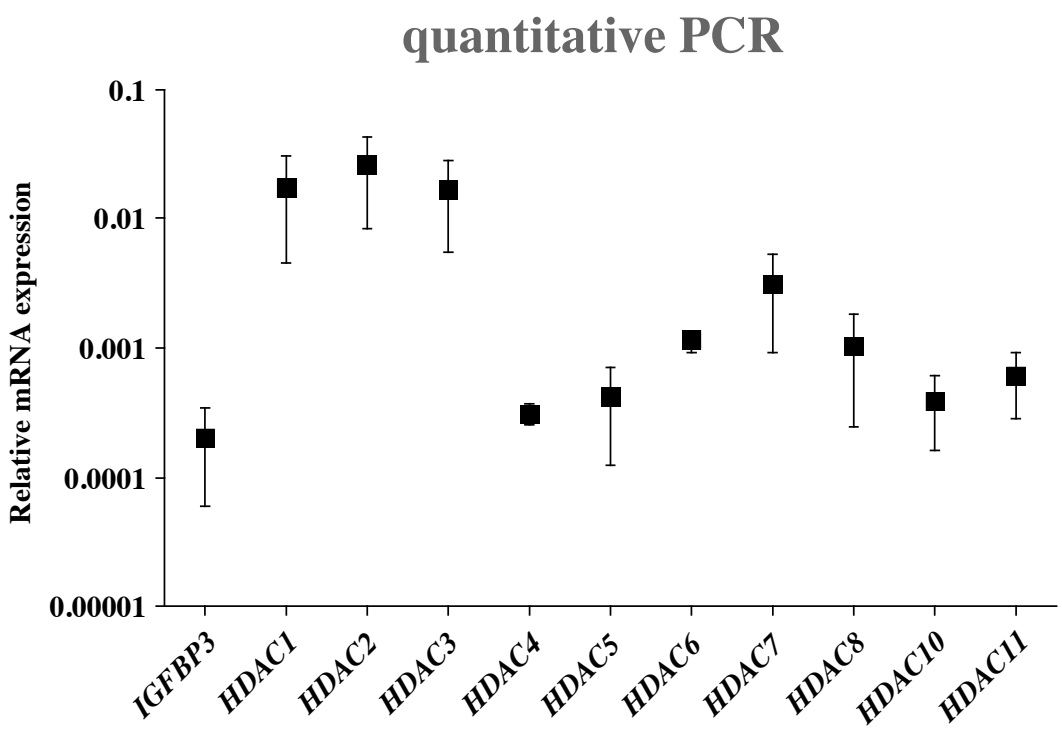
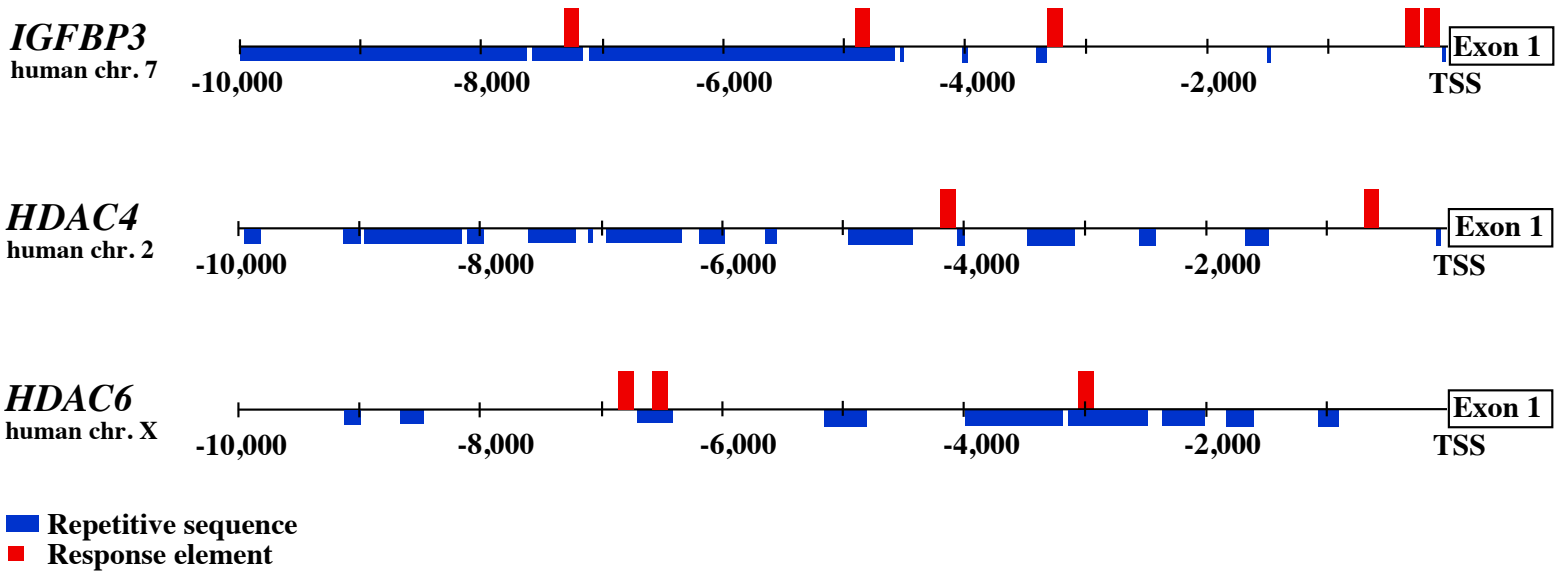

quantitative PCR
Relative mRNA expression
0.1
0.01
0.001
0.0001
0.00001
IGFBP3
HDAC1
HDAC2
HDAC3
HDAC4
HDAC5
HDAC6
HDAC7
HDAC8
HDAC10
HDAC11

Fig. S3

In silico screening



Gene	Chr	Start	End	Strand	Sequence
IGFBP3	chr 7	45927738	45927724	–	GGGTCAaggAGATCG
IGFBP3	chr 7	45927747	45927733	–	AGGGCGgcgGGGTCA
IGFBP3	chr 7	45930684	45930698	+	GGTTCAccgGGTGCA
IGFBP3	chr 7	45932200	45932186	–	AGGTCAagaAGTTTT
IGFBP3	chr 7	45934746	45934760	+	AGTTTAgaaAGTCCA
HDAC4	chr 2	239988326	239988340	+	CGGTCActgAGTGCG
HDAC4	chr 2	239991831	239991845	+	AGGACAtgaAGTTCA
HDAC6	chr X	48538627	48538613	–	AGTTCAcccAGGTGA
HDAC6	chr X	48538868	48538882	+	AGTTGCtttAGTTCA
HDAC6	chr X	48542429	48542415	–	AGGTCAggaATTTGA

Fig. S4

quantitative PCR

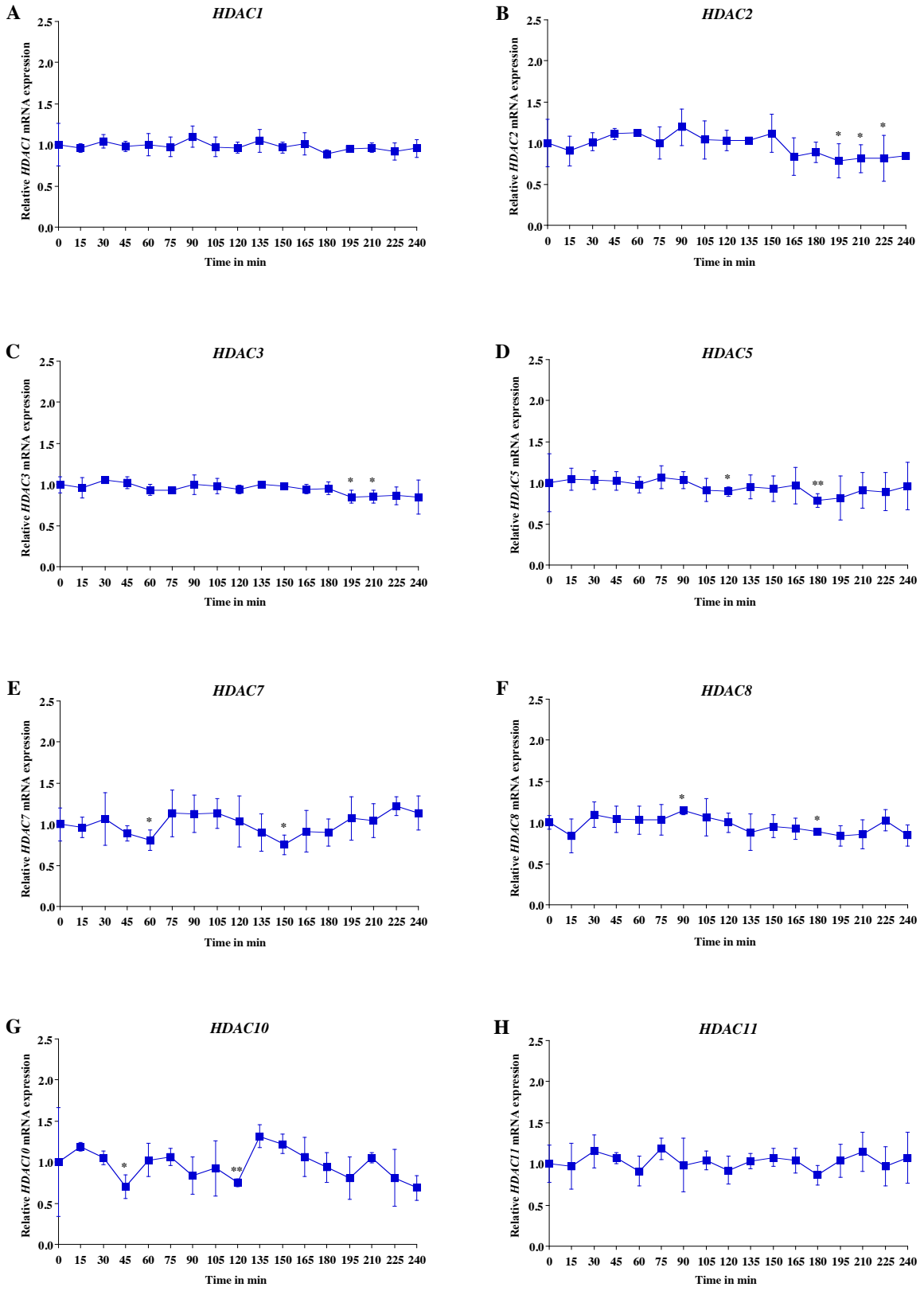


Fig. S5

quantitative PCR

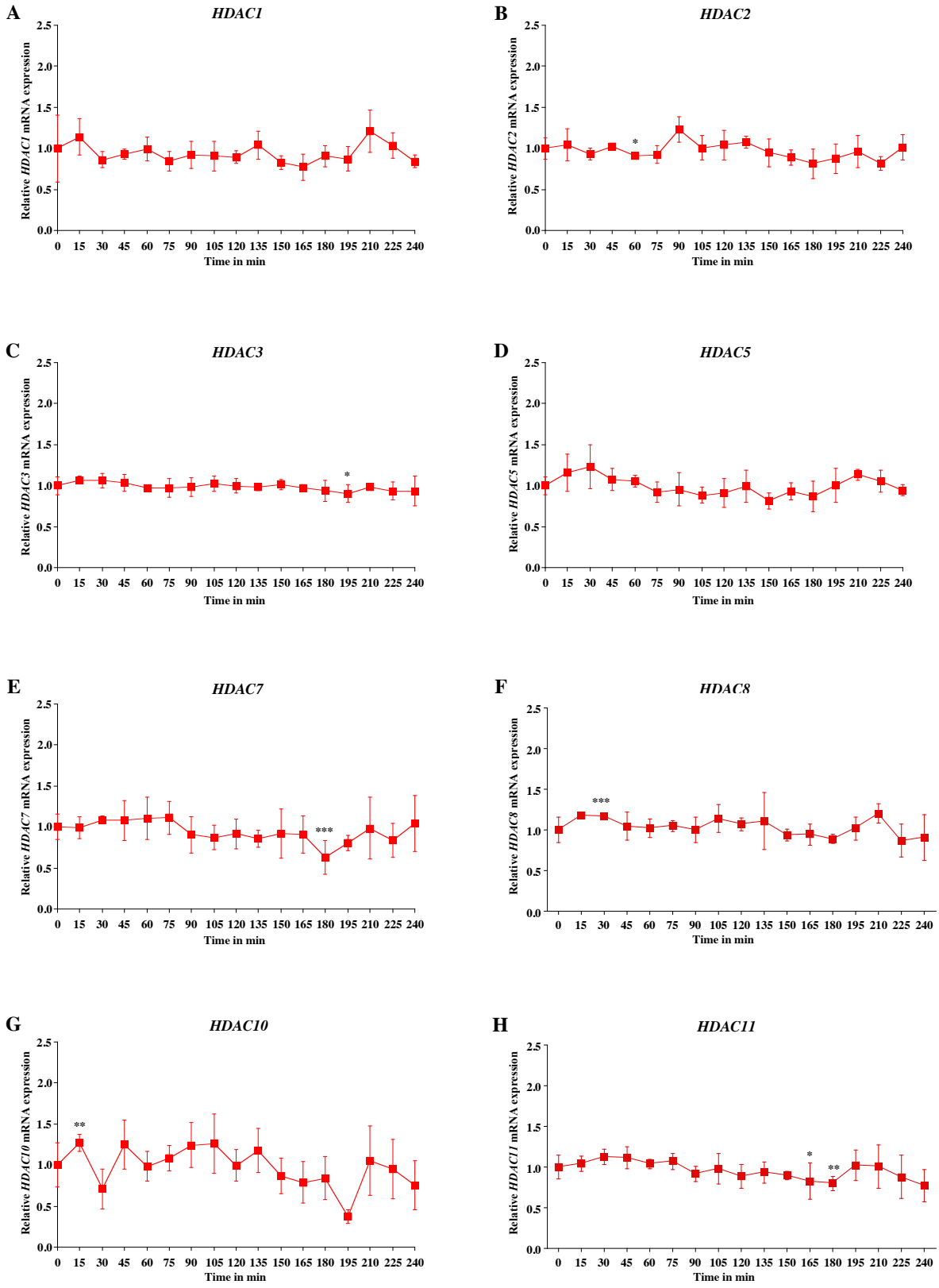


Fig. S6

quantitative PCR and Western blotting

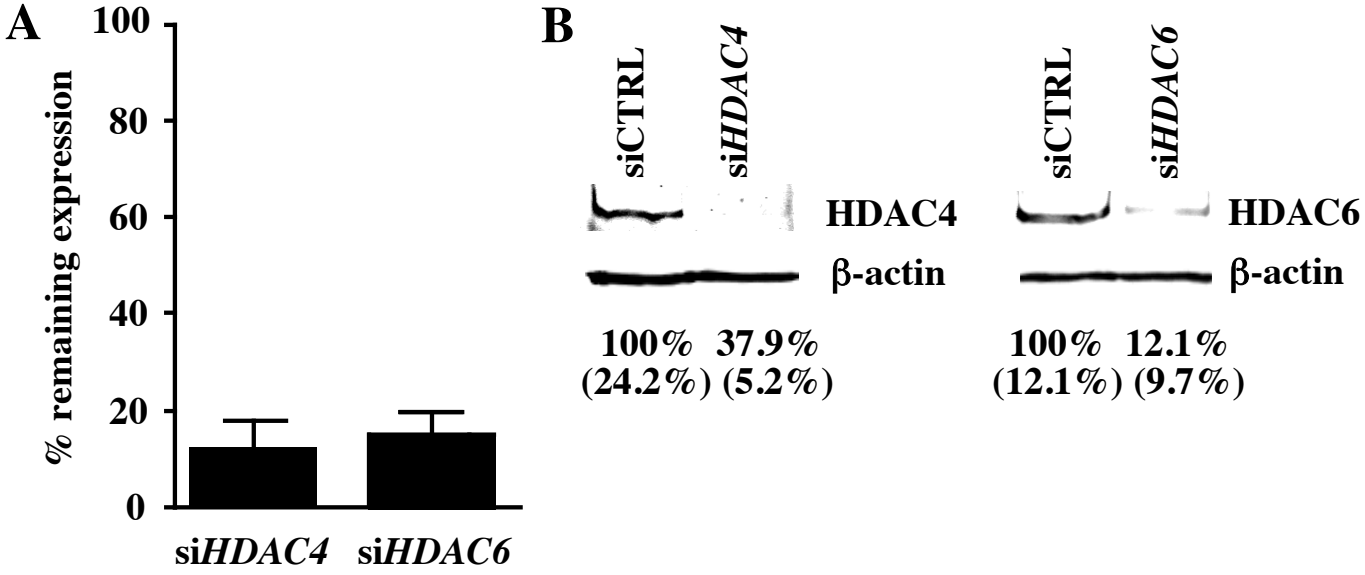


Fig. S7

Agarose gel electrophoresis

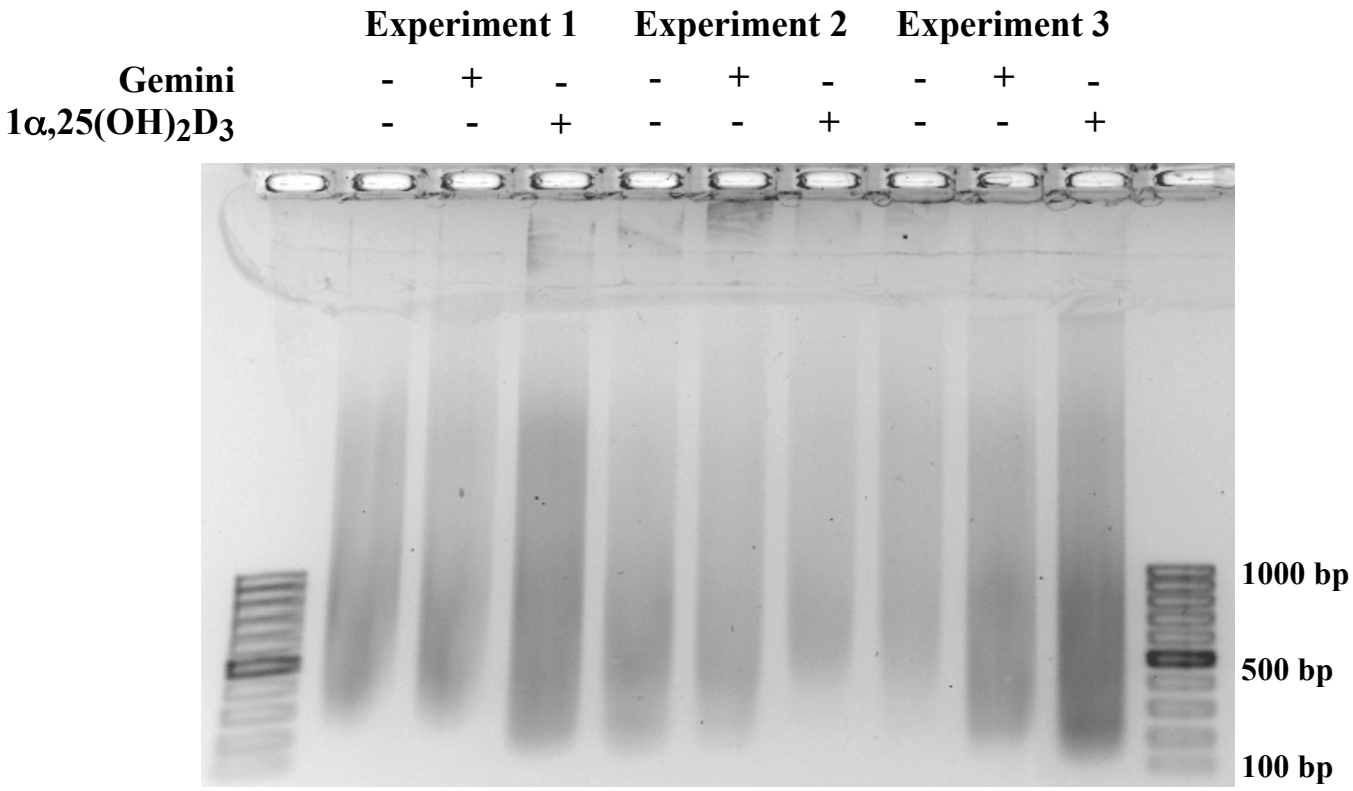


Fig. S8