Fig. S1

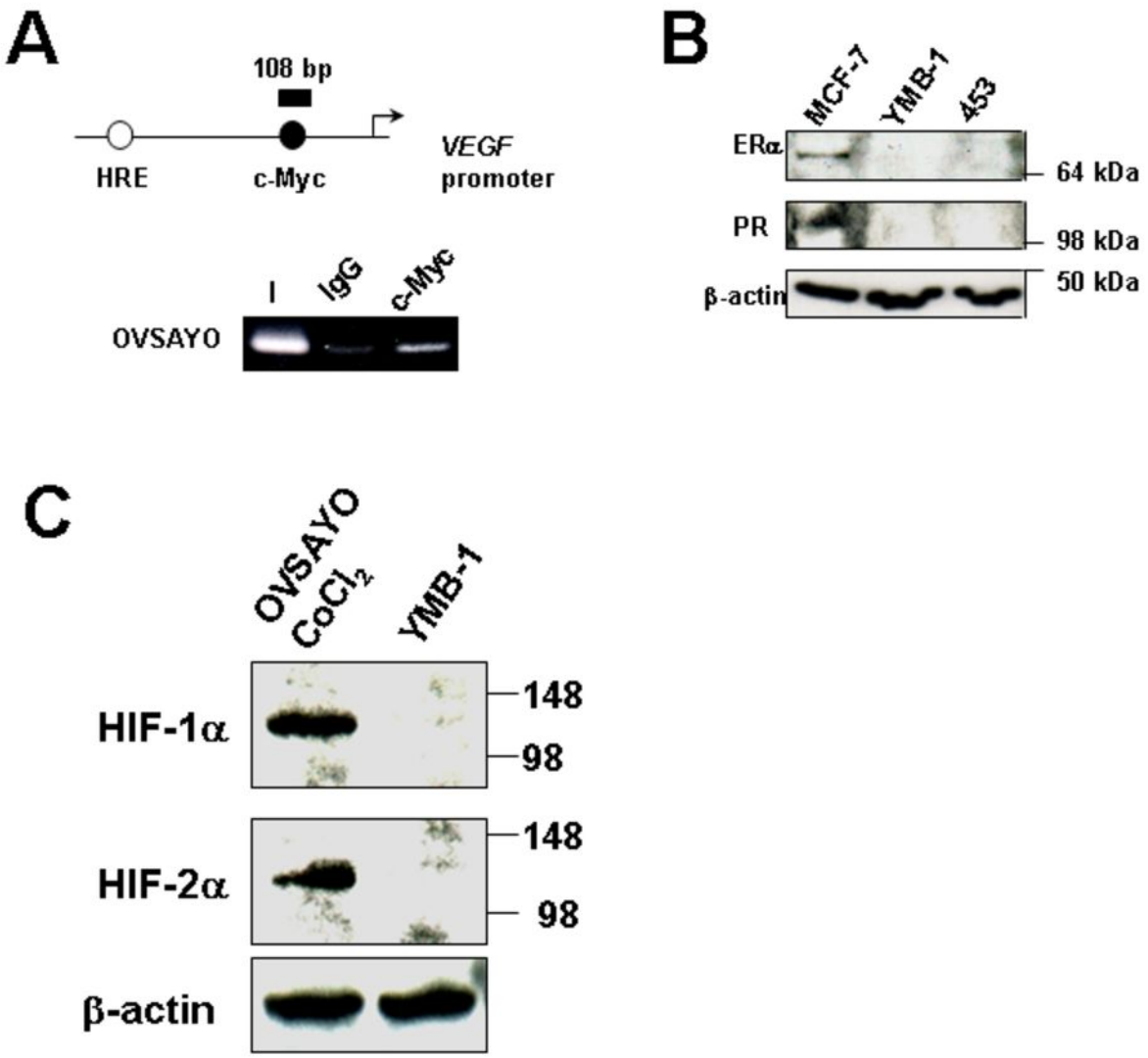

A
108 bp
HRE
c-Myc
VEGF promoter
I
IgG
c-Myc
OVSAYO
B
MCF-7
YMB-1
453
ERα
64 kDa
PR
98 kDa
β-actin
50 kDa
C
OVSAYO CoCl2
YMB-1
HIF-1α
148
98
HIF-2α
148
98
β-actin

Fig. S2

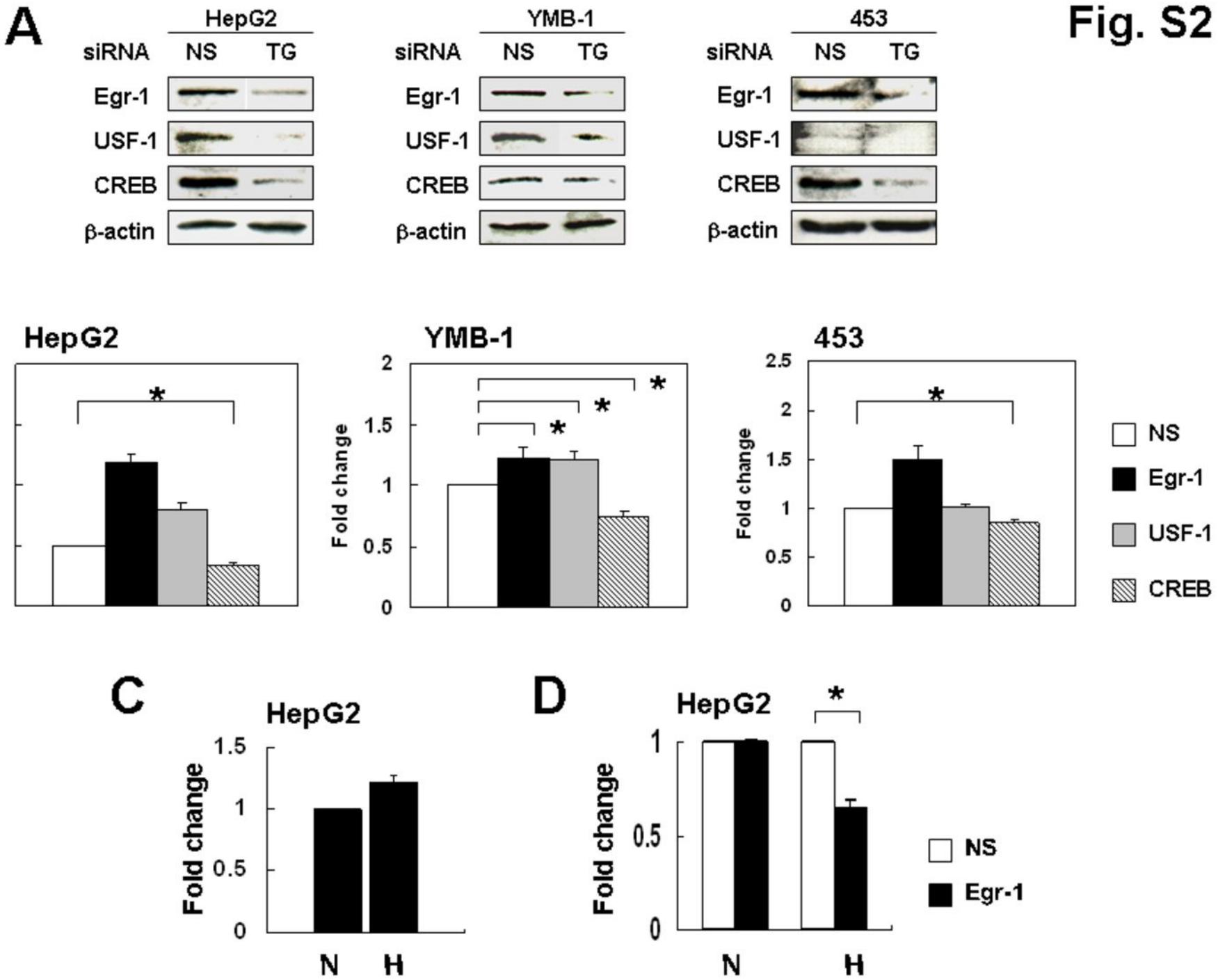
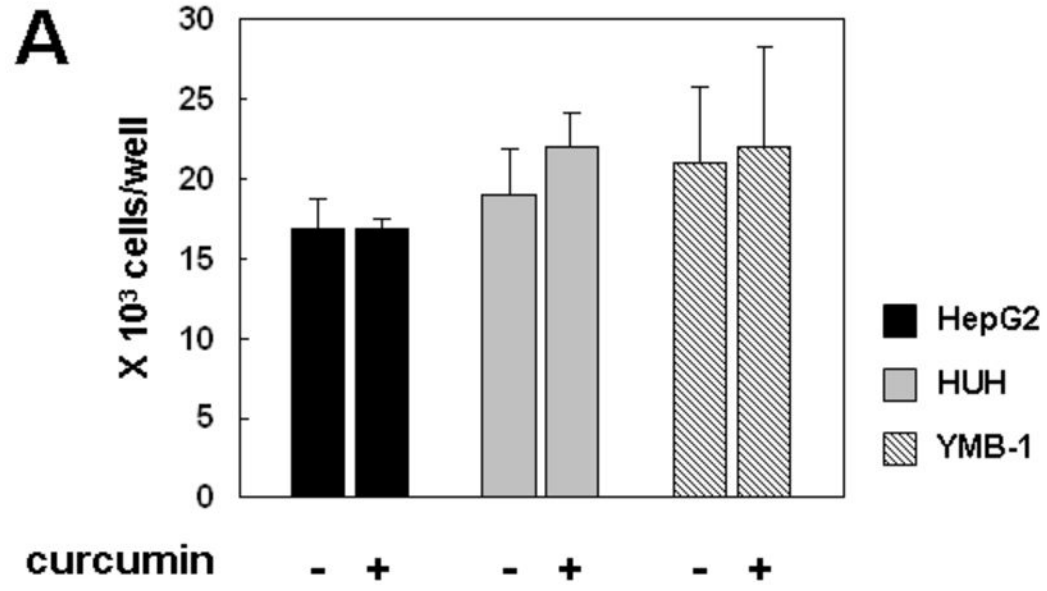

A
HepG2
YMB-1
453
siRNA
NS
TG
Egr-1
USF-1
CREB
β-actin
B
HepG2
YMB-1
453
Fold change
NS
Egr-1
USF-1
CREB
C
HepG2
Fold change
N
H
D
HepG2
Fold change
NS
Egr-1
N
H

Fig. S3


A
30
25
20
15
10
5
0
X 10³ cells/well
HepG2
HUH
YMB-1
curcumin
- + - + - +

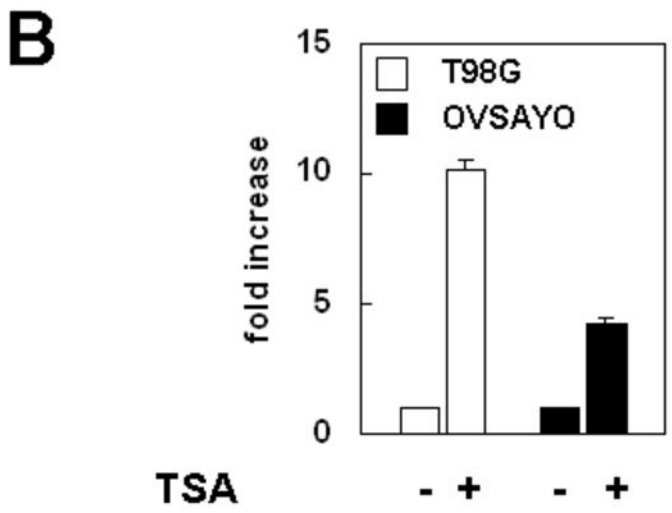

B
15
10
5
0
fold increase
T98G
OVSAYO
TSA
- + - +

Fig. S4

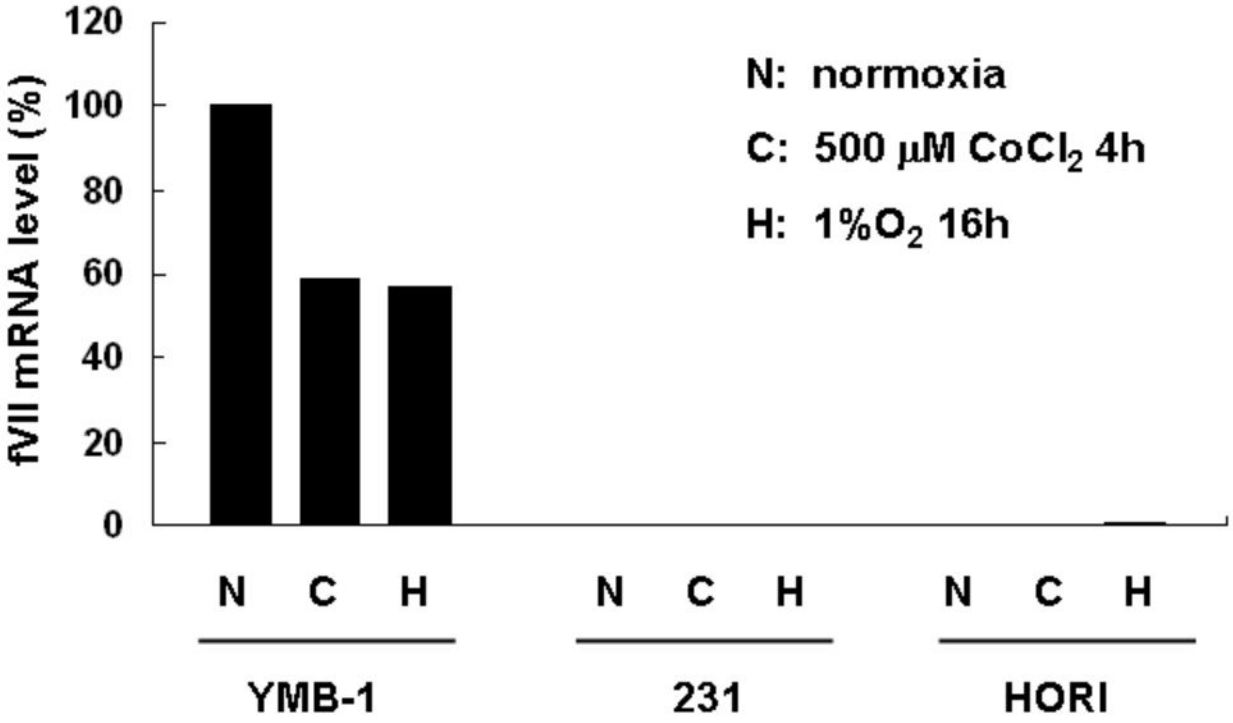
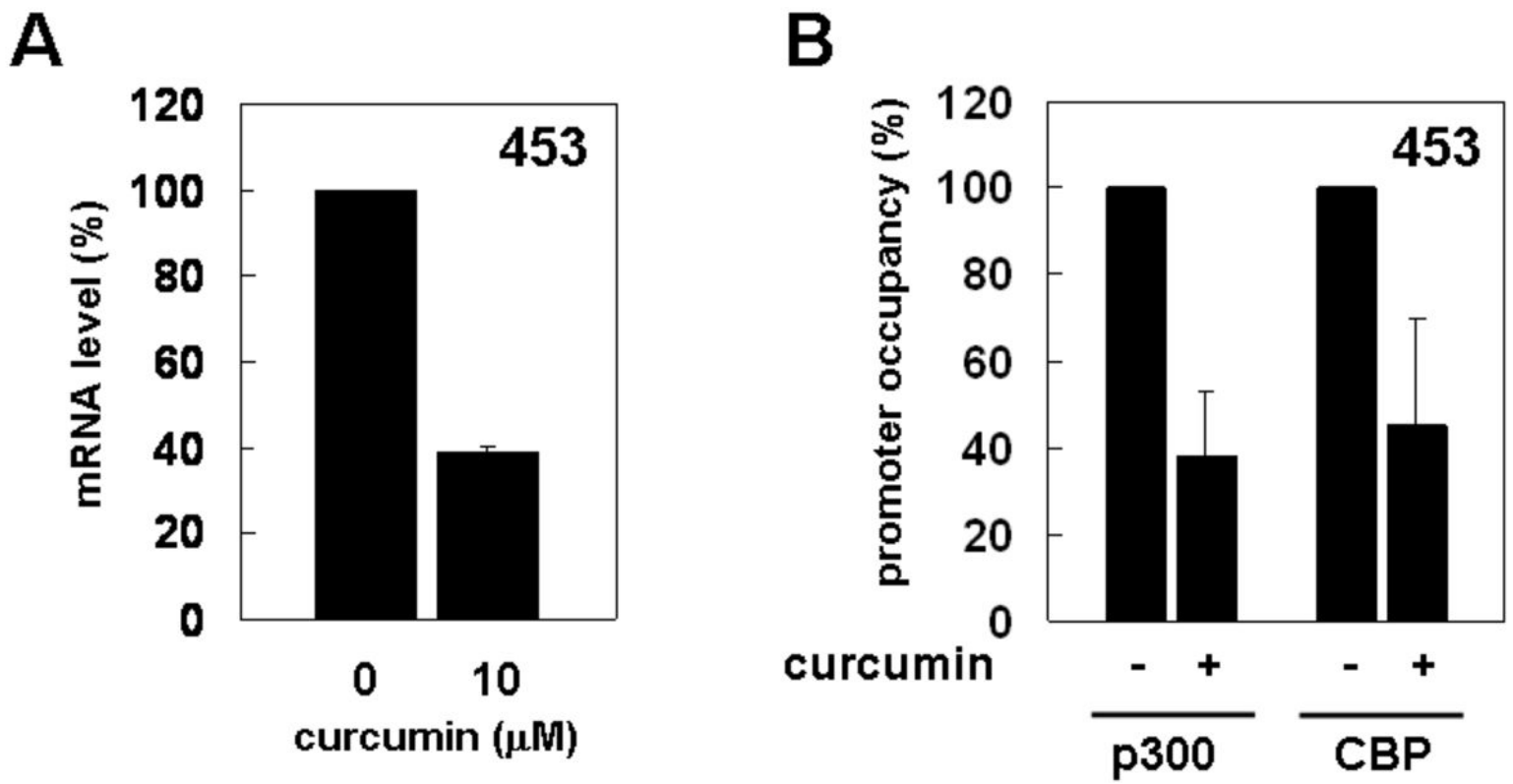

fVII mRNA level (%)
120
100
80
60
40
20
0
N: normoxia
C: 500 μM CoCl2 4h
H: 1%O2 16h
N C H
N C H
N C H
YMB-1
231
HORI

Fig. S5


A
120
100
80
60
40
20
0
mRNA level (%)
453
0
10
curcumin (μM)
B
promoter occupancy (%)
120
100
80
60
40
20
0
453
curcumin
-
+
-
+
p300
CBP

Legends for supplemental figures

Fig. S1. (A) ChIP analysis of c-Myc binding to the *VEGF* promoter region downstream of a hypoxia response element (HRE) in OVSAYO cells. A bent arrow is indicative of a transcription start site. (B) Western blotting analysis of ERα and PR expressions in breast cancer cells. MCF-7 cells were used as a positive control of ERα and PR expressions. (C) Western blot analysis of hypoxia inducible factors (HIFs) in YMB-1 cells under normoxia. HIFs expression in YMB-1 cells under normoxia was analyzed by immunoblotting. Lysate of OVSAYO cells treated with 500 μM $CoCl_2$ for 4 hours was also tested for positive control of HIFs expression. β-actin was examined as the protein-loading control.

Fig. S2. Effect of Egr-1, USF-1, or CREB on basal hepatocytic and ectopic *FVII* expression. (A) Knockdown of expression level of transcription factors by RNAi. Cancer cells (HepG2, YMB-1, and 453 cells) were transfected with siRNA for each transcription factor, then protein levels were analyzed by western blotting. (B) qPCR analysis of fVII mRNA expressions in cancer cells post-siRNA treatment. Fold change relative to non-specific siRNA-treated cells is shown for *FVII* expression. NS and TG

are indicative of nonspecific and targeted siRNAs, respectively. *Columns*, mean. *Bars*, SD (n = 3). *$P < 0.05$. We used the Student's *t*-test for statistical analysis. The siRNAs we used are described in "Supplemental materials".

After knockdown of each protein by siRNA transfection, fVII mRNA levels were compared with that from nonspecific siRNA-transfected cells by RT-PCR analysis. Silencing of Egr-1 or USF-1 expression enhanced fVII transcription in HepG2 and YMB-1 cells, although the fVII mRNA level was unchanged by knockdown of USF-1 expression in 453 cells. Silencing of CREB reduced *FVII* expression in all cell types.

We further tested the effect of Egr-1 on TF transcription by siRNA transfection (C, D) because Egr-1 is known to activate TF expression during hypoxia. (Rong, Y. et al. *Cancer Res.*, 66, 7067-74, 2006) HepG2 cells were cultured under normoxia or hypoxia (1% O_2) conditions for 24 h. TF mRNA level was determined by qRT-PCR. Hypoxia (1% O_2) slightly increased TF mRNA level (1.2-fold) in HepG2 cells (C) while VEGF transcription was enhanced approximately 5-fold compared to normoxia (data not shown). (D) HepG2 cells were transfected with siRNA for Egr-1 then cultured for 24 h. Cells were further cultured under normoxia or 1% O_2 conditions for 24 h. TF mRNA level was analyzed by qRT-PCR. Fold change relative to NS-siRNA–treated cells is shown for TF expression. N and H are indicative of normoxia and hypoxia, respectively.

Columns, mean. *Bars*, SD (n = 3). *$P < 0.05$. We used the Student's t-test for statistical analysis. As expected, knockdown of Egr-1 expression downregulated TF transcription under severe hypoxia (1% O_2) while leaving it unaffected under normoxia in HepG2 cells, suggesting that Egr-1 plays different roles in the transcription of the fVII and TF genes.

Fig. S3. (A) Cancer cells were seeded and cultured for 14 hours, then further cultured for 24 hours with or without 10 μM curcumin. Cell proliferation (viability) was measured using CellTiter96 AqueousOne Solution Cell Proliferation Assay (Promega). *Columns*, mean (n = 3); *Bars*, SD. (B) Effect of TSA on expression level of fVII in T98G and OVSAYO cells. mRNA level was analyzed by real-time PCR. fVII mRNA expression was normalized to the 18S ribosomal RNA level. *Columns*, mean. *Bars*, SD (n = 2).

Fig. S4. Detection of fVII mRNA from breast cancer cells cultured under hypoxia or hypoxia mimic condition. Total RNA extracted from fVII-high expressing (YMB-1) and very low expressing (231 and HORI) cells were analyzed by qRT-PCR analysis. Data were normalized as previously described.

Fig. S5. (A) Effect of curcumin on fVII mRNA expression level in 453 cells. Cells were cultured with or without curcumin for 24 h, then fVII mRNA level was analyzed by real-time PCR. mRNA expression was normalized to the 18S ribosomal RNA level. *Columns*, mean; *Bars*, SD (n = 3). (B) Effect of curcumin on *FVII* promoter occupancy by curcumin in 453 cells. Quantitative ChIP analysis was performed as previously described. Relative level of occupancy is shown as percentages of the curcumin (-) experiment. *Columns*, mean; *Bars*, SD (n = 3).

Supplemental materials

(I) Primers and probes used for TF expression analysis by real-time RT-PCR

Primers 5’-TAACCGGAAGAGTACAGACAGC-3’ and CACTCCTGCCTTTCTACACTTG-3’ and hybridization probes 5’-ATCATTGGAGCTGTGGTATTTGTGG-FITC-3’ and 5’-LCRed640-CATCATCCTTGTCATCATCCTGGC-3’.

(II) Primers and probes used for ChIP analysis

PCR primers used for real-time PCR analysis of *FVII* promoter region were 5’-CACTTGAGCTCAGTGGCTGG-3’ and 5’-ACATTCCCCATGGGACTGAC-3’. The hybridization probes used were 5’-GCCAGGTGCAGCTCTCAGCTG-FITC-3’ and 5’-LCRed640-GTGTTCAGAGGACGCCTGTGTCC-3’. PCR primers used for detection of c-Myc binding site within *VEGF* locus were 5’-GTCCGCACGTAACCTCACTTTC-3’ and 5’-GGTTCACAGCCTGAAAATTACC-3’.

(III) Small interfering RNAs

siRNAs for Egr-1 and USF-1 were synthesized by Qiagen and their sequences were

Egr-1: 5’-AGGCAUACCAAGAUCCACUUGdTdT-3’ and 5’-CAAGUGGAUCUUGGUAUGCCUdTdT-3’, USF: 5’-GACCCAACCAGUGUGGCUAdTdT-3’ and 5’-UAGCCACACUGGUUGGGUCdTdT-3’. siRNA for CREB was ON-TARGET plus SMART pool, CREB1 (Dharmacon, Lafayette, CA). Silencer Negative Control 1 RNAi (Ambion) was used for non-specific siRNA transfection.

(IV) Primary antibodies used for western blotting

HNF-4 (H-171, Santa Cruz Biotechnology, Santa Cruz, CA, USA), Sp1 (PEP2, Santa Cruz), Egr-1 (588, Santa Cruz), USF1 (H-86, Santa Cruz), c-Myc (N-262, Santa Cruz), CREB (Upstate Biotechnology, Lake Placid, NY, USA), ERα (H-184, Santa Cruz), PR (H-190, Santa Cruz), p300 (C-20, Santa Cruz), CBP (A-22, Santa Cruz), PCAF (H-369, Santa Cruz), SRC-1 (M-341, Santa Cruz), and fVII (Ref. 13). β-actin (AC-15, Sigma, St. Louis, MO, USA) was the antibody used for the protein-loading control.