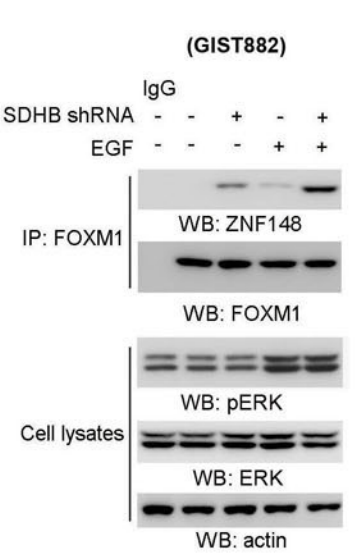
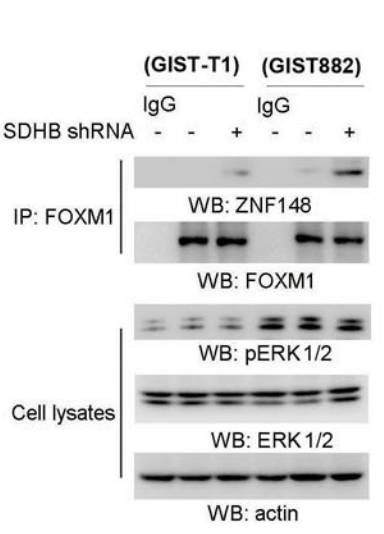
S1

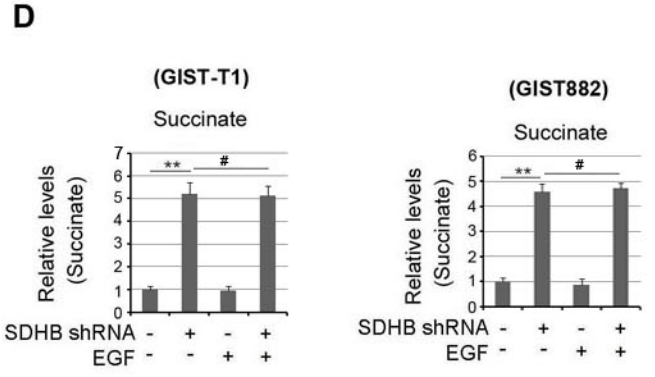
A

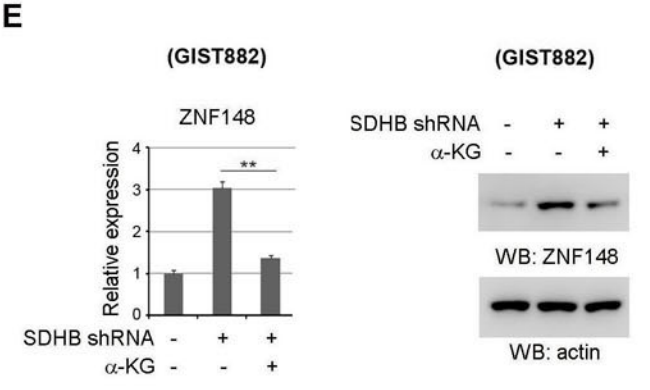
(GIST-T1)

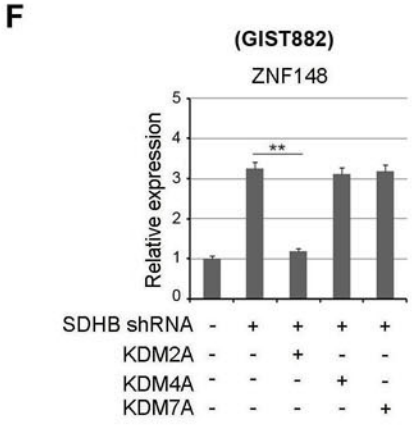
Peptides Hits		Protein Name
EGF(-)	(+)	
7	28	ZNF148
16	19	PRMT 5
6	8	MSH2
10	4	TRIM21
4	3	ACAT 1

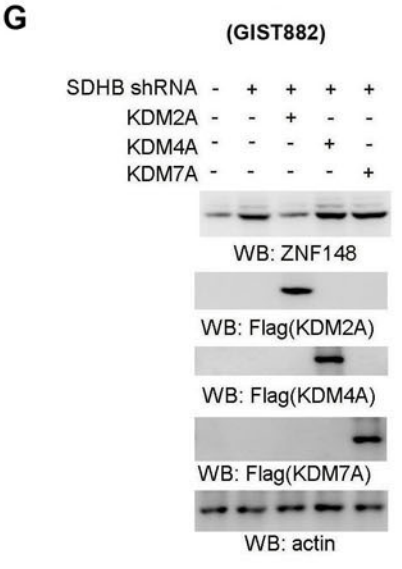


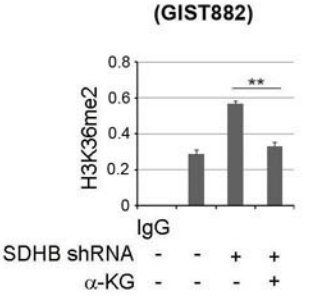


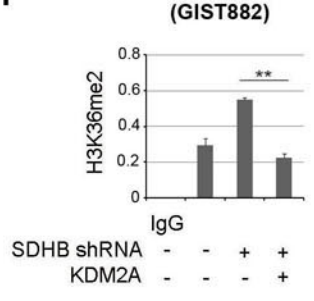


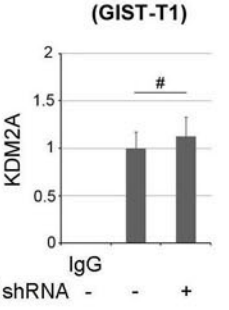












S1

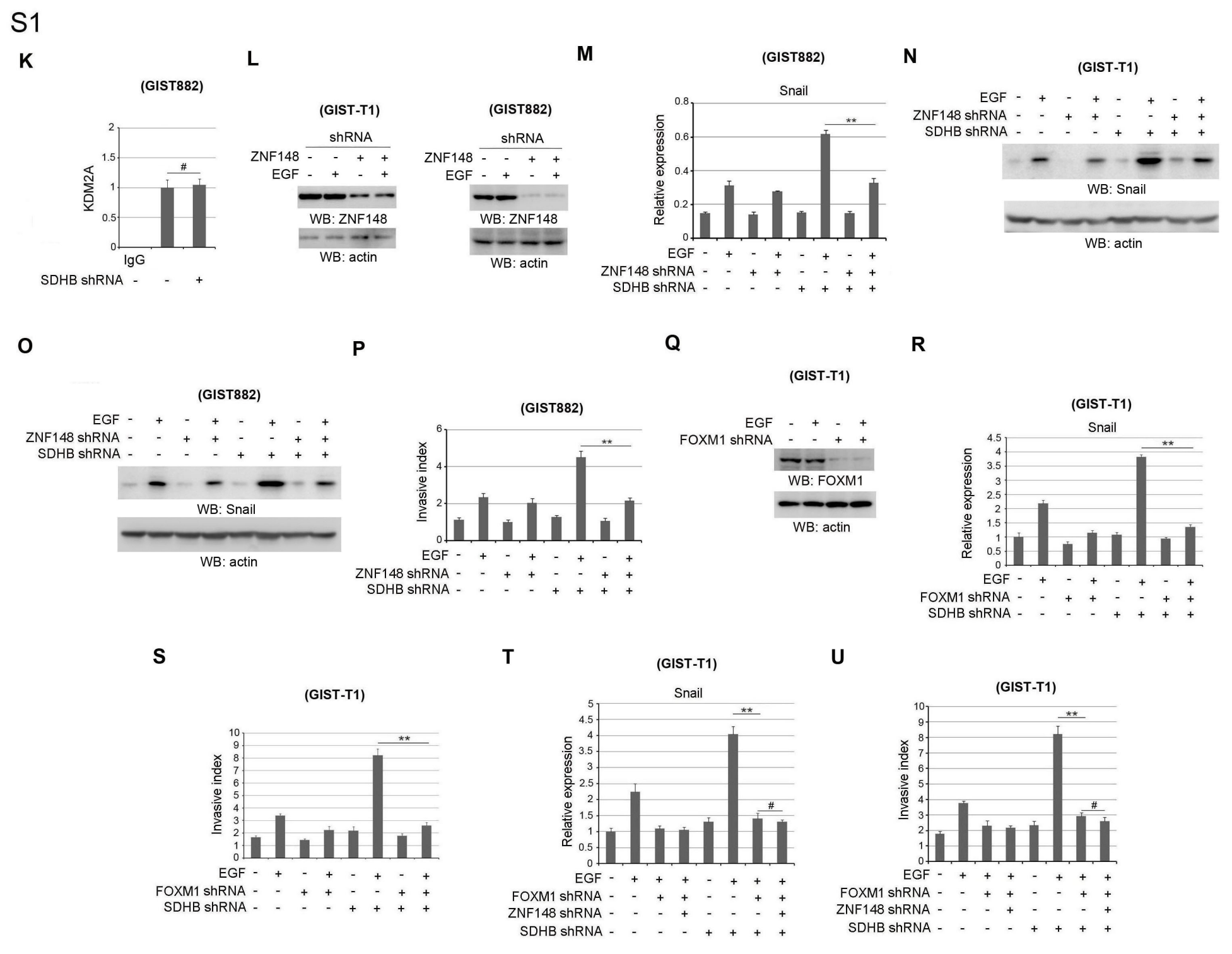

K
(GIST882)
KDM2A
IgG
SDHB shRNA
L
(GIST-T1)
(GIST882)
shRNA
ZNF148
EGF
WB: ZNF148
WB: actin
M
(GIST882)
Snail
Relative expression
EGF
ZNF148 shRNA
SDHB shRNA
N
(GIST-T1)
WB: Snail
WB: actin
O
(GIST882)
WB: Snail
WB: actin
P
(GIST882)
Invasive index
Q
(GIST-T1)
FOXM1 shRNA
WB: FOXM1
WB: actin
R
(GIST-T1)
Snail
Relative expression
S
(GIST-T1)
Invasive index
T
(GIST-T1)
Snail
Relative expression
U
(GIST-T1)
Invasive index

Figure S1. ZNF148 is upregulated by loss of SDH and interacts with FOXM1. **(A)** GIST-T1 cells transfected with or without plasmid for expressing SDHB shRNA were treated with or without EGF (100 ng/ml) for 1 h. Cellular extracts subjected to immunoprecipitation with an anti-FOXM1 antibody were analyzed by mass spectrometry. FOXM1-interacting proteins identified by mass spectrometry were shown. **(B)** GIST882 cells transfected with or without plasmid for expressing SDHB shRNA were treated with or without EGF (100 ng/ml) for 1 h. Cellular extracts subjected to immunoprecipitation with an anti-FOXM1 antibody. **(C)** GIST-T1 or GIST882 (right panel) cells were transfected with or without plasmid for expressing SDHB shRNA. Cellular extracts subjected to immunoprecipitation with an anti-FOXM1 antibody. **(D)** GIST-T1 or GIST882 (right panel) cells transfected with or without plasmid for expressing SDHB shRNA were treated with or without EGF (100 ng/ml) for 1 h. The abundance of intracellular Succinate was measured. **(E)** GIST882 cells transfected with or without plasmid for expressing SDHB shRNA were added with exogenous α-KG (20μM). The relative mRNA level of ZNF148 was analyzed by Q-PCR (left panel). Cellular extracts subjected to immunoblotting analyses (right panel). **(F)** GIST882 cells with or without SDHB shRNA expression were overexpressed with the KDM2A, KDM4A or KDM7A. The relative mRNA level of ZNF148 was analyzed by Q-PCR. **(G)** GIST882 cells with or without SDHB shRNA expression were overexpressed with the KDM2A, KDM4A or KDM7A. Cellular extracts subjected to immunoblotting analyses. **(H)** GIST882 cells with or without SDHB shRNA expression were added with exogenous α-KG (20μM). ChIP analyses with an anti-H3K36me2 antibody were performed. The primers covering ZNF148 gene promoter region were used for the Q-PCR. **(I)** GIST882 cells with or without SDHB shRNA expression were overexpressed with KDM2A. ChIP analyses with an anti-H3K36me2 antibody were performed. The primers covering ZNF148 gene promoter region were used for the Q-PCR. **(J)** GIST-T1 cells transfected with or without plasmid for expressing SDHB shRNA. ChIP analyses with an anti-KDM2A antibody were performed. **(K)** GIST882 cells transfected with or without plasmid for expressing SDHB shRNA. ChIP analyses with an anti-KDM2A antibody were

performed. **(L)** GIST-T1 or GIST882 cells transfected with or without the plasmid for expressing ZNF148 shRNA or/and SDHB shRNA were treated with or without EGF (100 ng/ml) for 12 h. Cellular extracts subjected to immunoblotting analyses. **(M)** GIST882 cells transfected with or without ZNF148 shRNA or/and SDHB shRNA were treated with or without EGF (100 ng/ml) for 12 h. The relative mRNA level of Snail was analyzed by Q-PCR. **(N,O)** GIST-T1 (N) or GIST882 (O) cells transfected with or without ZNF148 shRNA or/and SDHB shRNA were treated with or without EGF (100 ng/ml) for 12 h. Cellular extracts subjected to immunoblotting analyses. **(P)** GIST882 cells transfected with or without ZNF148 shRNA or/and SDHB shRNA were treated with or without EGF (100 ng/ml) for 12 h. Cell invasion assays were performed. **(Q)** GIST-T1 cells transfected with or without FOXM1 shRNA were treated with or without EGF (100 ng/ml) for 12 h. Cellular extracts subjected to immunoblotting analyses. **(R,S)** GIST-T1 cells transfected with or without FOXM1 shRNA or/and SDHB shRNA were treated with or without EGF (100 ng/ml) for 12 h. Relative mRNA levels were analyzed by Q-PCR (R). Cell invasion assays were performed (S). **(T,U)** GIST-T1 cells transfected with or without SDHB shRNA, FOXM1 shRNA or/and ZNF148 shRNA were treated with or without EGF (100 ng/ml) for 12 h. Relative mRNA levels were analyzed by Q-PCR (T). Cell invasion assays were performed (U). In B, C, E, G, L, N, O and Q, immunoblotting analyses were performed using the indicated antibodies and data represent 1 out of 3 experiments. In D-F, H-K, M, P, and R-U, the values are presented as mean ± s.e.m. (n=3 independent experiments), ** represents $P<0.01$ between the indicated groups, # represents $P>0.05$ between the indicated groups.

S2

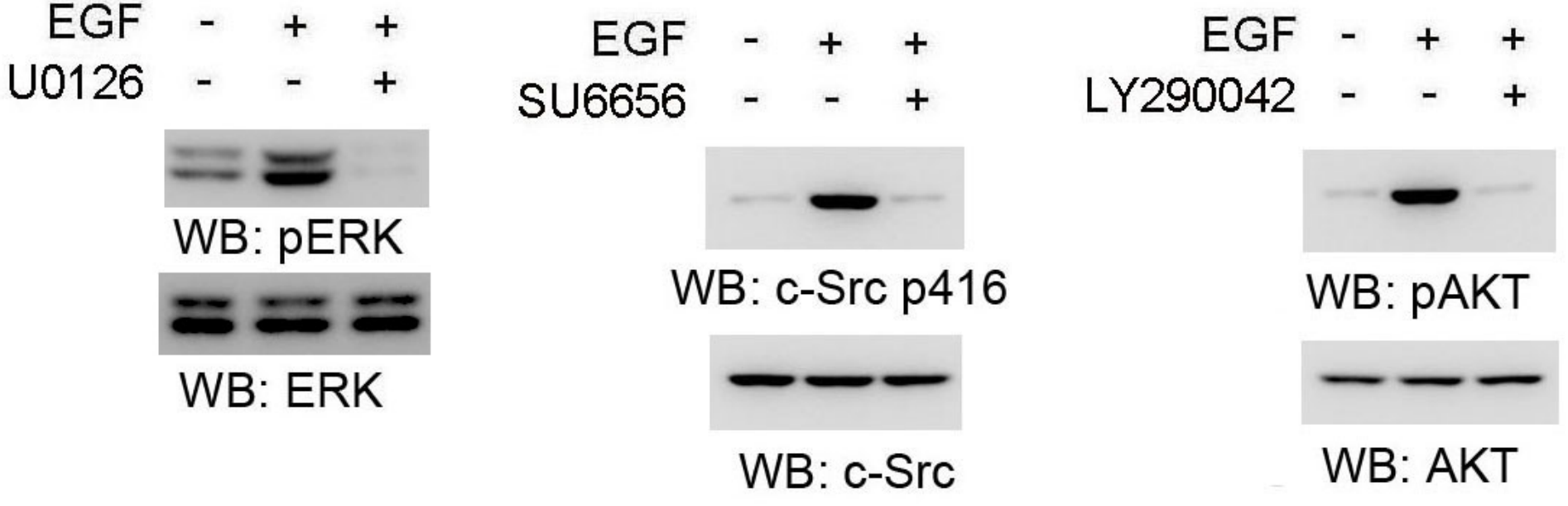

EGF - + +
U0126 - - +
WB: pERK
WB: ERK
EGF - + +
SU6656 - - +
WB: c-Src p416
WB: c-Src
EGF - + +
LY290042 - - +
WB: pAKT
WB: AKT

Figure S2. ERK activation is required for ZNF148-FOXM1 interaction. GIST-T1 cells with SDHB depletion were pretreated with U0126 (20μM) (left panel), SU6656 (10μM) (middle panel) or LY290042 (20μM) (right panel) for 1 h, prior to EGF treatment (100 ng/ml) for 1 h. Cellular extracts subjected to immunoblotting analyses.

S3

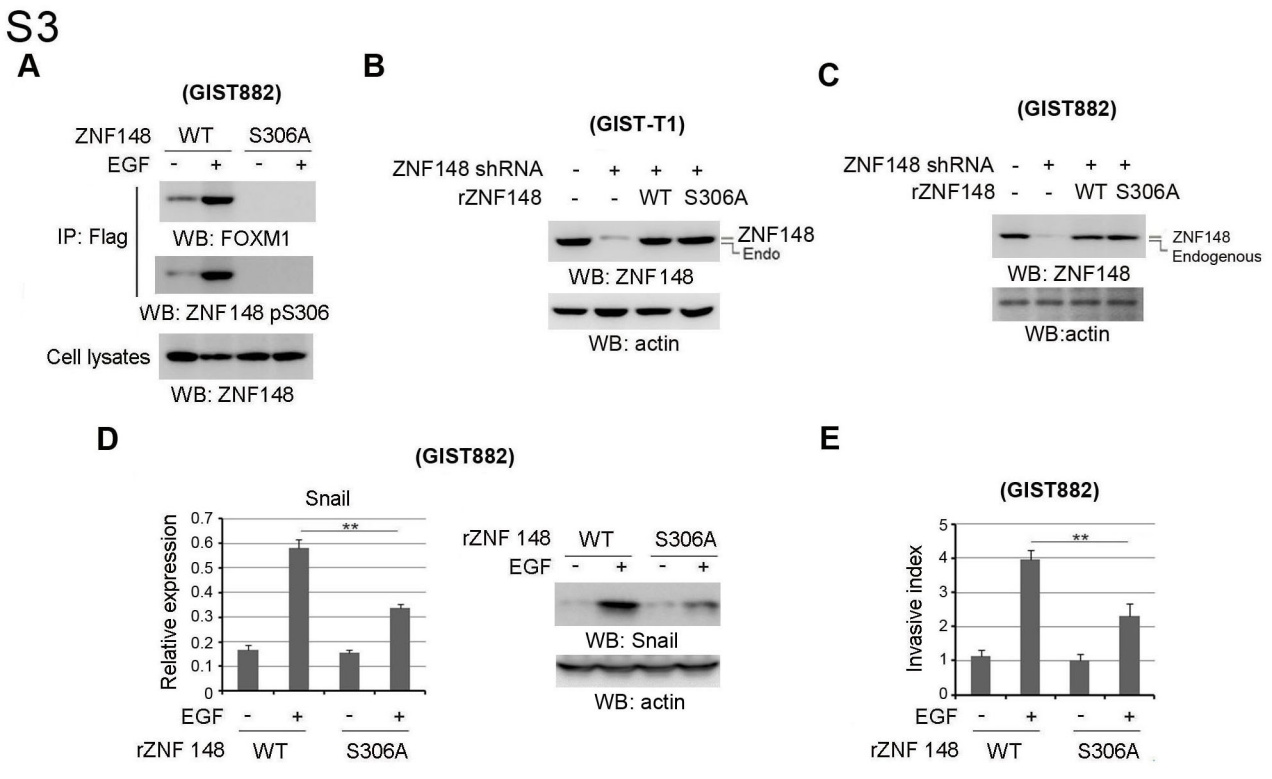

A
(GIST882)
ZNF148 WT S306A
EGF - + - +
IP: Flag
WB: FOXM1
WB: ZNF148 pS306
Cell lysates
WB: ZNF148
B
(GIST-T1)
ZNF148 shRNA - + + +
rZNF148 - - WT S306A
ZNF148
Endo
WB: ZNF148
WB: actin
C
(GIST882)
ZNF148 shRNA - + + +
rZNF148 - - WT S306A
ZNF148
Endogenous
WB: ZNF148
WB:actin
D
(GIST882)
Snail
Relative expression
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
EGF - + - +
rZNF 148 WT S306A
rZNF 148 WT S306A
EGF - + - +
WB: Snail
WB: actin
E
(GIST882)
Invasive index
5
4
3
2
1
0
**
EGF - + - +
rZNF 148 WT S306A

Figure S3. ERK phosphorylates ZNF148 at Ser306 (A) GIST882 cells expressed with Flag-tagged WT ZNF148 or ZNF148 S306A were treated with or without EGF for 1 h. Cellular extracts subjected to immunoprecipitation with an anti-Flag antibody. **(B)** GIST-T1 cells with SDHB depletion and ZNF148 depletion were reconstituted with expression of WT rZNF148 or r ZNF148 S306A. Cellular extracts subjected to immunoblotting analyses. **(C)** GIST882 cells with SDHB depletion and ZNF148 depletion were reconstituted with expression of WT rZNF148 or r ZNF148 S306A. Cellular extracts subjected to immunoblotting analyses. **(D)** GIST882 cells with SDHB depletion, ZNF148 depletion and reconstituted expression of WT ZNF148 or ZNF148 S306A were treated with or without EGF (100 ng/ml) for 1 h. The relative mRNA level of Snail was analyzed by Q-PCR (left panel). The protein level of Snail was analyzed by immunoblotting analysis (right panel). **(E)** GIST882 cells with SDHB depletion, ZNF148 depletion and reconstituted expression of WT ZNF148 or ZNF148 S306A were treated with or without EGF (100 ng/ml) for 1 h. Cell invasion assays were performed. In A-D, immunoblotting analyses were performed using the indicated antibodies. In D and E, the values are presented as mean ± s.e.m. (n=3 independent experiments), ** represents $P<0.01$ between the indicated groups.

S4

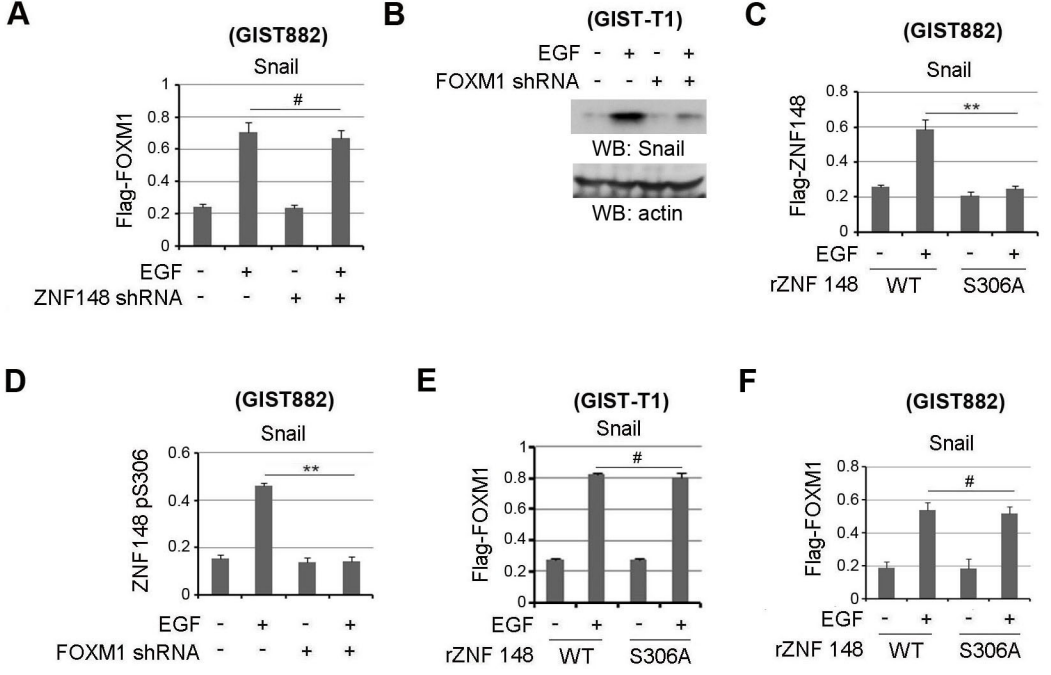
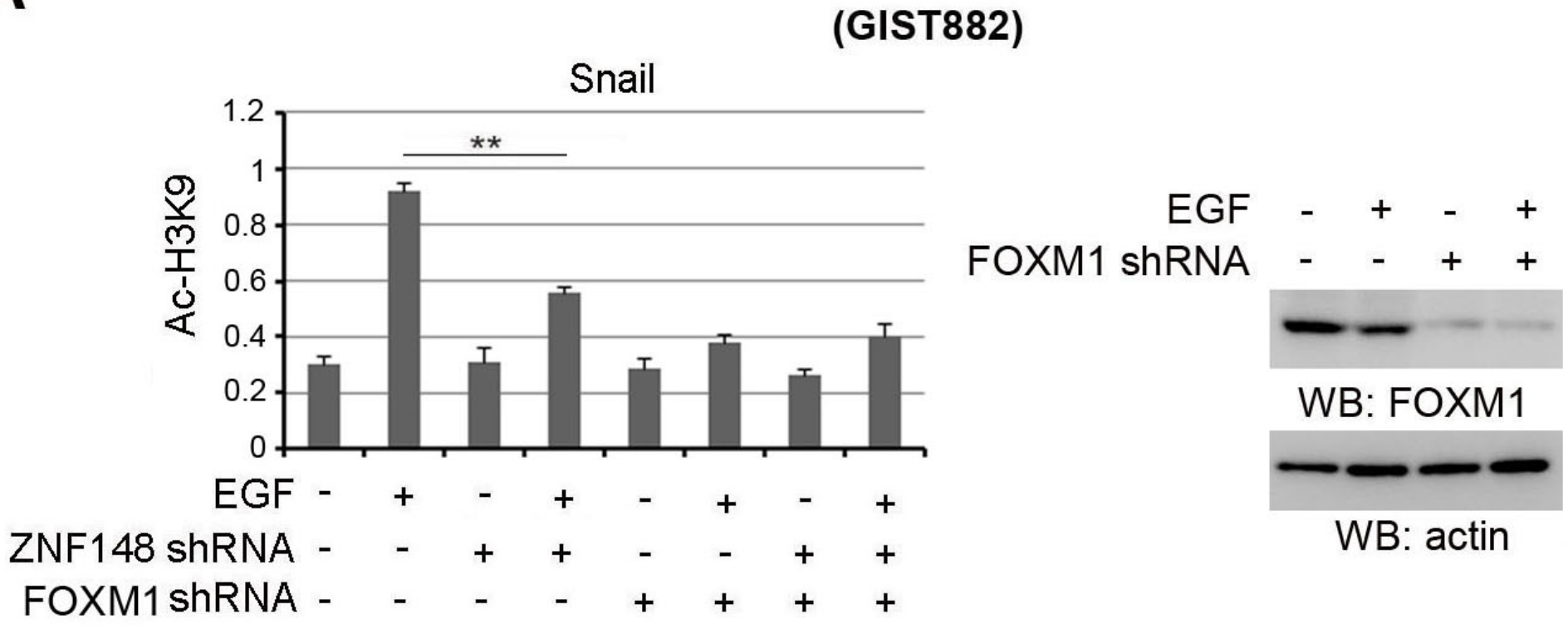

A
(GIST882)
Snail
Flag-FOXM1
1
0.8
0.6
0.4
0.2
0
#
EGF - + - +
ZNF148 shRNA - - + +
B
(GIST-T1)
EGF - + - +
FOXM1 shRNA - - + +
WB: Snail
WB: actin
C
(GIST882)
Snail
Flag-ZNF148
0.8
0.6
0.4
0.2
0
**
EGF - + - +
rZNF 148 WT S306A
D
(GIST882)
Snail
ZNF148 pS306
0.6
0.4
0.2
0
**
EGF - + - +
FOXM1 shRNA - - + +
E
(GIST-T1)
Snail
Flag-FOXM1
1
0.8
0.6
0.4
0.2
0
#
EGF - + - +
rZNF 148 WT S306A
F
(GIST882)
Snail
Flag-FOXM1
0.8
0.6
0.4
0.2
0
#
EGF - + - +
rZNF 148 WT S306A

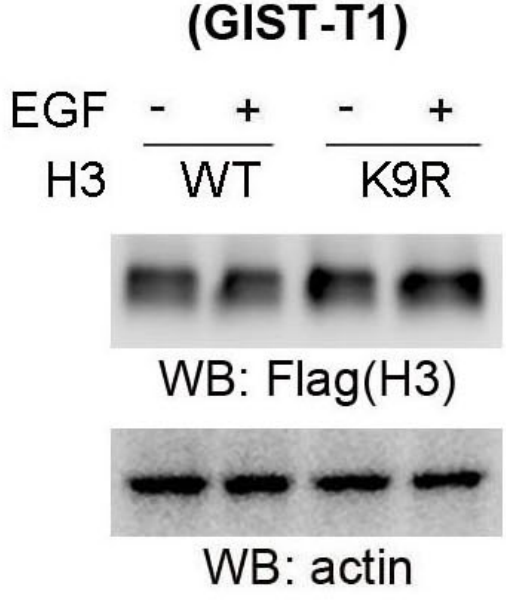
Figure S4. EGF induced ZNF148 recruitment to Snail promoter (A) GIST882 cells with SDHB depletion were transfected with or without the plasmid for expressing ZNF148 shRNA were treated with or without EGF (100 ng/ml) for 6 h. ChIP analyses with an anti-Flag antibody were performed. **(B)** GIST-T1 cells with SDHB depletion were transfected with or without the plasmid for expressing FOXM1 shRNA. Cellular extracts subjected to immunoblotting analyses with indicated antibodies. **(C)** GIST882 cells with SDHB depletion, ZNF148 depletion and reconstituted expression of WT ZNF148 or ZNF148 S306A were treated with or without EGF (100 ng/ml) for 6 h. ChIP analyses with an anti-Flag antibody were performed. The primers covering FOXM1 binding region of Snail gene promoter region were used for the Q-PCR. **(D)** GIST882 cells with SDHB depletion were transfected with or without the plasmid for expressing FOXM1 shRNA. Cells were treated with or without EGF (100 ng/ml) for 6 h. ChIP analyses with an anti-ZNF148 pS306 antibody were performed. The primers covering FOXM1 binding region of Snail gene promoter region were used for the Q-PCR. **(E, F)** GIST-T1 cells **(E)** or GIST882 cells **(F)** with SDHB depletion, ZNF148 depletion and reconstituted expression of WT ZNF148 or ZNF148 S306A were treated with or without EGF for 6 h. ChIP analyses with an anti-Flag antibody were performed. The primers covering FOXM1 binding region of Snail gene promoter region were used for the Q-PCR. In A-E, the values are presented as mean ± s.e.m. (n=3 independent experiments), ** represents $P<0.01$ between the indicated groups. # represents $P>0.05$ between the indicated groups.

S5

A


(GIST882)
Snail
Ac-H3K9
1.2
1
0.8
0.6
0.4
0.2
0
**
EGF - + - + - + - +
ZNF148 shRNA - - + + - - + +
FOXM1 shRNA - - - - + + + +
EGF - + - +
FOXM1 shRNA - - + +
WB: FOXM1
WB: actin

B


(GIST-T1)
EGF - + - +
H3 WT K9R
WB: Flag(H3)
WB: actin

C

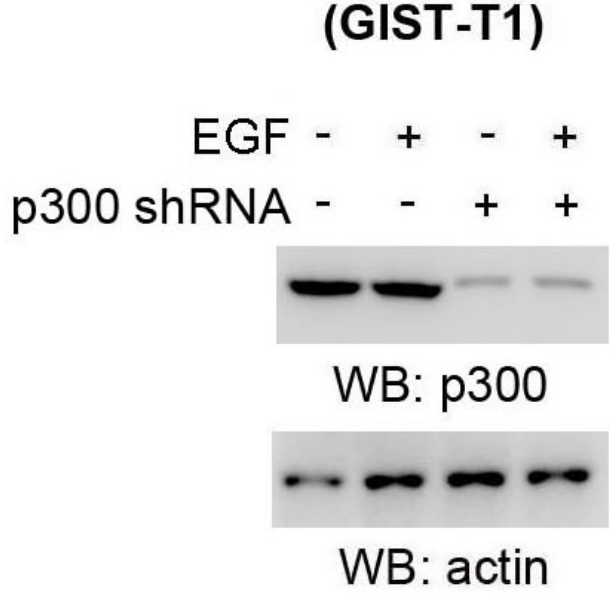

(GIST-T1)
EGF - + - +
p300 shRNA - - + +
WB: p300
WB: actin

Figure S5. ZNF148 promotes H3-K9 acetylation at Snail promoter. **(A)** GIST882 cells with SDHB depletion were transfected with or without the plasmid for expressing ZNF148 shRNA or/and FOXM1 shRNA. Cells were treated with or without EGF (100 ng/ml) for 6 h. ChIP analyses with an anti-Ac-H3K9 antibody were performed. The primers covering FOXM1 binding region of Snail gene promoter region were used for the Q-PCR. The values are presented as mean ± s.e.m. (n=3 independent experiments), ** represents $P<0.01$ between the indicated groups (left panel). Cellular extracts subjected to immunoblotting analyses (right panel). **(B)** SDHB-depleted GIST-T1 cells overexpressed with WT H3 or H3 K9R were treated with or without EGF (100 ng/ml). Cellular extracts subjected to immunoblotting analyses. **(C)** SDHB-depleted GIST-T1 cells expressed with or without p300 shRNA were treated with or without EGF (100 ng/ml). Cellular extracts subjected to immunoblotting analyses.