Supplementary Information

Supplementary figure legend

Figure S1. Hypoxic treatment increases FOXC1 mRNA and VEGF protein levels. (A) The mRNA levels of FOXC1 in A549 and CL1-5 cells with incubation of cobalt chloride (50 μM), which mimics hypoxia, for 24 hours. (B) The mRNA levels of FOXC1 in CL1-0 and CL1-5 cells at 24 hours after normoxic and hypoxic treatment (<1% O_2). (C) The protein levels of VEGF in A549 and CL1-5 cells with incubation of cobalt chloride (50 μM), which mimics hypoxia, for 24 hours. (D) Time course of VEGF expression after exposure of A549 cells to <1% O_2. *P <0.05 compared to control or wild type (WT) group.

Figure S2. The relative protein densities in Figure 3A (A and B), 3B (C and D), 3C (E), 3D (F and G), 3E (H and I), 5A (J), 5I (K and L). The expression was quantified by using Image J program and normalizing to that of β-actin. Error bars denote the standard deviation among triplicate experiments. *P <0.05 compared to control or wild type (WT) group.

Figure S3. (A) The mRNA levels of VEGF in A549 and CL 1-5 cells with or without FOXC1 knockdown. (B) The VEGF concentration in medium from A549 and CL 1-5 cells with or without FOXC1 knockdown. Data are means ±SD (n=9). *P < 0.001 compared to wild type (WT) cells, one-way ANOVA with Tukey multiple comparison test.

Figure S4. FOXC1 gain-of-function promotes cell growth, migration, invasion, angiogenesis and EMT in lung cancer cells. (A) Western blot analysis of FOXC1 overexpression in CL1-0 cells via the lentiviral-based FOXC1 overexpression. (B) Cell growth of CL1-0 cells with or without FOXC1 overexpression for 7 days. Cell growth was examined by trypan blue staining. The migration (C) and invasion (D) of CL1-0 cells with or without FOXC1 overexpression were measured by *in vitro* migration and invasion assay, respectively. The morphology characteristics (E) and quantification of total tubes (F) and tube length (G) for *in vitro* tube formation of HUVECs incubated with conditional medium from CL1-0 cells with or without FOXC1 knockdown. (H) The transcript levels of epithelial-mesenchymal transition (EMT) markers in CL1-0 cells with or without overexpression. Data are means ±SD (n=9). *P < 0.001 compared to wild type (WT) cells, one-way ANOVA with Tukey multiple comparison test.

Figure S5. Genetic manipulation of FOXC1 in lung cancer cells changes the

epithelial-mesenchymal transition (EMT). (A) Western blot analysis of FOXC1 overexpression and knockdown in A549 cells via the lentiviral-based FOXC1 overexpression and knockdown systems. (B) The morphology characteristics of A549 cells after FOXC1 overexpression and knockdown. (C) Immunofluorescence images from A549 cells with or without FOXC1 overexpression and knockdown immunostained to detect the E-cadherin (green), vimentin (red) and nuclear DNA (blue).

Figure S6. HIF-1-induced FOXC1 knockdown. **(**A) Graphic representation of 8 hypoxia-responsive elements (HRE)-driven FOXC1 shRNA construct. (B) Western blot analysis of hypoxia-induced FOXC1 knockdown in A549 cells.

Supplementary figures

Figure S1.

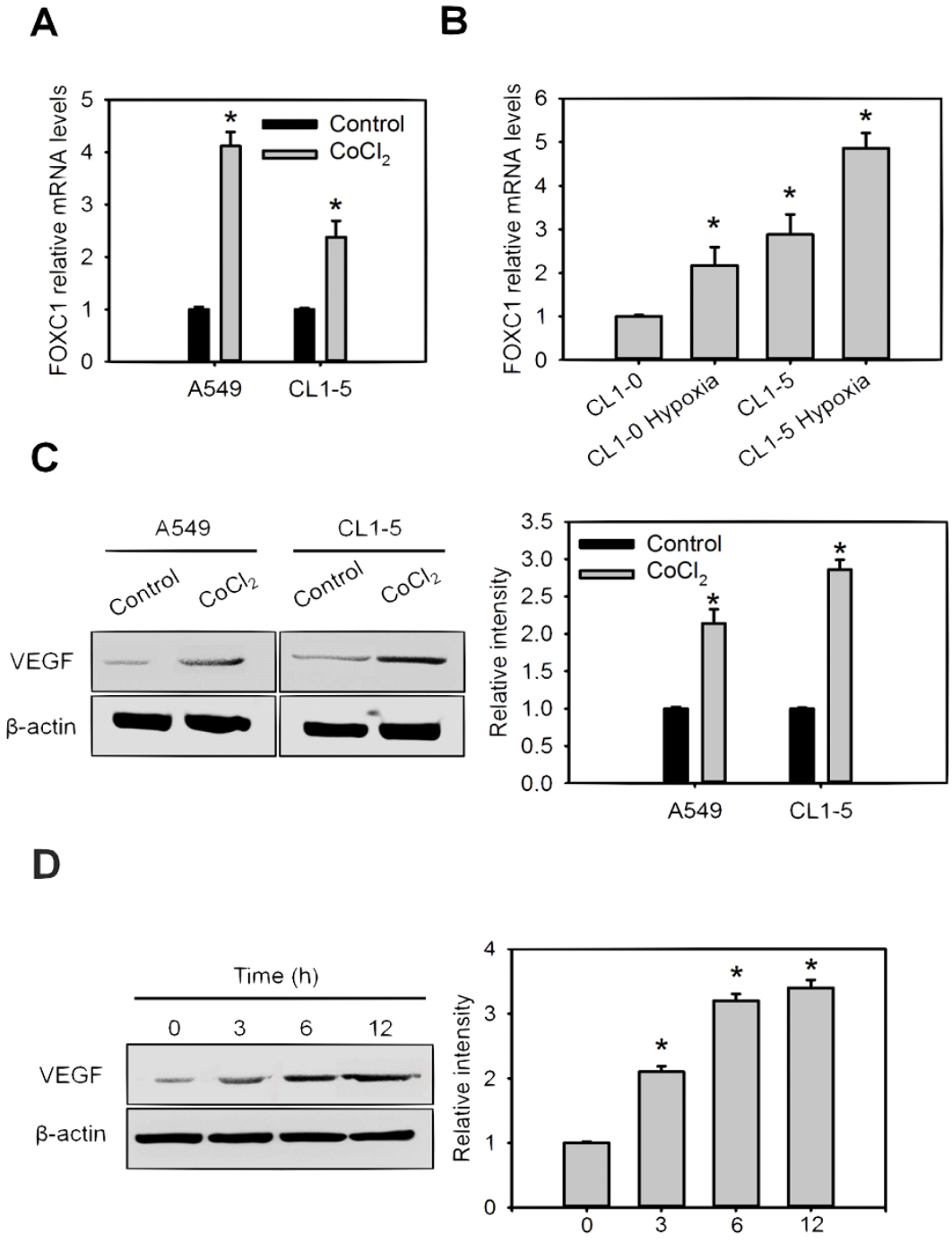


Figure S2

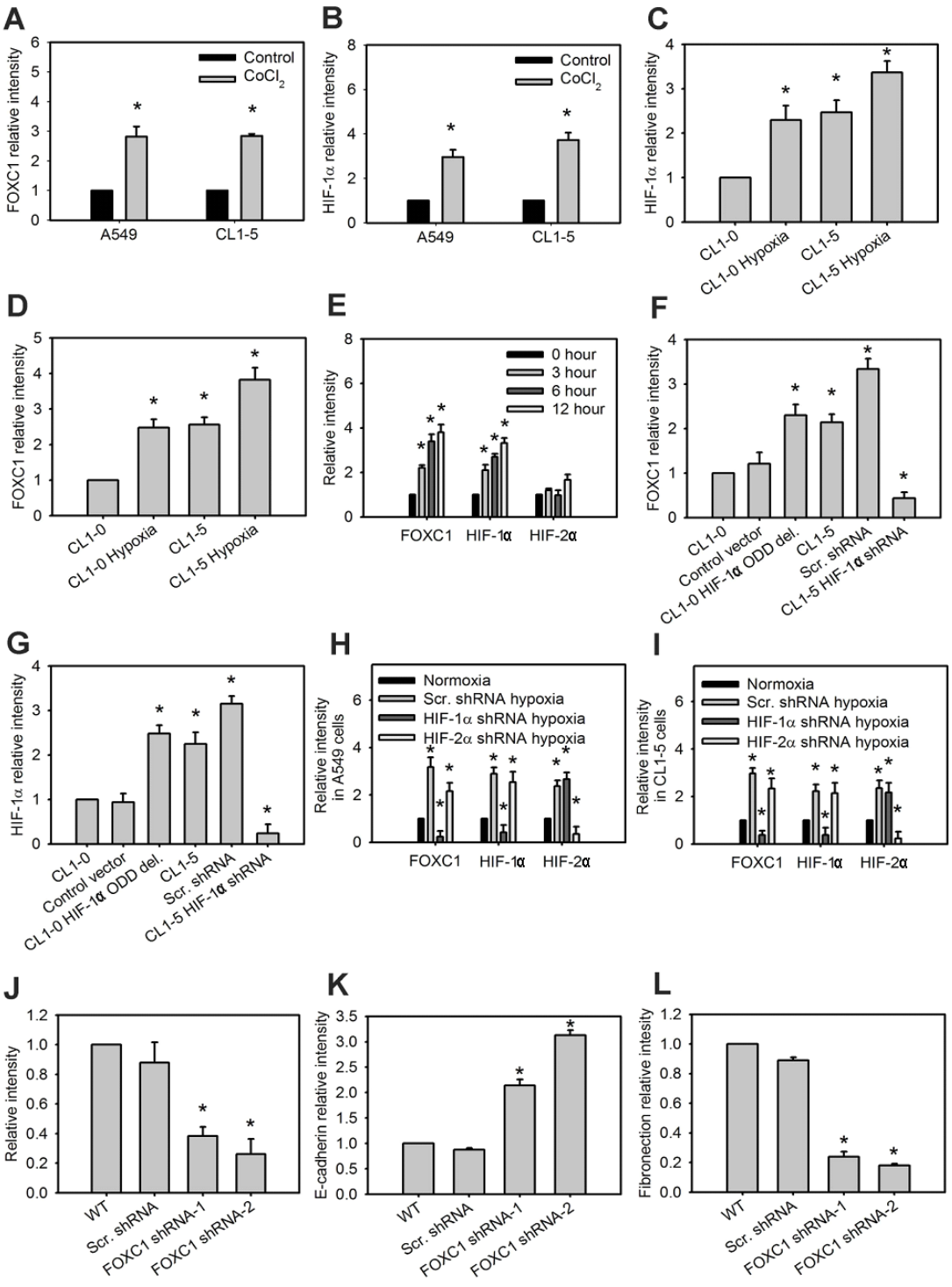

A
FOXC1 relative intensity
Control
CoCl2
A549
CL1-5
B
HIF-1α relative intensity
Control
CoCl2
A549
CL1-5
C
HIF-1α relative intensity
CL1-0
CL1-0 Hypoxia
CL1-5
CL1-5 Hypoxia
D
FOXC1 relative intensity
CL1-0
CL1-0 Hypoxia
CL1-5
CL1-5 Hypoxia
E
Relative intensity
0 hour
3 hour
6 hour
12 hour
FOXC1
HIF-1α
HIF-2α
F
FOXC1 relative intensity
CL1-0
Control vector
CL1-0 HIF-1α ODD del.
CL1-5
Scr. shRNA
CL1-5 HIF-1α shRNA
G
HIF-1α relative intensity
CL1-0
Control vector
CL1-0 HIF-1α ODD del.
CL1-5
Scr. shRNA
CL1-5 HIF-1α shRNA
H
Relative intensity in A549 cells
Normoxia
Scr. shRNA hypoxia
HIF-1α shRNA hypoxia
HIF-2α shRNA hypoxia
FOXC1
HIF-1α
HIF-2α
I
Relative intensity in CL1-5 cells
Normoxia
Scr. shRNA hypoxia
HIF-1α shRNA hypoxia
HIF-2α shRNA hypoxia
FOXC1
HIF-1α
HIF-2α
J
Relative intensity
WT
Scr. shRNA
FOXC1 shRNA-1
FOXC1 shRNA-2
K
E-cadherin relative intensity
WT
Scr. shRNA
FOXC1 shRNA-1
FOXC1 shRNA-2
L
Fibronection relative intesity
WT
Scr. shRNA
FOXC1 shRNA-1
FOXC1 shRNA-2

Figure S3

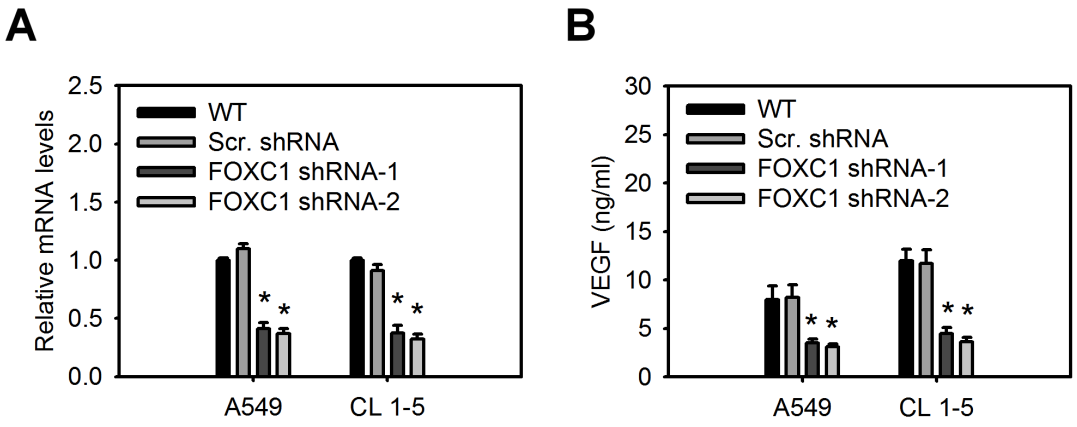
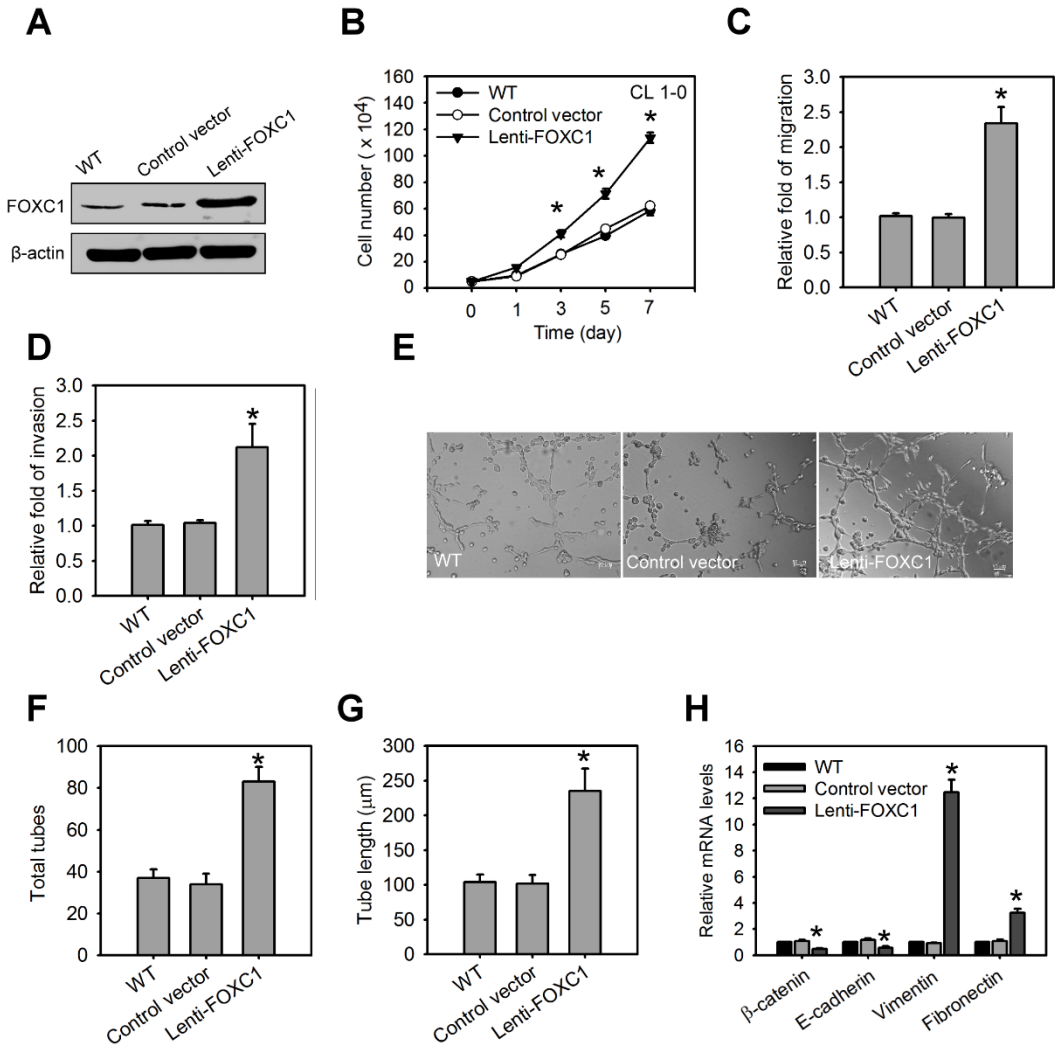

A
B
Relative mRNA levels
2.5
2.0
1.5
1.0
0.5
0.0
WT
Scr. shRNA
FOXC1 shRNA-1
FOXC1 shRNA-2
* *
* *
A549
CL 1-5
VEGF (ng/ml)
30
25
20
15
10
5
0
WT
Scr. shRNA
FOXC1 shRNA-1
FOXC1 shRNA-2
* *
* *
A549
CL 1-5

Figure S4


A
WT
Control vector
Lenti-FOXC1
FOXC1
β-actin
B
WT
Control vector
Lenti-FOXC1
CL 1-0
Cell number (x 10⁴)
Time (day)
C
Relative fold of migration
WT
Control vector
Lenti-FOXC1
D
Relative fold of invasion
WT
Control vector
Lenti-FOXC1
E
WT
Control vector
Lenti-FOXC1
F
Total tubes
WT
Control vector
Lenti-FOXC1
G
Tube length (μm)
WT
Control vector
Lenti-FOXC1
H
WT
Control vector
Lenti-FOXC1
Relative mRNA levels
β-catenin
E-cadherin
Vimentin
Fibronectin

.

Figure S5

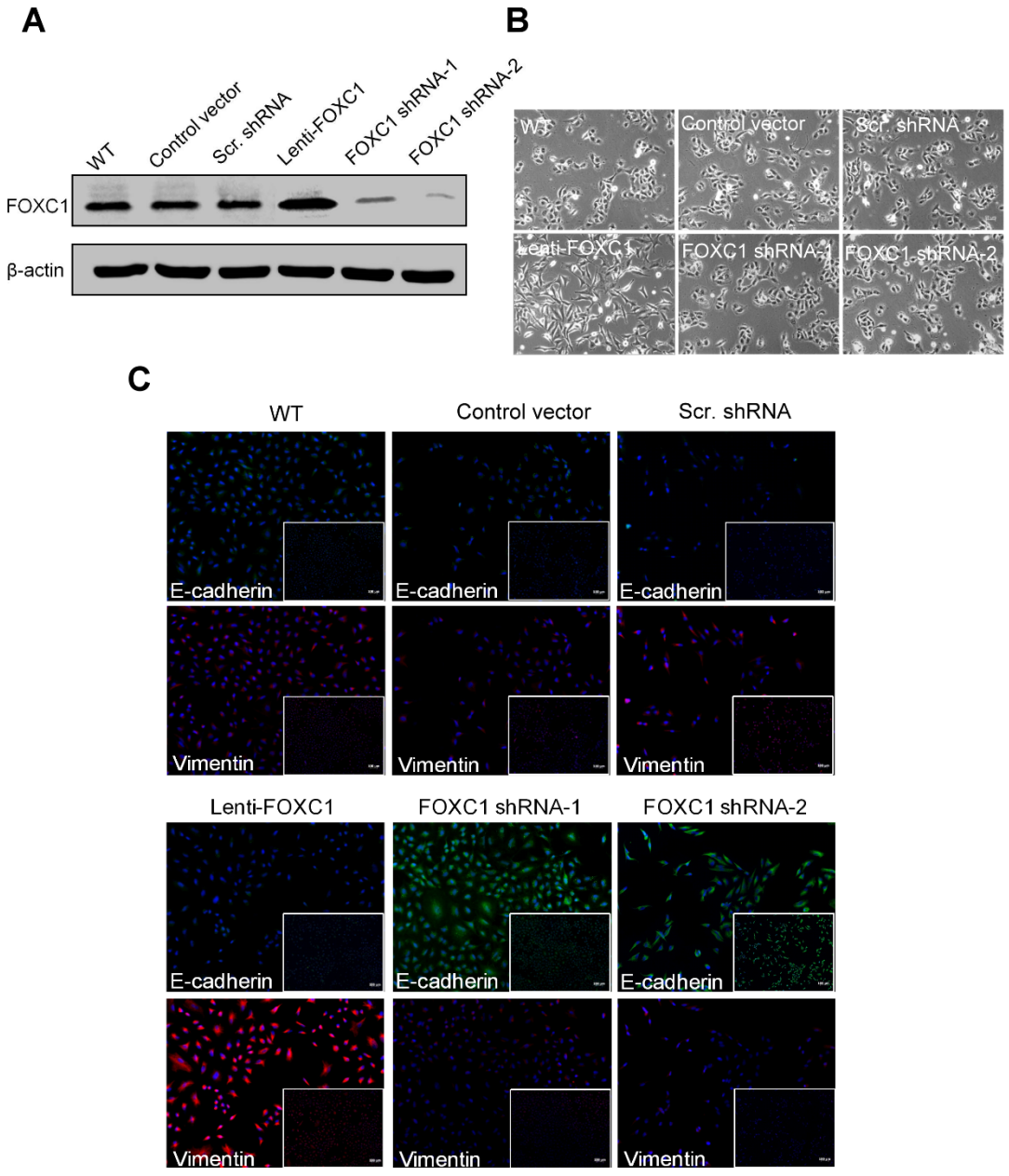
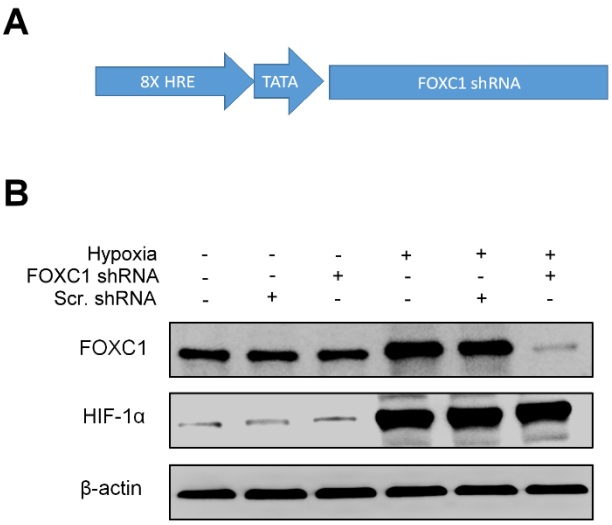

A
WT
Control vector
Scr. shRNA
Lenti-FOXC1
FOXC1 shRNA-1
FOXC1 shRNA-2
FOXC1
β-actin
B
WT
Control vector
Scr. shRNA
Lenti-FOXC1
FOXC1 shRNA-1
FOXC1 shRNA-2
C
WT
Control vector
Scr. shRNA
E-cadherin
Vimentin
Lenti-FOXC1
FOXC1 shRNA-1
FOXC1 shRNA-2

Figure S6


A
8X HRE
TATA
FOXC1 shRNA
B
Hypoxia - - - + + +
FOXC1 shRNA - - + - - +
Scr. shRNA - + - - + -
FOXC1
HIF-1α
β-actin