Supplementary Figure Legends

Figure 1. WNT5A is up-regulated by FOXC1 in TNBC cells. **A,** western blotting analysis of FOXC1 and WNT5A proteins in FOXC1-overexpressing or FOXC1-knockout TNBC cells. ACTIN was used as an internal control. The expression levels of WNT5A protein were quantified. The bar graph indicates mean ± SD, n = 3. ns, not significant, **, $p < 0.01$, ***, $p < 0.001$. **B** and **C,** real-time PCR analysis of WNT5A mRNA in FOXC1-overexpressing (**B**) and FOXC1-knockout (**C)** TNBC cells. Primers used were: WNT5A-forward: 5'-CCCTCGCCATGAAGAA GTCCA-3', WNT5A-reverse: 5'-CATACCTAGCGACC ACCAAGA-3'. The bar graph indicates mean ± SD, n = 3. ns, not significant, **, $p < 0.01$, ***, $p < 0.001$. **D,** western blotting analysis of WNT5A protein in different groups of MDA-MB-231 cells. ACTIN was used as an internal control. **E,** real-time PCR analysis of MMP7 mRNA expression in HCC1806 cells treated with different concentrations of WNT5A. The bar graph indicates mean ± SD, n = 3. **, $p < 0.01$, ***, $p < 0.001$.

Figure 2. *WNT5A* promoter is activated by a FOXC1 transactivator complex. **A,** Biotinylated oligonucleotide precipitation assays for the interaction of KHSRP and HNRNPH1 with the biotin-labeled *WNT5A* promoter oligo containing putative FOXC1-binding sites. Briefly, the 5'-biotinylated oligonucleotides were synthesized and annealed. Two micrograms of biotinylated double-stranded oligonucleotides were incubated with 20μg nuclear proteins at 4°C for 16h. DNA-bound proteins were precipitated using Streptavidin Agarose Beads (ThermoFisher) at 4°C for 2h, washed and detected by western blotting analysis using specific antibodies. The biotinylated oligonucleotide sequences of *WNT5A* promoter were: #1: Biotin-5'-forward: TTCGAAGACTGT AAAATGCCCACAG, Biotin-5'-reverse: CTGTGGGCATTTT ACAGTCTTCGAA. #2: Biotin-

5’-forward: GACTCAGGAGGT AAAGGGGAGATGT, Biotin-5’-reverse: ACATCTCCCCTTT ACCTCCTGAGTC.

Figure 3. WNT5A activates NF-κB signaling in TNBC cells. **A,** western blotting analysis of WNT5A protein in different groups of MDA-MB-436 cells. ACTIN was used as an internal control. **B,** real-time PCR analysis of MMP7 mRNA in the cells treated with the SRC inhibitor Dasatinib (Selleckchem) at different concentrations. The bar graph indicates mean ± SD, n = 3. ***, $p < 0.001$. **C,** western blotting analysis of p-SRC in different groups of MDA-MB-231 cells. Cells were treated with Dasatinib at different concentrations. ACTIN was used as an internal control. **D,** luciferase assay of HCC1806 cells transfected with NF-κB-responsive luciferase reporter construct and treated with recombinant WNT5A protein at different concentrations. The bar graph indicates mean ± SD, n = 3. *, $p < 0.05$, ***, $p < 0.001$. **E,** real-time PCR analysis of MMP7 mRNA in cells treated with the NF-κB inhibitor Bay 11-7082 at different concentrations. The bar graph indicates mean ± SD, n = 3. **, $p < 0.01$, ***, $p < 0.001$. **F,** real-time PCR analysis of KHSRP mRNA in cells treated with the NF-κB inhibitor Bay 11-7082 at different concentrations. The bar graph indicates mean ± SD, n = 3. **G,** western blotting analysis result of p65 protein in different groups of cells. ACTIN was used as an internal control. The p65 antibody was from Santa Cruz (1:500, sc-8008). pCMV empty vector and pCMV-p65 were gifts from Xin Lin (MD Anderson Cancer Center).

Figure 4. WNT5A mediates FOXC1-induced invasiveness of TNBC cells. **A,** proliferation assays in different groups of MDA-MB-231 cells. Cells were seeded into opaque 96-well plates at 2000 cells per well. The cell proliferation rate was assessed using the CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega) according to the manufacturer's instructions. **B** and **C,** migration (**B**) and invasion (**C**) assays in HCC1806 cells treated with different concentrations of recombinant

WNT5A protein. The bar graph indicates mean ± SD, n = 3. **, $p < 0.01$, ***, $p < 0.001$. **D** and **E,** real-time PCR analysis of MMP7 mRNA in MDA-MB-231 (**D**) and HCC1806 (**E**) cells. Cells were transfected with control or MMP7 siRNA. The bar graph indicates mean ± SD, n = 3. ***, $p < 0.001$. **F** and **G,** migration assay (**F**) and invasion (**G**) assays in MMP7-knockdown HCC1806 cells treated with different concentrations of recombinant WNT5A protein. The bar graph indicates mean ± SD, n = 3. ***, $p < 0.001$. **H** and **I,** western blotting analysis of MMP7 protein in different groups of MDA-MB-231 cells. pCMV-MMP7 (**H**) or pEGFP-C3-MMP7 (**I**) was transfected into FOXC1-WNT5A-KO MDA-MB-231 cells. ACTIN was used as an internal control.

Figure S1

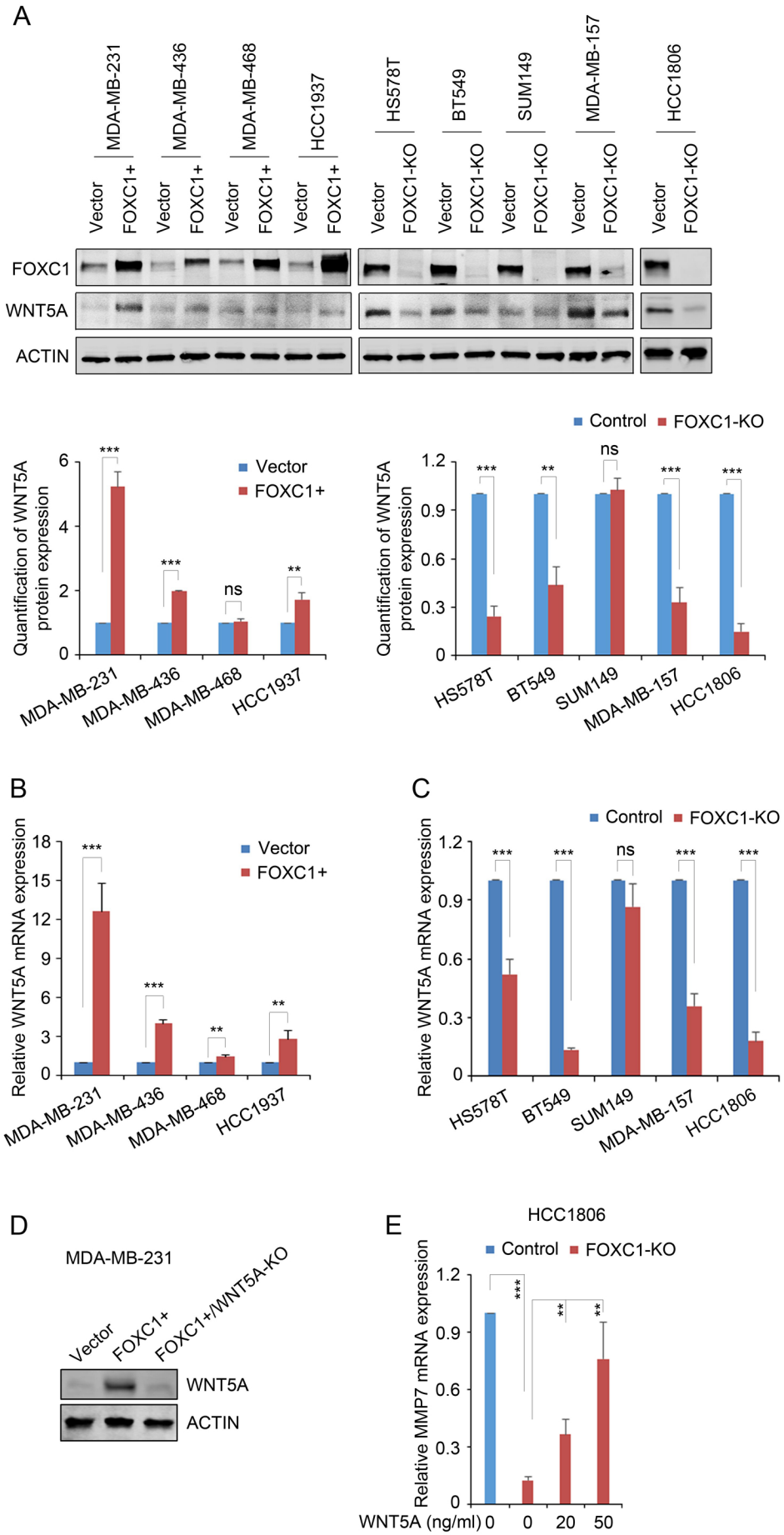

A
MDA-MB-231
MDA-MB-436
MDA-MB-468
HCC1937
HS578T
BT549
SUM149
MDA-MB-157
HCC1806
Vector
FOXC1+
FOXC1-KO
FOXC1
WNT5A
ACTIN
Quantification of WNT5A protein expression
Control
FOXC1-KO
B
Relative WNT5A mRNA expression
C
D
MDA-MB-231
FOXC1+/WNT5A-KO
E
HCC1806
Relative MMP7 mRNA expression
WNT5A (ng/ml) 0
0
20
50

Figure S2

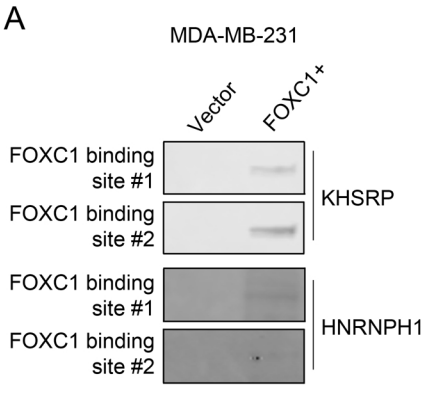

A
MDA-MB-231
Vector
FOXC1+
FOXC1 binding site #1
FOXC1 binding site #2
KHSRP
FOXC1 binding site #1
FOXC1 binding site #2
HNRNPH1

Figure S3

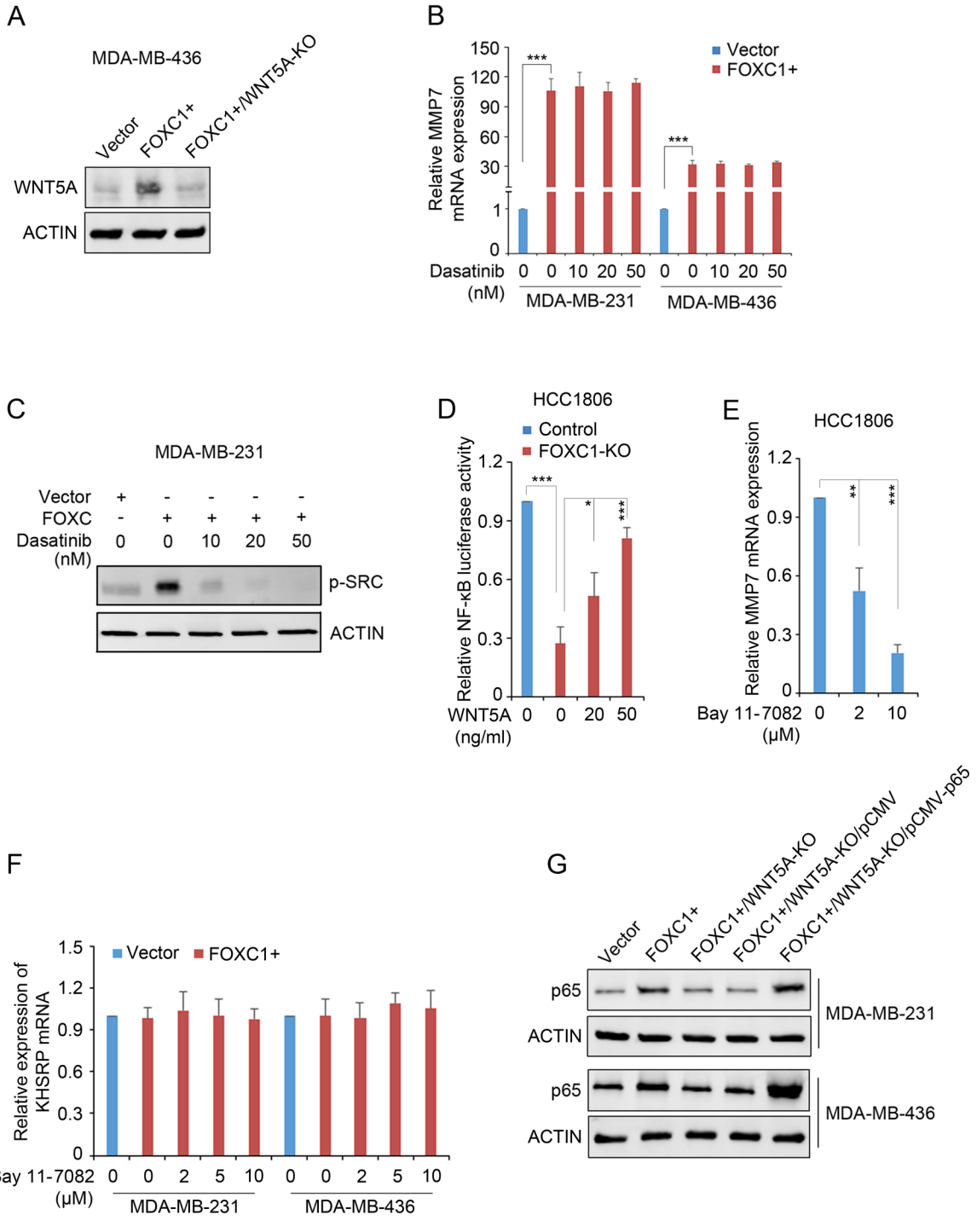
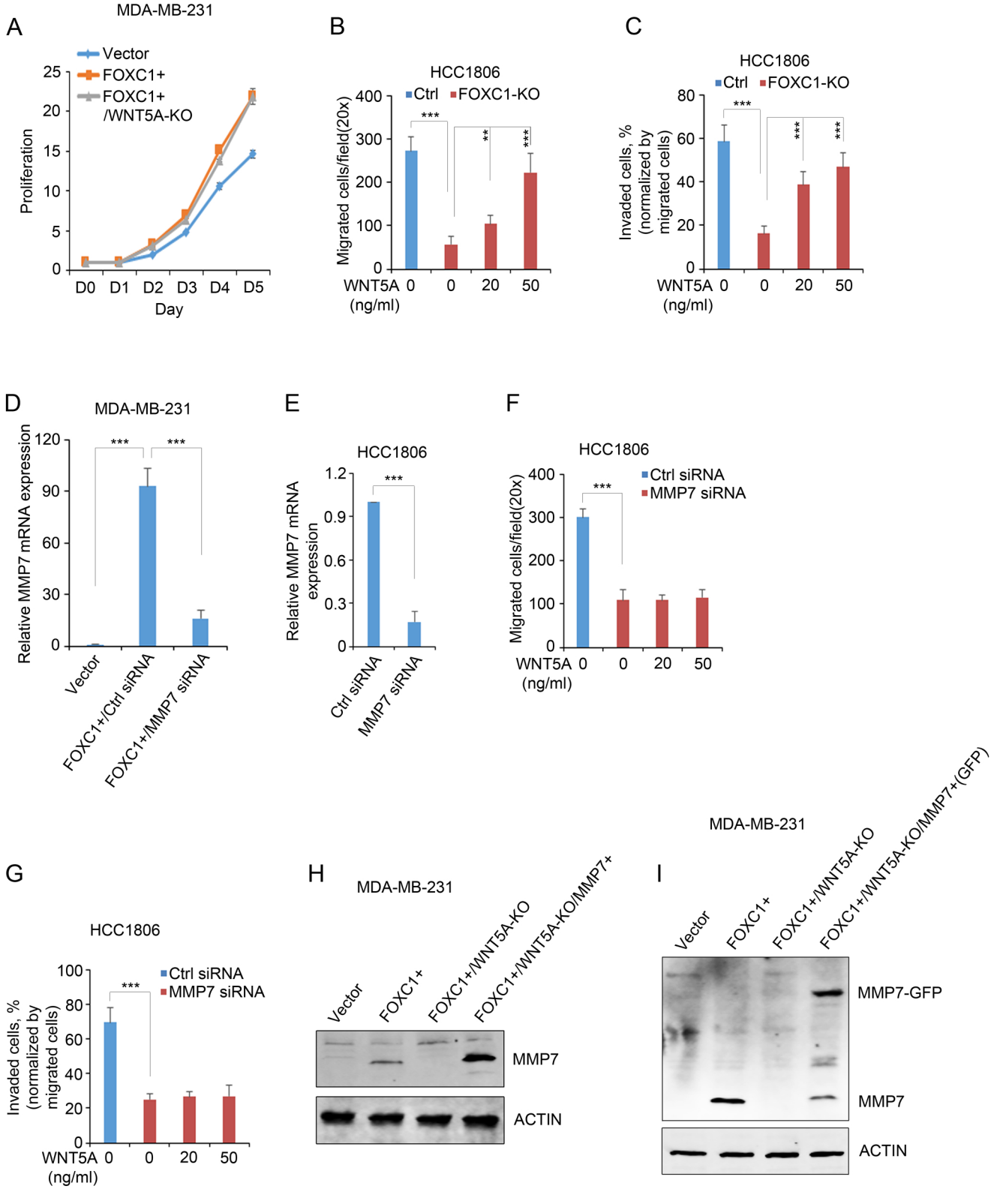

A
MDA-MB-436
Vector
FOXC1+
FOXC1+/WNT5A-KO
WNT5A
ACTIN
B
Relative MMP7 mRNA expression
Vector
FOXC1+
Dasatinib (nM)
MDA-MB-231
MDA-MB-436
C
MDA-MB-231
Vector
FOXC
Dasatinib (nM)
p-SRC
ACTIN
D
HCC1806
Control
FOXC1-KO
Relative NF-κB luciferase activity
WNT5A (ng/ml)
E
HCC1806
Relative MMP7 mRNA expression
Bay 11-7082 (μM)
F
Relative expression of KHSRP mRNA
Vector
FOXC1+
Bay 11-7082 (μM)
MDA-MB-231
MDA-MB-436
G
Vector
FOXC1+
FOXC1+/WNT5A-KO
FOXC1+/WNT5A-KO/pCMV
FOXC1+/WNT5A-KO/pCMV-p65
p65
ACTIN
MDA-MB-231
MDA-MB-436

Figure S4


A
MDA-MB-231
Vector
FOXC1+
FOXC1+ /WNT5A-KO
Proliferation
Day
D0 D1 D2 D3 D4 D5
B
HCC1806
Ctrl FOXC1-KO
Migrated cells/field(20x)
WNT5A (ng/ml)
C
HCC1806
Ctrl FOXC1-KO
Invaded cells, % (normalized by migrated cells)
WNT5A (ng/ml)
D
MDA-MB-231
Relative MMP7 mRNA expression
Vector
FOXC1+/Ctrl siRNA
FOXC1+/MMP7 siRNA
E
HCC1806
Relative MMP7 mRNA expression
Ctrl siRNA
MMP7 siRNA
F
HCC1806
Ctrl siRNA
MMP7 siRNA
Migrated cells/field(20x)
WNT5A (ng/ml)
G
HCC1806
Ctrl siRNA
MMP7 siRNA
Invaded cells, % (normalized by migrated cells)
WNT5A (ng/ml)
H
MDA-MB-231
Vector
FOXC1+
FOXC1+/WNT5A-KO
FOXC1+/WNT5A-KO/MMP7+
MMP7
ACTIN
I
MDA-MB-231
Vector
FOXC1+
FOXC1+/WNT5A-KO
FOXC1+/WNT5A-KO/MMP7+(GFP)
MMP7-GFP
MMP7
ACTIN