Supplementary Tables

Table S1: siRNA and shRNA target sequences used in this study

Target genes	**Sequences (5’ to 3’)**
Luciferase	CTTACGCTGAGTACTTCGA
KLF5 #5	CGATTACCCTGGTTGCACA
KLF5 #7	GATGTGAAATGGAGAAGTA
KLF5 #J	AAGCTCACCTGAGGACTCA

Table S2: Primer sequences used in this study

Names	**Sequences (5’ to 3’)**
U6	CGCAAGGATGACACGCAAATTC
miR-21	TAGCTTATCAGACTGATGTTGA
miR-152	TCAGTGCATGACAGAACTTGG
miR-153	TTGCATAGTCACAAAAGTGATC

Supplementary Figure Legends

Figure S1. MIF suppressed HCC1937 xenograft tumor cell proliferation *in vivo*.

HCC1937 xenograft tumors from the NOD SCID mice were fixed with 4% formaldehyde and embedded in paraffin. Antigens were retrieved by citrate buffer solution (0.05 M citric acid, pH 6.0). Then the anti-Ki67 (1:400 dilution, A) antibody and the anti-cleaved-caspase 3 (1:200, B) antibody were used for IHC after validation and optimization. A standard DAB staining protocol was used. Three xenograft tumors from each group were randomly selected for IHC staining. Representative images are shown, *, P<0.05, **, P<0.01, t-test.

Figure S2. MIF suppresses mammosphere formation in SUM149PT.

SUM149PT cells were plated in 6-well plate at 2×10^5/well and were treated with MIF at designed dosages. 48 h after MIF treatment, the cells were collected for FACS analysis or WB. Mammospheres were counted 14 days after plating.

A. MIF suppressed CSC ratio in a dosage-dependent manner.

B. MIF significantly suppressed mammosphere formation. *, P<0.05, **, p<0.01, t-test.

C. MIF suppressed KLF5 expression and induces PARP cleavage in a

dosage-dependent manner.

Figure S3. MIF suppresses KLF5 expression through inducing miR153 in PDX derived cells.

A-C. MIF suppresses ALDH+ cell populations in TNBC cell HCC1937 (A), PDX UM1(B) and MC1 (C) cells.

D&E. MIF suppressed KLF5 protein expression levels in both MC1 (D) and UM1 (E) in a dosage-dependent manner. The cells dissociated from MC1 and UM1 PDX tumors were plated in 12-well plate at 5×10^5/well and were treated with MIF. One day after MIF treatment, the cells were collected for WB.

F. MIF induced the miR-153 expression in UM1 cells. UM1 cells were treated with MIF as described in A&B. miR-153 level was detected by qPCR.

G. miR-153 suppressed KLF5 expression in UM1. UM1 cells dissociated from PDX tumors were plated in 6-well plate at a density of 1×10^6/well. One day after plating, the cells were transfected with miR-153 mimics at indicated concentrations. Two days after miR-153 transfection, the cells were collected for WB. Mcl-1 was detected as the positive control.

H. MIF suppresses KLF5 expression *in vivo*. Three HCC1937 xenograft tumors were randomly selected from each group for protein extraction. Tumor mass were ground and lysed using cell lysis buffer (50 mM Tris-HCl (pH7.4),

150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and protease inhibitor (P8340, sigma)). Forty microgram lysate was used for WB. The quantitative results are shown below. **P<0.01, t-test.

Figure S4. MIF induces miR-153 and *KLF5* mRNA expression in TNBC cells.

HCC1937 and MCF10A cells were treated with DMSO or 20 μM MIF for indicated time. The cells were then collected for RNA extraction and reverse transcription before *KLF5* mRNA and miR-153 detection.

A. *KLF5* mRNA was not suppressed by MIF treatment in HCC1937 and MCF10A cells.

B. miR-153 was induced by MIF in HCC1937 and MCF10A cells.

Figure S5. miR-153 suppresses HCC1937 cell mammosphere formation.

HCC1937 cells were plated in 12-well plate at 1.5×10^5/well and were transfected with 25 nM miR-153 mimics. Twelve hours after microRNA transfection, the cells were trypsinized and plated for mammosphere formation in ultra-low attachment plates.

A. miR-153 transfection decreased the KLF5 and FGF-BP protein levels.

B. miR-153 significantly suppressed mammosphere formation.

C.The quantitative data of mammosphere formation assays. The cells were cultured for 8 days. **, P<0.01, t-test.

Figure S6. GR knockdown does not block the KLF5 expression reduction induced by MIF.

HCC1937 and MCF10A cells were transfected GR siRNA for one day and treated with MIF (10-20 μM) for another day.

Figure S7. MIF suppresses the expression of CSC-related molecules.

HCC1937 (A) and SUM149PT (B) cells were plated at a density of 3-4$\times10^5$/well in 6-well plates. MIF was added to the cells one day after seeding at indicated doses. The cells were collected at 48h after MIF treatment for Western blotting. GAPDH was used as the loading control.

Figure S8. MIF suppresses basal type breast cancer cell survival.

A. HCC1937 and SUM149PT cells were plated at a density of 3×10^4/well in 48-well plates. MIF was added to the cells one day after seeding.

B. A clinical TNBC sample was dissociated and plated in 96-well plates at a

density of 5×10^4/well. The cells were treated with MIF at indicated dosages. All cells were fixed for SRB assay on day 3.

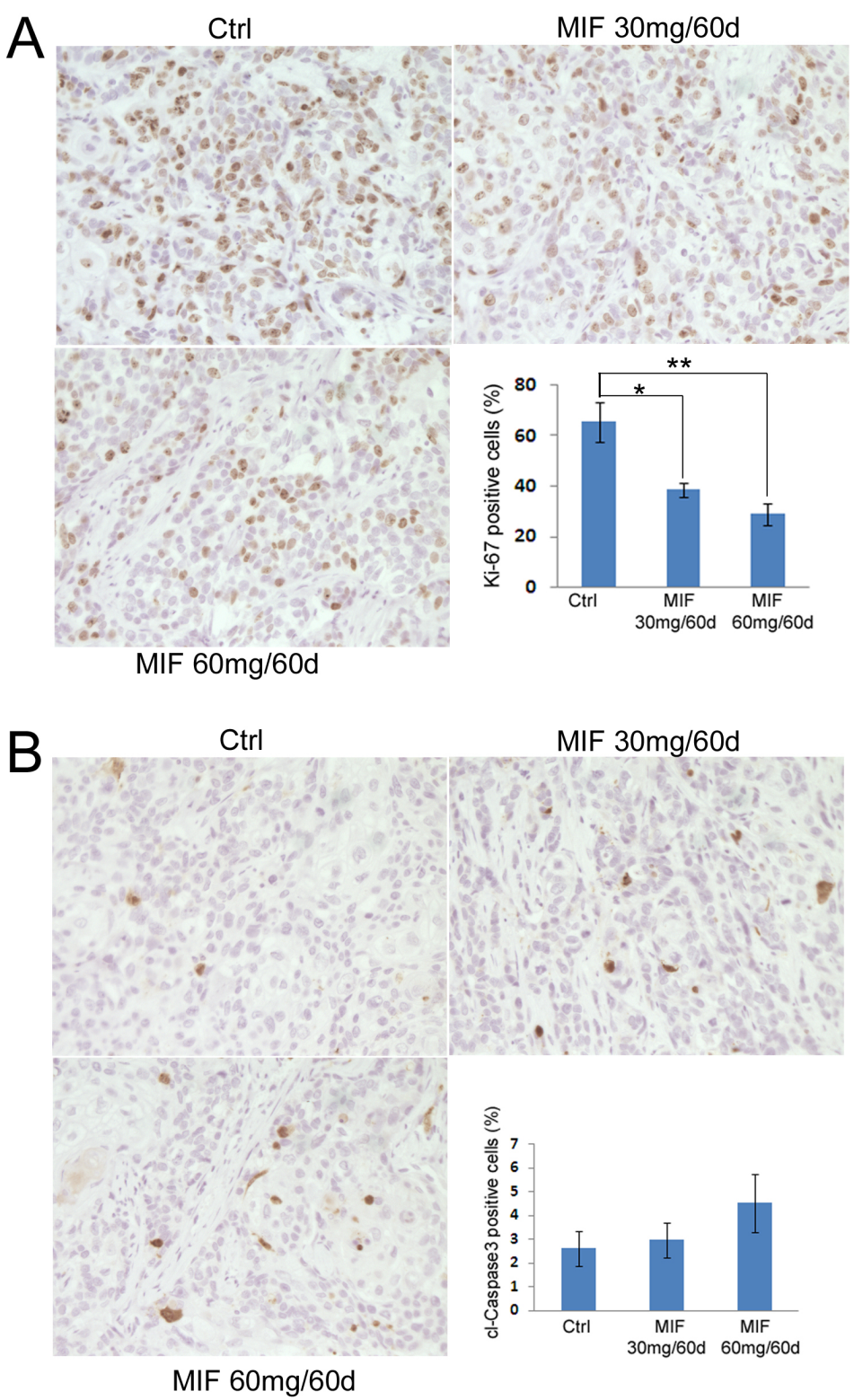
A
Ctrl
MIF 30mg/60d
MIF 60mg/60d
Ki-67 positive cells (%)
80
60
40
20
0
**
*
Ctrl
MIF 30mg/60d
MIF 60mg/60d
B
Ctrl
MIF 30mg/60d
MIF 60mg/60d
cl-Caspase3 positive cells (%)
7
6
5
4
3
2
1
0
Ctrl
MIF 30mg/60d
MIF 60mg/60d

Figure S1

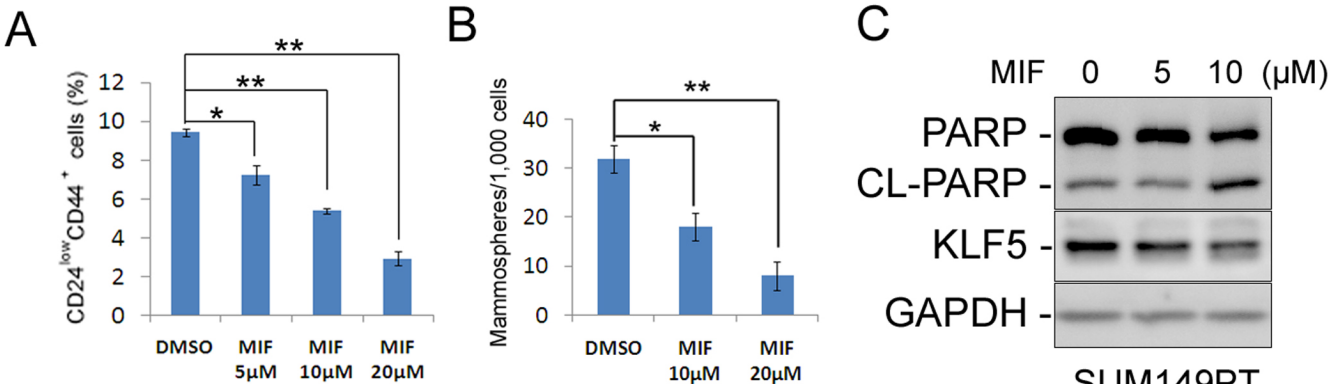
A
B
C
CD24low CD44+ cells (%)
Mammospheres/1,000 cells
DMSO
MIF 5μM
MIF 10μM
MIF 20μM
MIF 0 5 10 (μM)
PARP
CL-PARP
KLF5
GAPDH
SUM149PT

Figure S2

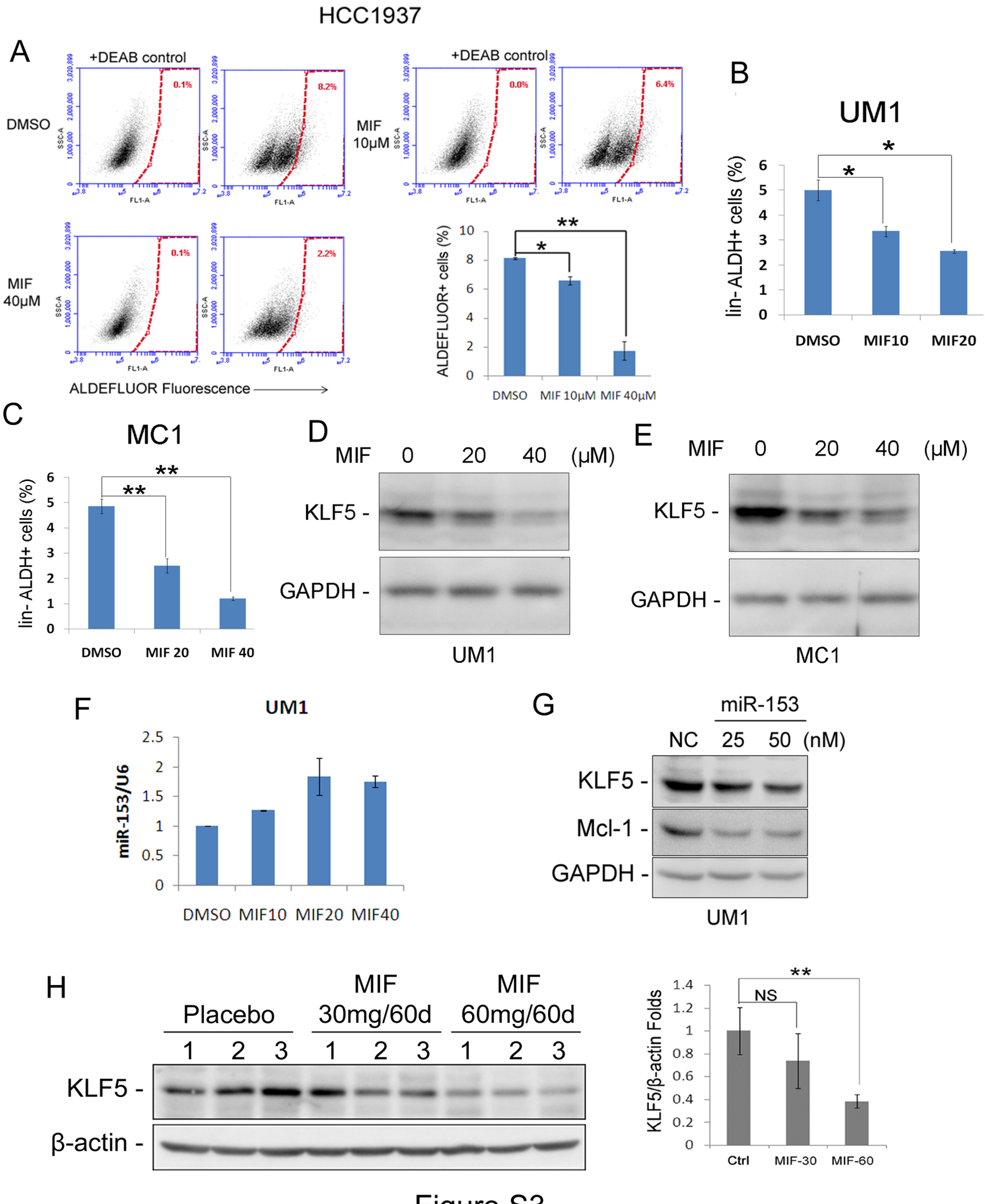
HCC1937
A
+DEAB control
DMSO
MIF 10μM
MIF 40μM
0.1%
8.2%
0.0%
6.4%
0.1%
2.2%
SSC-A
FL1-A
ALDEFLUOR Fluorescence
ALDEFLUOR+ cells (%)
DMSO
MIF 10μM
MIF 40μM
B
UM1
lin- ALDH+ cells (%)
DMSO
MIF10
MIF20
C
MC1
lin- ALDH+ cells (%)
DMSO
MIF 20
MIF 40
D
MIF 0 20 40 (μM)
KLF5 -
GAPDH -
UM1
E
MIF 0 20 40 (μM)
KLF5 -
GAPDH -
MC1
F
UM1
miR-153/U6
DMSO MIF10 MIF20 MIF40
G
miR-153
NC 25 50 (nM)
KLF5 -
Mcl-1 -
GAPDH -
UM1
H
Placebo
MIF 30mg/60d
MIF 60mg/60d
1 2 3 1 2 3 1 2 3
KLF5 -
β-actin -
KLF5/β-actin Folds
NS
Ctrl
MIF-30
MIF-60

Figure S3

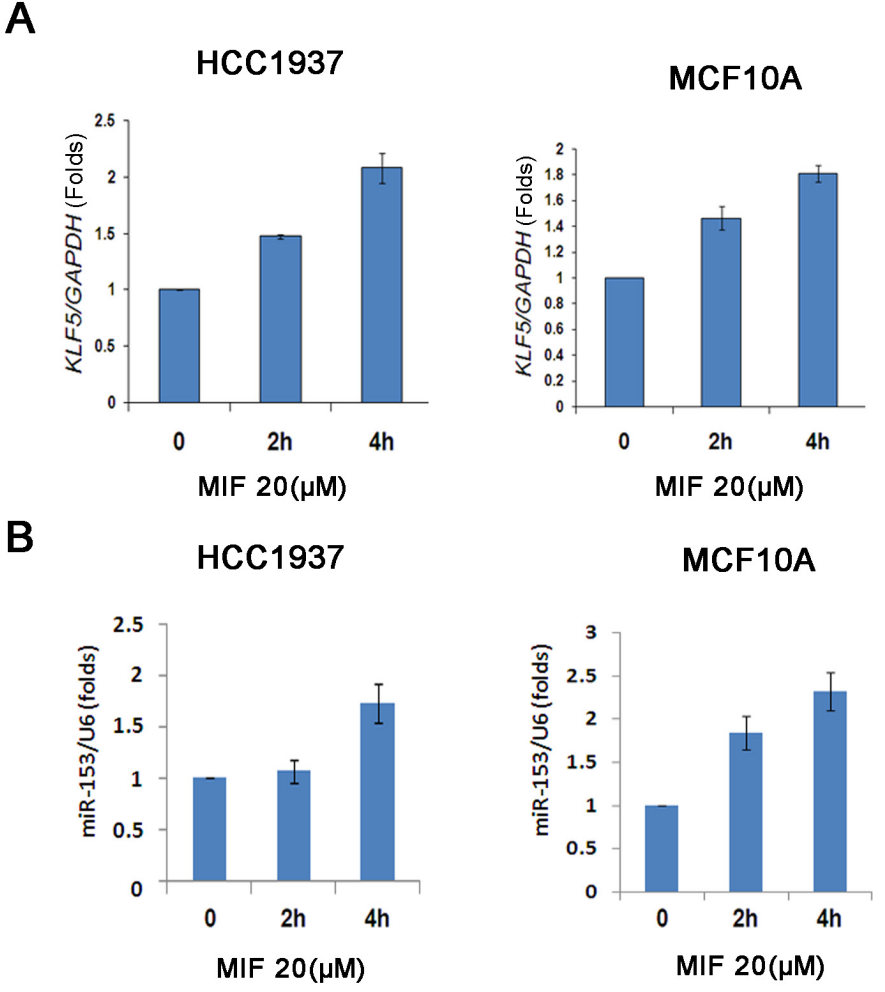
A
HCC1937
KLF5/GAPDH (Folds)
2.5
2
1.5
1
0.5
0
0
2h
4h
MIF 20(μM)
MCF10A
KLF5/GAPDH (Folds)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2h
4h
MIF 20(μM)
B
HCC1937
miR-153/U6 (folds)
2.5
2
1.5
1
0.5
0
0
2h
4h
MIF 20(μM)
MCF10A
miR-153/U6 (folds)
3
2.5
2
1.5
1
0.5
0
0
2h
4h
MIF 20(μM)

Figure S4

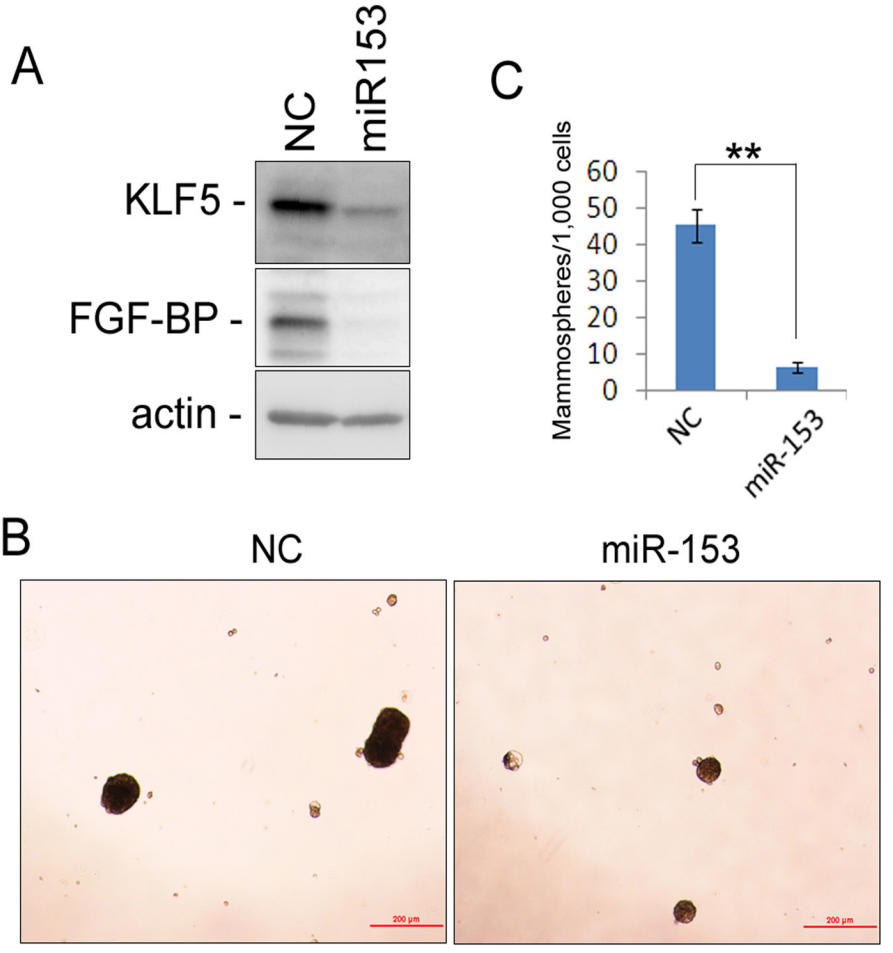
A
NC
miR153
KLF5 -
FGF-BP -
actin -
C
Mammospheres/1,000 cells
60
50
40
30
20
10
0
**
NC
miR-153
B
NC
miR-153
200 µm
200 µm

Figure S5

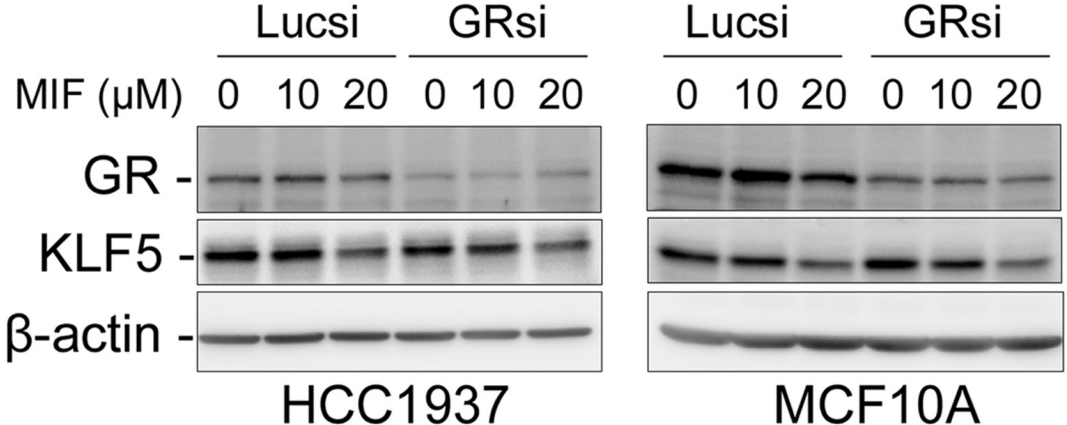
Lucsi
GRsi
Lucsi
GRsi
MIF (μM)
0 10 20 0 10 20
0 10 20 0 10 20
GR -
KLF5 -
β-actin -
HCC1937
MCF10A

Figure S6

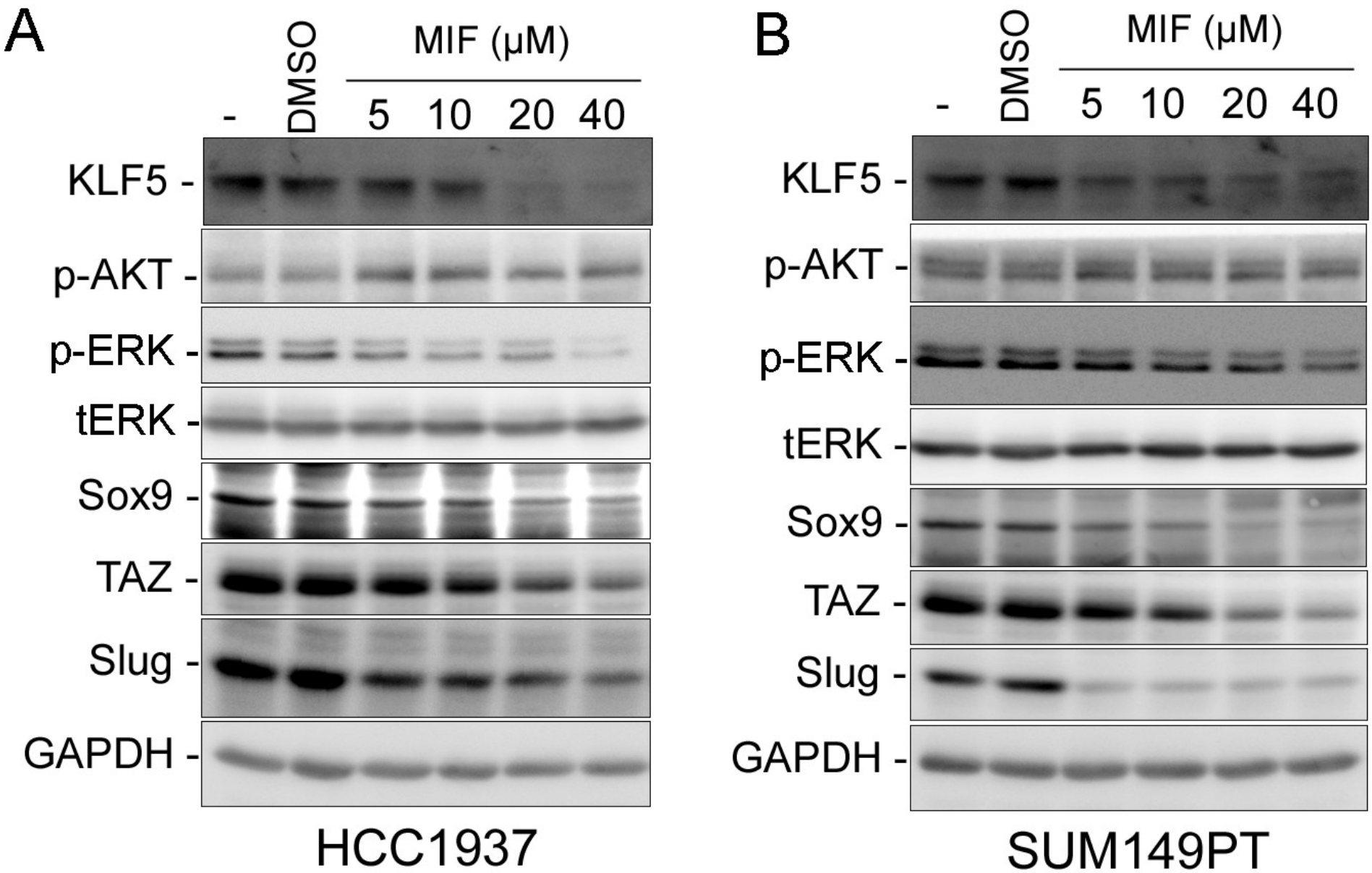
A
DMSO
MIF (μM)
-
5
10
20
40
KLF5
p-AKT
p-ERK
tERK
Sox9
TAZ
Slug
GAPDH
HCC1937
B
DMSO
MIF (μM)
-
5
10
20
40
KLF5
p-AKT
p-ERK
tERK
Sox9
TAZ
Slug
GAPDH
SUM149PT

Figure S7

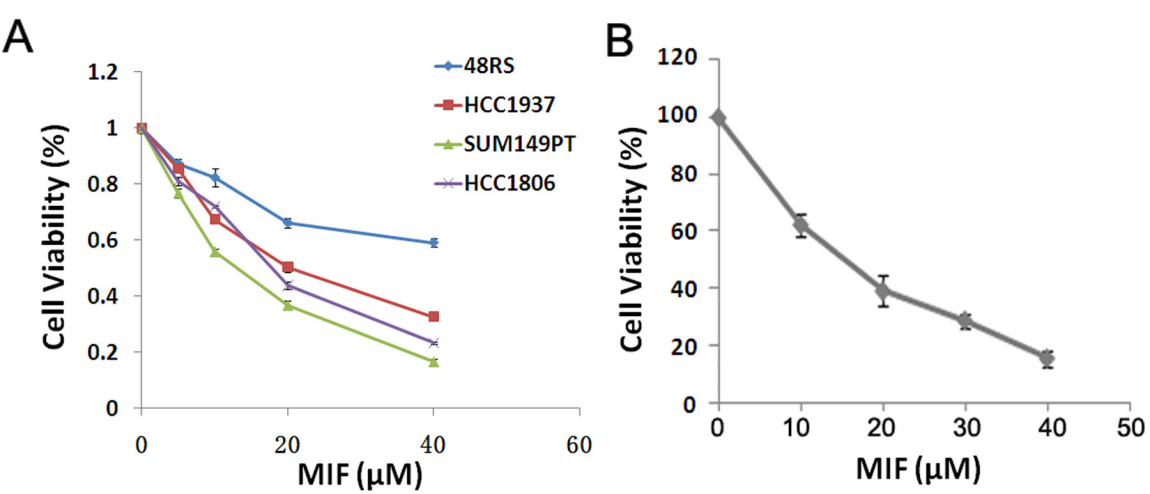
A
1.2
1
0.8
0.6
0.4
0.2
0
Cell Viability (%)
0
20
40
60
MIF (μM)
48RS
HCC1937
SUM149PT
HCC1806
B
120
100
80
60
40
20
0
Cell Viability (%)
0
10
20
30
40
50
MIF (μM)

Figure S8