Expanded View Figures

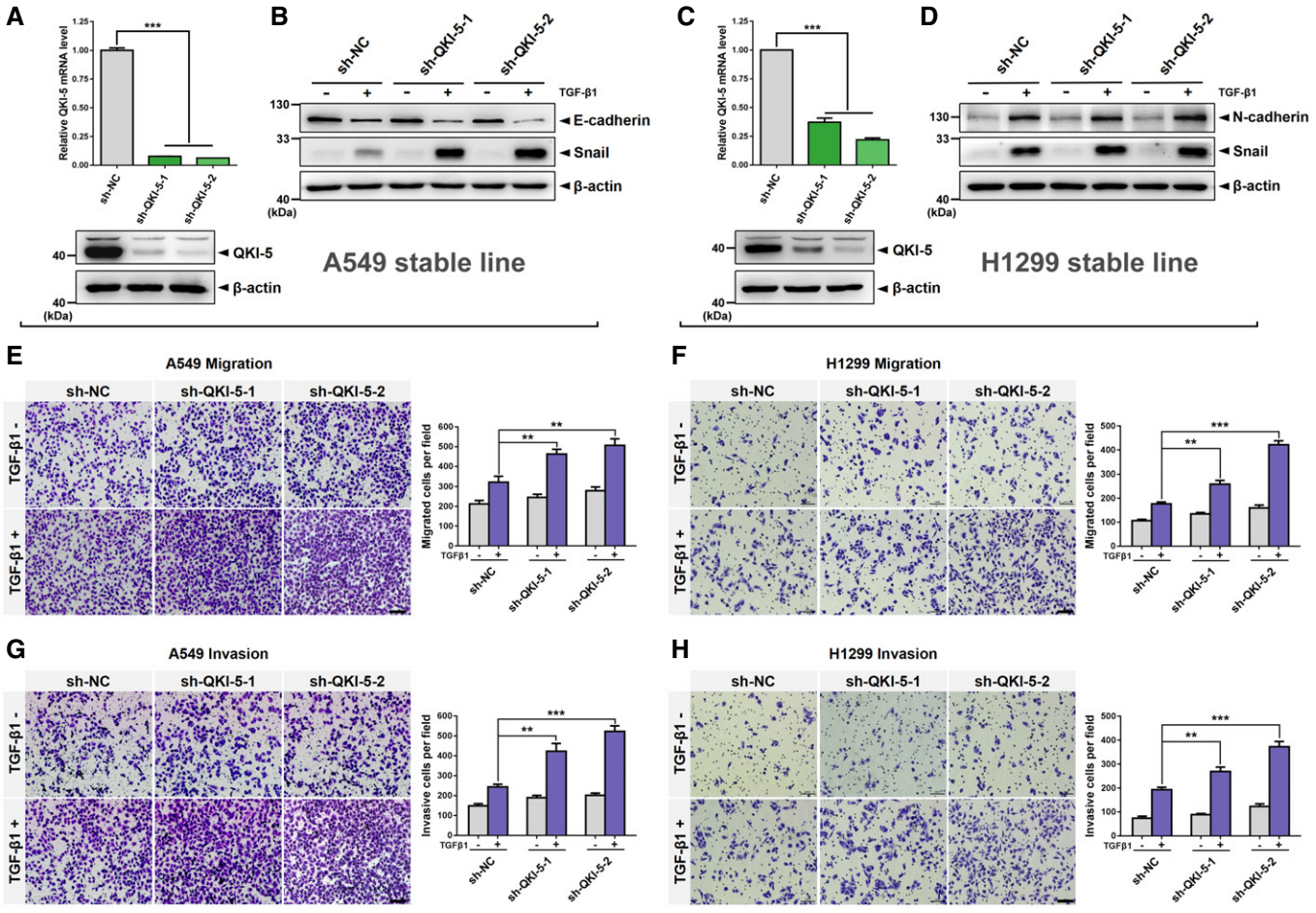

Figure EV1. Silencing of QKI-5 promotes TGF-β-induced EMT and LUAD cell invasion.

A–D After serum starvation for 24 h, QKI-5-silenced A549 cells and H1299 cells (A, C) were stimulated with or without TGF-β1 (5 or 10 ng/ml) for 24 h. Data are shown as the mean ± SD of $n = 3$ technical replicates. ***$P < 0.001$ compared to sh-NC by unpaired Student's t-test. The expression of EMT markers (E-cadherin or N-cadherin and Snail) was determined using Western blot. β-actin was used as an internal control (B, D).

E–H In the presence or absence of TGF-β1, the migratory and invasive abilities of QKI-5-silenced A549 and H1299 cells were evaluated by Transwell assays as described in Fig 4G–J. Migrated and invasive cells were stained and counted under a light microscope. Scale bar, 100 μm. Data are shown as the mean ± SD of $n = 3$ technical replicates. **$P < 0.01$ and ***$P < 0.001$ by unpaired Student's t-test.

Source data are available online for this figure.

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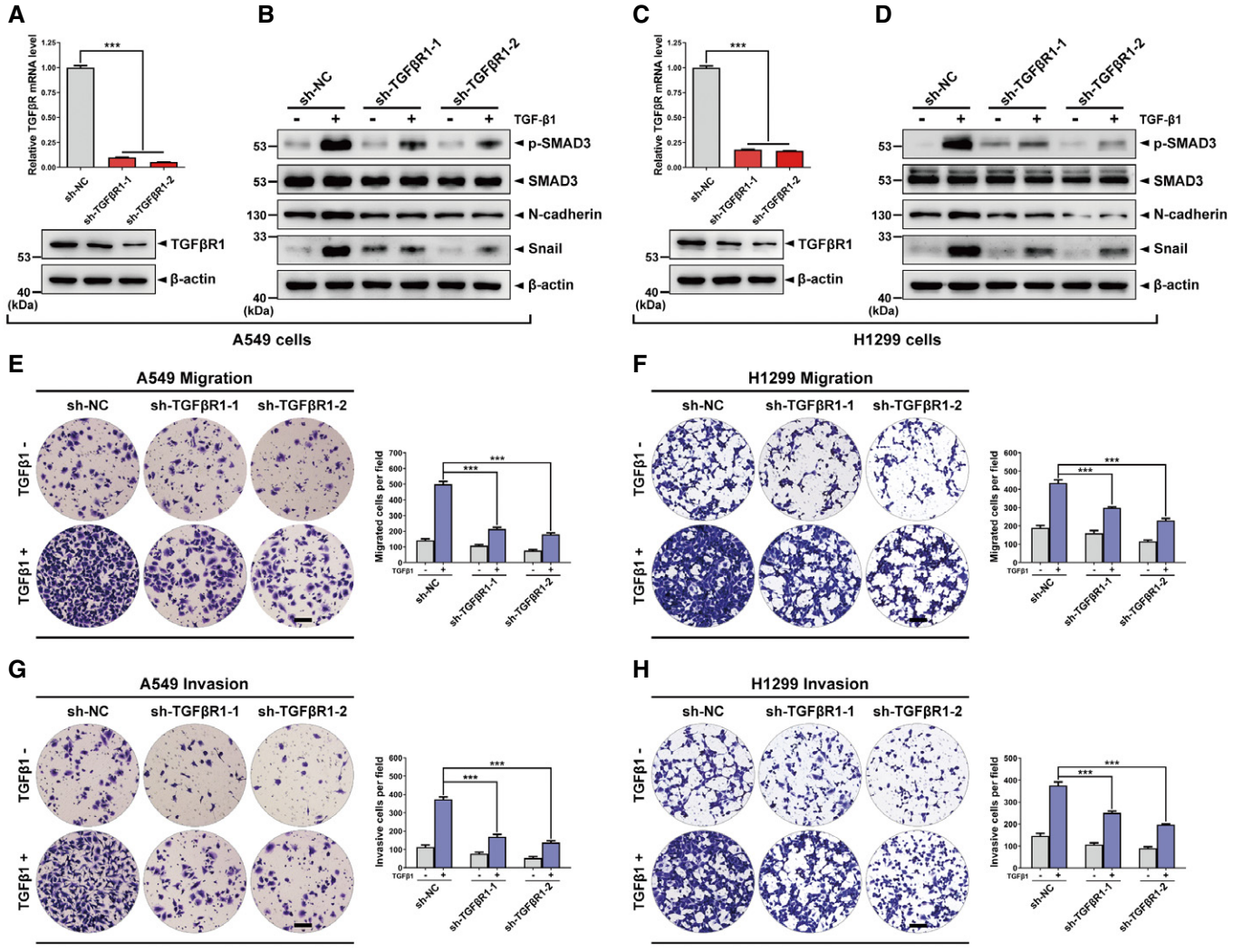

Figure EV2. Knockdown of TGFβR1 suppresses TGF-β-induced EMT and invasion of LUAD cells.

A–D After serum starvation for 24 h, TGFβR1-silenced A549 and H1299 cells (A, C) were stimulated with or without TGF-β1 (5 or 10 ng/ml) for 24 h. Data are shown as the mean ± SD of $n = 3$ technical replicates. ***$P < 0.001$ compared to sh-NC by unpaired Student's t-test. The expression of p-SMAD3, SMAD3, and EMT markers (N-cadherin and Snail) was determined using Western blot. β-actin was used as an internal control (B, D).

E–H In the presence or absence of TGF-β1, the migratory and invasive capabilities of TGFβR1-silenced A549 and H1299 cells were evaluated by Transwell assays as described in Fig 4G–J. Migrated and invasive cells were stained and counted under a light microscope. Scale bar, 100 μm. Data are shown as the mean ± SD of $n = 3$ technical replicates. ***$P < 0.001$ by unpaired Student's t-test.

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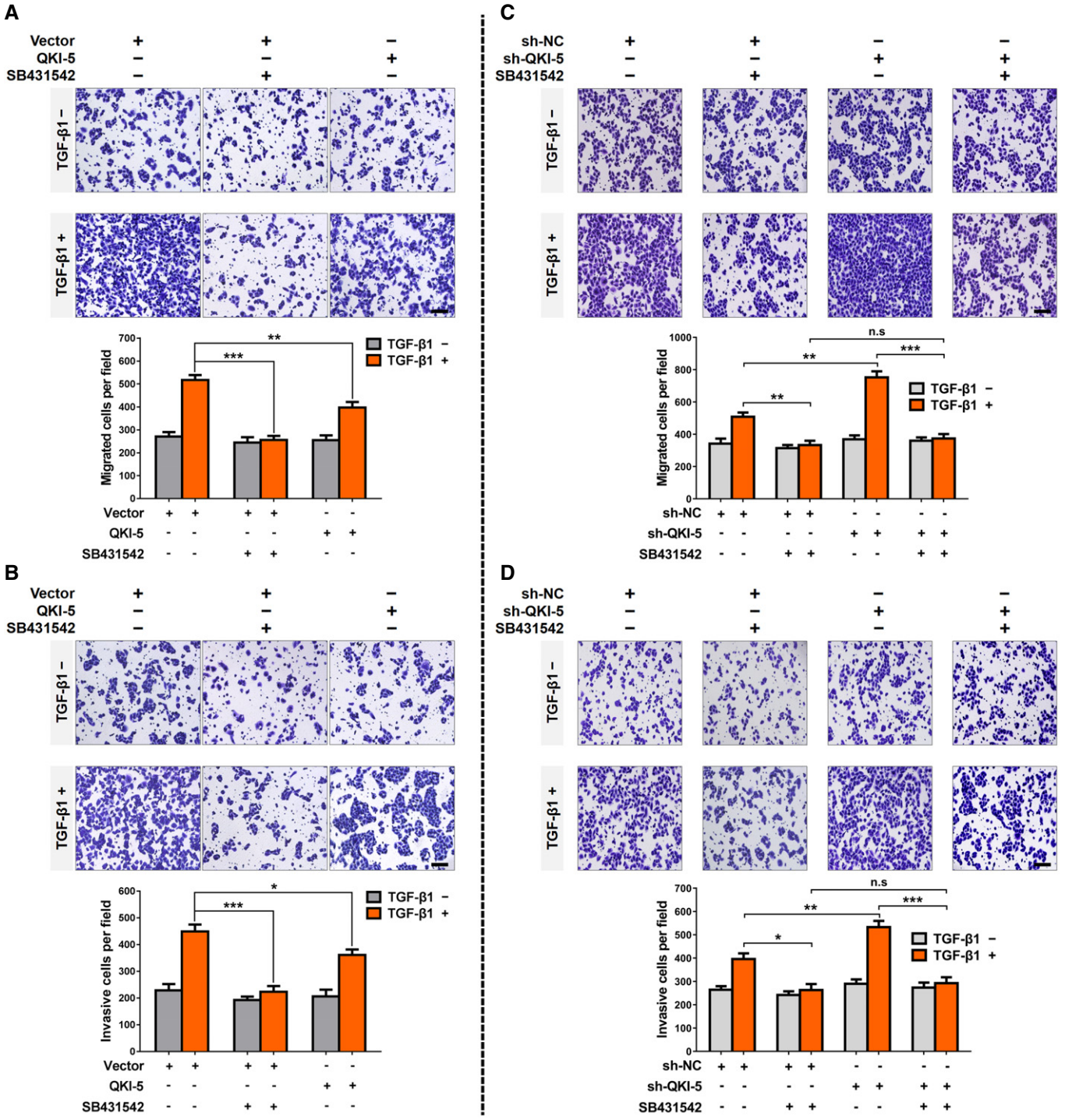

Figure EV3. QKI-5 inhibits TGF-β-induced EMT and LUAD cell invasion via a TGFβR1-dependent manner.

A, B QKI-5-overexpressing and control vector A549 cells treated with or without SB431542 (a specific TGFβR1 inhibitor, 10 mM) were stimulated with TGF-β1 (5 ng/ml) for 24 h and subjected to Transwell migration and invasion assays. Scale bar, 100 μm. Data are shown as the mean ± SD of $n = 3$ technical replicates. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ by unpaired Student's t-test.

C, D QKI-5-silenced and control sh-NC A549 cells treated with or without SB431542 were stimulated with TGF-β1 (5 ng/ml) for 24 h and subjected to Transwell migration and invasion assays. Scale bar, 100 μm. Data are shown as the mean ± SD of $n = 3$ technical replicates. n.s, not significant; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ by unpaired Student's t-test.

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Figure EV4. KLF6 knockdown promotes TGFβR1 expression and TGF-β-induced EMT as well as LUAD cell invasion in a QKI-5-dependent manner.

A, B A549 control cells were transfected with control siRNA or siRNA against KLF6 (si-KLF6-2), and A549 cells overexpressing QKI-5 were transfected with si-KLF6-2. Cells were then subjected to qRT–PCR and Western blot assays for detection of KLF6, QKI-5, and TGFβR1 expression. Data are shown as the mean ± SD of $n = 3$ technical replicates. $^{**}P < 0.01$ and $^{***}P < 0.001$ by unpaired Student's *t*-test.

C After serum starvation for 24 h, the above-mentioned A549 cells (A) were stimulated with or without TGF-β1 (5 ng/ml) for 24 h. The expression of p-SMAD3, SMAD3, and EMT markers (N-cadherin and Snail) was determined using Western blot. β-actin was used as an internal control. Densitometry values of each protein were normalized to β-actin and shown below the corresponding bands.

D, E A549 cells were treated as above and subjected to wound-healing migration assays. The wound healing was recorded (D) and quantitatively measured (E) at least six times. Scale bar, 100 μm. Data are shown as the mean ± SD of $n = 6$ technical replicates. $^{***}P < 0.001$ by unpaired Student's *t*-test.

F, G The migratory and invasive capabilities of A549 cells treated as above were determined by Transwell assays. Migrated (F) and invasive (G) cells were stained and counted in four microscopic fields. Scale bar, 100 μm. Data are shown as the mean ± SD of $n = 3$ technical replicates. $^{*}P < 0.05$ and $^{**}P < 0.01$ by unpaired Student's *t*-test.

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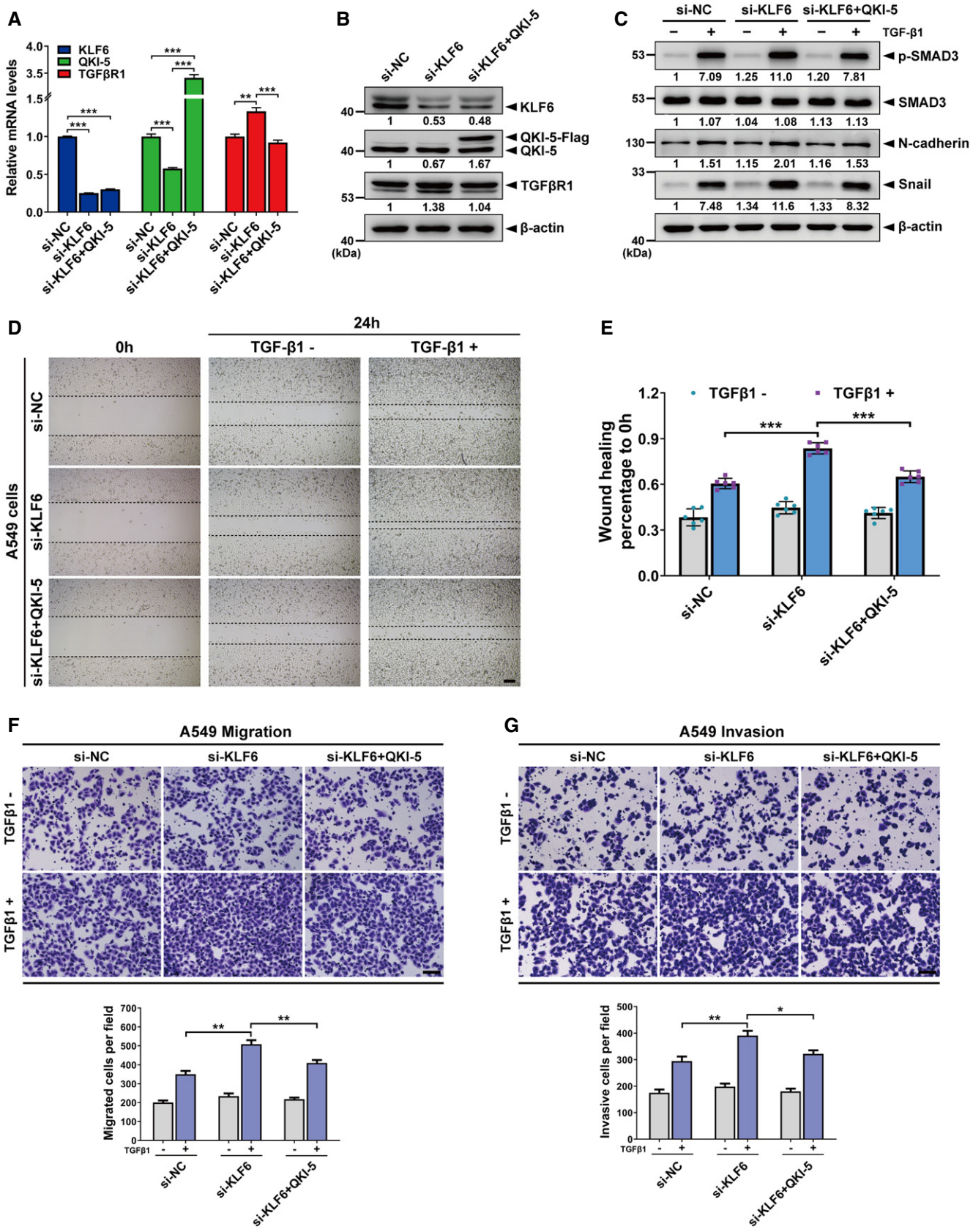
A
Relative mRNA levels
KLF6
QKI-5
TGFβR1
si-NC
si-KLF6
si-KLF6+QKI-5
B
si-NC
si-KLF6
si-KLF6+QKI-5
KLF6
QKI-5-Flag
QKI-5
TGFβR1
β-actin
(kDa)
C
si-NC
si-KLF6
si-KLF6+QKI-5
TGF-β1
p-SMAD3
SMAD3
N-cadherin
Snail
β-actin
(kDa)
D
0h
24h
TGF-β1 -
TGF-β1 +
A549 cells
si-NC
si-KLF6
si-KLF6+QKI-5
E
TGFβ1 -
TGFβ1 +
Wound healing percentage to 0h
si-NC
si-KLF6
si-KLF6+QKI-5
F
A549 Migration
si-NC
si-KLF6
si-KLF6+QKI-5
TGFβ1 -
TGFβ1 +
Migrated cells per field
TGFβ1
si-NC
si-KLF6
si-KLF6+QKI-5
G
A549 Invasion
si-NC
si-KLF6
si-KLF6+QKI-5
TGFβ1 -
TGFβ1 +
Invasive cells per field
TGFβ1
si-NC
si-KLF6
si-KLF6+QKI-5

Figure EV4.

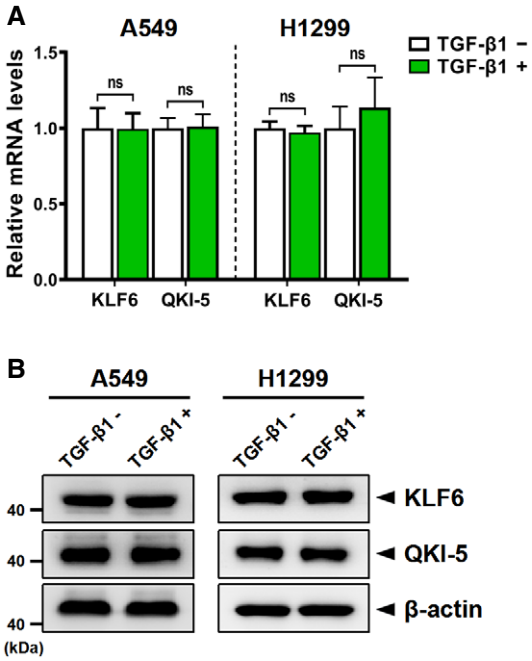

Figure EV5. KLF6 and QKI-5 expressions are not affected by TGF-β1 in LUAD cells.

A, B After being serum-starved for 24 h, A549 and H1299 cells were treated with or without TGF-β1 (5 or 10 ng/ml) for 24 h and subjected to qRT–PCR and Western blot assays for detecting the mRNA and protein expression of KLF6 and QKI-5. β-actin was used as an internal control. Data are shown as the mean ± SD of $n = 3$ technical replicates. ns, not significant; by unpaired Student's *t*-test.

Source data are available online for this figure.

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