SUPPLEMENTAL FIGURE LEGENDS:

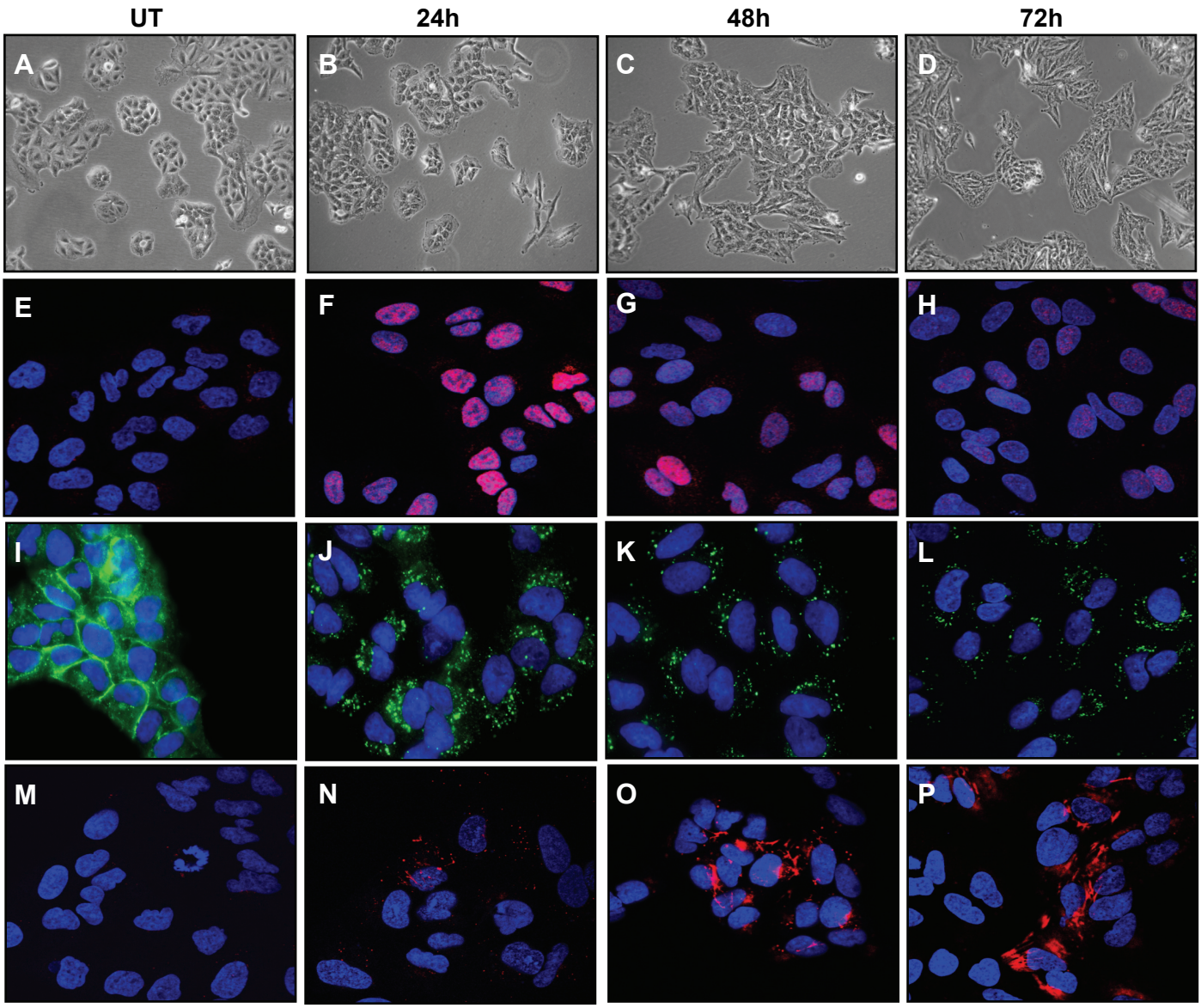
FIGURE S1. **TGF-β1 induces EMT in lung cancer A549 cells.** *A*-*D*, A549 cells were grown in the absence (UT) or presence of TGF-β1 for the indicated times. Phase contrast images are shown. *E*-*P*, cells were fixed and stained with antibodies against pSmad3 (red) (*E*-*H*), E-cadherin (green) (*I*-*L*) and fibronectin (red) (*M*-*P*), followed by incubation with fluorescent dye-tagged secondary antibodies. DAPI was used to stain nuclei. *R*, untreated (UT) or TGF-β1-treated A549 cells were lysed and cleared cell lysates were subjected to SDS-PAGE and immunoblotted with anti-pSmad3, anti-Smad3, anti-fibronectin or anti-E-cadherin antibody. Equal protein loading was confirmed by reprobing blots with an anti-β-actin antibody.

FIGURE S2. **Zyxin localizes at adherens junctions.** A549 cells grown in either serum free media or media supplemented with TGF-β1 for 24 h. Cells were fixed and immunostained with anti-zyxin (red) or anti-p120 (green) antibody. DAPI (blue) was used to stain nuclei. Scale bar: 20 μm.

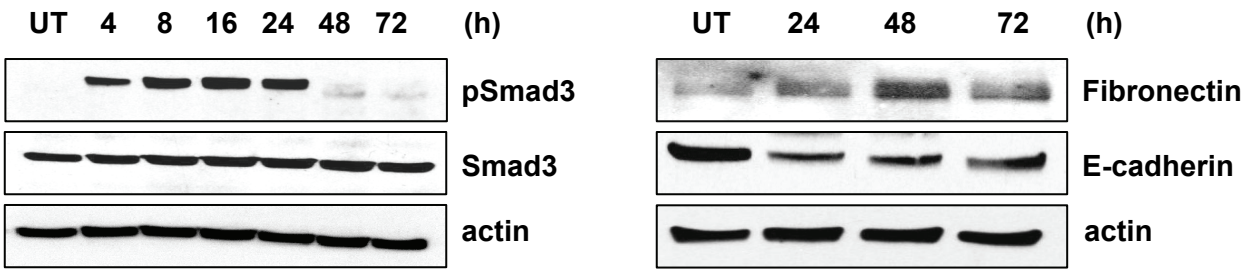
FIGURE S3. **TGF-β1-dependent zyxin expression in lung cancer cells.** NSCLC cells (A549, H2030, H441 and H1299) and SCLC cells (DMS273 and H82) were either untreated (UT) or treated with TGF-β1 for indicated times. Cells were lysed and Western blotting performed by using anti-pSmad3, anti-Smad3 or anti-zyxin antibodies. Anti-β-actin was used as a loading control. Representative blots are shown.

FIGURE S4. **Zyxin mediates integrin α5 expression.** *A*, cells were transfected with either control (ctr) or one of two independent siRNAs directed against zyxin. Transfected cells were treated with TGF-β1 for 24 h and whole-cell lysates prepared. Equal amounts of protein were analyzed by Western blotting using anti-zyxin antibody and anti-β-actin as a loading control. A549 cells were transfected with either control or zyxin specific siRNA. Control and zyxin-depleted A549 cells were grown in the absence or presence of TGF-β1 for 24 h. Cells were incubated with antibody against integrin α5 or immunoglobulin (IgG; gray) as isotype control and analysed by flow cytometry. Representative histograms are shown. *B*, A549 cells were transiently transfected with either control EGFP vector or zyxin-EGFP expression vector and then incubated in presence or absence of TGF-β1 for 24 h. The mRNA levels of both integrin α5 and integrin β1 were quantified by qRT-PCR. Data are presented as the mean ±SD of three independent experiments performed in duplicates.

Figure S1


UT
24h
48h
72h
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P

R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Smad3
actin
UT 24 48 72 (h)
Fibronectin
E-cadherin
actin

Figure S2

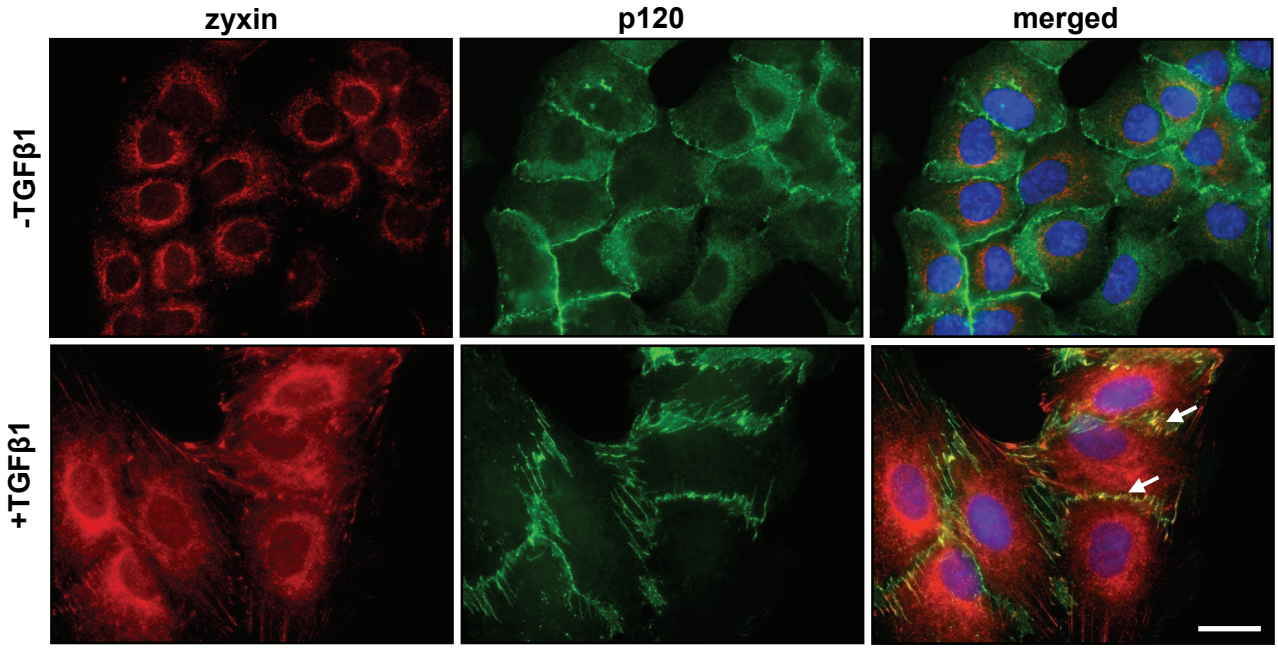


Figure S3

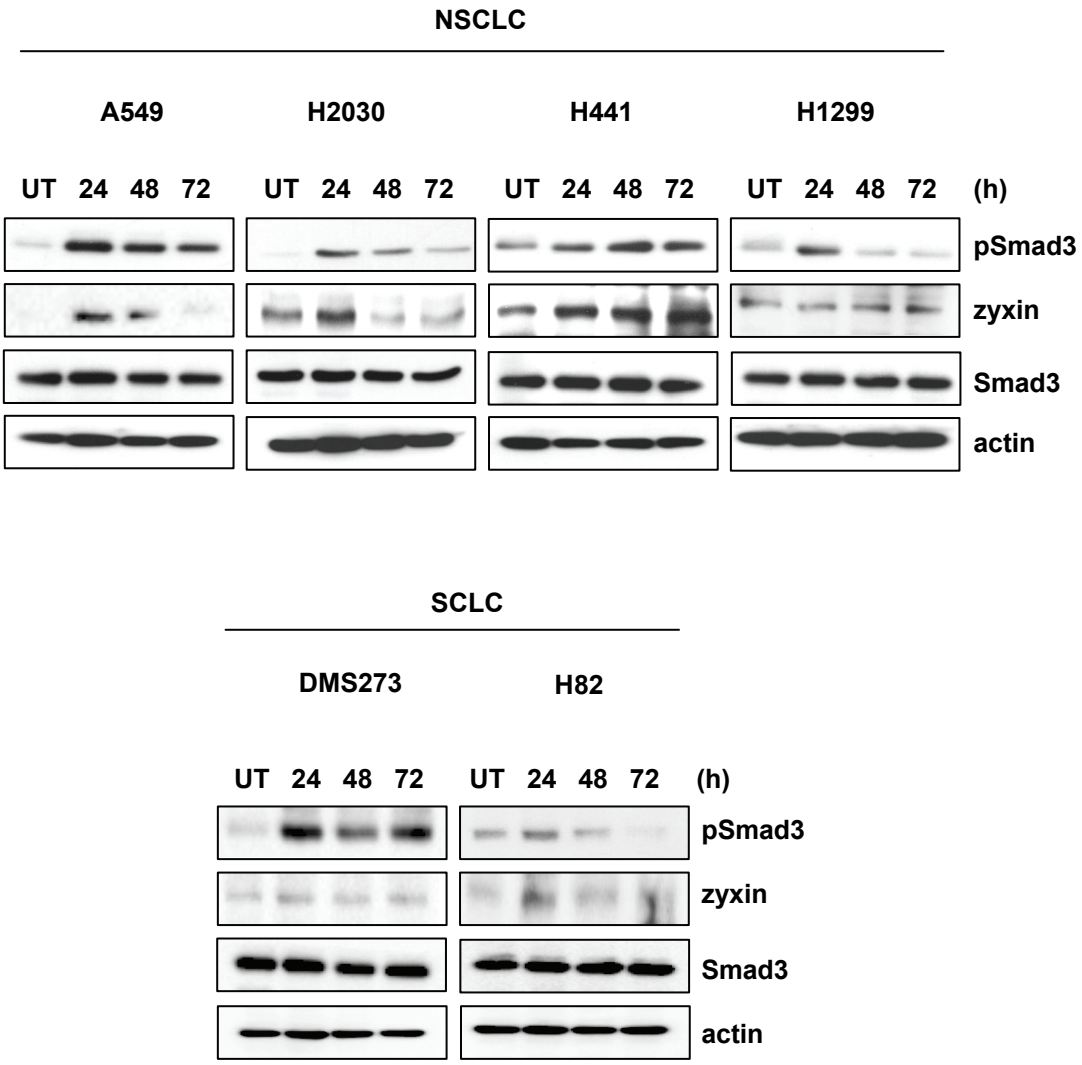
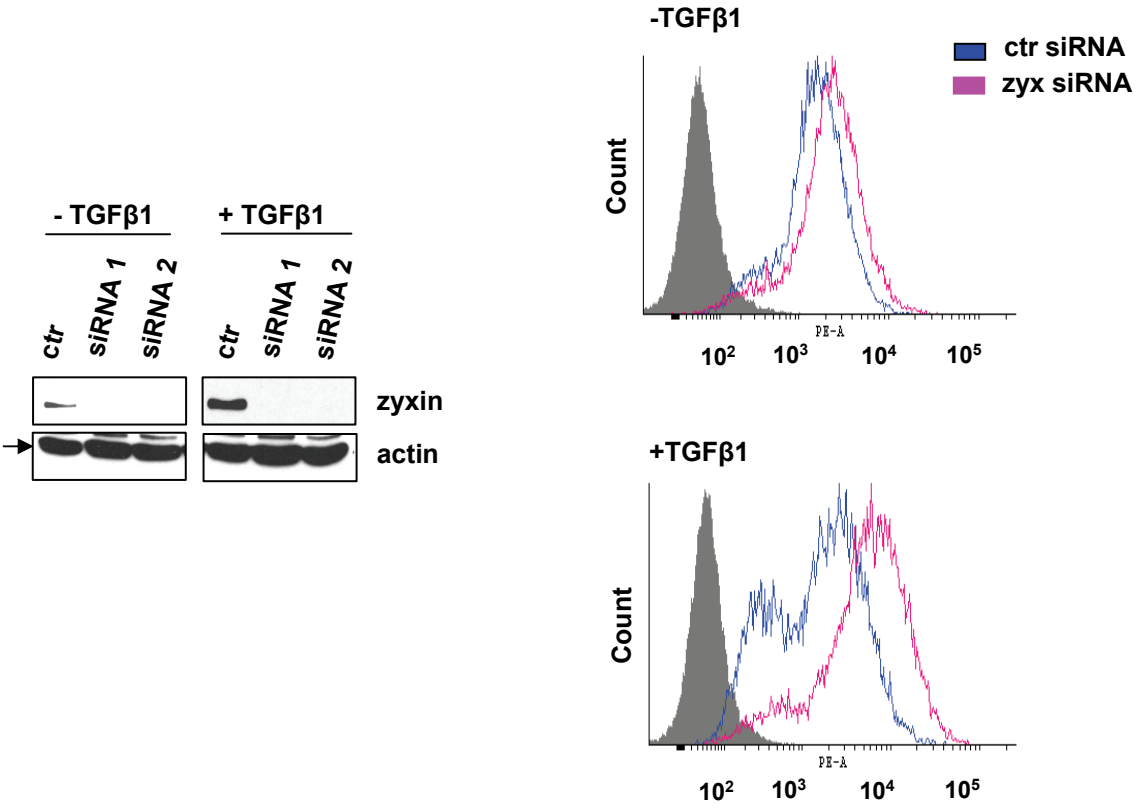

NSCLC
A549
H2030
H441
H1299
UT 24 48 72
UT 24 48 72
UT 24 48 72
UT 24 48 72
(h)
pSmad3
zyxin
Smad3
actin
SCLC
DMS273
H82
UT 24 48 72
UT 24 48 72
(h)
pSmad3
zyxin
Smad3
actin

Figure S4

A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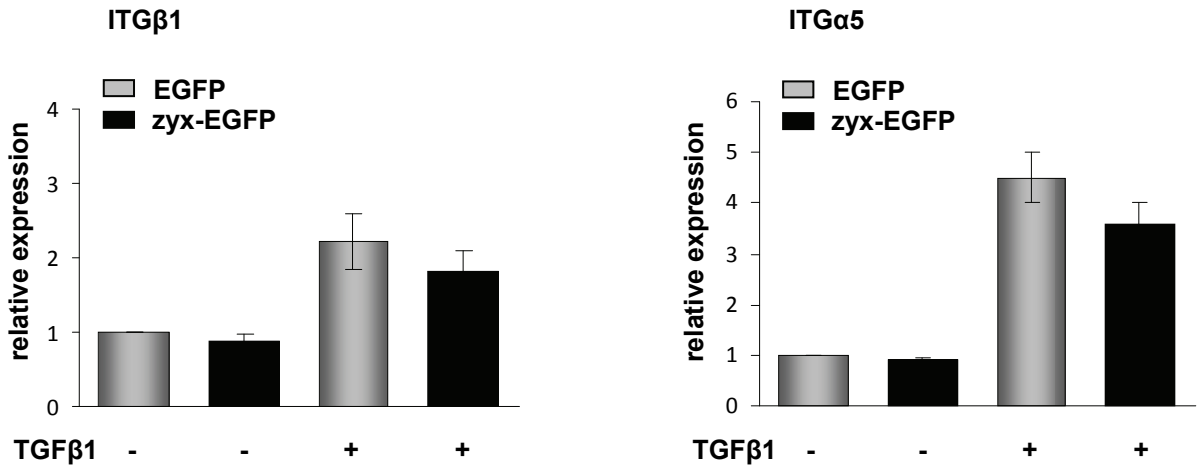
+ TGFβ1
ctr
siRNA 1
siRNA 2
ctr
siRNA 1
siRNA 2
zyxin
actin
-TGFβ1
ctr siRNA
zyx siRNA
Count
PE-A
10²
10³
10⁴
10⁵
+TGFβ1
Count
PE-A
10²
10³
10⁴
10⁵

B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EGFP
zyx-EGFP
relative expression
0
1
2
3
4
TGFβ1 - - + +
ITGα5
EGFP
zyx-EGFP
relative expression
0
1
2
3
4
5
6
TGFβ1 - - + +