

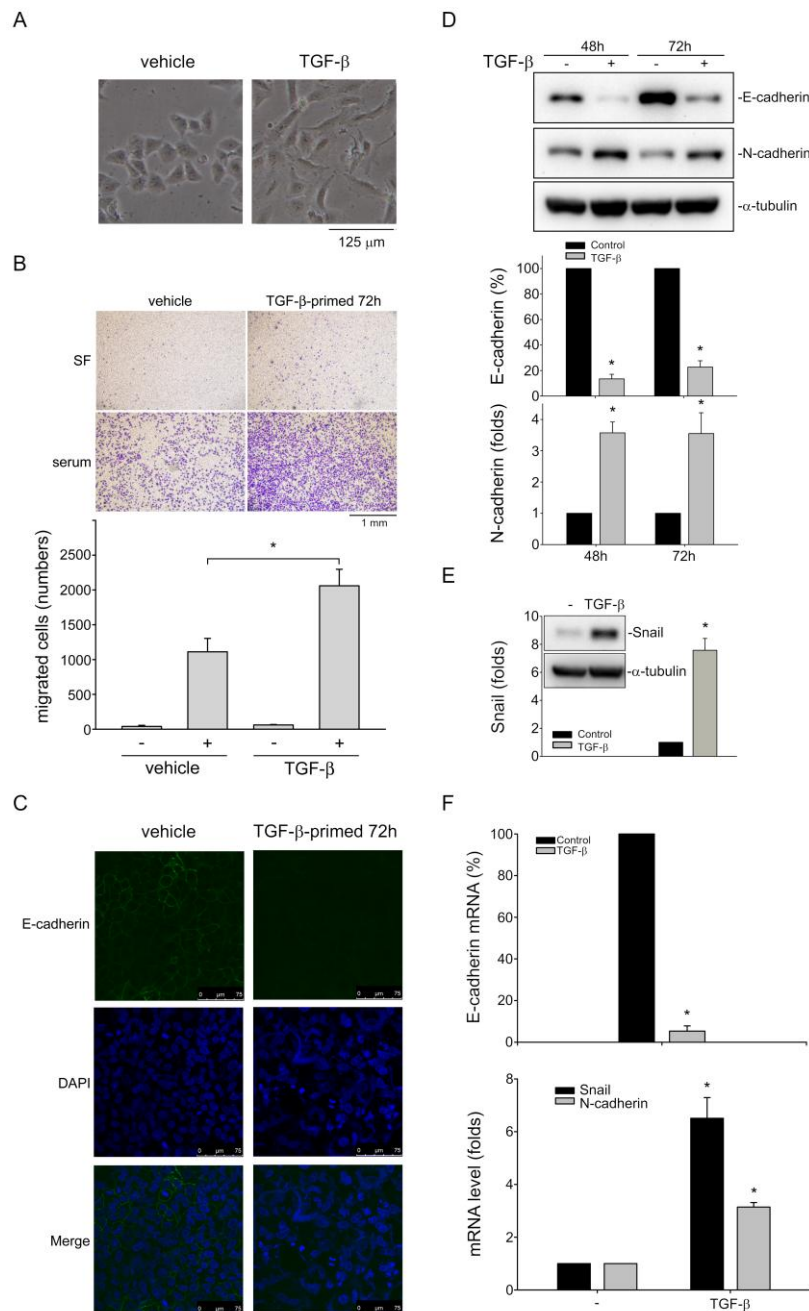
Supplement Information

Supplement Methods

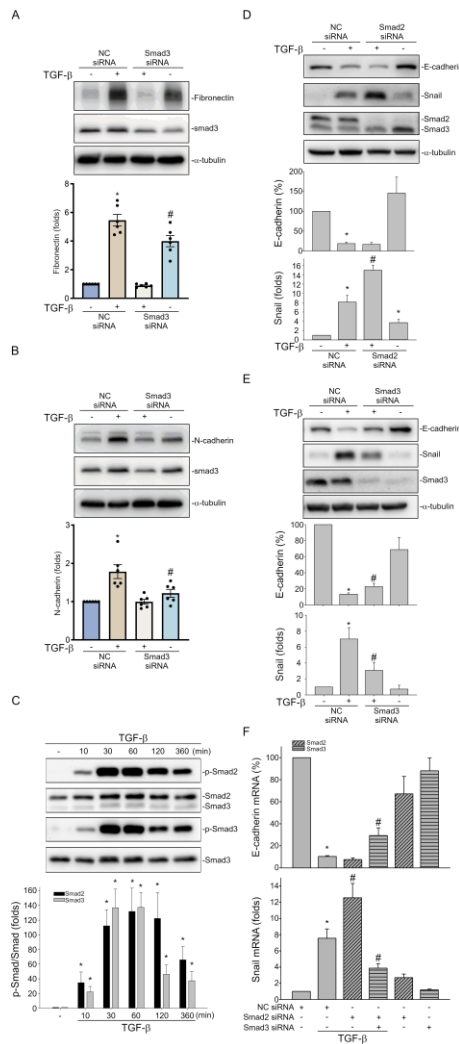
Chromatin immunoprecipitation (ChIP) assay

A ChIP assay was performed as previously described (Huang et al., 2025). Briefly, cells were cross-linked with 1% formaldehyde at 37 °C for 10 min, rinsed with ice-cold PBS, and lysed in SDS buffer. Chromatin was sonicated (4 x 15 s) and centrifuged (10 min), and the supernatant was diluted in a ChIP dilution buffer. After pre-clearing with protein A-agarose (60 µl, 1 h, 4 °C), an aliquot of each sample was used as “input” in the PCR analysis. The remaining soluble chromatin was incubated overnight at 4°C with either control normal IgG or Smad3 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunocomplexes were captured using protein A-agarose (20 µl, 2 h, 4 °C), followed by sequential washes in low-salt, high-salt, and LiCl immune complex washing buffers, and two washes in Tris-EDTA buffer. DNA was eluted, cross-links reversed (0.2 M NaCl, 65 °C, 4 h), and purified using GPTM DNA purification spin columns (Viogene, New Taipei City, Taiwan). PCR amplification of a 164-bp *snail* promoter fragment (-1052 to -889), a 166-bp *GLUT1* promoter fragment (-167 to -2), or a 222-bp *PFKFB3* promoter fragment (-2579 to -2358) was performed using Promega PCR Master Mix with primers as follows: For *snail* promoter fragment, sense: 5'-cctcagctgaaaatccttcg-3' and antisense: 5'-agaagtggcaactgcttcaga-3'; For *GLUT1* promoter fragment, sense: 5'-gatgcagatggatgcctca-3' and antisense: 5'-tctgggagaagtttgctttg-3'; For *PFKFB3* promoter fragment, sense: 5'-tgtgttcaccaaccagaaa-3' and antisense: 5'-tggtgaaacccgtctctac-3'; PCR conditions: 30 cycles (95 °C for 30 s, 56 °C for 30 s, 72 °C for 45 s). Products were analyzed by 1.5 % agarose gel electrophoresis

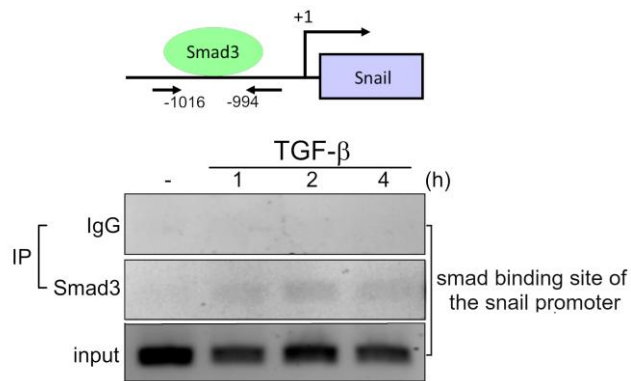
Supplement Figures



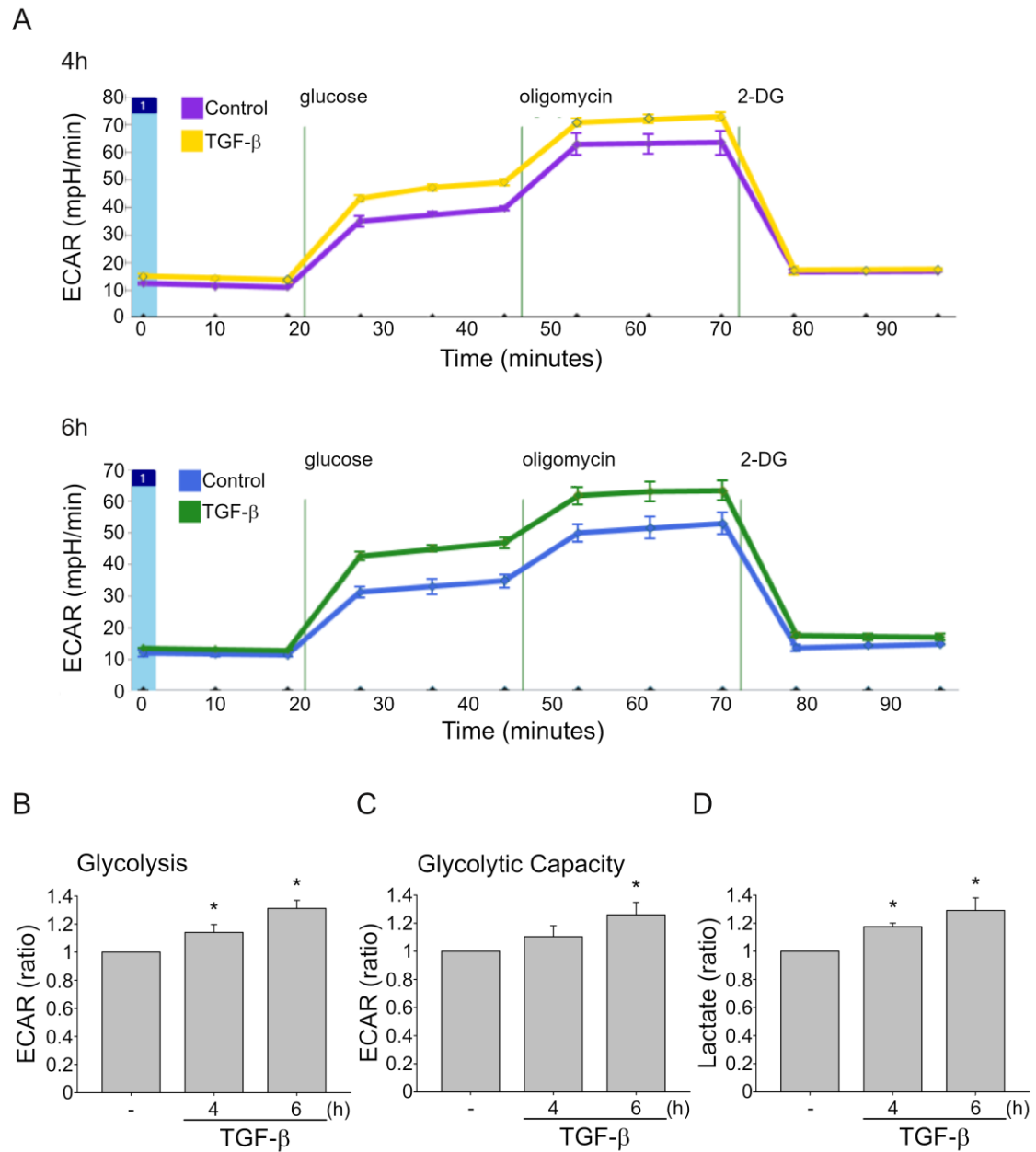
Supplement Figure S1. TGF- β induces EMT in A549 cells. (A) Representative phase-contrast microscopy images of A549 cells stimulated with TGF- β (10 ng/ml) for 72 h; scale bar = 125 μ m. (B) Transwell migration assay of A549 cells following 72 h of TGF- β stimulation (10 ng/ml) (n=4). (C) Confocal immunofluorescence analysis of E-cadherin distribution in A549 cells treated with TGF- β (10 ng/ml) for 72 h (n=3). (D, E) Immunoblot analysis of E-cadherin (48 or 72 h; D) and Snail (24 h; E) protein expression (n=6). (F) Relative mRNA levels of *E-cadherin*, *N-cadherin*, and *Snail* following 24 h of TGF- β treatment (10 ng/ml) as determined by qRT-PCR (n=6). Data are presented as mean $\pm$ SEM. *p<0.05, compared with the control group.



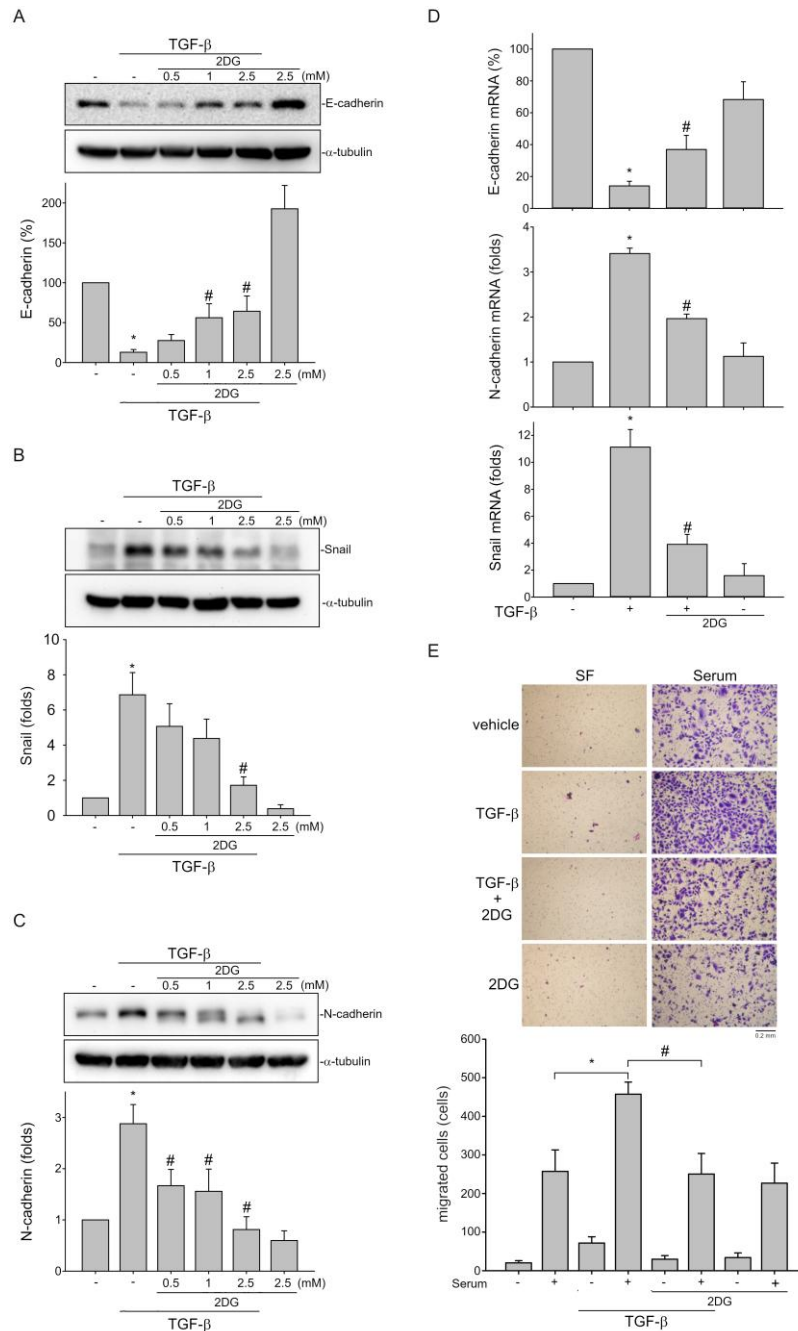
Supplement Figure S2. Smad3 mediates TGF- β -induced EMT in BEAS-2B or A549 cells. (A, B) Effects of Smad3 knockdown on mesenchymal markers, Fibronectin (A) and N-Cadherin (B) in BEAS-2B cells. Cells were transfected with negative control (siNC), or Smad3 siRNA for 48 h, followed by TGF- β stimulation (10 ng/ml) for 72 h. Protein levels were determined by immunoblotting (n=6). *p<0.05, compared with the control group; #p<0.05, compared with the negative control group in the presence of TGF- β . (C) Immunoblot analysis of Smad2 and Smad3 phosphorylation in A549 cells treated with TGF- β (10 ng/ml) for the indicated time course (n=6). (D, E) Effects of Smad2 (D) or Smad3 (E) knockdown on EMT markers in A549 cells. Cells were transfected with negative control (siNC), Smad2, or Smad3 siRNA for 48 h, followed by TGF- β stimulation (10 ng/ml) for 24 h (Snail) or 72 h (E-cadherin). Protein levels were determined by immunoblotting (n=6). (F) Relative mRNA levels of *Snail* (6 h) and *E-cadherin* (24 h) in A549 cells determined by qRT-PCR following siRNA transfection and TGF- β stimulation (n=6). Data are presented as mean $\pm$ SEM. *p<0.05, compared with the control group; #p<0.05, compared with the negative control group in the presence of TGF- β .



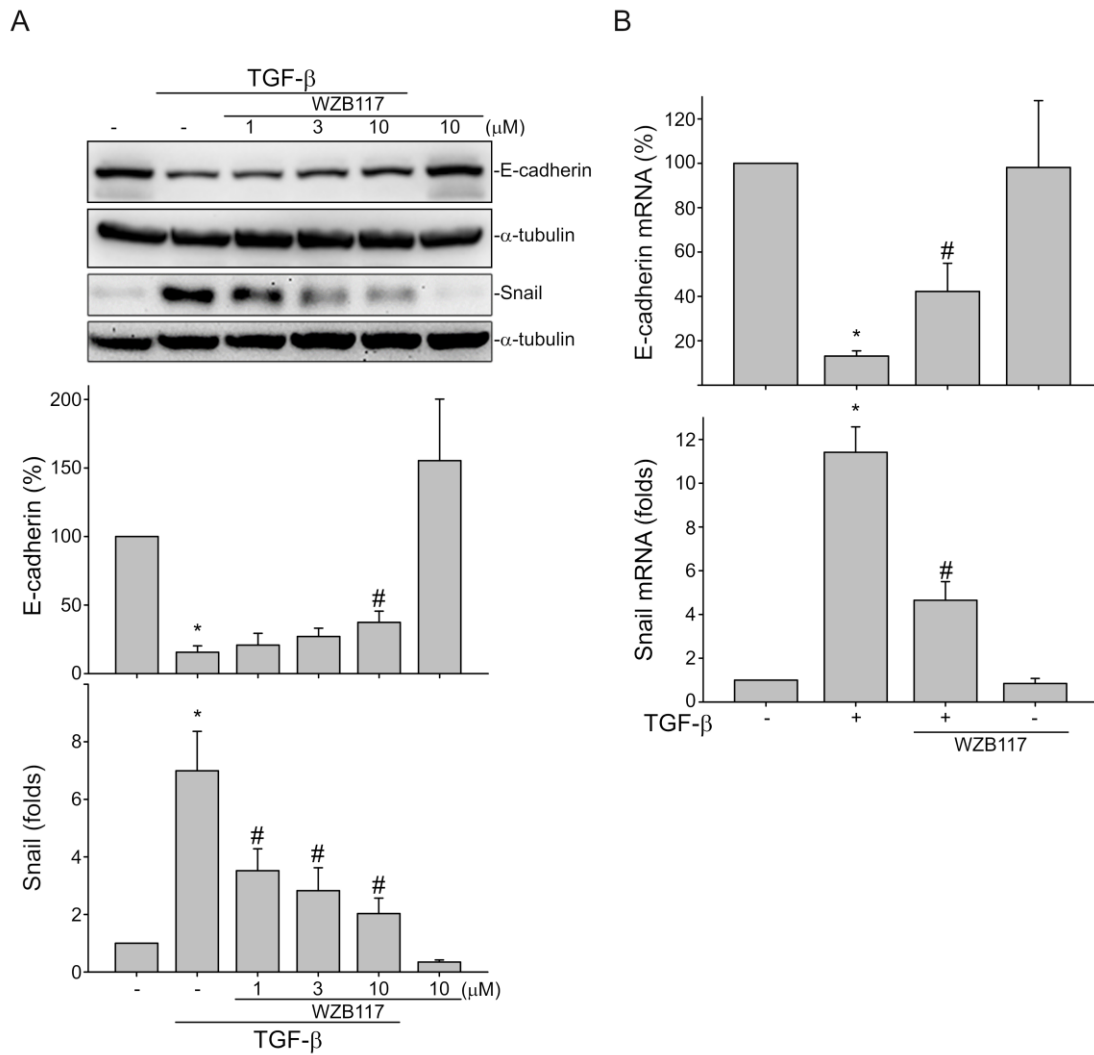
Supplement Figure S3. TGF- β promotes Smad3 binding to the Snail promoter in A549 cells. Chromatin immunoprecipitation (ChIP) analysis of Smad3 binding to the *Snail* promoter region in A549 cells stimulated with TGF- β (10 ng/ml) for the indicated time points. Representative PCR gel traces are shown from one of three independent experiments with similar results.



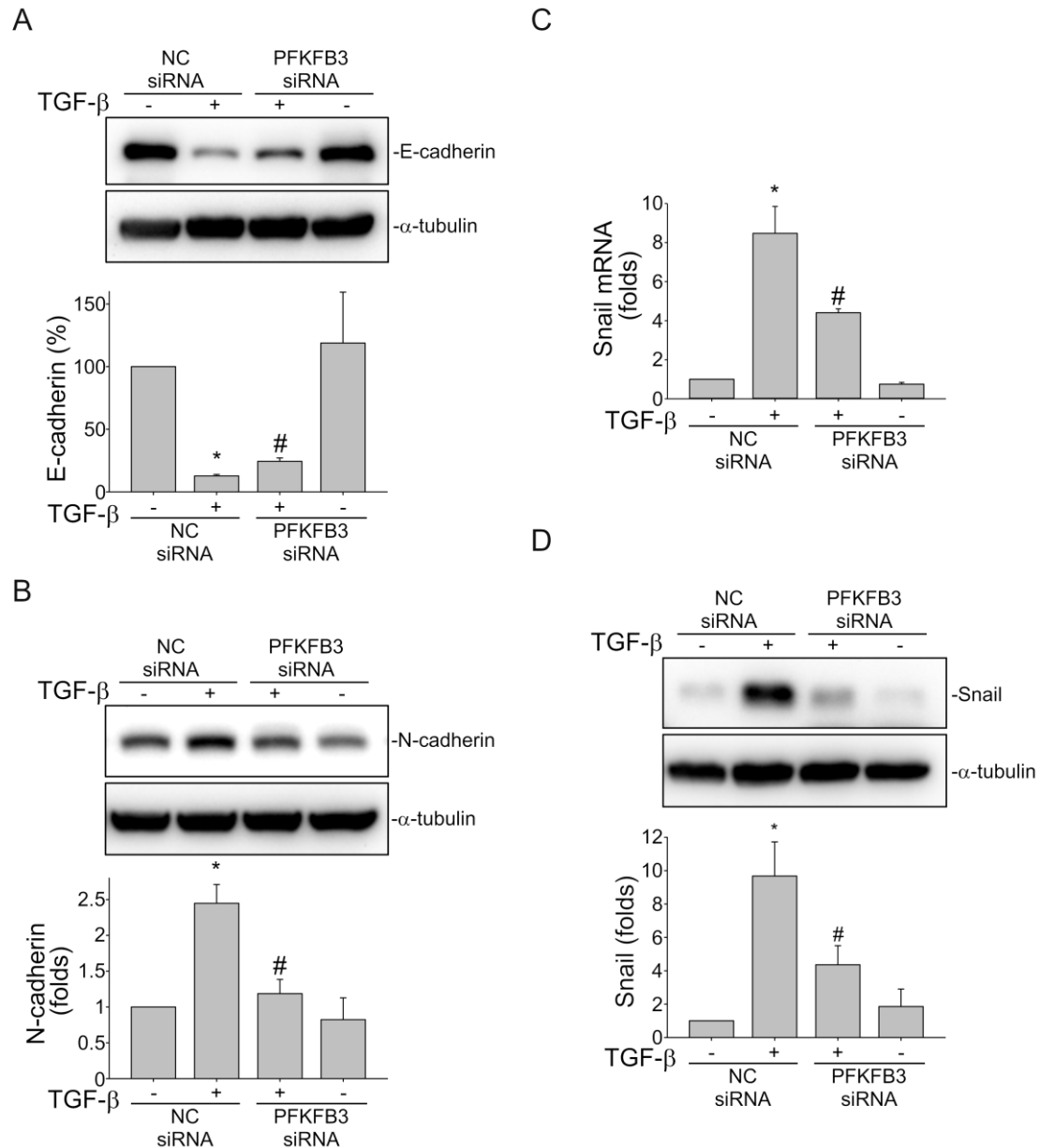
Supplement Figure S4. TGF- β promotes glucose uptake and glycolysis in A549 cells. (A) Real-time extracellular acidification rate (ECAR) in A549 cells treated with TGF- β (10 ng/ml) for 4 or 6 h, measured via Seahorse XFe24 analyzer. (B, C) Compiled results for glycolysis (B) and glycolytic capacity (C) derived from ECAR profiles (n=6). (D) Analysis of lactate production in A549 cells (left) and BEAS-2B cells (right) following TGF- β stimulation (10 ng/ml) for 4 or 6 h (n=5). Data are presented as mean $\pm$ SEM. * p <0.05, compared with the control group



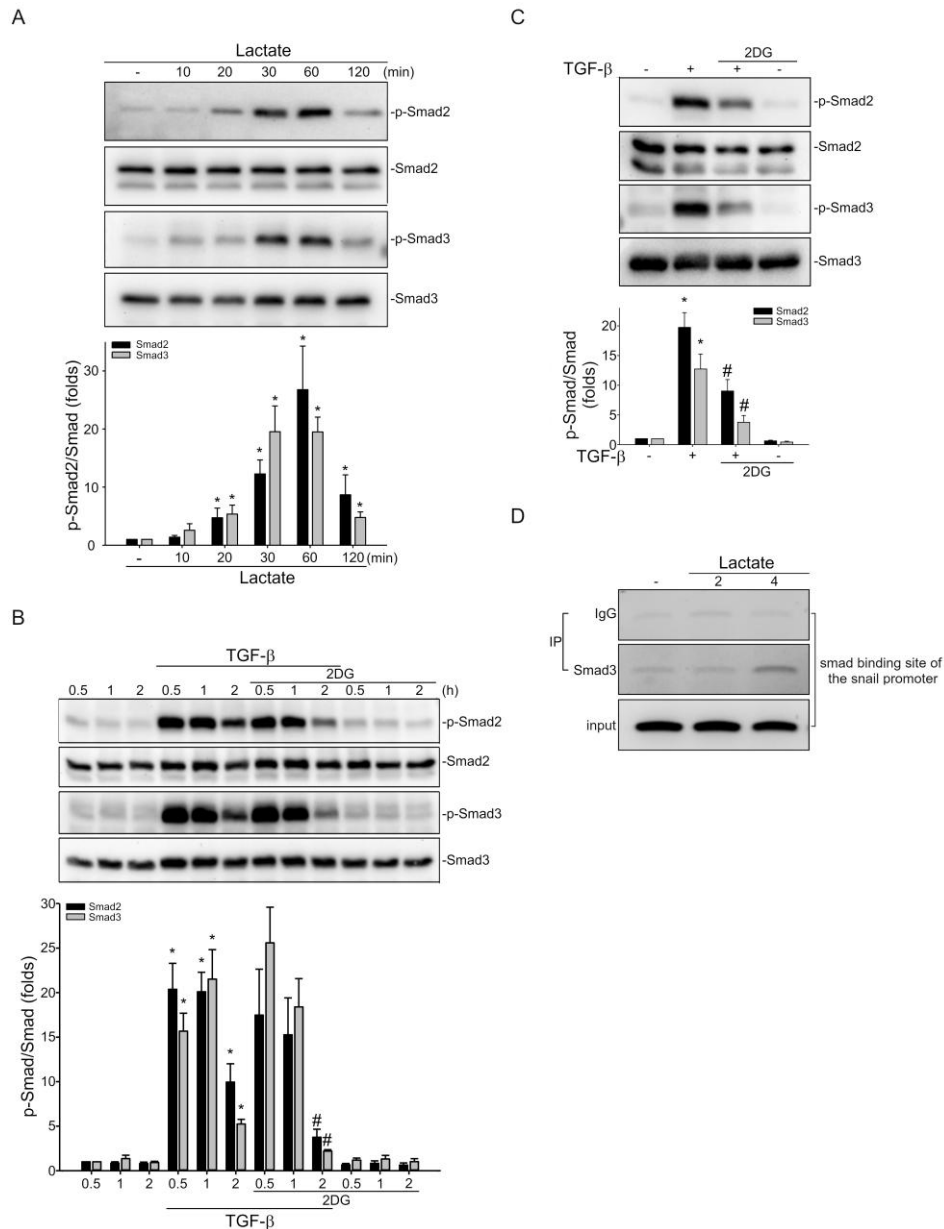
Supplement Figure S5. Glycolysis is required for TGF- β -induced EMT in A549 cells. (A–C) Immunoblot analysis of EMT markers in A549 cells pre-treated with vehicle or 2-deoxy-glucose (2-DG; 1.25 or 2.5 mM, 30 min) followed by TGF- β stimulation (10 ng/ml). Protein levels of E-cadherin (72 h; **A**), Snail (24 h; **B**), and N-cadherin (72 h; **C**) were determined (n=6). (**D**) Relative mRNA levels of *Snail* (6 h), *E-cadherin*, and *N-cadherin* (24 h) determined by qRT-PCR following 2-DG pre-treatment and TGF- β stimulation (n=6). (**E**) Transwell migration assay of A549 cells treated with 2-DG (2.5 mM) and TGF- β (10 ng/ml) for 72 h (n=8). Data are presented as mean $\pm$ SEM. *p<0.05, compared with the control group; #p<0.05, compared with the negative control group in the presence of TGF- β .



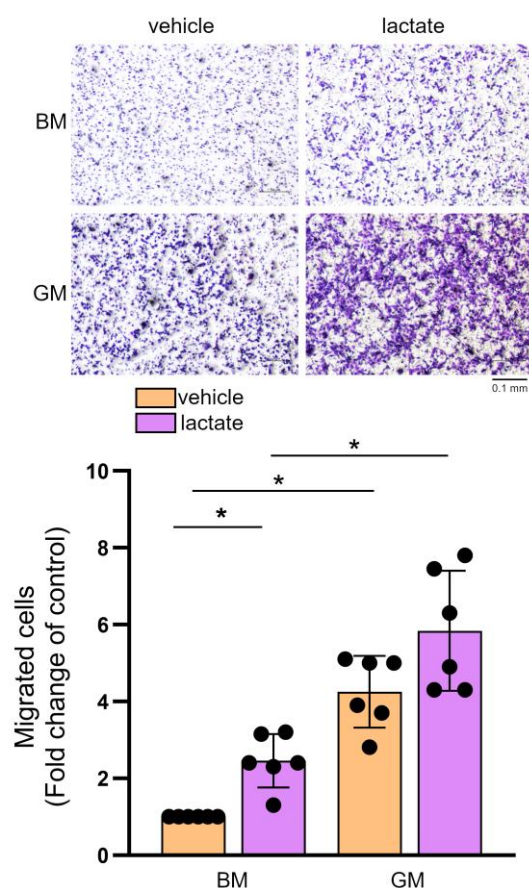
Supplement Figure S6. Pharmacological inhibition of GLUT1 attenuates TGF- β -induced EMT in A549 cells. (A, B) Analysis of E-cadherin and Snail expression in A549 cells pre-treated with vehicle or the GLUT inhibitor WZB117 (10 μ M, 30 min) followed by TGF- β stimulation (10 ng/ml). Protein levels were determined by immunoblotting at 24 h (A), and relative mRNA levels of *E-cadherin* and *Snail* were determined by qRT-PCR (B) (n=6). Data are presented as mean $\pm$ SEM. *p<0.05, compared with the control group; #p<0.05, compared with the negative control group in the presence of TGF- β .



Supplement Figure S7. PFKFB3 is required for TGF- β -induced EMT in A549 cells. (A, B) Immunoblot analysis of E-cadherin (A) and N-cadherin (B) expression in A549 cells transfected with negative control (siNC) or PFKFB3 siRNA for 48 h, followed by stimulation with TGF- β (10 ng/ml) for 48 h (n=6). (C) Relative mRNA levels of *E-cadherin* and *Snail* in PFKFB3-knockdown cells stimulated with TGF- β (10 ng/ml) for 24 h, as determined by qRT-PCR (n=6). Data are presented as mean $\pm$ SEM. *p<0.05, compared with the control group; #p<0.05, compared with the negative control group in the presence of TGF- β .



Supplement Figure S8. Lactate and glycolysis regulate Smad2/3 signaling in A549 cells. (A) Immunoblot analysis of Smad2 and Smad3 phosphorylation in A549 cells treated with lactate (20 mM) over the indicated time course (n=6). (B, C) Effect of glycolysis inhibition on Smad signaling. Cells were pre-treated with 2-DG (2.5 mM, 30 min) followed by TGF- β stimulation (10 ng/ml) for 0.5, 1, 2 h (B) or 6 h (C). Smad2 and Smad3 phosphorylation levels were determined by immunoblotting (n=6). (D) Chromatin immunoprecipitation (ChIP) analysis of Smad3 binding to the *Snail* promoter in A549 cells treated with lactate (20 mM) for the indicated times. Representative PCR gel traces are shown from one of five independent experiments with similar results. Data are presented as mean $\pm$ SEM. *p<0.05, compared with the control group; #p<0.05, compared with the negative control group in the presence of TGF- β .



Supplement Figure S9. Exogenous lactate treatment promotes cell migration in BEAS-2B cells. BEAS-2B cells were pretreated with vehicle or lactate (10 mM) for 72 hours. Following treatment, cells were harvested and resuspended in basal medium (BM). A total of 2×10^4 cells in BM were seeded into the upper chambers of the Transwell plate. To evaluate cell motility, either BM or complete growth medium (GM) was added to the lower chamber as a chemoattractant. After 24 hours of incubation, the BEAS-2B cells that had migrated through the membrane were fixed, stained and quantified as described in the “Materials and Methods” section. Each column represents the mean $\pm$ SEM of six independent experiments. * $p < 0.05$, compared with the indicated group;

References:

Huang, Y. H., Huang, Y. M., Huang, W. J., Yu, M. C., Chuang, C. H., Hsu, Y. F., ... Hsu, M. J. (2025). The hydroxamate based HDAC inhibitor WMJ-J-09 induces colorectal cancer cell death by targeting tubulin and downregulating survivin. *Sci Rep*, 15(1), 19590. doi:10.1038/s41598-025-04714-w