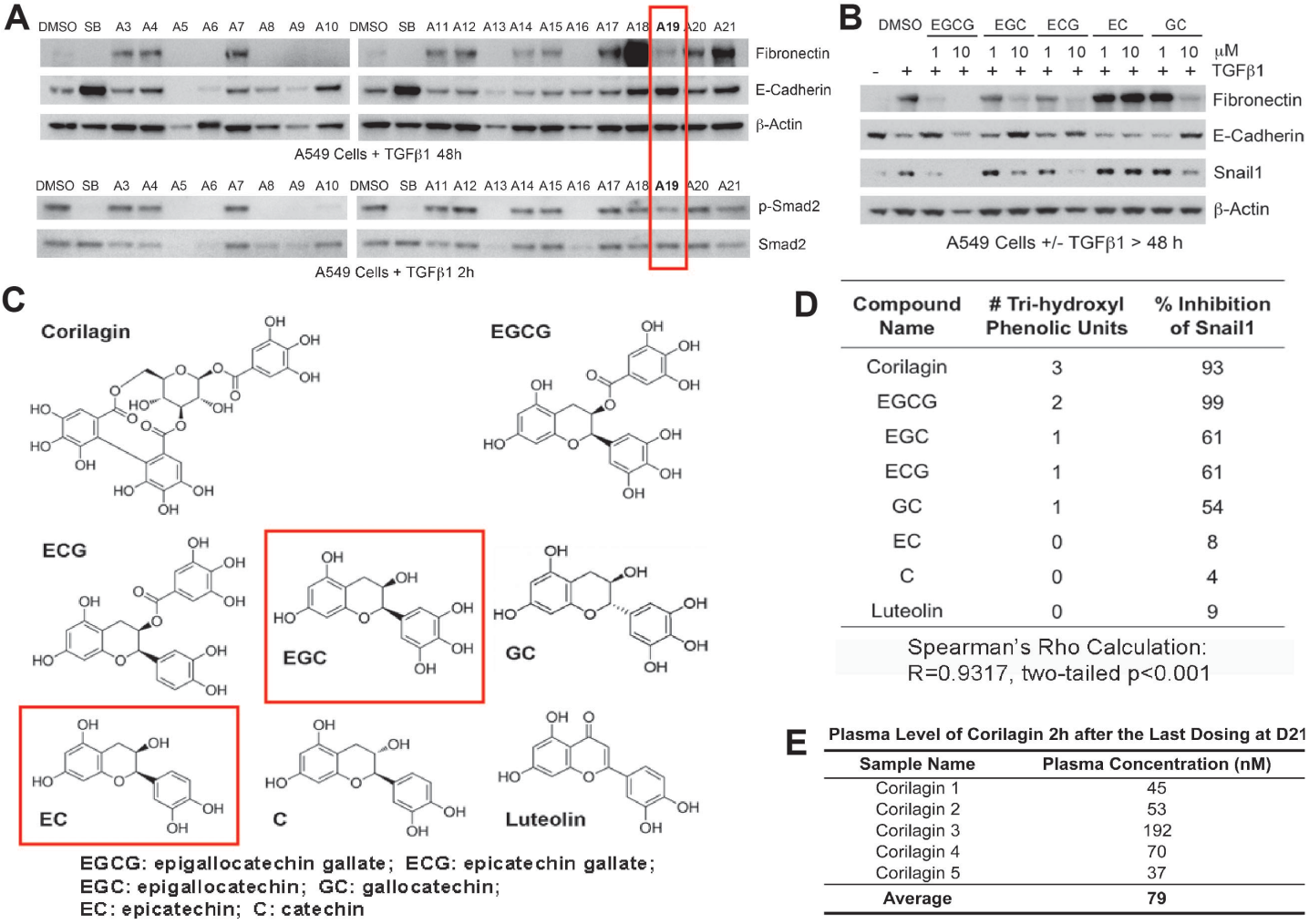
Supplementary Figures with Legends

Supplemental Figure S1



D

Compound Name	# Tri-hydroxyl Phenolic Units	% Inhibition of Snail1
Corilagin	3	93
EGCG	2	99
EGC	1	61
ECG	1	61
GC	1	54
EC	0	8
C	0	4
Luteolin	0	9

Spearman's Rho Calculation:
R=0.9317, two-tailed p<0.001

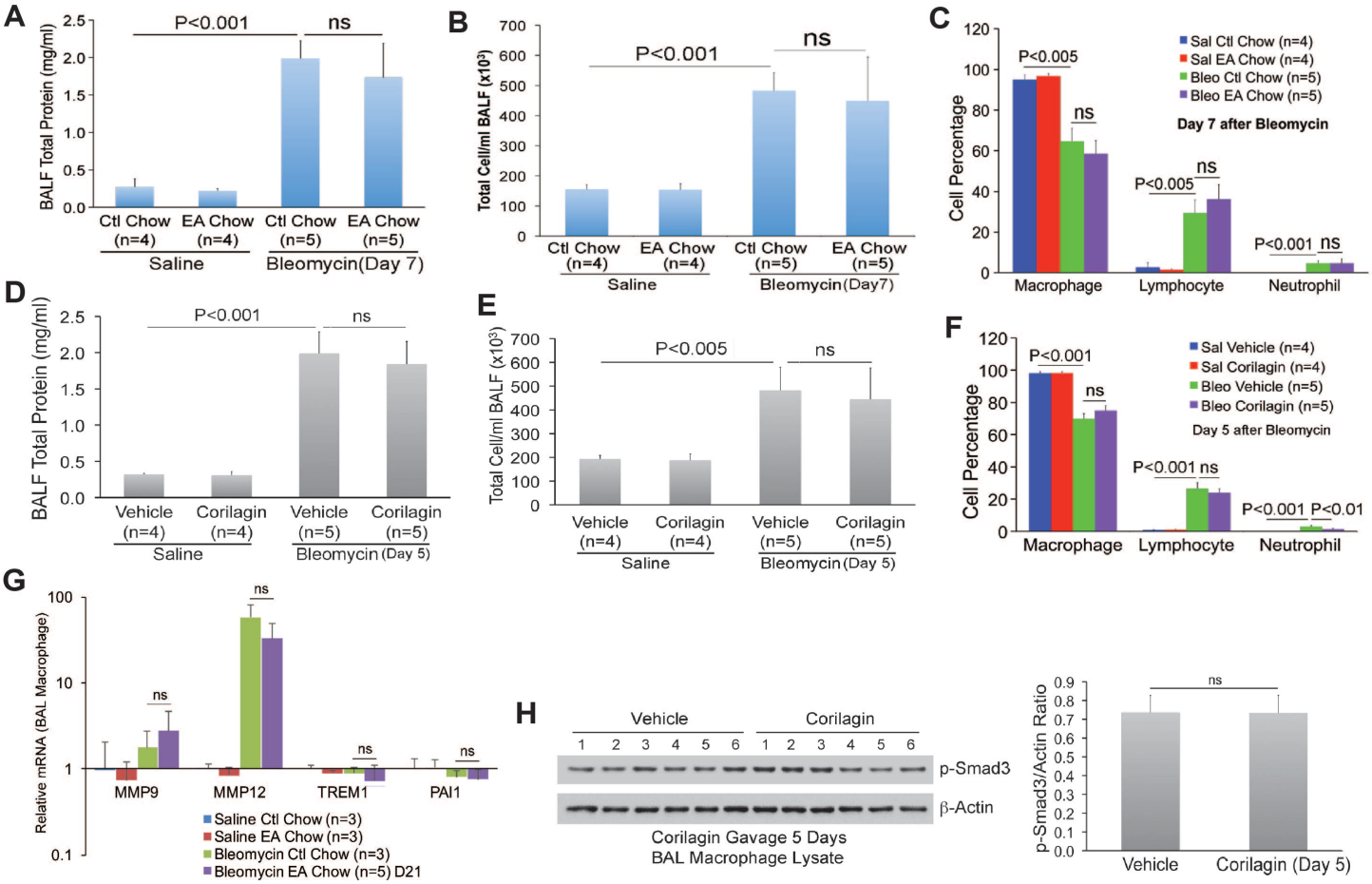
E

Plasma Level of Corilagin 2h after the Last Dosing at D21

Sample Name	Plasma Concentration (nM)
Corilagin 1	45
Corilagin 2	53
Corilagin 3	192
Corilagin 4	70
Corilagin 5	37
Average	**79**

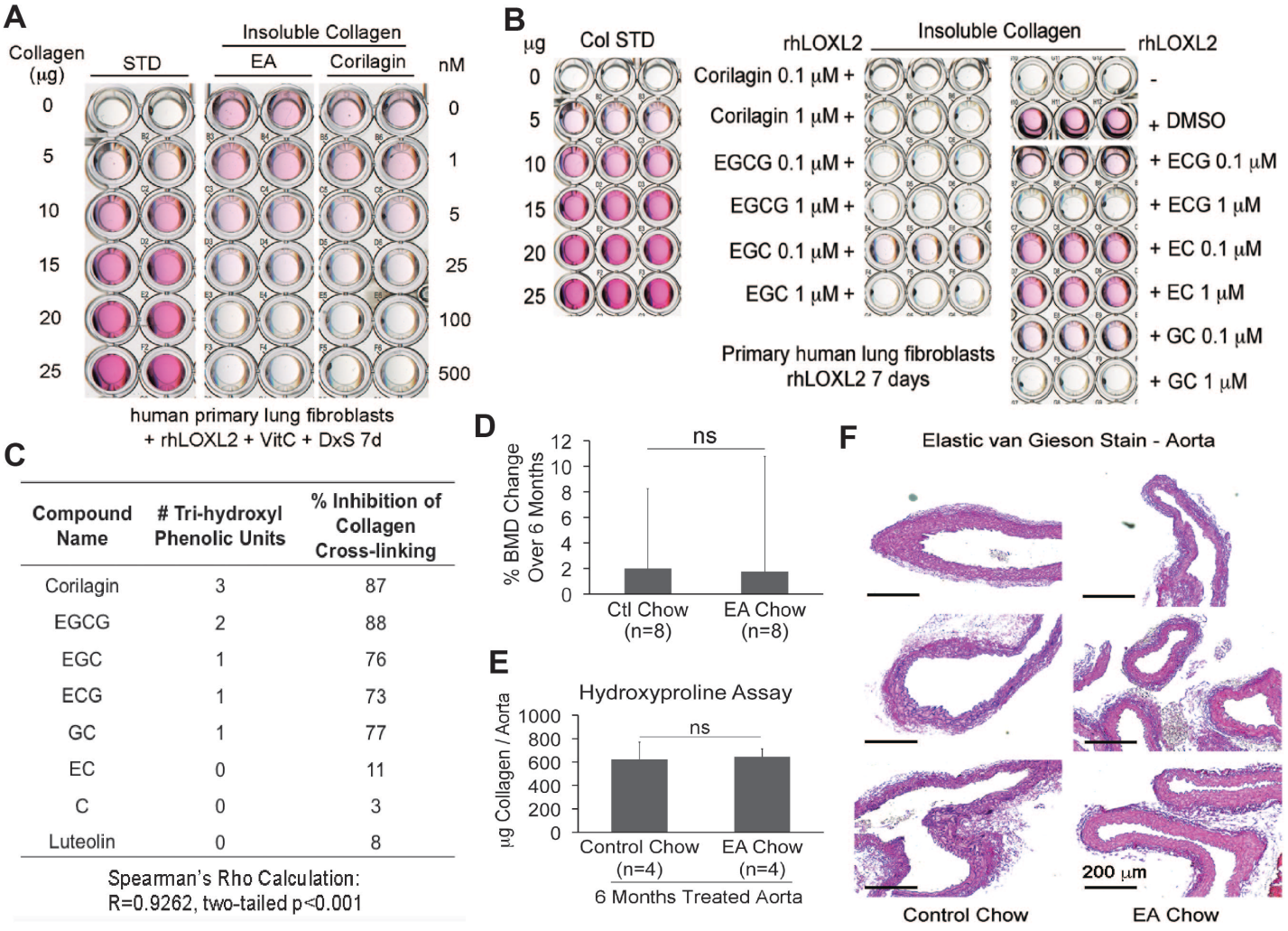
Supplemental Figure S1. Ellagic acid (EA) and other trihydroxyphenolic compounds inhibit TGFβ1-dependent EMT. (**A**) A549 cells were stimulated with TGFβ1 in the presence of ALK5 inhibitor SB431542 and 21 lead compounds from high-throughput screening for 48 hours. The lysates were blotted for fibronectin, E-cadherin, and β-actin. The cells treated for 2 hours were blotted for p-Smad2 and Smad2. Note, two lead compounds were excluded due to solubility issue. (**B**) A549 cells stimulated with TGFβ1 were treated with different catechins (1 and 10 μM) for 48 h and the lysates were blotted for fibronectin, E-cadherin, snail1, and β-actin. The data shown is a representative of at least three experiments with similar results. (**C**) Structure comparison of corilagin, catechins, and luteolin. (**D**) Correlation of trihydroxyphenolic motif and inhibition of TGFβ1-induced snail1. Data were expressed as percent inhibition of Snail1 by compounds vs DMSO control. Correlation was analyzed by Spearman's Rho Calculation. (**E**) Plasma levels of corilagin 2 hours after the last oral dosing of 100 mg/kg at day 21. n=5.

Supplemental Figure S2



Supplemental Figure S2. Ellagic acid and corilagin do not affect immune cell distribution or macrophage TGFβ1 response in vivo. (**A-C**) Total protein concentration (**A**), total cell counts (**B**), and immune cell distribution (**C**) from bronchoalveolar lavage fluid (BALF) of mice intratracheal injected with saline or bleomycin treated with ctl or EA chow. (**D-F**) Total protein concentration (**D**), total cell counts (**E**), and immune cell distribution (**F**) from BALF of mice intratracheal injected with saline or bleomycin treated with vehicle or corilagin. n=4-5. (**G**) Transcripts of TGFβ1–responsive genes were measured in RNA extracts by qRT-PCR from BALF pellets intratracheal injected with saline or bleomycin for 21 days and treated with EA or control pump (n=3-5/group). Metalloproteinase (MMP)9, MMP12, TREM1, and plasminogen activator type I (PAI)1 mRNA levels are normalized to that of β-actin. Data for **A-G** represent mean±SD. P value by one-way ANOVA with a Tukey post hoc test. "ns", not significant. (**H**) Airway macrophages from BALF of mice intratracheal injected with saline or bleomycin for 5 days and treated with vehicle or corilagin were lysed and blotted for p-Smad3 and β-actin (left). Quantification (mean±SD) of densitometry values of p-Smad3/β-actin ratio (n=6) was shown (right). P value by unpaired two-tailed t-test. "ns", not significant by t-test. The data from **A-F** are representative of at least three experiments with similar results.

Supplemental Figure S3

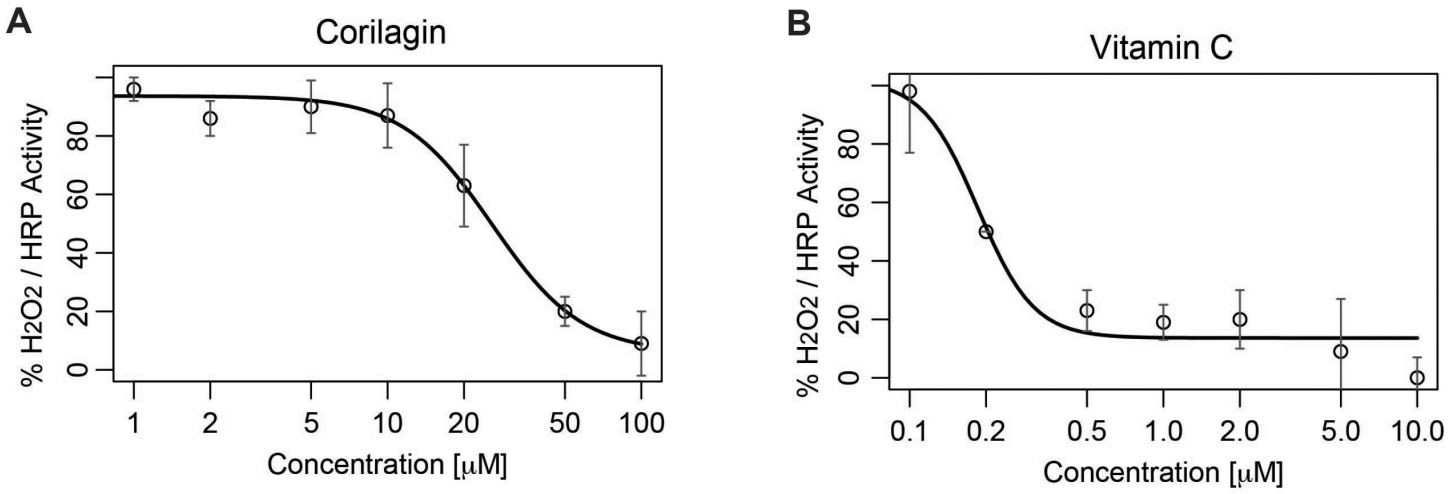


Compound Name	# Tri-hydroxyl Phenolic Units	% Inhibition of Collagen Cross-linking
Corilagin	3	87
EGCG	2	88
EGC	1	76
ECG	1	73
GC	1	77
EC	0	11
C	0	3
Luteolin	0	8

Spearman's Rho Calculation:
R=0.9262, two-tailed p<0.001

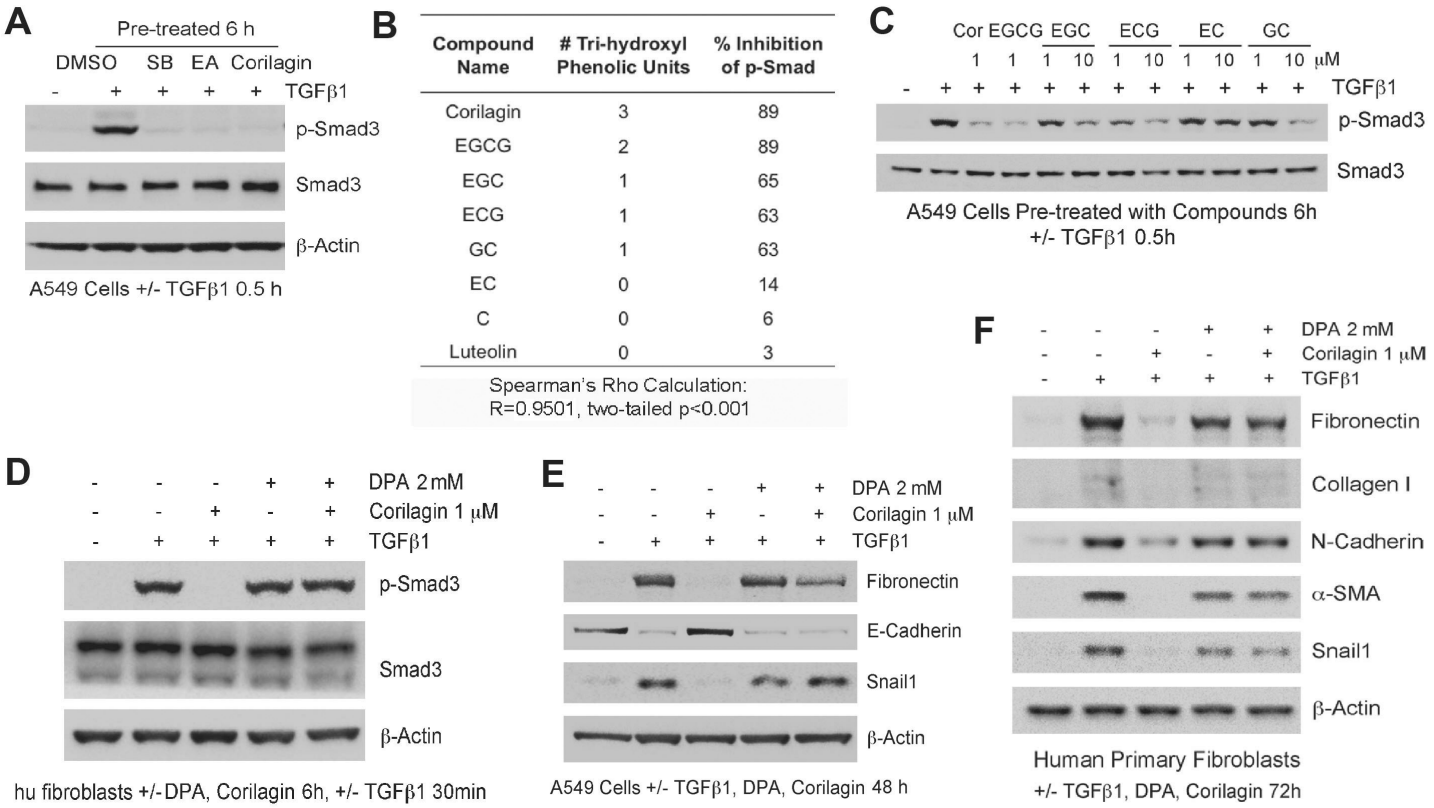
Supplemental Figure S3. Trihydroxyphenolic compounds inhibit LOXL2-dependent collagen cross-linking. (**A-B**) Primary human lung fibroblasts cultured in the presence of Vitamin C and Dextran Sulfate were treated with recombinant human LOXL2 (rhLOXL2) in the presence of different concentrations of EA or corilagin (0-500 nM) (**A**) or catechins (**B**) for 7 day. The insoluble cross-linked collagen was extracted and measured by Sircol assay. (**C**) Correlation of trihydroxyphenolic motif and inhibition of rhLOXL2-induced collagen cross-linking. Long-term EA chow treatment does not affect bone mineral density or aorta collagen content. Data were expressed as percent inhibition of collagen cross-linking by compounds vs DMSO control. Correlation was analyzed by Spearman's Rho Calculation. (**D**) Change in bone mineral density (% BMD Change Over 6 Months) from baseline at lumbar spine and proximal femur in mice treated with ctl chow (n=8) or EA chow (n=8). Data are expressed as mean±SD. "ns" indicates not significant (unpaired two-tailed t-test). (**E**) Hydroxyproline analysis of aortas isolated from mice treated with ctl chow (n=4) or EA chow (n=4) for 6 months. Data are expressed as mean±SD. "ns" indicates not significant (unpaired two-tailed t-test). (**F**) Elastic van Gieson stain of paraffin sections of aortas from mice treated with ctl chow (n=3) or EA chow (n=3) for 6 months. Scale bar, 200 μm. The data shown in **A** and **B** are representative of at least three experiments with similar results.

Supplemental Figure S4



Supplemental Figure S4. Corilagin does not function as an antioxidant. Hydrogen peroxide radical scavenging activity of corilagin (0-100 μM) (**A**) and Vitamin C (0-10 μM) (**B**). Data are presented as mean percent H_2O_2/HRP activity (vs no compound control)±SD of triplicates. A representative of three independent experiments is shown.

Supplemental Figure S5



Supplemental Figure S5. Trihydroxyphenolic compounds inhibit TGFβ1 but not EGF signaling. Corilagin inhibition of TGFβ1 responses is dependent on LOXL2 activity. (**A**) A549 cells pre-treated with different inhibitors for 6 h were stimulated with TGFβ1 (4 ng/ml) for 30 min and the cell lysates were blotted for p-Smad3, Smad3, and β-actin. (**B**) Correlation of trihydroxyphenolic motif and inhibition of TGFβ1 signaling. Data were expressed as percent inhibition of p-Smad3 by compounds vs DMSO control. Correlation was analyzed by Spearman's Rho Calculation. (**C**) A549 cells pre-treated with corilagin and catechins for 6 h were stimulated with TGFβ1 for 30 min and the cell lysates were blotted for p-Smad3 and Smad3. (**D**) Primary human lung fibroblasts were pre-treated with 1 μM corilagin with or without 2 mM penicilamine (DPA) for 6 h before TGFβ1 stimulation for 30 min. The cell lysates were blotted for p-Smad3, Smad3, and β-actin. (**E**) A549 cells were stimulated with TGFβ1 or left un-stimulated for 48 h in the presence or absence of 1 μM corilagin with or without 2 mM penicilamine (DPA) and the lysates were blotted for fibronectin, E-cadherin, Snail1, and β-actin. (**F**) Primary human lung fibroblasts were stimulated with TGFβ1 in the presence or absence of 1 μM corilagin with or without penicillamine (DPA) for 72 h. The lysates were blotted for fibronectin, collagen I, N-cadherin, α-SMA, Snail1, and β-actin. All the data shown are representative of at least three experiments with similar results.

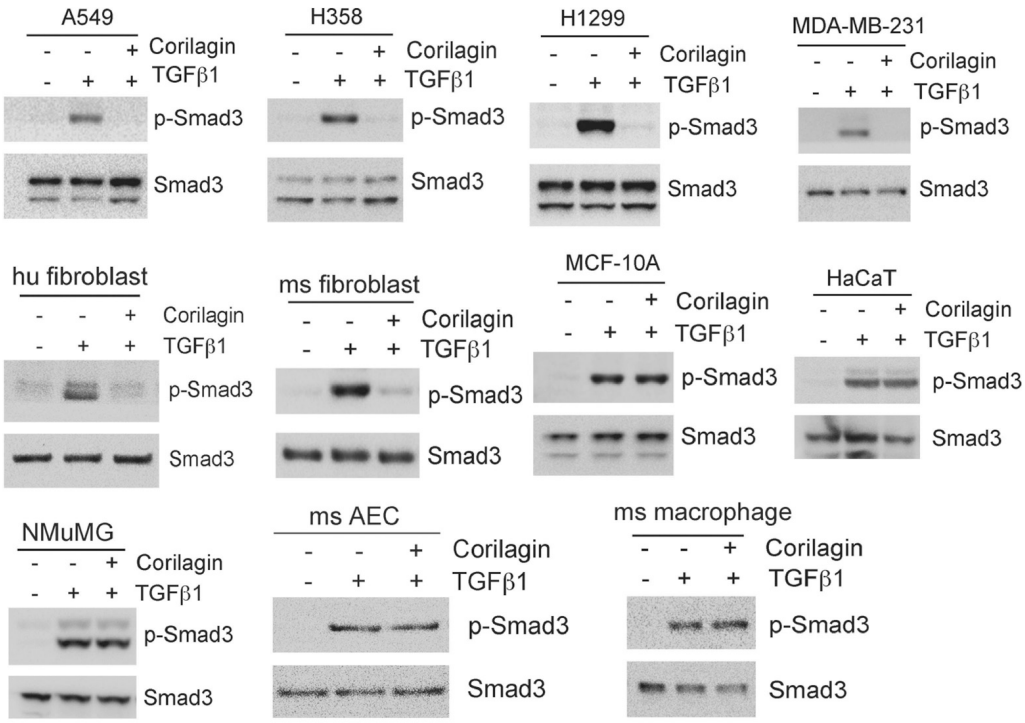
Supplemental Figure S6

A **Table I. Correlation of LOXL2 Level and Corilagin Inhibitory Effect on TGFβ1 Response**

Cell Type	Relative LOXL2 Level	Corilagin Inhibition of p-Smad3 (%) Mean (±SD) n=3
A549 (human lung adenocarcinoma)	3070	90 (1)
H358 (human lung adenocarcinoma)	3616	89 (5)
H1299 (human lung adenocarcinoma)	1641	78 (4)
MDA-MB-231 (human breast adenocarcinoma)	30574	93 (2)
Human primary lung fibroblasts	20171	94 (2)
Mouse primary lung fibroblasts	8903	90 (1)
MCF-10A (human mammary epithelial cells)	1	7 (2)
HaCaT (human keratinocytes)	226	8 (1)
NMuMG (mouse mammary epithelial cells)	388	10 (3)
Mouse primary alveolar type II cells	15	3 (1)
Mouse primary alveolar macrophages	6	2 (1)

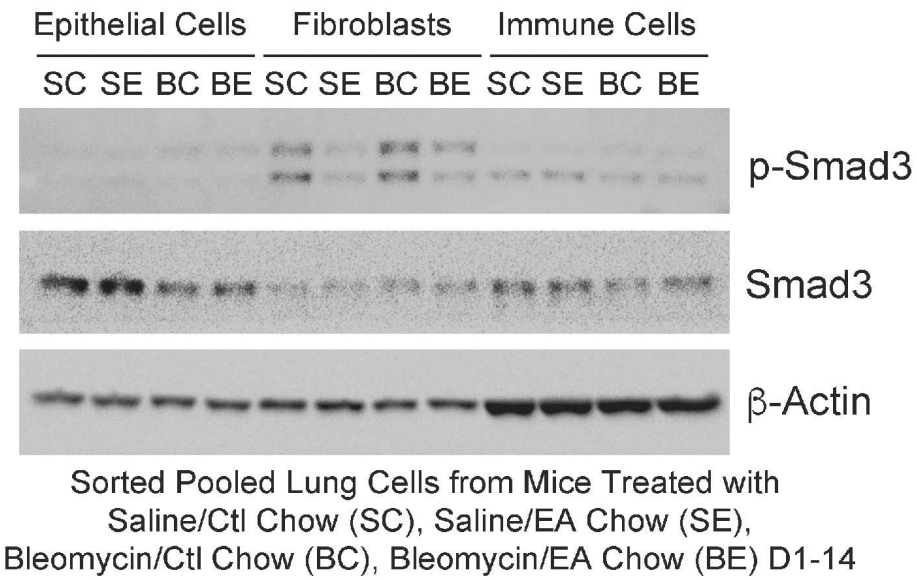
Spearman's Rho Calculation: R=0.9476, two-tailed p<0.001

B



Supplemental Figure S6. Corilagin inhibition of TGFβ1 signaling is dependent upon LOXL2 expression in target cells. (**A**) Correlation of cellular LOXL2 levels and corilagin inhibition of TGFβ1 signaling. LOXL2 levels were determined by qPCR and LOXL2 levels in each cell type were expressed as fold vs that in MCF-10A (1). P-Smad3 inhibition was quantified from three independent experiments and data represent mean percent inhibition by corilagin vs DMSO control±SD. n=3. Correlation was analyzed by Spearman's Rho Calculation. (**B**) Different cells were pre-treated with 1 μM corilagin for 6 h before stimulating with TGFβ1 for 30 min and the cell lysates were blotted for p-Smad3 and Smad3. Results from representative of three experiments for each cell type are shown.

Supplemental Figure S7



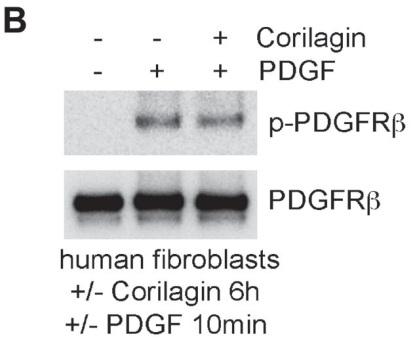
Supplemental Figure S7. Inhibition of TGFβ1 signaling by trihydroxyphenolics is specific to fibroblasts in vivo. Mouse lung epithelial cells, fibroblasts, and immune cells sorted from mice treated for 14 days with saline+control chow (SC, n=5), saline+EA chow (SE, n=5), bleomycin+control chow (BC, n=6), or bleomycin+EA chow (BE, n=3) were lysed and cell lysates blotted for p-Smad3, total Smad3, and β-Actin.

Supplemental Figure S8

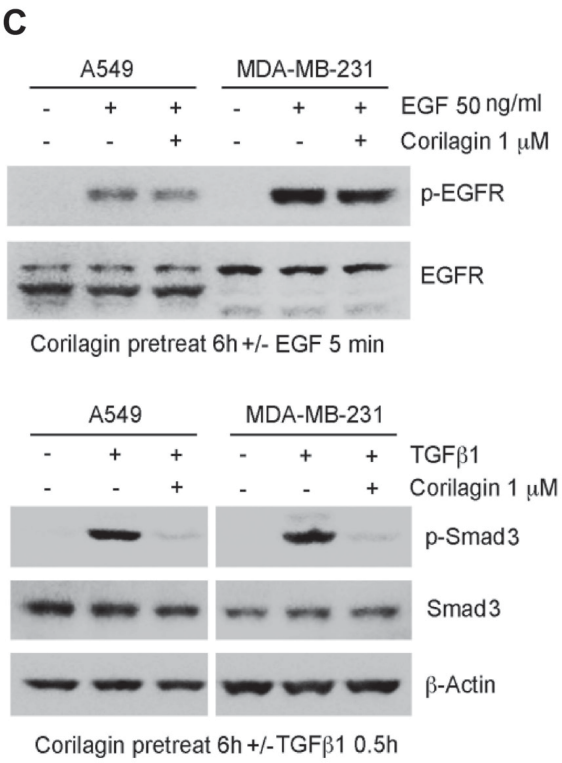
A

Kinase	Mean % Control (1μM Corilagin)	Mean % Control (10 μM Corilagin)
Abl	112	114
ALK6	112	111
ALK	85	90
AMPKα1	109	103
ASK1	104	102
Aurora-A	87	77
CaMKI	99	102
CDK1/cyclinB	107	94
CDK2/cyclinA	117	107
CDK6/cyclinD3	96	98
CDK7/cyclinH/MAT1	103	87
CDK9/cyclin T1	104	106
CHK1	72	89
CK1γ1	90	86
CK2α2	18	22
DRAK1	104	102
eEF-2K	101	104
EGFR	18	5
EphA5	77	22
EphB4	16	4
Fyn	72	3
GSK3β	96	100
IGF-1R	101	27
IKKα	100	98
JAK2	96	94
KDR	96	95
LOK	105	113
Lyn	85	8
MAPKAP-K2	90	80
MEK1	100	93
MKK7β	90	23
Mnk2	108	105
MSK2	110	107
MST1	114	116
mTOR	78	47
NEK2	84	72
p70S6K	92	99
PAK2	92	89
PDGFRβ	23	2
PI3 Kinase (p110α/p85α)	51	12
PI3 Kinase (p110β/p85α)	50	15
PI3 Kinase (p110δ/p85α)	48	2
PI3 Kinase (p120γ)	92	73
Pim-1	108	97
PKA	109	106
PKBα	99	94
PKCα	90	91
PKCθ	107	108
PKG1α	105	109
Plk3	104	98
PRAK	37	25
ROCK-I	109	117
Rse	60	25
Rsk1	141	141
SAPK2a	107	114
SRPK1	99	93
ACTR2	98	100
ALK1	106	113
ALK2	100	100
ALK4	100	105
ALK6	122	125
A-Raf	101	93
BMPR2	91	90
B-Raf	91	70
B-Raf(V599E)	85	59
c-RAF	82	55
c-RAF	109	78
IRAK1	92	89
IRAK4	88	88
IRAK4	94	88
LIMK1	98	94
LRRK2	98	96
MLK1	105	110
MLK1	109	111
MLK2	90	84
RIPK1	105	125
RIPK2	101	110
TAK1	108	106
TAK1	104	109
TGFBR1	104	97
TGFBR2	103	98
ZAK	94	73

B

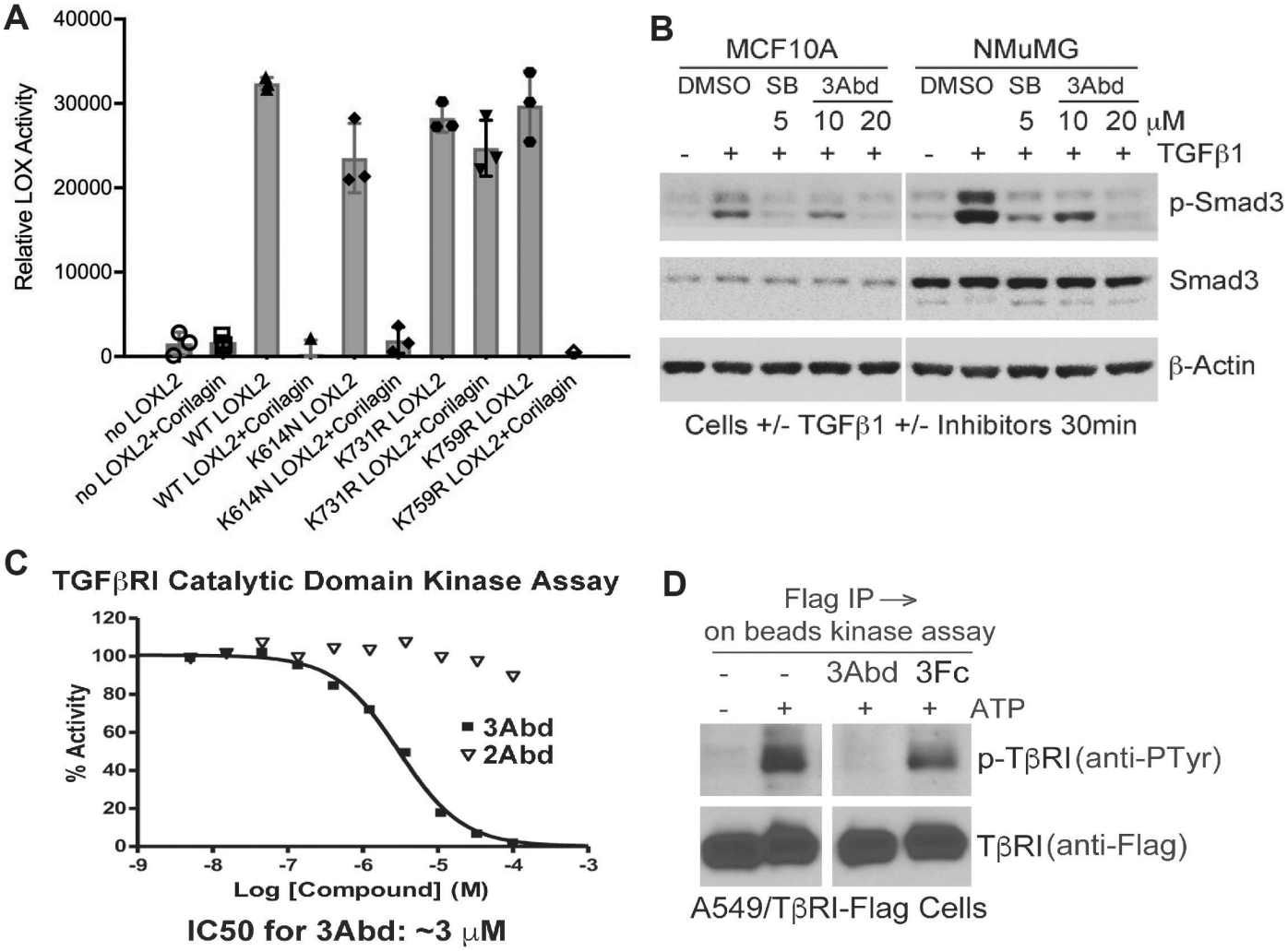


C



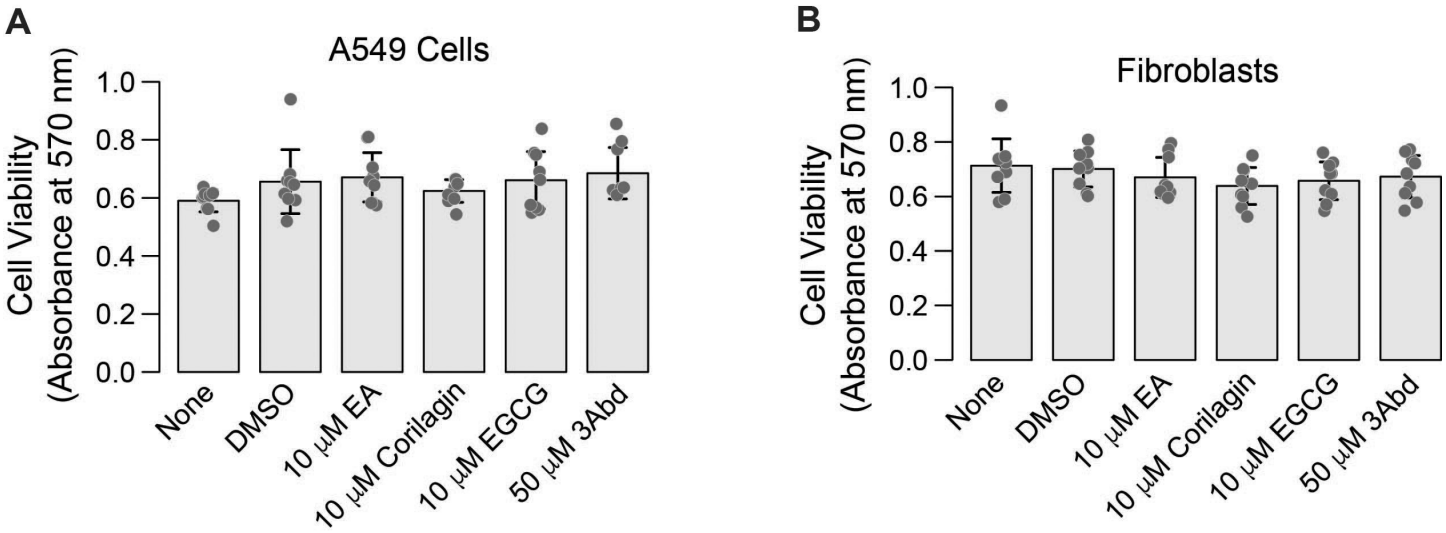
Supplemental Figure S8. Corilagin (1 μM) does not globally block kinase activity. (**A**) Corilagin (1 μM and 10 μM) effects on 82 purified kinase activities measured as percent ATP incorporation for each enzyme compared with DMSO controls. (**B**) A549 cells were pre-treated with 1 μM corilagin for 6 h before stimulating with PDGF (10 ng/ml) for 10 min and the cell lysates were blotted for p-PDGFRβ and total PDGFRβ. (**C**) A549 and MDA-MB-231 cells were pre-treated with 1 μM corilagin for 6 h before stimulating with EGF (50 ng/ml) for 5 min or with TGFβ1 for 30 min and the cell lysates were blotted for p-EGFR and total EGFR or p-Smad3 and Smad3, respectively. **B, C** are representative of three experiments with similar results.

Supplemental Figure S9



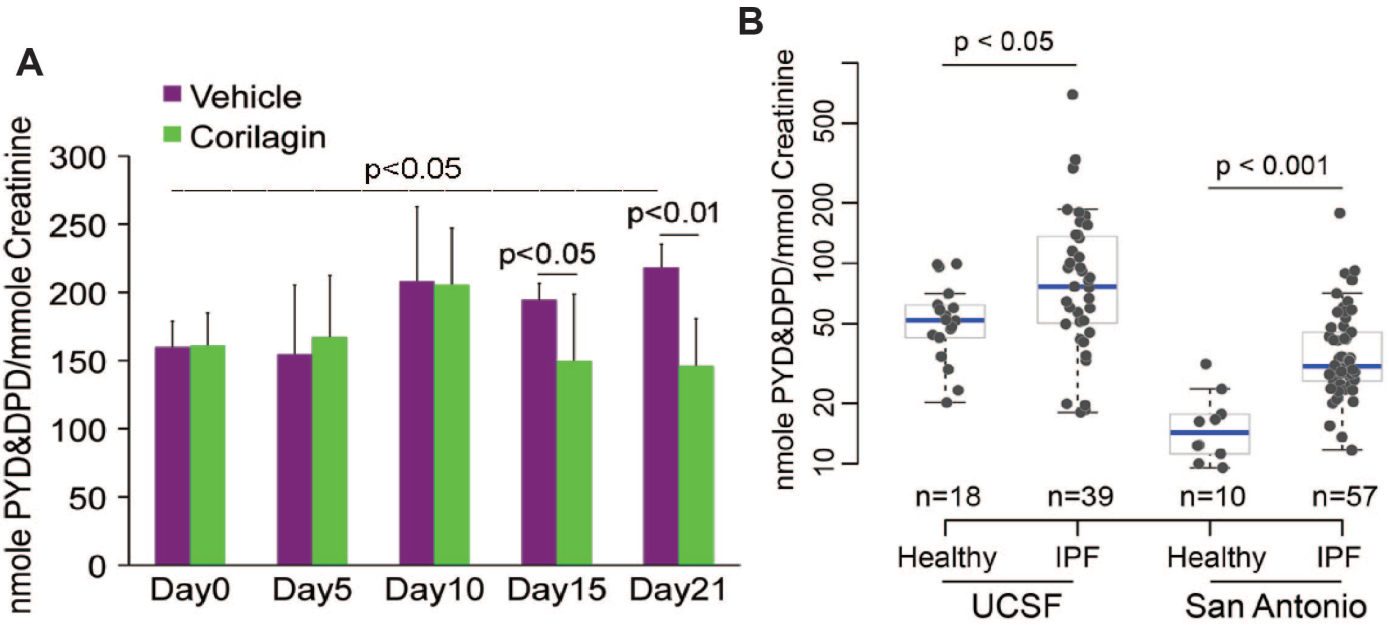
Supplemental Figure S9. Trihydroxyphenolic motif mimics the LTQ leading to auto-oxidation and generation of a novel inhibitor of TGFβR1 kinase. (**A**) NMuMG cells transiently transfected with wild-type or mutant human LOXL2 were treated with 1 μM corilagin or DMSO for 6 h. LOX activity of conditioned media from treated cells was measured. Data presented as relative intensity at Ex/Em 540/590 nm. Data represent mean±SD, n=3. (**B**) MCF-10A and NMuMG cells were stimulated with TGFβ1 for 30 min in the presence or absence of 3Abd (10 and 20 μM) without pre-incubation. Cell lysates were blotted for p-Smad3, Smad3, and β-actin. (**C**) Purified ALK5/TGFβRI catalytic domain kinase assay was carried out in the presence of ten doses of 3Abd or 2Abd starting from 100 μM. The kinase activity was indicated by ^{33}P-ATP signals and the IC50 of 3Abd was calculated. (**D**) A549 cells transfected with Flag-tagged TRI and TRII were immunoprecipitated with anti-Flag antibody and the in vitro kinase assay was performed on beads in the presence or absence of 20 μM 3Abd or 3Cc. The final reaction was eluted and analyzed by immunoblotting for phosphotyrosine and Flag. All the data shown are representative of at least three experiments with similar results.

Supplemental Figure S10



Supplemental Figure S10. Trihydroxyphenolics do not affect cell viability. Effect of EA (10 μM), Corilagin (10 μM), EGCG (10 μM), and 3Abd (50 μM) on cell viability of A549 (**A**) and human primary lung fibroblasts (**B**). Data are presented as mean absorbance at 570 nm±SD. n=9.

Supplemental Figure S11

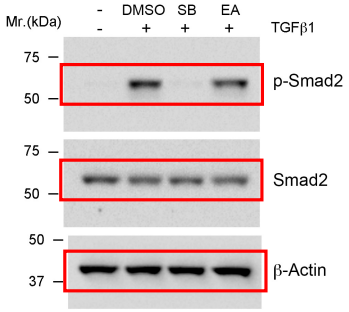


Supplemental Figure S11. Urinary PYD/DPD levels are higher in bleomycin-treated mice and IPF patients than that in controls. (**A**) Mouse pyridinoline (PYD) and deoxypyridinoline (DPD) were measured at different time points (D0-D21) in the urine samples pool-collected from mice injected with bleomycin and treated with vehicle (n=5) or corilagin (n=5). Three urine sample collections at each time point. P value by one-way ANOVA with a Tukey post hoc test. (**B**) Human PYD and DPD were measured in the urine samples collected independently from two cohorts of healthy donors and IPF patients from University of California at San Francisco (UCSF) and University of Texas Health Science Center at San Antonio (San Antonio). The data was analyzed by Mann-Whitney test. Data for **A** and **B** represent mean DPD and PYD/nmol/mmol creatinine±SD.

Supplemental Table 1. Primer and probe sequences for Taqman Quantitative PCR

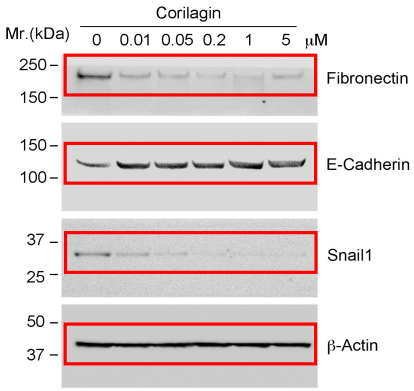
Name	Sequence
Mouse MMP9 F	5′-TTCCTGGTGGCAGCGC-3′
Mouse MMP9 R	5′-CGGCACGCTGGAATGATC-3′
Mouse MMP9 Probe	5′-/FAM/CCCAGTGCATGGCCGAACTCG/BHQ/-3′
Mouse MMP12 F	5′-TGTGGAGTGCCCGATGTACA-3′
Mouse MMP12R	5′-AGTGAGGTACCGCTTCATCCAT-3′
Mouse MMP12 Probe	5′-/FAM/CATCTTAGAGCAGTGCCCCAGAGGTCAA/BHQ/-3′
Mouse PAI1 F	5′-TGCATCGCCTGCCATTG-3′
Mouse PAI1 R	5′-GACATTTCCACAGTGGACCTTGA-3′
Mouse PAI1 Probe	5′-/FAM/TTGGCCCATGGCACCCTCCA/BHQ/-3′
Mouse Trem1 F	5′-CAAACCGCATCACCACAAAG-3′
Mouse Trem1 R	5′-ACGTGGTTCAGCCACTCCTC-3′
Mouse Trem1 Probe	5′-/FAM/CACTTGATCGCACCCAGAAGGCC/BHQ/-3′

Figure 1C



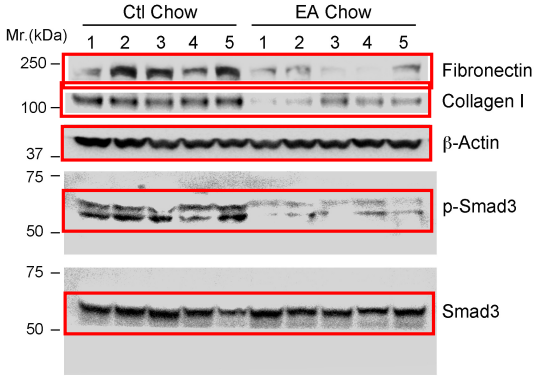
A549 Cells

Figure 1H



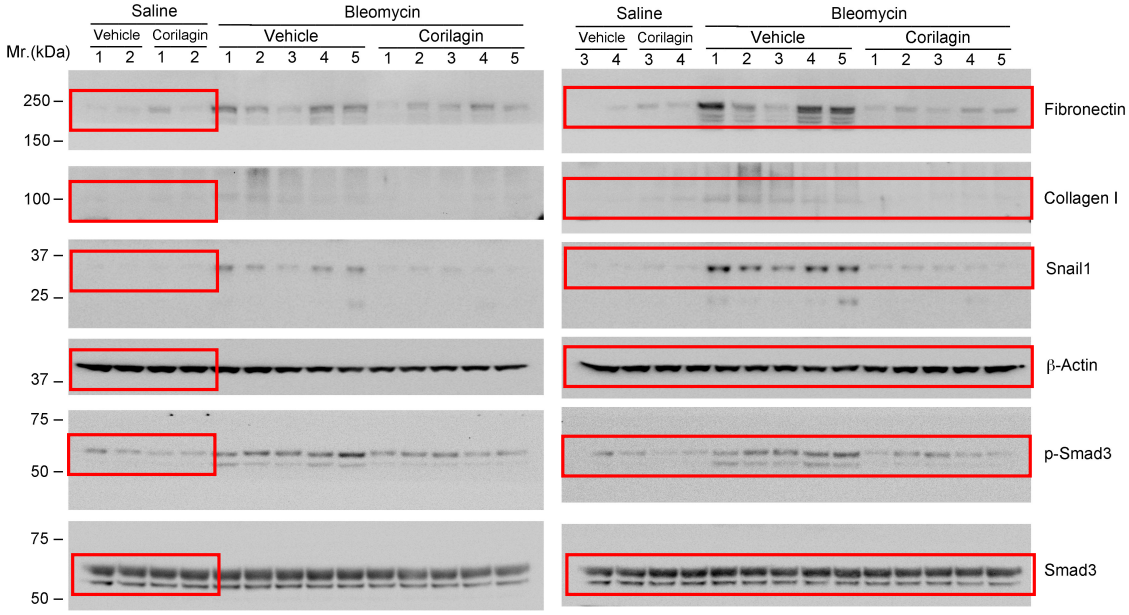
A549 Cells +TGFβ1 48 h

Figure 2G



344SQ Primary Tumor Lysate

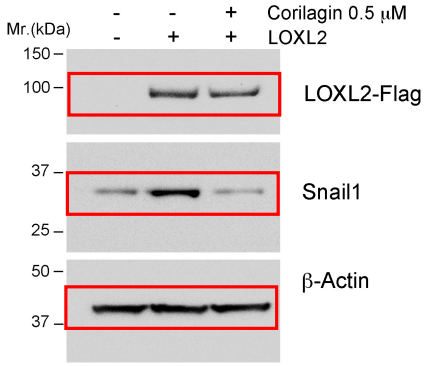
Figure 1K



Mouse Whole Lung Lysate
Corilagin gavage 100 mg/kg D10-D21

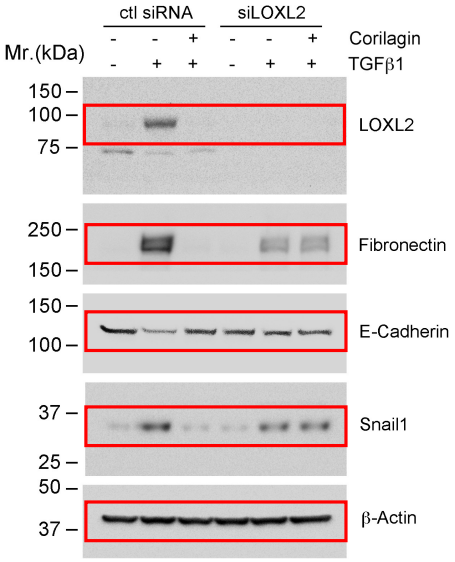
Mouse Whole Lung Lysate
Corilagin gavage 100 mg/kg D10-D21

Figure 3D



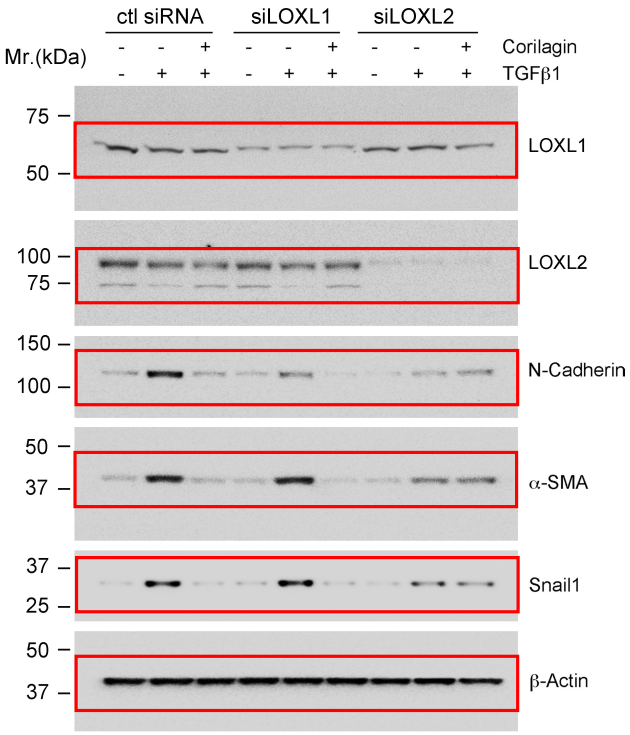
NMuMG±LOXL2±Corilagin 24 h

Figure 3E



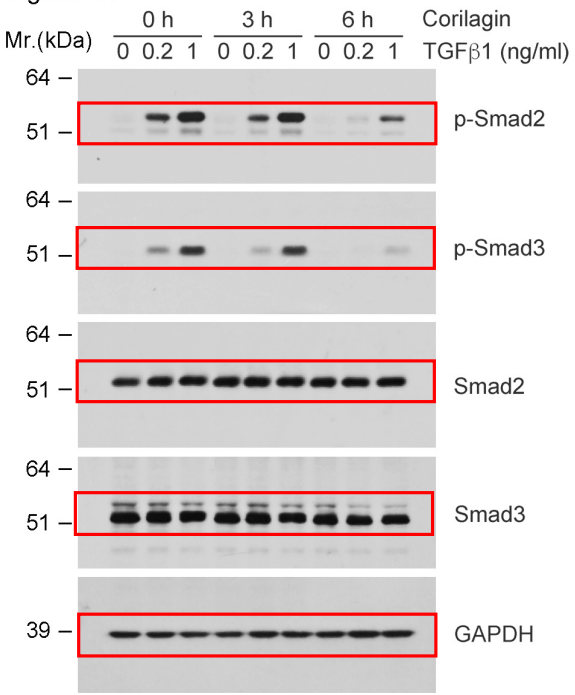
A549 Cells
siLOXL2 72h, TGFβ1 48h

Figure 3F



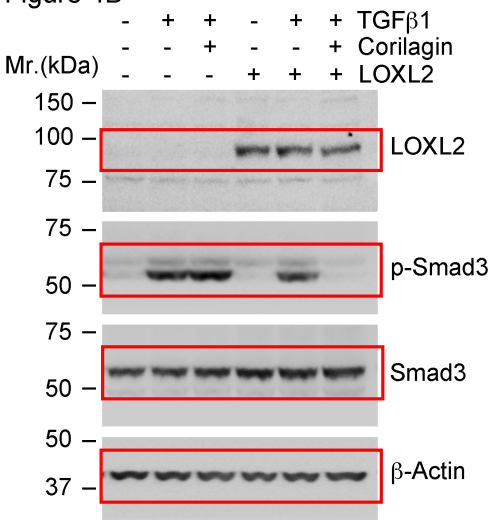
Human Primary Fibroblasts
siLOXL2 72h, TGFβ1 48h

Figure 4A



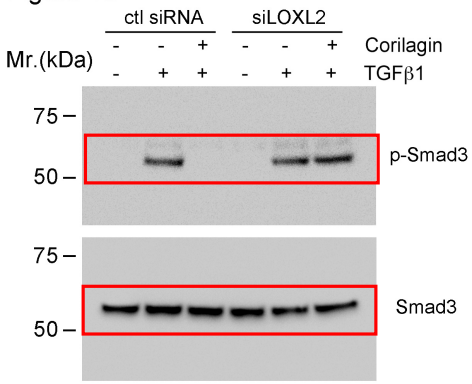
A549 Cells -/+ TGFβ1 30 min
Pretreat 1 μM Corilagin 0-6 h

Figure 4B



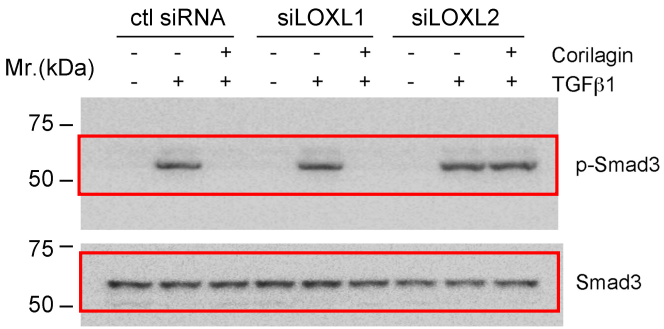
NMuMG±LOXL2 48h
±Corilagin 6h ±TGFβ1 30min

Figure 4C



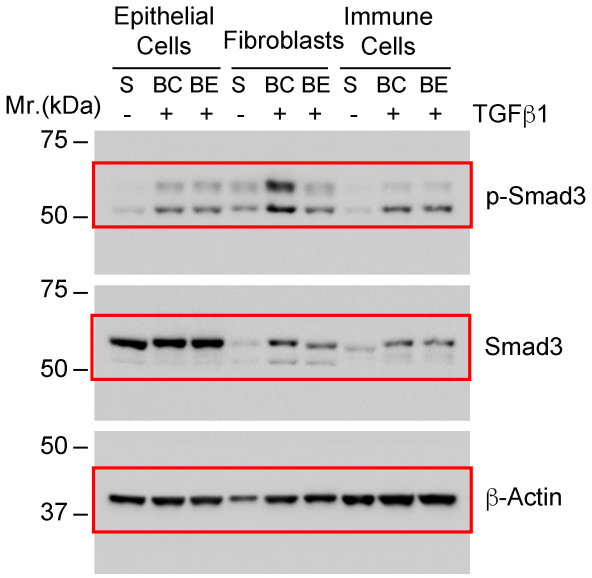
A549 Cells
siLOXL2 72h, TGFβ1 0.5h

Figure 4D



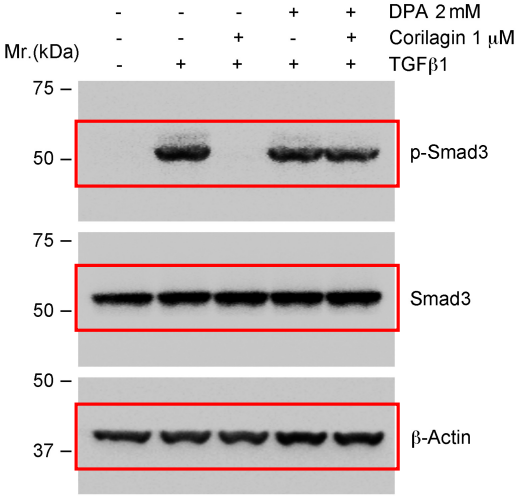
Human Primary Fibroblasts
siLOXL2 72h, TGFβ1 0.5h

Figure 4E



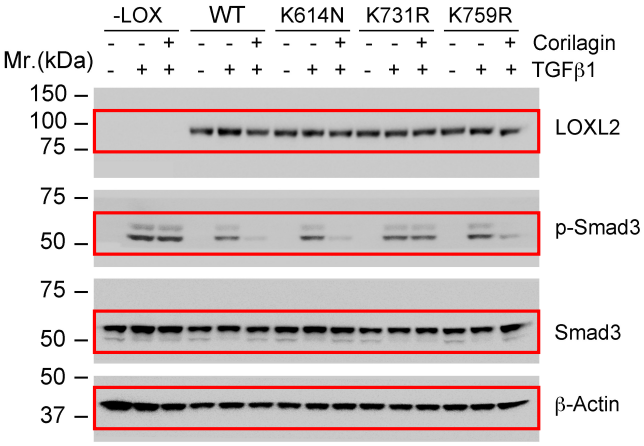
Sorted Pooled Lung Cells from Mice
Treated with Saline (S), Bleomycin
without (BC) or with EGCG (BE) D7-14

Figure 4F



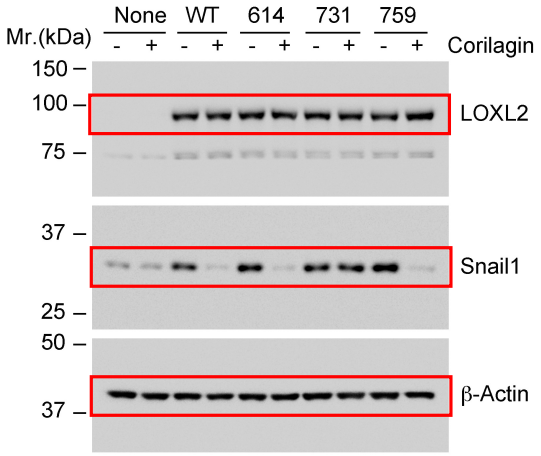
A549 Cells +/- DPA, Corilagin 6h, +/- TGFβ1 30min

Figure 5C



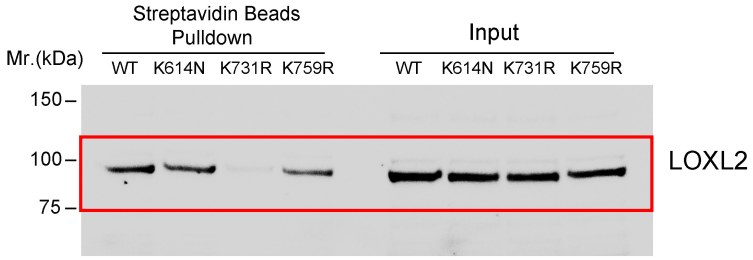
NMuMG Cells ± LOXL2 48h
± Corilagin 6h + TGFβ1 30min

Figure 5D



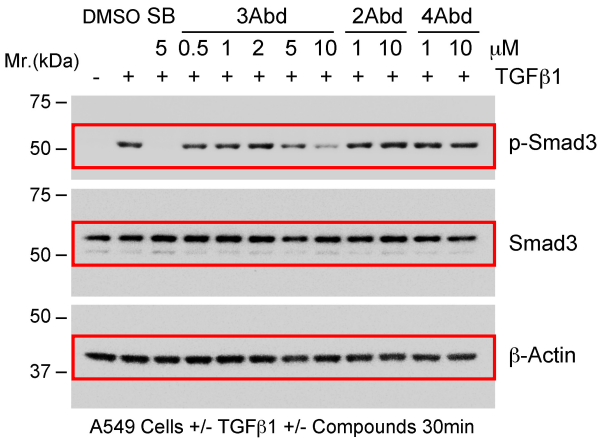
NMuMG Cells +/- WT or Mut LOXL2
+/- Corilagin 24h

Figure 5E



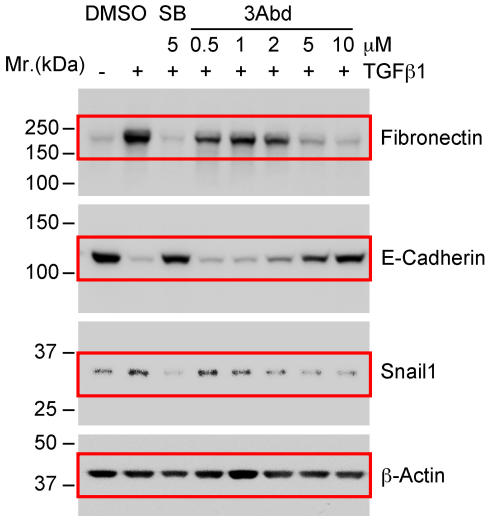
NMuMG Cells + WT or Mut LOXL2 + Corilagin + Biotin-Hydrazide
Streptavidin Magnetic Beads Pulldown and Anti-Flag Ab (M2) Blot

Figure 6B



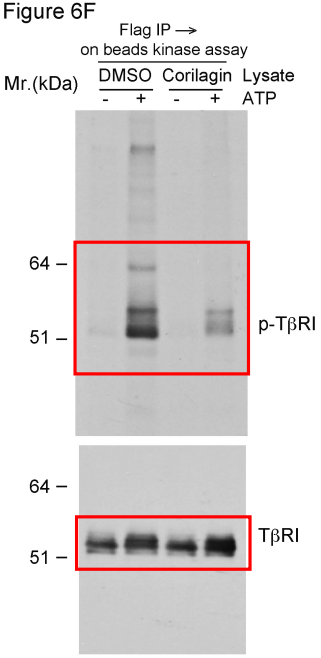
A549 Cells +/- TGFβ1 +/- Compounds 30min

Figure 6C



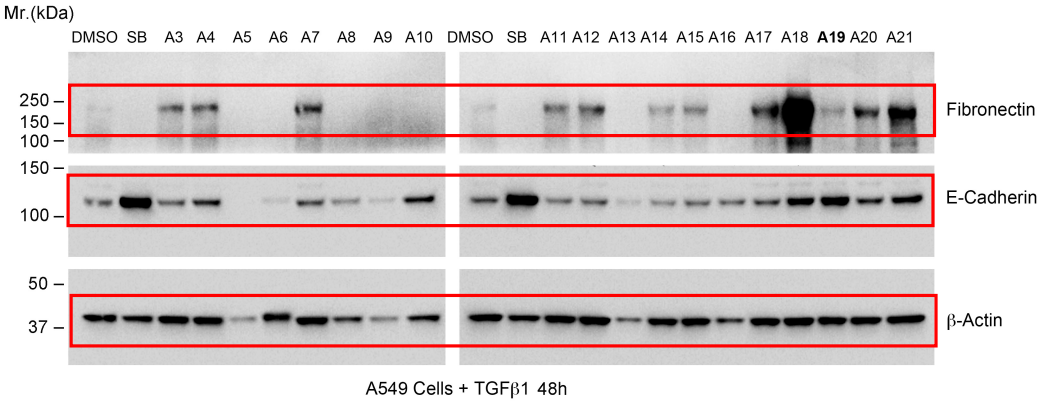
A549 Cells +/- TGFβ1 +/- Inh 48h

Figure 6F

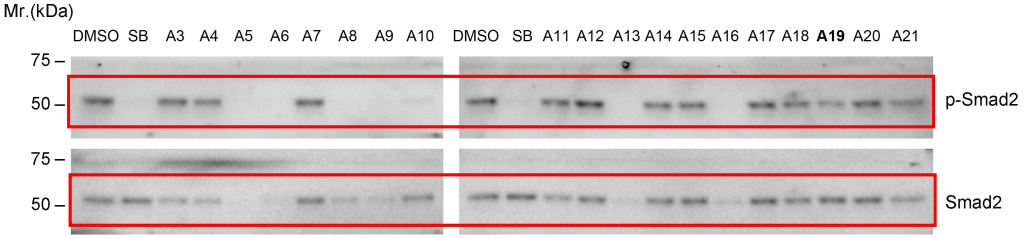


A549/TβRI-Flag Cells

Figure S1A

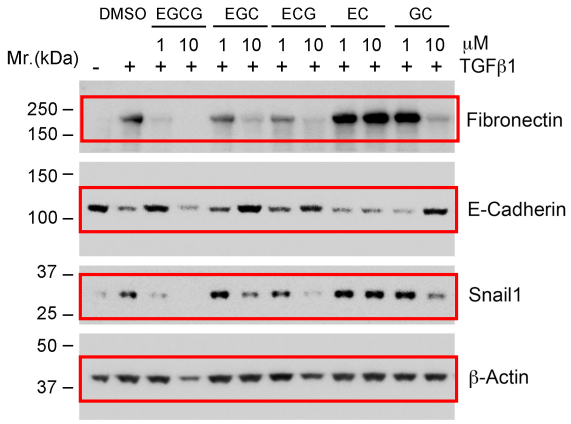


A549 Cells + TGFβ1 48h



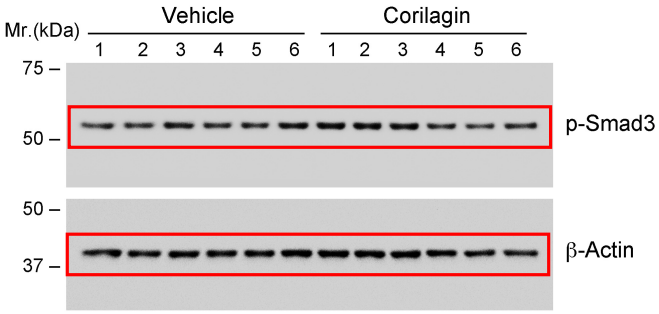
A549 Cells + TGFβ1 2h

Figure S1B



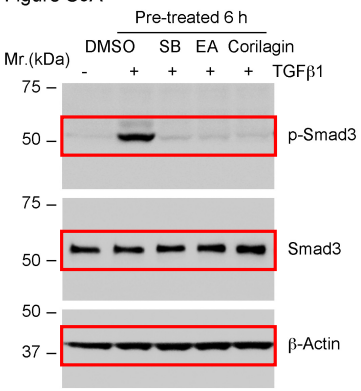
A549 Cells +/- TGFβ1 > 48 h

Figure S2H



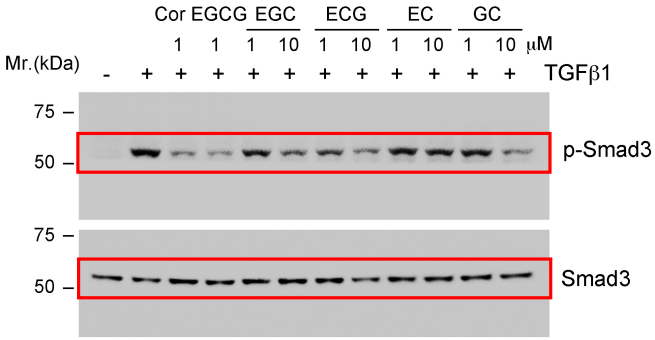
Corilagin Gavage 5 Days
BAL Macrophage Lysate

Figure S5A



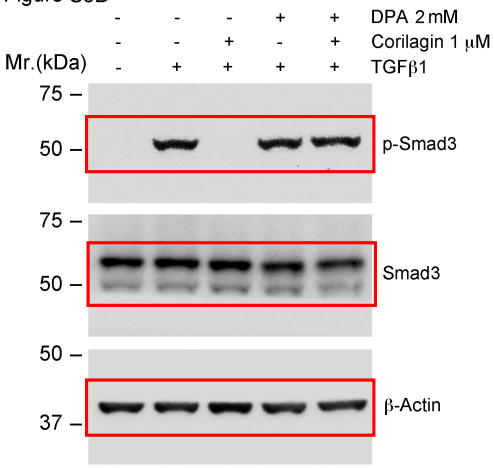
A549 Cells +/- TGFβ1 0.5 h

Figure S5C



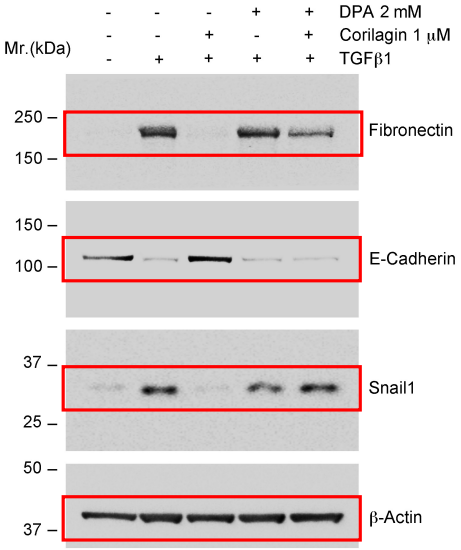
A549 Cells Pre-treated with Compounds 6h
+/- TGFβ1 0.5h

Figure S5D



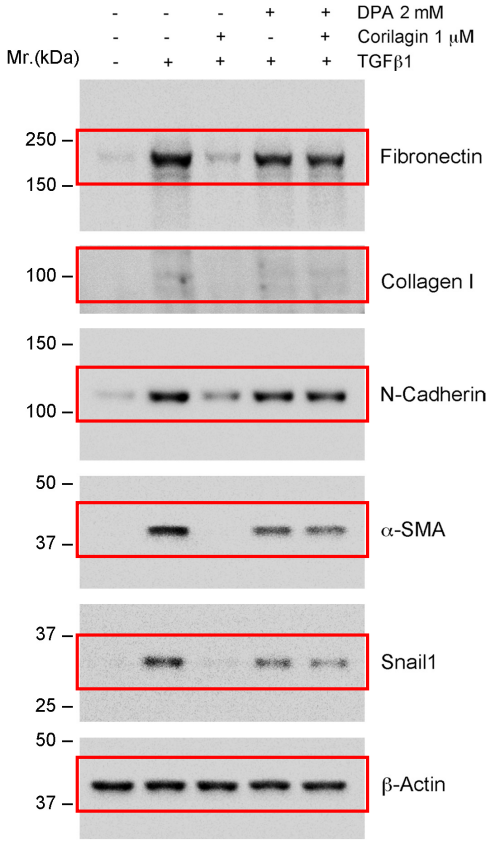
hu fibroblasts +/- DPA, Corilagin 6h, +/- TGFβ1 30min

Figure S5E



A549 Cells +/- TGFβ1, DPA, Corilagin 48 h

Figure S5F



Human Primary Fibroblasts
+/- TGFβ1, DPA, Corilagin 72h

Figure S6B

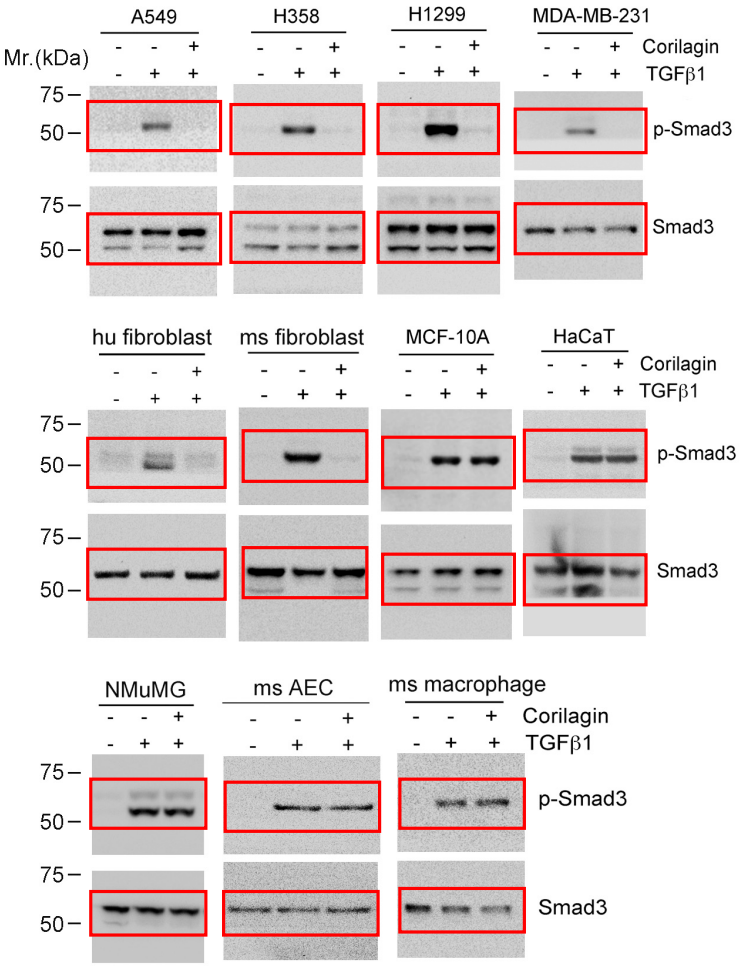
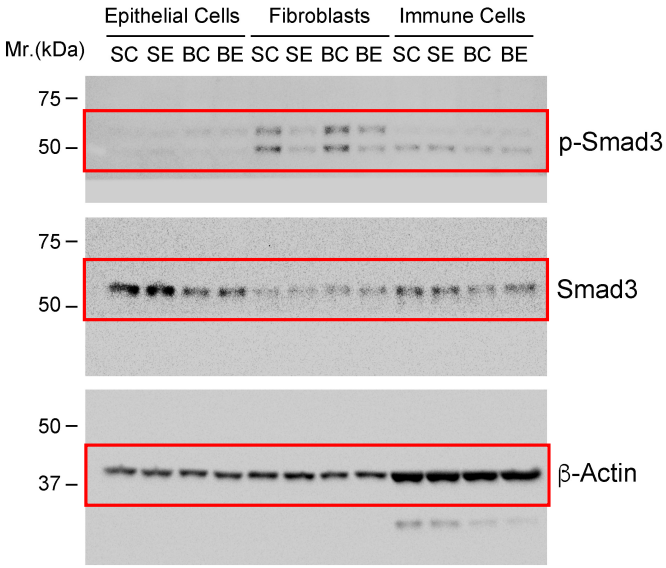
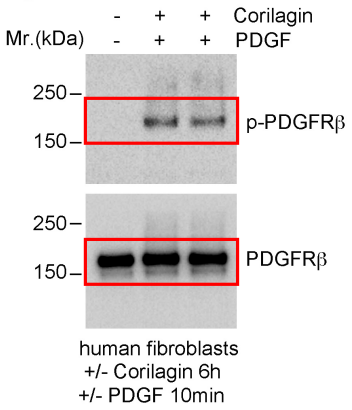


Figure S7



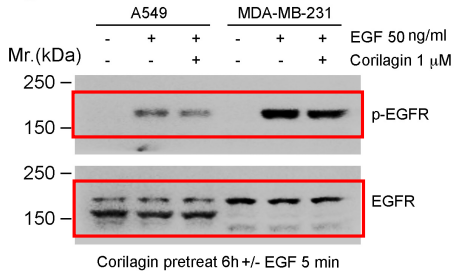
Sorted Pooled Lung Cells from Mice Treated with Saline/Ctl Chow (SC), Saline/EA Chow (SE), Bleomycin/Ctl Chow (BC), Bleomycin/EA Chow (BE) D1-14

Figure S8B

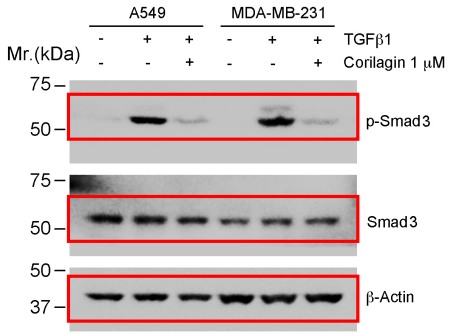


human fibroblasts
+/- Corilagin 6h
+/- PDGF 10min

Figure S8C

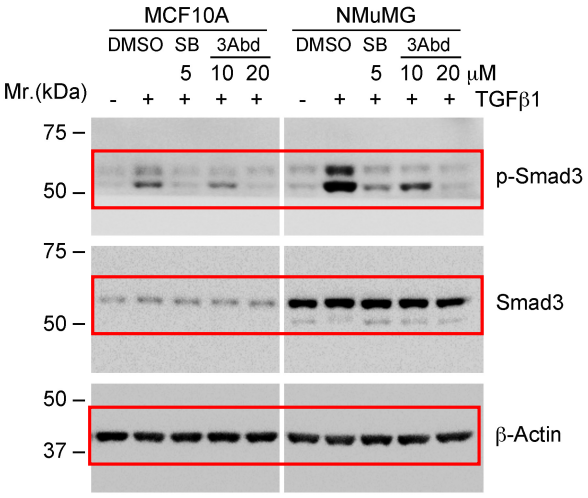


Corilagin pretreat 6h +/- EGF 5 min



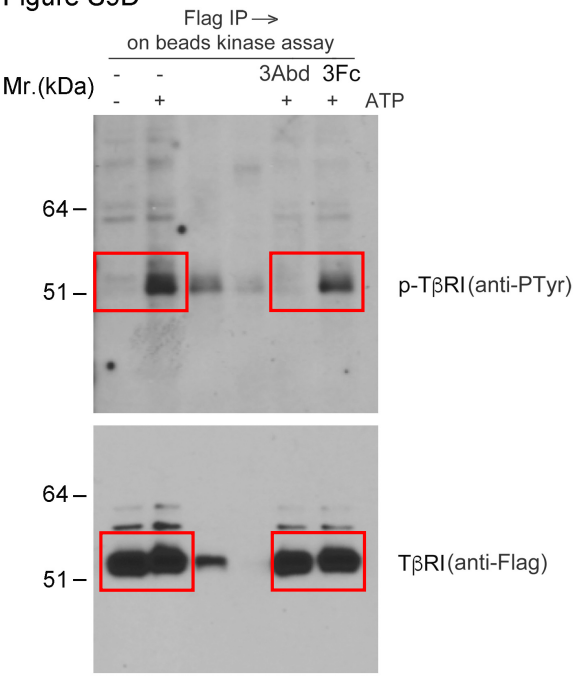
Corilagin pretreat 6h +/-TGFβ1 0.5h

Figure S9B



Cells +/- TGFβ1 +/- Inhibitors 30min

Figure S9D



A549/TβRI-Flag Cells