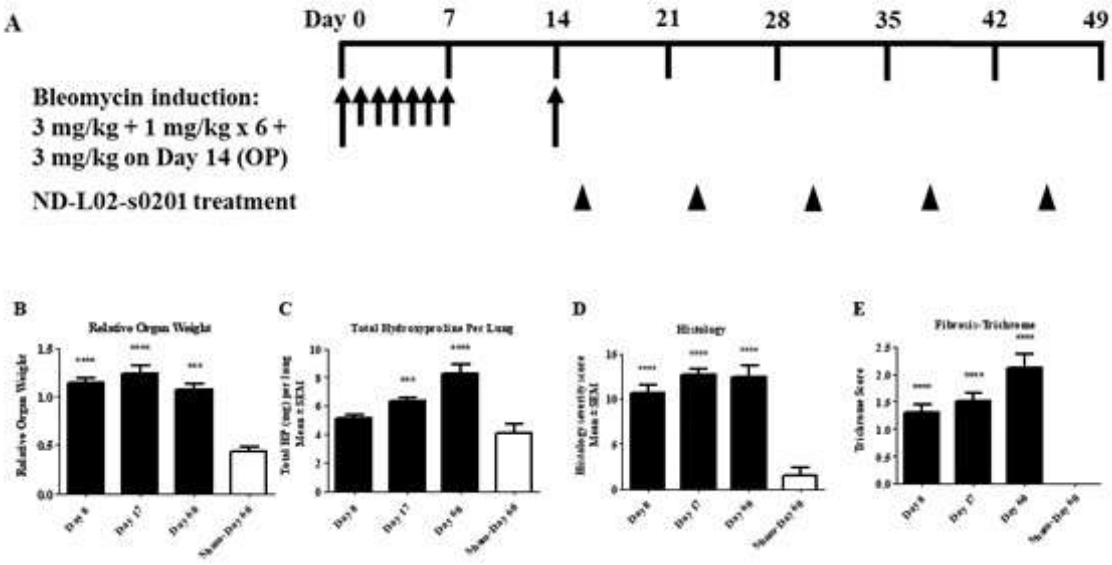
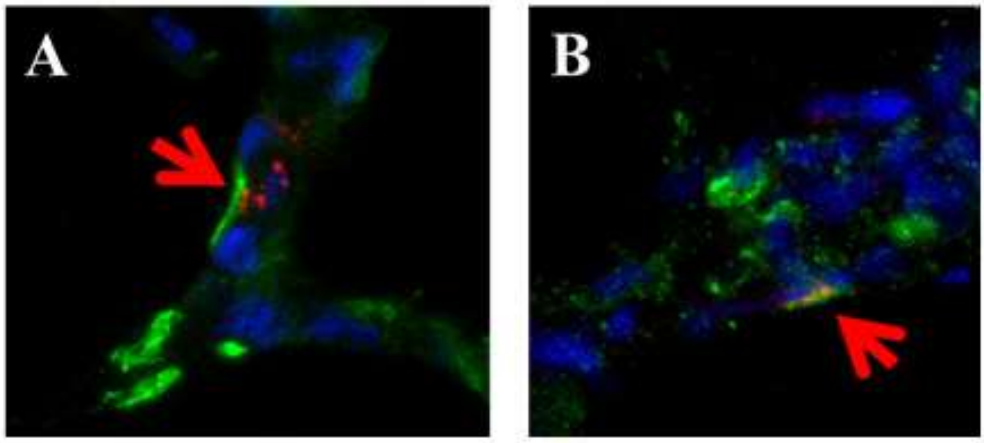
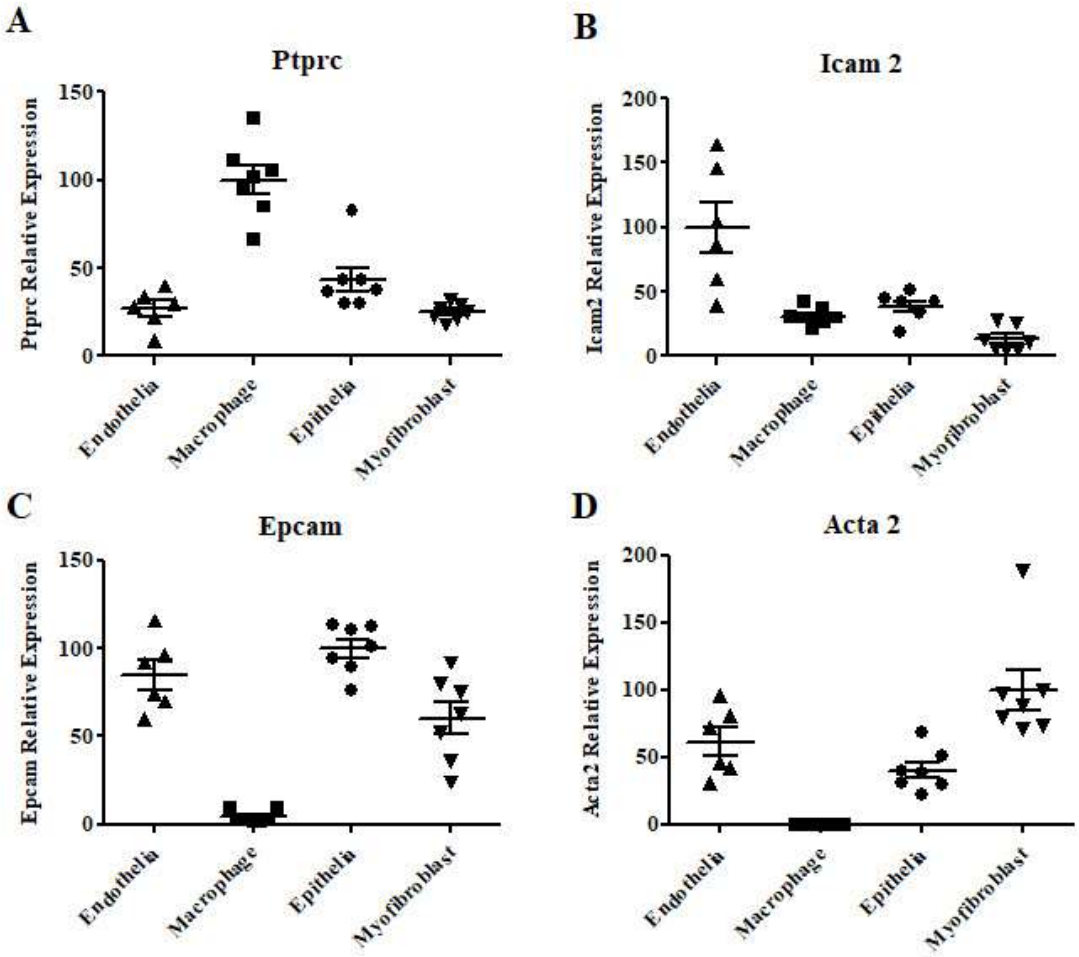


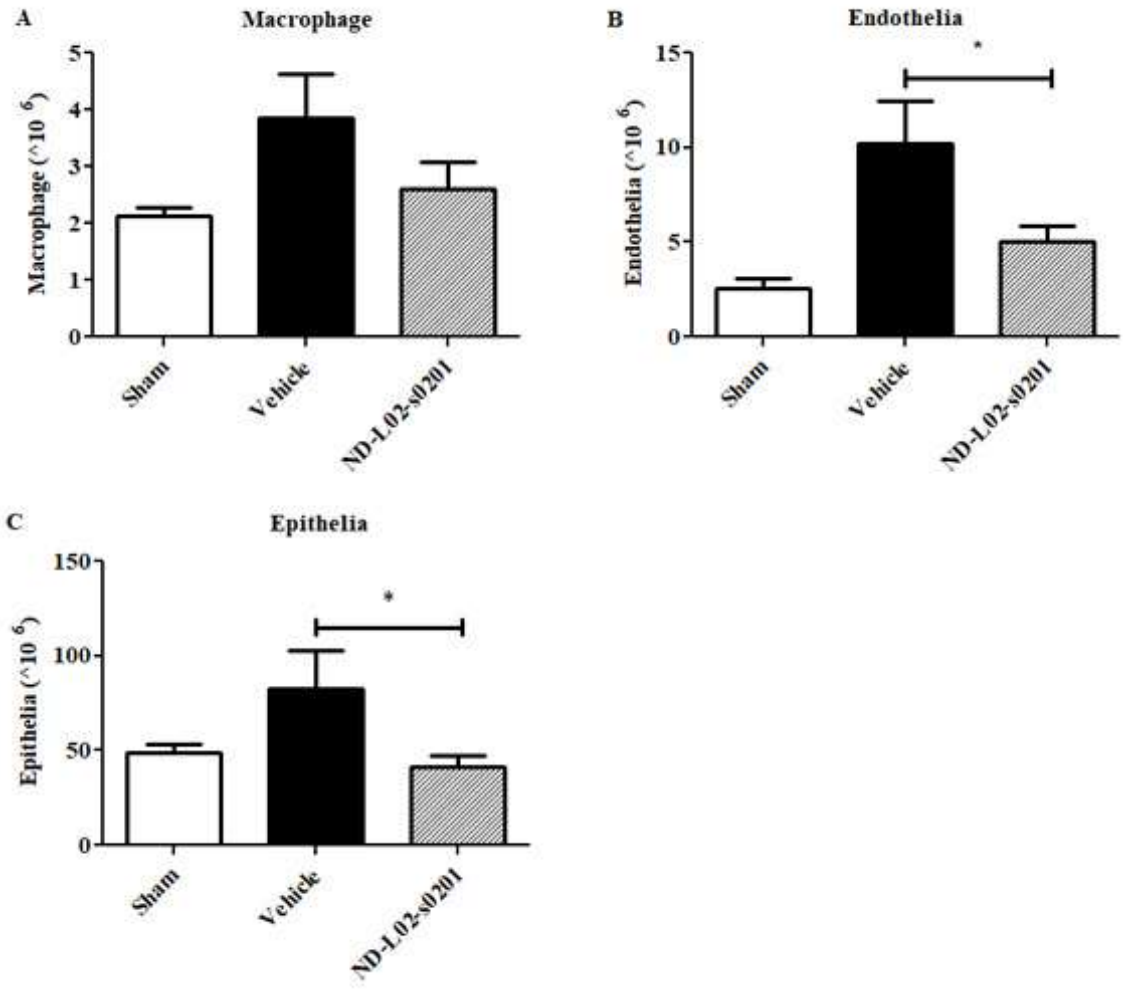
Supplement Fig. 1. BLM rat lung fibrosis model development and validation. (A) Male Sprague-Dawley rats were treated with BLM at 3 mg/kg (Day 0) followed by 1 mg/kg (Day 1 to 6) (0.5 mL/kg, OP) and boosted on Day 14 at 3 mg/kg. Subsets of animals were terminated on Day 6, 17 and 60 following BLM inductions respectively. Lung tissue collected from each animal were assessed on relative lung weight, collagen content, and fibrotic severity through histopathology evaluation. (B) Significantly increased relative lung weight was observed starting from Day 6 and throughout to Day 17 and 60. (C) Lung collagen deposition increased from Day 6 to 17 and 60, indicating the disease progression continued after the final doses of BLM on day 14. (D, E) Histopathology and Trichrome staining-based fibrosis evaluation confirmed this conclusion.



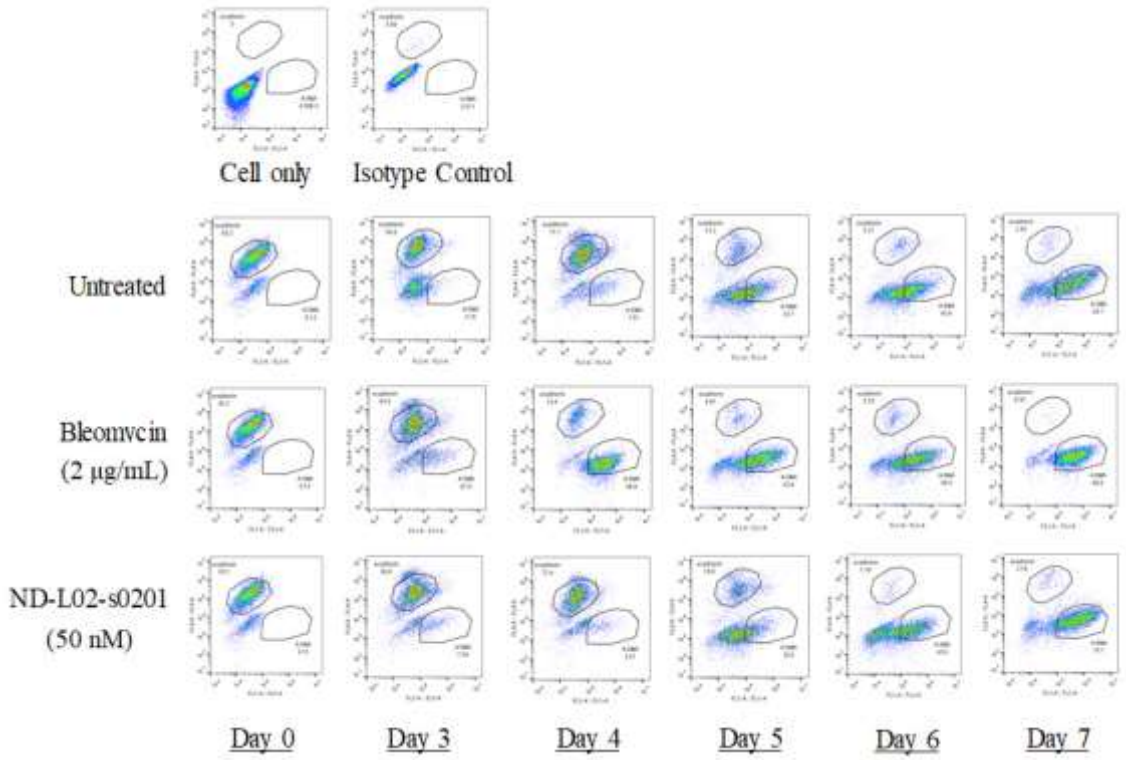
Supplement Fig. 2. Cellular uptake of ND-L02-s0201 in BLM treatment rat lung tissue. Male Sprague-Dawley rats were treated with BLM at 3 mg/kg (Day 0) followed by 1 mg/kg (Day 1 to 6) (0.5 mL/kg, OP) and boosted on Day 14 at 3 mg/kg. Animals received ND-L02-s0201 treatment once at 2 mg/kg and the tissue samples were collected four hours after injection for image process. Colocalization assessment demonstrates that formulation promoted the uptake into myofibroblasts as well as additional cell types like alveolar type-2 cells which can transform into myofibroblasts through EMT processing following BLM treatment. (A) ND-L02-s0201 with labeled siRNA (in red), fibroblasts visualized with anti α-SMA stain (in green), cell nuclei stained with DAPI (in blue). (B) ND-L02-s0201 with labeled siRNA (in red), alveolar type-2 visualized with anti proSP-C stain (in green), cell nuclei stained with DAPI (in blue).



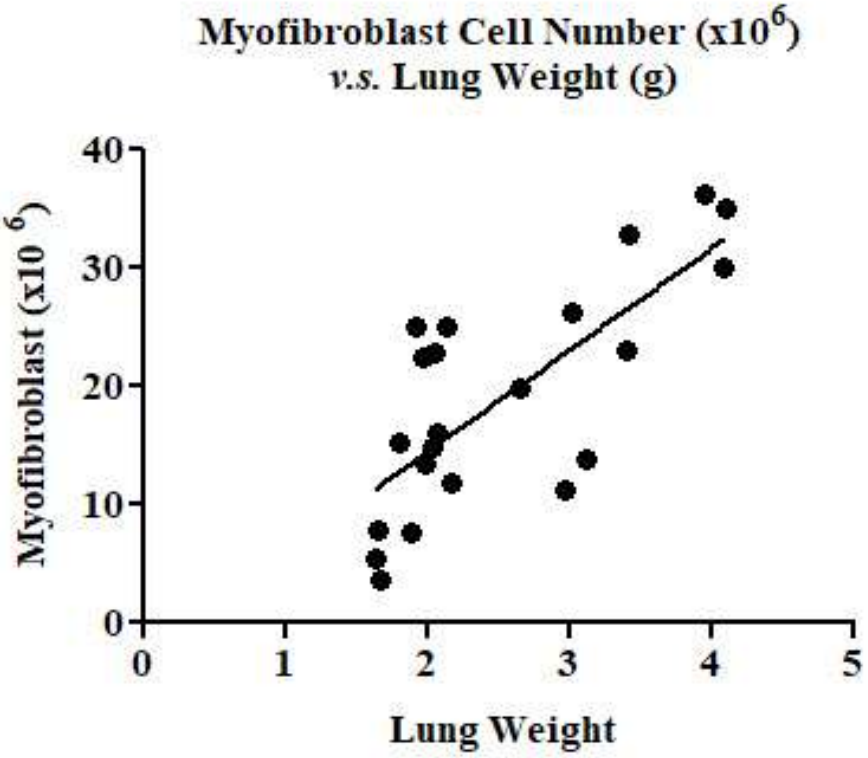
Supplement Fig. 3. Enrichment of cell subpopulation from lung tissue. For the four cell subpopulations isolated from naïve animals, Q-PCR analysis for four cell marker genes, (A) PTPRC (CD45) (Rn00709901_m1), (B) ICAM2 (Rn01461346_m1), (C) EPCAM (Rn01473202_m1), and (D) ACTA2 (Rn01759928_g1), were completed using housekeeping gene MRPL19 (Rn01425270_m1) as reference gene. As shown below, the relative expression levels for the respective gene marker were the highest among the four isolated cell populations, confirming the relative enrichment of the specific cell type following the cell isolation procedures.



Supplement Fig. 4. Increase in cell populations following BLM induction. The increase in myofibroblasts was more prominent following BLM induction, approximate 5.2 folds related to the Sham Group (Fig. 2B). Comparatively the changes in other cell populations macrophage (A), endothelia (B) and epithelia (C) were less significant, 1.8, 3.9 or 1.7 folds related to the Sham Group respectively. ND-L02-s0201 treatment (2 mg/kg/week, four weeks) resulted in the reduction of myofibroblast counts by 60% related to the Vehicle Group, towards the level of the Sham Group (Fig. 2B).

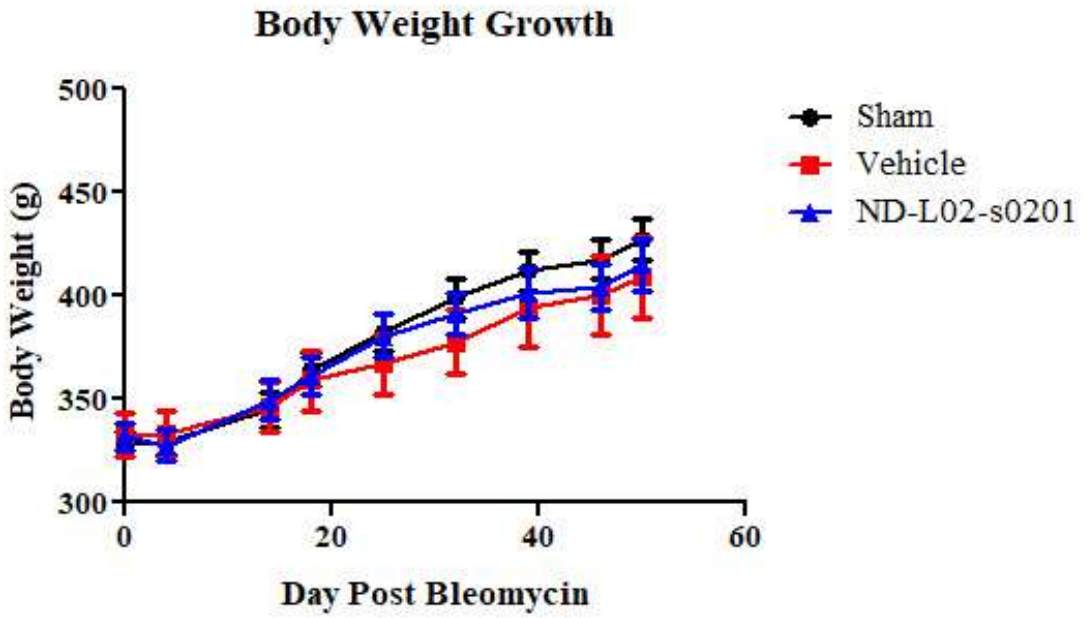


Supplement Fig. 5. EMT assay with primary rat epithelial cells. Culture of primary lung cells were treated with BLM and ND-L02-s0201 as described in Materials and Method. Anti α-SMA-FITC (Abcam, ab8211) and anti E-Cadherin-Alexa 647 (BD, 560062) double staining FACS (BD Accuri™ C6) were performed for cells collected from Day 0 and Days 3-7. Dot plot presented below showed distribution of E-cadherin positive (Y axis) and α-SMA positive (X axis) cells.

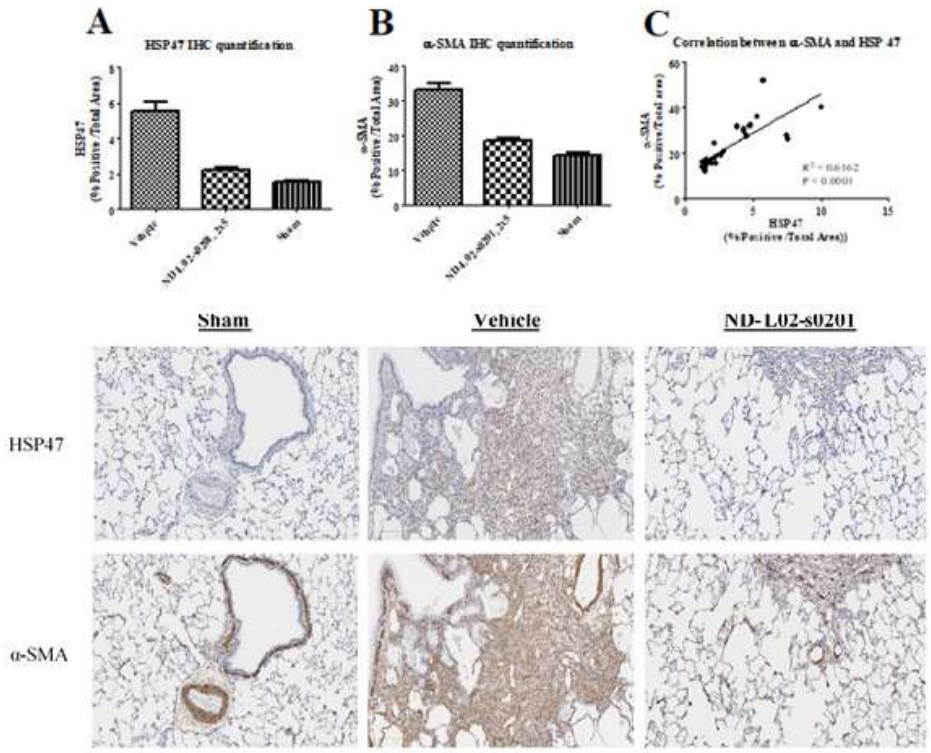


Number of XY Pairs	22
Pearson r	0.7457
95% confidence interval	0.4726 to 0.8881
P value (two-tailed)	< 0.0001
P value summary	****
Is the correlation significant? (alpha=0.05)	Yes
R square	0.5560

Supplement Fig. 6. Correlation between myofibroblast population and lung weight in BLM treated rats. Total twenty-two rats were treated with BLM (same regimen as Fig. 1) and the lung tissues were collected for myofibroblast isolation following lung weight measurement at different time-points after BLM induction (Day 7, 14, 21 and 28).



Supplement Fig. 7. Animal body weight growth during running endurance evaluation following BLM induction in rat chronic model of lung fibrosis. The test condition was described as in Fig. 5.



Supplement Fig. 8. Image quantification analyses of anti-HSP47 or α-SMA IHC staining from the sequential sections of BLM treated rat lung tissue. Male Sprague-Dawley rats were treated with BLM eight times at 3 mg/kg (Day 0, 14) and 1 mg/kg (Day 1 to Day 6) to induce chronic lung damage. ND-L02-s0201 was dosed IV at 2 mg/kg, QW x five weeks Day 18 following initial BLM induction. (A) Image quantification of HSP47 IHC stain, (B) Image quantification of α-SMA IHC stain and (C) Correlation analysis of IHC stains between HSP47 and α-SMA. Representative images of anti HSP47 (top panel) or α-SMA (low panel) IHC stain of sequential sections from BLM rat lung tissue (10 x).

Supplement Tab 1. Fibrosis RT^2 Profiler PCR Arrays (Qiagen) on eighty-four genes of rat lung tissues. Relative gene expression levels were expressed as fold-change following either BLM induction alone (n = 6) or BLM + ND-L02-s0201 (1.5 mg/kg/week, four weeks) treatment (n = 6) related to Sham Group (n = 4). Fold-Changes were calculated using 2^ (-ΔΔCt CT) formula.

Gene Symbol	Description	Gene Fold-Changes	
		BLM alone	BLM + ND-L02-s0201
Acta2	Smooth muscle alpha-actin	1.536	1.1159
Agt*	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	3.147	5.1047
Akt1	V-akt murine thymoma viral oncogene homolog 1	1.1504	-1.4265
Bcl2ξ	B-cell CLL/lymphoma 2	1.6407	-1.2021
Bmp7	Bone morphogenetic protein 7	-1.9549	-1.8196
Cav1ξ	Caveolin 1, caveolae protein	-1.415	1.3392
Ccl11ξ	Chemokine (C-C motif) ligand 11	-1.923	1.3112
Ccl12	Chemokine (C-C motif) ligand 12	1.8419	1.1433
Ccl3ξ	Chemokine (C-C motif) ligand 3	1.2206	-1.0601
Ccr2ξ	Chemokine (C-C motif) receptor 2	3.4077	1.6814
Cebpbξ	CCAAT/enhancer binding protein (C/EBP), beta	1.0668	-1.0679
Col1a2*,ξ	Collagen, type I, alpha 2	3.592	-1.0581
Col3a1*,ξ	Collagen, type III, alpha 1	3.4519	1.136
Ctgf*,ξ	Connective tissue growth factor	2.143	-1.4466
Cxcr4	Chemokine (C-X-C motif) receptor 4	1.5641	1.1641
Dcn*	Decorin	-2.8133	-1.7326
Edn1*	Endothelin 1	2.4282	1.0891
Egf*	Epidermal growth factor	-2.1586	-1.9671

Eng	Endoglin	1.0173	-1.78
Faslg	Fas ligand (TNF superfamily, member 6)	-1.5083	-2.844
Grem1[*, ξ]	Gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	6.2729	-1.7664
Hgf	Hepatocyte growth factor	1.4619	-1.2472
Ifng[ξ]	Interferon gamma	-1.432	1.1745
Il10	Interleukin 10	-1.1964	-1.2874
Il13[*]	Interleukin 13	-3.7558	-3.9919
Il13ra2[*]	Interleukin 13 receptor, alpha 2	-4.1883	-4.4439
Il1a[ξ]	Interleukin 1 alpha	1.2016	-1.4653
Il1b[ξ]	Interleukin 1 beta	1.1479	-1.0713
Il4[*]	Interleukin 4	-2.5329	-2.5989
Il5[ξ]	Interleukin 5	-1.8821	1.7433
Ilk	Integrin-linked kinase	1.5692	1.3277
Inhbe[*]	Inhibin beta E	-4.1883	-4.4439
Itga1	Integrin, alpha 1	-1.5497	-3.2797
Itga2[*, ξ]	Integrin, alpha 2	2.3786	-1.3272
Itga3	Integrin, alpha 3	1.7594	1.1128
Itgav[ξ]	Integrin, alpha V	1.821	-1.3337
Itgb1[*, ξ]	Integrin, beta 1	5.0062	2.7398
Itgb3	Integrin, beta 3	-1.0391	-1.2524
Itgb5	Integrin, beta 5	-1.0231	-2.1338
Itgb6	Integrin, beta 6	1.1853	-1.3146
Itgb8	Integrin, beta 8	1.7626	1.0147
Jun[*]	Jun oncogene	2.005	1.2567
Lox[*, ξ]	Lysyl oxidase	2.9497	-1.0426
Ltbp1[ξ]	Latent transforming growth factor beta binding protein 1	1.5587	-1.0862
Mmp13[*]	Matrix metallopeptidase 13	-3.1857	-4.0202
Mmp14[*]	Matrix metallopeptidase 14 (membrane-inserted)	2.6236	1.1419
Mmp1[*]	Matrix metallopeptidase 1a (interstitial collagenase)	-5.2566	-5.5774
Mmp2[*, ξ]	Matrix metallopeptidase 2	3.567	1.0756

Mmp3*	Matrix metallopeptidase 3	-2.7467	-4.4439
Mmp8	Matrix metallopeptidase 8	-1.0371	-1.0956
Mmp9	Matrix metallopeptidase 9	1.0432	1.0773
Myc	Myelocytomatosis oncogene	1.8077	1.7728
Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	1.5246	-1.0677
Pdgfa	Platelet-derived growth factor alpha polypeptide	1.0067	-1.0273
Pdgfbξ	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	1.1111	-2.1585
Plat*, ξ	Plasminogen activator, tissue	5.6391	3.3442
Plau*, ξ	Plasminogen activator, urokinase	2.2371	-1.0942
Plg*, ξ	Plasminogen	8.0086	4.5203
Serpina1	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	1.6863	2.2534
Serpine1*, ξ	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	12.3588	2.8291
Serpinh1* (HSP47)	Serine (or cysteine) peptidase inhibitor, clade H, member 1	2.2791	1.2243
Smad2	SMAD family member 2	-1.1286	-2.1036
Smad3ξ	SMAD family member 3	1.0536	-1.599
Smad4	SMAD family member 4	-1.9082	-1.7313
Smad6ξ	SMAD family member 6	1.193	-1.0832
Smad7	SMAD family member 7	-1.1216	-1.9593
Snai1*, ξ	Snail homolog 1 (Drosophila)	9.8467	6.0475
Sp1ξ	Sp1 transcription factor	1.4969	-1.1213
Stat1ξ	Signal transducer and activator of transcription 1	1.0245	-1.2164
Stat6	Signal transducer and activator of transcription 6	1.292	1.1881
Tgfb1ξ	Transforming growth factor, beta 1	1.1571	-1.2068
Tgfb2ξ	Transforming growth factor, beta 2	1.3323	-1.6495

Tgfb3[ξ]	Transforming growth factor, beta 3	1.278	-2.4358
Tgfbr1[ξ]	Transforming growth factor, beta receptor 1	1.6867	-1.6032
Tgfbr2[ξ]	Transforming growth factor, beta receptor II	1.9345	-1.0495
Tgif1[ξ]	TGFB-induced factor homeobox 1	1.0034	-1.4144
Thbs1[ξ]	Thrombospondin 1	2.1517	-1.7516
Thbs2[*, ξ]	Thrombospondin 2	22.7212	1.7471
Timp1[*]	TIMP metallopeptidase inhibitor 1	2.4288	1.274
Timp2[ξ]	TIMP metallopeptidase inhibitor 2	1.053	-1.1596
Timp3	TIMP metallopeptidase inhibitor 3	-1.033	-1.5895
Timp4[ξ]	Tissue inhibitor of metalloproteinase 4	-1.0028	1.6266
Tnf	Tumor necrosis factor (TNF superfamily, member 2)	-1.2244	-1.6372
Vegfa	Vascular endothelial growth factor A	-1.8622	-2.7792

[*] Fold-change cut-off > 2 following BLM induction *v.s.* Sham Group.

[ξ] Fold-change cut-off > 1.5 following BLM + ND-L02-s0201 *v.s.* BLM induction alone Group.