Supplementary Material

Specific Features of Fibrotic Lung Fibroblasts Highly Sensitive to Fibrotic Processes Mediated via TGF-β–ERK5 Interaction

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Table S1A. Upregulated genes (223) in fibrotic fibroblasts when compared to control fibroblasts

IP > Control (Q value < 0.05)							
Tracking ID	**Q value**	**Refseq**	**Gene ID**	**Tracking ID**	**Q value**	**Refseq**	**Gene ID**
A2M	0.0048375	NM_000014	2	JCHAIN	0.030287	NM_144646	3512
ABHD5	0.0471383	NM_016006	51099	KCNJ2	0.0048375	NM_000891	3759
ACPP	0.0048375	NM_001292037	55	KCNJ2-AS1	0.0264114	NR_036534	400617
ADAMTS12	0.0183316	NM_030955	81792	KISS1	0.0484813	NM_002256	3814
ADAMTS8	0.0048375	NM_007037	11095	KRBOX1	0.0284973	NM_001205272	100506243
ADH1B	0.0048375	NM_001286650	125	LAMA1	0.0183316	NM_005559	284217
ADM	0.0048375	NM_001124	133	LAMA3	0.0048375	NR_130106	3909
AGAP6	0.0264114	NM_001077665	414189	LAMA4	0.0048375	NM_002290	3910
AGT	0.0354203	NM_000029	183	LGALS9	0.00865342	NR_024043	3965
AKR1C1	0.0048375	NM_001353	1645	LINC00578	0.0048375	NR_047568	100505566
AKR1C3	0.0284973	NM_003739	8644	LINC00999	0.0484813	NR_024497	399744
ALDH1A1	0.0048375	NM_000689	216	LITAF	0.0048375	NR_024320	9516
AMPH	0.0411378	NM_139316	273	LMCD1	0.0048375	NM_014583	29995
APBB1IP	0.00865342	NM_019043	54518	LOX	0.0411378	NM_002317	4015
AQP11	0.0264114	NM_173039	282679	LRRC15	0.0048375	NM_130830	131578
ARHGAP20	0.00865342	NM_020809	57569	LRRN4CL	0.0048375	NM_203422	221091
ARHGEF3	0.0284973	NM_019555	50650	LSAMP	0.01215	NM_002338	4045
ARRDC4	0.0411378	NM_183376	91947	LY6K	0.0375759	NM_017527	54742
ATP6V1G1	0.0152262	NM_004888	9550	MAF	0.0048375	NM_005360	4094
BCL2L1	0.0499355	NM_138578	598	MAP1LC3A	0.030287	NM_181509	84557

BDKRB2	0.0048375	NM_000623	624	MAP1LC3B	0.0471383	NM_022818	81631
BEX1	0.0048375	NM_018476	55859	MAP2	0.00865342	NM_031847	4133
C15orf48	0.0048375	NM_197955	84419	MAP4K4	0.0455469	NM_145687	9448
C4B	0.0499355	NM_001002029_9		MEDAG	0.030287	NM_032849	84935
CASS4	0.0212162	NM_020356	57091	MFAP2	0.01215	NM_017459	4237
CCDC68	0.0048375	NM_025214	80323	MFAP5	0.0048375	NR_123734	8076
CCK	0.00865342	NM_001174138	885	MGLL	0.0411378	NM_007283	11343
CCL2	0.0048375	NM_002982	6347	MILR1	0.0284973	NM_001291317	284021
CCL26	0.0048375	NM_006072	10344	MME	0.0048375	NM_007289	4311
CCND1	0.0239029	NM_053056	595	MMP2	0.0394834	NM_004530	4313
CD248	0.0048375	NM_020404	57124	MOXD1	0.0048375	NM_015529	26002
CD36	0.0048375	NR_110501	948	MT1L	0.0435375	NR_001447	4500
CD82	0.0455469	NM_002231	3732	MTRNR2L1	0.0048375	NM_001190452	100462977
CDKN1A	0.0048375	NM_078467	1026	MYO1D	0.0152262	NM_015194	4642
CEBPB	0.0284973	NM_005194	1051	NABP1	0.0048375	NR_045623	64859
CES1	0.0048375	NM_001266_2		NEAT1	0.0152262	NR_131012	283131
CHI3L1	0.0048375	NM_001276	1116	NEDD9	0.0048375	NR_073131	4739
CHL1	0.0239029	NR_045572	10752	NNMT	0.0048375	NM_006169	4837
CHST15	0.0048375	NM_015892	51363	NPC1	0.0048375	NM_000271	4864
CLEC14A	0.0048375	NM_175060	161198	NPIPA1	0.0484813	NM_006985_4	
CLEC2B	0.0048375	NM_005127	9976	OSR2	0.0048375	NM_053001	116039
COL11A1	0.0048375	NM_080630	1301	PAM	0.0354203	NR_033440	5066
COLEC12	0.0048375	NM_130386	81035	PAMR1	0.0048375	NM_015430	25891

COX14	0.0152262	NM_032901	84987	PLEKHG1	0.0183316	NM_001029884	57480
COX7A1	0.0048375	NM_001864	1346	PLOD2	0.0048375	NM_182943	5352
CPM	0.0183316	NM_198320	1368	PMP22	0.00865342	NR_104018	5376
CPXM2	0.0394834	NM_198148	119587	PODN	0.0048375	NM_153703	127435
CRABP2	0.0048375	NM_001878	1382	POSTN	0.0048375	NM_006475	10631
CREG1	0.0455469	NM_003851	8804	PPAPDC1A	0.030287	NM_001030059	196051
CRLF1	0.0484813	NM_004750	9244	PPP1R14A	0.0048375	NM_033256	94274
CSDC2	0.0048375	NM_014460	27254	PPP1R3C	0.0048375	NM_005398	5507
CST1	0.0048375	NM_001898	1469	PTGDS	0.0048375	NM_000954	5730
CSTB	0.0471383	NM_000100	1476	PTGES	0.0048375	NM_004878	9536
CTSH	0.0152262	NM_004390	1512	PTGS1	0.0048375	NM_080591	5742
CTSK	0.0048375	NM_000396	1513	PTK7	0.0183316	NR_072998	5754
CTSL	0.0239029	NM_145918	1514	RAB3IL1	0.0048375	NM_013401	5866
CYB5A	0.01215	NM_148923	1528	RAI14	0.0239029	NM_015577	26064
CYBA	0.0394834	NM_000101	1535	RARRES1	0.0152262	NM_206963	5918
CYGB	0.0048375	NM_134268	114757	RARRES2	0.0048375	NM_002889	5919
DAB2	0.0048375	NM_001343	1601	RARRES3	0.0183316	NM_004585	5920
DDIT4	0.0048375	NM_019058	54541	RASL11A	0.0394834	NM_206827	387496
DHRS3	0.0048375	NM_004753	9249	RASL12	0.0048375	NM_016563	51285
DMKN	0.0048375	NR_033746	93099	RCAN2	0.0048375	NM_005822	10231
DPP4	0.0212162	NM_001935	1803	RNA5S9	0.0411378	NR_023371	100169760
DRAM1	0.0152262	NM_018370	55332	RND3	0.0048375	NM_005168	390
DSEL	0.0048375	NM_032160_2		S100A4	0.0048375	NM_019554	6275

EDNRB	0.0394834	NM_003991	1910	SAT1	0.0048375	NR_027783	6303
EIF4A1	0.0048375	NM_001416	1973	SDCBP	0.0048375	NM_005625	6386
EMP1	0.0484813	NM_001423	2012	SEC22B	0.00865342	NM_004892	9554
EPAS1	0.0048375	NM_001430	2034	SEPP1	0.0048375	NM_005410	6414
EPB41L3	0.0484813	NM_012307	23136	SERINC2	0.0239029	NM_178865	347735
EPDR1	0.0048375	NM_017549	54749	SERPINE2	0.0048375	NR_073116	5270
F3	0.0048375	NM_001993	2152	SERPINF1	0.0048375	NM_002615	5176
FAM105A	0.0048375	NM_019018	54491	SERPING1	0.0048375	NM_001032295	710
FAM150A	0.0048375	NM_207413	389658	SGIP1	0.0048375	NM_032291	84251
FAM155A	0.01215	NM_001080396	728215	SH3D19	0.0484813	NM_001243349	152503
FAM45A	0.0152262	NR_130122	404636	SH3KBP1	0.030287	NM_031892	30011
FAM84A	0.0048375	NM_145175	151354	SLC16A4	0.0327462	NM_004696	9122
FBLIM1	0.00865342	NM_017556	54751	SLC16A7	0.00865342	NR_073056	9194
FBLN1	0.0048375	NM_006487	2192	SLC40A1	0.0048375	NM_014585	30061
FBLN2	0.0048375	NM_001998	2199	SMPDL3A	0.0048375	NM_006714	10924
FBLN5	**0.0048375**	**NM_006329**	**10516**	SNCA	0.0048375	NM_007308	6622
FBN2	0.0048375	NM_001999	2201	SOD2	0.0048375	NM_001024466	6648
FBXO32	0.0048375	NM_148177	114907	SPON2	0.0048375	NM_012445	10417
FGF7	0.0048375	NM_002009	2252	SQRDL	0.0048375	NM_021199	58472
FGL2	0.0048375	NM_006682	10875	STEAP1	0.0048375	NM_012449	26872
FIBIN	0.00865342	NM_203371	387758	SULF1	0.0048375	NM_015170	23213
FRY	0.0048375	NM_023037	10129	SVEP1	0.0048375	NM_153366	79987
FTL	0.0048375	NM_000146	2512	SYT14	0.0327462	NR_027459	255928

GABARAPL1	0.0048375	NM_031412	23710	TBC1D2B	0.00865342	NM_144572	23102
GDF15	0.0048375	NM_004864	9518	TCF21	0.0048375	NM_198392	6943
GFRA1	0.00865342	NM_145793	2674	TEK	0.01215	NM_001290078	7010
GLRX	0.0048375	NM_002064	2745	TFPI	0.00865342	NM_006287	7035
GNG11	0.0048375	NM_004126	2791	TFPI2	0.0048375	NM_006528	7980
GOSR2	0.0264114	NM_054022	9570	TGM2	0.0048375	NM_198951	7052
GPNMB	0.0048375	NM_002510	10457	THBS2	0.0048375	NM_003247	7058
GPRC5B	0.0264114	NM_016235	51704	TMEM119	0.0048375	NM_181724	338773
GREM1	0.0048375	NM_013372_2		TMEM176B	0.01215	NM_014020	28959
HBA1	0.0499355	NM_000558	3039	TMEM35	0.0327462	NM_021637	59353
HEBP1	0.0048375	NM_015987	50865	TMEM51	0.030287	NM_018022	55092
HGF	0.0048375	NM_001010934	3082	TNFRSF21	0.01215	NM_014452	27242
HIST1H4K	0.0411378	NM_003541	8362	TP53I11	0.01215	NM_006034	9537
HNMT	0.0048375	NM_006895	3176	TRIM58	0.0239029	NM_015431	25893
HSD17B14	0.0375759	NM_016246	51171	U2AF1	0.0048375	NM_006758_2	
HSPB6	0.030287	NM_144617	126393	USP53	0.0048375	NM_019050	54532
HTATIP2	0.0048375	NM_006410	10553	VAMP8	0.0048375	NM_003761	8673
ICAM1	0.00865342	NM_000201	3383	VSTM4	0.0471383	NM_144984	196740
IFITM1	0.0048375	NM_003641	8519	VWA5A	0.0394834	NM_198315	4013
IFITM2	0.00865342	NM_006435	10581	WBSCR27	0.0212162	NM_152559	155368
IFITM3	0.0455469	NR_049759	10410	WFDC1	0.0048375	NM_021197	58189
IGFBP2	0.0048375	NM_000597	3485	ZNF106	0.0048375	NM_022473	64397
IL1R1	0.0264114	NM_001288706	3554				

Table S1B. Downregulated genes (56) in fibrotic fibroblasts when compared to control fibroblasts

IP < Control (Q value < 0.05)							
Tracking ID	**Q value**	**Refseq**	**Gene ID**	**Tracking ID**	**Q value**	**Refseq**	**Gene ID**
ADGRG1	0.0183316	NM_201525	9289	IGF2BP3	0.00865342	NM_006547	10643
APCDD1L	0.0212162	NR_130908	164284	KIF11	0.0471383	NM_004523	3832
BAALC	0.0048375	NM_024812	79870	LOC728392	0.01215	NM_001162371	728392
CADM1	0.0048375	NM_014333	23705	MALL	0.01215	NM_005434	7851
CCDC144B	0.0048375	NR_036647	284047	MCM2	0.00865342	NR_073375	4171
CDCA7	0.0048375	NM_145810	83879	MDGA1	0.0048375	NM_153487	266727
CHRDL1	0.0212162	NM_145234	91851	MGP	0.0327462	NM_001190839	4256
CLU	0.030287	NR_045494	1191	MICA	0.0152262	NM_001289154_5	
COL15A1	0.0048375	NM_001855	1306	MT1X	0.0284973	NM_005952	4501
CTSZ	0.0284973	NM_001336	1522	MYBL2	0.0048375	NM_002466	4605
CXCL12	0.0471383	NM_199168	6387	NPTX1	0.0048375	NM_002522	4884
DCLK2	0.0499355	NR_036614	166614	PCOLCE2	0.0435375	NM_013363	26577
EHD4	0.0152262	NM_139265	30844	PHGDH	0.0048375	NM_006623	26227
ELN	0.0048375	NM_001278939	2006	PLAC9	0.0239029	NM_001012973	219348
ENPP1	0.0048375	NM_006208	5167	POMGNT2	0.0284973	NM_032806	84892
EYA2	0.0048375	NM_172110	2139	PRDM8	0.0048375	NM_020226	56978
EZH2	0.0048375	NM_152998	2146	PRSS3	0.0212162	NM_007343	5646
FABP4	0.0048375	NM_001442	2167	PWP2	0.0411378	NM_005049_2	
FST	0.0048375	NM_013409	10468	RPL13A	0.0048375	NR_073024	23521
G0S2	0.0284973	NM_015714	50486	SAPCD2	0.030287	NM_178448	89958

GCOM1	0.0048375	NR_104371	145781	SEMA7A	0.030287	NM_003612	8482
GJA9-MYCBP	0.0048375	NR_037637	1E+08	SMAP2	0.01215	NM_022733	64744
GREM2	0.0048375	NM_022469	64388	STXBP6	0.0152262	NM_014178	29091
HERC2P10	0.0499355	NR_072991_2		SYNJ2	0.0048375	NM_003898	8871
HIST1H1D	0.0048375	NM_005320	3007	TRIM55	0.0212162	NM_184087	84675
HIST1H3B	0.0375759	NM_003537	8358	TUBGCP5	0.0499355	NM_052903_2	
HOMER2	0.0264114	NM_199330	9455	VAT1L	0.0375759	NM_020927	57687
IGF2BP1	0.0048375	NM_006546	10642	WRAP73	0.0375759	NM_017818	49856

Table S2. Key up or downregulated genes in fibrotic fibroblasts according to histological type

IP > Control		
Tracking ID	**Q value (IP > Control)**	**Q value (UIP > NSIP)**
ADAMTS8	0.0048375	0.0299362
CHL1	0.0239029	0.00938
COLEC12	0.0048375	0.00938
F3	0.0048375	0.00938
FBLN1	0.0048375	0.00938
GFRA1	0.00865342	0.00938
IGFBP2	0.0048375	0.00938
LRRC15	0.0048375	0.00938
MGLL	0.0411378	0.00938
SERINC2	0.0239029	0.00938
VWA5A	0.0394834	0.0237135
WFDC1	0.0048375	0.00938
U2AF1	0.0048375	0.00938
	Q value (IP > Control)	**Q value (UIP < NSIP)**
CCL26	0.0048375	0.0299362
CD36	0.0048375	0.00938
CHI3L1	0.0048375	0.00938
DPP4	0.0212162	0.00938
GOSR2	0.0264114	0.0237135
HSPB6	0.030287	0.00938
IFITM1	0.0048375	0.00938
IFITM2	0.00865342	0.00938
LSAMP	0.01215	0.00938
NNMT	0.0048375	0.00938
POSTN	0.0048375	0.00938
PPAPDC1A	0.030287	0.00938
SEPP1	0.0048375	0.00938
SERPING1	0.0048375	0.00938
TFPI2	0.0048375	0.00938
THBS2	0.0048375	0.0299362
IP < Control		
	Q value (IP < Control)	**Q value (UIP < NSIP)**
G0S2	0.0284973	0.00938
GCOM1	0.0048375	0.00938

	Q value (IP < Control)	**Q value (UIP > NSIP)**
CLU	0.030287	0.00938
FST	0.0048375	0.00938
HIST1H3B	0.0375759	0.0237135
IGF2BP3	0.00865342	0.00938
MDGA1	0.0048375	0.00938
PRDM8	0.0048375	0.00938
TRIM55	0.0212162	0.0237135

Fig. S1

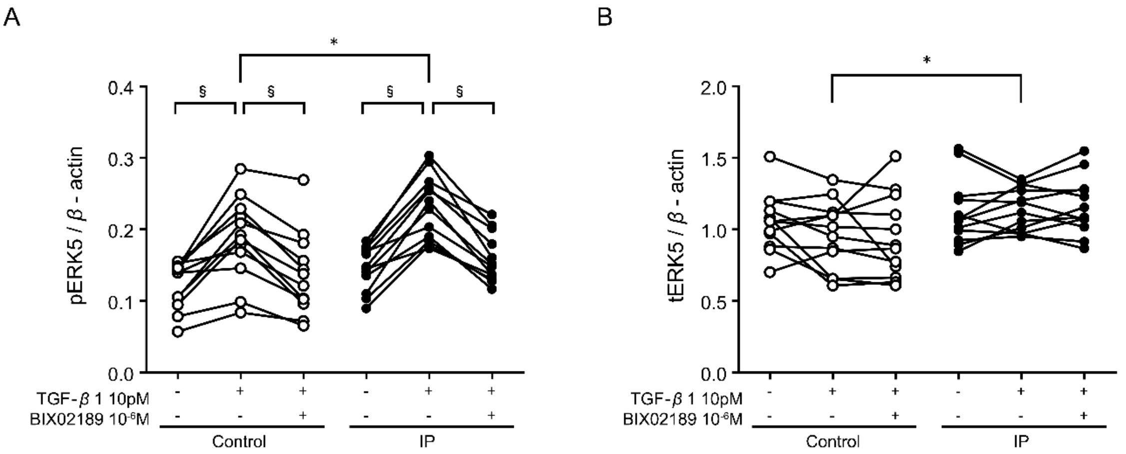

Fig. S1. Regulation of ERK5 signaling in lung fibroblasts. Effect of BIX02189 on transforming growth factor (TGF)-β1-induced Phospho-ERK5 (p-ERK5) (A) and total ERK5 (tERK5) (B). Sub-confluent fibroblasts were cultured in serum-free (SF)-DMEM for 24 h and then incubated in the presence or absence of TGF-β1 or BIX02189 for 8 h. The vertical axis shows the relative intensity of p-ERK5 and ERK5 vs. β-actin; the horizontal axis shows the conditions, control and lung fibrotic (interstitial pneumonia, IP) fibroblasts. The values represent the mean ± SEM of at least three independent experiments. *P < 0.05, §P < 0.001.

Fig. S2

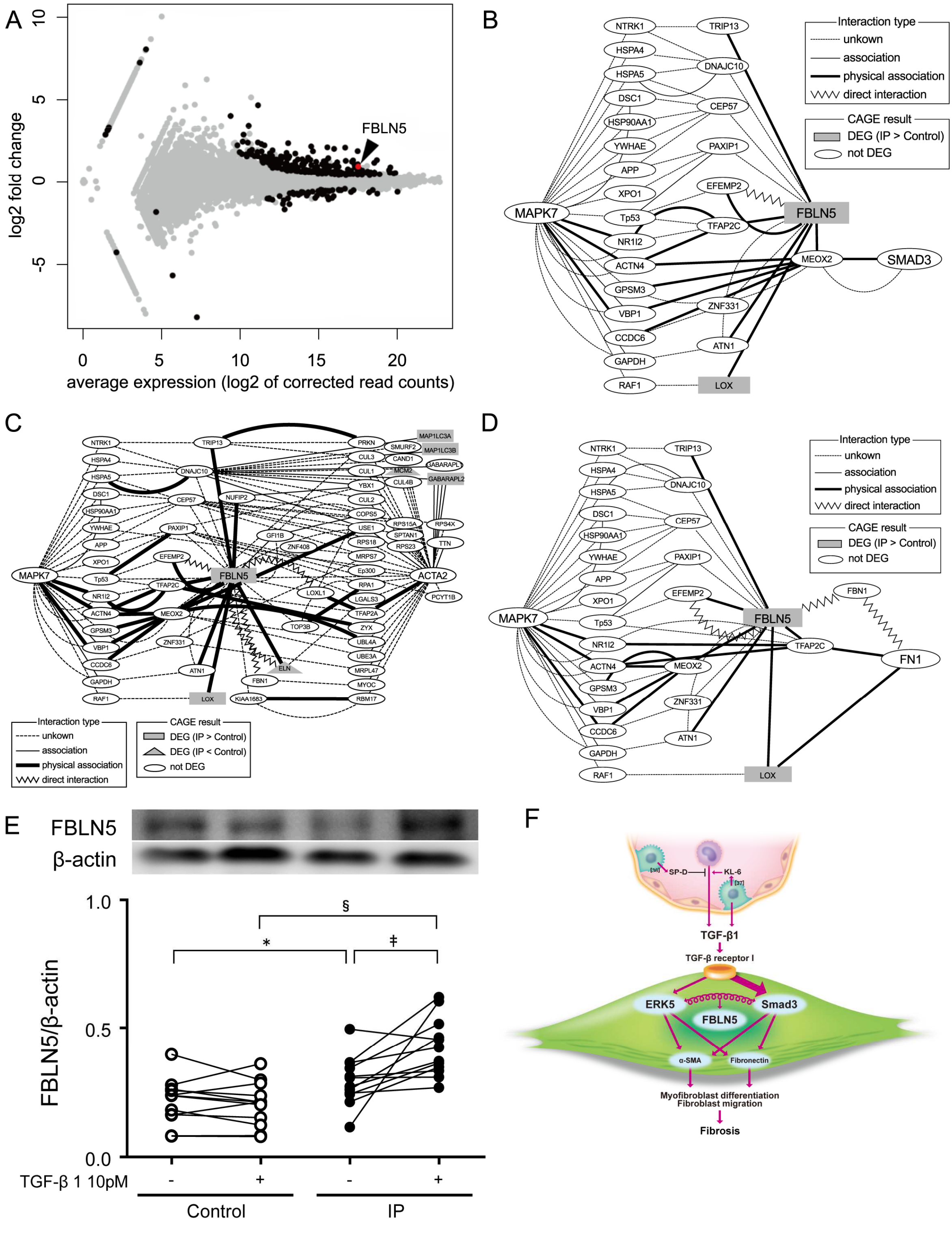

Fig. S2. Bioinformatic analysis of CAGE data to identify candidate markers. (A) MA plot of individual gene expression in lung fibrotic fibroblasts compared to that in control fibroblasts. The horizontal axis represents average expression levels based on cpm on a log2 scale; the vertical axis represents fold change on a log2 scale. Individual dots represent the activities of individual genes; gray and black dots indicate genes with non-differentially expressed and statistically significant differences (FDR < 0.05), respectively; and red dots indicate selected candidate markers. (B–D) extracellular-signal-regulated kinase (ERK5) (MAPK7)-TGF-β1 molecular interaction networks were constructed with VaProS software. Upregulation of fibulin-5 (FBLN5) was closely related to the ERK5 (MAPK7)-TGF-β1-mediated Smad3 (B), α-SMA (ACTA2) (C), and fibronectin (FN1) (D) networks. (E) Regulation of fibulin-5 in lung fibroblasts from control and interstitial pneumonia (IP) subjects. Sub-confluent fibroblasts were cultured in serum-free (SF)-DMEM for 24 h, and then incubated with or without TGF-β1 for 24 h. Total protein was extracted, and western blotting was performed with specific antibodies against fibulin-5. The vertical axis shows the relative intensity of fibulin-5 vs. β-actin; the horizontal axis shows the conditions. *$P < 0.05$, ‡$P < 0.005$, §$P < 0.001$. (F) Through the airway cell-fibroblast interaction, lung fibroblasts are continually exposed to TGF-β1, which is regulated by mediators released by airway cells under inflammatory conditions [36-37], resulting in the distinct phenotype of fibrotic fibroblasts. This high sensitivity to TGF-β1 along with upregulation of FBLN5 and activation of ERK5 signaling leads to the development of fibrosis in fibrotic fibroblasts.

Fig. S3

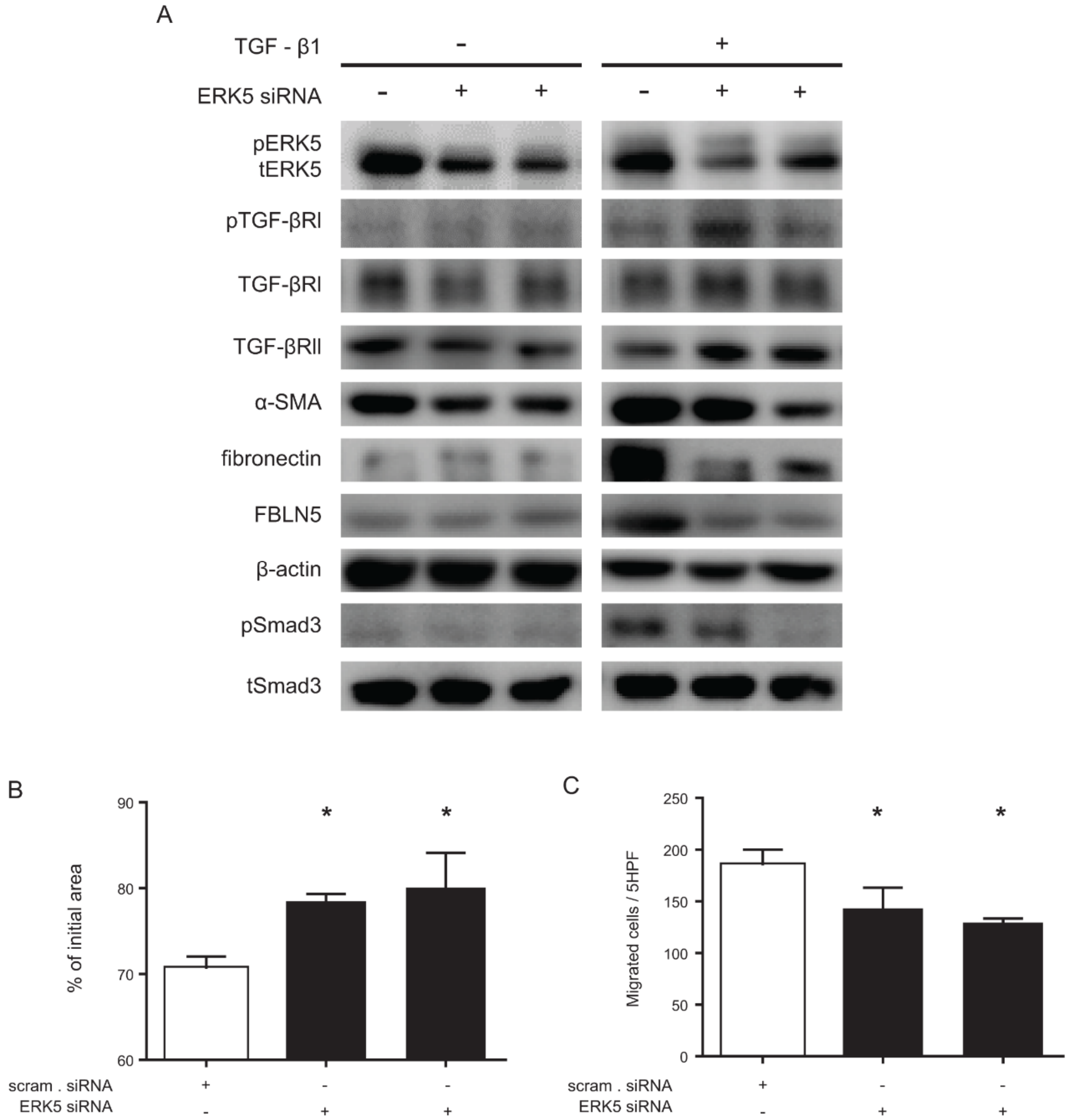

Fig. S3. Effects of the extracellular-signal-regulated kinase (ERK)5 siRNA on HFL-1 cells. (A) HFL-1 cells were transfected with 20 pmol/L control or ERK5 siRNA for 48 h and then treated with 10 pM transforming growth factor (TGF)-β1 for 8 h to detect the expression of targets related to fibrotic processes, i.e., TGF-βRI phosphorylation (p-TGF-βRI), TGF-βRI, TGF-βRII, α-SMA, fibronectin, and fibulin-5 expression. Alternatively, cells were treated with 10 pM TGF-β1 for 30min to detect Smad3 phosphorylation (p-Smad3) and total-Smad3 (t-Smad3) by using Western blot analysis. (B-C) ERK5-knocked down HFL-1 cells were examined for collagen gel contraction (B) and chemotaxis (C). Collagen gel contraction, the vertical axis shows the gel size measured after 3 days of contraction expressed as a percentage of the initial value. Chemotaxis, the vertical axis shows the number of migrated cells per five high-power fields (5 HPF). The horizontal axes show the treatments. The values represent the means ± SEMs of at least three independent experiments. $*P < 0.05$.

ONLINE DATA SUPPLEMENT

Specific features of fibrotic lung fibroblasts highly sensitive to fibrotic processes mediated via TGF-β–ERK5 interaction

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1. Supplementary Methods

1.1 Cell culture

Human fetal lung fibroblasts (HFL1, catalog no. CCL-153) were purchased from the American Type Culture Collection (Manassas, VA, USA). Primary lung fibroblasts were isolated from surgical lung specimens as previously described [1]. Briefly, for normal control lung fibroblasts, portions of lung parenchymal tissue as distal as possible from any tumor were obtained from patients who underwent lung cancer resection. For lung fibrotic fibroblasts, lung parenchymal tissue was isolated from the honeycomb-like fibrotic tissue. All tissues were freed of the pleural surface, minced under sterile conditions, and cultured in DMEM supplemented with 10% Fetal calf serum (FCS), 100 µg/ml penicillin, 250 µg/ml streptomycin, and 1 µg/ml amphotericin B in a humidified atmosphere of 5% CO_2. Cells directly derived from the tissue (referred to as "P0") exhibited a typical spindle-shaped fibroblast-like morphology and were positive for vimentin and negative for cytokeratin staining. The passages 4 to 6 of primary lung fibroblasts were used for the chemotaxis, three-dimensional collagen gel contraction, and enzyme-linked immunosorbent assays to exclude the effect of differences in passage number and culture conditions. Lung fibroblasts from 24 patients, including 12 subjects with lung fibrosis according to a definitive final diagnosis by a multidisciplinary team recognized as the gold standard [2] and 12 control subjects without clinical airway symptoms or lung functional abnormalities were used for the experiments (Table 1).

1.2 Measurement of fibronectin release

Sub-confluent lung fibroblasts grown in a 6-well plate were deprived of serum for 2 h and stimulated with TGF-β1 in the presence or absence of BIX02189. The supernatant of the monolayer culture was harvested after 24 h, frozen, and stored at −80 °C until analysis. Fibronectin production from lung fibroblasts was measured with a human fibronectin immunoassay kit (R&D Systems) according to the manufacturer's instructions.

1.3 Western blotting

To standardize culture conditions, cells were passaged at a density of 8×10^4/ml and cultured for 60 h, then collected for preparation of whole cell lysates. The medium was changed to DMEM without serum for 24 h, and cells were treated with 10 pM TGF-β1 or left untreated in the presence or absence of BIX02189 for 8 or 24 h. Primary antibodies against the following proteins were used for western blotting: Smad3 (cat. no. #9513), phospho-Smad3 (cat. no. #9520), ERK5 (cat. no. #3372), phospho-Smad1 (cat. no. #9553), and Smad1 (cat. no. #9743) (all from Cell Signaling Technology, Danvers, MA, USA); α-smooth muscle actin (α-SMA; Sigma-Aldrich; cat. no. A2547); TGF-βRII (cat. no. sc-17799) and BMP-3 (cat. no. sc-390046) (both from Santa Cruz Biotechnology, Santa Cruz, CA, USA); TGF-βRI (cat. no. #ab31013) and BMP-4 (cat. no. #ab39973) (both from Abcam, Cambridge, UK); β-actin (Wako Pure Chemical Industries; cat. no. #013-24553); Phospho-TGF-βRI (Thermo Scientific, Asheville, NC, USA; cat. no. #PA5-40298); and fibulin-5 (Proteintech, Rosemont, IL, USA; cat. no. #60081-1-lg). Phospho-ERK5 can be detected as the band with slower electrical migration that represents the active form of the protein as previously described [3-4]. The bound antibodies were visualized using peroxidase-conjugated secondary antibodies and enhanced chemiluminescence with a LAS4000 image analyzer (GE Healthcare Bio-Science AB, Uppsala, Sweden); band intensity was analyzed with an ImageQuant TL (GE Healthcare Bio-Science AB).

1.4 Small interfering RNA (siRNA)-mediated knockdown assays

Extracellular-signal-regulated kinase 5 (ERK5) siRNAs were purchased from Invitrogen (Invitrogen by Life Technologies, USA). The sequences of siRNAs were as follows: Stealth siRNAs HSS140814 sense: CCAUGGCAUGAACCCUGCCGAUAUU, anti-sense: AUAUCGGCAGGGUUCAUGCCAUGG. Stealth siRNAs HSS140815 sense: ACAGAUCCGCUUCCAGCCUUCUCUA, anti-sense: UAGAGAAAGGCUGGAAGCGGAUCUGU. The negative control siRNA were purchased from Invitrogen (cat. no. #12935400, Invitrogen by Life Technologies, USA). The siRNAs were transfected into HFL-1 human fetal lung fibroblasts using Lipofectamine 2000 reagent (Invitrogen by Life Technologies, USA) at a concentration of 20pmol per cm^2 dish. Transfected cells were grown at 37 °C in 5% CO_2 for 24 h before the experiments.

1.5 Lipofectamine transfection.

HFL-1 human fetal lung fibroblasts were plated onto 60 mm plates at 50-80% confluence. Transient transfection of siRNA was carried out using Lipofectamine 2000 (Invitrogen by Life Technologies, USA) reagent following the manufacturer's instructions. Briefly, HFL-1 were washed with serum-free medium and cultured for 48 h in serum-free medium without antibiotics. The transfection complex (siRNA and transfection reagent mixture) was added to the medium in a drop-wise manner and mixed gently by rocking the media back and forth. After 4–6 h, the culture medium was changed back to DMEM containing serum and antibiotics and incubated at 37 °C for 48 h. The cells were treated with 10pM TGF-β1 for 30 min or 8 h after transfection. Western blot analysis, collagen gel contraction assays, and chemotaxis assay were performed.

2. Online references

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