

Supplementary Data

Use of Patterned Collagen Coated Slides to Study Normal and Scleroderma Lung Fibroblast Migration

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Target Protein Name	Phospho Site (Human)	Full Target Protein Name	Globally Normalized Dermis-Like	Globally Normalized Linear	%Error Range Linear	Z-ratio (Linear, Dermis-Like)
Erk2	Pan-specific	Extracellular regulated protein-serine kinase 2 (p42 MAP kinase)	972	2826	10.88	2.03
CAMK2d	Pan-specific	Calcium/calmodulin-dependent protein-serine kinase 2 delta	2394	6789	8.43	1.98
CAMK2b	Pan-specific	Calcium/calmodulin-dependent protein-serine kinase 2 beta	2491	6515	14.21	1.83
Kit	Y936	Kit/Steel factor receptor-tyrosine kinase	1442	3701	3.66	1.79
CaMKK (CaMKK2)	Pan-specific	Calcium/calmodulin-dependent protein-serine kinase kinase	3373	8575	10.45	1.77
CASP7	Pan-specific	Caspase 7 (ICE-like apoptotic protease 3 (ICE-LAP3), Mch3)	2270	5724	2.16	1.76
CASP5	Pan-specific	Caspase 5 (ICH3 protease, ICE(rel)-III)	1219	3060	4.71	1.75
CaMK1d	Pan-specific	Calcium/calmodulin-dependent protein-serine kinase 1 delta	2016	5002	18.07	1.73
ErbB2 (HER2)	Pan-specific	ErbB2 (Neu) receptor-tyrosine kinase	1330	3287	14.68	1.72
CDK5	Pan-specific	Cyclin-dependent protein-serine kinase 5	3221	7838	2.34	1.69
CDK2	Pan-specific	Cyclin-dependent protein-serine kinase 2	1096	2614	19.84	1.65
Hsp90a/b	Pan-specific	Heat shock 90 kDa protein alpha/beta	5453	12997	2.33	1.65
CaMK4	Pan-specific	Calcium/calmodulin-dependent protein-serine kinase 4	3108	7236	3.65	1.60
CASP4	Pan-specific	Caspase 4 (ICH2 protease, ICE(rel)-II)	3704	8496	4.34	1.58
CDK4	Pan-specific	Cyclin-dependent protein-serine kinase 4	1440	3287	8.07	1.57
PKCa	Pan-specific	Protein-serine kinase C alpha	2886	1286	24.17	-1.54
PKA Cb	S339	cAMP-dependent protein-serine kinase catalytic subunit beta	2814	1251	2.46	-1.55
PKC	Pan-specific	Protein-serine kinase C alpha	3985	1764	1.30	-1.55
Vimentin	S34	Vimentin	4855	2113	2.85	-1.59
PTP1C	Pan-specific	Protein-tyrosine phosphatase 1C (SHP1, SHPTP1)	3535	1508	3.99	-1.62
PKA Ca/b	Pan-specific	cAMP-dependent protein-serine kinase catalytic subunit alpha/beta	3319	1376	26.59	-1.68
SOD (Cu/Zn)	Pan-specific	Superoxide dismutase 1	2877	1152	4.69	-1.74
VHR	Pan-specific	Dual specificity protein phosphatase 3	3899	1173	12.78	-2.29
PP2Cd	Pan-specific	Protein-serine phosphatase 2C - catalytic subunit - delta isoform	4181	1119	5.11	-2.51

Table S1. Phosphorylation profiling of aligned and non-aligned normal lung fibroblasts. Kinexus analysis showing phosphoproteins of increased (>1.5) or decreased (<1.5) abundance in migrating cells.

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Group	Gene Name	Unique peptides	Sequence coverage [%]	Mol. weight [kDa]	Intensity	Protein
Control only	CILP	1	1.3	132.56	412810	cartilage intermediate layer protein
	CSTB	2	24.5	11.139	155530	cystatin B
	LGALS7	2	18.4	15.075	296800	lectin, galactoside-binding, soluble, 7
	MMP14	2	3.8	65.893	245920	matrix metalloproteinase 14
	S100A16	1	10.7	11.801	96256	S100 calcium binding protein A16
	SERPINB10; SERPINB2	2	7.5	28.463	360250	serpin peptidase inhibitor, clade B (ovalbumin), member 10; serpin peptidase inhibitor, clade B (ovalbumin), member 2
	SERPINB6	4	16.3	43.024	606800	serpin peptidase inhibitor, clade B (ovalbumin), member 6
	TGM1	1	4.3	40.628	96502	transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase)
SSc only	ANXA7	1	1.9	50.315	199460	annexin A7
	COL1A2	6	6.2	129.15	1857500	collagen, type I, alpha 2
	COL3A1	2	1.8	138.44	300140	collagen, type III, alpha 1
	DPT	1	5.5	24.005	135510	dermatopontin
	EDIL3	1	3.8	52.747	119100	EGF-like repeats and discoidin I-like domains 3
	F10	1	2.3	54.731	256440	coagulation factor X
	F13A1	1	11.6	15.951	172310	coagulation factor XIII, A1 polypeptide
	F2	4	11.8	70.505	1745500	coagulation factor II (thrombin)
	LAMB1	1	0.6	200.48	136580	laminin, beta 1
	LAMC1	1	0.9	177.6	195810	laminin, gamma 1
	LEPRE1	5	8.2	78.921	2170400	leucine proline-enriched proteoglycan (leprecan) 1
	LEPREL2	1	2	81.836	135110	leprecan-like 2
	P4HA1	1	24	61.049	2208600	prolyl 4-hydroxylase, alpha polypeptide I
	PLOD1	10	20.5	83.549	2915800	procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1
	PLOD3	4	7.2	84.784	626960	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3
	PXDN	6	7.6	165.27	1873500	peroxidasin homolog
	S100A4	1	10.9	11.728	102590	S100 calcium binding protein A4
	SERPINA1	1	2.8	40.234	111420	serpin peptidase inhibitor, clade A (alpha-1 antitrypsin, antitrypsin), member 1
	SPARC	2	15.4	17.47	882830	secreted protein, acidic, cysteine-rich (osteonectin)
	THBS1	14	16.1	129.38	6713500	thrombospondin 1
	TIMP1	1	12.5	10.706	323820	TIMP metalloproteinase inhibitor 1

Table S2. Protein modification of extracellular matrices by SSc lung fibroblasts. SSc and control lung fibroblasts were cultured for 48 hours and then switched to serum-free conditions overnight before cells were detached using trypsin-EDTA. The remaining extracellular matrix was extracted, fractionated, digested and analysed using mass spectrometry and bioinformatics.

Supplementary Data

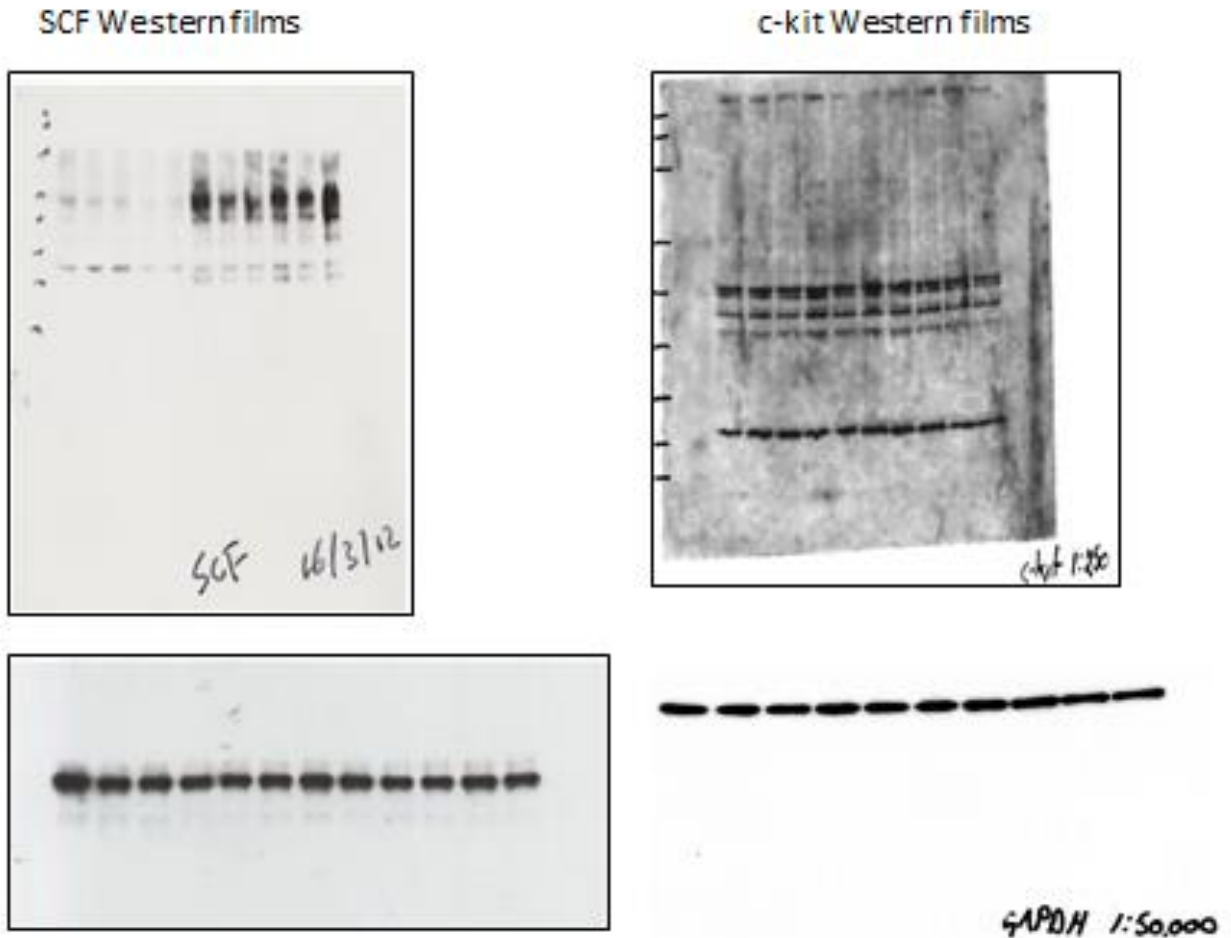


Figure S3. Scanned western blot films of stem cell factor, c-kit and their respective GAPDH. SSc and control lung fibroblasts both n=5-6, were cultured. Western blot showed that SCF protein was increased in SSc cells compared to the controls. There was no difference in c-kit protein amount between SSc and control fibroblasts.