

Functional Insights into the Sphingolipids C1P, S1P, and SPC in Human Fibroblast-Like Synoviocytes by Proteomic Analysis

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Table S1. Proteins reproducibly regulated by C1P, S1P and SPC in human FLSs.

Accession ID	Gene	Protein name	AR C1P	AR S1P	AR SPC	AR IL-1 β
P09341	CXCL1	Growth-regulated alpha protein	22.8 $\pm$ 38.8 *	22.3 $\pm$ 38.9	15.1 $\pm$ 37.4	17.3 $\pm$ 15.5 **
			(1.34 $\pm$ 0.70)	(1.54 $\pm$ 1.20)	(1.52 $\pm$ 0.92)	(482 $\pm$ 329)
P08254	MMP3	Stromelysin-1	1.70 $\pm$ 0.68 ***	1.14 $\pm$ 0.48	1.01 $\pm$ 0.45	15.2 $\pm$ 9.65 **
			(0.75 $\pm$ 0.37)	(1.21 $\pm$ 0.87)	(1.36 $\pm$ 0.85)	
Q7LBR1	CHMP1B	Charged multi-vesicular body protein 1b	1.49 $\pm$ 0.39 *	1.45 $\pm$ 0.44	1.23 $\pm$ 0.27	5.01 $\pm$ 2.31 **
			(1.23 $\pm$ 0.50)	(1.49 $\pm$ 0.77)	(1.19 $\pm$ 0.48)	
Q15043	SLC39A14	Metal cation symporter ZIP14	1.45 $\pm$ 0.27 **	1.16 $\pm$ 0.17	1.00 $\pm$ 0.13	7.73 $\pm$ 3.16 **
			(0.88 $\pm$ 0.25)	(0.98 $\pm$ 0.53)	(1.29 $\pm$ 0.47)	
P47712	PLA2G4A	Cytosolic phospholipase A2	1.32 $\pm$ 0.11 ***	1.20 $\pm$ 0.09	1.08 $\pm$ 0.08	3.43 $\pm$ 1.47 **
			(0.89 $\pm$ 0.16)	(0.84 $\pm$ 0.35)	(1.08 $\pm$ 0.32)	
Q4EZA9	MT-CO1	Cytochrome c oxidase subunit 1	1.32 $\pm$ 0.39	1.58 $\pm$ 0.33 **	1.39 $\pm$ 0.34	1.05 $\pm$ 0.28
			(1.03 $\pm$ 0.24)	(1.13 $\pm$ 0.32)	(0.99 $\pm$ 0.20)	
Q7Z7M4	SOD2	Superoxide dismutase (Fragment)	1.30 $\pm$ 0.20 *	1.23 $\pm$ 0.17	1.14 $\pm$ 0.16	4.35 $\pm$ 1.63 **
			*	*		
O60488	ACSL4	Long-chain-fatty-acid--CoA ligase 4	1.29 $\pm$ 0.18 *	1.15 $\pm$ 0.09	1.04 $\pm$ 0.11	4.76 $\pm$ 1.36 ***
			*	*		
Q5NKV8	ICAM1	Intercellular adhesion molecule 1	1.29 $\pm$ 0.17 **	1.17 $\pm$ 0.12	1.01 $\pm$ 0.03	4.17 $\pm$ 1.34 **
			**	*		
A0A0S2Z4X9	GFPT2	Glutamine-fructose-6-P transaminase (isomerizing)	1.26 $\pm$ 0.05 ***	1.15 $\pm$ 0.10	1.05 $\pm$ 0.07	3.94 $\pm$ 1.34 **
			***	*		

Proteins are ordered according to their mean abundance ratio (AR) in C1P-treated FLSs. The ARs of protein levels in FLSs treated with S1P, SPC and IL-1 β are shown relative to the abundance of their untreated controls. Accession IDs, gene symbols, and protein names were obtained from UniProt Knowledgebase. The ARs are presented as mean $\pm$ SD, and those reproducibly regulated in at least 11 of 14 replicates are shown in bold. The mRNA expression of select proteins was determined by RT-PCR using the 2 $^{-\Delta\Delta C_t}$ method. The data are presented in brackets and cursive style as mean $\pm$ SD of the fold-change in mRNA expression relative to untreated control (normalized to 1). *0.05 $\geq P > 0.01$; **0.01 $\geq P > 0.001$; *** $P \leq 0.001$.

Table S2. The 19 proteins reproducibly regulated by C1P, S1P and/or SPC in the presence of IL-1 β .

Accession ID	Gene	Protein name	AR C1P+IL-1 β	AR S1P+IL-1 β	AR SPC+IL-1 β	AR IL-1 β
P09341	CXCL1	Growth-regulated alpha protein	20.8 $\pm$ 18.2	22.5 $\pm$ 20.9 *	22.7 $\pm$ 21.8	17.3 $\pm$ 15.5
P02795	MT2A	Metallothionein-2A	(503 $\pm$ 256) 7.13 $\pm$ 2.55 **	(449 $\pm$ 275) 7.09 $\pm$ 3.15 **	(471 $\pm$ 321) 6.38 $\pm$ 3.24 *	(482 $\pm$ 329) 3.47 $\pm$ 1.47
P04733	MT1F	Metallothionein-1F	(4.34 $\pm$ 2.42) 6.23 $\pm$ 3.05 *	(4.19 $\pm$ 2.45) 6.76 $\pm$ 4.92 *	(4.06 $\pm$ 1.92) 6.23 $\pm$ 4.61 *	(4.44 $\pm$ 1.99) 3.89 $\pm$ 2.91
P09038-1	FGF2	Isoform 2 of Fibroblast growth factor 2	1.84 $\pm$ 0.46	1.95 $\pm$ 0.46 **	1.70 $\pm$ 0.35	1.49 $\pm$ 0.32
D6W5K2	TMSB10	Thymosin, β 10, isoform CRA_a (Fragment)	1.59 $\pm$ 0.77	1.71 $\pm$ 0.76 **	1.64 $\pm$ 0.52 *	1.31 $\pm$ 0.46
Q59EN5	PSAP	Prosaposin	1.59 $\pm$ 1.27	1.80 $\pm$ 1.44 **	1.51 $\pm$ 1.11	1.21 $\pm$ 1.07
A0A6I8PLD9	KRTCAP2	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit KCP2	(0.96 $\pm$ 0.36) 1.43 $\pm$ 0.40 **	(0.98 $\pm$ 0.28) 1.41 $\pm$ 0.38 **	(0.83 $\pm$ 0.22) 1.41 $\pm$ 0.41 *	(0.94 $\pm$ 0.24) 1.02 $\pm$ 0.21
O60476	MAN1A2	Mannosyl-oligosaccharide 1,2- α -mannosidase IB	1.38 $\pm$ 0.30	6.15 $\pm$ 3.33 *	1.76 $\pm$ 0.48	1.50 $\pm$ 0.23
P42677	RPS27	40S ribosomal protein S27	1.33 $\pm$ 0.26 ***	1.23 $\pm$ 0.28 ***	1.13 $\pm$ 0.24	1.05 $\pm$ 0.24
Q71UM5	RPS27L	40S ribosomal protein S27-like	1.19 $\pm$ 0.19 **	0.87 $\pm$ 0.12 **	0.77 $\pm$ 0.11	0.78 $\pm$ 0.12
A0A024QYX3	RBM3	RNA binding motif (RNP1, RRM) protein 3, isoform CRA_c	(0.47 $\pm$ 0.06) 1.19 $\pm$ 0.23 *	(0.47 $\pm$ 0.05) 1.18 $\pm$ 0.21 *	(0.52 $\pm$ 0.08) 1.17 $\pm$ 0.17 *	(0.50 $\pm$ 0.08) 0.87 $\pm$ 0.25
A8K644	SFRS4	Splicing factor, arginine/serine-rich 4, isoform CRA_b	1.18 $\pm$ 0.22	1.16 $\pm$ 0.17 *	1.15 $\pm$ 0.13 *	0.90 $\pm$ 0.17

P62841	<i>RPS15</i>	40S ribosomal protein S15	1.13 ± 0.25 **	1.13 ± 0.28 **	1.10 ± 0.22 *	0.90 ± 0.25
P30048	<i>PRDX3</i>	Thioredoxin-dependent peroxide reductase, mitochondrial	1.08 ± 0.12	1.11 ± 0.16 *	1.12 ± 0.17 *	0.89 ± 0.24
Q8N5Y3	<i>GYG1</i>	GYG1 protein (Fragment)	0.89 ± 0.25	0.83 ± 0.20 **	0.86 ± 0.17 *	1.17 ± 0.23
B4DLF7		cDNA FLJ60294	0.81 ± 0.20 *	0.80 ± 0.18 **	0.83 ± 0.18 *	2.95 ± 1.40
Q96FJ2	<i>DYNLL2</i>	Dynein light chain 2, cytoplasmic	0.68 ± 0.43 (0.70±0.29)	0.66 ± 0.44 * (0.88±0.24)	0.61 ± 0.41 * (0.77±0.28)	1.04 ± 0.76 (0.84±0.27)
A0A384M DR3	<i>FTL</i>	Ferritin	0.58 ± 0.18 **	0.58 ± 0.22 *	0.59 ± 0.26	0.80 ± 0.19
Q6NS36	<i>FTH1</i>	Ferritin (Fragment)	0.57 ± 0.16 * (0.93±0.13)	0.56 ± 0.15 ** (0.93±0.09)	0.58 ± 0.18 * (0.93±0.10)	0.91 ± 0.15 (1.05±0.18)

Proteins are ordered according to their mean abundance ratio (AR) in FLS treated with C1P in the presence of IL-1 β . The ARs of protein levels in human FLSs treated with C1P, S1P or SPC in the presence of IL-1 β and IL-1 β alone are shown relative to the abundance of their untreated controls. Accession IDs, gene symbols, and protein names were obtained from UniProt Knowledgebase. The ARs are presented as mean $\pm$ SD, and those reproducibly regulated above 1.2-fold or below 0.8-fold relative to FLS treated with IL-1 β alone in at least 11 of 14 replicates are shown in bold. The mRNA expression of select proteins was determined by RT-PCR using the $2^{-\Delta\Delta C_t}$ method. The data are presented in brackets and cursive style as mean $\pm$ SD of the fold-change in mRNA expression relative to untreated control (normalized to 1). Significantly different to the AR IL-1 β as indicated by the Holm-Sidak-adjusted P-value: *0.05 $\geq$ P > 0.01; **0.01 $\geq$ P > 0.001; ***P $\leq$ 0.001.

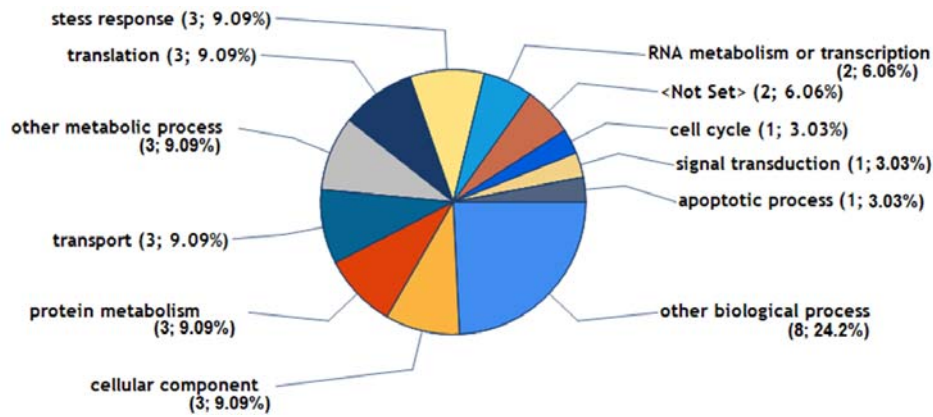


Figure S1. The biological processes of FLS being altered by C1P in the presence of IL-1 β . The 10 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold in FLS by C1P + IL-1 β during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

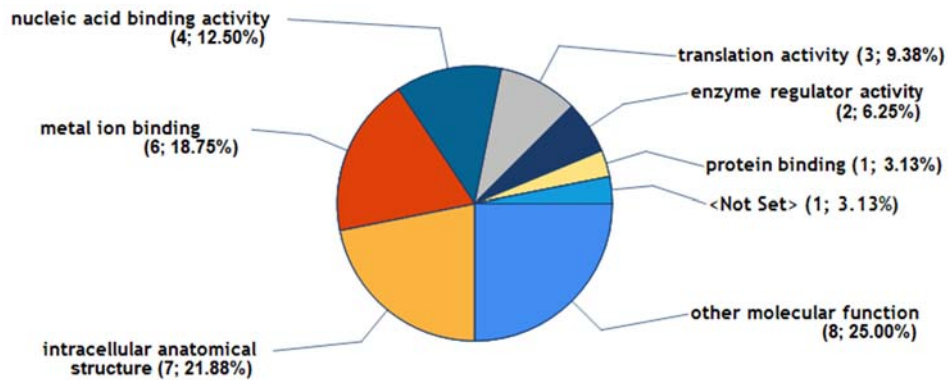


Figure S2. The molecular functions of FLS being altered by C1P in the presence of IL-1 β . The 10 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold by C1P + IL-1 β in FLS during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

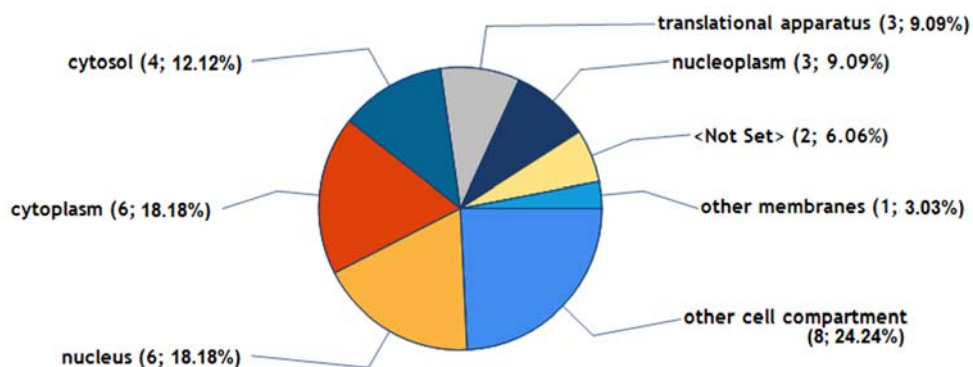


Figure S3. The cellular localization of 10 proteins being regulated by C1P in the presence of IL-1 β . The 10 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold in FLS by C1P + IL-1 β during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

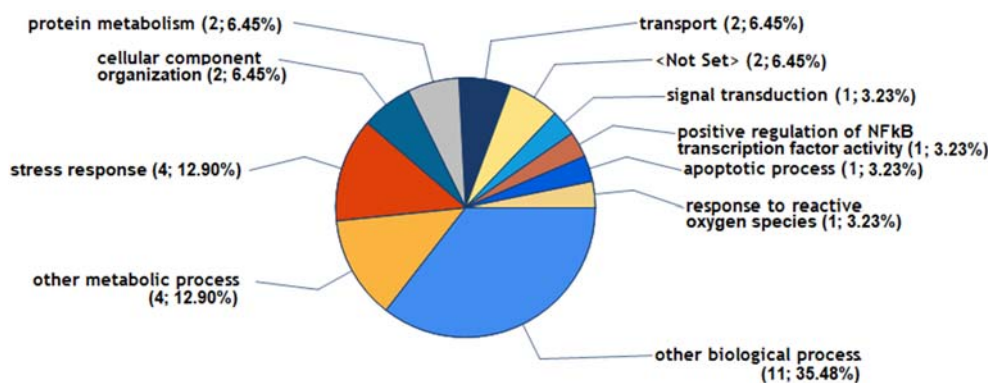


Figure S4. The biological processes of FLS being altered by S1P in the presence of IL-1 β . The 13 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold in FLS by S1P + IL-1 β during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

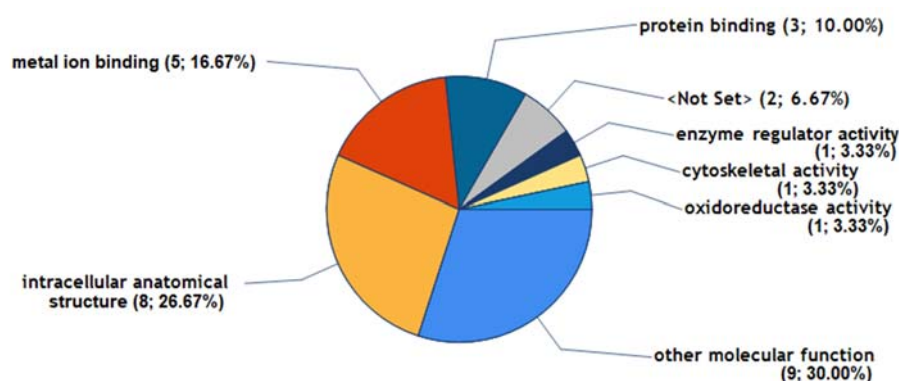


Figure S5. The molecular functions of FLS being altered by S1P in the presence of IL-1 β . The 13 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold by S1P + IL-1 β in FLS during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

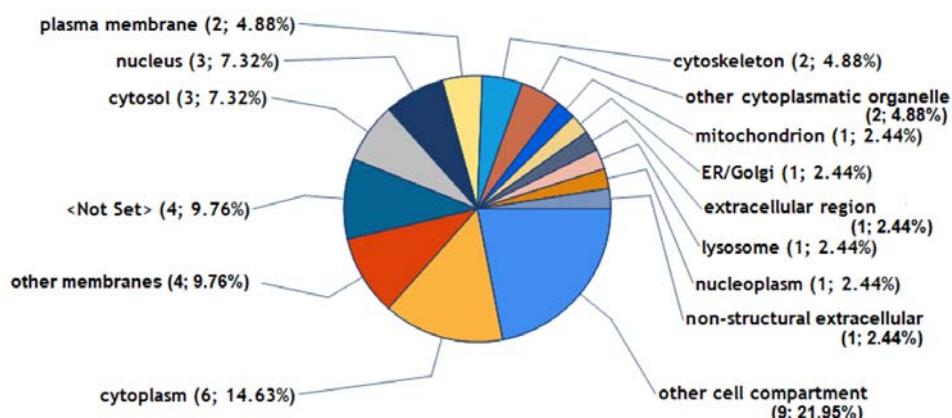


Figure S6. The cellular localization of 13 proteins being regulated by S1P in the presence of IL-1 β . The 13 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold in FLS by S1P + IL-1 β during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

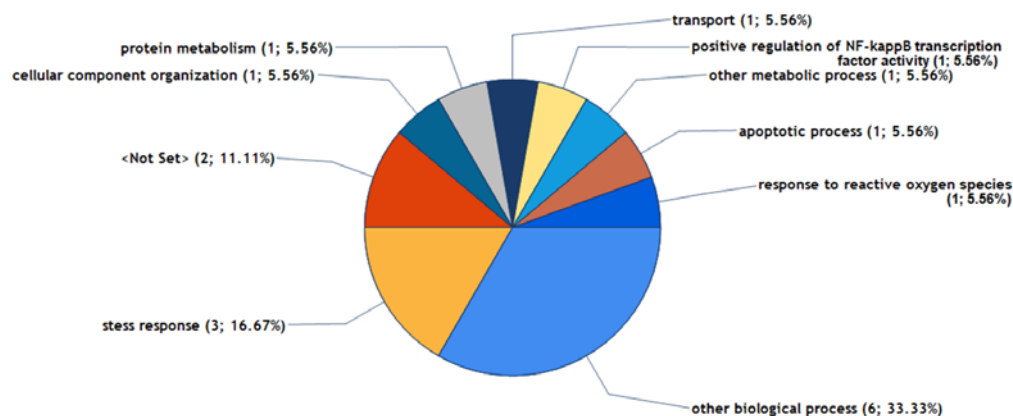


Figure S7. The biological processes of FLS being altered by SPC in the presence of IL-1 β . The 8 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold in FLS by SPC + IL-1 β during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

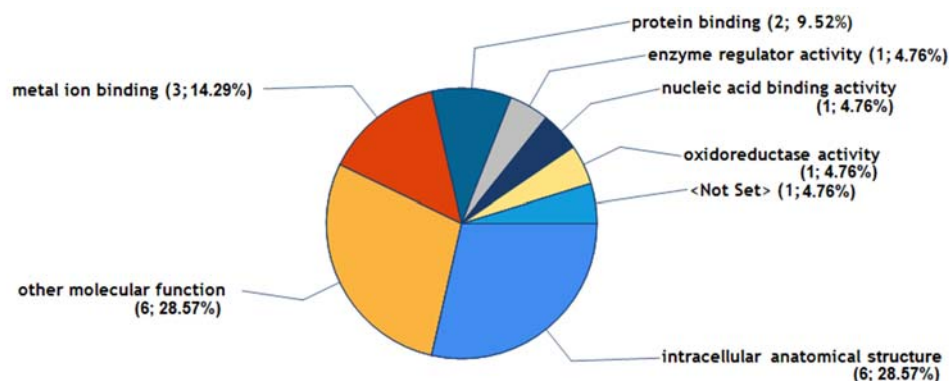


Figure S8. The molecular functions of FLS being altered by SPC in the presence of IL-1 β . The 8 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold by SPC + IL-1 β in FLS during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.

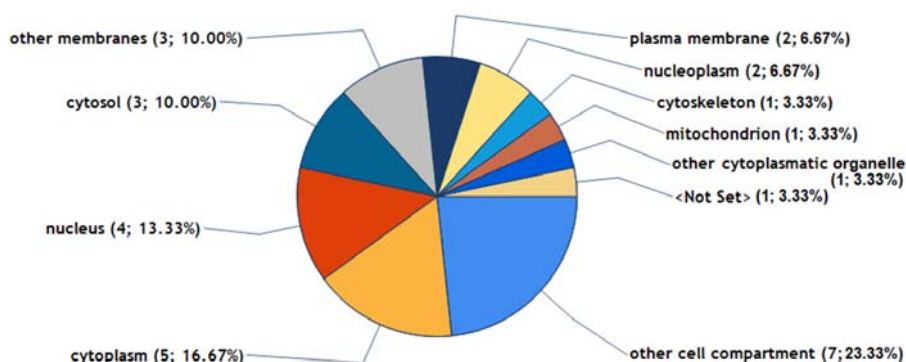


Figure S9. The cellular localization of 8 proteins being regulated by SPC in the presence of IL-1 β . The 8 proteins were reproducibly upregulated by at least 1.2-fold or downregulated by 0.8-fold in FLS by SPC + IL-1 β during 48 h of treatment relative to FLS treated with IL-1 β alone. Table S2 provides further data on these proteins. The Go Slim categories for proteins were generated by Proteome Discoverer 2.5 software using the Gene Ontology (GO) database.