

Supplemental data

Supplemental Table 1A

Effect of DUSP1 over-expression and IL1B on inflammatory gene expression. A549 cells were either not infected or infected with Ad5-DUSP1 or Ad5-Null (control) at a MOI of 10 for 24 h before either no stimulation (NS) or IL1B treatment (1 ng/ml). Cells were harvested after 6 h for real-time PCR analysis of the indicated genes and GAPDH. Data ($N = 4$), normalized to GAPDH, were expressed as a percentage of IL1B-stimulated cells. Data are shown as a heat map in Fig. 1A. Significance relative to IL1B-treated samples, was tested by ANOVA with a Dunnett's post-test. All analyses compare Ad5-DUSP1 to IL1B-treatment and in no case was there any significant effect of the null virus (Ad-null).

NS	IL1B	IL1B+Ad5-DUSP1	IL1B+Ad5-Null	HUGO gene	P value
0.046798	100	6.908991	96.9073	PI3	***
4.796774	100	8.169916	89.70302	TFF1	***
0.430232	100	14.65387	111.7821	CSF2	***
0.586421	100	14.932	97.17675	CXCL3	***
5.605619	100	17.13548	97.28996	PTGS2	***
1.600973	100	18.73996	122.4827	CXCL5	***
0.240848	100	21.38961	112.7739	IL8	***
0.100442	100	36.46495	103.4321	CCL20	**
11.8663	100	53.24498	83.30597	NFKBIA	***
20.7395	100	79.60745	92.86077	MAP3K8	
3.444836	100	80.86997	87.10033	BIRC3	
20.32855	100	81.52168	117.2474	IFNGR1	
1.784336	100	82.60778	116.4463	CCL2	
0.024887	100	84.04284	101.4327	CCL5	
0.115963	100	93.49159	83.47164	IL6	
5.563433	100	85.41597	104.6495	CCL4	**
2.735732	100	104.5693	94.8439	TNFAIP3	
18.02371	100	134.9119	92.17291	EFNA1	
0.698538	100	142.261	89.97934	ICAM1	**
16.0625	100	150.3968	86.24011	IFIT3iso1	**
4.592691	100	166.6928	73.39421	HELZ2	*
7.253281	100	187.8341	115.042	APOL6	***
2.341992	100	221.3489	93.69964	CFB	***
2.512993	100	237.123	92.23913	MX1	**
17.80476	100	248.1079	121.9886	STAT5A	**
0.46891	100	323.0425	104.0344	UBD	***
3.200933	100	519.4126	80.72658	CMPK2	***
6.335893	100	801.4803	96.54577	IFIT1	***
22.75595	100	3430.191	123.7676	TLR2	***
0.689279	100	3682.19	110.7226	IFIT3iso2	***
0.108521	100	3984.608	92.90648	CXCL10	***

Supplemental Table 1B

Effect of IL1B and IRF1-targeting siRNA on inflammatory gene expression. A549 cells were incubated with LMNA (control) or IRF1-targeting siRNAs prior to either no stimulation (NS) or IL1B treatment (1 ng/ml). Cells were harvested after 6 h for real-time PCR analysis of the indicated genes and GAPDH. Data ($N = 4$), normalized to GAPDH, were expressed as a percentage of IL1B+LMNA siRNA-stimulated cells. Data are shown as a heat map in Fig. 1F. For each IRF1-targeting siRNA, significance relative to IL1B+LMNA siRNA-treated samples, was tested by ANOVA with a Dunnett's post-test.

NS	IL1B+LMNAsiRNA	IL1B+IRF1siRNA1	IL1B+IRF1siRNA2	Gene	p value	
11.47971	100	6055.417	4361.353	CCL5	***	***
35.32663	100	2276.193	1854.385	CXCL5	***	***
0.058747	100	126.8368	168.0418	CCL20		
0.144538	100	115.5344	155.3629	CSF2		
9.234919	100	96.67167	149.5014	TFF1		
0.544161	100	128.9808	145.5629	TNF		
17.5615	100	93.34953	135.3033	EFNA1		
19.29435	100	109.7398	132.5686	TLR2		
0.244158	100	111.3051	125.8073	ICAM1		
5.607688	100	95.07089	122.0188	BIRC3		
6.396882	100	91.30604	115.4286	TNFAIP3		
5.871413	100	133.0294	115.107	GOS2		
23.92698	100	91.31629	112.6268	MAP3K8		
0.395436	100	110.514	112.5134	PI3		
1.197002	100	94.28899	96.69292	CCL2		
0.01547	100	85.99541	94.79675	IL6		
13.6009	100	145.3991	93.68611	STAT5A		
6.818868	100	87.02939	77.79903	PTGS2		
2.756561	100	59.18466	68.75256	CFB	*	*
0.349935	100	60.33389	52.90243	UBD	*	*
9.547711	100	39.80418	41.1086	IFIT3iso1	*	*
5.853933	100	41.83818	37.58789	HELZ2	*	**
6.243556	100	39.29731	29.18912	APOL6	***	***
2.018916	100	36.51087	25.29688	IFIT1	***	***
0.549646	100	26.75874	24.35754	MX1	***	***
0	100	33.65772	16.36983	CXCL10	*	**
0.884514	100	28.58642	12.78376	CMPK2	***	***
0.325002	100	19.42893	9.812231	IFIT3iso2	***	***

Supplemental Table 2

Effect of PS1145 on the mRNA expression of IRF1 and IRF1-dependent genes. A549 cells were either not stimulated (NS), treated with IL1B (1 ng/ml) or pre-treated with PS1145 at 10 μ M for 30 min prior to IL1B stimulation. Cells were harvested after 2 h (for IRF1) or 6 h (for IRF1-dependent genes) for real-time PCR analysis of the indicated genes and GAPDH. Data (n = 4) were normalized to GAPDH and presented as means \pm S.E. Significance, relative to IL1B-treated samples was tested using a paired t test.

Time (h)	Gene Symbol	Gene mRNA/GAPDH		
		NS	IL1B	IL1B + PS1145
2	IRF1	0.024 \pm 0.058	286.3 \pm 95	129.8 \pm 60*
6	APOL6	0.16 \pm 0.033	1.3 \pm 0.046	0.31 \pm 0.041***
	CFB	0.071 \pm 0.022	1.2 \pm 0.072	0.24 \pm 0.012***
	CMPK2	0.064 \pm 0.029	1.02 \pm 0.14	0.14 \pm 0.015***
	CXCL10	0.027 \pm 0.028	0.76 \pm 0.16	0.041 \pm 0.010**
	HELZ2	0.044 \pm 0.011	3.2 \pm 0.67	0.22 \pm 0.026**
	IFIT1	0.013 \pm 0.0081	7.9 \pm 1.4	0.11 \pm 0.040*
	IFIT3 iso1	0.14 \pm 0.012	1.0 \pm 0.10	0.27 \pm 0.025**
	IFIT3 iso2	0.035 \pm 0.021	0.92 \pm 0.054	0.16 \pm 0.020***
	MX1	0.011 \pm 0.0091	4.7 \pm 1.5	0.043 \pm 0.016**
UBD	0.0087 \pm 0.0060	3.9 \pm 1.0	0.36 \pm 0.13**	

Supplemental Table 3

(A) Primers used for PCR analysis

Forward (F) and reverse (R) primer sequences (5'-3') are shown in addition to the accession number for each gene. For genes with more than one splice variant, primers were designed to pick up all variants, with the exception of IFIT3 for which two sets of primers were designed. All primers were designed using Primer Express software (Applied Biosystems) and were synthesised by the DNA synthesis lab at the University of Calgary.

Target gene	Accession Number	Primer Sequences
APOL6	NM_030641.3	F: CCCTGCCAGACCAGGGGACC R: GGAGCGTCATCCTCATCCCTTGC
BCL2A1	NM_001114735.1; NM_004049.3	F: CCCC GGATGTGGATACCTA R: CTAGAAAAGTCATCCAGCCAGA
BIRC3	NM_001165.3; NM_182962.1	F: CCGTCAAGTTC AAGCCAGTTACCC R: AGCCATTTCACGGCAGCA
CCL2	NM_002982.3	F: GCTCGCTCAGCCAGATGCAA R: TCCTGAACCCACTTCTGCTTG
CCL4	NM_002984.3	F: CTTCTCGCAACTTTGTGGT R: GCTTGCTTCTTTTGGTTTG

Negative regulation of IRF1 by MAPKs

CCL5	NM_002985.2	F: TGCCTACATTGCCCGCCAC R: GGGTTGGCACACACTTGGCG
CCL20	NM_001130046.1; NM_004591.2	F: TGACATCAATGCTATCATCTTTTACA R: TTTGCGCACACAGACAACCTTT
CFB	NM_001710.5	F: ATGCCACATACCCCAAATTTGGGT R: GTTAGTCCCTGACTTCAACTTGTGGT
CMPK2	NM_207315.2	F: GCCGGGGCATGGAGAAGACC R: TGGAGGGGCTGGCATCAACCA
CSF2	NM_000758.2	F: CCATGATGGCCAGCCACTACAAGC R: ACTGGCTCCAGCAGTCAAAGG
CXCL1	NM_001511.2	F: TCAATCCTGCATCCCCCA R: CATAGAATCTTCAAACTAATGAATAAAT
CXCL3	NM_002090.2	F: TCATCGAAAAGATACTGAACAAGGG R: GAAGTGTCAATGATACGCTGATAAGC
CXCL5	NM_002994.4	F: CCCAAAATGATCAGTAATCTGCAA R: CAAATTTCTTCCCGTTCTTCA
CXCL10	NM_139089.1	F: TTCCTGCAAGCCAATTTTGTCT R: TCTTCTCACCCCTTCTTTTTTCATTGT
EFNA1	NM_004428.2; NM_182685.1	F: TCACAGTCCTCAGGCCCATGACA R: GTGGGGCAGCACTGTGACCG
FAM129A	NM_052966.2	F: GCTGGACGAGGGCAAGTGCG R: AGGCGCCAATGGTGGCTTGG
GAPDH	NM_002046	F: TTCACCACCATGGAGAAGGC R: AGGAGGCATTGCTGATGATCT
GOS2	NM_015714.3	F: GCGCCGTGCCACTAAGGTCA R: CACGCTGCCAGCACGTACA
ICAM-1	NM_000201.2	F: TGCCCTGATGGGCAGTCAACA R: GCAGCGTAGGGTAAGGTTCTTG
IFIT1	NM_001548.3	F: AACCTGCAGAACGGCTG R: TGAAAGTGACATCTCAATTGCTCC
IFIT3iso1	NM_001549.4	F: GCGTGCCCTACTCTCCCACC R: AGCTGTGGAAGGATTTTCTCCAGGG
IFIT3iso2	NM_001031683.2	F: TCAGAACTGCAGGGAAACAGCCA R: AGCTGTGGAAGGATTTTCTCCAGGG
IFNGR1	NM_000416.2	F: GAATTTGCTGTATGCCGAGATG R: TGATTTGCTTCTCCTCCTTTCTG
IKBa/NFKBIA	NC_000014.9	F: TGGTGCCTTGGGTGCTGAT R: GGCAGTCCGGCCATTACA
IL6	NM_000600.3	F: CCTGAGAAAGGAGACATGTAACAAGA R: GGAAGGTTCAAGTTGTTTTCTGC
IL8	NM_000584.2	F: GCAGCTCTGTGTGAAGGTGC R: AAAGGTTTGGAGTATGTCTTTATGCA
IL32	NM_001012631.1; NM_004221.4; NM_001012718.1; NM_001012636.1; NM_001012635.1; NM_001012634.1; NM_001012632.1; NM_001012633.1	F: GCAGCACCCAGAGCTCACTCC R: AGGCTCCTCGGTTGCGGGAT

IRF1	NM_002198.2	Cytoplasmic IRF1: F: CTCACTGCAGCCCCCTGCGTC R: TGGGCATGTTGGCTCTGCTGC Unspliced nuclear IRF1: F: GCGACCGCCGAATCG R: TTCGACCCCCCACTTCCT
ISG20	NM_002201.4	F: TCCCTGCGGGTGCTGAGTGA R: GCTCCATCGTTGCCCTCGCA
LAMB3	NM_000228.2; NM_001127641.1; NM_001017402.1	F: CAGCCAGGCTCCCCAACGTG R: GGCTCGGCTCCTGGCTTCCT
MAP3K8	NM_005204.3 NM_001244134.1	F: CGAAGAAAAGAATGGCGTGTA R: AGCCTGGATTTCCACATCAGA
MX1	NM_001144925.1; NM_002462.3	F: GGCAGCGGGATCGTGACCAG R: CCTTCCCCGGCGATGGCATT
NFKB2	NM_001077493.1; NM_002502.3; NM_001077494.1	F: AACCCAAGGAGCCAGCCCCA R: CAGCCATATCGAAATCGGAAG
NFKBIZ	NM_001005474.1; NM_031419.2	F: GGCTTCTGGCCAAGCTGTGGAT R: TCCCCGGGCGTTGGTGTGTTG
OLR1	NM_002543.3	F: TGGTGCTGGGCATGCAATTATCCC R: GCCGGGCTGAGATCTGTCCCT
PI3	NM_002638.3	F: AGCCTGGCTCCTGCCCCATT R: GCAAGGACCGGCTCCCTCTCA
PRIC285	NM_001037335.2; NM_033405.3	F: ACGGTCATTCAGGGCCCACCA R: GGCCTCAGCCTGCTCACTGT
PTGS2	NM_000963.2	F: GCTGGGCCATGGGGTGGACT R: CCTGCCCCACAGCAAACCGT
SOD2	NM_000636.2; NM_001024465.1; NM_001024466.1	F: CGTGGCTGTGGTGGCTTCGG R: CCTGCTGGTGCCGCACACT
TFF1	NM_003225.2	F: TCTGCGCCCTGGTCCTGGTG R: GCACACTGGGAGGGCGTGAC
TLR2	NM_003264.3	F: GCTGCTCGGCGTTCTCTC R: AAGCAGTGAAAGAGCAATGGG
TNF	NM_000594.2	Cytoplasmic TNF: F: GTGATCGGCCCCCAGAGGGAA R: TGGAGCTGCCCTCAGCTTGA
TNFAIP3	NM_006290.2	F: AGGCGCTGTTTCAGCACGCTC R: CGGGCCATGGGTGTGTCTGT
UBD	NM_006398.3	F: GGTTCCTGGCCCCTTGTCTGCAG R: ACGCTGTCATATGGGTTGGCATCA
U6	NR_004394.1	F: AATTGGAACGATACAGAGAAGATTAGC R: GGAACGCTTCACGAATTTGC

(B) Sequences for siRNA targeting

IRF-1 siRNA was supplied by Invitrogen, Canada and others were supplied by Qiagen, Canada.

Target name	Sequence
LMNA siRNA	5'-CTGTACTATCCTGTAAATATA-3'
DUSP1 siRNA 1	5'-TAGCGTCAAGACATTTGCTGA-3'
DUSP1 siRNA 2	5'-AACTGGACTTCCAGAAGAACA-3'
IRF1 siRNA 1	5'-GGGACAUCAACAAGGAUGCCUGUU U-3'
IRF1 siRNA 2	5'-CGGACAGCACCAGTGATCTGTACA A-3'

(C) Primers used for ChIP PCR analysis

Forward (F) and reverse (R) primer sequences (5'-3') are shown in addition to the accession number for each gene. Primer location for each gene was selected based on the presence of IRF1 binding site as shown in the custom tracks of UCSC genome browser database. All primers were designed using primer designing software (Integrated DNA technologies) and were synthesised by the DNA synthesis lab at the University of Calgary.

Target gene	Accession Number	Primer Sequences
CMPK2	NM_207315.2	F: GCCAGCGGAAACGAAAG R: AGGAGGAGAGGCCGGAA
CXCL10	NC_000004.12	F: ATTTCCCTCTGCTCCTCTTT R: GAATGGATTGCAACCTTTGTTT
IFIT1	NM_001548.3	F: CACCATTGGCTGCTGTTTAG R: CTCCTCTGAGATCTGGCTATTC
IFIT3	NC_000010.11	F: GTGGAAACCTCTTCAGCATTTG R: CAGAGAAGCAGGGACTATTTACC
hMYOD1(A)	NC_000011.10	F: TGCAGGAGATGAAATACTAAGCAAGTA R: AGATTGGAAACTGAGGACTTTAGTTAGAG
hOLIG3	NC_000006.12	F: GGCAAGGACAGAGACAATCATA R: CTCTGTGTTCTCGCTTTGGA
hMYOG	NC_000001.11	F: CCAATGAGACTGAGTGGGTTTTTC R: TCACCAGAGAAGACTGCTTTGC