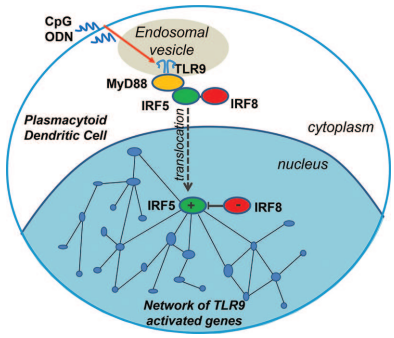
DOI: 10.1002/eji.201545911

Microarray plus RNAi studies demonstrate that IRF5 upregulates while IRF8 downregulates the same network of TLR9 activated genes in CAL-1 cells. IRF8 and IRF5 colocalize within the cytoplasm of resting human pDC and cotranslocate to the nucleus after CpG stimulation. Findings suggest that these two transcription factors coregulate TLR9 signaling in human pDCs.

IRF5 and IRF8 modulate the CAL-1 human plasmacytoid dendritic cell line response following TLR9 ligation

Folkert Steinhagen, Luis G. Rodriguez, Debra Tross, Poonam Tewary, Christian Bode and Dennis M. Klinman

European Journal of Immunology, 00 (0), 1–9



European Journal of Immunology

Supporting Information

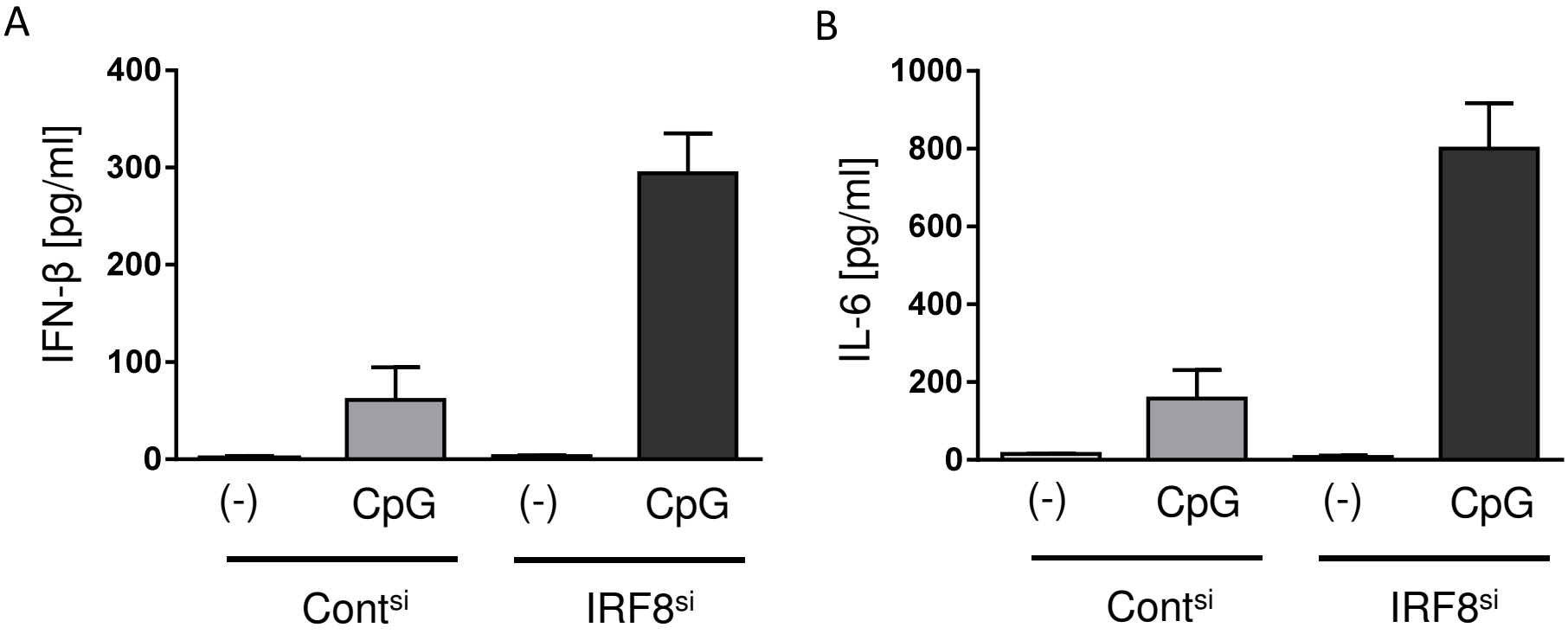
for

DOI 10.1002/eji.201545911

Folkert Steinhagen, Luis G. Rodriguez, Debra Tross, Poonam Tewary, Christian Bode and Dennis M. Klinman

IRF5 and IRF8 modulate the CAL-1 human plasmacytoid dendritic cell line response following TLR9 ligation

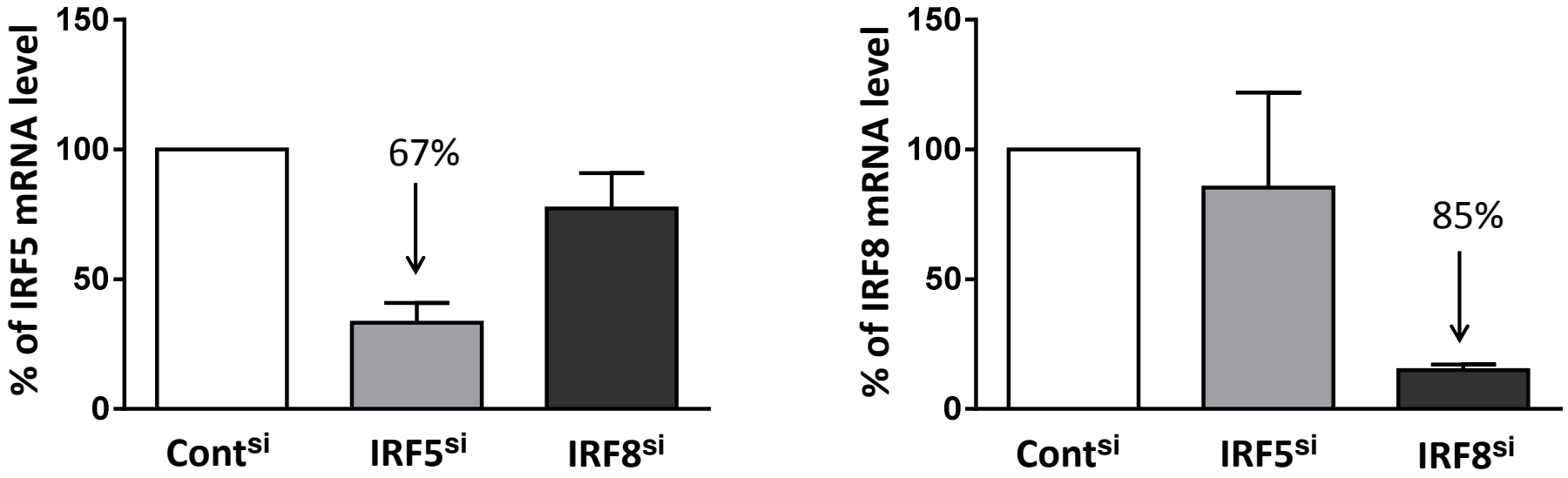
Supporting Information Figure 1



Cytokine production by siRNA transfected CAL-1 cells.

CAL-1 cells were transfected with 1 nM of control siRNA ($Cont^{si}$) or siRNA against IRF8 ($IRF8^{si}$) to silence gene expression as described in Figure 1. The siRNA transfected cells were stimulated for 8 h with 1 µM of CpG ODN and the amount of A) IFN-ß or (B) IL-6 in culture supernatants was determined by ELISA as described earlier [7]. Results represent the mean ± SD of two independent experiments performed in triplicate.

Supporting Information Figure 2



Effects of siRNA silencing on mRNA expression.

CAL 1 cells were transfected for microarray experiments with siRNA as described in Figure 2. The silencing efficacy of the indicated siRNAs was evaluated by analyzing mRNA levels by RT-PCR. Changes in mRNA levels were evaluated by comparison to cells transfected with control siRNA in each experiment. Results were determined by RT-PCR with GAPDH used as the endogenous control. Data represent the mean ± SD of 4 independent experiments.

Supplemental Table I. List of genes activated by CpG stimulation in IRF silenced CAL-1 cells

	Fold increase in mRNA level				Fold increase in mRNA level		
Gene Symbol	**Cont^{si}**	**IRF8^{si}**	**IRF5^{si}**	**Gene Symbol**	**Cont^{si}**	**IRF8^{si}**	**IRF-5^{si}**
A2ML1	1.52	2.61	1.54	C6orf120	1.62	2.5	1.22
ABLIM1	1.69	2.66	1.06	C6orf57	2.02	3.01	1.11
ACAD10	1.91	2.35	1.65	C7orf58	0.89	2.07	1.03
ACOT11	1.38	2.17	0.99	CCDC130	1.59	2.04	1.06
ACSS3	1.76	2.58	1.24	CCL1	9.09	43.17	4.56
ADAM19	2.27	3.47	0.79	CCL2	3.06	4.89	4.74
ADAM8	1.89	3.48	1.44	CCL22	2.36	2.42	1.66
ADAR	1.88	2.33	1.64	CCL4	7.46	42.64	1.3
AIF1	1.89	2.46	1.13	CCNG2	1.47	2.29	1.05
ALG2	1.36	2.42	1.03	CCNYL1	2.03	2.3	1.59
ANKRD24	1.9	2.55	1.61	CCR7	4.4	10.07	1.43
ANKRD33B	1.85	1.89	1.12	CD36	2.4	3.1	2.1
AOAH	1.75	2.34	1.1	CD38	2.88	2.03	1.71
APOBEC3G	2.0	1.61	1.26	CD40	6.14	17.97	2.97
ARHGAP24	2.33	3.22	1.38	CD44	1.4	2.06	0.97
ARHGEF3	2.18	3.39	1.29	CD48	2.01	3.4	1.55
ARL5B	1.71	2.06	1.63	CD70	3.82	7.19	2.0
AT64A	1.85	1.0	2.14	CD74	1.88	2.56	1.79
ATP2A1	1.36	2.48	1.16	CD83	2.52	5.36	1.58
B2M	1.77	2.36	1.36	CDH1	1.17	2.07	1.1
B2M	1.65	2.16	1.04	CDKN1A	2.24	3.55	1.5
B3GNT2	2.82	3.41	1.2	CEACAM4	1.95	2.57	1.14
B3GNT8	1.93	2.84	1.1	CFLAR	1.76	1.31	1.27
B4GALT5	2.15	4.06	1.65	CHMP5	2.15	3.5	1.88
BAIAP2	1.16	1.91	1.4	CHRM1	9.33	15.52	4.77
BCL2A1	6.47	18.02	2.55	CLEC2D	1.88	1.61	1.28
BCL3	1.43	2.0	1.08	CLEC4C	1.69	1.48	1.05
BID	2.18	2.55	1.81	CLIC4	1.21	1.84	1.26
BIRC3	4.54	7.3	3.1	COL22A1	2.39	2.94	1.65
BLZF1	2.17	6.81	1.63	CRCP	1.79	2.63	1.16
BRIP1	1.79	0.9	1.38	CREM	2.69	3.18	1.93
BRSK2	1.1	2.02	0.91	CTSC	2.52	3.2	1.64
BST2	1.37	2.76	1.11	CTSH	1.89	2.85	1.03
BTG1	1.59	3.35	1.02	CXCL10	1.26	2.24	1.05
BTG2	2.5	4.1	2.34	CXCR7	1.86	2.78	1.05
BTG3	1.97	1.1	1.64	CXorf21	1.61	0.95	0.9
BVES	1.97	3.59	1.22	CXXC1	1.49	1.84	1.09
C10orf129	2.05	2.36	1.43	CYBASC3	1.4	2.18	1.1
C12orf41	1.7	1.81	1.44	CYGB	1.23	2.23	0.73

C12orf45	1.15	2.46	1.2	CYTIP	2.31	3.03	2.24
C14orf169	1.4	2.2	0.86	DHX58	1.58	2.12	1.15
C16orf86	1.24	1.84	0.77	DIDO1	3.49	9.0	1.36
C1QA	1.87	1.63	0.79	DKFZp43	1.64	2.68	1.1
C20orf203	4.2	3.22	3.24	DKK2	1.51	2.21	1.96
C21orf58	1.49	1.86	1.15	DNAH12	1.97	2.66	1.44
DPPA5	1.34	1.99	1.07	HLA-E	2.22	3.91	1.25
DSE	2.32	4.14	2.07	HLA-F	2.3	4.15	1.88
DUSP5	2.86	6.51	1.32	HLA-G	2.21	4.51	1.6
EHD1	2.23	4.54	1.87	HLA-G	1.04	1.89	0.87
EHD4	5.11	3.31	3.72	HLA-H	1.22	2.52	0.91
EIF2AK2	2.61	4.75	2.52	HLA-J	1.62	4.45	1.17
EPB49	1.95	2.34	1.34	HMCN2	1.84	2.24	1.43
EPSTI1	6.21	20.77	2.65	HMGCS1	1.66	1.92	1.46
EXOSC9	2.16	1.55	1.44	hsa-mir-565	2.18	2.36	1.63
EXT1	1.38	2.56	0.96	HSD3B2	1.02	2.35	1.4
FAM126A	1.59	2.08	0.85	HSD3B7	2.52	3.59	1.65
FAM129A	3.43	5.68	2.15	HSH2D	2.18	3.32	1.36
FAM49A	1.8	2.45	1.06	ICAM1	1.85	2.17	1.5
FAM75C2	1.36	2.22	1.29	ID2B	1.76	2.04	1.21
FAM78A	2.18	3.37	1.25	IFI16	2.87	3.72	1.66
FCRL1	1.31	2.71	1.13	IFI27	3.24	19.91	1.52
FGFR4	1.38	2.22	1.13	IFI30	1.14	2.0	0.94
FOXE1	1.36	2.19	1.06	IFI35	1.91	3.71	1.09
FTSJD2	1.32	2.08	1.03	IFI44	2.31	11.82	1.79
FYTTD1	1.78	1.97	1.56	IFI44L	3.68	8.32	2.21
G6PC2	1.44	2.09	1.03	IFI6	3.61	12.09	2.28
GLOD5	2.16	3.03	1.34	IFIH1	2.4	5.65	1.96
GLT1D1	1.74	2.35	1.24	IFIT1	6.39	61.9	2.23
GMIP	1.74	2.7	1.35	IFIT2	2.6	14.59	1.4
GMPR	2.28	3.14	1.43	IFIT3	1.19	3.99	0.81
GNB4	1.63	2.09	1.6	IFIT5	4.29	9.92	2.78
GPR137B	1.78	3.46	1.55	IFITM1	11.9	40.02	7.91
GPR171	2.99	3.75	1.79	IFITM2	3.18	10.1	2.02
GPR183	3.59	6.03	1.56	IFNB1	2.2	6.38	0.95
GPR65	3.28	2.43	2.46	IGFBP4	2.84	2.57	1.18
GRHPR	0.92	2.01	0.78	IL10RA	3.26	5.04	2.8
GRIA4	1.75	2.08	0.88	IL18R1	2.47	2.76	1.5
GRIK1	1.95	2.36	1.1	IL1B	2.53	2.33	2.45
GRIK1-AS1	1.51	2.49	1.04	IL28RA	1.89	1.51	0.95
HERC5	2.2	6.92	1.09	IL4I1	3.1	6.33	1.94
HERC6	1.19	2.82	1.12	INPP4A	1.81	2.06	1.45
HIPK2	1.55	1.99	1.0	INPP4B	1.54	2.23	1.07
HIST1H3E	2.03	2.98	1.52	IQCD	1.55	2.31	1.09
HLA-A	1.09	2.18	1.0	IRF7	1.85	4.17	1.27
HLA-B	1.65	3.61	1.12	ISG15	2.93	5.96	1.5

HLA-C	1.89	3.62	1.37	ISG20	2.95	6.51	1.26
HLA-DPA1	1.58	3.06	1.56	JDP2	2.12	3.88	1.66
HLA-DPB2	1.59	3.13	1.42	KCNJ9	1.34	1.98	0.91
HLA-DQA1	2.16	3.95	1.62	KCNQ3	0.84	1.97	0.86
HLA-DQB1	1.98	3.31	1.88	KIAA0226	2.05	3.3	1.78
HLA-DQB2	1.67	2.77	1.65	KIAA0922	1.44	2.7	1.1
HLA-DRA	1.95	3.93	1.72	KLF6	1.51	2.42	1.37
HLA-DRB1	1.56	3.05	1.43	KRBA1	1.33	2.29	0.98
HLA-DRB3	2.07	3.72	2.11	KRT16P3	2.53	2.54	2.03
HLA-DRB5	2.42	4.54	2.67	LAIR1	2.26	2.42	1.69
HLA-DRB6	1.3	3.39	1.31	LAMA1	1.68	2.47	1.07
LAP3	2.98	4.31	2.15	OAS1	1.13	2.04	0.96
LGALS9	2.2	4.21	1.61	OAS2	6.98	14.43	4.11
LILRA1	1.82	1.9	0.98	OAS3	1.87	7.06	1.63
LILRA5	1.94	1.9	1.35	OPTN	2.78	2.93	1.85
LILRB5	1.35	1.86	1.02	OR2T4	1.65	3.4	0.79
LIMA1	2.64	1.75	1.55	OR4C13	1.88	2.34	1.67
LMNB1	1.45	1.94	1.25	OR51T1	1.7	3.27	0.99
LOC10029	2.01	2.12	1.35	OTOA	1.31	2.21	1.28
LOC10050	1.73	2.38	1.16	P2RX5	2.11	2.88	0.8
LOC10065	1.7	1.72	1.5	P2RY6	1.72	2.66	1.22
LOC338667	1.35	1.97	1.11	PARP12	3.02	9.83	1.61
LOC390705	2.31	3.84	1.4	PARP14	8.91	12.89	4.02
LOC645638	7.86	13.16	3.02	PARP9	2.51	5.63	1.43
LRRC14	2.32	3	1.52	PCDP1	1.28	1.88	0.95
LSG1	1.33	2.3	0.83	PDCD11	1.99	3.05	1.54
LST1	1.55	3.37	1.47	PDE7A	2.09	5.13	2.11
LTA	1.37	2.73	1.02	PDYN	1.85	1.68	1.13
LTB	1.53	3.3	1.03	PEA15	1.71	2.65	1.85
LYN	2.64	3.61	1.89	PFKL	1.44	2.12	1.01
MANEAL	2.36	3.07	2.24	PGPEP1	2.32	2.75	1.82
MAP3K8	3.75	5.42	2.2	PHF11	2.33	2.79	2.05
MARCKSL1	1.85	3.04	1.02	PIAS3	1.06	1.99	0.9
MARS2	1.94	2.5	1.62	PIK3R3	1.69	2.17	1.47
MAST2	1.7	2.54	0.96	PIM3	1.27	1.93	0.9
MCL1	2.83	3.19	1.78	PLD1	1.88	2.04	1.53
MEGF11	1.47	2.0	1.23	PLEKHO1	1.55	2.37	1.53
MFN1	1.79	2.22	1.31	PLSCR1	4.66	8.52	2.48
MGC23270	1.54	2.49	1.01	PMAIP1	2	2.53	1.25
MMGT1	1.84	2.36	1.46	PNOC	2.37	4.23	1.42
MRC1	1.63	2.28	1.37	PNPT1	1.93	3.0	1.34
MS4A7	2.52	2.93	2.43	PPM1K	1.63	2.34	1.13
MVP	1.28	1.94	0.8	PPPDE2	1.57	1.48	1.14
MX1	21.06	65.96	7.99	PRDM15	1.71	2.54	1.04
NAPA	1.39	1.77	1.05	PRKAR2B	1.79	1.89	1.3
NCF2	2.64	1.87	1.96	PSMB9	1.87	2.17	1.65

NCOA7	1.41	3.05	1.18	PTGER4	1.51	2.42	1.33
NEXN	2.36	9.9	1.42	PTPRR	1.9	2.82	1.42
NFAT5	1.98	2.62	1.07	PVRL1	1.52	2.06	1.05
NFKB1	2.73	4.32	1.67	RAB11FIP1	1.81	2.37	1.57
NFKBIA	2.27	3.4	1.79	RAB30	1.89	2.38	1.19
NFKBIE	1.75	2.63	1.61	RAB8B	2.94	3.0	2.58
NFKBIZ	1.38	2.03	1.0	RAB9A	1.5	1.87	1.08
NGRN	1.81	2.37	1.38	RAB9BP1	1.7	2.32	0.93
NLRP9	1.86	1.09	1.34	RABGAP1L	2.27	8.65	1.49
NMI	1.95	3.07	1.84	RAMP2	1.25	1.91	0.7
NP_0035.1	2.09	4.3	1.48	RAPGEF5	1.35	2.03	0.75
NR4A3	1.36	2.64	0.89	RBCK1	1.65	2.97	1.04
NT5C3	3.6	4.02	1.6	RBM41	1.76	2.16	1.34
NUDCD1	1.85	1.42	1.43	RBMS1	1.69	2.79	1.62
NUDT11	3.1	2.25	2.11	REL	2.65	7.33	1.49
RFTN1	1.73	2.36	1.13	TNFSF10	6.86	9.87	3.49
RELB	2.26	3.67	1.84	TNFSF13	2.08	5.52	1.27
REPS2	1.87	4.26	1.9	TNIP1	1.53	1.83	1.14
RGS1	3.99	5.7	1.47	TOR1AIP1	1.74	1.97	1.69
RHBDF2	1.49	2.18	1.07	TOR1B	2.24	2.37	1.67
RIPK2	1.17	2.17	0.89	TRAF1	2.49	5.11	1.44
RIT1	1.58	1.88	1.21	TRAF4	2.03	3.17	1.45
RNF213	1.15	2.05	1.01	TRAFD1	1.41	1.99	1.18
RRBP1	1.85	2.99	1.03	TRAPPC2L	1.55	2.6	1.22
RUNX3	2.06	1.58	1.54	TREX1	1.63	2.09	1.17
SAMD9	2.91	6.49	1.88	TRIB2	2.1	6.01	1.77
SAMD9L	8.07	20.01	2.77	TRIM21	1.39	1.95	1.2
SAT1	1.77	2.43	1.59	TRIM22	4.26	7.31	3.07
SCAF8	1.62	2.58	1.23	TRIM23	1.37	2.25	0.96
SEC61B	1.07	2.94	1.57	TRIM26	1.76	2.26	1.25
SERPINB1	4.17	6.01	1.6	TRIM34	1.65	3.22	1.19
SF3B4	1.36	2.08	0.98	TRIM36	1.56	2.37	1.55
SFT2D2	2.1	1.84	1.53	TRIM69	1.58	2.07	1.11
SGSM3	0.99	1.8	0.91	TTLL10	1.27	1.95	0.79
SLAMF7	3.77	3.7	1.55	TTLL2	2.86	6.97	2.21
SLC29A2	3.19	11.02	2.22	UBE2L6	4.47	11.08	2.17
SLC35A4	1.73	2.45	1.12	UCP3	1.8	2.2	0.88
SLC41A2	1.55	1.82	1.33	USE1	1.69	2.21	1.44
SOCS1	1.52	3.22	0.91	USP18	7.02	15.67	2.47
SP100	2.2	2.51	1.99	WFDC6	1.63	2.51	1.18
SP110	4.1	4.9	2.69	ZC3HAV1L	1.38	2.17	0.91
SPATS2L	1.95	2.67	1.24	ZCCHC2	1.71	3.03	1.28
SPDYA	1.93	2.2	1.27	ZFP57	1.91	2.99	1.09
SPPL2A	1.79	2.4	1.48	ZMAT4	1.45	2.0	1.17
SPPL3	1.34	2.06	1.19	ZMYND10	1.53	2.08	1.22
SRGAP2	1.89	2.49	1.62				

ST20	7.18	21.66	3.35
STARD4	2.16	1.92	2.08
STAT1	5.3	12.89	3.09
STAT2	2.03	3.3	1.4
STAT6	1.41	1.88	0.98
SUPT3H	1.34	2.16	1.19
SWAP70	1.58	3.25	1.29
TAGAP	1.53	2.75	0.9
TANC2	2.23	2.71	1.22
TANK	1.55	2.37	1.3
TAP1	1.58	1.92	1.13
TBX15	1.56	2.42	1.08
TCTN3	1.78	2.69	1.3
TDRD7	2.03	4.3	1.53
TICAM1	1.07	2.13	1.05
TLR7	1.62	1.59	0.83
TMCC3	2.34	2.76	1.76
TMEM229B	1.2	2.94	1.17
TMEM86B	1.64	2.49	1.05
TNF	2.04	3.04	1.3
ZNF205	1.24	1.92	0.96
ZNF295	1.6	2.37	1.16
ZNF385B	1.84	2.69	1.34

CAL-1 cells were transfected with siRNA targeting IRF5 ($IRF5^{si}$), IRF8 ($IRF8^{si}$) or with control siRNA ($Cont^{si}$) as described in Fig 2. They were then stimulated with 1 μM of CpG ODN for 9 h and gene expression monitored by microarray. All genes significantly up-regulated ($p < 10^{-3}$) in either siRNA treated group are shown. Levels of gene induction are expressed as fold increase compared to untreated cells transfected with $Cont^{si}$.