Supplementary Information

STAT3 and NF-κB are Simultaneously Suppressed in Dendritic Cells in Lung Cancer

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Supplementary Figures & Tables

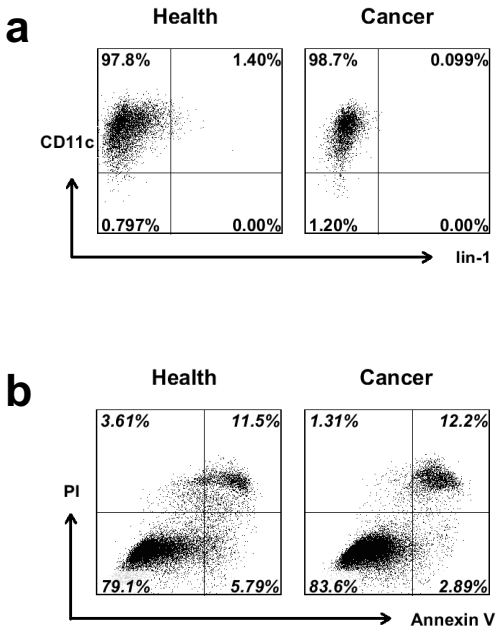


Figure S1. Tumour-induced DCs maintained normal differentiation. Human MoDCs were generated in health or cancer group. (a) FACS assay for DC population analysis. Cells were dual labeled with lin-1/CD11c, and FACS data showed typical MoDC generation in both cancer and healthy groups. (b) Apoptosis assay for DCs. Cells were harvested and incubated with AnnexinV-FITC and PI. Dot Plot showed MoDCs cultured in the presence of tumour sera showed no increased apoptosis compared to the healthy controls.

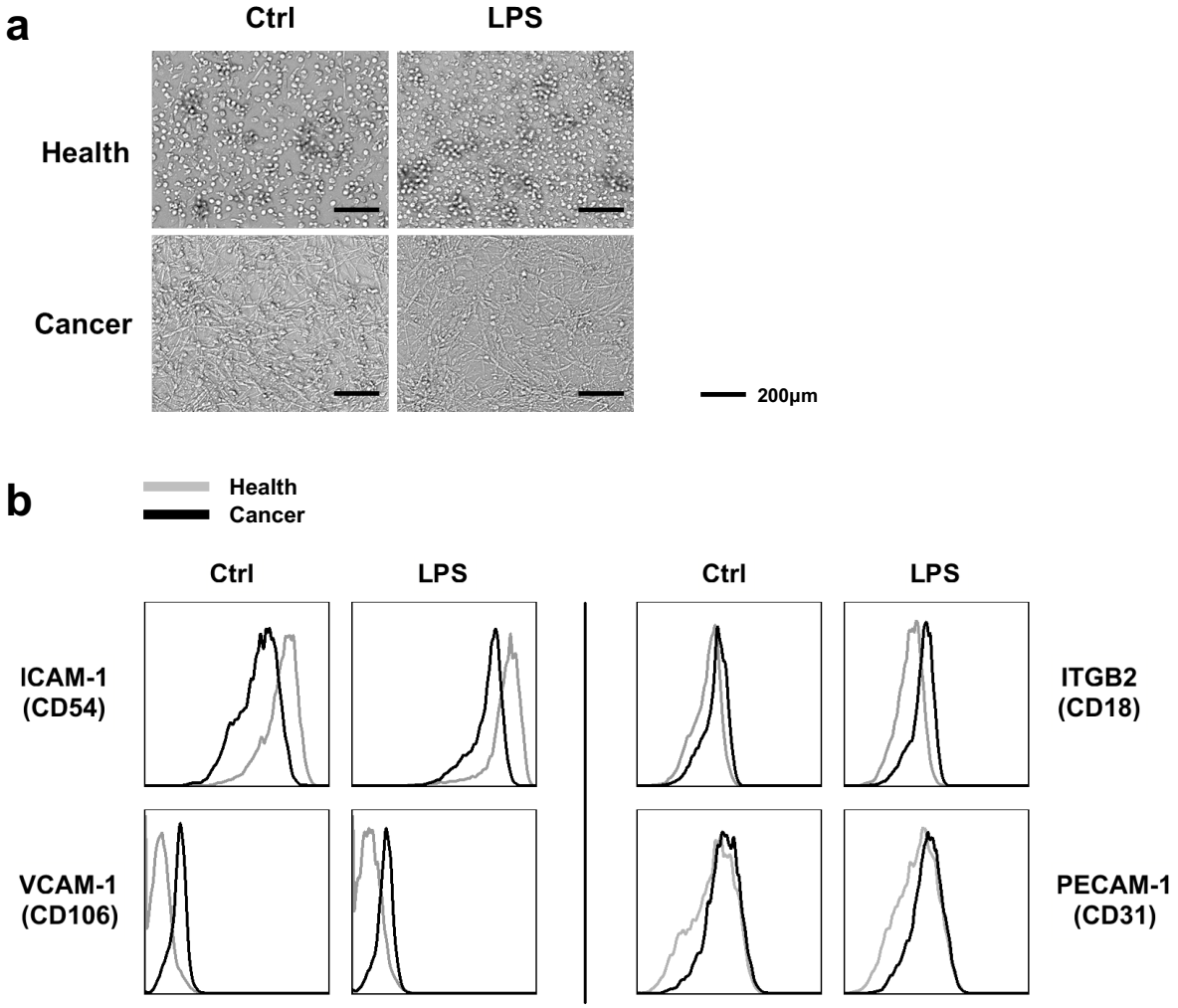


Figure S2. Tumour-induced DCs morphology changes. Human MoDCs were generated and further incubated with or without LPS for an additional 2 days. (a) Morphology of DCs in cancer or healthy group. Scale bars represent 200μm. (b) FACS assay for cell surface molecules of immature or mature DCs as indicated.

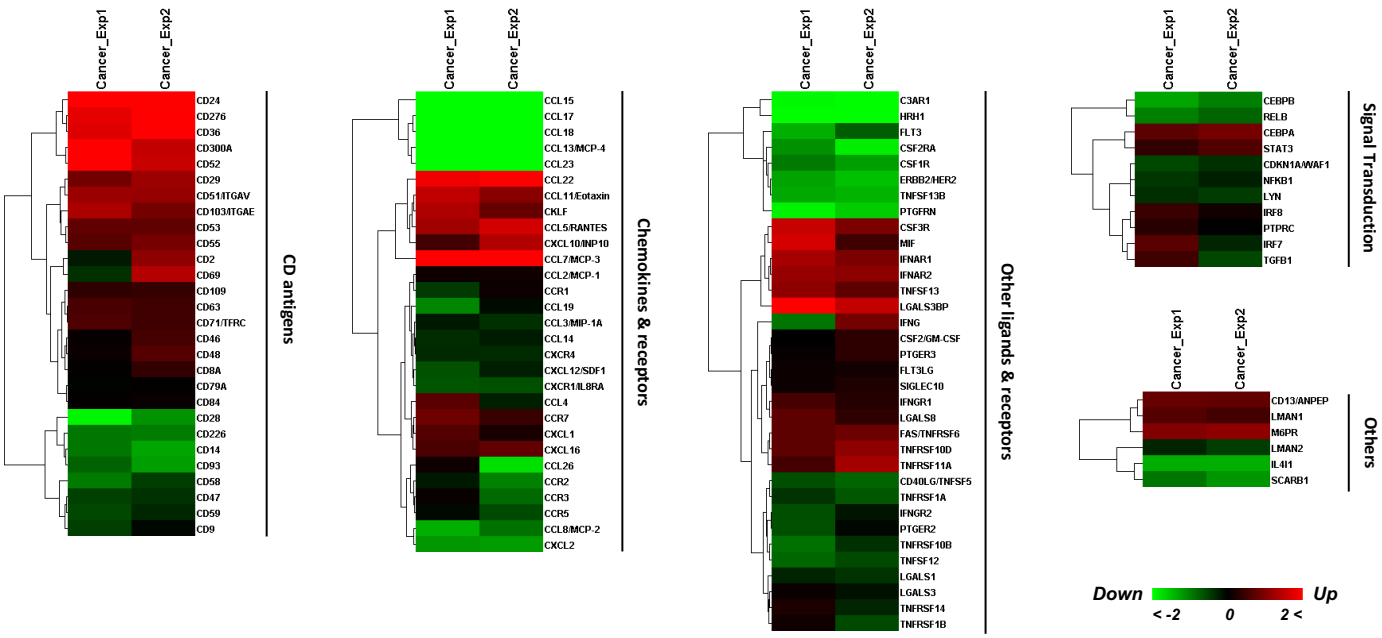
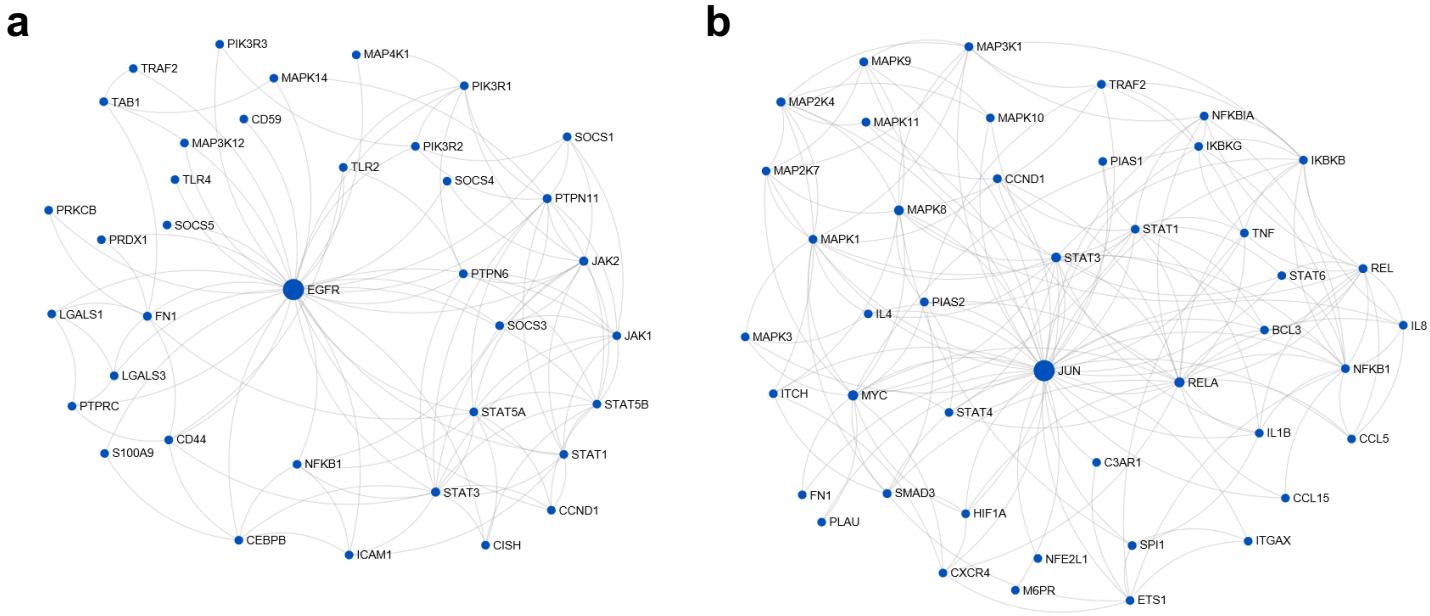


Figure S3. Clusters of immune-related DEGs altered in tumour-induced DCs. Heatmap for immune-related gene sets of DCs. Genes fold-changes were based on microarray data and shown in colour as indicated.



EGFR Pathway **JUN Pathway**

Figure S4. Sub-network of EGFR & JUN signaling pathways. To obtain a detailed view of Figure 4a, sub-networks were constructed by selecting the nodes connected with EGFR (a) or JUN (b).

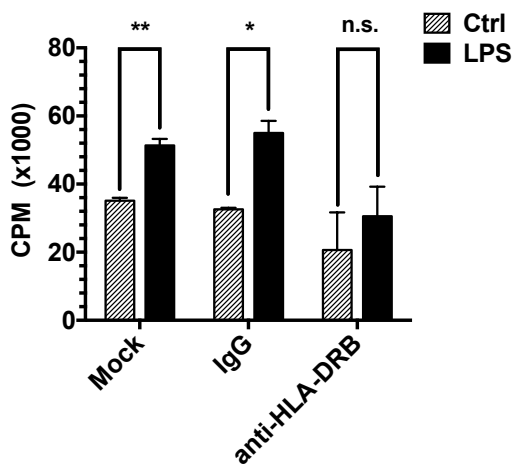


Figure S5. Competition MLR by HLA-DR blockade. MoDCs generated in FCS medium were co-cultured with PBMCs from another healthy donor at a ratio of 1:65, with HLA-DR blockading antibody or isotype IgG added. ^{3}H-TdR was added on day 4, and beta liquid scintillation was measured 12-18 hours later. Error bars, SEM. n.s., none significant; *, $P < 0.05$; **, $P < 0.01$.

Table.S1. List of genes found to be significantly regulated in microarray experiments

Gene ID	*Symbol*	*Location*	*Full Name*	*Cancer Exp1*	*Cancer Exp2*
Top 20 down-regulated genes					
6414	SEPP1	5q31	Selenoprotein P, Plasma, 1	0.0329	0.0567
7850	IL1R2	2q12-q22	Interleukin 1 Receptor, Type II	0.0536	0.0243
339390	CLEC4G	19p13.2	C-Type Lectin Domain Family 4, Member G	0.0993	0.0919
6362	CCL18	17q11.2	Chemokine (C-C Motif) Ligand 18 (Pulmonary And Activation-Regulated)	0.1227	0.2583
283316	CD163L1	12p13.3	CD163 Molecule-Like 1	0.1339	0.0752
5031	P2RY6	11q13.5	Pyrimidinergic Receptor P2Y, G-Protein Coupled, 6	0.1341	0.1066
6361	CCL17	16q13	Chemokine (C-C Motif) Ligand 17	0.1549	0.2886
23179	RGL1	1q25.3	Ral Guanine Nucleotide Dissociation Stimulator-Like 1	0.1572	0.1730
10462	CLEC10A	17p13.1	C-Type Lectin Domain Family 10, Member A	0.1577	0.1228
3957	LGALS2	22q12-q13|22q13.1	Lectin, Galactoside-Binding, Soluble, 2	0.1588	0.1544
27076	LYPD3	19q13.31	LY6/PLAUR Domain Containing 3	0.1604	0.3603
6035	RNASE1	14q11.2	Ribonuclease, RNase A Family, 1 (Pancreatic)	0.1712	0.1557
3269	HRH1	3p25	Histamine Receptor H1	0.1841	0.1603
3122	HLA-DRA	6p21.3	Major Histocompatibility Complex, Class II, DR Alpha	0.1954	0.1637
50486	G0S2	1q32.2-q41	G0/G1 Switch 2	0.2000	0.2014
4318	MMP9	20q11.2-q13.1	Matrix Metallopeptidase 9	0.2006	0.2749
64240	ABCG5	2p21	ATP-Binding Cassette, Sub-Family G (WHITE), Member 5	0.2059	0.1597
85019	TMEM241	18q11.2	Transmembrane Protein 241	0.2092	0.1686
1471	CST3	20p11.21	Cystatin C	0.2156	0.1570
2162	F13A1	6p25.3-p24.3	Coagulation Factor XIII, A1 Polypeptide	0.2192	0.1939
Top 20 up-regulated genes					
440992	RPS20P14	3q27.3	Ribosomal Protein S20 Pseudogene 14	13.6692	9.5598
118430	MUCL1	12q	Mucin-Like 1	12.8619	12.2210
390107	RPS24P15	11p13	Ribosomal Protein S24 Pseudogene 15	9.6442	6.2639
130773	RPL23AP37	2p14	Ribosomal Protein L23a Pseudogene 37	9.3945	5.4780
6286	S100P	4p16	S100 Calcium Binding Protein P	7.9978	6.0193
348	APOE	19q13.2	Apolipoprotein E	7.9822	8.9974
54627	MAP10	1q42.2	Microtubule-Associated Protein 10	7.5633	3.7377
55711	FAR2	12p11.22	Fatty Acyl CoA Reductase 2	7.2273	7.8296
27306	HPGDS	4q22.3	Hematopoietic Prostaglandin D Synthase	7.2152	7.5915
441533	RPL26P37	Yp11.2	Ribosomal Protein L26 Pseudogene 37	7.0451	3.9103
4828	NMB	15q22-qter	Neuromedin B	6.6957	6.0493
10114	HIPK3	11p13	Homeodomain Interacting Protein Kinase 3	6.6845	4.0924
341356	RPL31P50	12p11.21	Ribosomal Protein L31 Pseudogene 50	6.6412	2.9488
253013	RPL31P8	5q12.1	Ribosomal Protein L31 Pseudogene 8	6.5690	3.7312
597	BCL2A1	15q24.3	BCL2-Related Protein A1	6.4727	3.9652
314	AOC2	17q21	Amine Oxidase, Copper Containing 2 (Retina-Specific)	6.4507	8.3630
432369	ATP5EP2	13q12	,TP Synthase, H+ Transporting, Mitochondrial F1 Complex, Epsilon Subunit Pseudogene	6.3876	3.2278
112942	CFAP36	2p16.1	Cilia And Flagella Associated Protein 36	6.2681	4.8231
126235	RPS4XP21	19q13.11	Ribosomal Protein S4X Pseudogene 21	6.2045	3.9817
400410	ST20	15q25.1	Suppressor Of Tumorigenicity 20	6.1505	6.9169

Table.S2. KEGG pathway cluster of DEGs

ID	*KEGG Pathway*	*Count*	*PValue*	*Genes Symbol*
hsa04640	**Hematopoietic cell lineage**	28	2.98E-05	CD14, CD1A, CD1C, CD33, CD36, CD3D, CD3G, CD8B, CSF1R, CSF2RA, CSF3, CSF3R, FCER2, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, IL1R1, IL1R2, IL3RA, IL4, IL7R, ITGA2, ITGA2B, ITGA5, ITGAM, MME, THPO
hsa04612	**Antigen processing and presentation**	25	3.37E-04	CD74, CD8B, CTSB, HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HSPA1A, HSPA5, HSPA8, IFNA14, KIR2DL3, KIR2DL4, KIR2DS4, KLRC1, LGMN, PSME2, RFX5, TAP2
hsa04514	**Cell adhesion molecules (CAMs)**	33	1.21E-03	ALCAM, CD226, CD276, CD28, CD6, CD8B, CDH1, CDH15, CDH2, CDH4, CLDN14, HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, ICAM1, ITGAM, ITGAV, ITGB1, JAM3, L1CAM, NFASC, NLGN2, SDC3, SDC4, SELL, SPN
hsa04610	**Complement and coagulation cascades**	20	2.57E-03	C1QA, C1QB, C1QC, C1R, C2, C3AR1, C5, C8B, CFD, CFH, CFI, F13A1, F2, F3, F7, PLAU, PLAUR, SERPINA5, TFPI, VWF
hsa05322	**Systemic lupus erythematosus**	24	9.72E-03	C1QA, C1QB, C1QC, C1R, C2, C5, C8B, CD28, CTSG, FCGR2A, HIST1H2BC, HIST1H2BF, HIST1H2BO, HIST1H3G, HIST1H4C, HIST1H4I, HIST1H4J, HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5
hsa04060	**Cytokine-cytokine receptor interaction**	49	3.22E-02	ACVR2B, CCL11, CCL13, CCL17, CCL18, CCL20, CCL21, CCL22, CCL23, CCL3L1, CCL5, CCL7, CCL8, CCR8, CSF1R, CSF2RA, CSF3, CSF3R, CXCL2, CXCL5, EDA2R, EGFR, FIGF, IFNA14, IFNAR1, IFNAR2, IL18, IL1R1, IL1R2, IL1RAP, IL22RA2, IL28RA, IL3RA, IL4, IL7R, IL8, IL8RB, INHBC, LIFR, LTB, NGFR, PDGFB, PPBP, PRLR, TNFRSF4, TNFSF13B, XCL1, XCL2, ZFP91
hsa04620	**Toll-like receptor signaling pathway**	22	4.20E-02	AKT2, CCL5, CD14, IFNA14, IFNAR1, IFNAR2, IKBKB, IL8, IRAK1, JUN, LBP, MAP2K6, MAP3K7IP2, MAP3K8, MAPK10, MAPK12, MAPK3, NFKBIA, PIK3CG, RAC1, SPP1, TICAM2
hsa04062	**Chemokine signaling pathway**	36	4.69E-02	ADRBK1, ADRBK2, AKT2, BCAR1, CCL11, CCL13, CCL17, CCL18, CCL20, CCL21, CCL22, CCL23, CCL3L1, CCL5, CCL7, CCL8, CCR8, CSK, CXCL2, CXCL5, GNGT2, IKBKB, IL8, IL8RB, MAPK3, NFKBIA, PAK1, PIK3CG, PPBP, PRKCB1, RAC1, RAP1B, STAT2, TIAM1, XCL1, XCL2

Table.S3. Top Nodes in DEGs Signaling Nework

Id	*Label*	*Degree*	*Betweenness*
Q04206	RELA	65	2989.63
P42224	STAT1	50	218.86
P40763	STAT3	48	921.64
Q9Y4K3	TRAF6	44	1869.04
P05412	JUN	43	1648.73
P19838	NFKB1	43	654.75
O15111	CHUK	39	826.84
Q12933	TRAF2	38	724.53
P00533	EGFR	36	1300.43
P17676	CEBPB	34	449.98
Q9Y6K9	IKBKG	33	520.26
O14920	IKBKB	33	194.69
Q06124	PTPN11	32	546.11
P29350	PTPN6	31	947.64
Q04864	REL	30	93.3
P01106	MYC	29	1069.4
P27986	PIK3R1	29	0
P23458	JAK1	28	380.91
O43318	MAP3K7	28	0
P19438	TNFRSF1A	25	556.83
P42226	STAT6	25	51.36
O60674	JAK2	24	329.28
P02751	FN1	23	1054.3
Q13114	TRAF3	23	178.67
Q00653	NFKB2	23	177.76
O14543	SOCS3	22	1005.94
P28482	MAPK1	22	572.08
P45983	MAPK8	22	323.94
P45985	MAP2K4	22	180.66
Q99683	MAP3K5	22	153.37

Table.S4. Inhibitory receptor expression screen

Entrez ID	*Symbol*	*Other Name*	*Gene name*	*Cancer Exp1*	*Cancer Exp2*
80381	CD276	B7H3	CD276 molecule	2.574	3.293
84868	HAVCR2	Tim3	hepatitis A virus cellular receptor 2	2.199	2.475
5175	PECAM1	CD31	platelet/endothelial cell adhesion molecule (CD31 antigen)	1.557	1.427
4345	CD200	OX2	CD200 molecule	1.534	1.457
7301	TYRO3		TYRO3 protein tyrosine kinase	0.579	0.556
10288	LILRB2	ILT4	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	0.522	0.374
50856	CLEC4A	DCIR	C-type lectin domain family 4, member A	0.485	0.458
2212	FCGR2A	FcγRIIA	Fc fragment of IgG, low affinity IIa, receptor (CD32)	0.259	0.188
10462	CLEC10A	CD301	C-type lectin domain family 10, member A	0.158	0.123

Table.S5. qRT Primers

Gene	*Forward Primer*	*Reverse Primer*
Actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
GAPDH	CCATGTTCGTCATGGGTGTGAACCA	GCCAGTAGAGGCAGGGATGATGTTC
HLA-DMA	TGATCCAGCAAATAGGGCCA	CTCTGGACACCGGGATTTTC
HLA-DMB	AAAGACACCCTGATGCAGCG	TGTGGCACAATTCTGAAGCC
HLA-DOA	TGGCCCAGACCAGCTTCTAT	GGAACTTGCGGAACAAATGG
HLA-DPB	GGGACACAGCGCTTCCTGGAG	CAAGCAGGTTGTGGTGCTGCA
HLA-DQA	CTCTGACCACCGTGATGAGC	CTCTCCAGGTCCACGTAGAA
HLA-DQB	CGAGTACTGGAACAGCCAGAAGG	GGAGTCATTTCCAGCATCACCAGG
HLA-DRB	CACCAGACCACGTTTCTTGGAGT	CACGTTCTCCTCCTGGTTATGGA
CD74	TGGAGAACCTGCGCATGAAG	CTTGGTGGCATTCTGCATGG
CIITA	CCTGCTGTTCGGGACCTAAA	GGATCCGCACCAGTTTGG
IL1b	AGCTACGAATCTCCGACCAC	CGTTATCCCATGTGTCGAAGAA
IL1R1	ATGAAATTGATGTTCGTCCCTGT	ACCACGCAATAGTAATGTCCTG
IL1R2	ATGTTGCGCTTGTACGTGTTG	CCCGCTTGTAATGCCTCCC
IL1RAP	GGGGACTAGACACCATGAGG	ACCAGATCAGAGTAAGGCCAG
IL1RN	CATTGAGCCTCATGCTCTGTT	CACTGTCTGAGCGGATGAA
IL6	CCAGGAGCCCAGCTATGAAC	GATGCCGTCGAGGATGTACC
IL10	TCAAGGCGCATGTGAACTCC	GATGTCAAACTCACTCATGGCT
IFNG	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
TNF	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
NFKB1	AACAGAGAGGATTTCGTTTCCG	TTTGACCTGAGGGTAAGACTTCT
RelA	ATGTGGAGATCATTGAGCAGC	CCTGGTCCTGTGTAGCCATT
IRF8	AGTAGCATGTATCCAGGACTGAT	CACAGCGTAACCTCGTCTTC
MafB	GACGCAGCTCATTCAGCAG	CCGGAGTTGGCGAGTTTCT
STAT2	GAGCCAGCAACATGAGATTGA	GCCTGGATCTTATATCGGAAGCA
TAP1	TCTCCTCTCTTGGGGAGATG	GAGACATGATGTTACCTGTCTG
TRAF2	GCTCATGCTGACCGAATGTC	GCCGTCACAAGTTAAGGGGAA
TRAF3	GCGTGTCAAGAGAGCATCGTT	GCAGATGTCCCAGCATTAACT
BIC	TGGCTCTCCCACCCAATGG	AGAATTTAAACCACAGATTTCC