Supplementary Materials: IRF4 Mediates the Oncogenic Effects of STAT3 in Anaplastic Large Cell Lymphomas

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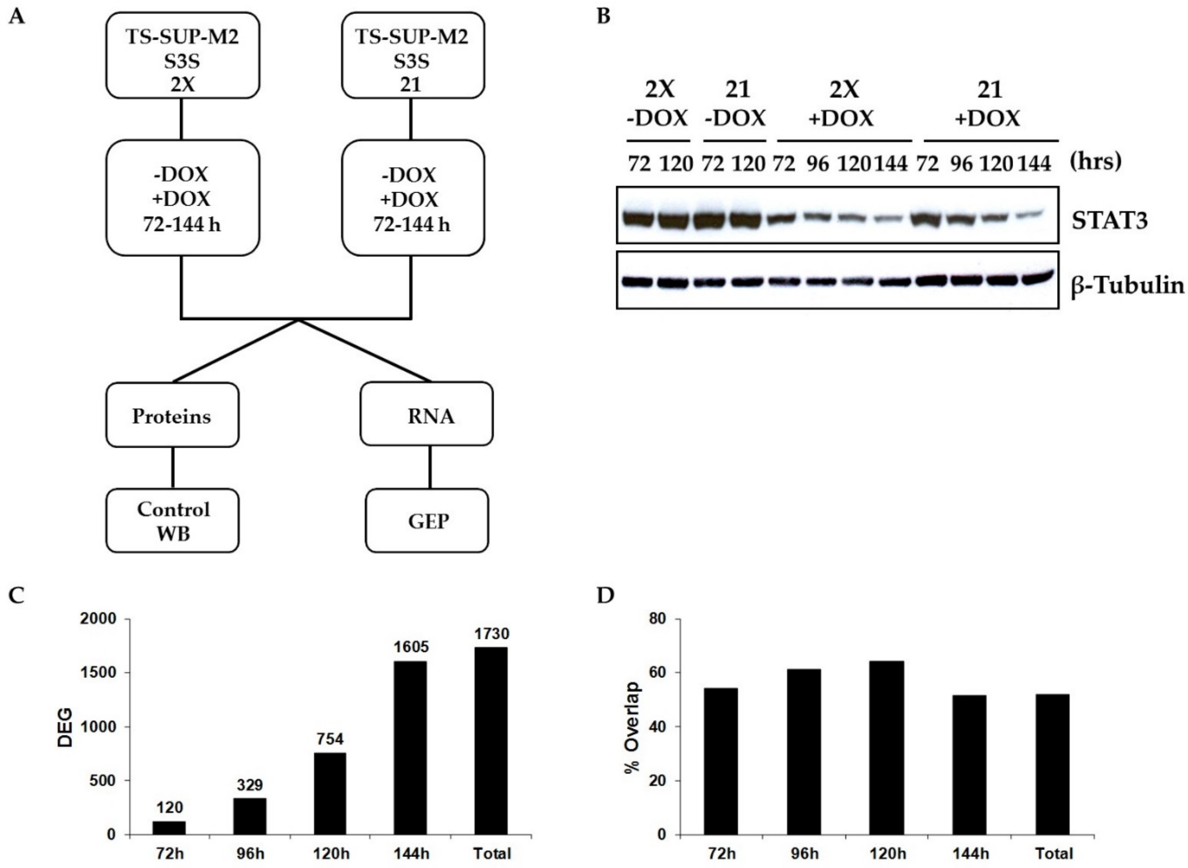
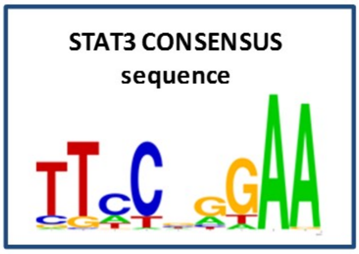
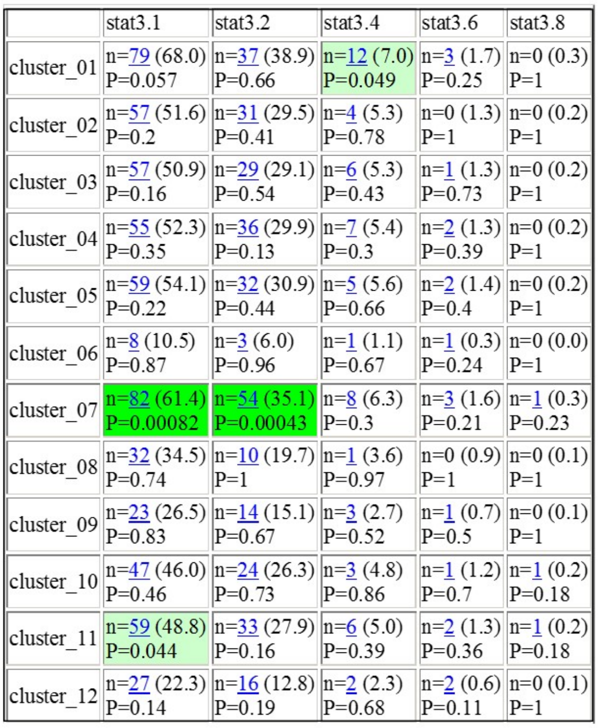


Figure S1. Kinetics of STAT3-regulated genes in the ALK-positive ALCL cell line TS-SUP-M2. **(A)** Experimental design of the gene expression profile (GEP) experiment. Two clones (2X and 21) of the ALKpositive ALCL cell line (TS-SUP-M2 S3S) expressing a specific doxycycline-inducible STAT3 short hairpin RNA (shRNA) were used [27]. Biological triplicates of TS-SUP-M2 S3S cells were cultured in the presence (+DOX) or absence (−DOX) of doxycycline (1 μg/mL) for 0, 72, 96, 120, 144 h, and harvested for RNA and protein extraction. RNA was labeled by Illumina Total Prep RNA Amplification Kit, and hybridized on Illumina HumanHT-12 BeadChip Array. **(B)** STAT3 silencing was confirmed by western blotting at the indicated time points. **(C)** Differential Expressed Genes (DEG) for each time point. In total 1730 genes were modulated after STAT3 KD. Differential score: $p < 0.001$ and FC > 2. **(D)** Percentage of overlapping DEG with a previous STAT3 knock down GEP experiment performed with 3 different shRNAs [30].

A



B



	stat3.1	stat3.2	stat3.4	stat3.6	stat3.8
cluster_01	n=79 (68.0) P=0.057	n=37 (38.9) P=0.66	n=12 (7.0) P=0.049	n=3 (1.7) P=0.25	n=0 (0.3) P=1
cluster_02	n=57 (51.6) P=0.2	n=31 (29.5) P=0.41	n=4 (5.3) P=0.78	n=0 (1.3) P=1	n=0 (0.2) P=1
cluster_03	n=57 (50.9) P=0.16	n=29 (29.1) P=0.54	n=6 (5.3) P=0.43	n=1 (1.3) P=0.73	n=0 (0.2) P=1
cluster_04	n=55 (52.3) P=0.35	n=36 (29.9) P=0.13	n=7 (5.4) P=0.3	n=2 (1.3) P=0.39	n=0 (0.2) P=1
cluster_05	n=59 (54.1) P=0.22	n=32 (30.9) P=0.44	n=5 (5.6) P=0.66	n=2 (1.4) P=0.4	n=0 (0.2) P=1
cluster_06	n=8 (10.5) P=0.87	n=3 (6.0) P=0.96	n=1 (1.1) P=0.67	n=1 (0.3) P=0.24	n=0 (0.0) P=1
cluster_07	n=82 (61.4) P=0.00082	n=54 (35.1) P=0.00043	n=8 (6.3) P=0.3	n=3 (1.6) P=0.21	n=1 (0.3) P=0.23
cluster_08	n=32 (34.5) P=0.74	n=10 (19.7) P=1	n=1 (3.6) P=0.97	n=0 (0.9) P=1	n=0 (0.1) P=1
cluster_09	n=23 (26.5) P=0.83	n=14 (15.1) P=0.67	n=3 (2.7) P=0.52	n=1 (0.7) P=0.5	n=0 (0.1) P=1
cluster_10	n=47 (46.0) P=0.46	n=24 (26.3) P=0.73	n=3 (4.8) P=0.86	n=1 (1.2) P=0.7	n=1 (0.2) P=0.18
cluster_11	n=59 (48.8) P=0.044	n=33 (27.9) P=0.16	n=6 (5.0) P=0.39	n=2 (1.3) P=0.36	n=1 (0.2) P=0.18
cluster_12	n=27 (22.3) P=0.14	n=16 (12.8) P=0.19	n=2 (2.3) P=0.68	n=2 (0.6) P=0.11	n=0 (0.1) P=1

Figure S2. Enrichment of putative STAT3 binding sites in cluster 7 (early down-regulated genes). (**A**) The position weight matrix (PWM) STAT3 sequence logo obtained as described by Vallania et al, 2009 [32]. (**B**) The number of genes carrying putative STAT3 binding sites is represented for each cluster of genes regulated by STAT3 inducible knockdown in the cell line TS-SUP-M2 S3S (P = *p* value). Each human gene is associated with a score which is the sum of the conservation scores of the STAT3 sites found in its promoter region (−2000 +500 from TSS).The enrichment is evaluated with respect to all the genes in the chip that were detected above background in at least one sample. Cluster 7 showed a strong overrepresentation of putative STAT3 targets. This cluster includes 82 genes bearing one STAT3 binding site (stat3.1; *p* value 0.00082) and 54 genes with two binding sites (stat3.2; *p* value 0.00043).

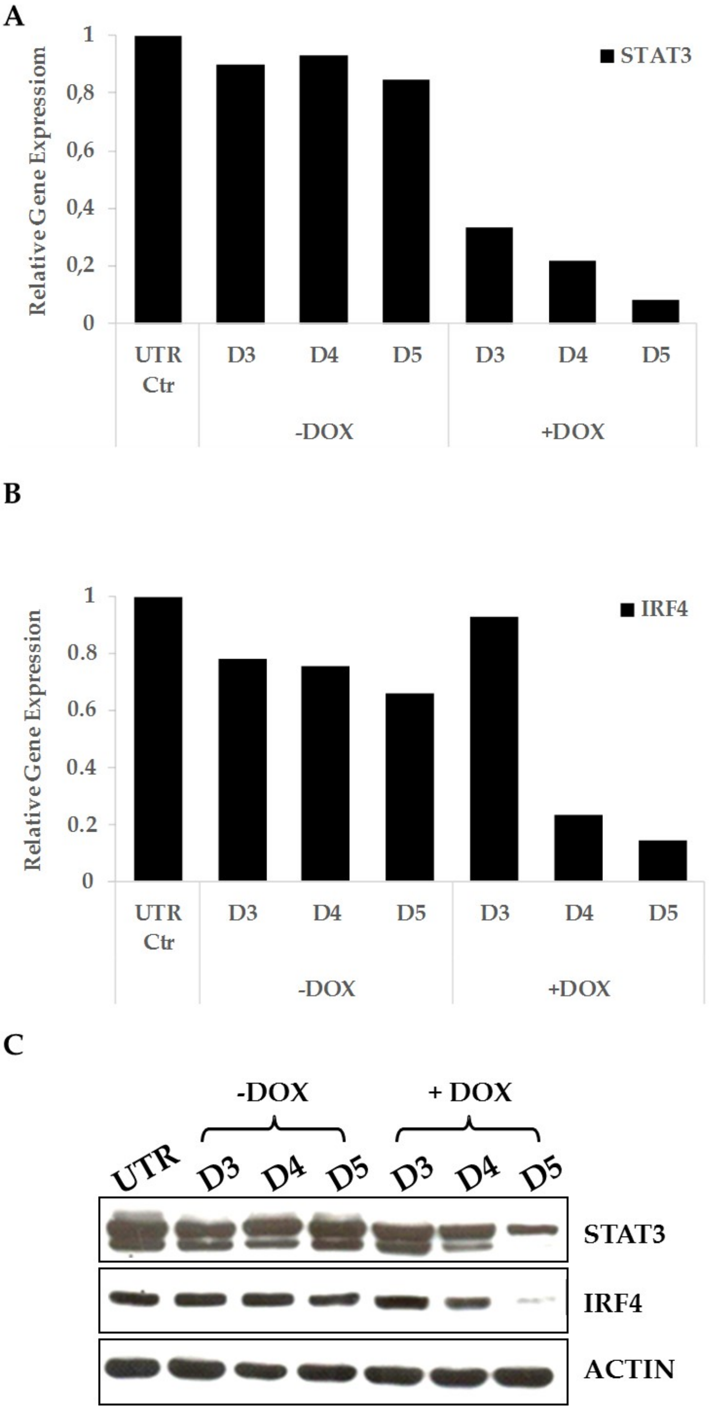


Figure S3. STAT3 regulates IRF4 expression in ALCL cells. TS-SUP-M2 S3S cells were grown in the absence (-DOX) or presence (+DOX) of doxycycline (1μg/mL) to induce STAT3 depletion. Pellet for RT-qPCR and western blot analyses were collected at 72 (D3), 96 (D4) and 120 (D5) h. (**A**–**B**) RT-qPCR analysis shows progressive decrease of STAT3 and IRF4 mRNA levels after 72 and 96 h of doxycycline treatment, respectively. (**B**) RT-qPCR analysis shows progressive decrease of IRF4 mRNA levels after 96 h of doxycycline treatment. Pellet were collected at 72 (D3), 96 (D4) and 120 (D5) h. TS-SUP-M2 S3S were grown in the presence (+DOX) or absence (−DOX) of doxycycline (1 μg/mL) for 120 h. TS-SUP-M2 S3S cells showed remarkable STAT3 and IRF4 downregulation after 5 days treatment with doxycycline. (**A**) STAT3 silencing after 5 days treatment with doxycycline detected by RT-qPCR. (**B**) IRF4 mRNA levels significantly decreased following STAT3 downregulation. (**C**) Western blot analysis showing progressive decrease of STAT3 and IRF4 protein levels after doxycycline treatment.

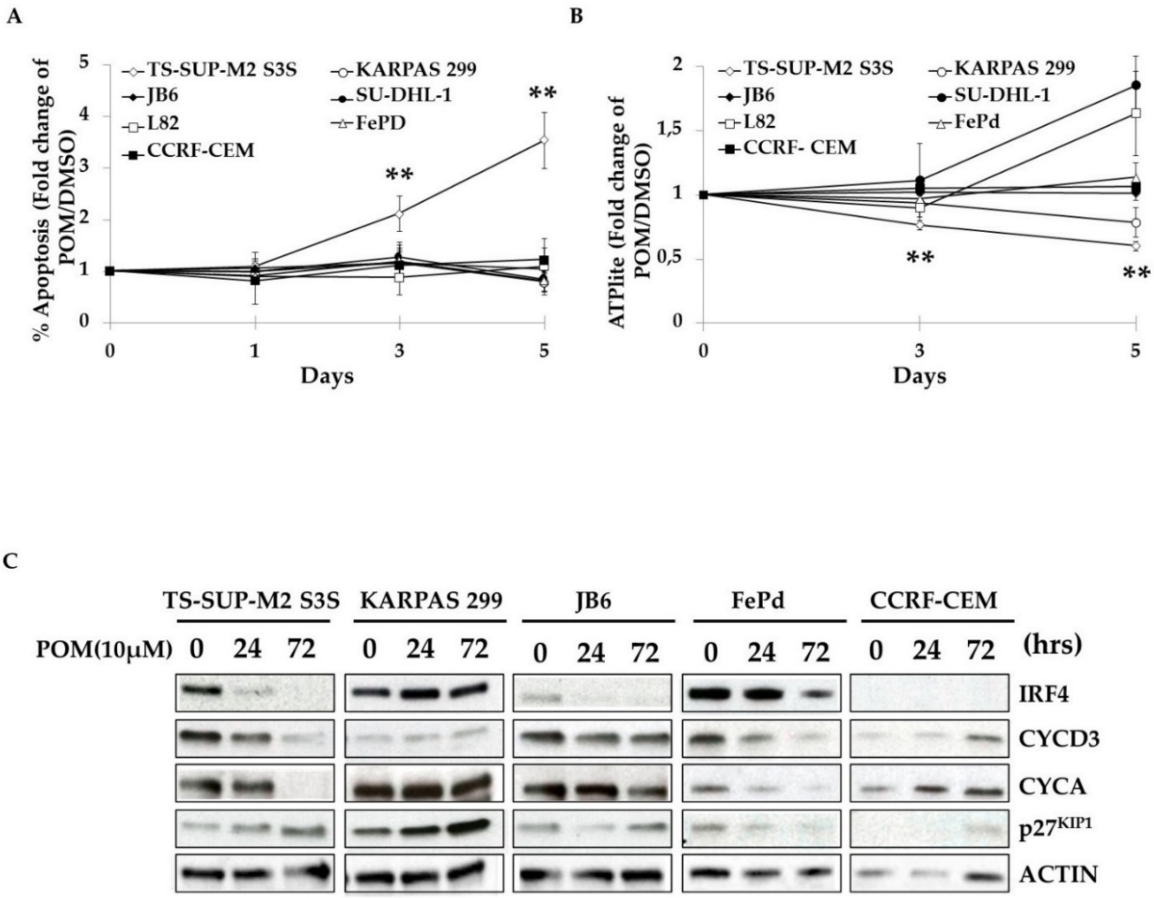


Figure S4. Effects of Pomalidomide treatment in ALCL cell lines. (**A**) Cell viability and (**B**) proliferation were measured in different ALCL cell lines at the indicated time points after DMSO or Pomalidomide (10μM) treatment. T-ALL CCRF-CEM cells were used as negative control. Data are presented as mean ± SD (*n* = 5). Analysis of cell death and cell proliferation revealed that only TS-SUP-M2 S3S cells were significantly sensitive to Pomalidomide treatment (***p* < 0.01;****p* < 0.001). (**C**) Western blot analysis of cells after Pomalidomide treatment at the indicated time points.

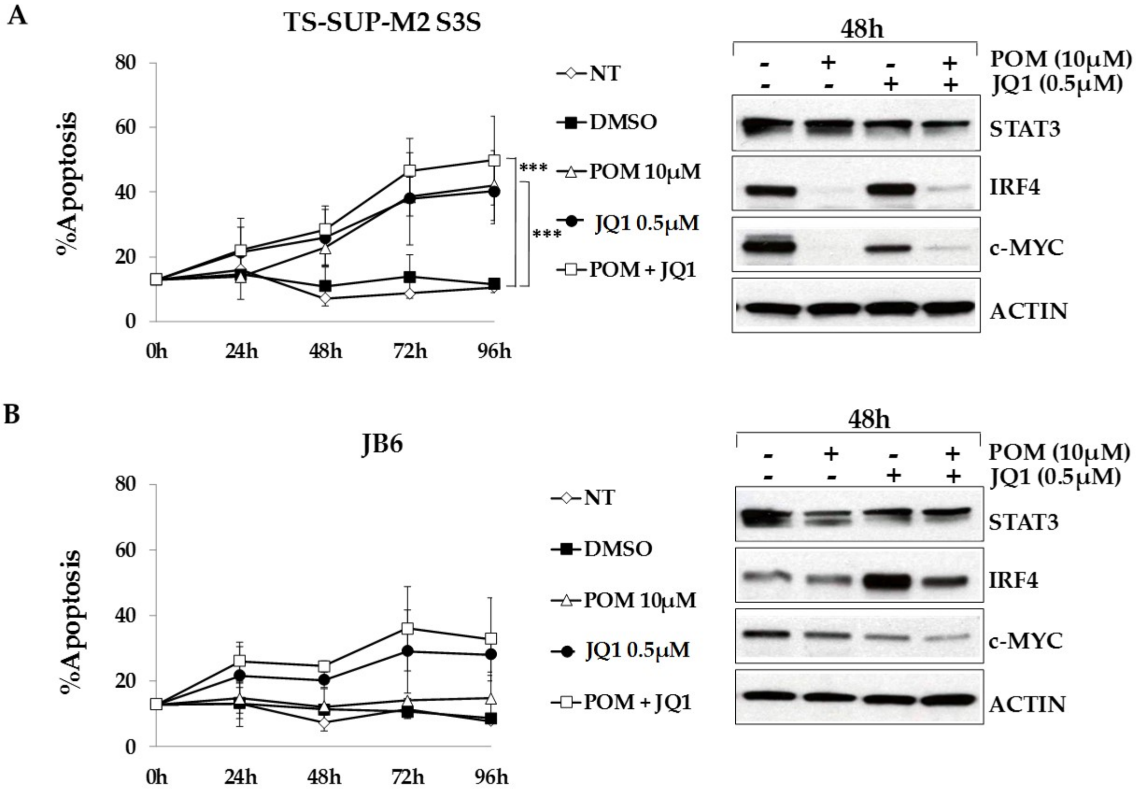


Figure S5. Effects of Pomalidomide combination with the BET family inhibitor JQ1 in TS-SUP-M2 S3S and JB6 cell lines. Apoptosis and western blot analysis of TS-SUP-M2 S3 (**A**) and JB6 (**B**) cells treated with DMSO, Pomalidomide, the BET inhibitor JQ1, or the combination of the two drugs. Error bars represent the s.d. of triplicate measurements (**$p < 0.01$;***$p < 0.001$). Pellet for western blot were collected 48 hours after treatments.

Table S1. List of Cluster 7 genes. Cluster 7 includes transcripts early down-regulated following inducible STAT3 KD in TS-SUP-M2cells

TargetID	ProbeID	SEARCH_KEY	CLUSTER ID	−DOX AVG_Sig	+DOX 72 h Log2R	+DOX 96 h Log2R	+DOX 120 h Log2R	+DOX 144 h Log2R	Oldexp
HS.527588	870047	ILMN_100916	7	3049	−2.78	−3.68	−3.95	−4.05	
C13ORF16	3390041	ILMN_19109	7	8732	−2.54	−3.52	−4.19	−5.08	
FGB	7210240	ILMN_13882	7	2650	−2.40	−3.24	−3.46	−3.84	−1.92
ZNF395	1980403	ILMN_17231	7	4559	−2.28	−1.62	−2.03	−2.81	−2.28
TNFRSF9	5670440	ILMN_13404	7	870	−2.13	−1.97	−2.18	−2.06	−0.74
LEF1	2810601	ILMN_30265	7	7327	−2.09	−2.90	−3.42	−3.80	−2.54
SLC2A5	7560541	ILMN_14785	7	6049	−2.08	−1.91	−1.75	−2.25	
ANKRD37	240682	ILMN_2423	7	2756	−2.07	−1.36	−1.32	−2.45	−1.65
UHRF2	2970612	ILMN_138390	7	2497	−2.02	−2.01	−1.64	−1.99	−1.30
LEF1	4570255	ILMN_30265	7	6489	−1.99	−2.97	−3.39	−3.82	−2.54
ICOS	2070037	ILMN_9996	7	13,683	−1.99	−3.00	−2.98	−3.38	−2.41
PSCD4	6510524	ILMN_16536	7	8740	−1.98	−2.30	−2.51	−2.20	−1.49
LOC196549	2710717	ILMN_17912	7	1588	−1.97	−1.57	−1.83	−2.44	−1.60
FCRLB	4730204	ILMN_24322	7	4927	−1.93	−1.88	−1.47	−1.74	−1.06
LOC145837	4050674	ILMN_30561	7	1311	−1.90	−2.33	−2.48	−2.49	
STARD13	3440184	ILMN_4567	7	2932	−1.89	−1.93	−2.38	−3.18	−1.13
IL6R	5870685	ILMN_6641	7	940	−1.89	−2.17	−2.21	−2.58	−1.36
SGK	4390450	ILMN_2451	7	5883	−1.85	−2.33	−2.56	−2.94	−2.20
STAT3	5090619	ILMN_29673	7	15,774	−1.80	−1.96	−2.32	−2.75	−1.94
SPTLC3	6590402	ILMN_90698	7	1223	−1.76	−1.77	−1.64	−2.17	
LOC158160	2030672	ILMN_21155	7	22,170	−1.76	−2.94	−3.47	−3.65	−0.81
SPTLC3	3780743	ILMN_7329	7	1177	−1.75	−1.84	−1.85	−2.16	
UPK1B	4490068	ILMN_11904	7	7281	−1.72	−2.23	−3.19	−3.63	−2.24
HS.560343	6400564	ILMN_114182	7	2546	−1.71	−1.90	−2.12	−1.86	

STARD13	3190411	ILMN_4466	7	1160	−1.69	−1.63	−1.91	−2.36	
STAT3	2100484	ILMN_170837	7	4524	−1.68	−1.77	−2.20	−2.68	
ALB	650431	ILMN_28973	7	857	−1.67	−2.09	−2.16	−2.37	
HS.93739	4540446	ILMN_74213	7	670	−1.65	−1.58	−1.42	−1.69	
STAT3	4250538	ILMN_29673	7	4178	−1.61	−1.95	−2.25	−2.64	−1.94
TMPRSS11D	2320168	ILMN_28873	7	2562	−1.60	−2.35	−2.57	−3.48	−1.94
MMP20	2480390	ILMN_1399	7	747	−1.56	−1.69	−1.81	−2.17	−1.50
HS.37648	4860280	ILMN_72502	7	1541	−1.53	−1.59	−1.69	−1.71	
IL10	6180093	ILMN_9173	7	878	−1.46	−1.63	−2.26	−2.25	
HSD17B7P2	7610142	ILMN_1267	7	4835	−1.44	−2.86	−3.41	−3.28	−2.21
HS.448059	2510671	ILMN_93404	7	2244	−1.42	−1.56	−1.48	−1.68	
SLCO2B1	6580441	ILMN_1897	7	820	−1.42	−1.81	−1.72	−1.82	−1.08
MEIS2	20358	ILMN_3095	7	1581	−1.41	−1.36	−1.07	−1.11	−1.36
AXUD1	7560041	ILMN_6524	7	9353	−1.40	−1.07	−0.95	−1.08	−0.79
IGSF11	5270102	ILMN_551	7	1617	−1.40	−1.45	−1.85	−2.38	
ABCA13	1780537	ILMN_19037	7	9378	−1.35	−0.64	−0.09	−0.07	−0.68
TNFRSF21	3780092	ILMN_9651	7	10,608	−1.35	−1.15	−1.53	−1.74	−1.50
HS.529631	7200286	ILMN_101314	7	1780	−1.33	−1.83	−1.59	−1.94	
FAM46C	6860347	ILMN_7706	7	8119	−1.32	−1.17	−0.69	−1.36	−0.84
CXCR5	3890400	ILMN_19896	7	577	−1.32	−1.37	−1.35	−1.67	
CMAH	5550066	ILMN_13868	7	5192	−1.31	−1.21	−1.33	−1.92	
NSL1	4640370	ILMN_164300	7	701	−1.31	−1.65	−1.94	−1.91	
CD59	1410201	ILMN_1905	7	953	−1.30	−1.51	−1.14	−0.88	
FBXO32	1990079	ILMN_3809	7	5857	−1.30	−1.06	−0.48	−0.70	−0.72
LOC642299	6020561	ILMN_31918	7	1926	−1.29	−1.51	−1.47	−1.72	
IQCG	70551	ILMN_26972	7	7879	−1.28	−1.91	−2.07	−1.88	−2.07
RORA	1110180	ILMN_13528	7	2362	−1.28	−1.77	−1.95	−2.10	−1.55
HS.36053	2940228	ILMN_72440	7	2285	−1.24	−1.86	−1.84	−2.24	
MYBPC2	5270100	ILMN_16419	7	10,920	−1.21	−1.91	−1.89	−2.22	−1.55
CD5	5050347	ILMN_29547	7	1644	−1.20	−1.68	−2.15	−2.22	−1.25
SOX2	5130156	ILMN_171554	7	1801	−1.20	−1.78	−1.66	−1.87	
NFIL3	5420564	ILMN_14880	7	3162	−1.19	−0.71	−0.69	−0.81	−1.19
PHF21A	6580164	ILMN_28019	7	8593	−1.18	−0.71	−0.22	−0.29	
HS.190748	1260356	ILMN_81073	7	612	−1.17	−1.29	−1.37	−1.23	
LOC651621	630095	ILMN_45641	7	2196	−1.17	−2.06	−2.26	−2.29	
TGM2	2940446	ILMN_8134	7	18,359	−1.16	−0.77	−0.69	−0.68	
CERK	3060692	ILMN_2275	7	3138	−1.16	−0.79	−0.63	−0.85	
GZMB	1850523	ILMN_23555	7	40,169	−1.15	−2.45	−3.11	−3.45	−1.34
VCL	70592	ILMN_26712	7	16,372	−1.13	−1.60	−1.21	−1.21	
CMAH	4250634	ILMN_13868	7	3642	−1.13	−1.36	−1.24	−1.83	
C9ORF38	50209	ILMN_36611	7	978	−1.12	−1.49	−1.48	−1.99	
SEMA4B	5080280	ILMN_25026	7	1832	−1.12	−0.47	−0.75	−0.70	
RGS16	1030102	ILMN_16445	7	5498	−1.11	−1.43	−2.25	−2.08	−1.65
TNFRSF10D	830113	ILMN_17600	7	1101	−1.10	−1.15	−0.71	−0.77	
STAMBPL1	7150059	ILMN_1387	7	6426	−1.10	−0.23	0.08	−0.65	
EDG1	160754	ILMN_138993	7	581	−1.10	−1.35	−1.43	−1.62	−1.78
CCDC46	6400161	ILMN_173004	7	2803	−1.10	−1.46	−0.98	−1.02	
RORA	3940703	ILMN_13528	7	808	−1.10	−1.59	−1.57	−1.72	−1.55
ERC2	7150050	ILMN_17447	7	605	−1.10	−1.24	−0.65	−0.39	−0.60
LOC440895	5310575	ILMN_44926	7	799	−1.10	−0.86	−0.82	−1.50	
ALB	5910010	ILMN_28973	7	581	−1.10	−1.58	−1.68	−1.44	
FLJ22447	1240653	ILMN_37423	7	1117	−1.09	−1.34	−1.43	−1.75	
CDK5RAP2	7570348	ILMN_9876	7	784	−1.09	−1.62	−1.69	−1.79	−0.98
TNFRSF8	4850253	ILMN_21182	7	6392	−1.08	−0.95	−1.77	−2.34	−1.36
C5ORF13	940471	ILMN_2442	7	13,584	−1.08	−1.11	−1.15	−1.18	−1.33
BIRC3	5080021	ILMN_3897	7	2415	−1.07	−0.81	−0.24	0.07	
CFH	380424	ILMN_12111	7	3249	−1.06	−0.78	−0.01	0.61	
ANKRD22	4150270	ILMN_7804	7	390	−1.06	−0.72	−0.37	0.12	
ERO1L	4780671	ILMN_4958	7	3416	−1.05	−1.04	−0.61	−1.02	−0.69
LOC650546	7210673	ILMN_35170	7	1731	−1.05	−1.30	−1.38	−2.39	
FCGR2A	1990278	ILMN_26366	7	558	−1.04	−1.16	−1.19	−1.14	
HS.368984	5490192	ILMN_87858	7	655	−1.02	−1.19	−1.01	−0.70	
N4BP2L1	620112	ILMN_24274	7	393	−1.01	−0.79	−0.71	−1.10	−0.74
MTUS1	3800017	ILMN_5924	7	1134	−1.00	−1.12	−1.51	−1.69	
SOX2	5080273	ILMN_13292	7	622	−1.00	−1.22	−1.22	−1.35	
KBTBD11	5050093	ILMN_20625	7	5058	−1.00	−1.78	−1.94	−2.14	−1.22

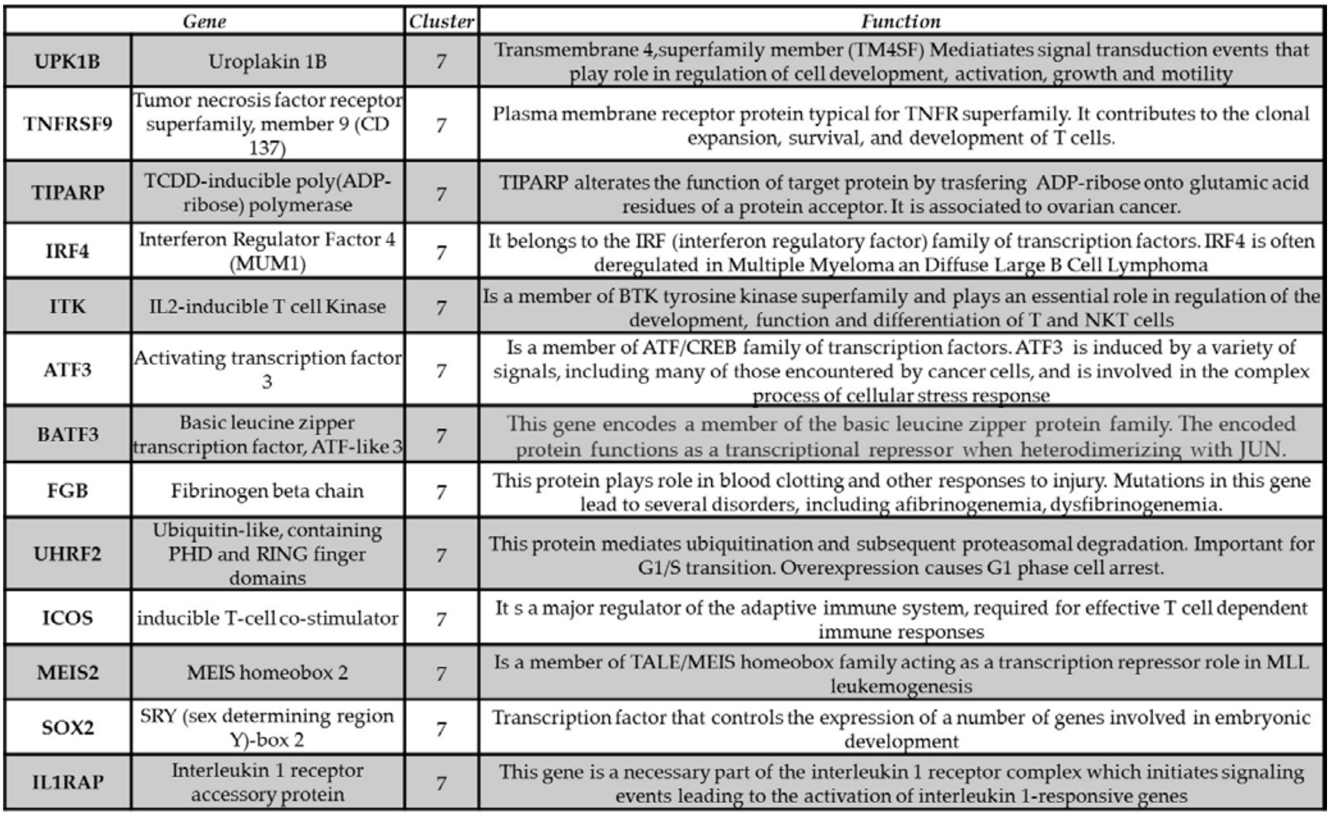
NIN	5310717	ILMN_3063	7	4443	−0.99	−1.05	−0.89	−1.01	−0.62
ZBTB32	7560273	ILMN_2617	7	2142	−0.99	−0.49	−0.74	−1.39	
KLF9	3390292	ILMN_2670	7	2736	−0.98	−1.40	−1.07	−1.08	−1.14
AQP9	160494	ILMN_15164	7	13,906	−0.97	−1.31	−1.44	−1.95	−1.63
HS.580444	840452	ILMN_132625	7	517	−0.97	−1.31	−1.40	−1.62	
DMBX1	2940187	ILMN_13566	7	1708	−0.97	−0.43	−1.01	−1.08	−1.23
MYO10	4670131	ILMN_28857	7	621	−0.97	−1.13	−0.79	−0.76	−1.70
IKZF1	730482	ILMN_22185	7	2966	−0.95	−0.65	−0.36	−1.26	−0.68
NIN	2190196	ILMN_3320	7	694	−0.94	−0.99	−1.08	−0.83	
PLOD2	7040477	ILMN_14675	7	14,800	−0.94	−0.81	−0.66	−1.06	−0.78
GPLD1	3180379	ILMN_20927	7	557	−0.93	−0.87	−1.00	−0.84	−0.81
C13ORF18	7000079	ILMN_19053	7	2630	−0.92	−1.57	−1.57	−1.99	−1.19
LOC647691	4920768	ILMN_46634	7	1159	−0.92	−0.82	−1.19	−1.53	
PRRX2	4150204	ILMN_9456	7	3810	−0.90	−1.14	−1.01	−1.51	−1.26
NLRP7	5670129	ILMN_2155	7	9786	−0.89	−0.45	−0.68	−1.02	
HELLS	7040161	ILMN_164061	7	1893	−0.89	−0.82	−1.44	−1.92	
KIAA1671	3120474	ILMN_42090	7	2311	−0.88	−1.51	−1.61	−1.40	
FLJ35880	1170241	ILMN_3282	7	744	−0.88	−0.87	−0.90	−1.42	
NLRP7	6180600	ILMN_10029	7	3380	−0.88	−0.59	−0.58	−1.18	
PLOD2	4640187	ILMN_25982	7	2357	−0.88	−0.93	−0.74	−1.41	
IRF4	6980370	ILMN_12414	7	1389	−0.88	−0.81	−1.48	−1.81	−1.67
HS.205745	2640497	ILMN_81914	7	713	−0.87	−1.11	−0.44	−0.34	
DICER1	5390433	ILMN_1996	7	1944	−0.87	−0.85	−0.55	−1.12	−0.94
HS.566469	1500133	ILMN_118789	7	408	−0.87	−0.90	−0.93	−1.19	
SLC19A3	4540593	ILMN_25079	7	926	−0.86	−0.98	−1.38	−1.92	−0.74
TIPARP	6760546	ILMN_4419	7	10,212	−0.86	−1.44	−1.69	−2.14	−1.13
SF3B3	1090239	ILMN_18316	7	9050	−0.85	−1.32	−1.33	−1.77	
LOC442597	2710121	ILMN_42881	7	69,455	−0.85	−1.33	−1.26	−1.51	
CACNB2	7100154	ILMN_2192	7	828	−0.85	−1.31	−1.64	−2.09	−1.24
SLC26A4	5900358	ILMN_23896	7	5740	−0.84	−0.57	−1.00	−1.57	−0.76
IL1RAP	3800427	ILMN_8626	7	497	−0.84	−1.15	−1.30	−1.36	−1.48
CDK5RAP2	4260017	ILMN_3896	7	26,180	−0.83	−0.98	−1.23	−1.07	−1.48
CSGALNACT2	1980092	ILMN_11419	7	1414	−0.83	−1.02	−0.80	−1.11	−1.26
LOC650546	4180148	ILMN_35170	7	2183	−0.83	−1.12	−1.51	−1.96	
HS.566966	670411	ILMN_119192	7	672	−0.82	−1.53	−1.67	−1.73	
ZNF672	6330646	ILMN_24640	7	4068	−0.82	−1.04	−0.85	−1.13	
ZDHHC19	830615	ILMN_27596	7	492	−0.82	−0.67	−0.97	−1.21	−0.76
SLCO2B1	1400121	ILMN_1897	7	439	−0.81	−1.02	−0.88	−0.66	−1.08
C13ORF18	2850286	ILMN_19053	7	3309	−0.81	−1.53	−1.57	−1.93	−1.19
ATF3	4780128	ILMN_182693	7	2392	−0.79	−1.12	−1.49	−0.96	
ITIH5	5050121	ILMN_9175	7	442	−0.78	−0.85	−1.03	−1.10	−0.95
CSF3R	6270114	ILMN_21047	7	1542	−0.78	−1.42	−1.04	−0.86	
CCDC34	3450408	ILMN_2645	7	10256	−0.77	−0.79	−1.19	−1.24	−1.55
FAM123A	2760291	ILMN_26739	7	3304	−0.76	−1.21	−1.55	−2.32	
HS.10862	1940563	ILMN_71180	7	17,896	−0.76	−0.67	−0.77	−1.15	
BATF3	5900253	ILMN_7180	7	26,954	−0.76	−1.14	−1.40	−1.66	−1.15
ASPH	2370450	ILMN_182157	7	2107	−0.76	−1.01	−1.49	−1.51	
TSHZ3	5870424	ILMN_19320	7	8306	−0.75	−0.66	−0.64	−1.26	−0.72
RNF144	3450136	ILMN_15740	7	3234	−0.74	−0.88	−0.70	−1.15	
AGTPBP1	4760427	ILMN_12151	7	2461	−0.74	−0.96	−1.18	−1.16	−1.14
BATF	6220195	ILMN_3307	7	6380	−0.73	−1.04	−1.15	−1.29	
RUNX1	7400368	ILMN_19125	7	1194	−0.72	−0.58	−0.60	−1.00	
PCCA	4730309	ILMN_6045	7	3934	−0.72	−0.93	−1.11	−0.94	
IL6R	1780603	ILMN_22419	7	329	−0.70	−0.96	−0.84	−1.00	−0.61
FAM113A	290296	ILMN_22766	7	2048	−0.70	−0.75	−0.83	−1.30	
DDX12	2120039	ILMN_33363	7	565	−0.70	−0.89	−1.16	−1.22	
C1ORF112	940240	ILMN_5134	7	2455	−0.68	−0.80	−0.94	−1.37	−0.95
MCM10	6580685	ILMN_2483	7	1471	−0.68	−0.94	−1.40	−1.86	−1.65
PASK	4150100	ILMN_19873	7	1133	−0.68	−0.85	−1.16	−1.60	−0.88
TNFAIP3	3360681	ILMN_2315	7	683	−0.68	−0.82	−1.08	−0.67	
VCL	2900068	ILMN_26712	7	797	−0.67	−0.79	−1.09	−0.93	
LOC653778	830639	ILMN_32201	7	6816	−0.67	−0.77	−0.66	−1.39	
CMTM7	2140239	ILMN_8486	7	24,363	−0.67	−0.74	−0.73	−1.20	−1.27
SLC1A3	4210403	ILMN_17250	7	494	−0.67	−1.21	−0.92	−1.45	−0.77
ACPP	2680725	ILMN_23186	7	836	−0.66	−1.03	−0.83	−0.73	−0.75
CACNB2	2850458	ILMN_2192	7	930	−0.66	−1.09	−1.32	−1.94	−1.24

GNAL	4200709	ILMN_29214	7	918	−0.65	−0.72	−1.17	−1.17	
CCDC34	7040184	ILMN_2645	7	32,783	−0.65	−0.84	−0.90	−1.35	−1.55
NFKBIA	4280113	ILMN_6745	7	20,541	−0.64	−1.25	−1.24	−1.01	−1.33
LOC654103	7320707	ILMN_37027	7	10,798	−0.64	−0.75	−0.71	−1.41	
FAM29A	4760615	ILMN_3594	7	637	−0.63	−0.82	−0.86	−1.18	−0.71
HS.566835	4880398	ILMN_119083	7	960	−0.63	−0.76	−0.70	−1.38	
ACMSD	20041	ILMN_21475	7	1130	−0.63	−1.42	−1.57	−1.36	
RAD54L	1980291	ILMN_1278	7	1080	−0.63	−1.04	−1.26	−1.69	−1.71
DPAGT1	2680176	ILMN_10306	7	1281	−0.63	−0.49	−0.76	−1.09	−0.65
CLIP1	2260066	ILMN_179647	7	8485	−0.62	−0.87	−1.03	−1.03	
LOC653513	5820167	ILMN_43919	7	1788	−0.62	−0.78	−1.23	−1.41	
ARID2	7100044	ILMN_163259	7	887	−0.61	−0.56	−0.59	−1.11	
MAN1C1	6280184	ILMN_12633	7	542	−0.60	−1.03	−1.15	−1.00	
LOC440359	5870307	ILMN_34340	7	10,799	−0.60	−0.67	−1.02	−1.50	
HNRNPA3	1440592	ILMN_5256	7	3138	−0.59	−1.09	−1.17	−1.63	−0.66
NOD1	7610390	ILMN_10741	7	603	−0.58	−1.07	−1.00	−0.97	−0.63
NBL1	5090156	ILMN_20462	7	6674	−0.58	−0.77	−0.98	−1.33	
USP32	5290369	ILMN_26659	7	1319	−0.57	−1.00	−1.18	−1.22	−1.20
MAPK14	4860600	ILMN_17267	7	929	−0.57	−0.47	−0.72	−1.06	−0.88
HELLS	4540768	ILMN_3656	7	977	−0.56	−1.06	−1.20	−1.64	−0.93
GADD45B	4920110	ILMN_138334	7	1555	−0.56	−0.78	−1.11	−1.09	
PIH1D1	5910202	ILMN_10897	7	3009	−0.56	−0.74	−0.90	−1.04	−0.88
ARID5B	1410408	ILMN_17477	7	6846	−0.56	−0.88	−1.21	−1.48	
ABHD3	5050241	ILMN_4359	7	1158	−0.56	−0.73	−0.59	−1.20	
FES	4120689	ILMN_27340	7	559	−0.56	−1.10	−1.50	−1.53	
PCNA	2510348	ILMN_6858	7	579	−0.56	−0.85	−0.99	−1.08	−0.81
AK3L1	160148	ILMN_28560	7	19,298	−0.55	−0.67	−0.77	−1.22	
POLD3	4590608	ILMN_24871	7	1295	−0.54	−1.04	−1.04	−1.63	−0.95
ANKRD32	6380747	ILMN_15362	7	1552	−0.54	−0.67	−0.57	−1.07	−0.60
DDX23	5090450	ILMN_2003	7	3819	−0.53	−0.52	−0.83	−1.05	−0.67
LOC654189	5700671	ILMN_30702	7	995	−0.52	−0.80	−0.62	−1.30	
CMTM7	4290403	ILMN_8951	7	1952	−0.52	−0.82	−0.92	−1.36	
SAPS3	2850273	ILMN_6618	7	2917	−0.51	−1.06	−1.01	−0.79	−0.73
IMPA2	2340241	ILMN_19881	7	6053	−0.51	−0.62	−0.92	−1.03	−1.18
MCC	2480669	ILMN_17732	7	434	−0.50	−0.89	−0.84	−1.02	
HSD17B7	3420328	ILMN_5318	7	30,246	−0.50	−0.71	−0.68	−1.09	
TOPBP1	870601	ILMN_24939	7	4008	−0.49	−0.90	−1.00	−1.22	−0.72
UCHL5IP	1090370	ILMN_27285	7	8570	−0.49	−1.15	−1.34	−1.78	
PMP22	7560138	ILMN_5694	7	14,585	−0.48	−0.64	−0.53	−1.02	−0.71
EME1	1260398	ILMN_3574	7	1147	−0.48	−0.72	−0.98	−1.40	−1.22
EXO1	2690458	ILMN_25997	7	707	−0.48	−1.00	−1.28	−1.36	
CCDC4	5890554	ILMN_1131	7	928	−0.47	−1.09	−1.29	−1.09	
ZNF138	360370	ILMN_36856	7	1182	−0.46	−0.71	−0.67	−1.23	
DICER1	5130091	ILMN_1996	7	8484	−0.46	−0.82	−0.95	−1.25	−0.94
HADH	6290709	ILMN_13258	7	8327	−0.45	−0.74	−0.67	−1.04	−0.73
TMEM106C	1430537	ILMN_7003	7	11,234	−0.45	−0.66	−0.92	−1.04	−0.64
PLOD2	460338	ILMN_14675	7	612	−0.44	−0.84	−0.56	−1.18	−0.78
PDS5B	5220575	ILMN_13136	7	2131	−0.44	−0.64	−0.73	−1.03	
PDS5B	870167	ILMN_20213	7	1213	−0.42	−0.78	−0.92	−1.20	−0.78
TNPO1	5080482	ILMN_18758	7	5142	−0.42	−1.08	−0.93	−1.42	
HS.135668	3830066	ILMN_77684	7	436	−0.41	−0.86	−0.70	−1.01	
CEP152	2340164	ILMN_570	7	1068	−0.41	−0.84	−0.94	−1.39	−1.47
FLJ13305	5870072	ILMN_5829	7	1312	−0.39	−0.62	−0.94	−1.08	
FBXO5	5670255	ILMN_9763	7	1243	−0.39	−1.22	−1.35	−1.46	−1.46
CHAF1A	2120097	ILMN_24131	7	775	−0.38	−0.87	−1.02	−1.32	−0.97
FANCD2	3610022	ILMN_12871	7	451	−0.38	−0.73	−1.02	−1.06	−0.93
SPRYD5	4880204	ILMN_14013	7	1048	−0.37	−1.04	−1.30	−1.18	
DNA2	6380452	ILMN_172479	7	872	−0.37	−0.69	−0.90	−1.13	
SIN3A	6420017	ILMN_14108	7	4662	−0.37	−0.69	−0.77	−1.10	−0.72
TGIF2	1340020	ILMN_25134	7	1540	−0.37	−0.71	−0.47	−1.00	
ITK	7560632	ILMN_23317	7	1572	−0.37	−0.69	−1.05	−0.79	
FAM80A	6840619	ILMN_12396	7	669	−0.36	−0.91	−0.92	−1.15	
CDK2	2350286	ILMN_12332	7	1377	−0.35	−0.90	−0.93	−1.14	−1.13
CACNB2	7210521	ILMN_24772	7	326	−0.35	−0.50	−0.68	−1.00	
RTTN	6400632	ILMN_5471	7	2581	−0.34	−0.88	−0.81	−1.10	
PDK3	110347	ILMN_15297	7	1622	−0.33	−0.67	−0.78	−1.07	

CCNF	3130541	ILMN_27253	7	7467	−0.31	−0.78	−0.80	−1.21	−0.96
MTHFS	6560066	ILMN_1014	7	14,508	−0.31	−0.61	−0.78	−1.04	−0.66
SLC39A10	5960332	ILMN_13415	7	1054	−0.31	−0.61	−0.61	−1.02	
POLA2	4920537	ILMN_8864	7	5588	−0.30	−0.58	−0.71	−1.01	−0.82
SLC37A4	5130577	ILMN_24454	7	5049	−0.28	−0.87	−0.82	−1.25	−0.84
NUCKS1	1450753	ILMN_17108	7	1290	−0.25	−0.97	−0.87	−1.38	
MASTL	580470	ILMN_7073	7	957	−0.23	−0.92	−0.84	−1.06	−1.06
SHMT1	2690528	ILMN_17710	7	3321	−0.19	−0.75	−0.64	−1.04	−0.71
FAM76B	6130273	ILMN_22478	7	1407	−0.18	−0.89	−0.69	−1.15	

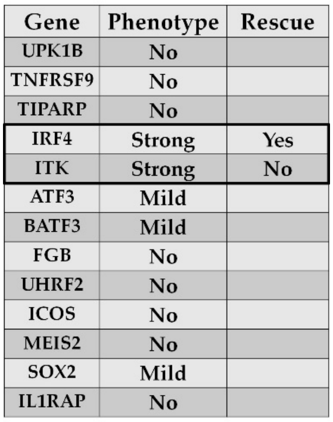
AVG: Average.

Table S2. Cluster 7 genes selected for functional screening with shRNA.



Gene		*Cluster*	*Function*
UPK1B	Uroplakin 1B	7	Transmembrane 4,superfamily member (TM4SF) Mediatiates signal transduction events that play role in regulation of cell development, activation, growth and motility
TNFRSF9	Tumor necrosis factor receptor superfamily, member 9 (CD 137)	7	Plasma membrane receptor protein typical for TNFR superfamily. It contributes to the clonal expansion, survival, and development of T cells.
TIPARP	TCDD-inducible poly(ADP-ribose) polymerase	7	TIPARP alterates the function of target protein by trasfering ADP-ribose onto glutamic acid residues of a protein acceptor. It is associated to ovarian cancer.
IRF4	Interferon Regulator Factor 4 (MUM1)	7	It belongs to the IRF (interferon regulatory factor) family of transcription factors. IRF4 is often deregulated in Multiple Myeloma an Diffuse Large B Cell Lymphoma
ITK	IL2-inducible T cell Kinase	7	Is a member of BTK tyrosine kinase superfamily and plays an essential role in regulation of the development, function and differentiation of T and NKT cells
ATF3	Activating transcription factor 3	7	Is a member of ATF/CREB family of transcription factors. ATF3 is induced by a variety of signals, including many of those encountered by cancer cells, and is involved in the complex process of cellular stress response
BATF3	Basic leucine zipper transcription factor, ATF-like 3	7	This gene encodes a member of the basic leucine zipper protein family. The encoded protein functions as a transcriptional repressor when heterodimerizing with JUN.
FGB	Fibrinogen beta chain	7	This protein plays role in blood clotting and other responses to injury. Mutations in this gene lead to several disorders, including afibrinogenemia, dysfibrinogenemia.
UHRF2	Ubiquitin-like, containing PHD and RING finger domains	7	This protein mediates ubiquitination and subsequent proteasomal degradation. Important for G1/S transition. Overexpression causes G1 phase cell arrest.
ICOS	inducible T-cell co-stimulator	7	It s a major regulator of the adaptive immune system, required for effective T cell dependent immune responses
MEIS2	MEIS homeobox 2	7	Is a member of TALE/MEIS homeobox family acting as a transcription repressor role in MLL leukemogenesis
SOX2	SRY (sex determining region Y)-box 2	7	Transcription factor that controls the expression of a number of genes involved in embryonic development
IL1RAP	Interleukin 1 receptor accessory protein	7	This gene is a necessary part of the interleukin 1 receptor complex which initiates signaling events leading to the activation of interleukin 1-responsive genes

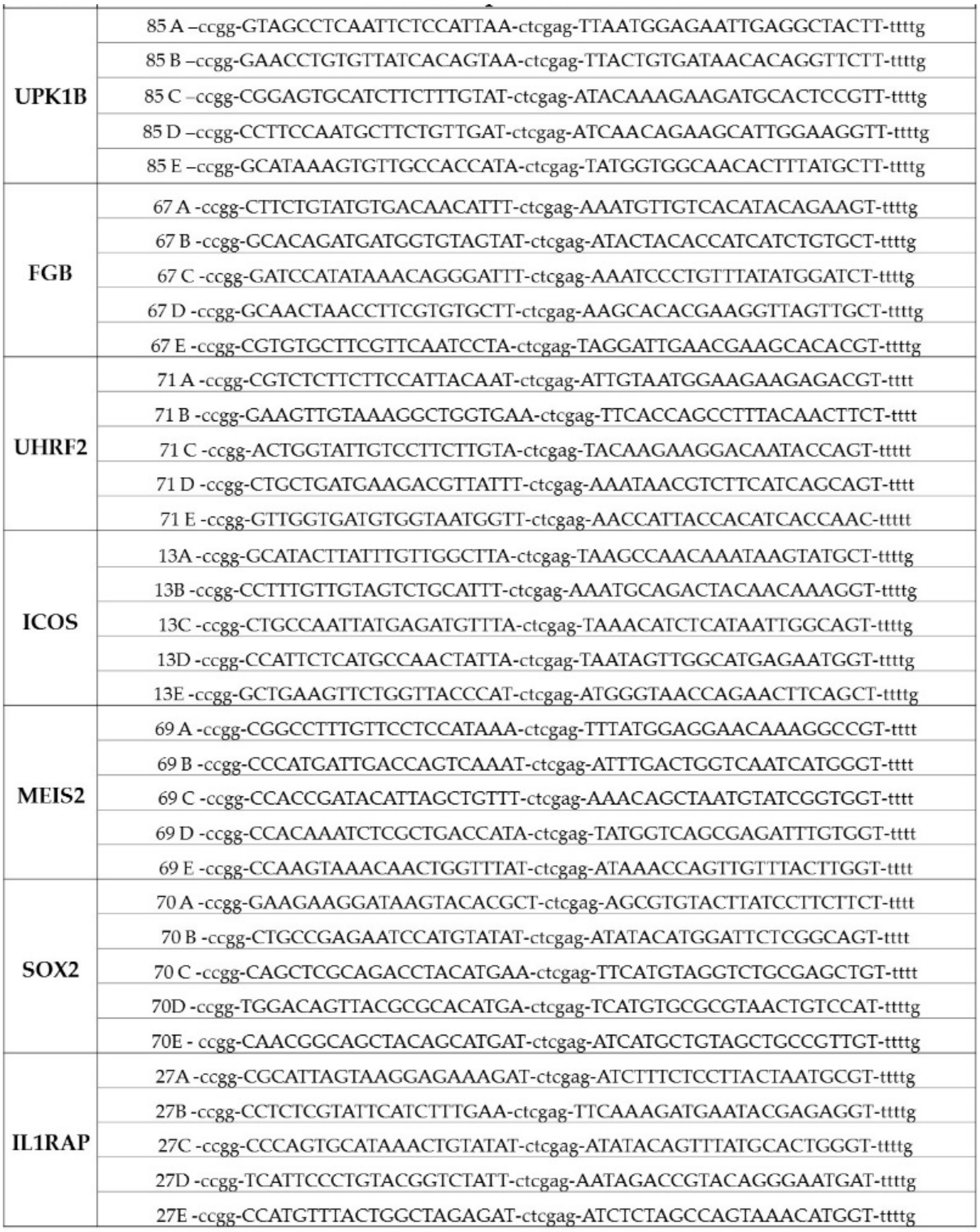
Table S3. Schematic result of the functional shRNA screening on cluster 7 genes. Positive hits were selected according to the following criteria: more than one shRNA sequence was able to reduce target mRNA levels by at least 70%; there was a correlation between the proportion of gene silencing and the phenotype. For genes showing "strong" phenotype, a "rescue" experiment expressing a shRNA-resistant open reading frame was performed (ITK and IRF4 highlighted in black box).



Gene	Phenotype	Rescue
UPK1B	No	
TNFRSF9	No	
TIPARP	No	
IRF4	Strong	Yes
ITK	Strong	No
ATF3	Mild	
BATF3	Mild	
FGB	No	
UHRF2	No	
ICOS	No	
MEIS2	No	
SOX2	Mild	
IL1RAP	No	

Table S4. List of shRNA sequences utilized in the screening. For each gene, 5 shRNA sequences from the TRC library (Sigma-Aldrich) were tested.

Gene	shRNA sequences from A to E
SNFT	3 A –ccgg-TGCTCAGAGAAGTCGGAAGAA-ctcgag-TTCTTCCGACTTCTCTGAGCA-tttt
	3 B –ccgg-GCTGACAAGCTCCATGAGGAA-ctcgag-TTCCTCATGGAGCTTGTCAGC-tttt
	3 C –ccgg-CCATGAGGAATATGAGAGCCT-ctcgag-AGGCTCTCATATTCCTCATGGT-tttt
	3 D –ccgg-GCACCTGACAGAGGCACTGAA- ctcgag-TTCAGTGCCTCTGTCAGGTGCT-tttt
	3 E –ccgg-CCCTATGAACTTTGTGCCAGT-ctcgag- ACTGGCACAAAGTTCATAGGGT-tttt
ATF3	12 A –ccgg-CCGCCTTTCATCTGGATTCTA-ctcgag-TAGAATCCAGATGAAAGGCGGT-tttt
	12 E –ccgg-CCTCTTTATCCAACAGATAAA-ctcgag-TTTATCTGTTGGATAAAGAGGT-tttt
	12 C –ccgg-CCTGAAGAAGATGAAAGGAAA-ctcgag-TTTCCTTTCATCTTCTTCAGGT-tttt
	12 D –ccgg-GCTGAACTGAAGGCTCAGATT-ctcgag-AATCTGAGCCTTCAGTTCAGCT-tttt
	12 B –ccgg-GCATTTGATATACATGCTCAA-ctcgag-TTGAGCATGTATATCAAATGCT-tttt
IRF4	45 A –ccgg-GCCCAAATTCTCCTCTCTAAA-ctcgag-TTTAGAGAGGAGAATTTGGGC-ttttt
	45 B –ccgg-GCCATTCCTCTATTCAAGAAT-ctcgag-ATTCTTGAATAGAGGAATGGC-ttttt
	45 C –ccgg-TGCGCTTTGAACAAGAGCAAT-ctcgag-ATTGCTCTTGTTCAAAGCGCAT-tttt
	45 D –ccgg-CCAGCAGGTTCACAACTACAT-ctcgag-ATGTAGTTGTGAACCTGCTGGT-tttt
	45 E –ccgg-GCTCTTTGACACACAGCAGTT-ctcgag-AACTGCTGTGTGTCAAAGAGCT-tttt
TNFRSF9	82 A –ccgg-CCGCAGATCATCTCCTTCTTT-ctcgag-AAAGAAGGAGATGATCTGCGGT-ttttg
	82 B –ccgg-GCAGAAAGAAACTCCTGTATA-ctcgag-TATACAGGAGTTTCTTTCTGCT-ttttg
	82 C –ccgg-GCAGGCAGTGTAAAGGTGTTT-ctcgag-AAACACCTTTACACTGCCTGCT-ttttg
	82 D –ccgg-GCTGGTACATTCTGTGATAAT-ctcgag-ATTATCACAGAATGTACCAGCT-ttttg
	82 E –ccgg-GCTCCGTTTCTCTGTTGTTAA-ctcgag-TTAACAACAGAGAAACGGAGCT-ttttg
TIPARP	83 A –ccgg-GAAGGCAAGCTACTCTCATAA-ctcgag-TTATGAGAGTAGCTTGCCTTCT-tttt
	83 B –ccgg-CCTTACTTACACTACTTACTT-ctcgag-AAGTAAGTAGTGTAAGTAAGGT-tttt
	83 C –ccgg-GAGCAATGTGAGGATTCTATT-ctcgag-AATAGAATCCTCACATTGCTCT-ttttg
	83 D –ccgg-AGGTCTTTGAGGCCAATATTA-ctcgag-TAATATTGGCCTCAAAGACCTT-ttttg
	83 E –ccgg-TAGCAATGTCAACTCTATTTA-ctcgag-TAAATAGAGTTGACATTGCTAT-ttttg
ITK	84 A –ccgg-TCAGTACACCAGTTCCACAGG-ctcgag-CCTGTGGAACTGGTGTACTGAT-tttt
	84 B –ccgg-GCCTTATATGACTACCAAACC-ctcgag-GGTTTGGTAGTCATATAAGGCT-tttt
	84 C –ccgg-CATCAACTATCACCAACATAA-ctcgag-TTATGTTGGTGATAGTTGATGTT-ttttg
	84 D –ccgg-GTGAGAACAATCCCTGTATAA-ctcgag-TTATACAGGGATTGTTCTCACTT-ttttg
	84 E –ccgg-GAAGACATCAGTACCGGATTT-ctcgag-AAATCCGGTACTGATGTCTTCTT-ttttg



UPK1B	85 A –ccgg-GTAGCCTCAATTCTCCATTAA-ctcgag-TTAATGGAGAATTGAGGCTACTT-ttttg
	85 B –ccgg-GAACCTGTGTTATCACAGTAA-ctcgag-TTACTGTGATAACACAGGTTCTT-ttttg
	85 C –ccgg-CGGAGTGCATCTTCTTTGTAT-ctcgag-ATACAAAGAAGATGCACTCCGTT-ttttg
	85 D –ccgg-CCTTCCAATGCTTCTGTTGAT-ctcgag-ATCAACAGAAGCATTGGAAGGTT-ttttg
	85 E –ccgg-GCATAAAGTGTTGCCACCATA-ctcgag-TATGGTGGCAACACTTTATGCTT-ttttg
FGB	67 A -ccgg-CTTCTGTATGTGACAACATTT-ctcgag-AAATGTTGTCACATACAGAAGT-ttttg
	67 B -ccgg-GCACAGATGATGGTGTAGTAT-ctcgag-ATACTACACCATCATCTGTGCT-ttttg
	67 C -ccgg-GATCCATATAAACAGGGATTT-ctcgag-AAATCCCTGTTTATATGGATCT-ttttg
	67 D -ccgg-GCAACTAACCTTCGTGTGCTT-ctcgag-AAGCACACGAAGGTTAGTTGCT-ttttg
	67 E -ccgg-CGTGTGCTTCGTTCAATCCTA-ctcgag-TAGGATTGAACGAAGCACACGT-ttttg
UHRF2	71 A -ccgg-CGTCTCTTCTTCCATTACAAT-ctcgag-ATTGTAATGGAAGAAGAGACGT-tttt
	71 B -ccgg-GAAGTTGTAAAGGCTGGTGAA-ctcgag-TTCACCAGCCTTTACAACTTCT-tttt
	71 C -ccgg-ACTGGTATTGTCCTTCTTGTA-ctcgag-TACAAGAAGGACAATACCAGT-ttttt
	71 D -ccgg-CTGCTGATGAAGACGTTATTT-ctcgag-AAATAACGTCTTCATCAGCAGT-tttt
	71 E -ccgg-GTTGGTGATGTGGTAATGGTT-ctcgag-AACCATTACCACATCACCAAC-ttttt
ICOS	13A -ccgg-GCATACTTATTTGTTGGCTTA-ctcgag-TAAGCCAACAAATAAGTATGCT-ttttg
	13B -ccgg-CCTTTGTTGTAGTCTGCATTT-ctcgag-AAATGCAGACTACAACAAAGGT-ttttg
	13C -ccgg-CTGCCAATTATGAGATGTTTA-ctcgag-TAAACATCTCATAATTGGCAGT-ttttg
	13D -ccgg-CCATTCTCATGCCAACTATTA-ctcgag-TAATAGTTGGCATGAGAATGGT-ttttg
	13E -ccgg-GCTGAAGTTCTGGTTACCCAT-ctcgag-ATGGGTAACCAGAACTTCAGCT-ttttg
MEIS2	69 A -ccgg-CGGCCTTTGTTCCTCCATAAA-ctcgag-TTTATGGAGGAACAAAGGCCGT-tttt
	69 B -ccgg-CCCATGATTGACCAGTCAAAT-ctcgag-ATTTGACTGGTCAATCATGGGT-tttt
	69 C -ccgg-CCACCGATACATTAGCTGTTT-ctcgag-AAACAGCTAATGTATCGGTGGT-tttt
	69 D -ccgg-CCACAAATCTCGCTGACCATA-ctcgag-TATGGTCAGCGAGATTTGTGGT-tttt
	69 E -ccgg-CCAAGTAAACAACTGGTTTAT-ctcgag-ATAAACCAGTTGTTTACTTGGT-tttt
SOX2	70 A -ccgg-GAAGAAGGATAAGTACACGCT-ctcgag-AGCGTGTACTTATCCTTCTTCT-tttt
	70 B -ccgg-CTGCCGAGAATCCATGTATAT-ctcgag-ATATACATGGATTCTCGGCAGT-tttt
	70 C -ccgg-CAGCTCGCAGACCTACATGAA-ctcgag-TTCATGTAGGTCTGCGAGCTGT-tttt
	70D -ccgg-TGGACAGTTACGCGCACATGA-ctcgag-TCATGTGCGCGTAACTGTCCAT-ttttg
	70E - ccgg-CAACGGCAGCTACAGCATGAT-ctcgag-ATCATGCTGTAGCTGCCGTTGT-ttttg
IL1RAP	27A -ccgg-CGCATTAGTAAGGAGAAAGAT-ctcgag-ATCTTTCTCCTTACTAATGCGT-ttttg
	27B -ccgg-CCTCTCGTATTCATCTTTGAA-ctcgag-TTCAAAGATGAATACGAGAGGT-ttttg
	27C -ccgg-CCCAGTGCATAAACTGTATAT-ctcgag-ATATACAGTTTATGCACTGGGT-ttttg
	27D -ccgg-TCATTCCCTGTACGGTCTATT-ctcgag-AATAGACCGTACAGGGAATGAT-ttttg
	27E -ccgg-CCATGTTTACTGGCTAGAGAT-ctcgag-ATCTCTAGCCAGTAAACATGGT-ttttg

Table S5. Primer sequences used in the present study.

Gene Name	Forward Primers	Reverse Primers
TPARP	5′-CCACACCACCCTCTAGCAAT-3′	5′-CTTTCAGACCCCGAGAGTTG-3′
ITK	5′-CGATTCCATCCCTCTTCTCA-3′	5′-TTCTGCCTCCCAAAACAAAC-3′
TNFRSF9	5′-CACTCTGTTGCTGGTCCTCA-3′	5′-GCGCTGGAGAAACTATTTGG-3′
UPK1B	5′-CCCTCCAAACAATGATGACC-3′	5′-CCTCTGCCGGAGAAACAG-3′
ATF3	5′-AAGGAAAAAGAGGCGACGAG-3′	5′-GCTGCTTCTCGTTCTTGAGC-3′
SNFT	5′-AGCCCTGAGGATGATGACAG-3′	5′-GTTTTCTTGCTCCAGGCTCTC-3′
IRF4	5′-ACCCCTACACCATGACAACG-3′	5′-GTCCAAACGTCATGGGACAT-3′
SOX2	5′-CTACGACGTGAGCGCCCTGC-3′	5′-GCCAAGAGCCATGCCAGGGG-3′
UHRF2	5′-ACTGGCTGGTGGATTTGCGGA-3′	5′-CCAGTCTTCCACCCGGTATCTGGA-3′
FGB	5′-GGGCTCGTCCAGCCAAAGCA-3′	5′-ACACCCCCAGGTCTGGGTCA-3′
MEIS2	5′-GCCAAACGTCCCCGGCAAGA-3′	5′-AGCGGGAACCCCTACTCCGT-3′
ICOS	5′-GTGCTCACTGGGAGTGGAAT-3′	5′-GTCAACTGGGGTTCAGCAAT-3′
Il1RAP	5′-TGGTTGTTCTAAGCCCCAAC-3′	5′-ACCTGCCCTGTGGATACTTG-3′
STAT3	5′-CTGCTCCAGGTACCGTGTGT-3′	5′-CCTCTGCCGGAGAAACAG-3′
TNFRSF8	5′-GTCACCCACCTGACATCACC-3′	5′-CCATGACATCCACTGTTCCA-3′
GAPDH	5′-AAGGAGAGCTCAAGGTCAGC-3′	5′-GGGAGTAGGGACCTCCTGTT-3′