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Supplementary Information for
Early B Cell Factor 4 modulates FAS-mediated apoptosis and promotes cytotoxic function in human immune cells

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Supplementary Materials and Methods

Whole genome CRISPR screen. The Human GeCKOv2 CRISPR knockout pooled library (Addgene, #1000000049) was used. The workflow is illustrated in Fig. 1a. The GeCKO library is divided into two sublibraries, A and B. Each library was transduced to Jurkat T cells at a low multiplicity of infection (0.3) to ensure that most cells receive only one viral construct. On day 3, Jurkat T cells were selected with 0.5 μg/mL puromycin. Then, cells were stimulated with 100 ng/ml FASL for 10 days. After treatment, genomic DNA was extracted to ensure over 500× coverage. The sgRNA sequences were amplified and deep sequencing was performed.

In Vitro Culture of Cells: Cells were cultured as described before (1). Briefly, 293T cells (CRL-3216) and Jurkat T cells, Clone E6-1 (TIB-152) were purchased from ATCC. 293T cells were cultured in complete DMEM. Jurkat T cells were cultured in complete RPMI. Primary T cells were cultured in complete RPMI with IL-2 and primary NK cells were cultured in complete RPMI with IL-2 and IL-15. Primary CD4, CD8 T cells and NK cells were isolated from PBMCs using the respective Miltenyi Pan Monocyte Isolation Kit. Macrophages were differentiated from CD14 monocytes by culturing with 50 ng/ml of GM-CSF and 50 ng/ml of M-CSF for 7 days.

Generation of EBF4 antibody. In collaboration with GenScript, the immunogen protein (sequence:

MDALPRSGLNLKEEPLLPAGLGSVRSWMQGAGILDASTAAQSGVGLARAHFEK

QPPSNLRKSNFFHFVLAMYDRQGQPVEVERTAFIDFVEKDREPGAEKTNNGIHY

RLRLVYNNGLRTEQDLYVRLIDSMSKQAIIYEGQDKNPEMCRVLLTHEIMCSRC

CDRKSCGNRNETPSDPVIIDRFFLKFFLKCNQNCLKNAGNPRDMRRFQVVVSTTV

SVDGHVLAVSDNMFVHNNSKHGRRARRLDPSEAATPCIKAISPGEGWTTGGATV

IVIGDNFFDGLQVVFGNVLVWSELITPHAIRVQTPPRHIPGVVEVTLSYKSKQFCK

GCPGRFVYTALNEPTIDYGFQRLQKVIPRHPGDPERLPKEVLLKRAADLAEALYG

VPGSNQELLLKRAADVAEALYSTPRAPGPLAPLAPSHPHPAVVGINAFSSPLAIAV

GDATPGPEPGYARSCSSASPRGFAPSPGSQQSGYGGGLGAGLGGYGAPGVAGLG

VPGSPSFLNGSTATSPFAIMPSSPPLAAASSMSLPAAAPTTSVFSFSPVNMISAVKQ

RSAFAPVLRPPSSPPQACPRAHGEGLPDQSFEDSDKFHSPARGLQGLAYSHHHHH

H) was synthesized. Two New Zealand rabbits were immunized with three injections by conventional protocol. 7 days after the 3rd immunization, immunized animal sera were tested by ELISA for immune response. 0.02% sodium azide was added as a preservative. The antibody specificity was confirmed by immunoblotting in EBF4 overexpression and knockout Jurkat T cells.

Immunoblot. Cells were lysed in either Triton X-100 or RIPA (EDTA-free) Lysis buffer containing Mini EDTA-free Protease Inhibitor Cocktail (Sigma) and Phosphatase Inhibitor Cocktail (Sigma). Lysates were standardized using the BCA Protein Assay Kit (Pierce). Proteins were resolved by SDS-PAGE, transferred onto nitrocellulose membranes and blocked in TBS-T (Tris Buffered Saline, 0.1% Tween-20) containing 5% milk before primary antibody incubation. Densitometric analysis was performed using the ImageJ software.

DISC assay. Nontarget and EBF4 KO Jurkat T cells were treated with and without 1 ug/ml APO-1-3 for 0 to120 minutes. Following stimulation, cells were collected and washed in ice-cold PBS to remove excess APO-1-3. Cells were lysed in 500 ul of TNTG Lysis Buffer (complete Triton-X Lysis Buffer with 10% glycerol) for 30 minutes on ice, and then centrifuged at 14000 xg for 14 minutes. The supernatant for each sample was collected to a new tube. For immunoprecipitation, Protein G magnetic Dynabeads were washed twice in Triton X lysis buffer, then resuspended in 50 ul of lysis buffer and added to the cell supernatants. 2 ul APO-1-3 was added to the untreated controls. Cell extract and beads were placed under rotating agitation at 4°C for 2 hours. Beads were washed 4 times using lysis buffer and a magnetic rack. Samples were heated at 95°C for 5 minutes in 20 ul of 2X SDS Sample Buffer. Resulting lysates were run on a 4-12% Bis-Tris gel, and immunoblotted for members of the DISC.

Cycloheximide (CHX) chase assay. Cells were collected and washed in PBS before being resuspended in RPMI at a concentration of 2 x 10^6 cells/ml. They were then treated with 10 µg/ml cycloheximide and were collected for Immunoblot at indicated time points.

CRISPR-Cas9 knockout. EBF4 knockout was performed as described before (1). EBF4 knockout plasmid was generated from the lentiCRISPRv2 vector (Addgene). Knockout plasmids and plasmids containing PAX2 and VSV-G (Addgene) were transfected into 293T cells using Lipofectamine 3000 reagent (Invitrogen). The culture supernatants containing lentivirus were harvested. Cell lines were seeded in complete medium supplemented with Polybrene (Sigma) and lentiviruses. Electroporation was also

performed for knockout (1). Briefly, cells were electroporated with a crRNA and tracrRNA (Integrated DNA Technologies) complex and Cas9 protein (Thermo fisher). After CRISPR screening, we used two different sgRNA constructs in subsequent experiments. Namely, the same sgRNA (AGTCGATGAGACGCACGTA) from the CRISPR screen and another sgRNA construct (ACCGGAAGAGCTGTGGCAAC).

Overexpression of EBF4. Lentiviral transduction was performed as described before (1). Briefly, the lentiviral EBF4 plasmid or FLAG tag EBF4 plasmid was generated from pLV-EF1a-IRES-Puro (Addgene) vector by replacing the puromycin fragment with a truncated EGFR protein. The lentiviral EBF4 plasmid and plasmids containing PAX2 and VSV-G (Addgene) were transfected into 293T cells using Lipofectamine 3000 reagent (Invitrogen).

FAS killing assay. 2 x 10^6 cells/ml were cultured in 96 well plate with FAS ligand. On day 1, cells were stained with propidium iodide (PI) and analyzed by flow cytometry analysis. The cells were counted for 30 seconds and the actual cell number of PI negative cells at each FASL dose were compared to that of unstimulated cells. For EBF4 overexpression cells, the cells were stained with EGFR and EGFR positive cells were considered as EBF4 overexpressor cells.

Quantitative RT-PCR. RT-qPCR was performed as described before (1). RNA was isolated and cDNA was synthesized. PCR with reverse transcription was performed in a

StepOnePlus detection system (Applied Biosystems). Relative Quantification of mRNAs were calculated by the double delta Ct method.

Bio-ID-mass spectrometry analysis.

Lentivirus were prepared by transfection of HEK293T cells according to manufacturer's instructions with pLKO-BioID2-EBF4:P2A:P2B = 4:3:1 ug respectively, and used for transduction of and puromycin selection Jurkat cells. pLKO-BioID2-EGFP and pLKP-BioID2_SBNO2 were also used for control virus production and transductions. Immunoblot confirmed correct expression of corresponding bioID2-fusion proteins in the transduced Jurkat cells. Jurkat cells stably expressing BioID2-EGFP, -EBF4, and -SBNO2 were correspondingly seeded with light, medium, and heavy stable isotope labeling amino acids in culture (SILAC) for 10 to 14 days. The cell cultures were then pulsed with 50 ug/mL of D-biotin (Invitrogen) for the last 24-hour culture before harvesting. Exact 100 x 10^6 cells from each SILAC cultured cells were pooled and washed with 40 mL cold PBS for 3 times. The pooled cell pellet was then lysed with 30 ul per million cells of SILAC-lysis buffer (50 mM Tris.Cl pH 7.5, 145 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.05% SDS, 10% glycerol, 1 x (Roshe) EDTA-free protease inhibitor cocktail), mixing well and on ice for 20 minutes. The lysates were centrifuged at 12,000 x g at 4 C for 10 minutes and the supernatant was transferred into a fresh tube for streptavidin pulling down. A volume of 300 uL of SILAC-lysis-buffer washed Dynabeads MyOne Streptavidin C1 (Invitrogen) into the cleared lysates, rocking in cold room for 4 hours. The beads were then washed by using magnetic separation module, washing with 1 mL of SILAC-lysis buffer for 3 rounds. The washed beads were resuspended in 50 uL of NuPage 4 x LDS loading dye with 1x

NuPage Sample Reducing Agent (Invitrogen). The samples were frozen at -80 before subjected to mass spectrometry analysis which was conducted basically as described previously (2). In brief, the samples were separated by SDS-PAGE and subjected to in-gel protein digestion with trypsin. The peptide mixtures were analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) on a UHPLC system coupled to a Q Exactive quadrupole-Orbitrap mass spectrometer via an electrospray ion source (all Thermo Fisher Scientific). For protein identification and SILAC-based quantitation the MS raw data was processed with the MaxQuant software (3). Forty candidates out of 2228 were determined by using a $\log_2$-fold change cutoff of 1.7 or higher (Fig. 3c and Supplemental table 2). These candidates were filtered using CRAPome/REPRINT (Resource for Evaluation of Protein Interaction Networks) to remove contaminants and background noise and then evaluated by the NIAID SIGNAL (Selection by Iterative pathway Group and Network Analysis Looping) database to classify KEGG biological pathways (4). Color intensity indicates the mean fold change (scale bottom).

Transcriptome analysis. Total RNA from un-stimulated Jurkat T cells (EBF4 knock out vs non-target guide RNA treated and Lentiviral transduction of EBF4 vs empty vector) was extracted and were used to prepare RNA-seq libraries. In collaboration with Novogene, the mRNA-seq was performed on the Illumina platform (PE150). RNA-seq data was processed using the Pipeliner workflow (https://github.com/CCBR/Pipeliner). Reads were trimmed using Cutadapt v1.18 and aligned to the human hg38 reference genome and Gencode release28 using two-pass STAR v2.7.0f (5, 6). RSEM v1.3.0 was used for gene-level

expression quantification, and limma v 3.38.3 was used for *voom* quantile normalization and differential expression analysis (7, 8).

Chromatin immunoprecipitation and DNA sequencing. FLAG tagged EBF4 Jurkat T cells were isolated by EGFR biotinylated antibody (R&D) and were cross-linked in 1% formaldehyde for 15 minutes at room temperature, quenched in 2.5 M glycine for 5 minutes, washed, and resuspended in 1st lysis buffer (50 mM HEPES-KOH, 140 mM NaCl, 1mM EDTA, 10% Glycerol, 0.5% NP-40, 0.25% TritonX-100, and proteinase inhibitor cocktail) and incubated 10 minutes. The cells were resuspended with 2nd lysis buffer (20 mM Tris-HCl, 200 mM NaCl, 2 mM EDTA, 0.5 mM EGTA, and proteinase inhibitor cocktail) and incubated 10 minutes. Cells were resuspended with 3rd lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1% TritonX-100, 0.1% Na-Deoxycholate, 0.1% SDS, and proteinase inhibitor cocktail) and incubated 10 minutes. Cross-linked chromatin was sonicated for 4 minutes using a Biruptor-Pico (30 seconds on/30 seconds off). Precleared chromatin extract was incubated overnight at 4°C with anti-FLAG antibody (Cell signaling) and immunoprecipitated with protein G sepharose beads. DNA was used to generate a standard Illumina sequencing library. The sequencing was performed on the Illumina platform (PE150).

ChIP-seq analysis. Reads were trimmed with Cutadapt v1.18. All reads aligning to the Encode hg38 v1 blacklist regions were identified by alignment with BWA v0.7.17 and removed with Picard SamToFastq (https://broadinstitute.github.io/picard/) (9, 10). The remaining reads were aligned to the hg38 reference genome using BWA. Reads with a

mapQ score less than 6 were removed with SAMtools v1.6 and PCR duplicates were removed with Picard MarkDuplicates (11). Data was converted into bigwigs for viewing and normalized by reads per genomic content (RPGC) using deepTools v3.0.1 using the following parameters: --binSize 25 --smoothLength 75 --effectiveGenomeSize 2700000000 --centerReads --normalizeUsing RPGC. RPGC-normalized input values were subtracted from RPGC-normalized ChIP values of matching cell type genome-wide using deepTools with --binSize 25 (12). Peaks were called using macsNarrow (macs v2.1.1) with the following parameter: -f "BAMPE" (13). Significant peaks were defined as those passing IDR across two biological replicates (14). Motif analyses were completed using the MEME suite v5.1.0 (15).

Assay for Transposase-Accessible Chromatin using sequencing. We followed the Omni-ATAC protocol (16). Briefly, 50000 cells were washed and lysed in lysis buffer (10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM MgCl2) containing 0.1% NP40, 0.1% Tween-20, and 0.01% Digitonin for 10 min on ice to prepare the nuclei. Immediately after lysis, nuclei were spun at 500 x g for 10 min to remove the supernatant. Nuclei were then incubated with Tn5 transposon and tagmentation buffer (20 mM Tris-HCl (pH 7.6), 10 mM MgCl2, 20% Dimethylformamide) containing 0.1% Tween-20 and 0.005% Digitonin at 37 °C for 30 min. Stop buffer was added directly into the reaction to end tagmentation. PCR was performed to amplify the library. Libraries were then purified with SPRI (Beckman) beads and deep sequencing was performed. The sequencing was performed on the Illumina platform (HiSeq PE150). Samples were trimmed for adapters using Cutadapt v 1.18 before alignment. The trimmed reads were aligned to the hg38 reference using Bowtie2 v2.3.4.1

with flag -k 10 (17). The peaks were called using Genrich v0.6 (https://github.com/jsh58/Genrich) with the following flags -j -y -r -v -d 150 -m 5 -e chrM,chrY. Genrich-produced bedgraphs were normalized by library size (reads per million sequenced reads, RPM) for visualization. Topologically associating domains for Jurkat T cells were identified using the platform of Yue lab (http://yuelab.org/) from published paper (18).

Reporter assay. Luciferase reporter assays were carried out using pNL2.1 [Nluc/Hygro] (Promega) or pGL4.23 [luc2/minP]. pNL2.1 with each promoter region sequence or pGL4.23 with 5 repeats of "CCCAGGGG" was co-transfected with Renilla vector and EBF4 overexpression vector or empty vector, into HEK293 cells in six-well plates using Lipofectamine 3000 Transfection Reagent (Invitrogen). EBF4 was tested in five different doses. After 24 hours of transfection, the cell lysate was processed for luciferase activity using the luciferase reporter system, according to the manufacturer's instructions (Promega Nano-Glo® Luciferase Assay System and Renilla Luciferase Assay System). Luciferase was measured and was calculated as relative light units normalized to transfection control (Renilla).

Single cell data analysis. Human and mouse white adipose tissue cell Seurat clusters and subclusters found on publicly available database were used to identify EBF4 positive cell populations (19). Quality control and downstream analysis conducted by the Rosen Lab and were as described in the study (19). Data was visualized with UMAP embedding using

the Seurat package in R version 4.1.3. Previously annotated cell types were grouped into larger categories as shown in Fig. 2D and Fig. 3F.

Ingenuity upstream regulator analysis and Gene set enrichment analysis (GSEA). Upstream regulator analyses were performed using QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) tools. Comparison between control vs overexpression cells, the genes were selected from a $\log_2$-fold change cutoff of 1.5 or higher or cutoff of -1.5 or lower. GSEA was performed using the WEB-based Gene SeT Analysis Toolkit (WebGestalt).(20)

Mice. Animal procedures were performed under protocols approved by the NIAID Animal Care and Use Committee. Ebf4-deficient mice were generated from Taconic C57BL/6 mice using the CRISPR-Cas9 genome-editing method. Briefly, 4-week-old C57BL/6 females were super-ovulated using 5 IU of pregnant mare serum gonadotropin followed 48 hr later with 5IU of human chorionic gonadotropin which was followed by mating with C57BL/6 males. The pregnant females were sacrificed, and the embryos were collected from the oviducts. The embryos were then electroporated with a mixture of Cas9 (200ng/ul, IDT DNA., Coralville, IA), sgRNAs (50ng/ul each, Synthego, Melano Park, CA) using NEPA21 electroporator (NapaGene, Japan) following manufacturer's guidelines. The embryos were then transferred into oviducts of CD1 pseudo-pregnant mothers. Once the pups were weaned at three weeks, PCR analysis to detect exon deletion was done from the ear punch biopsies. The followings are the sequences of sgRNAs. Ebf4_198f,

ccuacggcucccgcucccca; Ebf4_70r, agaccggccgaguccccccg; Ebf4_105f, uugggggcgcccacgaacug; Ebf4_232r, uuaccucugcgccgcggugu.

Statistical analysis. Data are represented as mean ± SD with a significant difference reported as * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$. Two-way comparisons were calculated using a two-tailed, paired or unpaired Student's *t* test.

Data Availability

All study data are included in the article and/or SI Appendix. All sequencing data will be deposited in the GEO database upon publication.

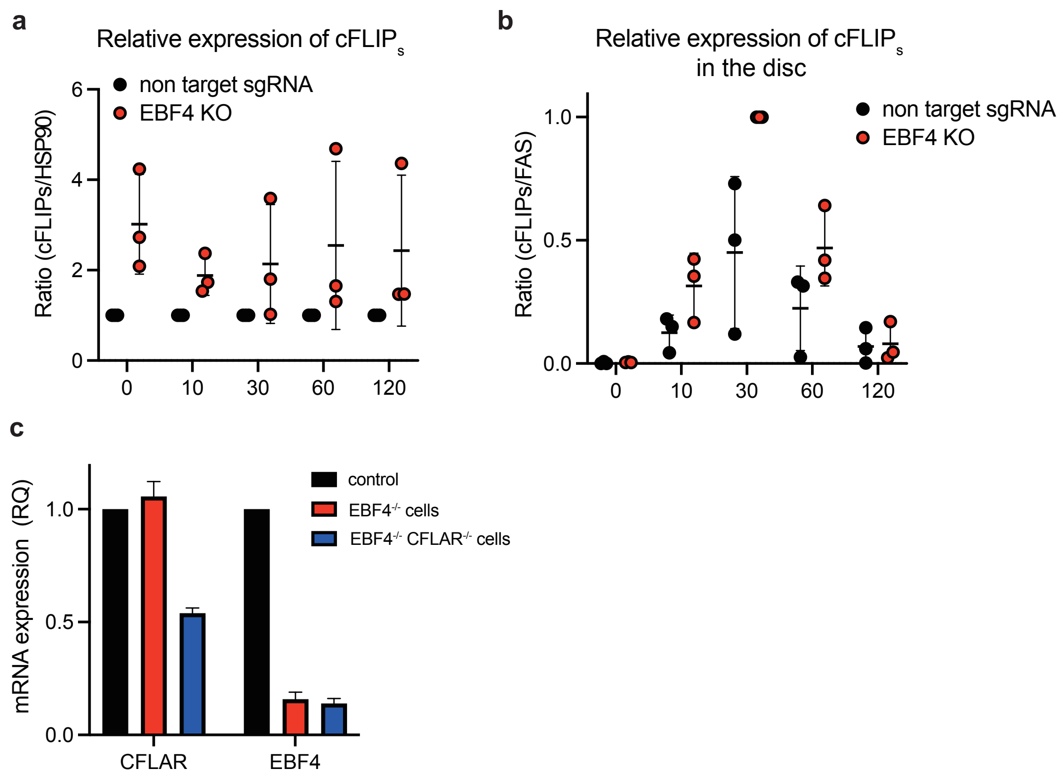

Figure S1. (**a**) Relative Intensity of c-FLIP protein levels in control and EBF4 KO Jurkat T cells to HSP90 loading control as quantified from immunoblots. **(b)** Relative intensity of c-FLIP protein levels at the DISC, standardized to pulldown by CD95, as quantified from immunoblots. **(c)** mRNA levels of CFLAR and EBF4 in EBF4 KO and CFLAR EBF4 double KO Jurkat T cells.

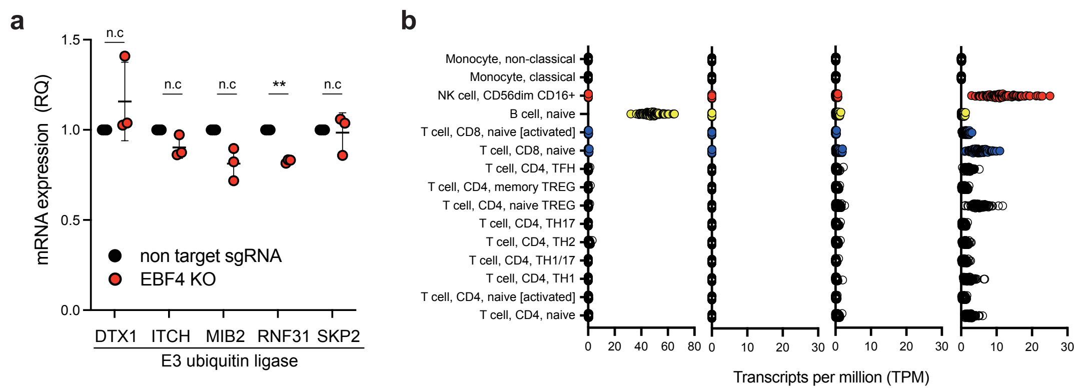

Figure S2. (a) mRNA levels of several E3 ubiquitin ligase involved in c-FLIP protein expression in control and EBF4 KO Jurkat T cells. **(b)** The EBF family members' gene expression by reanalysis of eQTL database (DICE; https://dice-database.org) are shown as boxplots. The levels of expression are depicted as transcripts per million (TPM).

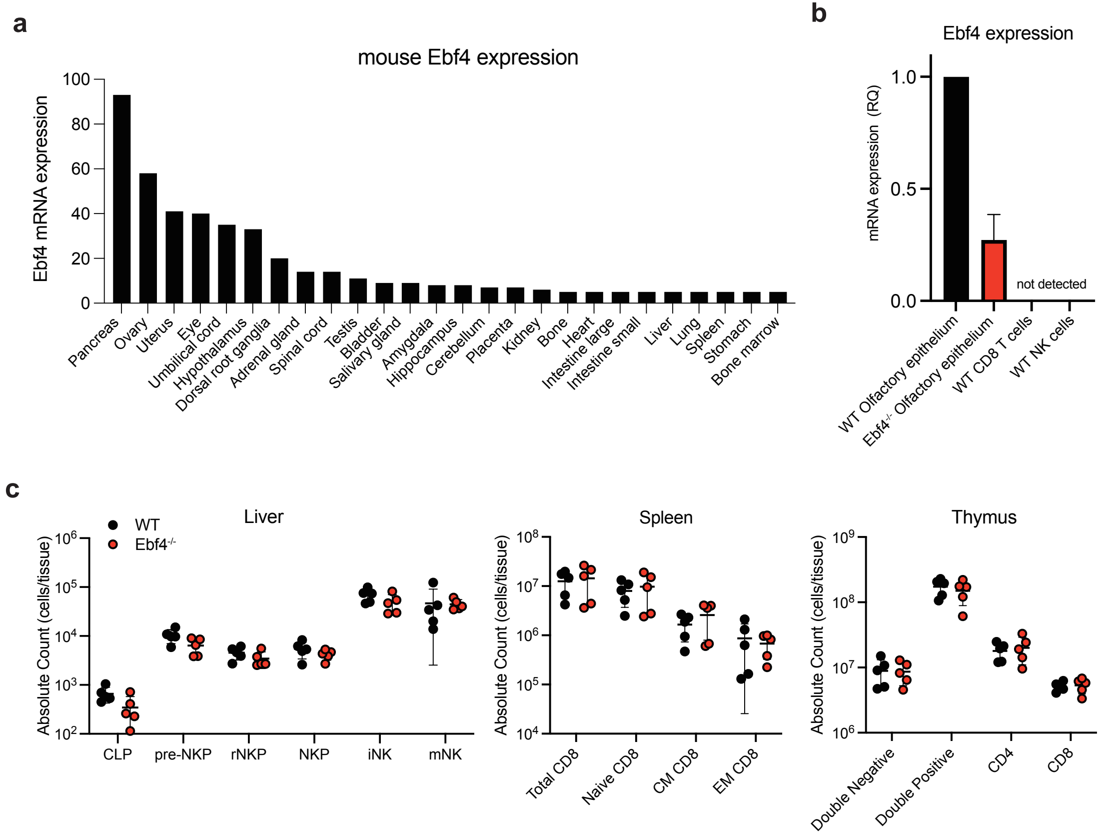

Figure S3. (a) EBF4 expression in different mouse tissues (http://ds.biogps.org). **(b)** EBF4 expression in WT and Ebf4$^{-/-}$ mouse olfactory epithelium, WT mouse CD8 T cells, and WT mouse NK cells as quantified by RT-qPCR. **(c)** Counts of NK and CD8 precursor populations in WT and Ebf4$^{-/-}$ mouse liver, spleen, and thymus.

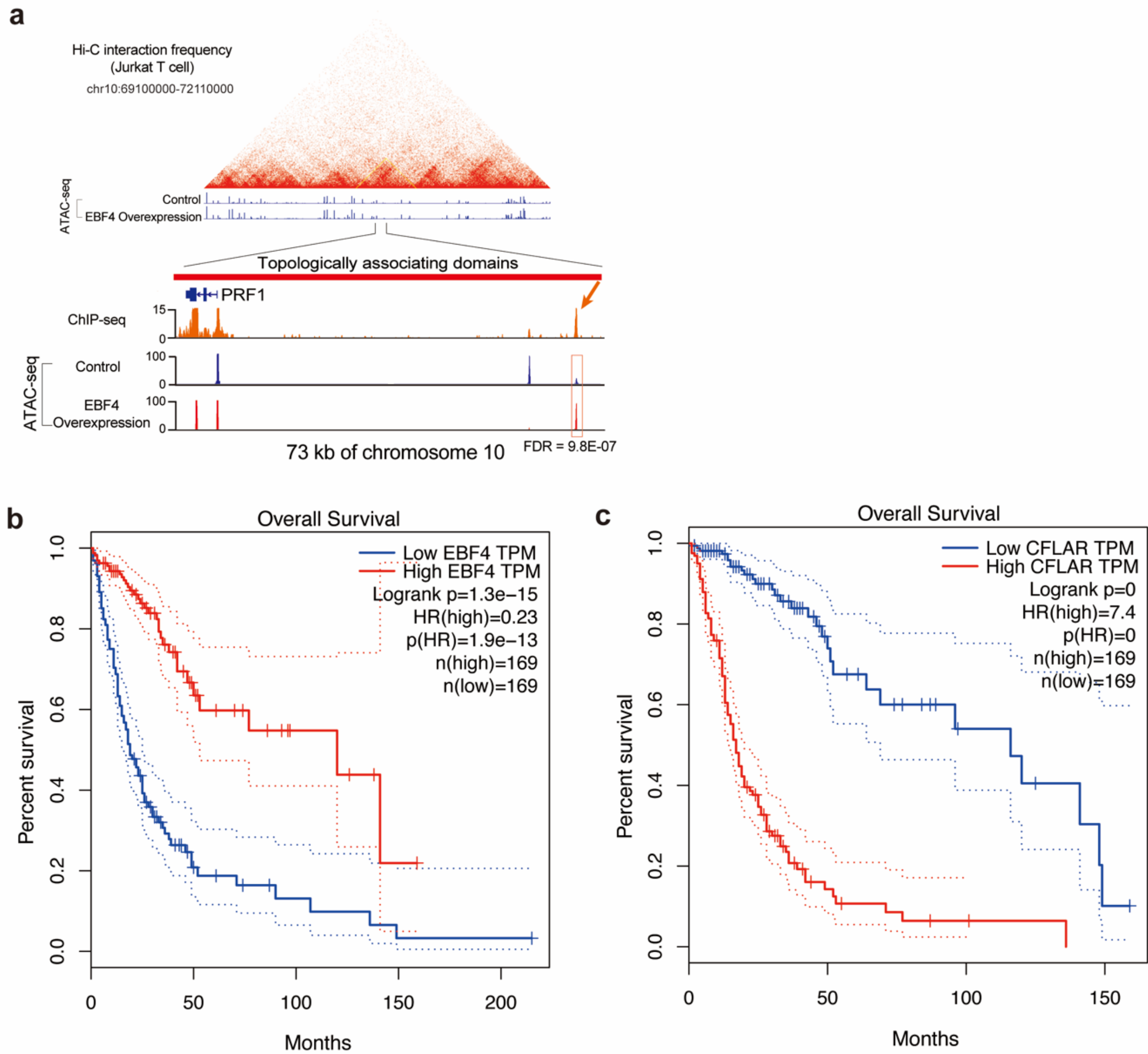

Figure S4. (a) ATAC-seq sequencing tracks from control and EBF4 overexpression Jurkat T cells for the PRF1 loci. (**b,c**) RNA expression of EBF4 with relation to survival data in the cancer genome atlas database. Kaplan-Meier survival curves of patients with glioma/glioblastomas stratified by high and low EBF4 expression (b) and CFLAR expression (c).

Supplemental table 1 The top 50 gene list found in whole genome screen

Rank	Gene name	Log10 FC
1	**FADD**	**3.4203626**
2	LSP1	2.84692256
3	**FAS**	**2.7040191**
4	Non Targeting Control	2.62317629
5	AMBRA1	2.59151057
6	OR2A12	2.49855713
7	AMTN	2.46072793
8	CKLF	2.42207039
9	**CASP8**	**2.33563044**
10	SH3RF2	2.31568199
11	EBF4	2.30179604
12	LIMD1	2.29598947
13	UAP1	2.26648976
14	SSH3	2.26330662
15	hsa-mir-2682	2.25405724
16	EPB42	2.24858955
17	PAX5	2.24035363
18	ARAP3	2.21899154
19	WWC2	2.21816474
20	hsa-mir-1915	2.21366668
21	POU5F1B	2.2090089
22	PPP2CA	2.15465709
23	NonTargetingControlGuideForHuman_0239	2.15432251
24	OSTF1	2.12279072
25	HOXB1	2.10619657
26	GMPPA	2.0957561
27	GLS2	2.0920836
28	MX2	2.06206166
29	OPN3	2.05691867
30	CYP8B1	2.05678985
31	hsa-mir-663b	2.05267605
32	ING5	2.04986709
33	IL18	2.04905338
34	GGT1	2.04351946
35	TAC3	2.04144849
36	hsa-mir-184	2.03588213
37	SNRPB	2.02186898
38	ARL5A	2.01604966
39	VWF	1.99858905
40	SRPK3	1.99576667
41	SGCZ	1.98581126
42	OR2K2	1.97515402
43	ADCK5	1.96714072
44	ZFP30	1.96707293
45	hsa-mir-4492	1.95880697
46	OR6A2	1.95654921
47	SUPT20HL1	1.95515116
48	IL17C	1.93466303
49	SPRY1	1.92601401
50	hsa-mir-4688	1.92448516

Supplemental table 2 The top 80 gene list found in Bio-ID-mass spectrometry analysis

Rank	gene name	FC	Rank	gene name	FC
1	GSTM3	3.25859487	41	VANGL2	1.84360042
2	FDPS	3.10729669	42	SLC25A1	1.84290027
3	ANXA1	3.01080652	43	DGKZ	1.8380003
4	ACADM	2.92940974	44	HSD17B11	1.8370998
5	UBASH3B	2.72030799	45	CCDC71L	1.8233999
6	ARHGDIB	2.50659689	46	VANGL1	1.82250023
7	RASSF2	2.49640153	47	PCIF1	1.81250001
8	DHCR7	2.44399274	48	DNAJA1	1.81230026
9	ARPC4	2.39849594	49	HSD17B7	1.79479989
10	DGCR8	2.33669591	50	SPTBN1	1.78830027
11	TMEM173	2.27980432	51	IKZF2	1.78760006
12	FAR1	2.27100361	52	ELMO2	1.78470051
13	RAB8B	2.22809385	53	PSMA6	1.78019959
14	TMEM97	2.20749474	54	DDX60	1.7779996
15	EBP	2.18999868	55	CLIC1	1.77629968
16	LCP2	2.18039573	56	MBNL1	1.77490032
17	KLHL7	2.15410713	57	PSMD5	1.77019955
18	CS	2.12530515	58	DUT	1.76559935
19	PSMB10	2.0934029	59	DNTTIP1	1.76130041
20	VIM	2.07370403	60	TAOK1	1.7541
21	MYH9	2.06579916	61	IDI1	1.75179991
22	ETFB	2.06269427	62	STAT5A	1.75059942
23	NUDT1	2.04959662	63	TAP1	1.75020025
24	IDH2	2.015098	64	TAP2	1.74450049
25	AP3S2	1.98659943	65	PPIA	1.72850047
26	SDCBP	1.97690014	66	MED12L	1.72260031
27	LGALS9	1.97300006	67	DTX3L	1.72220037
28	POLD3	1.97119978	68	PASK	1.71380029
29	DTX1	1.96379999	69	DDX39A	1.70290033
30	TAF5L	1.95909995	70	PTPN6	1.70130052
31	DIP2B	1.95330062	71	CDK2	1.69979999
32	CBX2	1.94780061	72	GMPPA	1.69419977
33	PHF8	1.94530046	73	RMI1	1.68250058
34	CLPTM1	1.93680002	74	MYL6	1.67930002
35	NSDHL	1.91629942	75	PLEKHA2	1.67790031
36	PHF5A	1.89409934	76	FDFT1	1.67740028
37	STAT3	1.87240043	77	YWHAQ	1.67480021
38	GIMAP7	1.86980005	78	AK4	1.67130032
39	MAPK3	1.86709974	79	PDK3	1.66739972
40	RHOC	1.86269956	80	PTK2B	1.65839998

Supplemental table 3 The genes whose promoter regions EBF4 binds to and are subjected to transcriptional regulation by EBF4

Gene name	vs EBF4 overexpression (FC)	vs. EBF4 KO (FC)	Baseline FPKM
ELL3	55.9059034	-1.1074002	1.15
PLCH2	45.4217373	-1.8826868	0.02
KREMEN2	10.3027371	-1.1168557	0.83
NKG7	7.55152783	-1.5700277	14.87
IZUMO4	7.04561091	-1.0972897	0.73
POU2AF1	7.02340483	-1.47127	5.99
CTTN	6.34063691	-1.2032746	3.02
TBX21	5.17378084	-1.5649186	0.35
FAM20C	4.97080433	-3.3789969	0.76
GZMA	4.91849643	-3.8540211	24.44
HR	4.85875337	-1.8012456	3.44
GRID1	4.33266456	-1.030178	1.8
SUSD4	3.85048379	-1.5963616	12.02
TNRC6C-AS1	3.54596354	-1.5127719	1.14
FGF9	3.38621943	-1.105297	1.77
SEMA6C	3.19376949	-1.2075929	3.35
SLC4A11	3.07539705	-1.0045343	5.59
JAML	3.07490843	-1.0238982	1.72
ARSG	3.04450665	-1.1878685	1.98
ADARB1	3.02044685	-1.2761288	12.57
GORASP1	2.98794254	-1.0923173	20.07
CHGB	2.96901078	-1.0182227	0.16
ADORA2A	2.93844618	-1.0227293	3.05
TSKU	2.89704773	-1.3594226	10.25
GALNT6	2.88683548	-1.0181728	9.85
CAMSAP2	2.85105266	-1.6714313	1.39
JAK3	2.83156953	-1.3928444	5.94
PRCP	2.7279387	-1.1174832	23.13
ATP11A	2.71238522	-1.0258531	8.21
GABRB3	2.66750574	-1.1742672	15.64
C11orf95	2.65846927	-1.0895501	10.61
AMPD2	2.64466698	-1.0371196	22.83
CUEDC1	2.58880146	-1.331303	1.38
LGALS3BP	2.58249978	-1.1245566	42.24
CCDC107	2.44603879	-1.0438341	16.52
PLEKHG5	2.4314816	-1.1032711	2.55
NHLRC4	2.3630333	-1.9065575	1.23
HCP5	2.35133787	-1.9352303	0.56
ZDHHC14	2.31663915	-1.1925932	3.76
GPR153	2.30846229	-1.0257735	9.89
OSCP1	2.27706711	-1.1407142	3.38
UCP2	2.18831364	-1.0402916	113.14
SNHG26	2.17778777	-1.1017922	0.35
AK4	2.14755761	-1.4368512	9.6
GALNT10	2.13186222	-1.0736459	6.99
TAPBP	2.09723823	-1.07264	27.75
GIMAP6	2.04068217	-1.0567418	38.47

Supplemental table 4 The genes whose intergenic or intron regions EBF4 binds to and are subjected to transcriptional regulation by EBF4

Gene name	vs EBF4 overexpression (FC)	vs. EBF4 KO (FC)	Baseline FPKM
EBF4	477.409164	-5.6941509	2.43
PLCH2	45.4217373	-1.8826868	0.02
COL15A1	25.8443564	-1.0282488	0.07
CD79A	25.3454439	-1.8490348	55.13
TC2N	20.6873964	-2.7943114	0.32
FOXO6	14.8252889	-1.0920284	4.38
ACSL6	13.0959366	-1.1025862	2.88
KCNK17	11.8709478	-3.1934107	0.1
GZMK	11.6157526	-2.4443275	1.23
PTGDR2	11.3841459	-1.5698488	1.26
LFNG	10.4037076	-1.0430988	0.63
ATF5	10.1906489	-1.0360922	16.03
MEGF6	9.9465795	-3.1236131	0.77
BTG2	9.58878225	-1.2087967	5.18
RGL4	9.25233068	-1.2439614	10.57
KCNC1	9.17072767	-2.1931563	0.07
TLR5	8.54847376	-1.4518112	1.18
IL2RB	8.35432524	-2.0369513	0.8
NT5E	8.32145596	-1.2165957	2.04
COL6A3	7.88326776	-1.2480621	0.08
DNAH17	7.75063552	-1.7776787	1.03
IZUMO4	7.04561091	-1.0972897	0.73
ZBTB7B	6.9792018	-1.3681513	0.37
VIPR2	6.85652501	-1.0513157	0.92
GRASP	6.82062031	-1.46542	13.43
LINC00689	6.42124295	-1.5301905	0.05
CTTN	6.34063691	-1.2032746	3.02
MAT1A	6.19257592	-2.1763992	6.8
SLC14A1	5.80544822	-2.3901095	1.44
GRAMD2A	5.35238008	-1.4219084	0.13
TBX21	5.17378084	-1.5649186	0.35
HS3ST3B1	5.08839602	-1.2790734	1.39
MYL4	5.00519552	-2.0213854	6.19
FAM20C	4.97080433	-3.3789969	0.76
COL6A2	4.83251204	-7.5595383	0.72
SLCO3A1	4.65167124	-1.4539571	6.19
MGLL	4.6113942	-1.956129	0.74
WNK2	4.43745331	-1.2464967	4.2
EOMES	4.33635189	-1.5260864	5.14
GRID1	4.33266456	-1.030178	1.8
GSDME	4.29209868	-1.0092355	4.22
DDIT4	4.24382277	-1.1821961	90.72
MICAL2	4.12742922	-1.7038706	5.93
DOC2A	4.10003506	-1.0656287	6.49
DCLK3	4.03167629	-1.5986213	1.08
CHRNA9	3.94895294	-1.2432474	12.81
RFLNA	3.88850227	-1.8950446	3.61
SUSD4	3.85048379	-1.5963616	12.02
UNC13A	3.82214683	-1.407711	0.24
GNGT2	3.66247929	-1.0219844	6.85
ITGB2	3.59020827	-1.4982292	84.1

RARA	3.5185188	-1.0535065	6.7
ZNF467	3.48424656	-2.053673	2.68
SPSB1	3.45615159	-1.2511615	5.46
MYL5	3.41612982	-1.0174761	5.55
CST7	3.39323855	-1.4129125	13
FGF9	3.38621943	-1.105297	1.77
LINC00963	3.37306238	-2.2362805	0.91
SYNGR1	3.31669378	-1.2942762	8.4
TNFRSF14	3.31286766	-1.1371355	7.22
DYNC1I1	3.2810702	-1.8063154	0.83
TAS1R3	3.25601983	-1.10221	0.14
TSC22D4	3.16525394	-1.0344277	40.73
RASSF2	3.15711394	-1.197257	11.07
PRF1	3.14733955	-1.7377274	4.98
ACOT11	3.11538185	-1.5317111	0.96
OAF	3.07388011	-1.1333528	9.87
RTN4R	3.05487448	-1.4686044	5.54
ARSG	3.04450665	-1.1878685	1.98
ADARB1	3.02044685	-1.2761288	12.57
ARHGEF4	3.0091869	-4.0541592	0.16

Supplemental table 5 The top genes for candidate E3 ligases found to have reduced expression in the EBF4 KO cells

Gene Symbol	vs EBF4 KO (FC)
RNF212	-1.990078
MID1	-1.8073896
ZNF521	-1.7029595
RNF125	-1.6116808
RNF157	-1.5364857
CHFR	-1.4684585
MEX3A	-1.3148871
TRAF5	-1.2581956
SMURF2	-1.2387773
ZNRF3	-1.1836951
CNOT4	-1.1552732
RNF44	-1.1422184
TRAF3	-1.0950743
PCGF3	-1.0817099
TRIM32	-1.0620113

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