

# BCAT1 is a NOTCH1 target and sustains the oncogenic function of NOTCH1

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## MATERIALS AND METHODS

**Cell lines and primary leukemia samples.** Human embryonic kidney (HEK) 293T cells were maintained in DMEM containing 10% fetal bovine serum (FBS) and 0.05 mg/ml penicillin/streptomycin. All T-ALL cell lines were maintained in RPMI-1640 media supplemented with 10% FBS and 0.05 mg/ml penicillin/streptomycin. We tested cell lines regularly for mycoplasma contamination. Primary T-ALL cells were expanded in vivo via i.v. injection in 6-8 weeks old female NOD SCID IL2R $\gamma$ null (NSG) immunodeficient mice. T-ALL cells from spleens of xenografted mice were cultured in vitro in MEM-alpha media supplemented with 10% human serum and cytokines for the duration of functional assays (48-72h). Patient derived xenograft (PDX) and cell line authentication was determined by analyzing several loci of short tandem repeats (STRs) using a commercial kit (PowerPlex 16 HS System, Madison, WI, USA). NOTCH1-induced T-ALL murine models were previously generated by transduction of bone marrow progenitors with activated forms of NOTCH1 oncogene (*NOTCH1 L1601P  $\Delta$ PEST* or  *$\Delta$ E-NOTCH1*)<sup>1</sup>. Spleens of diseased mice were used as a source of murine T-ALL cells for further studies. Thymuses from 6 weeks old normal C57/BL6 mice were obtained. Procedures involving animals and their care conformed with institutional guidelines and were authorized by local (OPBA) and national (Italian Ministry of Health) animal ethical committees.

**Mouse transplantation experiments.** Bcat1 knockout (-/-; KO) mice on a C57BL/6J background were generated using the CRISPR/Cas9 technology by Cyagen, (CA, USA). NOTCH1-induced T-ALL tumors were generated in mice as previously described<sup>2</sup>. Briefly, bone marrow (BM) cells were collected from 6- to 12-week-old WT and *Bcat1* KO C57BL/6 mice and BM progenitors (Lin<sup>-</sup>) were purified by negative selection using magnetic sorting (Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were cultured overnight in the presence of the following cytokines (all from Peprotech, London, U.K.): mIL-3 (10 ng/mL), mIL-6 (10 ng/mL), mFLT3L (50 ng/mL), mIL7 (100 ng/mL) and mSCF (50 ng/mL). The cells were then washed, resuspended in retroviral supernatant ( $\Delta$ E-NOTCH1), placed in the same cytokine cocktail containing polybrene (4  $\mu$ g/mL), and centrifuged at 1,290g for 90 minutes. A second round of spinoculation was performed the following day. After flow cytometric analysis of transduced progenitors, approximately  $50 \times 10^4$  Lin<sup>-</sup>/Sca1<sup>+</sup>/GFP<sup>+</sup> cells of each genotype were injected i.v. into lethally irradiated (9 Gy) recipients (6–8-week-old C57BL/6 female mice). Mice were bled after 2–3 weeks to monitor engraftment and evaluate the presence of circulating immature T cell progenitors by flow cytometry. Tumour bearing mice were euthanized and primary tumour cells were extracted from their spleens. For in vitro studies,  $\Delta$ E-NOTCH1 tumors were cultured in RPMI-1640 supplemented with 20% FBS, mIL-7 (10 ng/mL), mIL-2 (5ng/mL) and  $\beta$ -mercaptoethanol. Procedures involving animals and their care conformed with institutional guidelines and were authorized by local (OPBA) and national (Italian Ministry of Health) animal ethical committees.

**Flow cytometry and analysis of T-cell distribution.** Peripheral Blood (PB) and spleens were harvested from WT and KO mice. Red blood cell (RBC) lysis was performed using a hypotonic solution containing ammonium chloride for all samples. Briefly, the cells were blocked for 10 minutes with CD16/CD32 (mouse BD FC Block, BD Pharmingen, Oxford, U.K.) diluted 1:100 in PBS at 4°C and subsequently stained for 30 minutes with a combination of following panel of antibodies: Cd3e-BV421/BV510 (Biolegend, London., U.K.), Cd8-BV605/PE, Cd4-FITC/APC (all from BD Pharmingen). The fixable viability stain dye (FVS780; BD) was used to analyze only viable cells. Cells were analyzed on a BD LSR II flow cytometer and acquired data was analyzed with FlowJo (Tree Star Inc., Ashland, OR).

**Quantitative real-time PCR.** Total RNA from human and mouse samples were extracted using Trizol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). RNA from thymic samples were from a previous study<sup>3</sup>. These samples were obtained as surgical tissue discards from pediatric patients, ranging in age from 2 days to 5 years, undergoing cardiac surgery at the University Hospital of Padova, after informed consent. cDNA was generated with the Super Script First Strand Synthesis System for RT-PCR (Invitrogen) and analyzed by quantitative real-time PCR using SYBR Green PCR Master Mix (Applied Biosystems, Paisley, UK) and the HT 7900 Real-Time PCR System (Applied Biosystems). All primers were KiCqStart™ Primers from Sigma-Aldrich. Primer sequences are available upon request. Every sample was analyzed in triplicate and relative expression levels were normalized to RPL19 or  $\beta$ 2-microglobulin expression using the  $\Delta\Delta$ CT method.

**Total histone extraction.** Total histones were obtained using the EpiQuik total histone extraction kit (Epigentek, Farmingdale, NY, USA), according to the manufacturer's recommendations. Histone extracts were normalized for protein concentration using the Bradford method (Pierce).

**Immunohistochemistry.** All primary T-ALL (and T-cell lymphoblastic lymphoma; T-LBL) cases were retrieved from the archives of the Pathology Unit of Padua University Hospital (Padua-Italy). Patient derived xenograft (PDX) samples were previously generated<sup>4,5</sup>. Immunohistochemical (IHC) analysis was performed on 4  $\mu$ m-thick formalin-fixed paraffin-embedded (FFPE) tissue sections with the Bond Polymer Refine Detection kit in an automated immunostainer (BOND-MAX system; Leica Biosystems–Newcastle upon Tyne, UK), as previously described<sup>6</sup>. IHC analyses were run using the following primary antibodies: anti-BCAT1 (clone 51/ECA39, BD Pharmingen), anti-HES1 (clone D6P2U, Cell Signaling Technology), anti-BCAT2 (clone D8K3O, Cell Signaling Technology). Immunostains were performed on whole tissue sections. Appropriate positive and negative controls were also included. Thymic tissue (N=3) was run in parallel to assess BCAT1, BCAT2 and HES1 expression in normal T cell precursors. The following two-tiered scoring system was used to assess the expression of these markers: (i) low expressor: no staining (0) or weak positivity (+1) in <10% of tumor cells; (ii) high expressor: moderate to strong positivity (2+ to 3+) in  $\geq$ 10% of tumor cells. The scoring system was based on cytoplasmic (BCAT1/BCAT2) or nuclear expression (HES1) of each marker and intensity scores were defined by comparison with positive controls (i.e. *NOTCH1* mutated PDX samples with high BCAT1 and HES1 expression documented in western blot). Specifically, strong (score 3+) positivity was attributed to cases with protein expression comparable to that of the positive controls, moderate (score 2+) positivity to cases with protein expression slightly fainter than controls, and weak (score 1+) positivity to cases with barely detectable protein expression.

**Immunoprecipitation of acetylated proteins.** Immunoprecipitation of acetylated proteins was performed using the Signal-seeker Acetyl-Lysine detection kit (Cytoskeleton, Inc., Denver, CO, USA) according to the manufacturer's recommendations. Briefly, cells were lysed in diluted BlastR lysis buffer containing class I and II HDAC inhibitor (Trichostatin A; TSA) and class III HDAC inhibitor (Nicotinamide). Approximately 2 mg of protein was pre-cleared with Protein G–agarose (Santa Cruz Biotechnology) at 4°C for 30 min before being incubated with Acetyl-lysine Affinity beads or Acetyl-lysine IP control beads overnight at 4°C. Beads were washed three times with BlastR-2 wash buffer and bound proteins eluted with bead elution buffer. Immune complexes were analyzed by SDS-PAGE and immunoblot.

**Neutral comet assay.** T-ALL cells were treated with vehicle (DMSO) or etoposide (1  $\mu$ M) for 0 to 6h. The neutral comet assay was performed using the CometAssay Silver Kit (R&D Systems; #4251-050-K). Briefly, cells were

mixed with CometAssay LMAgarose (R&D Systems; 1:10[v/v]). Once the agarose had solidified, the cells were lysed with lysis solution (R&D Systems) overnight. The following day, we placed the slides briefly in neutral electrophoresis buffer (R&D Systems) before being placing them in an electrophoresis chamber containing neutral electrophoresis buffer. The slides were subjected to electrophoresis at 1V/cm for 1h at 4°C. We washed the slides in 70% ethanol. After drying the slides at room temperature, we stained the comets with SYBR-gold (ThermoFisher Scientific, #S11494). We viewed the comets by epifluorescence microscopy. For comet analysis we used the Open Comet software<sup>7</sup> to quantify the percentage of DNA in the tail in at least 50 comets per condition.

**Analysis of publicly available datasets.** Expression data for BCAA metabolic genes in primary T-ALL patients and thymic subpopulations were obtained from GSE46170<sup>8</sup>. Microarray data were also obtained from GSE12948<sup>9</sup>, GSE14959<sup>10</sup>, E-MTAB-9279<sup>1</sup>, CGAS00000000002<sup>11</sup>, GSE33469<sup>12</sup>, GSE33470<sup>13</sup>. RNA sequencing (RNA-seq) data of 264 pediatric T-ALL patients from St. Jude<sup>14</sup> was used. Gene expression data of B-cell chronic lymphocytic leukemia (B-CLL) patients analyzed with HGU133+2.0 Affymetrix GeneChip arrays (N = 107) was obtained from Gene Expression Omnibus (GSE22762)<sup>15</sup>. BCAT1 expression levels were extracted and used to generate Kaplan–Meier survival plots. The mean BCAT1 expression level was used as cut-off to define high and low BCAT1 expression.

**RNA-sequencing and gene-set enrichment analysis.** Total RNA from the spleens of  $\Delta E$ -NOTCH1 leukemia-bearing WT and *Bcat1* KO C57BL/6 mice was extracted using the RNAeasy Mini Kit (Qiagen, Hilden Germany), according to the manufacturer’s instructions. Library preparation and paired-end RNA sequencing using Illumina NextSeq 500, as well as downstream data analysis, were performed by Active Motif (Waterloo, Belgium). Sequenced reads were mapped to the genome using the STAR aligner with default settings and uniquely mapped reads were counted. Normalized counts per million and differential gene expression were determined with DESeq2. Hierarchical clustering of Z score and log fold-change expression values used in heatmaps was carried out using GenePattern software<sup>16</sup>. RNAseq data was also analyzed using iDEP<sup>17</sup>. Gene set enrichment analysis (GSEA) analysis was performed using gene sets from the Molecular Signature Database at the Broad Institute (<https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>) as previously described<sup>18</sup> using GenePattern software. Primary data has been deposited in GEO (GSE267966) and will be released September 1 2024.

**Steady state metabolite profiling.** Spleens (N=3) and thymuses (N=2) from  $\Delta E$ -NOTCH1 leukemia bearing mice and thymic tissue from 6 weeks old normal C57/BL6 mice (N=3-5) were obtained. Flash-frozen tissue (spleen or thymus) was subsequently analyzed by Capillary Electrophoresis Time-of-Flight Mass Spectrometry (CE-TOFMS;  $\Omega$ -scan analysis, HMT, Tokyo, Japan). For in vivo experiments, we analyzed flash-frozen tissue (spleen) from  $\Delta E$ -NOTCH1 leukemia bearing mice with WT (N=3) and *Bcat1* KO genotype (N=3) by CE-TOFMS (C-scope analysis, HMT, Tokyo, Japan). Further,  $\Delta E$ -NOTCH1 leukemia bearing mice (N=3 each) were injected intraperitoneally (i.p.) with three doses of ERG245 (30mg/kg), which is a potent BCAT1 inhibitor, at 8 h intervals or vehicle (PBS). Identification of known chemical entities was based on comparison to metabolomics library entries of purified standards and was performed by HMT. Heatmap representation of metabolites identified by CE-TOFMS in NOTCH1-induced ( $\Delta E$ -NOTCH1) leukemia cells treated with vehicle (PBS) or ERG245,  $\Delta E$ -NOTCH1 tumors versus normal thymic tissue or  $\Delta E$ -NOTCH1 tumors WT versus KO for *Bcat1* was performed using MetaboAnalyst<sup>19</sup>.

**Stable-isotope tracing experiments.** For the stable isotope-tracing experiments using primary cells, *ΔE-NOTCH1* leukemia bearing mice were injected i.p. with three doses of ERG245 (30mg/kg) at 8 h intervals or vehicle (PBS). Ten minutes before sacrifice mice were injected i.v. with <sup>13</sup>C<sub>6</sub> Leu (Cambridge Isotope laboratories, Tewksbury, MA, USA) and spleens were flash-frozen. In another set of experiments, *ΔE-NOTCH1* leukemia bearing mice (WT and *Bcat1* KO) were injected i.v. with <sup>13</sup>C<sub>6</sub> Leu (Cambridge Isotope laboratories) and spleens flash-frozen. Flash-frozen splenic tissue was subsequently analyzed by F-scope CE-TOFMS (HMT, Tokyo, Japan).

**Analysis of ChIP-seq databases.** The ChIP-seq data presented in this study were obtained from NCBI GEO under the following accession IDs: GSM959056 (HPB-ALL-NOTCH1)<sup>20</sup>, GSM2521494 (MOLT4-PolIII)<sup>21</sup>, GSM4271227 (MOLT4-H3K4me3)<sup>22</sup> and GSM3693104 (ATACseq MOLT4)<sup>23</sup>. Fastq files from these public ChIPseq and ATACseq datasets were downloaded and mapped to human reference genome (GRCH38/hg38) using Bowtie2 (version 2.5.0)<sup>24</sup> with default parameters. MACS2 (version 2.2.7.1)<sup>25</sup> was used to call peaks. Next, Bedgraphs generated by MACS2 were converted to BigWig files with UCSC – wigtobigwig tool (version 357) and displayed using pyGenomeTracks (version 3.7)<sup>26</sup>.

**Cell viability assays and flow cytometry.** We analyzed cell viability/proliferation in T-ALL cell lines via the bioluminescent method Vialight plus (Lonza, Basel, Switzerland) or by counting live cells after trypan blue staining. For assays evaluating the effects of drugs on T-ALL cell lines, viability was evaluated after 72h. In detail, human T-ALL cells ( $3 \times 10^5$ ) or mouse T-ALL cells ( $0.5 \times 10^5$ ) were seeded in 24-well flat-bottom plates and treated with increasing doses of the various compounds: Etoposide (50-500 nM), Cytarabine/Ara-C (25-100 nM), Doxorubicin hydrochloride (25-100 nM) all from Selleck (Selleck Chemicals LLC, Houston, TX), ERG245 (200-1000 μM; Ergon Pharmaceuticals, Washington DC, NW, USA). Dibenzazepine (DBZ; Syncom, Groningen, the Netherlands) was used in selected experiments. Di-β-hydroxybutyrate (3-HB; sodium salt) and sodium butyrate (NaB) were from Sigma-Aldrich. We analyzed apoptosis after 48-72h by flow cytometry (FACS) after staining with Annexin V-FITC (Roche) or Annexin V-PE (BD Biosciences, Milan, Italy) and SYTOX Red dead cell stain (Invitrogen). Apoptosis was defined as the sum of the percentage of Annexin V<sup>+</sup> and Annexin V<sup>+</sup>/ SYTOX Red<sup>+</sup> cells. Analysis of proliferation combined with cell cycle profile was performed using the Click-iT™ EdU Flow Cytometry Assay Kit (Life Technologies) according to the manufacturer's instructions. The samples were collected on a FACSCalibur (BD Biosciences) using Cell Quest software (BD Biosciences), and analysed with FlowJo (Tree Star Inc., Ashland, OR).

**Plasmids, lentiviral constructs and viral production.** For BCAT1 silencing experiments, HEK293T were transfected with pGipz non-silencing shRNA control, shBCAT1#1 (V3LH5-337223), shBCAT1#2 (V2LH5-64329) and appropriate packaging plasmids using JetPEI transfection reagent (Polyplus, Illkirch, France). Inactivation of human BCAT1 in T-ALL cells using the CRISPR-Cas9 technology was achieved using the guide sequence for BCAT1 (TATTAGGTCTTTAGCCTG; sgBCAT1)<sup>27</sup> which was cloned into LentiCRISPRV2 puro vector (Addgene #98290). BCAT1 over-expression was done using pLenti-BCAT1-Myc-DDK-P2A-Puro (RC219229L3; Origene, Rockville, MD, USA). Plasmids carrying the catalytic inactive mutant of BCAT1 (K222A) was synthesized and cloned in pLenti-Myc-DDK-P2A-Puro vector by Genewiz (ALENTA Life Sciences, Chelmsford, MA, USA). For viral production, viral supernatant from transfected cells was collected 48h after transfection, filtered and used to infect target cells. All infections of T-ALL cells were performed by spinoculation. After infection, T-ALL cells were selected for 3-7 days in puromycin before functional assays.

**Luciferase reporter experiments.** To perform reporter assays, BCAT1 promoter (-1407 to +195 relative to the TSS) was cloned into the pGL4.23[luc2/minP] vector (E841A, Promega, Madison, WI, USA) using Bgl II and Hind III restriction sites by Genewiz (ALENTA Life Sciences, Chelmsford, MA, USA). Constructs having the RBP-J binding site (TGGGAA) mutated or deleted were also generated. To measure the capacity of ICN1 transcription factor to induce BCAT1 expression, HEK293T cells were co-transfected with pGL4.23[luc2/minP] vector containing the above mentioned BCAT1 promoter (pGL4-BCAT1 promoter) and increasing amounts of pcDNA3-ICN1 (kind gift of A. Weng; Terry Fox Laboratory, BC Cancer Agency, Vancouver, Canada) and Renilla luciferase vector (Promega). In some experiments using HEK293T cells, pGL4-BCAT1 promoter construct with the RBP-J binding site (TGGGAA) mutated was used. To determine the effects of NOTCH1-inhibition on reporter activity, pcDNA3-ICN1 transfected cells were treated for 48h with increasing amounts of CB103 (Selleck). To evaluate the contribution of MYC to reporter activity, pcDNA3-ICN1 transfected cells were either transfected with MYC targeting hairpins (pLKO-shMYC#1 (TRCN0000039640), pLKO-shMYC#2 (TRCN0000174055)) or increasing amounts of the BRD4 inhibitor, JQ1 (Selleck). Experiments were repeated at least twice and performed at least in quadruplicate. Luciferase activity was measured 72h post-transfection by Dual-Glo Luciferase Reporter assay kit (Promega) according to the manufacturer's guidelines. For reporter assays in T-ALL cells, we resuspended  $1.5 \times 10^6$  HPB-ALL cells in 20  $\mu$ L of SF Nucleofector Solution (Lonza) with the addition of 700 ng of pGL4.23 vectors (BCAT1 promoter or control constructs) and 300 ng of pGL4.74 [hRluc/TK] Renilla luciferase reporter plasmid (Promega). Cells were electroporated (Amaxa Nucleofector; Lonza) using program CM130 and resuspended in 1 ml of RPMI / 20%FCS and incubated at 37°C/ 5% CO<sub>2</sub> for 48 hrs. In some experiments HPB-ALL cells were co-transfected with pcDNA3-ICN1 or treated with CB103. Experiments were repeated three times and performed at least in quadruplicate. Luciferase activity was measured 48-72h post-transfection by Dual-Luciferase Reporter assay kit (Promega). Relative luciferase activity was calculated as Firefly luciferase activity normalized against Renilla luciferase activity.

**Mouse studies.** NOTCH1-induced T-ALL tumors (generated using both *HD-APEST* and  $\Delta E$  alleles<sup>1</sup>) were secondarily transplanted intravenous (i.v.) into sub-lethally irradiated recipients (C57BL/6 females of 6-8 weeks). Tumor bearing mice were euthanized and primary tumor cells extracted from the spleens of leukemic mice. T-ALL PDX samples<sup>4</sup> were expanded in vivo via i.v. injection into female 6-8 weeks old NOD Rag1 null IL2R $\gamma$  null immunodeficient mice (NSG mice; Charles River, Wilmington, MA, USA). Tumor bearing mice were euthanized and tumor cells extracted from the spleens of leukemic mice and used in functional assays. For the evaluation of BCAT1 function in vivo, we infected MOLT4 or CCRF-CEM control (shCTRL) or BCAT1 deficient (shBCAT1#1/ shBCAT1#2) leukemic cells with lentiviral particles expressing luciferase (FUW-Cherry-LUC) and injected them i.v. into NSG mice ( $5 \times 10^6$  cells/mouse; N=5 per experimental group). We evaluated disease progression by in vivo bioimaging with the In Vivo Imaging System (IVIS, Xenogen, Grantham, UK). In therapy related experiments, PDX leukemic cells (T-ALL#19 or T-ALL#27) were infected with lentiviral particles expressing luciferase (FUW-Cherry-LUC) and injected i.v. into 6–8-week-old female NSG mice. After tumor engraftment (human CD45+ blasts  $\geq 1\%$  in peripheral blood), we treated homogeneous groups of animals (N=5) with vehicle (DMSO/PBS), Etoposide (10 mg/kg or 15 mg/kg twice a week; intraperitoneal), ERG245 (30 mg/kg three times a week; intraperitoneal) or the combination Etoposide (10 mg/kg or 15 mg/kg twice a week; intraperitoneal) + ERG245 (30 mg/kg three times a week; intraperitoneal) for 10-15 days. We evaluated disease progression and therapy response by in vivo bioimaging with the In Vivo Imaging System IVIS Spectrum (Xenogen), spleen weight and human CD45 analysis by flow cytometry. Xenografted mice were age- and sex-matched and randomly assigned to groups. No blinding methods were used. Procedures involving animals and their care conformed with institutional guidelines and were authorized by local (OPBA) and national animal ethical committees (Italian Ministry of Health; DGSAF 0006112; 177/2020-PR).



**BCAT1 promoter methylation testing by Methylation specific PCR (MSP).** Genomic DNA was extracted from T-ALL cell lines (DNeasy kit, Qiagen) according to the manufacturer's instructions. A total of 500 ng of genomic DNA was bisulfite modified using EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA, USA). Bisulfite modified DNA (20ng) was amplified with BCAT1 promoter specific primers (listed in *Online Supplementary Table S4*). A bisulphite-conversion specific actin beta (ACTB) PCR was performed (primers listed in *Online Supplementary Table S4*) to determine total amount of analyzed DNA. PCR conditions used are available upon request. PCR products were loaded on 2% agarose, stained with SYBR safe DNA gel stain (Thermo Fisher Scientific), and visualized under UV illumination.

**Table S1: Primer sequences for amplification of BCAT1 promoter following ChIP**

<b>TARGET LOCI</b>	<b>DIRECTION</b>	<b>SEQUENCE (5' to 3')</b>
BCAT1 <i>P1</i>	FORWARD	CTCTGGGAAAGAGATCGGCA
BCAT1 <i>P1</i>	REVERSE	CTGCATGCTGAGAGGACCAC
BCAT1 <i>P2</i>	FORWARD	AATCTTCGGGCTGGGAGAGA
BCAT1 <i>P2</i>	REVERSE	GCAGATCCCAAGGGTCGTAG
HES1 <i>P1</i>	FORWARD	AAGTTTCACACGAGCCGTTT
HES1 <i>P1</i>	REVERSE	GCTGTTATCAGCACCAGCTC
BCAT1 <i>NL</i>	FORWARD	GTATCGCTCTGCTGTGAGGG
BCAT1 <i>NL</i>	REVERSE	GTCAACACCGTGACCCGTTA

Table S2\_Differentially expressed genes between Bcat1KO and Bcat1WT NOTCH1-dependent leukemias

Ensemble ID	Symbol	DETMOR KO- log2FoldChange	DETMOR WT_padj	DETMOR WT#0_1	DETMOR WT#0_2	DETMOR WT#0_3	DETMOR WT#6_1	DETMOR WT#6_2	DETMOR WT#6_3	DETMOR KOH#_1	DETMOR KOH#_2	DETMOR KOH#_3	DETMOR KOH#_3	DETMOR KOH#_3	
ENSMUSG00000045010	Gm4779	10.64676018	6.46E-21	2.80947046	2.32265191	2.32279427	2.320152159	2.2	2.2	7.84426137	7.84063232	7.95125726	11.5428683	11.4973322	11.58069
Gm15446		7.21766548	1.55E-14	2.58618665	2	2	2	2	2	5.45829491	5.45829491	5.95140272	5.5837553	5.926222	6.20763
ENSMUSG00000046774	8030474K03Rik	6.937006211	1.12E-10	4.17376203	4.25078255	4.39582017	2.3198067	2.3198067	2.3198067	2.32024072	2.17869317	2.1743575	7.496925	10.905635	10.83097
ENSMUSG000000031125	3830403K18Rik	8.836535449	1.38E-08	2.58660819	2	2	4.51477944	4.31481119	3.80131961	4.14663134	7.6533566	7.3008695	10.645245	10.687365	10.629257
ENSMUSG00000095574	Trbv2-1	6.774001762	1.21E-15	3.32488899	3.32409484	2.58640584	2.3198067	2.320152159	2.32024072	5.99882775	6.0397503	5.9279464	9.1124347	9.0759588	9.231708
ENSMUSG00000064645	Trbv5	6.574131389	6.64E-08	6.46413927	6.57331899	6.73208315	4.29353538	4.452152566	4.45251622	13.2868952	13.400864	13.28948	7.2655037	7.3974477	7.4738522
ENSMUSG00000076873	Trdv5	6.308259504	6.57E-17	3.70385559	3.70294367	3.46218583	3.31555453	3.900368117	3.57933023	6.692374	6.6817489	6.2639549	10.216645	10.338571	10.242446
ENSMUSG000000046391	1700042O1Rik	5.92430055	2.22E-05	2.58660819	2	2	2	2	2	5.12817554	5.2050447	5.1266377	7.4249784	7.3003549	7.1780377
ENSMUSG00000018849	Wwc1	6.038304381	0.012429	2.58660819	2	2	2	2	2	6.7669798	6.7896504	6.9159751	2	2	2
ENSMUSG00000059256	Gzmd	5.92430055	2.22E-05	2.58660819	2	2	2	2	2	3.99906228	2.3209407	2.9985073	4.3912513	3.6980072	3.6980072
ENSMUSG00000076472	Trbv15	5.073432964	1.20E-13	8.53235509	8.3655153	8.6709762	8.75764398	8.879916979	8.7925193	10.9874367	10.980466	11.026306	14.622336	14.622336	14.594324
ENSMUSG00000093954	Gm16867	4.9949891262	1.78E-11	3.32488899	3.00180885	3.17232976	2.3198067	2.582001393	2.3198067	7.56863151	7.2713083	7.4889326	5.2842203	4.9969249	5.1275786
ENSMUSG000001029634	lghn5-5	4.679381387	0.000424	2.32291573	2.32265191	2.32279427	5.41661938	5.60653695	5.34992256	6.35636259	6.3876093	6.5517291	10.012709	9.9287142	9.8372877
ENSMUSG000000969306	H4c17	4.567884131	0.0001886	2	2	2	2	2	2	2.58214912	3.32117796	2.8067906	3.3198201	3.3198201	3.9054796
ENSMUSG00000039058	Ak5	4.56594415	0.0118454	2	2	2	2.58640584	2.3198067	2	2.8037367	5.5344488	5.698767	5.7251591	2.3216648	2.3215434
Gm15471		4.06539629	5.95E-05	2.58660819	2.8099055	2.58640584	3.31555453	3.45377331	3.16523297	7.10731028	7.0826646	7.0845638	3.1691935	3.5826201	3.4582072
ENSMUSG00000022156	Gzme	4.02999245	0.0002177	2.32265191	2.58640584	2.32279427	2.320152159	2.58214912	2.8068195	3.1671805	3.5829719	5.2842203	4.4565564	5.042703	5.042703
ENSMUSG00000036242	Arm4	3.932010222	0.0087328	3.00246783	3.22265191	3.22279427	2.80280525	2	2	2.58214912	6.0432195	5.8353786	5.6976827	2.3216648	2.8063055
ENSMUSG00000078161	Erf3	3.78649808	0.0037746	2.32265191	2.58640584	2.3198067	2.320152159	2	2	3.16923045	3.2309407	2.5839675	4.9057492	4.5206899	4.3203887
ENSMUSG00000034057	Myh3	3.757288127	9.04E-06	3.32488899	2.8099055	2.32279427	2.3198067	2	2	4.16895254	4.0836837	3.805222	5.3563635	5.2822481	5.4576822
ENSMUSG000000031137	Fgf13	3.681374957	7.68E-05	2.80947046	2.58618665	2.58640584	2.58142511	2.582001393	2.32024072	3.45863601	3.9962396	2.5839675	5.7801385	5.4307771	5.5528283
ENSMUSG00000001672	Marvd3	3.650379111	0.0385308	2	2	2	2.3198067	2	2	2.99937492	2.5862874	3.9977603	2.8067906	2	2.3215434
ENSMUSG00000074332	Rbm44	3.519759705	2.29E-05	2	3.17193469	3.46218583	2.80280525	3.694282913	3.31686005	6.30259365	7.2231634	6.9159751	3.9059249	4.520339	4.5219723
Gzmb		3.391219553	5.42E-35	3.32488899	4.9002284	3.91006404	4.79824119	4.577548304	4.38547538	7.75366023	6.7046433	7.5284591	7.1686446	6.939114	7.3379731
ENSMUSG00000020182	Dmc	3.373618911	1.67E-05	3.32488899	3.32409484	3.46218583	2.80280525	3.45377331	3.31686005	6.98747365	6.8025878	6.5970492	3.9059249	4.1671919	4.1684284
ENSMUSG00000040170	F2c	3.228087338	0.0058496	3.32488899	3.4617334	3.58784774	2.99469065	3.31659371	3.31686005	7.03221076	7.1767959	6.5970492	3.4585936	3.2821918	3.2315434
ENSMUSG00000023963	Cyp39a1	3.107736987	2.19E-50	5.00431594	4.70349952	4.70410076	4.5761027	4.452152566	4.24125532	7.63539933	7.7298887	7.8108474	7.3712226	7.3274893	7.2555137
ENSMUSG00000035868	Thrsp	3.094750268	0.0021854	3.32488899	3.00180885	3.22279427	2.99469065	2.582001393	2.32024072	5.99882775	5.8533786	5.9514022	2.9934167	3.4571958	3.688562
ENSMUSG00000006014	Prq4	3.05306881	0.0042104	3.91050864	3.9134969	3.32452507	2.3198067	2.582001393	2.8037367	6.14856176	6.0828102	4.88899	3.1691935	3.4571958	2.990381
ENSMUSG00000076492	Trbv2-1	3.04548074	0.0057546	8.02722352	8.09631967	8.0955453	5.44976368	5.576804784	4.69328731	7.7269362	7.7162491	7.7375809	11.92211	11.130276	11.084879
ENSMUSG00000019124	Srmd	3.020547911	0.001713	3.91050864	2.58618665	3.58784774	2.80280525	2.803546664	2.99577786	6.52263561	6.4215511	5.971999	3.3211338	3.8048451	3.3207737
ENSMUSG00000063458	Lcm1	2.932945811	1.53E-06	3.8108791	3.17193469	3.00216447	2.80280525	3.16498864	3.69459039	4.39130525	5.129405	4.2545969	6.3910631	6.2254914	6.0205618
ENSMUSG00000038233	Gask1a	2.874848371	1.46E-07	2.80947046	2.58618665	3.00216447	2.80280525	3.45377331	2.58214912	4.32092783	4.5194791	4.2456699	6.4135004	5.9511354	5.1682145
ENSMUSG000000030587	2200200D01Rik	2.771071831	2.04E-30	4.09123546	4.17273779	4.00324548	3.79976415	3.579034195	3.45405889	6.26560109	6.1450822	6.48899	6.0431569	6.3542087	6.2269699
ENSMUSG00000020890	Gucy2e	2.763132983	6.70E-19	3.588252	3.5873738	3.0912803	3.99202642	3.800122232	3.69459039	6.68529867	6.5982986	6.4568505	6.5063505	6.371694	6.4575947
ENSMUSG00000004276	Ccdc14	2.754692054	0.0003357	4.00370016	3.4617334	4.00324548	3.16042452	3.16042452	3.31686005	4.08650672	3.9496236	2.5456699	7.8009006	6.4600416	6.7530343
ENSMUSG000000021747	Clap20dc	2.700140424	0.0228972	4.39631085	4.3952449	5.133115	2.58142511	2.582001393	2.58214912	3.45863601	3.8038253	4.4568892	7.1939312	7.0627693	7.3110069
ENSMUSG000000058740	Kcnk1	2.697415794	0.0430233	3.32488899	2.32265191	3.17232976	2.3198067	2.80354666	2	5.49071386	5.2050447	4.7523434	2.9934167	2.3212258	2.5843213
ENSMUSG000000061451	Tmem151a	2.666964075	0.0199102	3.32488899	2.58618665	3.22279427	2.80280525	2.320152159	2.58214912	4.4568378	4.4568378	4.5827438	2.5845236	2.9982436	2.990381
ENSMUSG00000009513	Igfbp3	2.652023111	0.0385283	5.00431594	4.52654927	5.133115	3.4862151	4.809055268	8.47545387	11.0967839	11.028489	11.061088	7.7198071	7.6404116	7.7123577
ENSMUSG000000021587	Pcsk1	2.632305056	0.0001208	2.32291573	2.58618665	3.22279427	2.80280525	2.582001393	3.2024072	3.99906228	3.9026627	4.0851792	3.3211338	3.8048451	3.8058906
ENSMUSG00000018727	Csp4l	2.525442675	4.38E-05	5.28982865	5.61803048	5.98133135	4.51477944	4.08060667	4.1657958	6.16874406	6.2026998	6.1870014	8.2081541	8.0839996	8.3644211
ENSMUSG00000102222	Pcdh3a10	2.449578891	9.12E-07	2.58660819	3.4617334	2.58640584	2.58142511	2.31659371	3.16523297	4.4584062	4.3883165	4.0851792	4.9984727	4.9038448	5.0857649
ENSMUSG000000115970	8430426J06Rik	2.466152126	0.0038826	2	2.32265191	2	2.80280525	2.320152159	2.99577786	3.80646187	3.8166684	2.9985073	3.9985281	3.4571958	3.9054796
ENSMUSG00000076494	Trbv2-3	2.439532288	0.0346512	6.7729351	7.00352509	6.8998926	3.45267087	3.202152159	2.8037367	7.49063046	7.5187257	7.4371104	7.8302398	7.8406179	9.0987519
ENSMUSG00000047501	Cldn4	2.439420158	6.61E-08	7.86282756	7.83010029	7.86223222	5.8996936	5.969894122	5.79950395	9.56290567	9.5284125	9.5275679	6.8204662	6.9591729	6.8810795
ENSMUSG00000073535	Gm5532	2.430863336	5.35E-13	5.7324803	5.52686336	5.585469	5.07800818	5.277416118	5.41859685	7.14853192	7.1075762	6.8919313	5.2513657	8.3184571	8.1972625
ENSMUSG00000041789	2700046A07Rik	2.397176781	6.67E-05	3.32488899	3.17232976	2.80280525	2.582001393	2.32024072	3.45863601	4.5808347	4.6176023	5.0863008	4.4565564	4.8525203	4.9125203
ENSMUSG000000066677	Hn203	2.346690953	2.05E-30	6.55931079	6.19324212	6.15382946	5.57521373	5.418193514	5.41795896	8.42082892	8.4171786	8.3807531	8.133128	8.2397055	7.9530343
ENSMUSG00000022206	Irf3	2.325839848	0.0031753	3.91050864	4.00271242	4.00977189	4.45267087	3.90038817	3.99366215	6.69957415	4.2440258	4.0851792	6.6425919	6.6113385	6.7520209
ENSMUSG00000076525	Igkv1-9	2.306716522	0.008073	4.86223324	4.86109829	4.3258989	6.32973889	6.785835982	6.79920113	6.10734654	6.2629098	6.0415883	9.120226	8.993692	8.9924441
ENSMUSG000000110998	130028E13Rik	2.222183093	0.0127299	3.17266678	2.58618665	4.35640584	2.58142511	2.582001393	4.52229072	5.4523787	4.6533878	6.4143475	2.8067906	3.1679733	2.990381
ENSMUSG00000035299	1700044B02Rik	2.122534186	1.04E-34	5.46391545	6.06948093	6.04845802	5.7974791	5.968945122	5.82052842	7.8996371	7.899				

ENSMUSG0000015854	Cd5f	1.372277129	0.0112117	11.0547707	11.0690248	11.0575647	10.2771082	10.28689573	10.2987709	12.7625851	12.749111	12.771802	10.834736	10.810964	10.810257
ENSMUSG0000005470	Ilgp1	1.356098732	9.06E-30	8.04925097	8.3478669	8.4921176	8.19407762	8.12050891	8.01957931	9.73515736	9.6586386	9.6957321	9.2933211	9.4311336	9.4819021
ENSMUSG00000078190	Ndn3os	1.351121881	0.031713	4.25182216	4.0022628	4.0144603	4.94493511	4.850310455	4.74768681	6.20827055	6.5498556	6.6486517	4.6583541	4.9038448	4.5835988
ENSMUSG00000062380	Tubb3	1.347486232	4.97E-08	6.37634871	6.91035866	6.7456312	7.18932456	7.598611844	7.36678712	8.75364865	8.9959373	8.6336677	8.0967343	7.9390567	8.1928587
ENSMUSG00000025355	Mmp19	1.34601271	0.000798	5.7594523	6.1326921	6.8200878	6.61914967	6.540935812	6.515474	8.12288881	8.5951375	8.1107984	8.608065	6.6831222	6.8310331
ENSMUSG00000035042	Ccl5	1.319536213	0.0004292	5.17430934	5.36081511	5.9102831	6.23785108	6.483348273	6.38246458	7.72669362	7.6245127	7.6117839	6.7536193	6.8548574	6.6275099
ENSMUSG00000015947	Og1	1.313112517	0.0007776	6.19440344	6.64732718	6.6758614	6.95548763	7.024794023	7.16170391	8.68175632	8.591297	8.1719282	7.2178869	7.4228316	7.2605570
ENSMUSG00000030087	Klf15	1.308463239	0.0238154	5.32636716	5.55789694	5.7041056	5.16047251	5.349521258	5.0369515	7.22760162	6.9839667	6.962893	5.5366355	5.2822481	5.1752786
ENSMUSG000000102748	Pcdhgb2	1.298948604	0.0313441	3.70385559	2.8099055	2.45640074	3.5155543	4.080660662	2.99577786	4.39130525	3.9962936	6.9851792	3.9901203	4.0343246	4.0589914
ENSMUSG00000063415	Cyp2b1	1.279849405	0.0041252	5.3248043	5.46271875	5.82028078	6.0774525	5.968945212	6.07905	7.76695684	6.7317802	7.5669316	6.0648508	6.1464304	6.3200999
ENSMUSG00000027954	Ela2	1.267903381	0.0437339	5.7594523	5.83625203	5.73192013	5.0780801	5.944466378	3.99366215	5.55344488	5.7233502	5.8227219	7.1983912	7.0409858	7.117072
ENSMUSG00000071716	Apol7e	1.265367583	4.31E-07	6.95896994	6.73139962	6.60406041	5.71808778	5.968945212	6.2208143	7.91165967	7.8716573	7.4809856	7.3824188	7.5664147	7.6491655
ENSMUSG00000095028	Sirpb1b	1.25418795	0.023827	4.25182216	4.58797599	4.52713629	4.69144319	4.922214091	5.24036336	6.24674292	6.246128	6.3894728	5.3207428	4.5206589	4.9252608
ENSMUSG00000078922	Tgfp1	1.247814368	0.0002397	7.03820412	7.13279528	7.08101331	6.75793781	6.785835982	6.78626481	7.46638339	7.4468304	7.3721245	6.8097212	6.8149603	6.8983338
ENSMUSG00000079018	Lyc6c1	1.246490667	3.57E-05	7.94148901	7.8364421	7.86223222	7.59691102	7.506993378	7.44294829	9.40176898	9.4108268	9.3257065	8.3296162	8.1863578	8.3947032
ENSMUSG00000045502	Hcar2	1.233419489	0.0009736	6.86274267	7.10154208	7.26160061	6.84771084	6.719355474	6.99180835	8.76363266	8.6208151	8.7009453	7.3824188	7.3003549	7.4411063
ENSMUSG00000005413	Hmx1	1.226379242	0.0079159	11.6839566	11.7148479	11.7060601	11.7074791	11.75887927	11.7745512	13.5835951	13.57814	13.57279	11.808854	11.796175	11.831363
ENSMUSG00000078780	Gm5150	1.223223922	0.003342	5.9114938	5.75823544	5.9823432	5.87272847	5.692224792	5.72010376	7.5988682	7.37879	7.5745045	6.0431569	6.0627843	6.2649621
ENSMUSG00000069792	Wfdc17	1.206534765	7.84E-05	7.81219846	7.8364421	7.9866333	8.8032378	8.66361126	8.68511223	9.84581235	9.791188	9.9248033	9.1124347	9.1411681	9.1013773
ENSMUSG00000062661	Ncs1	1.205531226	0.0303741	4.52763697	4.81054442	4.32538969	4.94493511	5.175473404	4.24125532	4.45840862	4.85372	4.804795	6.0862233	6.0841549	6.4575947
ENSMUSG00000079018	Lyc6c1	1.199230674	7.82E-05	3.91050864	4.25078255	4.17329055	3.5787902	4.314811193	4.38547538	5.35642361	4.85372	4.907492	5.0043618	4.5681514	4.3585154
ENSMUSG00000106438	Gm32051	1.19244001	0.0313241	7.49667514	7.6473994	7.51993112	6.97832655	7.181167553	6.96992448	9.18688325	9.1894145	9.1133785	7.2081719	7.3094655	7.2724809
ENSMUSG00000028967	Erf1f1	1.188126433	0.0002816	6.7596366	6.3957609	6.4796499	6.0576959	7.09988163	6.89871662	8.48660354	8.4711786	8.2778222	7.2933373	7.4641701	7.4045896
ENSMUSG00000030088	Alph11h	1.177831261	0.001262	5.58948364	5.64718272	5.4633646	5.5966862	6.746316554	6.45136042	7.12806832	7.1347479	7.0953124	7.498558	7.263363	7.6054453
ENSMUSG00000073555	Gm4951	1.174891147	1.54E-05	6.02699627	5.93411597	5.6623661	6.48168928	6.450935812	6.6500968	7.88751393	7.9435035	7.7181689	6.8567094	7.1764912	7.2835271
ENSMUSG00000040359	Snt1	1.170911007	0.0113104	9.46842793	9.63477427	9.50414722	9.64941038	9.50719937	9.62458126	11.3974951	11.357555	11.325132	9.5980626	9.7902888	9.5924101
ENSMUSG00000027488	Spa1	1.162500152	0.0665175	5.7324805	5.93411597	5.73192013	5.87726108	6.100140373	6.92823673	7.64982615	7.6245127	7.5284591	6.1688812	6.2067717	6.1479311
ENSMUSG00000021453	Gadd45f	1.160299276	1.82E-30	8.53235509	8.61090871	8.70211559	8.3379026	8.417466598	8.10540797	9.57618494	9.5668347	9.5375367	6.9189095	6.9812507	8.082335
ENSMUSG00000047798	Cad300f	1.156680499	0.0014401	7.52052367	7.70398571	7.3124548	7.84752678	7.824145038	7.61376735	9.26554423	9.301149	9.3212122	7.9412189	8.0733527	8.68373
ENSMUSG00000057378	Ryr3	1.154887553	0.0229388	4.95849239	4.32482117	4.86171081	4.7458302	4.577548304	4.63675592	6.10734654	5.8706344	6.1818449	5.0863008	4.5206589	5.1275786
ENSMUSG00000056486	Chn1	1.15453982	0.0008282	3.588252	3.5873738	3.8104603	4.23953538	3.90038219	3.80131951	4.80628319	4.5786077	5.0842768	4.5838651	4.5820339	4.6422398
ENSMUSG00000023913	Slp2p	1.154129381	0.0207404	8.90273562	8.88316802	8.89895554	6.9504523	6.905835781	9.4591968	11.0835603	10.93639	11.087793	9.3385411	9.3995178	9.3107906
ENSMUSG00000066363	Pear3f	1.151086701	0.001003	7.16466507	6.88613607	6.69997156	6.98388262	7.957025524	8.28627212	8.75364865	8.9463808	8.8643174	8.723093	8.7514051	8.6201469
ENSMUSG00000022257	Lmx4	1.144331724	0.0026227	7.51261788	7.51923634	7.47996206	6.89608167	6.64966349	6.89871662	7.53015807	7.653566	7.676515	8.7865975	8.899591	8.7260141
ENSMUSG00000024529	Latp	1.140649138	0.0056506	5.98881011	6.21876127	6.14701072	6.46557494	6.759610343	6.4676579	7.83797521	8.087858	8.002681	6.5837004	6.7108646	6.6705764
ENSMUSG00000072720	Myo18b	1.136985497	0.0001606	4.3258746	5.21267838	5.1737707	4.9282119	4.314811193	4.00010939	6.10734654	5.8533786	6.1469286	5.8567548	5.4562365	5.245194
ENSMUSG00000052957	Gm15558	1.134132543	4.00E-07	6.49656573	6.51125134	6.88629232	6.97832655	7.17125337	6.89871662	8.21793488	8.2909419	8.161916	7.6207615	7.5815208	7.6491655
ENSMUSG00000050493	Fam167b	1.118621793	0.0155165	5.46391545	4.61283643	5.9882332	5.66626426	5.451341511	5.45174579	7.54567078	7.37879	7.0485638	5.9877653	6.1256991	6.0205618
ENSMUSG00000081219	Bambi-pb1	1.115384927	0.0003126	9.35141493	9.35674017	9.3892895	8.44069483	8.49899114	8.63187329	9.68700594	9.7195933	9.6822052	10.404806	10.419611	10.501907
ENSMUSG00000030148	Clec4a2	1.113191806	0.0243288	7.24320577	7.22688701	7.16047621	8.31592423	8.166147644	8.22527572	9.49860236	9.3916902	9.4747881	8.0210711	8.0463867	8.026011
ENSMUSG00000032942	Ucp3	1.110666211	0.0103659	5.52806525	5.3248572	5.30378569	5.38296568	5.1241051	5.0000365	6.84428313	6.8154103	6.5512791	5.2842203	5.6114821	5.5821809
ENSMUSG00000032246	Calml4	1.10835106	0.0027511	4.25182216	3.8099383	4.46297179	3.79976415	3.801002232	3.45405584	5.12815754	4.9489913	5.0417695	4.8584657	4.6974661	4.3907597
ENSMUSG00000052270	Zfp278	1.100479624	0.0166682	5.86257889	5.8601365	6.25205866	6.44927858	6.220393213	6.59207333	7.73348268	7.7960448	7.6835716	6.0862233	6.4232164	6.2949621
ENSMUSG00000051529	Pf2r	1.09595329	0.0058003	3.588252	3.70294367	4.17329055	3.57787902	3.900368117	3.57933023	4.32092783	4.2440258	4.5210949	4.9057492	4.9511354	4.6422398
ENSMUSG00000075410	Prd1	1.094051639	0.0031596	5.04872871	5.0475718	5.27521202	4.7458302	5.404446959	5.48415014	6.69629374	7.0540478	6.6955566	5.2467492	5.520353	5.4542194
ENSMUSG00000065291	Ilc10	1.092710689	0.0150737	5.67697062	5.70377736	5.5585469	6.17768502	6.499285457	6.38426458	7.61384494	7.6993366	7.6835716	6.5223022	6.5044343	6.4411076
ENSMUSG00000087975	Zfp703	1.091847333	0.0398543	3.70385559	3.32409844	3.60216447	3.16402452	3.36168005	3.80646187	4.3562874	3.9977603	4.3912513	4.4565564	4.3907597	4.3907597
ENSMUSG00000080595	Lyc6c1	1.074170266	1.38E-07	8.93858183	8.79148531	8.71853007	8.33822066	8.409055326	8.38396172	9.99875445	9.8989466	9.9870599	9.2631341	9.3731767	9.3132165
ENSMUSG00000026018	Zfp1073	1.073411635	5.99E-06	5.7324805	5.73126334	6.81137207	4.94493511	5.036574414	5.036574414	6.49065826	6.246128	6.4565805	6.1688812	6.4229134	6.3904846
ENSMUSG00000021710	Nln	1.068732402	5.48E-08	9.50674147	9.42985919	9.44931575	9.71221977	9.633347194	9.75811119	10.9161242	10.970914	10.100446	10.178597	10.325117	10.251929
ENSMUSG00000026011	Ctca9	1.066364867	0.0007163	6.29008118	6.55805062	6.6623661	6.7970971	6.886216252	6.6212426	7.12806832	7.0612931	7.0305273	8.0539852	8.1258183	8.1529207
ENSMUSG00000058818	Pirb	1.065180033	0.0186079	9.08171058	9.05887072	8.89302429	8.95234329	9.03008527	9.00841711	10.6417069	10.655064	10.695953	8.9557968	9.0188799	9.0802388
ENSMUSG00000031298	Nkam2	1.0633693													

ENSMUSG00000051379	Firt3	-1.232971136	0.048264	3.70385559	4.00271242	4.58856814	5.07808018	5.03657444	4.57791872	2.99937492	3.3189638	3.805222	2.9993416	4.3894726	3.8059806
ENSMUSG00000024617	Camk2a	-1.247272209	0.014383	8.51663015	8.4298382	8.41790296	6.92044908	6.83689877	6.957599	6.61351047	5.9022768	5.9971999	7.0009511	6.969595	7.0315575
ENSMUSG00000062675	Nrg2	-1.247818156	1.70E-16	7.09224949	7.14306434	7.12314247	7.50529491	7.258118628	7.59904762	6.32074021	6.0397500	6.0632807	6.0648508	6.1052135	9.9581959
ENSMUSG00000045441	Gprn3	-1.256048284	0.0004124	5.25234065	4.6689376	4.81106345	5.32895688	5.079611326	4.85069372	4.58392055	3.9032667	3.9778603	3.9059249	3.9043138	3.4582072
ENSMUSG00000054672	Scart2	-1.272688525	0.0227143	5.61923966	5.86601365	5.88667588	5.75454663	5.779162544	5.6881947	5.85681683	6.0282107	5.9795858	5.969224	5.6114821	7.1261413
ENSMUSG00000027718	H2t1	-1.275021283	0.0223871	4.46346769	4.9002284	5.00378569	5.1198645	5.516345674	5.45174579	3.32117796	3.9962366	3.1682663	3.8064143	4.4565564	4.5353589
ENSMUSG00000057777	Matb21	-1.278167186	0.0037991	4.81158291	4.95734574	5.04811968	4.23953538	4.385115654	4.6335188	2.99937492	3.5816684	3.5829719	3.8064143	3.6990072	4.0859914
ENSMUSG00000028051	Hcn3	-1.28126743	0.0083073	6.58988881	6.30721343	6.40770759	5.41661938	5.48374496	5.24036336	4.32092783	3.9032667	4.5824738	5.2467492	5.166801	5.3558154
ENSMUSG00000027408	Opk1	-1.283883948	0.0041451	8.78952164	8.60720365	8.71853007	10.3633465	10.31192685	10.3903122	8.47449705	8.1499347	8.2544399	8.6816904	8.4883772	8.7429273
ENSMUSG00000020310	Madcam1	-1.292966516	0.0003175	5.39677993	5.91026528	5.9562317	6.36491562	6.38349048	6.23991717	4.45840862	5.1655312	5.2067896	5.0863008	4.1671919	4.6422398
ENSMUSG0000018012	Gm46620	-1.298518976	1.75E-34	9.22406693	9.17851613	9.15680187	8.90233964	8.948272285	8.80219052	7.80612683	7.5725886	7.1804874	7.7198071	7.7380178	7.8989746
ENSMUSG00000078866	Zfp970	-1.309783736	0.0007998	7.26219058	7.59599763	7.45543808	9.01181164	8.642238916	8.71272291	6.64265579	7.8965044	6.9362468	7.1589122	7.0189611	7.301905
ENSMUSG00000006916	Lyz2	-1.313206334	0.0285637	11.5479567	11.5310875	11.5219016	12.9519016	13.14370405	13.1057775	10.1395838	10.077223	10.176932	11.807246	11.867624	11.720031
ENSMUSG00000041670	Rims1	-1.313804088	0.0064784	7.14434313	7.38723323	7.45543808	6.29368298	6.120891544	6.05813879	5.04329531	5.124905	4.7523434	5.9760466	5.927435	6.2649621
ENSMUSG00000089774	Slic5a3	-1.314438799	0.0052008	7.20446983	7.2129712	7.50407612	8.86903562	9.03008529	8.84648499	6.61351047	6.595173	6.1753407	6.275368	7.3974477	7.255137
ENSMUSG00000073008	Gpr174	-1.316685634	0.030136	5.43073817	4.8619829	4.3253969	6.17977121	5.968945122	5.96936166	3.80646187	3.5816684	3.977603	4.5224741	4.5718938	5.2637562
ENSMUSG00000030124	Denn2b	-1.320914846	0.022538	10.0561218	10.1199464	10.1199464	9.38939991	9.78388262	8.15612975	8.50258909	8.4087421	8.446524	7.995936	7.7176509	7.7947702
ENSMUSG00000052864	Limp	-1.322042432	1.99E-05	7.83139318	7.69703261	7.8180301	7.05576959	7.16127192	7.23017273	5.69928551	5.7767759	5.6119669	6.7266531	6.6970601	6.3904846
ENSMUSG00000023046	Sema12	-1.324243544	0.0009344	7.19462112	7.2129712	7.14375451	8.67596859	8.602214522	8.64267941	6.78015477	6.8407184	6.8172968	6.7800906	6.5202	9.981357
ENSMUSG00000078653	Yhd2	-1.325042391	4.70E-08	5.64839374	5.49514752	5.78889632	5.44976368	5.313919113	5.60694538	4.39130525	3.9032667	4.4569882	4.62475	4.5206589	4.3907597
ENSMUSG00000018554	Xnt1	-1.326498048	0.0087525	8.02722352	8.16346327	8.25306879	6.605073	6.450935812	6.74674493	5.99882775	5.7502104	5.6411084	6.7800906	6.7380836	6.4075571
ENSMUSG00000060924	Csm2d1	-1.32672121	0.0056076	4.46346769	4.00271242	4.17329055	4.7458302	4.850310455	5.12179534	3.80646187	3.8082253	4.0851792	3.321138	3.167933	3.1688562
ENSMUSG00000028459	Cd72	-1.333008415	1.10E-08	7.00477759	7.02587124	6.98146948	7.29341274	7.09988167	7.29553879	6.14856716	5.9022768	6.2852586	5.5837553	4.9038448	5.905945
ENSMUSG00000037341	Sncp2	-1.338289669	0.0001139	6.874076	6.9628416	6.94669973	7.3646584	7.172366832	7.49952437	5.45829491	5.1655312	5.4567164	6.5859491	6.4723766	6.1681075
ENSMUSG00000028979	Slic5a7	-1.339886848	0.0102341	4.00370016	4.46239037	4.58759317	3.57787902	3.99332894	4.0010044	3.16923045	3.1671805	2.9985073	3.5840846	3.4571958	3.688562
ENSMUSG00000020027	Srp3k	-1.342038303	0.0046419	5.46391545	5.28864725	5.47853717	4.5761027	4.314811199	4.74768881	3.45863601	3.4883165	3.1682663	3.458936	3.997364	4.1680424
ENSMUSG00000022865	Xcadr	-1.342835717	0.0017599	7.35353879	7.44647592	7.5047822	7.27503768	7.576246893	7.35807114	5.04329531	5.1655312	5.1626377	6.4701992	6.6548359	6.4904142
ENSMUSG00000041649	Klf8	-1.35702799	0.016501	5.36200308	5.83662503	5.6478363	7.05576959	6.772782752	6.79920113	4.8569031	5.3174793	4.8554004	5.0432368	5.1514821	4.9525203
ENSMUSG00000030724	Cd19	-1.360341068	0.0263231	8.58230872	8.41296217	8.513315	9.34795505	9.036574414	9.1567958	4.7317796	3.3189638	3.3201366	4.5224741	4.5820399	4.0859914
ENSMUSG00000024347	Ps2d	-1.364066944	0.0099863	5.39677993	5.64718272	5.04819618	5.41747944	5.036574414	5.1657958	4.7317796	3.3189638	3.3201366	4.5224741	4.5820399	4.0859914
ENSMUSG00000085876	Gm12409	-1.374251358	0.0126768	4.75980943	4.32482117	5.133115	6.34795505	6.47768881	6.20681915	4.74768881	3.3189638	2.9985073	3.3201366	4.2248746	4.2464084
ENSMUSG00000056602	Flyc	-1.385397548	5.13E-22	8.48060956	8.32999992	8.07350242	8.38180623	8.31763913	8.29543144	6.80614917	6.7502127	6.5512917	7.0648115	7.1962531	7.2649121
ENSMUSG00000031362	Xrd	-1.390301101	4.79E-07	5.88724069	5.77077736	5.9362317	6.8996396	5.77310734	5.54685383	4.7382239	4.5628274	4.4569882	4.62475	4.5178938	4.5219723
ENSMUSG00000024402	Lta	-1.39237647	0.0276214	4.25182216	4.25078252	3.9106044	3.34795505	4.899179037	3.8466829	4.32092783	3.562874	2.5839675	3.6952821	3.997364	3.5836798
ENSMUSG00000024910	Ctsv	-1.397046538	0.0287701	5.7324805	5.2511628	5.5886792	7.60428944	7.491141583	7.67820854	5.45829491	5.3878451	5.2607896	5.9582344	5.9306102	5.8808491
ENSMUSG00000033590	Myo5c	-1.397770734	0.0242525	3.70385559	4.0902284	4.00254548	5.27585864	4.899179037	5.34992256	3.99906228	2.9975301	3.805222	3.6952821	3.8048451	3.1688562
ENSMUSG00000058286	Spib	-1.416982019	1.85E-05	5.58948364	5.71326334	6.23291401	6.52897969	6.66387342	6.66430124	4.80628319	5.5459378	5.2067896	5.0863008	4.804326	4.3907597
ENSMUSG00000033805	Sufl1	-1.42192487	0.0053192	5.04872711	5.0475718	5.1737077	6.71322779	6.620815729	6.6373874	4.69938173	5.0400503	4.7523434	4.5838651	4.5206589	4.5807649
ENSMUSG00000088088	Rmp	-1.422219324	0.0186829	5.32636716	5.98066607	5.5585469	4.79824119	4.946446378	3.69459039	3.69957415	3.4562874	3.4575315	3.912513	4.6490942	4.0859914
ENSMUSG00000015420	Rmp2	-1.422219324	0.0186829	5.32636716	5.98066607	5.5585469	4.79824119	4.946446378	3.69459039	3.69957415	3.4562874	3.4575315	3.912513	4.6490942	4.0859914
ENSMUSG00000021047	Nov1a	-1.427812318	0.0250481	3.46257176	3.5873738	4.00324548	4.4507334	3.900368117	4.16335188	3.32117796	3.2029407	2.9985073	3.1691935	3.5826201	2.5842312
ENSMUSG00000071984	Fndc1	-1.443217626	0.0283793	4.25182216	3.9095428	3.17239297	3.99202864	3.90086817	4.16335188	3.32117796	3.2029407	2.9985073	3.1691935	3.4571958	3.688562
ENSMUSG00000046634	Pkd11	-1.44606215	0.021062	6.34453832	6.128736	6.3384577	5.31235716	4.946446378	4.5167958	4.80628319	3.5174793	3.4989999	3.5485936	3.4894726	3.6991077
ENSMUSG00000021803	Cd191	-1.457045945	0.0393785	3.91050864	4.32482117	3.58784774	4.89767527	5.036574414	5.12179534	3.32117796	2.5831664	2.9985073	3.1691935	4.1671919	4.0859914
ENSMUSG00000019852	Argef3	-1.462056199	0.019183	6.30846291	6.11939055	5.52751202	5.87272847	6.201034321	6.31389544	3.80646187	3.4562874	4.5219499	4.9530493	5.4562365	5.3201962
ENSMUSG00000054679	Srf12	-1.462581494	0.0350073	4.00370016	3.81990383	3.23525507	3.99202864	4.240904489	4.85069372	2.99937492	3.520274	3.321138	3.167933	2.8065309	2.8065309
ENSMUSG00000096780	Them181b-ps	-1.468965733	7.19E-10	10.3712387	10.3176208	10.4305779	10.4948979	10.3995444	10.3502204	9.07554443	8.9950448	8.7767721	8.7876797	8.8458263	8.4575947
ENSMUSG00000057337	Cns3	-1.481816547	0.0051232	7.37985225	7.09097207	7.24261601	6.6045073	6.957168369	6.77321143	4.45840862	4.4600812	4.6584684	6.265538	5.939884	6.1275864
ENSMUSG00000076608	Ikgf5	-1.481857805	0.0101061	4.91116549	4.81054442	5.0912803	6.58971479	6.635311692	6.81202249	4.24690442	4.3883165	4.804795	4.8568457	4.5718938	5.1275786
ENSMUSG00000029370	Rasf6	-1.497243503	0.0033329	5.13365172	4.81054442	4.88586814	6.05608822	5.77310734	5.45174579	3.45863601	3.3189638	4.1676023	4.7537657	4.5206589	3.9955659
ENSMUSG00000094094	Ikgf5-45	-1.503242865	0.0003327	6.94728585	6.78484383	7.24261601	8.20382462	8.348759273	8.20111131	6.06491344	5.6101701	5.8048185	6.8567904	6.4560766	6.6275909
ENSMUSG00000074024	4634247E19R1k	-1.506004703	0.0183732	3.91050864	5.2511628	3.57123629	3.57787902	4.45251622	3.32117796	3.32117796	3.1671805	2.9985073	5.5840846	6.9900782	3.6836798
ENSMUSG00000042474	Fmr1	-1.510012	0												

ENSMUSG00000073821	8030451A03Rik EN	-2.034526497	0.0015178	4.17376203	4.32482117	4.00324548	5.77149776	5.664223877	5.82502842	2.80681915	3.5816684	3.3201366	4.1689007	3.6980072	3.4582072
ENSMUSG00000013289	H13ra2	-2.058257339	0.0065722	5.64839374	5.39585892	5.00378569	7.75774193	7.568714194	7.58417614	6.69938173	4.2440258	3.4575315	5.4906532	5.2822424	5.6966634
ENSMUSG00000025905	Oprk1	-2.07340283	0.0362333	5.09181499	4.81054442	4.70410076	5.2863532	5.820948884	5.82301648	6.20827055	6.40468	6.1619669	4.8568457	5.3891376	4.4578572
ENSMUSG00000096899	Ighv1-81	-2.07808429	0.0143003	6.8874076	6.49530804	7.11272495	10.3934704	10.34755779	10.3380831	6.84428313	7.0826466	7.0953124	7.7866094	7.7123607	7.7395785
ENSMUSG00000034731	Dgkh	-2.07894396	0.0201005	6.82493613	6.77166524	7.17235151	9.55166062	9.576107386	9.44280291	6.47453922	6.7033341	6.4065447	6.67116	6.9154646	7.1376815
ENSMUSG00000048001	Hes5	-2.08880711	0.0248446	5.91116549	4.95734574	5.04819618	9.20866188	9.306304012	9.32475128	6.71304274	6.3703341	6.5970492	6.2082076	5.9508904	5.8804941
ENSMUSG00000039691	Span10	-2.100528015	0.0003964	5.09181499	5.13248572	4.9410628	6.31182357	6.27695841	6.40133334	2.99937492	3.3189638	3.3201366	4.6993254	4.5206589	4.9075997
ENSMUSG00000087670	9530306M11Rik	-2.114066048	0.0201062	3.32488899	2.58618665	3.17232976	3.79764151	3.579034159	3.57933023	2.58454581	2.3209407	2	2	2	2
ENSMUSG00000076680	Ighv6-6	-2.114257759	0.0200546	5.46391545	5.67575754	5.68200784	9.37959443	9.356287233	9.42830383	6.10734654	5.9022768	5.9279464	6.8690297	6.7106246	6.5861325
ENSMUSG00000067242	Lg1l	-2.200640717	0.0064262	4.52763697	4.3952449	4.32538969	7.14953168	7.295105165	7.20122591	5.08635957	4.5194791	4.6413475	3.9990123	3.9973646	3.9985569
ENSMUSG00000114203	Gm35279	-2.217445991	1.85E-05	4.00370016	4.52654927	4.39582017	4.89767527	4.577548304	4.80100044	3.45863601	3.3189638	3.213312	2.8079096	2.5837918	2.9990381
ENSMUSG00000048905	Bnip5	-2.229472198	0.0404492	7.95321895	8.05340948	7.89907086	9.49493511	8.899179037	4.69328731	3.45863601	3.4562874	3.1682663	5.8814153	5.7781007	5.7261413
ENSMUSG00000040536	Necab1	-2.231610422	0.0404492	7.36236337	7.23233783	7.37925104	11.6241862	11.6426097	11.5374844	8.18859126	8.3349672	8.1468022	8.6533321	8.7176176	8.5905525
ENSMUSG00000058246	Gm10037	-2.270456851	0.0003526	4.52763697	4.0902284	3.81044603	4.23953538	3.694282913	4.31516673	2.80681915	2.5833164	3.4575315	3.216648	2.982436	2
ENSMUSG00000091679	Vmn296	-2.275034215	0.0001118	4.39631085	4.32482117	3.46218583	4.38371152	4.314811193	4.51657958	2.99937492	2	2	2	2	2
ENSMUSG00000015599	Ttbk1	-2.282827469	0.0480931	3.17266678	3.00180885	3.22279427	3.31555453	3.99332894	3.90069389	2.80681915	2.3209407	2.3213312	2.8079096	2	2
ENSMUSG00000096638	Ighv2-9	-2.313943793	0.0120474	4.46346769	3.8099383	4.52654927	4.79739099	7.642327755	7.58417614	3.80646187	3.9962936	4.5827438	4.9988477	5.0413056	4.7532483
ENSMUSG00000103749	Gm135E5	-2.32083505	0.0062016	3.32488899	3.17193469	4.00254548	4.07934474	3.99332894	3.99366215	2.32167809	2.3209407	3.4575315	2.5845236	2	2
ENSMUSG00000034634	Lyd6	-2.323074219	0.0074472	10.1910288	10.1016343	10.0930981	13.29557	13.24981286	13.4378543	10.810127	10.829212	10.810081	8.6295157	8.9406057	8.9406057
ENSMUSG00000108187	4930511E03Rik	-2.326450441	0.0294049	4.64799974	5.64718272	4.81106345	3.31555453	3.202152159	3.45405584	2.58454581	2.3209407	2.5839675	2.8079096	3.4571958	3.3207737
ENSMUSG00000086638	4930405A21Rik	-2.349510243	0.0029462	3.17266678	3.8099383	3.42152076	4.16165755	3.45377331	3.80131951	2.58454581	2	2	2	2	2
ENSMUSG00000077431	Gm22591	-2.349610144	0.0018982	4.00370016	3.8099383	4.32538969	3.31555453	3.316593717	3.3168005	2.80681915	2.3209407	2.5839675	3.216648	2.8058495	3.2155434
ENSMUSG00000031297	Slc7a3	-2.353648776	0.0010718	4.00370016	4.3952449	4.52713629	6.5966682	6.066172633	6.54649305	3.45863601	3.4562874	3.4575315	3.9990123	4.4565564	3.9995659
ENSMUSG00000096461	Ighv14-130	-2.354895026	0.011291	4.70461358	4.70349952	4.17329055	8.0972963	7.939610226	8.7433013	4.69938173	4.5883163	3.4575315	5.1687544	5.0483618	5.4245194
ENSMUSG00000094866	Ccl21a	-2.373678255	7.02E-11	6.97055819	6.74494743	6.15943499	9.868046256	9.16758613	5.32080275	3.7030104	5.520835	5.2047492	4.8549613	4.5358154	4.5358154
ENSMUSG00000049097	Atp1a3	-2.385835463	0.0005949	6.13393312	5.81630348	5.9715695	9.8638612	9.821908906	9.02778156	5.69928551	6.0514409	5.8045815	5.867548	5.8796366	5.7795753
ENSMUSG00000096638	Pcdh10	-2.392215398	0.0024843	3.8108791	3.5873738	4.09071789	3.79764151	4.452152866	2.99577786	2.32167809	2.3209407	2	2	2	2
ENSMUSG00000011297	Gm30034	-2.413884508	0.0260061	2.80947046	2.58618665	3.17232976	5.19968984	5.546496599	5.349922526	2.58454581	3.3189638	3.213312	3.1691935	2.8058495	3.3207737
ENSMUSG00000012350	Ehf	-2.423718249	0.0057917	4.17376203	4.46239027	4.70410076	7.10713385	7.375016529	7.17168638	3.16923045	3.1671805	3.9977603	4.3912513	4.9511354	4.8563221
ENSMUSG00000095889	Ighv1-58	-2.435715517	0.0184221	4.58907321	4.32482117	3.58784774	7.82875198	7.939610226	8.00290208	3.584129	4.3883165	3.9047007	5.906532	5.1261702	5.0277371
ENSMUSG00000076577	Ighv8-30	-2.4403939	0.0002292	8.43532692	8.54660853	8.50412385	11.4674607	11.44077569	11.4504746	8.05405081	8.4748918	8.3677366	8.1124442	8.1206658	8.1206658
ENSMUSG00000094787	Ighv1-54	-2.454515356	0.0162564	4.86223324	5.003164	4.90071189	8.50518723	8.594816539	8.60265489	6.428059	3.9962936	4.804795	6.0862233	5.9739884	5.9905763
ENSMUSG00000043531	Sorcs1	-2.457804767	0.0042414	2.58680819	3.0010885	3.91006404	3.79976415	4.249094449	4.80100044	2.32167809	2.3209407	2.5839675	2.5845236	2.5837918	2.3215434
ENSMUSG00000094941	Ighv1-133	-2.496119535	0.0148366	4.70461358	4.52654927	4.17329055	9.64373022	9.404180173	8.07381756	3.80646187	3.4562874	3.9047007	5.0863008	5.7781007	5.3581514
ENSMUSG00000096670	Ighv2-6	-2.517592733	0.0307395	3.00246783	2.58618665	2.58645084	6.9323889	6.383840938	6.515474	3.45863601	3.5816684	3.5829719	3.1691935	3.4571958	3.9091077
ENSMUSG00000094051	Ighv1-31	-2.525454004	0.0049439	4.46346769	4.25078255	4.00324548	7.82875198	7.904135911	7.69214615	4.80628319	4.8031183	4.6413475	4.6993254	4.3894726	4.2464084
ENSMUSG00000030268	Bcal1	-2.542537577	3.80E-08	6.60479354	8.4589015	8.5892432	5.1737707	5.920570739	5.8381631	5.9022768	5.1266377	7.1983912	7.0518733	7.3818259	7.3818259
ENSMUSG00000095981	Ighv10-1	-2.561629899	0.0048259	4.95849239	5.13248572	4.84819618	8.44810599	8.46287401	8.51911228	4.45840862	4.1660812	4.2456699	5.699224	5.520353	5.9289391
ENSMUSG00000076550	Ighv4-63	-2.562292137	0.010586	4.90123546	4.3952449	4.58856814	8.27951699	8.041087013	8.58408826	5.08635957	5.8533786	5.167202	4.5224741	4.5820339	4.648284
ENSMUSG00000095700	Ighv10-3	-2.565443233	0.0100645	4.75908943	5.46271875	5.5854849	9.30684125	9.3310035	9.36880488	5.28428008	5.0831014	4.804795	6.5685941	6.5044343	6.3380206
ENSMUSG00000094561	Ighv1-22	-2.568267478	0.0115536	4.75908943	4.81045442	5.25179848	6.9278512	6.444419964	5.91318993	5.80619384	6.0397503	6.206268	6.1072388	6.0627431	6.3901958
ENSMUSG00000097276	4930525G20Rik	-2.583426162	0.0481825	3.00246783	2.8099055	3.00214647	3.5787902	3.900368117	3.3168005	2	2	2	2	2	2
ENSMUSG00000096915	Lyz1	-2.586054965	7.62E-07	5.58948364	5.67575754	5.1737707	7.81616229	6.620815729	6.34959091	3.16923045	3.3189638	2.9985073	4.2468879	4.6409042	4.3203887
ENSMUSG00000044453	F1ar1	-2.612772449	0.0093738	3.70385559	4.52654927	3.17232976	3.5778092	3.316593717	3.57933023	2.32167809	2.3209407	2	2	2	2
ENSMUSG00000096672	Ighv1-63	-2.615708106	0.0258084	4.25182216	4.32482117	3.58784774	7.2804926	7.161271392	7.29553879	2.32167809	2.9975301	2.5839675	4.5836651	4.5820339	5.1275786
ENSMUSG00000022899	Slc15a2	-2.650478955	1.05E-09	7.81219846	7.86773959	8.06232222	9.52274907	9.478988158	9.5461773	6.68529867	6.5802429	6.7113724	5.699224	6.0190694	5.9059045
ENSMUSG00000043230	Mfn124b	-2.668332141	0.0335007	3.00246783	2	2	2	2	2	2	2	2	2	2	2
ENSMUSG00000026616	Cr2	-2.69202042	0.0144066	5.55910126	5.42945425	5.1092831	2.99460605	2.995560606	5.28214912	2.99937492	3.4562874	2.5839675	2.3216648	3.1697332	2.9990381
ENSMUSG00000034486	Gbx2	-2.727323324	0.004927	3.70385559	3.17193469	3.81044603	3.99202864	2.995556056	4.80100044	2	2	2	2	2	2
ENSMUSG00000094345	Ighv14-126	-2.733466338	0.001965	5.2138525	5.2511628	5.133115	9.09534772	9.15355204	9.20348884	5.64270583	5.5804312	5.752122	5.5837553	5.4562655	5.6420858
ENSMUSG00000035429	Ptprh	-2.772185218	0.0027958	3.17266678	3.32488899	3.46218583	3.79976415	3.316593717	3.45405584	2.32167809	2.3209407	2	2	2	2
ENSMUSG00000038086	Hspb2	-2.805994106	0.0105016	4.0707267	5.8601264	6.09153444	2.58142511	3.801002232	3.2024072	2.80681915	3.1671805	2.9985073	3.1691935	3.4571958	2.9903081
ENSMUSG00000096498	Ighv2-5	-2.81457046	0.01312	3.70385559	4.0902284	3.32455007	8.00521758	8.001360358	8.27705428	3.32117796	3.8038253	4.2456699	5.3911259	4.9969249	4.9907585
ENSMUSG00000074892	B3gal1	-2.822115619	0.0081977	3.32488899	3.17193469	3.17232976	7.								

Table S3\_Hallmark pathways enriched in Bcat1 WT and KO leukemias

<b>Pathways enriched in Bcat1 WT leukemias</b>	ES	NES	NOM p-val	FDR q-val
HALLMARK_G2M_CHECKPOINT	0.42567	1.71925	0	0.006739
HALLMARK_MITOTIC_SPINDLE	0.41392	1.68235	0	0.006137
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.34885	1.423	0.004451	0.097323
HALLMARK_E2F_TARGETS	0.34738	1.40488	0.0118871	0.087318
HALLMARK_IL2_STAT5_SIGNALING	0.29979	1.21522	0.0751105	0.416291
HALLMARK_UV_RESPONSE_DN	0.31143	1.21287	0.1036496	0.353612
HALLMARK_COAGULATION	0.28995	1.13833	0.1869301	0.530567
HALLMARK_ESTROGEN_RESPONSE_EARLY	0.27199	1.10751	0.2315341	0.573327
HALLMARK_INFLAMMATORY_RESPONSE	0.2671	1.07843	0.2759104	0.6134
HALLMARK_HEDGEHOG_SIGNALING	0.34868	1.06397	0.3468697	0.604395
HALLMARK_TGF_BETA_SIGNALING	0.31186	1.04334	0.3811802	0.620579
HALLMARK_MYOGENESIS	0.25379	1.03423	0.3644444	0.600102
HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.23717	0.97192	0.5369318	0.76375
HALLMARK_SPERMATOGENESIS	0.25016	0.96383	0.5342262	0.735256
HALLMARK_KRAS_SIGNALING_DN	0.23191	0.94643	0.6017442	0.739215
HALLMARK_ESTROGEN_RESPONSE_LATE	0.2323	0.94442	0.6143057	0.69889
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.24033	0.91822	0.6308943	0.73008
HALLMARK_ANGIOGENESIS	0.26646	0.83742	0.744186	0.88554
HALLMARK_WNT_BETA_CATENIN_SIGNALING	0.21143	0.66439	0.9622642	0.992732

<b>Pathways enriched in Bcat1 KO leukemias</b>	ES	NES	NOM p-val	FDR q-val
HALLMARK_OXIDATIVE_PHOSPHORYLATION	-0.55955	-2.41774	0	0
HALLMARK_ADIPOGENESIS	-0.44633	-1.96541	0	0
HALLMARK_PEROXISOME	-0.42111	-1.68117	0.0026667	0.016598
HALLMARK_DNA_REPAIR	-0.39622	-1.6771	0	0.01317
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	-0.48062	-1.62967	0.0025063	0.014993
HALLMARK_XENOBIOTIC_METABOLISM	-0.36976	-1.60909	0	0.014463
HALLMARK_INTERFERON_ALPHA_RESPONSE	-0.40215	-1.58082	0.005102	0.016218
HALLMARK_APOPTOSIS	-0.35749	-1.53048	0	0.023055
HALLMARK_P53_PATHWAY	-0.34366	-1.52925	0.0030211	0.020832
HALLMARK_BILE_ACID_METABOLISM	-0.37796	-1.51214	0.0058651	0.021403
HALLMARK_IL6_JAK_STAT3_SIGNALING	-0.39037	-1.4898	0.0086207	0.023926
HALLMARK_FATTY_ACID_METABOLISM	-0.34599	-1.47731	0.0031847	0.02465
HALLMARK_HYPOXIA	-0.3309	-1.44721	0.006079	0.029479
HALLMARK_INTERFERON_GAMMA_RESPONSE	-0.3166	-1.39444	0.012945	0.045257
HALLMARK_PI3K_AKT_MTOR_SIGNALING	-0.3471	-1.38569	0.0258621	0.046325
HALLMARK_COMPLEMENT	-0.31305	-1.35672	0.0186916	0.056331
HALLMARK_ALLOGRAFT_REJECTION	-0.29664	-1.30317	0.020339	0.081336
HALLMARK_PANCREAS_BETA_CELLS	-0.37285	-1.26095	0.1234867	0.110291
HALLMARK_KRAS_SIGNALING_UP	-0.27289	-1.20407	0.070946	0.166313
HALLMARK_MTORC1_SIGNALING	-0.27663	-1.20235	0.0758621	0.160013
HALLMARK_APICAL_JUNCTION	-0.26799	-1.18999	0.0664452	0.170627
HALLMARK_GLYCOLYSIS	-0.26816	-1.18638	0.0827815	0.167523
HALLMARK_MYC_TARGETS_V1	-0.25788	-1.13489	0.1456954	0.234451
HALLMARK_HEME_METABOLISM	-0.25118	-1.09828	0.2024169	0.292568
HALLMARK_UV_RESPONSE_UP	-0.25492	-1.08273	0.2739274	0.316627
HALLMARK_PROTEIN_SECRETION	-0.26803	-1.05734	0.3164894	0.359791
HALLMARK_NOTCH_SIGNALING	-0.31323	-0.98176	0.4774347	0.565478
HALLMARK_CHOLESTEROL_HOMEOSTASIS	-0.249	-0.94304	0.5605263	0.671222
HALLMARK_ANDROGEN_RESPONSE	-0.22428	-0.88852	0.7147059	0.817348
HALLMARK_APICAL_SURFACE	-0.23808	-0.81848	0.8004751	0.938566
HALLMARK_MYC_TARGETS_V2	-0.16052	-0.5775	0.9976247	0.999054

**Table S4: Primer sequences for amplification of BCAT1 promoter using Methylation Specific PCR (MSP)**

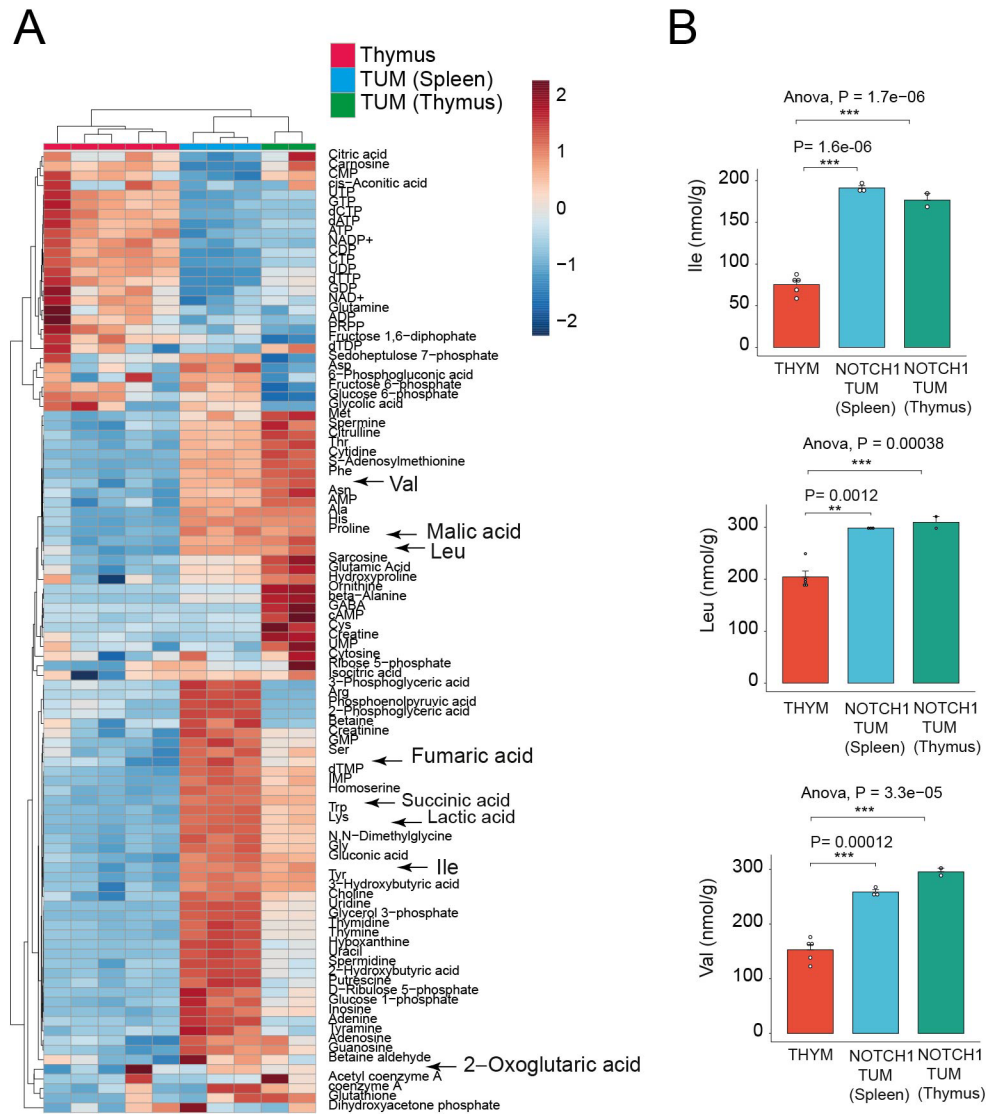
<b>TARGET LOCI</b>	<b>DIRECTION</b>	<b>SEQUENCE (5' to 3')</b>
BCAT1-prom-T1-M	FORWARD	GAGAGATTTTATTATTTGGGGGC
BCAT1-prom-T1-M	REVERSE	CTAACCGTATAAACCGAATCTACGA
BCAT1-prom-T1-U	FORWARD	GAGAGATTTTATTATTTGGGGGTG
BCAT1-prom-T1-U	REVERSE	TAACCATATAAACCAAATCTACAAC
BCAT1-prom-T1-WT*	FORWARD	GTGTCTTCCTGCTGATGCAA
BCAT1-prom-T1-WT	REVERSE	AGATCCCAAGGGTCGTAGC
ACTB	FORWARD	GTGATGGAGGAGGTTTAGTAAGTT
ACTB	REVERSE	AATTACAAAAACCACAACCTAATAAA

\*WT, represents unmodified or wild-type primers. M, methylation-specific primers; and U, unmethylated-specific primers.

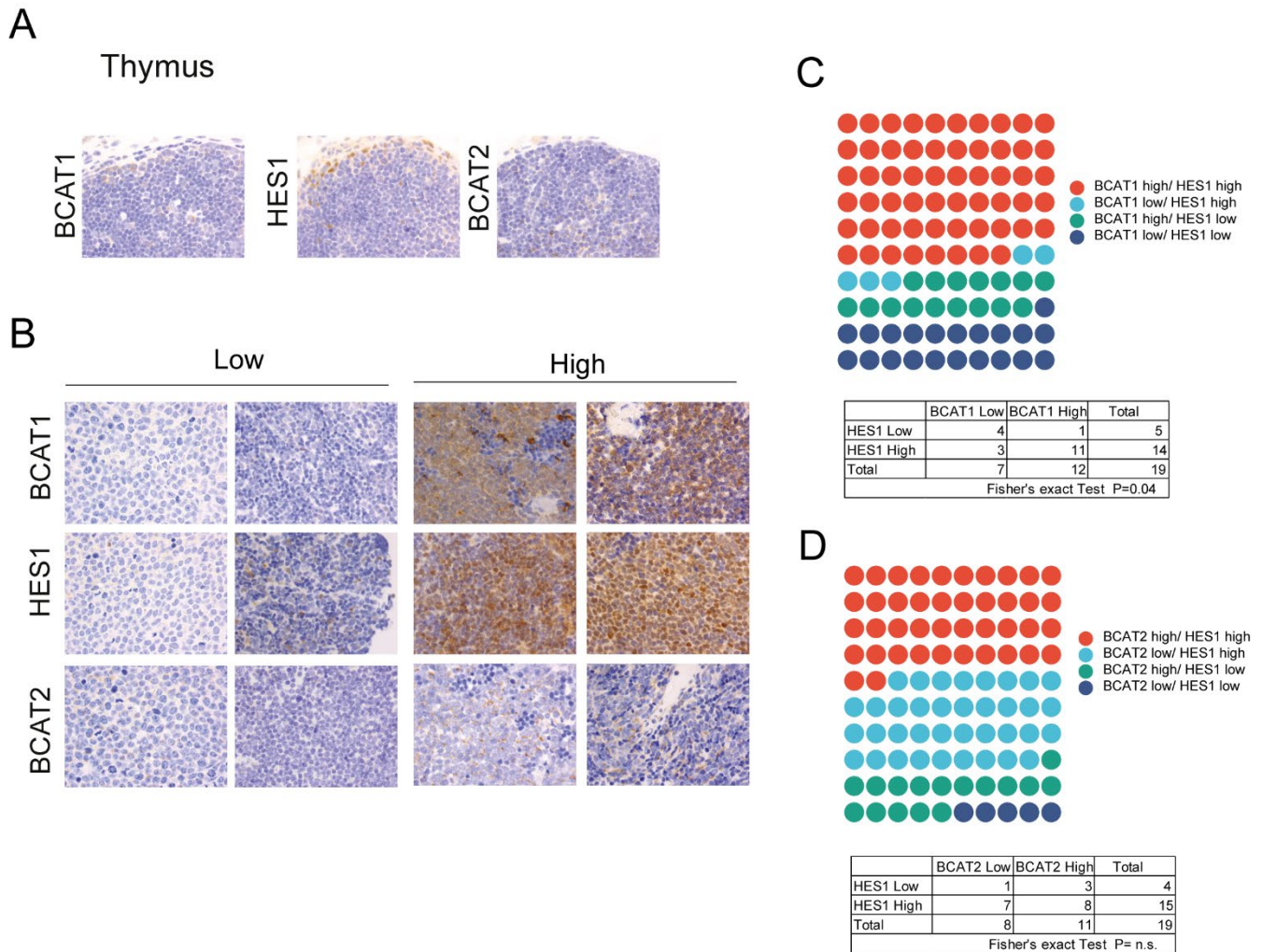




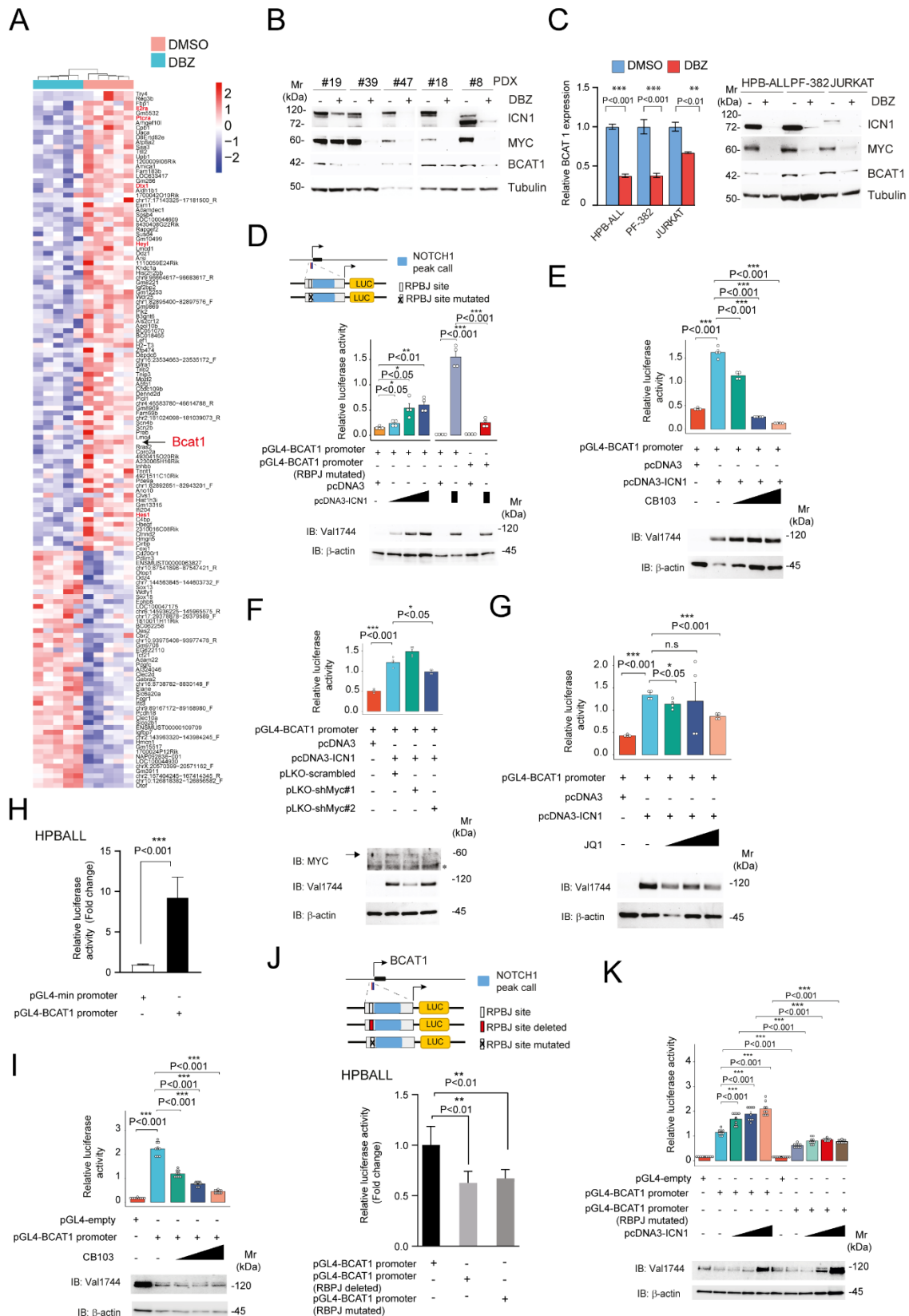
leukemic) DP cells and *ICN1*-induced DP leukemic cells (NIC Tumors). (B) Expression levels (qRT-PCR) of *Bcat2* in thymocytes obtained from 6-8 weeks old C57/Bl6 mice and leukemic cells from six  $\Delta E$ -*NOTCH1* T-ALL tumors (NOTCH1-T). Significance was calculated using an unpaired two-tailed t-test. ns= not significant. (C) Western blot (top) showing protein expression levels of Bcat2.  $\beta$ -actin and tubulin are shown as loading controls. Graphical representation of Bcat2/ $\beta$ -actin ratios (bottom). Bars represent mean values. Significance was calculated using an unpaired two-tailed t-test.  $**P < 0.01$ . (D) Heatmap representation of metabolites identified by capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) of thymic tissue (N=3) obtained from 6 weeks old C57/Bl6 mice and NOTCH1-induced  $\Delta E$ -*NOTCH1* tumor tissue (spleen; N=3). (E) Metabolite Set Enrichment Analysis (MSEA) was used to determine differentially enriched metabolite sets between normal thymic tissue and  $\Delta E$ -*NOTCH1* tumors. The top 25 enriched pathways are shown. (F) Quantification of tissue BCAA (isoleucine, leucine and valine) and BCKA (3-methyl-2-oxovaleric acid, KMV) in thymic tissue (N=3) obtained from 6 weeks old C57/Bl6 mice and NOTCH1-induced  $\Delta E$ -*NOTCH1* tumor tissue (spleen; N=3). Significance was calculated using an unpaired two-tailed t-test.  $*P < 0.05$ ,  $**P < 0.01$ . (G) Expression levels (qRT-PCR) of *Lat1* (*slc7a5*) in thymocytes obtained from 6-8 weeks old C57/Bl6 mice and leukemic cells from six  $\Delta E$ -*NOTCH1* T-ALL tumors (NOTCH1-T). Significance was calculated using an unpaired two-tailed t-test.  $**P < 0.01$ . (H) Heat map showing the expression levels of BCAT1 (two probes), BCAT2 and HES1 (surrogate of activated NOTCH1) between healthy human thymic subpopulations and total thymus and diagnostic pediatric T-ALL samples (N=32). SP8=CD8 single positive, DP= CD3 negative CD4 and CD8 double positive, SP4= CD4 single positive, Thy=total thymus, DP3= CD3 positive CD4 and CD8 double positive, ISP=intermediate single positive, DN= CD4 and CD8 double negative. (I) BCAT1 expression levels in immunophenotypically distinct T-ALL subtypes (pre-cortical, cortical and post-cortical). Significance was calculated using a nonparametric t-test (Mann-Whitney).  $*P < 0.05$ ,  $***P < 0.001$ . (J) BCAT1 expression levels in *NOTCH1/FBXW7* wild-type (wt) and *NOTCH1/FBXW7* mutated (mut) T-ALL in COG ALL TARGET cohort<sup>14</sup>. Significance was calculated using an unpaired two-tailed t-test.  $**P < 0.01$ . (K) BCAT1 expression levels in *NOTCH1/FBXW7* wt (wt) and *NOTCH1/FBXW7* mutated (mut) T-ALL in GSE14959 cohort (N=37)<sup>10</sup>. Two different probes are shown. Significance was calculated using a nonparametric t-test (Mann-Whitney).  $*P < 0.05$ ,  $**P < 0.01$ . (L) BCAT1 expression levels in NOTCH1 wild-type (wt) and NOTCH1 activated (*NOTCH1/FBXW7* mutated) T-ALL (adult and pediatric) (CGAS00000000002)<sup>11</sup>. Significance was calculated using an unpaired two-tailed t-test.  $*P < 0.05$ . (M) Western blot analysis of ICN1, BCAT1, HES1, MYC, BCAT2, PTEN in T-ALL cell lines. Tubulin and  $\beta$ -actin are shown as loading controls. Cell lines with un-mutated NOTCH1 (wt) or activated NOTCH1 signaling (mutated/translocated) are shown. Arrow indicates specific band. (N) Correlation analysis between expression levels of BCAT1 and HES1 in T-ALL cell lines (ALL-SIL, CCRF-HSB2, DND41, HPB-ALL, Jurkat, PF-382, P12-Ichikawa, RPMI8402, MOLT4, CCRF-CEM, MOLT-3, MOLT-16; N=12) from GSE168386<sup>28</sup>. (O) Schematic drawing of BCAT1 gene structure (left) with amplicon coordinates indicated (UCSC Genome browser GRCh38/hg38 version). Methylated cytosines residing in CpG sites are depicted as red lines. (P) Methylation specific PCR (MSP) of BCAT1 promoter T1<sup>29</sup>,<sup>30</sup> was set-up and used to evaluate methylation status of BCAT1 in T-ALL cell lines. Primer sets used for amplification are designated as unmethylated (U) and methylated (M). A bisulphite-conversion specific beta-actin gene (*ACTB*) PCR assay was performed as control assay to evaluate total amount of analyzed DNA. All DNA samples were bisulfite-treated. Relative BCAT1 transcript levels of the analyzed T-ALL cell lines are also shown (lower panel). BCAT1 expression is relative to a human thymocyte sample (#1).



**Figure S2.  $\Delta E$ -NOTCH1 tumors derived from infiltrated spleen and thymus have a similar metabolic profile.** (A) Heatmap representation of metabolites identified by capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) of thymic tissue (N=5) obtained from 6 weeks old C57/Bl6 mice and NOTCH1-induced  $\Delta E$ -NOTCH1 tumor tissue (spleen; N=3 or thymus; N=2). Selected metabolites differentially expressed between normal and leukemic tissue are remarked. (B) Quantification of tissue BCAA (isoleucine, leucine and valine) in thymic tissue (N=5) obtained from 6 weeks old C57/Bl6 mice and NOTCH1-induced  $\Delta E$ -NOTCH1 tumor tissue (spleen; N=3 or thymus; N=2). Significance was calculated using an unpaired two-tailed t-test or ANOVA test (normal versus leukemia). \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

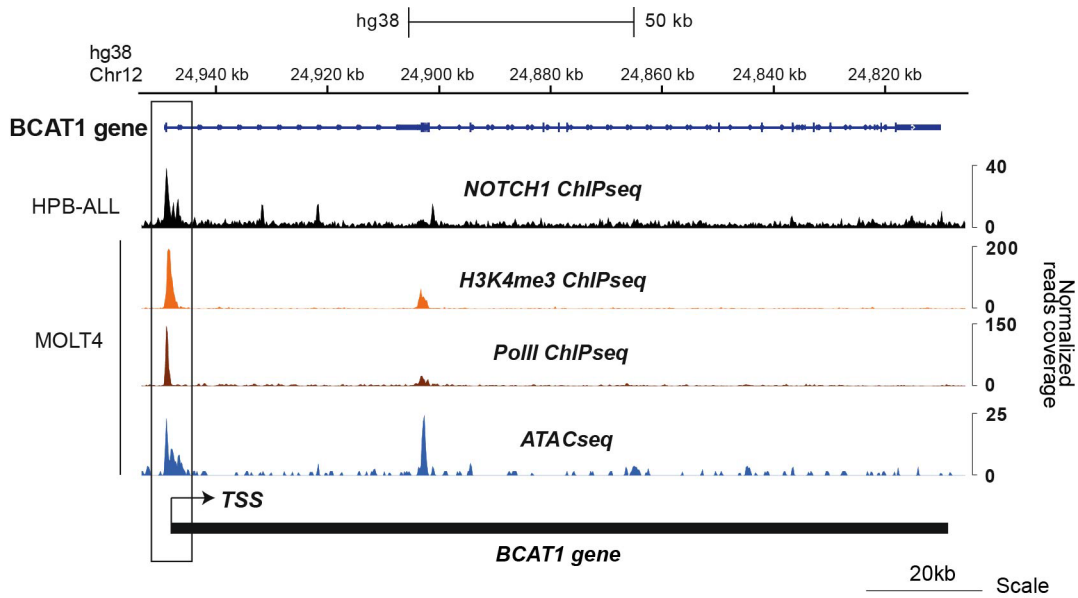


**Figure S3. BCAT1 expression associates with NOTCH1 activation in human T-ALL.** (A) BCAT1 (left), HES1 (middle) and BCAT2 (right) immunohistochemical staining of human thymus (top): original magnification 400 $\times$ . (B) Immunohistochemical staining for BCAT1 (top), HES1 (middle), and BCAT2 (bottom) in representative cases of T-ALL showing low (0, +1) and high (+2, +3) expression levels. Original magnification  $\times$ 400. (c) Circle waffle representation of BCAT1 immunohistochemical staining results obtained in T-ALL/T-LBL/PDX samples analysed (top) and correlation table for Fisher's exact test (bottom). (D) Circle waffle representation of BCAT2 immunohistochemical staining results obtained in T-ALL/T-LBL/PDX samples analysed (top) and correlation table for Fisher's exact test (bottom).

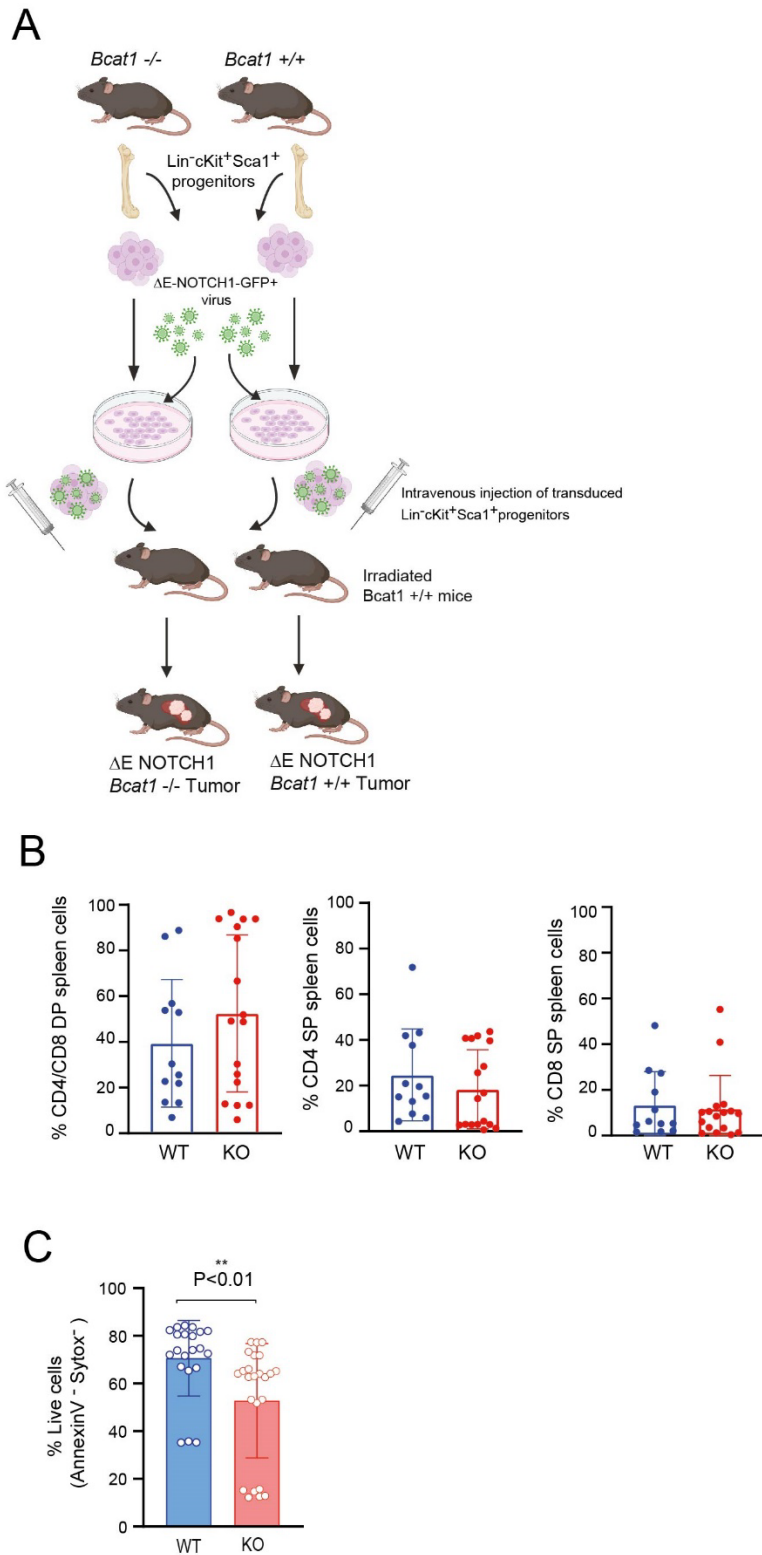


**Figure S4. BCAT1 is modulated upon NOTCH1 inhibition.** (A) Heat map representation of the top down-regulated genes following in vivo DBZ treatment of five  $\Delta E$ -NOTCH1 tumors. BCAT1 and selected known

NOTCH1 target genes are shown<sup>1</sup>. (B) PDX samples were treated in vivo with DBZ (10 µg/kg every 8 hours for a total of 3 injections) or vehicle (DMSO) for 24h before analysis of BCAT1 protein levels. ICN1 and MYC proteins are also shown. Tubulin is shown as loading control. (C) T-ALL cell lines were treated with DBZ (250 nM) or vehicle for 72h before analysis of BCAT1 transcript (left) or BCAT1 protein levels (right). ICN1 and MYC proteins are also shown. Tubulin is shown as loading control. Mean value and SD are shown. For statistical analysis, an unpaired t-test was used. \*\* $P < 0.01$ . \*\*\* $P < 0.001$ . (D) Schematic representation (top) of *BCAT1* promoter construct (BCAT1-Luc) containing the region -1407 to +195 from the transcription start site (TSS) including the proximal RBPJ binding site. The location of the NOTCH1 ChIP peak is also depicted on the reporter construct. HEK 293T cells were co-transfected with pGL4 luciferase reporter construct (BCAT1-Luc) and different amounts of pcDNA3-ICN1 or control plasmid. In some experiments, HEK 293T cells were also co-transfected with BCAT1-Luc having the RBPJ site mutated and ICN1 plasmid. Immunoblot shows expression levels of ICN1 in transfected cells.  $\beta$ -actin is shown as loading control. Error bars indicate  $\pm$  SD. Results from one of two independent experiments performed in quadruplicate are shown. Significance was calculated using an unpaired two-tailed t-test. \*  $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (E) HEK 293T cells were co-transfected with pGL4 luciferase reporter construct (BCAT1-Luc) and ICN1 or control plasmid. After 24h, ICN1 transfected cells were treated with increasing concentration of CB103 (10 nM-1 µM) or vehicle for 48h. Immunoblot shows expression levels of ICN1 in transfected cells.  $\beta$ -actin is shown as loading control. Error bars indicate  $\pm$  SD. Results from one of two independent experiments performed in quadruplicate are shown. Significance was calculated using an unpaired two-tailed t-test. \*\*\* $P < 0.001$ . (F) HEK 293T cells were co-transfected with pGL4 luciferase reporter construct (BCAT1-Luc) and ICN1 or control plasmid. After 24h, ICN1 transfected cells were transfected with plasmids silencing MYC (pLKO-shMYC#1, pLKO-shMYC#2) or non-silencing control (pLKO-shscrambled). Cells were harvested 48h later. Immunoblot shows expression levels of MYC and ICN1 in transfected cells.  $\beta$ -actin is shown as loading control. Arrow indicates specific band. Asterisc (\*) indicates non-specific band. Error bars indicate  $\pm$  SD. Results from one of two independent experiments performed in quadruplicate are shown. Significance was calculated using an unpaired two-tailed t-test. \*  $P < 0.05$ , \*\*\* $P < 0.001$ . (G) HEK 293T cells were co-transfected with pGL4 luciferase reporter construct (BCAT1-Luc) and ICN1 or control plasmid. After 24h, ICN1 transfected cells were treated with increasing concentrations of JQ1 (10 nM-1 µM) or vehicle for 48h. Immunoblot shows expression levels of ICN1 in transfected cells.  $\beta$ -actin is shown as loading control. Error bars indicate  $\pm$  SD. Results from one of two independent experiments performed in quadruplicate are shown. Significance was calculated using an unpaired two-tailed t-test. \*  $P < 0.05$ , \*\*\*  $P < 0.001$ . n.s=not significant. (H) Relative luciferase reporter activity in HPB T-ALL cells transfected with a BCAT1 promoter construct (BCAT1-Luc) or empty pGL4minP plasmid. Mean value and SD are shown (N=6). For statistical analysis, an unpaired t-test was used. \*\*\* $P < 0.001$ . The experiment was repeated three times with similar results. (I) Relative luciferase reporter activity in HPB T-ALL cells transfected with pGL4 luciferase reporter construct (BCAT1-Luc) or control plasmid. After 24h, transfected cells were treated with increasing concentration of CB103 (0.5 µM-5 µM) or vehicle for 48h. Immunoblot shows expression levels of ICN1 in transfected cells.  $\beta$ -actin is shown as loading control. Error bars indicate  $\pm$  SD. Results from one of two independent experiments performed is shown. Significance was calculated using an unpaired two-tailed t-test. \*\*\* $P < 0.001$ . (J) Relative luciferase reporter activity in HPB T-ALL cells transfected with BCAT1-Luc wt construct, BCAT1-Luc having the RBPJ site deleted or mutated. Mean value and SD are shown (N=6). For statistical analysis, an unpaired t-test was used. \*\* $P < 0.01$ . The experiment was repeated three times with similar results. (K) Relative luciferase reporter activity in HPB T-ALL cells transfected with BCAT1-Luc wt construct, BCAT1-Luc having the RBPJ site mutated or control vector. These cells were co-transfected with increasing concentrations of pcDNA3-ICN1 or control plasmid (100-500 ng). Error bars indicate  $\pm$  SD. For statistical analysis, an unpaired t-test was used. \*\*\* $P < 0.001$ . Immunoblot shows expression levels of ICN1 in transfected cells.  $\beta$ -actin is shown as loading control.



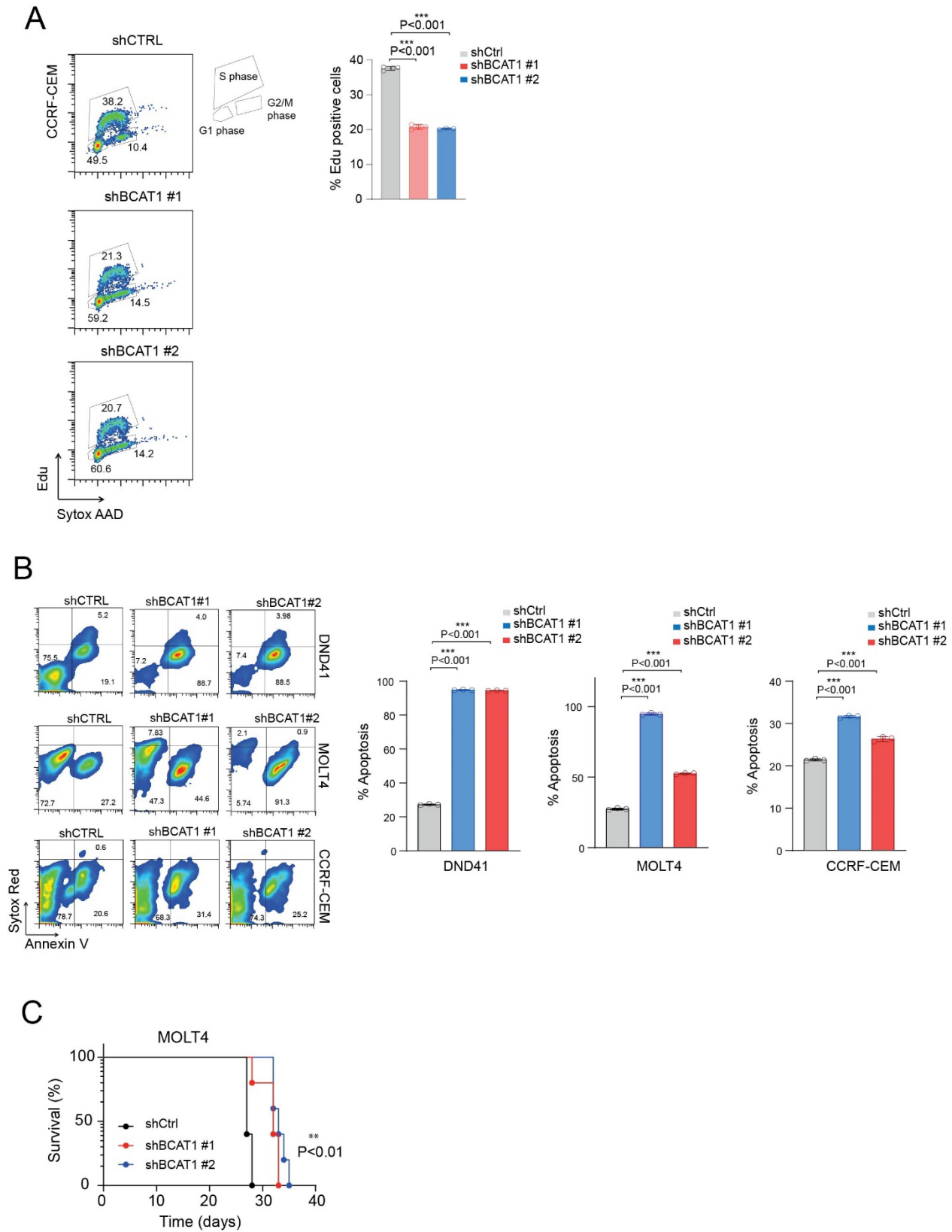
**Figure S5. The NOTCH1 binding region in the BCAT1 locus is associated with promoter features in T-ALL cells.** Profiles of H3K4me3, Pol II and NOTCH1 ChIP-seq binding in the BCAT1 locus in T-ALL cells. ATAC-seq data for the same region is also shown.



**Figure S6. *Bcat1* promotes NOTCH1-dependent leukemia onset.** (A) Schematic representation of the experimental procedure for the generation of  $\Delta E$ -NOTCH1 leukemias wild-type (WT; +/+) and null (KO; -/-) for *Bcat1*. Image was generated with BioRender software. (B) Immunophenotype distribution showing the expression levels of CD4 and CD8 in  $\Delta E$ -NOTCH1-induced leukemias at the moment of sacrifice. DN, double negative; DP,

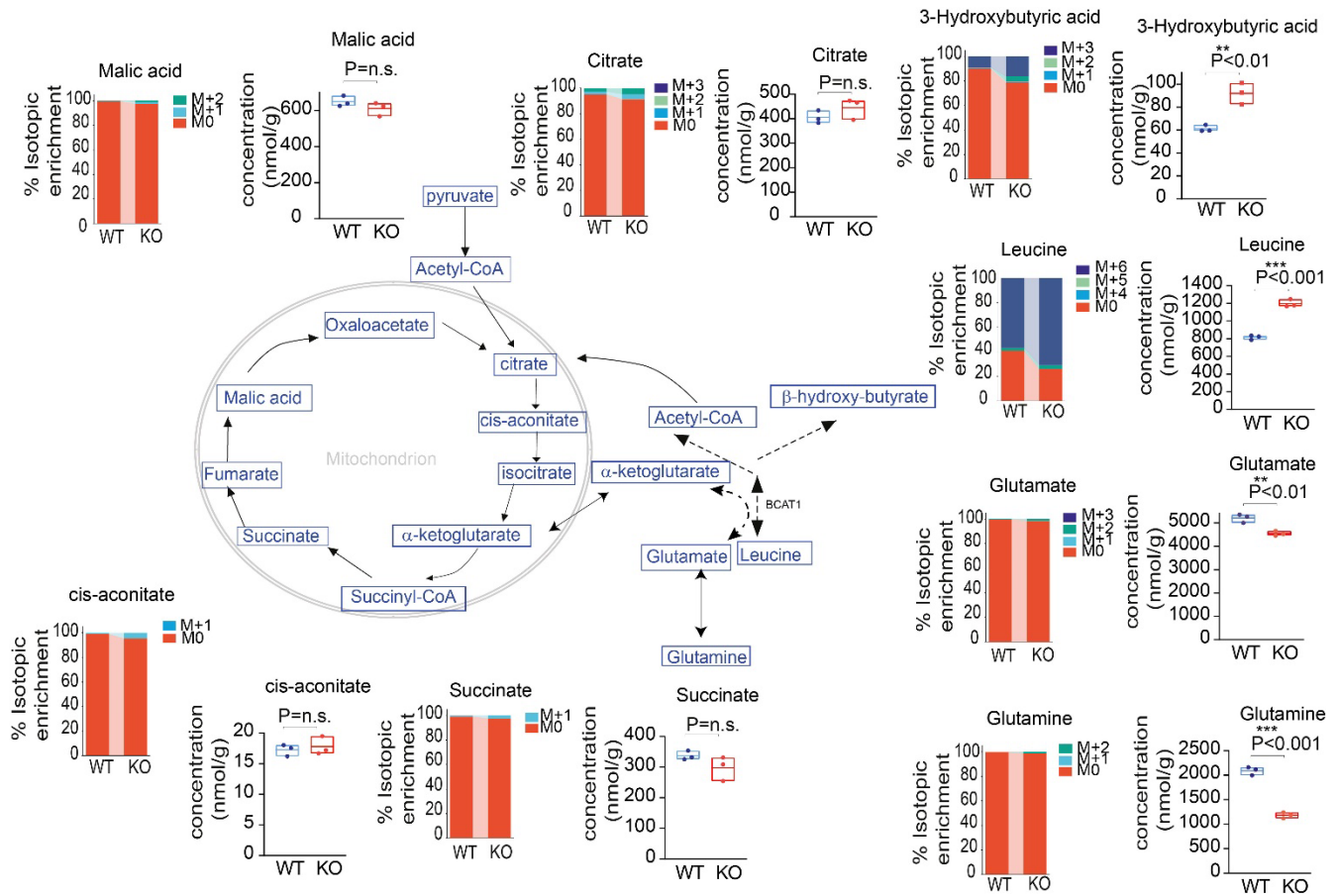


double positive; SP, single positive. WT, *Bcat1* wild-type; KO, *Bcat1* null. (C) Quantification of viable cells (Annexin V-Sytox Red<sup>+</sup>) in  $\Delta E$ -*NOTCH1* leukemias WT and null for *Bcat1* obtained ex vivo (bar graph). Data for bar graph is shown as mean  $\pm$  SD. Significance was calculated using an unpaired two-tailed t-test. \*\* $P < 0.01$ .

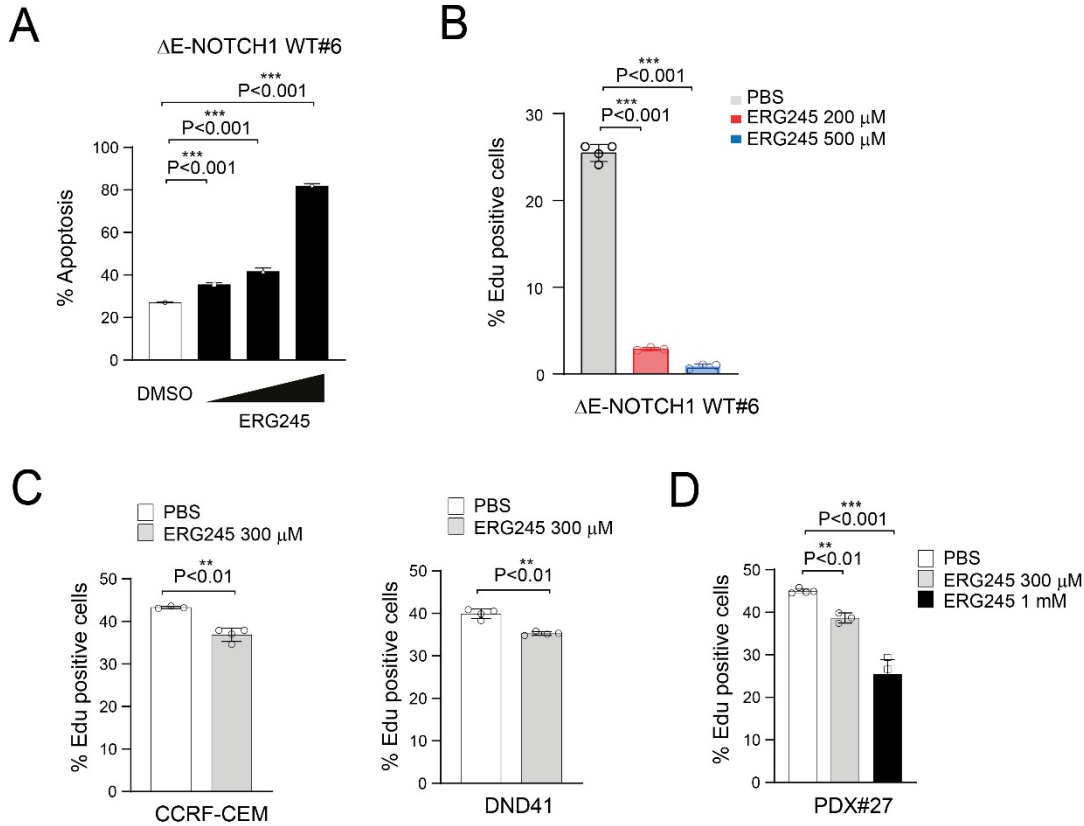


**Figure S7. Functional effects of BCAT1 depletion.** (A) Representative plots (left) and bar graph representation (right) of CCRF-CEM cells transduced with shCTRL, shBCAT1#1 or shBCAT1#2 twelve days post-puromycin

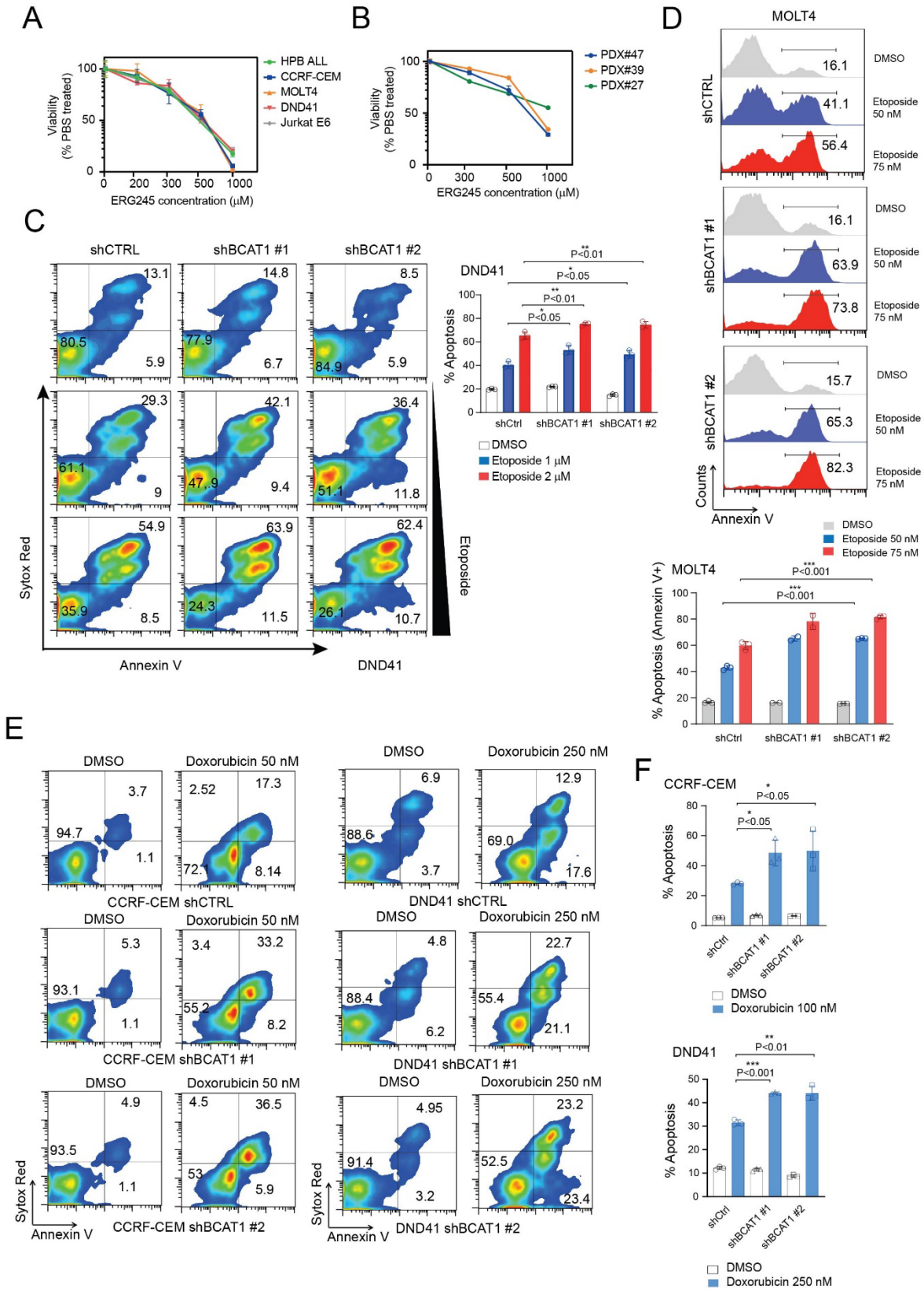
selection and assessed for EdU incorporation by fluorescence-activated cell sorting (FACS) analysis. Data for bar graph is shown as mean  $\pm$  SD. Significance was calculated using an unpaired two-tailed t-test. \*\*\* $P < 0.001$ . (B) Representative plots of apoptosis (left) and quantification of apoptosis (right) in DND41, MOLT4 and CCRF-CEM T-ALL cells transduced with shCTRL, shBCAT1 #1 and shBCAT1 #2 constructs 3-5 days post-puromycin selection. Data for bar graph is shown as mean  $\pm$  SD. Significance was calculated using an unpaired two-tailed t-test. \*\*\* $P < 0.001$ . (C) Kaplan-Meier survival curves of overall survival in NSG mice xenografted with MOLT4 cells expressing luciferase and transduced with shCTRL or shBCAT1 (#1 and #2). Log-rank test was performed to calculate  $P$  value. \*\* $P < 0.01$ .



**Figure S8. Metabolic impact of Bcat1 depletion on  $\Delta E$ -NOTCH1 leukemias.** Results for *in vivo* isotope-tracing experiments following i.v. administration of  $^{13}\text{C}_6$  Leu in  $\Delta E$ -NOTCH1 leukemias WT and KO for *Bcat1*. Mean percentages (from N=3 determinations) of  $^{13}\text{C}_6$  Leu derived: (i) TCA intermediates: citrate (M0, M+1, M+2, M3), cis-aconitate, (M0, M+1), malic acid (M0, M+1, M+2), succinate (M0, M+1); (ii) BCAA and derivatives: leucine (M0, M+4, M+5, M+6), glutamate (M0, M+1, M+2, M3), glutamine (M0, M+1, M+2) and beta-hydroxybutyrate (M0, M+1, M+2, M+3) are shown. F-scope metabolic quantification for selected metabolites in the same tumors is also shown. Changes in glutaminolysis, TCA cycle intermediates, and BCAA and intermediates are shown as floating bars representing mean  $\pm$  SD. Significance was calculated using a nonparametric t-test (Mann-Whitney). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . n.s.= not significant.

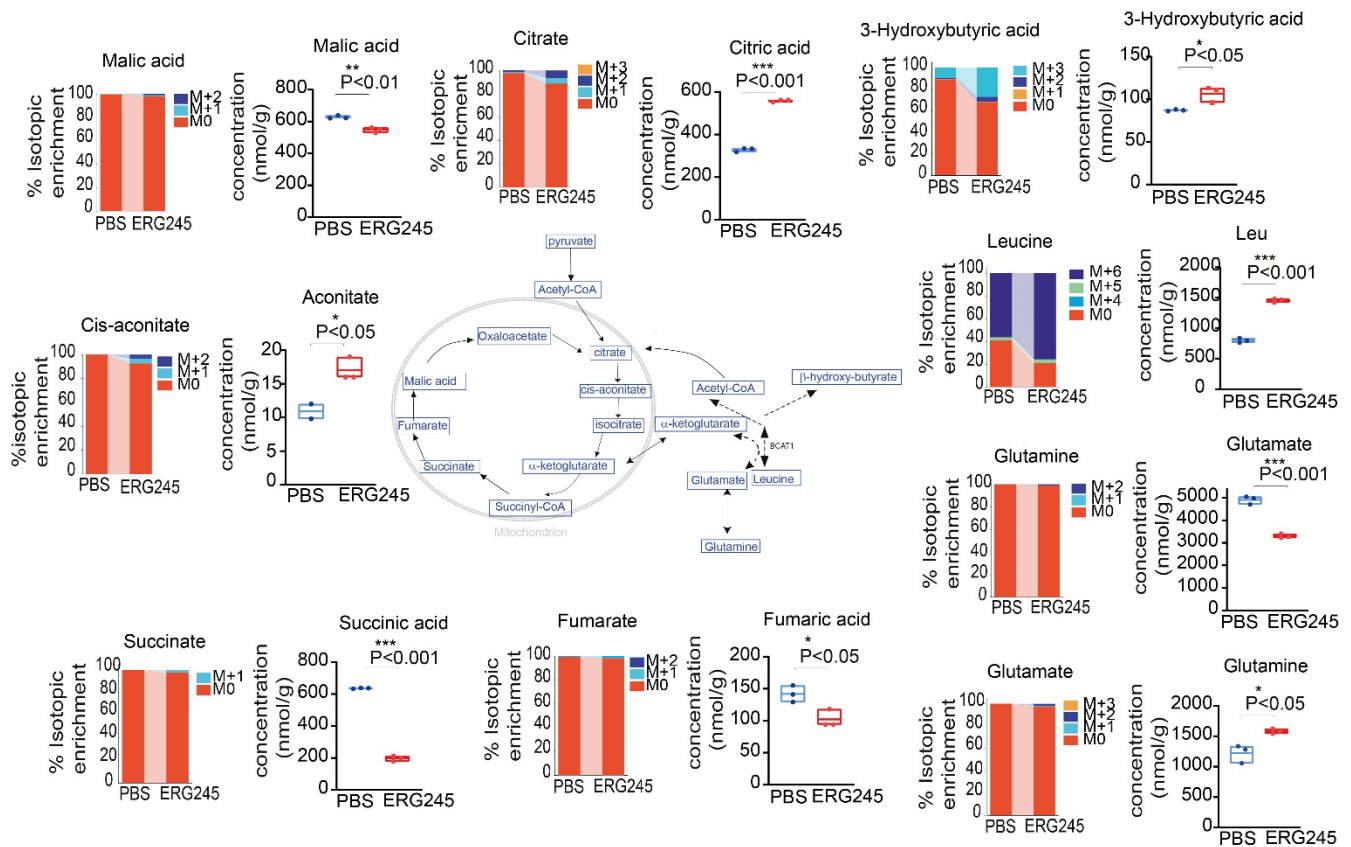


**Figure S9. Functional effects of a BCAT1 specific inhibitor, ERG245.** (A) Quantification of apoptosis in  $\Delta E$ -*NOTCH1* leukemia wild-type for *Bcat1* (WT#6) treated in vitro for 48h with PBS (vehicle) or increasing doses of ERG245 (200  $\mu$ M-1 mM). Data for bar graph is shown as mean  $\pm$  SD. Significance was calculated using an unpaired two-tailed t-test. \*\*\* $P$ < 0.001. (B) Quantification of EdU incorporation (S-phase cells) in  $\Delta E$  *NOTCH1* leukemia wild-type for *Bcat1* (WT#6) treated in vitro for 48h with PBS (vehicle) or increasing doses of ERG245 (200  $\mu$ M- 500  $\mu$ M). Data for bar graph is shown as mean  $\pm$  SD. Significance was calculated using an unpaired two-tailed t-test. \*\*\* $P$ < 0.001. (C) Quantification of EdU incorporation (S-phase cells) in T-ALL cell lines (CCRF-CEM, DND41) treated in vitro for 72h with PBS (vehicle) or ERG245 (300  $\mu$ M). Data for bar graph is shown as mean  $\pm$  SD. Significance was calculated using an unpaired two-tailed t-test. \*\* $P$ < 0.01. (D) Quantification of EdU incorporation (S-phase cells) in PDX#27 treated in vitro for 72h with PBS (vehicle) or increasing doses of ERG245 (300  $\mu$ M-1 mM). Data for bar graph is shown as mean  $\pm$  SD. Significance was calculated using an unpaired two-tailed t-test. \*\* $P$ < 0.01, \*\*\* $P$ < 0.001.



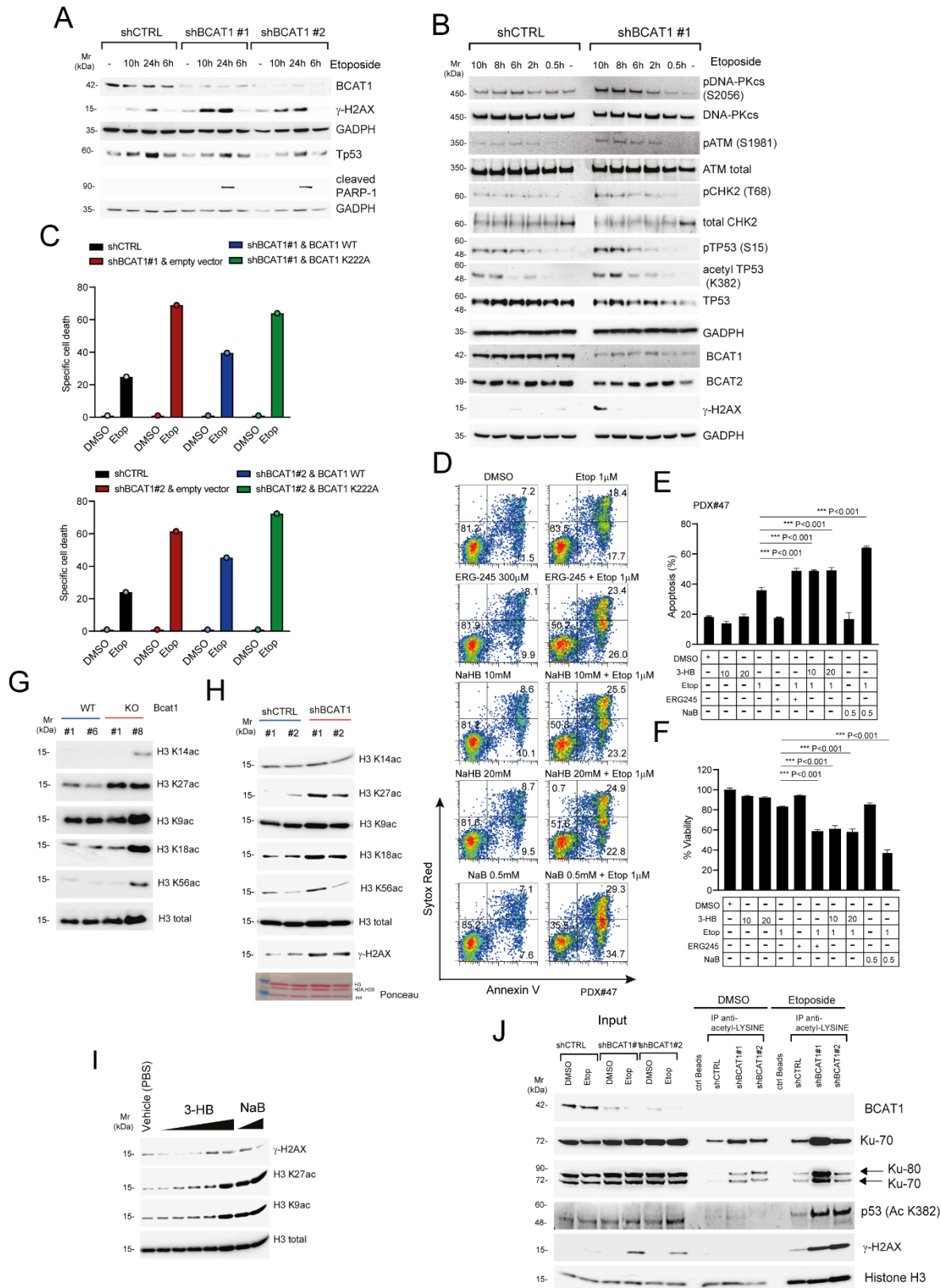
**Figure S10. BCAT1 specific inhibition has modest cytotoxic effects on human T-ALL, while BCAT1 depletion increases sensitivity to DNA damaging agents. (A) Cell viability analysis in T-ALL cell lines (HPB-**

ALL, CCRF-CEM, MOLT4, DND41, Jurkat E6). T-ALL cells were treated in vitro for 72h with PBS (vehicle) or increasing doses of ERG245 (200  $\mu$ M-1 mM). Data is shown as mean  $\pm$  SD. (B) Cell viability analysis in PDX samples (PDX#47, PDX#39, PDX#27). T-ALL cells were treated in vitro for 72h with PBS (vehicle) or increasing doses of ERG245 (300  $\mu$ M-1 mM). Data is shown as mean  $\pm$  SD. (C) Representative plots of apoptosis (left) in DND41 T-ALL cells transduced with shCTRL or shBCAT1 (#1 and #2) and treated in vitro for 48h with DMSO (vehicle) or etoposide (1  $\mu$ M or 2  $\mu$ M). Quantification of apoptosis (right) in DND41 T-ALL cells transduced with shCTRL or shBCAT1 (#1 and #2) and treated in vitro for 48h with DMSO (vehicle) or etoposide (1  $\mu$ M or 2  $\mu$ M). Significance was calculated using an unpaired two-tailed t-test. \*  $P < 0.05$ , \*\*  $P < 0.01$ . (D) Representative plots (top) and bar graph representation (bottom) of annexin V staining in MOLT4 T-ALL cells transduced with shCTRL or shBCAT1 (#1 and #2) and treated with vehicle (DMSO) or etoposide (Etop; 50-75 nM) for 48h. (E) Representative plots of apoptosis in CCRF-CEM (left) or DND41 (right) T-ALL cells transduced with shCTRL, shBCAT1#1 or shBCAT1#2 and treated with vehicle (DMSO) or doxorubicin (Doxo; 50 or 250 nM, respectively) for 48h. (F) Bar graph representation of apoptosis in CCRF-CEM (top) or DND41 (bottom) T-ALL cells transduced with shCTRL, shBCAT1#1 or shBCAT1#2 and treated with vehicle (DMSO) or doxorubicin (Doxo; 50 or 250 nM, respectively) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**Figure S11. Metabolic impact of BCAT1 inhibition on  $\Delta E$ -NOTCH1 leukemias.** Results for *in vivo* isotope-tracing experiments following i.v. administration of  $^{13}\text{C}_6$  Leu in primary  $\Delta E$ -NOTCH1 leukemic tissue (N=3) treated with vehicle (PBS) or BCAT1-specific inhibitor, ERG245 (30 mg/kg every 8 hours for 24h). Percentages of  $^{13}\text{C}_6$  Leu derived: (i) TCA intermediates: citrate (M0, M+1, M+2, M3), cis-aconitate, (M0, M+1, M+2), fumarate (M0, M+1, M+2), malic acid (M0, M+1, M+2), succinate (M0, M+1); (ii) BCAA and derivatives: leucine (M0, M+4, M+5, M+6), glutamate (M0, M+1, M+2, M3), glutamine (M0, M+1, M+2) and beta-hydroxybutyrate (M0, M+1, M+2, M3) are shown. F-scope metabolic quantification for selected metabolites in the same tumors is also shown. Changes in glutaminolysis, TCA cycle intermediates, and BCAA and intermediates are shown as floating bars representing mean  $\pm$  SD. Significance was calculated using a nonparametric t-test (Mann-Whitney). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . n.s.= not significant

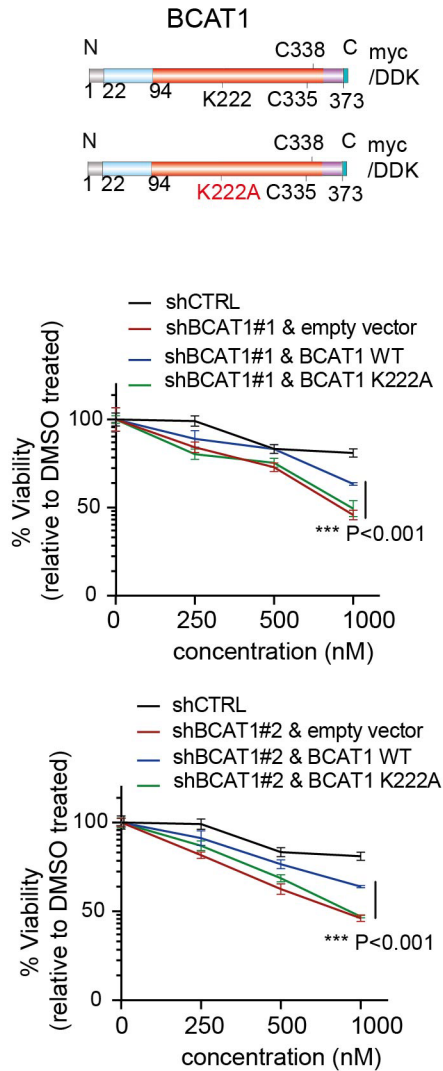




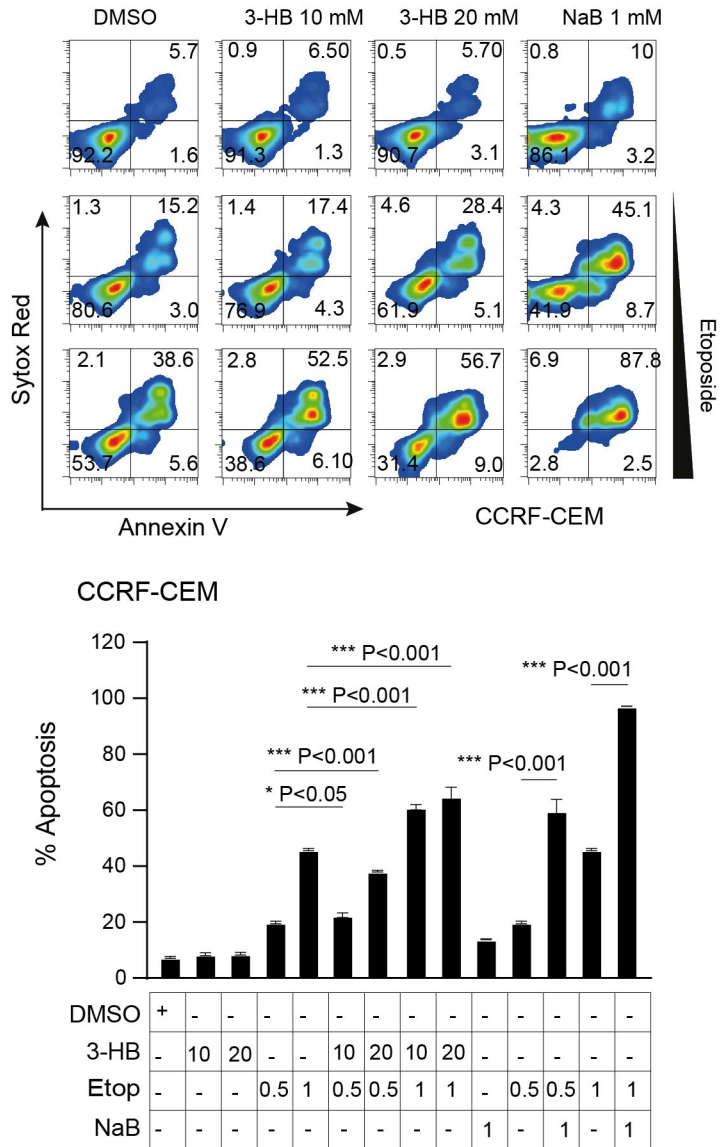
**Figure S12. Increased responsiveness to DNA damaging agents in BCAT1 depleted cells is associated with an altered DNA damage response and dependent on its catalytic activity. (A) CCRF-CEM T-ALL cells**

transduced with shCTRL or shBCAT1 (#1 and #2) were treated with 1  $\mu$ M etoposide for the indicated time. Subsequently, whole cell lysates were collected and analyzed by immunoblotting for proteins implicated in the DNA damage response and apoptosis ( $\gamma$ H2AX, TP53, cleaved PARP-1). GADPH is shown as loading control. (B) CCRF-CEM T-ALL cells transduced with shCTRL or shBCAT1 #1 were treated with 1  $\mu$ M etoposide for the indicated time (0-10 hours). Subsequently, whole cell lysates were collected and analyzed by immunoblotting for proteins implicated in the DNA damage response. Total DNA-PKcs, ATM, CHK2 and GADPH are shown as loading controls. (C) Specific apoptosis<sup>31</sup> analysis in BCAT1 depleted CCRF-CEM T-ALL cells (shBCAT1#1 or shBCAT1#2) engineered to express empty vector, wild-type (WT) or catalytic inactive (K222A) BCAT1 and treated in vitro for 48h with DMSO (vehicle) or etoposide (1  $\mu$ M). Specific apoptosis analysis of CCRF-CEM cells infected with a control shRNA (shCTRL) and treated in vitro for 48h with DMSO (vehicle) or etoposide (1  $\mu$ M) is also shown. Significance was calculated using an unpaired two-tailed t-test. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . (D) Representative plots of apoptosis in PDX#47 cells treated with vehicle (DMSO), 3-HB (10-20 mM), NaB (0.5 mM), ERG245 (300  $\mu$ M) etoposide (Etop; 1  $\mu$ M) or the combination (ERG245 + Etop or 3-HB + Etop or NaB + Etop) for 48h. (E) Quantification of apoptosis in PDX#47 cells treated in vitro with vehicle (DMSO), 3-HB (10-20 mM), NaB (0.5 mM), ERG245 (300  $\mu$ M), etoposide (Etop; 1  $\mu$ M) or the combination (3-HB + Etop or ERG245 + Etop or NaB + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*\*\*  $P < 0.001$ . (F) Cell viability analysis in PDX#47 cells treated in vitro with vehicle (DMSO), 3-HB (10-20 mM), NaB (0.5 mM), ERG245 (300  $\mu$ M), etoposide (Etop; 1  $\mu$ M) or the combination (3-HB + Etop or ERG245 + Etop or NaB + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*\*\*  $P < 0.001$ . (G) Total histones were extracted from tumors WT or KO for *Bcat1* and immunoblots were performed for acetylated histone H3 variants. Total H3 is shown as loading control. (H) Total histones extracted from CCRF-CEM cells transduced with shCTRL (#1, #2) or shBCAT1 (#1 and #2) were analyzed by immunoblotting for acetylated histone H3 variants and  $\gamma$ H2AX. Total H3 and Ponceau staining are shown as loading controls. (I) CCRF-CEM TALL cells were treated for 24h with vehicle or increasing concentrations of 3-HB (1-40 mM) or NaB (0.5-1 mM). Total histones were extracted and analyzed by immunoblotting for selected acetylated histone H3 variants (K27ac, K9ac) and  $\gamma$ H2AX. Total H3 is shown as loading control. (J) CCRF-CEM T-ALL cells transduced with shCTRL or shBCAT1 (#1 and #2) were treated with vehicle (DMSO) or 1  $\mu$ M etoposide for 24h, subsequently whole cell lysates were collected and immunoprecipitated using anti-acetyl-lysine affinity beads or control beads and probed for KU70, KU80,  $\gamma$ H2AX. Total H3 was used as loading control (for input).

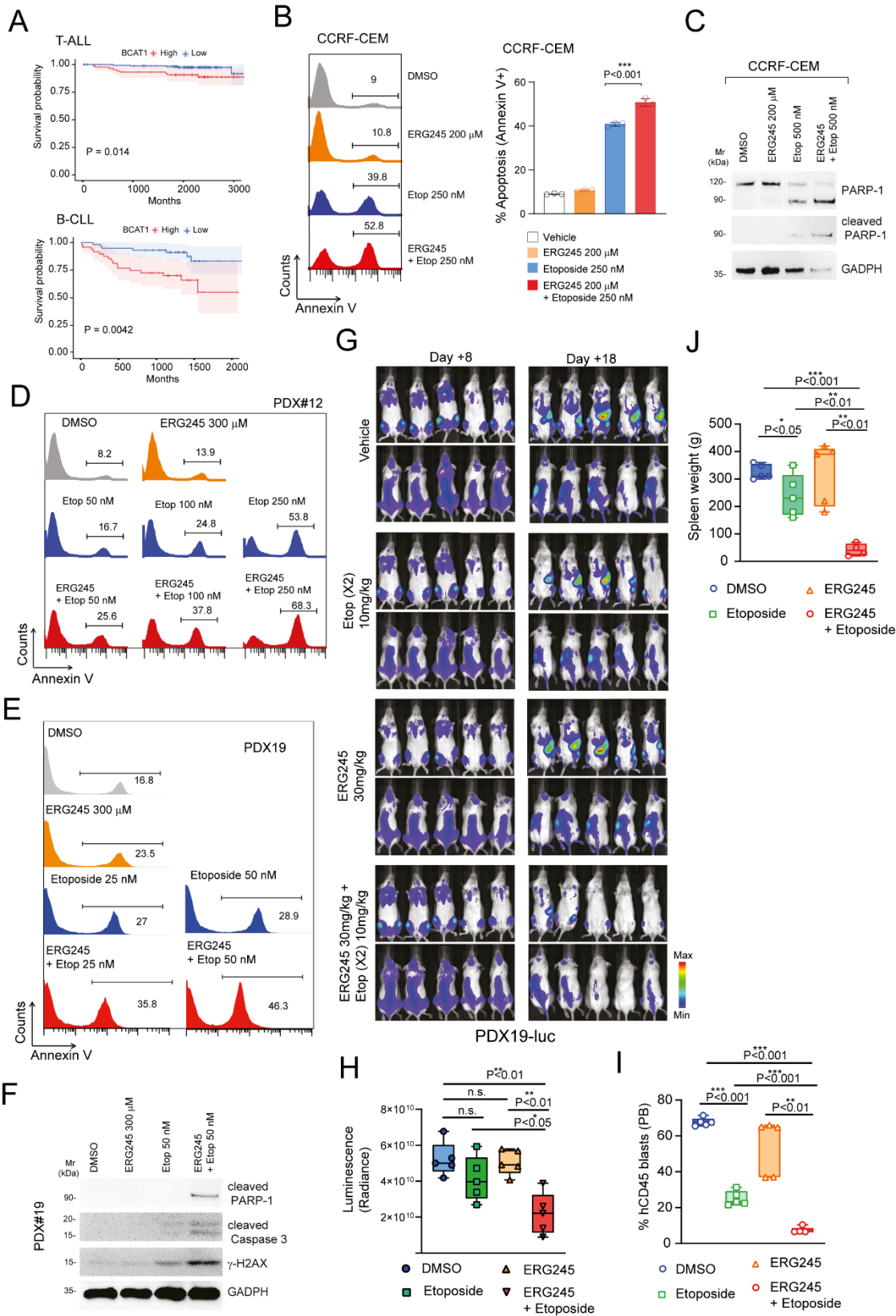
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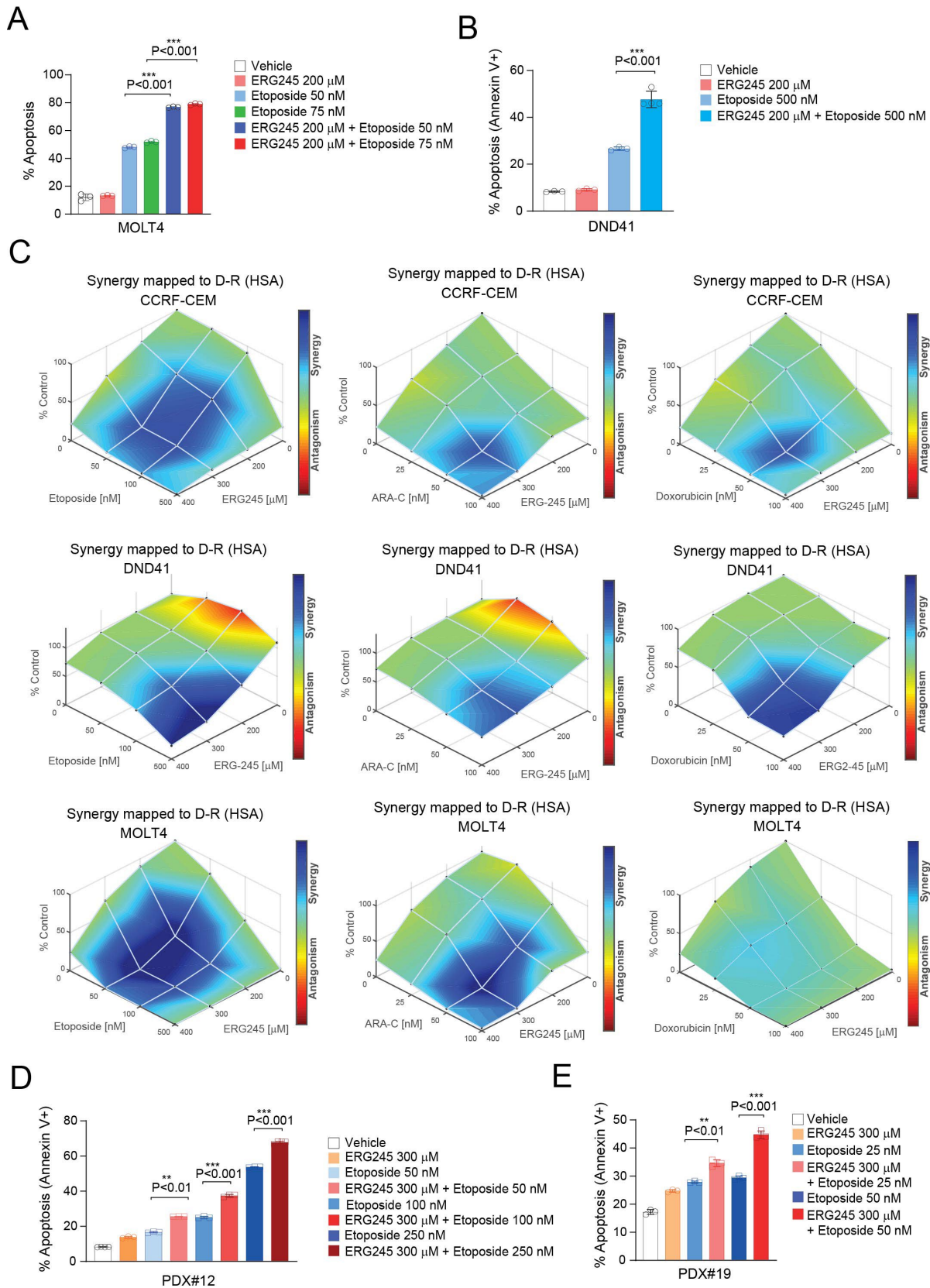


**Figure S13. Metabolic function of BCAT1 contributes in modulating the sensitivity to DNA damaging agents.** (A) Schematic representations (top) of the constructs encoding full-length (WT) and catalytic inactive mutant of BCAT1 (K222A). Cell viability analysis (lower panels) in BCAT1 depleted CCRF-CEM T-ALL cells (shBCAT1#1 or shBCAT1#2) engineered to express empty vector, wild-type (WT) or catalytic inactive (K222A) BCAT1 and treated in vitro for 48h with DMSO (vehicle) or etoposide (250 nM– 1 μM). Cell viability analysis of CCRF-CEM cells infected with a control shRNA (shCTRL) and treated in vitro for 48h with DMSO (vehicle) or etoposide (250 nM– 1 μM) is also shown. Significance was calculated using an unpaired two-tailed t-test. \*\*\*  $P < 0.001$ . (B) Representative plots of apoptosis (top) or quantification of apoptosis (bottom) in CCRF-CEM T-ALL cells treated with vehicle (DMSO), 3-HB (10-20mM), NaB (1mM), etoposide (Etop; 0.5-1 μM) or the combination (3-HB + Etop or NaB + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .



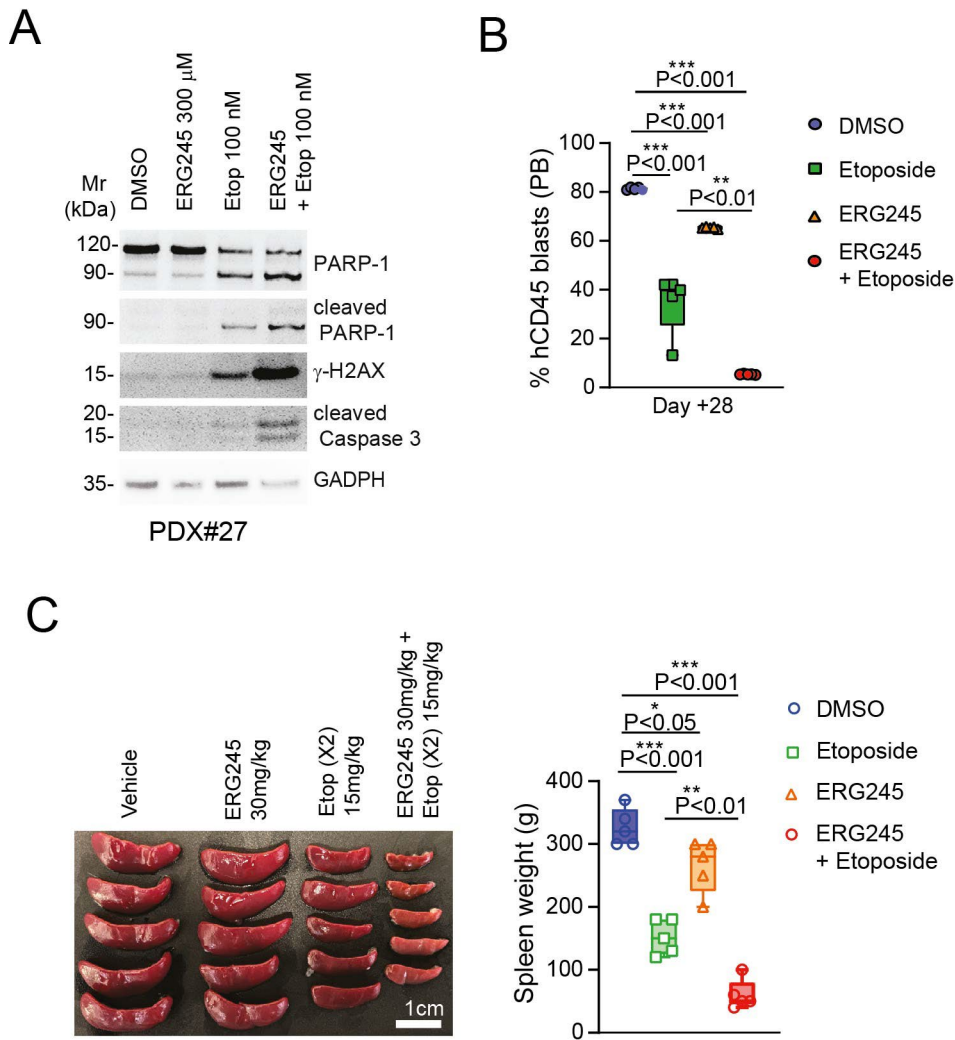
**Figure S14. BCAT1 expression correlates with prognosis in NOTCH1-dependent leukemias and represents a therapeutic target in T-ALL.** (A) Kaplan–Meier survival curves (top) of the entire series of 261 T-ALL patients

(with reported OS). BCAT1 high cases ( $>$  mean expression) or BCAT1 low cases ( $<$  mean expression). Log-rank Mantel-Cox test was performed to calculate  $P$  value.  $*P < 0.05$ . Shaded area represents 95% CI. Kaplan–Meier survival curves of a cohort of 107 B-CLL patients (bottom). BCAT1 (probe 22585\_at) high cases ( $>$  mean expression) or BCAT1 low cases ( $<$  mean expression). Log-rank Mantel-Cox test was performed to calculate  $P$  value.  $**P < 0.01$ . Shaded area represents 95% CI. (B) Representative plots (left) and bar graph representation (right) of annexin V staining in CCRF-CEM T-ALL cells treated with vehicle (DMSO), BCAT inhibitor (ERG245), etoposide (Etop) or the combination (ERG245 + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test.  $***P < 0.001$ . (C) Western blot analysis of PARP-1 (total or cleaved PARP-1) in CCRF-CEM cells treated for 48h with DMSO (vehicle), ERG245 (200  $\mu$ M), etoposide (Etop; 500 nM) or ERG245 + Etop. GADPH was used as protein loading control. (D) Representative plots of annexin V staining in PDX#12 T-ALL cells treated with vehicle (DMSO), BCAT inhibitor (ERG245; 300  $\mu$ M), etoposide (Etop; 50, 100, 250 nM) or the combination (ERG245 + Etop) for 48h. (E) Representative plots of annexin V staining in PDX#19 T-ALL cells treated with vehicle (DMSO), BCAT inhibitor (ERG245; 300  $\mu$ M), etoposide (Etop; 25, 50 nM) or the combination (ERG245 + Etop) for 48h. (F) Western blot analysis of cleaved PARP-1, cleaved caspase 3 and phosphorylated  $\gamma$ H2AX in PDX#19 cells treated for 48h with DMSO (vehicle), ERG245 (300  $\mu$ M), etoposide (Etop; 50 nM) or ERG245 + Etop. GADPH was used as protein loading control. (G) Representative images of bioluminescence in NSG mice xenografted with PDX#19 cells expressing luciferase (PDX#19-luc) and treated with vehicle (DMSO), BCAT inhibitor (ERG245; 30 mg/kg three times a week), etoposide (Etop; 10 mg/kg twice a week) or the combination (ERG245 + Etop). Analysis before (day 8 post-transplantation) and 10 days after start of treatment (day 18 post-transplantation) is shown. (H) Quantitative analysis of tumor load via in vivo bioluminescence imaging of NSG mice xenografted with PDX#19-luc 10 days after treatment with vehicle (DMSO), BCAT inhibitor (ERG245), etoposide (Etop) or the combination (ERG245 + Etop). Significance was calculated using an unpaired two-tailed t-test. n.s: not significant.  $*P < 0.05$ ,  $**P < 0.01$ . (I) Quantitative analysis of tumor burden in NSG mice xenografted with PDX#19-luc and treated with vehicle (DMSO), BCAT inhibitor (ERG245), etoposide (Etop) or the combination (ERG245+Etop), estimated by analyzing human CD45 expression in the blood (PB) at sacrifice (t=18 days). Significance was calculated using an unpaired two-tailed t-test.  $**P < 0.01$ ,  $***P < 0.001$ . (J) Analysis of spleen weight in NSG mice xenografted with PDX#19-luc and treated with vehicle (DMSO), BCAT inhibitor (ERG245), etoposide (Etop) or the combination (ERG245+Etop). Significance was calculated using an unpaired two-tailed t-test.  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ .



**Figure S15. BCAT1 inhibition synergizes with numerous chemotherapeutic drugs to reduce cell viability.** (A) Quantification of apoptosis in MOLT4 T-ALL cells treated with vehicle (DMSO), BCAT inhibitor (ERG245),

etoposide (Etop; 50-75 nM) or the combination (ERG245 + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*\*\*  $P < 0.001$ . (B) Quantification of apoptosis (Annexin V positive) in DND41 T-ALL cells treated with vehicle (DMSO), BCAT inhibitor (ERG245), etoposide (Etop) or the combination (ERG245 + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*\*\*  $P < 0.001$ . (C) CCRF-CEM, DND41 and MOLT4 T-ALL cells were incubated with different concentrations of etoposide (0- 500 nM, left panels), cytarabine/ara-C (0- 100 nM, middle panels) or doxorubicin (0- 100 nM, right panels) and ERG245 (0- 400  $\mu$ M) for 72h. After treatment, cell viability was assessed using a bioluminescent assay (Vialight plus). Analysis of combination efficacy and synergy for chemotherapeutic drugs (etoposide, ara-C and doxorubicin) and the BCAT inhibitor ERG245 was done using the HSA model with Combenefit software. (D) Quantification of apoptosis (Annexin V positive) in PDX#12 T-ALL cells treated with vehicle (DMSO), BCAT inhibitor (ERG245; 300  $\mu$ M), etoposide (Etop: 50, 100, 250 nM) or the combination (ERG245 + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . (E) Quantification of apoptosis (Annexin V positive) in PDX#19 T-ALL cells treated with vehicle (DMSO), BCAT inhibitor (ERG245; 300  $\mu$ M), etoposide (Etop: 25, 50 nM) or the combination (ERG245 + Etop) for 48h. Significance was calculated using an unpaired two-tailed t-test. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**Figure S16. BCAT1 inhibition synergizes with etoposide to reduce viability.** (A) Western blot analysis of PARP-1 (total or cleaved PARP-1), phosphorylated  $\gamma$ H2AX and cleaved caspase 3 in PDX#27 cells treated for 48h with DMSO (vehicle), ERG245 (300  $\mu$ M), etoposide (Etop; 100 nM) or ERG245 + Etop. GADPH was used as protein loading control. (B) Quantitative analysis of tumor burden in NSG mice xenografted with PDX#27-luc and treated with vehicle (DMSO), BCAT1 inhibitor (ERG245), etoposide (Etop) or the combination (ERG245+Etop), estimated by analyzing human CD45 expression in the blood (PB) at sacrifice. Significance was calculated using an unpaired two-tailed t-test. \*\* $P < 0.01$ , \*\*\*  $P < 0.001$ . (C) Representative images of spleens (left) and analysis of spleen weights (right) in PDX#27-luc xenografted mice at the end of treatment in NSG mice xenografted with PDX#27-luc and treated with vehicle (DMSO), BCAT1 inhibitor (ERG245), etoposide (Etop) or the combination (ERG245+Etop). Significance was calculated using an unpaired two-tailed t-test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\*  $P < 0.001$ .



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