1 ***Supplementary Information***

2

3 **Materials and Methods**

4

5 **Cell Culture**

6 All of the T-ALL cell lines were cultured in RPMI-1640 medium (BioWest, Kansas
7 City, MO) supplemented with 10% FBS (BioWest). 293T cells were maintained in
8 DMEM medium (BioWest) supplemented with 10% FBS and
9 penicillin/streptomycin (Life Technologies, Carlsbad, CA).

10

11 **Antibodies and Reagents**

12 The antibodies used for immunoblotting were anti-BCL2, BclxL, c-IAP1, caspase-
13 3, caspase-7, GATA3, MCL1, RUNX1, α-tubulin, XIAP (Cell Signaling Tech,
14 Danvers, MA), MYB, TAL1 (Millipore, Billerica, MA), TCF3/E2A (BD Biosciences,
15 San Jose, CA), and TRIB2 (Abcam, Cambridge, MA, USA). Antibodies for TAL1,
16 GATA3 and RUNX1 (Santa Cruz Biotech, Dallas, Texas) were used for ChIP
17 analysis as described previously.[1] DMSO, etoposide and MG-132 were
18 purchased from Sigma (St. Louis, MO), and doxorubicin was purchase from LC
19 Laboratories (Woburn, MA).

20

21 **shRNA Knockdown Analysis**

22 The control shRNAs that were designed to target *GFP* or *Luciferase (LUC)* and
23 shRNAs targeting *TRIB2* or a transcription factor gene were cloned into the

24 lentiviral vector pLKO.1-puro and used as reported previously.[1] Briefly, individual
25 shRNA constructs were co-transfected into 293T cells with the packaging
26 plasmids pMDLg/pRRE and pRSV-Rev together with the envelope plasmid
27 pMD2.G using the FuGENE 6 transfection reagent (Roche, Indianapolis, IN)
28 according to the manufacturer's instructions. Supernatants containing the
29 lentivirus were then collected and filtered through a 0.45 μm filter (Thermo,
30 Waltham, MA). shRNA knockdown experiments were then subsequently
31 conducted by first infecting T-ALL cells with lentivirus in the presence of
32 Polybrene (8 μg/ml: Millipore) by centrifugation at 1,300 rcf for 1.5 hrs. Cells
33 were selected by the addition of puromycin (0.7 μg/ml: Sigma) for at least 36 hrs
34 after infection. Finally, the cells were collected from 72 hrs post infection onwards
35 for various downstream experiments. shRNA sequences are described below:
36

Target Genes	**shRNA IDs**	**shRNA sequences**
GFP	shGFP	ACAACAGCCACAACGTCTATA
LUC	shLUC	CTTCGAAATGTCCGTTCGGTT
TRIB2	shTRIB2 #1 (main figure)	CGCCCGAGACTCCGAACTTGT
TRIB2	shTRIB2 #4 (Supplementary Figure)	GCGTTTCTTGTATCGGGAAAT
TAL1	shTAL1	GCTCAGCAAGAATGAGATCCT
GATA3	shGATA3	CATCCAGACCAGAAACCGAAA

RUNX1	shRUNX1	CGGCAGAAACTAGATGATCAG
TCF3/E2A	shE2A	GCAGCCTCTCTTCATCCACAT
TCF/12HEB	shHEB	GCAATCATTCAGTCCTGTCTA
LMO1	shLMO1	GCCACATTAGAACTTCTCCGT

37

38 **Rescue Study**

39 For the rescue study involving the overexpression of the XIAP or BCL2 protein,
40 the wild-type *XIAP* or *BCL2* cDNA was first cloned into the pMSCV-IRES-GFP
41 (“MIG”) retrovirus expression vector. A retrovirus expressing an empty vector
42 (EV) or *XIAP* or *BCL2* cDNA was propagated in 293T cells by co-transfection of
43 packaging plasmid pMD-MLV and envelope plasmid VSV-G using FuGENE 6
44 (Roche). Jurkat cells were first infected with either retrovirus expressing an MIG
45 (EV) or *XIAP* or *BCL2* cDNA in the presence of Polybrene (8 μg/ml: Millipore) by
46 centrifugation at 1,300 rcf for 1.5 h. After 24 hrs of retrovirus infection, the cells
47 were infected with lentivirus expressing *LUC* or *TRIB2* shRNA, as detailed above.

48

49 **Cell Viability Assay**

50 The cell viability of cells was measured using the CellTiter-Glo kit (Promega,
51 Madison, WI). Briefly, T-ALL cells were seeded in 96-well plates after lentivirus
52 infection. The cell viability was then measured according to the manufacturer’s
53 instructions using the Tecan Infinite® 200 PRO plate reader (Tecan, Seestrasse,
54 Switzerland). For treatment with chemical inhibitors, the cells were first plated

into 96-well plates and incubated for the indicated time points in either DMSO (control), etoposide or doxorubicin before the cell viability was measured.

Apoptosis Analysis

The cells were washed in PBS, incubated in staining buffer containing PE-labeled anti-AnnexinV antibody (MBL International, Woburn, MA) and analyzed by BD LSR II (BD Biosciences).

Protein Extraction and Immunoblotting

For protein extraction, T-ALL cell pellets were lysed in RIPA buffer [50 mM Tris (pH 8.0), 10 mM EDTA, 150 mM NaCl, 0.5% deoxycholic acid, 0.1% SDS, 1% NP-40, 1 mM PMSF, 1 mM sodium pyrophosphate, 1 mM sodium orthovanadate and protease inhibitor cocktail (Roche)]. For immunoblotting, the protein concentration was first measured using the DC protein assay kit (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein for each sample were diluted in Laemmli sample buffer (Bio-Rad Laboratories) including β-mercaptoethanol (Bio-Rad) and resolved on an SDS-PAGE gel. The proteins were then transferred onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories). The membrane was then blocked with 5% non-fat milk, probed with the designated primary and secondary antibodies and subsequently developed using the enhanced chemiluminescence method.

RNA Extraction and Gene Expression Analysis

78 The total RNA from cells was extracted using the RNeasy Mini Kit (QIAGEN,
79 Valencia, CA). In total, 1 μg of the purified total RNA was then reverse-
80 transcribed using QuantiTect (QIAGEN) according to the manufacturer's
81 instructions. The mRNA expression levels of the genes of interest were then
82 determined by conducting quantitative real-time qPCR carried out on the ABI
83 7000 Detection System (Applied Biosystems, Foster City, CA) using the Power
84 SYBR Green PCR Master Mix (Roche) according to the manufacturer's
85 instructions. The primer sequences of the genes of interest are described below:
86

Target Genes	**Direction**	**PCR primer sequences (5' to 3')**
TRIB2-CDS	Forward	GGAAGACGCCTACATTCTGC
	Reverse	GTAGCTGCCACTGGTGTTCA
TRIB2-3'UTR	Forward	TGGTCCTGTTTTTGGGTAGG
	Reverse	GTCTGCACATACATGCCACA
TAL1	Forward	TTCCCTATGTTCACCACCAA
	Reverse	AAGATACGCCGCACAACTTT
TCF3/E2A	Forward	TCATCCTGAACTTGGAGCAG
	Reverse	CAACCACACCTGACACCTTT
GATA3	Forward	TTCAGTTGGCCTAAGGTGGT
	Reverse	CGCCGGACTCTTAGAAGCTA
RUNX1	Forward	CTGGTGTCTTCAGCCAGATG
	Reverse	CGACTGTGTACCGTGGACTG
ALDH1A2	Forward	AGGCCCTCACAGTGTCTTCT

	Reverse	ACATCTTGAATCCCCCAAAG
GAPDH	Forward	CTCCTCTGACTTCAACAGCGACAC
	Reverse	TGCTGTAGCCAAATTCGTTGTCAT
JAK1	Forward	CCCAAATTCAGGATTGGTTC
	Reverse	AGAGTGGCCACAGGTTTGAC
LIG4	Forward	GTACGGAATTTGATCCATGC
	Reverse	CATTTGTTCCACGGTTTGAA
XIAP	Forward	AACCAAAACCCCAGGGATAG
	Reverse	TCGGAAACTGCTGGGATTAC
TCF12/HEB	Forward	TCTCCAGTTTCCATTGTTGG
	Reverse	TTCTTGCTGCTGGTTGAAAA
MYB	Forward	TGTTGCATGGATCCTGTGTT
	Reverse	AGTTCAGTGCTGGCCATCTT

87

88 **Construction of the *ALDH1A2* Enhancer Reporter Plasmid and the**
89 **Generation of a Stably Expressing Cell Line**

90 To clone the *ALDH1A2* enhancer reporter plasmid, a genomic region (887 bp in
91 size: Chr 15, 56,093,389-56,094,275, hg18) was identified via enriched binding
92 by the various transcription factors involved in the TAL complex, as observed in
93 the ChIP-seq data from Jurkat cells.[1] This region was amplified by PCR from
94 genomic DNA extracted from Jurkat cells using the QIAamp DNA Blood Mini Kit
95 (QIAGEN) and cloned into the pGL4.27 [luc2P/minP/Hygro] vector (Promega)
96 upstream of a minimal promoter and the *luciferase* gene. The following primers

97 were used for the PCR amplification: ALDH1A2 enh Fw: 5'-CAC CAC TAG TTA
98 GTT TGG TTA GAG T-3' and ALDH1A2 enh Rv: 5'-TAT CCA ACA GAC GTC
99 CCC TTT-3'. For the generation of cell lines stably expressing the *ALDH1A2*
100 enhancer reporter plasmid, Jurkat cells were transfected with the *ALDH1A2*
101 enhancer reporter plasmid using the NEON® Transfection System (Life
102 Technologies) and single clones expressing the reporter plasmid were selected
103 using 400 μg/ml hygromycin (Sigma-Aldrich). As a negative control, Jurkat cells
104 were transfected with the empty pGL4.27 vector and stable cell lines were also
105 selected with hygromycin as described.

106

107 **One-Glo™ + Tox Luciferase Reporter and Cell Viability Assay**

108 The Jurkat cells stably expressing either the empty pGL4.27 or the *ALDH1A2*
109 enhancer reporter plasmid were first infected with lentivirus expressing the
110 desired shRNAs as described above. After 72 hrs of infection, the luciferase
111 activity was measured using the One-Glo™ + Tox Luciferase Reporter and Cell
112 viability assay kit (Promega) following the manufacturer's instructions. Briefly, the
113 cell viability was first determined using the CellTiter-Fluor™ Assay, and the
114 fluorescence was measured using the Tecan Infinite® 200 PRO plate reader
115 (Tecan). The firefly luciferase activity was then determined using the One-Glo™
116 Luciferase Assay with the same samples, and the fluorescence (cell viability) was
117 also measured using the Tecan Infinite® 200 PRO plate reader (Tecan). The
118 relative luciferase gene expression of each sample was then calculated by
119 normalizing the luminescence readings to the fluorescence readings.

120

121 **Chromatinimmunoprecipitation (ChIP)**

122 Jurkat cells transduced with control or *TRIB2* shRNA were cross-linked in fresh
123 11% formaldehyde solution for 10 minutes at room temperature followed by
124 quenching with glycine. Cells were rinsed twice with 1xPBS, pelleted by
125 centrifugation and flash frozen in liquid nitrogen and stored at –80°C. Cell pellets
126 were lysed as previously described[1] and sonicated by Bioruptor's sonicator at a
127 high power for 50 x 30 second pulses (30 second pause between pulses).
128 Samples were kept at 4°C at all times. ChIP reaction was performed, as
129 previously described.[1] Quantitative PCR was carried out on the ABI 7000
130 Detection System (Applied Biosystems) using the Power SYBR Green PCR
131 Master Mix (Roche) and the following primers: ALDH1A2 CHIP-PCR Fw: 5'- GTT
132 CCA CCA AGT CTG CAA GC-3' and ALDH1A2 CHIP-PCR Rv: 5'- GCC TGG
133 CTT TCT CTC TTG GT-3 for *ALDH1A2* gene region bound by TAL1 complex.
134 LIG4 CHIP-PCR Fw: 5'- TGA CAG ACC ACT TTC CTG AAA CT-3' and LIG4
135 CHIP-PCR Rv: 5'- GAG CAA ATT TTG GGA GAT AGG GC -3' for *LIG4* gene
136 region bound by TAL1 complex.

137

138 **Microarray Gene Expression Analysis and Gene Set Enrichment Analysis**
139 **(GSEA)**

140 To analyze global gene expression changes after *TRIB2* knockdown, Jurkat cells
141 were transduced with control shRNA or shRNA targeting *TRIB2* in biological
142 duplicate. Total RNA was harvested using TRIzol reagent followed by a column

143 purification using the RNeasy Mini kit (Qiagen). A total of 4 RNA samples (2
144 controls and 2 *TRIB2* knockdowns) were used for microarray expression analysis.
145 Genome-wide RNA expression analysis was performed using the HG U133 plus
146 2.0 microarray chip (Affymetrix, Santa Clara, CA) at the Dana-Farber Cancer
147 Institute, Boston. The expression data can be found at
148 http://www.ncbi.nlm.nih.gov/geo/ under accession number GSE66013. After
149 normalization using the dChip software (Dana-Farber Cancer Institute),[2] 30,904
150 of 54,613 probes with presence-call >50% (2/4 arrays) were filtered. The genes
151 differentially expressed between the 2 controls and 2 knockdown samples were
152 selected at a lower 90% confidence bound of fold-change >2.0, P-value <0.05 by
153 two sample t-test (two-tailed) and mean difference >50. GSEA (Broad Institute,
154 Cambridge, MA)[3] was performed for the filtered genes using the "Ratio of
155 Classes" for metrics for ranking genes. The high-confidence target genes that are
156 downregulated by knockdown of *TAL1*, *GATA3* or *RUNX1* were identified
157 previously[1] and used as gene sets for GSEA. Heatmaps were created using the
158 Gene Pattern software (Broad Institute) (Figure 1) or GSEA (Supplementary
159 Figure 2a). For the analysis of primary T-ALL patient samples, a dataset reported
160 by Winter et al[4] (GEO accession number GSE14618) was used and normalized
161 by the dChip software. mRNA expressions of XIAP (228363_at) and TRIB2
162 (202478_at) on the Affymetrix U133 Plus 2.0 chip were analyzed.
163

164 **Published ChIP-seq Datasets Used in This Study**

165 A previously published ChIP-seq dataset was used and mapped to HG18, as
166 previously reported by us.[1]
167
168 **Statistical Analysis**
169 Significant values (*p*-values) were calculated using the two-tailed Student's t-test.
170 All *p*-values that were less than or equal to 0.05 were considered statistically
171 significant. Correlation of expression values between two genes was analyzed
172 by Pearson's r using Prism software (Graphpad software, La Jolla, CA).
173

174 **Supplementary Table 1. Genes significantly regulated after *TRIB2***
175 **knockdown in Jurkat cells.**
176
177 **Supplementary Table 2. Expression of apoptosis-related genes after *TRIB2***
178 **knockdown in Jurkat cells.**
179
180
181

182 **Supplementary Figure Legends**

183 **Supplementary Figure 1.** (a) T-ALL cell lines were lysed and whole-cell extracts
184 were subjected to Western blot analysis with antibodies specific for TAL1, TRIB2
185 or α-tubulin (loading control). (b) Global gene expression in Jurkat cells
186 transduced with *TRIB2* shRNA (shTRIB2) or control *GFP* shRNA (shGFP) were
187 measured by microarray analysis. The experiment was performed with biological
188 duplicates. The gene probes significantly down- or upregulated by *TRIB2*
189 knockdown (KD) compared to control were shown. (c) *TRIB2* shRNA (shTRIB2)
190 and control *GFP* shRNA (shGFP) were transduced by lentivirus infection into
191 Jurkat cells in biological duplicates ("dup1" and "dup2"). *TRIB2* mRNA
192 expression was measured by quantitative RT-PCR using two different primer sets
193 targeting the coding DNA sequence (CDS) or 3' UTR and normalized by *GAPDH*
194 expression. Gene expression compared to "shGFP-dup1" was shown as the
195 mean ± standard deviation (SD) of duplicate experiments. (d) mRNA
196 expressions of TRIB2 and XIAP were analyzed by microarray analysis using a
197 dataset reported by Winter el al[4]. $P<0.05$ by the Pearson's r. (e) The *TRIB2*
198 shRNA (#1 or #4) and control *GFP* shRNA were transduced by lentivirus infection
199 into Jurkat cells. Whole cell extracts were subjected to immunoblot analysis with
200 antibodies indicated. Cleaved forms of the caspase-3 and PARP were shown by
201 arrowheads. (f) T-ALL cell lines (Jurkat, RPMI-8402, CCRF-CEM, MOLT-4,
202 KOPT-K1 and DND-41) were transduced by lentivirus infection with the shTRIB2
203 or shGFP. The cell viability was measured at days 3 and 7 after lentivirus
204 infection. The growth rate (day 7/day 3) relative to control is shown as the mean

205 ± SD of triplicate experiments. ***p < 0.001 by two-sample, two-tailed t-test. (g,h)
206 Jurkat cells were first transduced by retrovirus infection with cDNA encoding the
207 *XIAP* or *BCL2* gene or an empty vector. The cells were then transduced by
208 lentivirus infection with *TRIB2* shRNA (shTRIB2) or *Luciferase* shRNA (shLUC:
209 control). (g) Apoptosis was analyzed at day 3 after lentiviral infection by flow
210 cytometric analysis of cells stained with AnnexinV-PE. (h) The cell viability was
211 measured at day 3 and 7 after lentivirus infection. The growth rate (day 7/3)
212 relative to control is shown as the mean ± standard deviation (SD) of triplicate
213 experiments; ***p<0.001 by two sample, two-tailed t-test. (i) Jurkat cells were
214 transduced by retrovirus infection with cDNA encoding the *TRIB2* gene or an
215 empty vector (EV). The cells were then treated with etoposide (10 μM) or
216 doxorubicin (100 nM) for 24, 48 or 72 hours. The cell viability was measured,
217 and the growth inhibition (%) compared to the DMSO-treated control was shown
218 ± SD of triplicate experiments; *p<0.05, ***p<0.001 by two sample, two-tailed t-
219 test.
220
221 **Supplementary Figure 2.** (a) The high-confident TAL1 target genes that were
222 downregulated by *TAL1* knockdown (KD) were determined previously[1]. The
223 expression profile of these genes after *TRIB2* knockdown (KD) in Jurkat cells
224 were analyzed by GSEA (Figure 2a, left). A heatmap generated is shown. (b, c)
225 Jurkat cells were transduced with the shTRIB2 or shGFP by lentivirus infection
226 (b) or with the cDNA encoding *TRIB2* (MIG-TRIB2) or an empty vector (MIG-EV)
227 (c). The mRNA expression of the TAL1 complex members (TAL1, RUNX1,

228 GATA3, MYB and E2A), the TAL1 targets (ALDH1A2 and LIG4) and a non-TAL1
229 target (JAK1) were measured by quantitative RT-PCR and normalized by
230 GAPDH expression. Gene expression compared to shGFP or EV-transduced
231 cells was shown as the mean ± standard deviation (SD) of duplicate experiments;
232 *$p<0.05$, **$p<0.01$, by two sample, two-tailed t-test. (d) Jurkat cells were
233 transduced by retrovirus infection with a MIG-EV or MIG-TRIB2. Whole cell
234 extracts were subjected to immunoblot analysis with antibodies indicated. (e,f)
235 Jurkat cells were first transduced by retrovirus infection with a MIG-EV or cDNA
236 encoding *XIAP* (MIG-XIAP: e) *or BCL2* (MIG-BCL2: f). The cells were then
237 transduced by lentivirus infection with the *TRIB2* shRNA or *Luciferase* shRNA
238 (shLuc: control). Whole cell extracts were subjected to immunoblot analysis with
239 antibodies specific for TRIB2, Caspase 3, PARP, XIAP, TAL1, GATA3, RUNX1,
240 MYB, E2A, BCL2, and α-tubulin. (g) Jurkat cells transduced with a MIG-EV or
241 MIG-TRIB2 were treated with MG-132 (30 μM) for 4 hrs. Whole cell extracts were
242 subjected to immunoblot analysis with antibodies indicated (left). The mRNA
243 expression of *E2A* was measured by quantitative RT-PCR and normalized by
244 *GAPDH* expression (right). Gene expression compared to DMSO-treated control
245 was shown as the mean ± SD of duplicate experiments. (h) ChIP-seq gene tracks
246 represent transcription factors bound at the *ALDH1A2* gene in Jurkat cells.
247 Bound transcription factors [TAL1, HEB, E2A, LMO1, GATA3, RUNX1, CBP, and
248 RNA polymerase 2 (RNAP2)] in Jurkat cells are shown. The *x*-axis indicates the
249 linear sequence of genomic DNA, and the *y*-axis indicates the total number of
250 mapped reads. The black horizontal bar indicates the genomic scale in kilobases

251 (kb). The blue boxes in the gene map represent exons, and the arrows indicate
252 the location and direction of the transcriptional start site. The *ALDH1A2* enhancer
253 is indicated by an arrowhead. (i) A schematic representation of the experimental
254 design. The *ALDH1A2* enhancer was introduced into the reporter construct
255 carrying a minimum promoter sequence (promoter) and a *luciferase* gene. The
256 *luciferase* reporter construct was transfected into Jurkat cells. The *luciferase*
257 activity was measured after knockdown of a transcription factor (TF). (j) ChIP
258 analysis was performed with specific antibodies for TAL1, GATA3, RUNX1 or
259 control IgG in Jurkat cells transduced with control (shGFP) or *TRIB2* shRNA
260 (shTRIB2). The amount of genomic DNA in ChIP and input (whole cell lysate)
261 samples were measured by quantitative PCR analysis using specific primers
262 targeting the regions within *ALDH1A2* and *LIG4* genes bound by the TAL1
263 complex. Each ChIP fraction was normalized to input and shown as fold-change
264 compared to IgG: mean ± SD of duplicate experiments. (k) TRIB2 contributes to
265 T-cell leukemogenesis by positively regulating the oncogenic TAL1 complex and
266 XIAP and by negatively regulating the tumor suppressor E2A.
267

268 **References**

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