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Supplementary Information

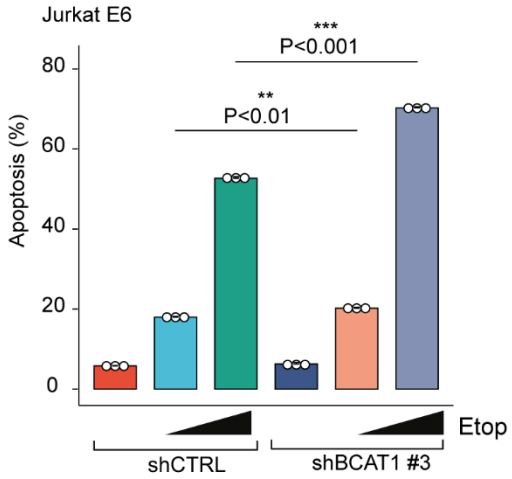
BCAT1 associates with DNA repair proteins KU70 and KU80 and contributes to regulate DNA repair in T-cell acute lymphoblastic leukemia (T-ALL)

Valeria Tosello, Chiara Rompietti, Adonia Papathanassiu, Giorgio Arrigoni and Erich Piovan

Supplementary Information

-Supplementary Table S1: Differentially expressed genes between CCRF-CEM shBCAT1 and control cells
-Supplementary Table S2: Differentially expressed genes between CCRF-CEM shBCAT1 treated with etoposide and control cells treated with etoposide
-Supplementary Table S3: BCAT1 interacting partners identified by Affinity purification mass spectrometry
-Supplementary Figure S1. BCAT1 loss sensitizes cells to apoptosis following etoposide treatment
-Supplementary Figure S2. Cellular distribution of BCAT1 and KU proteins in T-ALL cells
-Supplementary Figure S3. BCAT1 interacts with KU80 protein
-Supplementary Figure S4. BCAT1 depletion increases KU-70 acetylation

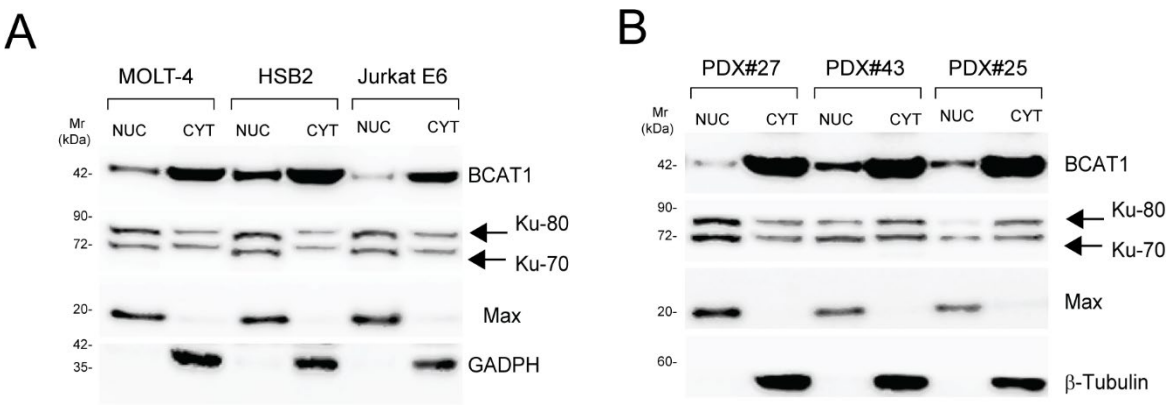
A



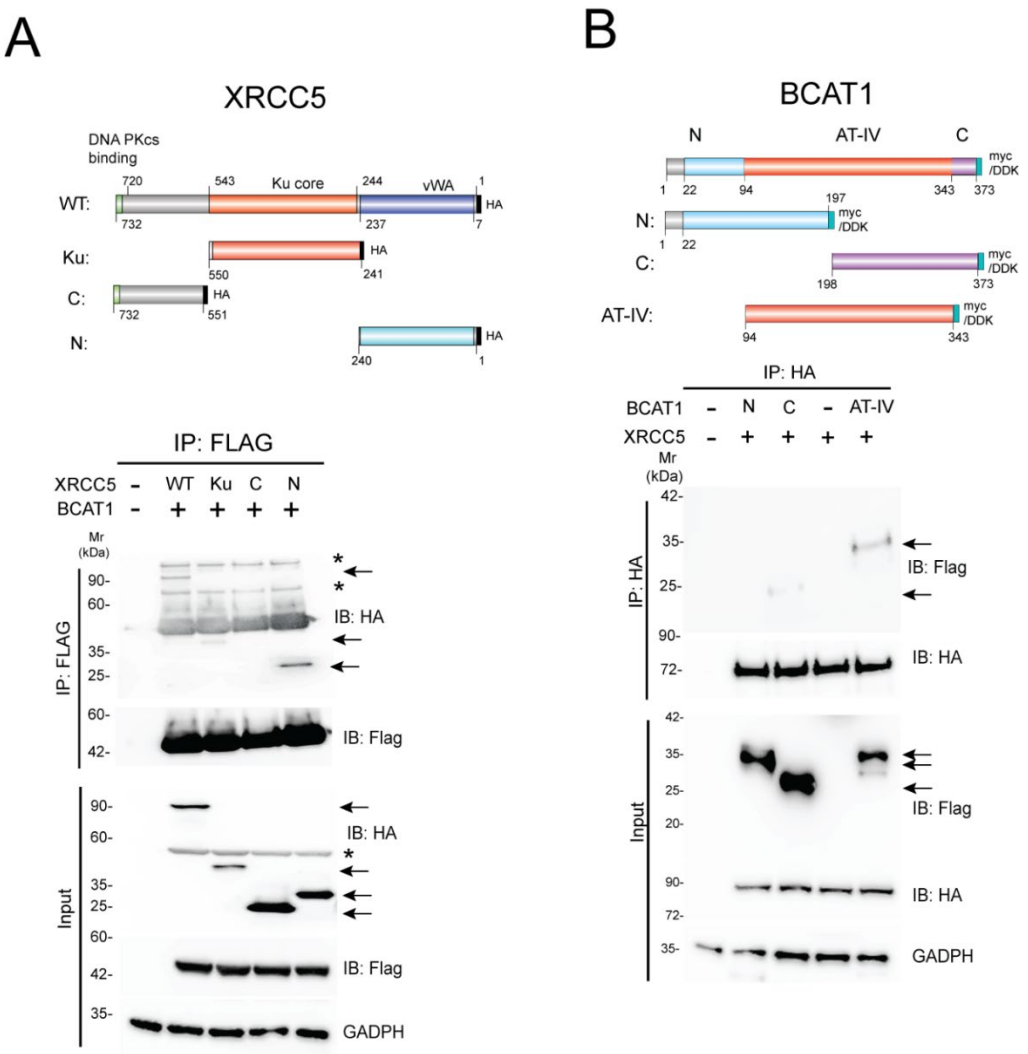
B

		IC_{50} (nM)	SD
CEM	shCTRL	3028.06	849.8292
	shBCAT1 #1	359.057	40.01659
	shBCAT1 #2	405.2155	26.97683
Jurkat	shCTRL	1446.52	ND
	shBCAT1 #3	617.73	ND

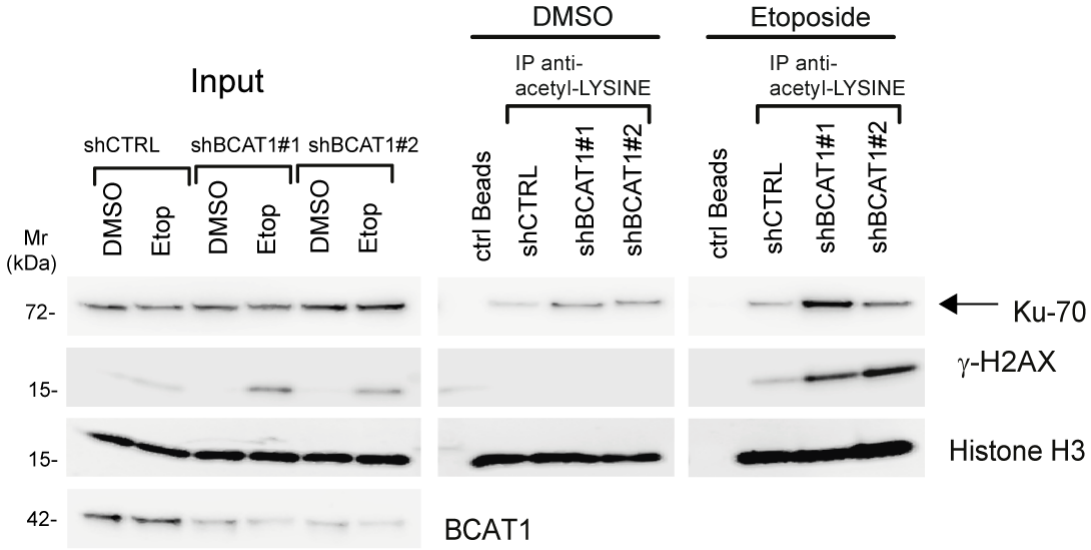
Supplementary Figure S1. BCAT1 loss sensitizes cells to apoptosis following etoposide treatment. (**A**) Quantification of apoptosis in Jurkat E6 T-ALL cells transduced with shCTRL or sh*BCAT1* (#3) and treated in vitro for 48h with DMSO (vehicle) or Etoposide (250 nM– 1 μM). Significance was calculated using an unpaired two-tailed t-test. ** $p < 0.01$, *** $p < 0.001$. (**B**) Half inhibitory concentration (IC_{50}) for Etoposide in CCRF-CEM and Jurkat T-ALL cell lines transduced with shCTRL or sh*BCAT1* targeting vectors. SD:Standard deviation of IC_{50} values obtained from two independent experiments. ND: Not determined.



Supplementary Figure S2. Cellular distribution of BCAT1 and KU proteins in T-ALL cells. (**A**) Cellular localization analysis of BCAT1, KU70, KU80 via Western blot analysis of nuclear and cytoplasmic cell fractions in cell lysates from MOLT-4, CCRF-HSB2, and Jurkat E6 T-ALL cells. GADPH and Max proteins are shown as controls for cytosolic and nuclear fractions. CYT: cytoplasmic fraction; NUC: nuclear fraction. (**B**) Cellular localization analysis of BCAT1, KU70, KU80 via Western blot analysis of nuclear and cytoplasmic cell fractions in cell lysates from patient derived xenograft cells (PDX; PDX#27, PDX#43, PDX#25). Tubulin and Max proteins are shown as controls for cytosolic and nuclear fractions. CYT: cytoplasmic fraction; NUC: nuclear fraction.



Supplementary Figure S3. BCAT1 interacts with KU80 protein. (**A**) Schematic representations of the plasmids encoding full-length (WT) and truncation mutants of XRCC5 (top). vWA: von Willebrand A domain. HEK 293T cells stably expressing epitope-tagged BCAT1 were transfected with the indicated plasmid. Cell lysates were subjected to IP with anti-FLAG beads followed by immunoblot analysis with the indicated antibodies. (**B**) Schematic representations of the plasmids encoding full-length (WT) and truncation mutants of BCAT1 (top). N: Branched chain amino acid aminotransferase-like N-terminal domain; AT-IV: aminotransferase class IV domain; C: Branched chain amino acid aminotransferase-like C-terminal domain. HEK 293T cells were transfected with HA-tagged XRCC5 and the indicated BCAT1 mutant plasmids. Cell lysates were subjected to IP with anti-HA beads followed by immunoblot analysis with the indicated antibodies. The arrows indicate expected positions of the respective proteins, and asterisks (*) indicate non-specific bands.



Supplementary Figure S4. BCAT1 depletion increases KU-70 acetylation. CCRF-CEM T-ALL cells transduced with shCTRL or shBCAT1 (#1 and #2) were treated with 1 μM etoposide for 24h, subsequently whole cell lysates were collected and immunoprecipitated using anti-acetyl-lysine affinity beads or control beads and probed for KU70, γH2AX. Total H3 was used as loading control (for input).