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Supplementary Materials for

A dual role for PSIP1/LEDGF in T cell acute lymphoblastic leukemia

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Other Supplementary Material for this manuscript includes the following:

Tables S1 to S6

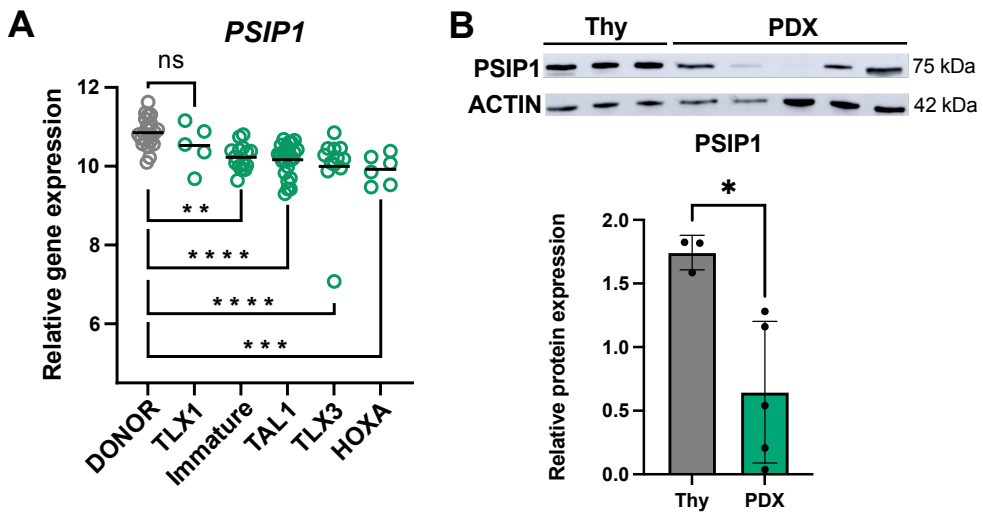

Fig. S1. PSIP1 is lower expressed in most T-ALL subtypes compared to T-cells of healthy donors. (A) All T-ALL molecular subgroups, except TLX1, express significantly lower levels of *PSIP1* compared to healthy donor material present in the microarray data of Clappier *et al.*(20) The data is plotted for one probe (X209337_s_at), but similar results were obtained for other probes (n = 3)(one-way ANOVA with Dunnett's multiple comparisons test). (B) Western blot analysis (top) and quantification (bottom) of PSIP1 and loading control ACTIN on healthy thymocyte samples (n=3) and T-ALL PDX samples (n=5)(Mann-Whitney test, *: $p < 0.05$).

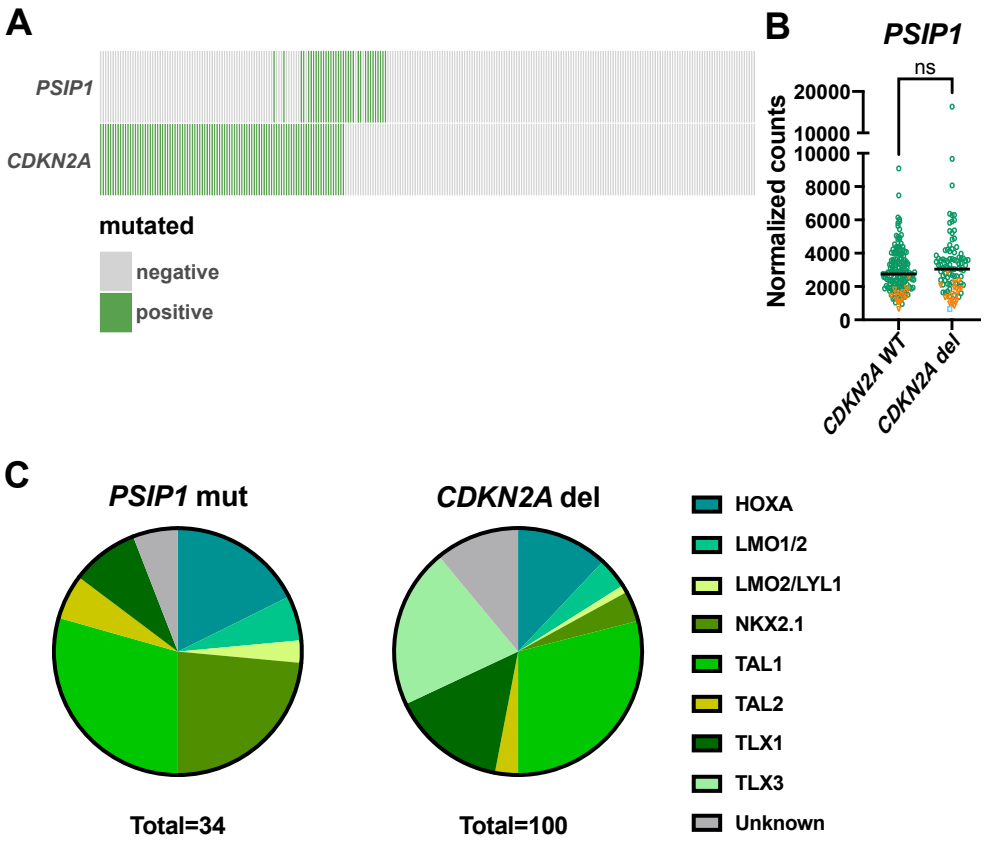

Fig. S2. *PSIP1* and *CDKN2A* deletions do not need to co-occur. (A) Genomic landscape of *PSIP1* and *CDKN2A* alterations in T-ALL. Each vertical line represents a T-ALL patient from the cohort described in Liu *et al.* (22), with a green line indicating the presence copy number loss and a grey line indicating that both wild type alleles are present. 56% (19/34) of the T-ALL patients with a copy number loss for *PSIP1* have a co-occurring *CDKN2A* mutation. Noteworthy, only 32% (6/19) of these patients had a single deletion covering both the PSIP1 and the *CDKN2A* locus. (B) Normalized counts for *PSIP1* mRNA for T-ALL cases in Liu *et al.* (22) with or without *CDKN2A* deletions (*CDKN2A* del or *CDKN2A* WT, respectively). Orange symbols represent cases with a copy number loss of PSIP1. The blue symbol represents a T-ALL patient who has a frameshift mutation (Y18fs) in one *PSIP1* allele and a deletion of the other *PSIP1* allele. (C) The occurrence of *PSIP1* deletions or mutation and *CDKN2A* deletion varies across different molecular subgroups of T-ALL.

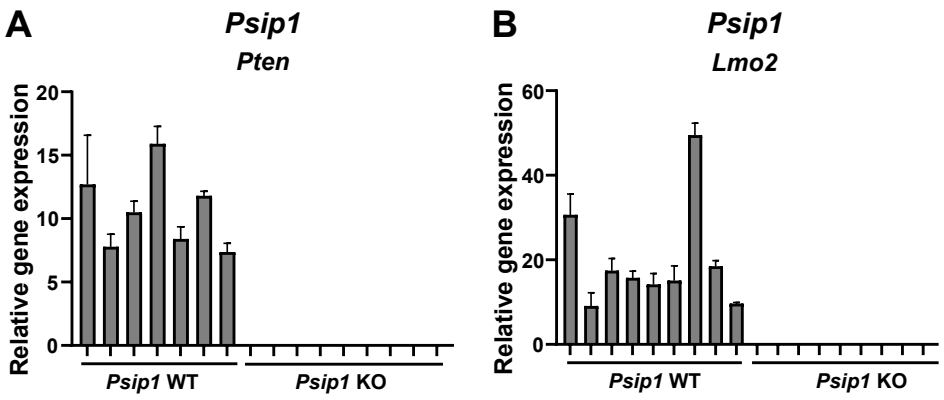

Fig. S3. Validation of Psip1 loss in spontaneous T-ALL mouse models. RT-qPCR validating the complete loss of Psip1 expression in thymoma samples of *Lck-Cre Pten (Pten)*(A) or *CD2-*i*Cre CD2-Lmo2 (Lmo2)*(B) mice, that are either wild-type (*Psip1* WT: *Psip1*$^{+/+}$*Lck-Cre*$^{tg/+}$*Pten*$^{fl/fl}$ or *Psip1*$^{fl/fl}$*CD2-*i*Cre*$^{+/+}$*CD2-Lmo2*$^{tg/+}$, respectively) or knockout (*Psip1* KO: *Psip1*$^{fl/fl}$*Lck-Cre*$^{tg/+}$*Pten*$^{fl/fl}$ or *Psip1*$^{fl/fl}$*CD2-*i*Cre*$^{tg/+}$*CD2-Lmo2*$^{tg/+}$, respectively).

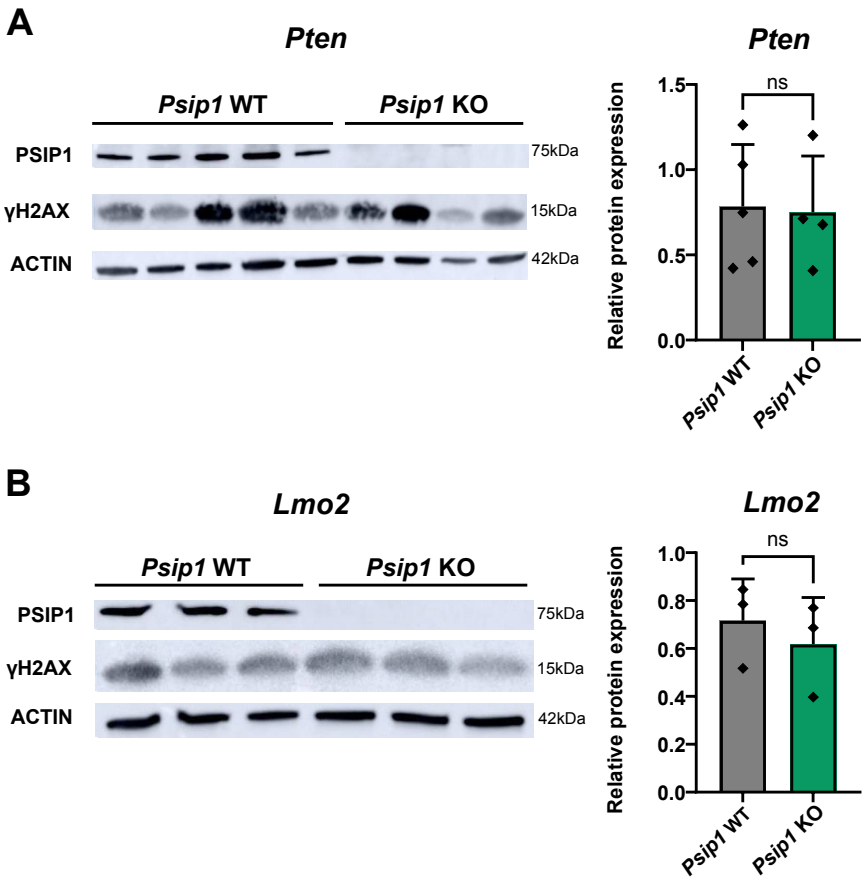

Fig. S4. Loss of Psip1 does not induce DNA damage in preleukemic samples. (A,B) Western blot analysis (left) and quantification (right) of the protein levels of PSIP1, γH2AX and Actin (loading control) in thymi of 42-day old Lck-Cre Pten (Pten)(A) or CD2-iCre CD2-Lmo2 (Lmo2)(B) model, that are either wild-type (Psip1 WT: Psip1+/+Lck-Cretg/+Ptenfl/fl or Psip1fl/flCD2-iCre+/+CD2-Lmo2tg/+, respectively) or knockout (Psip1 KO: Psip1fl/flLck-Cretg/+Ptenfl/fl or Psip1fl/flCD2-iCretg/+CD2-Lmo2tg/+, respectively). The protein level of γH2AX normalized against house-keeping gene β-Actin (Mann-Whitney test). ns: not significant

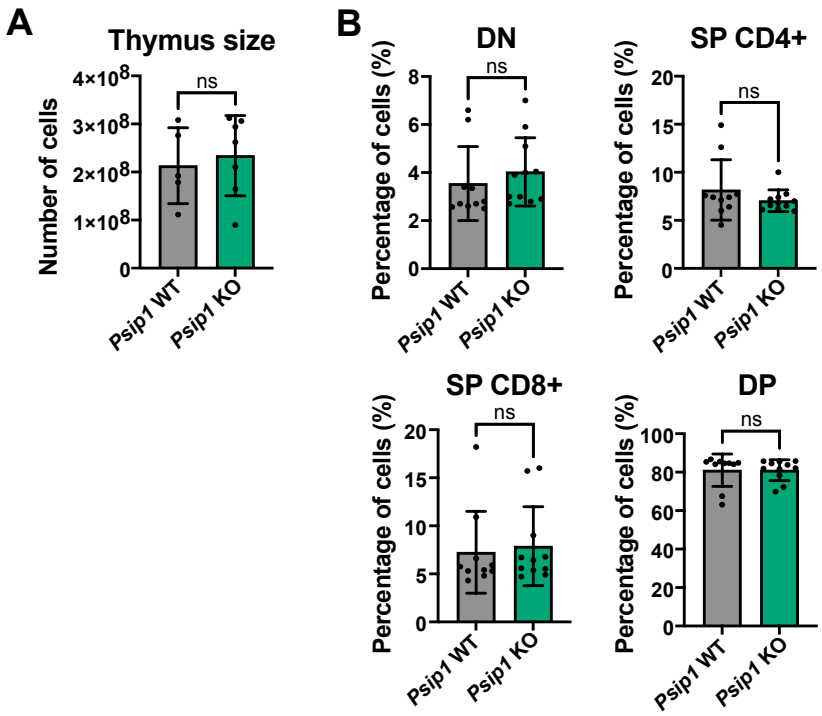

Fig. S5. T-cell specific Loss of *Psip1* does not alter T-cell development in mice. Thymi of 42 days old litter mates, that are either wild-type (*Psip1* WT: *Lck-Cre*$^{+/+}$ *Psip1*$^{fl/fl}$) or knockout for *Psip1* (*Psip1* KO: *Lck-Cre*$^{tg/+}$ *Psip1*$^{fl/fl}$), were collected and no difference was observed in the size of their thymi (A)(litters: 2, *Psip1* WT: n=5, *Psip1* KO: n=7). Additionally, there were no significant differences in T-cell fractions (B) between *Psip1* KO mice and their wild-type control littermates: double negative (DN) T-cells, single positive (SP) CD4+ T-cells, SP CD8+ cells and double positive (DP) T-cells (litters: 3, *Psip1* WT: n=10, *Psip1* KO= 11).

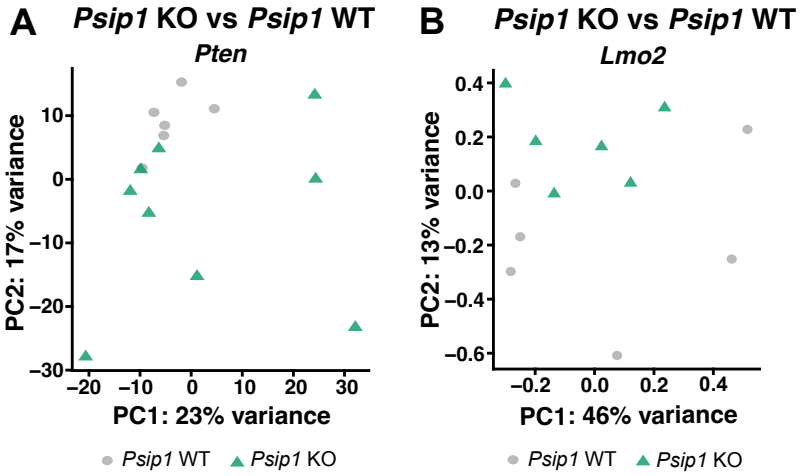

Fig. S6. Low degree of separation between Psip1 KO tumors versus Psip1 WT on principal component analysis (PCA). The PCA plots of RNAseq data on thymoma samples of Lck-Cre Pten (Pten)(A) or CD2-iCre CD2-Lmo2 (Lmo2)(B) mice, that are either wild-type (Psip1 WT, grey circles) or knockout (Psip1 KO, green triangles) for Psip1. There is a minimal separation between the Psip1 KO and Psip1 WT genotype in both T-ALL models.

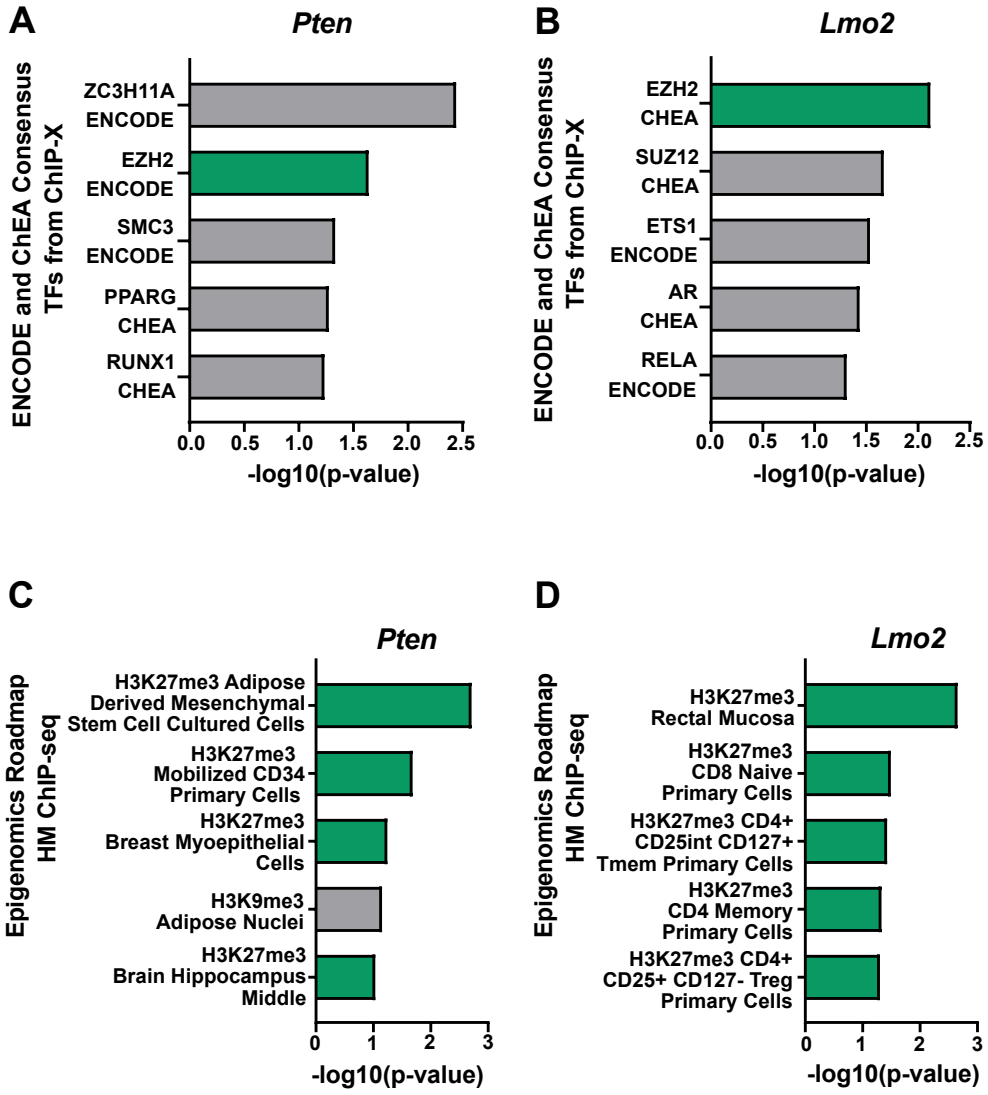

Fig. S7. Enrichment for EZH2 target genes and genes found in proximity of H3K27me3 histone marks in genes downregulated upon Psip1 loss in two different spontaneous T-ALL mouse models. Analysis of the downregulated genes upon Psip1 knockout in RNA-seq data from thymoma samples of the two different spontaneous T-ALL mice models, namely Lck-Cre Pten (Pten)(A,C) or CD2-iCre CD2-Lmo2 (Lmo2)(B,D) model, showed an enrichment for target genes of EZH2 in 'ENCODE and ChEA consensus transcription factors (TFs) from X-ChIP' (A, B) and an enrichment for the proximity of H3K27me3 in 'Epigenomics Roadmap HM ChIP-seq' (C,D).

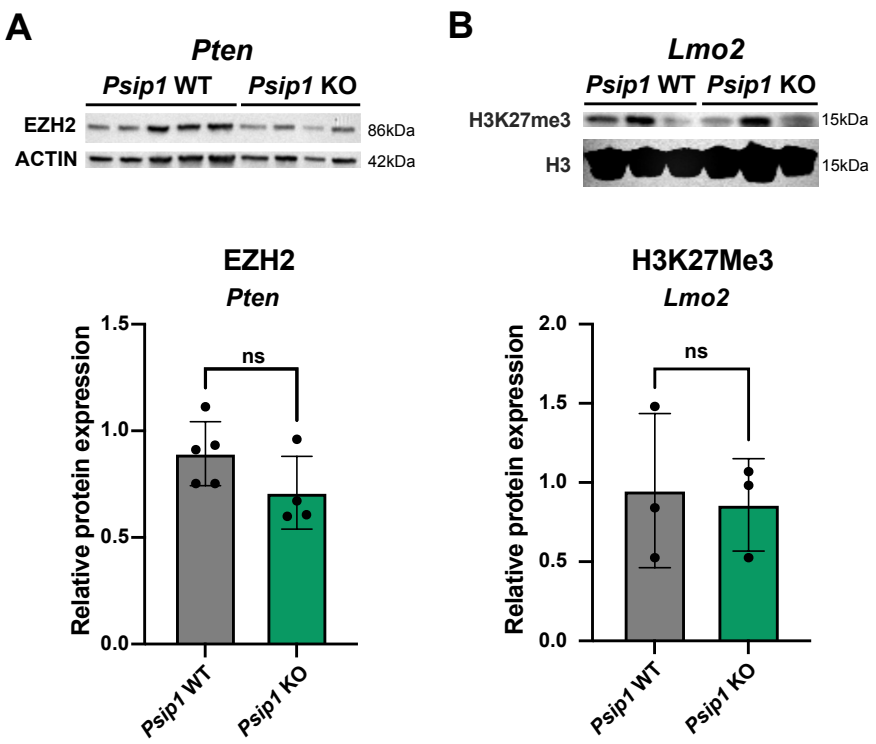

Fig. S8. Validation of EZH2 and H3K27me3 protein levels in preleukemic thymus samples. (A) Western blot analysis (top) and normalization (bottom) for EZH2 and Actin (loading control) in thymi of 6-week-old Lck-Cre Pten (Pten) mice. The protein level of EZH2 normalized against house-keeping gene β-Actin (Mann-Whitney test). (B) Western blot analysis (top) and normalization (bottom) for H3K27me3 in thymi of 6-week-old CD2-iCre CD2-Lmo2 (Lmo2) mice. The protein level of H3K27me3 was normalized against the total H3 protein level (Mann-Whitney test). ns: not significant.

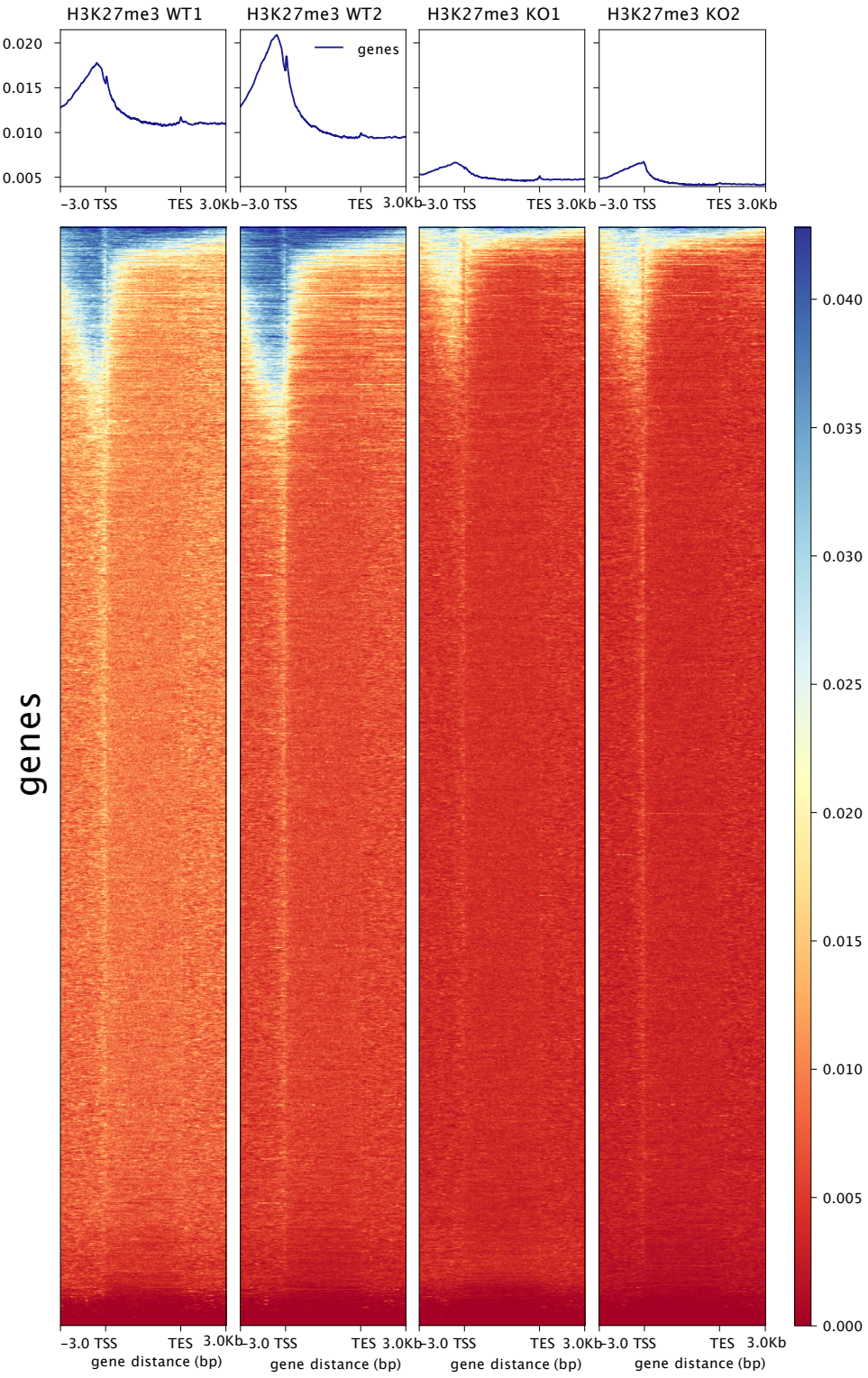

Fig. S9. Reduced H3K27me3 levels in thymus samples of Psip1 knockout mice. CUT&RUN analysis (Nextflow pipeline (56)) for H3K27me3 binding in preleukemic thymus samples of 6-week-old *Psip1*$^{fl/fl}$/*CD2*-i*Cre*$^{tg/+}$ (KO) or Cre-negative *Psip1*$^{fl/fl}$/*CD2*-i*Cre*$^{+/+}$ littermate control (WT) mice (n=2 for each group). Individual heatmaps (bottom) and metanalysis (top) of H3K27me3-binding profiles −3 kb and +3 kb around the transcription start site.

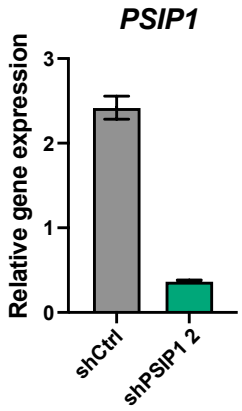

Fig. S10. Validation of doxycycline-inducible vectors. Jurkat cells were transduced with doxycycline-inducible shRNA vectors (shCtrl: a control hairpin, shPSIP1 2: shRNA 2 targetting PSIP1, same shRNA as in non-inducible vectors) and treated for 72h with doxycycline *in vitro*. RT-qPCR validation that the mRNA level of PSIP1 is downregulated upon doxycycline treatment.

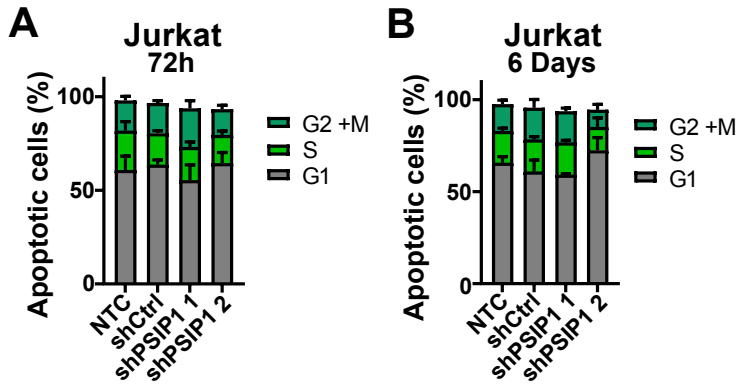

Fig. S11. Knockdown of PSIP1 expression does not impair cell cycle progression in Jurkat. Upon PSIP1 knockdown, no difference can be noted upon cell cycle analysis compared to the control cells, neither at a 72h (A) or 6-day (B) timepoint. NTC: non-transduced control

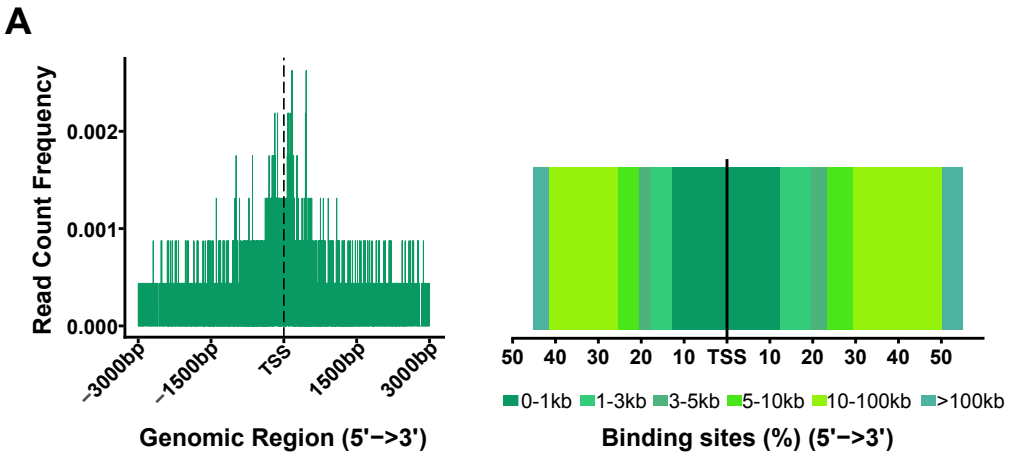

Fig. S12. PSIP1 binds mostly in proximity of the transcription start site. PSIP1 CUT&RUN data reveals that, as priorly described6, PSIP1 primarily binds in proximity of the transcription start site (TSS), which is indicated by the genomic localization of the detected reads.

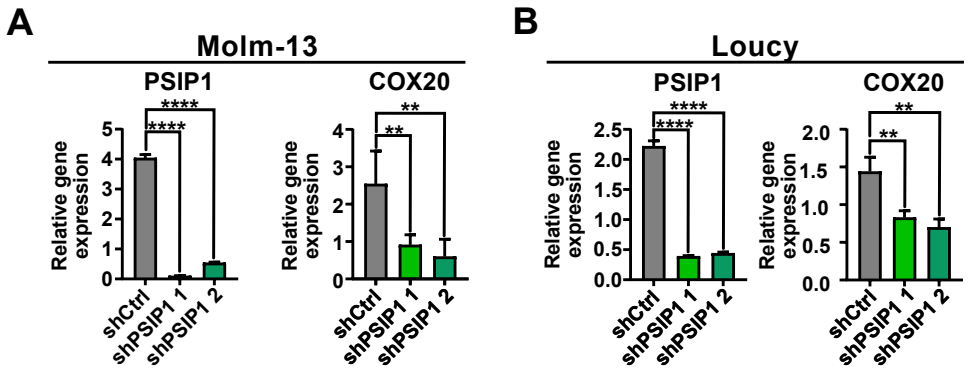

Fig. S13. PSIP1 knockdown leads to a downregulation of COX20 on mRNA level in leukemic cell lines. RT-qPCR validation that the mRNA level of COX20 is downregulated upon PSIP1 knockdown and is reproducible in multiple cell lines (Molm-13 (A), Loucy (B)) (n = 3; test: one-way ANOVA with Dunnett's multiple comparisons test). **: $p < 0.01$; ****: $p < 0.0001$

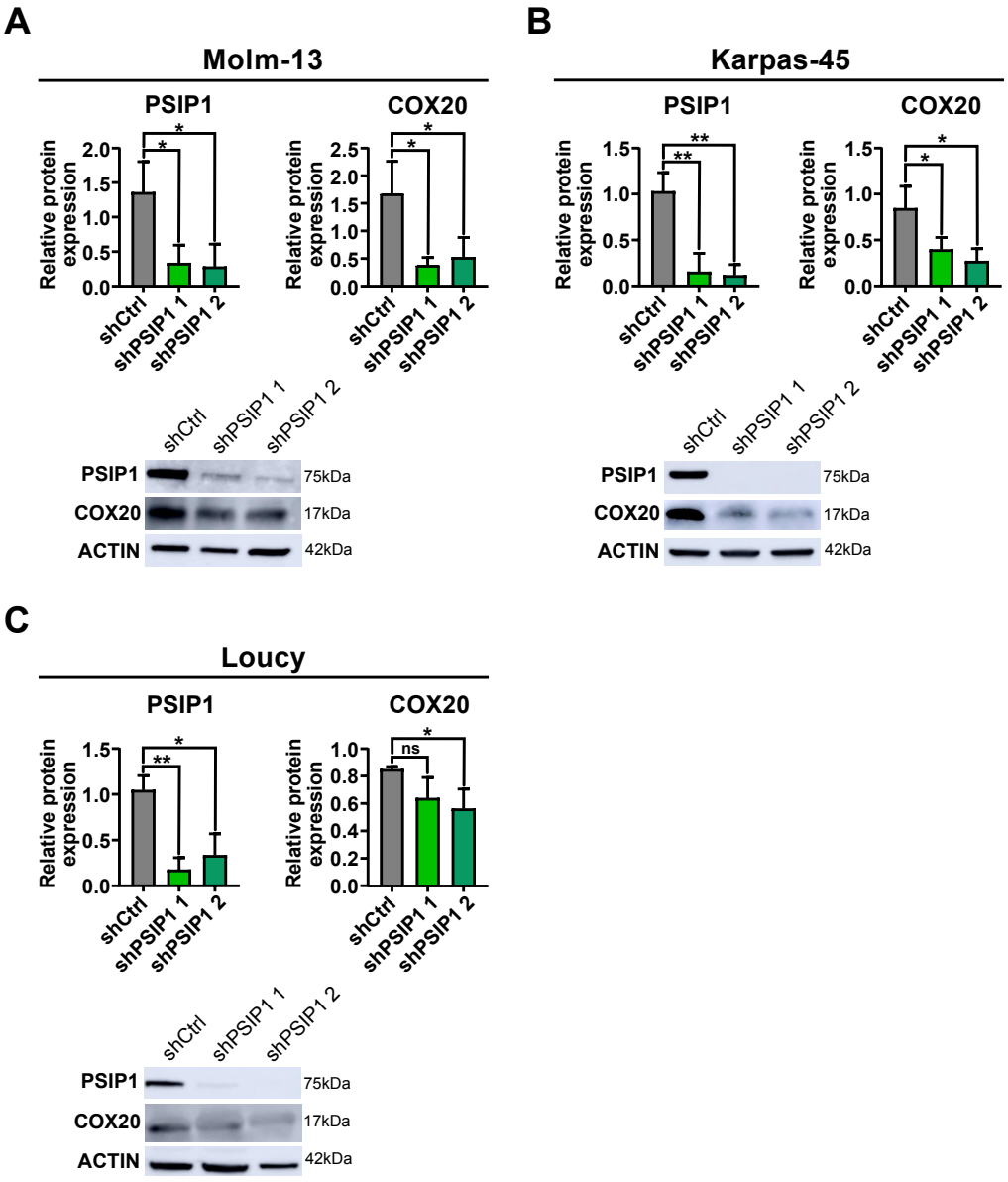

Fig. S14. PSIP1 knockdown leads to a downregulation of COX20 on protein level in leukemic cell lines. Western blot validation that the protein level of COX20 is downregulated upon PSIP1 knockdown and is reproducible in multiple cell lines (Molm-13 (A), Karpas-45 (B), and Loucy (C)) (one blot representative for 3 replicates, normalization: n = 3, one-way ANOVA with Dunnett's multiple comparisons test). ns: non-significant; *: $p < 0.05$; **: $p < 0.01$

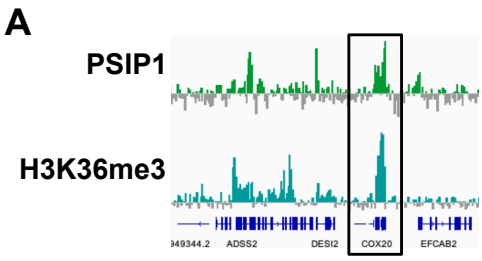

Fig. S15. Although no PSIP1 peak was called on the COX20 gene locus, PSIP1 binding signal was detected. IGV image of CUT&RUN data of PSIP1 and H3K36me3 binding in Jurkat. MACS2 was not able to significantly call a peak for PSIP1 at the COX20 gene locus. However, upon closer observation of the IgG-subtracted tracks in IGV, to some extent PSIP1 binding could be observed. A peak for H3K36me3, a histone mark recognized by PSIP1, could be called in the gene region.

Table S1. Tissue affected and immunophenotype tumors in T-ALL mouse models.

Table S2. Differentially expressed genes in *Lck-Cre Psip1 Pten* cohort

Table S3. Differentially expressed genes in *CD2-iCre Psip1 CD2-Lmo2* cohort

Table S4. Differentially expressed genes upon PSIP1 knockdown in Jurkat cells.

Table S5. Summary of HOMER results of H3K27me3 Cut&Run.

Table S6. Summary of MEME analysis of H3K27me3 Cut&Run.

shRNA nr	*Region*	*TRC nr*	*Target sequence*	*Backbone*
shCtrl	no target	SHC002	CAACAAGATGAAGAGCACCAA	TRC1
shPSIP1 1	CDS (exon 5)	TRCN0000074819	GCAGCAACTAAACAATCAAAT	TRC1
shPSIP1 2	CDS (exon 7-8)	TRCN0000074820	GCAGCTACAGAAGTCAAGATT	TRC1
shPsip1 1	CDS	TRCN0000012116	CGGTTCAAAGTCAGTCAAGTT	TRC1
shPsip1 2	CDS	TRCN0000012117	AGATGAAAGGTTATCCTCATT	TRC1
shCOX20 1	CDS	TRCN0000218942	GCAGCAATTGAACAATCTTGA	TRC2
shCOX20 1	CDS	TRCN0000234379	TGTTGGAGTAGGAGGGTTTAT	TRC2

Table S7. Short hairpins.

Gene	*Forward primer*	*Reverse primer*	Species
PSIP1	AAAACAGGGGTTACTTCAACCTC	GGCCTTTCAGCATATTCCTTCT	Human
COX20	TTCATTAGGATCTGTTGTGGCT	CCGTTGTGTTTTCTTTCAGGAT	Human
YWHAZ	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT	Human
HMBS	GGCAATGCGGCTGCAA	GGGTACCCACGCGAATCAC	Human
UBC	ATTTGGTGCGCGGTTCTTG	TGCCTTGACATTCTCGATGGT	Human
TBP	CACGAACCACGGCACTGATT	TTTTCTTGCTGCCAGTCTGGAC	Human
SDHA	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG	Human
Psip1	ACCTGGAGACCTCATCTTCG	AAAGCAGTCTCATGGGTTCC	Mouse
Hprt1	GGATTTGAATCACGTTTGTGT	TGGCAACATCAACAGGACTC	Mouse
Gapdh	CCCCAATGTGTCCGTCGTG	GCCTGCTTCACCACCTTCT	Mouse
G6pdh	ATGCAGAACCACCTCCT	TTCAACACTTTGACCTTCTCA	Mouse
Rpl13a	CCTGCTGCTCTCAAGGTTGTT	TGGTTGTCACTGCCTGGTACTT	Mouse
Matr3	TGGACCAAGAGGAAATCTGG	TGAACAACTCGGCTGGTTTC	Mouse
Eef1a1	TCGCCTTGGACGTTCTTTT	GTGGACTTGCCGGAATCTAC	Mouse
Tbp	TCTACCGTGAATCTTGGCTGTAAA	TTCTCATGATGACTGCAGCAAA	Mouse

Table S8. qPCR primers.