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Supplemental Information

Cooperative Enhancer Activation by TLX1 and STAT5 Drives Development of NUP214-ABL1/TLX1-Positive T Cell Acute Lymphoblastic Leukemia

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Table S1 (Related to Figure 1): NUP214-ABL1^{+} T-ALL cases from published studies

#	Gender	Adult/Pediatric	Age	Immune phenotype	ABL1	Other alterations
Graux et al., 2004						
1	M	A	52	cortical	NUP214-ABL1 fusion	TLX1
2	M	P	3	cortical	NUP214-ABL1 fusion	TLX1
3	M	A	23	cortical	NUP214-ABL1 fusion	TLX1
4	M	P	7	mature	NUP214-ABL1 fusion	TLX3
5	M	A	25	mature	NUP214-ABL1 fusion	TLX3
6	F	P	ped	cortical	NUP214-ABL1 fusion	TLX3
7	M	P	9	?	NUP214-ABL1 fusion	TLX3
8	M	P	4	?	NUP214-ABL1 fusion	TLX3
9	F	A	31	pre T	NUP214-ABL1 fusion	TLX1
Graux et al., 2008						
10	M	A	28	mature	NUP214-ABL1 fusion	TLX3
11	F	A	36	cortical	NUP214-ABL1 fusion	TLX1
12	M	P	3	pre T	NUP214-ABL1 fusion	TLX1
13	F	P	14	cortical	NUP214-ABL1 fusion	TLX3
14	F	P	6	?	NUP214-ABL1 fusion	TLX3
15	F	A	28	Cortical	NUP214-ABL1 fusion	TLX1
16	M	A	22	Cortical	NUP214-ABL1 fusion	TLX1
17	M	P	7	Cortical	NUP214-ABL1 fusion	TLX3
18	M	P	18	Cortical	NUP214-ABL1 fusion	TLX1
19	M	P	11	pre T	NUP214-ABL1 fusion	TLX3
20	M	A	26	Cortical	NUP214-ABL1 fusion	TLX3
21	M	P	5	pre T	NUP214-ABL1 fusion	TLX3
22	M	P	8	pre T	NUP214-ABL1 fusion	TLX3
23	M	P	17	pre T	NUP214-ABL1 fusion	TLX3
24	M	P	12	pre T	NUP214-ABL1 fusion	TLX3
25	M	A	40	pre T	NUP214-ABL1 fusion	TLX1
26	M	A	48	Cortical	NUP214-ABL1 fusion	TLX1
27	M	P	9	?	NUP214-ABL1 fusion	TLX3
28	M	P	11	?	NUP214-ABL1 fusion	TLX3
29	M	P	12	?	NUP214-ABL1 fusion	TLX3
30	F	P	14	?	NUP214-ABL1 fusion	TLX3
31	M	A	42	?	NUP214-ABL1 fusion	TLX1
32	M	P	2	?	NUP214-ABL1 fusion	TLX1
33	M	A	36	?	NUP214-ABL1 fusion	TLX1
Burmeister et al., 2006						
34	M	A	18	thymic	NUP214-ABL1 fusion	TLX3
35	M	A	20	thymic	NUP214-ABL1 fusion	/
36	M	A	39	thymic	NUP214-ABL1 fusion	TLX1
37	M	A	34	thymic	NUP214-ABL1 fusion	TLX1

38	M	A	28	thymic	NUP214-ABL1 fusion	TLX1
39	M	A	23	thymic	NUP214-ABL1 fusion	TLX1
40	M	A	18	thymic	NUP214-ABL1 fusion	/
41	M	A	18	early T	NUP214-ABL1 fusion	TLX3
42	F	A	27	thymic	NUP214-ABL1 fusion	TLX1
Liu et al., 2017						
43	M	P	13	Cortical	NUP214-ABL1 fusion	TLX1
44	M	P	4	?	NUP214-ABL1 fusion	TLX3
45	M	P	7	Cortical	NUP214-ABL1 fusion	TLX3
46	M	P	7	Pre-cortical	NUP214-ABL1 fusion	TLX3

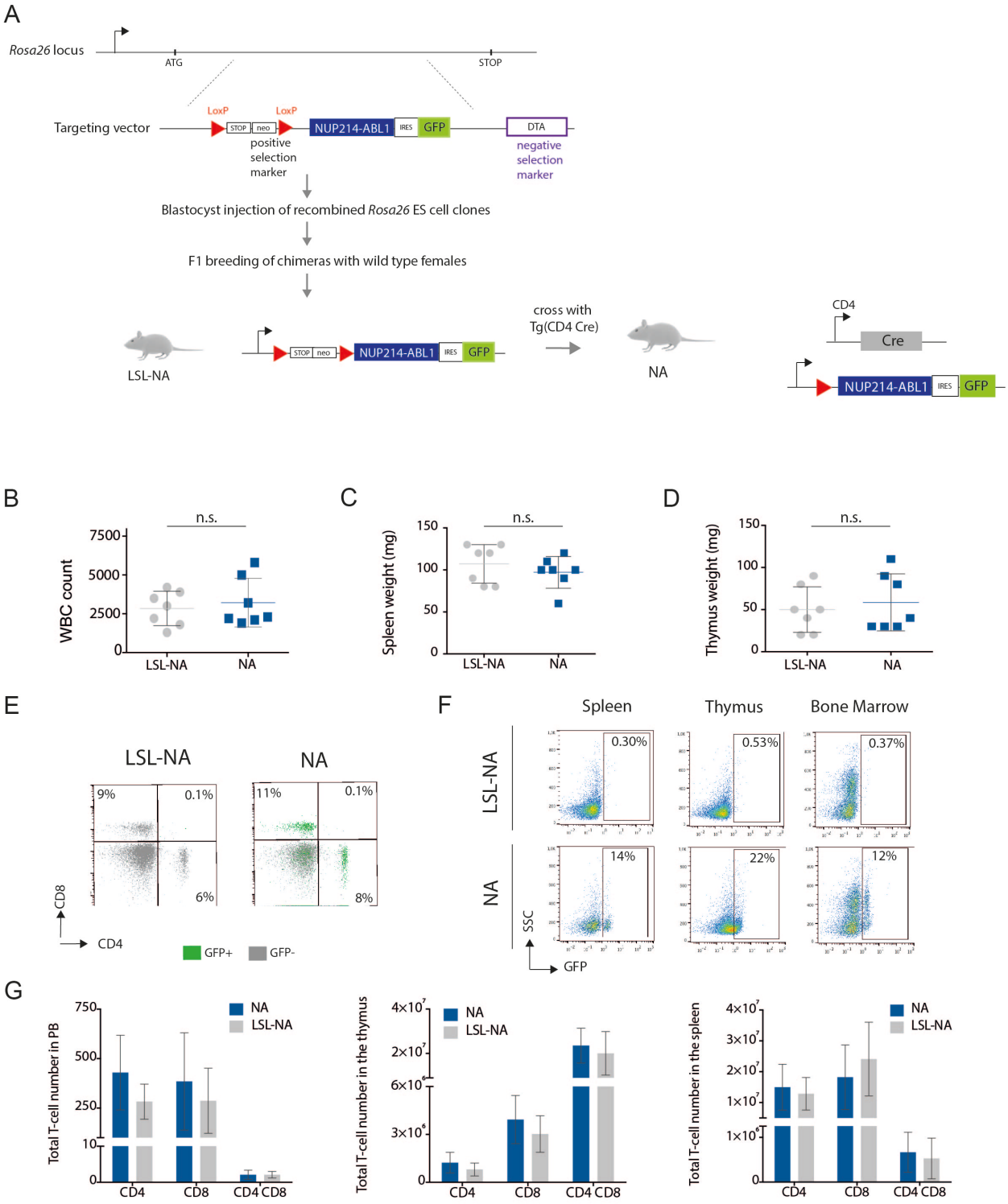

Figure S1 (Related to Figure 1): Expression of NUP214-ABL1 alone is not sufficient to cause T-ALL in a transgenic mouse model

(A) Schematic overview of the generation of the LSL-NA and the NA transgenic mouse models through homologous recombination in mouse embryonic stem (ES) cells. **(B-D)** White blood cell count (WBC, cells/µL) **(B)**, Spleen weight **(C)** and thymus weight **(D)** in LSL-NA and NA mice at end

stage (>360 days). Data are represented as mean ± SD Statistical significance was calculated using unpaired two tailed t-test with equal variance. **(E)** Peripheral blood staining for CD4 and CD8 T cells in LSL-NA and NA mice at end stage. **(F)** FACS analysis of spleen, thymus and bone marrow analyzing the frequency of GFP cells in LSL-NA and NA mice at end stage. **(G)** Total T cell numbers in LSL-NA and NA in peripheral blood (1 µL), thymus or spleen. Data are represented as mean ± SD. Statistical significance calculated using unpaired two tailed t-test with equal variance.

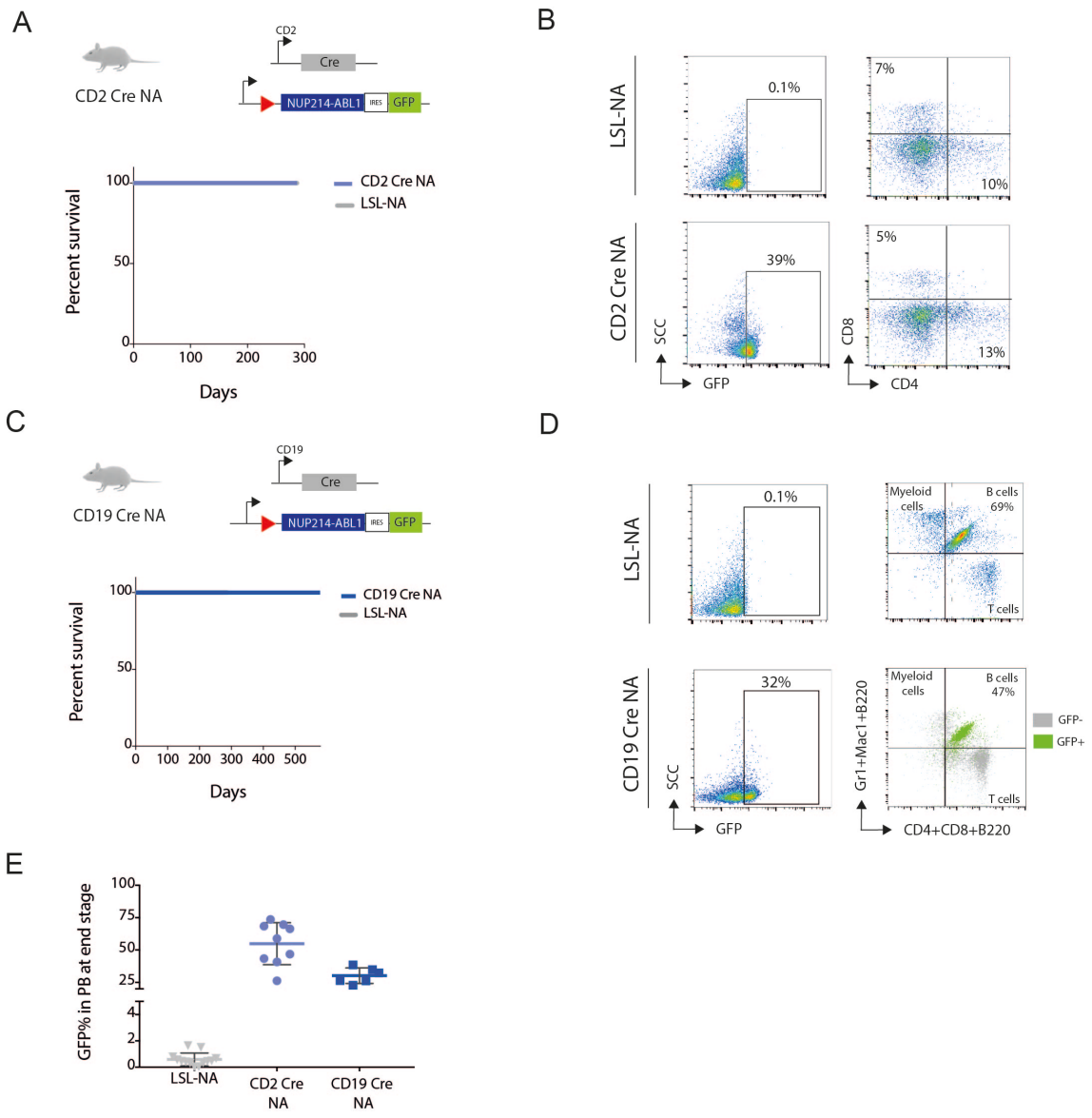

Figure S2 (Related to Figure 1): Expression of NUP214-ABL1 in lymphoid progenitors or B-cells is not sufficient to cause leukemia development

(A) Schematic of LSL-NA mice crossed with CD2 Cre mice (top) and associated Kaplan-Meier survival curve for CD2 Cre NA and LSL-NA mice (bottom). **(B)** FACS analysis of peripheral blood for GFP and CD4/CD8 staining of LSL-NA and CD2 Cre NA mice. **(C)** Schematic of LSL-NA mice crossed with CD19 Cre mice (top) and associated Kaplan-Meier survival curve for CD19 Cre NA and LSL-NA mice (bottom). **(D)** FACS analysis of peripheral blood for GFP and proportion of myeloid cells, B cells and T cells in CD19 Cre NA mice. **(E)** Percentage of GFP positive cells in peripheral blood of LSL-NA, CD2 Cre NA and CD19 Cre NA mice at end stage (>360 days). Data are represented as mean ± SD.

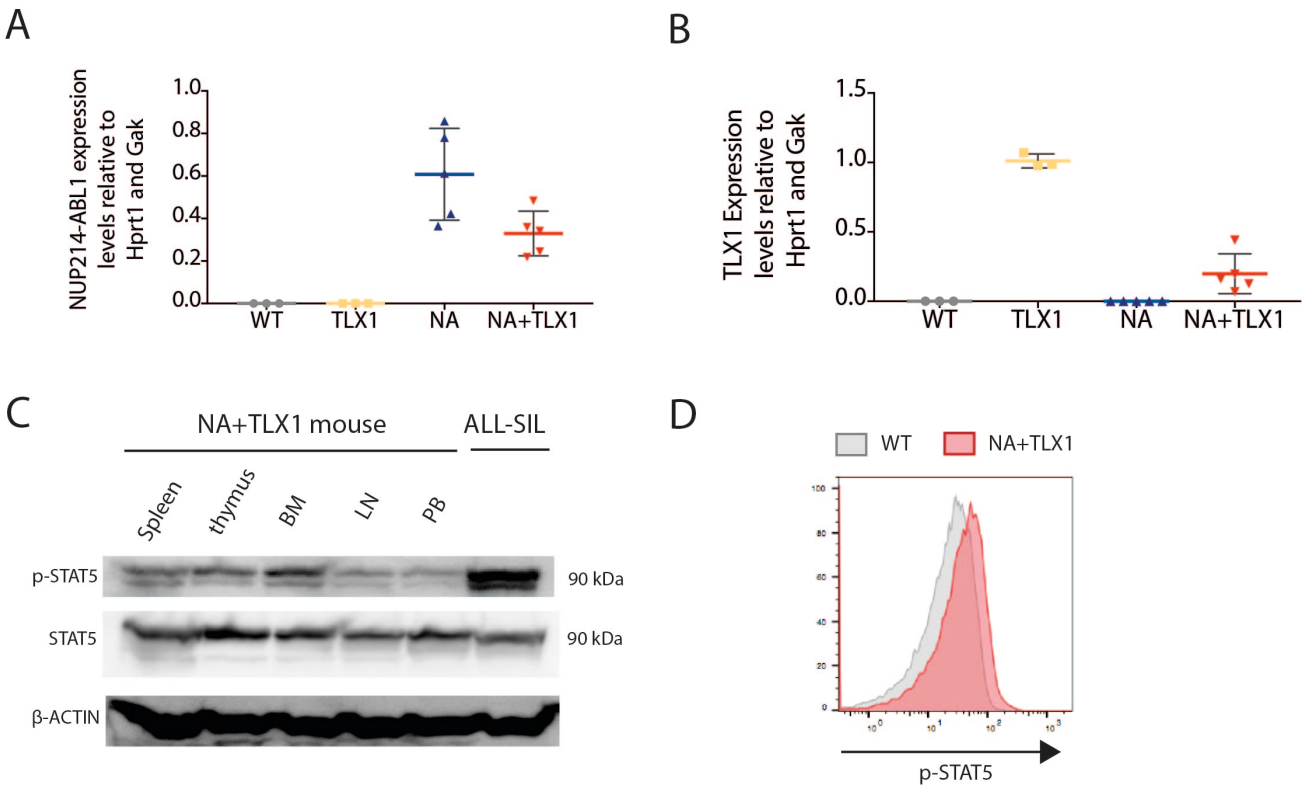

Figure S3 (Related to Figure 1): NUP214-ABL1 and TLX1 expression in the transgenic mouse models.

(A-B) qRT-PCR of NUP214-ABL1 (**A**) or TLX1 (**B**) expression in $CD4^{+}CD8^{+}$ thymus or spleen cells harvested from the different transgenic mouse models. Data are represented as mean ± SD. Statistical significance calculated using unpaired two tailed t-test with equal variance. **(C)** Western blot to show activation of STAT5 in different tissues of NA+TLX1 mice and in ALL-SIL cells. **(D)** phospho-flow for phospho-STAT5 (p-STAT5) in peripheral blood cells of NA+TLX1 mice.

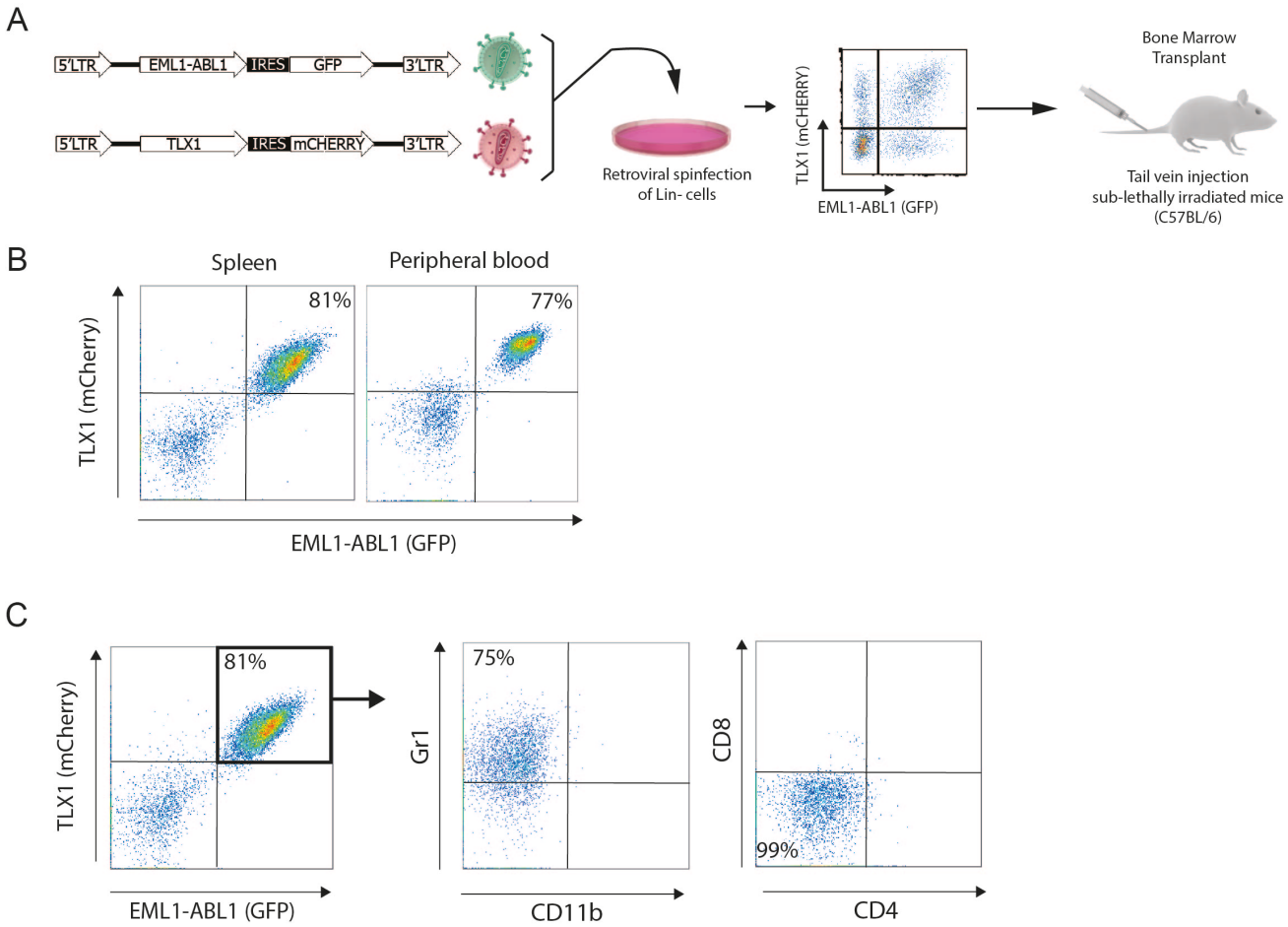

Figure S4 (Related to Figure 1): Cooperation between EML1-ABL1 and TLX1 in an ex vivo pro T cell system and in a mouse bone marrow transplant model.

(A) Schematic of the strategy followed for the bone marrow transplant assay **(B)** FACS analysis of spleen and peripheral blood for EML1-ABL1 (GFP) and TLX1 (mCHERRY) expression in spleen cells (left) and peripheral blood (right) from a EML1-ABL1+TLX1 leukemic mouse. **(C)** FACS analysis of EML1-ABL1+ TLX1+ spleen cells from a EML1-ABL1 + TLX1 leukemic mouse, showing expression of Gr1 and CD11b or CD4 and CD8.

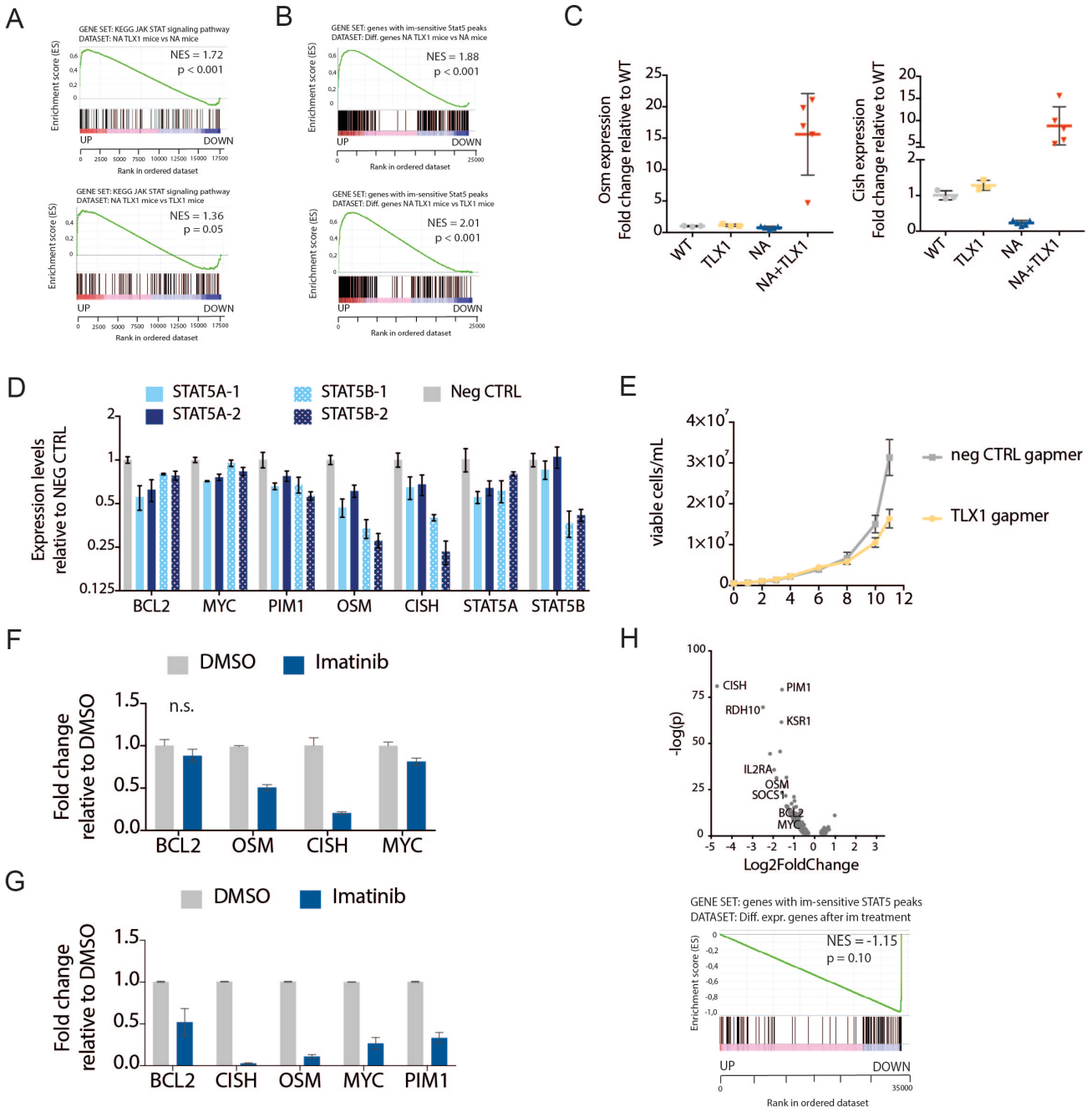

Figure S5 (Related to Figure 2): NUP214-ABL1 and TLX1 activate the JAK-STAT pathway through STAT5 signaling

(A) Gene set enrichment analysis (GSEA) showing enrichment of JAK-STAT pathway genes in the differentially expressed genes in NA+TLX1 compared to NA (top) or NA+TLX1 compared to TLX1 (bottom). (NES = normalized enrichment score). **(B)** GSEA showing enrichment of STAT5 target genes (as defined by ChIP-seq) in the differentially expressed genes in NA+TLX1 compared to NA (top) or TLX1 (bottom). (NES = normalized enrichment score). **(C)** qRT-PCR to show *Osm* (left) and *Cish* (right) expression in the different transgenic mouse models. Statistical significance calculated using unpaired two tailed t-test with equal variance. Data are represented as mean ± SD. **(D)** qRT-PCR of STAT5 target genes after 48 hr treatment with 10 μM STAT5 gapmers. Data are

represented as mean ± SD. **(E)** Growth curve of ALL-SIL cells treated with 10 µM TLX1 or negative control (neg CTRL) gapmer for 12 days. Data are represented as mean ± SD. **(F,G)** qRT-PCR of STAT5 target genes after 3 hr imatinib treatment in the NUP214-ABL1^{+}TLX1^{+} cell line PEER **(F)** or NUP214-ABL1^{+}TLX3^{+} patient-derived xenograft cells (X12) **(G)**. Data are represented as mean ± SD. **(H)** Volcano plot showing up- and downregulated genes (top) and GSEA to show enrichment of STAT5 target genes in differentially expressed genes (bottom) after imatinib treatment (500 nM imatinib or DMSO for 3 hr) in a NUP214-ABL1^{+}TLX3^{+} patient-derived xenograft sample X12 (n=3 – experiment was performed using human leukemic cells isolated from 3 separate xenograft NSG mice).

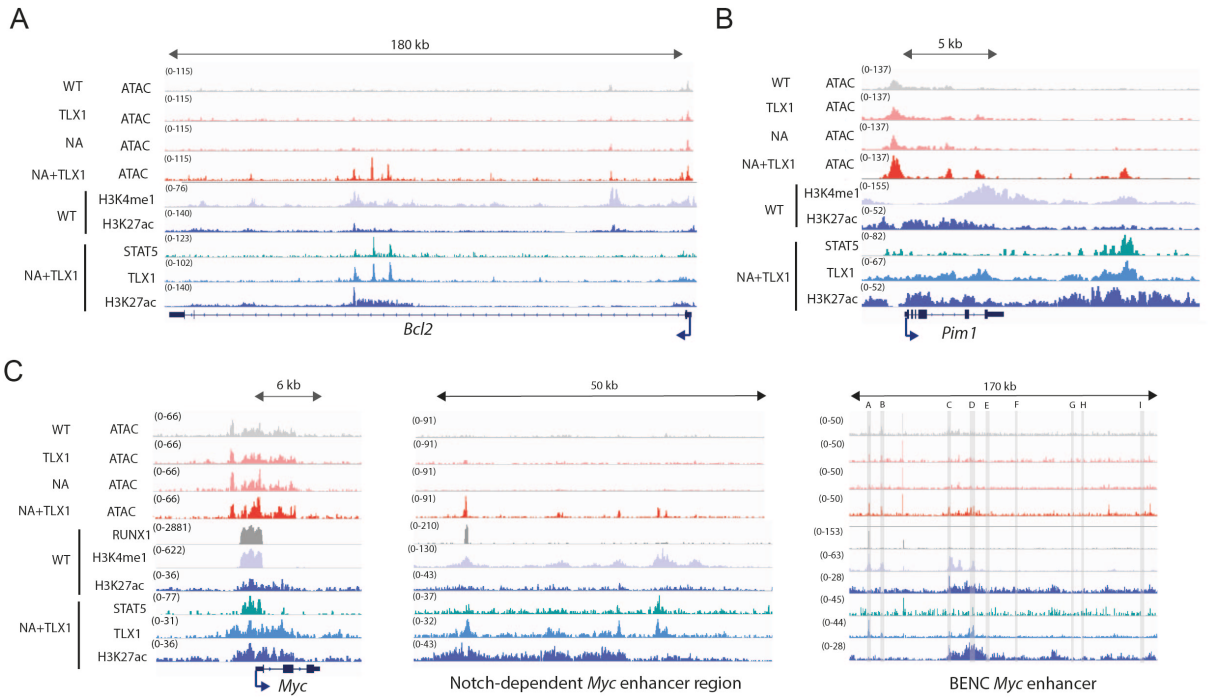

Figure S6 (related to Figure 4): TLX1 and STAT5 bind in newly accessible enhancer regions.
(A-C) ATAC-seq tracks (performed in $CD4^{+}CD8^{+}$ WT, NA, TLX1 and NA+TLX1 cells) and ChIP-seq tracks (H3K4me1, H3K27ac in WT cells, STAT5, TLX1, H3K27ac in NA+TLX1 cells) at the *Bcl2* locus **(A),** the *Pim1* locus **(B)** and at the *Myc* gene locus and enhancer loci **(C)**.

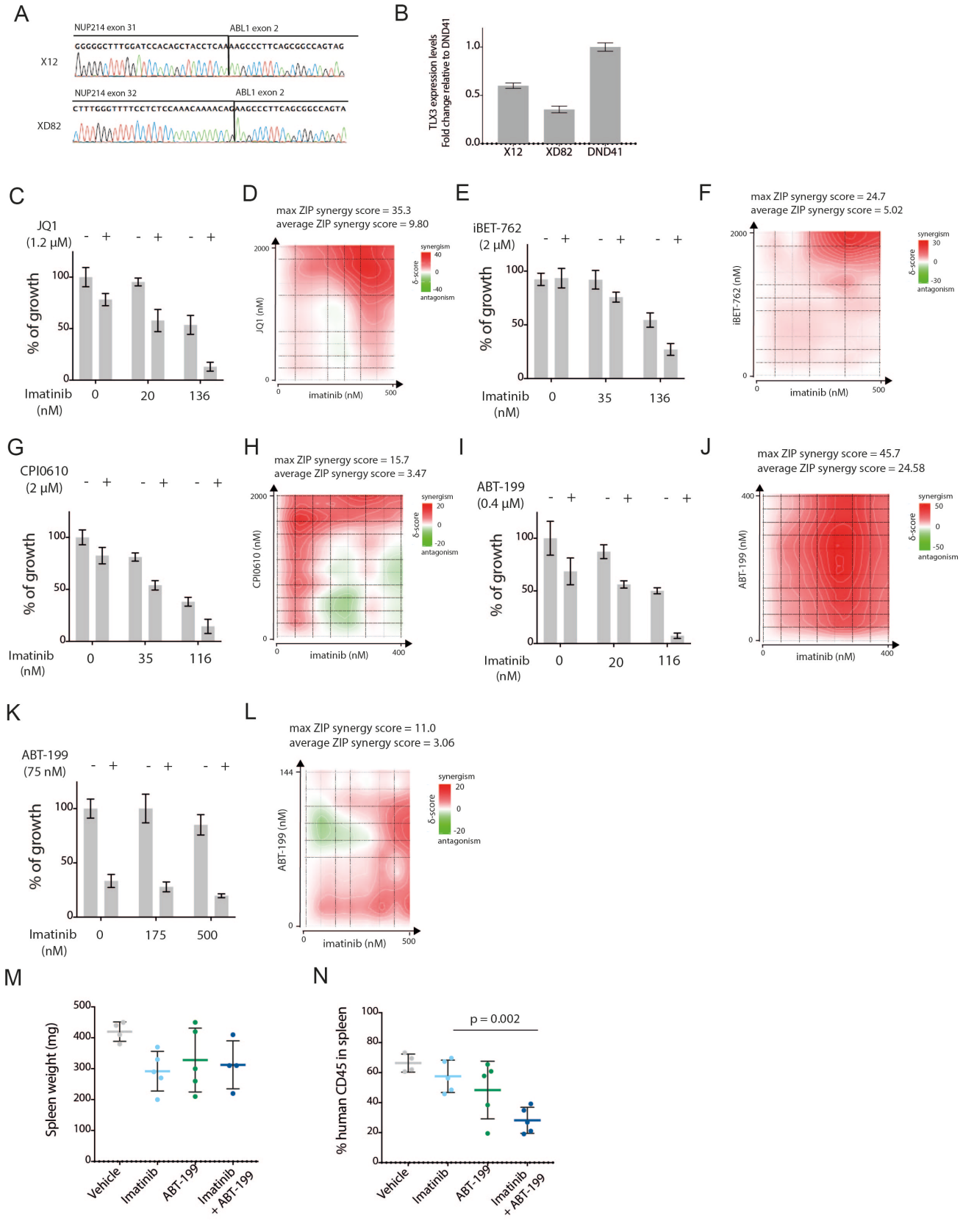

Figure S7 (Related to Figure 6): Downstream effectors of NUP214-ABL1 and TLX1 can be targeted to improve treatment strategies.

(A) Sanger sequencing profile of the *NUP214-ABL1* fusion detected in PDX samples X12 and

XD82. **(B)** qRT-PCR analysis of *TLX3* expression in T-ALL PDX samples X12 and XD82. Data are represented as mean ± SD. **(C)** Growth of ALL-SIL cells after 48 hr treatment with imatinib with or without JQ1 (1.2 μM). Data are represented as mean ± SD. **(D)** Synergy matrix plot showing δ-scores for ALL-SIL cells treated with imatinib + JQ1. **(E)** Growth of ALL-SIL cells after 48 hr treatment with imatinib with or without iBET-762 (2 μM). Data are represented as mean ± SD. **(F)** Synergy matrix plot showing δ-scores for ALL-SIL cells treated with imatinib + iBET-762. **(G)** Growth of ALL-SIL cells after 48 hr treatment with imatinib with or without CPI0610 (2 μM). Data are represented as mean ± SD. **(H)** Synergy matrix plot showing δ-scores for the combination treatment of ALL SIL cells with imatinib + CPI0610. **(I)** Growth of ALL-SIL cells after 48 hr treatment with imatinib with or without ABT-199 (0.4 μM). Data are represented as mean ± SD. **(J)** Synergy matrix plot showing δ-scores for the combination treatment of ALL SIL cells with imatinib + ABT-199. **(K)** Growth of NA+TLX1 mouse leukemic cells after 48 hr treatment with imatinib with or without ABT-199 (75 nM). Data are represented as mean ± SD. **(L)** Synergy matrix plot showing δ-scores for the combination treatment of NA+TLX1 mouse leukemic cells with imatinib + ABT-199. **(M)** Spleen weight of mice treated with ABT-199, imatinib or a combination of imatinib + ABT-199. Data are represented as mean ± SD. **(N)** % human CD45 cells detected by flow cytometry in spleen samples of mice treated with ABT-199, imatinib or a combination of imatinib + ABT-199. Statistical significance calculated using unpaired two tailed t-test with equal variance. Data are represented as mean ± SD.

Table S2 (Related to STAR methods): primers for genotyping of the transgenic mouse strains

LSL-NA	
LSL-NA Fw	5'-AGAGGGGGAGGTTTCTTCAGT-3'
LSL-NA Rv	5'-ACACCATTCCCCATTGTGATTAT-3'
WT Fw	5'-CAATACCTTTCTGGGAGTTCTCTGC-3'
WT Rv	5'-CTGCATAAAACCCCAGATGACTACC-3'
TLX1	
Fw	5'-AGGTACCCTCCTTGGTGGAG-3'
Rv	5'-AAAGTAGAAGGGGGAGGGGAGG-3'
CD4 Cre / CD19 Cre	
Fw	5'-GCGGTCTGGCAGTAAAAACTATC-3'
Rv	5'-GTGAAACAGCATTGCTGTCACTT-3'
CD2 iCre	
Fw	5'-AGATGCCAGGACATCAGGAACCTG-3'
Rv	5'-ATCAGCCACACCAGACACAGAGATC-3'

Table S3 (Related to STAR methods): primers for qRT-PCR

Mouse		
Myc	Fw	5'-AGAGCTCCTCGAGCTGTTTG-3'
	Rv	5'-TGAAGTTCACGTTGAGGGG-3'
Bcl2	Fw	5'-AGTACCTGAACCGGCATCTG-3'
	Rv	5'-AGGGTCTTCAGAGACAGCCA-3'
Osm	Fw	5'-TGCTCCAACTCTTCCTCTCAG-3'
	Rv	5'-CAGGTGTGTTCAGGTTTTGG-3'
Cish	Fw	5'-CAGAGAATGAACCGAAGGTG-3'
	Rv	5'-CCTCGCTGGCTGTAATAGAAC-3'
Human		
TLX1	Fw	5'-GACAAAGTGGAGACGGCAGA-3'
	Rv	5'-CTGTGCCAGGCTCTTCTGG-3'
NUP214-ABL1	Fw	5'-AGGAAAACCCAGTCAGGATG-3'
	Rv	5'-TGAGGCTCAAAGTCAGATGC-3'
MYC	Fw	5'-AAAACCAGCAGCCTCCCGCGA-3'
	Rv	5'-AATACGGCTGCACCGAGTCGT-3'
OSM	Fw	5'-CAGCTCCAGAAGCAGACAGA-3'
	Rv	5'-CCCTGCAGTGCTCTCTCAGT-3'
PIM1	Fw	5'-AGGGTCTCTTCAGAATGTCAGC-3'
	Rv	5'-TGGATCTCAGCAGTTTCCTG-3'
CISH	Fw	5'-CTGCTGTGCATAGCCAAGAC-3'
	Rv	5'-GTGCCTTCTGGCATCTTCTG-3'
BCL2	Fw	5'-GCCCTGTGGATGACTGAGTA-3'
	Rv	5'-AGGGCCAAACTGAGCAGAG-3'