SUPPLEMENTARY APPENDIX

A novel xenograft model to study the role of TSLP-induced CRLF2 signals in normal and malignant human B lymphopoiesis

Olivia L. Francis,[1*] Terry-Ann M. Milford,[1*] Shannalee R. Martinez,[1] Ineavely Baez,[1] Jacqueline S. Coats,[1] Karina Mayagoitia,[1] Katherine R. Concepcion,[1] Elizabeth Ginelli,[1] Cornelia Beldiman,[1] Abigail Benitez,[1] Abby J. Weldon,[1] Keshav Arogyaswamy,[1] Parveen Shiraz,[1] Ross Fisher,[1] Christopher L. Morris,[1] Xiao-Bing Zhang,[1] Valeri Filippov,[1] Ben Van Handel,[2] Zheng Ge,[3,4] Chunhua Song,[4] Sinisa Dovat,[4] Ruijun Jeanna Su,[1*] and Kimberly J. Payne[1*]

[1]Loma Linda University, CA, USA; [2]Novogenix Laboratories, LLC, 1425 San Pablo, CA, USA; [3]The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Department of Hematology, Nanjing 210029, China; and [4]Pennsylvania State University Medical College, Department of Pediatrics, Hershey, PA, USA

**OLF and TMM contributed equally to this work.*

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Correspondence: kpayne@llu.edu

Supplementary Materials

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[†]Olivia L. Francis,[1] [†]Terry-Ann M. Milford,[1] Shannalee R. Martinez,[1] Ineavely Baez,[1] Jacqueline S. Coats,[1] Karina Mayagoitia,[1] Katherine R. Concepcion,[1] Elizabeth Ginelli,[1] Cornelia Beldiman[1] Abigail Benitez,[1] Abby J. Weldon,[1] Keshav Arogyaswamy,[1] Parveen Shiraz,[1] Ross Fisher,[1] Christopher L. Morris,[1] Xiao-Bing Zhang,[1] Valeri Filippov,[1] Ben Van Handel,[2] Zheng Ge,[3,4] Chunhua Song,[4] Sinisa Dovat,[4] Ruijun Jeanna Su,[1*] and Kimberly J. Payne[1*]

[1]Loma Linda University, Department of Pathology and Human Anatomy, Loma Linda, CA 92350
[2] Novogenix Laboratories, LLC, 1425 San Pablo, BCC 205, LA CA 90033.
[3]The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Department of Hematology, Nanjing 210029, China
[4]Pennsylvania State University Medical College, Department of Pediatrics, Hershey, PA 17033

[†]OLF and TMM contributed equally to this work.

*Correspondence to:

Kimberly J. Payne, Department of Pathology and Human Anatomy, Center for Health Disparities and Molecular Medicine, First floor Mortensen Hall, 11085 Campus St., Loma Linda University Telephone: 909-558-4300 Ext. 81363 FAX: 909-558-0177

Ruijun Jeanna Su, Department of Pathology and Human Anatomy, Center for Health Disparities and Molecular Medicine, First floor Mortensen Hall, 11085 Campus St., Loma Linda University Telephone: 909-558-4300 Ext. 81372 FAX: 909-558-0177

Supplementary Methods

Cells and Cell Culture Media

The human stromal cell line HS-27a (HS27) was selected for use in our model because 1) HS27 is one of a limited number of human bone marrow-derived stromal cell lines, 2) HS27 secretes low levels of growth factors that do not support proliferation of isolated progenitor cells in co-cultures;[1] and 3) our laboratory has found that HS27 grow robustly in culture. HS27 stroma were maintained in RPMI 1640 medium (Irvine Scientific) supplemented with penicillin/streptomycin, L-glutamine, 50 μM 2-ME and 10% FBS (Omega Scientific). MUTZ5 and MHH-CALL4 were maintained in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) supplemented with penicillin/streptomycin, L-glutamine, 50 μM 2-ME and 20% FBS (Omega Scientific, Tarzana, CA). This media was also used for *in vitro* cultures with and without TSLP (both for phospho-flow assays and for gene expression profiling in response to TSLP).

Flow Cytometry

For detection of surface antigens, cells were stained using standard flow cytometry protocols, followed by fixation and permeabilization as described below to detect intracellular antigens. To assess intracellular IgM, the Fix & Perm® cell fixation and permeabilization kit (Life Technologies, Grand Island, NY)) was used according to manufacturer's instructions. To discriminate living from dying cells, in some experiments, cells were stained with fixable viability dyes (eBioscience, San Diego, CA), per manufacturer's instructions. All antibodies were monoclonal, anti-human antibodies, unless otherwise stated. Specific antibody clones and conjugates are shown below.

Antibody	**Clone**	**Manufacturer**
Ig κ light chain FITC	G20-193	BD Pharmingen (San Jose, CA)
Ig λ light chain FITC	JDC-12	
CD66b FITC	G10F5	
CD34 APC	8G12	BD Biosciences (San Jose, CA
CD56 PE	MY31	
CD79a PE	HM47	
IgM PE.Cy-5	G20-127	
Anti-Phospho AKT (pS473) Alexa Fluor 488	M89-61	
Anti-phospho STAT5 (pY694) PE	47/Stat5 (pY694)	
Anti-Phospho S6 (pS235/S236) Alexa Fluor 488	N7-548	
CD19 APC	HIB19	eBioscience (San Diego, CA)
CD19 PE.Cy7	SJ25C1	
CD45 PE.Cy7	HI30	
Ig κ light chain eFluor 450	TB28-2	
Ig λ light chain eFluor 450	1-155-2	
CD19 APC	LT19	Miltenyi Biotec (Auburn, CA)
CD29 PE	TS2/16	
CD90 APC	DG3	
CD127 PE (IL-7Ra)	MB15-18C9	
Anti-Mouse CD45 FITC	30F11	

CD10 APC.Cy7	HI10A	BioLegend (San Diego, CA)
CD14 APC	M5E2	
CD19 APC	HIB19	
CD19 Pacific Blue	HIB19	
CD34 PerCP	581	
CD38 Brilliant Violet 421	HIT2	
Ig κ light chain Pacific Blue	MHK-49	
Ig λ light chain Pacific Blue	MHL-38	
IgD PE	IA6-2	
TSLPR APC	1B4	

Statistical Analysis
Statistical analysis was performed using Students t test and data expressed as mean ± SEM. Statistical significance was defined as $p<0.05$.

Phospho-Flow Cytometry Staining
Human CRLF2 B-ALL cell lines were harvested from continuous culture and primary CRLF2 B-ALL cells were obtained as BM from patient-derived xenografts (Patient 1) or from primary patient sample (Patient 2). Cells were rested in culture without cytokines for 2 hrs, and then cultured with mTSLP, hTSLP or no cytokine for 30 mins (S6 and AKT) or 1 hour (STAT5). Cells were then harvested and stained with antibody to detect phosphorylated STAT5 (pSTAT5), AKT (pAKT) or S6 (pS6) as previously described for STAT5.[2] Primary CRLF2 B-ALL patient-derived samples were also stained with CD19 APC prior to fixation and permeabilization for detection of phospho proteins and CD19+ cells were gated for phospho-flow cytometry plots.

ELISA Assays of TSLP
Concentrations of hTSLP in the supernatant of transduced HS27 stromal culture and in sera collected from NSG mice injected with transduced stroma were measured using the Human TSLP ELISA MAX Kit (BioLegend, San Diego, CA). Stromal cell supernatants were harvested from confluent flasks when stromal cells were passaged (typically two times per week) and sera was obtained from mice at weekly time points at the time of weekly stromal cell injection. Data was collected using the uQuant microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT) and analyzed using the KC Junior, version 1.6 (BioTek Instruments, Inc., Winooski, VT)

Lentivirus Vector and TSLP-expressing Human Stromal Cells
TSLP vector was generated using standard molecular cloning techniques with complementary DNA encoding human TSLP (purchased from Thermo Scientific Open Biosystems). In this vector, the EF-1α promoter was used to drive TSLP expression. A control vector with GFP was also generated. The lentiviral vector packaging and titering were performed as previously described.[3] In brief, VSV-G–pseudotyped lentiviral vectors were prepared by calcium phosphate–mediated 3-plasmid transfection of 293T cells. Briefly, 27 μg transfer vector construct, 17.5 μg second-generation gag-pol packaging construct pCMV.R8.74, and 9.5 μg VSV-G expression construct pMD2.G were used for transfection of $12x10^6$ 293T cells overnight in 25 ml DMEM with 10% fetal bovine serum. The cells were treated with 10 mM sodium butyrate during the first of three 12-hour vector supernatant collections. The supernatant was filtered through 0.22-μm-pore-size filters and concentrated 100-fold by ultracentrifugation before

freezing and storing at –80° C. All vector stocks were titered by transducing HT1080 cells with analysis for GFP expression by flow cytometry or real-time PCR.

Processing of Samples from Xenograft Mice

Flow cytometry analysis of peripheral blood (PB) was used to establish chimerism with human cells prior to euthanasia. PB was collected using tail-tip excision (tail nick) and placed into a microtainer serum separator tube (BD Gold, 200/cs). Samples were centrifuged at 1500 rpm for 15 mins. Serum was removed for ELISA analysis. Blood cells were subjected to red blood cell lysis[4] and remaining white blood cells were stained for human surface markers. NSG mice were euthanized 5 weeks after CB CD34+ transplantation or 12 weeks after CRLF2 B-ALL transplantation. On the day of euthanasia fresh bone marrow (BM), spleen and PB were harvested. Single cell suspensions were obtained from the BM by using 26g syringe and PBS to flush the femora and humeri and by filtering cells with a 70 micron cell strainer (BD Biosciences, San Jose, CA). Cells obtained from all tissues were stained immediately for flow cytometry analysis or were frozen for future use.

Microarray Analysis of Gene Expression in CRLF2 B-ALL Cells

Mice engineered to be +T and –T by stromal cell injection were transplanted with primary CRLF2 B-ALL cells from Patient 1. Following transplant, mice received weekly intraperitoneal injection of $5X10^6$ +T or –T stroma from Transduction 1 (see Results section). Nine weeks post transplant, BM was harvested and frozen for later use. For microarray to evaluate *in vivo* TSLP-induced changes in gene expression, xenograft BM was thawed and human B-ALL cells were isolated by magnetic separation using the Human CD19 microbead kit (Miltenyi Biotec, Inc., San Diego, CA) according to manufacturer's protocol and rapidly frozen on dry ice (Samples E1-E3 and F1-F3 described below). Frozen cells were shipped to Miltenyi Biotec (Miltenyi Genomic Services) for RNA isolation, sample preparation, whole genome microarray, and discriminatory gene analysis (DGA) as follows:

Total RNA Isolation. Human total RNA was isolated using the NucleoSpin® RNA kit (Macherey-Nagel, Bethlehem, PA). RNA quality and integrity were determined using the Agilent RNA 6000 Nano Kit on the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA was quantified by measuring A260nm on the ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

RNA Amplification and Labelling. Sample labelling was performed as detailed in the "One-Color Microarray-Based Gene Expression Analysis protocol (version 6.6, part number G4140-90040). Briefly, 50 ng RNA was used for the amplification and labelling step using the Agilent Low Input Quick Amp Labelling Kit (Agilent Technologies). Yields of cRNA and the dye-incorporation rate were measured with the ND-1000 Spectrophotometer (NanoDrop Technologies).

Microarray Sample Key

Sample	Source of Cells
E1, E2, E3	CRLF2 B-ALL cells from +T mice
F1, F2, F3	CRLF2 B-ALL cells from –T mice

Hybridization of Agilent Whole Human Genome Oligo Microarrays. The hybridization procedure was performed according to the "One-Color Microarray-Based Gene Expression Analysis protocol (version 6.6, part number G4140-90040) using the Agilent Gene Expression Hybridization Kit (Agilent Technologies). Briefly, 0.6 µg Cy3-labeled fragmented cRNA in hybridization buffer was hybridized overnight (17 hours, 65 °C) to Agilent Whole Human

Genome Oligo Microarrays 8x60K v2 (AMADID 039494) using Agilent's recommended hybridization chamber and oven. Following hybridization, the microarrays were washed once with the Agilent Gene Expression Wash Buffer 1 for 1 min at room temperature followed by a second wash with preheated Agilent Gene Expression Wash Buffer 2 (37 °C) for 1 min.

Scanning and data analysis: Fluorescence signals of the hybridized Agilent Microarrays were detected using Agilent's Microarray Scanner System G2505C (Agilent Technologies). The Agilent Feature Extraction Software (FES) 10.7.3.1 was used to read out and process the microarray image files.

Gene Set Enrichment Analysis: Microarray data was evaluated at the level of gene sets to define and quantitate trends in gene expression similar to published data. Ranked gene lists were created and submitted to the online public repository provided by the BROAD Institute for Gene Set Enrichment Analysis (GSEA)[5, 6] to evaluate Oncogenic Signatures that are enriched in CRLF2 B-ALL cells expanded in +T as compared to –T mice (www.broadinstitute.org/gsea). For plots shown in Figures 5 and 6 the top half of the GSEA enrichment plot shows the enrichment score for each gene and the bottom half shows the values of the ranking metric moving down the list of the ranked genes.

Discriminatory gene expression analysis. After probe summarization, quantile normalization and log2 transformation, differentially expressed genes were identified by a combination of effect size and statistical significance. For the analysis of the individual comparisons, only reporters with an at least two-fold median up- or downregulation in +T samples compared to –T samples and an adjusted p-value (Student's t-test, two-tailed, equal variance, Benjamini-Hochberg correction for multiple testing) $p \leq 0.05$ were considered relevant. In an additional filtering step, only Agilent reporters with signal intensity values significantly above local background ($p<0.01$ as calculated by the Rosetta Resolver® gene expression data analysis system) in at least two samples of the group with higher median expression were selected.[7] All microarray data reported in this publication were deposited in the NCBI's Gene Expression Omnibus at National Center for Biotechnology Information and are accessible via the GEO series accession number GSE65274.

Real Time Quantitative RT-PCR

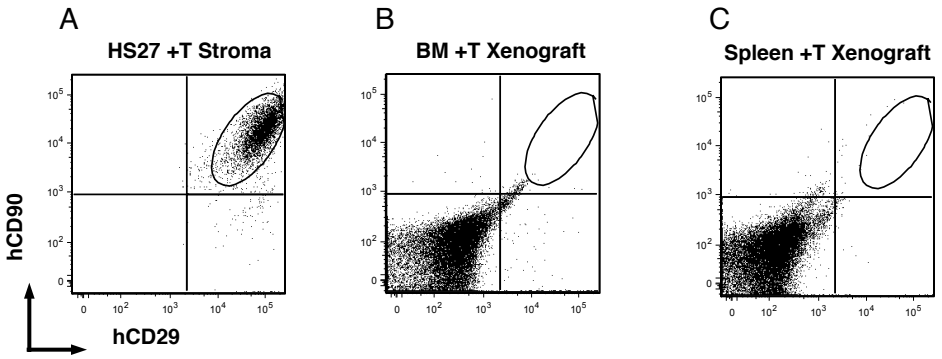
Expression of select genes observed in microarray results was validated using quantitative reverse transcriptase - polymerase chain reaction (qRT-PCR) using primers shown below. Total RNA (2.0µg) was reverse transcribed using poly d(T)20 primers and SuperScript II reverse transcriptase (Life Technologies, Grand Island, NY). The resulting cDNAs were used for qRT-PCR analysis of their mRNA levels on a StepOne Plus 7500 Real-time PCR system (Applied Bioscience Inc., Foster City, CA) using specific primers for each gene (see supplemental table 2 for primer sequences) following the manufacturer's instructions. The fluorescence threshold value was calculated and normalized to the values of 18s rRNA. The fold change in mRNA expression of genes between the TSLP-treated W31 cell group versus untreated group was achieved by the ratio of fluorescence threshold value in the treated groups versus that of the untreated group. The fold change for each gene was paired with that of microarray analysis. Linear regression analysis of the fold change versus that of microarray was performed with Origin 6.0. The graph shows the best-fit line, the formula for the best-fit line ($Y = a + bX$), its correlation coefficient (r), and the probability (P) that the r value is a false positive.

Gene	Forward Primer	Reverse Primer
BIVM	5'-GGCCAGAGGCAATGCAAAG	5'-TGAGGTCTAATACTTTCCGCTGT
PTH2	5'-CTGGTTCTCCACAGGTGATG	5'-CATGTACGAGTTCAGCCACT

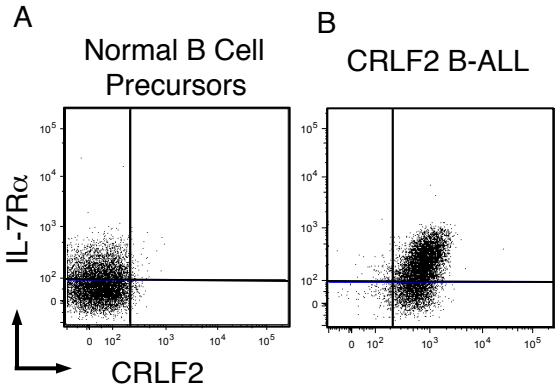
FOSL2	5'-AACACCCTGTTTCCTCTCCG	5'-ATCTACCCGGAATTTCTGCGG
RAMP1	5'-GCAGGACCATCAGGAGCTACA	5'-GCCTACACAATGCCCTCAGTG
SLC4A8	5'-TGGGTCCAGTAGGGAAAGGT	5'-ACCGTCACCTGGTCTAGGAA
NR4A2	5'-GTAACTCGGCTGAAGCCATGC	5'-GTCGTAGCCTGTGCTGTAGTT
KCNH8	5'- GCAAGATCAATTTGCATCCACTAT	5'- AAGGCAGGGCAGCGATTA
THSD7A	5'- CCATCTCGAGTCTTTGTTCACATT	5'- CATTTGGGAATTGTGCTTCTCA
RAB26	5'-CCAGGCCCTTCTGACTTTGT	5'-CTGCTTGTTGGGACTGTTGC
RGS1	5'-CTCCCTGGGTGAACAGCTTG	5'-GCTTCTTCAACTCTGCGCCT
NELL1	5'-GATATGAAGCCACCCGTGTT	5'-CTGAAGGTCAGGGTCCATCC
MDF1	5'-GAAGTTGCAGACACACCCATCTC	5'-GTCCAGGACGATGTTGCACAG
KREMEN2	5'-TGAGGACCCAGAGGCCC	5'-CAGACAGCTCCCCCAATCG
CPNE8	5'-CGCCTCCTCCCAATATGGAC	5'-ATTTCTGCAGGACACGGACA

RNA Sequencing and Analysis of Gene Expression in CRLF2 B-ALL Cells

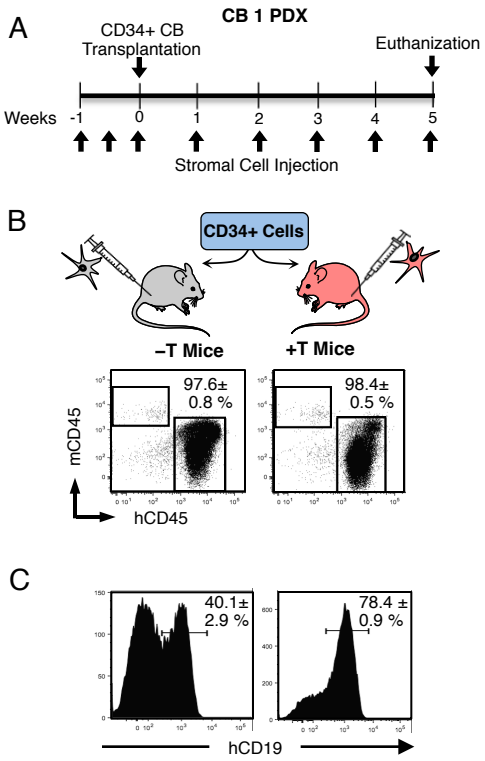
RNA libraries were prepared for sequencing by the UCLA Clinical Microarray Core using mRNASeq kits (Kapa) following RNA amplification (Ovation RNA-Seq V2, NuGen). Libraries were sequenced on a HiSeq 2000/2500 at 1 X 50 bp. Reads were mapped to the human genome (hg19) using RefSeq annotation; transcript levels were estimated using RNA-Seq by Expectation Maximization.[8] Pairwise differential expression assessments were performed using the method EBSeq;[9] genes were considered to be differentially expressed based on a False Discovery Rate of less than 5% (Posterior Probability of being Differentially Expressed < 0.05). Gene Ontology (GO) analysis was performed using DAVID. Gene Set Enrichment Analysis (GSEA) was performed using MSigDB. Ranked lists of genes having a fold change of greater than 1.5 were used as input for GSEA. The transcript per million (TPM) values from respective samples were clustered using hierarchical clustering with R. Spearman rank correlation was used as the distance metric followed by average linkage clustering. Significance of differences in correlation coefficients was determined using Fisher's r-to-z transformation.



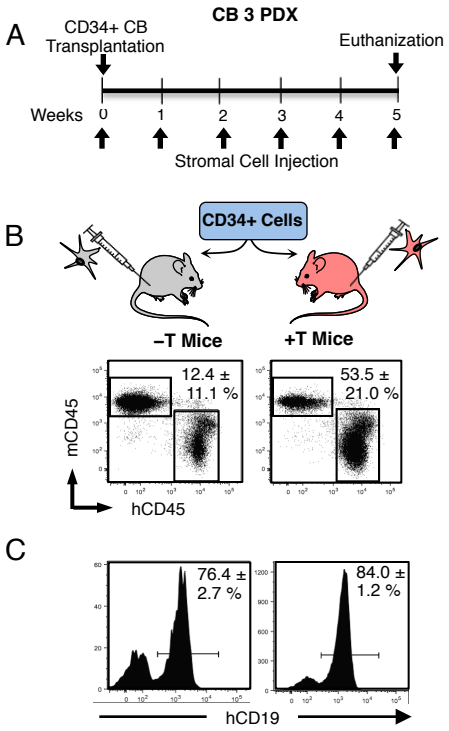
Supplementary Figure 1: HS27 stroma are not detectable in bone marrow or spleen of xenograft mice. (A) Flow cytometry plots showing human CD29 and CD90 expression on HS27 stromal cells transduced to express human TSLP (+T HS27 stroma). Similar staining was observed for HS27 stroma transduced with empty vector (–T HS27 stroma). +T or –T HS27 stroma were injected at weekly time points into sublethally irradiated NSG mice for 13 weeks. Mice were euthanized and bone marrow (BM) and spleen were harvested and stained for flow cytometry to detect human CD29 and CD90 co-expression as a means of identifying HS27 stroma in these tissues. Plotted are 25,000 events from (B) BM and (C) spleen of +T mice showing that CD29+CD90+ HS27 stroma are not detectable in BM or spleen of +T NSG mice. Similar data were obtained in –T NSG mice. Data shown are representative of n=5 +T mice and n=4 –T mice.



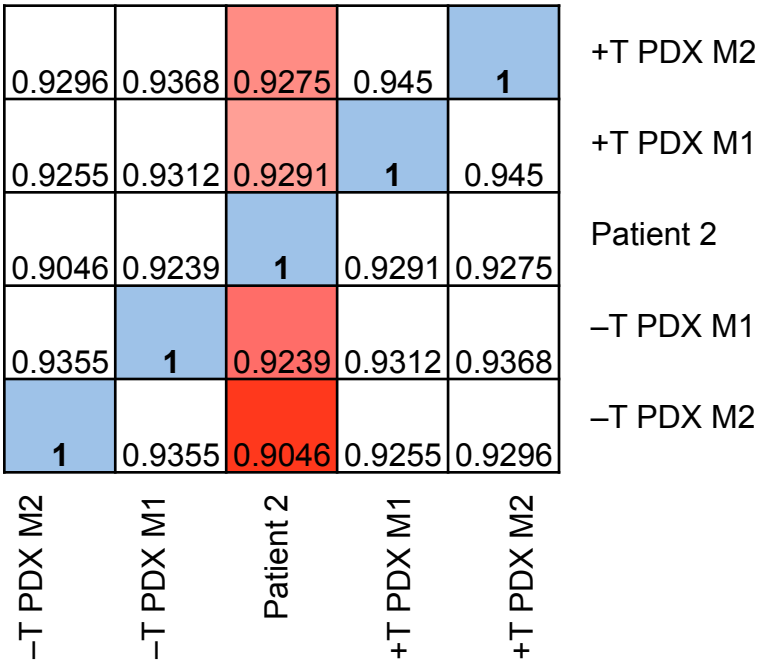
Supplementary Figure 2: Expression of CRLF2 and IL-7Rα on normal human B cell precursors and CRLF2 B-ALL harvested from xenograft mice. (A) Bone marrow was harvested from xenograft mice transplanted with human CD34+ cord blood cells and stained for flow cytometry. Plotted is CRLF2 and IL-7Rα expression on B cell precursors (hCD19+ and Ig kappa & lambda–) B) Bone marrow was harvested from a patient-derived xenograft generated using Patient 1 CRLF2 B-ALL cells. Plotted is the CRLF2 and IL-7Rα staining on human CRLF2 B-ALL (hCD19+) cells. Gates were set based on isotype control staining.



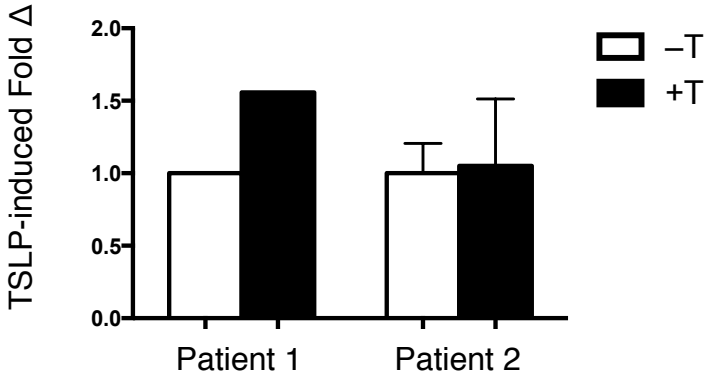
Supplementary Figure 3: Functional effects of hTSLP produced in +T xenograft mice on normal human B lineage cells generated from CB 1. A) –T and +T NSG mice were transplanted with 1 X 10^5 CD34+ human cord blood cells (CB 1) and injected with weekly doses of $1X10^7$ stromal cells (transduction 1 stroma). B) Five weeks post-transplant, mice were euthanized, BM harvested and cells were stained for flow cytometry to detect mouse CD45 (mCD45), human CD45 (hCD45), and hCD19. Total living cells were gated and mCD45 vs. hCD45 is plotted with gates to identify mouse and human leukocytes respectively, in the BM. C) hCD45+ cells were gated and hCD19 expression is shown by histogram. Percentages are mean ± SEM. Data shown in A-C were obtained from CB 1, n=3 –T PDX and n= 5 +T PDX.



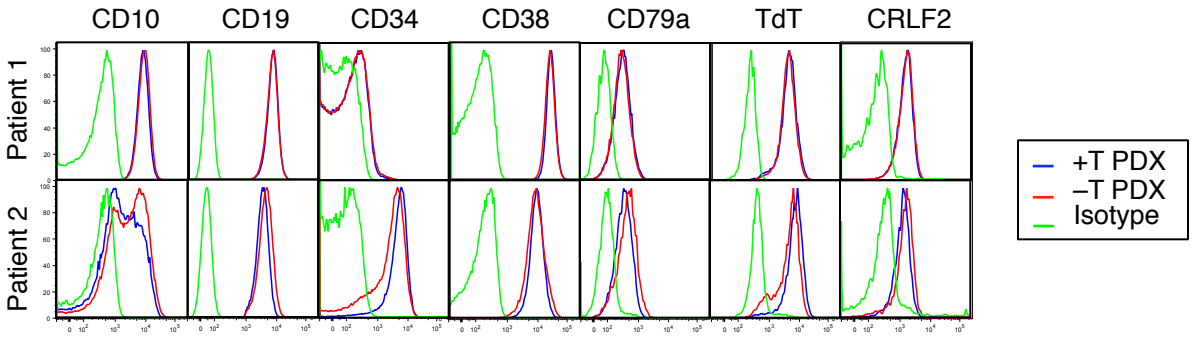
Supplementary Figure 4: Functional effects of hTSLP produced in +T xenograft mice on normal human B lineage cells generated from CB 3. A) –T and +T NSG mice were transplanted with 1 X 10^5 CD34+ human cord blood cells (CB 3) and injected with weekly doses of 5X10^6 stromal cells (transduction 2 stroma). B) Five weeks post-transplant, mice were euthanized, BM harvested and cells were stained for flow cytometry to detect mouse CD45 (mCD45), human CD45 (hCD45), and hCD19. Total living cells were gated and mCD45 vs. hCD45 is plotted with gates to identify mouse and human leukocytes respectively, in the BM. C) hCD45+ cells were gated and hCD19 expression is shown by histogram. Percentages are mean ± SEM. Data shown in A-C were obtained from CB 3, n=4 –T PDX and n= 5 +T PDX.



Supplementary Figure 5: Spearman rank coefficients for comparisons of gene expression in +T and –T PDX to original patient sample. A) CRLF2 B-ALL cells from Patient 2 were used to generated –T and +T PDX mice as described in Figure 6. RNA sequencing was performed on CRLF2 B-ALL cells isolated from 2 +T mice (+T PDX M1 and M2) and 2 –T PDX mice (–T PDX M1 and M2) and from the original Patient 2 primary sample used to generate them. Spearman rank coefficients are shown for comparison of gene expression in +T and –T PDX and in the primary patient sample. The comparisons between +T and –T PDX and the primary patient sample are highlighted to match the heatmap in Fig. 6E. Significance of differences in correlation coefficients ($p < .0001$) was determined using Fisher's r-to-z transformation.



Supplementary Figure S6: CRLF2 B-ALL leukemia cell burden in BM of –T and +T PDX. Bone marrow cells from +T and –T PDX generated from Patient 1 and Patient 2 and shown in Fig. 5 and 6 were harvested, counted by hemocytometer, and stained for flow cytometry to identify living CRLF2 B-ALL cells. Total living cell counts and percentages of living CRLF2 B-ALL cells within the total living cells were used to calculate the number of CRLF2 B-ALL cells per PDX. Graphed is the fold change in living leukemia cells in +T PDX as compare to –T PDX obtained by normalizing CRLF2 B-ALL cell numbers in +T animals to the cell numbers in –T animals for each experiment.



Supplemental Figure S7: Surface immunophenotype CRLF2 B-ALL from –T and +T PDX. +T and –T PDX were stained for flow cytometry to determine immunophenotype with respect to marker expression on the original primary CRLF2 B-ALL patient sample (see Online Supplementary Table 1). Plotted is expression of indicated markers on gated living human CD19+ leukemia cells in –T and +T PDX samples from Patient 1 and Patient 2.

Patient Designation	Gender	Age	Immunophenotypic Markers	*CRLF2 and IKZF1* Status	Ethnicity
Patient 1	M	10 years	CD10+ CD19+ CD20+ CD22 surface, dim CD22 cytoplasmic, dim CD34 partial CD38 partial CD45 dim CD58 dim CD79a+ HLA-DR+	*CRLF2 High* *No IKZF1* deletion	Hispanic
Patient 2	M	18 years	CD9+ CD10 partial CD19+ CD20 partial CD22 surface, dim CD22 cytoplasmic, dim CD34 partial CD38+ CD45 dim CD79a+ HLA-DR+ TdT+ CD3 cytyoplasmic– Kappa– Lambda– MPO–	*CRLF2 High* *IKZF1* deletion	Hispanic

Supplementary Table 1. CRLF2 B-ALL Patient Characteristics. *IKZF1* deletion = deletion of one allele of *IKZF1 (Ikaros)* was determine as described.[10] Both patients expressed high levels of CRLF2 as assessed by flow cytometry in Figure 1.

Gene	Microarray (fold change)	qRT-PCR (fold change)
BIVM	4.440	**6.907**
PTH2	3.477	**2.784**
FOSL2	2.793	**1.843**
RAMP1	2.725	**2.227**
SLC4A8	2.628	**6.417**
NR4A2	2.138	**3.548**
KCNH8	2.154	**2.355**
THSD7A	–3.628	**–6.648**
RAB26	–2.727	**–2.726**
RGS1	–2.749	**–2.498**
NELL1	–3.586	**–3.143**
MDF1	–2.168	**–7.621**
KREMEN2	–2.336	**–2.003**
CPNE8	–3.008	**–4.695**

Supplementary Table 2: **qRT-PCR validation of genes that are differentially regulated in CRLF2 B-ALL cells expanded in +T as compared to –T xenograft mice.** The table shows the list of genes that were selected from the microarray dataset for validation by qRT-PCR analysis and their respective fold changes as determined by microarray and by qRT-PCR.

Supplemental References

1. Graf L, Iwata M, Torok-Storb B. Gene expression profiling of the functionally distinct human bone marrow stromal cell lines HS-5 and HS-27a. Blood. 2002;100(4):1509-1511.
2. Parrish YK, Baez I, Milford TA, et al. IL-7 Dependence in human B lymphopoiesis increases during progression of ontogeny from cord blood to bone marrow. J Immunol. 2009;182(7):4255-4266.
3. Meng X, Neises A, Su RJ, et al. Efficient reprogramming of human cord blood CD34+ cells into induced pluripotent stem cells with OCT4 and SOX2 alone. Mol Ther. 2012;20(2):408-416.
4. Kang EM, Areman EM, David-Ocampo V, et al. Mobilization, collection, and processing of peripheral blood stem cells in individuals with sickle cell trait. Blood. 2002;99(3):850-855.
5. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet. 2003;34(3):267-273.
6. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545-15550.
7. Weng L, Dai H, Zhan Y, He Y, Stepaniants SB, Bassett DE. Rosetta error model for gene expression analysis. Bioinformatics. 2006;22(9):1111-1121.
8. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC bioinformatics. 2011;12(323.
9. Leng N, Dawson JA, Thomson JA, et al. EBSeq: an empirical Bayes hierarchical model for inference in RNA-seq experiments. Bioinformatics. 2013;29(8):1035-1043.
10. Song C, Gowda C, Pan X, et al. Targeting casein kinase II restores Ikaros tumor suppressor activity and demonstrates therapeutic efficacy in high-risk leukemia. Blood. 2015;126(15):1813-1822.