Aurora A kinase as a target for therapy in *TCF3-HLF* rearranged acute lymphoblastic leukemia

Jessica Leonard,[1] Joelle S.J. Wolf,[2] Michelle Degnin,[1] Christopher A. Eide,[1] Dorian LaTocha,[1] Kyle Lenz,[2] Beth Wilmot,[3] Charles G. Mullighan,[4] Mignon Loh,[5] Stephen P, Hunger,[6] Brian J. Druker,[1,7] Marc M. Loriaux,[8] Jeffrey W. Tyner[1,9] and Bill H. Chang[2]

[1]Division of Hematology and Medical Oncology, Department of Medicine and OHSU Knight Cancer Institute, OHSU, Portland, OR; [2]Division of Hematology and Oncology, Department of Pediatrics and OHSU Knight Cancer Institute, OHSU, Portland, OR; [3]Division of Bioinformatics and Computational Biology, Department of Medical Informatics and Clinical Epidemiology, and OHSU Knight Cancer Institute, OHSU, Portland, OR; [4]Department of Pathology, St Jude Children's Research Hospital and University of Tennessee Health Science Center, Memphis, TN; [5]Department of Pediatrics, Benioff Children's Hospital, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA; [6]Department of Pediatrics and Center for Childhood Cancer Research, Children's Hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [7]Howard Hughes Medical Institute, Chevy Chase, MD; [8]Department of Pathology and OHSU Knight Cancer Institute, OHSU, Portland, OR and [9]Department of Cell, Molecular, and Cancer Biology, and OHSU Knight Cancer Institute, OHSU, Portland, OR, USA

Correspondence: BILL H. CHANG - changb@ohsu.edu

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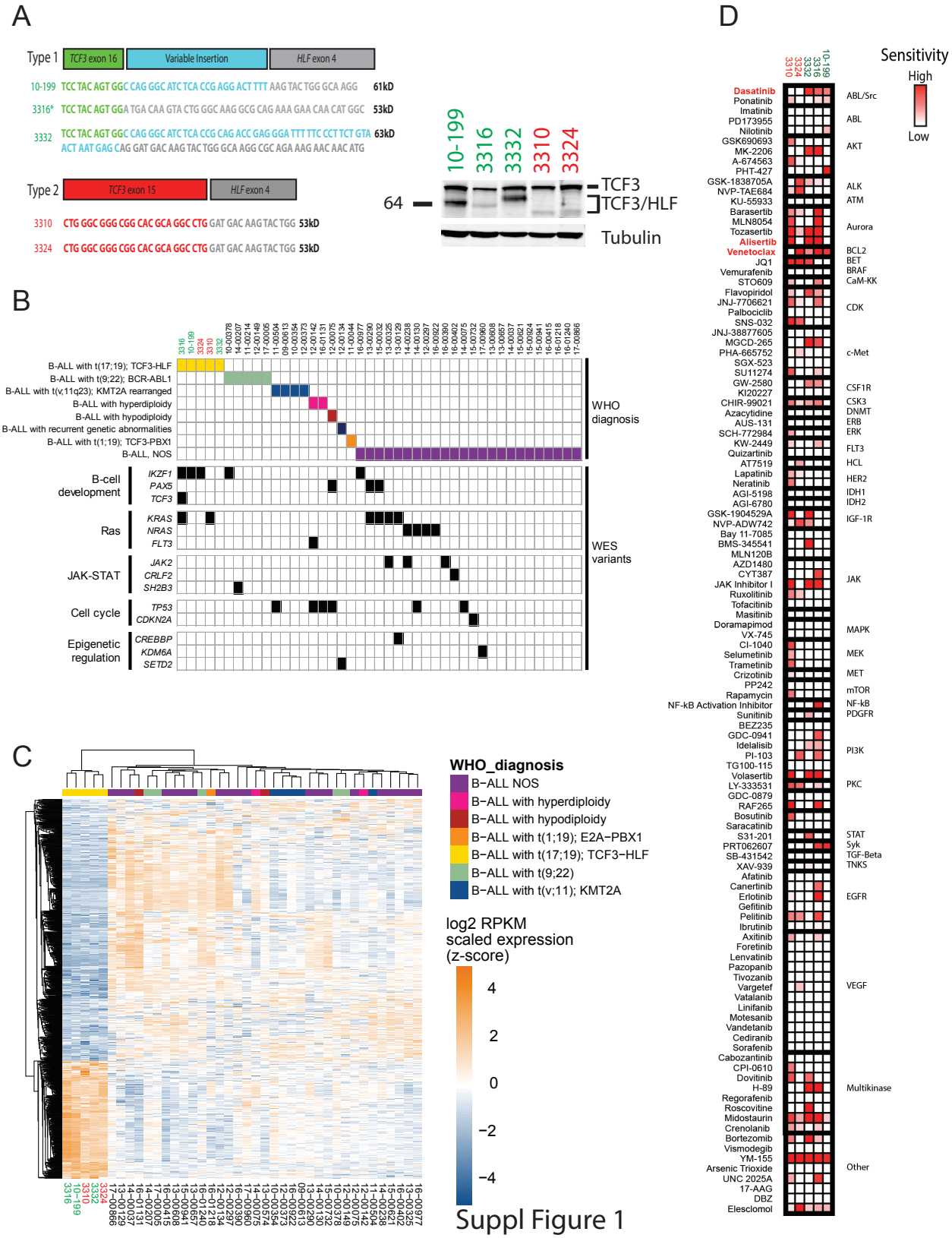
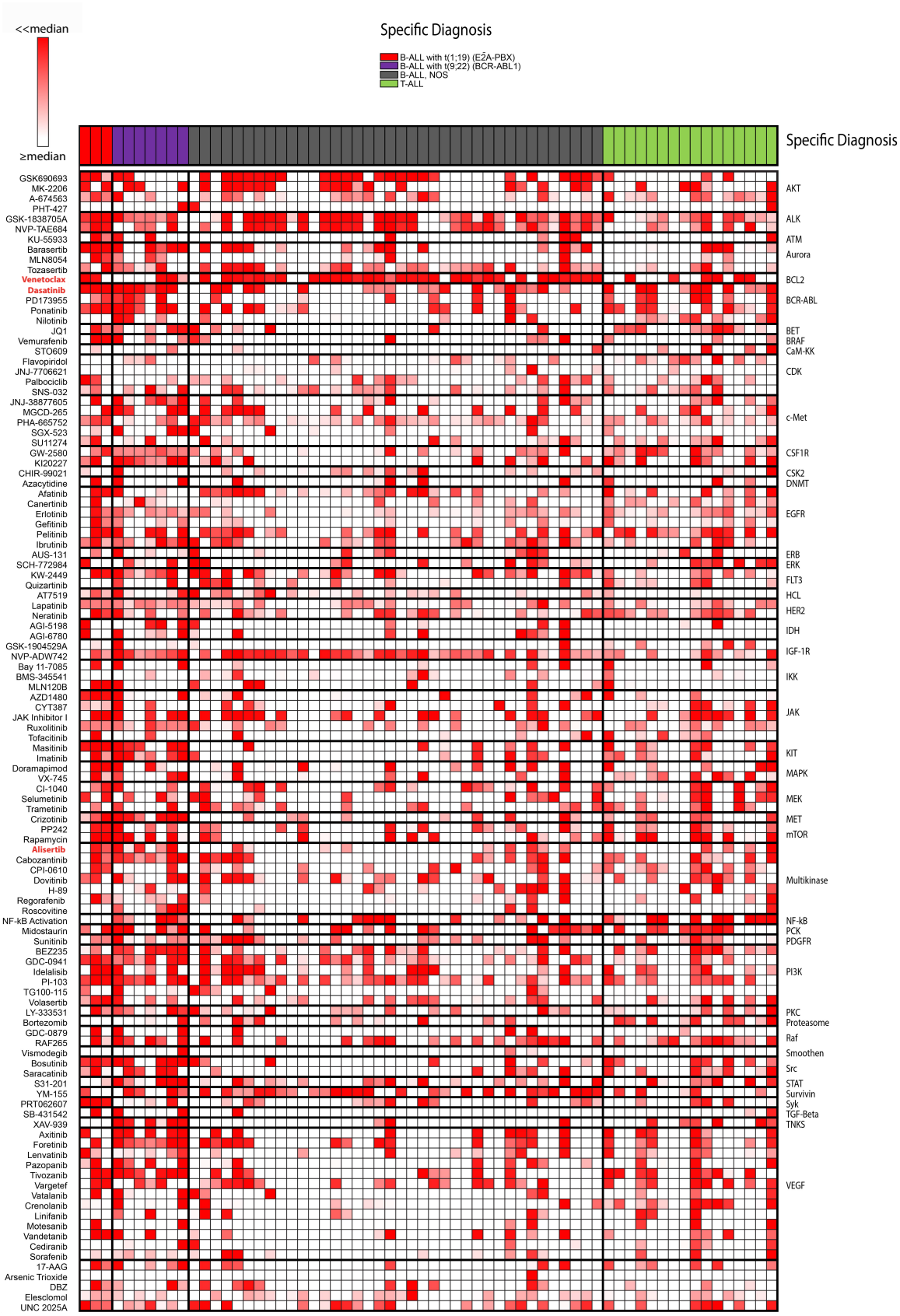
Leonard, et al Supplemental Data



Suppl Figure 1

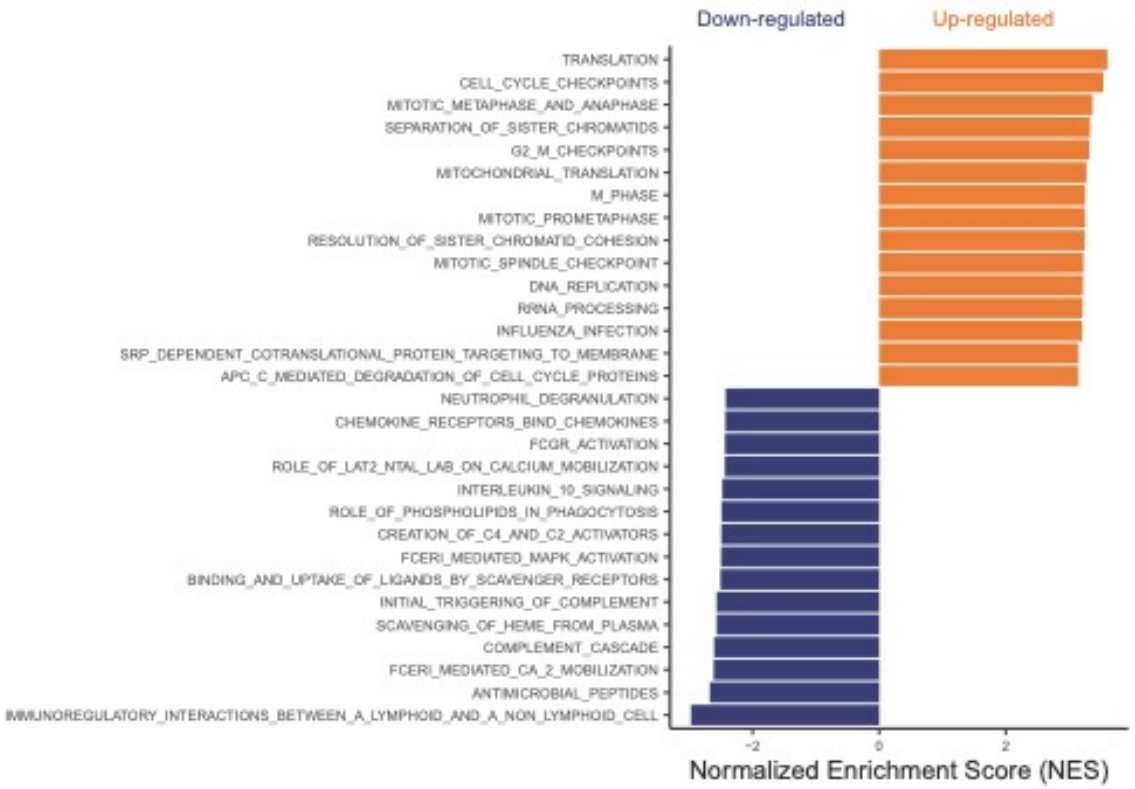
Supplemental Figure 1. Biochemical, molecular and functional interrogation of *TCF3-HLF* ALL. **A)** Sanger Sequencing results of five patient derived samples using standard RT-PCR from isolated RNA and corresponding immunoblot. *TCF3-HLF* fusions were amplified from first strand cDNA using Forward Primer: TGCACAACCACGCGGCCCTC; reverse primer: TGCCATGTTGTTCTTTCTGCG. PCR product then underwent standard sanger sequencing, while whole cell lysate from leukemic samples were separated by standard SDS-PAGE and subsequently immunoblotted for using anti-TCF3 with loading control using anti-α-tubulin. Three samples were identified to carry a type 1 translocation (10-199, 3316, and 3332 Green) with variable insertions corresponding to different protein length, while two others carried a type 2 translocation (3310, 3324 Red). *Although 3316 is predicted to have an early stop site at the end of exon 13 immunoblot analysis identified a larger protein product closer to 60kD suggesting a potential read-through effect overcoming the stop codon consistent with a preservation of *HLF* expression**.** The immunoblot shown is representative of three independent experiments. **B**) Whole exome sequencing identifies minimal pathological variants**.** Five *TCF3-HLF* ALL samples and thirty-eight B-cell ALL samples underwent whole exome sequencing using the Illumina Nextera RapidCapture Exome capture probes and protocol, which provided coverage of 37 Mb of genomic DNA coding regions. Variant filters excluded variants not resulting in an amino acid change and variants with ExAC (gnomAD) frequency >0.1%. Resultant variants were then compared to genes in the St. Jude PeCan variant database for patients with B-ALL which included variants that matched exactly with variants found in PeCan of that occurred in PeCan hotspot regions. *IKZF1* variant in *TCF3-HLF* ALL carried the predicted I125V mutation, *KRAS* variant in *TCF3-HLF* ALL carried the predicted G12V/S. **C)** Differential gene expression analysis. RNA-seq analysis was performed whereby samples were sequenced using the Agilent SureSelect Strand-Specific RNA Library Preparation Kit on the Bravo

robot (Agilent). The STAR aligner v020201 was used to align reads to the hg19 version of the human genome. These alignments were summarized at the gene-level relative to Ensembl 83 gene models using featureCounts and fusions were generated using STAR-Fusion v1.3.2. Normalization was performed using the conditional quantile normalization procedure which produced GC-content corrected log2 Reads Per Kilobase per Million mapped reads (RPKM) values. To identify genes whose expression was significantly up- or down-regulated in patients harboring the t(17;19) translocation, normalized expression (log2 RPKM) of 21,001 evaluable genes was compared between t(17;19)-positive (n=5) and –negative (n=35) ALL patient samples by two-tailed student's *t*-test using the GenePattern Comparative Marker Selection package (Broad Institute). Raw p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method. The top 1000 most significant differentially expressed genes were subjected to unsupervised, hierarchical clustering by Euclidean pairwise average linkage method (Gene Pattern Hierarchical Clustering package; Broad). **D)** t(17;19) ALL specimens subjected to *ex vivo* drug sensitivity and assessed for viability by MTS. Freshly isolated mononuclear cells from the spleens of xenografted animals were incubated with increasing concentrations of an array of small-molecule inhibitors (4-7 dose points in addition to no drug control). IC_{50} plots were calculated and compared to the median IC_{50} of over one thousand hematologic malignancies assessed by the assay. For a given sample, drug IC_{50} values were considered <<median if less than or equal to 5-fold below the median IC_{50} for all samples tested (red). Where an IC_{50} approached the median or was not achieved at a dose of 1μM, for a given drug, an IC_{50} value equal to the highest drug concentration used was arbitrarily assigned (white). Each column represents a t(17;19) ALL sample, each row identifies the compound name, and each block identifies the drug target. Venetoclax, dasatinib, and alisertib are highlighted in red.


<<median
≥median
Specific Diagnosis
B-ALL with t(1;19) (E2A-PBX)
B-ALL with t(9;22) (BCR-ABL1)
B-ALL, NOS
T-ALL
Specific Diagnosis
GSK690693
MK-2206
A-674563
PHT-427
GSK-1838705A
NVP-TAE684
KU-55933
Barasertib
MLN8054
Tozasertib
Venetoclax
Dasatinib
PD173955
Ponatinib
Nilotinib
JQ1
Vemurafenib
STO609
Flavopiridol
JNJ-7706621
Palbociclib
SNS-032
JNJ-38877605
MGCD-265
PHA-665752
SGX-523
SU11274
GW-2580
KI20227
CHIR-99021
Azacytidine
Afatinib
Canertinib
Erlotinib
Gefitinib
Pelitinib
Ibrutinib
AUS-131
SCH-772984
KW-2449
Quizartinib
AT7519
Lapatinib
Neratinib
AGI-5198
AGI-6780
GSK-1904529A
NVP-ADW742
Bay 11-7085
BMS-345541
MLN120B
AZD1480
CYT387
JAK Inhibitor I
Ruxolitinib
Tofacitinib
Masitinib
Imatinib
Doramapimod
VX-745
CI-1040
Selumetinib
Trametinib
Crizotinib
PP242
Rapamycin
Alisertib
Cabozantinib
CPI-0610
Dovitinib
H-89
Regorafenib
Roscovitine
NF-kB Activation
Midostaurin
Sunitinib
BEZ235
GDC-0941
Idelalisib
PI-103
TG100-115
Volasertib
LY-333531
Bortezomib
GDC-0879
RAF265
Vismodegib
Bosutinib
Saracatinib
S31-201
YM-155
PRT062607
SB-431542
XAV-939
Axitinib
Foretinib
Lenvatinib
Pazopanib
Tivozanib
Vargetef
Vatalanib
Crenolanib
Linifanib
Motesanib
Vandetanib
Cediranib
Sorafenib
17-AAG
Arsenic Trioxide
DBZ
Elesclomol
UNC 2025A
AKT
ALK
ATM
Aurora
BCL2
BCR-ABL
BET
BRAF
CaM-KK
CDK
c-Met
CSF1R
CSK2
DNMT
EGFR
ERB
ERK
FLT3
HCL
HER2
IDH
IGF-1R
IKK
JAK
KIT
MAPK
MEK
MET
mTOR
Multikinase
NF-kB
PCK
PDGFR
PI3K
PKC
Proteasome
Raf
Smoothen
Src
STAT
Survivin
Syk
TGF-Beta
TNKS
VEGF

Supplemental Figure 2. ALL specimens subjected to *ex vivo* drug sensitivity and assessed for viability by MTS. Freshly isolated primary patient samples were incubated with increasing concentrations of an array of small-molecule inhibitors (4-7 dose points in addition to no drug control). IC_{50} plots were calculated and compared to the median IC_{50} of over one thousand hematologic malignancies assessed by the assay. For a given sample, drug IC_{50} values were considered <<median if less than or equal to 5-fold below the median IC_{50} for all samples tested (red). Where an IC_{50} approached the median or was not achieved at a dose of 1μM, for a given drug, an IC_{50} value equal to the highest drug concentration used was arbitrarily assigned (white). Venetoclax, dasatinib, and alisertib are highlighted in red.

A



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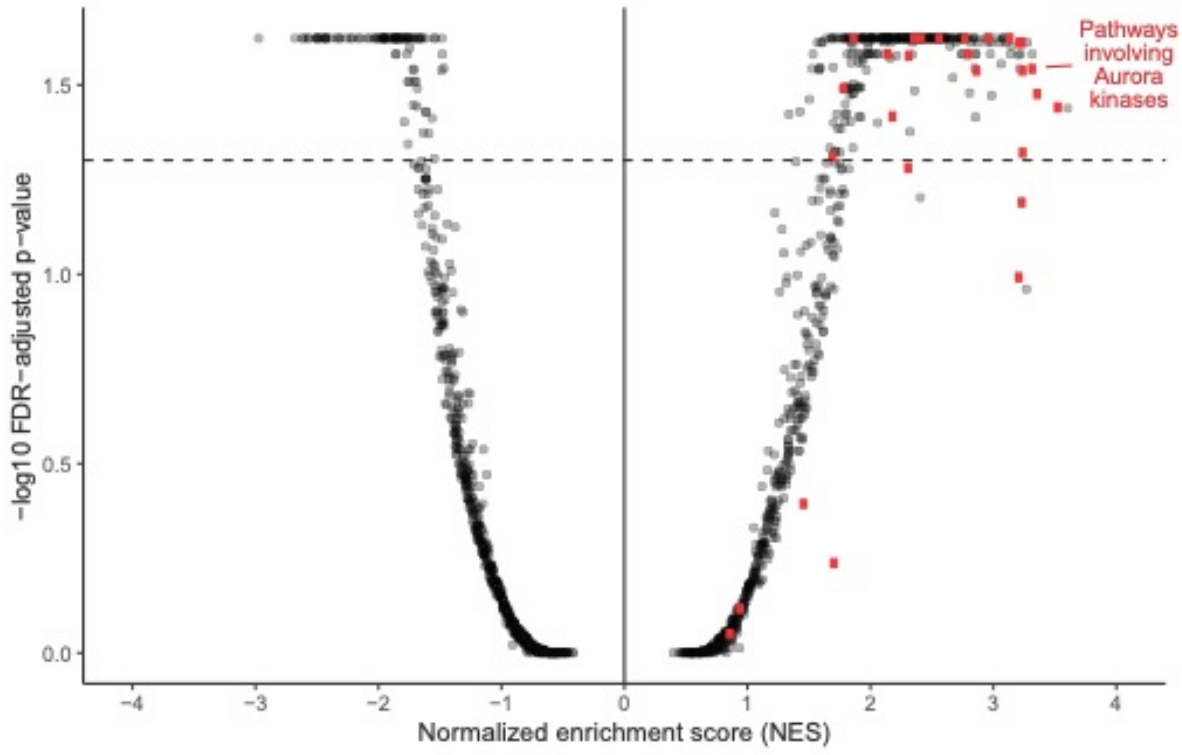

Supple Figure 3

Supplemental Figure 3. Gene set enrichment analysis (GSEA). Pre-ranked GSEA was run using the computed Wald statistic values for each gene from DE analysis and the Reactome library of curated gene sets to identify up- and down-regulated pathways using the fgsea R package. All p-values were adjusted for false discovery rate (FDR) by the Benjamini-Hochberg step-wise method; FDR-adjusted $p < 0.05$ was considered statistically significant. **A)** Top 15 up and 15 down-regulated Pathways from GSE by normalized enrichment scores (NES). Pathways involving cell cycle appear to be upregulated (orange) in *TCF3-HLF* ALL compared to other B-cell ALLs. **B)** Volcano plot of Enriched Reactome Pathways of *TCF3-HLF* ALL compared to other B-cell ALL. Plots compare Normalized expression scores (NES) compared to the −log10 FDR−adjusted p−value. Each point on the plot represents a reactome pathway gene set, with its x coordinate being the enrichment score (positive values = enriched in *TCF3-HLF* samples compared to other B-ALL samples; negative values = down in *TCF3-HLF* samples compared to other B-ALL samples) and its y-coordinate being the FDR-adjusted significance. The red highlighted dots are pathways whose gene sets contained at least one of the Aurora kinase family genes (AURKA, AURKB, AURKC).