

Oncogenic deregulation of *BCL11B* in lineage ambiguous leukemia

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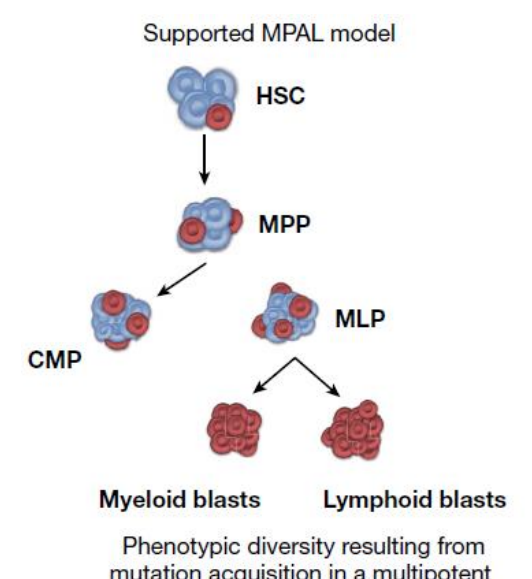
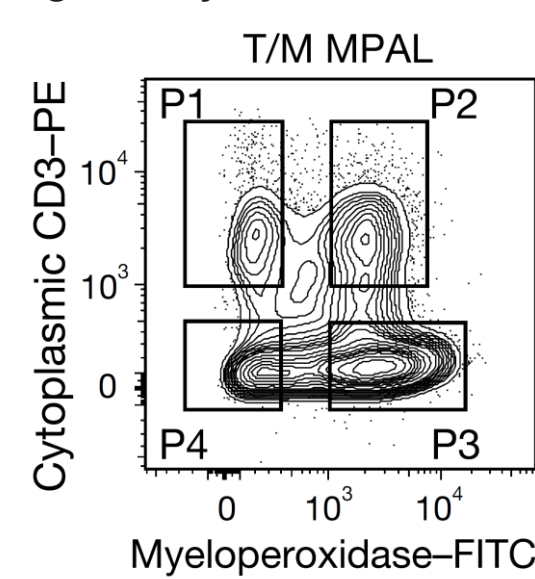
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ABSTRACT

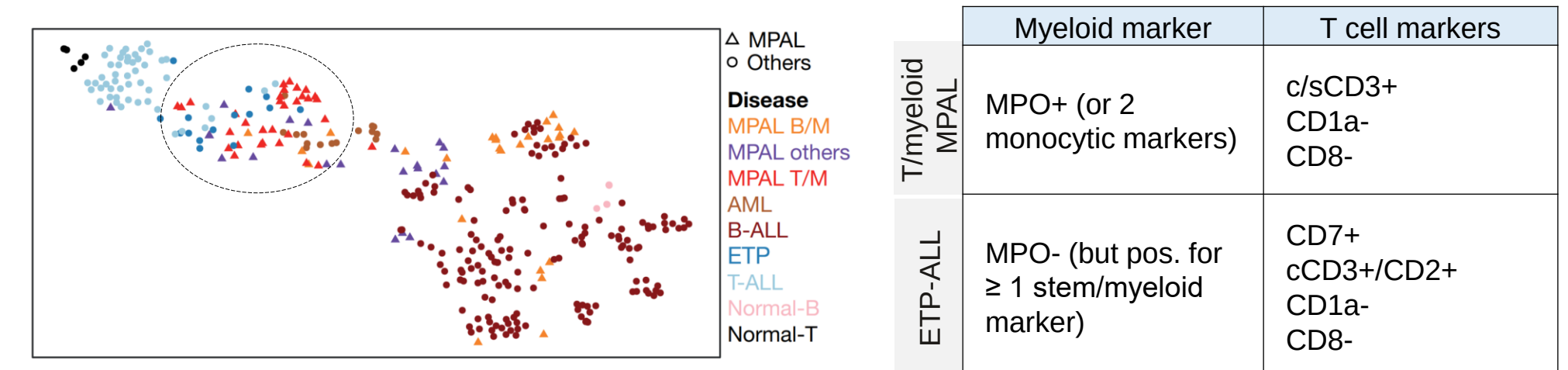
Acute leukemias of ambiguous lineage (ALAL) pose significant diagnostic and therapeutic challenges due to lack of clarity surrounding their cellular origins and driving genomic alterations, as well as poor long-term response to conventional chemotherapy. Most ALAL cases are diagnosed as mixed phenotype acute leukemia (MPAL) or acute undifferentiated leukemia (AUL); however, early T-cell precursor acute lymphoblastic leukemias (ETP-ALL) often express myeloid markers suggesting a degree of lineage ambiguity. Biological distinctions between these diseases remain vague, as T/myeloid MPAL and ETP-ALL exhibit similar mutational and immunophenotypic profiles, highlighting the need for improved understanding of ALAL etiology. Using gene expression profiling of a large cohort of leukemia samples, we identified a novel subtype with a distinct gene expression profile that included one-third of T/myeloid MPAL and ETP-ALL cases. Structural variants targeting the 14q32 locus, near the T cell transcription factor gene *BCL11B*, were identified in all cases with whole genome sequencing (WGS) data available. Using chromatin-based assays, we demonstrated that these SVs resulted in enhancer hijacking or *de novo* super-enhancer formation which served to aberrantly activate *BCL11B*, likely in a hematopoietic stem or early progenitor cell. Viral *BCL11B* expression in cord blood CD34⁺ HSPCs led to activation of T cell gene programs, repressed myeloid gene expression, and resulted in a population of cytoplasmic CD3⁺ cells *in vitro*. Collectively, these results demonstrate that *BCL11B* deregulation in primitive hematopoietic cells drives a subset of lineage-ambiguous leukemias that transcend conventional immunophenotypic and diagnostic distinctions.

BACKGROUND

- ALAL accounts for 3-5% of acute leukemia cases and outcomes are relatively poor compared to ALL¹.
- Most ALAL are diagnosed as MPAL, which is characterized by an immunophenotype including both myeloid and lymphoid markers (**left**). The genomic and biological basis for this lineage ambiguity remains incompletely understood. A hematopoietic stem or early progenitor cell has been implicated as the most likely cell of origin² (**right**); however, this has not been rigorously tested.



- Although classified as distinct diagnostic entities, T/myeloid MPAL and ETP-ALL share overlapping features, including gene expression profile² (**left**), genomic alterations^{2,3}, and are only distinguished immunophenotypically by the presence or absence of MPO⁴ (**right**).



In this work, we performed gene expression profiling on a large cohort of diverse leukemia samples to resolve biological heterogeneity among lineage ambiguous cases, irrespective of original diagnosis or immunophenotype.

References

- ¹Weinberg and Arber. *Leukemia* 2010
²Alexander et al. *Nature* 2018
³Liu et al. *Nature Genetics* 2017
⁴Arber et al. *Blood* 2016
⁵Zhang et al. *Molecular Cell* 2020
⁶Ha et al. *Leukemia* 2017

1. Gene expression profiling of 2,573 acute leukemia samples identified a new biological subgroup defined by *BCL11B* structural variants (SVs) spanning multiple diagnostic categories.

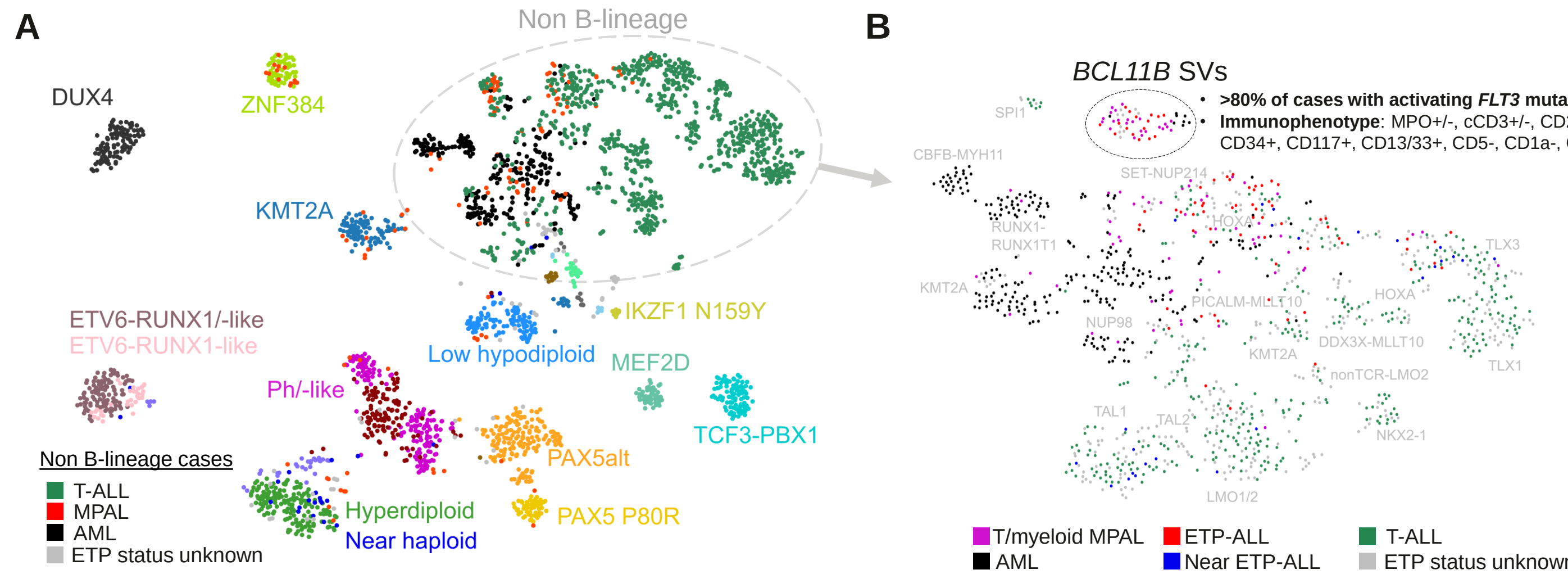


Figure 1 (A) tSNE analysis of 2,573 leukemia samples representative of all major lineages. All MPAL cases (shown in red) clustering with B-ALL subgroups were diagnosed as B/myeloid MPAL. The non-B lineage samples are circled. **(B)** tSNE analysis considering only the non-B cases identified a new subgroup with *BCL11B* SVs, including one-third of all T/myeloid MPAL and ETP-ALL cases. Activating FLT3 mutations were identified in >80% of these cases. The immunophenotype of these cases is shown; note that they are variable for cytoplasmic MPO. In both tSNE plots, the genomic subtype is indicated next to each cluster.

2. The *BCL11B* subgroup SVs involve at least 8 different chromosomes and occur near super-enhancers active in normal hematopoietic stem and progenitor cells.

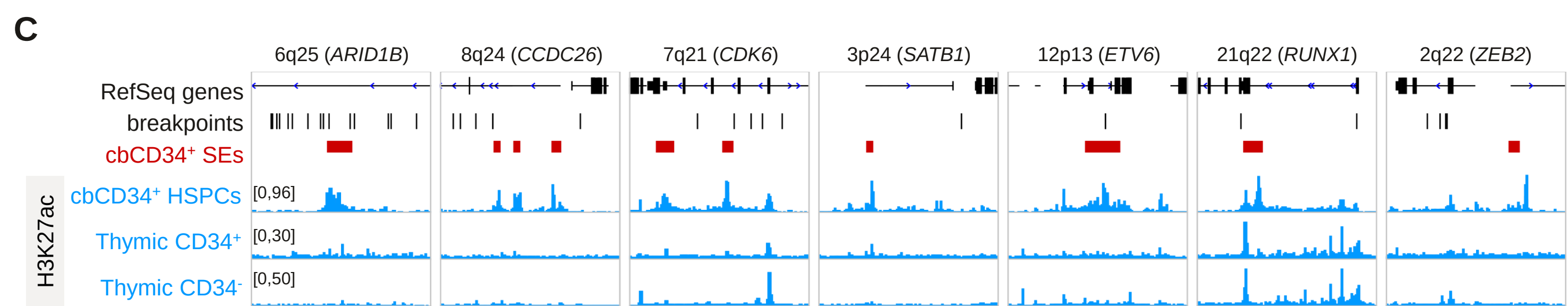
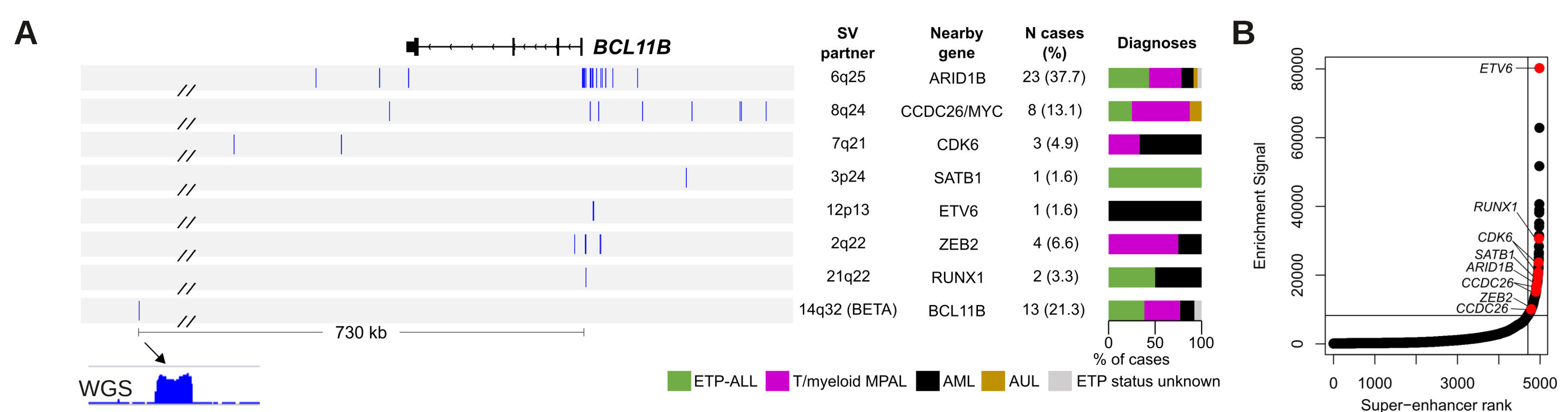


Figure 2 (A) Positions of SV breakpoints (blue tick marks) are shown for each patient according to rearrangement partner. The majority of the breakpoints occur up- or downstream of the *BCL11B* gene body and predominantly involve the 6q25 locus (near *ARID1B*), or a novel tandem amplification event (*BCL11B* Enhancer Tandem Amplification, BETA) 730 kb downstream of *BCL11B*. **(B)** H3K27ac ChIP-seq from normal human cord blood (cb) CD34⁺ hematopoietic stem and progenitor cells (HSPCs), CD34⁺ and CD34⁺ thymic progenitors⁵ showing that all breakpoints occur near super-enhancers active in HSPCs, which are diminished or absent in thymic progenitors, supporting an HSPC as the cell of origin.

RESULTS

3. Analysis of 3D genome structure demonstrated that *BCL11B* SVs result in enhancer hijacking or *de novo* enhancer formation

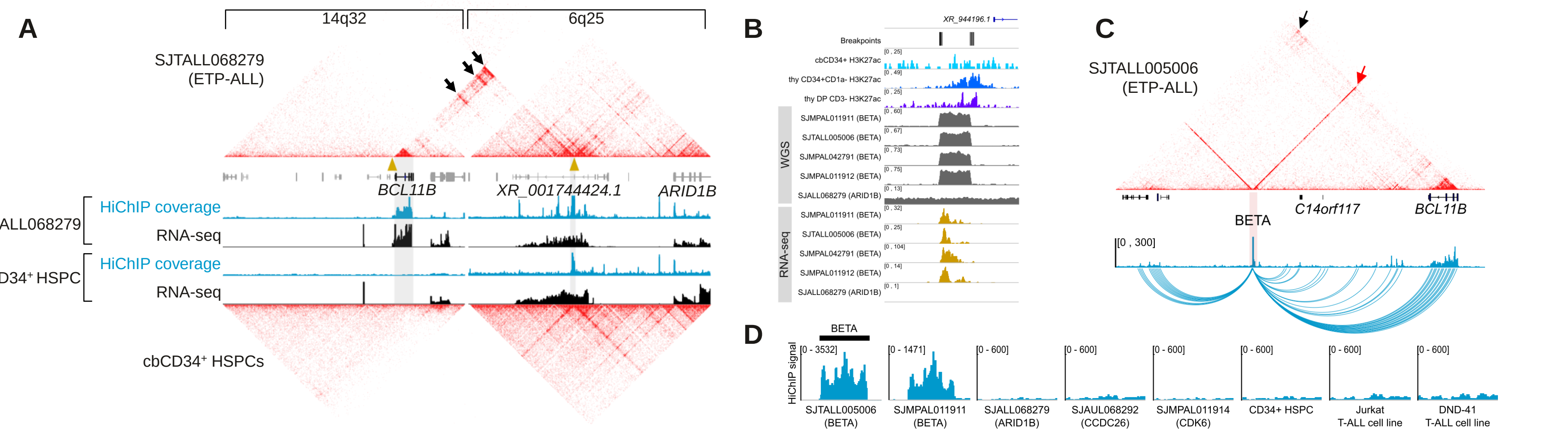
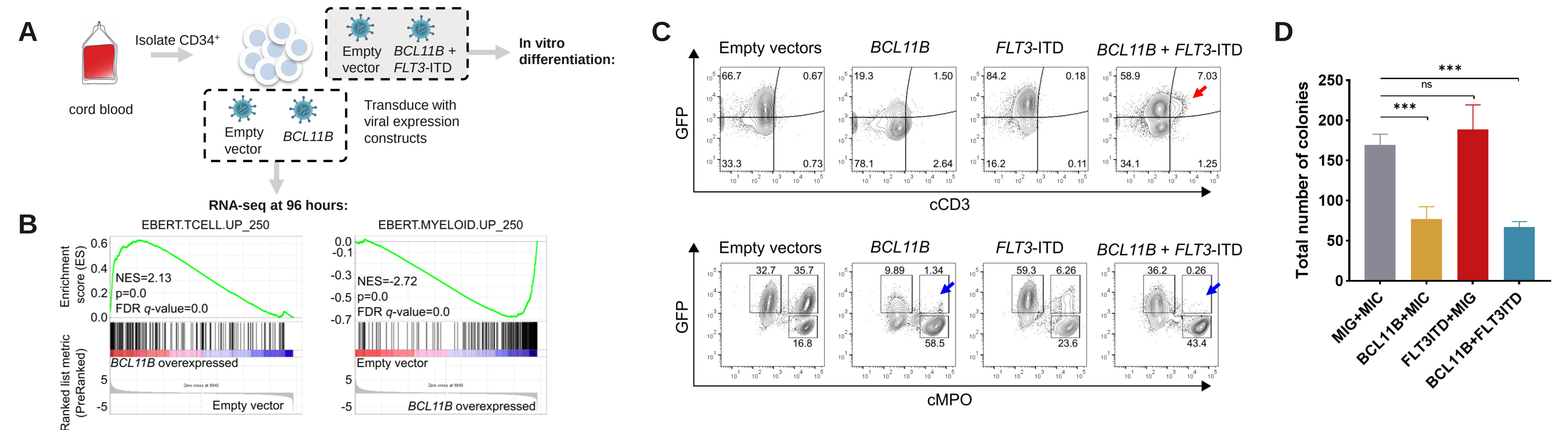


Figure 3 (A) H3K27ac HiChIP data from patient SJTALL068279 (ETP-ALL) with a t(6;14)(q25;32) translocation (top) and normal CD34⁺ HSPCs (bottom). Gold arrowheads point to the positions of each breakpoint and black arrows point to long-range chromatin interactions between enhancers at 6q25 and the *BCL11B* gene, visible only in the leukemic cells. **(B)** Genome browser snapshot of the genomic region containing BETA. H3K27ac ChIP-seq indicates weak enhancer properties in early thymic progenitor cells. WGS and RNA-seq tracks show the high copy number accompanied by evidence of transcription (possibly enhancer-associated RNA) only in samples with the amplification. **(C)** H3K27ac HiChIP in leukemic cells from patient SJTALL005006 (ETP-ALL) showing evidence of BETA looping to *BCL11B*. **(D)** HiChIP coverage at the BETA genomic region in an ETP-ALL and T/myeloid MPAL case in comparison to leukemic cells or normal cells lacking the amplification.

4. In vitro modeling supports a role for *BCL11B* in driving lineage aberrancy and promoting differentiation arrest.



CONCLUSIONS

- Gene expression profiling identified a novel subtype of lineage ambiguous leukemia defined by noncoding SVs near the *BCL11B* gene.
- These SVs occurred near super-enhancers active in normal HSPCs, but not more committed hematopoietic cell types, supporting the notion that the SVs must have occurred in an HSPC where the super-enhancer was active.
- Using HiChIP, we confirmed these super-enhancers remained active in primary leukemia samples and demonstrated direct enhancer-promoter interactions between the *BCL11B* gene and rearranged super-enhancers.
- BETA is a novel mechanism of oncogenic enhancer formation resulting from high copy tandem amplification in *cis* with *BCL11B*.
- Viral overexpression of *BCL11B* and *FLT3*-ITD in cbCD34⁺ HSPCs support a role for *BCL11B* in driving lineage aberrancy characteristic of this subgroup.
- Future work will investigate the efficacy of *FLT3* inhibitors in PDX models of *BCL11B* deregulated leukemia.

This work is currently in press at *Cancer Discovery* (DOI 10.1158/2159-8290.CD-21-0145)