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Novel ligand-binding domain truncated *CPSF7::RARA::CPSF7* tripartite fusion confers primary ATRA resistance in atypical acute promyelocytic leukemia

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Data availability statement

Data are available on request from Hongwei Wang(wanghw68@hotmail.com). The RNA-sequencing original data of the patient contact Zhifang Xu(Xu_zfang@163.com).

Authorship

Contribution: H.W.and Z.X. conceived and designed the study, reviewed the manuscript; H.L provided valuable discussion; K.D., Z.A., Z.J., X.W., B.Q., Y.N., Q.J.and Z.S. performed experiments and conducted preliminary analysis; X.Z. and H.L. conducted in-depth bioinformatics analysis and data interpretation; F.R., Y.Z., J.C., C.D and L.G. provided study materials or recruited patient. K.D. and Z.A. drafted the initial manuscript.

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To the editor

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia that is molecularly defined in the vast majority of cases by the t(15;17)(q24.1;q21.2) translocation, generating the PML::RARA fusion gene.[1] This oncogenic fusion disrupts retinoic acid receptor alpha (RARA)–mediated transcriptional regulation and blocks granulocytic differentiation, a defect therapeutically reversed by all-trans retinoic acid (ATRA) and arsenic trioxide (ATO). The introduction of differentiation therapy has transformed APL into one of the most curable acute leukemias. However, approximately 5–10% of cases lack PML::RARA and instead harbor rearrangements involving other individually rare retinoic acid receptor (RAR) genes, including RARA, RARB, and RARG, which are collectively referred to as atypical APL (aAPL).[2-5] These cases frequently exhibit primary resistance to ATRA and are associated with inferior outcomes.

Recent studies revealed that a subset of ATRA-resistant aAPL is not driven by simple bipartite *X*::*RAR* fusions, but by more complex *X*::*RAR*::*X*/*Y* tripartite fusion genes that truncate the RAR ligand-binding domain (LBD), particularly the helix 11_12 (H11_12) module encoded by the terminal exon.[4,5] This region is essential for ligand-induced conformational change, coactivator recruitment, and transcriptional activation. Loss of H11_12 or H12 disrupts allosteric signaling and provides a unifying molecular explanation for ATRA resistance. Within this emerging framework, we identified and functionally characterized a novel *CPSF7*::*RARA*::*CPSF7* tripartite fusion gene in an aAPL patient with primary resistance to ATRA-based therapy.

A 27-year-old man presented with fatigue, fever, gingival swelling, and weight loss. Physical examination showed pallor without organomegaly, lymphadenopathy, or bleeding.Laboratory evaluation revealed leukocytosis (white blood cell 44.89 × 10^9/L), anemia (hemoglobin 55 g/L), thrombocytopenia (platelet 58 × 10^9/L), and marked coagulopathy (prothrombin time 17.1 S, fibrinogen 1.79 g/L, D-dimer 8763 mg/L). Bone marrow examination showed 16.0% abnormal promyelocytes with marked size variation, coarse azurophilic granules, Faggot cells , and round to oval nuclei with

coarse chromatin and 1-2 distinct nucleoli (Supplemental figure 1A). Cytogenetic analysis revealed a complex karyotype, including -Y, der(11)t(11;13)(q11.3;q22),inv(13)(q11.2q21),and der(13)t(11;13)inv(13) (Supplemental figure 1B). Fluorescence in situ hybridization using dual-color, dual-fusion *PML*::*RARA* probes was negative (Supplemental figure 1C). Flow cytometry demonstrated expression of CD33, CD56, CD64, CD117, CD123, and cytoplasmic MPO, partial expression of CD13 and CD15, and absence of CD34 and HLA-DR, consistent with an APL-like immunophenotype (Supplemental figure 1D). A multiplex panel screening 43 common leukemia fusion genes yielded negative results.[6]

The diagnosis of PML::RARA-negative aAPL was established based on APL-like morphology and immunophenotype. The patient was immediately started on a high-risk regimen consisting of ATRA, ATO, and IA (idarubicin and cytarabine). This initial regimen was administered empirically based on the suspected diagnosis of aAPL prior to molecular confirmation; the course was not complicated by differentiation syndrome.Post-induction, pancytopenia persisted; bone marrow showed 14.0% blasts/promyelocytes with positive MRD by flow cytometry, confirming primary refractory disease. Subsequent progression (63.0% blasts) occurred.

To identify the underlying molecular abnormality, whole-transcriptome sequencing (WTS) of diagnostic bone marrow specimens was performed and revealed a novel in-frame *CPSF7*::*RARA*::*CPSF7* fusion transcript. This tripartite fusion comprises CPSF7 (MANE Select transcript NM_001142565.3) exons 1 to 5 fused to RARA (NM_000964.4) exons 3 to 8, followed by CPSF7 exons 6 to 9 (Supplemental figure 2 A, B). The breakpoint occurs at the canonical splice site of the CPSF7 exon 5/intron 5 boundary, resulting in truncation at the natural end of exon. Importantly, the rearrangement deletes *RARA* exon 9, resulting in truncation of its LBD through loss of H11_12 helices (Figure 1A). The tripartite configuration was independently confirmed by reverse transcription PCR and Sanger sequencing, which verified the cis arrangement of both fusion junctions (Figure 1B).

Given the absence of established treatment guidelines for this rare CPSF7::RARA::CPSF7 fusion, the patient received venetoclax-based HAE after molecular diagnosis. After several months of therapy, he achieved continuous remission, with bone marrow showing 0.5% blasts and promyelocytes, negative MRD by flow cytometry, and undetectable CPSF7::RARA::CPSF7 fusion transcript by qPCR, following which he underwent allogeneic HSCT. He has remained in continuous complete remission for 30 months post-transplant. At 24-month follow-up, blood counts were normal: WBC 11.00 × 10^9/L, Hb 154 g/L, PLT 329 × 10^9/L, ANC 3.92 × 10^9/L, ALC 6.20 × 10^9/L. Bone marrow showed 1.0% blasts and promyelocytes with negative MRD by flow cytometry， confirming molecular complete remission with full hematologic recovery.

To investigate the functional consequences of this novel fusion, we generated expression constructs encoding the CPSF7::RARA::CPSF7 tripartite fusion protein, a CPSF7::RARA-delH11_12 mutant, and a bipartite CPSF7::RARA fusion retaining an intact LBD. Functionally, CPSF7::RARA localized to the cytoplasm, whereas CPSF7::RARA::CPSF7 and the LBD-truncated mutant accumulated in the nucleus (Figure 2A). In luciferase assays, wild-type RARA and full-length CPSF7::RARA showed dose-dependent ATRA-induced transcriptional activation; the tripartite fusion and mutant did not (Figure 2B).

We next evaluated the biological effects of the fusion in hematopoietic cell models. Stable expression of the CPSF7::RARA::CPSF7 fusion or the LBD-truncated mutant in MV4-11 and HL-60 cells conferred a significant proliferative advantage compared with vector controls or cells expressing the full-length CPSF7::RARA fusion (Figure 3A). In differentiation assays, HL-60 cells expressing the intact CPSF7::RARA fusion exhibited partial impairment of ATRA-induced granulocytic differentiation. By contrast， cells expressing the CPSF7::RARA::CPSF7 tripartite fusion displayed near-complete resistance, as evidenced by markedly reduced upregulation of CD11b, CD15, and CD16 and failure to downregulate CD117 (Figure 3B). These results establish a clear structure–function relationship, indicating that truncation of the RARA-LBD, rather

than the identity of the fusion partner itself, is the dominant determinant of ATRA resistance.

Although PML::RARA-positive APL is highly curable, aAPL remains challenging.[7-10] More than 30 rare RAR fusion genes have been reported across over 200 aAPL cases, most initially classified as bipartite X::RAR fusions.[8-10] Yet the assumption that retention of an intact LBD would confer ATRA sensitivity has proven incorrect.

Recently, Zhou et al. identified an unexpected mechanism of ATRA resistance in aAPL, centered on tripartite *RAR* fusion genes that lead to truncation of its LBD.[4] In all *RARG*-rearranged aAPL cases analyzed in their study, the fusions uniformly adopted an *X*::*RARG*::*X*/*Y* tripartite configuration, in which aberrant 3′ splicing of *RARG* consistently resulted in loss of its LBD-H11_12. In contrast, the *RARA*-rearranged aAPL cases exhibited a more heterogeneous pattern: some harbored *X*::*RARA*::*X*/*Y* tripartite fusions with 3′ splicing–mediated deletion of its LBD-H11 or -H11_12, whereas others retained bipartite *X*::*RARA* fusions with intact LBD. Notably, whether a *RARA* fusion was bipartite or tripartite appeared to correlate with its 5′ fusion partner. The authors further demonstrated that loss of the LBD-H11_12 or -H12 segment caused by aberrant 3′ splicing constitutes a shared molecular basis for complete ATRA resistance among tripartite RAR fusion proteins.

Zhou et al. revealed a new dimension of *RAR* fusion diversity that extends beyond variable 5′ partners, identifying a shared structural mechanism responsible for ATRA resistance.[4] However, owing to limited sample size, this pioneering study encompassed only a subset of previously reported *RARA* fusion partners. Whether other, less-characterized *RAR* fusion genes adopt bipartite or tripartite configurations remains to be determined. Shortly thereafter, Wu et al. reported a case of *PML*::*RARG*::*LINE-L2a*, further supporting the association between tripartite *RARG* fusions and ATRA resistance.[5]

CPSF6 and CPSF7 are both components of the cleavage and polyadenylation specificity factor complex.[11] Building on the experimental evidence that CPSF6

generates tripartite RARG fusions, we herein identified a novel CPSF7::RARA::CPSF7 tripartite fusion in an ATRA-resistant aAPL patient. To our knowledge, this is the first report of CPSF7 as a fusion partner of RAR in aAPL, and notably, the fusion occurs in a tripartite configuration.

The *CPSF7* gene is located at chromosome 13q22. Although karyotyping revealed der(11)t(11;13)(q11.3;q22) and inv(13)(q11.2q21), no abnormalities involving 17q21.2, where the *RARA* gene resides, were detected in this case. The presence of the *CPSF7*::*RARA*::*CPSF7* tripartite fusion therefore indicates that *RARA* was incorporated through a cryptic or submicroscopic structural rearrangement not resolvable by conventional karyotype analysis.

Functional characterization confirmed that the CPSF7::RARA::CPSF7 fusion exerts its leukemogenic effect through RARA-LBD truncation, rendering it completely unresponsive to ATRA in luciferase assays—explaining the primary ATRA resistance in this case. We speculate that this CPSF7::RARA::CPSF7 fusion may be ATO-insensitive, given the absence of the PML moiety (the primary ATO target) and the poor clinical response to initial ATO-based therapy in this case. This preliminary finding warrants further investigation.

Although the use of non-APL cell lines represents a limitation, the consistent results across two independent myeloid models provide robust evidence that LBD truncation drives ATRA resistance.

Clinically, this case highlights the diagnostic challenges in aAPL. Definitive identification of the CPSF7::RARA::CPSF7 fusion required WTS, which guided successful venetoclax-based therapy and allo-HSCT, resulting in sustained remission. These findings underscore the critical role of comprehensive genomic profiling—particularly WTS—in suspected APL lacking PML::RARA, especially with primary ATRA resistance.

In summary, we identified and characterized a novel CPSF7::RARA::CPSF7 tripartite fusion in aAPL, further expanding the spectrum of RAR partners. Our findings provide

additional evidence that truncation of the LBD is a recurrent mechanism driving primary ATRA resistance, underscoring the clinical importance of identifying tripartite RAR fusions for predicting therapeutic response.

This study was approved by the Ethics Committee of the Second Hospital of Shanxi Medical University. Written informed consent was obtained.

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Figure1. identification of the CPSF7::RARA::CPSF7 fusion in the aAPL case.

(A) Schematic structure of the identified CPSF7::RARA::CPSF7 tripartite fusion gene.

(B) Experimental validation of the CPSF7::RARA::CPSF7 fusion breakpoint. The fusion junction is confirmed by Sanger sequencing of the specific ~1500 bp RT-PCR amplicon resolved by agarose gel electrophoresis.

Figure 2. Subcellular localization and transcriptional activity of CPSF7::RARA::CPSF7 and its mutants.

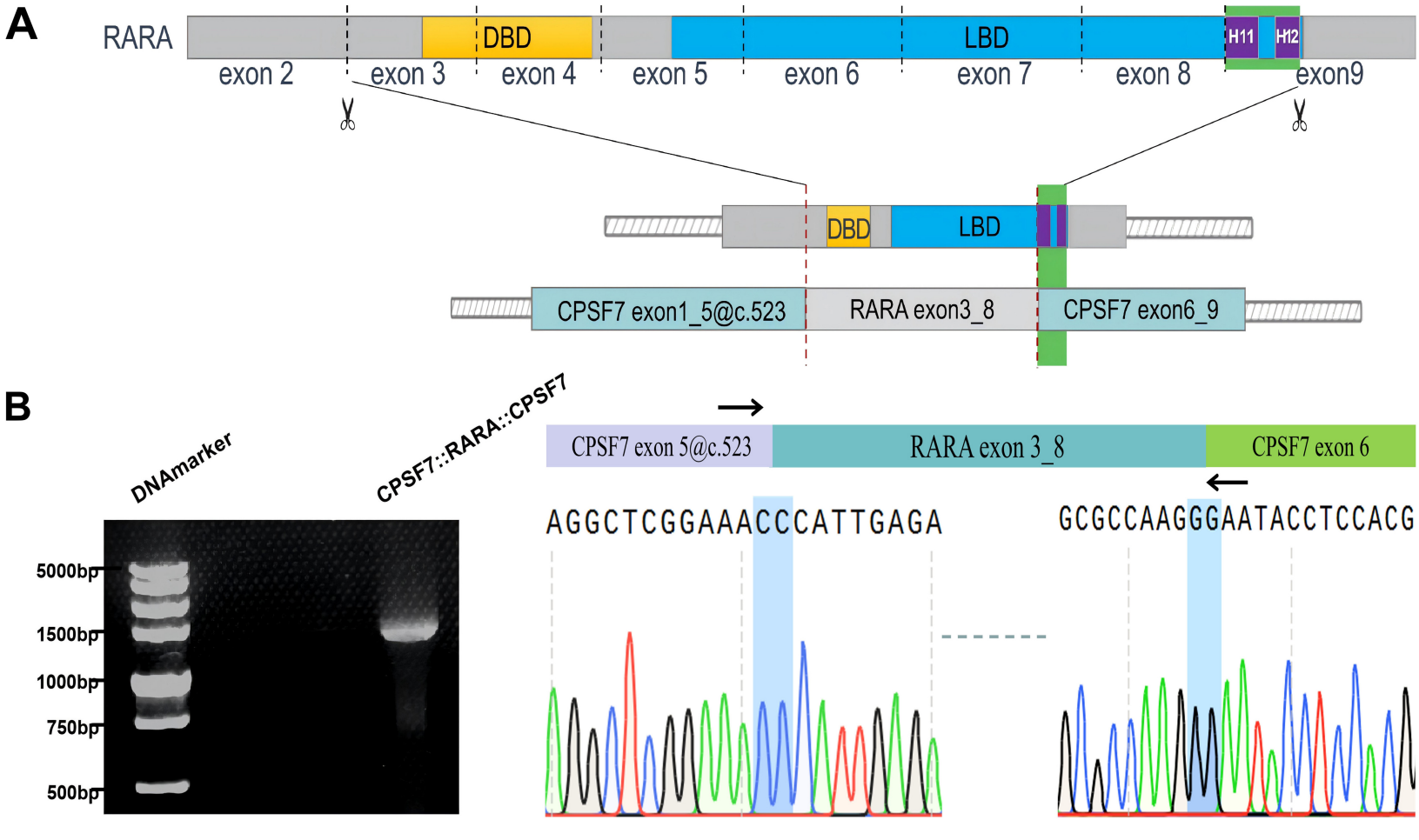
(A) Subcellular localization analysis in control cells and cells expressing the empty vector, CPSF7::RARA, CPSF7::RARA::CPSF7, CPSF7::RARA-delH11_12, wild-type CPSF7, or wild-type RARA.

(B) Dual-luciferase reporter assay evaluating RARE response element activity.

Figure 3. Impact of the CPSF7::RARA::CPSF7 fusion on myeloid cell proliferation and differentiation.

(A) Relative proliferation of stably transduced MV411 and HL-60 cells.

(B) Comparative analysis of surface differentiation markers (CD16, CD11b, CD15, CD34, CD117) expression in stably transduced HL-60 cells post-ATRA treatment.*P < 0.05, **P < 0.01, ***P < 0.001 （one-way ANOVA with Bonferroni post hot test)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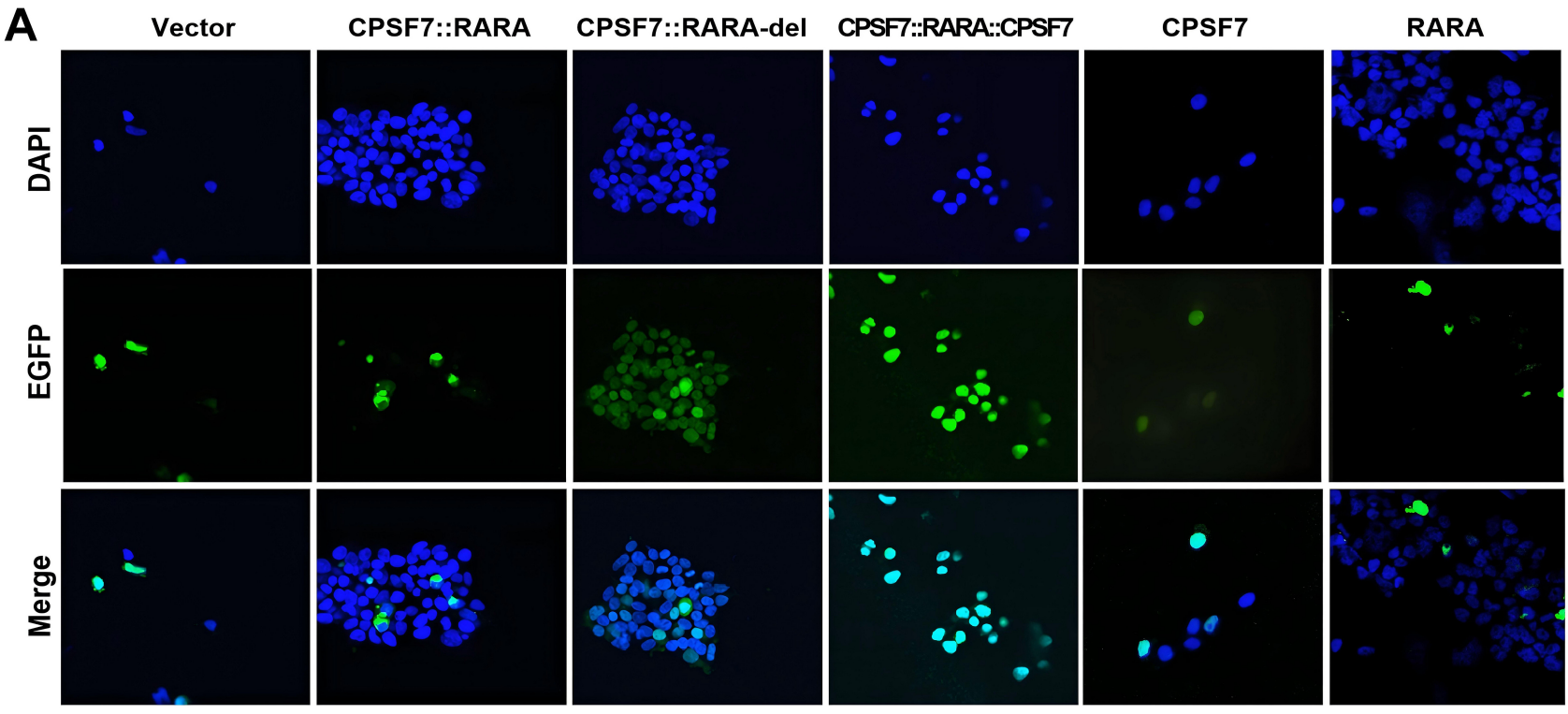
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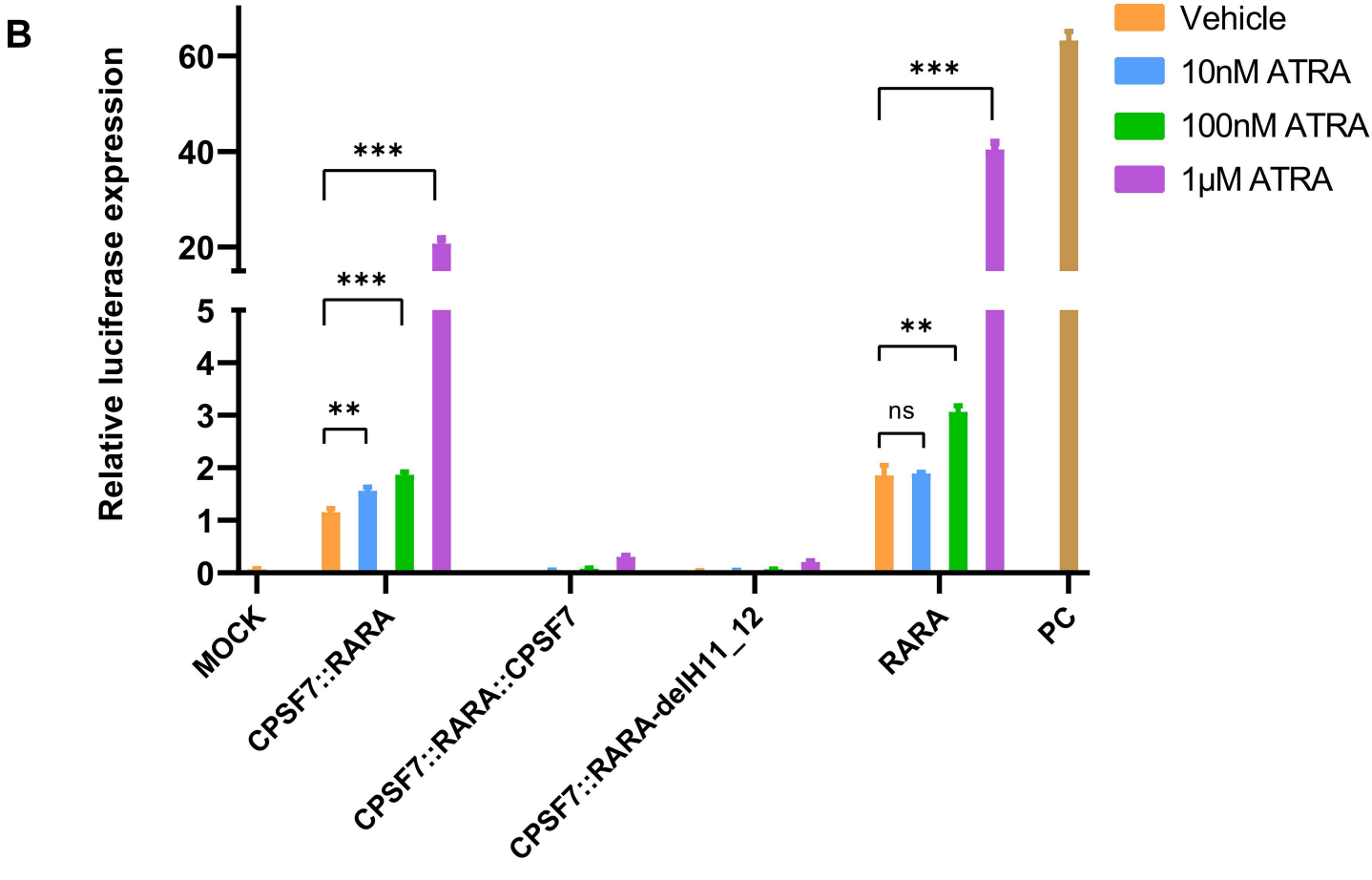
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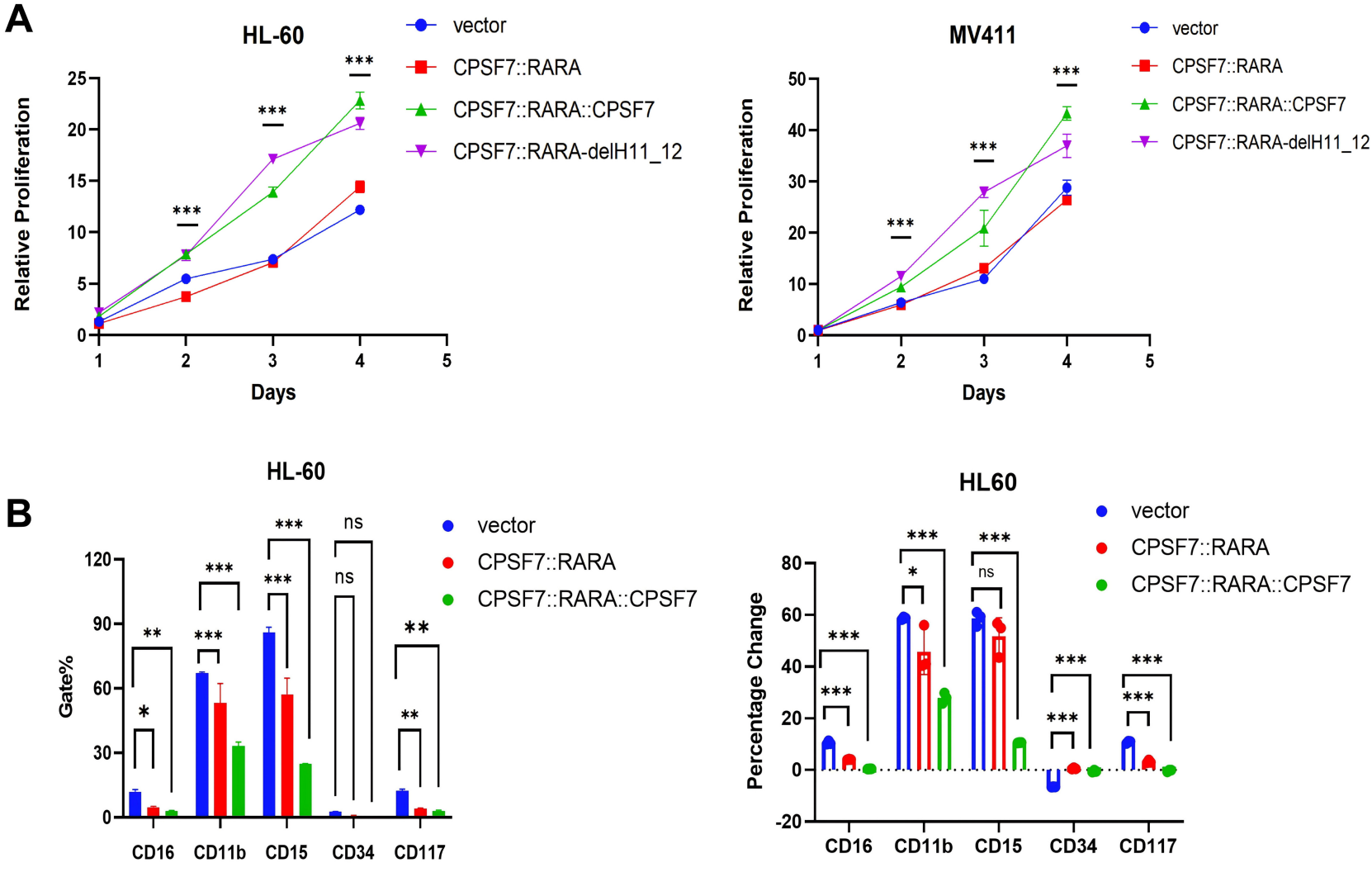
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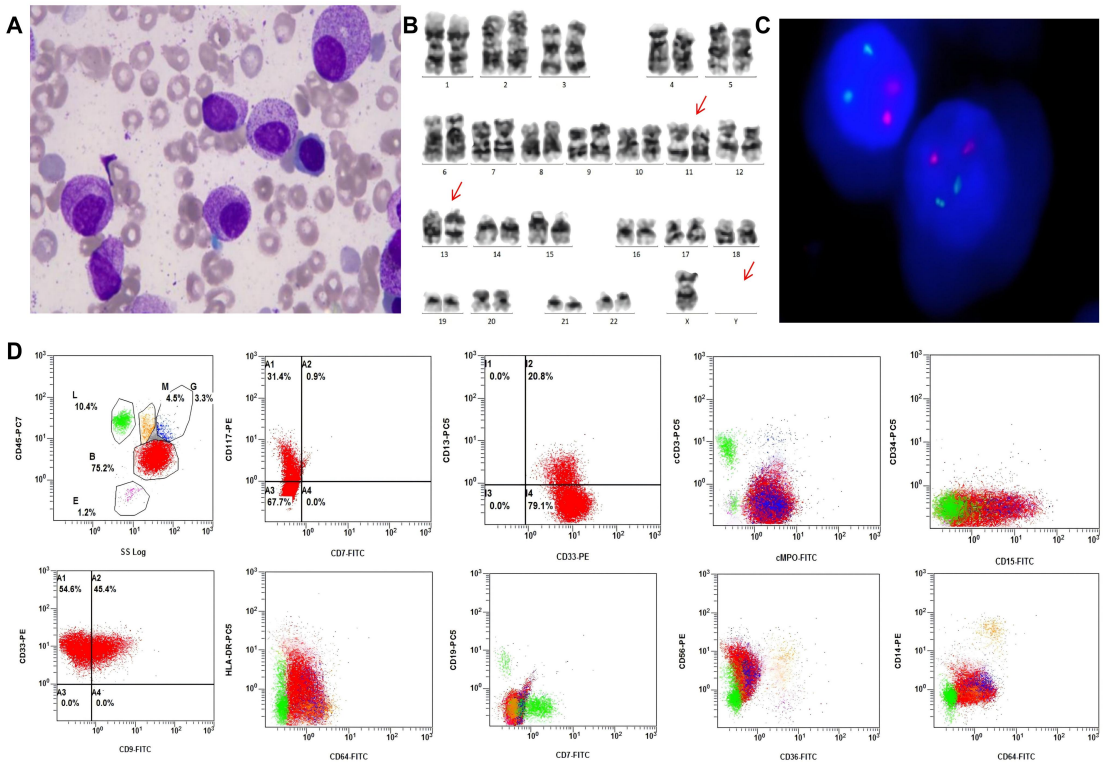


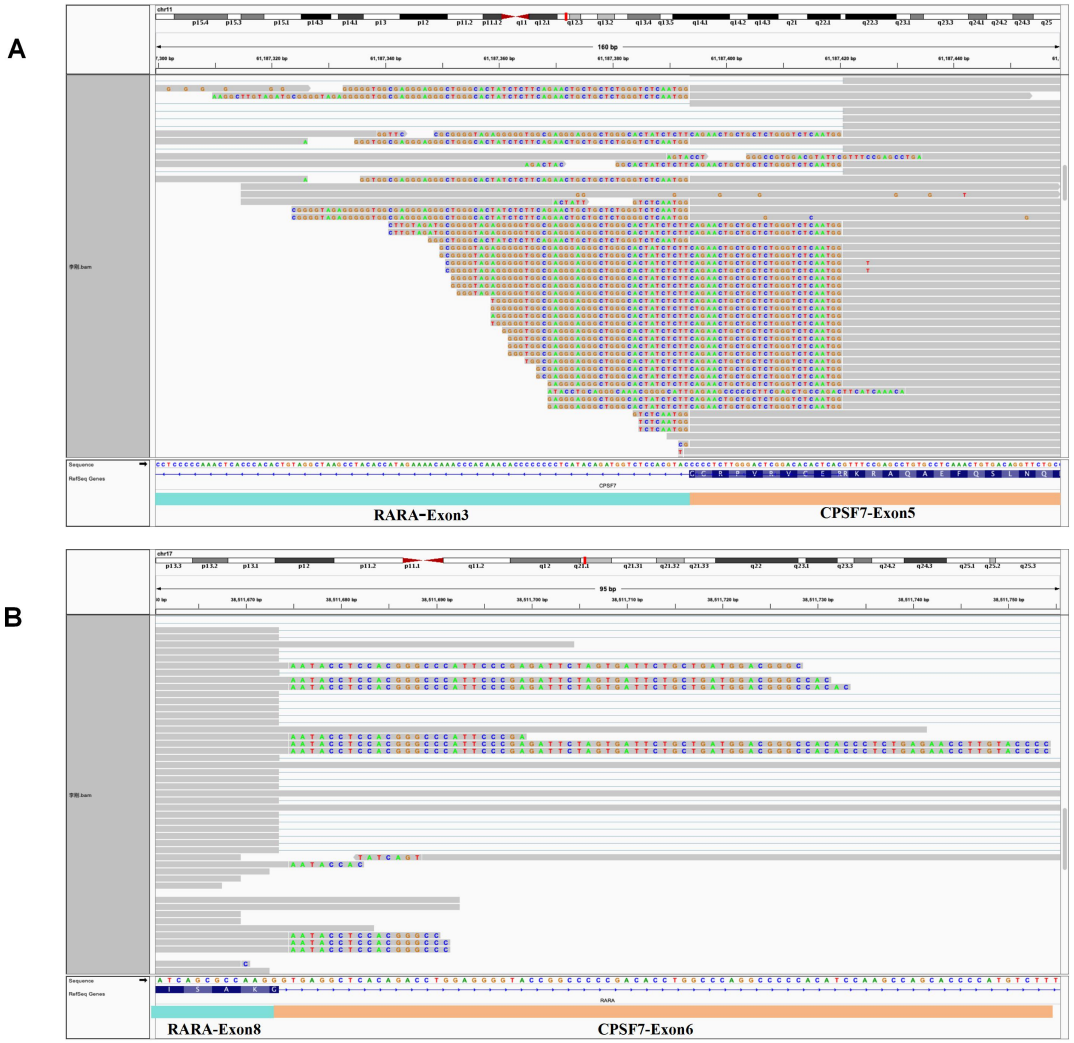
Supplemental figure 1. Diagnostic findings of the novel CPSF7::RARA::CPSF7 fusion gene in the aAPL case.

(A) Bone marrow smear morphology from the aAPL patient (Wright-Giemsa staining, ×1000).

(B) Representative karyotype of the patient.

(C) FISH analysis was negative for the PML::RARA fusion. Analysis was performed using a dual-color, dual-fusion translocation probe.

(D) Immunophenotypic analysis by flow cytometry.



Supplemental figure 2. Fusion gene analysis of the patient by whole transcriptome sequencing.

(A) Integrative Genomics Viewer view of the fusion of CPSF7 exon 5 with RARA exon 3.

(B) Integrative Genomics Viewer view of the fusion RARA exon8 with CPSF7 exon6.