

ARTICLE OPEN



RUNX1 aberrations in blast-phase chronic myeloid leukaemia induce the RNA-binding protein SPATS2L, which promotes growth, survival and stress granule assembly

D. A. Palmer^{1,2}, D. Ogilvie-Park³, A. L. Muir⁴, R. Craig⁴, O. Sevim^{3,5}, L. Stott³, A. Kakkar³, PA Lewis⁶, M. Wilson⁶, K. Heesom⁶, G. A. Horne⁴, M. Copland⁴, S. Mustjoki^{7,8,9}, S. Adnan-Awad^{7,10,11}, K. Porkka^{7,8,9,12}, S. Jain³, N. Bayat³, D. Famili^{1,2}, H. Webb³, M. J. West^{3,13}, F. K. Mardakheh¹⁴, T. Chevassut^{1,2}, A. Tonks¹⁵, S. G. Kellaway¹⁶, B. P. Towler³ and RG Morgan³✉

© The Author(s) 2026

RUNX1 is a critical regulator of hematopoiesis. In CML, somatic *RUNX1* mutations and *RUNX1::MECOM/EVI1*, are associated with TKI resistance and progression to BP-CML. To examine post-transcriptional dysregulation downstream of *RUNX1* aberrations, we used orthogonal organic phase separation to characterise the RNA-binding proteome of *RUNX1* deficient BP-CML cells. *RUNX1* depleted BP-CML cells exhibited significant alterations to RBPs involved in stress response pathways and translation/ribosome-biogenesis. Furthermore, *RUNX1* depletion or expression of *RUNX1::EVI1* in BP-CML cells induced expression and RNA binding activity of SPATS2L, a component of stress granules (SG). Whilst *RUNX1* depletion increased SG-assembly, SPATS2L depletion reduced SG-assembly and inhibited the growth/survival of multiple BP-CML cell lines. Homoharringtonine (HHT), used historically in TKI-resistant CML, ablated SG-assembly in BP-CML cells with *RUNX1* depletion, and primary BP-CML cells with LOF/hypomorphic *RUNX1* mutations were preferentially sensitised to HHT. Suppressing SPATS2L expression induced by *RUNX1* depletion, reduced SG-forming capacity and increased the HHT-sensitivity of *RUNX1* depleted BP-CML cells, suggesting SPATS2L contributes to therapeutic resistance in *RUNX1* mutated CML. Finally, we demonstrate that SGs may contribute more widely to TKI-resistance in this setting. This study suggests that SPATS2L/SG induction could be critical to *RUNX1*-mutant leukemias, and, provides preliminary evidence for a mutationally-targeted approach in CML with *RUNX1* aberrations.

Leukemia; <https://doi.org/10.1038/s41375-026-03161-4>

INTRODUCTION

Chronic myeloid leukemia (CML), is characterised by the *BCR::ABL1* mutation, which drives clonal proliferation and genomic instability [1]. Tyrosine kinase inhibitors (TKIs) have markedly improved survival rates in chronic-phase CML (CP-CML) [2–8]. However, in blast-phase CML (BP-CML), responses to single-agent TKIs are often short-lived [9], necessitating the use of intensive chemotherapy regimens consolidated with an allogeneic stem cell transplant to induce durable remissions [10]. The acquisition of secondary somatic mutations in CML, seen in the driver genes associated with myeloid malignancies, or additional chromosomal abnormalities (ACAs), accelerates transformation into the blast phase [11–14]. Somatic *RUNX1* mutations are enriched in BP-CML, occurring at a rate of 10%–30% [11, 12], but are seldom detected at diagnosis [13, 15], indicating they serve as critical molecular events driving TKI

resistance and disease transformation. Furthermore, the t(3;21) (q26.2;q22) translocation, generating *RUNX1::MECOM/EVI1* (*MDS1* and *EVI1* complex locus) transcripts, encountered in BP-CML as part of the high-risk 3q.26 ACA group [14], is also associated with TKI resistance and adverse survival [14, 16].

Myeloid malignancies are frequently enriched for *RUNX1* mutations and *RUNX1* fusion genes [17, 18]. *RUNX1* mutations are associated with chemoresistance and adverse survival, occurring as missense, frameshift (indels), nonsense and splice site mutations, frequently clustering in the DNA-binding runt homology domain (RHD) [11–13, 19–23]. In CML, somatic *RUNX1* mutations have also been shown to cooperate with *BCR::ABL1*, accelerating leukemogenesis [24]. Furthermore, *RUNX1* mutations in BP-CML are associated with the induction of interferon signalling, immune pathways and lymphoid antigen expression,

¹Clinical and Experimental Medicine, Brighton Sussex & Medical School, Brighton, UK. ²University Hospitals Sussex NHS Foundation Trust, Brighton, UK. ³School of Life Sciences, University of Sussex, Brighton, UK. ⁴Paul O' Gorman Leukaemia Research Centre, School of Cancer Sciences, University of Glasgow, Glasgow, UK. ⁵Faculty of Medicine, Pamukkale University, Denizli, Türkiye. ⁶University of Bristol Proteomics Facility, Bristol, UK. ⁷Hematology Research Unit Helsinki, Department of Hematology, University of Helsinki and Helsinki University Hospital Comprehensive Cancer Centre Helsinki, Helsinki, Finland. ⁸ICAN Digital Precision Cancer Medicine Flagship University of Helsinki and Helsinki University Hospital Comprehensive Cancer Centre Helsinki Finland, Helsinki, Finland. ⁹Translational Immunology Research Program University of Helsinki, Helsinki, Finland. ¹⁰Clinical Pathology Department, National Cancer Institute, Cairo University, Cairo 11796, Egypt. ¹¹MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine and National Institute of Health Research (NIHR), Oxford Biomedical Research Centre, University of Oxford, Oxford, UK. ¹²Department of Oncology, Department of Clinical Chemistry, HUS Diagnostic Centre, Helsinki University Hospital, University of Helsinki, Helsinki, Finland. ¹³School of Biological and Behavioural Sciences, Queen Mary University London, London, UK. ¹⁴Department of Biochemistry, University of Oxford, Oxford, UK. ¹⁵Department of Haematology, Division of Cancer and Genetics, Cardiff University, Cardiff, UK. ¹⁶Centre for Cancer Sciences, School of Medicine, University of Nottingham, Nottingham, UK. ✉email: rhys.morgan@sussex.ac.uk

Received: 16 April 2026 Revised: 8 September 2026 Accepted: 23 September 2026

Published online: 09 October 2026

and preclinical responses to novel therapies, including mTOR, BCL2 and VEGF inhibitors, have been shown [23]. This indicates *RUNX1* mutations in CML are associated with their own unique mechanisms for disease transformation, which could be amenable to targeting. *RUNX1::MECOM/EVI1* disrupts *RUNX1*- and *EVI1*-driven gene networks, affecting hematopoietic differentiation and lineage commitment [25–29]. *RUNX1::MECOM/EVI1* has also been shown to cooperate with *BCR::ABL1*, inducing leukemic progression [30]. Consequently, targeting *RUNX1* aberrations in CML remains an area of unmet clinical need. Research has predominantly focused on transcriptional dysregulation mediated by *RUNX1* mutations or fusion genes in myeloid malignancies [17, 18]; however, growing evidence suggests *RUNX1* is a regulator of key post-transcriptional processes, including splicing [30–33] and ribosome biogenesis (RiBi)/translation [34, 35].

To explore this, we characterised changes to the RNA-bound proteome in BP-CML cells with *RUNX1* depletion. Stress response pathways and RiBi/translation were enriched for differentially regulated RNA-binding proteins (RBP) upon *RUNX1* depletion. Furthermore, *RUNX1* depletion or expression of *RUNX1::EVI1* induced the RBP SPATS2L (Spermatogenesis Associated Serine Rich 2-Like), previously identified as upregulated in *RUNX1*-mutated primary BP-CML cells [23]. SPATS2L is a component of stress granules (SG) [36, 37], membraneless condensates protecting mRNAs/RBPs during stress, promoting cellular survival and contributing to therapeutic resistance [38, 39]. We found SPATS2L to be a critical regulator of SG-assembly in BP-CML cells with *RUNX1* depletion. Furthermore, we found HHT inhibited SG-assembly in BP-CML cells with *RUNX1* depletion, and primary BP-CML cells with LOF/hypomorphic *RUNX1* mutations were preferentially sensitised to HHT. These findings demonstrate that disrupting SG-assembly may represent a novel therapeutic strategy in *RUNX1*-mutated BP-CML.

METHODS

Orthogonal organic phase separation (OOPS)

OOPS was performed as described [40], adapted to suspension cells. For each experimental replicate, $\sim 10 \times 10^6$ cells were crosslinked with 300 mJ UV-C and then pelleted before resuspension in 1 mL of TRIzol™ LS Reagent (Invitrogen, ThermoFisher Scientific), followed by the addition of 200 μ L of UltraPure™ Chloroform (Invitrogen, ThermoFisher Scientific). Following vortexing and centrifugation at 4 °C, 12,000 $\times g$, for 15 min, the aqueous and organic phases were removed, isolating the interphase into 1 mL TRIzol™ LS with 200 μ L UltraPure™ Chloroform. The final interphase was centrifuged for 2 min at 4 °C, 12,000 $\times g$, after the addition of 1 mL ice-cold methanol (ThermoFisher Scientific). Samples were resuspended in 100 μ L RBP buffer containing 100 mM TEAB (Triethylammonium bicarbonate buffer; Merck-Millipore), 1 mM $MgCl_2$ (ThermoFisher Scientific), 1% SDS solution (Severn Biotech) diluted in a stock of RNAase-free water (ThermoFisher Scientific), and RNA digestion was carried out with 8 μ g RNase A/T1 enzyme (ThermoFisher Scientific). The next day TRIzol™ LS and UltraPure™ Chloroform were added followed by centrifugation (as above), and 400 μ L of the organic phase was added in a 1:4 ratio to 99 + % Acetone (ThermoFisher Scientific) and precipitated overnight at –20 °C. Samples were centrifuged at 17,000 $\times g$, 4 °C for 20 min, and resuspended in 50 μ L RIPA buffer (ThermoFisher Scientific). Protein was quantified in each replicate using an EZQ protein quantitation kit (ThermoFisher Scientific), and 10–15 experimental replicates for each cell line condition were combined into three replicates, each containing 20 μ g of protein for tandem-mass-tag (TMT)-labelling and LC-MS (described in supplementary methods). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD076395.

RESULTS

Induction of stress response pathways and dysregulated RiBi/translation is a feature of BP-CML cells with *RUNX1* depletion

To examine post-transcriptional disruption downstream of *RUNX1* loss, we first evaluated whether *RUNX1* depletion modified the

RNA-bound proteome. We used two unique shRNAs targeting the *RUNX1* NM_001754.4 transcript (isoform c) in KU812 and K562 myeloid BP-CML cells (Fig. 1A), followed by OOPS (a method of enriching RNA–protein interactions) coupled to TMT-labelled LC–MS. We first confirmed the successful execution of OOPS–MS by enriching RBPs to around 50% in both cell lines, using the GO search term “RNA-binding” (Supplemental Fig. S1A, B). In KU812 cells, 19.2% of detected RBPs (136/708) were significantly altered in abundance with *RUNX1* depletion compared to controls ($p < 0.05$; Fig. 1B), whilst fewer changes were observed in K562 cells (8/963), potentially reflecting the heterogeneity of the BP-CML cell lines used and the variation with OOPS efficiency (Supplemental datasheets 1 and 2). Thus, we focused our downstream analysis on KU812 cells. To establish pathways enriched for differentially regulated RBPs, we performed an analysis of canonical pathways (Fig. 1C, D). *RUNX1* depletion activated multiple pathways associated with the stress response, such as the *response of EIF2AK4 (GCN2) to amino acid deficiency*, *oxidative phosphorylation*, *NRF2–mediated oxidative stress response*, and the *unfolded protein response* (UPR). Furthermore, there was an enrichment of differentially regulated RBPs associated with RiBi/translation, including with *rRNA processing in the nucleolus and cytosol* and *translation-initiation -elongation and -termination*.

RUNX1-deficient HSCs have attenuated ER stress, an UPR and low rates of RiBi [34]. The enrichment of differentially regulated RBPs associated with stress response pathways and dysregulated RiBi/translation, found in KU812 cells with *RUNX1* depletion, led us to consider whether *RUNX1* regulates 5'TOP (terminal oligopyrimidine) motifs. Cells exposed to stress stall translation by downregulating the expression of mRNAs containing a 5'TOP motif, a region in the 5'UTR of mRNAs of multiple components of translation [41, 42]. *RUNX1* depletion in KU812 cells led to a significant difference ($p < 0.05$) in expression of 22% (17/78) of 5'TOP motifs, of which 58% were increased ribosomal subunit proteins (Fig. 1E). To expand these findings, we performed polysome profiling in KU812 and K562 cells with *RUNX1* depletion (Fig. 1F; Supplemental Fig. S2). Area under the curve (AUC) analysis showed an increase in monosomes and polysomes with *RUNX1* depletion, but no consistent increase in the polysome/monosome ratio, indicating *RUNX1* depletion increased ribosome abundance in BP-CML cells, without a shift towards actively translating polysomes.

RUNX1 depletion or expression of *RUNX1::EVI1* causes induction of SPATS2L in BP-CML cells

We next decided to interrogate an RBP with dysregulated RNA-binding activity/expression in the post-transcriptional processes identified, common to both cell lines. We identified the upregulation of SPATS2L, known as Stress Granule and Nucleolar Protein (SGNP; Fig. 2A, B), an RBP which regulates rRNA processing [36], found to be enriched 1.65 and 0.52 $\log_2$ -fold on RNA derived from K562 and KU812 cells upon *RUNX1* depletion, respectively. SPATS2L is predicted to have four isoforms, and we observed increased expression of multiple different SPATS2L isoforms from whole cell lysates derived from both cell lines with *RUNX1* depletion (Fig. 2C), indicating total expression was impacted. To understand whether *RUNX2/3* contribute to elevated SPATS2L expression, we examined their expression in response to *RUNX1* depletion. *RUNX2/3* expression was either low, unchanged or inconsistent across both cell lines, meaning they are unlikely to regulate SPATS2L induction (Supplemental Fig. S3). To consider other forms of *RUNX1* dysregulation on SPATS2L expression in the context of BP-CML, we evaluated whether expression of *RUNX1::EVI1* t(3;21), part of the 3q.26 cytogenetically rearranged group [14], which has a repressive effect over *RUNX1* [25–29], could also induce SPATS2L. We induced expression of *RUNX1::EVI1* via lentiviral transduction of K562 and KU812 cells, and *RUNX1* and

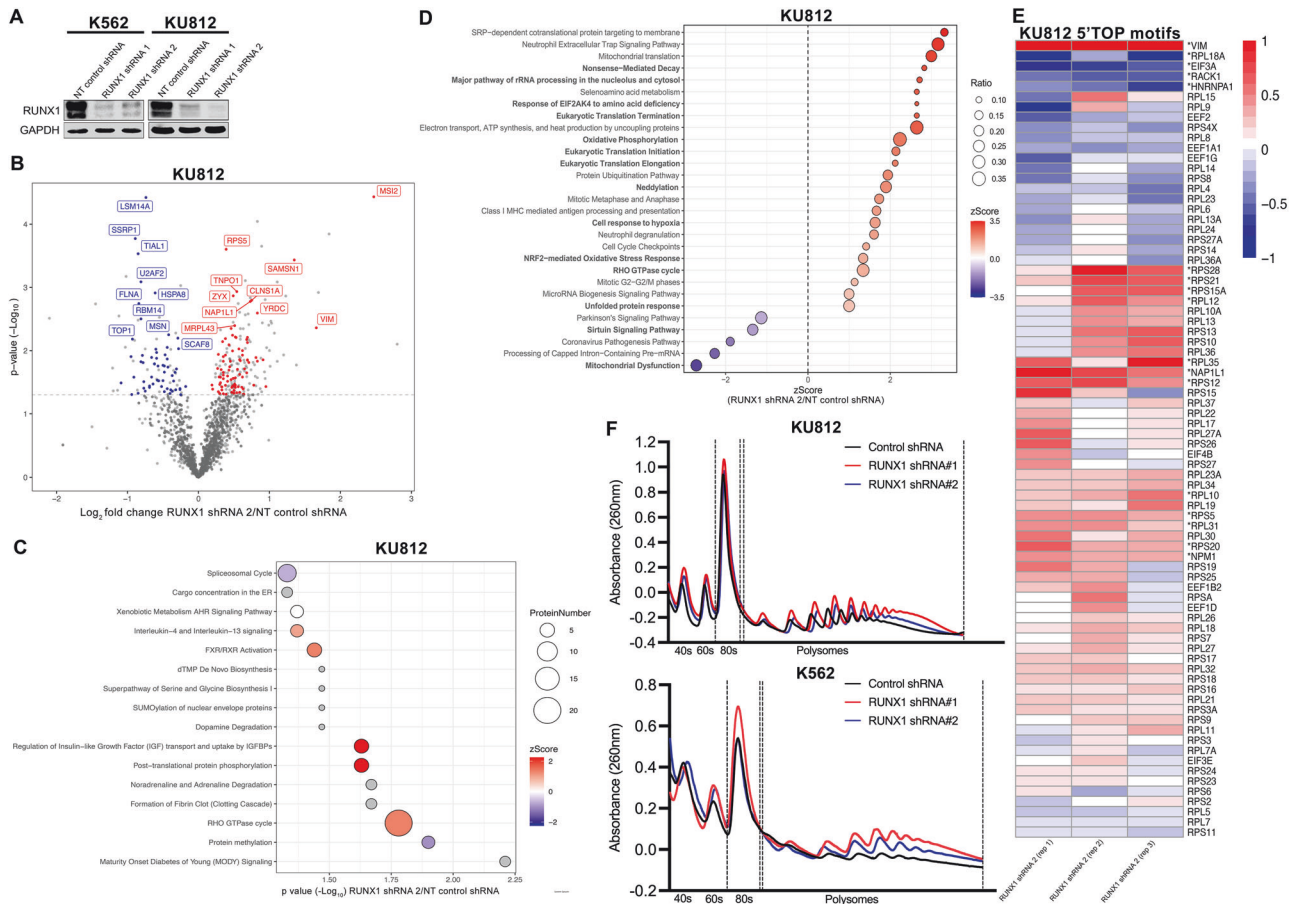


Fig. 1 RUNX1-depleted BP-CML cells are enriched for differentially regulated RBPs associated with stress response pathways and dysregulated RiBi/translation. **A** Western blots of K562 and KU812 cells transduced with two unique RUNX1-targeting shRNAs compared to a non-targeting (NT) control shRNA. GAPDH was used as a loading control. **B** Volcano plot depicting the most differentially regulated RBPs in RUNX1 depleted (shRNA 2) ($n = 3$) versus NT control-shRNA KU812 cells ($n = 3$). The most significantly enriched (red)/depleted (blue) RBPs are annotated. Horizontal dashed lines indicate the threshold for statistical significance ($p < 0.05$) on a $-\log_{10}$ scale (1.3). Dark grey dots are non-statistically significant RBPs and light grey dots are proteins which are non-RNA-binding. **C** A reactome of canonical pathways enriched for differentially regulated-RBPs in RUNX1-depleted (shRNA 2) versus NT control-shRNA KU812 cells. Pathways coloured in grey represent insufficient proteins significantly changed, with known activation/inhibition roles within that pathway to calculate a z-score. **D** A reactome of canonical pathways enriched for differentially regulated-RBPs in RUNX1 depleted (shRNA 2) versus NT control-shRNA KU812 cells based on z-Scores alone, comparing the direction of LogFC and its alignment to the expected direction of change, based upon activation/enrichment of that pathway. A z-score > -1 and < 1 was used as a cut-off, and pathways represented by ≤ 5 proteins were excluded. Relevant pathways have been highlighted. **E** A heat map containing all RBPs from OOPS in RUNX1-depleted (shRNA 2) KU812 cells with 5'TOP motifs. Each RUNX1 shRNA replicate is compared to its respective NT control-shRNA replicate ($n = 3$). LogFC is represented by the colour scale. Statistically significant proteins are annotated by $*p < 0.05$. **F** Polysome profiles of KU812 and K562 cells transduced with NT control-shRNAs (black) compared to RUNX1-targeting shRNAs (red/blue). Dashed vertical lines represent monosome and polysome fractions. The 40 s, 60 s, 80 s subunits, polysomes and absorbance readings (260 nm) are annotated.

EV11 appeared between 150 and 250 kDa, confirming the N-terminus of RUNX1 fused to EV11 (Fig. 2D). We found RUNX1::EV11 induced SPATS2L expression in each of these cells, comparable to endogenous levels present in SKH1 cells; a BP-CML cell line harbouring t(3;21) (Fig. 2D) [43]. To evaluate whether SPATS2L protein induction was also observed at the transcript level, we examined mRNA levels and demonstrated a significant increase in *SPATS2L* mRNA expression with both expression of RUNX1::EV11 and RUNX1 depletion (Fig. 2E, F). Given *SPATS2L* mRNA induction correlated with protein expression, we assessed relevance to primary BP-CML samples by interrogating the RNAseq dataset of Awad et al. [23]. *SPATS2L* was significantly higher in RUNX1 mutated (all RHD-mutant) versus RUNX1 wild-type primary BP-CML samples (Fig. 2G). Taken together, these findings demonstrate SPATS2L is induced in BP-CML cells upon different forms of RUNX1 aberration, implying a wider role in RUNX1 mutant BP-CML pathogenesis.

SPATS2L depletion inhibits growth, survival and SG-assembly in BP-CML cells

SPATS2L regulates the growth and survival of acute myeloid leukemia (AML) cells, and high expression is associated with inferior overall survival outcomes in AML patients [44]. To explore SPATS2L in the context of BP-CML, we depleted SPATS2L in K562, KU812 and SKH1 cells using two SPATS2L-targeting shRNAs, which targeted multiple SPATS2L isoforms, inferred by depletion of these bands within the predicted molecular weight range (50–75 kDa; Fig. 3A). SPATS2L depletion inhibited the growth and viability of all three BP-CML cell lines (Fig. 3B–D), further substantiating its pro-leukaemic role. SPATS2L has been shown to localise to [36] and coordinate SG-assembly [37]; membraneless foci which protect mRNAs/proteins from degradation and promote survival during conditions of acute stress [38]. Given these findings, we explored whether the capacity for SG-assembly was disrupted by SPATS2L depletion in BP-CML cells using a 1-h sodium arsenite

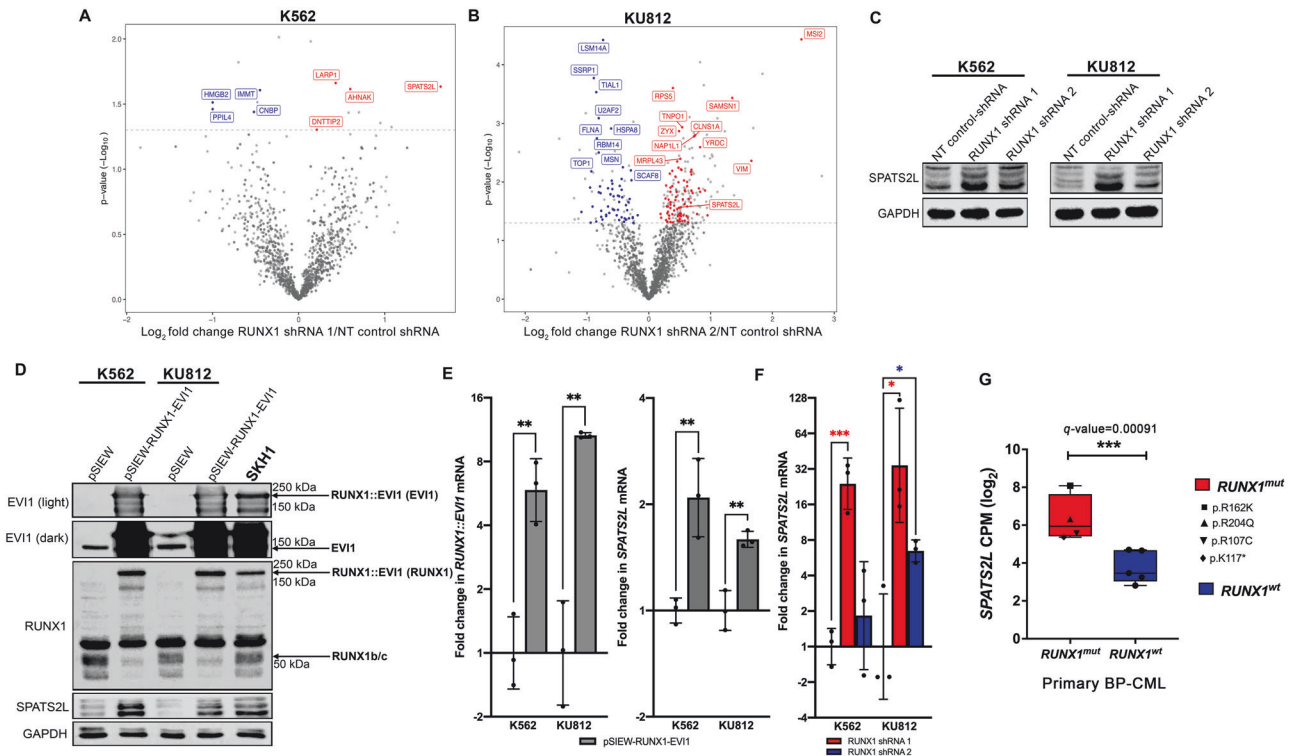


Fig. 2 **RUNX1 depletion and expression of RUNX1::EV11 induces SPATS2L in BP-CML cells.** **A, B** Volcano plots of the most differentially regulated RBPs in RUNX1-depleted K562 (shRNA 1) and KU812 (shRNA 2) cells ($n = 3$) versus NT-control shRNAs ($n = 3$). Each plot depicts the most significantly enriched (red)/depleted (blue) RBPs. Horizontal dashed lines indicate the threshold for statistical significance ($p < 0.05$) on a $-\log_{10}$ scale (1.3). SPATS2L has been annotated in each volcano plot. **C** Western blot showing the level of SPATS2L in K562 and KU812 cells with RUNX1 depletion compared to NT controls. GAPDH was used as a loading control in each blot. **D** Western blot showing the level of EV11, RUNX1 and SPATS2L in K562 and KU812 cells transduced with a pSIEW-RUNX1-EV11 or pSIEW (control) plasmid, alongside SKH1 cells. The darker EV11 image allows for visualisation of EV11 (145 kDa) in pSIEW-K562 and -KU812 cells. GAPDH was used as a loading control. **E, F** RT-qPCR of *RUNX1::EV11* and *SPATS2L* in K562 and KU812 cells transduced with pSIEW-RUNX1-EV11 (grey) compared to pSIEW controls, and RT-qPCR of *SPATS2L* in K562 and KU812 cells with RUNX1 depletion (red/blue) compared to controls. Data is represented by fold change on $\Delta\Delta C_t$ readings. **G** Box and whisker plot showing *SPATS2L* mRNA expression (counts per million) across *RUNX1* RHD mutant ($n = 4$) vs *RUNX1* wild-type ($n = 5$) primary BP-CML cells generated from the RNAseq dataset of Awad et al., *Leukemia*, 2021 [23]. Data represents mean $\pm$ 1 SD ($n = 3$) and statistical significance is denoted as $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$ from a two-tailed unpaired student's *t*-test.

(NaAsO₂) treatment, which enhances their formation for visualisation by CLSM (Supplemental Fig. S4). Using a G3BP1 Stress Granule Assembly Factor 1 (G3BP1) antibody (SG marker), we measured SG number/size in K562 and KU812 cells with SPATS2L depletion (Fig. 4A–D). This demonstrated a reduction in the average SG number per cell (but not size), which, taken together, demonstrates SPATS2L depletion inhibits growth, viability and SG-assembly in BP-CML cells.

RUNX1 depletion in BP-CML cells induces SG-assembly, which are nearly eradicated with HHT treatment

Upon induction of oxidative stress SPATS2L localises to SGs and the nucleolus, where it has been reported to regulate 5.8 s rRNA processing [36]. Given SPATS2L depletion reduced SG-assembly in BP-CML cells, we evaluated whether the subcellular distribution of SPATS2L was modified by stress induction. To do this we performed nuclear-cytoplasmic fractionations $\pm$ NaAsO₂ treatment to induce oxidative stress in K562 and KU812 cells with RUNX1 depletion. In keeping with our previous observations, we found an overall higher basal level of SPATS2L upon RUNX1 depletion versus controls, which was elevated still further upon NaAsO₂ exposure (Fig. 5A, B). Notably, NaAsO₂ exposure alone was sufficient to increase SPATS2L, suggesting induction of this RBP could be a general cellular stress response. We next considered whether RUNX1-depleted BP-CML cells had increased SG-forming capacity, given our findings that RUNX1 depletion induced multiple RBPs-associated with stress response pathways and

dysregulated RiBi/translation. We observed significantly increased SG-assembly in K562 cells with RUNX1 depletion, with a similar trend in KU812 cells (Fig. 5C–E). Canonical SG-assembly occurs following eIF2 α phosphorylation, which disrupts cap-dependent translation, leading to the sequestration of mRNAs into these cellular condensates, lacking the 60 s ribosomal subunit [38]. Harringtonine inhibits NaAsO₂-induced SG-assembly by freezing 80 s ribosomes onto mRNAs at the 60 s ribosomal-A site [45]. HHT has demonstrated in vitro efficacy in AML cells with RUNX1 mutations [35], and we found BP-CML cells with RUNX1 depletion had higher SG-forming capacity following NaAsO₂ exposure. Thus, we hypothesised that an additional mechanism by which HHT induces apoptosis in BP-CML with RUNX1 aberrations could be through SG inhibition. Supporting this concept, we found SG-assembly was nearly eliminated in K562 RUNX1 shRNA cells following a pretreatment with HHT and subsequent 1-h NaAsO₂ exposure (Fig. 5F, G). In summary, these data confirm SPATS2L is induced upon stress, and RUNX1 depletion increases SG-assembly in BP-CML cells, which is prevented with HHT treatment.

BP-CML cells with mutations in RHD of RUNX1 are preferentially sensitised to HHT

Our findings showing HHT nearly eliminated SG-assembly in BP-CML cells with RUNX1 depletion prompted us to further investigate this translation inhibitor in *RUNX1*-mutated BP-CML, a therapy with a history of clinical use in CML [46–49]. K562 and KU812 cells were sensitised to HHT with RUNX1 depletion, with a

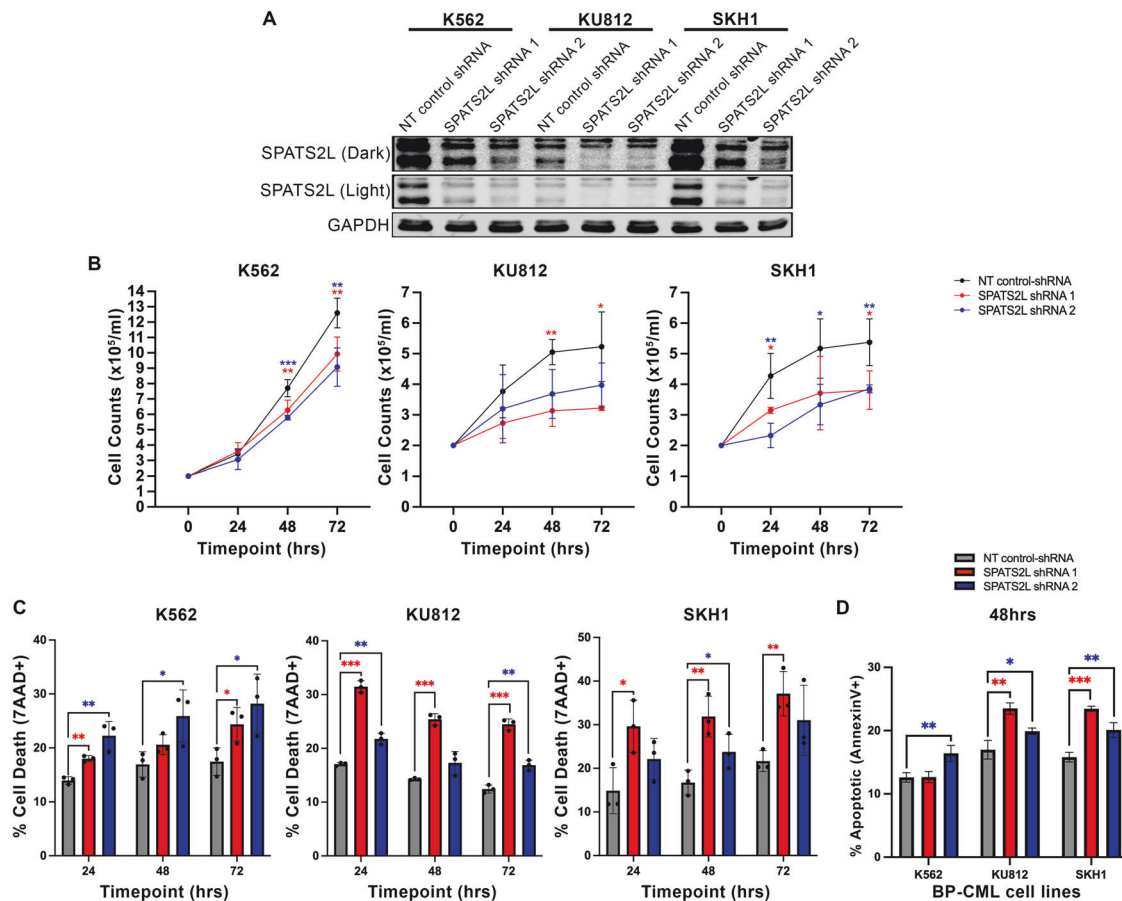


Fig. 3 SPATS2L depletion impairs the growth and survival of BP-CML cells. **A** Western blot showing SPATS2L level in response to two unique SPATS2L-targeting shRNAs in K562, KU812 and SKH1 cells. A dark and light SPATS2L exposure is depicted due to the lower expression in KU812 cells. GAPDH was used as a loading control. **B** Cell counts in K562, KU812 and SKH1 cells with SPATS2L depletion at 24- 48- and 72-h post-seeding. Data represents mean ± 1 SD ($n = 5$) in K562, ($n = 3$) in KU812 and ($n = 4$) in SKH1 cells, with statistical significance denoted by * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$ from a two-tailed unpaired student's t -test. **C** Cell viability in K562, KU812 and SKH1 cells was measured by 7AAD⁺ staining (dead cells) at 24- 48- and 72-h post-seeding. Data represents mean ± 1 SD ($n = 3$). Statistical significance is denoted by * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$ from a two-tailed unpaired student's t -test at each timepoint. **D** Percentage apoptotic (Annexin V positive staining for early/late-stage apoptosis) K562, KU812 and SKH1 cells with SPATS2L depletion versus NT-shRNA controls at 48 hours post-seeding. Data represents mean ± 1 SD ($n = 3$). Statistical significance is denoted by * $p < 0.05$ ** $p < 0.01$ or *** $p < 0.001$ from a two-tailed unpaired student's t -test.

heightened sensitivity after 48-h (Fig. 6A; Supplemental Fig. S5A). To explore the mechanism of cell death, we next used the RUNX1 shRNA1 cell lines, which demonstrated the greatest sensitivity to HHT, to establish whether HHT primarily induced apoptosis at 48-h. We found a significant increase in apoptosis with HHT treatment in BP-CML cells with RUNX1 depletion (Fig. 6B). However, a higher basal level of apoptosis existed in KU812 RUNX1 shRNA 1 cells compared to controls; therefore we also analysed the sensitivity of these cells using fold change (Supplemental Fig. S5B). Given the increased HHT-sensitivity observed with RUNX1 depletion, we next treated 5 RUNX1 mutated and 4 RUNX1 wild-type primary BP-CML samples with HHT (the clinical/molecular characteristics of these samples are provided in Supplemental Table S7). Despite a trend towards higher HHT-sensitivity amongst RUNX1-mutated samples, we found no significant differences between RUNX1-mutated and RUNX1-wild-type samples (Fig. 6C); however, given the heterogeneity of RUNX1 mutations, we further stratified our analysis between RHD or C-terminal mutations. From this, we observed statistically higher rates of HHT-induced apoptosis in BP-CML samples with RUNX1 RHD mutations versus RUNX1 wild-type samples (Fig. 6D, E), albeit with a small sample number. Overall, these findings demonstrate RUNX1 dysregulation sensitises BP-

CML cells to HHT through increased apoptosis, with mutations in the RHD demonstrating increased susceptibility.

SPATS2L inhibition in RUNX1-depleted BP-CML cells inhibits growth and viability and increases HHT sensitivity

SPATS2L amplification occurs with increasing HHT resistance in AML cells, and its subsequent depletion sensitises these cells to HHT [44]. We found dysregulated RiBi/translation and increased SG-assembly were features of BP-CML cells with RUNX1 depletion, which could sensitise these cells to HHT. However, SPATS2L could contribute to therapeutic resistance by sustaining SG-assembly in response to treatment. Thus, we next aimed to establish whether inhibition of SPATS2L induction observed upon RUNX1 depletion could impact the growth, viability and HHT-sensitivity of BP-CML cells. For this, we used the puromycin-selected K562 RUNX1 shRNA1 cell line, since it demonstrated superior SG-forming capacity, and co-transduced separately with two distinct GFP⁺ SPATS2L-targeting shRNAs. High transduction efficiency was confirmed through GFP⁺ assessment, indicating efficient SPATS2L-targeting/control shRNAs in K562 RUNX1 shRNA 1 cells (Supplemental Fig. S6). We next measured SPATS2L expression, which confirmed the previously observed SPATS2L induction upon RUNX1 depletion was abrogated in the double-transduced

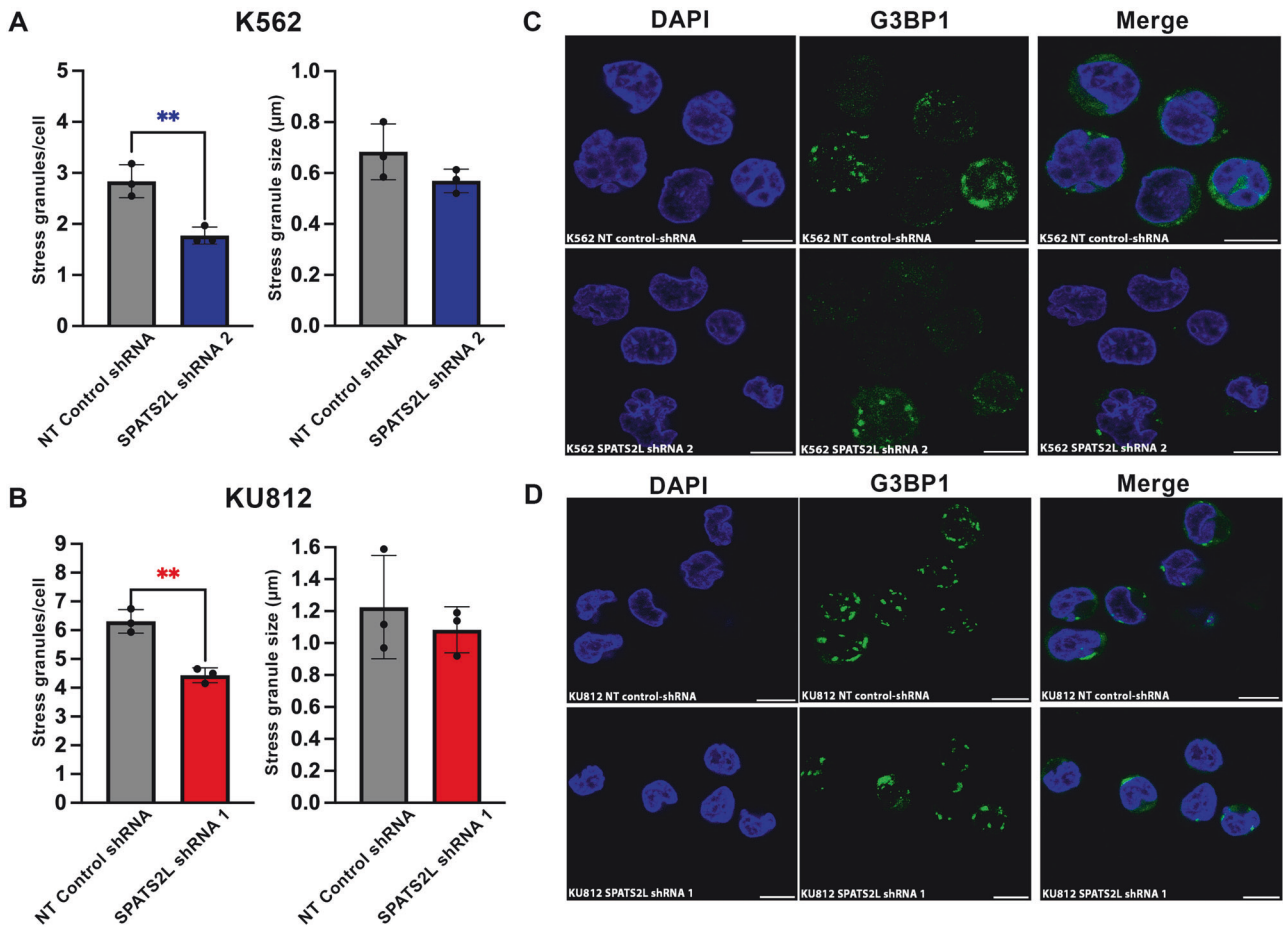


Fig. 4 SPATS2L depletion impairs SG-assembly in BP-CML cells. Summary bar graphs showing the average number/size (μm) of SG in SPATS2L-depleted (A) K562 and (B) KU812 cells relative to NT control-shRNA cells, following 1-h NaAsO₂ exposure. Data represents mean ± 1 SD ($n = 3$). Statistical significance is denoted by ** $p < 0.01$ from a two-tailed unpaired student's t -test. Representative CLSM images show the DAPI, G3BP1 and merged stains of (C) K562 and (D) KU812 cells transduced with an NT control-shRNA or SPATS2L-targeting shRNAs following 1 h NaAsO₂ exposure. White scale bars represent 10 μm.

K562 cells, most notably using SPATS2L shRNA2 (Fig. 7A). Thus, we used this K562 cell line for subsequent testing, which exhibited overall impaired growth and viability compared with the control K562 RUNX1 shRNA line capable of SPATS2L induction (Fig. 7B, C). To establish whether SPATS2L inhibition could further increase HHT sensitivity of RUNX1-depleted K562 cells, we treated double-transduced cells with an HHT-dose response for 48 hours. Given the higher basal level of death in K562 RUNX1 shRNA1/SPATS2L shRNA2 cells, we measured HHT sensitivity with fold change (Fig. 7D). This demonstrated K562 cells with RUNX1 depletion were further sensitised to HHT when SPATS2L induction was inhibited, which also increased imatinib sensitivity (Supplemental Fig. S7). Finally, we assessed SG-assembly in double-transduced cells to examine whether SPATS2L mediated the increased SG-forming capacity of RUNX1-depleted cells observed in Fig. 5B, C. Figure 7E, F demonstrated that whilst RUNX1 depletion alone significantly elevated SG number per cell, when combined with SPATS2L depletion, this SG-forming capacity was diminished back to control level. Overall, these results support the targeting of SPATS2L in the context of RUNX1 dysregulation in BP-CML and could imply the SPATS2L/SG axis contributes to therapeutic resistance in this setting.

Stress granules may contribute more widely to TKI resistance
Finally, to examine whether SGs could contribute more generally to TKI resistance in BP-CML, we assessed the chemosensitivity of a commercially available imatinib-resistant K562 cell line (K562-R)

with dual SG/TKI inhibition. To inhibit SG formation, we used the G3BP1 inhibitor FAZ-3532, thus far untested in leukemia. After first confirming that K562-R cells were significantly more resistant than parental K562 cells (Fig. 8A–C; Supplemental Fig. S8A, B), we ascertained the dose necessary to inhibit SG formation in K562 cells. We observed a marked reduction in SG-assembly at 5 μM (Fig. 8B, C) and confirmed FAZ-3532 was not cytotoxic to K562 cells when used in isolation (Supplemental Fig. S8C). However, when 5 μM FAZ-3532 was combined with different doses of imatinib, K562-R cells were re-sensitised, with significantly enhanced cell death across 24-, 48- and 72-h timepoints (Fig. 8D–F). These data indicate SGs could contribute more generally to TKI resistance in BP-CML.

DISCUSSION

Using the RNA-interactome capture method, OOPS, we characterised how RUNX1 loss causes post-transcriptional dysregulation in BP-CML cells. RUNX1 is a regulator of post-transcriptional processes, including splicing [31–33] and RiBi/translation [34, 35], prompting us to investigate whether RUNX1 loss altered the RNA-bound proteome. We found RUNX1 depletion in BP-CML cells impacted RBPs-associated with stress responses and RiBi/translation. Previous research has shown different RUNX1 mutations in the RHD cluster into LOF, hypomorphic and wild-type-like groups [50]; hypomorphic mutants being characterised by defective CBFβ interaction [51]. LOF/hypomorphic RUNX1 mutations are

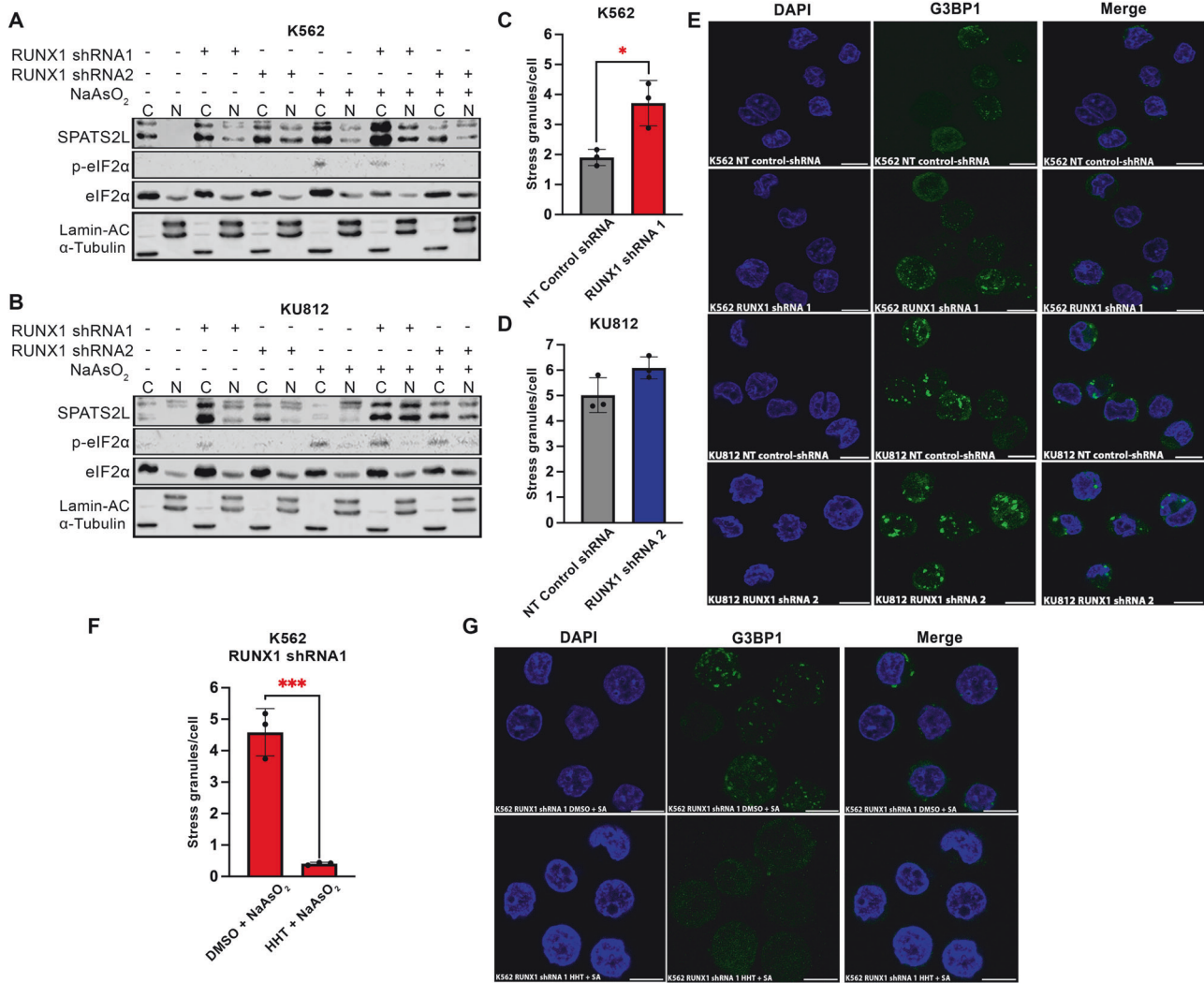


Fig. 5 RUNX1-depleted BP-CML cells have increased SG-forming capacity, which are eliminated with HHT treatment. Western blots showing the cytoplasmic (C) and nuclear (N) distribution of SPATS2L, eIF2α and phospho-eIF2α in **A** K562 and **B** KU812 cells with RUNX1 depletion +/- 0.5 M NaAsO₂ treatment for 1-h. Lamin A/C and α-tubulin indicate the level and purity of fractions, and p-eIF2α status indicates stress/SG-induction. Summary graphs showing the average SG number per cell in RUNX1-depleted versus NT control-shRNA **C** K562 and **D** KU812 cells. Data represents mean ± 1 SD ($n = 3$). Statistical significance is denoted by * $p < 0.05$ from a two-tailed unpaired student's t -test. **E** Representative CLSM images showing DAPI, G3BP1 and merged images of K562 and KU812 cells with RUNX1 depletion compared to NT controls. **F** Summary graph showing the average number of SG per cell in K562 RUNX1 shRNA 1 cells treated with DMSO+NaAsO₂ compared to HHT (50 ng/mL for 48-h) + NaAsO₂. Data represents mean ± 1 SD ($n = 3$). Statistical significance is denoted by *** $p < 0.001$ from a two-tailed unpaired student's t -test. **G** Representative CLSM images show the DAPI, G3BP1 and merged stains of K562 RUNX1 shRNA 1 cells treated with DMSO + SA compared to HHT + NaAsO₂. The white scale bar represents 10 μm.

associated with the enrichment of genes with stress response pathways and rRNA processing/RiBi [50], supporting our findings seen in KU812 cells with RUNX1 depletion, implying the stress response and dysregulated RiBi/translation are intrinsically linked. Consequently, the interaction between these processes made us consider whether RUNX1, a regulator of rRNA transcription [34], functions more broadly as a regulator of 5'TOP motifs. Cells exposed to stress downregulate cap-dependent translation through eIF2α phosphorylation, modifying the expression of any mRNA containing a 5'TOP motif [42]. We found multiple constituents of ribosomal subunits containing 5'TOP motifs were induced with RUNX1 depletion, with an increase in ribosome occupancy in polysomes. However, we found no increase in actively translating polysomes, suggesting global translation does not increase. As cells adapt to stress, the modification of ribosome production/function occurs, facilitating translational reprogramming, critical to cellular survival [52]. Thus, RUNX1 mutations in

CML could induce translational reprogramming, promoting cellular survival.

The disruption of cap-dependent translation leads to canonical SG-assembly and membraneless cytoplasmic foci which protect mRNAs/proteins, promoting survival and therapeutic resistance [38, 39]. We found RUNX1 depletion or expression of RUNX1::EV1 induced SPATS2L, a component of SGs [36, 37]. In BP-CML cells, we found SPATS2L depletion inhibited growth and survival and reduced SG-assembly, in keeping with previous research [37]. Supporting our findings, SPATS2L is upregulated in RUNX1-mutated, compared with RUNX1-wild-type, primary BP-CML cells [23]. However, it remains unclear whether RUNX1 regulation of SPATS2L is direct or indirect via the triggering of cellular stresses upon RUNX1 modulation. Indeed, RUNX1 ChIP-peaks are not consistently enriched at the SPATS2L promoter [53, 54], and NaAsO₂ (oxidative stress) exposure alone was sufficient to induce SPATS2L (Fig. 5A). Regardless, enhanced SG-assembly was

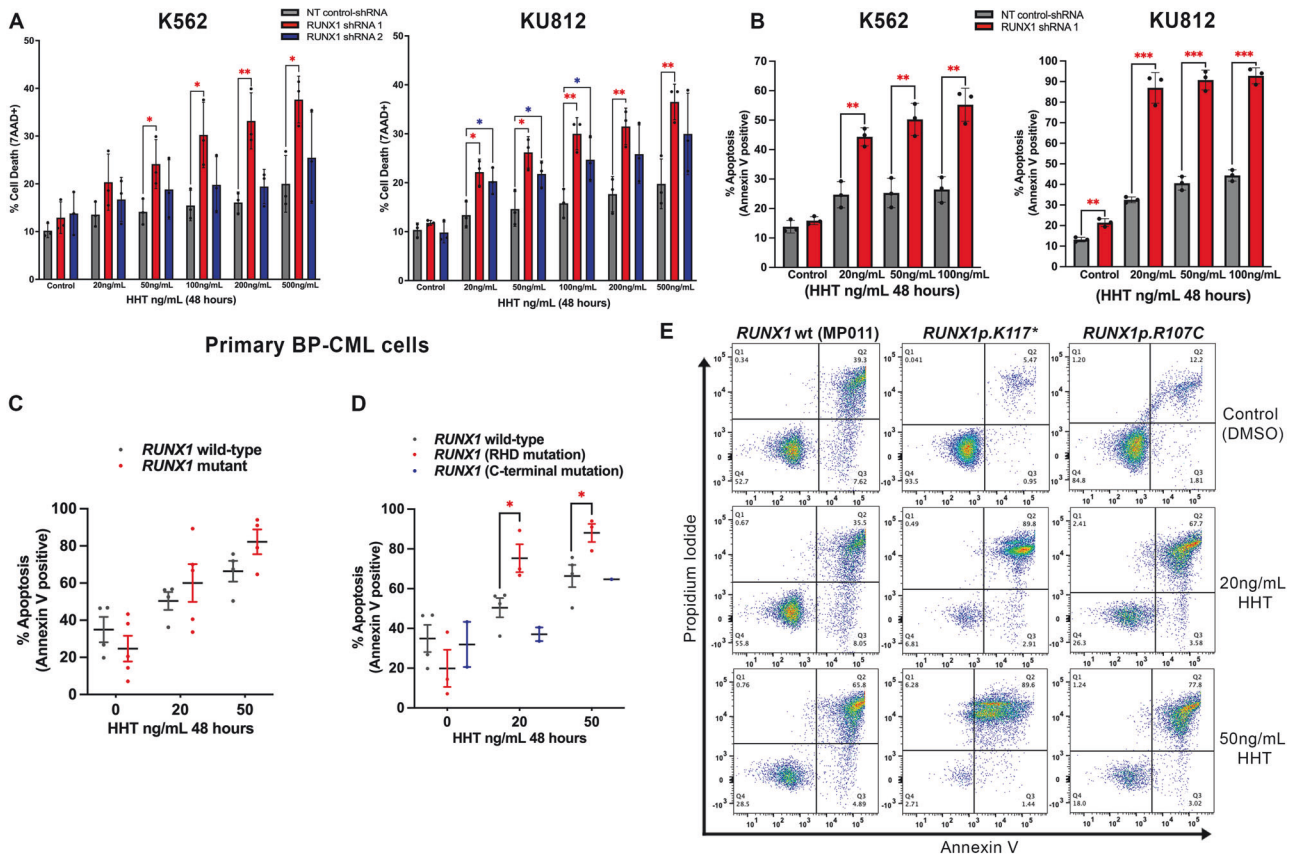


Fig. 6 *RUNX1*-depleted BP-CML cells and primary BP-CML cells with *RUNX1* RHD mutations exhibit enhanced HHT-sensitivity. **A** Summary bar graph showing percentage cell death determined by 7AAD positivity in K562 and KU812 cells with *RUNX1* depletion compared to NT controls, treated with a dose-response (20–500 ng/mL) of HHT for 48-h. Data represents mean $\pm$ 1 SD ($n = 3$). Statistical significance is denoted by * $p < 0.05$ or ** $p < 0.01$ from a two-tailed unpaired student's *t*-test. **B** Percentage apoptosis (Annexin V positive staining for early/late-stage apoptosis) by way of flow cytometry analysis in KU812 and K562 cells with *RUNX1* depletion (shRNA 1) compared to NT controls, treated with 20–100 ng/mL HHT at 48-h. Data represents mean $\pm$ 1 SD ($n = 3$). Statistical significance is denoted by ** $p < 0.01$ or *** $p < 0.001$ from a two-tailed unpaired student's *t*-test. **C** Percentage apoptosis (early/late-stage) determined by Annexin V positive staining in *RUNX1* mutated compared to *RUNX1* wild-type, patient-derived BP-CML cells, after treatment with 20 ng/mL or 50 ng/mL HHT at 48-h (compared to a control dose). Data represents mean $\pm$ SEM ($n = 5$) *RUNX1* mutated samples and ($n = 4$) *RUNX1* wild-type samples. One *RUNX1*-mutated sample (MP014) had insufficient cells for a 50 ng/mL HHT dose. **D** Percentage apoptosis (early/late-stage) determined by Annexin V positive staining in patient-derived BP-CML cells with/without *RUNX1* mutations, with *RUNX1* mutations grouped based on whether they occur in the RHD (red) or C-terminus (blue), treated with 20 ng/mL or 50 ng/mL HHT at 48-h (compared to a control dose). Data represents mean $\pm$ SEM ($n = 3$) *RUNX1* RHD mutated samples and ($n = 4$) *RUNX1* wild-type samples. Statistical significance is denoted by * $p < 0.05$ from a two-tailed unpaired student's *t*-test. **E** Representative flow cytometric pseudoplots of Annexin V staining are shown for a *RUNX1* wild-type (MP011) primary BP-CML sample and two primary *RUNX1* mutated BP-CML samples with mutations in the RHD (*RUNX1p.K117** and *RUNX1p.R107C*), treated with 20 ng/mL or 50 ng/mL HHT, compared to a control dose.

observed downstream of *RUNX1* modulation, suggesting SGs could be important for *RUNX1*-mutant pathogenesis.

The induction of SG-assembly with *RUNX1* depletion in BP-CML cells led us to consider whether their targeting represents a novel therapeutic strategy. HHT and its semisynthetic derivative Omacetaxine Mepessucinate (OM) inhibit translation by binding to the ribosomal-A site, preventing new ribosomes from entering elongation but allowing ribosomes already in elongation to run off mRNAs [55–57]. Harringtonine inhibits SG-assembly by freezing 80 s ribosomes on mRNAs, changing their conformational structure, and preventing the recruitment of “ribosome-free” mRNAs into these condensates [45, 55]. We found HHT ablated SG-assembly in *RUNX1*-deficient K562 cells. Notably, it has been suggested BCR::ABL1 localises to SGs, essential for its oncogenic function [58], implying the inhibition of SGs could disrupt BCR::ABL1 function. HHT/OM has a history of clinical use in CML [46–49, 59] and has demonstrated preclinical efficacy in *RUNX1*-mutated AML cells [35]. These findings were followed up with a phase I/II clinical trial where no responses were seen in relapse/refractory *RUNX1*-mutated AML patients treated with OM

+venetoclax [60]. However, doses of OM used were lower than historically used in CML [46–48], and two patients with untreated *RUNX1*-mutated MDS had complete responses, leading the authors to indicate that HHT/OM could be beneficial in non-heavily pre-treated *RUNX1*-mutated myeloid malignancies [60]. As CML represents a disease not heavily pre-treated before the blast phase, we considered whether BP-CML cells with *RUNX1* mutations could be HHT-sensitised.

RUNX1 mutations in BP-CML cells exhibit different sensitivities to novel therapies, highlighting their heterogeneity [23]. On testing primary *RUNX1*-mutated BP-CML cells with HHT, we found *RUNX1p.K117**, *RUNX1p.R107C* and *RUNX1p.S100F* (RHD-mutant) exhibited the greatest sensitivity. *RUNX1p.K117** (*RUNX1p.K90fsx101*- isoform b) causes haploinsufficiency through defective CBF β interaction, nuclear localisation, and transactivation [61]. *RUNX1p.R107C* (*RUNX1p.R80C*-isoform b) occurs at a “hotspot residue,” leading to defective CBF β interaction and transactivation [62]. Finally, *RUNX1p.S100F* (*RUNX1p.Ser73Phe*-isoform b) causes defective CBF β interaction and transactivation [63]. *RUNX1* RHD mutations clustering into LOF/hypomorphic

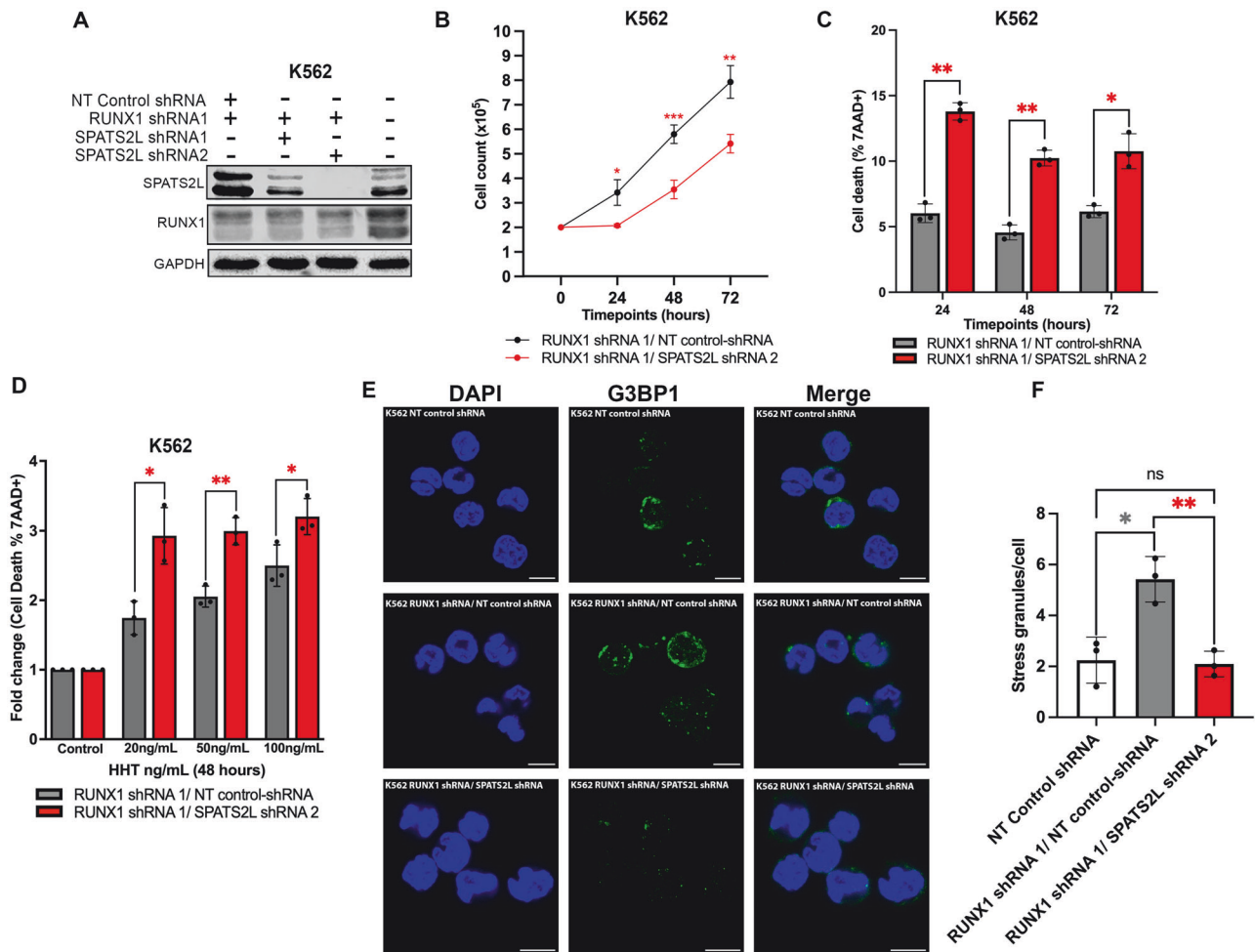


Fig. 7 Inhibiting SPATS2L induction in RUNX1-depleted K562 cells impairs growth and viability and increases HHT sensitivity. **A** Western blot showing the level of SPATS2L and RUNX1 in K562 RUNX1 shRNA 1 cells, transduced with two GFP⁺ SPATS2L-targeting shRNAs, compared to a GFP⁺ non-targeting shRNA. GAPDH was used as a loading control. **B** Line graph summarising cell growth rates of K562 RUNX1 shRNA 1 cells transduced with a GFP⁺ NT control-shRNA or SPATS2L shRNA 2 at 24- 48- and 72-h. **C** Summary bar graph showing viability of K562 RUNX1 shRNA1/NT control-shRNA and K562 RUNX1 shRNA1/SPATS2L shRNA2 cells as deduced by flow cytometric assessment of 7AAD⁺ (dead cells). Data represents mean $\pm$ 1 SD ($n = 3$), and statistical significance is denoted by * $p < 0.05$ or ** $p < 0.01$, from a two-tailed unpaired student's t -test. **D** Summary bar graph showing the fold change in percentage of dead cells in K562 RUNX1 shRNA1/NT control-shRNA cells compared with RUNX1 shRNA1/SPATS2L shRNA2 cells, following 48-h HHT treatment (20–100 ng/mL) as deduced by flow cytometric assessment of 7AAD⁺ events. Data from each cell line are normalised to the control dose, mean $\pm$ 1 SD ($n = 3$). Statistical significance is denoted by * $p < 0.05$ or ** $p < 0.01$ from a two-tailed unpaired student's t -test on log₂-transformed fold change values. **E** Representative CLSM images showing DAPI, G3BP1 and merged images of K562 NT control-shRNA, K562 RUNX1 shRNA1/NT control-shRNA or K562 RUNX1 shRNA1/SPATS2L shRNA2 cells following 0.5 M NaAsO₂ for 1-h. White scale bars represent 10 μ m. **F** Summary bar graph showing average SG number per cell in K562 NT control-shRNA, K562 RUNX1 shRNA1/NT control-shRNA, and K562 RUNX1 shRNA1/SPATS2L shRNA2 cells following induction with 0.5 M NaAsO₂ for 1-h. Data represents mean $\pm$ 1 SD ($n = 3$) and statistical significance is denoted by * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$ from a two-tailed unpaired student's t -test.

groups are enriched for a gene signature associated with stress response pathways and RiBi [50] (similar to the disrupted RBP-interactome we derived from RUNX1-depleted KU812 cells). This implies the increased HHT sensitivity we observed with the LOF/hypomorphic *RUNX1* mutations we tested could be related to the dysregulation of these processes. Supporting this, the *RUNX1* C-terminal mutations we tested, including *RUNX1p.R320** and *RUNX1p.Q274fs*His36* were less HHT-sensitive than BP-CML cells with wild-type *RUNX1*. The *RUNX1p.R320** mutation does not affect CBF β interaction [63] and exhibits similar genomic binding sites to RUNX1 [64], suggesting it drives leukemogenesis through altered mechanisms. Indeed, when modelled by Jayne et al. [64], using a CRISPR induced *RUNX1p.Arg320** mutation in K562 cells, RNA-Seq revealed no significant induction of *SPATS2L* versus *RUNX1* wild-type cells. Furthermore, *RUNX1* mutations leading to C-terminal deletions bind to DNA with a higher affinity than RUNX1, altering

the transcriptional programme by affecting the activation/repression of target genes [65]. Thus, the difference in HHT response between LOF/hypomorphic-RHD and C-terminal mutations could be related to differences in the induction of dysregulated post-transcriptional processes. Although RUNX1 depletion models can partially model the impact of reduced DNA-binding/CBF β interaction on HHT- sensitivity, they cannot completely recapitulate the molecular response observed with *RUNX1* mutations, so experiments with larger numbers of *RUNX1* mutant BP-CML samples are required.

LOF/hypomorphic *RUNX1* mutations are associated with the induction of stress response pathways, ER stress and PERK-regulated genes [50]. The induction of the integrated stress response (ISR), through PERK-activation, leads to eIF2 α phosphorylation [66], promoting SG-assembly. Furthermore, PERK-eIF2 α phosphorylation is upregulated in CML cells which have become

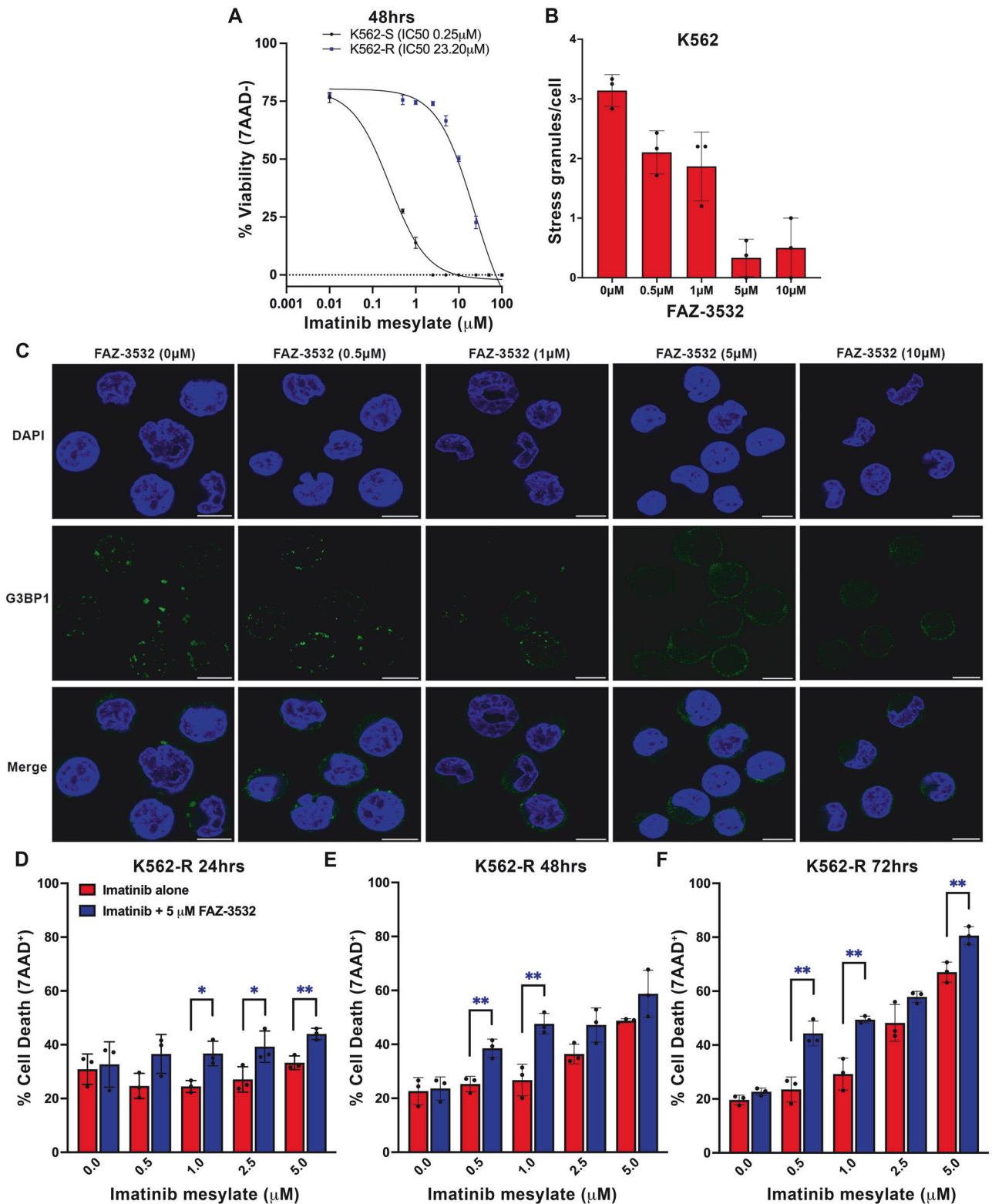


Fig. 8 Stress granules may contribute more generally to TKI resistance in CML. **A** Summary of imatinib mesylate dose-response curves exhibiting the IC50 (determined by 7AAD positivity) of sensitive parental K562 (K562-S) cells versus the commercially available imatinib-resistant K562 cell line (K562-R) at 48-h. **B** Bar graph summarising the average number of NaAsO₂ (0.5 M) induced SGs per cell in K562 cells pre-treated with 0–10 μ M FAZ-3532 for 24-h. Data represents mean $\pm$ 1 SD of three technical repeats. **C** Representative CLSM images showing DAPI, G3BP1 and merged images of K562-S cells treated with 0–10 μ M FAZ-3532 for 24-h prior to NaAsO₂ (0.5 M for 1-h) induced SG formation. White scale bars represent 10 μ m. Summary bar graphs showing the percentage cell death (deduced from 7AAD positivity) in K562-R cultures treated with 0–10 μ M imatinib $\pm$ 5 μ M FAZ-3532 at **D** 24-, **E** 48- and **F** 72-h. Data represents mean $\pm$ 1 SD (n = 3) and statistical significance is denoted by * p < 0.05, or ** p < 0.01 from a two-tailed unpaired student's t -test.

resistant to imatinib [67]. These findings could imply that SG induction is a feature of CML cells with LOF/hypomorphic *RUNX1* mutations, promoting TKI resistance. We found SPATS2L was induced with *RUNX1* depletion or expression of *RUNX1::EV11*. SPATS2L amplification occurs in AML cells with increasing resistance indices to HHT [44]. Thus, whilst the induction of stress response pathways, dysregulated RiBi/translation and SG-assembly, may sensitise BP-CML cells with LOF/hypomorphic *RUNX1* mutations to HHT, the continued amplification of SG-formation through a SPATS2L/SG axis could serve as a mechanism for therapeutic resistance. To examine this, we created a K562 cell line with SPATS2L depletion on a background of *RUNX1* depletion. K562 *RUNX1* shRNA 1 cells, already sensitised to HHT, were further sensitised with concurrent SPATS2L depletion, suggesting SGs contribute to therapeutic resistance. Indeed, this is the first published study to indicate a role for SGs in TKI-resistance (or therapeutic resistance more generally in leukaemia), although a pre-print suggests they promote AML LSC survival and stress adaptation [68]. SPATS2L has been implicated in drug resistance in AML previously; however, given the impact of its depletion alone on cell growth/viability, we can't exclude an additive effect with *RUNX1* suppression through induction of cellular stress/reduced cell fitness.

In summary, our study demonstrates *RUNX1* to be a critical regulator of the RNA-bound proteome in BP-CML cells. We revealed the induction of stress response pathways, dysregulated RiBi/translation and increased SG-assembly are a feature of BP-CML cells with *RUNX1* aberrations, and LOF/hypomorphic *RUNX1* mutations may be preferentially sensitised to HHT through these dysregulated post-transcriptional processes. Whilst a limitation of our work was testing a small number of primary samples, due to the increasing rarity of BP-CML, our findings provide preliminary evidence in support of a mutationally targeted approach to *RUNX1* aberrations and propose SPATS2L/SG targeting represents a novel therapeutic approach in this disease.

DATA AVAILABILITY

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD076395. All other data featured in this manuscript are available from the corresponding author upon reasonable request.

REFERENCES

- Quintás-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood*. 2009;113:1619–30.
- Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355:2408–17.
- Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. *N Engl J Med*. 2017;376:917–27.
- Cortes J, Kim DW, Raffoux E, Martinelli G, Ritchie E, Roy L, et al. Efficacy and safety of dasatinib in imatinib-resistant or-intolerant patients with chronic myeloid leukemia in blast phase. *Leukemia*. 2008;22:2176–83.
- Cortes JE, Kim DW, Pinilla-Ibarz JL, Le Coutre P, Paquette R, Chuah C, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2013;369:1783–96.
- Garg RJ, Kantarjian H, O'Brien S, Quintás-Cardama A, Faderl S, Estrov Z, et al. The use of nilotinib or dasatinib after failure to 2 prior tyrosine kinase inhibitors: long-term follow-up. *Blood*. 2009;114:4361–8.
- Khoury HJ, Cortes JE, Kantarjian HM, Gambacorti-Passerini C, Baccarani M, Kim DW, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. *Blood*. 2012;119:3403–12.
- Rea D, Mauro MJ, Boquimpani C, Minami Y, Lomaia E, Voloshin S, et al. A phase 3, open-label, randomized study of asciminib, a STAMP inhibitor, vs bosutinib in CML after 2 or more prior TKIs. *Blood*. 2021;138:2031–41.
- Copland M. Treatment of blast phase chronic myeloid leukaemia: a rare and challenging entity. *Br J Haematol*. 2022;199:665–78.
- Copland M, Slade D, McIlroy G, Horne G, Byrne JL, Rothwell K, et al. Ponatinib with fludarabine, cytarabine, idarubicin, and granulocyte colony-stimulating factor chemotherapy for patients with blast-phase chronic myeloid leukaemia (MATCH-POINT): a single-arm, multicentre, phase 1/2 trial. *Lancet Haematol*. 2022;9:e121–32.
- Branford S, Wang P, Yeung DT, Thomson D, Purins A, Wadham C, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. *Blood*. 2018;132:948–61.
- Grossmann V, Kohlmann A, Zenger M, Schindela S, Eder C, Weissmann S, et al. Deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. *Leukemia*. 2011;25:557–60.
- Ochi Y, Yoshida K, Huang YJ, Kuo MC, Nannya Y, Sasaki K, et al. Clonal evolution and clinical implications of genetic abnormalities in blastic transformation of chronic myeloid leukaemia. *Nat Commun*. 2021;12:2833.
- Wang W, Cortes JE, Tang G, Khoury JD, Wang S, Bueso-Ramos CE, et al. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. *Blood*. 2016;127:2742–50.
- Branford S, Kim DD, Apperley JF, Eide CA, Mustjoki S, Ong ST, et al. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. *Leukemia*. 2019;33:1835–50.
- Wang W, Cortes JE, Lin P, Beaty MW, Ai D, Amin HM, et al. Clinical and prognostic significance of 3q26.2 and other chromosome 3 abnormalities in CML in the era of tyrosine kinase inhibitors. *Blood*. 2015;126:1699–706.
- Sood R, Kamikubo Y, Liu P. Role of *RUNX1* in hematological malignancies. *Blood*. 2017;129:2070–82.
- Kellaway SG, Coleman DJ, Cockerill PN, Raghavan M, Bonifer C. Molecular basis of hematological disease caused by inherited or acquired *RUNX1* mutations. *Exp Hematol*. 2022;111:1–2.
- Greif PA, Konstantin NP, Metzler KH, Herold T, Pasalic Z, Ksienzyk B, et al. *RUNX1* mutations in cytogenetically normal acute myeloid leukemia are associated with a poor prognosis and up-regulation of lymphoid genes. *Haematologica*. 2012;97:1909.
- Tang JL, Hou HA, Chen CY, Liu CY, Chou WC, Tseng MH, et al. *AML1/RUNX1* mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. *Blood*. 2009;114:5352–61.
- Mendler JH, Maharry K, Radmacher MD, Mrózek K, Becker H, Metzler KH, et al. *RUNX1* mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and MicroRNA expression signatures. *J Clin Oncol*. 2012;30:3109–18.
- Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. *RUNX1* mutations in acute myeloid leukemia are associated with distinct clinicopathologic and genetic features. *Leukemia*. 2016;30:2160–8.
- Adnan Awad S, Dufva O, lanevski A, Ghimire B, Koski J, Maliniemi P, et al. *RUNX1* mutations in blast-phase chronic myeloid leukemia associate with distinct phenotypes, transcriptional profiles, and drug responses. *Leukemia*. 2021;35:1087–99.
- Zhao LJ, Wang YY, Li G, Ma LY, Xiong SM, Weng XQ, et al. Functional features of *RUNX1* mutants in acute transformation of chronic myeloid leukemia and their contribution to inducing murine full-blown leukemia. *Blood*. 2012;119:2873–82.
- Kellaway SG, Keane P, Kennett E, Bonifer C. *RUNX1-EV11* disrupts lineage determination and the cell cycle by interfering with *RUNX1* and *EV11* driven gene regulatory networks. *Haematologica*. 2021;106:1569.
- Kellaway SG, Keane P, Edginton-White B, Regha K, Kennett E, Bonifer C. Different mutant *RUNX1* oncoproteins program alternate haematopoietic differentiation trajectories. *Life Sci Alliance*. 2021;4:e202000864.
- Tokita K, Maki K, Mitani K. *RUNX1/EV11*, which blocks myeloid differentiation, inhibits CCAAT-enhancer binding protein a function. *Cancer Sci*. 2007;98:1752–7.
- Loke J, Assi SA, Imperato MR, Ptasińska A, Cauchy P, Grabovska Y, et al. *RUNX1-ETO* and *RUNX1-EV11* differentially reprogram the chromatin landscape in t (8; 21) and t (3; 21) AML. *Cell Rep*. 2017;19:1654–68.
- Izutsu K, Kurokawa M, Imai Y, Ichikawa M, Asai T, Maki K, et al. The t (3; 21) fusion product, *AML1/Evi-1* blocks *AML1*-induced transactivation by recruiting CtBP. *Oncogene*. 2002;21:2695–703.
- Cuenca GM, Ren R. Cooperation of BCR-ABL and *AML1/MDS1/EV11* in blocking myeloid differentiation and rapid induction of an acute myelogenous leukemia. *Oncogene*. 2001;20:8236–48.
- Dhir A, Ethell A, Watkins R, Lam C, Tur-Rodriguez K, Persinger J, et al. The splicing factor PTBP1 interacts with *RUNX1* and is required for leukemia cell survival: acute myeloid leukemia. *Leukemia*. 2025;10:1–4.
- Huang YJ, Yan M, Chen JY, Chen L, Kim E, Shima T, et al. *RUNX1* deficiency cooperates with SRSF2 mutation to further disrupt RNA splicing and exacerbate myelodysplastic syndromes in mouse models. *Blood*. 2018;29:1796.
- Grinev VV, Barneih F, Ilyushonak IM, Nakjang S, Smink J, van Oort A, et al. *RUNX1/RUNX1T1* mediates alternative splicing and reorganises the transcriptional landscape in leukemia. *Nat Commun*. 2021;12:520.
- Cai X, Gao L, Teng L, Ge J, Oo ZM, Kumar AR, et al. *Runx1* deficiency decreases ribosome biogenesis and confers stress resistance to hematopoietic stem and progenitor cells. *Cell Stem Cell*. 2015;17:165–77.

35. Mill CP, Fiskus W, DiNardo CD, Birdwell C, Davis JA, Kadia TM, et al. Effective therapy for AML with RUNX1 mutation by cotreatment with inhibitors of protein translation and BCL2. *Blood*. 2022;139:907–21.
36. Zhu CH, Kim J, Shay JW, Wright WE. SGPN: an essential stress granule/nucleolar protein potentially involved in 5.8 s rRNA processing/transport. *PLoS One*. 2008;3:e3716.
37. Asamitsu S, Yabuki Y, Matsuo K, Kawasaki M, Hirose Y, Kashiwazaki G, et al. RNA G-quadruplex organizes stress granule assembly through DNAPTP6 in neurons. *Sci Adv*. 2023;9:2035.
38. Marcelo A, Koppenol R, de Almeida LP, Matos CA, Nóbrega C. Stress granules, RNA-binding proteins and polyglutamine diseases: too much aggregation? *Cell Death Dis*. 2021;12:592.
39. Wang F, Li J, Fan S, Jin Z, Huang C. Targeting stress granules: a novel therapeutic strategy for human diseases. *Pharmacol Res*. 2020;161:105143.
40. Azman MS, Alard EL, Dodel M, Capraro F, Faraway R, Dermitt M, et al. An ERK1/2-driven RNA-binding switch in nucleolin drives ribosome biogenesis and pancreatic tumorigenesis downstream of RAS oncogene. *EMBO J*. 2023;42:e110902.
41. Philippe L, van den Elzen AM, Watson MJ, Thoreen CC. Global analysis of LARP1 translation targets reveals tunable and dynamic features of 5' TOP motifs. *Proc Natl Acad Sci USA*. 2020;117:5319–28.
42. Cockman E, Anderson P, Ivanov P. TOP mRNPs: molecular mechanisms and principles of regulation. *Biomolecules*. 2020;10:969.
43. Mitani K, Ogawa S, Tanaka T, Miyoshi H, Kurokawa M, Mano H, et al. Generation of the AML1-EVI-1 fusion gene in the t (3; 21)(q26; q22) causes blastic crisis in chronic myelocytic leukemia. *EMBO J*. 1994;13:504–10.
44. Li F, Ye W, Yao Y, Wei W, Lin X, Zhuang H, et al. Spermatogenesis associated serine rich 2 like plays a prognostic factor and therapeutic target in acute myeloid leukemia by regulating the JAK2/STAT3/STAT5 axis. *J Transl Med*. 2023;21:115.
45. Fedorovskiy AG, Burakov AV, Terenin IM, Bykov DA, Lashkevich KA, Popenko VI, et al. A solitary stalled 80S ribosome prevents mRNA recruitment to stress granules. *Biochemistry (Mosc)*. 2023;88:1786–99.
46. Quintás-Cardama A, Kantarjian H, García-Manero G, O'Brien S, Faderl S, Estrov Z, et al. Phase I/II study of subcutaneous homoharringtonine in patients with chronic myeloid leukemia who have failed prior therapy. *Cancer*. 2007;109:248–55.
47. Kantarjian HM, Talpaz M, Smith TL, Cortes J, Giles FJ, Rios MB, et al. Homoharringtonine and low-dose cytarabine in the management of late chronic-phase chronic myelogenous leukemia. *J Clin Oncol*. 2000;18:3513–21.
48. O'Brien S, Kantarjian H, Keating M, Beran M, Koller C, Robertson LE, et al. Homoharringtonine therapy induces responses in patients with chronic myelogenous leukemia in late chronic phase. *Blood*. 1995;86:3322–6.
49. Fang B, Li N, Song Y, Han Q, Zhao RC. Standard-dose imatinib plus low-dose homoharringtonine and granulocyte colony-stimulating factor is an effective induction therapy for patients with chronic myeloid leukemia in myeloid blast crisis who have failed prior single-agent therapy with imatinib. *Ann Hematol*. 2010;89:1099–105.
50. Ozturk K, Panwala R, Sheen J, Ford K, Jayne N, Portell A, et al. Interface-guided phenotyping of coding variants in the transcription factor RUNX1. *Cell Rep*. 2024;23:7.
51. Matheny CJ, Speck ME, Cushing PR, Zhou Y, Corpora T, Regan M, et al. Disease mutations in RUNX1 and RUNX2 create nonfunctional, dominant-negative, or hypomorphic alleles. *EMBO J*. 2007;26:1163–75.
52. Liu B, Qian SB. Translational reprogramming in cellular stress response. *Wiley Interdiscip Rev RNA*. 2014;5:301–5.
53. Luo Y, Hitz BC, Gabdank I, Hilton JA, Kagda MS, Lam B, et al. New developments on the encyclopedia of DNA elements (ENCODE) data portal. *Nucleic Acids Res*. 2020;8:882–9.
54. Chacon D, Beck D, Perera D, Wong JW, Pimanda JE. BloodChIP: a database of comparative genome-wide transcription factor binding profiles in human blood cells. *Nucleic Acids Res*. 2014;1:172–7.
55. Adivarahan S, Livingston N, Nicholson B, Rahman S, Wu B, Rissland OS, et al. Spatial organization of single mRNPs at different stages of the gene expression pathway. *Molecular cell*. 2018;72:727–38.
56. Ihn SJ, Farlam-Williams L, Palazzo AF, Lee HO. Cytoplasmic protein-free mRNA induces stress granules by two G3BP1/2-dependent mechanisms. *BioRxiv*. 2024. <https://doi.org/10.1101/2024.02.07.578830> [Preprint].
57. Ingolia NT, Lareau LF, Weissman JS. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell*. 2011;147:789–802.
58. Kashiwagi S, Fujioka Y, Kondo T, Satoh AO, Yoshida A, Fujioka M, et al. Localization of BCR-ABL to stress granules contributes to its oncogenic function. *Cell Struct Funct*. 2019;44:195–204.
59. Kantarjian HM, Talpaz M, Santini V, Murgo A, Cheson B, O'Brien SM. Homoharringtonine: history, current research, and future directions. *Cancer*. 2001;92:1591–605.
60. DiNardo CD, Jen WY, Montalban-Bravo G, Wang X, Loghavi S, Lavu S, et al. Omacetaxine and venetoclax in relapsed/refractory acute myeloid leukemia or myelodysplastic syndrome with mutant RUNX1. *Blood Neoplasia*. 2025:100145.
61. Michaud J, Wu F, Osato M, Cottles GM, Yanagida M, Asou N, et al. In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. *Blood*. 2002;99:1364–72.
62. Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2B B gene associated with myeloblastic leukemias. *Blood*. 1999;93:1817–24.
63. Tsai SC, Shih LY, Liang ST, Huang YJ, Kuo MC, Huang CF, et al. Biological activities of RUNX1 mutants predict secondary acute leukemia transformation from chronic myelomonocytic leukemia and myelodysplastic syndromes. *Clin Cancer Res*. 2015;21:3541–51.
64. Jayne ND, Liang Z, Lim DH, Chen PB, Diaz C, Arimoto KI, et al. RUNX1 C-terminal mutations impair blood cell differentiation by perturbing specific enhancer-promoter networks. *Blood Adv*. 2024;8:2410–23.
65. Matsuura S, Komeno Y, Stevenson KE, Biggs JR, Lam K, Tang T, et al. Expression of the runt homology domain of RUNX1 disrupts homeostasis of hematopoietic stem cells and induces progression to myelodysplastic syndrome. *Blood*. 2012;120:4028–37.
66. Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. The integrated stress response. *EMBO Rep*. 2016;17:1374–95.
67. Kusio-Kobialka M, Podszewalow-Bartnicka P, Peidis P, Glodkowska-Mrowka E, Wolanin K, Leszak G, et al. The PERK-eIF2α phosphorylation arm is a pro-survival pathway of BCR-ABL signaling and confers resistance to imatinib treatment in chronic myeloid leukemia cells. *Cell Cycle*. 2012;11:4069–78.
68. Tajik A, Tsao E, Jahangiri S, Seale B, Yee BA, Naritomi JT, et al. Stress granules underlie acute myeloid leukemia stem cell survival <https://doi.org/10.1101/2025.01.14.632811>. [Preprint].

ACKNOWLEDGEMENTS

The authors thank the patients involved for supplying samples for this research. The authors also thank the staff at the Paul O'Gorman Leukaemia Centre (Glasgow) for generous help with FACS and the staff at Queen Mary University of London for kind help with OOPS.

AUTHOR CONTRIBUTIONS

DP devised the study, designed and executed experiments, analysed the data and wrote the manuscript. DO-P optimised and performed SG analyses, whilst AM and RC assisted with primary BP-CML sample processing/analysis. OS, LS and AK executed molecular assays. PL, MW, KH performed proteomics analyses, whilst GH, MC, SM, SA, KP provided BP-CML samples access and TC provided mentorship and guidance. NB, SJ, and DF contributed experimental data, whilst HW and MJW assisted with *RUNX1::MECOM/EVI1* cloning. FKM guided OOPS experiments and further scientific expertise were provided by SGK, AT and BPT. RGM co-devised the study, designed experiments, analysed data, managed the project and edited the manuscript.

FUNDING

D.A.P. is directly funded by the University Hospitals Sussex (UHS) NHS Foundation Trust and the British Society of Haematology (Early-Stage Research Start-up Grant – Palmer). DAP, SGK and RGM are co-funded by Cancer Research UK (EDDPJT-Nov25/100039).

COMPETING INTERESTS

MC: research funding from Cyclacel Ltd and Incyte; advisory board member for Novartis, Incyte, Pfizer, Ascentage, Crossbow, Servier, Mendus BV; honouraria from Astellas, Jazz Pharmaceuticals, Novartis, Incyte, Pfizer, Servier, Azurity and Janssen. All other authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41375-026-03161-4>.

Correspondence and requests for materials should be addressed to RG Morgan.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026