Title: Dual targeting a LIN28B:β-catenin axis in acute myeloid leukaemia

Short title: Targeting a LIN28B:β-catenin axis in AML

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Abstract

Background

Wnt/β-catenin signalling is dysregulated in acute myeloid leukaemia (AML), where it lacks effective targeting strategies. Previously, we discovered that β-catenin interacts with several RNA-binding proteins (RBP), indicating post-transcriptional influence which is yet to be therapeutically interrogated in AML.

Methods

Co-immunoprecipitation confirmed protein interactions, and TCF/LEF reporters were used to assess Wnt signalling output in leukaemia cells. Regulatory crosstalk was assessed using immunoblotting and RT-qPCR approaches following lentiviral transduction of myeloid cell lines. Targeting of β-catenin and LIN28B was tested through combinations of genetic and pharmacological inhibition in AML cells.

Results

The most frequent RBP-binding motif amongst β-catenin-bound mRNAs was the GGAG motif targeted by oncofetal miRNA-regulating RBP; LIN28B. β-Catenin:LIN28B interactions were detected in lymphoid and myeloid cell lines, plus primary human $CD34^+$ fetal-liver HSCs. LIN28B positively regulated Wnt signalling output through *LEF1* regulation involving a post-transcriptional *let7* miRNA mechanism. Further miRNA sequencing of β-catenin- and LIN28B-depleted myeloid cells revealed potential cooperative and antagonistic function in miRNA regulation. Finally, dual-targeting both β-catenin and LIN28B through either genetic and/or pharmacological means preferentially reduced AML cell viability.

Conclusion

The β-catenin:LIN28B axis could represent a novel synthetically lethal relationship in AML which could be exploited in rare subtypes where LIN28B expression becomes reactivated.

Introduction

Canonical Wnt signalling is a transduction cascade critical for normal embryonic, fetal and adult development, and heavily dysregulated in human cancers.[1] The pathway regulates several key adult tissue stem cell systems including the gut, breast, hair follicle, liver and blood.[2] In particular, Wnt/β-catenin signalling plays a vital role in regulating the self-renewal,[3,4] and differentiation,[5-8] capacity of haematopoietic stem/progenitor cells (HSPC) in a dose-dependent fashion,[9,10] through both fetal and adult phases of haematopoiesis.[11-15] The importance of Wnt/β-catenin signalling to normal haematopoietic development means its activity is frequently hijacked in the development of haematological malignancies including acute myeloid leukaemia (AML). Indeed, disruption to the level, localisation and activity of the central mediator β-catenin has been observed across numerous AML subtypes,[15] and is known to contribute towards the emergence, maintenance of leukaemia stem cells (LSC), particularly those driven from *mixed-lineage leukaemia* (MLL) gene rearrangements.[16-19]

Despite extensive knowledge of aberrant Wnt signalling activity over three decades both in AML and human cancer beyond, effective β-catenin targeting strategies remain largely absent from the clinic. Previous approaches to drug Wnt/β-catenin signalling have shown promise with a number of agents reaching Phase I or II clinical trials predominantly in solid cancers,[20] as exemplified by WNT974 (PORCN inhibitor preventing Wnt ligand palmitoylation and secretion).[21,22] However, very few of these have been deployed in a haematological setting which might partly be explained by β-catenin's divergent molecular interactions in this context. For example, the well-characterised *APC*, *AXIN* and *CTNNB1* mutations which lead to Wnt signalling hyperactivation and initiate solid tumours such as colorectal cancer, are seldom reported in AML. Furthermore, β-catenin's well characterised alternative function at the cell membrane in cell-cell homotypic adhesion via cadherin complexes is likely not a prominent feature of haematopoietic tissue which is fluid in nature. Despite its extensive characterisation in epithelial tissues, β-catenin's molecular interactions/functions within haematological cells are much less well-defined and recent reports in AML suggest novel partnerships such as that with the transcription factor IRF7.[23] Our previous exploration of the β-catenin interactome in myeloid cells revealed the substantial enrichment of RNA binding proteins (RBP)[15,24] indicating a potential novel role for β-catenin in the post-transcriptional landscape of gene regulation in leukaemia cells.[25] Since this time we have characterised several β-catenin:RBP interactions including WT1,[26] TOE1,[27] and MSI2[28], and revealed β-catenin associates with an mRNA network of Wnt signalling and metabolic transcripts in leukaemia cells.[28] This study also suggested that β-catenin's interaction with RNA is likely indirect and probably through its various RBP partners. One putative interacting partner identified from our previous β-catenin interactome study was the oncofetal expressed, and miRNA regulating, RBP; LIN28B.[24]

Like OCT4 and SOX2, LIN28B is a critical pluripotency factor maintaining primitive stem cells,[29] through both its role as a suppressor of *let7* miRNA biogenesis,[30] and a direct binder of partner mRNAs.[31] In a normal haematopoietic setting, its expression is confined entirely to fetal HSPCs,[32] where it maintains stemness and suppresses differentiation through *let7*-Cbx2,[33] *let7*-Hmga2,[34] and IGF2BP3 axes.[35] Despite previous reports of β-catenin and LIN28/let7 crosstalk in several tissues including breast,[36] intestine,[37] retina,[38] and lung,[39-41] there are no studies examining the relationship of these two proteins in a haematopoietic context. This study aimed to examine the relationship between β-catenin and LIN28B in AML and evaluate any dual-targeting potential in human leukaemia.

Methods

Cell culture and drug treatments

The myeloid cell lines are used in this study are as follows: K562, HL60, HEL, U937, PLB-985, NOMO-1, OCI-AML3, EOL-1, ML-1, THP-1, KU812 (The European Collection of Authenticated Cell Cultures) and OCI-AML2, KG-1, KG1a, SET-2, NB4 and MONO-MAC6 (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH). Short-tandem repeat (STR) analysis was done for cell line authentication for these myeloid cell cultures. Myeloid cell lines were cultured in RPMI-1640 medium, supplemented with L-glutamine and streptomycin at 37°C with 5% CO_2. HEK293T cell line was utilized for lentivirus preparation and cultured in DMEM supplemented with L-glutamine and streptomycin at 37°C with 5% CO_2. β-Catenin stabilisation was achieved through the GSK-3β inhibitor CHIR99021 (Merk-

Millipore, Gillingham, Dorset) as previously described.[24] LIN28-let-7 interaction was globally blocked using compound 1632 (C1632)[42] (SML3673-10MG, Merck-Millipore). Six different inhibitors (BC2059 (MedChemExpress [MCE], HY-109103), MSAB (MCE, HY-120697), KYA1797K (MCE, HY-101090), LGK974 (MCE, HY-17545), Pyrvinium Pamoate (MCE, HY-A0293), XAV-939 (MCE, HY-15147)) were tested to achieve pharmacological inhibition of β-catenin expression in myeloid cell lines.

Primary samples

Bone marrow, peripheral blood or leukapheresis samples from patients diagnosed with AML (clinical information provided in Supplemental Table S1) were collected in accordance with the Declaration of Helsinki and with approvals of University Hospitals Bristol and Weston NHS Foundation Trust and London Brent Research Ethics committee (12/LO/1193). Human cord blood (CB) was obtained following informed consent from healthy mothers at full-term undergoing elective caesarean sections at Royals Sussex County Hospital and Princess Royal Hospital, with approval from University Hospitals Sussex NHS Foundation Trust, the East of England-Essex Research Ethics Committee, HRA and Health and Care Research Wales (18/EE/0403). Mononuclear cells (MNCs) with viability >80% following isolation via density gradient separation using Ficoll-Hypaque (Merck-Millipore, Gillingham, Dorset) were included in the study and cryopreserved in liquid nitrogen until experimental use.

Donated fetal tissue was provided for purposes of this research by the Human Developmental Biology Resource (www.hdbr.org), regulated by the UK Human Tissue Authority (www.hta.gov.uk) and covered under ethics granted by NHS Health Research Authorities: North East—Newcastle & North Tyneside Research Ethics Committee (REC: 18/NE/0290) and London—Fulham Research Ethics Committee (18/LO/0822). Informed consent was obtained from all participants, who donated human fetal tissue for research without receiving any monetary compensation. The $CD34^+$ HSPC fraction of human cord blood and fetal livers was enriched to >80% purity as previously described[43] using MiniMACS CD34 microbeads (Miltenyi Biotec, Woking, Surrey) according to the manufacturer’s instructions and confirmed by flow cytometric assessment of CD34 positivity.

Lentivirus preparation and transduction

MISSION lentiviral shRNA plasmids and lentiviral overexpression plasmids were obtained from Merck-Millipore and VectorBuilder, respectively (see Supplemental Table S2 for details). Plasmid DNAs were supplied as pre-transformed glycerol stocks, which were streaked onto agar plates to isolate single colonies. Colonies were incubated overnight at 37°C, and plasmid DNA was purified using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, Altrincham, Cheshire). HEK293T cells were used to produce lentivirus. Tissue culture–treated flasks were coated with 0.01% poly-L-lysine solution (Merck Millipore), after which 4–5 x 10^6 HEK293T cells were seeded to achieve ~70% confluency the following day. Lentiviral particles were generated using Lipofectamine 3000 Transfection Reagent (Thermo Fisher Scientific), Opti-MEM I (1X) (Thermo Fisher Scientific), and the appropriate envelope, packaging, and transfer plasmids. Viral supernatants were harvested 24- and 48-hours post-transfection. For transduction, myeloid cell lines were plated in non-treated 24-well tissue culture plates (Thermo Fisher Scientific). Plates were first coated with 40 µg/ml Retronectin (Takara Bio, London, UK) overnight at 4°C, then blocked with 1% BSA in PBS for 30 minutes. Virus-containing supernatants were centrifuged at 2000 × g for 90 minutes at room temperature to promote viral sedimentation. Subsequently, 1 ml of resuspended myeloid cells (2–5x10□) was added per well. Following 2 days of viral integration and cell expansion, cells were subjected to drug selection with 1 µg/ml puromycin using the corresponding parental cell line as control. Protein knockdown or overexpression was confirmed by immunoblotting.

Immunoblotting

Whole-cell lysates were prepared using ice-cold 10x cell lysis buffer (20 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1 mM EGTA; 1% Triton; 2.5 mM sodium pyrophosphate; 1 mM β-glycerophosphate; 1 mM Na_3VO_4; 1 µg/mL leupeptin - Cell Signaling Technology, Leiden, Netherlands) diluted with distilled water and supplemented with one tablet of Complete Mini Protease Inhibitor (Roche, Welwyn Garden City, Hertfordshire). Protein concentrations were determined using the Detergent-Compatible Colorimetric Assay Kit (BioRad) to ensure equal protein loading per sample. Lysates were diluted with 1x Laemmli buffer (Thermo Fisher Scientific) and distilled water, then heated at 95 °C for 5 minutes before immunoblotting. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) was performed to separate proteins, which were subsequently transferred onto Immobilon-P Hydrophobic PVDF Transfer Membranes. Gels ranging from 7.5% to 15% acrylamide were used depending on the size of the protein of interest. Membranes were probed overnight at 4 °C with antibodies against the target proteins. Primary antibodies used in this study are listed in Supplemental Table S3. Target proteins were visualized using the LumiGLO® Peroxidase Chemiluminescent Substrate Kit (Sera Care, Wembley, UK).

Cytoplasmic/nuclear fractionation

A total of 5×10^6 cells were harvested for cytoplasmic and nuclear fractionation. After washing the cells with ice-cold PBS, each pellet was resuspended in cytoplasmic lysis buffer (10mM Tris-HCl; pH 8, 10mM NaCl, 1.5mM $MgCl_2$, and 0.5% Igepal-CA630/NP40; supplemented with one tablet of Complete Mini Protease Inhibitor (Roche)). The samples were centrifuged at 800 x g for 5 minutes at 4°C, and the supernatant was collected as the cytoplasmic fraction. The pellet was then washed with ice-cold PBS, resuspended in nuclear lysis buffer (Cell Signalling Technology), and subjected to sonication. Following sonication, the nuclear lysates were centrifuged at 17,000 × g for 10 minutes at 4°C, and the supernatant was collected as the nuclear fraction. Both cytoplasmic and nuclear fractions were quantified and processed for immunoblotting as described for whole-cell lysates above.

Co-immunoprecipitation (Co-IP)

Protein G Dynabeads (Thermo Fisher Scientific) were coupled with 8 µg of LIN28B antibody (Cell Signalling Technology, 4196) or rabbit IgG (Cell Signalling Technology, 2729). The beads were crosslinked to the antibodies using 20 mM dimethyl pimelimidate. Next, 1 mg of whole-cell lysate was incubated overnight at 4 °C with the crosslinked bead–antibody complexes in Co-IP lysis buffer (20 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1 mM EGTA; 1% Triton; 2.5 mM sodium pyrophosphate; 1 mM β-glycerophosphate; 1 mM Na_3VO_4; 1 µg/mL leupeptin; 10% glycerol; and 0.5 mM DTT). After incubation, the samples were washed with Co-IP wash buffer (phosphate-buffered saline (PBS) containing 0.02% Tween-20). Each sample was then diluted with 2× Laemmli buffer (Thermo Fisher Scientific) and Co-IP lysis buffer, and incubated at 95°C for 5 minutes before immunoblotting. 20 µg/ml RNase A (Thermo Fisher Scientific) was added to the Co-IP lysates to remove RNA molecules.

RNA extraction and clean-up

Total RNA extraction was done using Zymo Research Quick-RNA™ Miniprep Kit (Cambridge Bioscience, Cambridge, Cambridgeshire) and the extracted total RNA was exposed to DNase treatment to remove genomic DNA from RNA samples through TURBO DNA-free™ Kit (Thermo Fisher Scientific). Manufacturer's guidelines were followed.

RT-qPCR

The abundance of target transcripts was detected using One-Step RT-qPCR Mix (APTO-GEN, London, UK). The RT-qPCR reactions was prepared as follows: RT+ reaction (5 µl 2x SyGr RT-qPCR mix, 0.5 µl 20x reverse transcriptase, 0.25 µl forward/reverse primer, 1 µl 10ng of RNA, 3 µl nuclease-free water) and RT- reaction (5 µl 2x SyGr RT-qPCR mix, 0.25 µl forward/reverse primer, 1 µl 10ng of RNA, 3.5 µl nuclease-free water). RT-qPCR was performed under the recommended thermocycler conditions: 1 cycle (50^{o}C, 10 minutes), 1 cycle (95^{o}C, 1 minutes) and 40 cycles (95^{o}C, 5 seconds & 60^{o}C 20 seconds). Forward and reverse primer sequences used in this study are listed in Supplemental Table S4 and were optimised to show efficiency between 90-110%.

Motif Analysis

Our previous β-catenin RIP-seq analysis identified the 190 common and significantly enriched transcripts in K562 and HEL cells. The full-length mature sequence of the longest isoform of each of these were used in motif analysis using the STREME tool within the MEME Suite searching for motifs between 6-15nt and the model of control sequences as a background.[44]

miRNA sequencing

$2x10^6$ cells were harvested for each of the three biological replicates per condition (β-catenin shRNA, LIN28B shRNA and non-targeting shRNA). Total RNA extraction was performed using the Quick-RNA™ Miniprep Kit (Cambridge Bioscience) as described in manufacturer's guidelines. Quality control of total RNA samples was done with the Agilent TapeStation 4150. Lastly, the RNA samples were sent for miRNA sequencing (miRNA-seq) to BGI Genomics (Shenzhen, China) for library preparation and sequencing.

BGI Genomics provided clean FASTQ files, and we pre-processed them in sRNAbench on the sRNAtoolbox platform,[45] with the following parameters: miRbase release 22.1 (as miRNA annotation reference database), Homo sapiens (hsa) (as short name from miRbase), human (GRCh38_p13) (as species), "Provided reads are already trimmed" (as sequencing library protocol), the rest of parameters as defaults. Raw read counts obtained after preprocessing was used to determine upregulated or downregulated miRNAs with β-catenin or LIN28B knockdown versus control using DESeq2 (version 1.44.0) package in R software.[46] Also, tidyverse (version 2.0.0) and ComplexHeatmap (version 2.20.0) packages were used for data manipulation and visualisation.[47]

RNA immunoprecipitation (RIP) / Cross-linking immunoprecipitation (CLIP)

RIP and CLIP assays were done as previously described.[48]

RNA stability assay

K562 cells were seeded at a density of $1x10^6$ cells/ml, and 5 µg/ml Actinomycin D (Merck Millipore) was added to the cells. Samples were collected at 0-, 4- and 8-hour time points, and RNA extraction, DNase treatment and RT-qPCR was performed as described above. The RNA stability of the target RNAs were calculated based on the RNA abundance in 0-time point for each cell line separately.

Flow cytometry

Wnt/β-catenin signalling activity was measured in $2x10^5$ myeloid cells using the BAR/fuBAR reporter system.[49] Cells were treated overnight with either DMSO or CHIR99021. TCF/LEF activity was assessed by flow cytometric analysis of Venus yellow fluorescent protein (YFP) intensity. Cell viability of myeloid cells with β-catenin knockdown after C1632 treatment was assessed using 7-AAD viability staining solution (Thermo Fisher Scientific), whereas cell viability was evaluated using the Zombie Violet Fixable Viability Kit (BioLegend, London, UK) when the β-catenin inhibitor pyrvinium pamoate was used. This was because pyrvinium pamoate exhibits red fluorescence, which interferes with the 7-AAD staining channel during flow cytometry. Data acquisition was performed on a CytoFLEX flow cytometer (Beckman Coulter, California, USA), with a minimum of 2x10□ debris-excluded events collected per sample. Flow cytometric data were analysed using FlowJo software (Tree Star Inc., Ashland, OR, USA).

Statistics

All experiments were performed in three biological replicates unless otherwise stated and statistical tests performed using GraphPad Prism (version 10; GraphPad, Boston, MA). Statistical analyses were conducted using Student's *t*-test or ANOVA, with a threshold of $p < 0.05$. Data are presented as mean ± 1 standard deviation unless otherwise stated.

Results

β-Catenin and LIN28B interact in myeloid leukaemia cell lines and fetal HSPC

Our previous exploration of the β-catenin interactome in haematopoietic cells revealed a dense RBP network,[24] and subsequent β-catenin RIP-seq of myeloid cells identified β-catenin enrichment with mRNAs encoding regulators of Wnt signalling pathway and metabolism.[28] These studies also suggested β-catenin has little direct RNA binding capacity and so RNA association is likely mediated through its many canonical RBP partners.[15,24] To shortlist the RBP partners most important for β-catenin's mRNA interactions, we analysed the most frequently occurring RBP binding motifs amongst the 190 commonly and significantly enriched transcripts derived from β-catenin RIP-seq in K562 and HEL cells. From this analysis we identified the GGAG motif to be the most frequently enriched binding motif (p=0.019) which is bound by the *let7* miRNA regulating and oncofetal expressed RBP; LIN28 (Figure 1A).[50] LIN28B was identified as a putative β-catenin interacting protein in myeloid cells from our original interactome study,[24] suggesting β-catenin influence in post-transcription could be mediated through a LIN28B interaction. To evaluate the merit of targeting this interaction in AML we explored *LIN28B* RNA expression across human leukaemias using the Leukaemia MILE Study dataset via BloodSpot[51] which indicated a small number of ALL, CML, and AML cases exhibit *LIN28B* reactivation (Figure 1B).

To interrogate this interaction further we examined the LIN28B and β-catenin expression across a panel of 18 myeloid cell lines. We observed variable expression of both the long and short LIN28B isoforms in approximately 50% of myeloid cell lines (Figure 1C), and co-expression of both LIN28B and β-catenin was detected in numerous cell lines which didn’t particularly align with any specific geno- or phenotypic features. Notably, the alternative LIN28 paralogue, LIN28A, was not detectable in any of the myeloid cell lines examined but was detected in the T47D breast cancer cell line, confirming antibody functionality (Supplementary Figure S1A). Based on this screen, we opted to validate the β-catenin:LIN28B interaction in the two cell lines used from the original TMT mass spectrometric analysis of the original β-catenin interactome study.[24] As observed in Figure 1D, LIN28B was efficiently Co-IP’d from both K562 and HEL cells under both basal and Wnt stimulated conditions (CHIR99021 treated) and exhibited β-catenin interaction in both contexts. We also confirmed the β-catenin:LIN28B interaction in lymphoid SEM cells suggesting this interaction extends beyond a myeloid context (Supplementary Figure S1B). Given the association of both LIN28B[52] and β-catenin[25] with RNA, we wanted to rule out non-specific interaction of both proteins due to RNA co-occupancy. Complete digestion of RNA in input lysates prior to co-IP through RNase A treatment, had no impact on the β-catenin:LIN28B interaction (Figure 1E), suggesting the two molecules may be complexed together regardless of RNA presence. However, AlphaFold modelling suggests against a direct interaction between the two proteins suggesting other common intermediate proteins or structures could mediate the association (Supplementary Figure S1C).

We next wanted to test whether this interaction could be detected in primary AML samples and screened LIN28B expression across primary AML patient samples we've previously examined for β-catenin expression.[26,28] We were unable to detect LIN28B in our 30 patient cohort (Supplementary Figure S1D) in keeping with low LIN28B expression in adult haematopoietic tissue.[53] Instead, given LIN28B's well characterised role as a regulator of fetal HSC emergence and development,[32-35] a setting also requiring active Wnt/β-catenin signalling,[11-13] we repeated the LIN28B Co-IP from human fetal liver (FL)-derived CD34$^+$ HSPC. After confirming enrichment of FL-CD34$^+$ HSPC to >85% purity (Figure 1F), and abundant levels of both proteins in input lysates, we confirmed β-catenin enrichment through LIN28B Co-IP (Figure 1G). Taken together, these findings identify LIN28B as a novel interacting partner of β-catenin in both myeloid and lymphoid cell lines, and primary human FL-CD34$^+$ HSPCs.

LIN28B modulates Wnt/β-catenin signalling output through LEF1 via a post-transcriptional mechanism

Given previous reports of LIN28B and Wnt signalling crosstalk in solid tissues,[36,37] we next wanted to assess the degree and direction of crosstalk in leukaemia cells. To examine this, we employed the β-catenin activated reporter (BAR) system adopted previously,[26,28,54] to assess TCF/LEF activity upon LIN28B modulation. Upon confirmation of efficient LIN28B knockdown using shRNA (Figure 2A), we observed a ~2-fold decrease in TCF/LEF activity in K562 cells upon Wnt signalling activation (Figure 2B and C, $p < 0.05$). Reciprocally, ectopic LIN28B promoted a ~2-fold increase in TCF/LEF activity under active Wnt signalling conditions (Figures D and E, $p < 0.05$). Collectively, these results indicate that LIN28B is a positive regulator of Wnt/β-catenin signalling in myeloid cells.

To investigate the underlying mechanism of LIN28B regulation of Wnt signalling output we first examined β-catenin expression and localization following LIN28B ablation. We observed no significant changes to overall β-catenin level (Figure 2F) or subcellular localization (Figure 2G). We next checked the level of the downstream Wnt transcription factors LEF1 and TCF4 (*TCF7L2*) and observed a consistent reduction in both *LEF1* mRNA and protein levels in K562 cells following LIN28B depletion (Figure 2F, G, and H). Reciprocally, ectopic expression of LIN28B in K562 cells led to substantially increased LEF-1 protein levels (Figure 2D). These data indicate LIN28B positively regulates Wnt/β-catenin signalling activity through modulation of the downstream transcription factor LEF-1.

We next aimed to determine whether LIN28B regulation of LEF-1 occurred through transcriptional mechanisms since LIN28B has been linked with transcriptional regulation previously.[55] To test this, we designed *LEF1* primers spanning intronic regions of *pre-LEF1*, and assessed abundance following LIN28B depletion. As a control, we first validated these intronic primers in K562 cells treated with CHIR99021 to induce Wnt signalling activation since *LEF1* is a known Wnt signalling target gene,[56] which confirmed a 4-fold increase compared to DMSO-treated cells (Figure 2I). Using these validated primers, we next measured *LEF1* levels in LIN28B-depleted K562 cells and observed no change versus control NT-shRNA K562 cells (Figure 2J), suggesting regulation through post-transcriptional means.

LIN28B likely regulates LEF1 via a let7 miRNA mechanism and has shared miRNA target regulation with β-catenin

We then aimed to investigate the mechanism through which LIN28B regulates LEF-1 expression in myeloid cells. Initially we hypothesised this could be through direct transcript binding since LIN28B is a well-known mRNA binding protein,[52] and such interactions influence target expression/stability as evidenced with TP53,[57] and several splicing factors (*FUS/TLS*, *hnRNP F*, *TDP-43* and *TIA-1*).[50] Adopting both LIN28B RIP and CLIP approaches from K562 cells, we first confirmed efficient enrichment of *LIN28B* mRNA as a positive control, since LIN28B is known to bind and autoregulate its own transcript (Supplementary Figure S2A-C).[50] We also confirmed the significant enrichment of *LEF1* mRNA (Supplementary Figure S2A-C), however subsequent assessment of *LEF1* mRNA stability in LIN28B-depleted cells failed to show any marked differences from control cells (Supplementary Figure S2B). We next considered whether regulation of *LEF1* could occur through *let-7*-

dependent mechanisms given the well-characterised role of LIN28B in the repression of *let-7* miRNA maturation.[30] To first evaluate if LIN28B impacted *let-7* expression in myeloid cells we performed miRNA-seq in K562 cells with LIN28B shRNA (Figure 3A). We also examined miRNA expression in β-catenin depleted cells (Figure 3A) given its previous link regulating *let-7* expression in breast cancer cells,[36] and to reveal any potential overlapping function with LIN28B. Clustering analysis revealed clear and distinct miRNA expression profiles across control, LIN28B and β-catenin shRNA samples (Figure 3B and E-MTAB-16028; ArrayExpress). Comparison of miRNA expression levels between both LIN28B-depleted and β-catenin–depleted cells relative to control (NT shRNA), revealed significantly upregulated and downregulated miRNAs in each condition (Figures 3C-H; see also Supplemental miRNA-seq data sheet). As expected, numerous *let-7* miRNAs, including *let-7d*, *let-7i* and *let-7f-1*, were significantly upregulated in response to LIN28B ablation (Figures 3C). In contrast, we observed no significantly differential *let-7* miRNA expression with β-catenin depletion in K562 cells (Figure 3D), suggesting β-catenin is not a major regulator of mature *let-7* miRNA expression in a myeloid setting. However, several other miRNA species were differentially regulated in response to β-catenin modulation (Figures 3E-H and Supplemental Table S5), including several overlapping with LIN28B depletion (Figures 3C-H and Supplemental Table S6), suggesting novel miRNA regulating roles in haematopoietic cells which could be mediated through cooperation with LIN28B.

Given that multiple *let-7* miRNAs were induced following LIN28B suppression we opted to globally inhibit their expression, rather than interrogating individual *let-7* family members, as a means of evaluating *LEF1* regulation. For this we deployed C1632, a small molecule inhibitor (SMI) which blocks LIN28:*let-7* interaction resulting in *let-7* induction.[42] Following overnight treatment with two doses of C1632 we observed substantially reduced levels of LEF-1 protein (Figure 4A) and *LEF1* exonic mRNA (Figure 4B), in both K562 and HEL cells which was significantly depleted at 100µM, however intronic *LEF-1* remained unchanged (Figure 4C) confirming post-transcriptional, rather than transcriptional regulation, by C1632. Examination of TCF/LEF activity in C1632-treated cells, also revealed dose dependent depletion of

Wnt signalling capacity in response to C1632 treatment in both K562 and HEL cells (Figure 4D). In summary, these findings indicate that LIN28B may regulate LEF-1 expression through a post-transcriptional *let-7* miRNA-mediated mechanism.

Dual β-catenin and LIN28B targeting decreases AML cell death viability

Public expression databases suggest LIN28B reactivation can occur in discrete human leukaemia subtypes including normal/complex karyotype and *MLL*-rearranged AML (Figure 1B), settings where aberrant β-catenin level, localisation or activity is also reported.[16-19,58,59] Therefore, dual-targeting of the LIN28B:β-catenin axis could be clinically viable given the molecular cooperation indicated from previous experiments. To test this, we initially deployed two doses of C1632 (10 and 100μM) against THP1 and HEL cells expressing either control or β-catenin shRNA (Figure 5A) across 24, 48 and 72 hours and assessed cell viability. As seen in Figures 5B and C, 100μM C1632 significantly reduced the viability of both β-catenin depleted AML cell lines relative to NT-shRNA controls at 72hrs treatment with both shRNAs, also impacting HEL cells from 48hrs onwards. However, no increased C1632 sensitivity was observed in other β-catenin depleted myeloid cell lines (K562 and KG-1 cells; Supplementary Figure S3), implying subgroup specificity for where the β-catenin:LIN28B axis promotes survival.

Reciprocally, we also assessed the impact of targeting β-catenin through SMIs in a LIN28B-depleted setting. We reasoned that compounds targeting β-catenin stability, rather than TCF/LEF or CBP/p300 interaction, were more applicable in this scenario given its interaction and subsequent miRNA regulation with LIN28B is likely post-transcriptional. We first needed to identify an efficient β-catenin degrader in leukaemia cells, since many SMIs were optimised in solid tissues where mechanisms of Wnt/β-catenin disruption differ from leukaemia cells.[15,25] To our surprise, many of the proposed β-catenin degraders were ineffective at reducing β-catenin stability across a 24hrs exposure in myeloid cells including LGK974, MSAB, KYA1797K, XAV-939, and BC2059 (Tegatrabetan), and the β-catenin targeted PROTAC xStAx-VHLL (Supplementary Figure S4). These data suggest β-catenin stability is governed by completely distinct mechanisms in leukaemia cells. The only agent mildly effective at reducing β-catenin stability from 5µM was pyrvinium pamoate (PP; Figure 6A) which is an FDA-approved Casein Kinase-1α (destruction complex component) agonist and anthelmintic known to also have anti-cancer activity, especially against B-ALL[60] and MLL-rearranged AML.[61,62] Therefore, we treated LIN28B shRNA expressing HEL and THP1 cells (Figure 6B) with 5µM PP and assessed cell viability 24-72hrs. As observed in Figure 6C, PP significantly increased the death of LIN28B-depleted HEL cells versus NT-shRNA control counterparts. THP1 cells were exquisitely sensitive to PP with NT-shRNA control cells exhibiting ≥ 90% cell death with a 5µM dose after just 24 hours making any cooperative cell killing challenging to observe (Supplementary Figure S5A). Therefore, we dropped the PP concentration to 0.5µM and assessed cell viability 24-72hrs but failed to observe differential PP sensitivity between THP1 cells with LIN28B shRNA versus NT-shRNA controls (Supplementary Figure S5B). However, it should be noted that upon immunoblotting of β-catenin levels, 0.5µM PP was insufficient to reduce β-catenin stability until 72hrs in THP1 cells (Supplementary Figure S5C) which was much quicker in HEL cells. Finally, to evaluate a proof-of-concept approach to targeting LIN28:β-catenin in AML cells we deployed a dual targeting strategy using both SMIs. As shown in Figure 6D, dual treatment of HEL cells with C1632 and PP led to more cell death in combination than using either drug individually. Taken together, these data support the notion that dual β-catenin:LIN28B targeting could be an effective strategy in discrete subgroups of AML where LIN28B is reactivated and β-catenin can be efficiently degraded.

Discussion

This study has demonstrated β-catenin and LIN28B interact for the first time in any context. AlphaFold modelling suggests this interaction is indirect, with our data suggesting an RNA-independent association that may be part of a wider protein complex. Indeed, this is now the third RBP interaction with β-catenin that we've demonstrated has regulatory capacity over LEF-1 expression,[27,28] and points to β-catenin constituting a wider post-transcriptional regulatory network that governs expression of this Wnt transcription factor. We've not been able to characterise the functional significance of the β-catenin:LIN28B interaction but our data suggests no capacity for either to regulate the stability/localisation of the other. Instead we speculate this interaction could impact miRNA expression since our miRNA-seq experiment identified co-regulated targets from LIN28B and β-catenin depletion, and cooperation between β-catenin and LIN28A in miRNA regulation has been reported in the expansion of breast cancer stem cells.[36] This study also demonstrated that LIN28B is able to regulate expression of LEF-1, likely through a post-transcriptional or translational mechanism. Crosstalk between Wnt/β-catenin signalling and LIN28 has been observed frequently in both normal development,[38,41,63-65] and cancer,[37,66] however this is the first report in a haematopoietic context and the first to implicate LEF-1 regulation. Our experiments using the LIN28:*let7* inhibitor C1632 suggest this regulation is primarily post-transcriptional in nature and potentially via miRNA regulation of *LEF1*. One previous study reports *let7* regulation of LEF-1 expression (via *let-7i-3p*),[67] however there are numerous miRNAs reported to regulate *LEF1* expression including miR-26b,[68,69] miR-34a,[70-74] miR-181a,[75] miR-218,[76] miR-223,[71] miR-381,[77] miR-557,[78] and miR-621,[79] suggesting further mechanistic characterisation in relation to LIN28B will be important in a haematopoietic setting. Interestingly, miR-181a and miR-34a were amongst the most significantly increased miRNAs following LIN28B and β-catenin depletion, respectively, in our study, indicating a potential novel route for *LEF1* regulation in haematopoietic cells.

LIN28B itself has an enigmatic and complex role in normal and malignant haematopoiesis. Expression is reportedly restricted to fetal haematopoiesis,[32] where it regulates fetal HSC development through *let-7*–Hmga2,[34] *let-7*-Cbx2,[33] and IGF2BP3 axes.[35] Active Wnt/β-catenin signalling also contributes to these early stages of haematopoiesis,[11-13] and our finding that β-catenin interacts with LIN28B in purified human fetal liver CD34$^+$ HSPCs raises the intriguing possibility of crosstalk/cooperation between these proteins in early haematopoietic development. Upon transition to adult haematopoiesis, LIN28B expression is rapidly downregulated (permitting mature *let-7* expression) with expression only reactivated in a small number of leukaemias. There is evidence for LIN28B serving both tumour suppressive and oncogenic functions in malignant haematopoiesis. *In vivo* models of MLL-derived myeloid leukaemogenesis (MLL-AF9 and MLL-ENL) have demonstrated that LIN28B expressing fetal progenitors have reduced transformation capacity, and only the reduction of LIN28B expression in these cell provides a leukaemia-permissive environment.[53,80] In myeloid neoplasms, LIN28B reactivation appears to have a pro-leukaemic role contributing to differentiation arrest,[81] growth/survival,[82] and promotion of a stem-like transcriptional programme.[83] In CML, LIN28B expression is enhanced in blast phase versus chronic phase, and promotes proliferation through a miR-181d-PDCD4 axis (miR-181d also upregulated in our β-catenin shRNA miRNA-seq) which involves a LIN28B DNA-binding function.[84] A novel fetal-like subgroup of juvenile myelomonocytic leukaemia (JMML) cases also exhibit LIN28B reactivation where its expression predicts inferior survival.[85,86] Collectively this implies whilst pre-existing LIN28B expression appears to inhibit leukaemogenesis, in rare number of AML cases where LIN28B becomes reactivated it can serve oncogenic roles likely through its ability to confer stem-like properties.

Although this study has not elucidated the functional role of the β-catenin:LIN28B interaction, we have shown that dual targeting of both molecules may have therapeutic merit in subsets of myeloid leukaemia where they are both expressed. Notably, one of the cell lines susceptible to the β-catenin shRNA/C1632 combination was THP-1 cells which is an MLL-rearranged myeloid cell line, and expression databases suggest LIN28B reactivation occurs in a small subset of MLL leukaemias of both myeloid and lymphoid origin (Figure 1B). LIN28B inhibitors remain in their infancy as the importance of this RBP in human cancer continues to emerge. Although C1632 has shown activity against several solid cancers,[87-91] it's not a particularly efficient compound requiring high doses such as those used in this study (100µM) to be effective. However, this evidence shows in principle that LIN28B targeting could be a worthwhile approach in the treatment of specific AML cohorts if efficacious inhibitors could be developed. Several new LIN28 inhibitors are currently in development,[92-95] such as ln268,[96] and if successful it's likely they'll exhibit a favourable toxicity profile in AML given LIN28B is not expressed in normal adult haematopoietic tissues limiting on-target toxicity.

Even if LIN28B inhibition can be refined, our study highlights that β-catenin drug targeting in leukaemia remains a formidable challenge. Many of the compounds proposed to reduce β-catenin level in solid tissues, including LGK974 (PORCN inhibitor), MSAB (β-catenin degrader), KYA1797K (Axin agonist), XAV-939 (Tankyrase inhibitor), BC2059 (Transducin β-Like 1 blocker), and xStAx-VHL (β-catenin PROTAC) were largely ineffective in leukaemia cells, pointing to alternative modes of β-catenin stabilisation/regulation in myeloid leukaemia yet to be defined. The only compound that showed any capacity for β-catenin degradation was PP which is an FDA-approved quinoline-derived cyanine dye and antihelminth drug that boosts the activity of Casein Kinase-1α, a critical destruction complex component responsible for providing the serine/threonine phosphorylation necessary for β-catenin's subsequent ubiquitination and proteasomal degradation. In recent years, PP has emerged as an anti-tumour compound with activity against leukaemias,[60-62] however PP is not a specific Wnt/β-catenin inhibitor, also acting through mitochondrial disruption, PI3K/AKT inhibition, and UPR activation.[97] Therefore, there remains an urgent and unmet need for specific and efficient β-catenin degraders in myeloid leukaemia.

In summary, we've identified an interaction between β-catenin and LIN28B, and shown LIN28B can impact Wnt signalling activity in haematopoietic cells through the post-transcriptional regulation of LEF-1 expression. This study has demonstrated that dual targeting of β-catenin and LIN28B could represent an novel and effective strategy in specific AML subgroups where both proteins are actively contributing to disease biology.

Author contributions

OS performed experiments, analysed data and co-wrote the manuscript. MW managed laboratory, performed experiments and provided experimental guidance. RL assisted with FL-CD34 HSC experiments whilst AG, DP, HP and KH generated contributing data, and AB provided primary AML samples. LC and SN provided reagents and experimental advice, while AR provided primary FL-CD34 HSCs and conceptual guidance. BT and RGM performed experiments, analysed data, co-wrote the manuscript and conceived/directed the study.

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References

1 Maurice, M. M. & Angers, S. Mechanistic insights into Wnt-beta-catenin pathway activation and signal transduction. *Nat Rev Mol Cell Biol* (2025). https://doi.org:10.1038/s41580-024-00823-y

2 Nusse, R. & Clevers, H. Wnt/beta-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* **169**, 985-999 (2017). https://doi.org:10.1016/j.cell.2017.05.016

3 Reya, T. *et al.* A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**, 409-414 (2003).

4 Willert, K. *et al.* Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* **423**, 448-452 (2003).

5 Sheng, Y. *et al.* Activation of wnt/beta-catenin signaling blocks monocyte-macrophage differentiation through antagonizing PU.1-targeted gene transcription. *Leukemia* **30**, 2106-2109 (2016). https://doi.org:10.1038/leu.2016.146

6 Danek, P. *et al.* beta-Catenin-TCF/LEF signaling promotes steady-state and emergency granulopoiesis via G-CSF receptor upregulation. *Blood* **136**, 2574-2587 (2020). https://doi.org:10.1182/blood.2019004664

7 Brown, A. L. *et al.* The GM-CSF receptor utilizes beta-catenin and Tcf4 to specify macrophage lineage differentiation. *Differentiation* **83**, 47-59 (2012). https://doi.org:S0301-4681(11)00126-5 [pii];10.1016/j.diff.2011.08.003 [doi]

8 Shooshtarizadeh, P. *et al.* Gfi1b regulates the level of Wnt/beta-catenin signaling in hematopoietic stem cells and megakaryocytes. *Nat Commun* **10**, 1270 (2019). https://doi.org:10.1038/s41467-019-09273-z

9 Luis, T. C., Ichii, M., Brugman, M. H., Kincade, P. & Staal, F. J. Wnt signaling strength regulates normal hematopoiesis and its deregulation is involved in leukemia development. *Leukemia* **26**, 414-421 (2012). https://doi.org:leu2011387 [pii];10.1038/leu.2011.387 [doi]

10 Luis, T. C. *et al.* Canonical wnt signaling regulates hematopoiesis in a dosage-dependent fashion. *Cell Stem Cell* **9**, 345-356 (2011).

11 Kwarteng, E. O., Hetu-Arbour, R. & Heinonen, K. M. Frontline Science: Wnt/beta-catenin pathway promotes early engraftment of fetal hematopoietic stem/progenitor cells. *J Leukoc Biol* **103**, 381-393 (2018). https://doi.org:10.1002/JLB.1HI0917-373R

12 Ruiz-Herguido, C. *et al.* Hematopoietic stem cell development requires transient Wnt/beta-catenin activity. *J Exp Med* **209**, 1457-1468 (2012). https://doi.org:10.1084/jem.20120225

13 Tran, H. T., Sekkali, B., Van Imschoot, G., Janssens, S. & Vleminckx, K. Wnt/beta-catenin signaling is involved in the induction and maintenance of primitive hematopoiesis in the vertebrate embryo. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 16160-16165 (2010). https://doi.org:10.1073/pnas.1007725107

14 Luis, T. C. *et al.* Wnt3a deficiency irreversibly impairs hematopoietic stem cell self-renewal and leads to defects in progenitor cell differentiation. *Blood* **113**, 546-554 (2009).

15 Wagstaff, M., Coke, B., Hodgkiss, G. R. & Morgan, R. G. Targeting beta-catenin in acute myeloid leukaemia: past, present, and future perspectives. *Biosci Rep* (2022). https://doi.org:10.1042/BSR20211841

16 Wang, Y. *et al.* The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. *Science* **327**, 1650-1653 (2010).

17 Yeung, J. *et al.* beta-Catenin mediates the establishment and drug resistance of MLL leukemic stem cells. *Cancer Cell* **18**, 606-618 (2010).

18 Dietrich, P. A. *et al.* GPR84 sustains aberrant beta-catenin signaling in leukemic stem cells for maintenance of MLL leukemogenesis. *Blood* **124**, 3284-3294 (2014). https://doi.org:10.1182/blood-2013-10-532523

19 Fong, C. Y. *et al.* BET inhibitor resistance emerges from leukaemia stem cells. *Nature* **525**, 538-542 (2015). https://doi.org:10.1038/nature14888

20 Park, W. J. & Kim, M. J. A New Wave of Targeting 'Undruggable' Wnt Signaling for Cancer Therapy: Challenges and Opportunities. *Cells* **12** (2023). https://doi.org:10.3390/cells12081110

21 Tabernero, J. *et al.* A Phase Ib/II Study of WNT974 + Encorafenib + Cetuximab in Patients With BRAF V600E-Mutant KRAS Wild-Type Metastatic Colorectal Cancer. *Oncologist* **28**, 230-238 (2023). https://doi.org:10.1093/oncolo/oyad007

22 Rodon, J. *et al.* Phase 1 study of single-agent WNT974, a first-in-class Porcupine inhibitor, in patients with advanced solid tumours. *Br J Cancer* **125**, 28-37 (2021). https://doi.org:10.1038/s41416-021-01389-8

23 Hosseini, A. *et al.* Perturbing LSD1 and WNT rewires transcription to synergistically induce AML differentiation. *Nature* **642**, 508-518 (2025). https://doi.org:10.1038/s41586-025-08915-1

24 Morgan, R. G. *et al.* LEF-1 drives aberrant beta-catenin nuclear localization in myeloid leukemia cells. *Haematologica* **104**, 1365-1377 (2019). https://doi.org:10.3324/haematol.2018.202846

25 Sevim, O., Park, H. & Morgan, R. G. Post-transcriptional control of gene expression by beta-catenin: expanding the non-canonical ARMoury. *Oncogene* (2025). https://doi.org:10.1038/s41388-025-03470-5

26 Wagstaff, M. *et al.* Crosstalk between beta-catenin and WT1 signaling activity in acute myeloid leukemia. *Haematologica* **108**, 283-289 (2023). https://doi.org:10.3324/haematol.2021.280294

27 Park, H. *et al.* TOE1 influences canonical Wnt signalling in myeloid leukaemia cells through LEF-1 modulation and regulates the proliferation of haematopoietic cells through PAK2. *bioRxiv*, 2025.2012.2011.691106 (2025). https://doi.org:10.64898/2025.12.11.691106

28 Wagstaff, M. *et al.* beta-Catenin interacts with canonical RBPs including MSI2 to associate with a Wnt signalling mRNA network in myeloid leukaemia cells. *Oncogene* (2025). https://doi.org:10.1038/s41388-025-03415-y

29 Zhang, J. *et al.* LIN28 Regulates Stem Cell Metabolism and Conversion to Primed Pluripotency. *Cell Stem Cell* **19**, 66-80 (2016). https://doi.org:10.1016/j.stem.2016.05.009

30 Piskounova, E. *et al.* Lin28A and Lin28B inhibit let-7 microRNA biogenesis by distinct mechanisms. *Cell* **147**, 1066-1079 (2011). https://doi.org:10.1016/j.cell.2011.10.039

31 Tsanov, K. M. *et al.* LIN28 phosphorylation by MAPK/ERK couples signalling to the post-transcriptional control of pluripotency. *Nat Cell Biol* **19**, 60-67 (2017). https://doi.org:10.1038/ncb3453

32 Yuan, J., Nguyen, C. K., Liu, X., Kanellopoulou, C. & Muljo, S. A. Lin28b reprograms adult bone marrow hematopoietic progenitors to mediate fetal-like lymphopoiesis. *Science* **335**, 1195-1200 (2012). https://doi.org:10.1126/science.1216557

33 Wang, D. *et al.* Developmental maturation of the hematopoietic system controlled by a Lin28b-let-7-Cbx2 axis. *Cell Rep* **39**, 110587 (2022). https://doi.org:10.1016/j.celrep.2022.110587

34 Copley, M. R. *et al.* The Lin28b-let-7-Hmga2 axis determines the higher self-renewal potential of fetal haematopoietic stem cells. *Nat Cell Biol* **15**, 916-925 (2013). https://doi.org:10.1038/ncb2783

35 Wang, S. *et al.* Enhancement of LIN28B-induced hematopoietic reprogramming by IGF2BP3. *Genes Dev* **33**, 1048-1068 (2019). https://doi.org:10.1101/gad.325100.119

36 Cai, W. Y. *et al.* The Wnt-beta-catenin pathway represses let-7 microRNA expression through transactivation of Lin28 to augment breast cancer stem cell expansion. *J Cell Sci* **126**, 2877-2889 (2013). https://doi.org:10.1242/jcs.123810

37 Tu, H. C. *et al.* LIN28 cooperates with WNT signaling to drive invasive intestinal and colorectal adenocarcinoma in mice and humans. *Genes Dev* **29**, 1074-1086 (2015). https://doi.org:10.1101/gad.256693.114

38 Yao, K. *et al.* Wnt Regulates Proliferation and Neurogenic Potential of Muller Glial Cells via a Lin28/let-7 miRNA-Dependent Pathway in Adult Mammalian Retinas. *Cell Rep* **17**, 165-178 (2016). https://doi.org:10.1016/j.celrep.2016.08.078

39 Zhang, T. *et al.* Glycogen synthase kinase-3beta promotes radiation-induced lung fibrosis by regulating beta-catenin/lin28 signaling network to determine type II alveolar stem cell transdifferentiation state. *FASEB J* **34**, 12466-12480 (2020). https://doi.org:10.1096/fj.202001518

40 Liu, X. *et al.* beta-Catenin/Lin28/let-7 regulatory network determines type II alveolar epithelial stem cell differentiation phenotypes following thoracic irradiation. *J Radiat Res* **62**, 119-132 (2021). https://doi.org:10.1093/jrr/rraa119

41 Thevarajan, I. *et al.* LIN28-mediated gene regulatory loops synchronize transitions throughout organogenesis. *Biochem Biophys Rep* **44**, 102226 (2025). https://doi.org:10.1016/j.bbrep.2025.102226

42 Roos, M. *et al.* A Small-Molecule Inhibitor of Lin28. *ACS Chem Biol* **11**, 2773-2781 (2016). https://doi.org:10.1021/acschembio.6b00232

43 Morgan, R. G. *et al.* gamma-Catenin is expressed throughout normal human hematopoietic development and is required for normal PU.1-dependent monocyte differentiation. *Leukemia* **27**, 2096-2100 (2013). https://doi.org:10.1038/leu.2013.96

44 Bailey, T. L. *et al.* MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res* **37**, W202-208 (2009). https://doi.org:10.1093/nar/gkp335

45 Aparicio-Puerta, E. *et al.* sRNAbench and sRNAtoolbox 2022 update: accurate miRNA and sncRNA profiling for model and non-model organisms. *Nucleic Acids Res* **50**, W710-W717 (2022). https://doi.org:10.1093/nar/gkac363

46 Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology* **15**, 550 (2014). https://doi.org:10.1186/s13059-014-0550-8

47 Gu, Z. Complex heatmap visualization. *Imeta* **1**, e43 (2022). https://doi.org:10.1002/imt2.43

48 Wagstaff, M. *et al.* beta-Catenin interacts with canonical RBPs including MSI2 to associate with a Wnt signalling mRNA network in myeloid leukaemia cells. *Oncogene* **44**, 2490-2503 (2025). https://doi.org:10.1038/s41388-025-03415-y

49 Biechele, T. L. & Moon, R. T. Assaying beta-catenin/TCF transcription with beta-catenin/TCF transcription-based reporter constructs. *Methods Mol.Biol.* **468**, 99-110 (2008).

50 Wilbert, M. L. *et al.* LIN28 binds messenger RNAs at GGAGA motifs and regulates splicing factor abundance. *Mol Cell* **48**, 195-206 (2012). https://doi.org:10.1016/j.molcel.2012.08.004

51 Gislason, M. H. *et al.* BloodSpot 3.0: a database of gene and protein expression data in normal and malignant haematopoiesis. *Nucleic Acids Res* **52**, D1138-D1142 (2024). https://doi.org:10.1093/nar/gkad993

52 Hafner, M. *et al.* Identification of mRNAs bound and regulated by human LIN28 proteins and molecular requirements for RNA recognition. *RNA* **19**, 613-626 (2013). https://doi.org:10.1261/rna.036491.112

53 Eldeeb, M. *et al.* A fetal tumor suppressor axis abrogates MLL-fusion-driven acute myeloid leukemia. *Cell Rep* **42**, 112099 (2023). https://doi.org:10.1016/j.celrep.2023.112099

54 Morgan, R. G. *et al.* gamma-Catenin is overexpressed in acute myeloid leukemia and promotes the stabilization and nuclear localization of beta-catenin. *Leukemia* **27**, 336-343 (2013). https://doi.org:10.1038/leu.2012.221

55 Tao, T. *et al.* LIN28B regulates transcription and potentiates MYCN-induced neuroblastoma through binding to ZNF143 at target gene promotors. *Proceedings of the National Academy of Sciences of the United States of America* **117**, 16516-16526 (2020). https://doi.org:10.1073/pnas.1922692117

56 Hovanes, K. *et al.* Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. *Nat Genet* **28**, 53-57 (2001). https://doi.org:10.1038/88264

57 Shi, J. *et al.* LIN28B inhibition sensitizes cells to p53-restoring PPI therapy through unleashed translational suppression. *Oncogenesis* **11**, 37 (2022). https://doi.org:10.1038/s41389-022-00412-8

58 Griffiths, E. A. *et al.* Pharmacological targeting of beta-catenin in normal karyotype acute myeloid leukemia blasts. *Haematologica* **100**, e49-52 (2015). https://doi.org:10.3324/haematol.2014.113118

59 Sheng, Y. *et al.* FOXM1 regulates leukemia stem cell quiescence and survival in MLL-rearranged AML. *Nat Commun* **11**, 928 (2020). https://doi.org:10.1038/s41467-020-14590-9

60 Nair, R. R. *et al.* Pyrvinium Pamoate Use in a B cell Acute Lymphoblastic Leukemia Model of the Bone Tumor Microenvironment. *Pharm Res* **37**, 43 (2020). https://doi.org:10.1007/s11095-020-2767-4

61 Wander, P. *et al.* High-throughput drug screening reveals Pyrvinium pamoate as effective candidate against pediatric MLL-rearranged acute myeloid leukemia. *Transl Oncol* **14**, 101048 (2021). https://doi.org:10.1016/j.tranon.2021.101048

62 Fu, Y. H. *et al.* Deciphering the Role of Pyrvinium Pamoate in the Generation of Integrated Stress Response and Modulation of Mitochondrial Function in Myeloid Leukemia Cells through Transcriptome Analysis. *Biomedicines* **9** (2021). https://doi.org:10.3390/biomedicines9121869

63 Bhattacharya, D., Rothstein, M., Azambuja, A. P. & Simoes-Costa, M. Control of neural crest multipotency by Wnt signaling and the Lin28/let-7 axis. *Elife* **7** (2018). https://doi.org:10.7554/eLife.40556

64 Wang, L. *et al.* NANOG and LIN28 dramatically improve human cell reprogramming by modulating LIN41 and canonical WNT activities. *Biol Open* **8** (2019). https://doi.org:10.1242/bio.047225

65 Zuo, Q. *et al.* BMP4 activates the Wnt-Lin28A-Blimp1-Wnt pathway to promote primordial germ cell formation via altering H3K4me2. *J Cell Sci* **134** (2021). https://doi.org:10.1242/jcs.249375

66 Ling, R. *et al.* Lin28/microRNA-let-7a promotes metastasis under circumstances of hyperactive Wnt signaling in esophageal squamous cell carcinoma. *Mol Med Rep* **17**, 5265-5271 (2018). https://doi.org:10.3892/mmr.2018.8548

67 Luo, Y. *et al.* The osteogenic differentiation of human adipose-derived stem cells is regulated through the let-7i-3p/LEF1/beta-catenin axis under cyclic strain. *Stem Cell Res Ther* **10**, 339 (2019). https://doi.org:10.1186/s13287-019-1470-z

68 Zhang, Z., Florez, S., Gutierrez-Hartmann, A., Martin, J. F. & Amendt, B. A. MicroRNAs regulate pituitary development, and microRNA 26b specifically targets lymphoid enhancer factor 1 (Lef-1), which modulates pituitary transcription factor 1 (Pit-1) expression. *J Biol Chem* **285**, 34718-34728 (2010). https://doi.org:10.1074/jbc.M110.126441

69 Eliason, S., Sharp, T., Sweat, M., Sweat, Y. Y. & Amendt, B. A. Ectodermal Organ Development Is Regulated by a microRNA-26b-Lef-1-Wnt Signaling Axis. *Front Physiol* **11**, 780 (2020). https://doi.org:10.3389/fphys.2020.00780

70 Liang, J. *et al.* LEF1 Targeting EMT in Prostate Cancer Invasion Is Regulated by miR-34a. *Molecular cancer research : MCR* **13**, 681-688 (2015). https://doi.org:10.1158/1541-7786.MCR-14-0503

71 Rodriguez-Ubreva, J. *et al.* C/EBPa-mediated activation of microRNAs 34a and 223 inhibits Lef1 expression to achieve efficient reprogramming into

macrophages. *Mol Cell Biol* **34**, 1145-1157 (2014). https://doi.org:10.1128/MCB.01487-13

72 Liu, X. *et al.* MicroRNA-34a Attenuates Metastasis and Chemoresistance of Bladder Cancer Cells by Targeting the TCF1/LEF1 Axis. *Cell Physiol Biochem* **48**, 87-98 (2018). https://doi.org:10.1159/000491665

73 Wang, X. *et al.* MiR-34a-5p Inhibits Proliferation, Migration, Invasion and Epithelial-mesenchymal Transition in Esophageal Squamous Cell Carcinoma by Targeting LEF1 and Inactivation of the Hippo-YAP1/TAZ Signaling Pathway. *J Cancer* **11**, 3072-3081 (2020). https://doi.org:10.7150/jca.39861

74 Wang, L., Xie, Y., Chen, W., Zhang, Y. & Zeng, Y. miR-34a Regulates Lipid Droplet Deposition in 3T3-L1 and C2C12 Cells by Targeting LEF1. *Cells* **12** (2022). https://doi.org:10.3390/cells12010167

75 Liang, J. *et al.* LEF1 targeting EMT in prostate cancer invasion is mediated by miR-181a. *Am J Cancer Res* **5**, 1124-1132 (2015).

76 Liu, Y. *et al.* MiR-218 reverses high invasiveness of glioblastoma cells by targeting the oncogenic transcription factor LEF1. *Oncol Rep* **28**, 1013-1021 (2012). https://doi.org:10.3892/or.2012.1902

77 Min, R. Q. & Ma, Q. MicroRNA-381 inhibits metastasis and epithelial-mesenchymal transition of glioblastoma cells through targeting LEF1. *Eur Rev Med Pharmacol Sci* **24**, 6825-6833 (2020). https://doi.org:10.26355/eurrev_202006_21672

78 Qiu, J. *et al.* MiR-557 works as a tumor suppressor in human lung cancers by negatively regulating LEF1 expression. *Tumour Biol* **39**, 1010428317709467 (2017). https://doi.org:10.1177/1010428317709467

79 Chen, X., Tu, J., Liu, C., Wang, L. & Yuan, X. MicroRNA-621 functions as a metastasis suppressor in colorectal cancer by directly targeting LEF1 and suppressing Wnt/beta-catenin signaling. *Life Sci* **308**, 120941 (2022). https://doi.org:10.1016/j.lfs.2022.120941

80 Okeyo-Owuor, T. *et al.* The efficiency of murine MLL-ENL-driven leukemia initiation changes with age and peaks during neonatal development. *Blood Adv* **3**, 2388-2399 (2019). https://doi.org:10.1182/bloodadvances.2019000554

81 Li, Y. *et al.* LIN28B promotes differentiation of fully transformed AML cells but is dispensable for fetal leukemia suppression. *Leukemia* **38**, 648-651 (2024). https://doi.org:10.1038/s41375-024-02167-0

82 Zhou, J. *et al.* Inhibition of LIN28B impairs leukemia cell growth and metabolism in acute myeloid leukemia. *J Hematol Oncol* **10**, 138 (2017). https://doi.org:10.1186/s13045-017-0507-y

83 Zhou, J. *et al.* LIN28B Activation by PRL-3 Promotes Leukemogenesis and a Stem Cell-like Transcriptional Program in AML. *Molecular cancer research : MCR* **15**, 294-303 (2017). https://doi.org:10.1158/1541-7786.MCR-16-0275-T

84 Zhou, M. *et al.* RNA-Binding Protein Lin28B Promotes Chronic Myeloid Leukemia Blast Crisis by Transcriptionally Upregulating miR-181d. *Molecular cancer research : MCR* **22**, 932-942 (2024). https://doi.org:10.1158/1541-7786.MCR-23-0928

85 Helsmoortel, H. H. *et al.* LIN28B overexpression defines a novel fetal-like subgroup of juvenile myelomonocytic leukemia. *Blood* **127**, 1163-1172 (2016). https://doi.org:10.1182/blood-2015-09-667808

86 Lai, J. *et al.* LIN28B hypomethylation drives oncogenic signaling and stratifies poor prognosis in juvenile myelomonocytic leukemia. *Transl Pediatr* **14**, 1541-1552 (2025). https://doi.org:10.21037/tp-2025-228

87 Chen, J. Y. *et al.* C1632 suppresses the migration and proliferation of non-small-cell lung cancer cells involving LIN28 and FGFR1 pathway. *J Cell Mol Med* **26**, 422-435 (2022). https://doi.org:10.1111/jcmm.17094

88 Chen, Y. *et al.* LIN28/let-7/PD-L1 Pathway as a Target for Cancer Immunotherapy. *Cancer Immunol Res* **7**, 487-497 (2019). https://doi.org:10.1158/2326-6066.CIR-18-0331

89 Zhang, Q. *et al.* C1632 inhibits ovarian cancer cell growth and migration by inhibiting LIN28 B/let-7/FAK signaling pathway and FAK phosphorylation. *Eur J Pharmacol* **956**, 175935 (2023). https://doi.org:10.1016/j.ejphar.2023.175935

90 Chen, H., Sa, G., Li, L., He, S. & Wu, T. In vitro and in vivo synergistic anti-tumor effect of LIN28 inhibitor and metformin in oral squamous cell carcinoma. *Eur J Pharmacol* **891**, 173757 (2021). https://doi.org:10.1016/j.ejphar.2020.173757

91 Xu, J. *et al.* Development of miRNA-based PROTACs targeting Lin28 for breast cancer therapy. *Sci Adv* **10**, eadp0334 (2024). https://doi.org:10.1126/sciadv.adp0334

92 Borgelt, L. *et al.* N-Biphenyl Pyrrolinones and Dibenzofurans as RNA-Binding Protein LIN28 Inhibitors Disrupting the LIN28-Let-7 Interaction. *ACS Med Chem Lett* **14**, 1707-1715 (2023). https://doi.org:10.1021/acsmedchemlett.3c00341

93 Borgelt, L. *et al.* Spirocyclic Chromenopyrazole Inhibitors Disrupting the Interaction between the RNA-Binding Protein LIN28 and Let-7. *Chembiochem* **24**, e202300168 (2023). https://doi.org:10.1002/cbic.202300168

94 Hommen, P. *et al.* Chromenopyrazole-Peptide Conjugates as Small-Molecule Based Inhibitors Disrupting the Protein-RNA Interaction of LIN28-let-7. *Chembiochem* **24**, e202300376 (2023). https://doi.org:10.1002/cbic.202300376

95 Radaeva, M. *et al.* Discovery of Novel Lin28 Inhibitors to Suppress Cancer Cell Stemness. *Cancers (Basel)* **14** (2022). https://doi.org:10.3390/cancers14225687

96 Matias-Barrios, V. M. *et al.* Developing novel Lin28 inhibitors by computer aided drug design. *Cell Death Discov* **11**, 5 (2025). https://doi.org:10.1038/s41420-024-02281-z

97 Schultz, C. W. & Nevler, A. Pyrvinium Pamoate: Past, Present, and Future as an Anti-Cancer Drug. *Biomedicines* **10** (2022). https://doi.org:10.3390/biomedicines10123249

Figure legends

Figure 1. (A) RBP binding motif analysis from transcripts commonly and significantly enriched from β-catenin-RIP-seq performed in K562 and HEL cells.[28] **(B)** RNA expression profiles from the Leukeamia MILE study on BloodSpot showing *LIN28B* mRNA expression across healthy bone marrow and numerous subtypes of human myeloid and lymphoid leukaemias. **(C)** Immunoblots showing β-catenin and LIN28B (long and short isoforms) expression across 18 myeloid leukaemia cell lines. GAPDH was used as a loading control. Immunoblots show β-catenin level in LIN28B Co-IPs from K562 and HEL whole cell lysates following overnight ± 5µM CHIR99021 treatment. **(D)** Immunoblots showing β-catenin levels in LIN28B Co-IPs from K562 and HEL whole-cell lysates following overnight treatment ± 5μM CHIR99021, without RNase. **(E)** Same as in **(D)**, performed with RNase A treatment (20μg/mL). **(F)** Flow-cytometric contour plot demonstrating the purity of CD34$^+$ HSCs isolated from human fetal liver. **(G)** Detection of β-catenin protein in LIN28B Co-IPs from human fetal liver CD34$^+$ HSCs. ID: immunodepleted lysate.

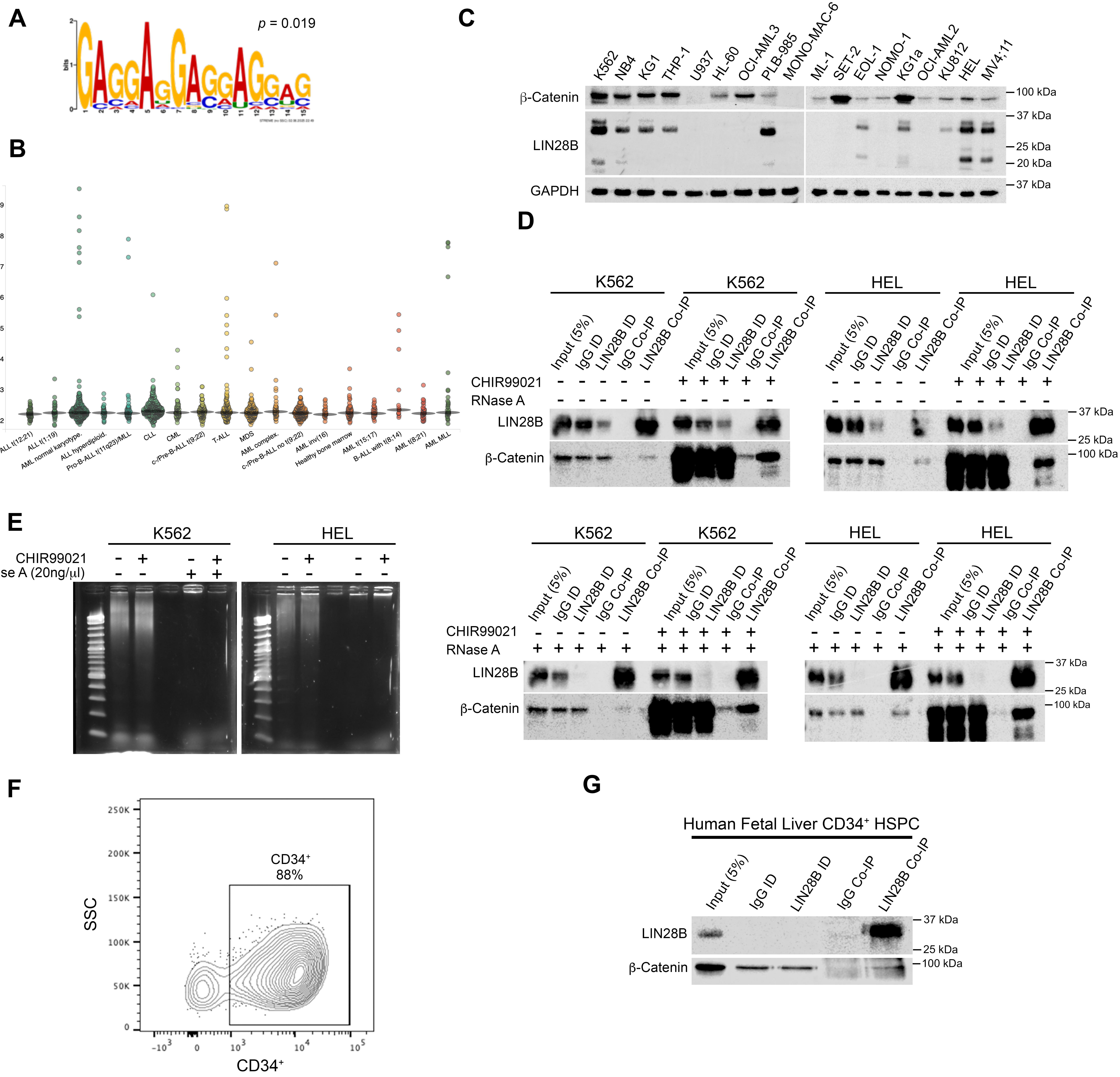
Figure 2. (A) Immunoblots showing LIN28B expression in K562 cells harbouring the BAR (β-catenin–activated reporter) or fuBAR (found unresponsive BAR; negative control) system with ± LIN28B shRNA. **(B)** Representative flow-cytometric histograms showing Venus Yellow Fluorescent Protein (YFP) intensity, reflecting TCF-dependent activity, in K562 cells with ± LIN28B shRNA following overnight treatment ± 5μM CHIR99021. The dashed histogram represents the fuBAR control, while the grey-filled histogram corresponds to non-targeting control shRNA. Blue and red histograms represent two LIN28B shRNAs. **(C)** Summary bar graph showing YFP MFI from the BAR/fuBAR system in K562 cells with ± LIN28B shRNA following overnight treatment ± 5μM CHIR99021. **D)** Immunoblot showing LIN28B and LEF-1 protein expression in K562 cells transduced with full-length human LIN28B cDNA (pLV-hLIN28B) versus an empty-vector (EV) control with GAPDH used as a loading control. **(E)** Summary bar graph showing YFP MFI from the BAR/fuBAR system in K562 cells ± pLV-hLIN28B following overnight treatment ± 5μM CHIR99021. **(F)** Immunoblots showing expression of LIN28B, β-catenin, LEF-1, and TCF4 in K562 cells with ± LIN28B shRNA. GAPDH was used as a loading control. **(G)** Immunoblots showing β-catenin, LEF-1, and TCF4 protein expression in cytoplasmic (C) and nuclear (N) fractions of K562 cells with ± LIN28B shRNA following ± 5μM CHIR99021. Lamin A/C and α-tubulin were used as fraction purity and loading controls. **(H)** Scatter plot showing fold changes in *LIN28B*, *LEF1*, *TCF7*, and *TCF7L2* mRNA levels in K562 cells with ± LIN28B shRNA, normalized to *GAPDH*. Summary graphs showing the fold change of immature *LEF1* mRNA levels assessed using primers spanning intronic regions of the *LEF1* gene in **(I)** K562 cells after overnight treatment with ± 5μM CHIR99021, and in **(J)** K562 cells with ± LIN28B shRNA. Data are presented as mean ± SD from three independent biological replicates. Statistical significance was assessed using Student's t-test (*$p < 0.05$).

Figure 3. (A) Immunoblots show β-catenin and LIN28B expression levels in K562 cells transduced with NT, β-catenin, or LIN28B shRNA. GAPDH was used as a loading control. **(B)** A heatmap derived from scaled and normalized miRNA expression values in K562 cells with NT, β-catenin, or LIN28B shRNA is shown. The top dendrograms illustrate clustering of samples by replicate and condition. Volcano plots display significantly upregulated or downregulated miRNAs in K562 cells comparing **(C)** LIN28B shRNA vs. NT shRNA and **(D)** β-catenin shRNA vs. NT shRNA. Multiple testing correction was performed using the Benjamini–Hochberg method, and adjusted *p*-values are reported (*p*-adj). Red dots indicate significantly upregulated miRNAs ($\log_2$ fold change > 1 and *p*-adj ≤ 0.05), whereas blue dots denote significantly downregulated miRNAs ($\log_2$ fold change < –1 and *p*-adj ≤ 0.05). The highlighted points represent the top 10 miRNAs in each comparison ranked by log2 fold change. Venn diagrams highlighting miRNAs commonly **(E)** upregulated or **(F)** downregulated in response to β-catenin or LIN28B shRNA in K562 cells. Venn diagrams highlighting potential antagonistically regulated miRNAs that are **(G)** downregulated by β-catenin shRNA but upregulated with LIN28B shRNA, and **(H)** upregulated by β-catenin shRNA but downregulated with LIN28B shRNA.

Figure 4. (A) Immunoblots showing the levels of LEF-1 protein in K562 and HEL cells following overnight treatment with 10μM or 100μM C1632. DMSO was used as a vehicle control. GAPDH served as a loading control. Bar graphs summarizing the fold change in *LEF1* mRNA levels in K562 and HEL cells after overnight treatment ± C1632 (10μM, 100μM) using primers to **(B)** exonic or (**C**) intronic regions, normalized to *GAPDH*. **(D)** Summary bar graphs showing MFI generated from BAR/fuBAR system in K562 and HEL cells following overnight treatment ± C1632 (10μM, 100μM) and ± 5μM CHIR99021. Data are presented as mean ± SD (standard deviation) from three independent biological replicates. Statistical significance was assessed using Student's t-test (*$p < 0.05$; **$p < 0.01$; **$p < 0.005$; ns: $p > 0.05$).

Figure 5. (A) Immunoblots showing the expression levels of β-catenin protein in HEL and THP1 cells with ± β-catenin shRNA. GAPDH served as a loading control. Bar graphs display the percentage of live cells in **(B)** HEL and **(C)** THP-1 cells with ± β-catenin shRNA following treatment with ± C1632 (10μM, 100μM) for 24, 48 and 72 hours. Data are presented as mean ± SD from three independent biological replicates. Statistical significance was assessed using Student's t-test (*$p < 0.05$; **$p < 0.01$; ns: $p > 0.05$).

Figure 6. (A) Immunoblots showing the expression level of β-catenin protein in HEL cells following overnight treatment with different concentrations of PP. **(B)** Immunoblots showing LIN28B protein expression level in HEL and THP-1 cells with ± LIN28B shRNA. GAPDH served as a loading control. **(C)** Bar graphs display the percentage of live cells in HEL cells with ± LIN28B shRNA following treatment with ± 5μM PP for 24, 48 and 72 hours. **(D)** Bar graphs display the percentage of live cells in HEL cells after treatment with DMSO, 100μM C1632, 5μM PP and 100μM C1632 & 5μM PP for 24, 48 and 72 hours. Data are presented as mean ± SD from three independent biological replicates. Statistical significance was assessed using Student's t-test (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; ns: non-significant).


A
p = 0.019
GAGGAGGAGGAGGAG
bits
B
Log₂ LIN28B mRNA Expression
ALL t(12;21)
ALL t(1;19)
AML normal karyotype.
ALL hyperdiploid.
Pro-B-ALL t(11q23)/MLL
CLL
CML
c-/Pre-B-ALL t(9;22)
T-ALL
MDS
AML complex.
c-/Pre-B-ALL no t(9;22)
AML inv(16)
Healthy bone marrow
AML t(15;17)
B-ALL with t(8;14)
AML t(8;21)
AML MLL
C
K562
NB4
KG1
THP-1
U937
HL-60
OCI-AML3
PLB-985
MONO-MAC-6
ML-1
SET-2
EOL-1
NOMO-1
KG1a
OCI-AML2
KU812
HEL
MV4;11
β-Catenin
LIN28B
GAPDH
100 kDa
37 kDa
25 kDa
20 kDa
37 kDa
D
K562
K562
HEL
HEL
Input (5%)
IgG ID
LIN28B ID
IgG Co-IP
LIN28B Co-IP
CHIR99021
RNase A
LIN28B
β-Catenin
37 kDa
25 kDa
100 kDa
E
K562
HEL
CHIR99021
RNase A (20ng/μl)
F
SSC
CD34⁺
CD34⁺
88%
250K
200K
150K
100K
50K
0
-10³
0
10³
10⁴
10⁵
G
Human Fetal Liver CD34⁺ HSPC
Input (5%)
IgG ID
LIN28B ID
IgG Co-IP
LIN28B Co-IP
LIN28B
β-Catenin
37 kDa
25 kDa
100 kDa

A
K562
BAR
fuBAR
LIN28B
GAPDH
37 kDa
25 kDa
37 kDa
LIN28B shRNA #1 - + - - + -
LIN28B shRNA #2 - - + - - +
B
= fuBAR
= BAR/Cont shRNA
= BAR/LIN28B shRNA #1
= BAR/LIN28B shRNA #2
K562
Normalised % events
Venus YFP
C
K562
NT-shRNA
LIN28B shRNA#1
LIN28B shRNA#2
Venus YFP MFI
BAR
fuBAR
D
K562
BAR
fuBAR
LIN28B
LEF1
GAPDH
37 kDa
25 kDa
50 kDa
37 kDa
pLV-EV + - + -
pLV-hLIN28B - + - +
E
K562
pLV-EV
pLV-hLIN28B
Venus YFP MFI
BAR
fuBAR
F
K562
LIN28B
β-Catenin
LEF-1
TCF-4
GAPDH
37 kDa
25 kDa
100 kDa
50 kDa
75 kDa
50 kDa
37 kDa
LIN28B shRNA #1 - + -
LIN28B shRNA #2 - - +
G
K562
DMSO
CHIR99021
C N C N C N
β-Catenin
TCF-4
LEF-1
Lamin A/C
α-Tubulin
100 kDa
75 kDa
50 kDa
50 kDa
75 kDa
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LIN28B shRNA#1 - - + + - - - - + + - -
LIN28B shRNA#2 - - - - + + - - - - + +
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K562
NT-shRNA
LIN28B-shRNA#1
LIN28B-shRNA#2
Fold Change (relative to NT-shRNA)
LIN28B
LEF1
TCF7L2
I
K562
LEF1 Intronic Primers
Fold Change (relative to DMSO)
DMSO
CHIR99021
J
K562
LEF1 Intronic Primers
Fold Change (relative to NT-shRNA)
NT-shRNA
LIN28B-shRNA#1
LIN28B-shRNA#2

A
K562
β-Catenin
LIN28B
GAPDH
100 kDa
37 kDa
25 kDa
37 kDa
β-Catenin shRNA − + −
LIN28B shRNA − − +
B
replicate
condition
Z-score
2
1
0
-1
-2
replicate
1
2
3
condition
β-Catenin_sh
NT_sh
LIN28B_sh
LIN28B_sh_Rep1
LIN28B_sh_Rep2
LIN28B_sh_Rep3
NT_sh_Rep1
NT_sh_Rep2
NT_sh_Rep3
β-Catenin_sh_Rep2
β-Catenin_sh_Rep1
β-Catenin_sh_Rep3
-Log10(padj)
Log2 Fold Change
C
LIN28B vs NT shRNA
hsa-let-7d-5p
hsa-let-7i-5p
hsa-miR-98-5p
hsa-miR-218-5p
hsa-let-7f-1-3p
hsa-miR-516b-5p
hsa-miR-3689b-5p
hsa-miR-523-3p
hsa-miR-202-5p
hsa-miR-3689a-5p
hsa-miR-3689e
hsa-miR-222-3p
hsa-miR-520h
hsa-miR-4772-3p
hsa-miR-4772-5p
hsa-miR-135a-3p
hsa-miR-6503-3p
hsa-miR-4704-5p
hsa-miR-4704-3p
hsa-miR-222-5p
125
100
75
50
25
0
-2.5
0.0
2.5
5.0
D
β-Catenin vs NT shRNA
hsa-miR-100-5p
hsa-miR-181c-5p
hsa-miR-181d-5p
hsa-miR-518b
hsa-miR-517-5p
hsa-miR-193b-3p
hsa-miR-516b-5p
hsa-miR-210-3p
hsa-miR-181c-3p
hsa-miR-518a-3p
hsa-miR-520a-5p
hsa-miR-935
hsa-miR-410-3p
hsa-miR-520h
hsa-miR-1270
hsa-miR-520e-3p
hsa-miR-363-3p
hsa-miR-193a-3p
hsa-miR-5189-3p
hsa-miR-373-3p
20
15
10
5
0
-5.0
-2.5
0.0
2.5
E
Upregulated miRNAs
β-Catenin shRNA
LIN28B shRNA
7
71
miR-1301-3p
miR-488-3p
miR-935
miR-193a-3p
miR-100-5p
miR-100-5p
miR-373-3p
miR-449b-5p
F
Downregulated miRNAs
β-Catenin shRNA
LIN28B shRNA
39
28
miR-4772-3p
miR-3681-5p
G
Downregulated in β-catenin shRNA &
Upregulated in LIN28B shRNA
β-Catenin shRNA
LIN28B shRNA
20
57
miR-3689a-5p
miR-516b-5p
miR-516a-5p
miR-517a-3p
miR-512-3p
miR-518a-3p
miR-520e-3p
miR-520c-3p
miR-498-5p
miR-517-5p
miR-517b-3p
miR-518b
miR-520f-3p
miR-3689e
miR-520h
miR-520a-5p
miR-520g-3p
miR-519d-3p
miR-944
miR-523-3p
miR-3689b-5p
H
Upregulated in β-catenin shRNA &
Downregulated in LIN28B shRNA
β-Catenin shRNA
LIN28B shRNA
14
0
30

A
C1632
K562
HEL
DMSO
10 µM
100 µM
DMSO
10 µM
100 µM
LEF-1
GAPDH
50 kDa
37 kDa
B
K562
LEF1
HEL
LEF1
Fold Change
(relative to DMSO)
2
1
-2
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2
1
-2
-4
-8
ns
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DMSO
10 µM
100 µM
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K562
LEF1 Intronic Primers
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LEF1 Intronic Primers
Fold Change
(relative to DMSO)
1.0
0.5
0.0
-0.5
-1.0
DMSO
10 µM
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K562
HEL
● DMSO
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▲ 100 µM C1632
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40000
20000
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ns
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BAR
fuBAR

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HEL
THP-1
β-Catenin
GAPDH
100 kDa
37 kDa
β-Catenin shRNA#1 − + −
β-Catenin shRNA#2 − − +
B
HEL
24 Hours
48 Hours
72 Hours
Live Cells (%)
0
20
40
60
80
100
NT-shRNA
β-Cat sh#1
β-Cat sh#2
**
*
*
ns
● DMSO
■ 10 μM C1632
▲ 100 μM C1632
C
THP-1
24 Hours
48 Hours
72 Hours
Live Cells (%)
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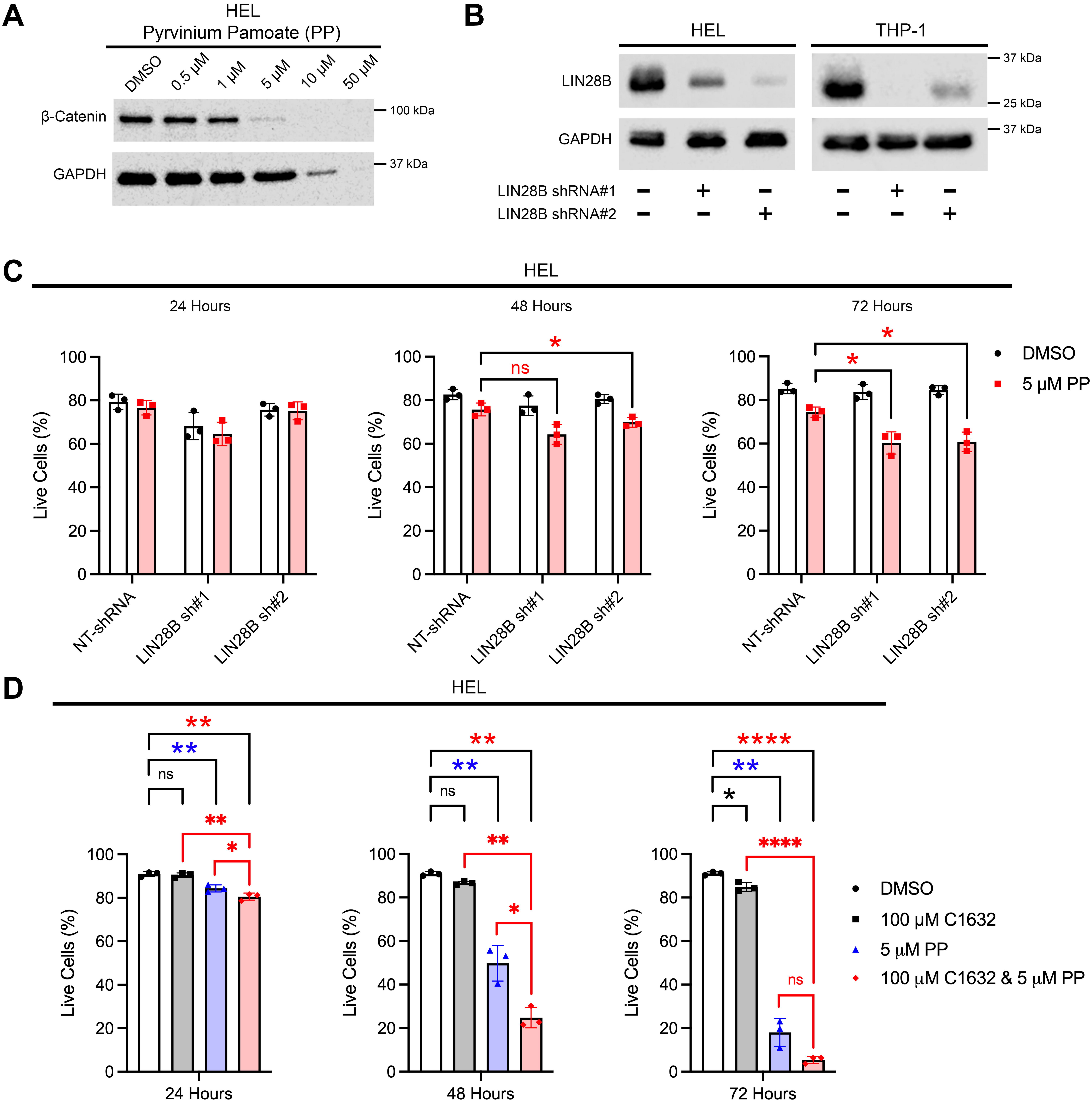
**
● DMSO
■ 10 μM C1632
▲ 100 μM C1632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DMSO
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1 μM
5 μM
10 μM
50 μM
β-Catenin
GAPDH
100 kDa
37 kDa
B
HEL
THP-1
LIN28B
GAPDH
37 kDa
25 kDa
37 kDa
LIN28B shRNA#1 − + − − + −
LIN28B shRNA#2 − − + − − +
C
HEL
24 Hours
48 Hours
72 Hours
Live Cells (%)
0
20
40
60
80
100
NT-shRNA
LIN28B sh#1
LIN28B sh#2
ns
*
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DMSO
5 μM PP
D
HEL
Live Cells (%)
24 Hours
48 Hours
72 Hours
ns
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DMSO
100 μM C1632
5 μM PP
100 μM C1632 & 5 μM PP