SUPPLEMENTARY METHODS

Flow cytometry

For detection of intracellular proteins and cell cycle analysis, cells were incubated with cell surface markers before incubation with anti-phosphoprotein antibodies using the PerFix EXPOSE kit (Beckman Coulter) or with Ki67, isotype control and DAPI (Sigma-Aldrich) as previously described (1). Analysis and cell sorting were performed using FACS LSR Fortessa or Gallios and FACS ARIAII or ARIA SORP and data were analyzed using Diva, Flow Jow or Kalusa softwares.

Antibodies used for flow cytometry are listed below.

Reactivity	Conjugate	Clone	Supplier
Anti-mouse CD45	APC eFluor780	30-F11	eBioscience
Anti-mouse CD45	AF700	30-F11	BD Biosciences
Anti-human CD11b	PE	Bear1	Beckman–Coulter
Anti-human CD14	APC H7	M0P9	BD Biosciences
Anti-human CD33	FITC	HIM3-4	BD Biosciences
Anti-human CD33	FITC	D3HL60.251	Beckman–Coulter
Anti-human CD34	PC7	581	Beckman–Coulter
Anti-human CD34	PC7	581	BD Biosciences
Anti-human CD38	eFluor450	HB7	eBioscience
Anti-human CD38	PE	LS198-4-3	Beckman–Coulter
Anti-human CD41	PE	P2	Beckman–Coulter
Anti-human CD41	Alexa Fluor700	MEM-06	ExBio
Anti-human CD45	APC-Cy7	2D1	BD Biosciences
Anti-human CD45	PE	HI30	BD Biosciences
Anti-human CD45	KrOr	J.33	Beckman–Coulter
Anti-human CD45	V450	HI30	BD Biosciences
Anti-human CD45RA	APC-Alexa Fluor 750	2H4	Beckman–Coulter
Anti-human CD90	Pacific Blue	Thy-1/310	Beckman–Coulter
Anti-human CD117	PC5.5	104D2D1	Beckman–Coulter
Anti-human CD123	FITC	7G3	BD Biosciences
Anti-human CD123	PC5.5	SSDCLY107D2	Beckman–Coulter
Anti-human JAM-C	APC	208212	R&D System
Anti-human Ki67	FITC	35	BD Biosciences
Anti-human phospho-SFK (Tyr416)	purified		Cell Signaling

Anti-human phospho-ERK1/2 (Thr202/tyr204)	purified	Cell Signaling
Anti-Rabbit	AlexaFluor 594	Jackson immunoresearch
DRAQ7	Alexa Fluor700	biostatus
Fixable viability	eFluor506	eBioscience

Cumulative relapse incidence

The cumulative relapse incidence (CRI) was defined as the duration from the date of complete remission to the date of relapse or death or last follow-up or allograft stem cell transplantation in first complete remission, by considering the death as a competing risk of the relapse. The CRI curves were estimated by the Prentice method and compared using the Gray test.

Mass spectrometry

20.10^6 cells were solubilized in 500ml of lysis buffer (50 mM HEPES pH 7.3, 10% glycerol, 0.1 mM EDTA, 150 mM NaCl, 1% Triton X100 and protease inhibitors). Cell lysates were pre-cleared and incubated with protein G beads coupled to 4G10 anti-Phosphotyrosine antibody. The beads were washed 5 times in lysis buffer, boiled in Laemmli buffer, and proteins were loaded on acrylamide gels. To visualize proteins by silver staining, 10% of the denatured protein extracts were loaded in a 4-12% Bis-Tris gradient pre-cast NuPAGE™ gel and run with MOPS buffer according to the manufacturer's instructions (Invitrogen). For mass spectrometry analysis, 90% of the denatured protein extracts were loaded in a 4-12% Bis-Tris acrylamide gel. Protein-containing bands were stained with Imperial Blue (Thermo Scientific), cut from gel at the expected size (i.e. 80kDa+/-5kDa), and following reduction and iodoacetamide alkylation, digested with high sequencing grade trypsin (Promega). The extracted peptides were further concentrated before analysis. Mass spectrometry analysis was conducted by liquid chromatography-tandem mass spectrometry (LC-MSMS) using a LTQ-Velos-Orbitrap (Thermo Scientific) online with a nanoLC RSLC Ultimate 3000 chromatography system (Dionex). Five microliters corresponding to $1/5^{th}$ of the whole sample was injected into the system in triplicate. After pre-concentrating and washing the sample on a Dionex Acclaim PepMap 100 C18 column (2 cm × 100 µm i.d., 100 Å, 5 µm particle size), the peptides were separated on a Dionex Acclaim PepMap

RSLC C18 column (15 cm × 75 µm i.d., 100 Å, 2 µm particle size) at a flow rate of 300 nL/min with a two-step linear gradient (4-20% acetonitrile/H_2O; 0.1% formic acid for 90 min and 20-45-45% acetonitrile/H_2O; 0.1% formic acid for 30 min). For peptide ionization using the nanospray source, the spray voltage was set at 1.4 kV, and the capillary temperature was 275°C. All of the samples were measured in data-dependent acquisition mode. Each run was preceded by a blank MS run to monitor system background. The peptide masses were measured using a full scan survey (scan range of 300-1700 m/z, with 30 K FWHM resolution at m/z=400, target AGC value of 1.00×10^6 and maximum injection time of 500 ms). In parallel to the high-resolution full scan in Orbitrap, the data-dependent CID scans of the 10 most intense precursor ions were fragmented and measured in the linear ion trap (normalized collision energy of 35%, activation time of 10 ms, target AGC value of 1.00×10^4, maximum injection time of 100 ms, isolation window of 2 Da). Parent masses obtained in the Orbitrap analyzer were automatically calibrated using a locked mass of 445.1200. The fragment ion masses were measured in the linear ion trap to obtain the maximum sensitivity and the maximum amount of MS/MS data. Dynamic exclusion was implemented with a repeat count of 1 and exclusion duration of 30 s. Raw files (triplicates) generated from mass spectrometry analysis were processed with Proteome Discoverer 1.4 (ThermoFisher Scientific). This software was used to search the data using an in-house Mascot server (version 2.4.1, Matrix Science Inc.) against the human subset of the SwissProt database. Database searches were performed using the following settings: a maximum of two trypsin miscleavages allowed, methionine oxidation and N-terminal protein acetylation as variable modifications, and cysteine carbamido-methylation as a fixed modification. A peptide mass tolerance of 6 ppm and fragment mass tolerance of 0.8 Da were used for the search analysis. Only peptides with high stringency Mascot score threshold (identity, FDR < 1%) were used for protein identification.

References

1. Coppin E, De Grandis M, Pandolfi PP, Arcangeli ML, Aurrand-Lions M, Nunes JA. Dok1 and Dok2 Proteins Regulate Cell Cycle in Hematopoietic Stem and Progenitor Cells. J Immunol. 2016 May 15;196(10):4110-21.