

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection <https://www.cbioportal.org/> (version 3.4.1); SigProfilerSingleSample (version 1.3); Bambino (version 1.6); Strelka (version 2.8.2); CONCERTING (version 1.0); CREST (version 1.0); NGS: illumina Hiseq X10; Sequencing: ABI3730; ddPCR: BioRad QX200; IC50: BioTek Synergy; Flow cytometry: BD FACS Canto Plus; Western Blot: LI-COR Odyssey; Microscopy: Leica SP8

Data analysis <https://pecan.stjude.cloud/>; Bowtie2 (version 2.2.6); MACS2 (version 2.1.0); deeptools (version 3.4.3); R package pheatmap (version 1.0.12); QuantaSoft (version 1.7.4); Image J (version 1.53a); Microsoft Excel (version 16.0.4266.1001); GraphPad Prism (version 8.0.1); FlowJo X (version 10.5.3); LAS AF Lite (version 1.0.0); SPSS (version 25.0.0)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

There was no active patient recruitment or prospective clinical trial data for the present study, but rather reanalysis of existing datasets, and clinical and genomics data were obtained as follows - Trials registered at ClinicalTrials.gov: AALL0232 (NCT00075725), AALL0331 (NCT00103285), ALL-BFM-2000 (NCT00430118), COALL 07-03 (NCT00343369), ALL-REZ BFM 2002 (NCT00114348). Trials registered at the Chinese Clinical Trial Registry: CCCG-ALL-2015 (ChiCTR-IPR-14005706). Trials not registered at ClinicalTrials.gov or at an equivalent agency (initiated before ClinicalTrials.gov release in 2000): XH-ALL-99 (China), SCMC-ALL-2005/2009 (China),

CCLG-ALL-2008 (China), ALL-BFM 95 (Germany), COALL 06-97 (Germany), ALL-REZ BFM 95/96 (Germany). TARGET is a cooperative collaboration, initiated in 2006, between NCI, the Children's Oncology Group, University of Colorado Cancer Center, University of New Mexico Cancer Center, and St. Jude Children's Research Hospital, as described here: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000218.v23.p8. TARGET raw sequencing data are available through dbGaP using accession phs000218. Obtaining access to TARGET data requires an Authorized Access request on the dbGaP web portal, which is reviewed by the NCI Data Access Committee, to ensure protection of patient data. Data from "TARGET" datasets needs controlled access to ensure patient data protection, and requests to access these datasets should be directed to "dbGaP web portal" and access will be supervised by "the NCI Data Access Committee" ethics/data access committee. PCGP raw data were originally deposited on EGA (EGAD00001000261, EGAD00001001864, EGAD00001000260, EGAD00001000161, EGAD00001000259, EGAD00001000164, EGAD00001000160, EGAD00001000163, EGAD00001000268, EGAD00001000162, EGAD00001000085, EGAD00001000165, EGAD00001000135, EGAD00001000159) and reanalyzed using bam files and somatic VCF files obtained from St. Jude Cloud Genomics Platform (<https://platform.stjude.cloud/data/cohort#>) by selecting the study "Pediatric Cancer Genome Project (PCGP)." PCGP data raw data are available on St. Jude Cloud at www.stjude.cloud under controlled access to protect patient data, and data access requests are reviewed by the PCGP Steering Committee. TCGA/PCAWG SNV and indel data were obtained through the ICGC Data Portal at <https://dcc.icgc.org/> by using the "Advanced Search" option, selecting "PCAWG" for "Study", "WGS" for "Donor Analysis Type", and "SSM" (SNVs and indels) for "Available Data Type", and data are available without controlled access, no accession number is needed. TP53 mutation data in different cancer types were downloaded from Cbioportal. ChIP-seq data was submitted to Genome Sequence Archive (GSA), with accession code HRA000386. All other data that support the findings of this study are available from the corresponding author upon request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size has been stated in the figure legends. This was chosen based on previous experience with similar experiments in the literature. We used minimal numbers of animals, which could still allow for generation of statistically meaningful data. No statistical methods were used to predetermine sample size. For mutational signature analysis, acute lymphoblastic leukemia samples were chosen based on the availability of matched diagnosis and relapse samples, and availability of whole-genome sequencing data from previous studies.
Data exclusions	For mutational signature analysis, samples with low signature reconstruction cosine similarity (below 0.85) were excluded as noted in the manuscript. Exclusion criteria were not pre-established.
Replication	All experimental findings in this study have been reproduced at least three times and sometimes even more. In the repeated experiments, similar results were obtained. Use of statistical methods have been described in relevant figure legends. All attempts at replication were successful.
Randomization	For experiments involving mice, randomization of mice was performed. For example, when we prepare 3 different cell lines for xenotransplantation models, we used a total of 30 mice. Therefore, we will randomly divide these 30 mice into 3 groups, each with 10 mice, which are used to inject 3 different cell lines. Mice that were used in all experiments were age and sex-matched where possible. For cell line experiments, samples were grouped based on the genotypes. For experiments other than those involving mice, samples were grouped based on genotypes and do not involve randomization.
Blinding	This study does not require investigators to be blinded to group allocation during data collection and/or analysis. Samples were analyzed with the same protocol by different investigators. The data collection was not subjective.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Western blots were analyzed with the following antibodies: mouse anti-actin (1:1,000; sc8432, Santa Cruz Biotechnology), mouse anti-p53 (1:2,000; DO1, ab1101, Abcam), rabbit anti-p53-phosphoS15 (1:2,000; #9284, Cell Signaling Technology), rabbit anti-p53-phosphoS20 (1:2,000; ab157454, Abcam), rabbit anti-γH2AX-phosphoS139 (1:5,000; 2212-1, Epitomics Biotechnology), rabbit anti-H2AX (1:5,000; #7631, Cell Signaling Technology), rabbit anti-CHK2-phosphoT68 (1:1,000; #2661, Cell Signaling Technology), rabbit anti-CHK1-phosphoS345 (1:1,000; #2348, Cell Signaling Technology), rabbit anti-CHK1 (1:1,000; #2360, Cell Signaling Technology), rabbit anti-CHK2 (1:5,000; 3428-1, Epitomics Biotechnology), rabbit anti-p21 (1:1,000; 3733-1, Epitomics Biotechnology), rabbit anti-PARP (1:1,000; #9542, Cell Signaling Technology), rabbit anti-PUMA (1:1,000; #98672, Cell Signaling Technology) rabbit anti-MSH2 (1:1,000; 15520-1-AP, Proteintech Group, Inc).
Validation	All the antibodies are commercial available. The specificity were validated by the manufactures as indicated on their websites. MSH2 antibody: western blot of MCF10A cells. All the other antibodies were used for western blots of Nalm6 and Reh cells. All the antibodies are suitable for human species reactivity.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Nalm6 was purchased from DSMZ. Reh and MCF10A were purchased from ATCC.
Authentication	All cell lines were identified by STR authentication, and kept at low passages to maintain their identity.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	NOD/Shi-scid/IL-2R ^γ null (NOG) mice (6–8 weeks) were grown under specific pathogen free (SPF) condition. All animal experiments were performed using age-matched female mouse littermates.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiment protocols were approved by the Medical Ethical Committee of Shanghai Jiao Tong University and Shanghai Children's Medical Center (SCMC)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	In SCMC-ALL-2005/2009, the 1085 evaluable patients ranged from 3 months to 18 years of age (median 4.83 years). There were 667 boys and 418 girls, yielding a gender ratio of 1.6:1. The immunophenotype: 966 B-cell precursor and 119 T-cell. The median leukocyte count was 7.1e9/L (range 0.1e9 to 908.0e9/L). 17.1% t(12;21)(ETV6-RUNX1) positive, 4.4% t(1;19)(TCF3-PBX1) positive, 3.5% t(9;22)(BCR-ABL1) positive, 2.3% t(4;11)(KMT2A-AFF1) positive. In the ALL-REZ BFM cohort (564 pediatric patients with first relapse of ALL), age ranged from 3 weeks to 22 years (median 5.38 years). The cohort included 340 boys and 224 girls (male-to-female ratio 1.5:1). Immunophenotype distribution: 482 B-cell precursor ALL relapses and 82 T-lineage ALL relapses. Genetic ALL subtype distribution: 14.9% t(12;21)(ETV6-RUNX1) positive, 2.1% t(9;22)(BCR-ABL1) positive and 1.6% t(4;11)(KMT2A-AFF1) positive cases. In the PCGP ALL cohort, ALL diagnosis ages ranged from 1 week to 22.8 years (median 6.2). Ages were unknown for 6 patients. The male-to-female ratio was 1.33:1. In the TARGET ALL cohort, ALL diagnosis ages ranged from 1.1 year to 30.0 years (median 7.3). Age and gender were unknown for 3 patients. The male-to-female ratio was 1.16:1.
Recruitment	There was no active patient recruitment or prospective clinical trial data for the present study, but rather reanalysis of existing datasets from different trials and institutions. From 2005 to 2014, 1085 evaluable patients with ALL aged 1 to 18 years old were enrolled and treated using SCMC-ALL-2005/2009 risk-stratified protocol. Mature B-cell leukemia patients were not included in this study. The diagnosis of ALL was based on morphologic evaluation of bone marrow smears in combination with immunophenotyping using panels of monoclonal antibodies directed toward lineage-associated antigens.

The ALL-REZ BFM cohort (pediatric relapsed ALL, total n = 564) included 531 patients selected from 793 evaluable German patients with first relapse of ALL from the ALL-REZ BFM 2002 trial and 33 patients selected from 731 evaluable German patients with first relapse of ALL from the ALL-REZ BFM 95/96 trial. Selection criteria included bone marrow blast count (extramedullary relapses excluded), immunophenotype (mature B-cell leukemia excluded) and sample availability. Patients from the ALL-REZ BFM 95/96 trial were additionally selected for T-lineage ALL. The selection criteria do not affect the results of the present study.

PCGP samples were obtained from a variety of institutions, including St. Jude Children's Research Hospital and COG member institutions. PCGP samples were previously analyzed as part of multiple studies analyzing individual ALL subtypes, including <https://www.nature.com/articles/ng.3691>, <https://www.nature.com/articles/ng.3230>, <https://www.nejm.org/doi/full/10.1056/NEJMoa1403088>, and <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3267575/>, among others.

In the TARGET ALL cohort, patients were analyzed from multiple institutions. TARGET is a cooperative collaboration, initiated in 2006, between NCI, the Children's Oncology Group, University of Colorado Cancer Center, University of New Mexico Cancer Center, and St. Jude Children's Research Hospital, as described here: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000218.v22.p8.

Ethics oversight

The SCMC-ALL-2005/2009 trial was approved by the SCMC institutional review board, and informed written consent was obtained from each patient's guardian. The study protocols were approved by the Medical Ethical Committee of Shanghai Jiao Tong University and Shanghai Children's Medical Center (SCMC).

The ALL-REZ BFM 95/96 and ALL-REZ BFM 2002 trials were approved by the institutional review board of the Charité-Virchow Medical Center, Medical faculty of the Humboldt-University of Berlin, Germany. Written informed consent to human research was obtained from patients or guardians.

PCGP genomic analysis was approved by the institutional review board at St. Jude Children's Research Hospital. Written informed consent was provided by a parent or guardian of each child or by a patient who was 18 years of age or older.

Genomic sequencing and informed consent of TARGET samples was coordinated by the NCI along with multiple institutions, and IRB approval is not required for analysis of the data (please see https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000218.v22.p8).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

Trials registered at ClinicalTrials.gov: AALL0232 (NCT00075725), AALL0331 (NCT00103285), ALL-BFM-2000 (NCT00430118), COALL 07-03 (NCT00343369), ALL-REZ BFM 2002 (NCT00114348).

Trials registered at the Chinese Clinical Trial Registry: CCCG-ALL-2015 (ChiCTR-IPR-14005706).

Trials not registered at ClinicalTrials.gov or at an equivalent agency (initiated before ClinicalTrials.gov release in 2000): XH-ALL-99 (China), SCMC-ALL-2005/2009 (China), CCLG-ALL-2008 (China), ALL-BFM 95 (Germany), COALL 06-97 (Germany), ALL-REZ BFM 95/96 (Germany).

Study protocol

There was no active patient recruitment or prospective clinical trial data for the present study, but rather reanalysis of existing datasets. Please refer to the methods part of the manuscript for the references of clinical trial protocol.

For the German trials ALL-BFM-2000, COALL 06-97, COALL 07-03, ALL-REZ BFM 2002 and ALL-REZ BFM 95/96, full trial protocols can be requested either from the study chair (see contacts information at ClinicalTrials.gov) or from the corresponding authors of publications referenced in the manuscript or from the German Society for Pediatric Oncology (GPOH – Geschäftsstelle, Chausseestraße 128/129, DE-10115 Berlin, Mail: info@kinderkrebsinfo.de)

For the Chinese trials SCMC-ALL-2005, SCMC-ALL-2009, CCLG-ALL-2008 and CCCG-ALL-2015 full trial protocols can be requested from the corresponding authors of publications referenced in the manuscript or from caijiaoyang@scmc.com.cn.

For the American trials AALL0232 and AALL0331, full trial protocols can be requested either from the study chair (see contacts information at ClinicalTrials.gov) or from the corresponding authors of publications referenced in the manuscript.

Data collection

There was no active patient recruitment or prospective clinical trial data for the present study, but rather reanalysis of existing datasets. Please refer to the methods part of the manuscript for the detailed information.

For the German ALL-REZ BFM cohort (pediatric relapsed ALL), data used for the present study was retrieved from the central clinical databases of the consecutive ALL-REZ BFM 95/96 and ALL-REZ BFM 2002 trials held at the Charité Berlin, Germany, in July 2019. Both trials were multicenter randomized phase III trials for relapsed pediatric ALL conducted in Germany. The ALL-REZ BFM 95/96 trial recruited patients from July 1995 until Dec 2001 (including a pilot phase from July 1995 until Jan 1997). The ALL-REZ BFM 2002 trial recruited from Jan 2002 until July 2012 (including a pilot phase from Jan 2002 until July 2003).

For the Chinese cohorts, the Pediatric Oncology Network Database (POND; <http://www.pond4kids.org>), which is an online pediatric oncology database developed by St. Jude Children's Research Hospital, was used to save and retrieve data for patients treated with the SCMC-ALL-2005 protocol. For the CCCG-ALL-2015 study, the National Children's Medical Center Leukemia Registry and Management System was used to save and retrieve patient data. The SCMC-ALL-2005/2009 trial recruited patients from May 2005

until Dec 2014. The SCMC-ALL-2015 trial recruited from Jan 2015 until Dec 2019.

For the previously published cohorts from TP53 studies of Yu et al., Stengel et al. and Wada et al. data used in the present study was retrieved from the publications referenced in the manuscript.

Outcomes

Outcome analysis was performed for the German ALL-REZ BFM cohort only. The primary outcome measure used in the present study was response of relapsed ALL patients to ALL-REZ BFM 95/06 and ALL-REZ BFM 2002 treatment. The trial protocols defined response as achievement of a second complete remission. Complete remission was assessed by conventional bone marrow cytology and defined as regenerating bone marrow with less than 5% leukemic blasts, peripheral blood without blasts and with evidence of regeneration, and absence of extramedullary leukemia manifestation.

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

ChIP-seq data was submitted to Genome Sequence Archive (GSA), the accession code is HRA000386.
Shared URL: <https://bigd.big.ac.cn/gsa-human/s/XRoalO00>

Files in database submission

WT.GRCh37.md.bam
R110H.GRCh37.md.bam
R158C.GRCh37.md.bam
R158H.GRCh37.md.bam
R175H.GRCh37.md.bam
R196G.GRCh37.md.bam
G245R.GRCh37.md.bam
R248P.GRCh37.md.bam
R248Q.GRCh37.md.bam
R249S.GRCh37.md.bam
R267Q.GRCh37.md.bam
R267W.GRCh37.md.bam
R273C.GRCh37.md.bam
R282P.GRCh37.md.bam
R337C.GRCh37.md.bam

Genome browser session (e.g. [UCSC](#))

No longer applicable

Methodology

Replicates

ChIP-seq for p53 were processed at least three times of biological replicates.

Sequencing depth

For each ChIP-seq sample, at least 20M raw reads of PE150 were sequenced.

Antibodies

The antibody used in ChIP-seq assay was mouse anti-p53 monoclonal antibody DO-1, purchased from Abcam, cat No. ab1101.

Peak calling parameters

macs2 callpeak -f BAMPE -g hs -B

Data quality

Fastqc and fastqc_screen were used for data quality control.

Software

bwa aln; macs2

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

For flow cytometry assay, cells were collected and washed with pre-cooling FACS buffer (1xPBS buffer with 2.5% FBS (v/v)).

Sample preparation	Then resuspended cells with diluted fluorescence-conjugated antibodies in FACS buffer and incubated at room temperature for 10 minutes. Washed the cells with FACS buffer and resuspended in 100-200 microliter FACS buffer for flow cytometry.
Instrument	Samples were acquired on a FACSCanto™ II Cell Analyzer (Becton Dickinson); Samples were sorted on a MoFlo XDP Cell Sorter (Beckman Coulter)
Software	FACSCanto™ II was used to collect the flow cytometry data. FlowJo X 10.0.7R2 was used to analyze the flow cytometry data.
Cell population abundance	Due to the low abundance of the relevant cell populations, the abundance of the populations in the post-sort fraction was not assessed.
Gating strategy	Cell apoptosis analysis: all cells were gated and assessed by staining PI and AnnexinV-APC, we gated on AnnexinV positive and PI negative cells as apoptotic cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.