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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

The sample size was determined by our experience that the injected mice develop leukemia in 30 days with a standard deviation of 10 days. If the time to occurrence of leukemia is extended to 40 days with a power of 90% and a significance level of 5%, then the difference to be detected is 10 days and the group size n=1 +21(10/10)^2=22; animals in each group for transplantation with MLL-AF9, DHFR-WDR5 and DHFR-WDR5 mutant respectively (3x22=66). These mice were divided into two groups, with 11 mice in each group, for TMS treated and untreated. The number of animals, (N=11) per experimental condition has been chosen to provide a sufficient number of mice to develop statistically significant survival curves (to time of euthanasia)(Reference: Dmitry Borkin et al., Pharmacologic Inhibition of the Menin-MLL Interaction Blocks Progression of MLL Leukemia In Vivo, 2015).

2. Data exclusions

Describe any data exclusions.

No data was excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Experimental findings were reliably reproduced.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

40 of 4-6 week old female C57BL/6 mice were randomly grouped after lethal irradiation (900rads) with each group (MAF9-S4, MAF9-S4mut and MAF9) containing 12 mice. The remaining 4 mice were using as control for lethal irradiation. The lethally irradiated mice were transplanted through tail vein intravenous injection with about 5X106 MAF9 cells, MAF9-S4 and MAF9-S4mut cells for each group. This experiment was repeated one more time.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Single blinding was used for the animal experiments. The experimental performers were unaware of the subject.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Phenix v1.9-1692, Coot v0.8.2, MOLREP v11.0.05, FlowJo v9.9.6, Prism v7, CCP4 v7.0, PROCHECK v3.5, Molprobity v4.4, Image Lab v5.2.1,PyMol v1.7.2.3. All are from respective third-party developers.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	All unique materials generated and used in this manuscript will be readily available from the authors.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Rabbit polyclonal anti-Ash2L antibody (Supplier Name: Bethyl, Catalog No. A300-489A; 20 citations provided on the manufacturer's website) Rabbit polyclonal anti-RbBP5 antibody (Supplier Name: Bethyl, Catalog No. A300-109A; 87 citations provided on the manufacturer's website) Rabbit polyclonal anti-Mll1 antibody (Supplier Name: Bethyl, Catalog No. A300-374A; 19 citations provided on the manufacturer's website) Anti-mouse APC-c-Kit antibody (Supplier Name: Biolegend, Catalog No. 105812, clone No. 2B8, lot No. B170596; 28 citations provided on the manufacturer's website) Anti-mouse APC-Gr-1 antibody (Supplier Name: Biolegend, Catalog No. 108412, clone No. RB6-8C5, lot No. B215461; 67 citations provided on the manufacturer's website) Anti-mouse FITC Sca-1 antibody (Supplier Name: Biolegend, Catalog No. 108105, clone No. D7, lot No. B138326; 20 citations provided on the manufacturer's website) Anti-mouse/human Pacific Blue CD11b antibody (Supplier Name: Biolegend, Catalog No. 101224, clone No. M1/70, lot No. B196387; 70 citations provided on the manufacturer's website).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Cell line prepared by transducting mouse bone marrow derived cells with MLL-AF9 fusion protein was used in the study. This cell line was further transduced with retroviral vectors encoding our monobody inhibitor.

b. Describe the method of cell line authentication used.

Stable cell line expresses mCherry protein constitutively and was used as a marker for cell line authentication.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cell lines were tested to be free of mycoplasma contamination using MycoAlert PLUS mycoplasma detection kit (Lonza, catalog number LT07-710). In addition, all media were supplemented with Gibco's antibiotic-antimycotic (Catalog number: 15240062)

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

80 4-6 week old female C57BL/6 mice (weight mean 22.28g with s.d. 1.07g) were purchased from Charles River laboratories and the Unit for Laboratory Animal Medicine (ULAM) of University of Michigan provided husbandry and housing for these mice. The mice used in the study were specific pathogen free. Immortalized MLL-AF9 leukemia cells that expressing DHFR fused with WDR5-targeting monobody or monobody mutant under the control of the TMS-inducible promoter (5 million cells) with the volume of 100ul were injected into tail vein of the lethally irradiated mouse, which is expected to develop leukemia within 4-6 weeks after transplantation. Immediately after irradiation, the mice were fed with 0.2mg/mL of enrofloxacin (Baytril) water for 1 week to prevent irradiation caused infection. The water supply was monitored every 3-4 days. Mice were closely monitored daily for sign of illness (e.g. reduced motility, hind limb paralysis, pale paws, hunched back, reduced weight), which will be end point of the study. About two weeks after transplantation the animals were randomized and treated with TMS via oral gavages at 20mg/20g twice daily for 10 days. ULAM provided this oral gavages service. Body weight was monitored daily during the treatment and at least 3 times a week after treatment. Peripheral blood (20 to 40 ul per sample) was checked twice a week by Fluorescence Activated Cell Sorting (FACS) to detect leukemia progression by assessment of GFP+ cells, increase in white blood cell count, and presence of immature forms of blood. Animals were euthanized by carbon dioxide overdose when they became moribund as described above. Spleen, liver, hind leg bone and bone marrows were collected. Leukemia was confirmed based on flow cytometry analysis and by histopathology. Note: TMS is commonly used in rodents for infection treatment.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.