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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.

For sufficient statistical power, sample size was defined by analyzing Tn tag data available from a previous study (Sun et al. Nature 2014). No further determination was made a posteriori.

2. Data exclusions

Describe any data exclusions.

Some data were excluded based on the quality of library sequences. Samples that presented sequencing results with poor quality (based on FastQC score), DNA libraries that presented mostly sequences without transposon (>70%) and samples with less than 10% of the predicted number of Tn tags were excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Experimental findings were reliably reproduced. We did find a large variability in some of our observations, and these are indicated where appropriate.

4. Randomization

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

Weaned mice from a Tn/Tn x M2/M2 HSB/HSB cross were selected randomly and separated into male and female cages. These mice were labeled with doxycyclin and randomly chosen at different time points for BM isolation.

5. Blinding

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

No blinding was performed during data collection.

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.	Custom code required for Tn tag integration analysis has been previously published (Sun et al. Nature 2014)

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.	N/A

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All antibodies were purchased from Biolegend or BD Biosciences, and are well characterized and validated by providers. Antibodies used and their concentrations are described in methods (page 10)

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	293T cells were obtained ATCC and were used as previously described (Sun et al. Nature 2014)
b. Describe the method of cell line authentication used.	N/A
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were regularly tested for mycoplasma contamination.
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.	Cells were used mainly for the purpose of validation of the technique of Tn integration quantitation, and not for describing any physiological features.

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.	Tn and HSB mice were generated as previously described (Sun et al. Nature 2014(. Tn/Tn homozygous mice (backcrossed into the C57BL/6J background) were crossed to M2/M2 HSB/HSB homozygous mice (mixed C57BL/6J x 129/SvJ background). To induce Tn mobilization, 8-10 weeks old male or female mice with the M2/HSB/Tn genotype were fed with 2mg/ml Dox together with 5mg/ml sucrose in drinking water for 48h. Thereafter, Dox was removed and successful labelling was verified by retroorbital sinus peripheral blood collection and analysis (70 ul) after 1 week. All animal procedures were approved by the Boston Children's Hospital Institutional Animal Care and Use Committee.

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.	N/A

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Cells were prepared from whole bone marrow, lineage-depleted using quadroMACS (LS columns), and filtered (40 um, BD) before FACS.
6. Identify the instrument used for data collection.	FACSAria Ilu (BD), LSRII (BD), Astrios XP (Beckman)
7. Describe the software used to collect and analyze the flow cytometry data.	FlowJo (TreeStar)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Cells were sorted with Purity modes at 75-80% efficiency. Post sort fractions analyzed were at least 98% pure.
9. Describe the gating strategy used.	This information is included in the Methods section (page 9-10) and Supplementary Information (Figures 1-3).

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