nature research

Corresponding author(s): Bana Jabri

# 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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

## Experimental design

1. Sample size

   Describe how sample size was determined.

   The majority of experiments were repeated at least three times to obtain data for indicated statistical analyses. Mice were allocated to experimental groups on the basis of their genotype and randomized within the given sex- and age-matched group. Given that our mice were inbred and matched for age and sex, we always assumed similar variance between the different experimental groups. We did not perform an a priori sample size estimation but always used as many mice per group as possible in an attempt to minimize type I and type II errors.

2. Data exclusions

   Describe any data exclusions.

   All experimental and control animals were littermates and none were excluded from the analysis at the time of harvest.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   The majority of experiments were repeated at least three times to ensure reproducibility. Statistical analysis were done to illustrate significance. All attempts to replicate experiments were sucessful

4. Randomization

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

   Mice were allocated to experimental groups on the basis of their genotype and randomized within the given sex- and age-matched group.

5. Blinding

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

   Investigators were not blinded during experiments and outcome assessment, except for micr oscopic analysis of fluorescent immunostaining, which has been performed blinded

Note: all in vivo studies must report how sample size was determined and 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<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☐ | ☒ Test values indicating whether an effect is present<br>*Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation) |

*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. | Flowjo, GraphPad Prism, Excel, ImageJ with relevant plug-ins, Metamorph, BLASTN, For RNA-seq analysis: Trim Galore, TopHat2 software package, HTSeq, R package limma, ComBAT, 16S analysis: illumina-utils, GAST, pheatmap library for R, R package vegan (see Methods for details) |

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 third party. | No unique materials were used |

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 validated by manufacturers and in previous publications. For flow cytometry the following antibodies were used:
(clone, dilution, company, catalog number, LOT, reactivity): Gr-1 PerCP-Cy5.5 (RB6-8CA, 1:400, eBioscience, 45-5931-80, 4310362, mouse), c-Kit APC (2B8, 1:100, BD Biosciences, 553356, 7251822, mouse), CD11b PE-Cy7 (M1/70, 1:400, eBioscience, 25-0112-82, 4277098, mouse), CD34 FITC (RAM34, 1:80, eBioscience, 11-0341-85, E00265-1634, mouse), CD16/CD32 PE-Cy7 (FcγRIII/II) (93, 1:200, eBioscience, 25-0161-81, 4334851, mouse), CD19 BV605 (1D3, 1:200, BD Biosciences, 563148, 7193806, mouse), CD3 BV605 (17A2, 1:100, BD Biosciences, 564009, 6070666, mouse), B220 BV605 (RA3-6B2, 1:200, BD Biosciences, 563708, 6175527, mouse), TER-119 BV605 (TER-119, 1:200, BD Biosciences, 563323, 7061844, mouse), CD48 APC-Cy7 (HM48-1, 1:100, BD Biosciences, 561242, 7026741, mouse), CD135 BV421 (A2F10.1, 1:200, BD Biosciences, 562898, 5308961, mouse), Sca-1 PE (D7, 1:400, Biolegend, 108108, B234288, mouse), CD150 PerCP-Cy5.5 (TC15-12F12.2, 1:100, Biolegend, 115922, B220585, mouse), CD126 BV421 (IL-6Ralpha) (D7715A7, 1:50, BD Biosciences, 740038, 7310516, mouse), phospho-Stat3 PE (pY705, 1:10, BD Biosciences, 612569, 7093820, mouse/human), CD45 APC-Cy7 (30-F11, 1:200, Biolegend, 103116, B237400, mouse), F4/80 APC (BM8, 1:100, eBioscience, 17-4801-82, E029032, mouse)
For in-vivo and in-vitro neutralization the following antibody was used:
IL-6 (MP5-20F3, Bio X Cell, BE0046, 654517M2, mouse)
For immunofluorescence staining the following antibodies were used:
ZO-1 (ZO1-1A12, 1:1000, ThermoFisher Scientific, 33-9100, SA241427, mouse/human/dog/rat), Cleaved caspase 3 (Asp175, 1:500, Cell Signaling Technology, 9661S, mouse/human/rat)
See Materials & Methods for additional details.

10. Eukaryotic cell lines

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

HEKBlue-TLR2 were kindly provided by the Gajewski lab

b. Describe the method of cell line authentication used.

Cell lines were authenticated using the relevant stimulation. (TLR2 ligand Pam3CSK4 for HEKBlue-TLR2 reporter cell line)

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

Cell lines were testes negative 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.

No commonly misidentified cell line was 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 all relevant details on animals and/or animal-derived materials used in the study.

Tet2-/- mice were previously described (see REF in manuscript). Tet2f (B6;129S-Tet2tm1.1Iaai/J) mice were crossed with the hematopoietic-specific Vav-cre line (B6.Cg-Tg(Vav1-icre)A2Kio/J), the villus and crypt small and large intestinal epithelial cell-specific Villin-cre line (B6.Cg-Tg(Vil1-cre)997Gum/J), and the myeloid cell lineage-specific LysM-cre line (Lyz2tm1(cre)Ifo)), respectively. For the experiments, > 20 week (wk) old Tet2-/- mice and littermate controls were used, unless indicated otherwise. Both female and male mice were used for experiments; no notable sex-dependent differences were found for the reported experiments. Mice were housed at the University of Chicago animal facilities under specific pathogen-free (SPF) conditions, where cages were changed on a weekly basis; ventilated cages, bedding, food and water (non-acidified) were autoclaved before use, ambient temperature maintained at 23ºC, and 5% Clidox-S was used as a disinfectant. Experimental breeding cages were randomly housed on two different racks in the vivarium, and all cages were kept on automatic 12-h light/dark cycles. Germ-free (GF) Tet2-/- mice were generated by two-stage embryo transfer, as previously described 35, bred to GF C57BL/6 WT mice to generate littermate controls, and maintained in flexible film isolators in McMaster's Axenic Gnotobiotic Unit. Animal husbandry for both SPF and GF facilities, and experimental procedures were performed in accordance with Public Health Service policy and approved by the University of Chicago Institutional Animal Care and Use Committees and the McMaster University Animal Care 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.

The study did not involve human research participants

nature research

Corresponding author(s): Bana Jabri

# 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. | For flow cytometry single cell suspensions were obtained from murine peripheral blood, spleen, and bone marrow. To obtain a single cell suspension spleens and bone marrow were mashed through a 70µm cell strainer. Erylysis of spleen, bone marrow and peripheral blood was performed using the Mouse Erythrocyte Lysing Kit (R&D Systems). For analysis of splenic and bone marrow hematopoietic precursors the mouse Lineage Cell Depletion Kit (Miltenyi Biotec) was used. See Materials & Methods for further details. |
| 6. Identify the instrument used for data collection. | Flow cytometry analysis was performed with a 9-color BD FACSCanto (BD Biosciences) and Aria Fusion (BD Biosciences; for cell sorting) |
| 7. Describe the software used to collect and analyze the flow cytometry data. | FACSDiva software (BD Biosciences) was used to collect flow cytometry data FlowJo software (Treestar) was used to analyze flow cytometry data |
| 8. Describe the abundance of the relevant cell populations within post-sort fractions. | Purity of post-sorted cell fractions was >98%. Do determine purity of post-sorted cell fractions an aliquot of post-sorted cells was re-sorted. |
| 9. Describe the gating strategy used. | All samples are FSC-A and SSC-A gated, followed by Live/Dead gating to select viable cells. Subsequent relevant gating was conducted as shown in figures and described in figure legends. |

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