nature research

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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, 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.

   No statistical methods were used to predetermine sample size. To study the consequences of BRAFV600E on the behavior a cohort of mice (n=10 per group) was studied and initial analysis was performed using Log-rank and 2-way ANOVA statistical analyses. Results from these experiments indicated that the phenotype was robust (100% genotype to phenotype penetrance) and subsequent experiments were done with a sample size of 4-7 mice per group.

2. Data exclusions

   Describe any data exclusions.

   No data was excluded

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   All attempts for replication were successful.

4. Randomization

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

   BRAFWT and BRAFVE male and female littermates were assigned randomly into the control or treated group.

5. Blinding

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

   Initial scoring (score 1) of mice was performed blinded. The investigators were not blinded to allocation during following experiments and outcome assessment.

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 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 summary of the descriptive 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.	n/a

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

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.	No restrictions

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 published, and titrated prior to initial experiments.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	n/a
b. Describe the method of cell line authentication used.	n/a
c. Report whether the cell lines were tested for mycoplasma contamination.	n/a
d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	n/a

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.	Both male and female mice were used, the phenotypes observed were not depended on sex. Progeny from either C57BL/6 or F1 generation from C57BL/6 and FVB/N was used for experiments. The age ranged from embryonic stages (E10.5-E14.5) to adult stages 1 month to 9 months of age.

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 involves stainings on histological slides form brain samples from patients followed at MSKCC and Freiburg according to institutional ethical guidelines and protocols. Details on age, gender and diagnosis are given in Supplementary Table 4.

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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.	For flow cytometry experiments, organs were incubated in PBS containing 1mg/ml collagenase D (Roche), 100U/ml DNase I (Sigma), 2.4mg/ml of dispase (Invitrogen) and 3% FCS (Invitrogen) at 37ºC for 30 min prior to mechanical disruption. For embryonic tissue incubation time at 37ºC was reduced to 20 min. For cell sorting, tissues were digested for 30min at RT in PBS containing 2 mg/ml of collagenase D (Roche), 200U/ml DNase I (Sigma), 4.8 mg/ml of dispase (Invitrogen), 3% FCS (Invitrogen) and 1uM of flavopiridol (Sigma) followed by mechanical disruption under a 100um filter. Cell suspensions were centrifuged at 320g for 7 min, resuspended in FACS buffer (PBS, 0.5% BSA and 2 mm EDTA) containing purified anti-CD16/32 (1:100 dilution) and 5 % normal mouse, 5 % normal rat and 5 % normal rabbit serum and incubated for 15min at 4ºC. Samples were immunostained with antibodies mixes for 30 min at 4ºC.
6. Identify the instrument used for data collection.	ARIA III, LSRFortessa
7. Describe the software used to collect and analyze the flow cytometry data.	Data was collected using DiVa 8.0.1 Software. Subsequent analysis was performed with FlowJo_v9.9.4.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Post-sort fractions for microglia and Kupffer cells were >95%
9. Describe the gating strategy used.	FSC/SSC gates and dead cell stain (DAPI or Hoechst) were used to define single live cells. Doublets were removed based on FSC-A/FSC-W. In primary experiments all gates were defined based on FMO controls. In consecutive experiments, gates in fluorescence channels were drawn based on clear separation between negative and positive populations as shown in Supplementary Information.

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