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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	QuantaSoft (BioRad Laboratories, v1.7.4), Simplicity (Berthold Detection Systems, v4.20), Softmax Pro (MDS Analytical Technologies, v5.3), Chemo Star Professional (Intas, v4.22), FACS Diva (BD, v6.1.2), ImmunoSpot (Cellular Techology Limited, v5.2.12)
Data analysis	QuantaSoft Pro (BioRad Laboratories, v1.0.596), Prism (GraphPad software, v6.01), Excel (Microsoft Office, v2010), AIDA Array Analyzer (raytest, v4.23), FlowJo (FlowJo, v10), ImmunoSpot (Cellular Techology Limited, v6.0.0.2)

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/reviewers upon request. 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

All data generated and analysed during the study are included in the manuscript. Data supporting the findings presented in the study are available upon request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	For analysis of MCMV replication in vivo , three to four mice / time point of analysis (n=3,4) were infected for each genotype. For in vitro analysis of MCMV-luciferase replication, BMDMs of 4 mice/ genotype (n=4) were infected. For WB analysis of SAMHD1 in BMDMs, cells of 4 WT mice (n=4) were analyzed/experiment. All in vitro infection experiments using luciferase reporter virus were done in quadruplicates.
Data exclusions	For in vitro MCMV WT control infection of 3T3 shCTRL cells, analysis of d4 has been excluded due to luciferase reads being out of range.
Replication	All experiments were successfully repeated as indicated.
Randomization	Not applicable.
Blinding	Not applicable.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials (e.g. cell lines) are available from the authors.

Antibodies

Antibodies used	Rabbit polyclonal anti-pT592, ProSci, Cat# 8005 Mouse monoclonal anti-SAMHD1 (clone 3F5), novusbio, Cat# NBP2-03285 Mouse monoclonal anti-IE-1 (Chroma 101), Rijeka University Rabbit polyclonal anti-cyclin A (clone C-19), Santa Cruz Biotechnology Inc., Cat# sc-596

Mouse polyclonal anti-cyclin E (clone M-20), Santa Cruz Biotechnology Inc., Cat# sc-481
Rabbit monoclonal anti-cyclin D1 (clone EPR2241), abcam, Cat# ab134175
Mouse monoclonal anti-Hsp90 α/β, Santa Cruz Biotechnology Inc., Cat# sc-13119
Mouse monoclonal anti-HA.11 (clone 16B12), BioLegend, Cat# 901502
Rat monoclonal anti-HA (clone 3F10), Roche, Cat# 11867423001
Mouse monoclonal anti-myc (clone 9B11), Cell Signaling, Cat# 2276
Horse anti-mouse IgG, HRP-linked, Cell Signaling, Cat# 7076
Goat anti-rabbit IgG, HRP-linked, Cell Signaling, Cat# 7074
Goat polyclonal anti-mouse IgG (H&L), Dylight 488, Thermo/Pierce, Cat# 35502
Rabbit monoclonal anti-mouse IgG (Light Chain Sp.) (clone D3V2A), HRP-linked, Cell Signaling, Cat# 58802S
Mouse monoclonal anti-CD11b eFluor 450 (clone M1/70), eBioscience, Cat# 48-0112-82
Mouse monoclonal anti-F4/80 APC (clone BM8), eBioscience, Cat# 17-4801-82

Validation

Validation is available on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK 293T (Prof. Manfred Marschall, Institute of Virology, FAU Erlangen, Germany)
HEK 293T shSAMHD1, this paper
MEF (Dr. Rayk Behrendt, Institute of Immunology, TU Dresden, Germany)
MEF SAMHD1 KO (Dr. Rayk Behrendt, Institute of Immunology, TU Dresden, Germany)
MEF SAMHD1 KO p6NST56 mucoSAMHD1 Iso1, this paper
MEF SAMHD1 KO p6NST56 mucoSAMHD1 T603A, this paper
NIH-3T3 (ATCC CRL-1658)
NIH-3T3 shCTRL, this paper
NIH-3T3 shSAMHD1 4, this paper
NIH-3T3 shSAMHD1 5, this paper
NIH-3T3 shSAMHD1 5 p6NST56 vector, this paper
NIH-3T3 shSAMHD1 5 p6NST56 mucoSAMHD1 WT, this paper
NIH-3T3 shSAMHD1 5 p6NST56 mucoSAMHD1 T603A, this paper

Authentication

Cell lines produced in this paper were authenticated by immunoblot analysis.

Mycoplasma contamination

HEK 293T, HEK 293T shSAMHD1, MEF, MEF SAMHD1 KO and NIH-3T3 cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

C57BL/6NCRL mice, male and female, between the age of 8 and 14 weeks
C57BL/6NCRL SAMHD1 knockout mice, male and female, between the age of 8 and 14 weeks
C57/6NCRL IFNAR knockout mice, male and female, between the age of 8 and 14 weeks
C57BL/6NCRL SAMHD1 knockout mice, male and female, between the age of 8 and 14 weeks
C57/6NCRL SAMHD1/IFNAR knockout mice, male and female, between the age of 8 and 14 weeks

Wild animals

No wild animals were involved in this study.

Field-collected samples

No samples collected in the field were used in this study.

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

Peritoneal cells from C57BL/6NCRL mice and SAMHD1 knockout mice isolated by PBS lavage, cells were stained with F4/80 and CD11b, fixed in 2 % PFA and analyzed.

Instrument	BD LSRII
Software	FACS Diva (BD, v6.1.2), FlowJo (FlowJo,v10)
Cell population abundance	Flow cytometry analysis was performed to define the F4/80 positive peritoneal macrophage population, which was about 50 %.
Gating strategy	By gating FSC/SSC, the living cell population was defined, which was further limited to single cells. The positive peritoneal macrophage population was selected by gating F4/80 against SSC.

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