

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 our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the 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
- ☐ ☒ A statement on 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 statistical parameters 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

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Flow cytometry data were collected using MACSQuantify version 2.1. Extracellular flux data were collected using Seahorse Wave Controller version 2.6. Light microscopy was performed with Olympus cellSens Standard imaging software version 1.17

Data analysis

-PET-CT images were uploaded into Amide (www.sourceforge.com) and volumetric regions-of-interest were drawn around the tumors.
-Flow cytometry data were analyzed using FlowJo v10.7.1
-Extracellular Flux (Seahorse) Data were analyzed in Agilent Wave software version 2.6
-Nanostring data were analyzed using NACHOV1.0.1, an R package for parsing, visualization, quality control, and normalization designed for NanoString nCounter data. Principal component analysis was performed using "FactoMineRv2.3" package in R. Hierarchical clustering of genes in Nanostring studies across samples and genes using default settings with the 'seaborn' package (version v0.11.0) in Python. Based on the hierarchical clustering, we grouped genes and performed gene set enrichment analyses using gProfiler9 with Reactome gene sets compared to all genes. Differentially expressed genes between the rapamycin and vehicle treated samples for each cell type were identified using "edgeR" (version 3.28.1).
-Graphs and statistical tests were generated using GraphPad Prism 9 unless otherwise noted.

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 and reviewers. 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

The normalized mRNA transcript data from this experiment has been deposited in Geo and can be accessed at GSE165223 and has been made publicly available.

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size	Sample sizes were determined by previous experiments and were logistically limited to ~10 mice per experiment for 18F nutrient uptake assays. For smaller effect sizes such as the effect of rapamycin on FDG uptake, data multiple experiments which all independently demonstrated the same phenotypic trends were merged to achieve statistical significance.
Data exclusions	Data were excluded only for systematic quality control purposes. Tumor samples with very low viability (<15%), MC38 tumor samples contaminated with lymph node tissue as suggested by elevated B cell abundance by flow cytometry (rare), FDG uptake samples with raw CPM less than 2-standard deviations above background CPM (rare), and 2 mRNA samples which did not pass quality control were excluded from analysis. For some experiments, biological replicates were merged into a new single biological replicate to ensure sufficient cell numbers. Combined biological replicates causes some figures to have unequal samples sizes across cell populations, treatments, and/or tissues.
Replication	As noted in figure legends, experiments were independently replicated at least twice and demonstrated the same phenotypes unless noted.
Randomization	Mice were randomly assigned to drug treatments immediately prior to treatment initiation, and mice in the same cage were given different treatments.
Blinding	No blinding experiments were involved in this study due to the authors' perceived low likelihood of bias in final readouts.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Mouse and cross-reactive antibodies: Fc block (1:50, BD 553142), CD45 BV510 (1:1600, 30-F11, BioLegend 103138), B220 e450 (1:400, RA3-6B2, ThermoFisher 48-0452-82), CD11b e450 (1:1600, M1/70, ThermoFisher 48-0112-82), CD11b FITC (1:1600, M1/70, BioLegend 101206), CD8a AF488 (1:1600, 53-6.7, BioLegend 100723), CD8a BV510 (1:600, 53-6.7, BD 563068), Ly6C FITC (1:4000, HK1.4, BioLegend 128006), CD11c PE (1:1000, N418, BioLegend 117308), FOXP3 PE (1:125, FJK-16s, ThermoFisher 12-5773-82), pS6 Ser235/236 PE (1:100, D57.2.2E, Cell Signaling 5316S), CD4 PerCP-Cy5.5 (1:600, RM4-5, BioLegend 100540), Ly6G PerCP-Cy5.5 (1:800, 1A8, BioLegend 127616), F4/80 PE-Cy7 (1:800, BM8, BioLegend 123114), NKp46 PE-Cy7 (1:200, 29A1.4, BioLegend 137618), CD3 PE-Cy7 (1:200, 17A2, BioLegend
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100220), CD3 FITC (1:200, 17A2, BioLegend 100204), CD3 APC (1:200 17A2, BioLegend 100236), CD206 APC (1:500, C068C2, BioLegend 141708), GLUT1 AF647 (1:500, EPR3915, Abcam ab195020), EPCAM PE (1:1500, G8.8, BioLegend 118206), Thy1.1 PerCP-Cy5.5 (1:2000, HIS51, ThermoFisher 45-0900-82), CD45 PE (1:1600, 30-F11, ThermoFisher 12-0451-83), Ly6C BV570 (1:400, HK1.4, BioLegend 128030), CD68 BV605 (1:200, FA-11, BioLegend 137021), HK1 AF647 (1:100, EPR10134(B), Abcam ab197864), HK2 AF647 (1:200, EPR20839, Abcam EPR20839), CD71 APC (1:100, RI7217, BioLegend 113820), CD98 PE (1:400, RL388, ThermoFisher 12-0981-81), MHCII I-A/I-E APC (1:4000, M5/114.15.2, BioLegend 107614), CD103 PE-Cy7 (1:200, 2E7, BioLegend 121425), LAG3 e450 (1:100, eBioC9B7W, ThermoFisher 48-2231-82), PD1 PE (1:100, 29F-1A12, BioLegend 135206), TIM3 APC (1:100, RMT3-23, BioLegend 119706), IFN γ APC (1:250, XMG1.2, BioLegend 505810), CD25 e450 (1:500, PD61.5, ThermoFisher 48-0251-82), CD44 PE-Cy7 (1:1000, IM7, BioLegend 103030), and CD62L APC (1:200, MEL-14, ThermoFisher 17-0621-82).

Human antibodies:

CD45 BV421 (1:400, HI30, BioLegend 304032), CD3 APC (1:200, UCHT1, BioLegend 300439), CD11b PerCP-Cy5.5 (1:200, ICRF44, BioLegend 301328), CD14 BV510 (1:200, M5E2, BioLegend 301842), CA9 AF647 (1:200, 303123, R&D Systems FAB2188R-100UG), and Human Fc Block (1:50, BD 564220)

Validation

Validation information from manufacturer of each antibody:

Mouse Antibody Validation

-CD45 BV510 (1:1600, 30-F11, Biolegend 103138), Positive staining observed on C57BL/6 mouse splenocytes when staining with CD45 (clone 30-F11) Brilliant Violet 510™.

-B220 e450 (1:400, RA3-6B2, ThermoFisher 48-0452-82), This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated Staining of C57BL/6 splenocytes with Anti-Mouse CD3e FITC and 0.25 μ g and 0.25 μ g of Anti-Human/Mouse CD45R (B220) eFluor® 450 results in the expected two single positive populations.

-CD11b e450 (1:1600, M1/70, ThermoFisher 48-0112-82), Positive Staining observed on BALB/c bone marrow cells when using Anti-Mouse CD11b eFluor® 450 (Product # 48-0112-82). Lymphoid cells from the bone marrow do not demonstrate staining.

-CD11b FITC (1:1600, M1/70, Biolegend 101206), Positive staining observed when staining C57B6 bone marrow in comparison to rat IgG2b k FITC isotype control.

-CD8a AF488 (1:1600, 53-6.7, Biolegend 100723), Positive staining observed when staining C57B6 splenocytes in comparison to rat IgG2b k Alexa Fluor® 488 isotype control.

-CD8a BV510 (1:600, 53-6.7, BD 563068), Double positive staining observed when Splenic leucocytes were stained simultaneously with PE Hamster Anti-Mouse CD3e antibody and with CD8a BV510. No double positive events were observed when staining with BD Horizon™ BV510 Rat IgG2a, κ Isotype Control.

-Ly6C FITC (1:4000, HK1.4, Biolegend 128006), Positive staining observed on the myeloid population from C57BL/6 bone marrow cells.

-CD11c PE (1:1000, N418, BioLegend 117308), Double Positive staining observed when C57BL/6 mouse splenocytes were stained with APC anti-mouse I-A/I-E (clone M5/114.15.2) and PE N418 in comparison to single positive staining PE Armenian hamster IgG isotype control. This supports that N418 is staining professional antigen presenting cells.

-FOXP3 PE (1:125, FJK-16s, ThermoFisher 12-5773-82), Positive staining of only the CD4 splenocytes were seen when using the Foxp3/Transcription Factor Staining Buffer Set and protocol. FOXP3 was not detected in the CD8+ stained in the same well.

-pS6 Ser235/236 PE (1:100, D57.2.2E, Cell Signaling 5316S), Positive staining observed in Jurkats. This signal decreased with cocktail of MEK/PI3K inhibitors (LY294002, Wortmannin, and U0126).

-CD4 PerCP-Cy5.5 (1:600, RM4-5, BioLegend 100540), Double positive staining observed on C57BL/6 mouse splenocytes stained with CD3e APC and CD4 (clone RM4-5) PerCP/Cyanine5.5, in comparison to single positive staining with rat IgG2a, κ PerCP/Cyanine5.5 isotype.

-Ly6G PerCP-Cy5.5 (1:800, 1A8, BioLegend 127616), Positive staining observed with C57BL/6 bone marrow cells stained with 1A8 PerCP/Cyanine5.5.

-F4/80 PE-Cy7 (1:800, BM8, BioLegend 123114), Positive staining observed with Thioglycolate-elicited BALB/c mouse peritoneal macrophages stained with BM8 PE/Cyanine7.

-NKp46 PE-Cy7 (1:200, 29A1.4, BioLegend 137618), Double positive staining observed when C57/BL6 mouse splenocytes were stained with NK1.1 Brilliant Violet 421™ and CD335 (clone 29A1.4) PE/Cyanine7 in comparison to single staining rat IgG2a, κ isotype control PE/Cyanine7. This matches the immunophenotype of NK Cells.

-CD3 PE-Cy7 (1:200, 17A2, BioLegend 100220), Positive staining observed when C57BL/6 splenocytes stained with 17A2 PE/Cyanine7.

-CD3 FITC (1:200, 17A2, BioLegend 100204), Double positive staining observed when C57BL/6 splenocytes stained with 17A2 FITC and CD3e clone 145-2C11 PE.

-CD3 APC (1:200 17A2, BioLegend 100236), Positive staining observed when C57BL/6 mouse splenocytes were stained with CD3 (clone 17A2) APC in comparison to negative staining with rat IgG2b, κ isotype control.

-CD206 APC (1:500, C068C2, BioLegend 141708), Double positive staining observed when staining Thioglycollate-elicited BALB/c peritoneal macrophages with CD107b (Mac-3) PE, and then intracellularly stained with CD206 (clone C068C2) APC. No positive staining observed with rat IgG2a, κ APC isotype control.

-GLUT1 AF647 (1:500, EPR3915, Abcam ab195020), unconjugated antibody has been validated on WT and KO lysates from A549 lung cancer cells.

-EPCAM PE (1:1500, G8.8, BioLegend 118206), Positive staining observed when staining TE-71 (mouse thymic epithelial stromal cell line) with CD326 (clone G8.8) PE. No staining seen with rat IgG2a, κ PE isotype control.

-Thy1.1 PerCP-Cy5.5 (1:2000, HIS51, ThermoFisher 45-0900-82), Positive staining seen on rat thymocytes when stained with Anti-Mouse/Rat CD90-1 (Thy-1-1) PerCP-Cyanine5-5. No staining seen with Mouse IgG2a K Isotype Control PerCP-Cyanine5-5.

-CD45 PE (1:1600, 30-F11, ThermoFisher 12-0451-83), Single positive staining seen with staining C57BL/6 mouse bone marrow cells with TER-119 Monoclonal Antibody, FITC and CD45 Monoclonal Antibody, PE. No PE positive events seen when staining with right Rat IgG2b κ Isotype Control, PE.

-Ly6C BV570 (1:400, HK1.4, BioLegend 128030), Myeloid cells from C57BL/6 bone marrow cells demonstrated positive staining with Ly-6C (clone HK1.4) Brilliant Violet 570 in comparison to rat IgG2a, κ Brilliant Violet 570™ isotype control.

-CD68 BV605 (1:200, FA-11, BioLegend 137021), thioglycolate-elicited BALB/c macrophages demonstrated positive signal when fixed, permeabilized, and then stained with CD68. No staining was observed with rat IgG2a, κ Brilliant Violet 605™ isotype control.

-HK1 AF647 (1:100, EPR10134(B), Abcam ab197864), unconjugated antibody is knockout validated in HK1 knock out HELA cells in comparison to other cancer cell lines like A549.

-HK2 AF647 (1:200, EPR20839, Abcam EPR20839), unconjugated antibody is knockout validated in wild type and HK2 knock out HCT116s.

-CD71 APC (1:100, RI7217, BioLegend 113820), Double positive staining seen in C57BL/6 mouse bone marrow cells stained with

Ter-119 PE and CD71 (clone RI7217) APC. This is the expected staining pattern of red blood cells.

- CD98 PE (1:400, RL388, ThermoFisher 12-0981-81), The RL388 antibody has been tested by flow cytometric analysis of activated and resting mouse splenocytes, demonstrating positive staining to conjugated IgG control.
- MHCII I-A/I-E APC (1:4000, M5/114.15.2, BioLegend 107614), Positive staining observed on C57BL/6 mouse splenocytes with anti-mouse I-A/I-E. No staining seen with rat IgG2b, κ APC isotype control.
- CD103 PE-Cy7 (1:200, 2E7, BioLegend 121425), Positive staining observed on C57BL/6 mouse splenocytes were stained with CD3 APC and CD103 (clone 2E7) PE/Cyanine7 (top) in comparison to Armenian hamster PE/Cyanine7 isotype control.
- LAG3 e450 (1:100, eBioC9B7W, ThermoFisher 48-2231-82), Positive staining observed on mouse T cells activated in culture for three days with anti-CD3/CD28 in comparison to Rat IgG1 K Isotype Control eFluor® 450.
- PD1 PE (1:100, 29F-1A12, BioLegend 135206), Double positives observed with Concanavalin A and IL-2 stimulated C57BL/6 splenocytes for 3 days when stained with 29F.1A12 PE and CD3 APC.
- TIM3 APC (1:100, RMT3-23, BioLegend 119706), Positive staining observed when mouse TIM3 transfected cells were stained with this antibody (Tim-3, clone RMT3-23) in comparison to rat IgG2a, κ APC isotype control.
- IFN γ APC (1:250, XMG1.2, BioLegend 505810), Positive staining observed 6 hours after PMA/ionomycin-stimulation of C57BL/6 mouse splenocytes stained with XMG1.2.
- CD25 e450 (1:500, PD61.5, ThermoFisher 48-0251-82), This antibody was verified by Cell treatment to ensure that the antibody binds to the antigen stated. Positive staining observed on CD8⁺ splenocytes after two days of IL2 and aCD3/CD28.
- CD44 PE-Cy7 (1:1000, IM7, BioLegend 103030), Positive staining observed on C57BL/6 mouse splenocytes stained with IM7.
- CD62L APC (1:200, MEL-14, ThermoFisher 17-0621-82). Positive staining observed on C57BL/6 mouse splenocytes stained with MEL-14.

Human Antibodies:

- CD45 BV421 (1:400, HI30, BioLegend 304032), Positive staining observed when Human peripheral blood lymphocytes were stained with CD45 (clone HI30) Brilliant Violet 421™. No Positive staining seen when staining same cells with mouse IgG1, κ Brilliant Violet 421™ isotype control.
- CD3 APC (1:200, UCHT1, BioLegend 300439), Positive staining observed when staining Human peripheral blood lymphocytes stained with UCHT1 APC.
- CD11b PerCP-Cy5.5 (1:200, ICRF44, BioLegend 301328), Positive staining observed on Human peripheral blood mononuclear cells were with CD11b (clone ICRF44) PerCP/Cyanine5.5 (top). No positive events seen with mouse IgG1, kapp PerCP/Cyanine5.5 isotype control.
- CD14 BV510 (1:200, M5E2, BioLegend 301842), Positive staining observed when Human peripheral blood monocytes were stained with CD14 (clone M5E2) Brilliant Violet 510™.
- CA9 AF647 (1:200, 303123, R&D Systems FAB2188R-100UG), Antibody tested for flow cytometry on HeLa cells. s. This antibody is specific based on direct ELISAs performed by manufacturer. This antibody clone does not cross-react with recombinant mouse (rm) CA9 or with rhCA1, 2, 3, 4, 5A, 6, 7, 8, 10, 12, 13, or 14.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	MC38 and CT26 were obtained from Barbara Fingleton lab at Vanderbilt. Renca was obtained from ATCC.
Authentication	Cell lines were previously validated by STR profiling.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	None

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	C57BL/6J (000664), BALB/cJ (000651), Rag1 KO (002216) and OTI transgenic (003831) mice were obtained from the Jackson Laboratory. PyMT GEMM mice were bred by crossing male transgenic mice expressing the polyoma virus middle T antigen (PyMT) oncoprotein under the MMTV-LTR (Jackson Laboratory 022974) with wildtype females on a similar B6/FVB mixed background. The GEMM mice were from a colony in which all mice expressed two Vhl alleles in which exon 1 is flanked by loxP sites (Jackson Laboratory 012933) but did not express a Cre transgene and were thus effectively wildtype. For injectable tumor models, 8-20 week old male and female mice were used. Mice were on 12 hour light/dark cycles which coincided with daylight in Nashville, TN. The mouse housing facility was maintained at 68-76°F and 30-70% humidity.
Wild animals	None
Field-collected samples	None
Ethics oversight	Vanderbilt University Medical Center Institutional Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Samples from recently resected tumor and matched PBMCs were analyzed. Patient characteristics are provided in Supplemental Information Table 1.
Recruitment	Patients sign a separate consent allowing tissue studies to be conducted on resected tumor and PBMC. Patients were treated at Vanderbilt University Medical Center and were uniformly recruited at the time leading up to surgery. The authors do not believe any selection biases are evident in patient recruitment which would make results non-representative.
Ethics oversight	Vanderbilt University Medical Center IRB

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	Single cell suspensions obtained from tumors and spleens were incubated in Fc block for 10min at room temp, stained for surface markers for 15min at room temp, washed with FACS buffer (PBS +2% FBS) once, and resuspended in FACS buffer for analysis on a Miltenyi MACSQuant Analyzer 10 or 16. For intracellular staining, the eBioscience™ Fcγ3/transcription factor staining buffer kit (Fisher 00-5523-00) was used. For intracellular cytokine staining, tumor single cell suspensions were incubated for 4hr at 37°C 5% CO2 in supplemented RPMI with PMA (50ng/mL, Sigma Aldrich P8139-1MG), ionomycin (750ng/mL, Sigma Aldrich I0634-1MG), and GolgiPlug (1:1000, BD 555029), and processed using the BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (ThermoFisher BDB554722). Surface staining was performed as described above, cells were fix/permed for 20min at 4°C, and then stained for intracellular markers for at least 30min at 4°C. Ghost Dye Red 780 viability dye (1:4000, Cell Signaling 18452S) was used identically to surface antibodies.
Instrument	Miltenyi MACSQuantAnalyzer 10 or 16
Software	FlowJo version 10.7.1
Cell population abundance	Purity, viability, and yield of all magnetic bead sorted cells were measured by flow cytometry and trypan staining. Purity of flow-sorted cells for mRNA analysis was confirmed by transcript levels of cell identification markers.
Gating strategy	Singlets were gated using FSC-Area vs FSC-Height. Then, dead cells were excluded using viability dye 780 vs FSC. Additional low FSC / low SSC debris were excluded by gating on FSC and SSC compared to splenocytes or PBMC as highly viable lymphocyte controls. From this total live cell gate, CD45+ immune cells were then gated versus CD45- cells. CD45- cells were additionally gated on high FSC and SSC before subsequent analysis to gate on cancer cells more specifically. For more specific gates, see Extended Data Figure 5h and 8a.

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