

SI Appendix

Supplementary Figure Legends

Supplementary Figure S1. xCT supports tumor cell viability and restores xCT-deficient MC38 tumor growth. (A-B) Viability of tumor cells measured by flow cytometry following 3 days of culture. Cells were first gated on singlets. Values represent mean +SEM from four independent experiments. (C-E) MC38 WT or xCT^{-/-} (clone 2-1) tumor cells were transduced with control (LvNeg) or Slc7a11 (LvSlc7a11) expression vectors. (C) Lysates were probed with anti-xCT or anti-vinculin (loading control) antibodies. xCT levels normalized to vinculin and relative to WT LvNEG are shown below. (D) Proliferation of MC38 cells was assessed by sulforhodamine B (SRB) staining at indicated time points. Data represent mean ± SD. One representative experiment from at least two replicates is shown. (E) Kaplan-Meier survival curves of C57BL/6 mice bearing MC38 tumors. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, (A, B, D unpaired 2-tailed t test) (E log-rank test).

Supplementary Figure S2. Cystine is required for T cell proliferation in vitro. Splenic T cells were freshly isolated from WT C57BL/6 mice and stimulated with anti-CD3/anti-CD28 beads. (A-B) Surface expression of CD25 (A) and CD69 (B) 24 hours post-stimulation as measured by flow cytometry. (C) Proliferation of CFSE-stained T cells as measured by CFSE dilution 3 days post-stimulation. T cells were first gated on viable singlets. One representative experiment from at least three replicates is shown. (D) Viability of T cells 3 days post-stimulation. Cells were first gated on singlets. Values

represent mean +SEM from four independent experiments. (E) RT-qPCR for *Slc7a11* normalized to *Gapdh* and shown relative to unstimulated (Unstim.) T cells at 24 hours post-stimulation. Error bars represent SEM. ***P<0.001, ****P<0.0001 (unpaired 2-tailed t test). One representative experiment from at least three replicates is shown.

Supplementary Figure S3. Generation of xCT^{-/-} mice. (A) Schematic diagram depicting CRISPR-Cas9-mediated deletion in exon 1 of the *Slc7a11* gene. Numbered black boxes indicate exons (not to scale). (B) xCT protein level in membrane fractions of MEFs from WT or xCT^{-/-} mice as measured by mass spectrometry. (C) Cystine uptake in MEFs cultured in medium with or without 100 uM BME measured as in Fig. 1B. (D) Proliferation of MEFs was assessed by sulforhodamine B (SRB) staining at indicated time points. Data represent mean ± SD. One representative experiment from at least three replicates is shown. (E-F) Blood was collected in the presence of N-ethylmaleimide (NEM) to prevent the oxidation of cysteine. Cysteine-NEM (E) and cystine (F) concentrations were measured by mass spectrometry. Each point represents one mouse (n=5) and error bars represents SEM. ****P<0.0001, ***P<0.001, **P<0.01, ns=not significant. (B, C, E, F) unpaired 2-tailed t test. (D) Linear regression analysis.

Supplementary Figure S4. Immunophenotyping of xCT^{-/-} mice. Flow cytometry on freshly isolated thymuses (A), spleens, blood or pooled inguinal and axillary lymph nodes (B) from WT or xCT^{-/-} mice. (C) Distribution of CD62L and CD44 expressing

CD8⁺ or CD4⁺ T cells in spleen, blood and lymph nodes (LN). Error bars indicate SEM. N=5 mice/group. Gating strategy is indicated on each graph.

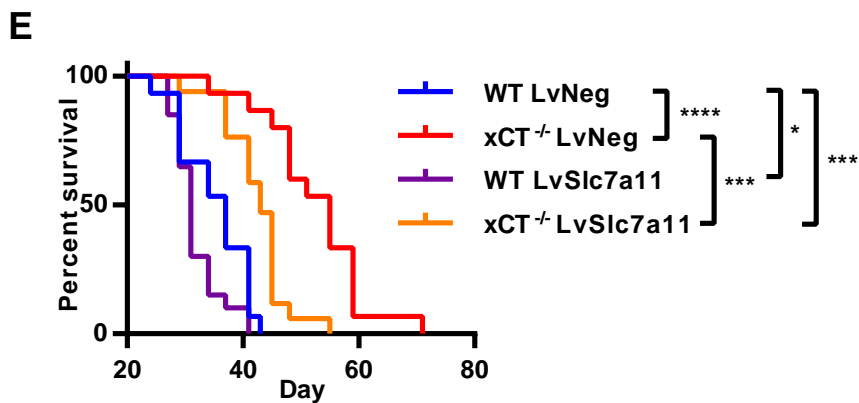
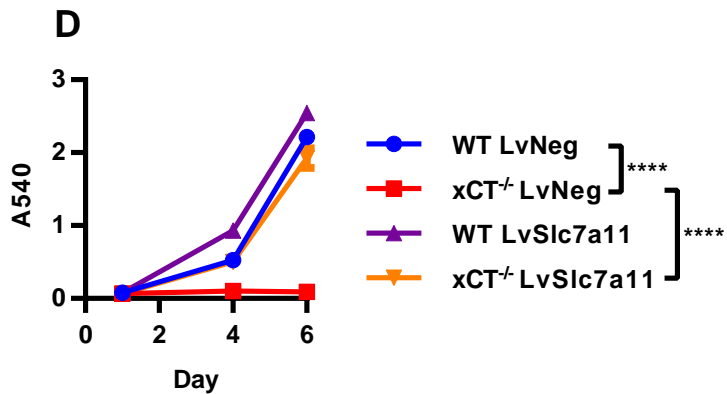
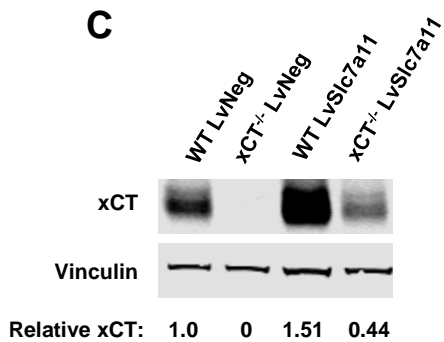
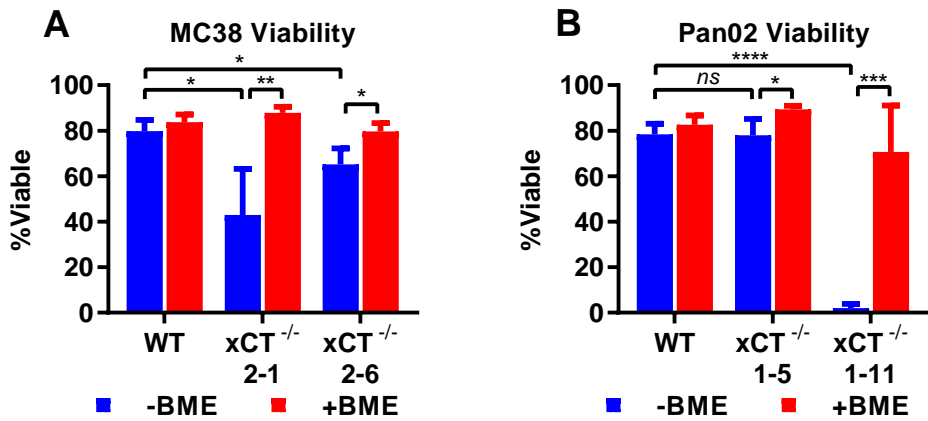
Supplementary Figure S5. xCT supports T cell viability in vitro. (A) Viability of anti-CD3/anti-CD28 stimulated T cells measured by flow cytometry following 3 days of culture. Cells were first gated on singlets. Values represent mean +SEM from four independent experiments. **P<0.01, ***P<0.0001 (unpaired 2-tailed t test).

Supplementary Figure S6. Host xCT is not required for T cell infiltration into tumors. (A) Representative images from Fig. 4A. MC38 tumors grown in WT (left panels) or xCT^{-/-} (right panels) mice. (Top panels) Brown indicates CD3⁺ cells. (Bottom panels) Brown indicates CD4⁺ cells and green indicates CD8⁺ cells.

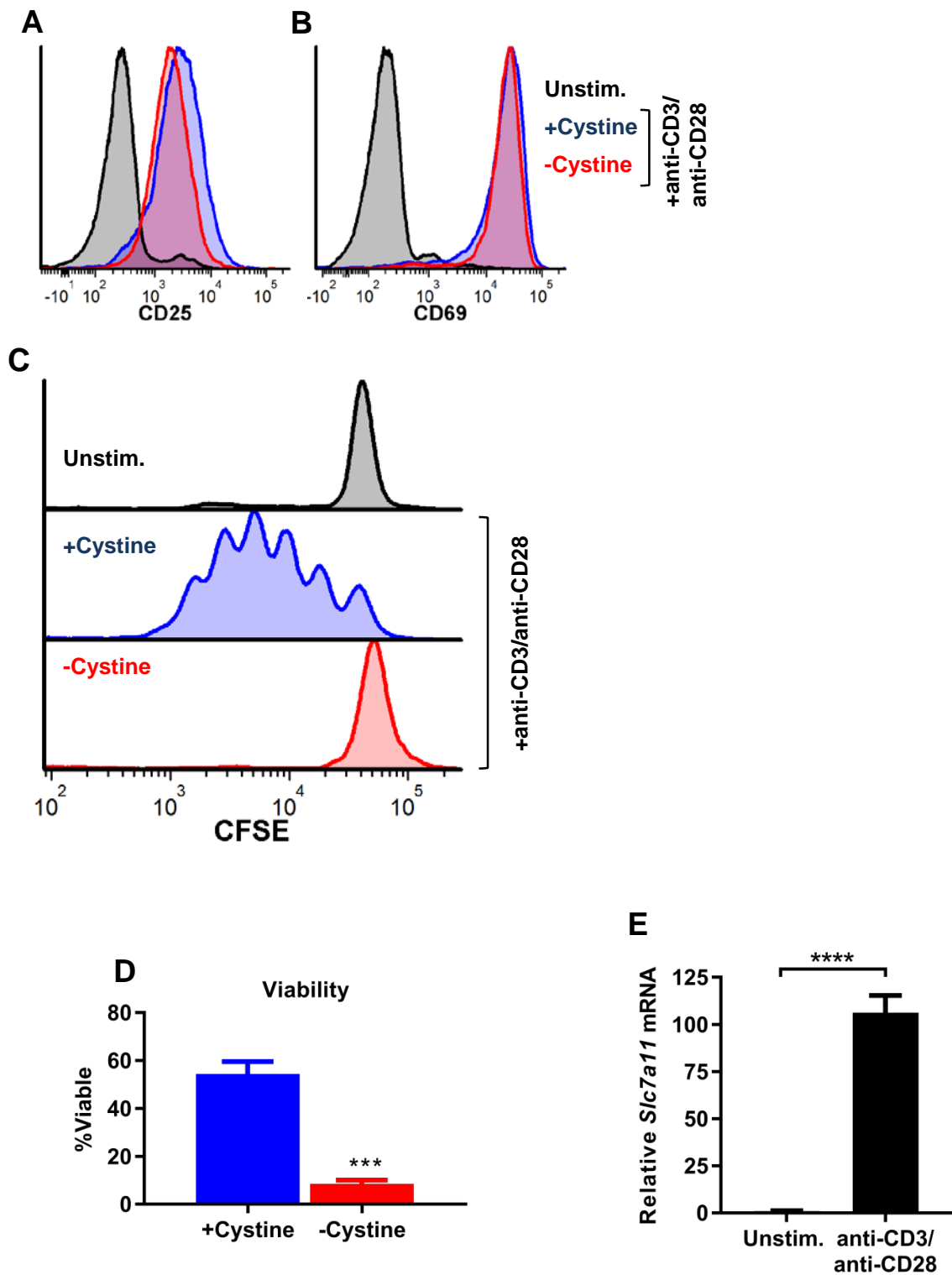
Supplementary Figure S7. xCT is not required for anti-tumor immunity. (A) Splenic T cells were isolated from WT or xCT^{-/-} OT-I mice, CFSE stained and stimulated with anti-CD3/anti-CD28 beads. Proliferation was assessed 3 days post-stimulation. T cells were first gated on viable singlets. One representative experiment from two replicates is shown. (B-C) MC38 (B) or B16OVA (C) cells were grown as xenografts in naïve WT or xCT^{-/-} mice. Growth curves of individual tumors are shown. Slope analysis was used to compare the growth rates of WT vs xCT^{-/-} tumors. (D) WT or xCT^{-/-} mice were immunized with irradiated B16OVA cells and 3 weeks later were challenged with live

B16OVA cells. Individual tumor growth curves are shown, and number of tumor-free mice is indicated below. ns= not significant (2-sided Fisher's exact test).

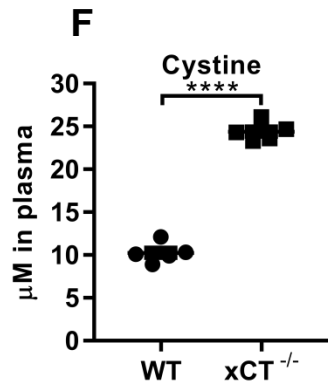
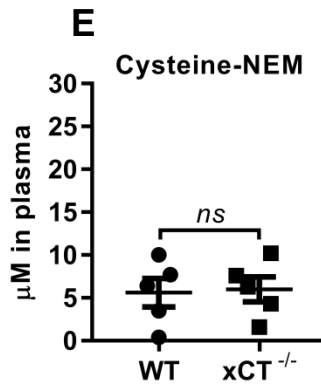
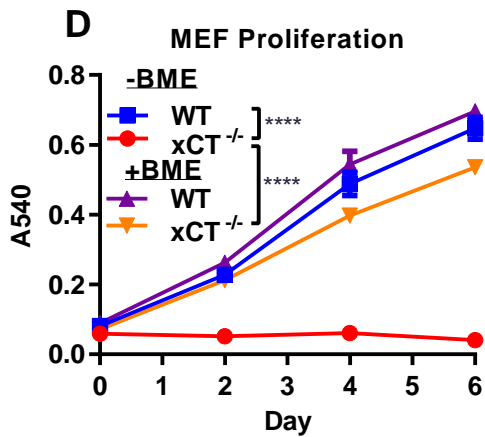
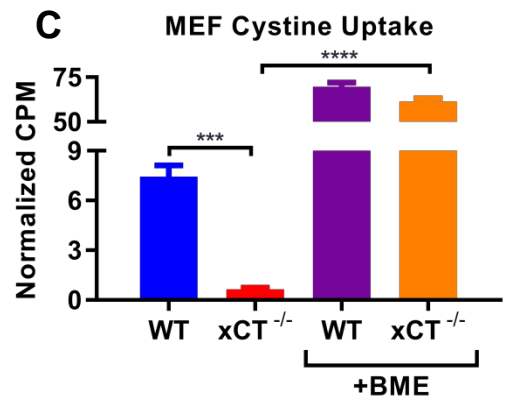
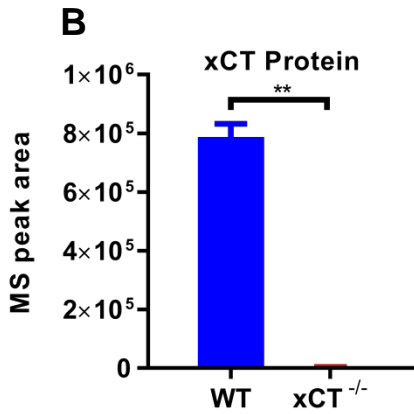
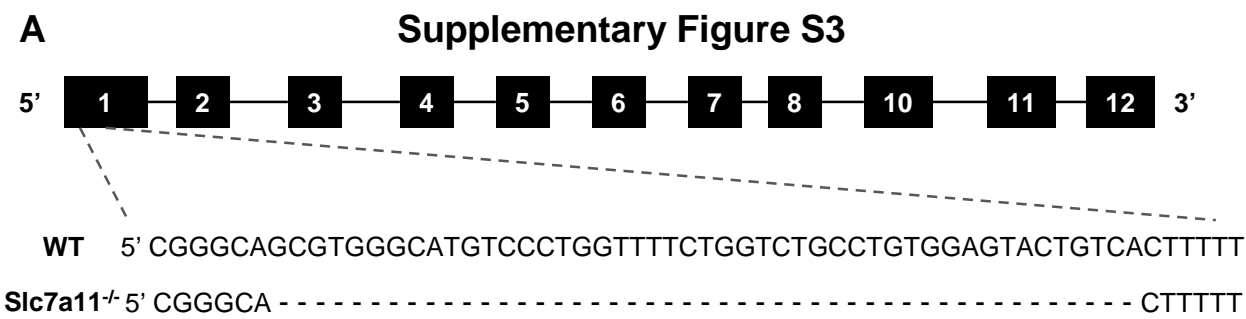
Supplementary Figure S1



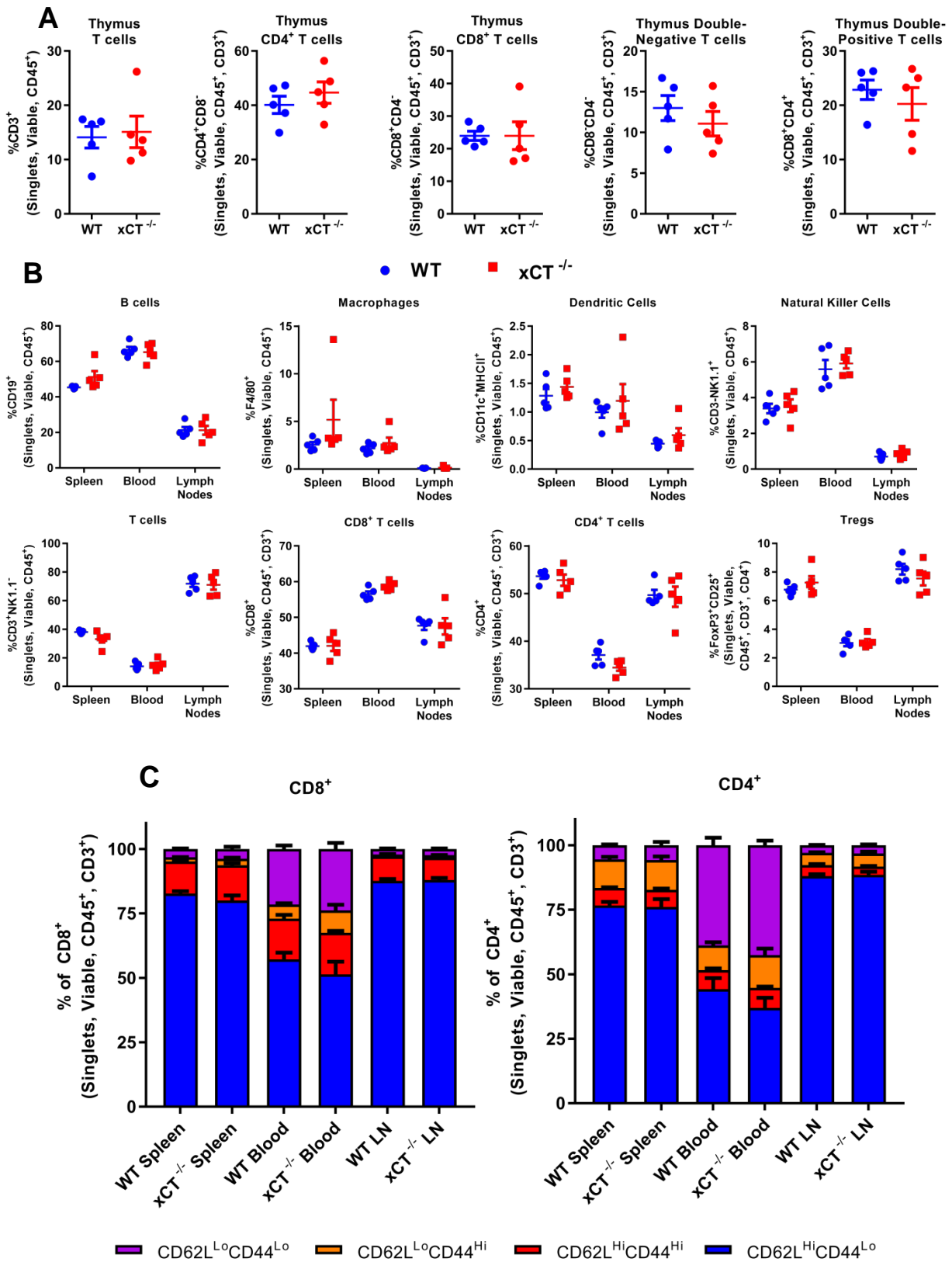
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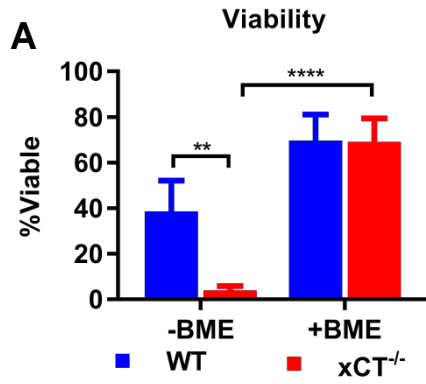
Supplementary Figure S3



Supplementary Figure S4



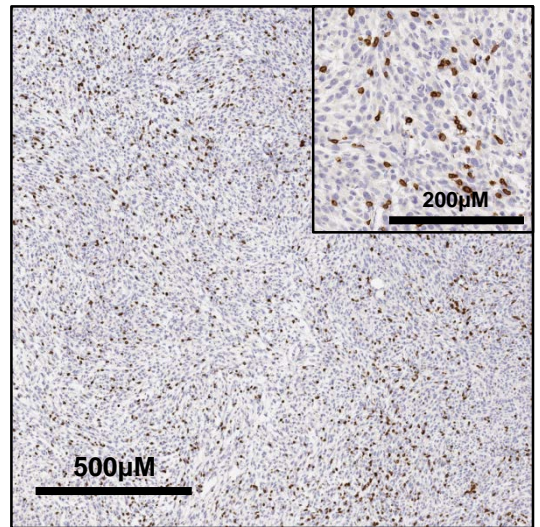
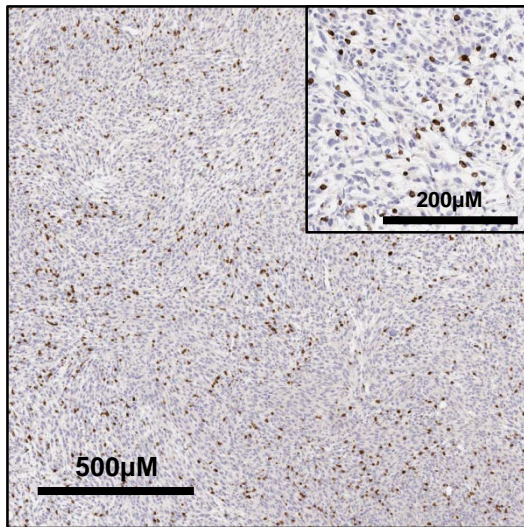
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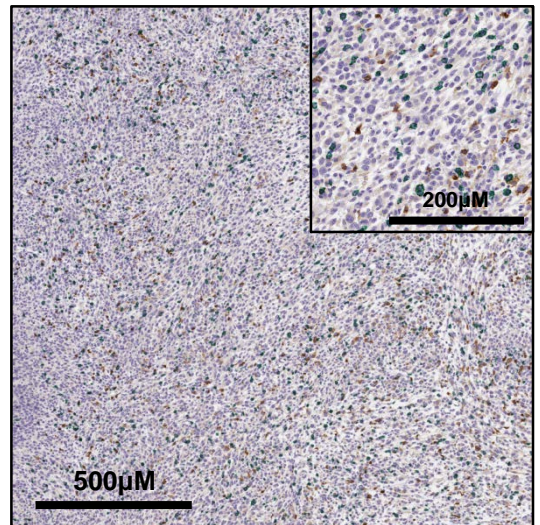
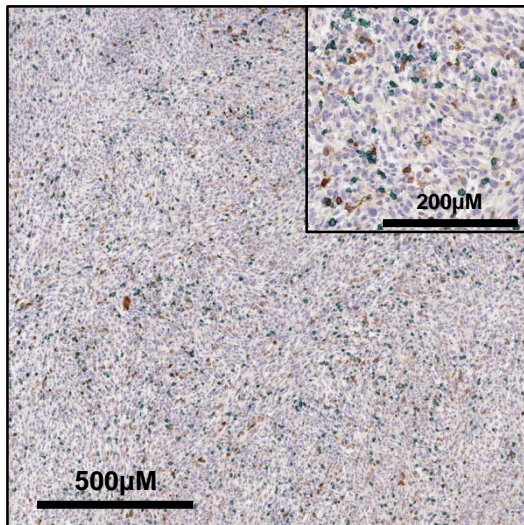
Supplementary Figure S6

A WT xCT^{-/-}

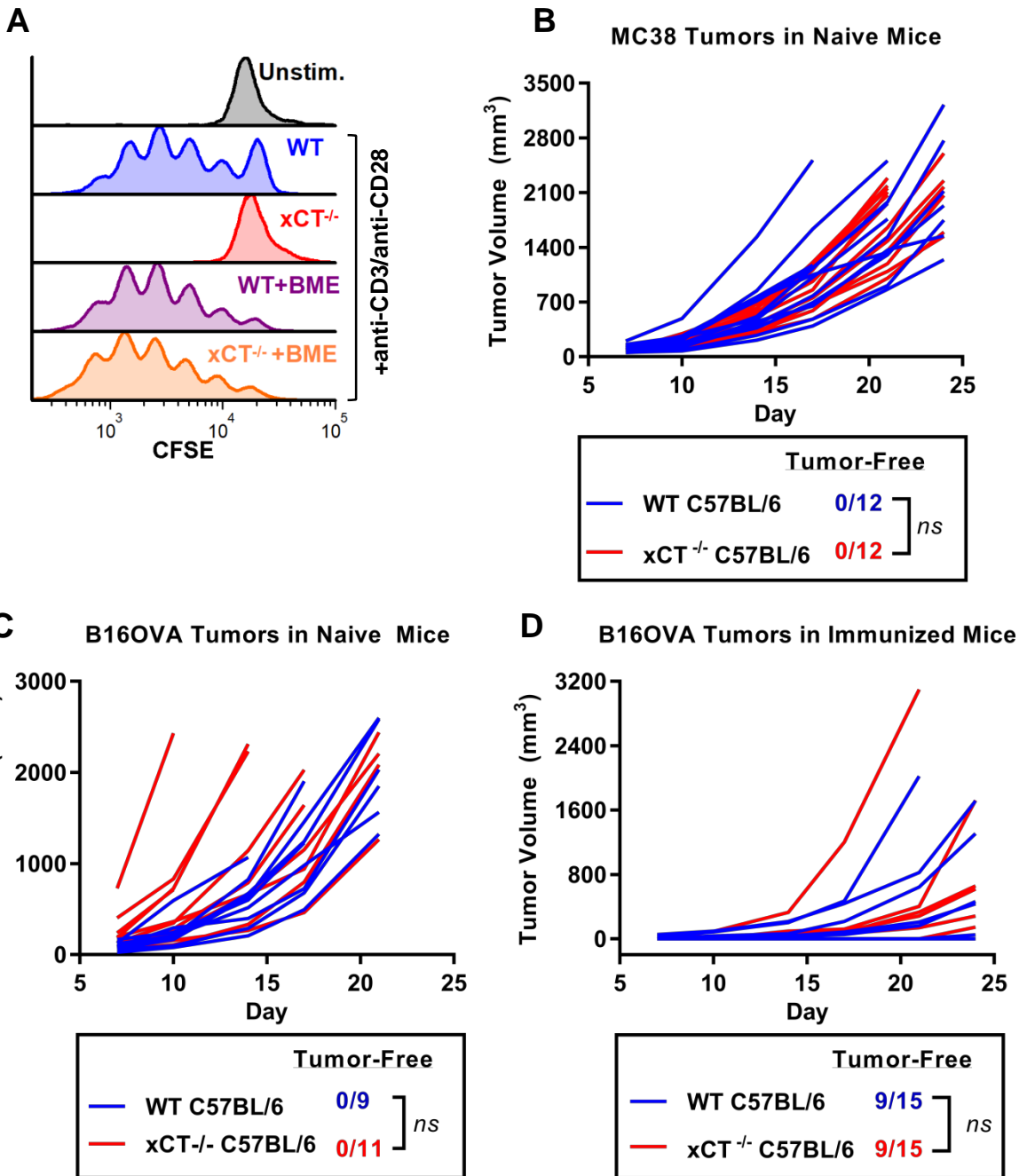
CD3



CD4/CD8



Supplementary Figure S7



Supplementary Methods

Cell Culture

Mouse embryonic fibroblasts (MEFs) were isolated as previously described (1). Briefly, 13-14 day old embryos were harvested from pregnant mice, minced in PBS, dissociated in 0.25% Trypsin-EDTA, and cultured in DMEM with 10% FBS and penicillin/streptomycin. Proliferation of MEFs and Slc7a11 overexpressing cancer cell lines was assessed by sulforhodamine B (SRB) staining (Sigma) as previously described (2).

xCT-deficient murine tumor cell lines were generated at HD Biosciences (Shanghai, China) by cloning the following guide sequences into the LentiCRISPR-v2 vector:

Pan02 clone 1-11 5'ATCGGCACCGTCATCGGATCAGG,

Pan02 clone 1-5 5' ATCGGCACCGTCATCGGATCAGG,

MC38 clone 2-1 5'ATCATCATCGGCACCGTCATCGG,

MC38 clone 2-6 5'ATCATCATCGGCACCGTCATCGG.

Stable cell lines were generated by lentiviral transduction with the constructs above, selection with puromycin, and isolation of single cell clones. All xCT-deficient cell lines were maintained in 100 uM BME.

Slc7a11 expression vectors were prepared at GeneCopoeia (Rockville, MD) by cloning murine Slc7a11 (NM_011990.2) from the Gateway PLUS shuttle vector GC-Mm06469 into the lentiviral expression vector pReceiver-Lv-115. Stable cell lines were generated

by lentiviral transduction with the Slc7a11 expression vector (LvSlc7a11) or the empty pReceiver-Lv-115 vector (LvNeg) followed by selection with hygromycin.

Tumor cell proliferation was assessed by plating 2,000 cells/well in 48-well plates and measuring confluence every 2 hours with the IncuCyte imaging system (Essen Bioscience).

Viability was measured after culturing cells for 72 hours in medium with or without 100 μ M BME. For tumor cells, supernatant was first collected and pooled with adherent cells that were acquired by trypsinization. Tumor cells or T cells were washed in DPBS, incubated with Fixable Viability Dye eFluor™ 780 (ThermoFisher #65-0865-14) diluted 1:1000 in DPBS for 15 minutes at 4°C, and washed in FACS buffer (0.5% FBS, 2mM EDTA in DPBS) prior to analysis by flow cytometry.

Cystine uptake assays were performed at HD Biosciences (Shanghai, China). 14 C cystine (Perkin Elmer #NEC845050UC) was diluted to 0.2 μ Ci/ml in a sodium-free buffer (25mM HEPES, 140mM N-methyl-D-glucamine chloride, 5.4mM KCl, 1.8mM CaCl₂, 0.8mM MgSO₄, 5mM glucose, pH7.5) and added to cells for 1 hour. Cells were washed in buffer without 14 C cystine, and radioactive cystine uptake was monitored as counts per minute (CPM) with a MicroBeta liquid scintillation counter (PerkinElmer). A parallel plate of cells was treated with CellTiter-Glo (Promega) per manufacturer's instructions and luminescence (RLU) was read on a luminometer. To normalize for differences in cell number, normalized CPM was calculated by (CPM/RLU)*10000.

GSH was measured with GSH-Glo (Promega), per manufacturer's instructions, after culturing cells for 7 hours in the presence or absence of 100 μ M BME. A parallel plate of cells was treated with CellTiter-Glo in order to normalize for differences in cell number. Values are shown relative WT cells without BME.

T cell activation and proliferation assays

For T cell activation and proliferation, 2×10^5 T cells were plated in 96-well U-bottom plates and stimulated with 2×10^5 Mouse T-Activator CD3/CD28 Dynabeads (ThermoFisher #11452D). Activation was assessed 24 hours post stimulation by cell surface expression of CD25 and CD69, or IL-2 secretion. IL-2 was measured in supernatants from T cell cultures with Mouse IL-2 Quantikine ELISA Kit (R&D Systems #M2000). T cell proliferation was assessed by CFSE staining as previously described (3). Briefly, T cells were stained with 40 μ M CFSE (ThermoFisher #65-0850-84) dissolved in complete DMEM at a concentration of 10^8 cells/ml. Cells were rocked at room temperature for 5 minutes, washed 3 times in complete DMEM, and plated as described above. CFSE dilution was measured by flow cytometry 3 days post stimulation. T cells were also stimulated in vitro with 1 μ g/ml Staphylococcal Enterotoxin B (Toxin Technology) by plating 10^6 freshly prepared CFSE-stained splenocytes in 96-well U-bottom plates. CFSE dilution in SEB-responsive T cells (CD3+V β 8+) was assessed 4 days post-stimulation.

For in vivo T cell proliferation assays, 50×10^6 CFSE-stained splenocytes (in 200 μ l RPMI) were transferred via tail-vein injection into wild type C57BL/6, $xCT^{-/-}$ C57BL/6 or CD45-congenic B6.SJL-Ptprca Pepcb/BoyJ mice (The Jackson Laboratory, stock #002014). One day later, mice were intraperitoneally injected with 25 μ g SEB or PBS vehicle. Three days later, splenocytes were harvested and CFSE dilution in SEB-responsive ($V\beta 8^+$) T cells was assessed by flow cytometry. To measure proliferation of OT-I T cells, mice were first implanted with 5×10^5 B16OVA tumor cells. After 7 days, 25×10^6 CFSE-stained splenocytes from WT OT-I or $xCT^{-/-}$ OT-I mice (in 200 μ l RPMI) were transferred via tail-vein injection to CD45-congenic B6.SJL-Ptprca Pepcb/BoyJ mice. Spleens, tumor draining lymph nodes and tumors were harvested 4 days after OT-I transfer. Tissues were processed as described below for flow cytometry. Prior to flow cytometry, tumor infiltrating lymphocytes were enriched using CD45 (TIL) microbeads (Miltenyi Biotec #130-110-618) and an autoMACS Pro Separator (Miltenyi Biotec), per manufacturer's instructions. Proliferation index was calculated using FCS Express (De Novo Software).

ELISPOT

Spleens were harvested from naïve or tumor-bearing mice 26 days post-tumor implant. 1×10^6 manually dissociated splenocytes were plated with or without 50,000 irradiated (50 Gy) MC38 tumor cells in complete medium with 100 μ M BME and incubated overnight at 37°C in a 5% CO₂ humidified incubator. Mouse IFN- γ ELISPOT Set (BD

Biosciences #551083) was used to detect tumor reactive T cells, per manufacturer's instructions. Spots were detected using an ImmunoSpot analyzer.

Immunoblotting

Cells were lysed in NuPAGE LDS buffer with reducing agent (ThermoFisher) followed by sonication to fragment DNA. Equal amounts of protein, quantified using the RC DC protein assay kit (Bio-Rad), were separated on NuPAGE 4-12% Bis Tris Midi gels, transferred to nitrocellulose membranes with iBlot (Invitrogen), blocked in TBS Odyssey Blocking Buffer (LI-COR), probed with primary antibodies (*SI Appendix*, Supplementary Table S1) diluted to 1:1000 in TBS Odyssey Blocking Buffer with 0.2% Tween, washed in TBS with 0.2% Tween, probed with IRDye 800 secondary antibodies (LI-COR), and imaged on an Odyssey Infrared Imager (LI-COR). Image Studio software version 4.0 was used for quantification of protein levels.

RT-qPCR

Samples were collected 24 hours post stimulation in vitro or in vitro. RNA was isolated using RNeasy kit (Qiagen #74104), cDNA was synthesized using SuperScript IV VILO Master Mix (ThermoFisher #11756050), and qPCR was performed with TaqMan Gene Expression Master Mix (ThermoFisher #4369016) and TaqMan primers for *Slc7a11* (Mm00442530_m1) or *Gapdh* (Mm99999915_g) with a C1000 ThermalCycler and CFX96 Real-Time System (Biorad). For analysis of SEB-stimulated T cells, SEB-

responsive T cells were isolated using anti-V β 8 antibody (*SI Appendix*, Supplementary Table S1) with Anti-Rat Kappa MicroBeads (Miltenyi Biotec #130-047-401) on an autoMACS Pro Separator (Miltenyi Biotec), per manufacturer's instructions.

Flow Cytometry

Isolated thymuses, spleens and lymph nodes were dissociated manually in complete DMEM. Dissociated splenocytes and whole blood were treated with ACK lysis buffer (Lonza #10-548E) to remove red blood cells. Tumors were dissociated using the mouse tumor dissociation kit (Miltenyi Biotec #130-096-730) and the gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec #130-096-427), according to manufacturer's instructions. Cells were stained for viability (see above), followed by Fc block with Ultra-LEAF™ Purified anti-mouse CD16/32 (Biolegend #101330) in FACS buffer for 10 minutes at 4°C. Cells were stained with antibodies (*SI Appendix*, Supplementary Table S1) against cell surface markers diluted 1:100 in FACS buffer for 15 minutes at 4°C. Intracellular staining was performed using Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher #00-5523-00), per manufacturer's instructions, with antibodies (*SI Appendix*, Supplementary Table S1) diluted 1:20. For experiments where MHC dextramers for detection of H-2Kb/SIINFEKL-specific T cells (Immudex #JD2163) were used, Fc block was applied and then 2 ul dextramer was added to 50 ul cells (in FACS buffer) and incubated for 20 minutes at room temperature. Fifty ul cell surface antibodies (diluted 1:50 in FACS buffer) were added, incubated for 20 minutes at room temperature, and washed in FACS buffer prior to flow cytometry. ROS were measured

following 24 hours in culture by resuspending trypsinized cells in DPBS with 5uM CM-H₂DCFDA (ThermoFisher # C6827) for 30 minutes at 37°C, followed by viability stain and resuspending in FACS buffer. All samples were run on a BD LSRFortessa X-20 (BD Biosciences).

Immunohistochemistry and Digital Image Analysis

Excised tumors were fixed in 10% neutral buffered formalin for 48 hours prior to being paraffin embedded. Five micron sections were deparaffinized in xylene and rehydrated through a graded series of alcohols to deionized water. Sections underwent heat-induced epitope retrieval in Borg Decloaker (Biocare Medical) for 30 minutes, enzyme block in Peroxidazed (Biocare Medical) for ten minutes, and protein block with Background Punisher (Biocare Medical) for ten minutes. Anti-CD3 (Abcam #ab135372) was applied at 1:200 for 60 minutes followed by MACH2 Rabbit HRP-Polymer (Biocare Medical) for 30 minutes, and Betazoid DAB (Biocare Medical) for five minutes. For dual staining, anti-CD4 (Sino Biological #50134-R001) was first applied for 60 minutes at 1:10,000, followed by MACH2 Rabbit HRP-Polymer for 30 minutes, and Betazoid DAB for five minutes. Sections underwent enzyme block in Peroxidazed (Biocare Medical) for ten minutes and protein block with Background Punisher for ten minutes. Anti-CD8 (Cell Signaling Technology #98941) was then applied at 1:300 for 60 minutes followed by MACH2 Rabbit HRP-Polymer for 30 minutes, and Vina Green (Biocare Medical) for 12 minutes. After chromogen staining, slides were rinsed in dH₂O, counterstained for 10 seconds in Tacha's hematoxylin (Biocare Medical), dehydrated in 100% alcohol, and

finished in xylene. Slides were scanned on a Leica/Aperio AT2 whole slide digital scanner and analyzed using custom algorithms created in Visiopharm software. Visiopharm IHC marker applications with threshold parameters were applied uniformly to identify CD3, CD4, and CD8 chromogen-positive area in viable regions of tumor sections.

Mass Spectrometry

Cysteine and cystine concentrations were measured in mouse plasma after N-ethylmaleimide (NEM) derivatization adapted from (4). Blood was collected via cardiac puncture in K3 EDTA tubes and immediately mixed with NEM at a final concentration of 10 mM. Blood was incubated at 4°C for 10 minutes and centrifuged at 1300 RCF for 10 minutes. Fifty microliters of plasma was added to 250 μ l cold methanol and stored at -80°C until mass spectrometry analysis. LC-MS/MS was used to measure the concentration of cysteine-NEM and cystine with external calibration curves of cysteine-NEM and cystine standard solutions. Five μ l sample was injected on an Imtakt Scherzo SM-C18 150 \times 2.0 mm column coupled with ASCIEX API4000. Mobile phase A of 100% water containing 0.1% formic acid and mobile phase B of 100% acetonitrile containing 0.1% formic acid were used at the flow rate of 0.3 mL/min and 30°C of column oven temperature. The following gradient was used: 0-6 min 0% B, 6-11 min 25% B, 11-12 min 25-95% B, 12-14 min 95% B, 14-15 min 95-0% B, and 15-25 min 0% B. Selected reaction monitoring was performed through the transitions m/z 247 (Q1) \rightarrow 184 (Q3) for Cysteine-NEM and m/z 241 (Q1) \rightarrow 152 (Q3) for cystine. The MS peak

area of Q3/Q1 184 /247 at retention time of 4.7 min for Cysteine-NEM and Q3/Q1 152/241 at retention time of 1.2 min for cystine were quantified using MAVEN (5).

Relative quantitation of proteins from MEFs was performed by dissociation of MEFs in 1.0 mL 100 mM ice-cold sodium carbonate buffer, pH 11.5, using TissueLyzer II (Qiagen) for 0.5 min at a rate of 0.30 repetitions three times and stored on ice for 30 sec between the dissociation cycles, followed by incubating on ice for 1 hour. The protein lysate was adjusted to pH 8.0 by the addition of Tris-HCl buffer (pH 7.0), and MgCl₂ was added to a final concentration of 2 mM. Nuclear DNA was fragmented by incubating with Universal Nuclease (ThermoFisher) for 30 min on ice. The membrane fraction was isolated by centrifugation at 20,000 g for 60 minutes at 4°C. The precipitated membrane-protein enriched fraction was solubilized in RIPA buffer (25 mM TrisHCl, pH 7.6, 150 mM NaCl, 1% SDS, 1% sodium deoxycholate, 1% NP-40 and 1X protease inhibitor) to extract membrane proteins, followed by determination of protein concentration by BCA assay. The membrane-protein enriched fraction was processed using filter-assisted sample preparation (FASP). Briefly, 50 ug of protein was loaded on a PES 100 kDa filter (ThermoFisher), and washed 4 times with 8 M Urea in 100 mM Tris-HCl buffer (pH 8.5) by centrifugation at 15,000 g for 20 min at room temperature. Proteins were reduced with 5 mM DTT (freshly prepared) in 8 M Urea and alkylated in the dark with 10 mM iodoacetamide in 8 M Urea. Samples were then washed four times with freshly prepared 25 mM ammonium bicarbonate buffer by centrifugation at 15,000 g for 20 min at room temperature and digested with trypsin/LysC with protein:enzyme at a 25:1 weight ratio overnight at 30°C. Following digestion, the peptides were separated

from undigested protein by ultrafiltration using centrifugation at 15,000 g for 20 min. The pH of the resulting peptide solution was adjusted by adding 10% formic acid to the final concentration of 0.25% formic acid. An aliquot (6.4 μ l) of each sample was injected and data were acquired on a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer fitted with a Dionex nano liquid chromatography and EASY-Spray™ Ion source. Specifically, the tryptic digested peptides were loaded onto a reversed-phase pre-column (C18 trap column, Acclaim PepMap 100, 100 μ m x 2 cm, ThermoFisher). Peptide separation was conducted via nano-LC using a 75 μ m x 50 cm PepMap C18 EASY-Spray column (3 μ m, 100 Å particles, ThermoFisher). Mobile phase A was HPLC grade water containing 0.1% formic acid and mobile phase B was HPLC grade acetonitrile containing 0.1% formic acid. The gradient elution program was as follows: 2 to 22.5% mobile phase B over 207 min, followed by 22.5 to 32% B over 30 min, 32 to 80% B over 10 min and a hold at 80% B for the last 10 min, all at a fixed flow rate of 300 nl/min in an Ultimate 3000 RSLCnano system (ThermoFisher). Q Exactive runs were operated with data dependent top 10 method and the parameters were as follows: resolution 70,000 at m/z 200 for MS1 with a scan range of m/z 300-1650, a predictive AGC target of 3×10^6 and a maximum injection time of 80 ms; 17,500 at m/z 200 for dd-MS2 with a predictive AGC target of 2×10^5 , maximum injection time 120 ms, isolation window of 1.6 m/z, NCE of 25, charge exclusion of unassigned, 1, 8, and > 8, 30s dynamic exclusion and underfill ratio 0.3%. Data was searched using MyriMatch spectral-peptide matching algorithm (6) against SwissProt mouse database (57,208 entries, May 2017 version) with the following parameter: semi-tryptic, precursor and fragment tolerance = 10 ppm, static modification 57.0215 on cysteine and dynamic

modification of 15.995 on methionine. A parsimonious protein summary was compiled using IDPicker with peptide inclusion set at 1% false-discovery rate (FDR) and protein inclusion requiring minimum of 1 unique peptide and 1 additional peptide. IDPQuantify (7), which is a part of the IDPicker program, was used to integrate and compute MS1 peak intensity. Differential expression was evaluated for each protein by a relative intensity-based absolute quantification metric (riBAQ, metrics from IDPicker) (8, 9) among each treatment conditions with two biological replicate samples. Slc7a11 was identified and displayed differential expression when comparing riBAQ. Slc7a11 unique peptide $^{13}\text{GGYLQGNMSGR}_{23}$ was detected with false-discovery rate (FDR) of 0.4%, passing databased FDR cut-off of 1% with FDR calculated using decoy database approach. The matches of MS/MS spectrum to peptide $^{13}\text{GGYLQGNMSGR}_{23}$ fragment ions were also manually validated. To improve the detection of this peptide and to allow this peptide serve as potential indicator of Slc7a11 total protein, a targeted MS1 proteomic experiment was designed to limit MS1 acquisition to the scan range m/z 568-574 that includes the mass-to-charge ratio of $^{13}\text{GGYLQGNMSGR}_{23}$ (m/z = 570.2668, doubly charged ion) with resolution 70,000 at m/z 200, predictive AGC target of 1×10^5 and a maximum injection time of 500 ms. The targeted MS1 proteomic experiment was operated with data dependent top 3 method and the dd-MS2 parameters were set as resolution 17,500 at m/z 200 with a predictive AGC target of 2×10^5 , maximum injection time 300 ms, isolation window of 2 m/z, NCE of 25, charge exclusion of unassigned, 1, 8, and > 8, 20s dynamic exclusion and underfill ratio 1%. MS peak area of peptide $^{13}\text{GGYLQGNMSGR}_{23}$ at m/z 570.2668 was extracted using MAVEN to indicate potential relative Slc7a11 protein levels.

References

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Supplementary Table S1. List of primary antibodies

Target	Catalog Number	Vendor	Application
xCT	98051	Cell Signaling Technology	WB
vinculin	V9264	Sigma	WB
CD25	561779	BD Biosciences	FC
CD69	562920	BD Biosciences	FC
CD45	553080	BD Biosciences	FC
CD25	562606	BD Biosciences	FC
CD11c	562782	BD Biosciences	FC
CD45.2	562895	BD Biosciences	FC
CD8a	563046	BD Biosciences	FC
Ki67	563462	BD Biosciences	FC
NK1.1	108737	Biolegend	FC
CD62L	104407	Biolegend	FC
CD44	103039	Biolegend	FC
CD19	115545	Biolegend	FC
CD3	100312	Biolegend	FC
CD4	61-0042-80	ThermoFisher	FC
V β 8.1/8.2	46-5813-82	ThermoFisher	FC
FoxP3	12-5773-82	ThermoFisher	FC
F4/80	61-4801-82	ThermoFisher	FC
MHCII	130-102-898	Miltenyi Biotec	FC
CD3	ab135372	Abcam	IHC
CD4	50134-R001	Sino Biological	IHC
CD8a	98941	Cell Signaling Technology	IHC

WB: Western Blot

FC: Flow Cytometry

IHC: Immunohistochemistry