Supplementary Methods

Viral Production and Transductions

Validated CAR-plasmid constructs were co-transfected together with pCL-Ampho retroviral packaging vector into Phoenix Ampho packaging cells using FuGENE HD (Promega) according to manufacturer's instructions. 48 hours post-transfection, the supernatants from the transfected packaging cells were harvested. Lymphocytes were isolated from peripheral blood using a Lymphoprep gradient (STEMCELL Technologies) and enriched from the white cell layer using Pan T cell isolation kit (Miltenyi Biotec). T lymphocytes were then transduced with CAR containing retroviral vectors 48 hours after stimulation with anti -CD3 (OKT3) and -CD28 antibodies (ThermoFisher Scientific) or anti-CD3/-CD28 Dynabeads (Gibco) plus human interleukin2 (h-IL2) (Peprotech) at 300U/ml. The supernatant was replaced with RPMI-1640 (Gibco), containing 100 U/mL penicillin and streptomycin (Gibco), 1% human serum (Merck Millipore), 10% fetal bovine serum (Sigma), 1 mmol/L sodium pyruvate (Gibco), and 2 mmol/L L-glutamine (Gibco), h-IL2 100U/ml supplemented with 0.1% β-mercaptoethanol (Thermo Fisher Scientific). For Jurkat-CAR cells no anti-CD3/CD28 stimulation was required. CAR-T cells were FACS sorted exploiting the truncated-CD34 tag added to the construct using a fluorophore conjugated anti-CD34-APC antibody (Biolegend).

Flow cytometry

To determine the expression of T-cell markers, conditioned cells were washed in ice cold PBS then stained with anti-CD69, anti-PD-1, anti-TIM3, anti-LAG3 antibodies (BioLegend) on ice for 30 min. To assess the effect on intracellular arginase expression, transporter modified CAR-T cells were stained with anti-human CD34 antibody for 20 min at 4^{o}C followed by fixation and permeabilisation using the Fixation/Permeabilization Concentrate and Diluent Kit (eBioscience). Cells were washed 4 times with Permeabilization Buffer and stained with unconjugated anti-human ARGII (CST), or anti-human ARGI

(CST) followed by donkey anti-rabbit APC conjugated secondary antibody (BioLegend) according to manufacturer's instructions. Fluorescence data was acquired using a CytoFLEX cytometer (Beckman Coulter). Normalised population statistics including the geometric mean fluorescence intensities were determined using FlowJo (BD Biosciences, formerly developed by FlowJo LLC).

Patient samples

In accordance with the Declaration of Helsinki, patient samples were obtained after written, informed consent prior to inclusion in the study. Heparinized blood samples were obtained from healthy donors at the University of Birmingham Health Partners, UK following Regional Ethics Committee approval (REC Number 10/H0501/39 and healthy leukocyte cones were provided by the NHSBT Blood Bank (Birmingham, UK).

Cell lines

Cell lines were originally obtained from ATCC and validated for authenticity by DNA short tandem repeats in line with American National Standards Institute ASN-0002-2011 (Northgene). $CD33^+$ AML cell lines (HL60, K562, KG1A, U937, NOMO or THP1), GD2+ tumour cell lines (KELLY or LAN1), or Jurkat (T-ALL line) were routinely cultured in RPMI 1640 medium supplemented with 10% v/v fetal bovine serum (FBS, Sigma) (R10%, 100 U/mL penicillin and streptomycin (Gibco), 1 mmol/L sodium pyruvate (Gibco), and 2 mmol/L L-glutamine (Gibco). All experiments were performed between passages 3-9, and cells were routinely confirmed as Mycoplasma negative by PCR analysis (LookOut, Sigma).

Cell culture assays

AML cell lines were incubated in R10% or amino acid-free RPMI 1640 (PAN Biotech, UK) supplemented with 10% *v/v* dialysed FBS (ThermoFisher Scientific). To test the effects of SLC7A5 inhibition or SLC7A11 inhibition where indicated JPH203 (0-1000μM, gift of J-Pharma, Japan) or sulfasalazine (0-1000μM) were added to cultures. After 72 hours the numbers of viable cells was assessed by flow cytometry using propidium iodide staining.

CAR-T cell functional assays

The expression of target antigens on tumour cells was confirmed by flow cytometry using antibodies against CD33 (BioLegend), and GD2 (PE) (Biolegend). 4-hour ^{51}Chromium-release assays at 4:1 ratios or flow cytometry were performed to test CAR-T cell cytotoxicity against target cells. CAR T effector cells were incubated with antigen-positive tumour target cells and antigen-negative controls.[23]

Proliferation Assays

T and CAR-T cells were cultured in 96-well flat bottom plates coated with either anti-CD3 (OKT3) antibody (3 μg/mL) and anti-CD28 antibody (2 μg/mL) (ThermoScientific) or target cells. Cells were cultured in 200μL of R10%, or in tumour conditioned media supplemented with 0.1% β-mercaptoethanol (Thermo Fisher Scientific) at 37 °C, 5% CO2. Where indicated, cells were cultured in low amino acid media (25% standard R10% FBS combined with 75% tryptophan/cystine/arginine depleted media (TCS Biosciences) and 10% dialysed FBS; v/v). For SLC7A11 related assays no β-mercaptoethanol was added to culture media. Tryptophan or Cystine (50μM; Sigma) were similarly added to relevant amino acid free media as indicated. Proliferation was determined by flow cytometry using CD34 antibody to gate on CAR-T cells and propidium iodide to discriminate dead cells. Proliferation was also determined by dye dilution assays. Briefly washed T cells suspended in PBS were labelled with 1μM carboxyfluorescein succinimidyl ester (CFSE) staining solution (Molecular Probes, ThermoFisher Scientific). Stained cells were washed three times in RPMI-1640 and rested for 10 minutes in complete media. Labelled cells were then cultured at 37 °C, 5% CO2 and analysed after 72 hours on a CytoFLEX Flow Cytometer (Beckman Coulter). Distinct populations of proliferating cells are depicted as histograms generated from data analysis using the FlowJo Software. Where indicated CAR-T cell proliferation was assessed by ^{3}H-thymidine labelling. Cells were incubated at 37^{o}C, 5% CO2 for 7 days and then 1μCi/well ^{3}H-thymidine (Perkin Elmer Life Sciences, Beaconsfield, UK) was added for 12-16 hours. ^{3}H-thymidine incorporation was measured using a TopCount reader (Perkin Elmer).

Immunoblotting

Cells were washed in PBS and lysed with RIPA lysis buffer (20nM Tris-HCl pH7.5, 150nM NaCl, 2mM EDTA, 1.0% Triton X-100) containing cOmplete™ EDTA-free protease inhibitors (Roche) and PhosSTOP™ phosphatase inhibitors (Sigma). Total protein concentraion in cell lysates were quantified by Bradford assay. Equal amounts of protein per test condition were electrophoresed in separate wells of a 4-20% Tris-Glycine SDS-PAGE gel (BioRad) at 150V for 1 hour. The separated proteins were transferred to PVDF membranes using the TransBlot Turbo system (BioRad). Protein bands were detected using primary antibodies to SLC7A5(CST), SLC7A11 (Sigma Aldrich), ARGI (CST), or ARGII (CST) where were indicated. Actin or GAPDH were used as a loading control (Cell Signalling) HRP-conjugated secondary antibodies of either goat anti-rabbit (CST) or horse anti-mouse (CST) were used for primary antibody detection. Blots were developed using ECL substrate (BioRad), exposed to CL-Xposure X-ray film (ThermoFisher Scientific) or documented using the ChemidocMP system (BioRad).

Cytokine analysis

Cytokines concentration in culture supernatants was determined using using the LEGENDPlex™ Human Inflammation Panel 1 bead-based flow cytometry assay Kit as per manufactures instructions (BioLegend). Acquited data was analysed using the The LEGENDplex™ Data Analysis Software Suite as per kit instructions (BioLegend).

Supernatant and murine sera amino acid analysis

Control or SLC7A5- or SLC7A11 modified anti-CD33 CAR-T cells were cultured in the presence of THP1 cells for 72hours as above. Cell culture supernatants were harvested. Tryptophan concentrations were measured using the Tryptophan Assay Kit (Alpha Diagnostic). In brief, culture supernatants, standards and controls were derivatised as per kit manufacturer's instructions using Tryptophan anti-sera and enzyme conjugate. Samples were then loaded unto the antibody solid phase of the kit supplied microtiter plate. After washing, and following TMB oxidation by peroxidase conjugates, and

acidification by sulfuric acid, the residual antibody bound analyte was detected as a measure of the absorbance at 450nm using the Biorad iMark microplate reader. Tryptophan concentrations in supernatants was extrapolated from standard curves of known concentrations. For murine sera samples were similarly studied using the Tryptophan Assay Kit (Abbexa).

Cysteine concentrations were similarly measured using the Human Cysteine (CYS) ELISA Kit assay (BlueGene, supplied by Biognosis Ltd) in culture supernatants or murine sera as per manufacturer's instructions. This included the addition of 20mM 2-carboxyethyl)phosphine (TCEP) to reduce free cystine to cysteine. No background cysteine is present in cell media.

AML and GD2+ tumour cell line murine xenografts

All experimental protocols were approved and monitored by Axis Bioservices Animal Welfare and Ethical Review Board (I2AE50C0) in compliance with the UK Home Office Animals (Scientific Procedures) Act 1986, the United Kingdom National Cancer Research Institute guidelines for the welfare of animals in cancer research, and the ARRIVE guidelines.

NOD/Shi-scid/IL-2R SCIDγnull (NSG) mice aged 10-14 weeks were injected with $1x10^6$ HL60 leukaemia cells into the tail vein. Following AML engraftment (5-10% of AML blasts in the bone marrow confirmed by bone marrow biopsy in each mouse), $2.5x10^6$ anti-CD33 CAR-T from 3 human donors were injected i.v. Bone marrow was harvested at the end of the experiment. AML engraftment was defined by the detection of human $CD45^+CD33^+$ cells using flow cytometry.

Nude mice aged 10-14 weeks were injected with $2.5x10^6$ GD2+ KELLY cells (1:1 in Matrigel) into the rear flank, subcutaneously. Following palpable neuroblastoma engraftment, $2.5x10^6$ anti-GD2 CAR-T were injected i.v. Calipers were used to measure tumour diameter on two orthogonal axes 2-3 times per week. Volume was calculated using the equation; $v = (lengthxwidth^2)/2$. Tumours were harvested at the end of the experiment for subsequent analysis.

PCRs

RNA was extracted using the RNeasy Mini kit (Qiagen). First strand cDNA synthesis was performed using SuperScript™ III Reverse Transcriptase (ThermoFisher) following the manufacturer's instructions. For quantification of gene expression, RT-Q-PCR was performed using the FAST SYBR Green Master Mix and the Applied Biosystems 7500 Fast Real-Time PCR system (ThermoFisher). Gene specific primer sequences were: For detection of CAR in murine tissue, CAR specific primers targeting the spacer region of the GD2 spacer region (Forward: ACTCCAAACTCACCGTGG AC, Reverse: TTTTTGCCGGGTGAGAGTGA) or the CD33 scFV (Forward: CGGGATCCAACATCATGCTGAC Reverse: AGGTAGTTCTTCTGGGAGCTGC) and 18s (housekeeping gene: Forward ACCCGTTGAACCCCATTCGTGA, Reverse GCCTCACTAAACCATCCAATCGG) were used. A No Reverse transcriptase control and a no-template-control (NTC) were used as negative controls of PCR amplification. Gene copy numbers were calculated using $2^{-\Delta CT}$method. ΔCT = CT (a target gene) −CT (housekeeping gene).

RNA-Sequencing

Sets of unmodified and modified CAR-T cells from human donors were cultured in 75% L-arginine free medium (anti-GD2 ARGI/ARGII/control CAR-Ts) or 75% tryptophan/cystine free conditions (anti-CD33 SLC7A5/SLC7A11/control CAR-Ts). RNA was isolated from the FACS sorted CAR-T cells using the Micro RNEasy® plus micro isolation Kit (Qiagen). Samples were prepared with the Illumina TruSeq RNA Sample Preparation Kit v2. They were sequenced on the Illumina HiSeq2000 platform using TruSeq v3 chemistry, over 76 cycles. Sequencing reads were aligned to GRCh37 human genome using STAR RNA-Seq aligner software.[24] Reads mapping to transcripts were counted by the same software.[25]

Arginase activity assays

Arginase activity in CAR-T cells was determined colourimetrically by quantifying urea produced in the stoichiometric conversion from arginine in the presence of α-isonitrosopropiophenone (ISPF). Briefly,

cells were pelleted and lysed with 50 μl of lysis buffer containing 0.1% Triton X-100, 5 μg pepstatin, 5 μg aprotinin and 5 μg antipaina. The samples were placed on a 37°C heat block for 30 minutes before centrifugation at 14,000 rpm and collection of the supernatants. To activate the arginase enzyme, buffer containing Tris-HCl (25 mM) and $MnCl_2$ (10 mM) was added and heated to 56°C for 10 minutes. L-arginine (0.5M, Sigma) was added and the samples were heated for 1 hour at 37°C. The hydrolysis of arginine was stopped with 800 μl of an acid solution mixture (H_2SO_4:H_3PO_4:H_2O, 1:3:7). The amount of urea produced was determined using 9% ISPF (m/v in 100% ethanol) and compared to a standard curve with absorbance measured at 540nm.

Gas chromatography – Mass Spectrometry (GC-MS)

Transduced Jurkat cells were cultured for 48h in L-arginine-restricted environment (i.e. 75% L-Arginine free medium) prior to seeding at 1.5 x10^6 cells/well onto a 12-well plate in 1 ml of SILAC RPMI 1640 Flex (Fisher Scientific, A2494201). The medium was supplemented with 10% dialysed FBS, 2mM L-Glutamine, 10mM D-Glucose, 200μM L-Lysine (all Sigma) and 500μM U-$^{13}C_6$-arginine. Cells were kept in flux media for 48 h. Then, cells were washed twice with saline and the plate was extracted with 500 μL of dry-ice cold methanol (Fisher Scientific), 200 μL of ice-cold distilled water with D6-glutaric acid standard (2,5 μg/ml) and 500 μL of chloroform (Fisher Scientific). The upper phase was transferred into a clean safe-lock tube and dried in a speedvac (45°C for 2-3h).

Dried samples were derivatized using a two-step protocol. Samples were resuspended in 2% methoxamine in pyridine, (40 μl, 1 h at 60°C), followed by addition of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide with 1% tert-butyldimethylchlorosilan (50 μl, 1 h at 60°C). Derivatised samples were transferred to glass vials for analysis by gas chromatography mass spectrometry (GC-MS).

An Agilent 7890B GC equipped with a polydimethylsiloxane column (Rxi-5ms, Restek, UK) connected to a 5977A MSD (Agilent Technologies UK Limited, Stockport, UK) was used for analysis of the

derivatized polar metabolites. Compound detection was carried out in scan mode and analyte ion counts were normalized to the internal standard. A natural abundance correction was performed, and Mass Isotopomer Distributions (MIDs) calculated using in-house MATLAB scripts. Data was normalized to protein amount.

Cellular Bioenergetics

Jurkat-CAR cells were prepared as above. 3 hours prior to Seahorse analysis the Jurkat-CAR cells were cultured in tryptophan, cystine or arginine low (75% free) media before counting and plating onto Cell-Takcoated (Corning) Agilent Seahorse XFe96 cell culture microplates (V3-PS, Agilent Technologies) at a density of $2x10^5$ cells/well in 175µl/well XF RPMI (#103576-100, Agilent Technologies), supplemented with 5mM glucose, 2mM L-Glutamine and 1mM sodium pyruvate and 5mM HEPES. All samples had an un-starved control (R10%). Following a 1-hour incubation at 37°C under air (non-CO_2 incubator), and calibration of the XFe96 sensor cartridge, the cell culture microplate was transferred to the Seahorse XFe96 extracellular flux analyzer (controlled at 37°C). Baseline oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) were measured for 4 cycles consisting of a 3-minute mix and 3-minute measure period before sequential addition of 2 µg/ml oligomycin, 3 µM BAM15, a mixture of 2 µM rotenone plus 2 µM antimycin A to determine rates of ATP coupled respiration, maximal and spare respiratory capacities, or non-mitochondrial cellular oxygen uptake respectively. Proton efflux rate was calculated from ECAR using the following formula: PER (pmol H^+/min) = ECAR (mpH/min) x BF (mmol H^+/L/pH) x Vol $_{\text{XF microchamber}}$ x Kvol; Kvol = 1.6, BF = 2.6 and Vol XF microchamber = 2.3. To establish glycolytic PER (glycoPER), PER were corrected for mitochondrial acidification (mitoPER) by multiplying mitochondrial OCR by the CO_2 contribution factor (0.61).

Statistical Analysis

Parametric and non-parametric student t-tests were used to determine the statistical significance of the difference in paired or unpaired observations between groups (GraphPad Prism, USA). All p values are two-tailed and p values <0.05 were considered to represent statistically significant events.

Supplementary Figure Legends

Supplementary Figure 1: Amino acid uptake through SLC7A5 and SLC7A11 promotes AML viability

A) Gene expression profile of SLC7A5, SLC7A11 and GAPDH on AML blasts from 562 patients at diagnosis, from *Li et al. 2013*, held within the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl) **B)** Representative Western blot of 6 AML cell lines demonstrating variable expression of SLC7A5 or SLC7A11. GAPDH shown as a loading control **C)** AML cell line viability is significantly reduced following culture for 72hours in cystine or tryptophan free media compared to R10%, as measured by flow cytometry. n=3 biological repeats per cell line **D)** AML cell line viability is significantly reduced by increasing concentrations of the SLC7A5 inhibitor JPH203 following culture for 72hours, as measured by flow cytometry. Mean and SD of n=3 biological repeats per cell line **E)** AML cell line viability is significantly reduced by increasing concentrations of the SLC7A11 inhibitor sulfasalazine following culture for 72hours, as measured by flow cytometry. Mean and SD of n=3 biological repeats per cell line **F-H)** Gene expression profile of SLC3A2, SLC7A5, SLC7A11 and GAPDH on $CD4^+$ or $CD8^+$ T cells isolated from the blood of AML patients (n=10) and age-matched healthy donors (n=10), from *Le Dieu et al. 2009*, held within the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl)

Supplementary Figure 2: SLC7A5 and SLC7A11 modified CAR-T cells

A) Schematic of CAR-T constructs containing the basic anti-CD33-CAR scFv-CD8 hinge-41BB-CD3ζ in concert with SLC7A5 or SLC7A11. A truncated CD34 is expressed for CAR-T identification and purification **B)** Representative flow cytometric staining demonstrating CAR-T cells post transduction and expansion, as identified by CD34 positive staining in 2 individual donors **C)** Representative flow cytometric staining demonstrating purity of transporter modified anti-CD33 CAR-T cells post sorting **D)** Expression of SLC7A5 by anti-CD33-CAR-T cells as demonstrated by western blot, with actin loading control. N=3 donors shown **E)** Representative expression of SLC7A11 by anti-CD33-CAR-T cells as demonstrated by western blot, with actin loading control. N=3 donors shown **F)** SLC7A5 expressing CAR-T cells induce a greater depletion of tryptophan in culture supernatants compared to anti-CD33 CAR-T cells after 72hours, as measured by colorimetric assay. N=3 donors shown. **G)** SLC7A11 expressing CAR-T cells induce a greater depletion of cystine in culture supernatants compared to anti-CD33 CAR-T cells after 72hours, as measured by colorimetric assay. (Cystine was reduced by TCEP to cysteine for detection in the colorimetric assay. Controls showing media and supernatants contain no background levels of cysteine also displayed). N=3 donors shown.

Supplementary Figure 3: Transporter expression increases CAR-T cell activation with no negative impact on exhaustion markers in vitro

A and B) CD69 activation marker is upregulated on in SLC7A5 or SLC7A11 modified anti-CD33 CAR-T cells after re-stimulation with THP1 target cells in vitro on days 4, 6, and 9 , as measured by flow cytometry. N=3 donors shown **C-H)** Expression of immune checkpoint exhaustion markers LAG3, PD-1, and TIM3 are not significantly changed in SLC7A5 or SLC7A11 modified anti-CD33 CAR-T cells after re-stimulation with THP1 target cells in vitro on days 4,6, and 9 , as measured by flow cytometry. N=3 donors shown.

Supplementary Figure 4: Cytokine profiles of transporter modified CAR-T cells in vitro

A) Transporter modified CAR-T cells release significantly less TNF-α into culture supernatants after re-stimulation with THP1 target cells in vitro on days 4,6, and 9, as measured by flow cytometry immunoassay. N=3 donors shown **B)** SLC7A5 modified CAR-T cells release significantly less IL-1β into culture supernatants after re-stimulation with THP1 target cells in vitro, as measured by flow cytometry immunoassay. N=3 donors shown **C-F)** Concentrations of IL-6, IL-10, MCP-1, and IL-23 in the culture supernatants after SLC7A5 or SLC7A11 modified anti-CD33 CAR-T cells are re-stimulated with THP1 target cells in vitro on days 4,6, and 9, as measured by flow cytometry immunoassay. N=3 donors shown **G)** Concentrations of Tryptophan and Cystine in the serum of leukaemia-bearing mice.

Supplementary Figure 5: Transporter modified CAR-T Jurkat cells

A) Flow cytometric staining demonstrating purity of transporter modified anti-CD33 Jurkat CAR-T cells post sorting **B)** Expression of transporter modifications in Jurkat CAR-T cell lines as demonstrated by western blot, with actin loading control. Positive controls for SLC7A5 are HEPG2a cell line and positive control for SLC7A11 are THP1 cell lines **C-E)** Basal mitochondrial respiration linked to ADP phosphorylation and glycolytic proton efflux rate (glycoPER) in SLC7A5 and SLC7A11 J-CAR-T cells starved from tryptophan or cysteine, respectively, compared to unmodified respective amino acid starved controls **F)** Significant increase in ATP supply rates within amino acid starved SLC7A5- or SLC7A11 modified J-CAR-T cells, compared to unmodified anti-CD33 CAR-T cell controls suggesting ATP-linked oxidative and glycolytic flux are less affected by amino acid starvation in cells expressing SLC7A5 or SLC7A11 modifications (pool of n=4 individual experiments performed in quadruplet biological replicates).

Supplementary Figure 6: SLC7A5 or SLC7A11 modified anti-CD33 CAR-T cells upregulate intracellular Arginase

Representative flow cytometry staining showing intracellular Arginase I **(A)** or Arginase II **(B)** enzyme expression. Arginase isoforms are upregulated in CAR-T cells following culture with THP1 target cells. Arginase isoforms are further increased by SLC7A5- or SLC7A11- expression under R10%, tryptophan low (75% free) or cystine low (75% free) conditions as measured by flow cytometry in CAR-T cells. N=3 human donors

Supplementary Figure 7: Arginase modified anti-GD2 CAR-T cells

A) Flow cytometric staining demonstrating purity of Arginase modified anti-GD2 CAR T cells post sorting **B-D)** Expression of immune checkpoint exhaustion markers PD-1, LAG3, and TIM3 are not significantly different in Arginase modified anti-GD2 CAR-T cells, as measured by flow cytometry. N=10 donors shown **E)** IFN-γ release by Arginase modified anti-GD2 CAR-T cells into culture supernatants after 96hours in the presence of anti-CD3/anti-CD28 antibodies, as measured by ELISA. N=8 donors shown **F)** IFN-γ release by Arginase modified anti-GD2 CAR-T cells into culture supernatants after 96hours in the presence of $GD2^+$ LAN1 tumour cells, as measured by ELISA. N=4 donors shown

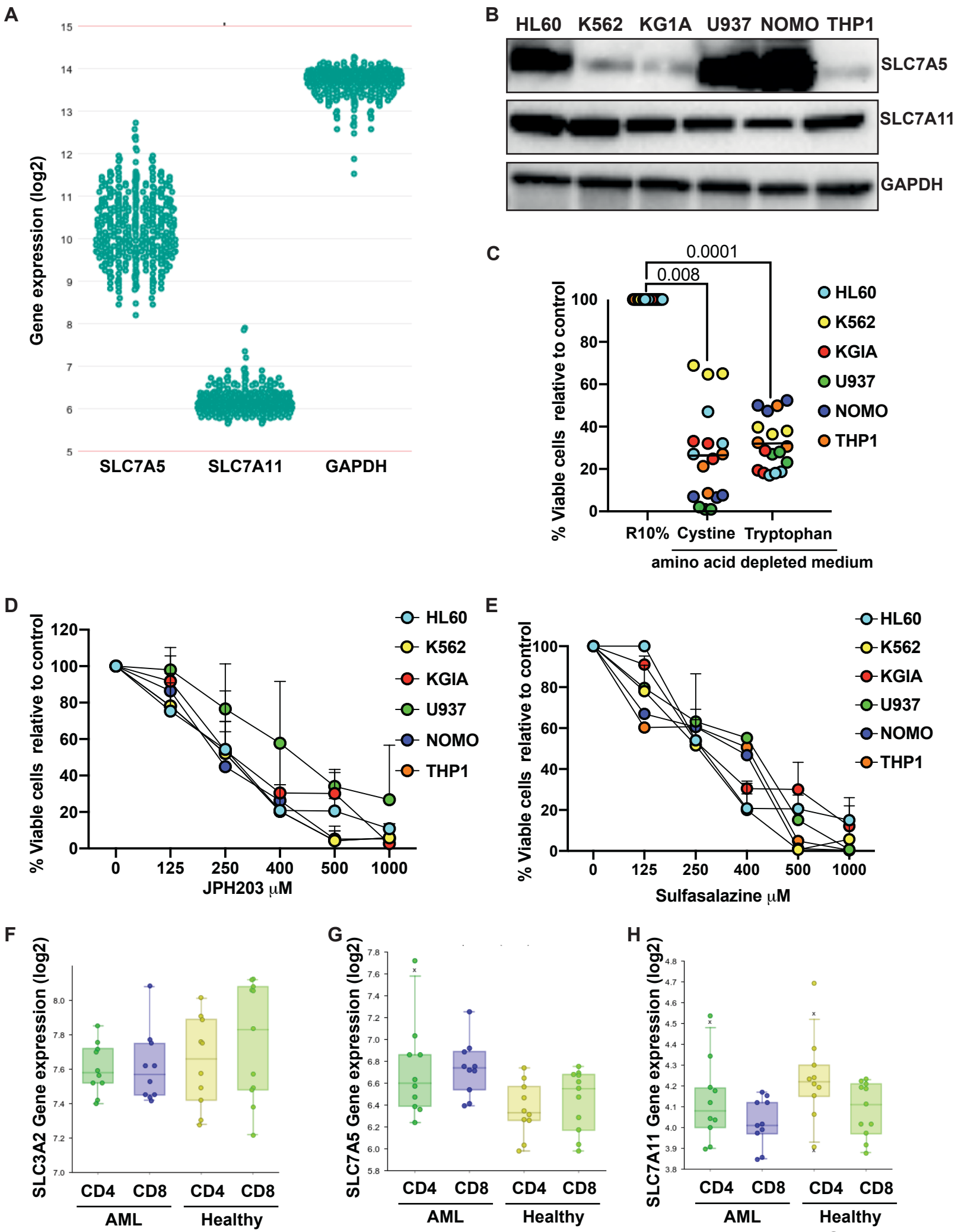
Supplementary Figure 8: Arginase modified anti-GD2 Jurkat CAR-T cells

A) Flow cytometric staining demonstrating purity of Arginase modified anti-GD2 Jurkat CAR-T cells post sorting **B)** Expression of Arginase I and Arginase II enzymes in Jurkat CAR-T cell lines as demonstrated by western blot, with actin loading control **C)** Arginase enzyme activity, measured by catabolism of arginine into ornithine and urea, is increased in ARGI- or ARGII- modified anti-CD33 CAR-T cells compared to unmodified CAR-T cells. Data from n=10 ARGI and n=7 ARGII human CAR-T cell repeats respectively.

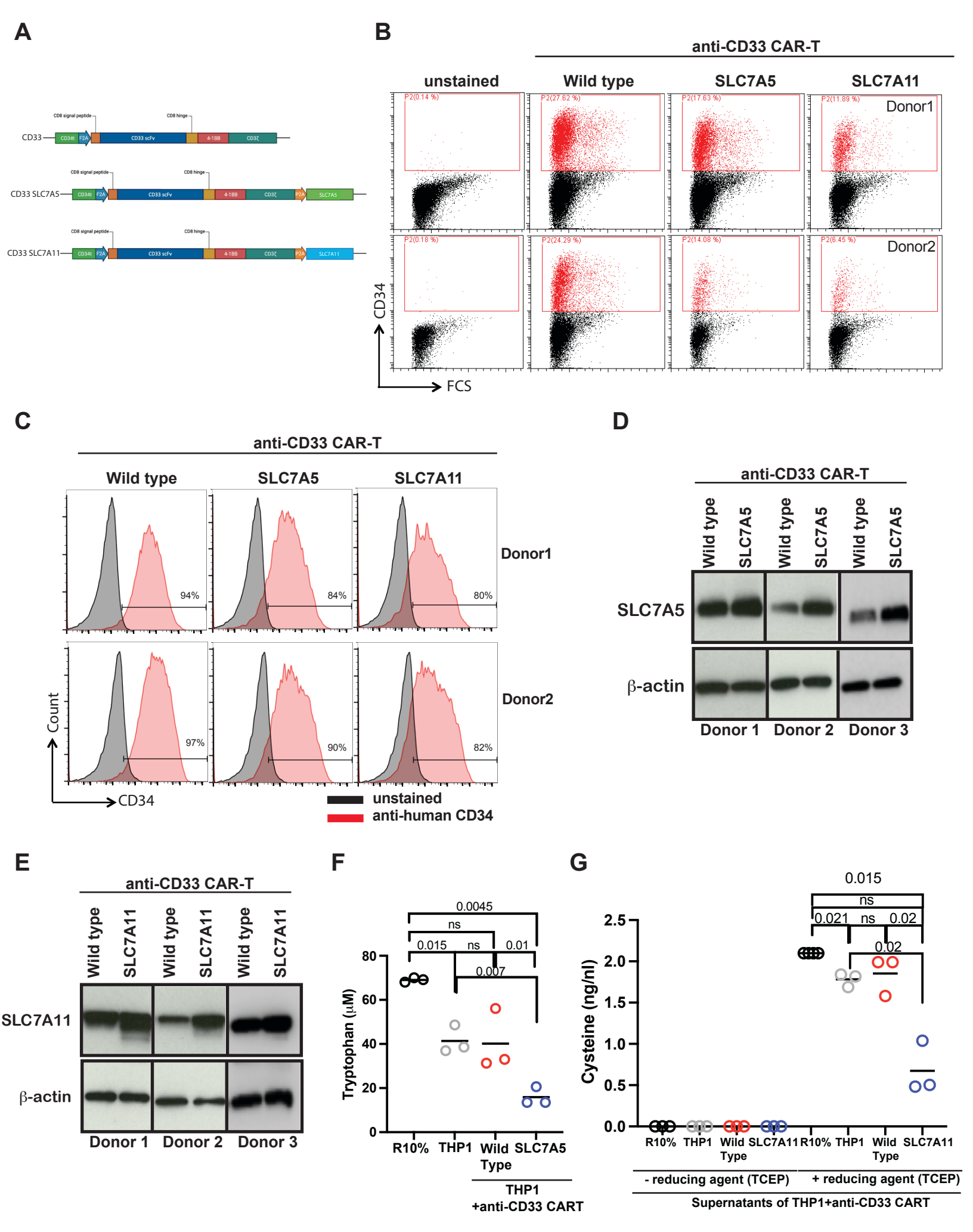
Supplementary Figure 9: Downstream changes in Arginase modified anti-GD2 CAR-T cells

A-F) Maximal respiratory capacity and consequent spare respiratory capacity are increased in ARGII modified anti-GD2 Jurkat CAR-T cells compared to unmodified anti-GD2 Jurkat CAR-T cells, consistent with increases in ATP supply rates within ARG modified Jurkat CAR T cells (pool of n=4 individual experiments performed in quadruplet biological replicates) **G)** Significant changes in intracellular metabolites as detected by GC-MS in Arginase modified anti-GD2 Jurkat CAR-T cells compared to unmodified anti-GD2 Jurkat CAR-T cells. (n=3 individual experiments performed in triplicate) H**)** Adjusted p-values for the genes with highest significant differential gene expression comparing Arginase I modified anti-GD2 CAR-T cells to unmodified anti-GD2 CAR-T cells from n=3 human donors **I)** p-values for the genes with highest significant differential expression comparing Arginase II modified anti-GD2 CAR-T cells to unmodified anti-GD2 CAR-T cells from n=3 human donors.

Supplementary Figure 10: Schematic depicting competition between tumour cells and CAR-T cells for limited amino acids, which can be overcome through SLC7A5/SLC7A11/ or ARGI/ARGII modifications



Supp Figure 1



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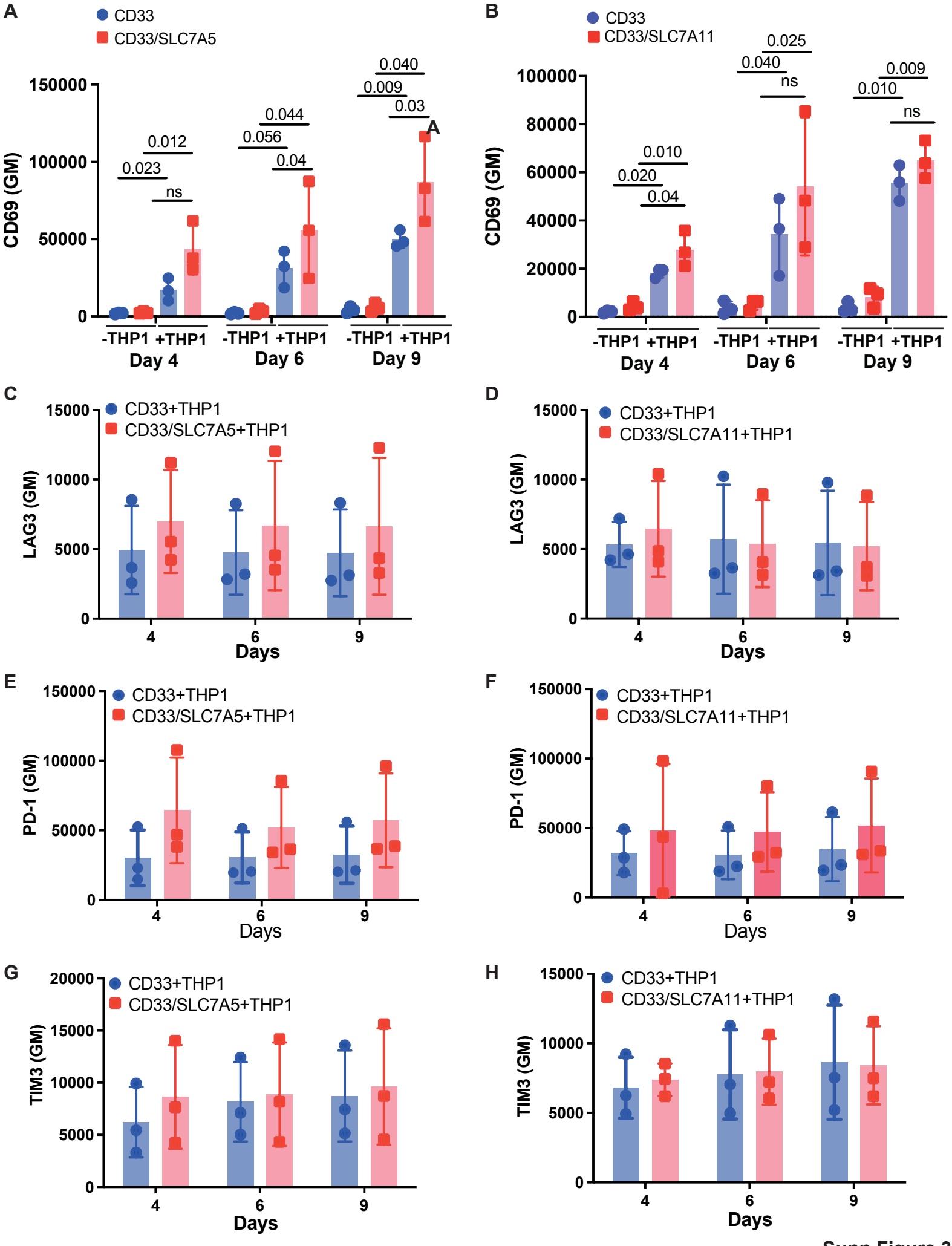

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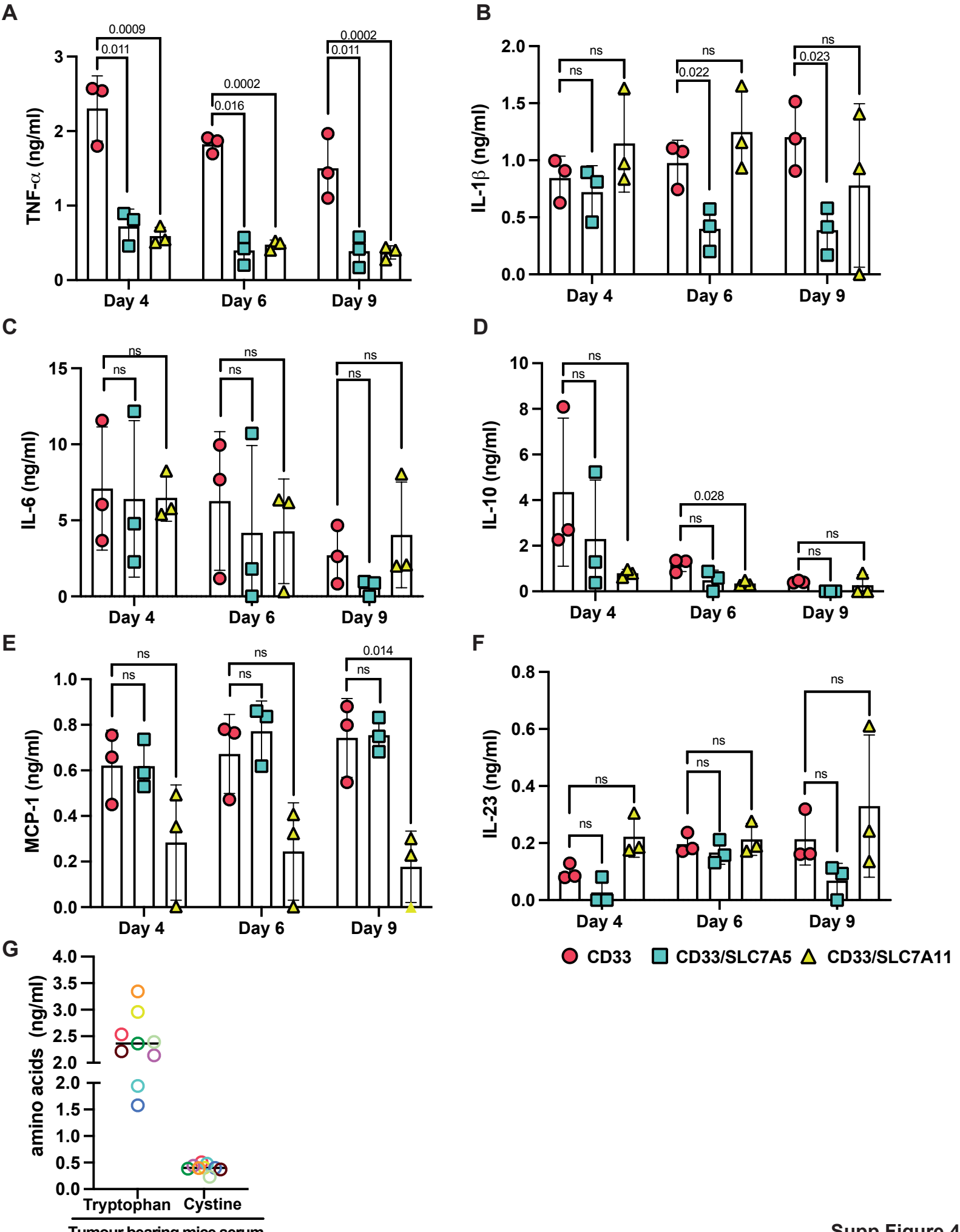

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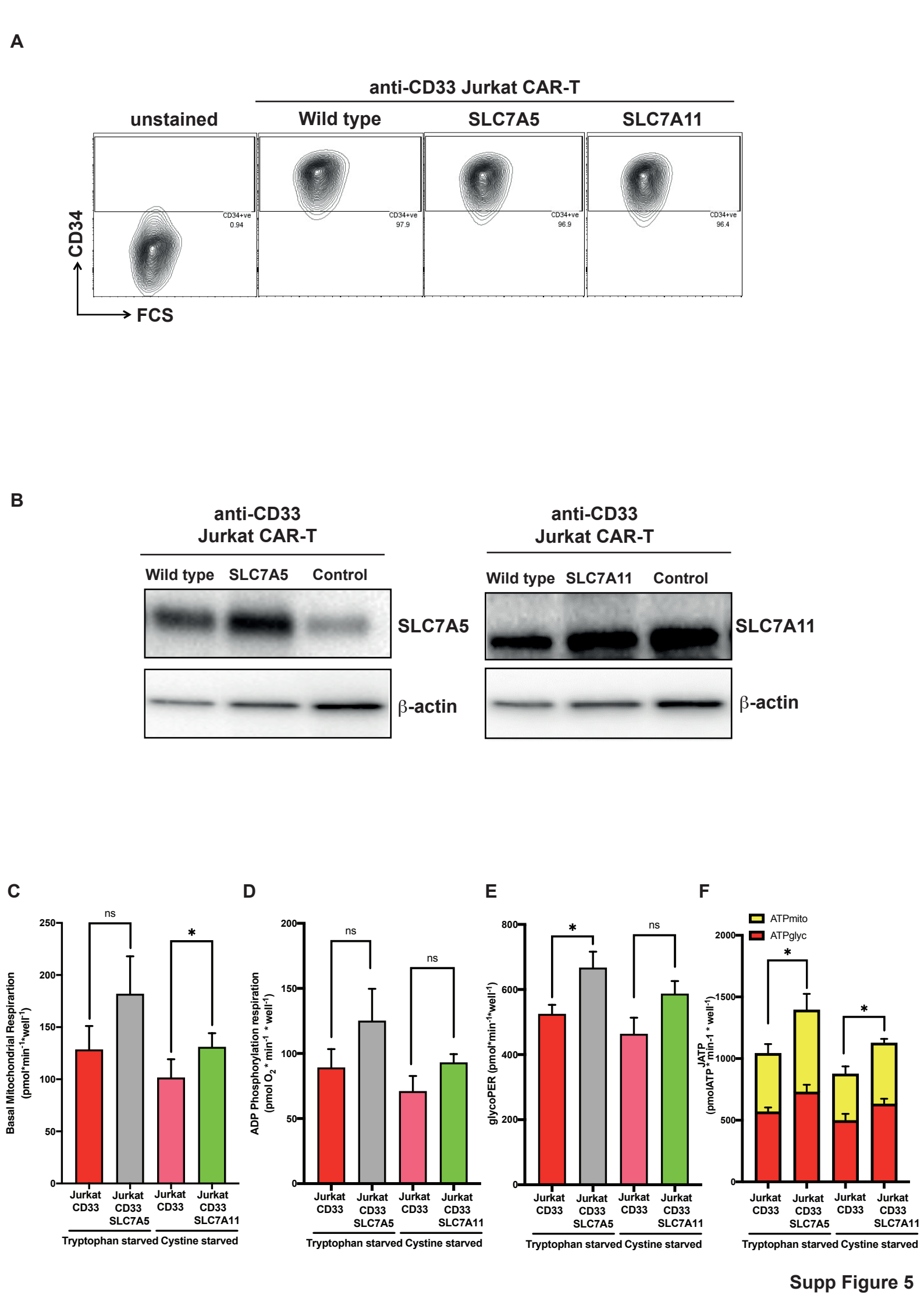

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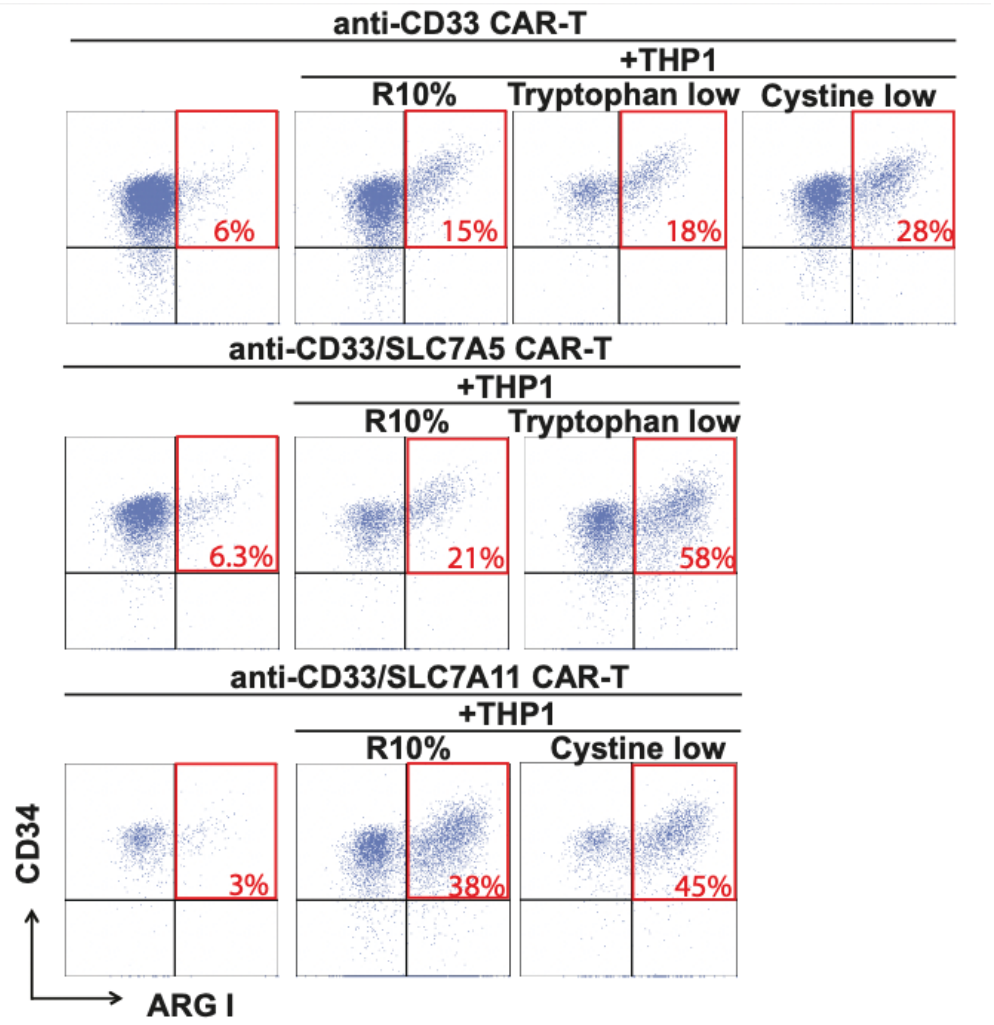

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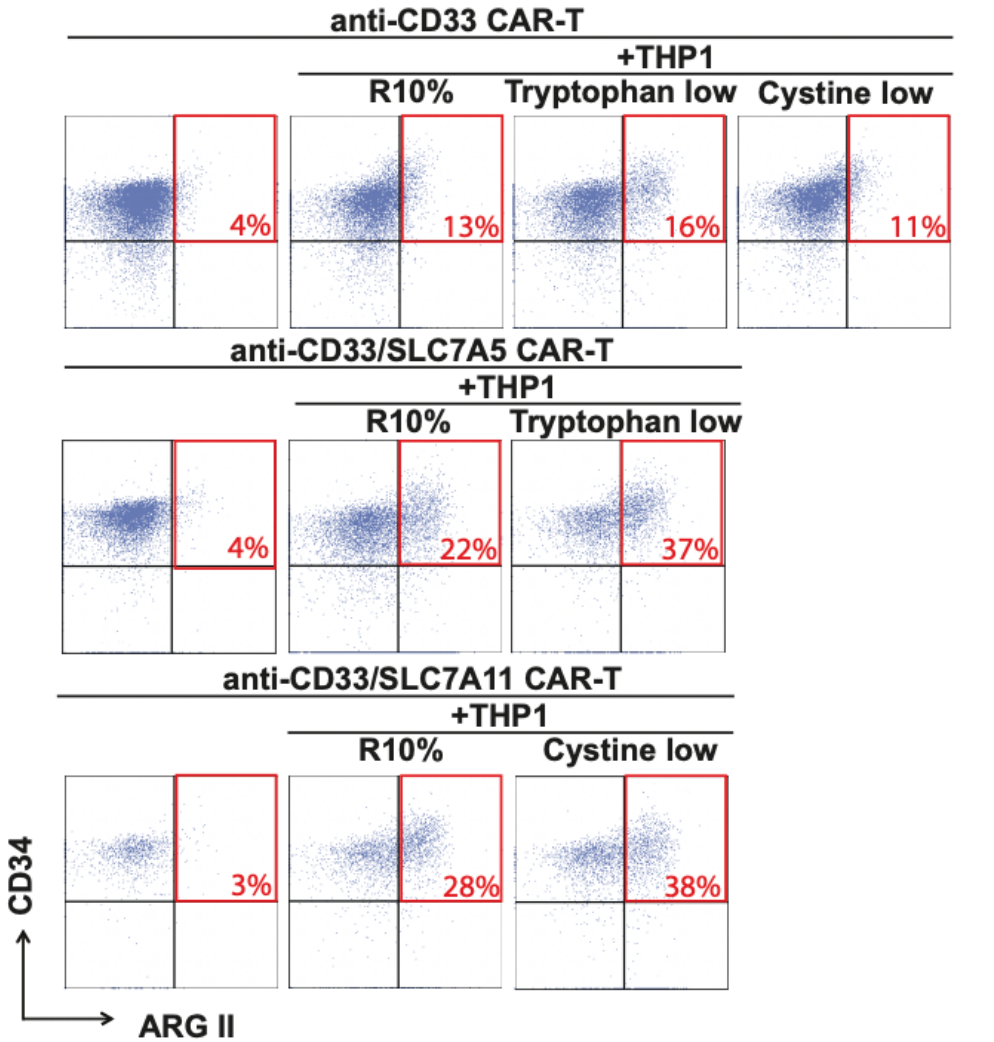

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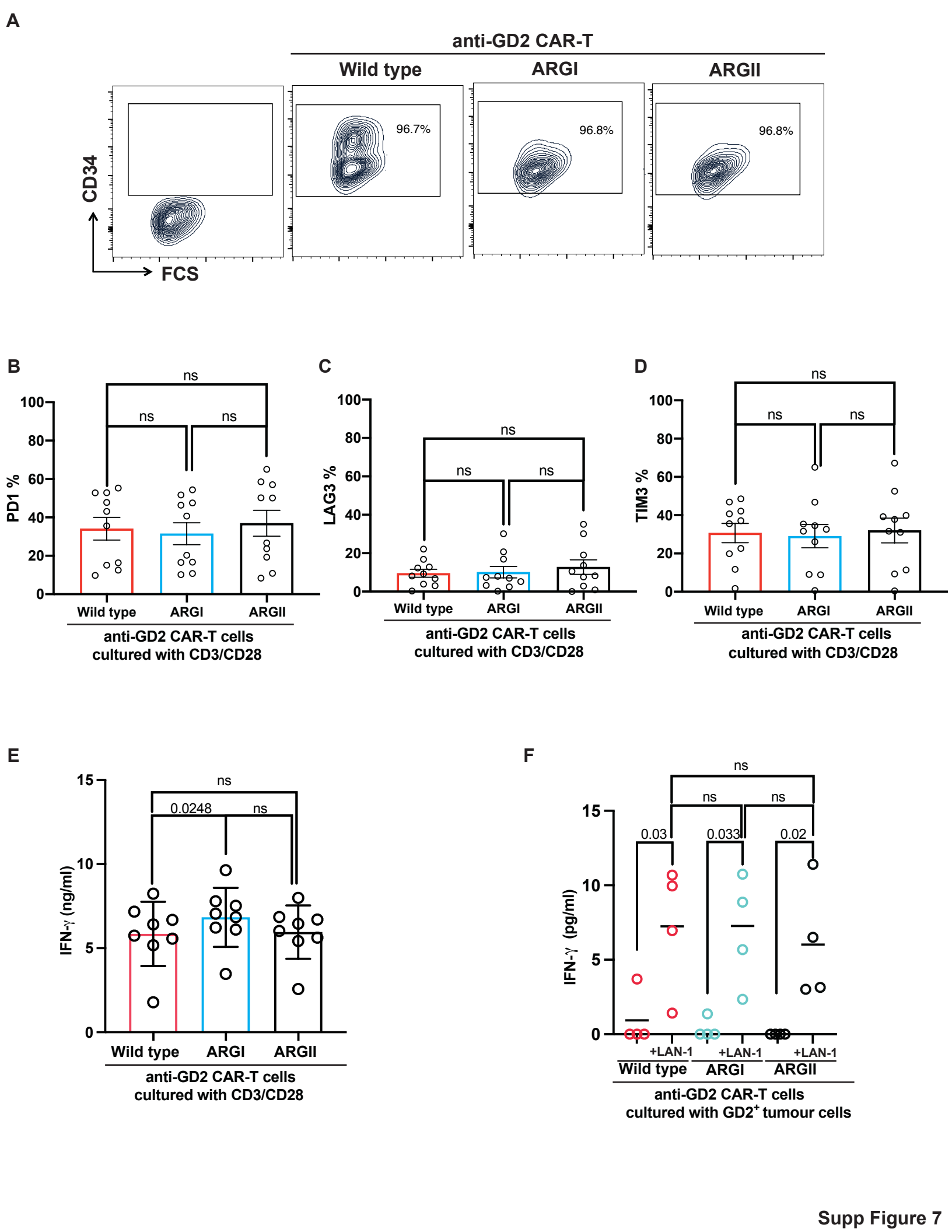

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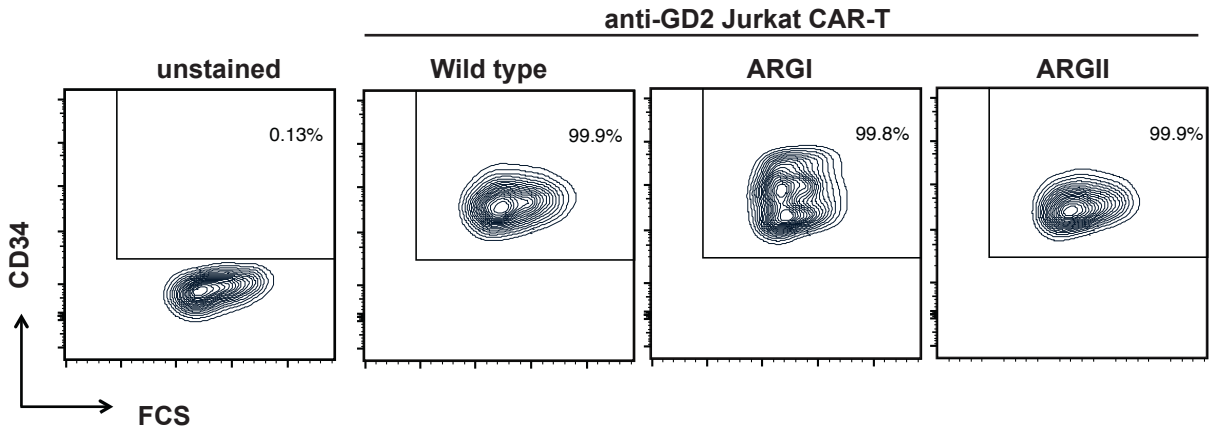

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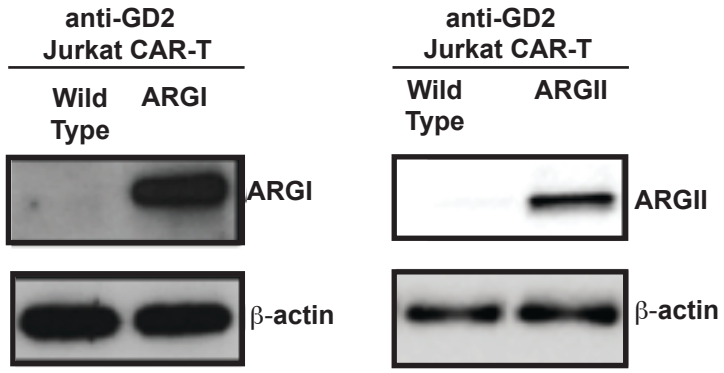

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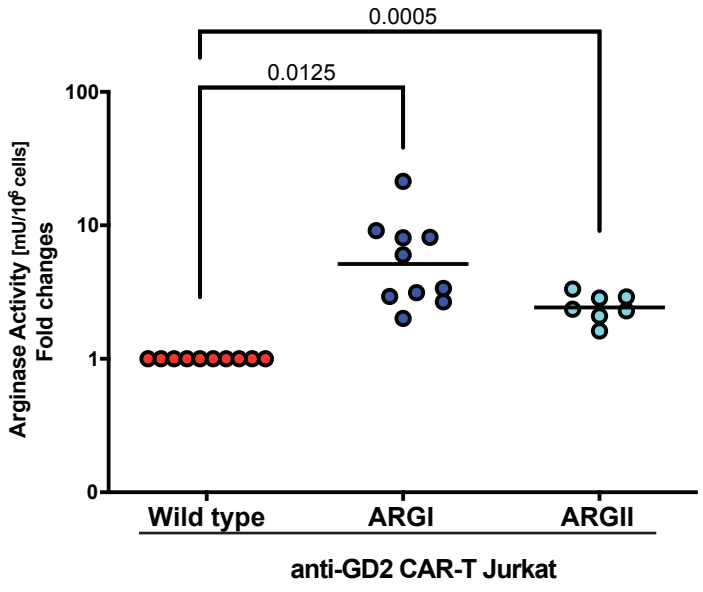
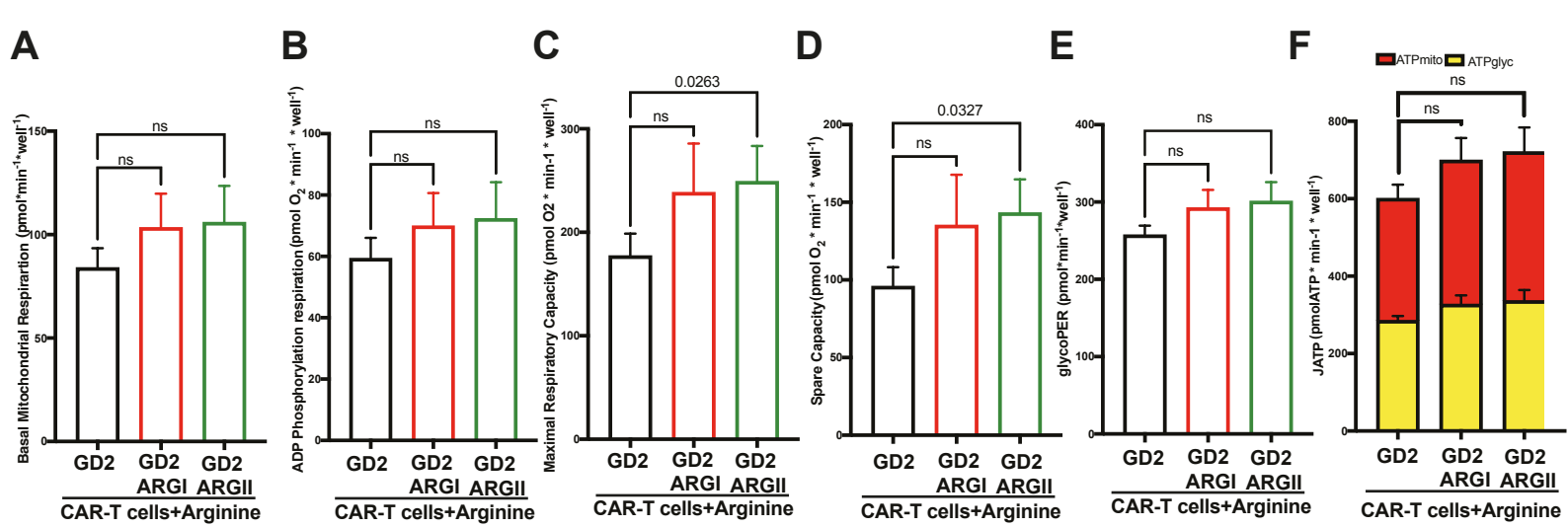
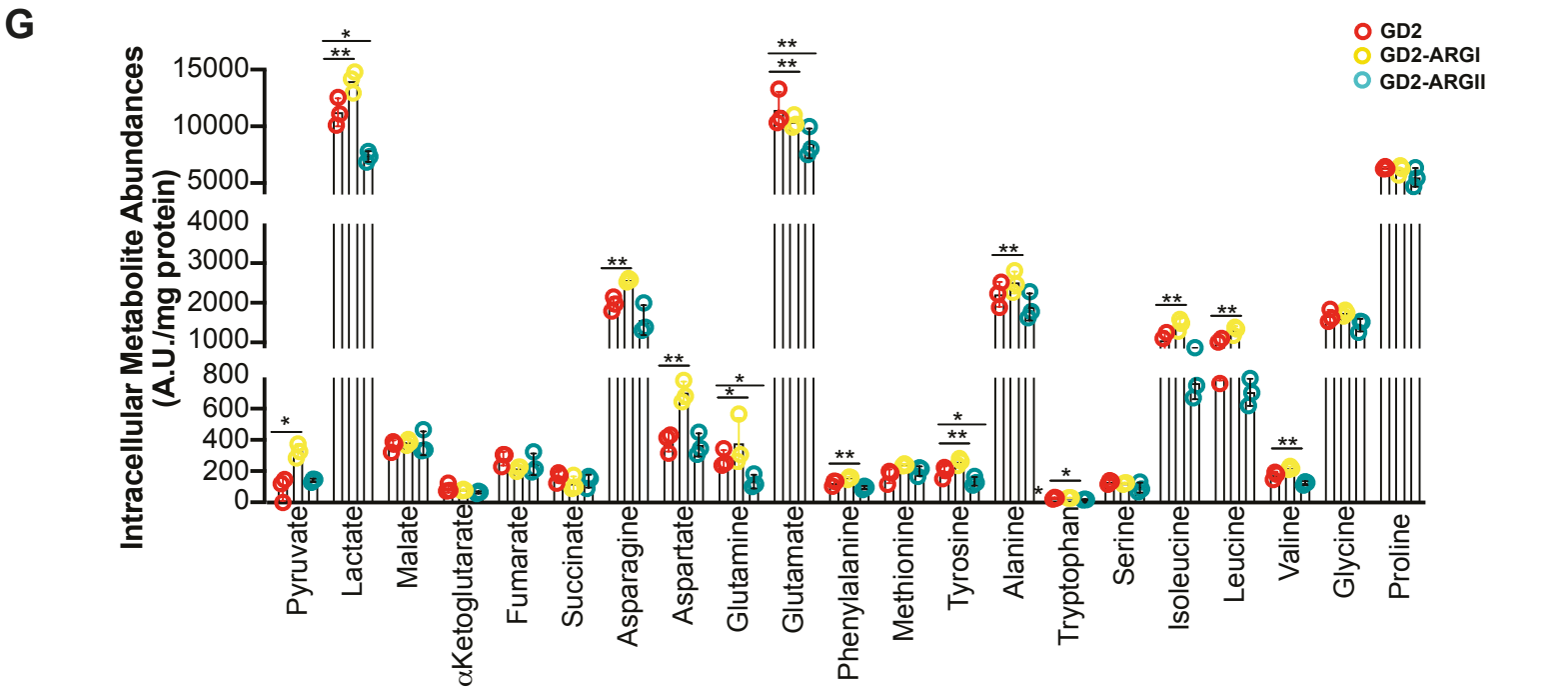
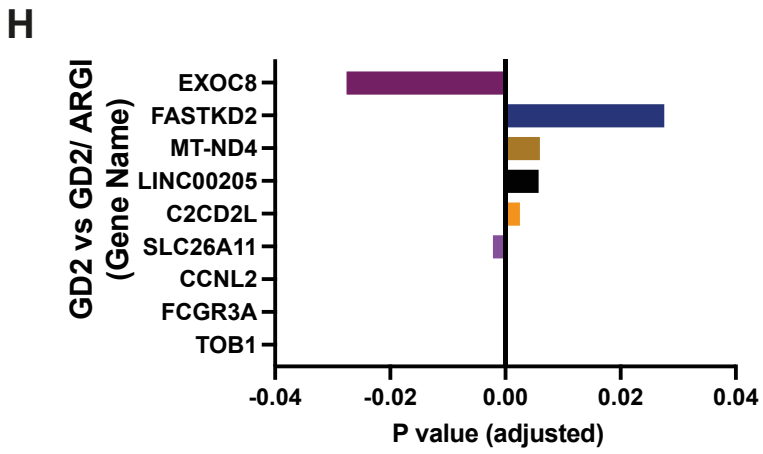
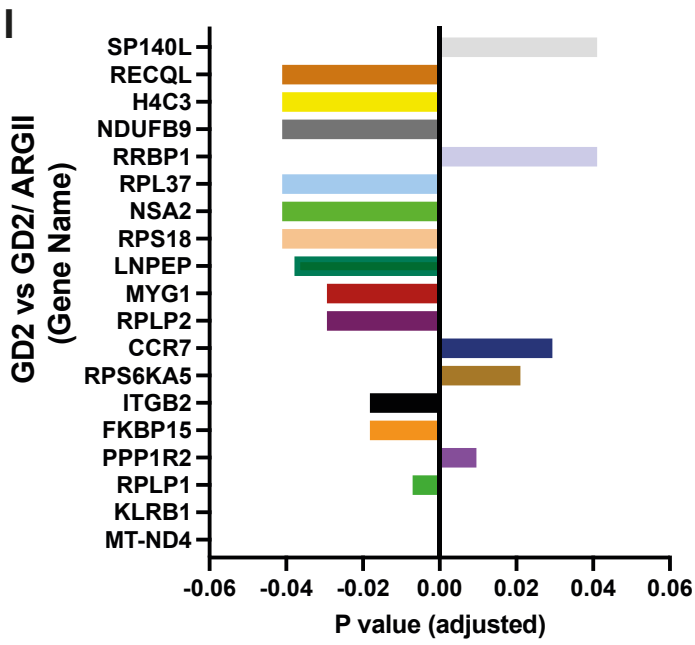


Supp Figure 8

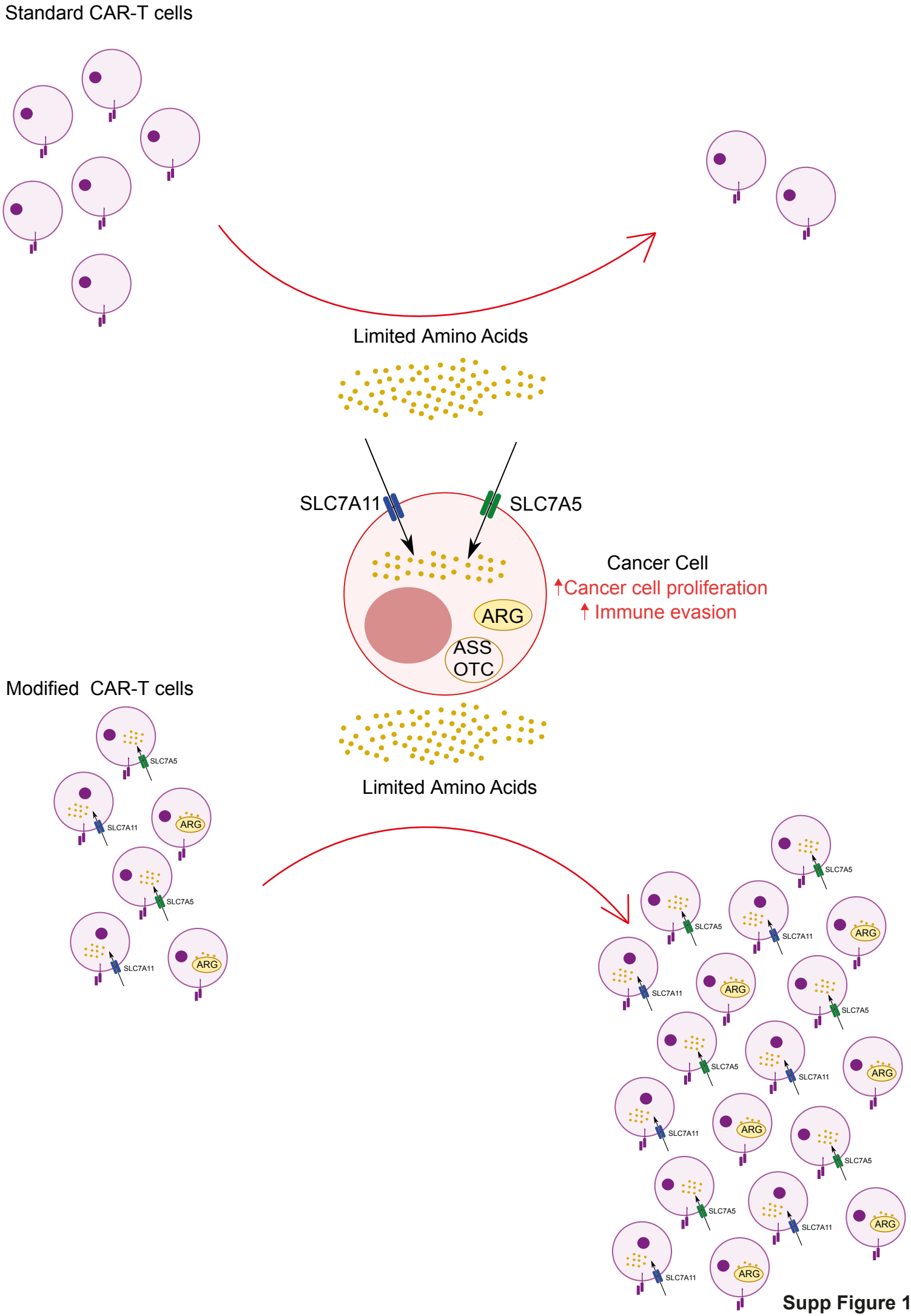








Supp Figure 9



Supp Figure 10