PNAS RESEARCH ARTICLE | IMMUNOLOGY AND INFLAMMATION

Glycogen-fuelled metabolism supports rapid mucosal-associated invariant T cell responses

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Edited by Craig Thompson, Memorial Sloan Kettering Cancer Center, New York, NY; received January 11, 2023; accepted May 12, 2023

Mucosal-associated invariant T (MAIT) cells are a subset of unconventional T cells which recognize a limited repertoire of ligands presented by the MHC class-I like molecule MR1. In addition to their key role in host protection against bacterial and viral pathogens, MAIT cells are emerging as potent anti-cancer effectors. With their abundance in human, unrestricted properties, and rapid effector functions MAIT cells are emerging as attractive candidates for immunotherapy. In the current study, we demonstrate that MAIT cells are potent cytotoxic cells, rapidly degranulating and inducing target cell death. Previous work from our group and others has highlighted glucose metabolism as a critical process for MAIT cell cytokine responses at 18 h. However, the metabolic processes supporting rapid MAIT cell cytotoxic responses are currently unknown. Here, we show that glucose metabolism is dispensable for both MAIT cell cytotoxicity and early (<3 h) cytokine production, as is oxidative phosphorylation. We show that MAIT cells have the machinery required to make (GYS-1) and metabolize (PYGB) glycogen and further demonstrate that that MAIT cell cytotoxicity and rapid cytokine responses are dependent on glycogen metabolism. In summary, we show that glycogen-fueled metabolism supports rapid MAIT cell effector functions (cytotoxicity and cytokine production) which may have implications for their use as an immunotherapeutic agent.

MAIT cells | immunometabolism | cytotoxicity

Significance

Mucosal-associated invariant T (MAIT) cells are a population of innate T cells capable of rapid effector responses. Here, we provide evidence which shows human MAIT cells can make, store and metabolize glycogen. Furthermore, we show that glycogen can fuel rapid MAIT cell responses including targeted cytotoxicity. Thus, this study highlights a novel metabolic pathway in human MAIT cells which may have implications for their use an immunotherapeutic agent.

Mucosal-associated invariant T (MAIT) cells are a population of unconventional T cells which are important in the immune defense against bacterial and viral infections (1–6). MAIT cells are restricted by the MHC like molecule MR1 (5) and recognize a limited set of bacterially derived antigens (7). MAIT cells are primed to respond and display an inherent 'innateness,' with higher levels of effector molecule mRNA at the steady than conventional T cells (8). MAIT cells can be activated either via T cell Receptor (TCR) triggering or innate cytokine stimulation, after which they are capable of producing a range of cytokines and lytic molecules, including IFNγ and granzyme B (9, 10). These rapid effector responses allow MAIT cells to initiate and amplify the immune response, as well as directly targeting infected or transformed cells (11–14). Robust anticancer responses, the ability to activate other anticancer cells (14) and their absence of MHC restriction has highlighted MAIT cells as attractive candidates for immunotherapy (14–16).

Several studies have identified tumor-infiltrating MAIT cells in primary and metastatic lesions (13, 17–19), but often report diminished effector function and loss of key cytokines including IFNγ (13, 17). Therefore, it is important to fully understand the molecular pathways regulating MAIT cell effector responses. Our previous work has demonstrated that MAIT cells undergo metabolic reprogramming in order to provide the energy and biosynthetic intermediates needed to support their robust effector functions (20, 21). We and others have demonstrated that human MAIT cells activated via their TCR for 18 h favor exogenous glucose as their carbon source, and engage in glycolytic metabolism as their primary metabolic program (20, 22). This is mediated by the activation of the critical metabolic regulators mTOR and MYC, which control the expression of nutrient transporters and key enzymes involved in metabolism of glucose (20).

Currently, the metabolic requirements for rapid MAIT cell effector responses such as cytotoxicity are unknown and were the focus of the current study. We show that MAIT cells cocultured with cancer cells pulsed with cognate antigen rapidly (within 2 h) degranulate and induce cell death. We demonstrate that these rapid responses are independent of glucose-fueled glycolytic metabolism, and show for the first time that MAIT cells contain the machinery required to synthesize and metabolize glycogen. We demonstrate that MAIT cells have glycogen stores, and inhibition of glycogenolysis inhibits MAIT cell

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Author contributions: F.C.C., N.K.-M., R.B., A.W., A.B., B.J.J., N.J., L.V.S., D.O'.S., and A.E.H. designed research; F.C.C., N.K.-M., R.B., A.W., A.B., B.B., E.B., B.J.J., O.K.R., and N.J. performed research; F.C.C., N.K.-M., R.B., A.W., A.B., B.B., E.B., B.J.J., O.K.R., N.J., L.V.S., D.O'.S., and A.E.H. analyzed data; and F.C.C., O.K.R., N.J., L.V.S., D.O'.S., and A.E.H. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2300566120/-/DCSupplemental.

Published June 12, 2023.

cytotoxicity and early cytokine responses, which may have implications for the therapeutic use of MAIT cells.

Results

MAIT Cell Respond Rapidly with Target Cell Lysis and Cytokine Production. We first assessed the expression of MR1 expression on two human cancer cell lines, and identified both the K562 myelogenous leukemia and the A549 lung carcinoma cell lines as MR1+, and furthermore, we demonstrate that the addition of 5-ARU-MG increased the expression of MR1 on the surface of K562 (Fig. 1*A*). Next, we demonstrate that MAIT cells respond to both A549 and K562 cells by degranulating (CD107a expression) and this is significantly boosted with the addition of 5-ARU-MG (Fig. 1 *B* and *C*). To confirm that MR1 was required for MAIT cell degranulation in response to K562 cells loaded with 5-ARU-MG, we next blocked MR1 and observed significantly reduced degranulation (Fig. 1 *D* and *E*). To build on these findings, and to confirm if MAIT cells can induce target cell death, we moved to a direct cytotoxicity assay, and demonstrate that MAIT cells can rapidly (within 2 h) kill K562 cells pulsed with 5-ARU-MG, and in a dose-dependent manner (Fig. 1*F*). In addition to cytotoxicity, we also show that MAIT cells can upregulate IFNγ expression within 3 h in response to TCR stimulation (Fig. 1 *G* and *H*).

MAIT Cell Cytotoxicity Is Not Dependent on Glucose Metabolism or Oxidative Phosphorylation (OxPhos). Our previously published data demonstrated that MAIT cells favor exogenous glucose as their carbon source, which is metabolized via glycolysis metabolism (21). We and others have reported that glucose metabolism is critical for MAIT cell IFNγ and granzyme production after 18 h (20, 22). To investigate if MAIT cell cytotoxicity was dependent on glucose metabolism, we utilized the glycolytic inhibitor 2deoxy-D-glucose (2DG) and show no effect on either MAIT cell degranulation or cytotoxicity at 3 h (Fig. 2 *A*–*C*). Studies in other T cell subsets have demonstrated that expression of the glucose transporter (GLUT1) can take up to 6 h (23). So, we next investigated expression of GLUT1 on TCR stimulated MAIT cells, and demonstrate detectable expression takes 6 h (Fig. 2*D*), further supporting the concept that rapid MAIT cell cytotoxicity is not supported by exogenous glucose metabolism. Additionally, we found that rapid upregulation of IFNγ was also not inhibited by addition of 2DG (Fig. 2*E*). Another major metabolic pathway utilized by some T cell subsets is OxPhos. To investigate whether OxPhos supports MAIT cell cytotoxicity we utilized the specific ATP synthase inhibitor oligomycin and show no effect on either MAIT cell degranulation or lysis of target cells (Fig. 2 *F* and *G*).

MAIT Cells Contain the Machinery to Synthesize and Metabolize Glycogen. We next investigated other potential metabolic pathways which may support early MAIT cell cytotoxicity by interrogating our recently published MAIT cell proteomic dataset (21) and identified that MAIT cells express the enzyme glycogen synthase (GYS-1) which is required to synthesize glycogen (Fig. 3*A*). We next investigated whether MAIT cells expressed the enzymes required for the breakdown of glycogen and found the brain isoform of glycogen phosphorylase (PYGB) in our proteomics dataset (Fig. 3*B*). To confirm the expression of these enzymes we utilized flow cytometry and show robust expression of both, with no change upon activation (Fig. 3 *C*–*F*). Finally, we verified expression via western blotting (Fig. 3 *G*–*I*). To investigate whether PYGB was active in MAIT cells we utilized a glycogen PYGB activity assay and observed increased PYGB activity in TCR stimulated MAIT cells (Fig. 3*J*). Finally, we show that MAIT cells contain stored glycogen, and that upon stimulation glycogen is content significantly is reduced (Fig. 3 *K* and *L*).

Glycogen Supports MAIT Cell Cytotoxicity and Early Cytokine Responses. To investigate if glycogen supports MAIT cell antitumor responses, we utilized the glycogen phosphorylase (PYG) inhibitor CP91149. We first investigated if CP91149 inhibited degranulation against A549 cells with or without the addition of 5-ARU-MG, and show that CP91149 significantly limited MAIT cell degranulation triggered by 5-ARU-MG pulsed A549 cells (Fig. 4 *A* and *B*). To confirm this finding, we investigated MAIT cell degranulation in response to 5-ARU-MG pulsed K562 cells, and again demonstrate the inhibition of PYG-limited degranulation (Fig. 4 *C* and *D*). Next, we utilized another glycogen phosphorylase inhibitor (GPI) (23), and again observed reduced MAIT cell degranulation (Fig. 4*E*). We next investigated if inhibiting the breakdown of glycogen limited MAIT cell target cell lysis and demonstrate a significant reduction in killing (Fig. 4*F*). Another protective function of MAIT cells is their robust production of effector molecules like IFNγ and granzyme B. Previous work in mice, demonstrated that memory T cell cytokine production is dependent on glycogen metabolism, we confirm these findings in human memory T cell (*SI Appendix*, Fig. S1). To this end we investigated if early IFNγ cytokine production (<3 h) by MAIT cells is dependent on the metabolism of glycogen and show that rapid IFNγ production is dependent on glycogen breakdown (Fig. 4 *G* and *H*). Similarly, granzyme B secretion was also inhibited with the addition of CP91149 (Fig. 4*I*). Since glycogen is metabolized into G6P and then fed into the glycolytic machinery, we blocked the glycolytic machinery further down that pathway using the GAPDH inhibitor heptelidic acid and show diminished MAIT cell degranulation (Fig. 4*J*). To further support the concept that glycogen supports early MAIT cell metabolic process we performed seahorse analysis on CP91149-treated MAIT cells after 3 h of stimulation and show that early glycolysis is dependent on the breakdown of glycogen (Fig. 4 *K* and *L*).

Discussion

MAIT cells are a subset of unconventional T cells which due to their potent effector functions and abundance have been shown to play an important role in the host defense against pathogens and malignancies (1, 24) and are now under investigation as a potential immunotherapeutic agent (15, 25). MAIT cells have been detected in both primary cancers and metastatic sites, however they are dysfunctional, losing their antitumor functions (13, 17, 18, 26). Therefore, it is critical to understand the molecular and metabolic requirements for MAIT cell effector responses.

In the current study, we confirm the robust cytotoxic potential of MAIT cells, with rapid degranulation and dose-dependent killing of both A549 and K562 target cells. We also demonstrate that MAIT cell cytotoxicity of cancerous cells is dependent on MR1 and boosted in the presence of antigen, confirming work in the setting of bacterial and virally infected cells (9, 12). Although cancer specific antigens for MAIT cells have yet to be identified (27), the loading of cancer metabolites onto MR1 has been described (28, 29). In addition, there is evidence emerging for microbial activation of tumor-infiltrating MAIT cells (30).

Currently, data on the molecular regulation of MAIT cell cytotoxicity remains unclear and will be necessary as they move toward therapeutic targets. Our group and others have previously highlighted the importance of glucose metabolism for MAIT cell effector functions such as cytokine production and proliferation (20, 22). We have also reported how altered MAIT cell metabolism underpins defective functions in obesity, potentially driving pathogenic MAIT

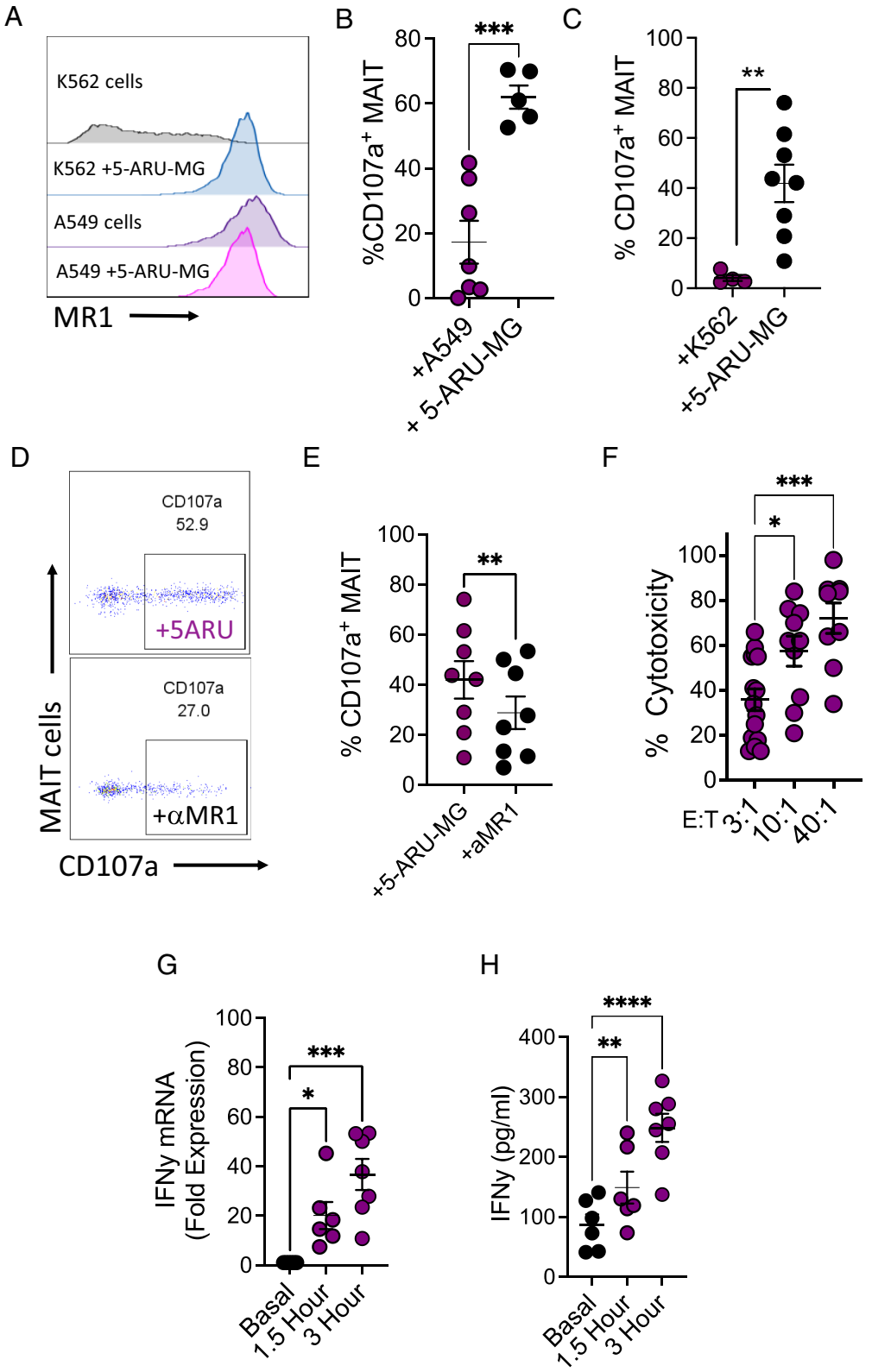

Fig. 1. MAIT cell respond rapidly with target cell lysis and cytokine production. (*A*) Flow cytometry histogram displaying MR1 expression on the surface of either K562 cells or A549 cells in the absence or presence of exogenous 5-ARU-MG. (*B*) Scatter plot showing CD107a expression on MAIT cells cultured with A549 cells or A549 cells pulsed with 5-ARU-MG. (*C*) Scatter plot showing CD107a expression on MAIT cells cultured with K562 or K562 pulsed with 5-ARU-MG. (*D* and *E*) Flow cytometry dot plots and scatter plot showing CD107a expression on MAIT cells cultured with K562 pulsed with 5-ARU-MG in the absence or presence of MR1 blocking antibody. (*F*) Scatterplot showing dose-dependent (effector to target ratio) cytotoxicity of IL-2 expanded MAIT cells in their targeting of K562 cells pulsed with 5-ARU-MG. (*G* and *H*) Scatter plots of IFNγ mRNA and secreted protein levels from IL-2 expanded MAIT cells stimulated with TCR beads (antiCD3/CD28) for either 1.5 h or 3 h. ns = not significant, *= $P > 0.05$, **= $P < 0.01$, ***= $P < 0.001$, ***= $P < 0.0001$ as measured by paired *t* test, Friedman test or mixed-effects analysis where appropriate.

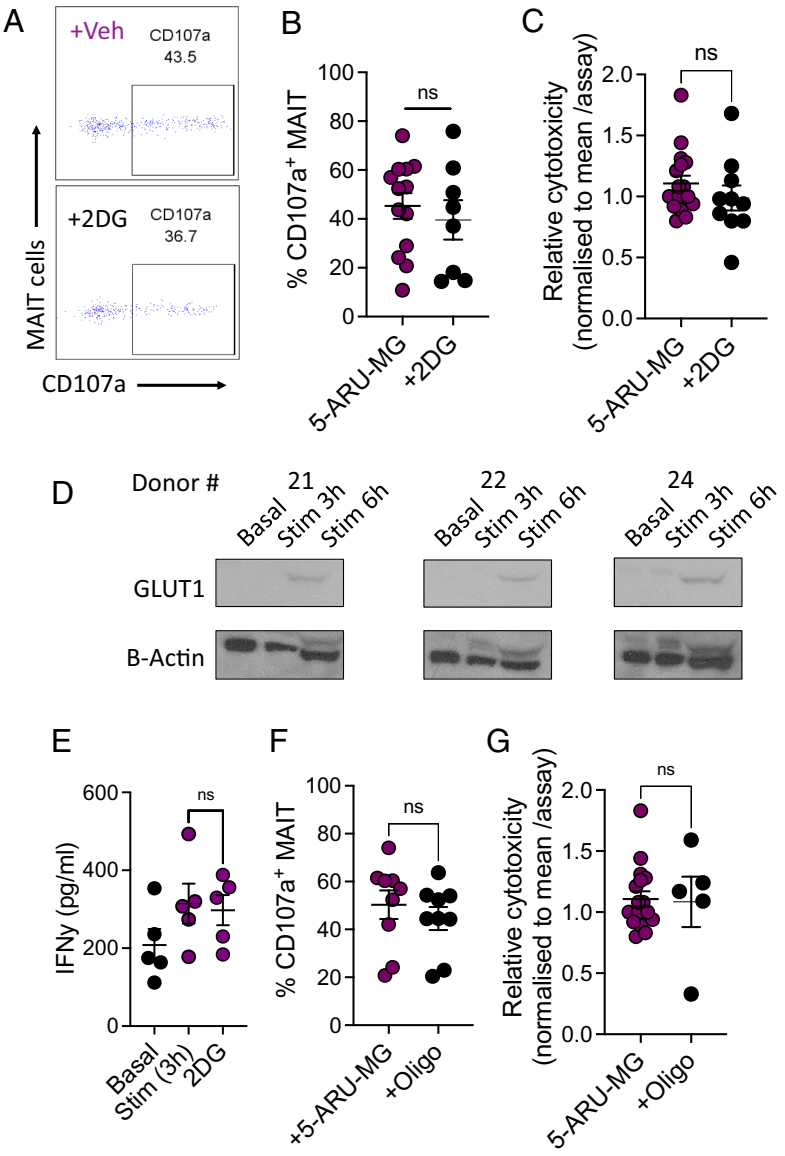

Fig. 2. MAIT cell cytotoxicity is not dependent on glucose metabolism or oxidative phosphorylation. (*A* and *B*) Flow cytometry dot plot and scatter plot showing CD107a expression on ex vivo MAIT cells in response to stimulation with K562 cells (pulsed with 5ARU-MG) with or without the addition of glycolytic inhibitor 2DG. (*C*) Scatterplot showing the impact of 2DG treatment on cytotoxic capacity of IL-2 expanded MAIT cells, displayed as fold change per sample. (*D*) Western Blot of GLUT1 protein expression by IL-2 expanded MAIT cells (three individual donors) at rest (basal) and after 3 h or 6 h of TCR bead stimulation. (*E*) Scatter plot showing the impact of 2DG treatment on early IFNγ secretion by IL-2 expanded MAIT cells stimulated with TCR beads. (*F*) Scatter plot showing CD107a expression on ex vivo MAIT cells cultured with K562 (pulsed with 5ARU-MG) in the absence or presence of the oxidative phosphorylation inhibitor oligomycin. (*G*) Scatterplot the impact of oligomycin treatment on the cytotoxic capacity of IL-2 expanded MAIT cells, displayed as fold change per sample. ns = not significant, as measured by paired *t* test, Wilcoxon test or Mann-Whitney test as appropriate.

cells (20, 31, 32). Here, we demonstrate that rapid MAIT cell cytotoxicity (and early cytokine production) is independent of exogenous glucose metabolism. In our search for an alternative carbon source, we observed that MAIT cells have the molecular machinery to synthesize and metabolize glycogen. Glycogen is the main energy storage form of glucose in the body, stored as a quickly mobilized multi-branched polysaccharide (33). Recent work by Zhang and colleagues reported that murine memory CD8+ T cells but not naïve CD8+ T cells could also synthesize and metabolize glycogen (23). We confirmed these findings in human memory T cells and hypothesized that MAIT cells, due to their "innateness" (8) might utilize glycogen to support their rapid functional responses. Using a series of experiments, we show that MAIT cell cytotoxicity and rapid cytokine responses (<3 h) are dependent on the breakdown of glycogen, supporting the concept that stored glycogen fuels rapid responses in innate effector T cells like MAIT cells and memory T cells. This is further supported by work in another innate immune subset, dendritic cells, which also utilize glycogen to fuel their rapid responses (34). Our data suggests that TCR triggering activates PYGB to break down glycogen, which then feeds glycolysis. This again is supported by the recent publication in murine memory CD8+ T cells, where the inhibition of glycolysis at the first enzyme (hexokinase) had no impact but inhibition further down the glycolytic pathway limited cellular responses (23).

Understanding the carbon sources required fuel MAIT cell effector functions may be of particular importance in the setting of cancer where limited glucose has been shown to impair T cell responses (35, 36). The ability of human MAIT cells to use stored glycogen to fuel their cytotoxicity, paired with their rapid functional responses, unrestricted properties and relative abundance further highlights their potential as an exciting candidate for cancer immunotherapy. In conclusion, we describe for the first time

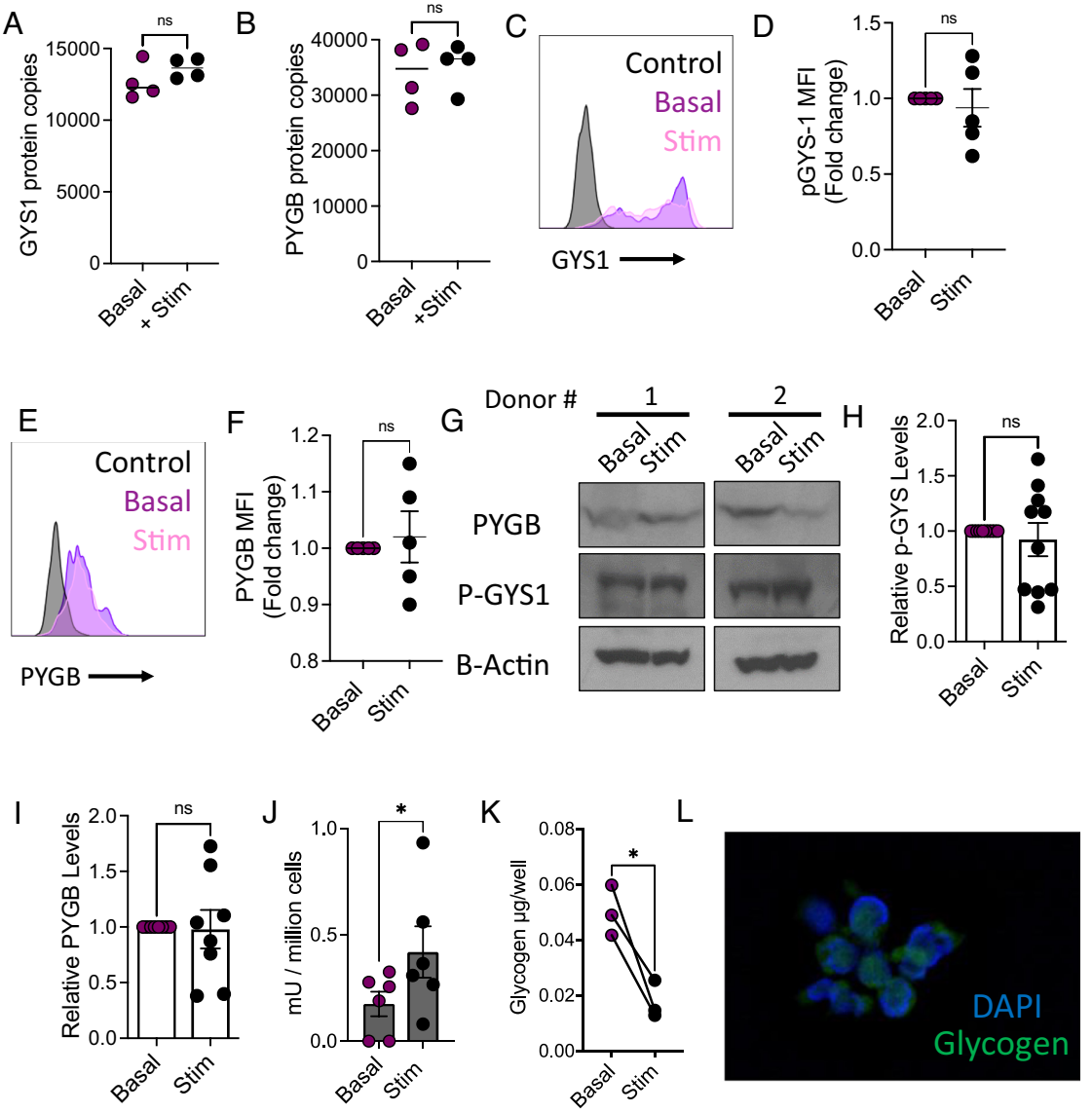

Fig. 3. MAIT cells contain the machinery to synthesize and metabolize glycogen. (*A* and *B*) Scatter plot showing the expression of GYS1 or PYGB in IL-2 expanded MAIT cells, either basal or stimulated for 18 h with anti-CD3/CD28 TCR beads and IL-18 for 18 h (data extrapolated from published proteomic dataset). (*C–F*) Representative flow cytometric histograms and scatter plots showing the expression of GYS1 or PYGB in ex-vivo MAIT cells, either basal or stimulated with anti-CD3/CD28 TCR beads for 18 h (*G–I*) Western blot and densitometry scatter plots showing the expression of phosphorylated GYS1 or PYGB in IL-2–expanded MAIT cells either basal or stimulated with anti-CD3/CD28 TCR beads for 6 h. (*J*) Scatter plot showing glycogen phosphorylase activity in TCR-stimulated MAIT cells. (*K*) Scatter plot showing glycogen levels in IL-2 expanded MAIT cells at rest (basal) or stimulated with TCR beads for 3 h. (*L*) Florescent microscopy image demonstrating the presence of glycogen in MAIT cells. ns = not significant, $P > 0.05$, *= $P < 0.05$ as measured by paired *t* test.

a novel metabolic pathway in human MAIT cells necessary for their rapid effector responses, further supporting the rationale for their use as an immunotherapeutic.

Materials and Methods

Study Cohorts and Ethical Approval. Full ethical approval was obtained from both St Vincent's University Medical Ethics Committee and Maynooth University Ethics Committee. We recruited a cohort of healthy adult donors from St Vincent's Healthcare Group. Inclusion criteria included ability to give informed consent, 18 to 55 y of age and a BMI<28. Exclusion criteria included current or recent (<2 wk) infection, current smoker, use of immunomodulatory or anti-inflammatory medications. All participants provided full consent.

Preparation of Peripheral Blood Mononuclear Cells (PBMC) and Expanded MAIT Cells. PBMC samples were isolated by density centrifugation over ficoll from fresh peripheral blood samples. PBMCs were either stored at −70 °C or used for MAIT cell expansion using 5-ARU-MG and IL-2 as previously described (21).

MAIT Cell Degranulation Assay. PBMCs were thawed and rested before addition of either metabolic inhibitors or vehicle control (i.e., 1 mM 2DG, 100 µM CP91149, 50 µM GPI (CP316819), 5 µM Heptelidic acid or dimethyl sulfoxide (DMSO)/water). A549 cells or K562 cells with or without pre-treatment with 5-ARU-MG were then cocultured with the PBMCs at a ratio of 10:1 PBMC:Target plus CD107a antibody (Miltenyi). After 30 min, protein transport inhibitor cocktail (Invitrogen) was added and cultured for further 2 h. MAIT cells were identified by flow cytometry with staining using specific surface monoclonal antibodies, namely CD3, CD161, and TCRVα7.2 (all Miltenyi), and degranulation assessed according to percentage of MAIT cells expressing CD107a. Cell populations were acquired using a Attune NXT flow cytometer and analyzed using FlowJo software (Treestar). Results are expressed as a percentage of the parent population as indicated and determined using flow minus-1 (FMO) and unstained controls.

MAIT Cell Cytotoxicity Assay. IL-2–expanded MAIT cells (21) were co-cultured with Calcein AM labelled K562 cells at a ratio of 3:1, MAIT cells to targets (and other ratios for dose curve) in the absence or presence of metabolic inhibitors or vehicle control (i.e., 1 mM 2DG, 100 µM CP91149, 50 µM GPI (CP316819),

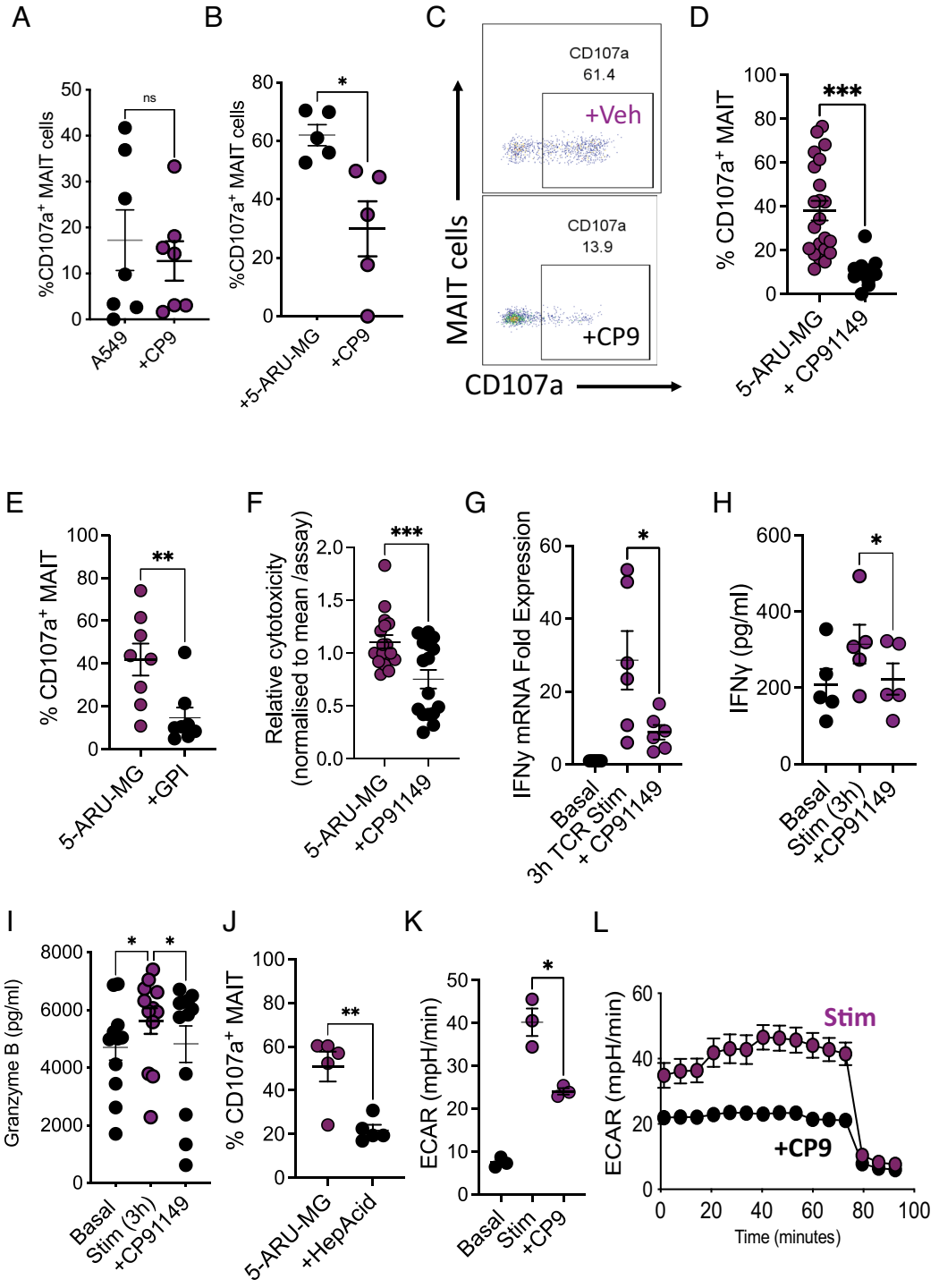

Fig. 4. Glycogen supports MAIT cells cytotoxicity and early cytokine responses. (*A* and *B*) Scatter plots showing CD107a expression on MAIT cells cultured with A549 cells alone or A549 cell pulsed with 5ARU-MG, in the absence or presence of the glycogen phosphorylase inhibitor CP91149. (*C* and *D*) Flow cytometry dot plot and scatter plot showing CD107a expression on MAIT cells cultured with K562 cells (pulsed with 5ARU-MG) in the absence or presence of the glycogen phosphorylase inhibitor CP91149. (*E*) Scatter plot showing CD107a expression on MAIT cells cultured with K562 cells (pulsed with 5ARU-MG) in the absence or presence of the glycogen phosphorylase inhibitor GPI. (*F*) Scatterplot showing the impact of CP91149 on cytotoxic capacity of MAIT cells against K562 cells, displayed as fold change per sample. (*G* and *H*) Scatter plot showing IFNγ mRNA levels or secreted protein from IL-2 expanded MAIT cells stimulated with TCR beads (for 3 h) in the absence or presence of CP91149. (*I*) Scatter plot showing granzyme B secreted protein from IL-2 expanded MAIT cells stimulated with TCR beads (for 3 h) in the absence or presence of CP91149. (*J*) Scatter plot showing CD107a expression on MAIT cells in response to stimulation with K562 (pulsed with 5ARU-MG) in the absence or presence of the GAPDH inhibitor heptelidic acid. (*K* and *L*) Scatter plot and Seahorse trace displaying ECAR rates in TCR bead-stimulated (3 h) IL-2 expanded MAIT cells treated with CP91149. *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$ as measured by Wilcoxon test, paired *t* test or Mann–Whitney test where appropriate.

5 μM Heptelidic acid or DMSO/water). After 2 h of coculture, supernatant was analyzed using a Spectramax plate reader to measure supernatant fluorescence at 485 nm excitation and 525 nm emission and percentage killing calculated as a proportion of max killing by Triton X.

MAIT Cell Cytokine Analysis. IFNγ mRNA measured in IL-2 expanded MAIT cells [with or without stimulation with CD3/CD28 beads (Gibco)] and their culture supernatant by rtPCR. Secreted IFNγ and granzyme B protein was measured in IL-2 expanded MAIT cells [with or without stimulation with CD3/CD28 beads (Gibco)]

and their culture supernatant by ELISA. To investigate the metabolic requirements of early cytokine responses, activated MAIT cells were treated with metabolic inhibitors or vehicle control (i.e., 1 mM 2DG, 100 μM CP91149 or DMSO/water). mRNA was extracted from MAIT cells using TRIzol according to the manufacturer's protocol. The synthesis of cDNA was performed using qScript cDNA Synthesis kit (QuantaBio). Real time RT-qPCR was performed using PerfeCTa SYBR Green FastMix Reaction Mix (Green Fastmix, ROX™) (QuantaBio) and KiCqStart primer sets (Sigma). Enzyme-Linked Immunosorbent Assay (ELISA) was performed as per the manufacturer's instructions (R&D Systems).

MAIT Cell Glycogen Machinery Analysis. The identification of GYS-1 and PYGB in MAIT cells was based on in silico analysis of a published MAIT cell proteomic dataset (21). The expression was confirmed using both flow cytometry on ex vivo MAIT cells and via western blotting on IL-2–expanded MAIT cells, both stimulated with CD3/CD28 TCR dynabeads for 6 h. For flow cytometry, PBMC were stimulated as described then surface-stained for MAIT cells before fix/perm using True-Nuclear Transcription Factor Buffer Set (Biolegend) then intracellularly stained with antibodies specific for p-GYS-1 (cell signaling) or PYGB (cell signaling). For western blotting, cells were lysed in NP-40 lysis buffer [50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 1% (w/v) IgePal, and complete protease inhibitor mixture (Roche)]. Samples were resolved using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes before analysis with anti-GYS-1 (cell signaling), PYGB (cell signaling) and anti-b-Actin (Sigma) antibodies. Protein bands were visualized using enhanced chemiluminescence.

MAIT Cell Glycogen Content and PYGB Activity Analysis. Glycogen content in MAIT cells [either basally or stimulated for 3 h with anti-TCR beads (Gibco)] was measured using BioVision glycogen kit according to the manufacturer's instructions, and by fluorescent microscopy using a previously published method (37). Glycogen PYGB activity in MAIT cells [either basally or stimulated for 3 h with anti-TCR beads (Gibco)] was measured using Sigma-Alrich Glycogen PYGB Colorimetric Assay Kit according to the manufacturer's instructions.

MAIT Cell Seahorse Analysis. Expanded MAIT cells were treated with metabolic inhibitors or vehicle control (i.e., 1 mM 2DG, 100 μM CP91149, or DMSO/water) and then stimulated with CD3/CD28 Dynabeads. After 3 h of stimulation, Seahorse metabolic flux analysis was performed according to the Seahorse instruction manual.

Statistics. Statistical analysis was completed using GraphPad Prism 9 Software (USA). Data are expressed as mean ± SEM. We determined differences between two groups using student *t* test and Mann–Whitney *U* test where appropriate. Analysis across three or more groups was performed using ANOVA. Statistical significance was defined as $P < 0.05$.

Data, Materials, and Software Availability. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD041544 (21). All study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. This study is supported by the National Children's Research Centre. N.K.-M. is supported by Health Research Board (ILP-POR-2019-110). Financial support for the Attune NxT, BMG Clariostar multi-mode microplate reader & Seahorse Analyzer was provided to Maynooth University Department of biology by Science Foundation Ireland (16/RI/3399).

1. D. I. Godfrey, H. F. Koay, J. McCluskey, N. A. Gherardin, The biology and functional importance of MAIT cells. *Nat. Immunol.* **20**, 1110–1128 (2019).
2. L. Le Bourhis *et al.*, Antimicrobial activity of mucosal-associated invariant T cells. *Nat. Immunol.* **11**, 701–708 (2010).
3. B. van Wilgenburg *et al.*, MAIT cells are activated during human viral infections. *Nat. Commun.* **7**, 11653 (2016).
4. E. Treiner *et al.*, Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* **422**, 164–169 (2003).
5. L. Kjer-Nielsen *et al.*, MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* **491**, 717–723 (2012).
6. A. J. R. Cooper, J. Clegg, F. C. Cassidy, A. E. Hogan, R. M. McLoughlin, Human MAIT cells respond to staphylococcus aureus with enhanced anti-bacterial activity. *Microorganisms* **10**, 148 (2022).
7. A. J. Corbett *et al.*, T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* **509**, 361–365 (2014).
8. M. Gutierrez-Arcelus *et al.*, Lymphocyte innateness defined by transcriptional states reflects a balance between proliferation and effector functions. *Nat. Commun.* **10**, 687 (2019).
9. A. Kurioka *et al.*, MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. *Mucosal Immunol.* **8**, 429–440 (2015).
10. B. van Wilgenburg *et al.*, MAIT cells are activated during human viral infections. *Nat. Commun.* **7**, 11653 (2016).
11. N. M. Provine *et al.*, MAIT cell activation augments adenovirus vector vaccine immunogenicity. *Science* **371**, 521–526 (2021).
12. H. Flament *et al.*, Outcome of SARS-CoV-2 infection is linked to MAIT cell activation and cytotoxicity. *Nat. Immunol.* **22**, 322–335 (2021).
13. A. M. Melo *et al.*, Mucosal-associated invariant T cells display diminished effector capacity in oesophageal adenocarcinoma. *Front. Immunol.* **10**, 1580 (2019).
14. E. V. Petley *et al.*, MAIT cells regulate NK cell-mediated tumor immunity. *Nat. Commun.* **12**, 4746 (2021).
15. D. I. Godfrey, J. Le Nours, D. M. Andrews, A. P. Uldrich, J. Rossjohn, Unconventional T cell targets for cancer immunotherapy. *Immunity* **48**, 453–473 (2018).
16. P. Sundström *et al.*, Tumor-infiltrating mucosal-associated invariant T (MAIT) cells retain expression of cytotoxic effector molecules. *Oncotarget* **10**, 2810–2823 (2019).
17. P. Sundström *et al.*, Human mucosa-associated invariant T cells accumulate in colon adenocarcinomas but produce reduced amounts of IFN-γ. *J. Immunol.* **195**, 3472–3481 (2015).
18. M. Duan *et al.*, Activated and exhausted MAIT cells foster disease progression and indicate poor outcome in hepatocellular carcinoma. *Clin. Cancer Res.* **25**, 3304–3316 (2019).
19. T. Yao, P. Shooshtari, S. M. M. Haeryfar, Leveraging public single-cell and bulk transcriptomic datasets to delineate MAIT cell roles and phenotypic characteristics in human malignancies. *Front. Immunol.* **11**, 1691 (2020).
20. A. O'Brien *et al.*, Obesity reduces mTORC1 activity in mucosal-associated invariant T cells, driving defective metabolic and functional responses. *J. Immunol.* **202**, 3404–3411 (2019).
21. N. Kedia-Mehta *et al.*, The proliferation of human mucosal-associated invariant T cells requires a MYC-SLC7A5-glycolysis metabolic axis. *Sci Signal* **16**, eabo2709 (2023).
22. M. E. Zinser *et al.*, Human MAIT cells show metabolic quiescence with rapid glucose-dependent upregulation of granzyme B upon stimulation. *Immunol. Cell Biol.* **96**, 666–674 (2018).
23. H. Zhang *et al.*, TCR activation directly stimulates PYGB-dependent glycogenolysis to fuel the early recall response in CD8(+) memory T cells. *Mol. Cell* **82**, 3077–3088.e3076 (2022).
24. C. O'Neill, F. C. Cassidy, D. O'Shea, A. E. Hogan, Mucosal associated invariant T cells in cancer-friend or foe? *Cancers (Basel)* **13**, 1582 (2021).
25. T. Parrot *et al.*, Expansion of donor-unrestricted MAIT cells with enhanced cytolytic function suitable for TCR-redirection. *JCI Insight* **6**, e140074 (2021).
26. L. Ling *et al.*, Circulating and tumor-infiltrating mucosal associated invariant T (MAIT) cells in colorectal cancer patients. *Sci. Rep.* **6**, 20358 (2016).
27. L. Kjer-Nielsen *et al.*, An overview on the identification of MAIT cell antigens. *Immunol. Cell Biol.* **96**, 573–587 (2018).
28. M. D. Crowther *et al.*, Genome-wide CRISPR-Cas9 screening reveals ubiquitous T cell cancer targeting via the monomorphic MHC class I-related protein MR1. *Nat. Immunol.* **21**, 178–185 (2020).
29. M. Lepore *et al.*, Functionally diverse human T cells recognize non-microbial antigens presented by MR1. *Elife* **6**, e24476 (2017).
30. S. Li *et al.*, Human tumor-infiltrating MAIT cells display hallmarks of bacterial antigen recognition in colorectal cancer. *Cell Rep. Med.* **1**, 100039 (2020).
31. A. O. Brien *et al.*, Targeting mitochondrial dysfunction in MAIT cells limits IL-17 production in obesity. *Cell Mol. Immunol.* **17**, 1193–1195 (2020).
32. R. Bergin *et al.*, Mucosal-associated invariant T cells are associated with insulin resistance in childhood obesity, and disrupt insulin signalling via IL-17. *Diabetologia* **65**, 1012–1017 (2022).
33. M. M. Adeva-Andany, M. Gonzalez-Lucan, C. Donapetry-Garcia, C. Fernandez-Fernandez, E. Ameneiros-Rodriguez, Glycogen metabolism in humans. *BBA Clin.* **5**, 85–100 (2016).
34. P. M. Thwe *et al.*, Cell-intrinsic glycogen metabolism supports early glycolytic reprogramming required for dendritic cell immune responses. *Cell Metab.* **26**, 558–567.e555 (2017).
35. C. H. Chang *et al.*, Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* **162**, 1229–1241 (2015).
36. P. C. Ho *et al.*, Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* **162**, 1217–1228 (2015).
37. M. Ovecka *et al.*, A sensitive method for confocal fluorescence microscopic visualization of starch granules in iodine stained samples. *Plant Signal Behav.* **7**, 1146–1150 (2012).