Glycogen fuelled metabolism supports rapid Mucosal Associated Invariant T cell responses

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Abstract

Mucosal Associated Invariant T (MAIT) cells are a subset of unconventional T cells which recognise 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 hours. 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 hours) 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-fuelled metabolism supports rapid MAIT cell effector functions (cytotoxicity and cytokine production) which may have implications for their use as an immunotherapeutic agent.

Significance Statement

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.

65 **Introduction**

66 Mucosal Associated Invariant T (MAIT) cells are a population of unconventional T
67 cells which are important in the immune defence against bacterial and viral
68 infections[1, 2, 3, 4, 5, 6]. MAIT cells are restricted by the MHC like molecule MR1[5], and
69 recognise a limited set of bacterially derived antigens[7]. MAIT cells are primed to
70 respond and display an inherent "innateness", with higher levels of effector
71 molecule mRNA at the steady than conventional T cells[8]. MAIT cells can be activated
72 either via TCR triggering or innate cytokine stimulation, after which they are capable
73 of producing a range of cytokines and lytic molecules, including IFNγ and granzyme
74 B[9, 10]. These rapid effector responses allow MAIT cells to initiate and amplify the
75 immune response, as well as directly targeting infected or transformed cells[11, 12, 13,
76 14]. Robust anti-cancer responses, the ability to activate other anti-cancer cells[14] and
77 their absence of MHC restriction has highlighted MAIT cells as attractive candidates
78 for immunotherapy[14, 15, 16].

79

80 Several studies have identified tumour infiltrating MAIT cells in primary and
81 metastatic lesions[13, 17, 18, 19], but often report diminished effector function and loss
82 of key cytokines including IFNγ[13, 17]. Therefore, it is important to fully understand the
83 molecular pathways regulating MAIT cell effector responses. Our previous work has
84 demonstrated that MAIT cells undergo metabolic reprogramming in order to provide
85 the energy and biosynthetic intermediates needed to support their robust effector
86 functions[20, 21]. We and others have demonstrated that human MAIT cells activated
87 via their TCR for 18 hours favour exogenous glucose as their carbon source, and
88 engage in glycolytic metabolism as their primary metabolic program[20, 22]. This is
89 mediated by the activation of the critical metabolic regulators mTOR and MYC,
90 which control the expression of nutrient transporters, and key enzymes involved in
91 metabolism of glucose[20, 23].

92

93 Currently, the metabolic requirements for rapid MAIT cell effector responses such as
94 cytotoxicity are unknown and were the focus of the current study. We show that
95 MAIT cells co-cultured with cancer cells pulsed with cognate antigen rapidly (within 2
96 hours) degranulate and induce cell death. We demonstrate that these rapid

97 responses are independent of glucose-fuelled glycolytic metabolism, and show for
98 the first time that MAIT cells contain the machinery required to synthesize and
99 metabolize glycogen. We demonstrate that MAIT cells have glycogen stores, and
100 inhibition of glycogenolysis inhibits MAIT cell cytotoxicity and early cytokine
101 responses, which may have implications for the therapeutic use of MAIT cells.
102
103 **RESULTS**
104 **MAIT cell respond rapidly with target cell lysis and cytokine production**
105 We first assessed the expression of MR1 expression on two human cancer cell lines,
106 and identified both the K562 myelogenous leukaemia and the A549 lung carcinoma
107 cell lines as MR1+, furthermore we demonstrate that the addition of 5-ARU-MG
108 increased the expression of MR1 on the surface of K562 (Figure 1A). Next, we
109 demonstrate that MAIT cells respond to both A549 and K562 cells by degranulating
110 (CD107a expression) and this is significantly boosted with the addition of 5-ARU-MG
111 (Figure 1B-C). To confirm that MR1 was required for MAIT cell degranulation in
112 response to K562 cells loaded with 5-ARU-MG, we next blocked MR1 and observed
113 significantly reduced degranulation (Figure 1D-E). To build on these findings, and to
114 confirm if MAIT cells can induce target cell death, we moved to a direct cytotoxicity
115 assay, and demonstrate that MAIT cells can rapidly (within 2 hours) kill K562 cells
116 pulsed with 5-ARU-MG, and in a dose-dependent manner (Figure 1F). In addition to
117 cytotoxicity, we also show that MAIT cells can upregulate IFNγ expression within 3
118 hours in response to TCR stimulation (Figure 1G-H).
119
120 **MAIT cell cytotoxicity is not dependent on glucose metabolism or oxidative**
121 **phosphorylation.**
122 Our previously published data demonstrated that MAIT cells favour exogenous
123 glucose as their carbon source, which is metabolised via glycolysis metabolism[23]. We
124 and others have reported that glucose metabolism is critical for MAIT cell IFNγ and
125 granzyme production after 18 hours[20, 22]. To investigate if MAIT cell cytotoxicity was
126 dependent on glucose metabolism, we utilized the glycolytic inhibitor 2deoxy-D-
127 glucose (2DG) and show no effect on either MAIT cell degranulation or cytotoxicity
128 at 3 hours (Figure 2A-C). Studies in other T cell subsets have demonstrated that

129 expression of the glucose transporter (GLUT1) can take up to 6 hours[24]. So, we next
130 investigated expression of GLUT1 on TCR stimulated MAIT cells, and demonstrate
131 detectable expression takes 6 hours (Figure 2D), further supporting the concept that
132 rapid MAIT cell cytotoxicity is not supported by exogenous glucose metabolism.
133 Additionally, we found that rapid upregulation of IFNγ was also not inhibited by
134 addition of 2DG (Figure 2E). Another major metabolic pathway utilized by some T
135 cell subsets is oxidative phosphorylation (OxPhos). To investigate if OxPhos supports
136 MAIT cell cytotoxicity we utilized the specific ATP synthase inhibitor oligomycin and
137 show no effect on either MAIT cell degranulation or lysis of target cells (Figure 2F-G).
138

139 **MAIT cells contain the machinery to synthesize and metabolize glycogen.**

140 We next investigated other potential metabolic pathways which may support early
141 MAIT cell cytotoxicity by interrogating our recently published MAIT cell proteomic
142 dataset[23] and identified that MAIT cells express the enzyme glycogen synthase (GYS-
143 1) which is required to synthesize glycogen (Figure 3A). We next investigated if MAIT
144 cells expressed the enzymes required for the breakdown of glycogen and found the
145 brain isoform of glycogen phosphorylase (PYGB) in our proteomics dataset (Figure
146 3B). To confirm the expression of these enzymes we utilized flow cytometry and
147 show robust expression of both, with no change upon activation (Figure 3C-F).
148 Finally, we verified expression via western blotting (Figure 3G-I). To investigate if
149 PYGB was active in MAIT cells we utilized a glycogen phosphorylase activity assay
150 and observed increased PYGB activity in TCR stimulated MAIT cells (Figure 3J).
151 Finally, we show that MAIT cells contain stored glycogen, and that upon stimulation
152 glycogen is content significantly is reduced (Figure 3K-L).
153

154 **Glycogen supports MAIT cell cytotoxicity and early cytokine responses.**

155 To investigate if glycogen supports MAIT cell anti-tumour responses we utilized the
156 glycogen phosphorylase (PYG) inhibitor CP91149. We first investigated if CP91149
157 inhibited degranulation against A549 cells with or without the addition of 5-ARU-
158 MG, and show that CP91149 significantly limited MAIT cell degranulation triggered
159 by 5-ARU-MG pulsed A549 cells (Figure 4A-B). To confirm this finding, we
160 investigated MAIT cell degranulation in response to 5-ARU-MG pulsed K562 cells,

161 and again demonstrate the inhibition of PYG-limited degranulation (Figure 4C-D).
162 Next, we utilized another glycogen phosphorylase inhibitor (GPI)[24], and again
163 observed reduced MAIT cell degranulation (Figure 4E). We next investigated if
164 inhibiting the breakdown of glycogen limited MAIT cell target cell lysis and
165 demonstrate a significant reduction in killing (Figure 4F). Another protective function
166 of MAIT cells is their robust production of effector molecules like IFNγ and granzyme
167 B. Previous work in mice, demonstrated that memory T cell cytokine production is
168 dependent on glycogen metabolism, we confirm these findings in human memory T
169 cell (Figure S1). To this end we investigated if early IFNγ cytokine production (<3
170 hours) by MAIT cells is dependent on the metabolism of glycogen and show that
171 rapid IFNγ production is dependent on glycogen breakdown (Figure 4G-H). Similarly,
172 granzyme B secretion was also inhibited with the addition of CP91149 (Figure. 4I).
173 Since glycogen is metabolized into G6P and then fed into the glycolytic machinery,
174 we blocked the glycolytic machinery further down that pathway using the GAPDH
175 inhibitor heptelidic acid and show diminished MAIT cell degranulation (Figure 4J). To
176 further support the concept that glycogen supports early MAIT cell metabolic
177 process we performed seahorse analysis on CP91149 treated MAIT cells after 3
178 hours of stimulation and show that early glycolysis is dependent on the breakdown
179 of glycogen (Figure 4K-L).
180
181 **Discussion.**
182 MAIT cells are a subset of unconventional T cells which due to their potent effector
183 functions and abundance have been shown to play an important role in the host
184 defence against pathogens and malignancies[1, 25] and are now under investigation as
185 a potential immunotherapeutic agent[15, 26]. MAIT cells have been detected in both
186 primary cancers and metastatic sites, however they are dysfunctional, losing their
187 anti-tumour functions[13, 17, 18, 27]. Therefore, it is critical to understand the molecular
188 and metabolic requirements for MAIT cell effector responses.
189
190 In the current study, we confirm the robust cytotoxic potential of MAIT cells, with
191 rapid degranulation and dose-dependent killing of both A549 and K562 target cells.
192 We also demonstrate that MAIT cell cytotoxicity of cancerous cells is dependent on

193 MR1 and boosted in the presence of antigen, confirming work in the setting of
194 bacterial and virally infected cells[9, 12]. Although cancer specific antigens for MAIT
195 cells have yet to be identified[28], the loading of cancer metabolites onto MR1 has
196 been described[29, 30]. In addition, there is evidence emerging for microbial activation
197 of tumour-infiltrating MAIT cells[31].
198
199 Currently, data on the molecular regulation of MAIT cell cytotoxicity remains unclear
200 and will be necessary as they move towards therapeutic targets. Our group and
201 others have previously highlighted the importance of glucose metabolism for MAIT
202 cell effector functions such as cytokine production and proliferation[20, 22, 23]. We have
203 also reported how altered MAIT cell metabolism underpins defective functions in
204 obesity, potentially driving pathogenic MAIT cells[20, 32, 33]. Here, we demonstrate that
205 rapid MAIT cell cytotoxicity (and early cytokine production) is independent of
206 exogenous glucose metabolism. In our search for an alternative carbon source, we
207 observed that MAIT cells have the molecular machinery to synthesize and
208 metabolise glycogen. Glycogen is the main energy storage form of glucose in the
209 body, stored as a quickly mobilised multibranched polysaccharide[34]. Recent work by
210 Zhang and colleagues reported that murine memory CD8+ T cells but not naïve CD8+
211 T cells could also synthesize and metabolize glycogen[24]. We confirmed these findings
212 in human memory T cells and hypothesized that MAIT cells, due to their
213 "innateness"[8] might utilize glycogen to support their rapid functional responses.
214 Using a series of experiments, we show that MAIT cell cytotoxicity and rapid cytokine
215 responses (<3 hours) are dependent on the breakdown of glycogen, supporting the
216 concept that stored glycogen fuels rapid responses in innate effector T cells like
217 MAIT cells and memory T cells. This is further supported by work in another innate
218 immune subset, dendritic cells, which also utilize glycogen to fuel their rapid
219 responses[35]. Our data suggests that TCR triggering activates PYGB to break down
220 glycogen, which then feeds glycolysis. This again is supported by the recent
221 publication in murine memory CD8+ T cells, where the inhibition of glycolysis at the
222 first enzyme (hexokinase) had no impact but inhibition further down the glycolytic
223 pathway limited cellular responses[24].
224

225 Understanding the carbon sources required fuel MAIT cell effector functions may be
226 of particular importance in the setting of cancer where limited glucose has been
227 shown to impair T cell responses[36, 37]. The ability of human MAIT cells to use stored
228 glycogen to fuel their cytotoxicity, paired with their rapid functional responses,
229 unrestricted properties and relative abundance further highlights their potential as
230 an exciting candidate for cancer immunotherapy. In conclusion, we describe for the
231 first time a novel metabolic pathway in human MAIT cells necessary for their rapid
232 effector responses, further supporting the rationale for their use as an
233 immunotherapeutic.
234

235 **Materials & methods**

236 **Study cohorts & ethical approval** Full ethical approval was obtained from both St
237 Vincent's University Medical Ethics Committee and Maynooth University Ethics
238 Committee. We recruited a cohort of healthy adult donors from St Vincent's
239 Healthcare Group. Inclusion criteria included ability to give informed consent, 18-55
240 years of age and a BMI<28. Exclusion criteria included current or recent (<2 weeks)
241 infection, current smoker, use of immunomodulatory or anti-inflammatory
242 medications. All participants provided full consent.
243

244 **Preparation of peripheral blood mononuclear cells (PBMC) and expanded MAIT**
245 **cells**

246 PBMC samples were isolated by density centrifugation over ficoll from fresh
247 peripheral blood samples. PBMCs were either stored at -70°C or used for MAIT cell
248 expansion using 5-ARU-MG and IL-2 as previously described[23].
249

250 **MAIT cell degranulation assay** PBMCs were thawed and rested before addition of
251 either metabolic inhibitors or vehicle control (i.e. 1mM 2DG, 100µM CP91149, 50µM
252 GPI (CP316819), 5µM Heptelidic acid or DMSO / water). A549 cells or K562 cells with
253 or without pre-treatment with 5-ARU-MG were then cocultured with the PBMCs at a
254 ratio of 10:1 PBMC:Target plus CD107a antibody (Miltenyi). After 30 minutes,
255 protein transport inhibitor cocktail (Invitrogen) was added and cultured for further 2
256 hours. MAIT cells were identified by flow cytometry with staining using specific

257 surface monoclonal antibodies namely; CD3, CD161 and TCRVα7.2 (all Miltenyi), and
258 degranulation assessed according to percentage of MAIT cells expressing CD107a.
259 Cell populations were acquired using a Attune NXT flow cytometer and analysed
260 using FlowJo software (Treestar). Results are expressed as a percentage of the
261 parent population as indicated and determined using flow minus-1 (FMO) and
262 unstained controls.
263 **MAIT cell cytotoxicity assay**. IL-2 expanded MAIT cells[23] were co-cultured with
264 Calcein AM labelled K562 cells at a ratio of 3:1, MAIT cells to targets (and other
265 ratios for dose curve) in the absence or presence of metabolic inhibitors or vehicle
266 control (i.e. 1mM 2DG, 100µM CP91149, 50µM GPI (CP316819), 5µM Heptelidic acid
267 or DMSO / water). After 2 hours of co-culture, supernatant was analysed using a
268 Spectramax plate reader to measure supernatant fluorescence at 485nm excitation
269 and 525nm emission and percentage killing calculated as a proportion of max killing
270 by Triton X.

271 **MAIT cell cytokine analysis.** IFNγ mRNA measured in IL-2 expanded MAIT cells (with
272 or without stimulation with CD3/CD28 beads (Gibco)) and their culture supernatant
273 by rtPCR. Secreted IFNγ and granzyme B protein was measured in IL-2 expanded
274 MAIT cells (with or without stimulation with CD3/CD28 beads (Gibco)) and their
275 culture supernatant by ELISA. To investigate the metabolic requirements of early
276 cytokine responses, activated MAIT cells were treated with metabolic inhibitors or
277 vehicle control (i.e. 1mM 2DG, 100µM CP91149 or DMSO / water). mRNA was
278 extracted from MAIT cells using Trizol according to the manufacturer's protocol.
279 Synthesis of cDNA was performed using qScript cDNA Synthesis kit (QuantaBio). Real
280 time RT-qPCR was performed using PerfeCTa SYBR Green FastMix Reaction Mix
281 (Green Fastmix, ROX™) (QuantaBio) and KiCqStart primer sets (Sigma). ELISA were
282 performed as per the manufacturer's instructions (R&D Systems).

283 **MAIT cell glycogen machinery analysis.**
284 The identification of glycogen synthase (GYS-1) and phosphorylase (PYGB) in MAIT
285 cells was based on *in silico* analysis of a published MAIT cell proteomic dataset[23] .
286 Expression was confirmed using both flow cytometry on ex-vivo MAIT cells and via

287 western blotting on IL-2 expanded MAIT cells, both stimulated with CD3/CD28 TCR
288 dynabeads for 6 hours. For flow cytometry, PBMC were stimulated as described then
289 surface stained for MAIT cells before fix/perm using True-Nuclear Transcription
290 Factor Buffer Set (Biolegend) then intracellularly stained with antibodies specific for
291 p-GYS-1 (Cell Signalling) or PYGB (Cell Signalling). For western blotting, cells were
292 lysed in NP-40 lysis buffer (50mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 1%
293 (w/v) IgePal, and complete protease inhibitor mixture (Roche)). Samples were
294 resolved using SDS-PAGE and transferred to nitrocellulose membranes before
295 analysis with anti-GYS-1 (Cell Signalling), PYGB (Cell Signalling) and anti-β-Actin
296 (Sigma) antibodies. Protein bands were visualised using enhanced
297 chemiluminescence.
298
299 **MAIT cell glycogen content and PYGB activity analysis**
300 Glycogen content in MAIT cells (either basally or stimulated for 3 hours with anti-TCR
301 beads (Gibco)) was measured using Biovision glycogen kit according to the
302 manufacturer's instructions, and by fluorescent microscopy using a previously
303 published method[38]. Glycogen phosphorylase activity in MAIT cells (either basally or
304 stimulated for 3 hours with anti-TCR beads (Gibco)) was measured using Sigma-
305 Alrich Glycogen Phosphorylase Colorimetric Assay Kit according to the
306 manufacturer's instructions.
307
308 **MAIT cell seahorse analysis** Expanded MAIT cells were treated with metabolic
309 inhibitors or vehicle control (i.e. 1mM 2DG, 100µM CP91149, or DMSO / water) and
310 then stimulated with CD3/CD28 Dynabeads. After 3 hours of stimulation Seahorse
311 metabolic flux analysis was performed according to the Seahorse instruction manual.
312
313 **Statistics** Statistical analysis was completed using Graph Pad Prism 9 Software (USA).
314 Data is expressed as mean±SEM. We determined differences between two groups
315 using student t-test and Mann Whitney U test where appropriate. Analysis across 3
316 or more groups was performed using ANOVA. Statistical significance was defined as
317 $p<0.05$.

318 **Contributors Statement:** FCC, NKM, EB, AB, BJ, AW and RB performed the
319 experiments and carried out analysis and approved the final manuscript as
320 submitted. OR and DOS recruited peripheral blood donors. AEH, LVS, NJ, DOS & FCC
321 conceptualized and designed the study, analyzed the data, drafted the manuscript,
322 and approved the final manuscript as submitted.

323

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460
461

462 **Figure Legends**
463
464 **Figure 1 MAIT cell respond rapidly with target cell lysis and cytokine production**.
465 **(A.)** Flow cytometry histogram displaying MR1 expression on the surface of either
466 K562 cells or A549 cells in the absence or presence of exogenous 5-ARU-MG. **(B)**
467 Scatter plot showing CD107a expression on MAIT cells cultured with A549 cells or
468 A549 cells pulsed with 5-ARU-MG. **(C)** Scatter plot showing CD107a expression on
469 MAIT cells cultured with K562 or K562 pulsed with 5-ARU-MG. **(D-E)** Flow cytometry
470 dot plots and scatter plot showing CD107a expression on MAIT cells cultured with
471 K562 pulsed with 5-ARU-MG in the absence or presence of MR1 blocking antibody.
472 **(F)** Scatterplot showing dose-dependent (effector to target ratio) cytotoxicity of IL-2
473 expanded MAIT cells in their targeting of K562 cells pulsed with 5-ARU-MG. **(G-H)**
474 Scatter plots of IFNγ mRNA and secreted protein levels from IL-2 expanded MAIT
475 cells stimulated with TCR beads (antiCD3/CD28) for either 1.5 hours or 3 hours. ns =
476 not significant, * = $p > 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ as
477 measured by paired t test, Friedman test or mixed-effects analysis where
478 appropriate.
479
480 **Figure 2 MAIT cell cytotoxicity is not dependent on glucose metabolism or**
481 **oxidative phosphorylation. (A-B)** Flow cytometry dot plot and scatter plot showing
482 CD107a expression on *ex-vivo* MAIT cells in response to stimulation with K562 cells
483 (pulsed with 5ARU-MG) with or without the addition of glycolytic inhibitor 2DG. **(C)**
484 Scatterplot showing the impact of 2DG treatment on cytotoxic capacity of IL-2
485 expanded MAIT cells, displayed as fold change per sample. **(D)** Western Blot of
486 GLUT1 protein expression by IL-2 expanded MAIT cells (3 individual donors) at rest
487 (basal) and after 3hours or 6 hours of TCR bead stimulation. **(E)** Scatter plot showing
488 the impact of 2DG treatment on early IFNγ secretion by IL-2 expanded MAIT cells
489 stimulated with TCR beads. **(F)** Scatter plot showing CD107a expression on *ex-vivo*
490 MAIT cells cultured with K562 (pulsed with 5ARU-MG) in the absence or presence of
491 the oxidative phosphorylation inhibitor oligomycin. **(G)** Scatterplot the impact of
492 oligomycin treatment on the cytotoxic capacity of IL-2 expanded MAIT cells,
493 displayed as fold change per sample. ns = not significant, as measured by paired t
494 test, Wilcoxon test or Mann-Whitney test as appropriate.
495
496 **Figure 3 MAIT cells contain the machinery to synthesize and metabolize glycogen.**
497 **(A-B)** Scatter plot showing the expression of GYS1 or PYGB in IL-2 expanded MAIT
498 cells, either basal or stimulated for 18 hours with anti-CD3/CD28 TCR beads and IL-
499 18 for 18 hours (data extrapolated from published proteomic dataset). **(C-F)**
500 Representative flow cytometric histograms and scatter plots showing the expression
501 of GYS1 or PYGB in *ex-vivo* MAIT cells, either basal or stimulated with anti-CD3/CD28
502 TCR beads for 18 hours **(G-I)** Western blot and densitometry scatter plots showing
503 the expression of phosphorylated GYS1 or PYGB in IL-2 expanded MAIT cells either

504 basal or stimulated with anti-CD3/CD28 TCR beads for 6 hours. **(J)** Scatter plot
505 showing glycogen phosphorylase activity in TCR-stimulated MAIT cells. **(K)** Scatter
506 plot showing glycogen levels in IL-2 expanded MAIT cells at rest (basal) or stimulated
507 with TCR beads for 3 hours. **(I)** Florescent microscopy image demonstrating the
508 presence of glycogen in MAIT cells. ns = not significant, $p > 0.05$, * = $p < 0.05$ as
509 measured by paired t test.
510
511 **Figure 4 Glycogen supports MAIT cells cytotoxicity and early cytokine responses.**
512 **(A -B)** Scatter plots showing CD107a expression on MAIT cells cultured with A549
513 cells alone or A549 cell pulsed with 5ARU-MG, in the absence or presence of the
514 glycogen phosphorylase inhibitor CP91149. **(C-D)** Flow cytometry dot plot and
515 scatter plot showing CD107a expression on MAIT cells cultured with K562 cells
516 (pulsed with 5ARU-MG) in the absence or presence of the glycogen phosphorylase
517 inhibitor CP91149. **(E)** Scatter plot showing CD107a expression on MAIT cells
518 cultured with K562 cells (pulsed with 5ARU-MG) in the absence or presence of the
519 glycogen phosphorylase inhibitor GPI. **(F)** Scatterplot showing the impact of CP91149
520 on cytotoxic capacity of MAIT cells against K562 cells, displayed as fold change per
521 sample. **(G-H)** Scatter plot showing IFNγ mRNA levels or secreted protein from IL-2
522 expanded MAIT cells stimulated with TCR beads (for 3 hours) in the absence or
523 presence of CP91149. **(I)** Scatter plot showing granzyme B secreted protein from IL-2
524 expanded MAIT cells stimulated with TCR beads (for 3 hours) in the absence or
525 presence of CP91149. **(J)** Scatter plot showing CD107a expression on MAIT cells in
526 response to stimulation with K562 (pulsed with 5ARU-MG) in the absence or
527 presence of the GAPDH inhibitor heptelidic acid. **(K-L)** Scatter plot and Seahorse
528 trace displaying ECAR rates in TCR bead-stimulated (3 hours) IL-2 expanded MAIT
529 cells treated with CP91149. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ as measured
530 by Wilcoxon test, paired t test or Mann-Whitney test where appropriate.
531
532