Modulation of glucose metabolism by 2-Deoxy-D-Glucose (2DG) promotes IL-17 producing human T cell subsets

Xin Chen[1,2,3], Lina Kozhaya[1], Chuxi Wang[1,2], Lindsey Placek[1], Ece Karhan[1], Derya Unutmaz[1,2]

[1] Jackson Laboratory for Genomic Medicine, Farmington, Connecticut, USA.

[2] Department of Immunology, University of Connecticut School of Medicine, Farmington, CT USA.

[3] Institute for Immunity, Transplantation, and Infection, Stanford University, Stanford, CA USA.

* Corresponding author E-mail: derya@mac.com

Abstract

Activation and differentiation of T cells are closely linked to their cellular metabolic programs. Glycolysis and mitochondrial metabolism are thought to be critical in modulating T cell function. Here we asked to what extent inhibition of glycolysis, using 2-Deoxy-D-Glucose (2DG), regulate activation, effector function, or differentiation of human T cell subsets. We found that glycolysis is required for T cell receptor (TCR)-mediated activation and proliferation of human naive CD4+ T cells but had less of an impact on memory subsets. CD4+ T cells cultured in the presence of 2DG displayed higher level of IL-17-secreting cells (Th17) from memory or *in vitro* differentiated naïve regulatory T cell (Tregs) subsets. Moreover, the mucosal associated invariant T (MAIT) cell subset survived or expanded better and secreted higher IL-17 in the presence of 2DG. Remarkably, we found that the 2DG effect was reversed by mannose but not by glucose. Collectively, these findings suggest that 2DG could enrich IL-17 secreting human effector T cell subsets and their cellular functions. Our finding provides a framework to manipulate glycolytic pathways in human T cells in infectious diseases such as COVID19 and in enhancing cancer immunotherapy.

30 ## Introduction

31 Metabolism has been recognized as an important regulator in T cell activation and lineage
32 differentiation (Araki et al., 2010; Chapman et al., 2020; Jacobs et al., 2008; MacIver et al., 2013;
33 Palmer et al., 2015; van der Windt & Pearce, 2012). Upon T cell activation, TCR signals and co-
34 stimulation activate the phosphatidtyl-inositide-3 kinase (PI3K)/Akt/mTORC1 signaling pathway
35 for the increased anabolic needs of effector T cells, which become more sensitive to metabolic
36 regulation (Ho et al., 2015; Palmer et al., 2015; Sena et al., 2013). Importantly, an efficient immune
37 response involves various T cell subsets, which have different metabolic requirements for
38 development and effector functions (MacIver et al., 2013; Pearce et al., 2013). It is still unclear
39 how metabolic pathways regulate the functions of T cell subsets specifically, but understanding
40 the underlining mechanisms would allow modulation of immunity in chronic disease and cancer
41 (Johnson et al., 2018).

42 Subsets of T cells that secrete IL-17 are the critical mediators in antimicrobial and pro-
43 inflammatory responses, as well as in pathogenesis in autoimmune or chronic inflammatory
44 diseases (Bettelli et al., 2006; Damasceno et al., 2020; Platt et al., 2020; Xu et al., 2020; Yasuda
45 et al., 2019). Th17 cells and a subset of Tregs are the major source of IL-17 secretion, which are
46 also characterized by the expression of the transcription factor RORC and the chemokine receptor
47 CCR6 (Singh et al., 2008; Valmori et al., 2010; Wan et al., 2011). Several mouse studies have
48 revealed a role of glucose metabolism in Th17 differentiation, via mTORC1, Myc, and HIF1a
49 signaling (Kastirr et al., 2015; Perl, 2016; Sasaki et al., 2016; Shen & Shi, 2019; Shi et al., 2011).
50 Inhibition of glycolysis with 2-Deocy-Glucose (2DG) or 3-bromopyruvate in mice for a short period
51 of time impaired Th17 differentiation, phenocopying the effect of HIF1a deficiency (Okano et al.,
52 2017; Shi et al., 2011). However, how glycolytic metabolism regulates the differentiation and
53 functions of human Th17 and IL-17 secreting Tregs remains unclear.

Another source of IL-17 producing T cells are mucosal associated invariant T (MAIT) cells (Coulter et al., 2017; Willing et al., 2018). Human MAIT cells can be identified by a semi-invariant T cell receptor(TCR) alpha chain(Va7.2) and CD161 expression (Chiba et al., 2018). MAIT cells can be activated by a broad range of bacteria and yeasts, and can discriminate and fine-tune their functional responses to complex human microbiota (Constantinides et al., 2019; Corbett et al., 2020; Hinks & Zhang, 2020; Ioannidis et al., 2020; Tastan et al., 2018). Thus, IL17-secreting MAIT cells plays a role in infections and autoimmune diseases such as Tuberculosis and Multiple Sclerosis (Coulter et al., 2017; Willing et al., 2018). While the frequency and distribution of MAIT cells are currently under intense investigation, there is still much to understand in the metabolic regulation of the function of the human MAIT cell subset.

In this study, we utilized 2DG, an analog and potent glycolysis inhibitor, to investigate the role of glucose metabolism in the activation, differentiation, and effector function of human T cell subsets. We showed that 2DG suppressed human T cell activation, cytokine production, and proliferation upon early T cell receptor (TCR) activation. However, 2DG treated naive T cells showed a greater reduced proliferative capacity and glycolytic function compared to memory T cells. Remarkably, 2DG treatment greatly enriched IL-17 producing CD4+ T cells in long term cultures and during *in vitro* differentiation of naive or naive regulatory T cells (TNreg) or from the MAIT cell subset. Together, our findings suggest a differential impact of glycolysis in different subsets and differentiation stages of human T cells.

76 # Results

77 **Inhibitor of glucose uptake, 2DG, suppresses early activation of CD4+ T cells**

78 It has been reported that 2DG inhibits glucose metabolism (Xi et al., 2014), which may in turn
79 regulate T cell activation (Buck et al., 2015; Palmer et al., 2015). Therefore, we first examined the
80 effect of 2DG on T cell activation by assessing the expression of the IL-2 receptor alpha (CD25)
81 and the glucose transporter 1 (GLUT1), as both are upregulated upon T cell activation for IL-2
82 signaling and glucose uptake, respectively (Chapman et al., 2019). Human primary CD4+ T cells
83 were activated with anti-CD3/CD28 beads in media alone or in media supplemented with 0.3mM,
84 1mM, or 3mM 2DG. The expression of CD25 and GLUT1 in T cells was significantly reduced by
85 3mM 2DG compared to the control (**Figure 1A-B**), but lower concentrations of 2DG (0.3mM or
86 1mM) did not reach statistical significance (data not shown). Because 2DG suppresses glycolysis
87 (Xi et al., 2014), we also activated T cell subsets in the glucose-free media as a comparison. In
88 contrast to the effect seen with 2DG, T cells activated in glucose-free-media showed higher
89 GLUT1 expression and CD25 expression comparable to the control condition (**Figure 1A-B**). We
90 also observed a reduced expression of program cell death 1 (PD1) and Lymphocyte Activating 3
91 (LAG3) on 2DG-treated T cells, which are known to be induced upon TCR activation
92 (Lichtenegger et al., 2018) (**Figure 1C-D**). However, the expression of PD1 or LAG3 was not
93 significantly different between T cells activated in glucose-free or regular media (**Figure 1C-D**).

94 TCR-activation of T cells leads to a rapid production of cytokines such as TNF, IFNγ, and IL-2. As
95 such, to determine whether 2DG also suppresses cytokine production, we collected activated T
96 cell supernatants 2 days post CD3/CD28 activation and analyzed the cytokine levels using a
97 cytometric bead assay. We found that the levels of TNF and IFNγ secretion were significantly
98 reduced but there was no difference in IL-2 secretion in the presence of 2DG culture compared
99 to control media (**Figure 1E**). Together, these data suggest that 2DG suppresses early human T

100 cell activation and cytokine production.

101 **2DG has different effects on activation or effector functions of naive and memory T cells.**

102 It has been reported that naive and memory T cells may have different metabolic requirements
103 for activation and proliferation (Almeida et al., 2016; MacIver et al., 2013; van der Windt & Pearce,
104 2012). To determine whether 2DG modulates the activation of naive vs memory CD4+ T cells
105 differently, we sorted these subsets from human CD4+ T cells using CCR7+CD45RO- (naive)
106 and CCR7+/-CD45RO+ (memory) markers and activated with anti-CD3/CD28 beads in media
107 alone, in 3mM 2DG, or in glucose-free media. Both T cell subsets displayed a similar trend of
108 reduction in GLUT1 and CD25 expression on day 1 post activation **(Figure 2A)**; however, on day
109 4 post-activation, 2DG-treated memory T cells upregulated GLUT1 to levels comparable to control
110 cells whereas most naïve T cells remained GLUT1 negative **(Figure 2B-C)**. Similar to unsorted
111 CD4+ T cells (**Figure 1A**), activation of naive or memory T cells in glucose-free media did not
112 significantly affect the expression of GLUT1 or CD25 (**Figure 2A-C**). Addition of IL-2 in these
113 activation experiments did not restore the 2DG-mediated down-regulation of GLUT1 or CD25
114 expression in either naive or memory T cells (**Supplemental Figure 1A-C**). Since we have
115 observed that 2DG suppresses T cell activation and glucose uptake, we hypothesized that 2DG
116 would also reduce T cell proliferative capacity. To address this, we labeled naive and memory T
117 cells with Celltrace violet (CTV) dye, and then activated with anti-CD3/CD28 coated beads.
118 Indeed, in 2DG-treated or in glucose-free media, naive or memory T cell subsets proliferated less
119 compared to control media (**Figure 2E**). Addition of IL-2 did not restore the low proliferation of
120 2DG-treated T cell subsets (S**upplemental Figure 1D**).

121 We next monitored the expansion of these activated T cells during 2-week culture in IL-2.
122 Expansion of 1 mM 2DG treated naive T cells were 30 fold after 14 days, compared to 150 fold
123 in the control, whereas memory T cells in the presence of 1mM 2DG expanded 85 fold compared

124 to 110 fold in the control media. We then asked whether 2DG has differential effects in the
125 glycolytic function of naive and memory subsets by using the Seahorse glucose stress test assay.
126 After T cell expansion in 2DG or control media for 14 days, 2DG was washed away and cells were
127 resuspended in Seahorse XF base media, and the metabolic functions were analyzed by the
128 Seahorse XFe96 Analyzer. The extracellular acidification rate (ECAR) was assessed after the
129 addition of glucose (gluc), oligomycin (oligo), and 2DG at indicated times and the glycolytic
130 capacity was determined. 2DG treated-memory T cells were comparable glycolytic function to the
131 control groups; however, 2DG-treated naive T cells showed significantly reduced glycolysis in
132 response to addition of glucose (**Figure 2F-G**).

133 **2DG enhances IL-17 producing CD4+ memory subsets.**

134 We next further explored which effector T cell subsets are modulated by suppressing glycolysis.
135 Since CCR6+ Th17 cells are reported enriched in the CD161+ subset as this subpopulation
136 produces more IL-17 upon *ex vivo* stimulation compared with CCR6+CD161- cells (Acosta-
137 Rodriguez et al., 2007; Cosmi et al., 2008; Wan et al., 2011), we hypothesized that 2DG would
138 preferentially affect the function of CD161+ T cell subsets. Indeed, after two weeks in culture with
139 2DG, we observed that CCR6+ and CD161+ populations were increased compared to cells in
140 control media, but interestingly, there was no difference in the low glucose condition (**Figure 3A**
141 **and 3B**). After 2DG was removed from the culture and cells reactivated with phorbol myristate
142 acetate (PMA) and calcium ionophore (ionomycin), we determined intracellular cytokine secretion
143 in the presence of brefeldin a (GolgiStop). We found that the frequency of cells that expressed IL-
144 17, IFNγ, IL-4 and IL-21 were significantly increased (**Figure 3C and 3D**), but no effect was seen
145 in IL-2 and TNF production from CD4+ T cells (**Supplemental Figure 3**). Taken together, these
146 data demonstrate that 2DG-treatment can modify the cytokine production of lineage-committed
147 cells in long term *in vitro* cultures.

148 **2DG enriches IL-17+ cells within Th17 lineage-committed CCR6+ memory T cell subset.**

149 Since we observed a significant increase in IL-17 cytokine production from 2DG-treated T cells,
150 we hypothesized that 2DG could enhance the IL-17 production from Th17 cells compared with
151 other effector T cells. To test this hypothesis, we sorted CD4+ T cells into CCR6- and CCR6+
152 memory T cell subsets, activated them in the presence or absence of 2DG, and expanded for two
153 weeks in IL-2, as described above (**Figure 3A**). Consistent with our prior results, 2DG-treated
154 CCR6- and CCR6+ CD4+ T cells displayed higher CD161 expression than control cells (**Figure**
155 **4A**). IL-17 production from purified and expanded CCR6+ T cells were increased in the presence
156 of 2DG (**Figure 4B**). Notably, IFNγ+ and IL-21+ producing CCR6+ T cells were also increased by
157 2DG (**Figure 4B-E**). Interestingly, the percentage of IL-10+ and IL-17+ T cells was significantly
158 increased in 2DG-treated CCR6+ T cells (**Figure 4F and 4G)**. Therefore, we concluded that 2DG
159 increased CD161+ within both CCR6+ and CCR6- T cells, and also enhanced IL-17, IFNγ, IL-21,
160 and IL-10 production from the sorted CCR6+ memory T cell subset.

161 **2DG increases *in vitro* generation of IL-17-producing Tregs from human naive regulatory**
162 **T cell (TNreg) precursors**

163 A subset of Tregs that secretes IL-17 can preferentially arise from human naive precursors
164 (CD25+CD45RO-CCR7+ cells, termed TNreg) in the presence of polarizing-cytokines IL-1β, IL-
165 23, and TGF-β (Mercer et al., 2014; Valmori et al., 2010). To explore how 2DG regulates Th17
166 cell differentiation from TNregs, we used this cytokine polarization protocol (**Figure 5A**) to
167 differentiate and expand IL-17-secreting cells from highly purified naive or precursors naive
168 regulatory T cells (TNregs) in media alone or in the presence of 1 mM 2DG. The Foxp3+ cells
169 during polarization of TNregs with 2DG were statistically similar in frequency (**Figure 5B and 5C**).
170 However, 2DG significantly increased generation of IL-17+ Tregs from TNreg precursors (**Figure**
171 **5D**). Since HELIOS expression defines a phenotypically distinct population of Tregs (Thornton et

172 al., 2019), and *in vitro*-generated IL-17+ cells from TNreg precursors did not express HELIOS
173 (Mercer et al., 2014), we then asked whether 2DG could induce IL-17, IFNγ, and IL-21 production
174 from HELIOS+ or HELIOS- populations of naive and TNregs. We also compared the 2DG effects
175 between HELIOS+ and HELIOS- population of naïve cells since HELIOS plays a role in naive T
176 cell differentiation (Ng et al., 2019). The frequency of IL-17+ T cells witihn HELIOS- naive T cells
177 was increased by 2DG in polarizing condition, but not within HELIOS+ subset (**Figure 5F**). 2DG
178 also significantly increased IL-17 and IFNγ populations only from HELIOS- population from
179 TNregs in the polarization condition (**Figure 5E-G**). Notably, when comparing the intracellular IL-
180 21 from HELIOS+ and HELIOS- subset of Tregs, the frequency of IL-21+ cells was significantly
181 higher in 2DG-treated HELIOS- subset (**Figure 5H**). But there was no significant difference in IL-
182 21 cytokines secretion in HELIOS+ or HELIOS- Treg subsets in 2DG-treated naive T cells
183 compared to controls (**Figure 5I**). Intracellular IL-10 was also increased in HELIOS- population of
184 TNregs in polarizing cytokines, and was significantly increased in HELIOS+ and HELIOS-
185 population from naive T cells (**Figure 5J and 5K**). In addition, increased cytokine production of
186 IL-17A, IL-17F, IL-10, and IFNγ was also confirmed by corresponding levels of cytokines detected
187 in the culture supernatants (**Figure 5L**). Taken together, 2DG enhanced the generation of IL-17-
188 producing cells from both naive and TNregs, which was restricted to HELIOS- subsets. 2DG also
189 increased the production of IFNγ, IL-21, and IL-10 from TNregs in the polarizing condition.

190 **Mannose reverses or rescues 2DG effects on T cell activation and effector functions.**

191 2DG has previously been shown to inhibit *N*-linked glycosylation (Andresen et al., 2012; Xi et al.,
192 2014), which can be modulated through a branch of the glucose metabolism pathway called the
193 hexosamine biosynthetic pathway (HBP) (Akella et al., 2019). Further, mannose, which is a major
194 component of sugar moeity in glycoproteins, can reverse the apoptotic effect of 2DG on cancer
195 cells (Ahadova et al., 2015; Gu et al., 2017; Kurtoglu et al., 2007). Thus, we sought to determine

196 whether mannose can also reverse the 2DG effects on short term activation of T cells (**Figure 2**).
197 Accordingly, we activated CD4+ naive T cells with anti-CD3/CD28 beads in media alone, with
198 3mM 2DG or mannose alone, or with 3mM 2DG supplemented with 3mM mannose. While we did
199 not observe any difference in CD25 and GLUT1 expression on day 1 post activation
200 (**Supplemental Figure 5**), mannose reversed the 2DG-mediated downregulation of CD25 and
201 GLUT1 expression on day 4 (**Figure 6 A and 6B**). Additional equimolar glucose did not reverse
202 the 2DG effects (**Figure 6B**). Equal molar of mannose significantly reverses the proliferative
203 capacity from 1 fold expansion with 2DG alone to 50 fold expansion with 2DG and mannose
204 comparing to 157 fold cell expansion in control media (**Figure 6C**). However, after long-term
205 culture, mannose did not rescue the impaired glycolytic function of 2DG-treated naive T cells
206 (**Figure 6 D and 6E**).

207 Next, we asked whether mannose could reverse 2DG effects on IL-17 enrichment. After 14 days
208 cell expansion, we observed a reduction of CCR6+ and CD161+ population in a mannose-does
209 dependent manner (**Figure 6 F-G**). In addition, the 2DG mediated-reduction of IL-17, IFNγ, and
210 IL-21 were also reversed by mannose (**Figure 6G**). Addition of equimolar glucose into 2DG
211 culture did not reverse the 2DG-mediated enrichment of IL-17 production (**Figure 6F and 6G**).
212 Together these findings suggest that the inhibitory effects of T cells activation and IL-17
213 production on 2DG-treated human T cells are mannose-dependent.

214

215 Discussion

216 In this study, we demonstrated that the glucose analog 2DG have remarkably different effects on
217 activation and differentiation of human T cell subsets. 2DG suppresses early human T cell
218 activation through the TCR, as assessed by cytokine production, and proliferation. However, in

219 long term cultures 2DG also profoundly increases the frequency of IL-17 production from lineage-
220 committed memory CCR6+CD4+ (Th17) T cells, MAIT cells and Tregs. In addition, inhibition of
221 glycolysis by 2DG in these memory subsets (Th17, MAIT and Tregs) does not affect their
222 expansion upon activation. On the other hand, naïve CD4+ T cells are more sensitive to the
223 inhibition of glycolysis by 2DG and therefore have reduced their survival and function. Importantly,
224 we also demonstrated that the 2DG-mediated IL-17 enrichment could be reversed by addition of
225 equimolar mannose.

226 A major question derived from our findings is that how inhibition of glycolysis can significantly
227 increase the IL-17 production from already lineage-committed/differentiated memory Th17 cells?
228 Several mice studies have revealed a role of glucose metabolism in Th17 differentiation (Kastirr
229 et al., 2015; Perl, 2016; Sasaki et al., 2016; Shen & Shi, 2019; Shi et al., 2011) but showed
230 discrepancies on the effect of inhibiting glycolysis in Th17 differentiation and functions. For
231 example, in one study Tregs treated with 0.2mM 2DG for 5 days reduced IL-17 production *in vitro*
232 (Li et al., 2019) and in another study reduced production of IL-17 by T cells after 5 day-2DG
233 treatment was observed (Shi et al., 2011). However, another study reported an enhancement of
234 Th17 cell polarization and IL-17 production after treatment of 2mM 2DG (Brucklacher-Waldert et
235 al., 2017). In our study, we observed reduced IL-17 production by 2DG treated T cells for during
236 early activation period. However, frequency of IL-17 producing subset was greately increased
237 after long-term T cell culture (12-14 days), during reactivation in the absence of 2DG. Th17 can
238 be characterized as “pathogenic” and “non-pathogenic” Th17 cells (Wu et al., 2018). Pathogenic
239 Th17 cells express more effector molecules such as CXCL3, CCL4, CCL5, IL-3, and IL-22,
240 whereas non-pathogenic Th17 cells exhibit upregulation of immune suppressive molecules and
241 cytokines such as IL-10 (Gaublomme et al., 2015; Lee et al., 2012). We hypothesized that the
242 increased subset of IL-17-secreting Th17 cells belongs to the “non-pathogenic” subset, as we
243 also found an increase in IL-10 production from the same cells. Since IL-17+ Th17 cells are crucial

244 for mediating mucosal immunity against fungi and bacteria infection in human subjects (Brembilla
245 et al., 2018; McDonald, 2012), our findings may suggest that the metabolic changes affect the
246 function of Th17 against commensal microbes in the gastrointestinal tract which would lead to
247 mucosal damage and mucosal-relative diseases such as inflammatory bowel disease (IBD)
248 (Galvez, 2014). Since the functions of Th17 cells in the small intestine can be closely regulated
249 by gut microbiome (Evans-Marin et al., 2018; Garidou et al., 2015), our finding also suggest that
250 a dysregulated metabolites from dysbiotic ileum microbiota (Visconti et al., 2019) could impact
251 Th17 homeostasis, and were sufficient to induce metabolic disease such as Type 2 Diabetes
252 (T2D) (Garidou et al., 2015).

253 We found that 2DG also increased the frequency of IL-17+ Tregs and IL-17 cytokine secretion
254 from these cells in the presence of polarizing cytokines *in vitro* differentiation. These IL-17-
255 producing Tregs were found to be accumulated in the inflamed joints of patients with rheumatoid
256 arthritis and were functionally suppressive (Afzali et al., 2013; Jung et al., 2017). However, we did
257 not observe a significant difference in suppressive capacity of 2DG-treated Tregs in proliferation
258 assay (data not shown), which is consistent with a human Tregs study *in vitro* (Tanimine et al.,
259 2019). Inhibition of glycolysis may also contribute to mucosal diseases, as IL-17+Tregs may
260 contribute to the development of colon cancer (Knochelmann et al., 2018; Marshall et al., 2016),
261 and possibly to IBD (Galvez, 2014).

262 As noted, an increased level of IL-10 from memory T cells (such as Tregs in the presence of
263 polarizing cytokines, CCR6- and CCR6+ memory T cells) was also observed throughout the
264 experiments. IL-10 is an anti-inflammatory cytokines, plays a central role in limiting the regulation
265 of immune responses (Iyer & Cheng, 2012). Elevated IL-10 signaling can inhibit antigen
266 presenting cells maturation, and chemokine secretion of the host during chronic viral infection
267 (Granelli-Piperno et al., 2004) and autoimmune disease (Braat et al., 2003). It can be highly
268 produced by a subset of Tregs, called Type 1 regulatory cells (Tr1) (Zeng et al., 2015), which is

269 associated with autoimmune diseases such as IBD, multiple sclerosis(MS), and type 1 diabetes
270 mellitus when their frequency was found reduced (Jia et al., 2019). Thus our data may suggest a
271 potential role of glycolysis in the function of IL-10 secreting subsets and related diseases.

272 Another subset of cells, where found differential and specific effect of 2DG were MAIT cells, which
273 are characterized with expression of CD161 and a semi-invariant Va7.2+TCR, are activated by a
274 ligand from riboflavin metabolism called 5-ARU in the context of MR-1 molecule (Godfrey et al.,
275 2019). MAIT cells are also unique in that they can be CD8+CD4- or CD8-CD4- and can also
276 produce IL-17 and mostly migrate to mucosal regions, thus potentially play an important role in
277 mucosal homeostasis and microbiome regulation (Oh & Unutmaz, 2019). In our experiments, we
278 had found that 2DG-treated cells compared to control induced higher frequency of CD161+ cells,
279 which is also highly expressed on MAIT cells (CD161hiVa7.2+) (Cosmi et al., 2008; Kleinschek
280 et al., 2009). We also identified a distinct subset exhibiting medium-expression of CD161 which
281 is also enhanced by 2DG, but is neither Va7.2+ (MAIT) nor CCR6+ (Th17) cells. The identity of
282 this subset is not fully clear, but Klenerman et al reported a distinct functional subset in human T
283 cells which had mid-level CD161 expression, produced IL-17/IFNγ, and were found to be highly
284 enriched in chronic inflammation (Billerbeck et al., 2010). Future studies will be needed to better
285 characterize this subset and its functions, as these have also shown different glucose tolerance
286 than other effector cells.

287 Our findings that 2DG enhances IL-17 production also in MAIT cells, in addition to Th17 cells,
288 suggest a shared metabolic pathways that regulate the effector functions of these mucosa-
289 associated subsets. Interestingly IL-17+ MAIT cells are found specifically enriched from the
290 peripheral blood of multiple sclerosis (MS) patient, implicating a proinflammatory role in
291 autoimmune diseases (Willing et al., 2018). IL-17+ MAIT are also associated with inflamed
292 mucosal tissue, and are found activated and produce more IL-17 in IBD patients (Serriari et al.,

293 2014). In addition to the gut, CD8+ MAIT cells have also been shown to be resident in normal
294 skin and are thought to play a role in skin-associated inflammations, such as psoriasis and
295 dermatitis herpetimorfis (Willing et al., 2018) and tissue repair (Constantinides et al., 2019).
296 Therefore, it is tempting to speculate that 2DG or similar analogs of glucose could be used to
297 modulate pathogenic responses of these T cell subsets.

298 An increase in IL-21 production in 2DG-treated memory T cells was also shown significant in our
299 study. IL-21 has been documented to regulate the differentiation and function of several CD4+ T
300 cells subsets, including Th17 cells (Leonard & Wan, 2016; Nurieva et al., 2007; Zhou et al., 2007),
301 Th2 (Frohlich et al., 2007; Lajoie et al., 2014), and T follicular helper cells (TFH) (Vogelzang et
302 al., 2008). Thus, the enhanced IL-17 and IL-4 cytokine production in 2DG treated T cells may be
303 mediated partially by IL-21 signaling. IL-21 was also reported to induce IL-10 production in Th17
304 polarizing condition in mice (Spolski et al., 2009), which is consistent with our observation that
305 differentiation of IL-17 secreting subsets from TNreg with 2DG increase IL-21 and IL-10 secretion.
306 Furthermore, the functional significance of IL-21 in regulating effector functions of CD8+ T cells
307 is highlighted by its critical role in sustaining anti-viral CD8+T cells during chronic LCMV infections
308 (Cui et al., 2011; Elsaesser et al., 2009) and its potent effects to induce and expand cytotoxic
309 CD8+ T cells for cancer immunotherapy (Davis et al., 2015; Santegoets et al., 2013). An increase
310 production of IL-21 and IL-4 cytokines may suggest that 2DG enhance TFH cells, which have high
311 expression level of CXCR5 and produce high level of IL-21 (Crotty, 2014). This hypothesis is also
312 supported by that exogenous Ag-specific TFH cells do not require glycolysis (Choi et al., 2018).
313 TFH is critical for the formation and maintenance of germinal centers and provide help for B cells
314 generating antibody responses after immunizations (Bentebibel et al., 2013; Duan et al., 2014;
315 Spolski & Leonard, 2010). Therefore, regulating TFH cells with glucose metabolism would
316 potentially enhance development of new or improved vaccines (Crotty, 2014).

317 One potential mechanism of an increase frequency of IL-17-secreting T cell subsets in 2DG-
318 treated cells is that 2DG alters T cells metabolism from glucose to alternative energy sources or
319 metabolic pathways such as fatty acid oxidation or pentose phosphate pathway. Previous studies
320 showed an inhibition of glucose metabolism with a glucose transporter inhibitor (CG-5) promoted
321 fatty acid oxidation and the pentose phosphate pathway in CD4+ T cells in a similar manner as
322 with 2DG (Li et al., 2019). Fatty acid metabolism has also been suggested to be involved in Th17
323 inflammation, as blockade of carnitine palmitoyltransferase 1 (CPT1), an enzyme responsible for
324 catalyzing the breakdown of long-chain fatty acids, inhibits Th17-associated cytokine production
325 from type 2 diabetes (T2D) patients (Nicholas et al., 2019).

326 One of our important findings towards mechanism of action of 2DG in our system is that 2DG-
327 mediated increases in IL-17-secreting cells can be reversed by addition of equimolar mannose
328 (Berthe et al., 2018; Kurtoglu et al., 2007). This suggests that another possible mechanism of
329 2DG is inhibiting the initial step of glycolysis affects the downstream metabolic pathway of
330 mannose. Since mannose is a dominant monosaccharide in N-linked glycosylation (Imperiali &
331 O'Connor, 1999), our observation may further suggest that part of the 2DG effects on T cell
332 function are through modifying sugar moieties generated in central carbon metabolism (e.g. N-
333 linked glycosylation). N-linked glycosylation plays a role in modulating activation and cytokine
334 signaling (Baum & Cobb, 2017; Dean et al., 1979; Hauser et al., 2016), and thereby may affect
335 differentiation and function of T cell subsets. An evidence to support this notion was our finding
336 that mannose reversed 2DG-mediated down-regulation of CD25 expression upon activation.
337 Similarly, another inhibitor of N-linked glycosylation, glucosamine, attenuated CD25 surface
338 retention on T cells (Chien et al., 2015), and down-regulation of N-linked glycosylation promotes
339 Th17 differentiation (Chien et al., 2015). However, surprisingly, addition of equimolar glucose
340 could not reverse 2DG-mediated downregulation of CD25. We reason that 2DG efficiently inhibits
341 glycolysis, therefore 2DG-treated cells could not utilize additional glucose. This speculation could

342 be supported by seahorse glycolytic analysis showing that neither additional mannose nor
343 glucose could reverse 2DG-mediated glycolytic capacity. Overall, it is conceivable that 2DG may
344 have dual mechanism in enhancing the survival and effector functions of IL-17 secreting subsets.

345 Recently, 2DG has also been considered as a potential therapy during viral infections such as
346 COVID-19 (Yang et al., 2021). Indeed, 2DG was approved by the Indian Council of Medical
347 Research (OCMR) to treat COVID-19 patients in India (Sahu & Kumar, 2021). 2DG shorten the
348 time of COVID patients recovered from the infection and there was a higher proportion of patients
349 with improved symptoms who were free from supplemental oxygen dependence (Sahu & Kumar,
350 2021) . The underling mechanism of the effect of 2DG in these COVID patients is still unclear, but
351 it was suggested that inhibiting glycolysis with 2DG could affected viral life cycle (Passalacqua et
352 al., 2019) and an increased glucose level could enhance the suppressive function of monocytes
353 and therefore enhanced SARS-CoV-2 infection (Codo et al., 2020). Given our findings, we also
354 speculate that 2DG effects may have been through ameloriation of excessive or uncontrolled
355 immune response during COVID19, which is observed during later severe disease stages. 2DG
356 has also been tested to treat advanced cancer and proven safe in phase I studies (Raez et al.,
357 2013; Stein et al., 2010). Our data shows long-term culture of human primary T cells with 2DG
358 enriched IL-17 secreting T cells, which have been reported to exhibit pathogenic features (Basdeo
359 et al., 2015). However, a significant increase in IFNγ production by 2DG may enhance therapeutic
360 anti-tumor activity (Medrano et al., 2017). Taking together, our study provides insights for future
361 clinical trials and strategies for development of 2DG-related cancer therapies.

362

363

364

365 ## Materials and Methods

366 **T cell purification, activation, and culture**

367 PBMCs from healthy individuals (New York Blood Center, New York, NY) were isolated using
368 Ficoll-Paque plus (GE Healthcare). CD4+ T cells were isolated using Dynal CD4+ isolation kits
369 (Invitrogen) and were 99% pure. Purified CD4+ cells were sorted in some experiments by flow
370 cytometry (FACSAria; BD Biosciences) based on CD45RO, CCR7, CD25, and chemokine
371 receptors expression into: 1) naive T cells (CD45RO-CCR7+CD25-), 2) memory T cells
372 (CD45RO+CD25-), 3) naive regulatory T cells (Tregs; CD45RO-CD25+), 4) Th17 cells
373 (CD45RO+CCR6+). Sorted subsets were 98% pure. All purified cells were kept at 37°C and 5%
374 CO_2 in complete RPMI 1640 media (RPMI 1640 supplemented with 10% FBS; Atlanta Biologicals,
375 Lawrenceville, GA), 8% GlutaMAX (Life Technologies), 8% sodium pyruvate, 8% MEM vitamins,
376 8% MEM nonessential amino acid, and 1% penicillin/streptomycin (all from Corning Cellgro). To
377 activate cells for expansion *in vitro*, anti-CD3/CD28 Dynabeads (Invitrogen) were used at a bead:
378 cell ratio of 1:2 and cultured in complete RPMI 1640 medium (Thermo Fisher Scientific) or
379 complete 1640 RMPI no glucose medium supplemented with IL-2 (10 ng/ml). For MAIT
380 experiments, MAIT cells were activated by adding riboflavin metabolite 5ARU (Toronto Research,
381 Ontario, Canada) into PBMC culture.
382

383 **Flow cytometry staining and analysis**

384 Cells were stained with fluorochrome-conjugated Abs in FACS buffer(PBS+2% FCS and 0.1%
385 sodium azide) for 30 min at 4°C (or room temperature for CCR6 staining). Abs used in surface
386 staining are IL-2R (CD25), PD1, CD161, Va7.2, CCR6 (from BioLegend), LAG3, CD3, and CD8
387 (from Invitrogen). For intracellular cytokine staining, cells were stimulated for 4 h at 37°C with
388 PMA (10ng/ml for PBMC and CD4+ T cells for 40 ng/ml) and ionomycin (500 ng/ml) (both from

389 Sigma-Aldrich) together with GolgiStop (BD Biosciences). Cells were then stained with fixable
390 viability dye (eBioscience) in PBS and surface marker Abs in PBS or FACS for 30 minutes, then
391 fixed and permeabilized using eBioscience fixation/permeabilization buffers for 30 min at 4°C
392 according to the manufacturer's instructions, before staining for intracellular cytokines for 30 min
393 at 4°C. Abs used for intracellular cytokines are IFNγ, TNF, IL-17A, IL-10, and IL-21(BioLegend).
394 Ab used for Regulatory T cells transcription factor intracellular staining are Foxp3 and Helios Abs
395 (BioLegend). Flow cytometry analyses were performed using an LSRFortessa X-20 flow
396 cytometer (BD Biosciences) and SP6800 spectral cell analyzer (Sony Biotechnology).

397 **Seahorse metabolism assay**

398 Seahorse assays were performed according to the manufacturer's instructions to analyze
399 glycolysis metabolism using the Seahorse XF Glycolysis Stress Test Kit (Agilent Technologies).
400 Briefly, 14 day post activation, naïve and memory T cells expanded in indicated conditions were
401 washed and resuspended in glucose-free media (Gibco). 300k cells per well were spun down
402 onto plates coated with Cell-Tak (Fisher scienctific). Four replicates were set up for each
403 condition. Glucose, oligomycin, and 2DG were serially injected to measure metabolic function.
404 Plates were analyzed using an XF^e 24 Extracellular Flux Analyzer (Agilent Technologies).
405 Glycolysis was calculated as average post-glucose ECAR values minus average basal ECAR
406 values.
407

408 **In vitro cytokine polarization assay**

409 Sorted TN and TNreg were activated with anti-CD3/CD28 beads and cultured in complete RPMI
410 media containing IL-2 10ng/ml (Chiron), together with IL-1β (10ng/ml), TGF-β (10ng/ml), and IL-
411 23 (100ng/ml) (R&D Systems). Cells were expanded for 2 weeks in media replenished for 2DG
412 and IL-2.
413

414 **Statistical analysis**

415 Data recorded by flow cytometry were analyzed using FlowJo (Tree Star, Ashland, OR). Statistical
416 analyses were performed using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA).
417 Error bars represent SEM. Results were compared using two-tailed t tests. Bonferroni corrections
418 were applied for multiple comparisons. For all experiments, significance was defined as *$p < 0.05$,
419 **$p < 0.01$, ***$p<0.001$

420

421 ## Conflict of Interest Statement

422 The authors declare that the research was conducted in the absence of any commercial or
423 financial relationships that could be construed as a potential conflict of interest.

424

425 ## Acknowledgements

426 The research in this study was supported by National Institute of Health (NIH) grants
427 U54NS105539 and R01AI121920 to D.U.

428

429

430

431

432

433

434

435 ## Figures Legends:

436 **Figure 1. 2DG suppresses early activation of CD4+ human T cells.** T cell subsets were
437 activated with aCD3/CD28 beads in media alone, 3mM 2DG or in glucose-free media. (A) Surface
438 expression of CD25 and GLUT1 on resting and 24h-activated CD4+ T cells with indicated
439 conditions. (B) Fold difference in GLUT1 expression and mean florescent intensity (MFI) of CD25
440 on 24h-activated CD4+ T cells in 3mM 2DG or in glucose-free media compared to control. (C)
441 Flow cytometry plot of LAG3 and PD1 expression on resting and activated CD4+ T cell in media
442 alone, 3mM 2DG, or in glucose-free media for 24h. (D) Statistical analysis of fold difference in
443 PD1, LAG3, and double positive expression of CD4+ T cells activated for 24 h in 3mM 2DG and
444 in glucose-free media. (E) CD4+ T cells were activated with anti-CD3/CD28 beads. Cell
445 supernatant were collected from the indicated conditions after 2 days post beads activation. The
446 levels of TNF, IFNγ, and IL-2 cytokines were determined by FlowCytomix Multiplex bead assay.
447 Data represent three independent experiments. **$p < 0.01$, ***$p < 0.001$

448

449 **Figure 2. 2DG has differential effects on activation of naive and memory T cell subsets.** (A)
450 Naive and memory CD4+ T cells were activated with anti-CD3/CD28 beads in media alone, 3mM
451 2DG, or in glucose-free media. Media was not supplemented with IL-2. Representative flow
452 cytometry plots of GLUT1 and CD25 surface expression on resting, and day 1(A)- or day 4(B)-
453 post activated Naive (top) and memory T cells (bottom) in indicated conditions were shown. (C)
454 Fold difference in frequency of GLUT1 expression or mean fluorescence intensity of CD25
455 expression on naive or memory T cells on day 1 (black)- and day 4 (grey)-post activation in
456 indicated conditions. (D) Naive and memory CD4+ T cells were labeled with CellTrace violet
457 (CTV) dye followed by anti-CD3/CD28 beads activation in media alone (control), 3mM 2DG, or in
458 glucose-free media. Representative histogram plot of CTV-labeled naive (up) and memory

459 (bottom) CD4+ T cells in 3mM 2DG (red) or in glucose-free media (blue) after day 4 and day 6
460 post TCR activation. (E) Representative fold expansion of naive and memory CD4+ T cells in 1
461 or 3mM 2DG or in glucose-free media after 2 week T cells expansion. (F) Naive and memory
462 CD4+ T cells were activated and expanded in media alone (control, black) or 1mM 2DG (red) for
463 14 days. Metabolic functions were analyzed using Seahorse glucose stress test assay. The
464 extracellular acidification rate (ECAR) was assessed after the addition of glucose (gluc),
465 oligomycin (oligo), and 2DG at indicated times and the glycolytic capacity was determined. (G)
466 Under glycolytic stress test, naive CD4+ T cell treated with 2DG demonstrated reduced glycolysis
467 when compared to control. Each set of data is representative of three donors. *$p < 0.05$, **$p <$
468 0.01, ***$p<0.001$
469
470 **Figure 3. 2DG enhances IL-17 production in primary CD4+ T cells.** Purified CD4+ T cells
471 were activated with aCD3/CD28 beads in media alone, 3mM 2DG, and in media with 0.5mM
472 glucose. (A) The representative flow cytometry plot of CCR6 and CD161 expression on day14
473 post- activated T cells were shown. (B) Statistical analysis of fold difference in frequency of
474 CCR6+ and CD161+ T cells in indicated conditions. (C) After 14 days' cell culture, T cells activated
475 and expanded in indicated condition were re-stimulated with PMA/ionomycin for 4h, followed by
476 intracellular staining as described in the method section. The percentage of IL-17 production from
477 CD161+ and CCR6+ T cells were shown. (D) Statistical analysis of fold difference in frequency of
478 IL17+, IFNγ, IL-4, and IL-21+ T cells from indicated conditions was shown. Each set of data is
479 representative of three donors. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$
480

481 **Figure 4: 2DG enriches the frequency of IL-17 from sorted CCR6+ memory T cells.** (A) sorted
482 CCR6+ and CCR6- memory cells were activated with aCD3/CD28 beads in media alone or with
483 3mM 2DG for 14 days. The frequency of CD161+ cells in day-14 post activated sorted CCR6+

484 and CCR6- subsets in 3mM 2DG compared to control were shown. (B) 14 days post activated T
485 cells were re-stimulated with PMA and ionomycin for 4h, followed by surface and intracellular
486 staining. The frequency of IFNγ and IL-17 was determined from sorted CCR6- and CCR6+ T cells.
487 (C) Statistic analysis of the frequency of IL-17+, IFNγ +, and IL17+ IFNγ+ T cells from CCR6- and
488 CCR6+ T cells as in Figure 4B. The representative plot (D) and the statistical analysis (E) of the
489 frequency of IL-21, IL-10, and IL-17 was determined from sorted CCR6- and CCR6+ T cells after
490 14 days expansion and re-activation with PMA and ionomycin. The representative plot (F) and
491 the statistical analysis (G) of the frequency of IL-10 and IL-17 was determined from sorted CCR6-
492 and CCR6+ T cells after 14 days expansion as in Figure 4D and E. Each set of data is the
493 representative of three donors. *$p < 0.05$, ***$p < 0.001$

494 **Figure 5. 2DG enhances *in vitro* generation of IL-17-producing cells from TNreg cells.** (A)
495 Polarization protocol with Naive or TNreg cells. (B) Representative FOXP3/HELIOS expression
496 of TNreg in two-week polarization cultures with or without 1mM 2DG. (C) Fold difference of IL-17
497 production from 1mM 2DG treated TNreg cells with polarizing cytokines (P.C.) or without (-)
498 compared with control after 14-day expansion. (D) Representative flow cytometry plots of
499 intracellular IFNγ and IL-10 secretion within HELIOS+ and HELIOS- gated populations of TNreg
500 after 14-day expansion. (E) Statistical analysis of IL-17 production of indicated subsets from 1mM
501 treated naive T cell (left)/TNreg(right) with or without polarizing cytokines. (F) Statistical analysis
502 of IFNγ from 1mM treated TNreg in polarizing cytokines (G) Representative flow cytometry plots
503 of intracellular IL-21 and IL-17 secretion within HELIOS+ and HELIOS- gated populations of
504 TNreg after 14-day expansion. (H) Statistical analysis of IL-21 production of indicated subsets
505 from 1mM treated naive T cell (left)/TNreg(right) with or without polarizing cytokines. (I)
506 Representative flow cytometry plots of intracellular IL-10 and IL-17 secretion within HELIOS+ and
507 HELIOS- gated populations of TNreg after 14-day expansion. (J) Statistical analysis of fold
508 difference of IL-10 production of indicated subsets from 1mM treated naïve T cell

509 (left)/TNreg(right) with or without polarizing cytokines. (K) Day-14 expanded TNreg with or without
510 polarization condition were re-stimulated with anti-CD3/CD28 beads. Cell supernatant were
511 collected from the indicated conditions after 2 days post beads activation. IL-17A, IL-17F, IFNγ,
512 and IL-10 productions from supernatant were determined by FlowCytomix Multiplex bead assay.
513 Data represent three independent experiments. *p < 0.05, **p < 0.01, ***p<0.01

514 **Figure 6. Mannose reverses or rescues 2DG effects on T cell subsets.** (A) Naive CD4+ T
515 cells were activated with aCD3/CD28 beads in media alone, 3mM 2DG alone, 3mM 2DG plus
516 1mM or 3mM mannose. Representative flow cytometry plots of GLUT1 and CD25 surface
517 expression on naive T cells in indicated conditions were shown. (B) Statistical analysis of fold
518 difference in GLUT1 and Mean Florescent Intensity (MFI) of CD25 on activated naive T cells as
519 in Figure 6A. (C) Representative fold expansion of naive CD4+ T cells in 3mM 2DG supplemented
520 with indicated concentration of mannose and glucose after 2-week T cells expansion. (D and E)
521 Naive T cells were activated and expanded in the indicated conditions for 14 days. 2DG was
522 washed away and the metabolic functions were analyzed using Seahorse glucose stress test
523 assay. The extracellular acidification rate (ECAR) was assessed after the addition of glucose
524 (gluc), oligomycin (oligo), and 2DG at indicated times and the glycolytic capacity was determined.
525 (E) CD4+ T cells were activated with aCD3/CD28 beads in media alone, 3mM 2DG alone, 3mM
526 2DG plus 1mM or 3mM mannose. Representative plot of PD1 and LAG3 expression of day-14
527 post activated CD4+T cells in indicated conditions. (F) CD4+ T cells were activated with
528 aCD3/CD28 beads in media alone, 3mM 2DG alone, 3mM 2DG plus 1mM or 3mM mannose. 14-
529 days post activated T cells were re-stimulated with PMA and ionomycin for 4h, followed by surface
530 and intracellular staining. Representative flow cytometry plots of T cells contained with CCR6,
531 CD161, and IL-17. (G) Statistical analysis of CD161, IFNγ, IL-17, and IL-21 expression of T cells
532 activated and expanded in indicated conditions for 14 days. Data represent three independent
533 experiments. *p < 0.05, **p < 0.01, ***p<0.01

534 ## Suppmental Figure Legends

535

536 **Supplemental Figure 1. Addition of IL-2 did not restore 2DG effects on naive and memory**
537 **T cell subsets.** (A) Naive and memory CD4+ T cells were activated with aCD3/CD28 beads in
538 media alone, 3mM 2DG, or in glucose-free media. Media was supplemented with IL-2.
539 Representative flow cytometry plots of GLUT1 and CD25 surface expression on resting, and day
540 1-(A) or day 4- post(B) activated Naïve (top) and memory T cells (bottom) in indicated conditions
541 were shown. (C) Fold difference in frequency of GLUT1 expression or mean fluorescence
542 intensity of CD25 expression on naive or memory T cells on day 1- and day 4-post activation in
543 media without IL-2. (D) Naive and memory CD4+ T cells were labeled with CTV dye followed by
544 aCD3/CD28 beads activation in media alone (control), 3mM 2DG, or in glucose-free media.
545 Representative histogram plot of CTV-labeled naïve (up) and memory (bottom) CD4+ T cells in
546 3mM 2DG (Red) or in glucose-free media(blue) after day 4 and day 6 post beads activation. Data
547 represent three independent experiments. ***$p<0.01$

548

549 **Supplemental Figure 2. GLUT1 expression was down-regulated on naive T cells but not**
550 **memory T cells after long-term culture.** Naive and memory CD4+ T cells were activated with
551 aCD3/CD28 beads in media alone (control in grey), or 1mM 2DG (in black). Media was not
552 supplemented with IL-2. Fold difference in frequency of GLUT1 expression on naïve or memory
553 T cells on day 14-post activation was shown. Data represent three independent experiments.
554 ***$p<0.01$

555

556 **Supplemental Figure 3. 2DG did not change IL-2 and TNF production by CD4+ T cells.**
557 Purified CD4+ T cells were activated with aCD3/CD28 beads in media alone, with various doses
558 of 2DG, and in glucose-free media. After 14 days' cell culture, T cells activated and culture in

559 indicated condition were re-stimulated with PMA/ionomycin for 4h, followed by intracellular
560 staining as described in the method section. The percentage of IL-2 and TNF production from
561 CD4+ T cells were shown. Data represent three independent experiments.
562
563 **Supplemental Figure 4. 2DG enhances *in vitro* generation of IL-17-producing cells from**
564 **naive T cells.** Fold difference of IL-17 production from 1mM 2DG treated naive T cells with
565 polarizing cytokines (P.C.) or without (-) compared with control after 14-day expansion.
566
567 **Supplemental Figure 5. Mannose did not reverse GLUT1 expression 2DG-treated naive T**
568 **cells.** Naive CD4+ T cells were activated with aCD3/CD28 beads in media alone, 3mM 2DG
569 alone, 3mM 2DG plus 1mM or 3mM mannose. Representative flow cytometry plots of GLUT1 and
570 CD25 surface expression on naive T cells activated for 24 h in indicated conditions were shown.
571 Data represent three independent experiments.
572
573

574 ## References

575 Acosta-Rodriguez, E. V., Rivino, L., Geginat, J., Jarrossay, D., Gattorno, M., Lanzavecchia, A.,
576 Sallusto, F., & Napolitani, G. (2007). Surface phenotype and antigenic specificity of human
577 interleukin 17-producing T helper memory cells. *Nat Immunol*, *8*(6), 639-646.
578 https://doi.org/10.1038/ni1467

579 Afzali, B., Mitchell, P. J., Edozie, F. C., Povoleri, G. A., Dowson, S. E., Demandt, L., Walter, G.,
580 Canavan, J. B., Scotta, C., Menon, B., Chana, P. S., Khamri, W., Kordasti, S. Y., Heck,
581 S., Grimbacher, B., Tree, T., Cope, A. P., Taams, L. S., Lechler, R. I., . . . Lombardi, G.
582 (2013). CD161 expression characterizes a subpopulation of human regulatory T cells that
583 produces IL-17 in a STAT3-dependent manner. *Eur J Immunol*, *43*(8), 2043-2054.
584 https://doi.org/10.1002/eji.201243296

585 Ahadova, A., Gebert, J., von Knebel Doeberitz, M., Kopitz, J., & Kloor, M. (2015). Dose-dependent
586 effect of 2-deoxy-D-glucose on glycoprotein mannosylation in cancer cells. *IUBMB Life*,
587 *67*(3), 218-226. https://doi.org/10.1002/iub.1364

588 Akella, N. M., Ciraku, L., & Reginato, M. J. (2019). Fueling the fire: emerging role of the
589 hexosamine biosynthetic pathway in cancer. *BMC Biol*, *17*(1), 52.
590 https://doi.org/10.1186/s12915-019-0671-3

591 Almeida, L., Lochner, M., Berod, L., & Sparwasser, T. (2016). Metabolic pathways in T cell
592 activation and lineage differentiation. *Semin Immunol*, *28*(5), 514-524.
593 https://doi.org/10.1016/j.smim.2016.10.009

594 Andresen, L., Skovbakke, S. L., Persson, G., Hagemann-Jensen, M., Hansen, K. A., Jensen, H.,
595 & Skov, S. (2012). 2-deoxy D-glucose prevents cell surface expression of NKG2D ligands
596 through inhibition of N-linked glycosylation. *J Immunol*, *188*(4), 1847-1855.
597 https://doi.org/10.4049/jimmunol.1004085

598 Araki, K., Youngblood, B., & Ahmed, R. (2010). The role of mTOR in memory CD8 T-cell
599 differentiation. *Immunol Rev*, *235*(1), 234-243. https://doi.org/10.1111/j.0105-
600 2896.2010.00898.x

601 Basdeo, S. A., Moran, B., Cluxton, D., Canavan, M., McCormick, J., Connolly, M., Orr, C., Mills,
602 K. H., Veale, D. J., Fearon, U., & Fletcher, J. M. (2015). Polyfunctional, Pathogenic

603 CD161+ Th17 Lineage Cells Are Resistant to Regulatory T Cell-Mediated Suppression in
604 the Context of Autoimmunity. *J Immunol*, *195*(2), 528-540.
605 https://doi.org/10.4049/jimmunol.1402990

606 Baum, L. G., & Cobb, B. A. (2017). The direct and indirect effects of glycans on immune function.
607 *Glycobiology*, *27*(7), 619-624. https://doi.org/10.1093/glycob/cwx036

608 Bentebibel, S. E., Lopez, S., Obermoser, G., Schmitt, N., Mueller, C., Harrod, C., Flano, E.,
609 Mejias, A., Albrecht, R. A., Blankenship, D., Xu, H., Pascual, V., Banchereau, J., Garcia-
610 Sastre, A., Palucka, A. K., Ramilo, O., & Ueno, H. (2013). Induction of
611 ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza
612 vaccination. *Sci Transl Med*, *5*(176), 176ra132.
613 https://doi.org/10.1126/scitranslmed.3005191

614 Berthe, A., Zaffino, M., Muller, C., Foulquier, F., Houdou, M., Schulz, C., Bost, F., De Fay, E.,
615 Mazerbourg, S., & Flament, S. (2018). Protein N-glycosylation alteration and glycolysis
616 inhibition both contribute to the antiproliferative action of 2-deoxyglucose in breast cancer
617 cells. *Breast Cancer Res Treat*, *171*(3), 581-591. https://doi.org/10.1007/s10549-018-
618 4874-z

619 Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Weiner, H. L., & Kuchroo, V.
620 K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector
621 TH17 and regulatory T cells. *Nature*, *441*(7090), 235-238.
622 https://doi.org/10.1038/nature04753

623 Billerbeck, E., Kang, Y. H., Walker, L., Lockstone, H., Grafmueller, S., Fleming, V., Flint, J.,
624 Willberg, C. B., Bengsch, B., Seigel, B., Ramamurthy, N., Zitzmann, N., Barnes, E. J.,
625 Thevanayagam, J., Bhagwanani, A., Leslie, A., Oo, Y. H., Kollnberger, S., Bowness, P., .
626 . . Klenerman, P. (2010). Analysis of CD161 expression on human CD8+ T cells defines
627 a distinct functional subset with tissue-homing properties. *Proc Natl Acad Sci U S A*,
628 *107*(7), 3006-3011. https://doi.org/10.1073/pnas.0914839107

629 Braat, H., Peppelenbosch, M. P., & Hommes, D. W. (2003). Interleukin-10-based therapy for
630 inflammatory bowel disease. *Expert Opin Biol Ther*, *3*(5), 725-731.
631 https://doi.org/10.1517/14712598.3.5.725

632 Brembilla, N. C., Senra, L., & Boehncke, W. H. (2018). The IL-17 Family of Cytokines in Psoriasis:
633 IL-17A and Beyond. *Front Immunol*, *9*, 1682. https://doi.org/10.3389/fimmu.2018.01682

634 Brucklacher-Waldert, V., Ferreira, C., Stebegg, M., Fesneau, O., Innocentin, S., Marie, J. C., &
635 Veldhoen, M. (2017). Cellular Stress in the Context of an Inflammatory Environment
636 Supports TGF-beta-Independent T Helper-17 Differentiation. *Cell Rep*, *19*(11), 2357-
637 2370. https://doi.org/10.1016/j.celrep.2017.05.052

638 Buck, M. D., O'Sullivan, D., & Pearce, E. L. (2015). T cell metabolism drives immunity. *J Exp Med*,
639 *212*(9), 1345-1360. https://doi.org/10.1084/jem.20151159

640 Chapman, N. M., Boothby, M. R., & Chi, H. (2019). Metabolic coordination of T cell quiescence
641 and activation. *Nat Rev Immunol*. https://doi.org/10.1038/s41577-019-0203-y

642 Chapman, N. M., Boothby, M. R., & Chi, H. (2020). Metabolic coordination of T cell quiescence
643 and activation. *Nat Rev Immunol*, *20*(1), 55-70. https://doi.org/10.1038/s41577-019-0203-
644 y

645 Chiba, A., Murayama, G., & Miyake, S. (2018). Mucosal-Associated Invariant T Cells in
646 Autoimmune Diseases. *Front Immunol*, *9*, 1333.
647 https://doi.org/10.3389/fimmu.2018.01333

648 Chien, M. W., Lin, M. H., Huang, S. H., Fu, S. H., Hsu, C. Y., Yen, B. L., Chen, J. T., Chang, D.
649 M., & Sytwu, H. K. (2015). Glucosamine Modulates T Cell Differentiation through Down-
650 regulating N-Linked Glycosylation of CD25. *J Biol Chem*, *290*(49), 29329-29344.
651 https://doi.org/10.1074/jbc.M115.674671

652 Choi, S. C., Titov, A. A., Abboud, G., Seay, H. R., Brusko, T. M., Roopenian, D. C., Salek-
653 Ardakani, S., & Morel, L. (2018). Inhibition of glucose metabolism selectively targets
654 autoreactive follicular helper T cells. *Nat Commun*, *9*(1), 4369.
655 https://doi.org/10.1038/s41467-018-06686-0

656 Codo, A. C., Davanzo, G. G., Monteiro, L. B., de Souza, G. F., Muraro, S. P., Virgilio-da-Silva, J.
657 V., Prodonoff, J. S., Carregari, V. C., de Biagi Junior, C. A. O., Crunfli, F., Jimenez
658 Restrepo, J. L., Vendramini, P. H., Reis-de-Oliveira, G., Bispo Dos Santos, K., Toledo-
659 Teixeira, D. A., Parise, P. L., Martini, M. C., Marques, R. E., Carmo, H. R., . . . Moraes-
660 Vieira, P. M. (2020). Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte

661 Response through a HIF-1alpha/Glycolysis-Dependent Axis. *Cell Metab*, *32*(3), 437-446
662 e435. https://doi.org/10.1016/j.cmet.2020.07.007

663 Constantinides, M. G., Link, V. M., Tamoutounour, S., Wong, A. C., Perez-Chaparro, P. J., Han,
664 S. J., Chen, Y. E., Li, K., Farhat, S., Weckel, A., Krishnamurthy, S. R., Vujkovic-Cvijin, I.,
665 Linehan, J. L., Bouladoux, N., Merrill, E. D., Roy, S., Cua, D. J., Adams, E. J., Bhandoola,
666 A., . . . Belkaid, Y. (2019). MAIT cells are imprinted by the microbiota in early life and
667 promote tissue repair. *Science*, *366*(6464). https://doi.org/10.1126/science.aax6624

668 Corbett, A. J., Awad, W., Wang, H., & Chen, Z. (2020). Antigen Recognition by MR1-Reactive T
669 Cells; MAIT Cells, Metabolites, and Remaining Mysteries. *Front Immunol*, *11*, 1961.
670 https://doi.org/10.3389/fimmu.2020.01961

671 Cosmi, L., De Palma, R., Santarlasci, V., Maggi, L., Capone, M., Frosali, F., Rodolico, G., Querci,
672 V., Abbate, G., Angeli, R., Berrino, L., Fambrini, M., Caproni, M., Tonelli, F., Lazzeri, E.,
673 Parronchi, P., Liotta, F., Maggi, E., Romagnani, S., & Annunziato, F. (2008). Human
674 interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. *J Exp Med*,
675 *205*(8), 1903-1916. https://doi.org/10.1084/jem.20080397

676 Coulter, E. H., Miller, L., McCorkell, S., McGuire, C., Algie, K., Freeman, J., Weller, B., Mattison,
677 P. G., McConnachie, A., Wu, O., & Paul, L. (2017). Validity of the activPAL3 activity
678 monitor in people moderately affected by Multiple Sclerosis. *Med Eng Phys*, *45*, 78-82.
679 https://doi.org/10.1016/j.medengphy.2017.03.008

680 Crotty, S. (2014). T follicular helper cell differentiation, function, and roles in disease. *Immunity*,
681 *41*(4), 529-542. https://doi.org/10.1016/j.immuni.2014.10.004

682 Cui, W., Liu, Y., Weinstein, J. S., Craft, J., & Kaech, S. M. (2011). An interleukin-21-interleukin-
683 10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. *Immunity*,
684 *35*(5), 792-805. https://doi.org/10.1016/j.immuni.2011.09.017

685 Damasceno, L. E. A., Prado, D. S., Veras, F. P., Fonseca, M. M., Toller-Kawahisa, J. E., Rosa,
686 M. H., Publio, G. A., Martins, T. V., Ramalho, F. S., Waisman, A., Cunha, F. Q., Cunha,
687 T. M., & Alves-Filho, J. C. (2020). PKM2 promotes Th17 cell differentiation and
688 autoimmune inflammation by fine-tuning STAT3 activation. *J Exp Med*, *217*(10).
689 https://doi.org/10.1084/jem.20190613

690 Davis, M. R., Zhu, Z., Hansen, D. M., Bai, Q., & Fang, Y. (2015). The role of IL-21 in immunity
691 and cancer. *Cancer Lett*, *358*(2), 107-114. https://doi.org/10.1016/j.canlet.2014.12.047

692 Dean, R. H., Polterauer, P., Hunter, C. E., Erath, H., Meng, H. C., & Scott, H. W., Jr. (1979). Effect
693 of intestinal venous diversion on lipid metabolism. *Surg Forum*, *30*, 85-86.
694 https://www.ncbi.nlm.nih.gov/pubmed/538706

695 Duan, Z., Chen, X., Liang, Z., Zeng, Y., Zhu, F., Long, L., McCrae, M. A., Zhuang, H., Shen, T.,
696 & Lu, F. (2014). Genetic polymorphisms of CXCR5 and CXCL13 are associated with non-
697 responsiveness to the hepatitis B vaccine. *Vaccine*, *32*(41), 5316-5322.
698 https://doi.org/10.1016/j.vaccine.2014.07.064

699 Elsaesser, H., Sauer, K., & Brooks, D. G. (2009). IL-21 is required to control chronic viral infection.
700 *Science*, *324*(5934), 1569-1572. https://doi.org/10.1126/science.1174182

701 Evans-Marin, H., Rogier, R., Koralov, S. B., Manasson, J., Roeleveld, D., van der Kraan, P. M.,
702 Scher, J. U., Koenders, M. I., & Abdollahi-Roodsaz, S. (2018). Microbiota-Dependent
703 Involvement of Th17 Cells in Murine Models of Inflammatory Arthritis. *Arthritis Rheumatol*,
704 *70*(12), 1971-1983. https://doi.org/10.1002/art.40657

705 Frohlich, A., Marsland, B. J., Sonderegger, I., Kurrer, M., Hodge, M. R., Harris, N. L., & Kopf, M.
706 (2007). IL-21 receptor signaling is integral to the development of Th2 effector responses
707 in vivo. *Blood*, *109*(5), 2023-2031. https://doi.org/10.1182/blood-2006-05-021600

708 Galvez, J. (2014). Role of Th17 Cells in the Pathogenesis of Human IBD. *ISRN Inflamm*, *2014*,
709 928461. https://doi.org/10.1155/2014/928461

710 Garidou, L., Pomie, C., Klopp, P., Waget, A., Charpentier, J., Aloulou, M., Giry, A., Serino, M.,
711 Stenman, L., Lahtinen, S., Dray, C., Iacovoni, J. S., Courtney, M., Collet, X., Amar, J.,
712 Servant, F., Lelouvier, B., Valet, P., Eberl, G., . . . Burcelin, R. (2015). The Gut Microbiota
713 Regulates Intestinal CD4 T Cells Expressing RORgammat and Controls Metabolic
714 Disease. *Cell Metab*, *22*(1), 100-112. https://doi.org/10.1016/j.cmet.2015.06.001

715 Gaublomme, J. T., Yosef, N., Lee, Y., Gertner, R. S., Yang, L. V., Wu, C., Pandolfi, P. P., Mak,
716 T., Satija, R., Shalek, A. K., Kuchroo, V. K., Park, H., & Regev, A. (2015). Single-Cell
717 Genomics Unveils Critical Regulators of Th17 Cell Pathogenicity. *Cell*, *163*(6), 1400-1412.
718 https://doi.org/10.1016/j.cell.2015.11.009

719 Godfrey, D. I., Koay, H. F., McCluskey, J., & Gherardin, N. A. (2019). The biology and functional
720 importance of MAIT cells. *Nat Immunol*, *20*(9), 1110-1128.
721 https://doi.org/10.1038/s41590-019-0444-8

722 Granelli-Piperno, A., Golebiowska, A., Trumpfheller, C., Siegal, F. P., & Steinman, R. M. (2004).
723 HIV-1-infected monocyte-derived dendritic cells do not undergo maturation but can elicit
724 IL-10 production and T cell regulation. *Proc Natl Acad Sci U S A*, *101*(20), 7669-7674.
725 https://doi.org/10.1073/pnas.0402431101

726 Gu, L., Yi, Z., Zhang, Y., Ma, Z., Zhu, Y., & Gao, J. (2017). Low dose of 2-deoxy-D-glucose kills
727 acute lymphoblastic leukemia cells and reverses glucocorticoid resistance via N-linked
728 glycosylation inhibition under normoxia. *Oncotarget*, *8*(19), 30978-30991.
729 https://doi.org/10.18632/oncotarget.16046

730 Hauser, M. A., Kindinger, I., Laufer, J. M., Spate, A. K., Bucher, D., Vanes, S. L., Krueger, W. A.,
731 Wittmann, V., & Legler, D. F. (2016). Distinct CCR7 glycosylation pattern shapes receptor
732 signaling and endocytosis to modulate chemotactic responses. *J Leukoc Biol*, *99*(6), 993-
733 1007. https://doi.org/10.1189/jlb.2VMA0915-432RR

734 Hinks, T. S. C., & Zhang, X. W. (2020). MAIT Cell Activation and Functions. *Front Immunol*, *11*,
735 1014. https://doi.org/10.3389/fimmu.2020.01014

736 Ho, P. C., Bihuniak, J. D., Macintyre, A. N., Staron, M., Liu, X., Amezquita, R., Tsui, Y. C., Cui,
737 G., Micevic, G., Perales, J. C., Kleinstein, S. H., Abel, E. D., Insogna, K. L., Feske, S.,
738 Locasale, J. W., Bosenberg, M. W., Rathmell, J. C., & Kaech, S. M. (2015).
739 Phosphoenolpyruvate Is a Metabolic Checkpoint of Anti-tumor T Cell Responses. *Cell*,
740 *162*(6), 1217-1228. https://doi.org/10.1016/j.cell.2015.08.012

741 Imperiali, B., & O'Connor, S. E. (1999). Effect of N-linked glycosylation on glycopeptide and
742 glycoprotein structure. *Curr Opin Chem Biol*, *3*(6), 643-649.
743 https://www.ncbi.nlm.nih.gov/pubmed/10600722

744 Ioannidis, M., Cerundolo, V., & Salio, M. (2020). The Immune Modulating Properties of Mucosal-
745 Associated Invariant T Cells. *Front Immunol*, *11*, 1556.
746 https://doi.org/10.3389/fimmu.2020.01556

747 Iyer, S. S., & Cheng, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation
748 and autoimmune disease. *Crit Rev Immunol*, *32*(1), 23-63.
749 https://doi.org/10.1615/critrevimmunol.v32.i1.30

750 Jacobs, S. R., Herman, C. E., Maciver, N. J., Wofford, J. A., Wieman, H. L., Hammen, J. J., &
751 Rathmell, J. C. (2008). Glucose uptake is limiting in T cell activation and requires CD28-
752 mediated Akt-dependent and independent pathways. *J Immunol*, *180*(7), 4476-4486.
753 https://doi.org/10.4049/jimmunol.180.7.4476

754 Jia, X., Zhai, T., Wang, B., Yao, Q., Li, Q., Mu, K., & Zhang, J. A. (2019). Decreased number and
755 impaired function of type 1 regulatory T cells in autoimmune diseases. *J Cell Physiol*,
756 *234*(8), 12442-12450. https://doi.org/10.1002/jcp.28092

757 Johnson, M. O., Wolf, M. M., Madden, M. Z., Andrejeva, G., Sugiura, A., Contreras, D. C.,
758 Maseda, D., Liberti, M. V., Paz, K., Kishton, R. J., Johnson, M. E., de Cubas, A. A., Wu,
759 P., Li, G., Zhang, Y., Newcomb, D. C., Wells, A. D., Restifo, N. P., Rathmell, W. K., . . .
760 Rathmell, J. C. (2018). Distinct Regulation of Th17 and Th1 Cell Differentiation by
761 Glutaminase-Dependent Metabolism. *Cell*, *175*(7), 1780-1795 e1719.
762 https://doi.org/10.1016/j.cell.2018.10.001

763 Jung, M. K., Kwak, J. E., & Shin, E. C. (2017). IL-17A-Producing Foxp3(+) Regulatory T Cells and
764 Human Diseases. *Immune Netw*, *17*(5), 276-286.
765 https://doi.org/10.4110/in.2017.17.5.276

766 Kastirr, I., Crosti, M., Maglie, S., Paroni, M., Steckel, B., Moro, M., Pagani, M., Abrignani, S., &
767 Geginat, J. (2015). Signal Strength and Metabolic Requirements Control Cytokine-
768 Induced Th17 Differentiation of Uncommitted Human T Cells. *J Immunol*, *195*(8), 3617-
769 3627. https://doi.org/10.4049/jimmunol.1501016

770 Kleinschek, M. A., Boniface, K., Sadekova, S., Grein, J., Murphy, E. E., Turner, S. P., Raskin, L.,
771 Desai, B., Faubion, W. A., de Waal Malefyt, R., Pierce, R. H., McClanahan, T., & Kastelein,
772 R. A. (2009). Circulating and gut-resident human Th17 cells express CD161 and promote
773 intestinal inflammation. *J Exp Med*, *206*(3), 525-534.
774 https://doi.org/10.1084/jem.20081712

775 Knochelmann, H. M., Dwyer, C. J., Bailey, S. R., Amaya, S. M., Elston, D. M., Mazza-McCrann,
776 J. M., & Paulos, C. M. (2018). When worlds collide: Th17 and Treg cells in cancer and

777 autoimmunity. *Cell Mol Immunol*, *15*(5), 458-469. https://doi.org/10.1038/s41423-018-
778 0004-4

779 Kurtoglu, M., Gao, N., Shang, J., Maher, J. C., Lehrman, M. A., Wangpaichitr, M., Savaraj, N.,
780 Lane, A. N., & Lampidis, T. J. (2007). Under normoxia, 2-deoxy-D-glucose elicits cell death
781 in select tumor types not by inhibition of glycolysis but by interfering with N-linked
782 glycosylation. *Mol Cancer Ther*, *6*(11), 3049-3058. https://doi.org/10.1158/1535-
783 7163.MCT-07-0310

784 Lajoie, S., Lewkowich, I., Herman, N. S., Sproles, A., Pesce, J. T., Wynn, T. A., Grusby, M. J.,
785 Hamid, Q., & Wills-Karp, M. (2014). IL-21 receptor signalling partially mediates Th2-
786 mediated allergic airway responses. *Clin Exp Allergy*, *44*(7), 976-985.
787 https://doi.org/10.1111/cea.12341

788 Lee, Y., Awasthi, A., Yosef, N., Quintana, F. J., Xiao, S., Peters, A., Wu, C., Kleinewietfeld, M.,
789 Kunder, S., Hafler, D. A., Sobel, R. A., Regev, A., & Kuchroo, V. K. (2012). Induction and
790 molecular signature of pathogenic TH17 cells. *Nat Immunol*, *13*(10), 991-999.
791 https://doi.org/10.1038/ni.2416

792 Leonard, W. J., & Wan, C. K. (2016). IL-21 Signaling in Immunity. *F1000Res*, *5*.
793 https://doi.org/10.12688/f1000research.7634.1

794 Li, W., Qu, G., Choi, S. C., Cornaby, C., Titov, A., Kanda, N., Teng, X., Wang, H., & Morel, L.
795 (2019). Targeting T Cell Activation and Lupus Autoimmune Phenotypes by Inhibiting
796 Glucose Transporters. *Front Immunol*, *10*, 833. https://doi.org/10.3389/fimmu.2019.00833

797 Lichtenegger, F. S., Rothe, M., Schnorfeil, F. M., Deiser, K., Krupka, C., Augsberger, C., Schluter,
798 M., Neitz, J., & Subklewe, M. (2018). Targeting LAG-3 and PD-1 to Enhance T Cell
799 Activation by Antigen-Presenting Cells. *Front Immunol*, *9*, 385.
800 https://doi.org/10.3389/fimmu.2018.00385

801 MacIver, N. J., Michalek, R. D., & Rathmell, J. C. (2013). Metabolic regulation of T lymphocytes.
802 *Annu Rev Immunol*, *31*, 259-283. https://doi.org/10.1146/annurev-immunol-032712-
803 095956

804 Marshall, E. A., Ng, K. W., Kung, S. H., Conway, E. M., Martinez, V. D., Halvorsen, E. C.,
805 Rowbotham, D. A., Vucic, E. A., Plumb, A. W., Becker-Santos, D. D., Enfield, K. S.,

806 Kennett, J. Y., Bennewith, K. L., Lockwood, W. W., Lam, S., English, J. C., Abraham, N.,
807 & Lam, W. L. (2016). Emerging roles of T helper 17 and regulatory T cells in lung cancer
808 progression and metastasis. *Mol Cancer*, *15*(1), 67. https://doi.org/10.1186/s12943-016-
809 0551-1

810 McDonald, D. R. (2012). TH17 deficiency in human disease. *J Allergy Clin Immunol*, *129*(6), 1429-
811 1435; quiz 1436-1427. https://doi.org/10.1016/j.jaci.2012.03.034

812 Medrano, R. F. V., Hunger, A., Mendonca, S. A., Barbuto, J. A. M., & Strauss, B. E. (2017).
813 Immunomodulatory and antitumor effects of type I interferons and their application in
814 cancer therapy. *Oncotarget*, *8*(41), 71249-71284.
815 https://doi.org/10.18632/oncotarget.19531

816 Mercer, F., Khaitan, A., Kozhaya, L., Aberg, J. A., & Unutmaz, D. (2014). Differentiation of IL-17-
817 producing effector and regulatory human T cells from lineage-committed naive precursors.
818 *J Immunol*, *193*(3), 1047-1054. https://doi.org/10.4049/jimmunol.1302936

819 Ng, M. S. F., Roth, T. L., Mendoza, V. F., Marson, A., & Burt, T. D. (2019). Helios enhances the
820 preferential differentiation of human fetal CD4(+) naive T cells into regulatory T cells. *Sci*
821 *Immunol*, *4*(41). https://doi.org/10.1126/sciimmunol.aav5947

822 Nicholas, D. A., Proctor, E. A., Agrawal, M., Belkina, A. C., Van Nostrand, S. C., Panneerseelan-
823 Bharath, L., Jones, A. R. t., Raval, F., Ip, B. C., Zhu, M., Cacicedo, J. M., Habib, C., Sainz-
824 Rueda, N., Persky, L., Sullivan, P. G., Corkey, B. E., Apovian, C. M., Kern, P. A.,
825 Lauffenburger, D. A., & Nikolajczyk, B. S. (2019). Fatty Acid Metabolites Combine with
826 Reduced beta Oxidation to Activate Th17 Inflammation in Human Type 2 Diabetes. *Cell*
827 *Metab*, *30*(3), 447-461 e445. https://doi.org/10.1016/j.cmet.2019.07.004

828 Nurieva, R., Yang, X. O., Martinez, G., Zhang, Y., Panopoulos, A. D., Ma, L., Schluns, K., Tian,
829 Q., Watowich, S. S., Jetten, A. M., & Dong, C. (2007). Essential autocrine regulation by
830 IL-21 in the generation of inflammatory T cells. *Nature*, *448*(7152), 480-483.
831 https://doi.org/10.1038/nature05969

832 Oh, J., & Unutmaz, D. (2019). Immune cells for microbiota surveillance. *Science*, *366*(6464), 419-
833 420. https://doi.org/10.1126/science.aaz4014

834 Okano, T., Saegusa, J., Nishimura, K., Takahashi, S., Sendo, S., Ueda, Y., & Morinobu, A. (2017).
835 3-bromopyruvate ameliorate autoimmune arthritis by modulating Th17/Treg cell
836 differentiation and suppressing dendritic cell activation. *Sci Rep*, *7*, 42412.
837 https://doi.org/10.1038/srep42412

838 Palmer, C. S., Ostrowski, M., Balderson, B., Christian, N., & Crowe, S. M. (2015). Glucose
839 metabolism regulates T cell activation, differentiation, and functions. *Front Immunol*, *6*, 1.
840 https://doi.org/10.3389/fimmu.2015.00001

841 Passalacqua, K. D., Lu, J., Goodfellow, I., Kolawole, A. O., Arche, J. R., Maddox, R. J., Carnahan,
842 K. E., O'Riordan, M. X. D., & Wobus, C. E. (2019). Glycolysis Is an Intrinsic Factor for
843 Optimal Replication of a Norovirus. *mBio*, *10*(2). https://doi.org/10.1128/mBio.02175-18

844 Pearce, E. L., Poffenberger, M. C., Chang, C. H., & Jones, R. G. (2013). Fueling immunity:
845 insights into metabolism and lymphocyte function. *Science*, *342*(6155), 1242454.
846 https://doi.org/10.1126/science.1242454

847 Perl, A. (2016). Activation of mTOR (mechanistic target of rapamycin) in rheumatic diseases. *Nat*
848 *Rev Rheumatol*, *12*(3), 169-182. https://doi.org/10.1038/nrrheum.2015.172

849 Platt, M. P., Bolding, K. A., Wayne, C. R., Chaudhry, S., Cutforth, T., Franks, K. M., & Agalliu, D.
850 (2020). Th17 lymphocytes drive vascular and neuronal deficits in a mouse model of
851 postinfectious autoimmune encephalitis. *Proc Natl Acad Sci U S A*, *117*(12), 6708-6716.
852 https://doi.org/10.1073/pnas.1911097117

853 Raez, L. E., Papadopoulos, K., Ricart, A. D., Chiorean, E. G., Dipaola, R. S., Stein, M. N., Rocha
854 Lima, C. M., Schlesselman, J. J., Tolba, K., Langmuir, V. K., Kroll, S., Jung, D. T., Kurtoglu,
855 M., Rosenblatt, J., & Lampidis, T. J. (2013). A phase I dose-escalation trial of 2-deoxy-D-
856 glucose alone or combined with docetaxel in patients with advanced solid tumors. *Cancer*
857 *Chemother Pharmacol*, *71*(2), 523-530. https://doi.org/10.1007/s00280-012-2045-1

858 Sahu, K. K., & Kumar, R. (2021). Role of 2-Deoxy-D-Glucose (2-DG) in COVID-19 disease: A
859 potential game-changer. *J Family Med Prim Care*, *10*(10), 3548-3552.
860 https://doi.org/10.4103/jfmpc.jfmpc_1338_21

861 Santegoets, S. J., Turksma, A. W., Powell, D. J., Jr., Hooijberg, E., & de Gruijl, T. D. (2013). IL-
862 21 in cancer immunotherapy: At the right place at the right time. *Oncoimmunology*, *2*(6),
863 e24522. https://doi.org/10.4161/onci.24522

864 Sasaki, C. Y., Chen, G., Munk, R., Eitan, E., Martindale, J., Longo, D. L., & Ghosh, P. (2016).
865 p((7)(0)S(6)K(1)) in the TORC1 pathway is essential for the differentiation of Th17 Cells,
866 but not Th1, Th2, or Treg cells in mice. *Eur J Immunol*, *46*(1), 212-222.
867 https://doi.org/10.1002/eji.201445422

868 Sena, L. A., Li, S., Jairaman, A., Prakriya, M., Ezponda, T., Hildeman, D. A., Wang, C. R.,
869 Schumacker, P. T., Licht, J. D., Perlman, H., Bryce, P. J., & Chandel, N. S. (2013).
870 Mitochondria are required for antigen-specific T cell activation through reactive oxygen
871 species signaling. *Immunity*, *38*(2), 225-236.
872 https://doi.org/10.1016/j.immuni.2012.10.020

873 Serriari, N. E., Eoche, M., Lamotte, L., Lion, J., Fumery, M., Marcelo, P., Chatelain, D., Barre, A.,
874 Nguyen-Khac, E., Lantz, O., Dupas, J. L., & Treiner, E. (2014). Innate mucosal-associated
875 invariant T (MAIT) cells are activated in inflammatory bowel diseases. *Clin Exp Immunol*,
876 *176*(2), 266-274. https://doi.org/10.1111/cei.12277

877 Shen, H., & Shi, L. Z. (2019). Metabolic regulation of TH17 cells. *Mol Immunol*, *109*, 81-87.
878 https://doi.org/10.1016/j.molimm.2019.03.005

879 Shi, L. Z., Wang, R., Huang, G., Vogel, P., Neale, G., Green, D. R., & Chi, H. (2011). HIF1alpha-
880 dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation
881 of TH17 and Treg cells. *J Exp Med*, *208*(7), 1367-1376.
882 https://doi.org/10.1084/jem.20110278

883 Singh, S. P., Zhang, H. H., Foley, J. F., Hedrick, M. N., & Farber, J. M. (2008). Human T cells that
884 are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol*, *180*(1), 214-
885 221. https://doi.org/10.4049/jimmunol.180.1.214

886 Spolski, R., Kim, H. P., Zhu, W., Levy, D. E., & Leonard, W. J. (2009). IL-21 mediates suppressive
887 effects via its induction of IL-10. *J Immunol*, *182*(5), 2859-2867.
888 https://doi.org/10.4049/jimmunol.0802978

889 Spolski, R., & Leonard, W. J. (2010). IL-21 and T follicular helper cells. *Int Immunol*, *22*(1), 7-12.
890 https://doi.org/10.1093/intimm/dxp112

891 Stein, M., Lin, H., Jeyamohan, C., Dvorzhinski, D., Gounder, M., Bray, K., Eddy, S., Goodin, S.,
892 White, E., & Dipaola, R. S. (2010). Targeting tumor metabolism with 2-deoxyglucose in
893 patients with castrate-resistant prostate cancer and advanced malignancies. *Prostate*,
894 *70*(13), 1388-1394. https://doi.org/10.1002/pros.21172

895 Tanimine, N., Germana, S. K., Fan, M., Hippen, K., Blazar, B. R., Markmann, J. F., Turka, L. A.,
896 & Priyadharshini, B. (2019). Differential effects of 2-deoxy-D-glucose on in vitro expanded
897 human regulatory T cell subsets. *PLoS One*, *14*(6), e0217761.
898 https://doi.org/10.1371/journal.pone.0217761

899 Tastan, C., Karhan, E., Zhou, W., Fleming, E., Voigt, A. Y., Yao, X., Wang, L., Horne, M., Placek,
900 L., Kozhaya, L., Oh, J., & Unutmaz, D. (2018). Tuning of human MAIT cell activation by
901 commensal bacteria species and MR1-dependent T-cell presentation. *Mucosal Immunol*,
902 *11*(6), 1591-1605. https://doi.org/10.1038/s41385-018-0072-x

903 Thornton, A. M., Lu, J., Korty, P. E., Kim, Y. C., Martens, C., Sun, P. D., & Shevach, E. M. (2019).
904 Helios(+) and Helios(-) Treg subpopulations are phenotypically and functionally distinct
905 and express dissimilar TCR repertoires. *Eur J Immunol*, *49*(3), 398-412.
906 https://doi.org/10.1002/eji.201847935

907 Valmori, D., Raffin, C., Raimbaud, I., & Ayyoub, M. (2010). Human RORgammat+ TH17 cells
908 preferentially differentiate from naive FOXP3+Treg in the presence of lineage-specific
909 polarizing factors. *Proc Natl Acad Sci U S A*, *107*(45), 19402-19407.
910 https://doi.org/10.1073/pnas.1008247107

911 van der Windt, G. J., & Pearce, E. L. (2012). Metabolic switching and fuel choice during T-cell
912 differentiation and memory development. *Immunol Rev*, *249*(1), 27-42.
913 https://doi.org/10.1111/j.1600-065X.2012.01150.x

914 Visconti, A., Le Roy, C. I., Rosa, F., Rossi, N., Martin, T. C., Mohney, R. P., Li, W., de Rinaldis,
915 E., Bell, J. T., Venter, J. C., Nelson, K. E., Spector, T. D., & Falchi, M. (2019). Interplay
916 between the human gut microbiome and host metabolism. *Nat Commun*, *10*(1), 4505.
917 https://doi.org/10.1038/s41467-019-12476-z

918 Vogelzang, A., McGuire, H. M., Yu, D., Sprent, J., Mackay, C. R., & King, C. (2008). A
919 fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity*,
920 *29*(1), 127-137. https://doi.org/10.1016/j.immuni.2008.06.001

921 Wan, Q., Kozhaya, L., ElHed, A., Ramesh, R., Carlson, T. J., Djuretic, I. M., Sundrud, M. S., &
922 Unutmaz, D. (2011). Cytokine signals through PI-3 kinase pathway modulate Th17
923 cytokine production by CCR6+ human memory T cells. *J Exp Med*, *208*(9), 1875-1887.
924 https://doi.org/10.1084/jem.20102516

925 Willing, A., Jager, J., Reinhardt, S., Kursawe, N., & Friese, M. A. (2018). Production of IL-17 by
926 MAIT Cells Is Increased in Multiple Sclerosis and Is Associated with IL-7 Receptor
927 Expression. *J Immunol*, *200*(3), 974-982. https://doi.org/10.4049/jimmunol.1701213

928 Wu, X., Tian, J., & Wang, S. (2018). Insight Into Non-Pathogenic Th17 Cells in Autoimmune
929 Diseases. *Front Immunol*, *9*, 1112. https://doi.org/10.3389/fimmu.2018.01112

930 Xi, H., Kurtoglu, M., & Lampidis, T. J. (2014). The wonders of 2-deoxy-D-glucose. *IUBMB Life*,
931 *66*(2), 110-121. https://doi.org/10.1002/iub.1251

932 Xu, H., Agalioti, T., Zhao, J., Steglich, B., Wahib, R., Vesely, M. C. A., Bielecki, P., Bailis, W.,
933 Jackson, R., Perez, D., Izbicki, J., Licona-Limon, P., Kaartinen, V., Geginat, J., Esplugues,
934 E., Tolosa, E., Huber, S., Flavell, R. A., & Gagliani, N. (2020). The induction and function
935 of the anti-inflammatory fate of TH17 cells. *Nat Commun*, *11*(1), 3334.
936 https://doi.org/10.1038/s41467-020-17097-5

937 Yang, C., Zuo, Q., Liu, X., Zhao, Q., Pu, H., Gao, L., Zhao, L., Guo, Z., Lin, Y., Liu, J., Bi, J., &
938 Yin, G. (2021). Small molecule screening identified cepharanthine as an inhibitor of
939 porcine reproductive and respiratory syndrome virus infection in vitro by suppressing
940 integrins/ILK/RACK1/PKCalpha/NF-kappaB signalling axis. *Vet Microbiol*, *255*, 109016.
941 https://doi.org/10.1016/j.vetmic.2021.109016

942 Yasuda, K., Kitagawa, Y., Kawakami, R., Isaka, Y., Watanabe, H., Kondoh, G., Kohwi-
943 Shigematsu, T., Sakaguchi, S., & Hirota, K. (2019). Satb1 regulates the effector program
944 of encephalitogenic tissue Th17 cells in chronic inflammation. *Nat Commun*, *10*(1), 549.
945 https://doi.org/10.1038/s41467-019-08404-w

946 Zeng, H., Zhang, R., Jin, B., & Chen, L. (2015). Type 1 regulatory T cells: a new mechanism of
947 peripheral immune tolerance. *Cell Mol Immunol*, *12*(5), 566-571.
948 https://doi.org/10.1038/cmi.2015.44

949 Zhou, L., Ivanov, II, Spolski, R., Min, R., Shenderov, K., Egawa, T., Levy, D. E., Leonard, W. J.,
950 & Littman, D. R. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential
951 engagement of the IL-21 and IL-23 pathways. *Nat Immunol*, *8*(9), 967-974.
952 https://doi.org/10.1038/ni1488
953

Figure 1

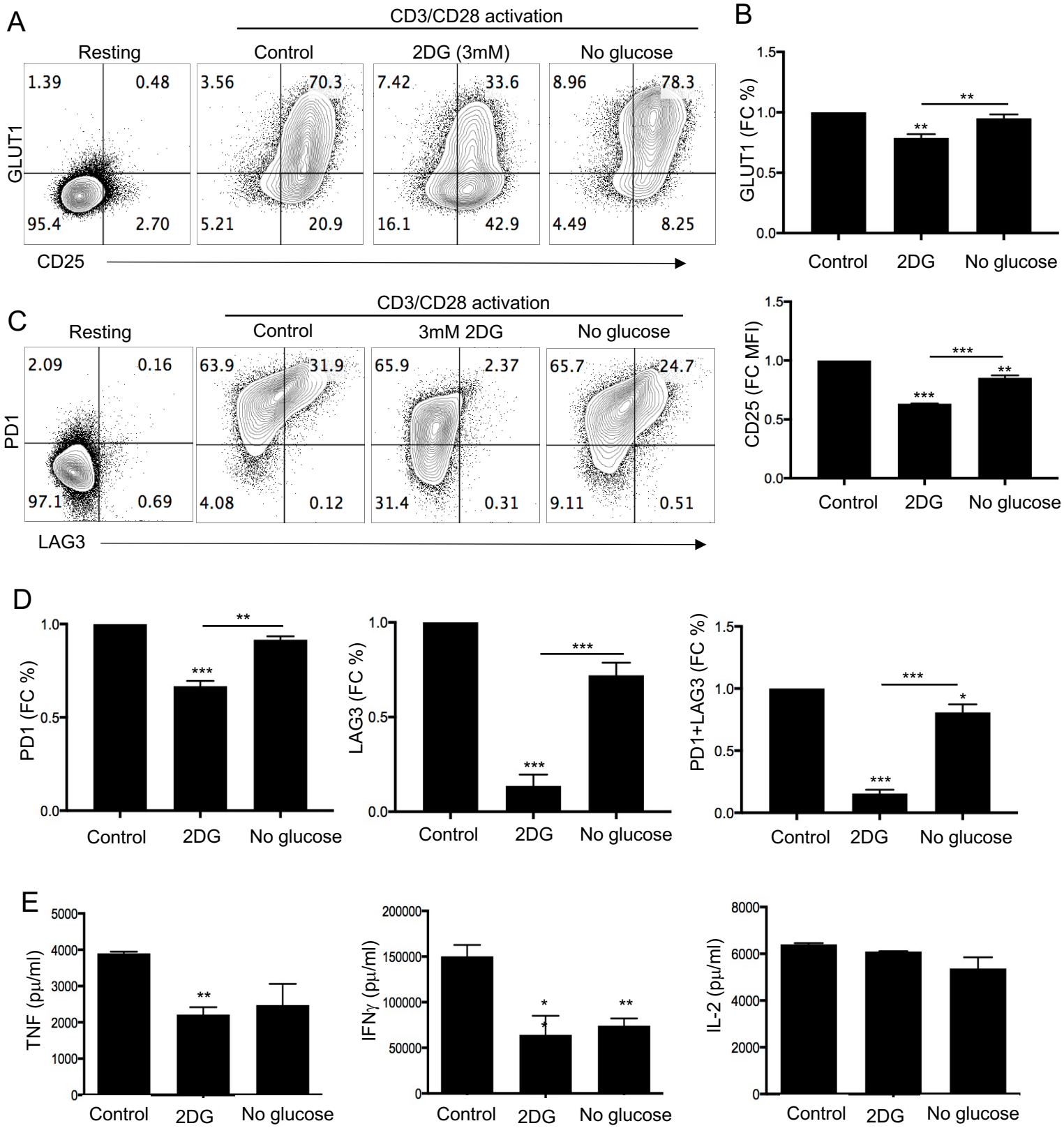

A
CD3/CD28 activation
Resting
Control
2DG (3mM)
No glucose
GLUT1
CD25
1.39
0.48
95.4
2.70
3.56
70.3
5.21
20.9
7.42
33.6
16.1
42.9
8.96
78.3
4.49
8.25
B
GLUT1 (FC %)
Control
2DG
No glucose
C
CD3/CD28 activation
Resting
Control
3mM 2DG
No glucose
PD1
LAG3
2.09
0.16
97.1
0.69
63.9
31.9
4.08
0.12
65.9
2.37
31.4
0.31
65.7
24.7
9.11
0.51
CD25 (FC MFI)
D
PD1 (FC %)
LAG3 (FC %)
PD1+LAG3 (FC %)
E
TNF (pμ/ml)
IFNγ (pμ/ml)
IL-2 (pμ/ml)

Figure 2

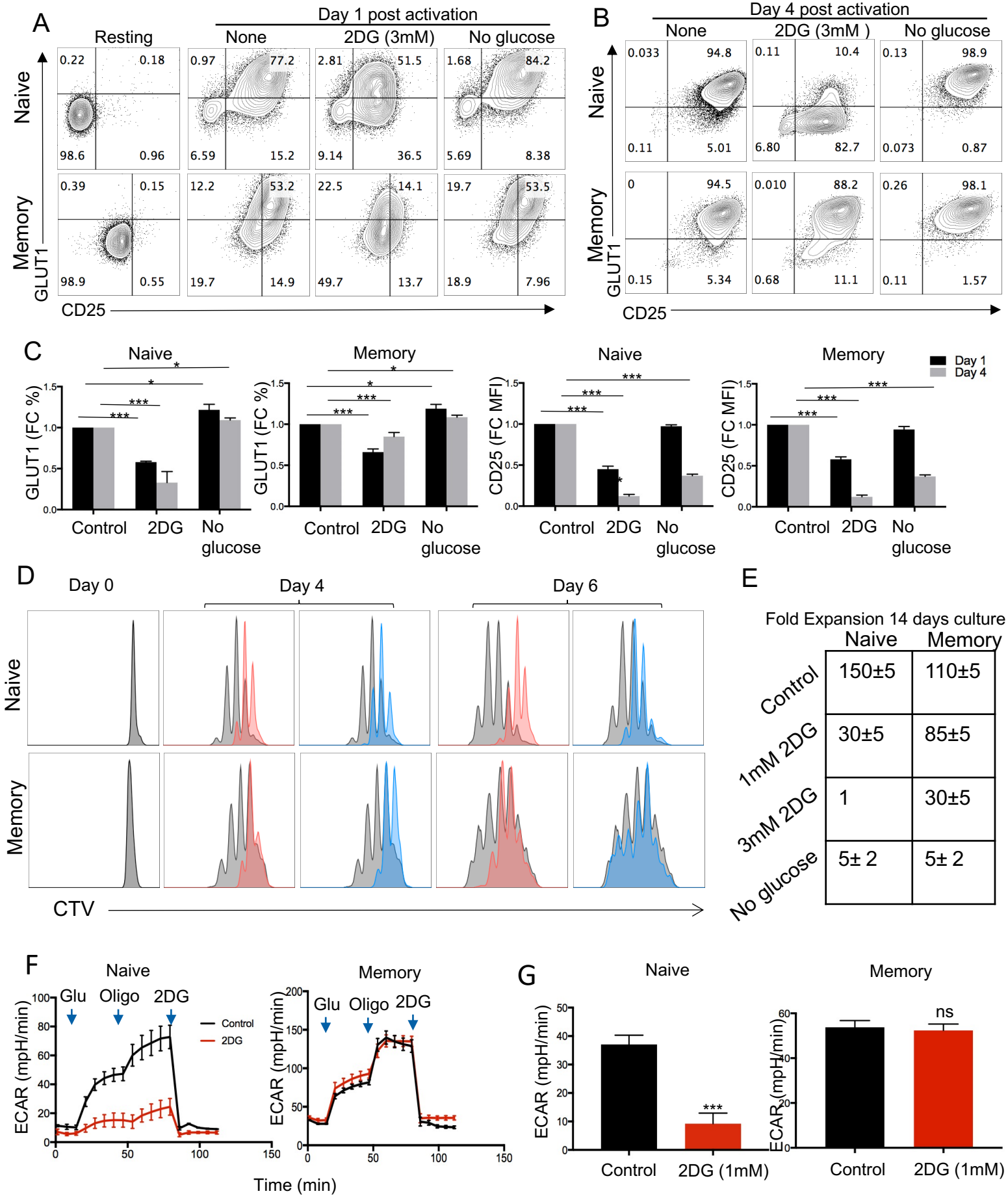

A
Day 1 post activation
Resting
None
2DG (3mM)
No glucose
Naive
Memory
GLUT1
CD25
0.22
0.18
98.6
0.96
0.97
77.2
6.59
15.2
2.81
51.5
9.14
36.5
1.68
84.2
5.69
8.38
0.39
0.15
98.9
0.55
12.2
53.2
19.7
14.9
22.5
14.1
49.7
13.7
19.7
53.5
18.9
7.96
B
Day 4 post activation
None
2DG (3mM)
No glucose
0.033
94.8
0.11
5.01
0.11
10.4
6.80
82.7
0.13
98.9
0.073
0.87
0
94.5
0.15
5.34
0.010
88.2
0.68
11.1
0.26
98.1
0.11
1.57
C
Naive
Memory
GLUT1 (FC %)
CD25 (FC MFI)
Day 1
Day 4
Control
2DG
No glucose
D
Day 0
Day 4
Day 6
Naive
Memory
CTV
E
Fold Expansion 14 days culture
Naive
Memory
Control
150±5
110±5
1mM 2DG
30±5
85±5
3mM 2DG
1
30±5
No glucose
5± 2
5± 2
F
Naive
Memory
Glu
Oligo
2DG
Control
2DG
ECAR (mpH/min)
Time (min)
G
Naive
Memory
ECAR (mpH/min)
ns
Control
2DG (1mM)

Figure 3

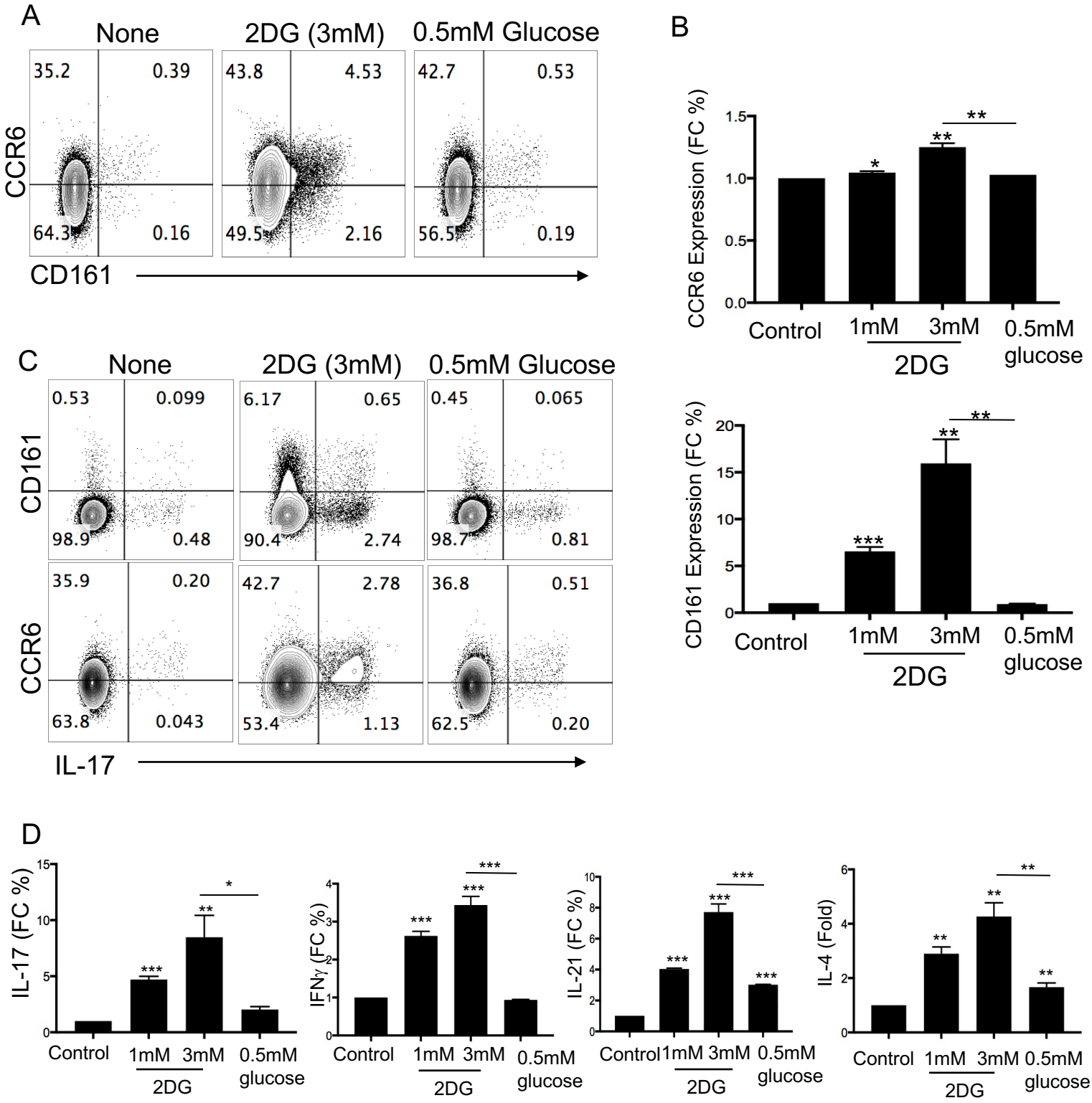

A
None
2DG (3mM)
0.5mM Glucose
CCR6
CD161
35.2
0.39
64.3
0.16
43.8
4.53
49.5
2.16
42.7
0.53
56.5
0.19
B
CCR6 Expression (FC %)
Control
1mM
3mM
0.5mM glucose
2DG
CD161 Expression (FC %)
C
CD161
CCR6
IL-17
0.53
0.099
98.9
0.48
6.17
0.65
90.4
2.74
0.45
0.065
98.7
0.81
35.9
0.20
63.8
0.043
42.7
2.78
53.4
1.13
36.8
0.51
62.5
0.20
D
IL-17 (FC %)
IFNγ (FC %)
IL-21 (FC %)
IL-4 (Fold)

Figure 4

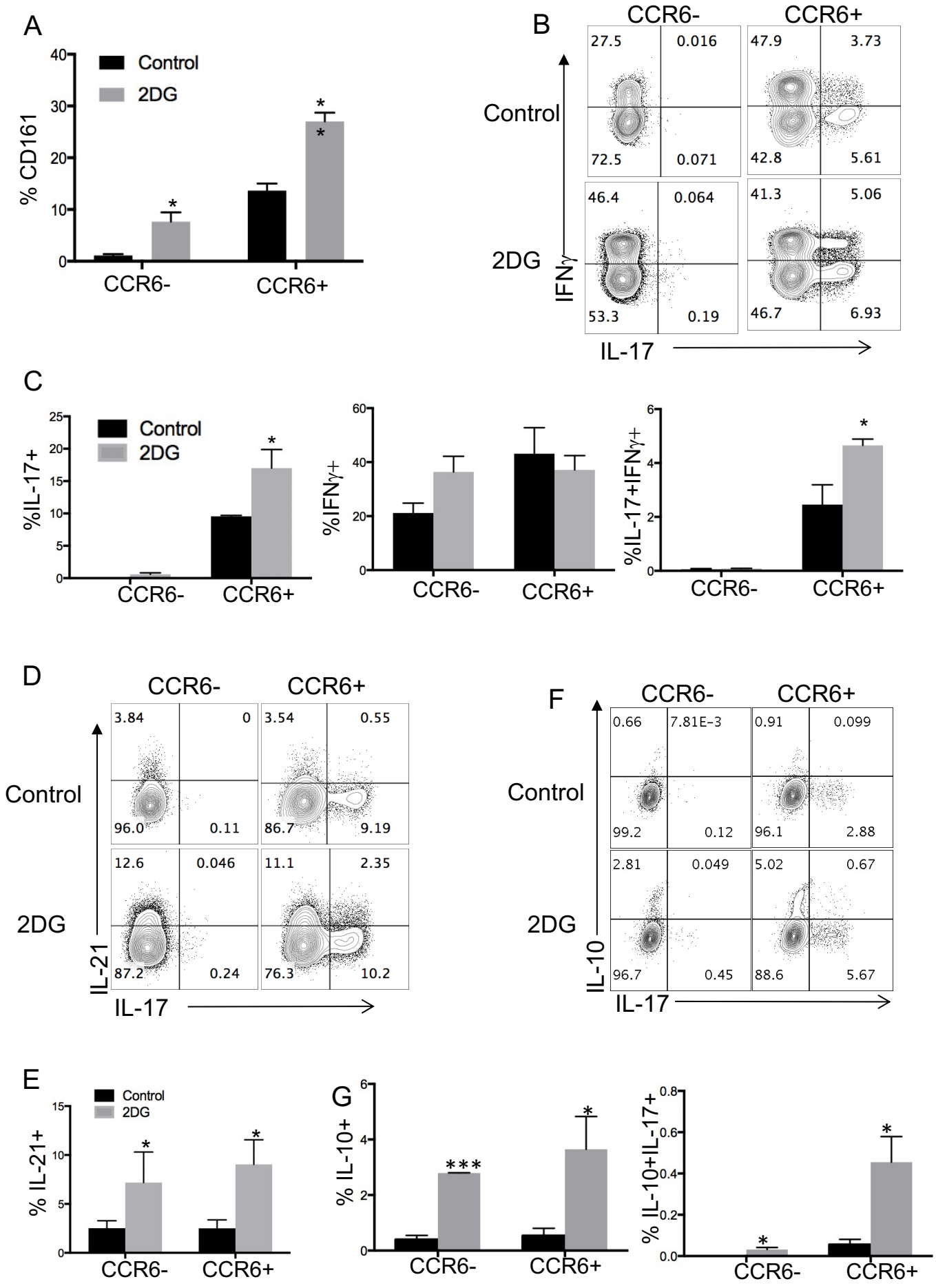

A
Control
2DG
% CD161
CCR6-
CCR6+
B
CCR6-
CCR6+
Control
2DG
IFNγ
IL-17
27.5
0.016
72.5
0.071
47.9
3.73
42.8
5.61
46.4
0.064
53.3
0.19
41.3
5.06
46.7
6.93
C
Control
2DG
%IL-17+
%IFNγ+
%IL-17+IFNγ+
CCR6-
CCR6+
D
CCR6-
CCR6+
Control
2DG
IL-21
IL-17
3.84
0
96.0
0.11
3.54
0.55
86.7
9.19
12.6
0.046
87.2
0.24
11.1
2.35
76.3
10.2
F
CCR6-
CCR6+
Control
2DG
IL-10
IL-17
0.66
7.81E-3
99.2
0.12
0.91
0.099
96.1
2.88
2.81
0.049
96.7
0.45
5.02
0.67
88.6
5.67
E
Control
2DG
% IL-21+
CCR6-
CCR6+
G
% IL-10+
% IL-10+IL-17+
CCR6-
CCR6+

Figure 5

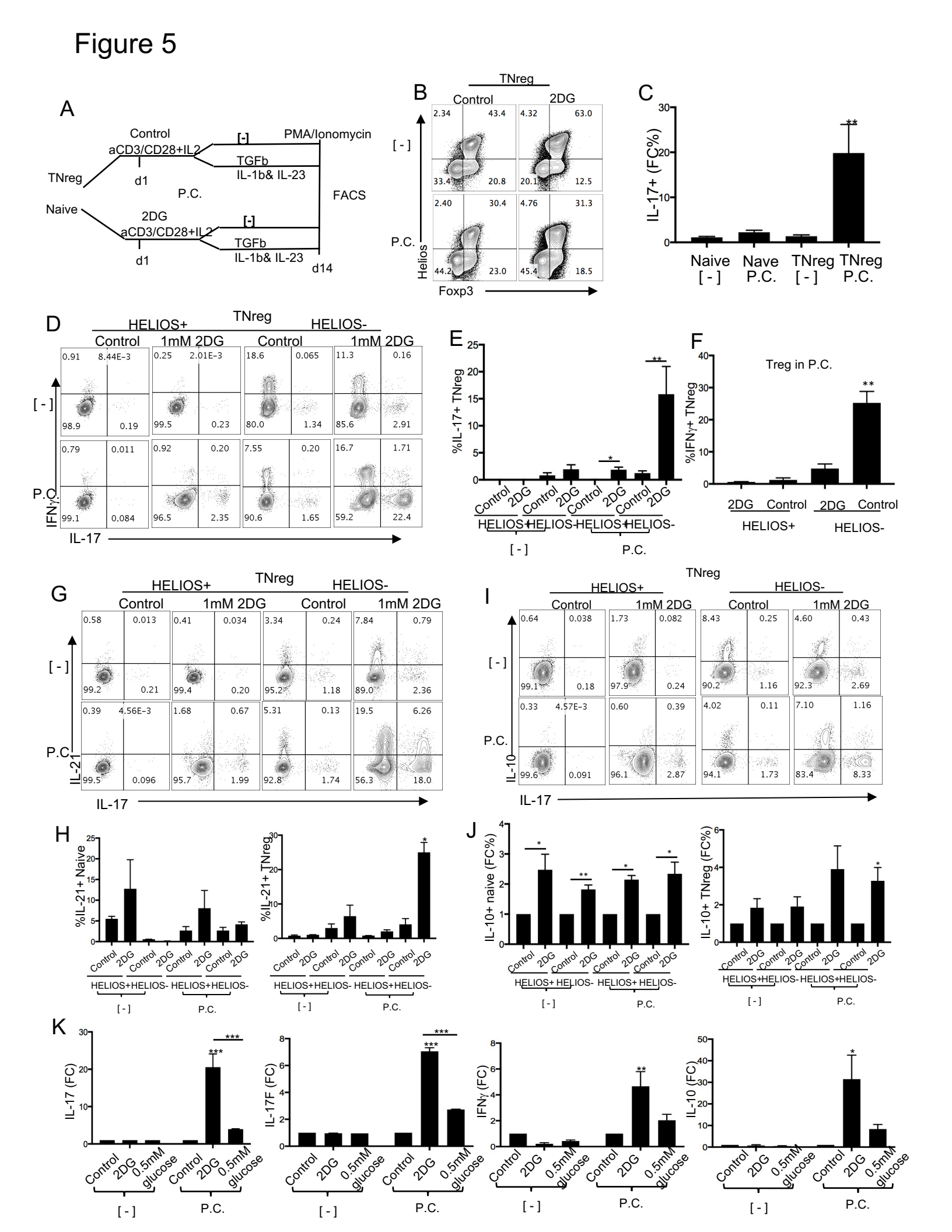
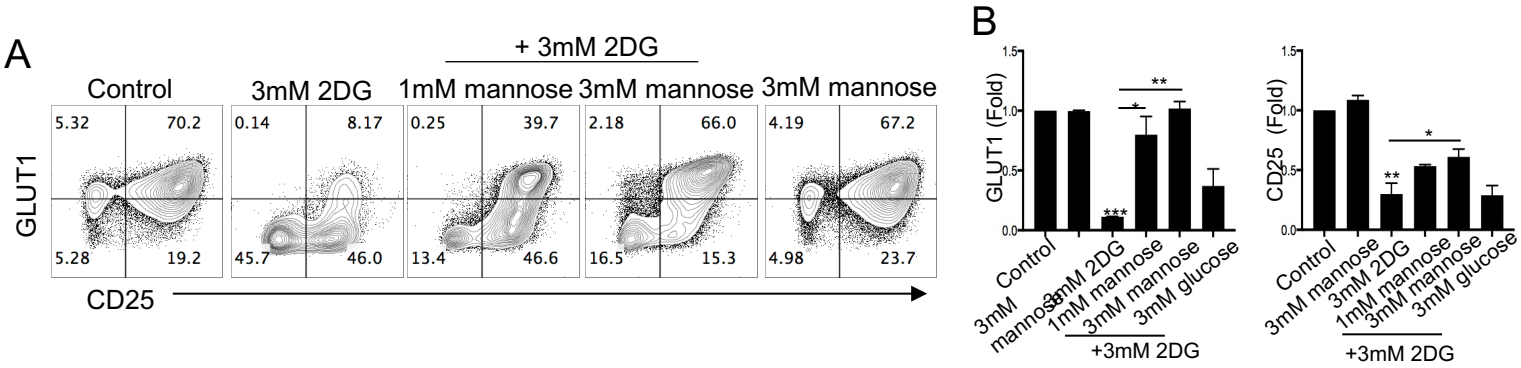
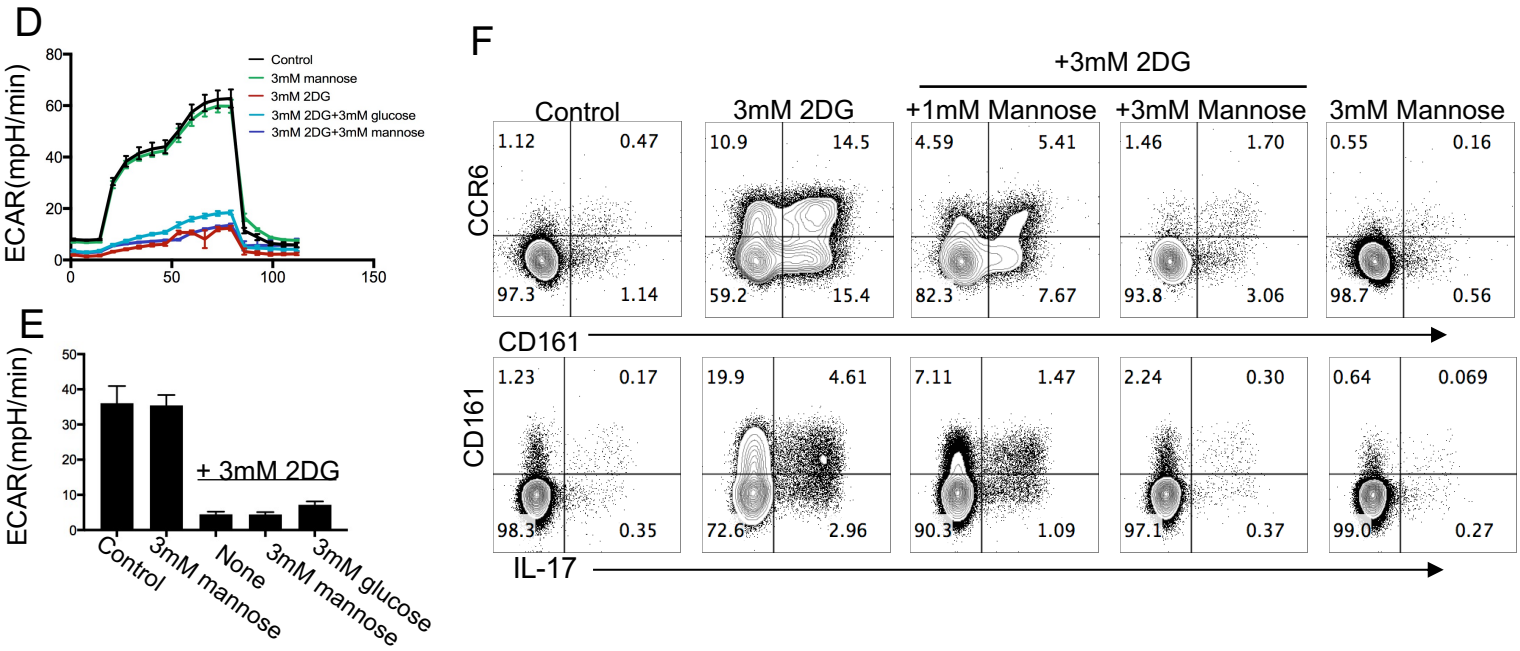

A
Control
aCD3/CD28+IL2
[-]
PMA/Ionomycin
TGFb
IL-1b& IL-23
TNreg
d1
P.C.
FACS
Naive
2DG
aCD3/CD28+IL2
[-]
TGFb
IL-1b& IL-23
d1
d14
B
TNreg
Control
2DG
[-]
P.C.
Helios
Foxp3
2.34
43.4
33.4
20.8
4.32
63.0
20.1
12.5
2.40
30.4
44.2
23.0
4.76
31.3
45.4
18.5
C
IL-17+ (FC%)
Naive [-]
Nave P.C.
TNreg [-]
TNreg P.C.
D
TNreg
HELIOS+
HELIOS-
Control
1mM 2DG
Control
1mM 2DG
[-]
P.C.
IFNγ
IL-17
0.91
8.44E-3
98.9
0.19
0.25
2.01E-3
99.5
0.23
18.6
0.065
80.0
1.34
11.3
0.16
85.6
2.91
0.79
0.011
99.1
0.084
0.92
0.20
96.5
2.35
7.55
0.20
90.6
1.65
16.7
1.71
59.2
22.4
E
%IL-17+ TNreg
Control
2DG
HELIOS+
HELIOS-
[-]
P.C.
F
Treg in P.C.
%IFNγ+ TNreg
2DG
Control
HELIOS+
HELIOS-
G
TNreg
HELIOS+
HELIOS-
Control
1mM 2DG
Control
1mM 2DG
[-]
P.C.
IL-21
IL-17
0.58
0.013
99.2
0.21
0.41
0.034
99.4
0.20
3.34
0.24
95.2
1.18
7.84
0.79
89.0
2.36
0.39
4.56E-3
99.5
0.096
1.68
0.67
95.7
1.99
5.31
0.13
92.8
1.74
19.5
6.26
56.3
18.0
I
TNreg
HELIOS+
HELIOS-
Control
1mM 2DG
Control
1mM 2DG
[-]
P.C.
IL-10
IL-17
0.64
0.038
99.1
0.18
1.73
0.082
97.9
0.24
8.43
0.25
90.2
1.16
4.60
0.43
92.3
2.69
0.33
4.57E-3
99.6
0.091
0.60
0.39
96.1
2.87
4.02
0.11
94.1
1.73
7.10
1.16
83.4
8.33
H
%IL-21+ Naive
%IL-21+ TNreg
Control
2DG
HELIOS+
HELIOS-
[-]
P.C.
J
IL-10+ naive (FC%)
IL-10+ TNreg (FC%)
Control
2DG
HELIOS+
HELIOS-
[-]
P.C.
K
IL-17 (FC)
IL-17F (FC)
IFNγ (FC)
IL-10 (FC)
Control
2DG
0.5mM glucose
[-]
P.C.

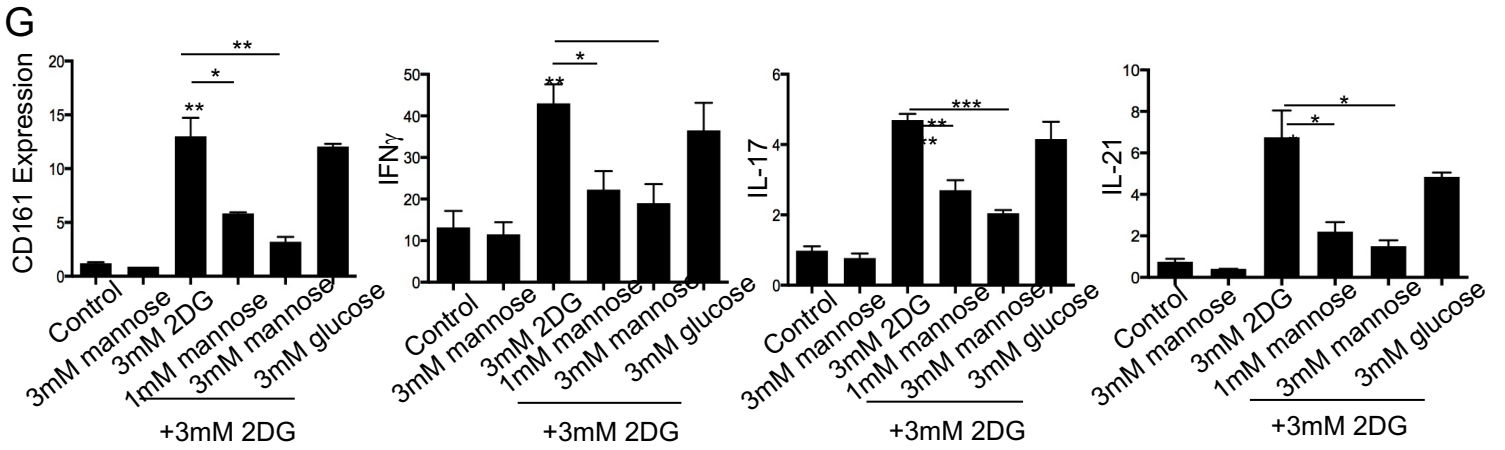
Figure 6



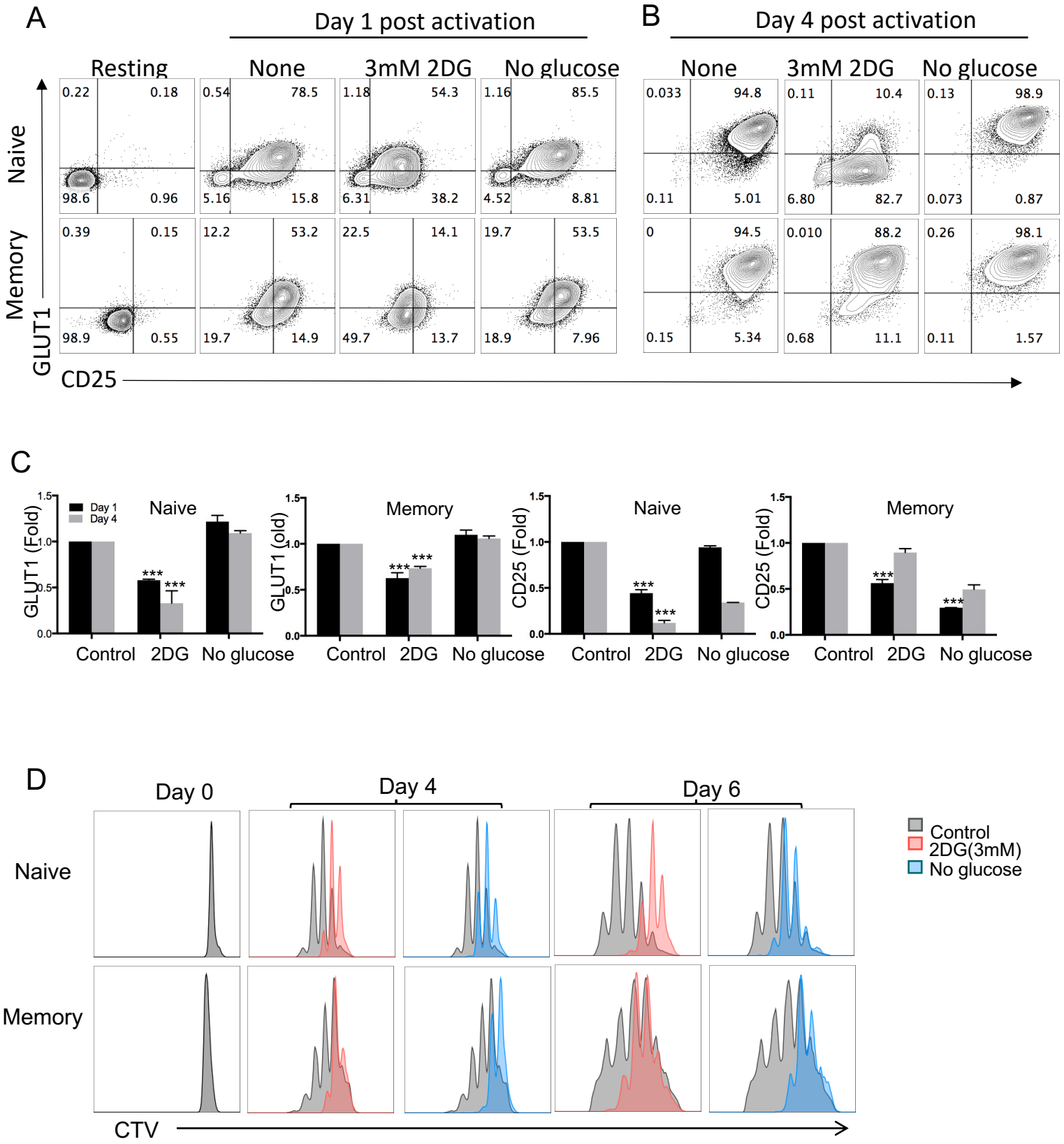
C Naive T Cell Expansion after 2 weeks culture

Control	3mM mannose	+ 3mM 2DG			
		None	1mM mannose	3mM mannose	3mM glucose
157±5	100±5	1	30±5	50±5	16±5

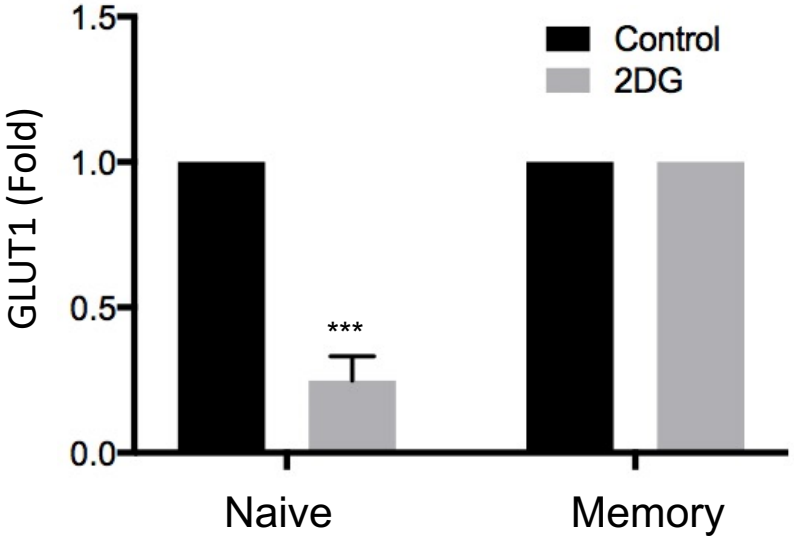




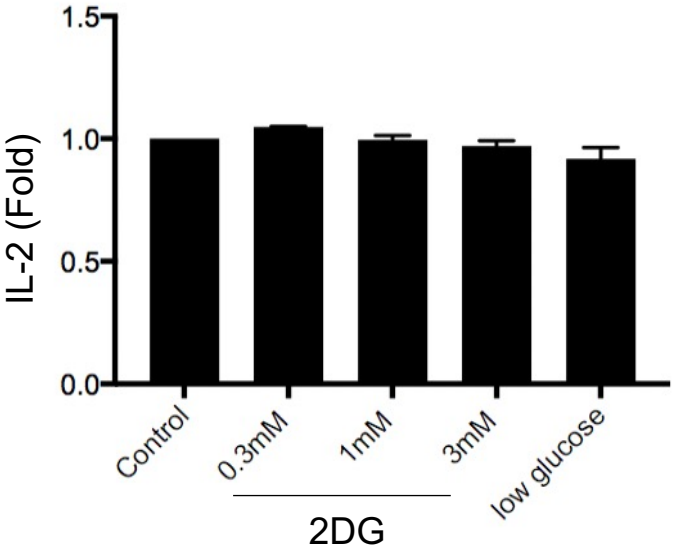
Supplemental Figure 1

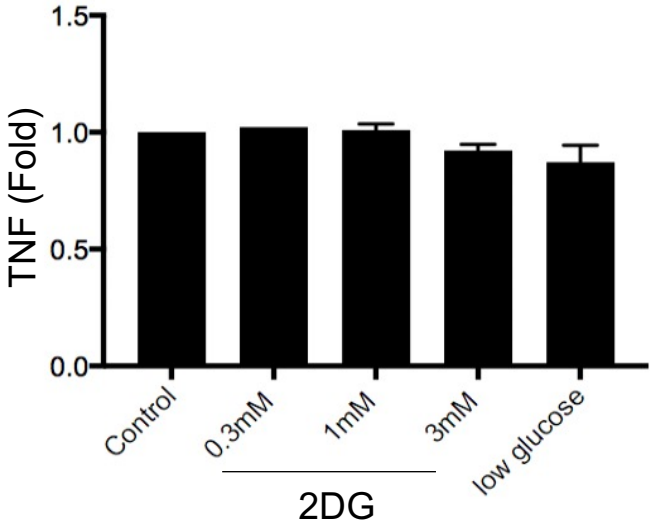


Supplemental Figure 2

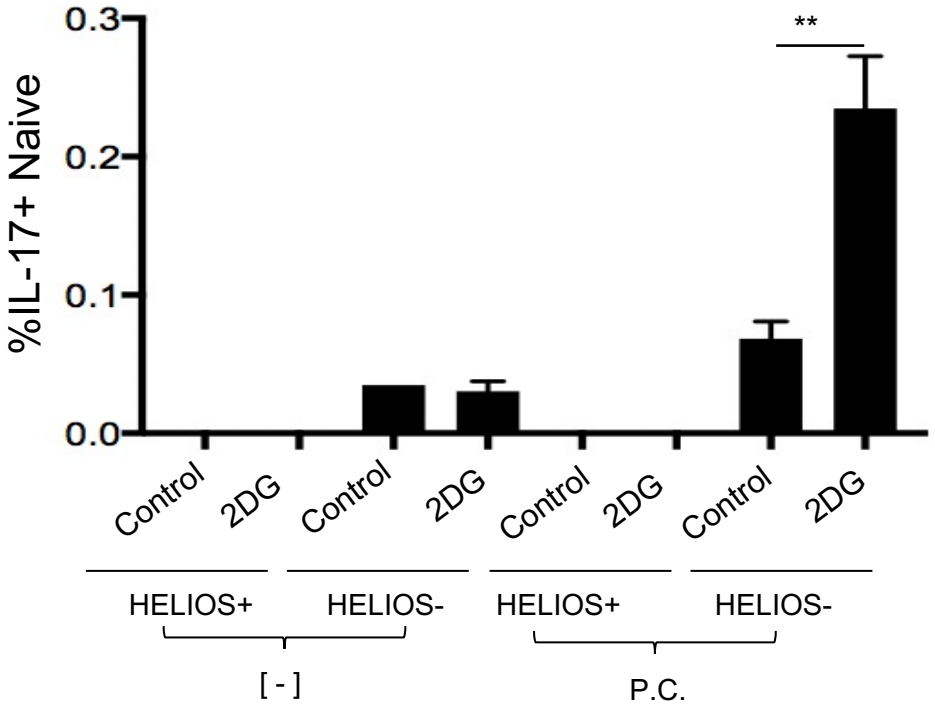
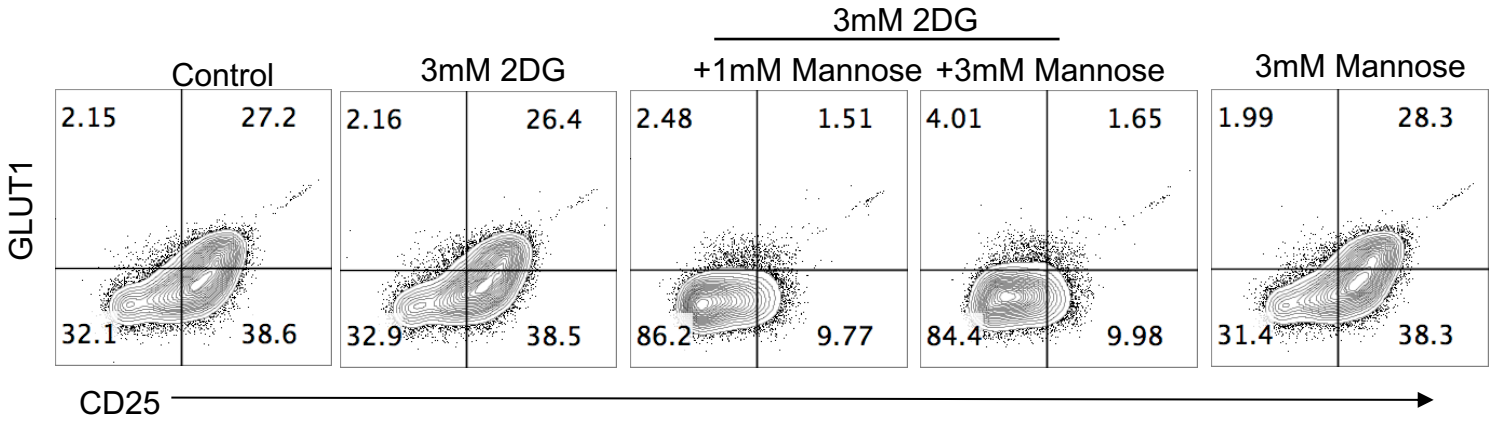


Supplemental Figure 3





Supplemental Figure 4



Supplemental Figure 5