

A Th17 cell-intrinsic glutathione/mitochondrial-IL-22 axis protects against intestinal inflammation

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Executive summary

- GSH-regulated Th17 cell-derived IL-22, but not IL-17 is required to maintain intestinal barrier integrity and to prevent lethality following *C. rodentium* infection.
- *GCLC* expression in IBD patients correlates positively with expression of genes related to gut integrity.
- *Gclc*-deficient Th17 cells accumulate mitochondrial ROS, which is linked to impaired mitochondrial function, dysregulated PI3K/AKT/mTOR signaling and impaired translation of IL-22.
- ROS-scavenging, IL-22 reconstitution or T cell-specific expression of IL-22 in *Gclc*-deficient T cells rescues mutant mice from the lethal infection outcome *in vivo*.

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47

48 **Summary**

49 Although the intestinal tract is a major site of reactive oxygen species (ROS) generation, the
50 mechanisms by which antioxidant defense in gut T cells contribute to intestinal homeostasis
51 are currently unknown. Here we show, using T cell-specific ablation of the catalytic subunit of
52 glutamate cysteine ligase (*Gclc*), that the ensuing loss of glutathione (GSH) impairs the
53 production of gut-protective IL-22 by Th17 cells within the lamina propria. Although *Gclc*
54 ablation does not affect T cell cytokine secretion in the gut of mice at steady-state, infection
55 with *C. rodentium* increases ROS, inhibits mitochondrial gene expression and mitochondrial
56 function in *Gclc*-deficient Th17 cells. These mitochondrial deficits affect the PI3K/AKT/mTOR
57 pathway, leading to reduced phosphorylation of the translation repressor 4E-BP1. As a
58 consequence, the initiation of translation is restricted, resulting in decreased protein synthesis
59 of IL-22. Loss of IL-22 results in poor bacterial clearance, enhanced intestinal damage, and high
60 mortality. ROS-scavenging, reconstitution of IL-22 expression or IL-22 supplementation *in vivo*
61 prevent the appearance of these pathologies. Our results demonstrate the existence of a
62 previously unappreciated role for Th17 cell-intrinsic GSH coupling to promote mitochondrial
63 function, IL-22 translation and signaling. These data reveal an axis that is essential for
64 maintaining the integrity of the intestinal barrier and protecting it from damage caused by
65 gastrointestinal infection.

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67

68 Introduction

69 T helper (Th) cells are essential for the protection of mucosal surfaces. In the gastrointestinal
70 (GI) tract, Th cells and in particular Th17 cells, maintain gut homeostasis by inducing tolerance
71 to the microbiome and by defending against intestinal pathogens (van Wijk and Cheroutre,
72 2010). Although Th17-derived cytokines such as IL-17 and IL-22 have been linked to
73 inflammatory bowel disease (IBD), increasing evidence points to beneficial effects of these
74 cytokines during intestinal inflammation (Eken et al., 2014, Kamanaka et al., 2011, Fujino et
75 al., 2003, Brand et al., 2006, Monteleone et al., 2012). IL-22 and IL-17 induce antimicrobial
76 peptide (AMP) production by intestinal epithelial cells (IECs) and protect the intestinal barrier
77 against pathogens that directly efface the intestinal epithelium (Liang et al., 2006, Kim et al.,
78 2012, Tsai et al., 2017, Keir et al., 2020, Lee et al., 2015). Accordingly, neutralization or genetic
79 ablation of IL-22 results in severe disease and increased epithelial cell damage in mouse colitis
80 models (Sugimoto et al., 2008, Zheng et al., 2008, Basu et al., 2012). In human IBD, damage to
81 the epithelial layer and increased permeability have been proposed as primary defects
82 (Shorter et al., 1972, Corridoni et al., 2014). Such intestinal damage is often causally linked to
83 reactive oxygen species (ROS), in line with a striking reduction in intestinal antioxidant capacity
84 and a rise in oxidative stress (Sido et al., 1998, Buffinton and Doe, 1995, Lih-Brody et al., 1996,
85 Kruidenier et al., 2003, Aviello and Knaus, 2017). However, how antioxidant defense provided
86 by gut T cells contributes to intestinal integrity is unclear.

87 The main antioxidant produced by activated T cells is glutathione (GSH) (Mak et al., 2017).
88 The rate-limiting reaction of GSH synthesis is catalyzed by glutamate cysteine ligase (GCL), a
89 complex of catalytic (GCLC) and modifier (GCLM) subunits (Meister, 1983, Chen et al., 2005).
90 Here, we use a T cell-specific *Gclc*-deficient mouse model to show that a lack of *Gclc* impairs
91 Th17 cell production of IL-22 in response to the intestinal pathogen *Citrobacter rodentium* (*C.*
92 *rodentium*), leading to defective bacterial clearance, enhanced intestinal damage, and high
93 mortality. Our results demonstrate that GSH regulates mitochondrial function and ROS in Th17
94 cells, which links the mitochondrial activity to the expression of IL-22 and is critical for
95 combatting a GI infection.

96

97

98 Results

99 ***Gclc* ablation in murine T cells results in high mortality during GI infection**

100 *C. rodentium* causes colitis symptoms in mice and shares pathogenic mechanisms with
101 human *E. coli* intestinal pathogens (Collins et al., 2014, Mullineaux-Sanders et al., 2019). We
102 infected wild-type (WT) C57BL/6 mice with *C. rodentium* and isolated colonic lamina propria
103 (LP) cells at day 7 post-infection (p.i.) in order to investigate antioxidant responses in these
104 cells. Flow cytometric analysis revealed that CD4⁺ T cells of infected WT mice produced more
105 thiols than those of uninfected WT mice (Figure 1A), indicating a greater antioxidant capacity.
106 The heightened metabolic activity of activated T cells drives increased ROS production, which
107 is in line with the expanded antioxidant capacity of T cells during *C. rodentium* infection (Gülow
108 et al., 2005, Yi et al., 2006, Sena et al., 2013). We hypothesized that LP CD4⁺ T cells increase
109 their production GSH, the main intracellular antioxidant and thiol, to neutralize the rising
110 oxidative stress caused by infection. Indeed, intracellular GSH was increased in sorted LP CD4⁺
111 cells isolated from infected WT mice (Figure 1B). Cytosolic ROS levels were comparable in LP
112 CD4⁺ cells isolated from uninfected and infected WT mice (Figure 1C), reflecting superior ROS
113 scavenging by the elevated GSH in the infected animals.

114 These data prompted us to investigate whether GSH is necessary to buffer ROS in LP T cells
115 of *C. rodentium*-infected mice. We took advantage of a mutant mouse strain harboring a T
116 cell-specific deletion of *Gclc* (*Cd4Cre Gclc^{fl/fl}* mice); T cells of these mutants cannot synthesize
117 GSH (Mak et al., 2017). As expected, we observed significantly lower levels of intracellular
118 thiols and GSH in LP CD4⁺ cells of infected *Cd4Cre Gclc^{fl/fl}* mice compared to those of infected
119 *Gclc^{fl/fl}* littermate controls (Figure 1D, 1E). Consequently, cytosolic ROS levels were increased
120 in LP CD4⁺ cells of infected mutants compared to controls (Figure 1F), confirming that GSH loss
121 reduces ROS buffering capacity during infection. Interestingly, cytosolic ROS in T cells of
122 uninfected *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice were comparable (Figure S1A), consistent with our
123 previous finding that higher ROS occur in *Gclc*-deficient splenic CD4⁺ T cells only after
124 activation (Mak et al., 2017). Thus, GSH is not needed to control ROS in T cells at steady-state
125 but becomes crucial upon infection.

126 To investigate physiological consequences of GSH loss in T cells, we monitored weights of
127 littermate *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice following *C. rodentium* infection. *Cd4Cre Gclc^{fl/fl}*

128 mice showed significant weight loss by day 11 p.i., whereas control animals were unaffected
129 (Figure 1G). Over 95% of mutant mice succumbed to the infection by day 20 p.i., whereas no
130 mortality was observed among controls (Figure 1H). No weight loss or spontaneous disease
131 development was observed in uninfected mutant mice up to age 12 months (data not shown).
132 While bacterial clearance, as determined by measuring *C. rodentium* load in feces, was
133 achieved by day 20 p.i. in control mice, the mutants showed a steady increase in bacterial load
134 until death (Figure 1I, left). At day 14 p.i., fecal *C. rodentium* shedding in mutant mice was
135 increased by $>10^5$ -fold relative to controls (Figure 1I, right). Anatomically, infected *Cd4Cre*
136 *Gclc^{fl/fl}* mice displayed a marked shortening of colon length on day 12 p.i. (Figure 1J).
137 Moreover, these colons exhibited ulcers and thickening that were not visible on colons from
138 infected *Gclc^{fl/fl}* mice (Figure 1J, S1B). Intestinal crypt loss, ulceration and necrosis of the
139 intestinal epithelium were present in distal colons of infected *Cd4Cre Gclc^{fl/fl}* mice but not in
140 those of infected controls (Figure 1K). Of note, intestinal microbiota composition was
141 comparable in uninfected *Cd4Cre Gclc^{fl/fl}* mice and controls (Figure S1C). Thus, *Gclc* ablation in
142 T cells renders mice unable to control *C. rodentium* infections, leading to intestinal damage
143 and high mortality. These data suggest a protective role for T cell-derived GSH during GI
144 infection.

145

146 **Pathogenic Th17 cells are the dominant Th subset responding to *C. rodentium***

147 *C. rodentium* infection elicits a robust T cell response in mice that ensures bacterial clearance
148 and survival (Simmons et al., 2003, Bry and Brenner, 2004, Bry et al., 2006). Accordingly, we
149 observed higher frequencies and total numbers of activated/memory CD4⁺ T cells (CD62L⁻
150 CD44⁺), and reduced frequencies of naïve CD4⁺ T cells (CD62L⁺CD44⁻), in colonic LP isolated
151 from infected *Gclc^{fl/fl}* mice at day 7 p.i. compared to uninfected controls (Figure S2A, S2B). To
152 determine which T cell subsets contribute to this response, we analyzed cytokine production
153 in colonic CD4⁺ T cells from infected and uninfected *Gclc^{fl/fl}* mice by flow cytometry. We found
154 comparable frequencies of Th22 cells (CD4⁺IL-17⁻IL-22⁺) and conventional Th17 cells producing
155 IL-17 but not IL-22 (CD4⁺IL-17⁺IL-22⁻) (Figure 2A-C). However, the frequency of so-called
156 “pathogenic” Th17 cells producing both IL-17 and IL-22 (CD4⁺IL-17⁺IL-22⁺) (Ghoreschi et al.,
157 2010) was drastically increased in *C. rodentium*-infected *Gclc^{fl/fl}* mice (Figure 2A, 2D), as was
158 the frequency of “pathogenic” Th17 cells producing IL-17 and IFN- γ (CD4⁺IFN- γ ⁺IL-17⁺)

159 (Krausgruber et al., 2016) (Figure S2C). In contrast, the frequency of LP Th1 cells producing
160 IFN- γ but not IL-17 (CD4⁺IFN- γ ⁺IL-17⁻) was not increased in infected *Gclc*^{fl/fl} mice (Figure 2E).
161 Importantly, IL-17⁺IL-22⁺ pathogenic Th17 cells showed a ~40-fold increase in absolute cell
162 numbers in response to infection, compared to a ~4 fold rise in numbers of conventional Th17
163 cells, Th22 cells, and Th1 cells and a ~14 fold increase in numbers of IL-17⁺IFN- γ ⁺ pathogenic
164 Th17 cells (Figure 2F, S2C). Thus, the response to *C. rodentium* infection in the LP is mediated
165 mainly by pathogenic IL-17⁺IL-22⁺ Th17 cells.

166 Retinoid orphan receptor ROR γ T is the master transcription factor (TF) driving Th17 cell
167 differentiation and IL-17 expression, while Tbet is the TF responsible for Th1 cell
168 differentiation and IFN- γ production (Ivanov et al., 2006, Szabo et al., 2000). A bacteria-driven
169 colitis is often associated with pathogenic Th17 cells, which co-express ROR γ T and Tbet
170 (Krausgruber et al., 2016, Ghoreschi et al., 2010). In colonic LP of *C. rodentium*-infected *Gclc*^{fl/fl}
171 mice, the frequency of Tbet⁺ROR γ T⁺ pathogenic Th17 cells rose sharply, whereas no increase
172 was observed in the frequencies of Tbet⁻ROR γ T⁺ conventional Th17 cells or Tbet⁺ROR γ T⁻ Th1
173 cells (Figure 2G). Total numbers of Tbet⁺ROR γ T⁺ pathogenic Th17 cells also increased markedly
174 after infection (Figure 2H). These results confirm the previous finding that *C. rodentium* elicits
175 stronger Th17 than Th1 responses (Mangan et al., 2006) and show that specifically the
176 pathogenic Th17 subset, producing both IL-17 and IL-22, is dominant in the T cell response
177 against this pathogen.

178

179 **GSH is crucial for the IL-17⁺IL-22⁺ Th17 response during *C. rodentium* infection**

180 *Cd4Cre Gclc*^{fl/fl} mice show normal thymocyte development (Mak et al., 2017). Frequencies
181 and absolute numbers of activated/memory T cells, as well as pathogenic and conventional
182 Th17 cells, are comparable in the LP of uninfected *Cd4Cre Gclc*^{fl/fl} mice and controls (Figure
183 S2D-F). Thus, *Gclc* is not necessary to maintain these cell populations in mice at steady-state.
184 However, analysis of CD4⁺ T cell subsets from colonic LP of *C. rodentium*-infected *Cd4Cre*
185 *Gclc*^{fl/fl} and *Gclc*^{fl/fl} mice on day 7 p.i. showed striking reductions in the frequencies of
186 conventional (IL-17⁺IL-22⁻) and pathogenic (IL-17⁺IL-22⁺ and IFN- γ ⁺IL-17⁺) Th17 cells in the
187 mutants (Figure 2I, S2G). The frequency of Th22 cells (IL-17⁻IL-22⁺) was unchanged in infected
188 mutants, and that of Th1 cells (IFN- γ ⁺IL-17⁻) was only slightly reduced (Figure S2H, S2I). While

189 absolute numbers of all subsets were decreased in infected *Cd4Cre Gclc^{fl/fl}* mice compared to
190 controls, the most prominent effect was on IL-17⁺IL-22⁺ pathogenic Th17 cells (Figure S2J).
191 Frequencies and absolute numbers of Tbet⁺RORγT⁺ pathogenic and Tbet⁺RORγT⁺ conventional
192 Th17 cells were also decreased in LP of infected *Cd4Cre Gclc^{fl/fl}* mice (Figure 2J, S2K). No
193 decrease in frequency or absolute number of Tbet⁺RORγT⁻ Th1 cells occurred in infected
194 mutant mice (Figure S2L).

195 Thus, absence of *Gclc* profoundly affects the IL-17⁺IL-22⁺ pathogenic Th17 cells that
196 dominate responses to *C. rodentium* infection, indicating that *Gclc* is critical for mounting Th17
197 cell responses to GI infections.

198

199 ***Gclc* ablation does not impair the innate lymphoid cell responses to *C. rodentium***

200 Although IL-17 and IL-22 are signature Th17 cytokines, these mediators are also co-produced
201 by innate lymphoid cells-type 3 (ILC3s) (Satoh-Takayama, 2015). While most ILC3s do not
202 express CD4, a subset called lymphoid tissue inducer (LTi) cells does express CD4 and so would
203 be targeted by *Cd4Cre*-driven *Gclc* deletion in our mouse model. CD4⁺ LTi cells are a critical
204 source of IL-22 in the gut during the early stages of *C. rodentium* infection, when bacteria have
205 just colonized the caecum and innate immunity is dominant (Geddes et al., 2011, Sonnenberg
206 et al., 2011). To determine if loss of LTi function contributed to the phenotype of our infected
207 *Cd4Cre Gclc^{fl/fl}* mice during early infection, we analyzed caecal LP CD4⁺ LTi cells (Lineage⁻CD3⁻
208 Nkp46⁻CD4⁺) from infected *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice at day 4 p.i. by flow cytometry.
209 Neither the frequency nor absolute number of LTi cells nor their cytokine production was
210 affected by *Gclc* ablation (Figure S3A, S3B). The same results were obtained for colonic LP LTi
211 cells at day 4 p.i. (Figure S3C, S3D). When we examined both T cell (CD3⁺CD4⁺) and LTi cell
212 populations at day 7 p.i., when bacteria have reached the colon, the frequencies and absolute
213 numbers of total LTi cells as well as IL-17⁺IL-22⁻, IL-17⁻IL-22⁺ and IL-17⁺IL-22⁺ LTi cells were
214 identical in infected mutant and control mice (Figure 3A-C). In contrast, and consistent with
215 our earlier findings, the frequency and absolute number of IL-17⁺IL-22⁺ Th17 cells in colonic
216 LP were drastically reduced in infected mutants compared to controls (Figure S3E). *Gclc*
217 ablation in colonic LP LTi cells did not affect their intracellular ROS levels, whereas *Gclc*-
218 deficient colonic LP T cells showed highly increased intracellular ROS (Figure 3D, S3F). Thus,

219 contrary to the abrogated IL-22 production seen in Th17 cells, *Gclc* loss does not result in
220 oxidative stress in LP LTi cells and therefore doesn't interfere with their IL-17 and IL-22
221 production in *C. rodentium*-infected mice.

222 To investigate if an effect of *Gclc* ablation in LTi cells might be masked by an adaptive immune
223 response, we crossed *Cd4Cre Gclc^{fl/fl}* mice with *Rag1^{-/-}* mice to generate a *Rag1^{-/-}Cd4Cre Gclc^{fl/fl}*
224 strain lacking the adaptive immune system (Mombaerts et al., 1992). Once again, the
225 frequencies and absolute numbers of IL-17⁺IL-22⁻, IL-17⁻IL-22⁺ and IL-17⁺IL-22⁺ LTi cells in
226 colonic LP, as well as their intracellular ROS levels, were identical in *C. rodentium*-infected
227 *Rag1^{-/-}Cd4Cre Gclc^{fl/fl}* and control *Rag1^{-/-}Gclc^{fl/fl}* mice (Figure S3G, S3H). When we compared
228 the course of disease in infected *Rag1^{-/-}Cd4Cre Gclc^{fl/fl}*, *Rag1^{-/-}Gclc^{fl/fl}*, *Cd4Cre Gclc^{fl/fl}* and
229 *Gclc^{fl/fl}* mice, we observed that both *Rag1^{-/-}Cd4Cre Gclc^{fl/fl}* and *Rag1^{-/-}Gclc^{fl/fl}* mice exhibited
230 severe weight loss and succumbed to the infection by day 15-20 p.i. (Figure 3E, 3F), consistent
231 with the documented importance of the adaptive response in controlling the later stages of
232 *C. rodentium* infection (Vallance et al., 2002, Bry and Brenner, 2004, Sonnenberg et al., 2011).
233 However, no differences in survival, weight loss, or fecal bacterial load were observed
234 between *Rag1^{-/-}Cd4Cre Gclc^{fl/fl}* and *Rag1^{-/-}Gclc^{fl/fl}* mice at day 4 p.i., when protection is
235 primarily mediated by innate immune cells (Figure 3E-3G). In addition, colon appearance and
236 length were comparable between infected *Rag1^{-/-}Cd4Cre Gclc^{fl/fl}* and *Rag1^{-/-}Gclc^{fl/fl}* mice at day
237 7 p.i. (Figure 3H). Thus, the innate response is intact in *Cd4Cre Gclc^{fl/fl}* mice and their
238 vulnerability to *C. rodentium* infection is due to their lack of T cell function.

239

240 **GSH-regulated IL-22 is essential for clearance of *C. rodentium***

241 IL-17, IL-22, IFN- γ and TNF- α all contribute to controlling the spread of *C. rodentium* within
242 the body and limiting intestinal epithelial damage (Mangan et al., 2006, Ishigame et al., 2009,
243 Basu et al., 2012, Zheng et al., 2008, Simmons et al., 2002, Shiomi et al., 2010, Gonçalves et
244 al., 2001). However, while mice lacking IL-17, IFN- γ or TNF- α all show increased histological
245 disease scores and high fecal bacterial titers, only mice lacking IL-22 are actually unable to
246 clear *C. rodentium* and succumb to the infection (Basu et al., 2012, Zheng et al., 2008, Ota et
247 al., 2011). Because IL-17⁺IL-22⁺ Th17 cells were greatly reduced in the colonic LP of our
248 infected *Cd4Cre Gclc^{fl/fl}* mice, we hypothesized that loss of Th17 cell-derived IL-22 caused their

249 high mortality. We therefore treated infected *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice with either a
250 recombinant IL-22-Fc fusion protein or a control-Fc fragment and monitored mouse weight
251 and survival. Infected mutant mice treated with control-Fc showed severe weight loss and
252 100% mortality, whereas recombinant IL-22-Fc treatment completely prevented weight loss
253 and fully restored survival (Figure 4A, 4B). Strikingly, IL-22-Fc treatment also blocked the
254 intestinal ulceration and crypt loss observed in control-Fc-treated *Cd4Cre Gclc^{fl/fl}* mice (Figure
255 4C). We therefore speculated that, because the *Gclc*-deficient T cells in the LP of infected
256 mutants produced very little IL-22, systemic effects were triggered that led to a fatal outcome.

257

258 ***Gclc*-dependent IL-22 regulates intestinal permeability during gut infection**

259 IL-22 is involved in many pathways that influence anti-bacterial immunity, including those
260 affecting pathogen virulence (Sakamoto et al., 2017, Sanchez et al., 2018). To investigate
261 whether *C. rodentium* virulence was altered in infected *Cd4Cre Gclc^{fl/fl}* mice, we determined
262 mRNA levels of the bacterial virulence factors EspA, EspG, EspF, EspI, Tir and Map in colonic
263 tissues of *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice at day 7 or 10 p.i. However, we found no differences
264 between mutant and control mice (Figure S4A) indicating that IL-22 lacking in *Cd4Cre Gclc^{fl/fl}*
265 T cells does not impact bacterial virulence.

266 IL-22 also regulates IEC production of AMPs such as Reg and β -defensins as well as lipocalin-
267 2 (Liang et al., 2006, Zheng et al., 2008, Raffatellu et al., 2009, Dixon et al., 2016). We therefore
268 measured mRNA levels of RegIII β , RegIII γ , β -defensin-2 and lipocalin-2 in caecum and distal
269 colon of infected *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice. Again, no major differences were detected
270 between mutant and control mice (Figure S4B, S4C). Moreover, lipocalin-2 levels in feces were
271 comparable between mutant and control mice throughout the infection (Figure S4D).

272 IL-22 has also been linked to intestinal barrier integrity and permeability (Keir et al., 2020,
273 Rendon et al., 2013). To measure intestinal permeability, we orally administered fluorescein
274 isothiocyanate (FITC)-Dextran to *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice on day 10 p.i. and measured
275 FITC-Dextran accumulation in blood plasma. Permeation of FITC-Dextran from the intestinal
276 lumen into the plasma was greatly enhanced in infected mutant mice compared to controls
277 (Figure S4E), but comparable in uninfected mutant and control mice (Figure S4F). This increase
278 in intestinal permeability was prevented by IL-22-Fc treatment of *Cd4Cre Gclc^{fl/fl}* mice,

279 indicating restoration of an intact intestinal layer (Figure 4D). By day 12 p.i., we detected high
280 bacterial titers in the blood, spleen and liver of infected *Cd4Cre Gclc^{fl/fl}* mice, whereas infected
281 control mice showed a minimal bacterial burden (Figure S4G, 4E). Again, IL-22-Fc treatment
282 of infected mutant mice reduced their bacterial loads back to control levels (Figure 4E). These
283 data point to a mechanism of *Gclc*-dependent regulation of T cell-intrinsic IL-22 production
284 that protects intestinal integrity and so prevents bacterial spread from the intestinal lumen to
285 the periphery.

286

287 ***Gclc*-regulated IL-22 bolsters intestinal tight junctions and mucus layer in *C. rodentium*-** 288 **infected mice**

289 IL-22 contributes to intestinal integrity by regulating the expression and intracellular location
290 of tight junction proteins such as the claudins, which maintain the epithelial barrier (Kim et
291 al., 2012, Tsai et al., 2017). Intact tight junctions are crucial for protection against infection by
292 attaching/effacing pathogens such as *C. rodentium*, which specifically target tight junction
293 structural proteins (Guttman et al., 2006, Garber et al., 2018, Xia et al., 2019). To investigate
294 whether *Gclc* ablation in T cells decreases claudin proteins, we immunoblotted distal colon
295 sections of *Cd4Cre Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice at day 12 p.i. Indeed, colons from infected mutants
296 showed lower claudin-2, claudin-3 and claudin-15 protein levels compared to colons from
297 infected controls (Figure 5A). In line, immunofluorescence microscopy of cross-sections of
298 colonic crypts showed abnormally diffused claudin-2 localization in infected *Cd4Cre Gclc^{fl/fl}*
299 mice (Figure 5B). Proper localization of claudin-2 in colonic crypts of infected *Cd4Cre Gclc^{fl/fl}*
300 mice, as well as normal lumen diameters, were entirely restored after IL-22-Fc treatment
301 (Figure 5B).

302 Another key function of IL-22 associated with intestinal pathogen exclusion is promotion of
303 mucin production by intestinal goblet cells (Sugimoto et al., 2008). The mucin-containing
304 mucus layer that protects the intestinal epithelium from enteric contents is critical for mouse
305 survival of *C. rodentium* infection (Sugimoto et al., 2008, Bergstrom et al., 2010, Desai et al.,
306 2016). The mucin protein MUC2 is the main component of the intestinal mucus layer and most
307 abundant in the colon (Johansson et al., 2008). We performed immunofluorescence
308 microscopy of cross-sections of intestinal crypts and observed a great reduction in MUC2 in

309 IECs of infected *Cd4Cre Gclc^{fl/fl}* mice on day 12 p.i. (Figure 5C). Again, normal MUC2 levels were
310 completely restored by IL-22-Fc treatment (Figure 5C). Alcian blue staining of transverse
311 sections of distal colons revealed a drastic decrease in total luminal mucus and the adherent
312 mucus layer in colons of infected *Cd4Cre Gclc^{fl/fl}* mice compared to controls (Figure 5D).
313 Moreover, the amount of mucin stored inside colonic IECs was lower in control-Fc-treated
314 *Cd4Cre Gclc^{fl/fl}* mice; especially in sites of ulceration (Figure 5D). Once again, mucus erosion
315 was prevented in infected mutant mice by IL-22-Fc injection (Figure 5D). These data indicate
316 the existence of a crucial axis between T cell-intrinsic GSH synthesis and IL-22 production that
317 is indispensable for the protective function of the intestinal epithelial barrier.

318

319 **GCLC expression in IBD patients correlates positively with expression of genes related to gut** 320 **integrity**

321 Our results above prompted us to investigate whether *GCLC* expression could be linked to
322 intestinal integrity in IBD patients. To this extend, we analyzed publicly available RNA
323 sequencing dataset (GSE109142) of rectal biopsies from patients with ulcerative colitis (UC), a
324 subtype of IBD (Haberman et al., 2019). MAYO scores (ranging from 0-12) were used to define
325 the disease severity as normal/mild (score <6), moderate (score <11) and severe (score ≥11)
326 (Teixeira et al., 2015). We then classified the rectal tissues of these patients as having low or
327 high *GCLC* expression (Figure S5A). In patients with moderate or severe disease, we identified
328 a positive correlation between *GCLC* expression and that of *CLDN15* (claudin-15) and *CLND2*
329 (claudin-2) (Figure 5E, S5B). *MUC2* expression correlated positively with *GCLC* in patients with
330 mild or moderate disease; patients with severe pathology likewise displayed a positive slope
331 but the correlation was not significant (Figure 5E, S5B). These data from IBD patients are in
332 line with our mouse results and further support our hypothesis that *Gclc* expression plays a
333 key role in preventing gut pathology.

334 In mouse models, IL-17 and IL-22, which can be largely produced by Th17 cell, have been
335 linked to the regulation of claudins and mucins production (Lee et al., 2015, Tsai et al., 2017,
336 Kim et al., 2012, Sugimoto et al., 2008). To investigate this relationship in the human setting,
337 we defined a Th17 cell gene expression signature that included *IL-17A*, *IL-17F*, *IFN-γ*, *CD3E*,
338 *CD4*, *STAT3*, *STAT5a*, *STAT1*, *STAT4*, *STAT6*, *AHR*, *RORC*, *RORA* and *TBX21*. We observed a

339 strong positive correlation between the Th17 gene signature score and *CLDN15*, *CLND2* and
340 *MUC2* levels in patients with moderate or severe pathology (Figure 5F, S5B). This score also
341 correlated positively with *IL-22* expression, indicating that most of the Th17 cells analyzed
342 expressed both IL-17 and IL-22 (Figure S5C). Thus, these results suggest a link between the
343 expression of Th17-related genes and intestinal epithelial integrity in IBD patients. Notably,
344 although *GCLC* expression did not correlate with disease severity, the expression of
345 glutathione synthase (*GSS*), which catalyzes the second step of GSH synthesis, was significantly
346 reduced in the gut of UC patients with severe pathology (Figure S5D). Moreover, there was a
347 strong positive correlation between *GCLC* expression and the Th17 gene signature score in IBD
348 patients (Figure 5G). These findings are in line with our murine data and indicate a strong
349 association between *GCLC*, Th17 cell markers, and genes related to gut integrity in the
350 intestine of IBD patients.

351

352 **T cell-intrinsic IL-22 is sufficient to restore survival of *C. rodentium*-infected *Cd4^{Cre}-Gclc^{fl/fl}*** 353 **mice**

354 We showed that systemic injection of recombinant IL-22-Fc prevented *C. rodentium*-induced
355 intestinal damage and mortality in *Cd4Cre Gclc^{fl/fl}* mice. To confirm that it is indeed GSH-
356 controlled IL-22 production by T cells that is the protective mechanism, we generated a novel
357 Cre recombinase-inducible IL22 transgenic mouse model (*IL22^{ind}* mice) by genetic targeting of
358 embryonic stem cells (Figure S6A, S6B). Animal derived from these cells harbor a transgene in
359 the *Rosa26* locus that contains cDNAs for IL22 and the EGFP reporter preceded by a loxP-
360 flanked STOP cassette and the CAG promoter (Figure S6A, S6B). After Cre-dependent removal
361 of the STOP sequence, IL22 and EGFP can be constitutively expressed. To reconstitute IL-17
362 production as well, we took advantage of an IL-17 transgenic mouse model where IL-17 and
363 EGFP expression could be controlled in a similar way (Haak et al., 2009). After Cre-dependent
364 removal of the STOP sequence, IL-22 or IL-17A and EGFP can be constitutively expressed. To
365 generate mice with T cell-specific IL-22 or IL-17A expression, we crossed *IL-22^{ind}* and *IL-17A^{ind}*
366 mice with *Cd4Cre* mice. EGFP and IL-22 were expressed in T cells of *Cd4Cre IL-22^{ind/+}* mice, but
367 not in *IL-22^{ind/+}* controls (Figure S6C, S6D). Similarly, EGFP and IL-17A were expressed in T cells
368 of *Cd4Cre IL-17A^{ind/+}* mice, but not in *IL-17A^{ind/+}* controls (Figure S6F). We then crossed *IL-22^{ind}*
369 and *IL-17A^{ind}* mice with *Cd4Cre Gclc^{fl/fl}* mice to generate *Cd4Cre Gclc^{fl/fl} IL-22^{ind/+}* and *Cd4Cre*

370 *Gclc^{fl/fl} IL-17A^{ind/+}* mice, which undergo T cell-specific *Gclc* deletion in parallel with induction of
371 EGFP and IL-22 or IL-17A, respectively. This approach uncouples IL-22 and IL-17A expression
372 from its regulation by *Gclc* in endogenous CD4⁺ cells (Figure S6E, S6G).

373 To investigate whether reinstating IL-22 production specifically in T cells of *Cd4Cre Gclc^{fl/fl}*
374 mice was sufficient to prevent infection-induced mortality, we infected *Cd4Cre Gclc^{fl/fl} IL-22^{ind/+}*
375 and *Cd4Cre Gclc^{fl/fl}* mice, along with *Gclc^{fl/fl}* and *Gclc^{fl/fl} IL-22^{ind/+}* controls, with *C. rodentium*.
376 Indeed, transgenic expression of IL-22 expression in mutant T cells (*Cd4Cre Gclc^{fl/fl} IL-22^{ind/+}*)
377 rescued these mice from the lethal infection outcome to a large extent and mice were
378 protected from weight loss (Figure 6A, S6H). When not completely protected from infection-
379 induced lethality *Cd4Cre Gclc^{fl/fl} IL-22^{ind/+}* showed a prolonged survival compared to infected
380 *Cd4Cre-Gclc^{fl/fl}* mice (Figure 6A). *Cd4Cre-Gclc^{fl/fl} IL-22^{ind/+}* mice were able to clear *C. rodentium*
381 infection by around day 60 p.i. (Figure S6I). In contrast to IL-22, reinstating IL-17 expression in
382 mutant T cells did not increase survival, nor could it limit weight loss (Figure 6B, S6J, K).
383 Importantly, T cell-specific expression of IL-22, in mutant T cells prevented bacterial spreading
384 to spleen and liver, which was in stark contrast to the transgenic expression of IL-17 in *Gclc*-
385 deficient (Figure 6C). These perplexing results indicate that it is the GSH-controlled T cell-
386 derived IL-22 and not the T cell-derived IL-17, which is sufficient to support full recovery of
387 these mutants. However, bacterial clearance in *Cd4Cre Gclc^{fl/fl} IL-22^{ind/+}* took more than twice
388 as long as in controls. This emphasizes the existence of additional factors that are under
389 control of *Gclc*, which contribute to optimal bacterial clearance. Nevertheless, our data
390 indicate that GSH-regulated IL-22 production by T cells appears to be the major factor
391 protecting the gut from detrimental bacterial infections.

392

393 **Mitochondrial function is impaired in *Gclc*-deficient Th17 cells**

394 To get more mechanistic insights how GSH regulates IL-22 production in Th17 cells we took
395 advantage of *in vitro* differentiated Th17 cells. Naïve CD4⁺ T cells from *Cd4Cre Gclc^{fl/fl}* mice
396 were activated in the presence of IL-6, IL-23 and IL-1 β and skewed to Th17 cells. These
397 conditions are known to induce naïve CD4⁺ T cells to differentiate into a pathogenic Th17 cell
398 subset co-producing IL-17 and IL-22 (Budda et al., 2016, Ghoreschi et al., 2010). In contrast,
399 conventional Th17 cells producing mainly IL-17 are induced if naive CD4⁺ T cells are activated

400 in the presence of IL-6 and TGF- β , since TGF- β inhibits IL-22 production (Rutz et al., 2011). In
401 line with our *in vivo* data, we found that *Cd4Cre-Gclc^{fl/fl}* CD4⁺ T cells differentiated into
402 pathogenic Th17 cells produced less IL-22 and IL-17 than controls (Figure S7A, B). In addition,
403 mutant Th17 cells showed increased cytosolic and mitochondrial ROS due to a lack of ROS
404 buffering caused by their GSH deficiency (Figure 6D, E). Accordingly, cytosolic and
405 mitochondrial ROS were greatly increased in *Gclc*-deficient LP T cells isolated from mice at 7
406 days p.i. with *C. rodentium* (Figure S7C, D). The addition of the antioxidant N-acetyl-cysteine
407 (NAC) during *in vitro* differentiation of the mutant cells restored ROS to control levels (Figure
408 6D, E). The same results were obtained when conventional Th17 cells were generated (Figure
409 S7E), implying that ROS accumulate in *Gclc*-deficient Th17 cells regardless of the
410 differentiation conditions.

411 Accumulating ROS can impact the mitochondrial dynamics and function (Chakrabarty and
412 Chandel, 2021). To assess the functionality of mitochondria in mutant Th17 cells, we measured
413 the oxygen consumption rate (OCR) in cultures of these cells. Basal and maximal OCR
414 measurements were highly reduced in *Gclc*-deficient pathogenic Th17 cells, but both
415 parameters were partially restored through ROS-scavenging by NAC (Figure 6F, 6G). We then
416 assessed mitochondrial mass (MM) and membrane potential (indicating activity; MMP) in
417 these cells by using MitoTracker Green (MG) and MitoTracker Deep Red (MDR), respectively.
418 Intriguingly, the mutant Th17 cells showed a drastic increase in MM, whereas MMP was
419 dramatically decreased (Figure 6H). The MDR/MG ratio indicates MMP/MM and represents
420 mitochondrial capacity (Pendergrass et al., 2004, Yu et al., 2020b). We found that MDR/MG
421 was decreased in *Gclc*-deficient Th17 cells, suggesting that their mitochondrial capacity is
422 impaired (Figure 6I). A decreased MDR/MG ratio was also observed in *Gclc*-deficient LP T cells
423 isolated at day 7 p.i. (Figure S7F). In addition, most control *Gclc^{fl/fl}* Th17 cells constituted a
424 MDR/MG^{hi} population, whereas mutant Th17 cells formed a MDR/MG^{lo} population (Figure 6J),
425 which suggests their mitochondria being depolarized and dysfunctional (Yu et al., 2020b).
426 Mitochondrial polarization and mass in mutant Th17 cells were almost completely restored
427 by NAC (Figure 6H-6J), indicating that mitochondrial activity in these cells depends on ROS
428 control. These results prompted us to investigate whether ROS-scavenging *in vivo* might
429 restore the functionality of mutant Th17 cells. Therefore, we administered NAC in the drinking
430 water of *Cd4Cre-Gclc^{fl/fl}* and *Gclc^{fl/fl}* mice starting at 7 days before *C. rodentium* infection and

431 continuing throughout the infection. In line with our *in vitro* data, the mortality of NAC-treated
432 infected mutant mice was drastically reduced (Figure 6K).

433

434 ***Gclc* regulates the mitochondrial-encoded components of the electron transport chain of** 435 **Th17 cells**

436 Our data so far indicate that mitochondrial dynamics and activity are affected by the absence
437 of *Gclc* in Th17 cells. To investigate the components of mitochondrial machinery on a larger
438 scale, we conducted bulk RNA sequencing of *in vitro* differentiated pathogenic Th17 cells.
439 Although, mutant Th17 cells showed decreased OXPHOS most of the genes that encoded for
440 subunits of the mitochondrial electron transport chain (ETC) appeared to be significantly
441 upregulated when compared to control cells (Figure 7A). Intriguingly, a closer examination
442 exposed that the ETC genes encoded within mitochondrial DNA were specifically down-
443 regulated in the mutant Th17 cells (Figure 7A). Notably, treating *Gclc*-deficient Th17 cells with
444 NAC restored the expression levels of these mitochondria-encoded ETC components (Figure
445 7A). This emphasizes that specifically mitochondrial gene expression is negatively impacted by
446 the absence of GSH in Th17 cells.

447 Mitochondrial gene expression is regulated by the mitochondrial transcription factor A
448 (TFAM), which is crucial for OXPHOS (Desdín-Micó et al., 2020, Fu et al., 2019, Baixauli et al.,
449 2015). In line with the reduced OXPHOS and the decreased expression of the mitochondrial
450 encoded ETC genes, we also observed that expression of TFAM is downregulated in *Gclc*-
451 deficient pathogenic Th17 cells, which can be restored by ROS-scavenging by NAC (Figure
452 7B). This suggests the existence of a yet unappreciated feedback loop where mitochondrial
453 respiration largely controls the generation of metabolic ROS (Dan Dunn et al., 2015), which
454 when not scavenged by GSH negatively affects TFAM, mitochondrial ETC genes and decreases
455 OXPHOS. In line with this ROS-induced negative mitochondrial cascade, ATP production was
456 decreased in *Gclc*-deficient *in vitro*-differentiated pathogenic Th17 cells (Figure 7C). Again,
457 ROS-scavenging by NAC could restore cellular ATP levels (Figure 7C). ATP is transported out of
458 the mitochondria and fosters many cellular pathways. Especially, cellular signaling is
459 dependent on ATP as it serves as a general kinase substrate. Consequently, low ATP
460 concentrations can influence kinase activities depending on their affinity for ATP (K_m -ATP).

461 We wondered whether the reduced ATP concentrations found in mutant Th17 cells and their
462 inability to express IL-22 are linked. The K_m -ATP of PI3K δ , which is an upstream kinase in the
463 mTOR activation pathway, is relatively high (118 mM), indicating that full activation of PI3K
464 requires substantial amounts of ATP (Somoza et al., 2015). In line with the reduced ATP, we
465 found that activation of PI3K was decreased in *Gclc*-deficient Th17 cells in a manner reversed
466 by ROS-scavenging (Figure 7D). Moreover, specific inhibition of PI3K lead to downregulation
467 of IL-22 production in the *in vitro*-differentiated WT pathogenic Th17 cells suggesting that
468 these pathways might be linked (Figure 7E). Interestingly, we did not observe a
469 downregulation of IL-22 encoding mRNA in mutant Th17 cells (Figure 7F). However, IL-22
470 protein expression was largely impacted in a ROS-dependent manner in *Gclc*-deficient
471 pathogenic Th17 cells, ruling out a transcriptional regulation of this pathway (Figure 7G). On
472 the contrary, IL-17 production was affected on both mRNA and protein levels (Figure S7G, H).
473 This data suggests a ROS- and PI3K-dependent translational control of IL-22 production in Th17
474 cells. PI3K can stimulate protein translation by the PI3K/AKT/mTOR signaling pathway. In line,
475 both phosphorylation of AKT and mTOR were significantly down-regulated in *Gclc*-deficient
476 Th17 cells, which were restored by ROS-scavenging (Figure 7H-I). mTOR is not only crucial for
477 host resistance to *C. rodentium* (Lin et al., 2016), but it also regulates translation. One mTOR
478 target is the repressor of translation 4E-BP1. mTOR-dependent phosphorylation of 4E-BP1
479 induces the dissociation from translation initiation factor eIF4E, which then initiates protein
480 translation (Sonenberg and Hinnebusch, 2009). In line with decreased mTOR and IL-22, 4E-
481 BP1 phosphorylation was decreased in *Gclc*-deficient Th17 cells, while 4E-BP1
482 phosphorylation remained at control levels upon ROS-scavenging (Figure 7J).

483 Collectively, these results indicate that the reduced IL-22 production by *Gclc*-deficient Th17
484 cells is linked to reduced protein translation and mitochondrial function. Thus, physiological
485 control of ROS is necessary for the generation of the IL-22-producing Th17 cells needed to
486 confer protective immunity during GI infections. Taken together, our data reveal a T cell-
487 intrinsic GSH-IL-22 signaling axis that is fundamental to the integrity of the gut intestinal
488 barrier and depends on the control of mitochondrial ROS.

489 Discussion

490 The GI tract is constantly exposed to microbial antigens of commensal and pathogenic
491 organisms. Th cells in the gut maintain immune tolerance to the microbiome and mount
492 immune responses against pathogens (van Wijk and Cheroutre, 2010). The GI tract is also a
493 major site of ROS generated by food processing and immune cell interactions (Aviello and
494 Knaus, 2017). Although low ROS support functions of T cells, elevated oxidative stress
495 interferes with their effector actions (Devadas et al., 2002, Gülow et al., 2005, Jackson et al.,
496 2004, Sena et al., 2013, Yi et al., 2006, Mak et al., 2017, Lang et al., 2013). Accordingly, T cells
497 contain antioxidants such as GSH that scavenge intracellular ROS and limit their accumulation
498 (Mak et al., 2017, Lian et al., 2018, Liang et al., 2006). In conventional T cells, GSH is essential
499 for the metabolic reprogramming triggered by activation (Mak et al., 2017). In contrast, GSH-
500 deficient Tregs show increased metabolic activity and are hyperproliferative but less
501 suppressive (Kurniawan et al., 2020). Thus, GSH has T cell subset-specific functions that all
502 contribute to immune homeostasis. The present study has revealed the role of GSH in Th17
503 cells, which are central effectors in mucosal immune responses. Loss of GSH-mediated ROS-
504 buffering in Th17 cells compromises the IL-22 production needed to defend against bacterial
505 GI infections.

506 Although several Th subsets contribute to immunity against *C. rodentium* including Th1,
507 Th22 and Th17 cells, this pathogen elicits stronger Th17 responses (Higgins et al., 1999, Basu
508 et al., 2012, Backert et al., 2014, Mangan et al., 2006). In line with this finding, we have shown
509 that the dominant Th cells responding to *C. rodentium* infection are CD4⁺IL-17⁺IL-22⁺ Th17
510 cells. WT LP CD4⁺ T cells increased their GSH production in response to *C. rodentium* infection,
511 suggesting a key role for GSH in these T cells. Consequently, *Gclc* deficiency had an important
512 effect on IL-17⁺IL-22⁺ Th17 cells. Our *in vitro* and *in vivo* studies have shown that ablation of
513 *Gclc* in Th17 cells is associated with the dysfunctionality of this important Th cell subset
514 resulting in a fatal infection outcome.

515 Our work has demonstrated that mitochondria in *Gclc*-deficient LP Th17 cells, as well as
516 mitochondria in *in vitro*-differentiated mutant Th17 cells, show compromised mitochondrial
517 capacity, which has been shown to impair Th17 cell function (Kaufmann et al., 2019).
518 Moreover, we observed dysregulation of expression of mitochondria-encoded ETC encoded
519 genes and their transcription regulator TFAM. TFAM has been linked to the stabilization of

520 mitochondrial DNA (mtDNA), its replication and OXPHOS (Ekstrand et al., 2004, Baixauli et al.,
521 2015). In T cells, TFAM deletion has been shown to skew these cells towards a pro-
522 inflammatory response (Desdín-Micó et al., 2020), specifically affecting immune surveillance
523 by Tregs (Fu et al., 2019). Our data is line with these findings as mitochondrial dysfunction
524 seems to affect mostly protective T cell responses, such as IL-22 production. Moreover, it has
525 been suggested that TFAM in ROR γ T⁺ lymphocytes plays a key role in small intestine
526 homeostasis (Fu et al., 2021). However, some questions still remain – is mROS dysregulation
527 leading to mutation of mitochondria-encoded genes, leading to altered ETC activity as
528 suggested for metastatic tumor cells (Ishikawa et al., 2008, Woo et al., 2012), or is the ROS-
529 induced halt of mitochondrial transcription the sole mechanism leading to dysfunctional
530 mitochondria? Our data, especially our immediate ROS-scavenging experiments suggest the
531 latter could be the case.

532 Due to abnormal mitochondrial function, decreases in OCR and total ATP were observed in
533 cultures of *Gclc*-deficient Th17 cells. Low ATP concentrations have recently been linked to
534 decreased PI3K activation (Xu et al., 2021), and the PI3K/AKT/mTOR signaling pathway couples
535 metabolic and transcriptional responses (Chi, 2012). We found that PI3K, AKT and mTOR
536 activation were all decreased in mutant Th17 cells *in vitro*, in line with a previous report that
537 oxidative stress inhibits PI3K/AKT/mTOR signaling in melanoma cells (Hambricht et al., 2015).
538 We therefore propose that a lack of ROS buffering in *Gclc*-deficient Th17 cells interferes with
539 TFAM expression and transcription of mitochondrial encoded genes of the ETC. This leads to
540 reduced OXPHOS and reduced mitochondrial ATP production resulting in lower cellular ATP
541 concentrations associated with decreased PI3K/AKT/mTOR activation in Th17 cells. Activated
542 mTORC1 enables release of the translation initiation factor eIF4E by phosphorylating the
543 repressor of translation 4E-BP1 (Sonenberg and Hinnebusch, 2009). The the low 4E-BP1
544 phosphorylation in *Gclc*-deficient Th17 cells is indeed associated with reduced production of
545 IL-22. Consistently, interference with mitochondrial translation has been previously
546 connected with T cell cytokine production (Almeida et al., 2021, Colaço et al., 2021). *In vitro*
547 as well as *in vivo*, we have shown that ROS-accumulation in Th17 cells is decisive for the
548 mitochondrial dysfunction and impaired cytokine production. Accordingly, the lethality of *C.*
549 *rodentium*-infected *Cd4^{Cre}-Gclc^{fl/fl}* mice could be prevented by *in vivo* ROS-scavenging.

550

551 Although CD4-expressing LTi cells also co-produce IL-22 and IL-17, we found no impact of
552 *Gclc* deletion on cytokine production by caecal or colonic LTi cells during *C. rodentium*
553 infection. In addition, the course of *C. rodentium* infection was nearly identical in *Rag1*^{-/-}
554 *Cd4Cre Gclc*^{fl/fl} and control *Rag1*^{-/-}*Gclc*^{fl/fl} mice, indicating that loss of *Gclc* in LTi cells has no
555 effect on the severe outcomes of infection in *Cd4Cre Gclc*^{fl/fl} mice. Intracellular ROS levels in
556 LTi cells were unaffected by *Gclc* deletion, suggesting that, unlike in T cells, LTi cell function
557 does not depend on *Gclc*-mediated ROS buffering. Indeed, mitochondrial metabolism and the
558 impact of mitochondrial ROS in ILC3s are known to diverge from that in Th17 cells. Whereas
559 Th17 cells maintain low levels of mitochondrial ROS to avoid detrimental effects, high
560 mitochondrial ROS promote ILC3 effector function, and antioxidants impair ILC3 cytokine
561 production (Di Luccia et al., 2019). Consistent with this report, we found that intracellular ROS
562 were much higher in T cells than in LTi cells after *Gclc* ablation (Figure 3D, S3F). Lastly, unlike
563 T cells, ILCs and other innate immune cells could rely more on alternative antioxidants to
564 compensate for loss of GSH-mediated ROS-buffering capacity (Yang et al., 2020).

565 In line with the finding that only mice lacking IL-22 are unable to clear *C. rodentium* infections
566 (Basu et al., 2012, Zheng et al., 2008, Ota et al., 2011, Ishigame et al., 2009, Guo et al., 2014),
567 we showed that IL-22-Fc treatment *in vivo* protected infected *Cd4Cre Gclc*^{fl/fl} mice from
568 lethality and that genetic restoration of T cell-mediated IL-22 production, but not IL-17, in
569 *Cd4Cre Gclc*^{fl/fl} mice allowed them to clear the pathogen. Although IL-22 controls AMP
570 expression (Liang et al., 2006, Zheng et al., 2008, Raffatellu et al., 2009, Dixon et al., 2016), we
571 saw no differences in these peptides between mutant and control mice. It may be that ILC-
572 derived IL-22 induces adequate AMP expression in *Gclc*-deficient mice. However, it is T cell-
573 derived IL-22 that is essential to regulate tight junction integrity and mucus production, which
574 protects the intestine from *C. rodentium*-induced damage during the late stages of *C.*
575 *rodentium* infection (Zindl et al., 2021). Accordingly, infected *Cd4Cre Gclc*^{fl/fl} mice exhibited
576 severely disorganized tight junctions, reduced IEC MUC2 content and a thin mucus layer. As a
577 result, gut permeability was increased in the mutants, allowing bacterial spread to the
578 peripheral organs and blood. Injection of IL-22-Fc preserved normal tight junctions, IEC MUC2
579 content and a robust mucus layer in infected mutants. Pertinently, loss of intestinal barrier
580 function, reduced mucin production, increased oxidative stress and decreased antioxidant
581 capacity have all been linked to chronic inflammation in IBD patients (Shorter et al., 1972,

582 Corridoni et al., 2014, Sido et al., 1998, Buffinton and Doe, 1995, Lih-Brody et al., 1996,
583 Kruidenier et al., 2003). In line, we saw a positive correlation between *GCLC* expression and
584 epithelial integrity markers in rectal tissue sections of UC patients. The expression of Th17 cell
585 markers correlated positively with *GCLC* expression, hinting that *GCLC* regulates Th17 cell
586 functions and thus intestinal epithelial integrity in humans with GI pathology. Our results
587 provide a rationale for exploring antioxidant treatment as a strategy to regulate Th17-derived
588 IL-22 production in these disorders.

589 In conclusion, our findings imply the existence of a previously unappreciated axis between
590 GSH, mitochondrial function and IL-22 signaling within Th17 cells that operates during GI
591 infections. If ROS accumulate in Th17 cells, TFAM, mitochondrial activity and PI3K/AKT/mTOR
592 are dysregulated, impairing IL-22 protein production. IL-22 is critical for intestinal barrier
593 integrity and mouse survival during bacterial GI infection. Further study of this newly
594 described GSH-dependent regulatory circuit in LP Th17 cells may yield new insights into how
595 increased oxidative stress affects the function of this T cell subset and leads to intestinal
596 pathology. Our results may also point to novel therapeutic strategies for modulating Th17 cell
597 function in the context of human GI disorders.

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619

620 **Author Contributions**

621 L.B. performed most experiments assisted by V.H., L.G., H.K, D.G.F., L.S-B., M.G., A.E, C.B., C.V.,
622 and S.F. Lamina propria isolations were carried out by L.B. and L.G., H.K., L.B., L.S-B. and D.G.F.
623 performed Seahorse flux assays. RNA-sequencing data analysis was performed by E.K. and J.L.
624 and guided by E.L. J-J.G. performed H&E and Alcian Blue histology stainings. IL-22^{ind} mice were
625 generated by S.S. and B.B. T.K., C.D., Y.C., V.V, B.B., I.S.H., P.A.L, A.W., and M.M. provided
626 reagents and expert comments. D.B. supervised the study. L.B., and D.B. conceptualized the

627 work, together with V.H. designed all experiments, analyzed the data, and wrote the
628 manuscript. All authors reviewed and edited the final manuscript.

629

630 **Declaration of Interests**

631 The authors declare no competing interests.

632 **Figure Legends**

633 **Figure 1: *Gclc* in LP T cells controls ROS and protects mice from lethal *C. rodentium* infection.**

634 (A) Left: Flow cytometric analysis (FCA) of intracellular thiol levels as detected by mBBr
635 staining of CD4⁺ cells in colonic LP, which was isolated from uninfected and *C. rodentium*-
636 infected *C57BL/6* mice at day 7 p.i. MFI, mean fluorescence intensity. Right: Quantification of
637 left panel results. Data are mean±SEM (n=3-4).

638

639 (B) Quantification of luminescence-based assay of intracellular GSH in FACS-sorted colonic LP
640 CD4⁺ cells isolated as in (A). Data are mean±SEM of relative luminescence units (RLU) (n=3).

641 (C) Left: FCA of intracellular ROS in CD4⁺ cells of the colonic LP, which was isolated as in (A)
642 and subjected to DCF-DA staining. Right: Quantification of left panel results. Data are
643 mean±SEM (n=3).

644 (D) Left: FCA of intracellular thiol levels as detected by mBBr staining in CD4⁺ cells of the
645 colonic LP, which was isolated from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice at
646 day 7 p.i. Right: Quantification of left panel results. Data are mean±SEM (n=3) and
647 representative of 2 trials.

648 (E) Quantification of luminescence-based assay of intracellular GSH in FACS-sorted CD4⁺ cells
649 from colonic LP isolated as in (D). Data are mean±SEM of RLU (n=3).

650 (F) Left: FCA of intracellular ROS in CD4⁺ cells of the colonic LP, which was isolated as in (D)
651 and subjected to DCF-DA staining. Right: Quantification of left panel results. Data are
652 mean±SEM (n=4).

653 (G) Change in whole body weight of *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice infected with *C. rodentium*
654 on day 0 and assayed on the indicated days p.i. Data are mean±SEM (n=3-4) and
655 representative of 5 trials.

656 (H) Survival of *Gclc^{fl/fl}* (n=14) and *Cd4Cre Gclc^{fl/fl}* (n=23) mice infected with *C. rodentium* on day
657 0. Data are pooled from 5 independent trials.

658 (I) Left: CFU of *C. rodentium* in feces of *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice at the indicated days
659 p.i. Data are mean±SEM (n=3-4) and representative of 5 trials. Right: Statistical analysis of left
660 panel data at day 14 p.i.

661 (J) Left: Macroscopic views and lengths of colons isolated from *C. rodentium*-infected *Gclc^{fl/fl}*
662 and *Cd4Cre Gclc^{fl/fl}* mice at day 12 p.i. Right: Quantification of colon lengths from left panel
663 results. Data are mean±SEM (n=5); 2 trials.

664 (K) Top: H&E-stained sections of distal colon from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre*
665 *Gclc^{fl/fl}* mice at day 12 p.i. Data are representative of 3 mice/genotype. Scale bars, 500µm.
666 Bottom: Higher magnification views of the boxed areas in the top panels. Scale bars, 100µm.

667

668 **Figure 2: *Gclc* is essential for Th17 cell responses during *C. rodentium* infection.**

669 (A) Intracellular FCA of IL-17 and IL-22 in CD4⁺ cells of the colonic LP, which was isolated from
670 uninfected and *C. rodentium*-infected *Gclc^{fl/fl}* mice at day 7 p.i. LP cells were stimulated with
671 PMA/calcium ionophore/Brefeldin A for 5 hr before staining. Plots are representative of 4
672 mice/genotype.

673 (B-E) Frequencies of IL-17⁻IL-22⁺ Th22 cells (B), IL-17⁺IL-22⁻ conventional Th17 cells (C), IL-17⁺IL-
674 22⁺ pathogenic Th17 cells (D), and IFN- γ ⁺IL-17⁻ Th1 cells (E) among the colonic LP CD4⁺ cells in
675 (A). Data are mean \pm SEM (n=4); 3 trials.

676 (F) Quantification of FCA of the indicated CD4⁺ T cell subsets among colonic LP cells from the
677 mice in (A). Relative fold increase represents the ratio of cell numbers in infected/uninfected
678 mice. Data are mean \pm SEM (n=4); 3 trials.

679 (G) Quantification of intracellular FCA of Tbet and ROR γ T in CD4⁺ cells of the colonic LP, which
680 was isolated from uninfected and *C. rodentium*-infected *Gclc^{fl/fl}* mice at day 7 p.i. Data are
681 frequencies of Tbet⁺ROR γ T⁺ (left), Tbet⁻ROR γ T⁺ (middle) and Tbet⁺ROR γ T⁻ (right) cells among
682 colonic LP CD4⁺ cells. Data are mean \pm SEM (n=3-6); 2 trials.

683 (H) Quantification of FCA of the indicated CD4⁺ T cell subsets among colonic LP cells from the
684 mice in (G). Relative fold increase represents the ratio of cell numbers in infected/uninfected
685 mice. Data are mean \pm SEM (n=3-6); 2 trials.

686 (I) Left: Intracellular FCA of IL-17 and IL-22 in CD4⁺ cells of the colonic LP, which was isolated
687 from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice at day 7 p.i. Middle: Frequencies
688 of IL-17⁺IL-22⁻ conventional Th17 cells from left panel results. Right: Frequencies of IL-17⁺IL-
689 22⁺ pathogenic Th17 cells from left panel results. Data are mean \pm SEM (n=4); 3 trials.

690 (J) Quantification of intracellular FCA of Tbet and ROR γ T in CD4⁺ cells of the colonic LP, which
691 was isolated from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice at day 7 p.i. Data are
692 frequencies of Tbet⁺ROR γ T⁺ (left) and Tbet⁻ROR γ T⁺ (right) cells among colonic LP CD4⁺ cells.
693 Data are mean \pm SEM (n= 6); 2 trials.

694

695 **Figure 3: Absence of *Gclc* in LTi cells does not impair cytokine production or immunity to *C.***
696 ***rodentium*.**

697 (A) Left: Intracellular FCA of IL-17 and IL-22 in colonic LP LTi cells of *C. rodentium*-infected
698 *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice at day 7 p.i. LP cells were stimulated with PMA/calcium
699 ionophore/Brefeldin A for 5 hr before staining. Right: Frequencies of the indicated
700 subpopulations from left panel results. Data are mean \pm SEM (n=5); 3 trials.

701 (B) Total cell numbers of the indicated subpopulations from the data in (A). Data are
702 mean \pm SEM (n=5); 3 trials.

703 (C) Frequencies (left) and total numbers (right) as determined by FCA of LTi cells among colonic
704 LP cells isolated from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice at day 7 p.i. Data
705 are mean \pm SEM (n=5); 3 trials.

706 (D) Quantification of FCA of intracellular ROS in CD3⁻CD4⁺ LTi cells of the colonic LP, which was
707 isolated from *C. rodentium*-infected *Gclc*^{fl/fl} and *Cd4Cre Gclc*^{fl/fl} mice at day 7 p.i. and subjected
708 to DCF-DA staining. Data are mean±SEM (n=4-5).

709 (E) Change in whole body weight of *Rag1*^{-/-}*Gclc*^{fl/fl}, *Rag1*^{-/-}*Cd4Cre Gclc*^{fl/fl}, *Gclc*^{fl/fl} and *Cd4Cre*
710 *Gclc*^{fl/fl} mice during *C. rodentium* infection. Data are mean±SEM (n=6-9); 3 trials.

711 (F) Survival of *Rag1*^{-/-}*Gclc*^{fl/fl} (n=9), *Rag1*^{-/-}*Cd4Cre Gclc*^{fl/fl} (n=9), *Gclc*^{fl/fl} (n=6) and *Cd4Cre Gclc*^{fl/fl}
712 (n=6) mice during *C. rodentium* infection. Data are representative of 3 trials.

713 (G) Left: CFU of *C. rodentium* in feces of *Rag1*^{-/-}*Gclc*^{fl/fl}, *Rag1*^{-/-}*Cd4Cre Gclc*^{fl/fl}, *Gclc*^{fl/fl} and
714 *Cd4Cre Gclc*^{fl/fl} mice at the indicated time points after infection on day 0. Right: Statistical
715 analysis of left panel results at day 14 p.i. Data are mean±SEM (n=6-9); 3 trials.

716 (H) Left: Macroscopic views and lengths of colons isolated from *C. rodentium*-infected *Rag1*^{-/-}
717 *Gclc*^{fl/fl} and *Rag1*^{-/-}*Cd4Cre Gclc*^{fl/fl} mice at day 7 p.i. Right: Quantification of colon lengths from
718 left panel results. Data are mean±SEM (n=4).

719

720 **Figure 4: IL-22 reconstitution rescues mice with T cell-specific GSH deficiency from lethal *C.***
721 ***rodentium* infection and prevents increased intestinal permeability.**

722 (A) Change in whole body weight of *C. rodentium*-infected *Gclc*^{fl/fl} and *Cd4Cre Gclc*^{fl/fl} mice
723 treated i.v. with recombinant IL-22-Fc or control-Fc at days -1, 3, 6, 9, 12 and 15 p.i. Mice were
724 infected on day 0. Data are mean±SEM (n=4-5); 2 trials.

725 (B) Survival of the mice in (A). Data are representative of 2 trials.

726 (C) Top: H&E-stained sections of distal colon of *C. rodentium*-infected *Gclc*^{fl/fl} and *Cd4Cre*
727 *Gclc*^{fl/fl} mice that were treated i.v. with IL-22-Fc or control-Fc as in (A, B) and examined at day
728 12 p.i. Data are representative of 3 mice/genotype. Scale bars, 500µm. Bottom: Higher
729 magnification views of the boxed areas in the top panels. Scale bars, 200µm.

730 (D) Quantification of plasma FITC-Dextran levels in mice of the indicated genotypes that were
731 infected with *C. rodentium* and treated i.v. with IL-22-Fc or control-Fc at days -1, 3, 6, 9, p.i. At
732 day 10 p.i., mice were gavaged with FITC-Dextran. FITC-Dextran was measured in plasma 4 hr
733 later. Data are mean±SEM (n=6).

734 (E) CFU of *C. rodentium* in liver (left), spleen (middle) and blood (right) of *C. rodentium*-infected
735 *Gclc*^{fl/fl} and *Cd4Cre Gclc*^{fl/fl} mice treated i.v. with IL-22-Fc or control-Fc at days -1, 3, 6, 9 p.i.
736 Organs and blood were collected at day 12 p.i. Data are mean±SEM (n=6).

737

738 **Figure 5: *Gclc* deficiency is linked to defects in intestinal tight junctions and mucus in *C.***
739 ***rodentium*-infected mice and IBD patients.**

740 (A) Representative immunoblots to detect the indicated claudin proteins in distal colon
741 segments of *C. rodentium*-infected *Gclc*^{fl/fl} and *Cd4Cre Gclc*^{fl/fl} mice at day 12 p.i. Actin, loading
742 control. Data are representative of 3 mice/genotype.

743 (B) Left: Top and middle: Immunofluorescence microscopy of cross-sections of colonic crypts
744 from distal colon segments that were collected at day 12 p.i. from *C. rodentium*-infected
745 *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice treated i.v. with IL-22-Fc or control-Fc at days -1, 3, 6, 9, p.i.
746 Sections were stained to detect claudin-2 (red), F-actin (green) and DNA (blue). Data are
747 representative of 6 mice/genotype. Scale bars, 100 μ m. Bottom: “Zoom” panels are higher
748 magnification views of boxed areas in the middle panels. Right: Quantification of lumen
749 diameters of 24-43 crypts measured per mouse in the left panel. Data are representative of 6
750 mice/genotype.

751 (C) Top and middle: Immunofluorescence microscopy of cross-sections of colonic crypts from
752 distal colon segments in (B) were stained to detect MUC2 (red), F-actin (green) and DNA (blue).
753 Data are representative of 6 mice/genotype. Scale bars, 100 μ m. Bottom: “Zoom” panels are
754 higher magnification views of boxed areas in the middle panel.

755 (D) Top: Alcian blue-stained cross-sections of distal colon segments that were collected at day
756 12 p.i. from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice treated i.v. with IL-22-Fc or
757 control-Fc at days -1, 3, 6, 9, p.i. Data are representative of 3 mice/genotype. Scale bars,
758 500 μ m. Middle: “Zoom” panels are higher magnification views of the boxed areas in the top
759 panels. Scale bars, 200 μ m. Bottom: H&E staining of the sections in the middle panels. Scale
760 bars, 200 μ m.

761 (E, F) Bioinformatics analyses of RNA sequencing data of rectal biopsies from ulcerative colitis
762 (UC) patients. The provided MAYO scores were used to define the disease severity as
763 normal/mild, moderate and severe disease (Teixeira et al., 2015). Significance and r^2 values
764 are indicated in the lower right corner of each frame. (E) Correlation of *GCLC* expression with
765 the epithelial integrity markers claudin-15 (*CLDN15*) (top), claudin-2 (*CLDN2*) (middle), and
766 mucin 2 (*MUC2*) (bottom). (F) Correlation of the Th17 score with the epithelial integrity
767 markers claudin-15 (*CLDN15*) (top), *CLDN2* (middle), and *MUC2* (bottom). The Th17 score was
768 defined as the mean of scaled log₂ gene expression values composing the signature (*IL-17A*,
769 *IL-17F*, *IFN- γ* , *CD3E*, *CD4*, *STAT3*, *STAT5a*, *STAT1*, *STAT4*, *STAT6*, *AHR*, *RORC*, *RORA* and *TBX21*).

770 (G) Bioinformatics analysis of RNA sequencing data of UC rectal biopsies as in (E,F) showing
771 correlation of *GCLC* expression with Th17 score.

772

773 **Figure 6: T-cell intrinsic IL-22 but not IL-17 expression rescues *Gclc*-dependent susceptibility**
774 **to *C.rodentium*.**

775 (A) Survival of *Gclc^{fl/fl}* (n=5), *Cd4Cre Gclc^{fl/fl}* (n=6), *Gclc^{fl/fl}IL-22^{ind/+}* (n=5) and *Cd4Cre Gclc^{fl/fl}IL-*
776 *22^{ind/+}* (n=8) mice infected with *C. rodentium* on day 0 and assayed on the indicated days p.i.
777 Data are mean \pm SEM (n=5-8).

778 (B) Survival of of *Gclc^{fl/fl}* (n=6), *Cd4Cre Gclc^{fl/fl}* (n=9), *Gclc^{fl/fl}IL-17A^{ind/+}* (n=6) and *Cd4Cre*
779 *Gclc^{fl/fl}IL-17A^{ind/+}* (n=7) mice infected with *C. rodentium* on day 0 and assayed on the indicated
780 days p.i. Data are mean \pm SEM (n=6-9).

781 (C) CFU of *C. rodentium* in spleen (left) and liver spleen (right) of *C. rodentium*-infected *Gclc^{fl/fl}*,
782 *Cd4Cre Gclc^{fl/fl}*, *Gclc^{fl/fl}IL-22^{ind/+}*, *Cd4Cre Gclc^{fl/fl}IL-22^{ind/+}*, *Gclc^{fl/fl}IL-17A^{ind/+}* and *Cd4Cre Gclc^{fl/fl}IL-*
783 *17A^{ind/+}* mice (n=4), day 10 p.i.

784 (D-J) Naïve T cells were sorted from spleen and lymph nodes of *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice
785 and induced to differentiate *in vitro* into pathogenic Th17 cells by culture with anti-CD3, anti-
786 CD28, IL-6, IL-1 β and IL-23. Cells were treated with 10mM NAC as indicated.

787 (D) Left: FCA of DCF-DA staining to detect cytosolic ROS in *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* *in vitro*-
788 differentiated Th17 cells. Right: Quantification of left panel results. Data are mean \pm SEM (n=3);
789 2 trials.

790 (E) Left: FCA of MitoSOX to detect mitochondrial ROS in *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* *in vitro*-
791 differentiated Th17 cells. Right: Quantification of left panel results. Data are mean \pm SEM (n=3);
792 2 trials.

793 (F, G) Seahorse quantification of basal OCR (E) and maximal OCR (F) in cultures of *Gclc^{fl/fl}* and
794 *Cd4Cre Gclc^{fl/fl}* *in vitro*-differentiated Th17 cells. Data are mean \pm SEM (n=3); 2 trials.

795 (H) Quantification of FCA of *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* *in vitro*-differentiated Th17 cells that
796 were treated with (left) MitoTracker Deep Red to assess mitochondrial activity, or (right)
797 MitoTracker Green to assess mitochondrial mass. Data are mean \pm SEM (n=3); 3 trials.

798 (I) Determination of fold change in the MDR/MG ratio from the data in (B). Data are
799 mean \pm SEM (n=3); 3 trials.

800 (J) Representative contour plots (left) and frequencies (right) of MDR/MG^{hi} and MDR/MG^{lo} cell
801 subpopulations among *in vitro*-differentiated *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* Th17 cultures as
802 determined by FCA. Data are mean \pm SEM (n=3); 3 trials.

803 (K) Survival of *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice (n=5-9) that were left
804 untreated or treated with 40mM NAC in drinking water. Data are pooled from 2 independent
805 trials.

806

807 **Figure 7: *Gclc* connects mitochondrial gene expression and mitochondrial function with IL-**
808 **22 protein translation in Th17 cells.**

809 (A-J) Naïve T cells were sorted from spleen and lymph nodes of *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* mice
810 and induced to differentiate *in vitro* into pathogenic Th17 cells by culture with anti-CD3, anti-
811 CD28, IL-6, IL-1 β and IL-23. Cells were treated with 10mM NAC as indicated.

812 (A-B) Bulk RNA seq was performed. (A) Heatmap representing GO:0022900 Electron transport
813 Chain. (B) Tfam expression. (n=3).

814 (C) Quantification of total ATP levels in *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* *in vitro*-differentiated Th17
815 cells as measured in RLU. Data are mean \pm SEM (n=3); 2 trials.

816 (D) Quantification of FCA of p-PI3K in *in vitro*-differentiated *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* Th17
817 cells. Data are mean \pm SEM (n=3); 2 trials.

818 (E) IL-22 protein concentrations as measured by ELISA in culture supernatants of *in vitro*-
819 differentiated C57BL/6J WT Th17 cells treated with PI3K inhibitor (LY294002) for last 24 hours
820 of differentiation. Data are mean±SEM (n=3).

821 (F) Quantification of qPCR determination of IL-22 mRNA expression of *in vitro*-differentiated
822 *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* Th17 cells. Data are mean±SD (n=3-4).

823 (G) IL-22 protein concentrations as measured by ELISA in culture supernatants of *in vitro*-
824 differentiated *Gclc^{fl/fl}* and *Cd4Cre Gclc^{fl/fl}* Th17 cells. Data are mean±SEM (n=3); 2 trials.

825 (H) Quantification of FCA of p-AKT in *in vitro*-differentiated *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* Th17
826 cells. Data are mean±SEM (n=3); 2 trials.

827 (I) Left: FCA to detect p-mTOR in *in vitro*-differentiated *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* Th17 cells.
828 Right: Quantification of left panel results. Data are mean±SEM (n=3); 3 trials.

829 (J) Quantification of FCA of p-4E-BP1 in *in vitro*-differentiated *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* Th17
830 cells. Data are mean±SEM (n=3); 2 trials.

831

832 **Supplementary Figure Legends**

833

834 **Supplementary Figure 1: T cell-specific ablation of *Gclc* does not affect the gut microbiome.**

835 (A) Quantification of flow cytometric analysis of DCF-DA staining to detect intracellular ROS in
836 CD4⁺ cells of the colonic LP, which was isolated from uninfected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}*
837 mice. MFI, mean fluorescence intensity. Data are mean±SEM (n=3); 2 trials.

838 (B) Macroscopic views and lengths of colons from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-*
839 *Gclc^{fl/fl}* mice at day 12 p.i. Images are representative of 5 mice per genotype; 2 trials.

840 (C) Quantification of qPCR determinations of DNA of the indicated bacterial genera in feces of
841 uninfected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice. $\Delta\Delta$ Ct values were normalized to total Eubacteria.
842 Data are mean±SEM (n=4); 2 trials.

843

844 **Supplementary Figure 2: T cell-specific ablation of *Gclc* does not alter steady-state levels of 845 IL-22 and IL-17 in colonic LP T cells.**

846 (A) Frequencies (left) and total numbers (right) of CD62L⁻CD44⁺ cells as determined by flow
847 cytometric analysis of CD4⁺ cells of the colonic LP, which was isolated from uninfected and *C.*
848 *rodentium*-infected *Gclc^{fl/fl}* mice at day 7 p.i. Data are mean±SEM (n=4); 2 trials.

849 (B) Frequencies (left) and total numbers (right) of CD62L⁺CD44⁻ cells as determined by flow
850 cytometric analysis of CD4⁺ cells of the colonic LP, which was isolated as in (A). Data are
851 mean±SEM (n=4); 2 trials.

852 (C) Frequencies (left) and total numbers (right) of IFN- γ ⁺IL-17⁺ cells as determined by flow
853 cytometric analysis of CD4⁺ cells of the colonic LP, which was isolated as in (A). LP cells were
854 stimulated with PMA/calcium ionophore/Brefeldin A for 5 hr before staining. Data are
855 mean \pm SEM (n=4); 3 trials.

856 (D) Frequencies (left) and total numbers (right) of CD62L⁻CD44⁺ cells as determined by flow
857 cytometric analysis of colonic LP CD4⁺ cells isolated from uninfected *Gclc*^{fl/fl} and *Cd4Cre-Gclc*^{fl/fl}
858 mice. Data are mean \pm SEM (n=4); 2 trials.

859 (E) Frequencies (left) and total numbers (right) of IL-17⁺IL-22⁺ cells as determined by
860 intracellular flow cytometric analysis of colonic LP CD4⁺ cells isolated as in (D). LP cells were
861 stimulated with PMA/calcium ionophore/Brefeldin A for 5 hr before staining. Data are
862 mean \pm SEM (n=4); 3 trials.

863 (F) Frequencies (left) and total numbers (right) of IL-17⁺IL-22⁻ cells as determined by
864 intracellular flow cytometric analysis of colonic LP CD4⁺ cells isolated as in (D). LP cells were
865 stimulated as in (E). Data are mean \pm SEM (n=4); 3 trials.

866 (G) Frequencies of IFN- γ ⁺IL-17⁺ cells as determined by flow cytometric analysis of CD4⁺ cells of
867 the colonic LP, which was isolated from *C. rodentium*-infected *Gclc*^{fl/fl} and *Cd4Cre-Gclc*^{fl/fl} mice
868 at day 7 p.i. LP cells were stimulated with PMA/calcium ionophore/Brefeldin A for 5 hr before
869 staining. Data are mean \pm SEM (n=4); 3 trials.

870 (H) Frequencies of IL-17⁺IL-22⁺ cells as determined by flow cytometric analysis of CD4⁺ cells of
871 the colonic LP, which was isolated as in (G). LP cells were stimulated as in (G). Data are
872 mean \pm SEM (n=4); 3 trials.

873 (I) Frequencies of IFN- γ ⁺IL-17⁻ cells as determined by flow cytometric analysis of CD4⁺ cells of
874 the colonic LP, which was isolated and treated as in (G). LP cells were stimulated as in (G). Data
875 are mean \pm SEM (n=4); 3 trials.

876 (J) Total cell numbers as determined by intracellular flow cytometric analysis of the indicated
877 CD4⁺ T cell subsets among colonic LP cells, which were isolated from *C. rodentium*-infected
878 *Gclc*^{fl/fl} and *Cd4Cre-Gclc*^{fl/fl} mice at day 7 p.i. LP cells were stimulated as in (G). Data are
879 mean \pm SEM (n=4); 3 trials.

880 (K) Total cell numbers as determined by intracellular flow cytometric analysis of
881 CD4⁺Tbet⁺ROR γ T⁺ (left) and CD4⁺Tbet⁻ROR γ T⁺ (right) cells of the colonic LP, which was isolated
882 from *C. rodentium*-infected *Gclc*^{fl/fl} and *Cd4Cre-Gclc*^{fl/fl} mice at day 7 p.i. Data are mean \pm SEM
883 (n= 6); 2 trials.

884 (L) Frequencies (left) and total numbers (right) of Tbet⁺ROR γ T⁻ cells as determined by
885 intracellular flow cytometric analysis of CD4⁺ cells in the colonic LP, which was isolated as in
886 (K). Data are mean \pm SEM (n= 6); 2 trials.

887

888 **Supplementary Figure 3: *Gclc* regulates IL-17 and IL-22 expression in T cells but not in LTi**
889 **cells.**

890 (A) Quantification of flow cytometric analysis of frequencies (left) and total numbers (right) of
891 LTi cells in caecal LP isolated from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice at day
892 4 p.i. Data are mean±SEM (n=4-5).

893 (B) Quantification of flow cytometric analysis of frequencies (left) and total numbers (right) of
894 the indicated LTi subpopulations from the data in (A) at day 4 p.i. LP cells were stimulated with
895 PMA/calcium ionophore/Brefeldin A for 5 hr before staining. Data are mean±SEM (n=4-5).

896 (C) Frequencies (left) and total cell numbers (right) as determined by flow cytometric analysis
897 of total LTi cells among colonic LP cells isolated from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-*
898 *Gclc^{fl/fl}* mice at day 4 p.i. Data are mean±SEM (n=4-5).

899 (D) Frequencies (left) and total cell numbers (right) as determined by intracellular flow
900 cytometric analysis of the indicated LTi subpopulations among colonic LP cells isolated as in
901 (C). LP cells were stimulated as in (B). Data are mean±SEM (n=4-5).

902 (E) Frequencies (left) and total cell numbers (right) of the indicated populations as determined
903 by flow cytometric analysis of intracellular IL-17 and IL-22 staining in CD3⁺CD4⁺ T cells among
904 colonic LP cells of *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice at day 7 p.i. LP cells
905 were stimulated as in (B). Data are mean±SEM (n=5); 3 trials.

906 (F) Quantification of flow cytometric analysis of intracellular ROS in CD3⁺CD4⁺ T cells of the
907 colonic LP, which was isolated from *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice at
908 day 7 p.i. and subjected to DCF-DA staining. Data are mean±SEM (n=4-5).

909 (G) Frequencies (left) and total cell numbers (right) as determined by intracellular flow
910 cytometric analysis of the indicated subpopulations of LTi cells among colonic LP cells isolated
911 from *C. rodentium*-infected *Rag1^{-/-}Gclc^{fl/fl}* and *Rag1^{-/-}Cd4Cre-Gclc^{fl/fl}* mice at day 7 p.i. LP cells
912 were stimulated as in (B). Data are mean±SEM (n=5).

913 (H) Quantification of flow cytometric analysis of intracellular ROS in CD3⁻CD4⁺ LTi cells of the
914 colonic LP, which was isolated as in (G) and subjected to DCF-DA staining. Data are mean±SEM
915 (n=5).

916

917 **Supplementary Figure 4: Ablation of *Gclc* in T cells does not affect either bacterial virulence**
918 **factors of *C. rodentium* or intestinal anti-microbial peptides (AMPs).**

919 (A) RT-qPCR determination of mRNA levels of the indicated bacterial virulence factors in
920 colonic tissues of *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice at day 7 and 10 p.i.
921 $\Delta\Delta$ Ct values were normalized to Rps17 expression. Data are mean±SEM (n=3-4); 2 trials.

922 (B) RT-qPCR determination of mRNA levels of the indicated AMPs in caecal or colonic tissues
923 of *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice at day 7 p.i. $\Delta\Delta$ Ct values were
924 normalized to Hprt expression. Data are mean±SEM (n=3-4); 3 trials.

925 (C) RT-qPCR determination of mRNA levels of lipocalin-2 in caecal or colonic tissues of *C.*
926 *rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice at day 10 p.i. $\Delta\Delta$ Ct values were normalized
927 to Hprt expression. Data are mean±SEM (n=4); 2 trials.

928 (D) ELISA determinations of lipocalin-2 protein in supernatants of fecal homogenates of *C.*
929 *rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice collected at the indicated time points after
930 infection. Data are mean±SEM (n=4); 3 trials.

931 (E) Quantification of plasma FITC-Dextran levels in *C. rodentium*-infected *Gclc^{fl/fl}* and *Cd4Cre-*
932 *Gclc^{fl/fl}* mice that were gavaged with FITC-Dextran at day 10 p.i. FITC-Dextran was measured 4
933 hr later. Data are mean±SEM (n=4-5); 3 trials.

934 (F) Quantification of plasma FITC-Dextran levels in uninfected *Gclc^{fl/fl}* and *Cd4Cre-Gclc^{fl/fl}* mice
935 that were gavaged with FITC-Dextran. FITC-Dextran was measured 4 hr later. Data are
936 mean±SEM (n=3).

937 (G) CFU of *C. rodentium* in liver (left) and spleen (right) of *C. rodentium*-infected *Gclc^{fl/fl}* and
938 *Cd4Cre-Gclc^{fl/fl}* mice at day 10 p.i. Data are mean±SEM (n=6); 3 trials.

939

940 **Supplementary Figure 5: GSS expression is decreased in rectal tissues of IBD patients with**
941 **severe pathology.**

942 (A) Bioinformatics analysis of RNA sequencing data of rectal biopsies from ulcerative colitis
943 (UC) patients (Haberman et al., 2019). The lower and upper quartile values of *GCLC* expression
944 were used to classify the patients into low, intermediate and high expressors.

945 (B) Heat-map representation of selected signature genes of interest in the low and high *GCLC*-
946 expressing UC patients in (A). The provided MAYO scores were used to define the disease
947 severity as normal/mild, moderate and severe disease (Teixeira et al., 2015).

948 (C) Bioinformatics analysis of RNA sequencing data of rectal biopsies from the UC patients in
949 (A,B) examining the correlation between Th17 score and *IL-22* expression. Significance and r^2
950 values are indicated in the lower right corner of each frame. The Th17 score was defined as
951 the mean of scaled log2 gene expression values composing the signature (*IL-17A*, *IL-17F*, *IFN-*
952 γ , *CD3E*, *CD4*, *STAT3*, *STAT5a*, *STAT1*, *STAT4*, *STAT6*, *AHR*, *RORC*, *RORA* and *TBX21*).

953 (D) Violin plots of *GCLC* (left) and *GSS* (right) expression according to UC disease severity
954 (showing normal/mild versus severe scores) from the data in (A). Significance was assessed
955 using the Wilcoxon signed rank test.

956

957 **Supplementary Figure 6: T cell-derived IL-22 is sufficient for mice with GSH-deficient T cells**
958 **to survive and clear *C. rodentium* infection.**

959 (A) Schematic representation of the vector bearing the mouse *IL-22* (or *IL-17*) and *EGFP* genes
960 into the *Rosa26* locus by homologous recombination. Upon Cre recombinase-mediated
961 excision of the stop cassette, IL-22 (or IL-17) and EGFP are expressed under the CAG promoter.
962 Southern blotting probes and fragments (after digestion with EcoRI) are depicted in black, with
963 screening PCR primers depicted in red. SA/LA, short/long homology arm; CAG, CMV early
964 enhancer/chicken β actin promoter; pA, poly A; stop, Westphal stop sequence; NeoR,

965 neomycin resistance; EGFP, enhanced green fluorescent protein; ES cells, embryonic stem
966 cells.

967 (B) Southern blot analyses of 7 positive ES cell clones identified by PCR using the external 5'
968 and 3' probes and the Neo probe indicated in (A). WT bands are 15.6 kb for both probes,
969 targeted bands are 8.2 or 11.5 kb for 5' and 3' probe, respectively, and 8.2 kb for the neo
970 probe.

971 (C) Flow cytometric analysis of EGFP expression by total live cells and by live CD4⁺ and CD8⁺
972 cells isolated from spleens of naïve *IL-22^{ind/+}* and *Cd4Cre IL-22^{ind/+}* mice. Plots are
973 representative of 3 mice per genotype.

974 (D) Flow cytometric analysis of EGFP and IL-22 expression by live CD4⁺ cells isolated from
975 spleens of naïve *IL-22^{ind/+}* and *Cd4Cre IL-22^{ind/+}* mice. Cells were activated overnight by culture
976 with soluble anti-CD3/anti-CD28 (5µg/mL) and restimulated with PMA/calcium
977 ionophore/Brefeldin A for 5 hr before cytokine staining. Plots are representative of 3 mice per
978 genotype.

979 (E) Flow cytometric analysis of EGFP and IL-22 expression by live CD4⁺ cells isolated from
980 spleens of naïve *Gclc^{fl/fl}*, *Cd4Cre Gclc^{fl/fl}*, *Gclc^{fl/fl}IL-22^{ind/+}* and *Cd4Cre Gclc^{fl/fl}IL-22^{ind/+}* mice. Cells
981 were activated by culture overnight with plate-bound anti-CD3 (5µg/mL) and soluble anti-
982 CD28 (1µg/mL) and restimulated for cytokine staining as in (D). Plots are representative of 3
983 mice per genotype.

984 (F) Flow cytometric analysis of EGFP and IL-17A expression by live CD4⁺ cells isolated from
985 spleens of naïve *IL-17A^{ind/+}* and *Cd4Cre IL-17A^{ind/+}* mice. Cells were activated overnight by
986 culture with soluble anti-CD3/anti-CD28 (5µg/mL) and restimulated with PMA/calcium
987 ionophore/Brefeldin A for 5 hr before cytokine staining. Plots are representative of 3 mice per
988 genotype.

989 (G) Flow cytometric analysis of EGFP and IL-17A expression by live CD4⁺ cells isolated from
990 spleens of naïve *Gclc^{fl/fl}*, *Cd4Cre Gclc^{fl/fl}*, *Gclc^{fl/fl}IL-17A^{ind/+}* and *Cd4Cre Gclc^{fl/fl}IL-17A^{ind/+}* mice.
991 Cells were activated by culture overnight with plate-bound anti-CD3 (5µg/mL) and soluble
992 anti-CD28 (1µg/mL) and restimulated for cytokine staining as in (D). Plots are representative
993 of 3 mice per genotype.

994 (H) Change in whole body weight of *Gclc^{fl/fl}* (n=5), *Cd4Cre Gclc^{fl/fl}* (n=6), *Gclc^{fl/fl}IL-22^{ind/+}* (n=5)
995 and *Cd4Cre Gclc^{fl/fl}IL-22^{ind/+}* (n=8) mice infected with *C. rodentium* on day 0 and assayed on the
996 indicated days p.i. Data are mean±SEM (n=5-8).

997 (I) CFU of *C. rodentium* in feces of *Gclc^{fl/fl}*, *Cd4Cre Gclc^{fl/fl}*, *Gclc^{fl/fl}IL-22^{ind/+}* and *Cd4Cre Gclc^{fl/fl}IL-
998 22^{ind/+}* mice. Mice were infected with *C. rodentium* on day 0 and feces were collected for
999 analysis on the indicated days. Data are mean±SEM (n=5-8).

1000 (J) Change in whole body weight of *Gclc^{fl/fl}* (n=6), *Cd4Cre Gclc^{fl/fl}* (n=9), *Gclc^{fl/fl}IL-17A^{ind/+}* (n=6)
1001 and *Cd4Cre Gclc^{fl/fl}IL-17A^{ind/+}* (n=7) mice infected with *C. rodentium* on day 0 and assayed on
1002 the indicated days p.i. Data are mean±SEM (n=6-9).

1003 (K) CFU of *C. rodentium* in feces of $Gclc^{fl/fl}$, $Cd4Cre\ Gclc^{fl/fl}$, $Gclc^{fl/fl}IL-17A^{ind/+}$ and $Cd4Cre$
1004 $Gclc^{fl/fl}IL-17A^{ind/+}$ mice. Mice were infected with *C. rodentium* on day 0 and feces were
1005 collected for analysis on the indicated days. Data are mean±SEM (n=6-9).

1006

1007 **Supplementary Figure 7: *Gclc* regulates mitochondrial function, ROS and IL-17.**

1008 (A-B) Naïve T cells were sorted from spleen and lymph nodes of $Gclc^{fl/fl}$ and $Cd4Cre\ Gclc^{fl/fl}$
1009 mice and induced to differentiate *in vitro* into pathogenic Th17 cells by culture with anti-CD3,
1010 anti-CD28, IL-6, IL-1β and IL-23. (A) IL-22 and (B) IL-17 were quantified by ELISA. Data are
1011 mean±SEM (n=3); 2 trials.

1012 (C) Left: Flow cytometric analysis of DCF-DA staining to detect intracellular ROS in CD3⁺CD4⁺
1013 cells of the colonic LP, which was isolated from *C. rodentium*-infected $Gclc^{fl/fl}$ and $Cd4Cre$
1014 $Gclc^{fl/fl}$ mice at day 7 p.i. Right: Quantification of the data in the left panel. Data are mean±SEM
1015 (n=4).

1016 (D) Quantification of MitoSOX staining to detect mitochondrial ROS in CD3⁺CD4⁺ cells of the
1017 colonic LP, which was isolated from *C. rodentium*-infected $Gclc^{fl/fl}$ and $Cd4Cre\ Gclc^{fl/fl}$ mice at
1018 day 7 p.i. Data are mean±SEM (n=4).

1019 (E) Naïve CD4⁺ T cells were sorted from spleen and lymph nodes of $Gclc^{fl/fl}$ and $Cd4Cre\ Gclc^{fl/fl}$
1020 mice and induced to differentiate *in vitro* into conventional Th17 cells by culture with anti-
1021 CD3, anti-CD28, IL-6, and TGF-β. Cells were treated with 10mM NAC as indicated. Left: Flow
1022 cytometric analysis of DCF-DA to detect ROS in $Gclc^{fl/fl}$ and $Cd4Cre\ Gclc^{fl/fl}$ *in vitro*-
1023 differentiated conventional Th17 cells. Right: Quantification of the data in the left panel. Data
1024 are mean±SEM (n=3); 2 trials.

1025 (F) Determination of MDR/MG ratio to assess mitochondrial activity per mitochondrial mass
1026 in the cells in (A, B). Data are mean±SEM (n=4).

1027 (G) Quantification of qPCR determination of IL-17A mRNA expression of *in vitro*-differentiated
1028 $Gclc^{fl/fl}$ and $Cd4Cre\ Gclc^{fl/fl}$ pathogenic Th17 cells. Data are mean±SD (n=3-4).

1029 (H) IL-17A protein concentrations as measured by ELISA in culture supernatants of *in vitro*-
1030 differentiated $Gclc^{fl/fl}$ and $Cd4Cre\ Gclc^{fl/fl}$ pathogenic Th17 cells. Data are mean±SEM (n=3); 2
1031 trials.

1032 **Materials and Methods**

1033 **Mice**

1034 *Cd4Cre Gclc^{fl/fl}* mice have been previously described (Mak et al., 2017). *Rag1^{-/-}Cd4Cre Gclc^{fl/fl}*
1035 mice were obtained by crossing *Cd4Cre Gclc^{fl/fl}* with *Rag1^{-/-}* mice. *Rag1^{-/-}* and C57BL/6J mice
1036 were purchased from The Jackson Laboratory, *IL-17A^{ind}* mice (Haak et al., 2009) were provided
1037 by Ari Waisman (Mainz, Germany). All mice were bred in the specific pathogen-free (SPF)
1038 facility of the Luxembourg Institute of Health (LIH).

1039 *IL-22^{ind}* mice [C57BL6/J-Gt(ROSA)26Sor<tm1(IL-22-ind)TgFl] were generated by Sabine Spath
1040 in the laboratory of Burkhard Becher at the Institute of Experimental Immunology (University
1041 of Zurich, Switzerland). The *Rosa26* targeting vector containing homology arms for the *Rosa26*
1042 locus was generated by Tobias Heinen in the laboratory of Ari Waisman (Mainz, Germany).
1043 The cDNA containing *IL-22* and *EGFP* was integrated by conventional cloning and is preceded
1044 by a loxP-flanked stop cassette which, when removed by Cre-mediated recombination, allows
1045 expression of IL-22 and EGFP from the upstream CAG promoter. The verified targeting vector
1046 was linearized and purified for injection using phenol/chloroform extraction.

1047 Homologous recombination and targeting of the *Rosa26* locus were performed by the
1048 transgenic facility of the B.S.R.C. "Alexander Fleming" in Greece using conventional targeting
1049 by electroporation in Bruce4 C57BL/6 ES cells. ES cells were screened by PCR for integration
1050 of the construct: primer forward, external *Rosa26* (TAG GTA GGG GAT CGG GAC TCT), primer
1051 reverse, mutant *Rosa26* (GCG AAG AGT TTG TCC TCA ACC). Using the Taq PCR Core Kit
1052 (Qiagen), the following touchdown PCR was performed: 2 cycles each at 60°C, 59°C and 58°C,
1053 then 35 cycles at 57°C. A band at 1300 bp indicated homologous recombination. Correct
1054 integration was further confirmed by Southern blot (c.f. Figure S6B) in which genomic DNA
1055 was digested with EcoRI and separated on a 0.7% agarose gel. The following probes were used:
1056 *Rosa26* external 5' probe 'Orkin' (restriction digest with EcoRI and PaeI, 698 bp), modified from
1057 (Mao et al., 1999) and *Rosa26* external 3' probe 'Soriano' (restriction digest with EcoRI, 700
1058 bp), adapted from (Awatramani et al., 2001, Soriano, 1999). Probes (50 ng) were labeled with
1059 ³²P using the Ladderman DNA labeling kit (TaKaRa) according to the manufacturer's protocol,
1060 and Southern blotting was performed according to standard protocols.

1061 Clone E2 was selected for downstream procedures. Chimeras were generated by injecting
1062 targeted ES cells into C57BL/6 albino mouse blastocysts. Routine genotyping of *IL-22^{ind}* mice

1063 was performed using the following primers at 58°C: Primer *Rosa26* FW (AAA GTC GCT CTG AGT
1064 TGT TAT), Primer *Rosa26* RW (GGA GCG GGA GAA ATG GAT ATG), Primer SpliceAcB (CAT CAA
1065 GGA AAC CCT GGA CTA CTG). The WT band appeared at 600 bp and the recombinant band at
1066 250 bp.

1067 *Cd4Cre IL-22^{ind}* mice were obtained by crossing *IL-22^{ind}* mice with *Cd4Cre* mice purchased
1068 from The Jackson Laboratory. *Cd4Cre Gcl^{fl/fl}IL-22^{ind}* mice were generated at the Luxembourg
1069 Institute of Health (Luxembourg) by crossing *IL-22^{ind}* with *Cd4^{Cre}-Gcl^{fl/fl}* mice.

1070 *Cd4Cre IL-17A^{ind}* mice were obtained by crossing *IL-17A^{ind}* mice with *Cd4Cre* mice purchased
1071 from The Jackson Laboratory. *Cd4Cre Gcl^{fl/fl}IL-17A^{ind}* mice were generated at the Luxembourg
1072 Institute of Health (Luxembourg) by crossing *IL-17A^{ind}* with *Cd4Cre Gcl^{fl/fl}* mice.

1073 All experiments used sex- and age-matched mice (8-12 weeks old) and were carried out using
1074 littermate controls. All animal experimentation protocols were approved and conducted
1075 according to the LIH Animal Welfare Structure guidelines.

1076

1077 ***Citrobacter rodentium* culture and infection**

1078 The nalidixic acid-resistant *Citrobacter rodentium* strain DBS100 was kindly provided by Dana
1079 Philipott (Toronto, Canada). Bacteria were suspended in 2.5% sterilized Miller's Luria Bertani
1080 (LB) Broth (VWR) containing 75µg/mL nalidixic acid (Sigma-Aldrich) and cultured in a shaking
1081 incubator at 37°C with 160rpm rotation. When bacterial density achieved 0.6 OD₆₀₀, mice were
1082 infected with 10⁸ CFU of *C. rodentium* in 100µL by oral gavage. The same bacterial culture was
1083 used to gavage all mice used in the same experiment. Food was withdrawn from mouse cages
1084 16 hr prior to infection.

1085 For *in vivo* administration of NAC (Sigma-Aldrich), experimental mice were supplied with
1086 drinking water containing 40 mM NAC whereas control groups received regular water. NAC
1087 administration commenced 7 days prior to infection, and fresh NAC-containing drinking water
1088 was provided every 3 days over the whole course of the infection.

1089 For *in vivo* administration of IL-22, mice were injected intravenously (i.v.) with
1090 100µg/100µL/mouse of either IL-22-Fc fusion protein (Genentech) or an isotype-matched
1091 IgG2A control-Fc protein (Ragweed:9652 10D9.W.STABLE mIgG2a; Genentech). Injections
1092 commenced one day before *C. rodentium* infection (day -1) and were repeated on days 3, 6,
1093 9, 12 and 15 p.i.

1094

1095 ***Citrobacter rodentium* quantification in mouse feces, organs and blood**

1096 To determine CFUs of *C. rodentium* in feces and organs, fresh samples of feces and organs
1097 were harvested from infected mice at various time points (as indicated in the Figure). Samples
1098 were weighed and homogenized in 1mL cold PBS by continuous vortexing (feces) or with the
1099 help of a syringe plunger (organs). To measure CFUs of *C. rodentium* in blood, blood was
1100 withdrawn by cardiac puncture at the desired time points and collected in BD Microtainer®
1101 tubes (BECTON DICKINSON; 365986). Blood samples and homogenates of feces and organs
1102 were plated in serial dilutions (up to 10⁹) on LB agar composed of 2.5% sterilized LB Broth
1103 (Miller) (VWR) plus 1.5% of agar (Bioscience) containing 75µg/mL nalidixic acid (Sigma-
1104 Aldrich). Plates were incubated overnight at 37°C in a bacterial incubator. Numbers of CFUs in
1105 feces, organs and blood were determined by blinded counting of bacterial colonies. Results
1106 were normalized to the dilution and weight of the organ or fecal pellets or the volume of blood
1107 used.

1108

1109 **Fluorescein isothiocyanate (FITC)-Dextran gut permeability assay**

1110 To assess gut permeability, mice were orally gavaged with 150 µL of an 80 mg/mL solution
1111 of 3-5 kDa-FITC-Dextran (Sigma-Aldrich) in PBS. Mice were sacrificed at 4 hr post-gavage, and
1112 blood was collected in BD Microtainer® tubes (BECTON DICKINSON; 365986) and centrifuged
1113 at 5000 rpm for 10 min at 4°C. Plasma was diluted 5-fold and FITC-Dextran was quantified
1114 using a Mithras LB 940 (Berthold Technologies) fluorometer at an excitation wavelength of
1115 485 nm and an emission wavelength of 535. Food was withdrawn from mouse cages 16 hr
1116 prior to FITC-Dextran administration.

1117

1118 **Colonic tissue histology and histochemistry**

1119 For histological analysis of mouse colons, distal colons containing fecal content were isolated
1120 at day 12 p.i. and fixed for 3 hr at room temperature (RT) in freshly prepared Carnoy's fixative
1121 [60% anhydrous methanol (Sigma-Aldrich), 30% chloroform (Sigma-Aldrich), 10% glacial acetic
1122 acid (Sigma-Aldrich)]. Fixed colons were transferred to fresh Carnoy's fixative and fixed again
1123 overnight. Fixed samples were washed in anhydrous methanol for 2 hr followed by transfer to
1124 fresh methanol and storage at 4°C until further use. Using a Tissue-Tek VIP processor
1125 (SAKURA), samples were subjected to consecutive washes with 100% denatured ethanol

1126 (VWR), treatment with the clearing agent toluene (VWR), and paraffin embedment (Leica).
1127 Sections (3 μ m) were cut using a Rotary Microtome Microm HM 340E (Thermo Fisher
1128 Scientific). Paraffin-embedded sections were either stained with hematoxylin (Meditate) plus
1129 eosin (VWR) (H&E), or Alcian Blue (Dako). Alcian Blue staining was performed using an Artisan
1130 Link Pro Special Staining System (Dako). Slides were scanned with an automated digital slide
1131 creation and viewing system (Philips IntelliSite Pathology Solution; 760001).

1132

1133 **Immunofluorescence staining and microscopy**

1134 To visualize tight junction localization and epithelial mucus content, distal colons were
1135 isolated on day 12 p.i. and feces were flushed out with cold PBS. Colons were cut open
1136 longitudinally and embedded flat, with the lumen facing downwards, in Tissue-Tek O.C.T.
1137 compound (SAKURA; 4583) in cryomolds and snap-frozen in liquid nitrogen. Cryosections
1138 (5 μ m) were prepared using a CM1850 UV Cryostat (Leica Biosystems) at -18°C and mounted
1139 on glass slides. For immunofluorescent analysis of colonic crypts, sections on glass slides were
1140 prepared as previously described (Lee et al., 2015). Briefly, samples were dried for 2 hr and
1141 fixed in 100% absolute ethanol (VWR) for 30 min at 4°C. Fixed samples were transferred to
1142 100% acetone (cooled at -20°C) (Sigma-Aldrich) and incubated for 3 min at RT, followed by
1143 blocking with PBS containing 10% FBS (Biochrom GmbH) for 30 min at RT and washing twice
1144 in PBS. Primary antibodies recognizing either claudin-2 (Abcam) or MUC2 (Abcam) were
1145 diluted 1:200 in PBS+10% FBS and incubated with slides overnight at 4°C. After two washes in
1146 PBS, samples were stained overnight at 4°C with secondary goat anti-rabbit IgG-Alexa Fluor
1147 594 antibody (Abcam) and Alexa Fluor 488 Phalloidin (Thermo Fisher Scientific). Washing was
1148 repeated and nuclei were stained with DAPI Fluoromount-G® medium (SouthernBiotech;
1149 0100-20) for 24 hr at RT. Stained sections were visualized using a ZEISS Axio Observer
1150 microscope, and images were analyzed with ZEISS ZEN Blue 3.0 software. Colonic lumen
1151 diameters (for claudin-2 staining) were calculated using Fiji imageJ.

1152

1153 **Preparation of lamina propria of colon and caecum**

1154 Lamina propria of colon or caecum was isolated using the Lamina Propria Dissociation Kit
1155 (Miltenyi) following the manufacturer's protocol. Briefly, colon or caecum tissues were
1156 resected and cut open longitudinally, and residual fat tissue was removed. Tissues were
1157 washed in HBSS without Ca²⁺, Mg²⁺ (Westburg) containing 10mM HEPES (Sigma-Aldrich).

1158 Washed tissues were cut into small pieces and predigested in HBSS without Ca^{2+} , Mg^{2+}
1159 containing 10mM HEPES, 5mM EDTA (Sigma-Aldrich), 5% FBS (Biochrom GmbH), 1mM
1160 dithiothreitol (DTT) (Sigma-Aldrich) for 20 min at 37°C using a rotator mixer (Intelli-Mixer,
1161 ELMI). Tissues were recovered in a 100µm strainer and re-incubated in fresh predigestion
1162 solution. After collection in a 100µm strainer, tissues were washed in HBSS without Ca^{2+} , Mg^{2+}
1163 containing 10mM HEPES for 20 min at 37°C under continuous rotation. Again, tissues were
1164 recovered in a 100µm strainer and transferred into a C Tube (Miltenyi Biotec; 130-096-334)
1165 containing HBSS with Ca^{2+} , Mg^{2+} (Westburg) and an enzyme mix (enzyme D, enzyme R, enzyme
1166 A) prepared from the Lamina Propria Dissociation Kit (Miltenyi) components. C Tubes were
1167 transferred into the GentleMACS Octo Dissociator with Heaters (Miltenyi Biotec; # 130-096-
1168 427) and the “37C_m_LPDK_1” program was run. Cold PBS containing 0.5% BSA (Sigma-
1169 Aldrich) was added to stop the reaction. Samples were passed through 40µm strainers,
1170 pelleted at 300xg for 10 min at 4°C, and resuspended in medium consisting of RPMI 1640
1171 (Westburg) supplemented with 10% FBS (Biochrom GmbH), 1% penicillin/streptomycin
1172 (Gibco), 1% L-glutamine (Westburg), and 55µM 2-mercaptoethanol (Gibco). Lamina propria
1173 lymphocytes were analyzed by flow cytometry (see below).

1174

1175 **Naïve T cell isolation and *in vitro*-differentiation**

1176 Naïve CD4^{+} T cells were isolated from mouse spleen and lymph nodes by magnetic bead
1177 sorting using the Naïve CD4^{+} T cell isolation kit (Miltenyi Biotec) following the manufacturer’s
1178 protocol. Negative magnetic bead sorting was performed using the autoMACS® pro Separator
1179 (Miltenyi Biotec). Cell numbers were determined using a CASY cell counter (Omni Life Science).
1180 To induce *in vitro* differentiation, naïve T cells were cultured at 2×10^6 cells/mL for 3 days in
1181 medium consisting of IMDM (Westburg) supplemented with 10% FBS (Biochrom GmbH), 1%
1182 penicillin/streptomycin (Gibco) and 55µM 2-mercaptoethanol (Gibco), and in the presence of
1183 Th cell subtype-specific cytokine mixes (see below).

1184 For the induction of conventional Th17 cells, naïve T cells were cultured in the presence of
1185 $\text{TGF-}\beta$ (2ng/µL; Bio-Techne), IL-6 (30ng/mL; Miltenyi Biotec), anti-IFN- γ (5µg/mL; BD
1186 Biosciences), soluble anti-CD28 (1µg/mL; Biolegend) and plate-bound anti-CD3 antibody (5
1187 µg/mL; Biolegend). For the induction of IL-22-expressing Th17 cells, naïve T cells were cultured
1188 in the presence of IL-6 (30ng/mL; Miltenyi Biotec), soluble anti-CD28 (1µg/mL; Biolegend),

1189 plate-bound anti-CD3 antibody (5µg/mL; Biolegend or BD), IL-1β (50ng/mL; Miltenyi Biotec)
1190 and IL-23 (20ng/mL; Miltenyi Biotec).

1191 For NAC experiments, the 3-day incubation period of *in vitro* T cell differentiation was
1192 conducted in culture medium containing 10mM of the antioxidant N-acetyl-cysteine (Sigma-
1193 Aldrich). For PI3K inhibitor treatment, 2 or 20 µM LY294002 or DMSO for control was added
1194 to culture medium for last 24h of the differentiation.

1195

1196 **DNA and RNA extractions**

1197 DNA from frozen fecal samples was isolated using the NucleoSpin DNA Stool kit (Macherey-
1198 Nagel) following manufacturer's protocol.

1199 For isolation of RNA from colon and caecum, tissues were collected in 1mL phenol-based
1200 TRIZOL (Thermo Fisher Scientific) and homogenized in a TissueLyser II (Qiagen) using Stainless
1201 Steel Beads (Qiagen; 69989). Samples were incubated at RT for 5 min, followed by addition of
1202 0.2 mL chloroform (Sigma-Aldrich) per sample. Samples were shaken for 15 seconds,
1203 incubated for 3 min at RT, and centrifuged at 12,000xg for 15 min at 4°C. The aqueous phase
1204 containing the RNA was collected and 0.5 mL isopropanol (VWR) was added per sample.
1205 Samples were transferred onto NucleoSpin RNA columns (Macherey-Nagel) to purify RNA
1206 using the NucleoSpin RNA Kit protocol. Briefly, after binding to columns, RNA was treated with
1207 DNase, washed and eluted in RNase-free water.

1208 For isolation of RNA from *in vitro* cultured Th17 cells, NucleoSpin RNA XS kit (Macherey-
1209 Nagel) was used according to protocol.

1210 DNA and RNA concentrations were quantified using a NanoDrop 2000c Spectrophotometer
1211 (Thermo Fisher Scientific).

1212

1213 **Real-time reverse transcription polymerase chain reaction (RT-qPCR)**

1214 For qPCR of stool DNA samples, 6µL DNA (50 ng) was mixed with 10µL SYBR™ Fast SYBR™
1215 Green Master Mix (FISHER SCIENTIFIC), 2pmol of forward primer and 2pmol of reverse primer
1216 (please see list of primers in Reagents). Reactions were run on a ABI 7500HT Fast qRT-PCR
1217 instrument.

1218 For RT-qPCR of RNA samples, 2µL RNA (150 ng) was mixed with 5µL Master Mix (Luna
1219 Universal One-Step RT-qPCR Kits; Bioké), 0.3µL reverse transcriptase, 2.7µL H₂O, 2pmol of

1220 forward primer and 2pmol of reverse primer (cf. Reagents). Reactions were run on a CFX384
1221 instrument (Bio-Rad).

1222 Transcript data were normalized to total Eubacteria (for bacterial DNA in feces), rps17 (for
1223 bacterial virulence factors), and hprt (for antimicrobial peptides), and analyzed using the $\Delta\Delta Ct$
1224 method as previously described (Mak et al., 2017).

1225

1226 **Flow cytometry**

1227 Flow cytometric staining and analyses were performed as previously described (Cossarizza
1228 et al., 2019). T cells were identified as either CD3⁺CD4⁺ or CD4⁺ alone (see Figure Legends). LTi
1229 cells were identified as Lineage⁻ [i.e. (Ter119, CD19, CD11b, CD5, Ly6G/Ly6C)⁻] CD3⁻Nkp46⁻
1230 CD4⁺.

1231 To stain surface molecules, cells were incubated in FACS buffer (PBS containing 1% FBS and
1232 5mM EDTA, pH 8.0) in the presence of antibodies (cf. Reagents; section Antibodies).
1233 Antibodies were diluted 1:200 and incubated for at least 30 min at 4°C, protected from light.
1234 Stained cells were washed in FACS buffer prior to flow cytometric analysis.

1235 For intracellular staining to detect p-mTOR, p-PI3K and p-AKT(T308), cells were either fixed
1236 and permeabilized using BD Cytotfix/Cytoperm Fixation/Permeabilization kit (BD) or fixed in
1237 2% formaldehyde (Sigma-Aldrich) for 10 min at RT, permeabilized in 0.01% saponin (Sigma-
1238 Aldrich), and incubated for 30 min with antibody (diluted 1:200 in saponin) at 4°C in the dark.
1239 Stained cells were washed and resuspended in saponin. For intracellular staining of p-4E-BP1
1240 (Thr37/46), cells were fixed with 4% formaldehyde (Sigma-Aldrich) for 15 min at RT,
1241 resuspended in 10 ul of PBS and permeabilized with ice-cold MetOH (Sigma-Aldrich) for 20
1242 min on ice, protected from light, washed 3x in PBS and stained in FACS buffer with antibodies
1243 for 1h at 4°C and washed in FACS buffer before flow cytometry analysis. For intracellular
1244 staining of transcription factors (including ROR γ T, Tbet), cells were fixed for 40 min at 4°C
1245 using the eBioscience™ Foxp3/Transcription Factor Fixation kit (Thermo Fisher Scientific) and
1246 permeabilized using the kit's permeabilization buffer. Cells were incubated for 30 min with
1247 antibodies (diluted 1:200 in permeabilization buffer) at 4°C in the dark. Stained cells were
1248 washed in permeabilization buffer before flow cytometry analysis.

1249 For intracellular staining of cytokines, cells were stimulated *in vitro* for 5 hr with phorbol 12-
1250 myristate 13-acetate (PMA; Sigma-Aldrich, 50ng/mL), calcium ionophore A23187 (Sigma-
1251 Aldrich, 750ng/mL), and BD GolgiPlug™ Protein Transport Inhibitor (BECTON DICKINSON,

1252 1:1000 dilution). Stimulated cells were washed once in FACS buffer before fixation for 20 min
1253 at 4°C using the BD Cytotfix/Cytoperm solution and permeabilization using BD Perm/Wash™
1254 buffer (BD Biosciences). Permeabilized cells were incubated for 30 min with antibodies
1255 (diluted 1:200 in BD Perm/Wash) at 4°C in the dark. Stained cells were washed in
1256 permeabilization buffer before flow cytometry analysis.

1257 To stain intracellular thiols, cells were processed as described previously (Franchina et al.,
1258 2022). Briefly, cells were stained at 37°C for 30 min in either complete RPMI 1640 (10% FBS,
1259 1% penicillin/streptomycin, 1% L-glutamine, 55µM 2-mercaptoethanol) for lamina propria
1260 cells, or in complete IMDM (10% FBS, 1% penicillin/streptomycin, 55µM 2-mercaptoethanol)
1261 for *in vitro*-differentiated Th17 cells. Monobromobimane (mBBr) (Thermo Fisher Scientific)
1262 was added 10min before washing off the supernatant. Stained cells were washed twice and
1263 resuspended in cold PBS for flow cytometric acquisition.

1264 For determination of intracellular or mitochondrial ROS levels, cells were stained at 37°C for
1265 30 min with 2 µM 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF-DA; Thermo Fisher
1266 Scientific) or 1 µM MitoSOX (Thermo Fisher Scientific), respectively. Cells were washed and
1267 resuspended as above. To quantify mitochondrial membrane potential or mass, cells were
1268 stained at 37°C for 30 min with 100nM MitoTracker Deep Red or 10nM MitoTracker Green
1269 (Thermo Fisher Scientific), respectively. Cells were washed and resuspended as above.

1270 Dead cells were excluded from flow cytometric analyses by staining with either DAPI
1271 (Thermo Fisher Scientific), LIVE/DEAD® Fixable Near-IR dye (Biolegend), LIVE/DEAD® Fixable
1272 Green dye (Biolegend) or 7-AAD (Thermo Fisher Scientific). Flow cytometry was performed
1273 using a BD Fortessa instrument (BD Biosciences), and results were analyzed using FlowJo
1274 v10.6.2 software (Tree Star).

1275

1276 **ELISA measurement of cytokines and lipocalin-2**

1277 IL-17 or IL-22 levels in culture supernatants were quantified using the Mouse IL-17 DuoSet
1278 ELISA kit (Bio-Techne) or the Mouse IL-22 DuoSet ELISA kit (Bio-Techne), respectively. The
1279 Mouse Lipocalin-2/NGAL DuoSet ELISA kit (Bio-Techne) was used to determine lipocalin-2
1280 concentrations in fecal supernatants obtained by homogenization of fecal pellets in PBS. All
1281 assays were performed following the manufacturer's instructions.

1282 Briefly, plates were coated with target-specific antibody overnight, washed in wash buffer,
1283 and blocked by incubating with reagent diluent for at least 1 hr. After washing, samples and

1284 the appropriate standards were added to plates and incubated for 2 hr. After washing, the
1285 corresponding biotinylated detection antibody was added for 2 hr. After washing, streptavidin
1286 coupled to horseradish peroxidase (HPR) was added for 20-30 min. Plates were washed and a
1287 substrate solution containing hydrogen peroxide was added. The HPR reaction was stopped
1288 by adding sulfuric acid. Optical density was determined using a Versa Max microplate reader
1289 (Molecular Devices) with SoftMax Pro7.1 software set to 450nm. Wavelengths were corrected
1290 by subtraction of background measurements at 570nm. Protein concentrations were
1291 determined and normalized to total cell numbers per culture (for IL-17 and IL-22) or fecal
1292 weight (for lipocalin-2).

1293

1294 **Luminescence assays**

1295 For quantification of GSH content in LP CD4⁺ cells, viable CD4⁺ cells were FACS-sorted using
1296 a FACSAria III (BD Biosciences) instrument according to published cell sorting guidelines
1297 (Cossarizza et al., 2019). Cells (1.5x10⁴/well) were subjected to a GSH/GSSG luminescence-
1298 based assay (Promega) following the manufacturer's protocol. For quantification of ATP levels
1299 in *in vitro*-differentiated Th17 cells, cells (1x10⁵) were subjected to the CellTiter-Glo[®] assay
1300 (Promega) following the manufacturer's protocol. Luminescence intensities were quantified
1301 using a Mithras LB 940 instrument (Berthold Technologies).

1302

1303 **Measurement of oxygen consumption rate (OCR) by Seahorse analysis**

1304 Seahorse analyses were performed using an XFe96 Extracellular Flux Analyzer (Agilent).
1305 Briefly, *in vitro*-differentiated Th17 cells were seeded in XF Seahorse DMEM medium (Agilent
1306 Technologies) supplemented with 1mM sodium pyruvate (Gibco), 2mM glutamine
1307 (Westburg), and 25mM glucose (Sigma-Aldrich) at a density of 2x10⁵ cells/well on a Seahorse
1308 XFe96 cell culture plate (Agilent Technologies; 101085-004). Plates were pre-coated with
1309 Corning™ Cell-Tak Cell and Tissue Adhesive (Thermo Fisher Scientific) containing 0.1M of
1310 sodium bicarbonate (Sigma-Aldrich). OCR was measured using the XF Cell Mito Stress Test
1311 (Agilent) according to manufacturer's protocol. Sequential injections of 1μM oligomycin A
1312 (Sigma-Aldrich), 3μM carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (Sigma-
1313 Aldrich), and 1μM antimycin A/rotenone (Sigma-Aldrich) were performed, with three
1314 measurements taken after each treatment.

1315 Basal OCR was calculated from raw OCR values measured just before oligomycin injection.
1316 Maximal OCR was calculated from raw OCR values obtained from the second measurement
1317 after FCCP injection.

1318

1319 **Immunoblot analysis**

1320 For detection of claudin-2, claudin-3 and claudin-15 proteins, distal colons were cut open
1321 and washed in cold PBS. The tissue was cut into very small pieces using a clean razor blade
1322 and transferred into 0.3 mL RIPA buffer containing 50 mM Tris-HCl pH7-8 (Sigma-Aldrich), 150
1323 mM NaCl (Sigma-Aldrich), 0.5% sodium deoxycholate (Sigma-Aldrich), 1% NP-40 (Abcam),
1324 0.1% SDS (Carl Roth), 2mM EDTA (Sigma-Aldrich) and Protease/Phosphatase Inhibitor Cocktail
1325 (100x) (Bioké). Samples were incubated for 30 min on ice, then sonicated for 20 min, followed
1326 by another 30 min incubation on ice. Samples were centrifuged at 16,000xg at 4°C for 20 min
1327 and the clear supernatant was collected. This procedure was repeated. Lysates were diluted
1328 1:3 in sample buffer containing 187.5 mM Tris-HCl pH 6.8, 6% SDS, 30% glycerol (Carl Roth),
1329 0.03% bromophenol blue (Sigma-Aldrich) and 10% 2-mercaptoethanol. Samples were
1330 incubated for 5 min at 95°C, loaded on a Novex™ WedgeWell™ 16% Tris-Glycine gradient gel
1331 (Thermo Fisher Scientific; XP00162), and run at 100V for 90-100 min. Proteins were
1332 transferred onto a nitrocellulose membrane (iBlot2 Transfer Stacks, Nitrocellulose Mini; Fisher
1333 Scientific, 15239296) using an iBlot2 machine (Thermo Fisher Scientific) and blocked with 5%
1334 milk (Carl Roth) for 1hr. After washing with PBS-Tween (PBS-T), primary antibodies recognizing
1335 claudin-2 (1:1000; Abcam), claudin-3 (1:200; Thermo Fisher Scientific), claudin-15 (1:200;
1336 Thermo Fisher Scientific), or actin (1:5000; Sigma-Aldrich), which were diluted in PBS-T
1337 containing 5% BSA, were added to blots and incubated overnight at 4°C. Membranes were
1338 washed and secondary mouse anti-rabbit IgG-HRP (Santa Cruz Biotechnology), which was
1339 diluted 1:5000 in PBS-T with 5% milk, was added for 1 hr at RT. Proteins were visualized using
1340 Luminata™ Crescendo Western HRP substrate (Thermo Fisher Scientific) and an INTAS ECL
1341 Chemocam Imager.

1342

1343 **Bulk RNA sequencing & data analysis**

1344 RNA extraction of *in vitro* cultured Th17 cells was performed with the NucleoSpin RNA XS Kit
1345 (Macherey-Nagel) according to the manufacturer's protocol. RNA concentrations and integrity
1346 were measured using a RNA 6000 NanoKit (Agilent) on a 2100 Bioanalyzer (Agilent).

1347 Sequencing was performed by the Sequencing Platform of the Luxembourg Center for Systems
1348 Biomedicine (LCSB) of the University of Luxembourg. Samples were prepared using an
1349 Illumina Stranded mRNA library prep kit (Illumina) with the addition of IDT for Illumina
1350 DNA/RNA UD indexes (Illumina). Paired-end sequencing was executed using an Illumina
1351 NextSeq2000 machine with a read length of 2 x 50 bp.

1352 RNA-seq transcript alignment was performed with Salmon (Patro et al., 2017) against the
1353 Mouse Transcriptome from Genecode release M30 assembly GRCm39 (Frankish et al., 2018).
1354 Subsequent analysis was conducted in R, and Tximeta (Love et al., 2020) was used to assign
1355 transcripts to genes before differential analysis with DESeq2 (Love et al., 2014). Gene set
1356 enrichment analysis (GSEA) was performed using ClusterProfiler (Yu et al., 2012).

1357

1358 **Bioinformatics analysis of RNA sequencing dataset GSE109142**

1359 The publicly available dataset GSE109142, which contains RNA sequencing TPM counts of
1360 rectal biopsies from pediatric ulcerative colitis (UC) patients (Haberman et al., 2019), was
1361 downloaded from the Gene Expression Omnibus (GEO) repository and processed using R
1362 version 4.1.0. Before applying the log₂ transformation, for each gene of interest, half of the
1363 smallest non-zero value was added to the TPM counts. The lower and upper quartile values of
1364 *GCLC* expression were used to classify the patients according to low, intermediate and high
1365 expression of *GCLC*. The heat-map representation was generated using the ComplexHeatmap
1366 package (Gu et al., 2016) in R. The provided IBD MAYO scores (ranging from 0 to 12) were used
1367 to define disease severity as normal/mild (score < 6), moderate (score < 11) and severe (score
1368 ≥ 11) (Teixeira et al., 2015). The Th17 score was defined as the mean of scaled log₂ gene
1369 expression values composing the signature (*IL-17A*, *IL-17F*, *IFN-γ*, *CD3E*, *CD4*, *STAT3*, *STAT5α*,
1370 *STAT1*, *STAT4*, *STAT6*, *AHR*, *RORC*, *RORA* and *TBX21*). To analyze the correlation between gene
1371 expression values or scores, the Pearson's correlation coefficient (*r*) were calculated and
1372 significances (p-value) as well as *r*² values shown.

1373

1374 **Quantification and statistical analysis**

1375 Data are expressed as the mean ± SEM with at least n=3 per group (refer to Figure Legends
1376 for detailed information). P values were determined by unpaired Student's t test, one-way
1377 ANOVA or two-way ANOVA using Prism 8.0 (GraphPad). P values of ≤0.05 were considered

1378 statistically significant and are indicated with one or more asterisks (* $p \leq 0.05$; ** $p \leq 0.01$;
1379 *** $p \leq 0.001$; **** $p \leq 0.0001$; ns: not significant).

1380 Reagents

Reagent	Source
Antibodies	
CD4-APC Clone GK1.5 (1:200)	Biolegend
CD3ε-PE-Cy7 Clone 145-2C11 (1:200)	Biolegend
CD4-PE Clone GK1.5 (1:200)	Biolegend
CD4-BV785 Clone GK1.5 (1:200)	Biolegend
CD4-BUV737 Clone GK1.5 (RUO) (1:200)	BD Biosciences
IL-17A-BV605 Clone TC11-18H10 (1 :200)	BD Biosciences
IL-22-PE Clone Poly5164 (1:200)	Biolegend
IFN-γ-APC Clone XMG1.2 (1:200)	Biolegend
CD4-BUV805 Clone L3T4 (1:200)	BD Biosciences
RORγT-BV421 Clone Q31-378 (1:200)	BD Biosciences
Nkp46 (CD335)-BV785 Clone 29A1.4 (1:200)	Biolegend
TER119-APC-eFluor 780 Clone TER-119 (1:200)	Thermo Fisher Scientific
CD19-APC-eFluor 780 Clone eBio1D3 (1:200)	Thermo Fisher Scientific
CD11b-APC-eFluor 780 Clone M1/70 (1:200)	Thermo Fisher Scientific
CD5-APC-eFluor 780 Clone 53-7.3 (1:200)	Thermo Fisher Scientific
Ly6G/Ly6C-APC-eFluor 780 Clone RB6-8C5 (1:200)	Thermo Fisher Scientific
AHR-PE Clone 4MEJJ (1:200)	Thermo Fisher Scientific
HIF-1 alpha-PE Clone Mgc3 (1:200)	Thermo Fisher Scientific
p-STAT3-Alexa®Fluor 647 Clone 13A3-1 (1:200)	Biolegend
p-mTOR-Pacific Blue Clone MRRBY (1:200)	Biolegend
p-PI3K p85/p55 (Tyr458, Tyr199)-PE Clone PI3KY458-1A11	Thermo Fisher Scientific
p-AKT(T308)-Alexa Fluor® 647 Clone D25E6	CST
p-4E-BP1 (Thr37/46)-Alexa Fluor® 647 Clone 236B4	CST
CD3ε-PerCP/Cy5.5 Clone 145-2C11 (1:200)	Biolegend
Anti-Claudin 2 antibody (1:200 or 1:1000)	Abcam
Recombinant Anti-MUC2 antibody [EPR23479-47]	Abcam
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594)	Abcam
Alexa Fluor™ 488 Phalloidin	Thermo Fisher Scientific
Ultra-LEAF™ Purified anti-mouse CD3ε Clone 145-2C11	Biolegend
BD Pharmingen™ Purified NA/LE Hamster Anti- Mouse CD3e Clone 145-2C11	BD
Ultra-LEAF™ Purified anti-mouse CD28 Clone 37.51	Biolegend
Purified NA/LE Rat Anti-Mouse IFN-γ Clone XMG1.2	BD Biosciences
Claudin 3 Polyclonal Antibody (1:200)	Thermo Fisher Scientific
Claudin 15 Polyclonal Antibody (1:200)	Thermo Fisher Scientific

Anti-Actin antibody produced in rabbit	Sigma-Aldrich
Mouse anti-rabbit IgG-HRP	Santa Cruz
Nalidixic acid	Biotechnology
LB broth (Miller)	Sigma-Aldrich
Agar-Agar	VWR
N-Acetyl-L-cysteine (NAC)	Bioscience
LY290004 (PI3Ki)	Sigma-Aldrich
IL-22-Fc fusion protein	Sigma-Aldrich
Isotype control-Fc	Genentech
(Ragweed:9652 10D9.W.STABLE mIgG2a)	Genentech
PBS (1X) without Ca ⁺⁺ , Mg ⁺⁺ , 500ml	Westburg
FBS Superior Lot: 0193F	Biochrom GmbH
Penicillin-Streptomycin (10,000 U/mL)	Gibco
L-Glutamine	Westburg
2-Mercaptoethanol	Gibco
Fluorescein isothiocyanate–dextran	Sigma-Aldrich
Methanol	Sigma-Aldrich
Chloroform	Sigma-Aldrich
2-Propanol ≥98%	VWR
Acetic acid	Sigma-Aldrich
Ethanol absolute	VWR
Ethanol eurodenatured	VWR
Toluene	VWR
Hematoxylin	Medite
Eosin	VWR
Alcian Blue	Dako
Paraffin	Leica
Acetone	Sigma-Aldrich
DAPI Fluoromount-G®	IMTEC DIAGNOSTICS
Hanks' BSS, with Phenol Red without Ca, Mg	Westburg
Hanks' BSS (1X) with phenol red, Ca ⁺⁺ and Mg ⁺⁺	Westburg
HEPES	Sigma-Aldrich
EDTA	Sigma-Aldrich
DL-Dithiothreitol solution (DTT)	Sigma-Aldrich
Bovine Serum Albumin (BSA)	Sigma-Aldrich
RPMI 1640 (without L-Glutamine)	Westburg
IMDM with HEPES and L-Glutamine	Westburg
Recombinant human TGF-β	Bio-Techne
Mouse IL-6, research grade	Miltenyi Biotec

Mouse IL-1 β , research grade	Miltenyi Biotec
Mouse IL-23, research grade	Miltenyi Biotec
Ambion™ TRIzol™ Reagent	Thermo Fisher Scientific
SYBR™ Fast Green Master Mix	Thermo Fisher Scientific
Luna Universal One-Step RT-qPCR Kit	Bioké
Formaldehyde	Sigma-Aldrich
Saponin	Sigma-Aldrich
Monobromobimane (mBBR)	Thermo Fisher Scientific
H ₂ DCFDA	Thermo Fisher Scientific
MitoSOX™ Red Mitochondrial Superoxide Indicator	Thermo Fisher Scientific
MitoTracker™ Deep Red FM	Thermo Fisher Scientific
MitoTracker Green FM	Thermo Fisher Scientific
DAPI (1:2000)	Thermo Fisher Scientific
Zombie NIR™ Fixable Viability Kit	Biolegend
Zombie Green™ Fixable Viability Kit	Biolegend
7-AAD	Thermo Fisher Scientific
Corning™ Cell-Tak Cell and Tissue Adhesive	Thermo Fisher Scientific
Oligomycin A	Sigma-Aldrich
FCCP	Sigma-Aldrich
Antimycin A	Sigma-Aldrich
Rotenone	Sigma-Aldrich
Sodium pyruvate	Gibco
Phorbol 12-myristate 13-acetate (PMA)	Sigma-Aldrich
Calcium Ionophore A23187	Sigma-Aldrich
D-(+)-Glucose solution	Sigma-Aldrich
GolgiPlug™ (Protein Transport Inhibitor)	BD Biosciences
Tween 20	Sigma-Aldrich
TRIS HCl	Sigma-Aldrich
NaCl	Sigma-Aldrich
Sodium deoxycholate	Sigma-Aldrich
Sodium bicarbonate solution	Sigma-Aldrich
NP-40	Abcam
20% SDS	Carl Roth
Protease/Phosphatase Inhibitor Cocktail (100x)	Bioké
Glycerol	Carl Roth
Bromophenol Blue sodium salt	Sigma-Aldrich
Powdered milk	Carl Roth
Luminata™ Crescendo Western HRP substrate	Thermo Fisher Scientific
Seahorse XF DMEM medium	Agilent Technologies
Naive CD4+ T Cell Isolation Kit, mouse	Miltenyi Biotec

Lamina Propria Dissociation Kit, mouse	Miltenyi Biotec
NucleoSpin RNA	Macherey-Nagel
NucleoSpin RNA XS	Macherey-Nagel
RNeasy Micro Kit (50)	Qiagen
Foxp3/Transcription Factor Staining Buffer Set	Thermo Fisher Scientific
BD Cytofix/Cytoperm Fixation/Permeabilization kit	BD Biosciences
Mouse IL-17 DuoSet ELISA	BIO-TECHNE
Mouse IL-22 DuoSet ELISA	BIO-TECHNE
Mouse Lipocalin-2/NGAL DuoSet ELISA	BIO-TECHNE
GSH/GSSG-Glo™ Assay	Promega
CellTiter-Glo®	Promega
Seahorse XFe96 Fluxpak	Agilent Technologies

Experimental Models: Organisms/Strains

<i>Gclc</i> ^{fl/fl} : B6	(Mak et al., 2017)
Rag1 ^{-/-} : B6	The Jackson laboratory
Rag1 <i>Gclc</i> ^{fl/fl} : B6	This paper
C57BL/6J	The Jackson laboratory
<i>Citrobacter rodentium</i> (DBS100)	(Geddes et al., 2011)

Oligonucleotides (Forward (F) and Reverse (R))

Eubacteria	
F : ACTCCTACGGGAGGCAGCAGT	(Gareau et al., 2011)
R : ATTACCGCGGCTGCTGGC	
Enterobacteriaceae	
F : GTGCCAGCMGCCGCGGTAA	(Gareau et al., 2011)
R : GCCTCAAGGGCACAACCTCCAAG	
Firmicutes	
F : GCTGCTAATACCGCATGATATGTC	(Gareau et al., 2011)
R : CAGACGCGAGTCCATCTCAGA	
Bacillus	
F : GCGGCGTGCCTAATACATGC	(Gareau et al., 2011)
R : CTTCACTCACGCGGCGT	
Bacteroides	
F : GAGAGGAAGGTCCCCAC	(Gareau et al., 2011)
R : CGCTACTTGGCTGGTTTCAG	
Lactobacillus/Lactococcus	
F : AGCAGTAGGGAATCTTCCA	(Gareau et al., 2011)
R : CACCGCTACACATGGAG	
SFB	
F : GACGCTGAGGCATGAGAGCAT	(Gareau et al., 2011)
R : GACGGCACGGATTGTTATTCA	

Rps17

F : CGCCATTATCCCCAGCAAG (Sanchez et al., 2018)

R : TGTCGGGATCCACCTCAATG

EspA

F : AGTGATCTTGCGGCTGAGTT (Sanchez et al., 2018)

R : ATCCACCGTCGTTGTCAAAT

EspG

F : CAATCGCCACATGCCATAC (Xia et al., 2019)

R : CTTTGAATTGCCGAGTCCC

EspF

F : GCTTAATGGAATTGGTCAGGCC (Xia et al., 2019)

R : GCGAGAGGGAGTTAATGACG

Espl

F : AGATGAAGGCCTGCTCTCAG (Sanchez et al., 2018)

R : ATATGCCTGGAACGGAAC TG

Tir

F : CTTCAGGAATGGGAGATGGA (Sanchez et al., 2018)

R : CAACCGCCTGAACAATACCT

Map

F : AGCGGTTGAAAGCGTGATAC (Sanchez et al., 2018)

R : CTTTACCGCACTGCTCATCA

Hprt:

F : TCAGTCAACGGGGGACATAAA (Kurniawan et al., 2020)

R : GGGGCTGTACTGCTTAACCAG

RegIII β

F : CCCTCCGCACGCATTAGTT (Mohanani et al., 2018)

R : CAGGCCAGTTCTGCATCAA

RegIII γ

F : ATGCTTCCCCGTATAACCATCA (Mohanani et al., 2018)

R : ACTTCACCTTGACCTGAGAA

β -defensin-2 :

F : AAGTATTGGATACGAAGCAG (Yu et al., 2020a)

R : TGGCAGAAGGAGGACAAATG

Lipocalin-2 :

F : TGGCCCTGAGTGTCATGTG (Feng et al., 2019)

R : CTCTTG TAGCTCATAGATGGTGC

Tbp:

F : GAAGAACAATCCAGACTAGCAGCA (Kurniawan et al., 2020)

R : CTTTATAGGGAACCTCACATCACAG

mIL17a : (Liu et al., 2017)

F : TTAACTCCCTTGGCGCAAAA

R : CTTTCCCTCCGCATTGACAC

mIL22 :

F : GCTCAGCTCCTGTACATCA

designed using Primer

Blast (Ye et al., 2012)

R : CAGTTCCCAATCGCCTTGA

FlowJo Software

Tree Star

Graphpad Prism

GraphPad Software, Inc

Image J

Fiji

Zen Blue 3.0

Zeiss

Wave Software

Agilent

Adobe Illustrator

Adobe systems

1381

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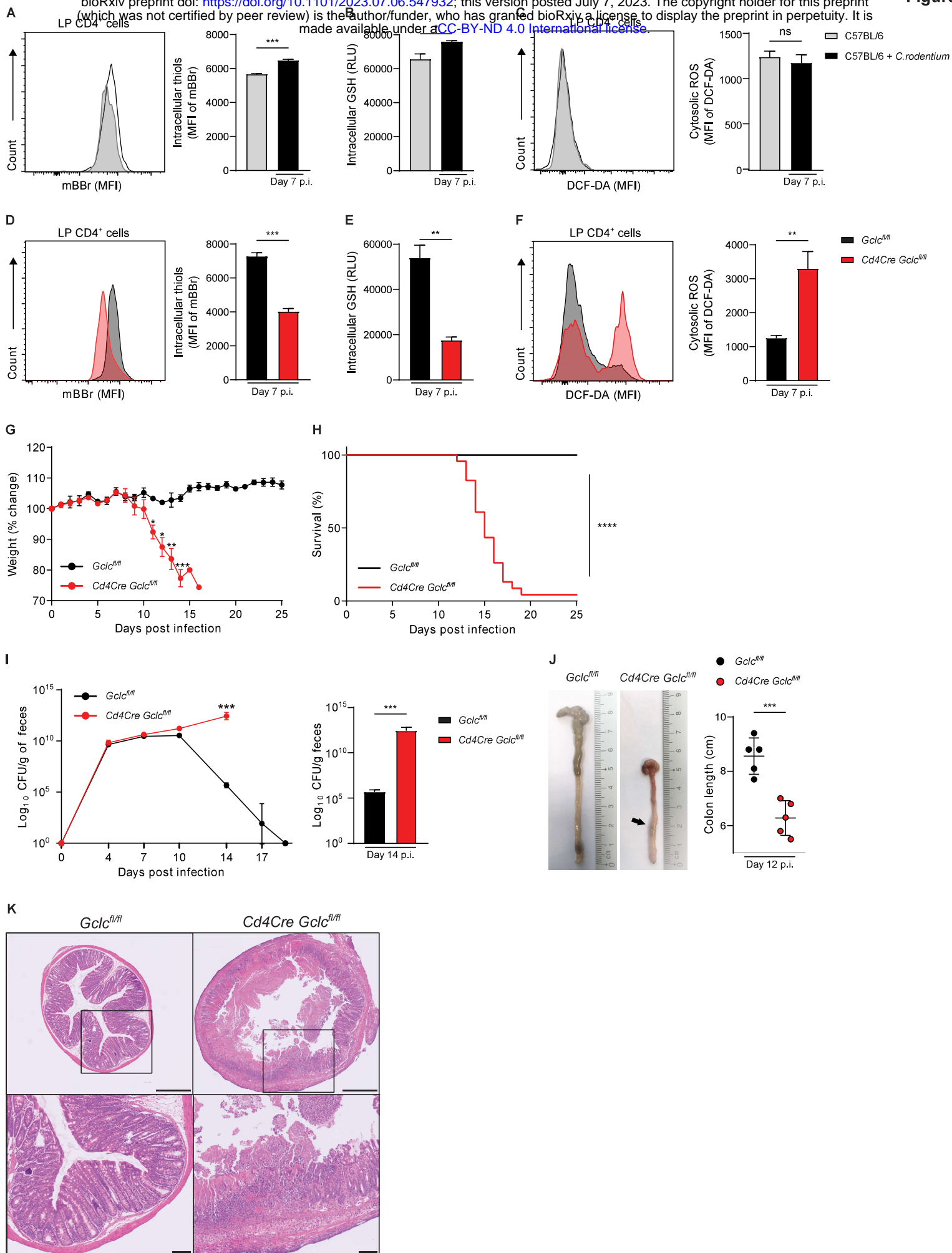
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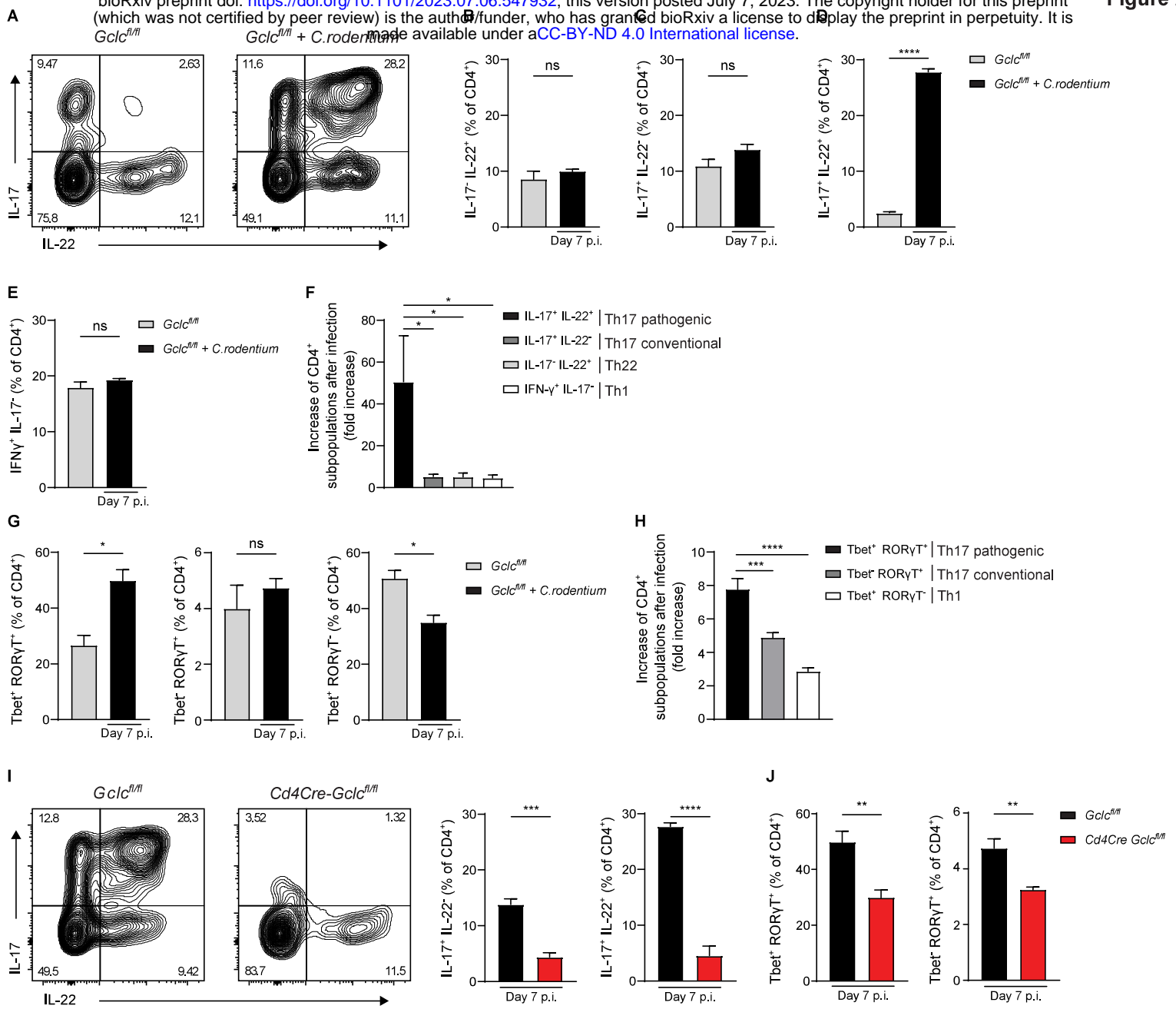
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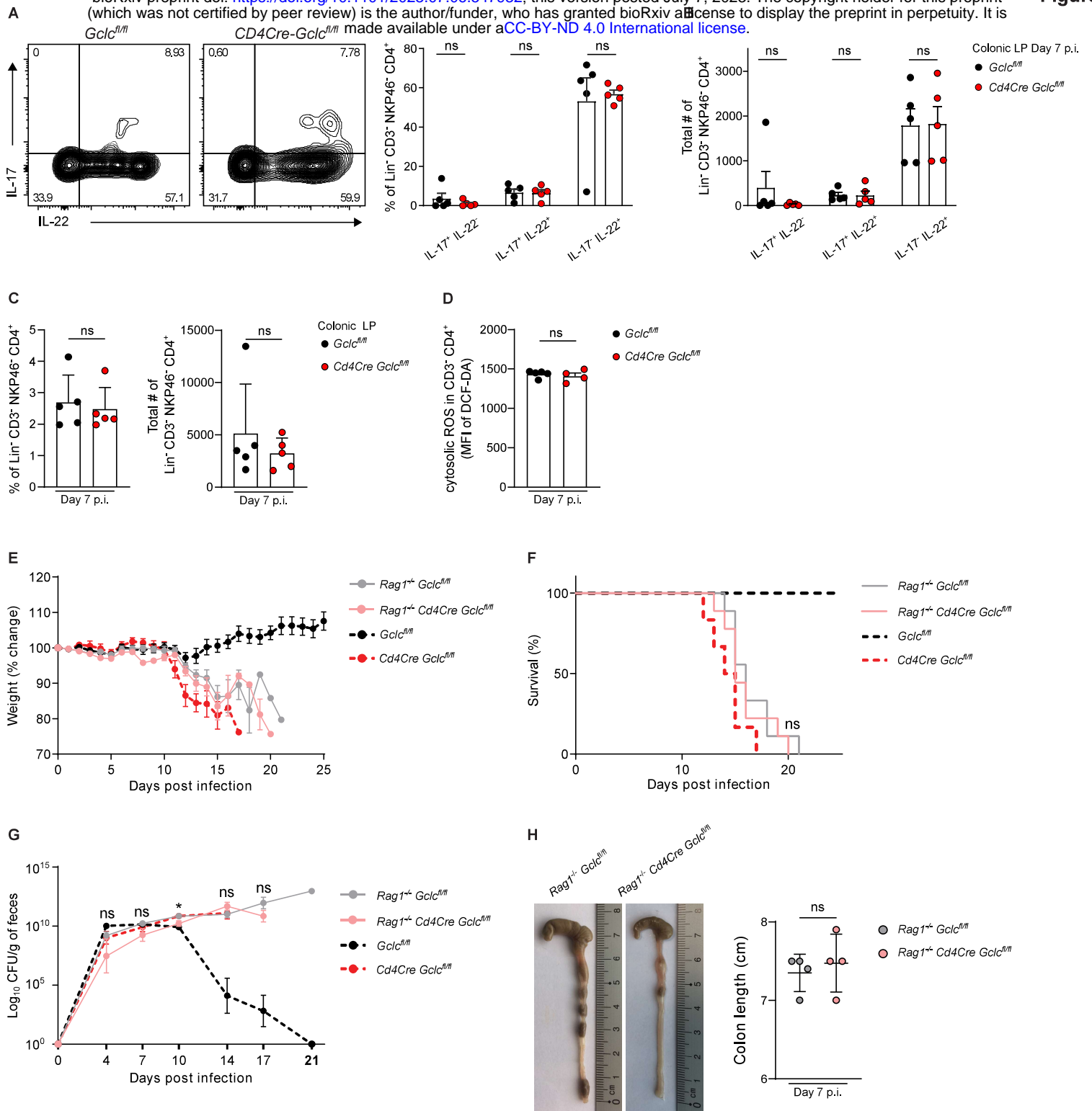
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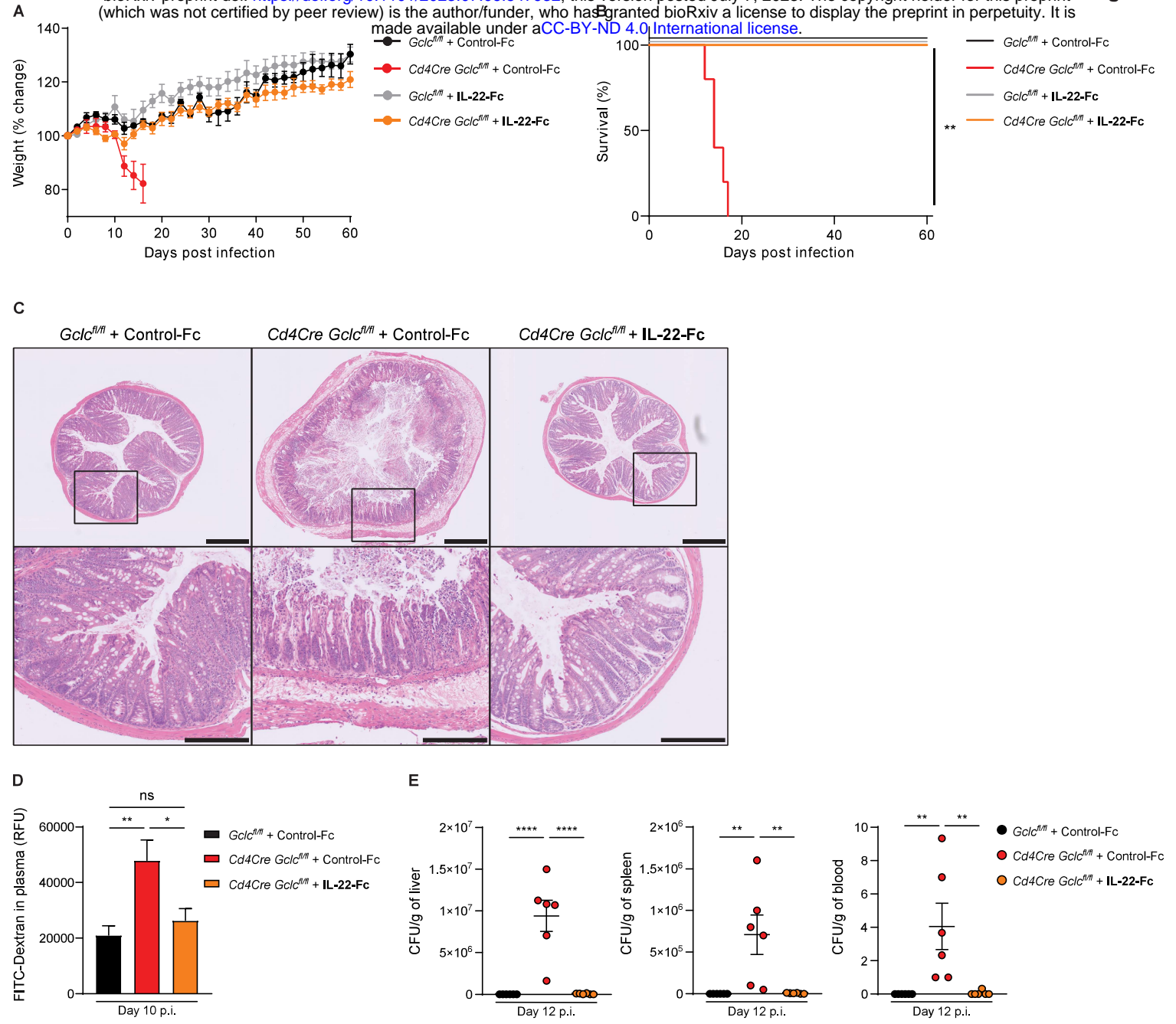




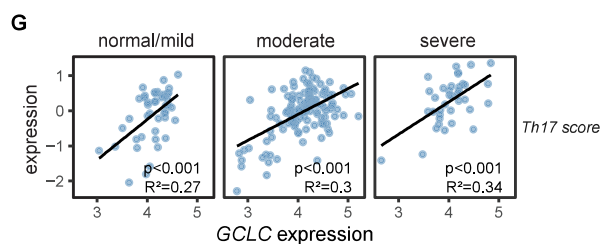
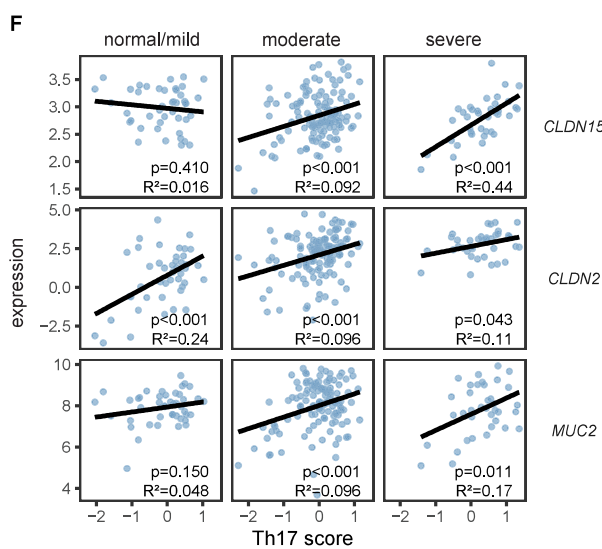
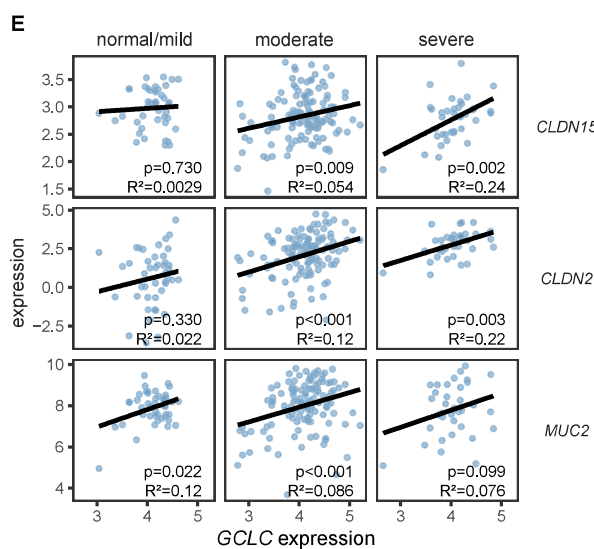
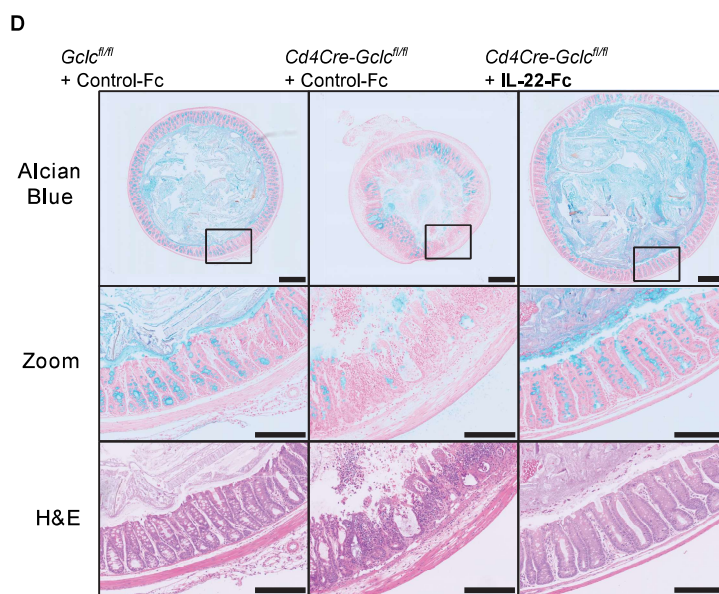
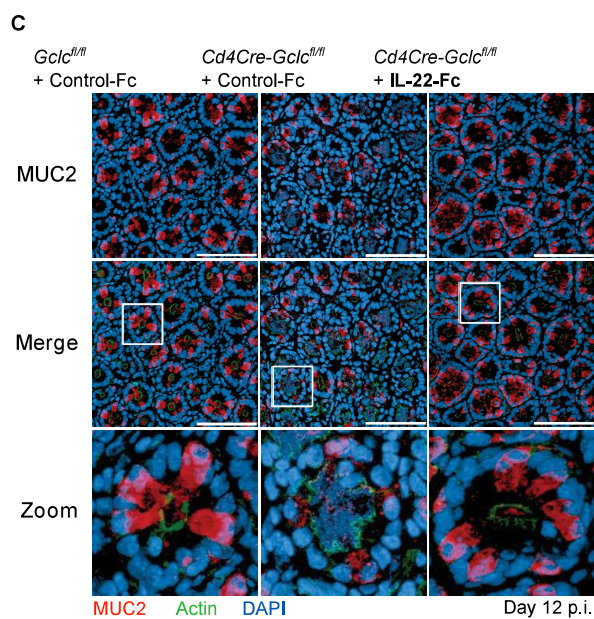
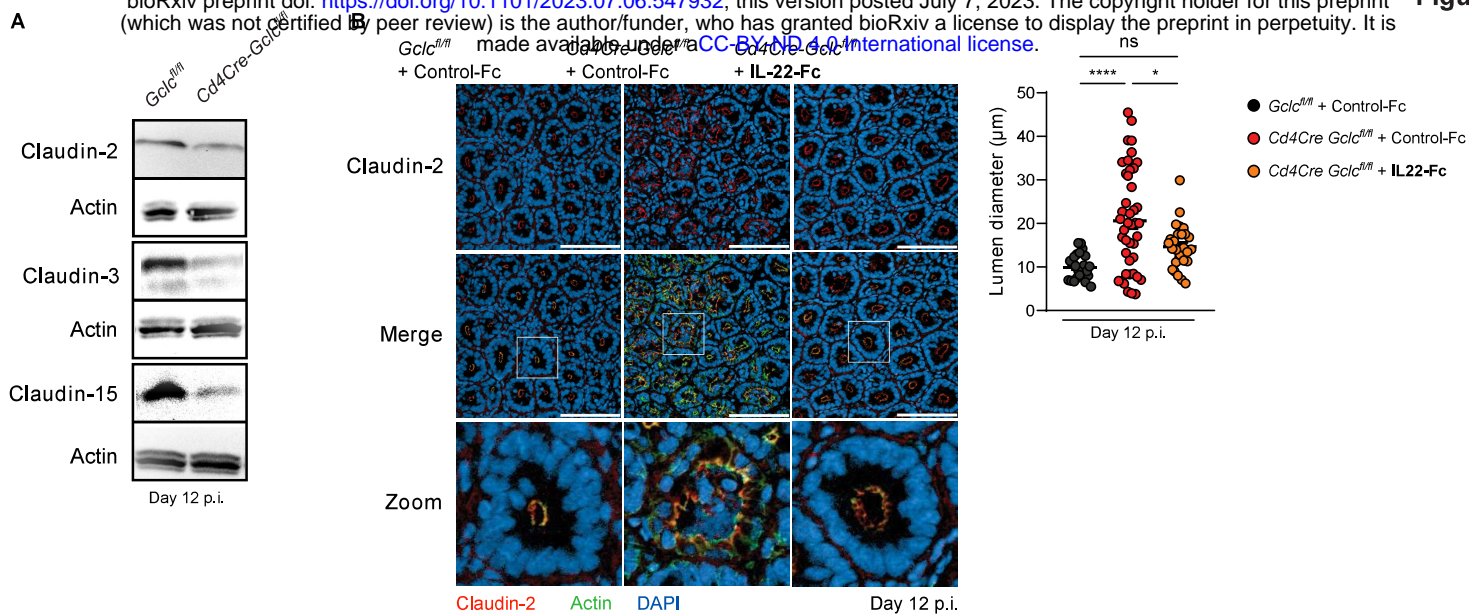
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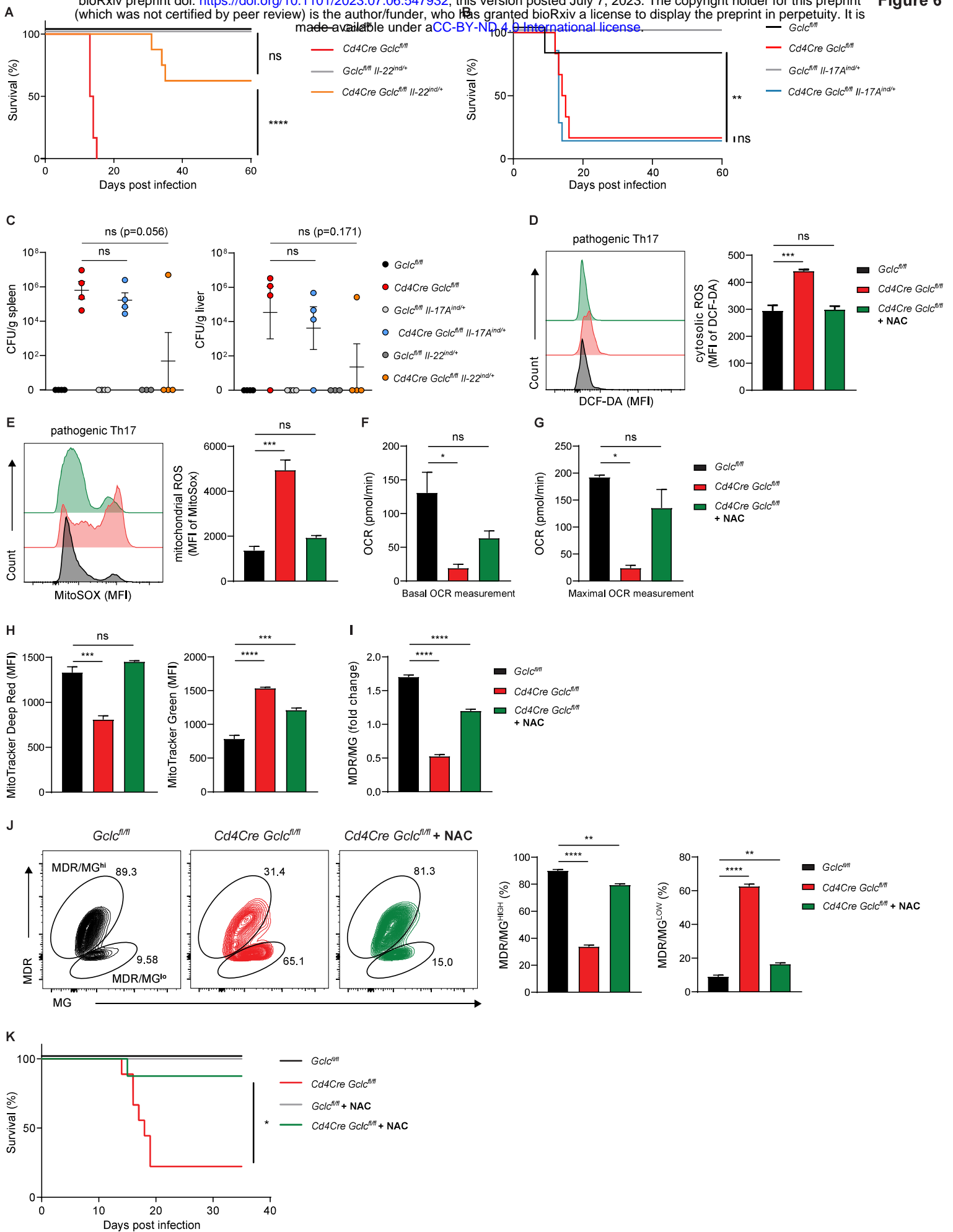


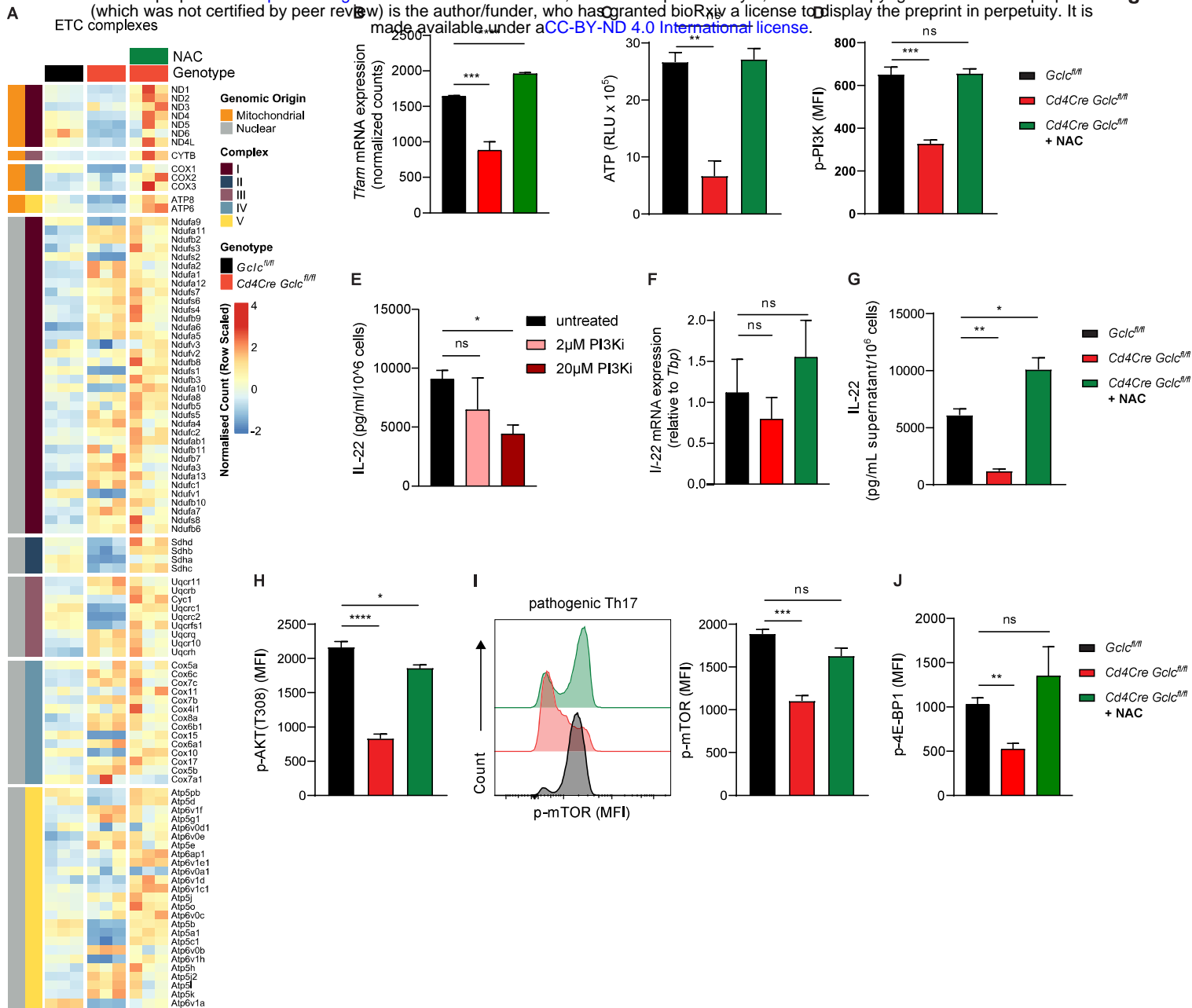
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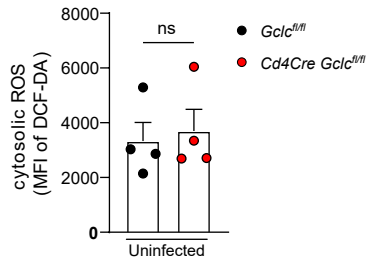




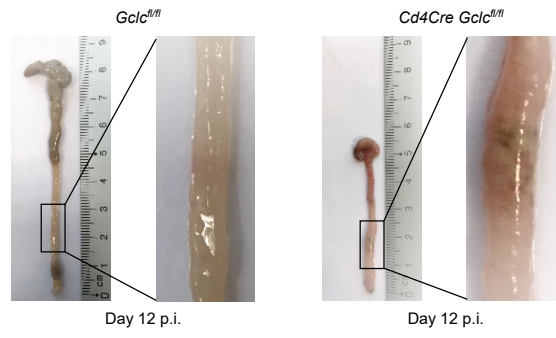


Supplementary Figure 1

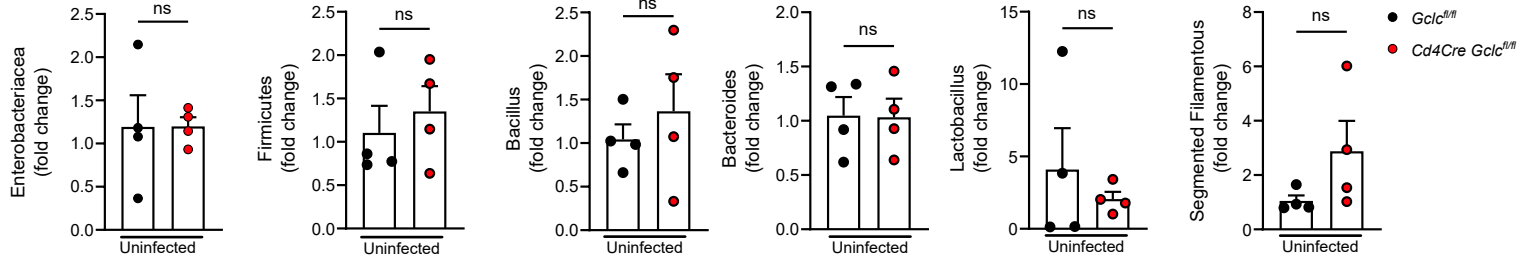
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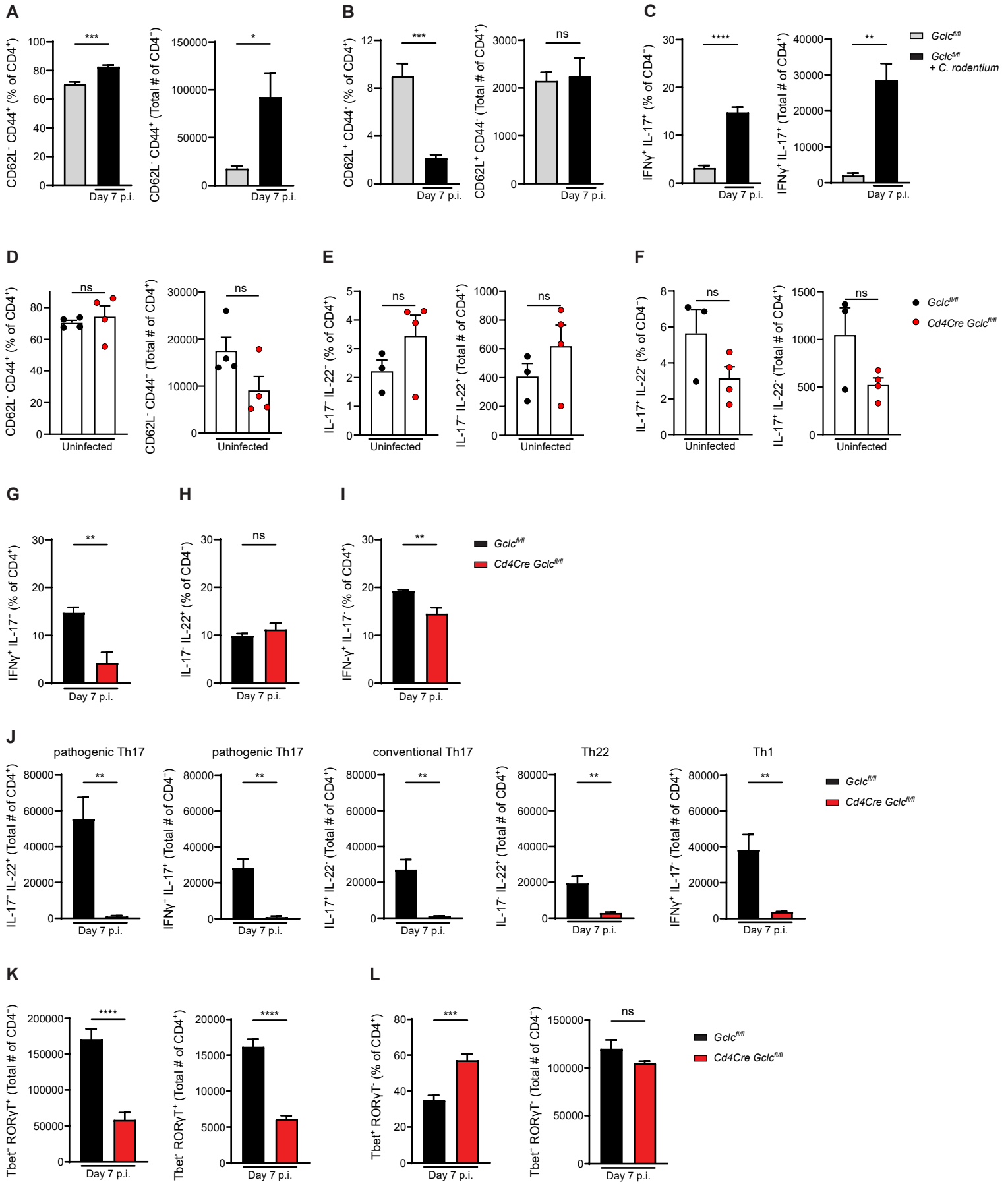
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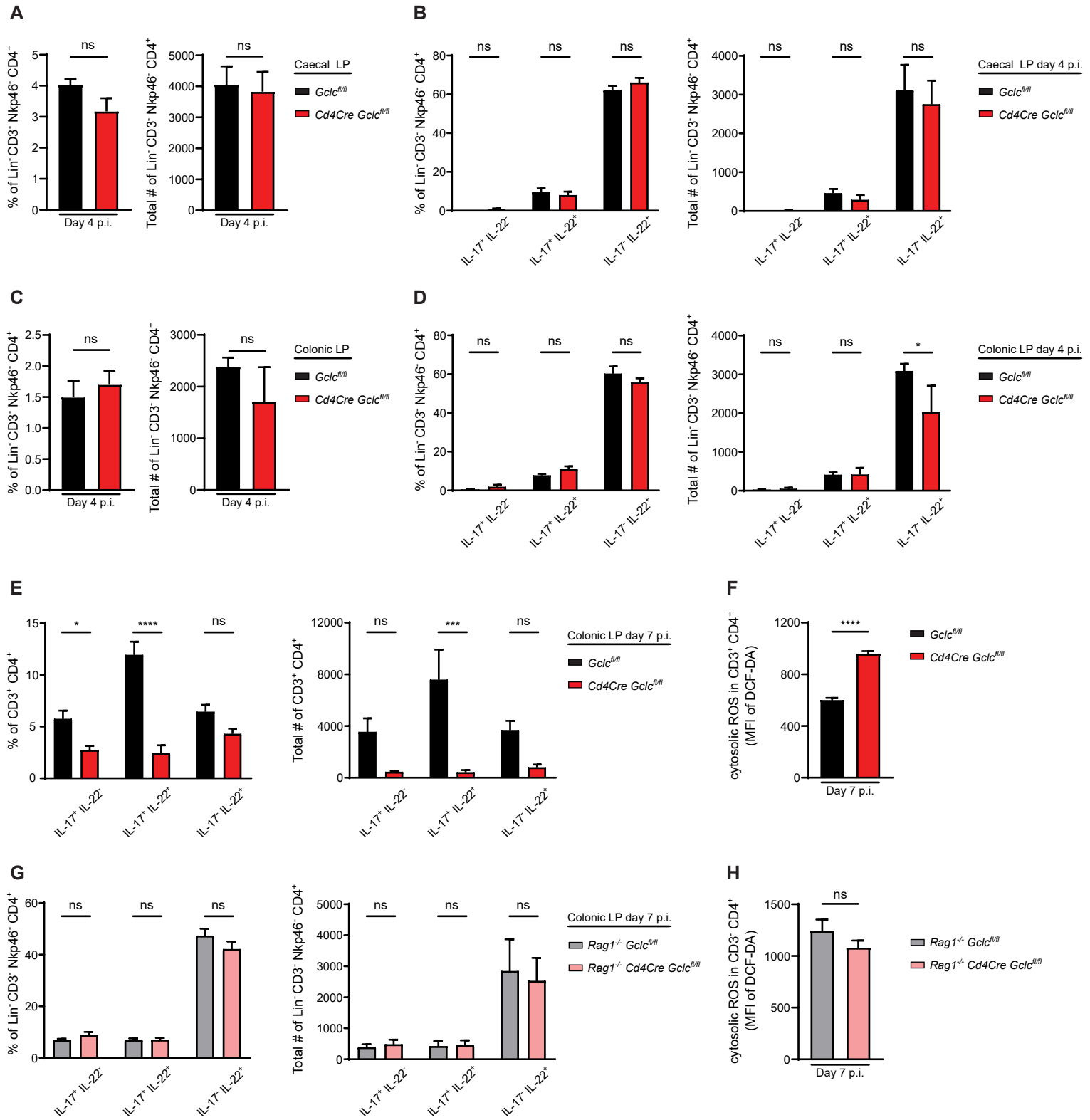
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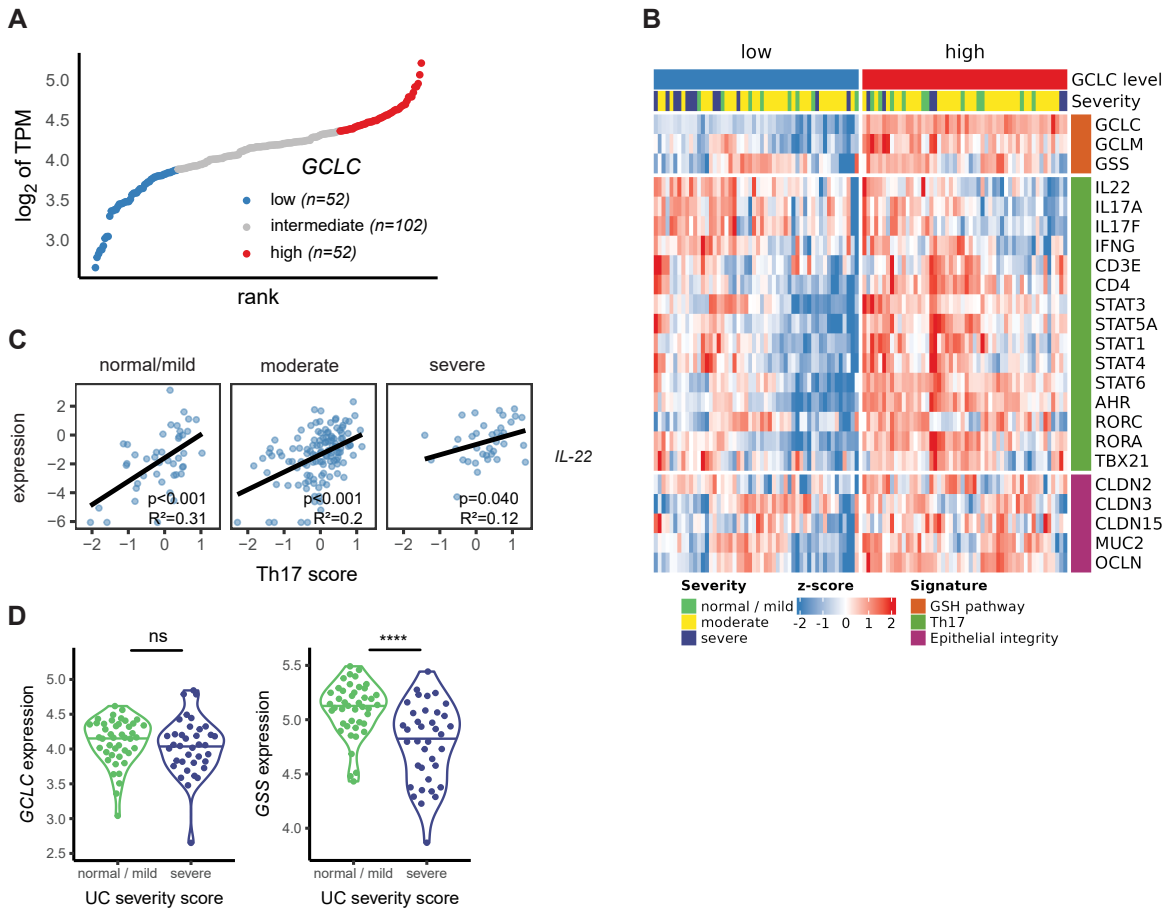
Supplementary Figure 2



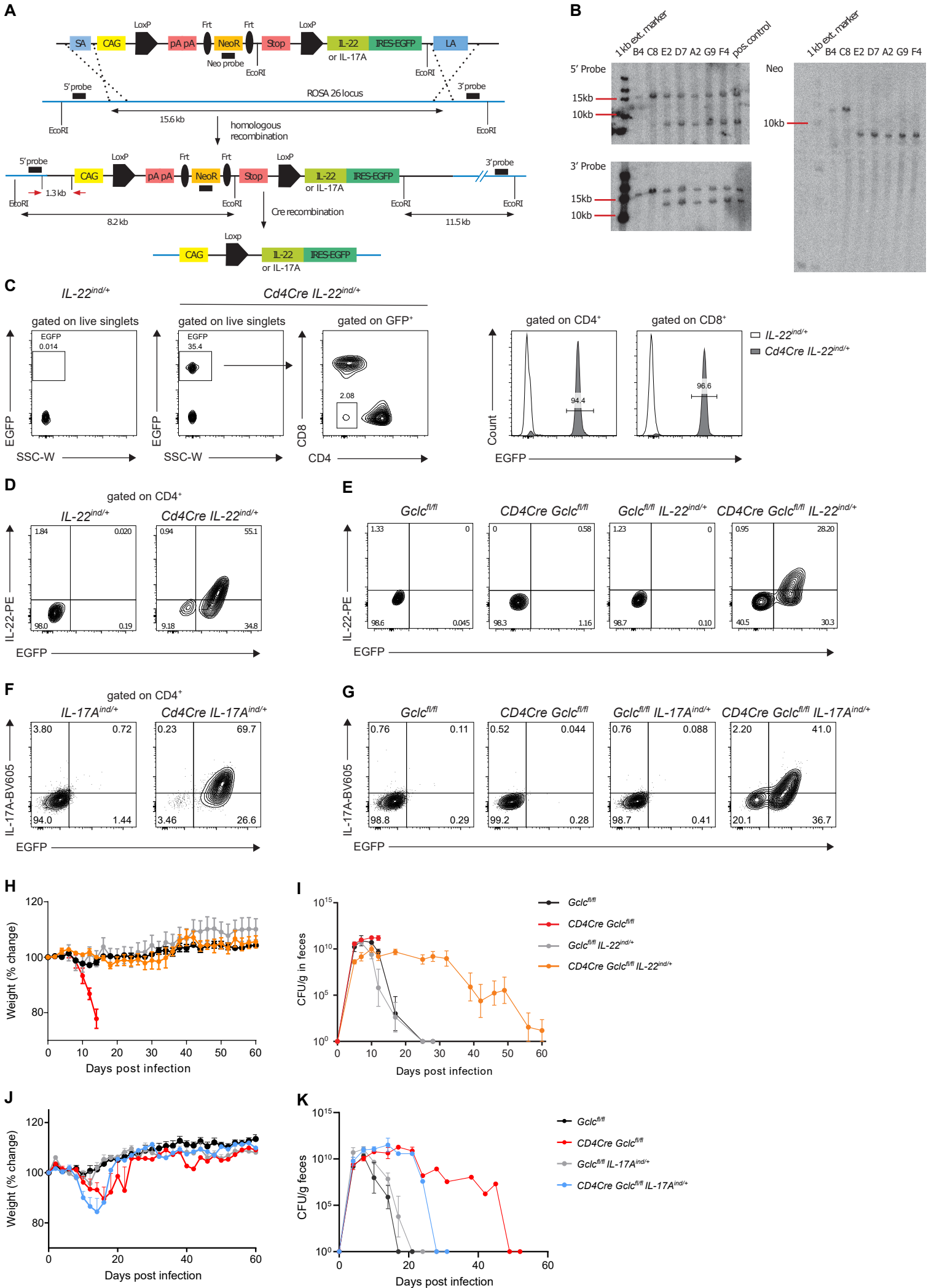
Supplementary Figure 3



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7

