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## Dual role of Mucosal-Associated Invariant T cells in type 1 diabetes

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26 **Abstract**

27 Type 1 diabetes is an autoimmune disease resulting from the destruction of pancreatic- $\beta$   
28 cells by the immune system involving innate and adaptive immune cells. Mucosal-associated  
29 invariant T (MAIT) cells are innate-like T-cells recognizing bacterial riboflavin-precursor  
30 derivatives presented by the MHC-I related molecule, MR1. Since T1D is associated with gut  
31 microbiota modification, we investigated MAIT cells in this pathology. In T1D patients and  
32 non-obese (NOD) diabetic mice, we detected MAIT cell alterations, including increased  
33 granzyme B production, which occur before disease onset. Analysis of NOD mice deficient  
34 for MR1, and therefore lacking MAIT cells, revealed a loss of gut integrity, increased anti-islet  
35 responses associated with exacerbated diabetes. Altogether our data highlight the role of  
36 MAIT cells in the maintenance of gut integrity and the control of anti-islet autoimmune  
37 responses. MAIT cell monitoring could represent a new biomarker in T1D while their  
38 manipulation may open new therapeutic strategies.

39

40

41 **Introduction**

42 Type 1 Diabetes (T1D) is an auto-immune disease characterized by the selective destruction  
43 of pancreatic islet  $\beta$  cells producing insulin, in the context of an underlying multigenetic  
44 inheritance<sup>1</sup>. When most of the  $\beta$  cells are destroyed or non-functional, the consecutive lack  
45 of insulin results in hyperglycemia and requires a life-long insulin replacement therapy<sup>1</sup>. The  
46 physiopathology of T1D involves both innate and adaptive immune systems that are  
47 inappropriately activated inducing a loss of self-tolerance and islet destruction<sup>2-5</sup>. T1D is  
48 characterized by the presence of anti-islet autoantibodies and autoreactive T cells. Innate  
49 immune cells are involved at various stages of the disease and are particularly important for  
50 the initiation of the local immune response in the pancreas and the pancreatic lymph  
51 nodes<sup>2,4</sup>. Recent data have highlighted the role of the intestinal microbiota in T1D by  
52 transfer experiments in NOD mice<sup>6-9</sup> and gut microbiota differences in children associated  
53 with T1D development<sup>10-12</sup>. Several studies also described gut mucosa alterations in NOD  
54 mice and T1D patients<sup>13-17</sup>.

55 MAIT cells are innate-like T cells recognizing bacterial metabolites, derived from the  
56 synthesis of riboflavin, presented by the monomorphic major-histocompatibility-complex-

57 class-I-related protein MR1<sup>18-20</sup>. These non-conventional T cells express a conserved  $\alpha\beta$ -TCR,  
58 consisting of an invariant TCR $\alpha$  chain, V $\alpha$ 7.2-J $\alpha$ 33/20/12 in humans and V $\alpha$ 19-J $\alpha$ 33 in mice,  
59 with a restricted set of TCR $\beta$  chains. MAIT cells produce various cytokines such as TNF- $\alpha$ ,  
60 IFN- $\gamma$ , IL-17 and granzyme B (GzB) that could participate to tissue inflammation and cell  
61 death<sup>18,21-31</sup>. The near absence of MAIT cells in germ-free mice<sup>18,32</sup> and their physiological  
62 localization at mucosal sites including the gut<sup>18,23</sup> suggest a strong interaction with the  
63 microbiota. Here for the first time we described MAIT cell alteration in T1D patients and our  
64 mouse data reveal the protective role of MAIT cells against T1D. The localization and the  
65 function of MAIT cells highlight their key role in the maintenance of gut integrity, thereby  
66 controlling the development of autoimmune responses against pancreatic  $\beta$  cells.

67

68

## 69 **Results**

### 70 **Alteration of blood MAIT cell frequency and phenotype in children with recent onset T1D**

71 We first began the investigation of MAIT cells in T1D by analyzing MAIT cell frequency and  
72 phenotype in fresh peripheral blood samples from children with recent onset T1D and  
73 children with established T1D as compared to age-matched control children (**Supplementary**  
74 **Tables 1 and 2**). MAIT cells can be identified in human blood as CD4<sup>-</sup> T lymphocyte  
75 expressing V $\alpha$ 7.2 TCR $\alpha$  gene segment and CD161<sup>high</sup><sup>19,20,24,33,34</sup> (**Fig. 1a**). MAIT cell frequency  
76 and number was decreased (3-fold) in the blood of recent onset T1D children whereas no  
77 significant difference was observed in children with established disease as compared to  
78 control children (**Fig. 1a and Supplementary Fig. 1a**). Decreased frequency was observed in  
79 both CD8<sup>+</sup> and double negative (DN) MAIT cell subsets (**Supplementary Fig. 1b**). Of note  
80 there was no difference in the frequencies of conventional CD4 and CD8 T cells, and of  
81 V $\alpha$ 7.2<sup>+</sup>CD161<sup>-</sup> T cells between the three children populations confirming that the decrease of  
82 MAIT cell frequency at the onset of T1D was **not secondary to changes** in other T cell  
83 populations nor to down-regulation of the CD161 marker (**Supplementary Fig. 2a-b**).  
84 Analysis of MAIT cell phenotype showed a decreased frequency of MAIT cells expressing  
85 tissue recruitment/adhesion molecules (CCR6, CD56) at the onset of the disease, an  
86 increased frequency of MAIT cells expressing the activation/exhaustion markers CD25 and  
87 PD1, and a decreased frequency of MAIT cells expressing the anti-apoptotic molecule Bcl-2  
88 (**Fig. 1b-c**). Multi-parametric analysis of MAIT cells in the children with established T1D

89 highlighted the intermediate phenotype of MAIT cells between those from recent onset T1D  
90 and control children (**Fig. 1c**). Interestingly in recent onset children the frequency of MAIT  
91 cells expressing migratory CCR6<sup>+</sup> or anti-apoptotic Bcl-2 molecules were positively correlated  
92 with the frequency of MAIT cells, whereas MAIT cell CD25 expression was negatively  
93 correlated with MAIT cell frequency (**Supplementary Fig.3**). These data suggest that  
94 decreased blood MAIT cell frequency could reflect their migration to inflamed tissues and/or  
95 their death by apoptosis subsequent to their activation.

96

### 97 **Alteration of blood MAIT cell function in children with recent onset T1D**

98 Cytokine and GzB production by fresh blood MAIT cells was analyzed after PMA-ionomycin  
99 stimulation. MAIT cells from children with recent onset T1D produced less IFN- $\gamma$ , whereas  
100 their production of TNF- $\alpha$ , IL-4, and GzB was increased as compared with MAIT cells from  
101 control children (**Fig. 2a-b**). Of note, among these effector molecules only the frequency of  
102 GzB correlated with the frequency of MAIT cells, the higher GzB production was observed in  
103 patients with the lower frequency of MAIT cells (**Supplementary Fig.4**). Multi-parametric  
104 analysis of cytokines and GzB production by MAIT cells also showed an intermediate status  
105 of blood MAIT cells from children with established T1D, between those from control children  
106 and recent onset T1D children, as already observed for MAIT cell surface phenotype (**Fig.**  
107 **2b**). We next analyzed the ability of MAIT cells to respond to specific TCR activation by the  
108 ligand 5-OP-RU<sup>20</sup>. Upon stimulation, MAIT cells from control children up-regulated CD69 and  
109 CD25 activation markers. Addition of blocking MR1 mAb confirms that this activation was  
110 TCR-dependent. Interestingly, MAIT cell activation was significantly reduced in children with  
111 recent onset T1D (**Fig. 2c**). Together, these results highlight functional alteration of MAIT  
112 cells in children with recent onset T1D.

113

### 114 **Cytotoxic function of MAIT cells on human $\beta$ cells**

115 We next investigated potential links between phenotype and functional alterations of MAIT  
116 cells and clinical characteristics of children with recent onset T1D (**Supplementary Tables 1**  
117 **and 2**). Interestingly, the frequency of GzB<sup>+</sup> MAIT cells was negatively associated ( $r=-0.71$ ,  
118  $P<0.0001$ ) with children's age at diagnosis (**Fig. 3a**), which is in agreement with the current  
119 view that T1D is more aggressive in the youngest children<sup>35,36</sup>. We speculate that production  
120 of GzB by MAIT cells, reflecting their cytotoxic potential, could be involved in the

121 physiopathology of T1D. Other MAIT cell parameters, such as their frequency, CCR6 and Bcl-  
122 2 expression, were also associated with the age at diagnosis (**Supplementary Fig. 5**). No  
123 significant correlations between MAIT cell parameters and age were observed in controls  
124 and children with established T1D (data not shown). Production of GzB by MAIT cells  
125 inversely correlated with HbA1c level at the onset of the disease but not in children with  
126 established T1D (**Fig. 3a**). Indeed, a more aggressive disease associated with sustained MAIT  
127 cell abnormalities suggests a shorter time of hyperglycemia before the onset thereby lower  
128 levels of HbA1c<sup>36</sup>.

129 The strong inverse correlation between GzB production by MAIT cells and the age of the  
130 children at the time of onset suggested that MAIT cells could participate to  $\beta$ -cell death.  
131 Interestingly, inflammatory cytokines usually produced in inflamed islets during T1D  
132 progression (IL1- $\beta$ , IFN- $\gamma$  and TNF- $\alpha$ ) induce MR1 up-regulation on the human  $\beta$ -cell line,  
133 EndoC- $\beta$ H1 cell (**Fig. 3b**). Moreover, co-culture experiments demonstrated the direct killing  
134 of EndoC- $\beta$ H1 cells<sup>37</sup> by purified MAIT cells from four healthy donors. Cell-sorted MAIT cells  
135 were cultured in presence of IL-7, then co-cultured with Endo- $\beta$ H1 cells pre-incubated with  
136 inflammatory cytokines, or not. The data in **Figure 3c-d** show that MAIT cells induced MR1-  
137 dependent EndoC- $\beta$ H1 cell apoptosis (annexin V<sup>+</sup> PI<sup>-</sup>), which was more efficient with Endo-  
138  $\beta$ H1 cells pre-incubated with inflammatory cytokines. During co-cultures MAIT cells were  
139 activated (CD25 up-regulation) and degranulated (CD107a<sup>+</sup>) (**Fig. 3e**). Altogether these data  
140 suggest that in the inflamed pancreas during diabetes progression MAIT cells can participate  
141 to  $\beta$ -cell destruction.

142

### 143 **MAIT cell alterations at different disease stages**

144 To further explore the link between MAIT cell parameters and clinical characteristics, 15 of  
145 the children with recent onset T1D were analyzed one year later. Although MAIT cell  
146 frequency among T cells remain to a similar level after one year of insulin, both CCR6<sup>+</sup> and  
147 Bcl-2<sup>+</sup> MAIT cell frequencies significantly increased. Conversely the frequencies of CD25<sup>+</sup>,  
148 PD1<sup>+</sup>, IL-17A<sup>+</sup>, and to some extent GzB<sup>+</sup>, MAIT cells decreased to levels observed in the  
149 control children (**Fig. 4a**). This longitudinal analysis strengthened the data obtained in the  
150 transversal analysis showing that MAIT cell phenotype was more similar to controls in the  
151 children under insulin therapy than at disease onset.

152

### 153 **MAIT cells as a new biomarker of T1D**

154 Canonical analysis was performed to compare MAIT cell alterations in the three groups of  
155 children (controls, recent onset and established T1D). This analysis revealed that MAIT cell  
156 parameters were sufficient to discriminate the three groups of children analyzed (**Fig. 4b**).  
157 Parameters involved in this discrimination are shown in **supplementary Figure 6**. Moreover,  
158 a statistical regression analysis identified four surface markers (CCR6, CD25, PD1, CD56) that  
159 define a predictive model for the diagnosis of the disease tested on the ROC curve (**Fig. 4c**).  
160 Importantly, we confirmed in another cohort of children from Milano that frequency and  
161 phenotype alteration of MAIT cell was observed in recent onset T1D as compared to age-  
162 matched control children (**Supplementary Table 3 and supplementary Fig. 7**). However  
163 technical difficulties impacting CD56 analysis on these frozen cells did not allow to applying  
164 the predictive model.

165 Finally, to test whether MAIT cell alterations could be detected before the onset of  
166 diagnosis, we characterized MAIT cells in adults at risk to develop T1D defined as direct  
167 relatives of T1D patients with at least two positive autoantibodies (**Supplementary Table 4**).  
168 As compared to the control group, there were increased frequencies of CD25<sup>+</sup> and PD1<sup>+</sup>  
169 MAIT cells and a trend toward a lower CCR6<sup>+</sup> MAIT cell frequency, even though the number  
170 of individuals analyzed were limited (**Fig. 4d**). Altogether our data in patients suggest that  
171 MAIT cells represent a new biomarker in T1D and they could play a role in the pathogenesis  
172 of T1D. Therefore, we investigated MAIT cells in mouse models, which allow analysis in  
173 tissues at different stages of disease development and could be manipulated to determine  
174 whether MAIT cells are involved in T1D physiopathology.

175

### 176 **MAIT cell characterization in NOD mice**

177 The NOD mouse is the most studied animal model of T1D because it shares many features  
178 with the human disease<sup>3,4</sup>, thus we investigated whether any of the MAIT cell alterations  
179 observed in T1D patients were evident in this model. MAIT cells were characterized, in 10  
180 week-old NOD and C57BL/6 mice, using mouse-MR1 tetramers loaded with the riboflavin  
181 derivative 5-OP-RU, whereas control tetramers were loaded with the non-agonist folate  
182 derivative Ac-6-FP<sup>20,27,32,38</sup> (**Fig. 5a**). The frequency and absolute number of MAIT cells were  
183 lower in NOD than C57BL/6 mice; 3 to 10-fold lower in spleen and pancreatic lymph nodes

184 (PLN). However MAIT cells were present in pancreatic islets of NOD mice and were more  
185 abundant in the ileum of NOD than C57BL/6 mice (**Fig. 5b**). Of note circulating MAIT cells  
186 were below detection limit in the blood from NOD mice. Mouse MAIT cells can be divided in  
187 three subsets, CD4<sup>+</sup>, CD8<sup>+</sup> or DN<sup>38</sup> (**Fig. 5c-d**). As previously shown, MAIT cells in peripheral  
188 tissues of C57BL/6 mice were mainly DN and expressed CD44 (**Fig. 5d supplementary Fig. 8**),  
189 corresponding to functionally mature MAIT cells<sup>32</sup>. In contrast, the proportion of DN MAIT  
190 cells was lower in NOD than C57BL/6 mice (**Fig. 5c-d**). Analysis of activation markers on MAIT  
191 cells from NOD mice revealed that CD69 was highly expressed in pancreatic islets and ileum,  
192 and CD25 was elevated in PLN and islets (**Fig. 5e-f**). While CD69 was also elevated on ileum  
193 MAIT cells from C57BL/6 mice, CD25 was not up-regulated in PLN from C57BL/6 mice (**Fig. 5f**  
194 **and supplementary Fig. 9**). Of note CD44 was more expressed on ileal MAIT cells from NOD  
195 than C57BL/6 mice. Regarding cytokine production in NOD mice, in all tissues MAIT cells are  
196 strong producers of IL-17A and TNF- $\alpha$ , whereas IFN- $\gamma$  and IL-22 was detected in only few  
197 cells (**Fig. 5g-h and supplementary Fig. 10**). Moreover, a majority of MAIT cells in PLN and  
198 ileum from NOD mice express CD44 and they produce large amount of cytokines.  
199 Interestingly, similar results were obtained for TNF- $\alpha$ , IFN- $\gamma$  and IL-22 cytokine production in  
200 both C57BL/6 and NOD mice (**Fig. 5h and supplementary Fig. 11**). Of note, higher frequency  
201 of MAIT cells from NOD produce IL-17, compare to C57BL/6, which could contribute to  
202 inflammation and diabetes progression. As in C57BL/6 mice<sup>34,38,39</sup>, most MAIT cells in NOD  
203 mice express PLZF, ROR- $\gamma$ t and T-bet, transcription factors characteristic of this innate-like T  
204 cell lineage<sup>23</sup> (**supplementary Fig. 12**). Altogether these data show that spleen MAIT cells are  
205 less frequent and mature in NOD than in C57BL/6 mice, however they express an activated  
206 phenotype and produce cytokines in PLN, islets and ileum from NOD mice.

207

### 208 **MAIT cell accumulation and dual function during diabetes development in NOD mice**

209 We next analyzed MAIT cells in NOD mice, at three stages of disease development, early  
210 stage (6-7 weeks old), pre-diabetic (15-17 weeks old) and mice at the onset of the disease  
211 (**Fig. 6a**). These three stages correspond respectively to mild peri-insulinitis characterized by  
212 moderate infiltration of inflammatory cells around pancreatic islets, to insulinitis associated to  
213 partial  $\beta$ -cell destruction and to severe destructive insulinitis resulting in insufficient insulin  
214 production<sup>4,5</sup> and hyperglycemia. During disease progression, MAIT cell frequency increased



215 in pancreatic islets and transiently in spleen and ileum (**Fig. 6a**). These data show that  
216 disease progression is associated to modification of MAIT cell tissue distribution. Since MAIT  
217 cells are effector cells and can migrate to inflamed tissues, we analyzed their migration by  
218 transfer experiments. MAIT cells were transferred into congenic NOD mice at two stages of  
219 disease development. Interestingly there is a higher recruitment of MAIT cells in the  
220 pancreas of 20-25 week old NOD mice than in younger (8-10 week) mice (**Fig. 6 b**). In  
221 contrast, MAIT cell recruitment in ileum decreased with aging, supporting the decreased  
222 frequency of MAIT cells in this tissue in diabetic NOD mice.

223 To further explore the function of MAIT cells in T1D, their cytokine production was analyzed  
224 in various tissues at the three stages of disease development. For that purpose, MAIT cells  
225 were purified by cell-sorter after MR1-tetramer staining, and level of GzB, IFN- $\gamma$ , IL-17A and  
226 IL-22 mRNA was analyzed by RT-qPCR (**Fig. 6c-d**). Interestingly, in pancreatic islets and ileum,  
227 MAIT cell cytokine profiles evolved with disease progression. In islets, MAIT cell production  
228 of IFN- $\gamma$  was detected in diabetic mice, and GzB was already detectable at pre-diabetic stage  
229 and further increased in diabetic mice (**Fig. 6c**). Both GzB and IFN- $\gamma$  from MAIT cells could  
230 participate to pancreatic  $\beta$ -cell death<sup>4</sup>. This increased production of IFN- $\gamma$  and GzB in islets  
231 was confirmed at protein levels (**Fig. 6e**). In ileum from NOD mice, T1D development was  
232 associated with decreased level of IL-17A and IL-22 mRNA in MAIT cells at the diabetic stage  
233 (**Fig. 6d**). Diminished IL-17A and IL-22 production by ileal MAIT cells from diabetic NOD mice  
234 was confirmed by intra-cellular staining (**Fig. 6f**). Altogether these results suggest that MAIT  
235 cells could play a dual role in NOD mice according to their tissue localization, promoting  $\beta$ -  
236 cell death in the pancreas whereas participating in gut mucosa homeostasis.

237

### 238 **Gut mucosa alterations during T1D progression in NOD mice**

239 Since ROR- $\gamma$ t is the master transcription factor for IL-17A<sup>40</sup> and is involved in IL-22  
240 production<sup>41,42</sup>, we analyzed ROR- $\gamma$ t expression in MAIT cells in the ileum of ROR- $\gamma$ t-GFP NOD  
241 mice (**Fig. 7a**). According to the decreased level of IL-17A and IL-22 in diabetic mice, ROR- $\gamma$ t  
242 expression was also impacted at the onset of the disease. Decreased IL-17, IL-22 and ROR- $\gamma$ t  
243 expression by MAIT cells in diabetic NOD mice prompt us to analyze IL-23 expression, a key  
244 cytokine inducing the production of these cytokines. Interestingly, at diabetes onset IL-23  
245 transcript level was decreased in the ileum (**Fig. 7b**), which could contribute to the

246 decreased IL-17/IL-22 production by MAIT cells. Of note, this defect in cytokine production is  
247 associated with significant increased gut permeability, as measured by in vivo FITC-dextran  
248 assay (**Fig. 7c**).

249 To investigate whether gut microbiota could directly impact MAIT cell function, we  
250 measured at different stages of disease development the level of bacterial agonist ligand in  
251 intestinal content using an in vitro biological assay (**Fig. 7d-g**). Intestinal contents activate  
252 purified MAIT cells in a dose dependent manner, as 5-OP-RU positive control (**Fig. 7d-e**). This  
253 activation was MR1 specific since it was inhibited by the addition of Ac-6-FP antagonist  
254 ligand (**Fig. 7d-f**). MAIT cell activation by intestinal contents from young, pre-diabetic and  
255 diabetic NOD mice did not reveal significant modification of MAIT cell ligand concentration  
256 (**Fig. 7g**). Altogether, these data suggest that MAIT cell defective IL-17 and IL-22 production  
257 might be due to lower IL-23 level in diabetic mice in association to increased gut  
258 permeability.

259

#### 260 **Absence of MAIT cells impacts diabetes development and intestinal homeostasis.**

261 To determine the role of MAIT cells in the pathogenesis of T1D, we generated MR1<sup>-/-</sup> NOD  
262 mice, since MR1 is required for thymic development of MAIT cells<sup>18,32,33,43</sup>. Our analysis was  
263 performed on MR1<sup>-/-</sup> and MR1<sup>+/-</sup> littermate NOD mice in co-housed condition to exclude  
264 possible impact of different gut microbiota on MAIT cell function. As expected MR1<sup>-/-</sup> NOD  
265 mice lacked MAIT cells, whereas there were no significant differences in the frequency of  
266 Foxp3<sup>+</sup> CD4 Treg, iNKT and  $\gamma\delta$ T cells in the spleen and PLN from both lines (**Fig. 8a and**  
267 **supplementary Fig. 13**). Most interestingly, MR1<sup>-/-</sup> NOD mice developed exacerbated  
268 diabetes compared to their littermate MR1<sup>+/-</sup> controls indicating a protective role of MAIT  
269 cells in T1D development (**Fig. 8b**). Of note, we also observed the protective role of MAIT  
270 cells against T1D using multi-low dose streptozotocin treatment, commonly used to induce  
271 T1D in C57BL/6 mice<sup>44</sup>. As shown in the **supplementary Fig. 14**, MR1<sup>-/-</sup> B6-MAIT<sup>CAST</sup> mice  
272 were more susceptible to T1D development than their MR1<sup>+/-</sup> B6-MAIT<sup>CAST</sup> littermates.  
273 Corroborating incidence results in NOD mice, there was an elevated frequency of anti-islet  
274 CD8<sup>+</sup> T cells specific for islet-specific glucose-6-phosphatase catalytic subunit-related protein  
275 (IGRP)<sup>45</sup> and IFN- $\gamma$ <sup>+</sup> anti-IGRP CD8 T cells, respectively in the PLN and pancreatic islets, from  
276 MR1<sup>-/-</sup> NOD mice compared to their littermate controls (**Fig. 8c-d**). Since PLN dendritic cells  
277 (DC) play a key role in anti-islet T cell priming<sup>46,47</sup>, DC phenotype was analyzed in both lines

278 of NOD mice. Supporting increased anti-islet T cell response, CD11c<sup>+</sup>CD11b<sup>+</sup>CD103<sup>+</sup> DC<sup>48-50</sup>  
279 frequency was elevated in PLN from MR1<sup>-/-</sup> NOD mice and these DC expressed higher level of  
280 MHC class II molecules and CD86 reflecting their more activated status (**Fig. 8e**). Since gut  
281 mucosa integrity controls immune cell activation<sup>44,51,52</sup> and MAIT cells are abundant and  
282 activated in this tissue, we explored gut mucosa of MR1<sup>-/-</sup> NOD mice. The lack of MAIT cells  
283 resulted in a significant increased intestinal permeability evaluated by FITC-dextran level in  
284 the blood after oral gavage (**Fig. 8f**). Reduced mRNA expression of occludin and mucin-2  
285 confirmed defective gut epithelial cell function and histology analysis showed an abnormal  
286 mucus accumulation in goblet cells of MR1<sup>-/-</sup> NOD mice (**Fig. 8g-h**). These abnormalities were  
287 associated with increased lymphoid cell infiltration in the lamina propria (**Fig. 8i**). Regarding  
288 the role of MAIT cells in the control of gut integrity and DC priming in the PLN, significant  
289 increased level of bacterial DNA 16S was detected in the PLN of MR1<sup>-/-</sup> versus control  
290 littermates (**Fig. 8j**). Altogether, our data reveal a link between MAIT cells, the gut mucosa  
291 and the increased anti-islet T cell responses.

292

293

## 294 **Discussion**

295 This study reveals MAIT cell alteration in T1D patients as well as in NOD mice. MAIT cell  
296 frequency is lower in the peripheral blood of patients compared to healthy children. Such  
297 decreased frequency could reflect their migration from the blood to inflamed tissues as  
298 described in other inflammatory and autoimmune diseases<sup>24-26,29,53-55</sup> and as supported by  
299 their increased frequency and migration in the pancreas of NOD mice during T1D  
300 development. Lower frequency of blood MAIT cells could also result from their sustained  
301 activation leading to cell exhaustion as indicated by their expression of Bcl-2, CD25 and PD1  
302 and by their defective *in vitro* response to Ag specific stimulation, in contrast to their  
303 increased cytokines and GzB production upon PMA-ionomycin. Of note MAIT cell defect in  
304 T1D might not only be a consequence of diabetes development but also might reflect  
305 intrinsic differences. Indeed in NOD mice, as compared to C57BL/6 mice, MAIT cells are less  
306 frequent and express a less mature phenotype, based on CD44 expression.

307 Our study in NOD mice points out the different phenotype and function of MAIT cells  
308 according to their tissue localization. This heterogeneity of MAIT cells observed in mice

309 might explain the mosaic of MAIT cell populations detected in the blood from control  
310 individuals and T1D patients. It would be interesting to further investigate the recirculation  
311 of MAIT cells between peripheral tissues and the blood. However study of blood MAIT cells  
312 in NOD mice was not performed due to their extremely low frequency.

313 The analysis of peripheral tissues from NOD mice highlights the role of MAIT cells in two  
314 tissues, the pancreas and the gut mucosa. In the pancreas, not only MAIT cell frequency  
315 increased with diabetes development but also their production of GzB and IFN- $\gamma$ , which  
316 could participate to the destruction of  $\beta$ -cells<sup>4,5</sup>. Most interestingly, in the youngest children  
317 with recent onset T1D, usually associated to a more aggressive disease<sup>35,36</sup>, circulating MAIT  
318 cells express the highest level of GzB. This parameter has the strongest correlations with  
319 clinical characteristics, age at diagnosis and % of HbA1c. Of note, increased GzB production  
320 was not observed in other T cell populations (data not shown). Moreover co-culture  
321 experiments show that MAIT cells are able to directly kill human  $\beta$  cell line.

322 In contrast to the pancreas, in the gut mucosa MAIT cells might play a protective role  
323 through their production of IL-17A and IL-22, two key cytokines in intestinal  
324 homeostasis<sup>41,42,56,57</sup>. The presence of gut alterations as T1D progress in NOD mice  
325 underscores the importance of MAIT cells in maintaining gut mucosa homeostasis. Moreover  
326 the lack of MAIT cells in MR1<sup>-/-</sup> NOD mice is associated with a loss of gut integrity shown by  
327 FITC-dextran assay, decreased expression of tight junction proteins and abnormal mucus  
328 distribution. Absence of MAIT cells in NOD mice favors gut leakiness, associated with  
329 translocation of bacteria components, such as 16S bacterial DNA, from the gut to peripheral  
330 tissues. The presence of bacterial DNA could promote T1D development through DC  
331 activation via TLR receptors in PLN and increased anti-islet pathogenic T cell responses.  
332 Altogether, our data support the model in which MAIT cell deficiency aggravates gut mucosa  
333 alterations, thereby promoting T1D development. These data are reminiscent of recent  
334 reports showing a critical link between the gut mucosa and the development of  
335 inflammatory and autoimmune diseases including T1D<sup>44,51,52</sup>. MAIT cells could act as a sensor  
336 of environmental changes by responding to alteration of gut mucosa by modulating the host  
337 immune system. Their abundance and activation status (CD69, cytokine production) in the  
338 gut could explain the prominent regulatory role of MAIT cells in the intestinal mucosa of  
339 NOD mice.

340 Overall the present study reveals MAIT cell alteration in T1D patients as well as in NOD mice  
341 and highlights the protective role of MAIT cells against diabetes, despite their infiltration in  
342 the pancreas of NOD mice and their production of GzB and IFN- $\gamma$ . These data lead us to  
343 propose that MAIT cells are at the crossroad between gut mucosa, inflammation and T1D  
344 (**supplementary Fig. 15**). Interestingly, MAIT cell alteration in both patients and mice has  
345 been detected before the onset of diabetes, indicating that MAIT cell could represent a new  
346 biomarker of T1D development. Moreover, our data suggesting the protective role of MAIT  
347 cells in NOD mice at the level of the gut mucosa could pave the way for the development of  
348 new therapeutic strategies based on their local triggering in this tissue at early phase of  
349 disease development.

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375

376 **Author contributions**

377 OR, JDS, LB, IN performed most of the experiments and data analyses; CT, BK, LC performed  
378 experiments; MO and MD participated to experiments with EndoC-β1 cell line; JR, JMC  
379 provided mouse MR1 tetramers; OL provided MR1<sup>-/-</sup> C57BL/6 and B6-MAIT<sup>CAST</sup> mice and

380 reagents; MB, MP and JB characterized patients and provided human samples; OR, IN, AL wrote  
381 the manuscript; JR, JMC, RS and OL provided intellectual input. AL supervised the work.

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- 509

## 510 **Online Methods**

511 **Human samples.** Peripheral blood samples were obtained from control children and from  
512 T1D children admitted in the Pediatric Endocrinology department of Necker hospital, Paris,  
513 France at T1D onset (i.e. within 10 days from first insulin injection), or with established  
514 disease. All control children, were children presenting at the hospital for growth retardation,  
515 short stature or precocious puberty. None of them had any other medical conditions or  
516 history of autoimmune disease. Patients with growth retardation or short stature were  
517 admitted for a growth hormone stimulation test. All of them were previously screened for  
518 coeliac disease or autoimmune thyroiditis that can be an etiology of short stature. This  
519 screening was negative for all patients. All of these "control" children were diagnosed with  
520 idiopathic growth hormone deficiency or idiopathic short stature. Patients with precocious  
521 puberty didn't have either any story of autoimmune disease. They were admitted for a  
522 gonadotropin-releasing hormone stimulation test and were diagnosed with idiopathic  
523 central precocious puberty. As reported in the method section, all "control" patients were  
524 screened for T1D related antibodies that were negative. Non-inclusion clinical following  
525 parameters contain: infection during the admission and associated other autoimmune  
526 diseases. The Ethics Committee (Comité de protection des personnes (CPP) Ile-de-France)  
527 approved the clinical investigations and written informed consent was obtained from all the  
528 parents. For the Milan cohort, peripheral blood from healthy control subjects and patients at  
529 T1D onset (i.e., within 10 days from first insulin injection) were collected. The study was  
530 approved by the San Raffaele Hospital Ethic Committee (protocol: DRI-003). At risk subjects  
531 were enrolled in the Type 1 Diabetes TrialNet Pathway to Prevention Trial (TN01 trial, former  
532 TrialNet Natural History Study)<sup>58,59</sup>. The overall objective of this study is to perform baseline  
533 and repeated assessments over time of the immunologic and metabolic status of individuals  
534 who are at risk for T1D (first/second degree relatives of patients with T1D). The study was  
535 approved by the San Raffaele Hospital Ethics Committee (protocol: NHPROTOCOL32803  
536 TN01). Our local study was approved by the TrialNet Ancillary Studies Subcommittee. All  
537 subjects included in this study signed the informed consent prior to blood donation.

538

539 **Mice.** MR1-/+ NOD mice was generated by backcrosses (>15 times) of MR1<sup>-/-</sup> C57BL/6J<sup>18,33</sup>  
540 on the NOD background, 17 insulin-dependent diabetes (Idd) loci associated with

541 susceptibility to T1D have been checked on all chromosomes. MR1<sup>-/-</sup> NOD mice were  
542 generated by intercross of MR1<sup>-/+</sup> NOD mice and the analyses were performed with MR1<sup>-/-</sup>  
543 and MR1<sup>-/+</sup> NOD littermates kept in co-housed cages. Transgenic ROR- $\gamma$ t-GFP NOD mouse  
544 was generated by microinjection of ROR- $\gamma$ t-GFP BAC construct<sup>60</sup> into NOD embryos. B6-  
545 MAIT<sup>CAST</sup> mice were provided by O. Lantz<sup>39</sup>. All mice were bred under specific pathogen-free  
546 conditions and the study was performed on females, except the steptozotocin experiments.  
547 NOD mice were tested weekly (daily in steptozotocin experiments) and considered diabetic  
548 after two consecutive positive urine glucose tests (DIABUR-TEST 5000, Roche), confirmed by  
549 a glycemia > 200 mg/dl (ACCU-CHEK, Roche). This study was approved by the ethics  
550 committee on animal experimentation CEEA 34 (APAFIS N°2015102016444419).

551

552 **Cell preparations.** Human peripheral blood mono-nucleated cells (PBMC) of patients from  
553 Necker Hospital were isolated from fresh blood samples by Ficoll-Paque (Leucosep) or  
554 samples from San Raffaele hospital were defrosted in RPMI with 10% FCS (Fetal Cow Serum).  
555 Mouse cells were prepared from different tissues as described below. For Pancreatic islets  
556 isolation, mice were sacrificed and pancreas was perfused with 3 mL of a collagenase P  
557 solution (0.8 mg/mL, Roche), which was then isolated and set free from surrounding tissues  
558 and lymph nodes. Digestion of the pancreas was performed at 37°C for 10 min and was  
559 stopped by adding large volume of cold 5% FCS RPMI before extensive washes. Pancreatic  
560 islets were then purified on a Ficoll discontinuous gradient. For immunofluorescence  
561 analysis, islets were dissociated with a non-enzymatic cell-dissociation solution (Sigma). For  
562 Intestine cell isolation, Miltenyi Lamina Propria Dissociation Kit for mouse was used to  
563 prepare epithelial and lamina propria cells. Mice were sacrificed and intestine was removed  
564 from mice. Intestine was cleared of feces, fat tissue and Peyer's patches with HBSS without  
565 Ca<sup>2+</sup> and Mg<sup>2+</sup> containing 10 mM HEPES solution. After passage through 100  $\mu$ m filter, the  
566 cell suspension was subjected to Percoll (GE Healthcare) density gradient of 40% and 80%  
567 and the interface between the layers containing lamina propria lymphocytes were collected  
568 and suspended in PBS containing 2% FCS and 0.1% sodium azide. For the preparation of  
569 mouse dendritic cells from lymph nodes and spleen, mice were sacrificed and organ were  
570 cut in small pieces and then digested at 37°C for 30 min with 2 mL of collagenase D (1.0

571 mg/mL, Roche) solution. Digestion was stopped by adding a large volume of cold 5% FCS  
572 RPMI, and cells were washed and filtered on cell strainer (40  $\mu$ m, BD Falcon) before staining.

573

574 **Flow cytometry and antibodies.** Cells were stained in PBS containing 5% FCS and 0.1%  
575 sodium azide. For human PBMC the following antibodies were used: CD3 (OKT3), CD4  
576 (OKT4), V $\alpha$ 7.2 (3C10), CD161 (HP-3G10), CCR6 (G034E3), CD56 (HCD56), CD69 (FN50), Bcl-2  
577 (100), IFN- $\gamma$  (4S B3), IL-17A (BL168), TNF- $\alpha$  (MAb11) and MR1 (26.5) mAbs from BioLegend;  
578 CD8 (SK1), PD1 (MIH4), CD25 (M-A251), IL-4 (8D4-8), granzyme B (GB11) mAbs and CD107a  
579 (HYA3) from BD Biosciences; CD3 (REA), CD161 (REA) mAbs from Miltenyi and CD4 (RPA-T4)  
580 mAb from eBiosciences. According to the PBCM number obtained from each patient, surface  
581 staining was always performed, and when possible due to the number of PBMC  
582 intracytoplasmic staining of cytokines and GzB after PMA-ionomycin stimulation was  
583 analyzed, and then PBMC bcl-2 expression was analyzed by intracytoplasmic staining  
584 (without stimulation). Data acquisition was performed using BD Biosciences LSR-Fortessa  
585 cytometer or FACS ARIA III cytometer for cells from patients from Necker hospital and  
586 Beckman Coulter Gallios for cells from patients from San Raffaele hospital.

587 Staining of mouse cells was performed with the following antibodies: TCR $\beta$  (H57), TCR-  
588  $\gamma\delta$  (GL3), CD45 (30F11), CD8 $\alpha$  (53-6.7), CD103 (M290), CD44 (IM7), I-Ak (10-3.6CD45), CD86  
589 (GL1), IL-17A (TC11-18H10) and IFN- $\gamma$  (XMG1.2) mAbs, were from BD Biosciences; CD19  
590 (6D5), CD4 (GK1.5), CD11b (M1/70), PLZF (9E12), T-bet (4B10), CD25(PC61), CD69 (HI-2F3),  
591 CD304 (3E12), and CXCR3 (SA011F11) mAbs, were from Biolegend; CD11c (N418), CD170  
592 (1RNM44N), F4/80 (BM8), ROR- $\gamma$ t (B2D) and FOXP3 (FJK-16s) mAbs were from eBioscience.  
593 Alpha-Galactosylceramide-CD1d tetramer was prepared by the laboratory and coupled to  
594 streptavidin-BV421 (Biolegend). NRP-V7 tetramer (IGRP206-214 (KYNKANVFL) reactive T cell)  
595 and TUM tetramer (TUM (KYQAVTTTL) reactive T cell, were provided by the National  
596 Institutes of Health tetramer core facility. Biotinylated mouse MR1 tetramers loaded with  
597 the active ligand (5-OP-RU) were used to specifically identify MAIT cells and biotinylated  
598 MR1 tetramers loaded with the non-activating ligand 6-formyl-pterin (6-FP) were used as a  
599 negative control. MR1 tetramers were coupled to streptavidin-PE (BD Biosciences) or  
600 Streptavidin-BV421 (Biolegend) and cells were stained with tetramers for 45 min at RT  
601 before surface staining with mAbs. Data acquisition was performed using a BD Biosciences

602 LSRFortessa or FACS-ARIA III cytometer. Flow cytometric analyses were performed with the  
603 FlowJo analysis software V10.1 (Tree Star) and the Spice software V5.3 was used for studying  
604 the multiparametric combination of cellular staining.

605

606 **In vitro cell stimulation.** For ligand stimulation of human MAIT cells, 5-OP-RU solution was  
607 obtained after incubating 1 molar equivalent of 5-A-RU with 2 molar equivalent of  
608 methylglyoxal (Sigma-Aldrich). Co-culture of Hela, expressing MR1 molecules, and PBMC in  
609 media containing RPMI supplemented with 10% FCS were done to analyze MAIT cell  
610 activation from both controls and recent onset T1D children in presence of MAIT cell ligand  
611 (5-OP-RU). Briefly, cells were plated at final concentration of  $1 \times 10^6$  PBMC/mL and  $1 \times 10^6$   
612 Hela/mL (1:1 ratio). In these cultures, MAIT cells were stimulated O.N. by the addition of  
613 their agonist ligand (5-OP-RU) at various concentration (0-5 nmol/L), in presence of blocking  
614 anti-MR1 (26.5 mAb) or not. Then, MAIT were stained with appropriate antibodies and cell  
615 surface activation markers were analyzed by flow cytometry.

616

617 **Intra-cellular staining.** For human Bcl-2 and mouse transcription factor staining, after  
618 surface staining lymphocytes were resuspended in fixation/permeabilization buffer (Foxp3  
619 staining kit from eBioscience) and incubated at 4°C in the dark then, cells were washed with  
620 PERM Wash buffer (eBioscience) and labeled with appropriate mAbs. For cytokine and  
621 granzyme B analysis of human MAIT cells, PBMC obtained from fresh samples, were  
622 analyzed after stimulation for 6 h at 37°C in RPMI medium supplemented with 10% FCS with  
623 PMA (25 ng/ml) and ionomycin (1 µg/ml), in the presence of Brefeldin A (10 µg/ml). For  
624 cytokine staining of mouse lymphocytes, cells were stimulated with PMA (10 ng/mL) and  
625 ionomycine (1 µg/mL) in the presence of Brefeldin A (10 µg/mL) for 4 h at 37°C (all reagents  
626 from Sigma-Aldrich) or cells were stimulated in vivo by i.v. injection of flagellin (FLA-PA) at 3  
627 µg/mouse 2 h before analysis, as previously described<sup>61</sup>. After surface staining, cells were  
628 fixed and permeabilized with Cytofix/Cytoperm kit (BD Biosciences) washed and incubated  
629 at 4°C in the dark for 30 min with anti-cytokines.

630



631  **$\beta$ -cell death induction by MAIT cells.** EndoC- $\beta$ H1<sup>37</sup> was provided and cultured by R.  
632 Scharfmann laboratory. EndoC- $\beta$ H1 were plated at final concentration of  $0.35 \times 10^6$  cells/mL  
633 during 24 h in specifically formulated DMEM media containing 5.6 mM glucose, 2% BSA  
634 fraction V (Roche diagnostics), 50  $\mu$ M 2-mercaptoethanol, 5  $\mu$ g/ml transferrin (Sigma-  
635 Aldrich), 6.7 ng/ml selenite (Sigma-Aldrich), 100 U/mL penicillin, 100  $\mu$ g/mL streptomycin.  
636 One day later, when indicated cytokines (TNF- $\alpha$  1.100 U/mL, IL-1 $\beta$  1.000 U/mL, IFN- $\gamma$  2.000  
637 U/mL) were added in EndoC- $\beta$ H1 cultures for 24 h. In parallel MAIT cells from donors were  
638 purified by negative isolation with Dynabeads Goat anti-Mouse IgG (Invitrogen), then stained  
639 with appropriate antibodies and sorted by FACS-ARIA. After sorting, MAIT cells were  
640 cultured in complete medium containing RPMI supplemented with 10% FCS, 1% penicillin/  
641 streptomycin, 1% HEPES and supplemented with 25 ng/mL recombinant human IL-7 (R&D  
642 Systems) for 48 h. Then, MAIT cells were added to EndoC- $\beta$ H1 cultures at 1:1 ratio and  
643 cultured 24 h in presence of different concentrations of 5-OP-RU. Blocking MR1 mAb (26.5)  
644 was added when indicated. MAIT cells were recovered in the supernatants and analyzed by  
645 flow cytometry for their surface expression of CD25 and CD107a. Adherent EndoC- $\beta$ H1 cells  
646 were harvested and stained with APC Annexin V Apoptosis Detection Kit with PI (Biolegend)  
647 according to the manufacturer's instruction. Cells were analyzed using LSRFortessa  
648 cytometer.

649

650 **Isolation of RNA and real-time reverse PCR analysis.** Mouse cells were lysed in RLT buffer  
651 with 1%  $\beta$ -mercaptoethanol, and mRNA was purified from lysed cells using RNeasy Mini Kit  
652 (Qiagen). cDNA was produced using the Superscript III reverse transcriptase (Invitrogen).  
653 Quantitative-PCR analysis was performed with SYBR Green (Roche) and analyzed with a  
654 LightCycler 480 (Roche). The relative expression was calculated using the  $2^{-\Delta\Delta Ct}$  method and  
655 normalized to the expression of the housekeeping gene GAPDH. The stability of GAPDH  
656 expression was confirmed by comparison with HPRT mRNA. The primers used for qPCR are  
657 described in **Supplementary Table 5**.

658

659 **MAIT cell transfer experiment.** CD45.2 MAIT cells were obtained from 9-10 week-old V $\alpha$ 19  
660 transgenic C $\alpha$ -/- NOD mice. T cells were isolated from splenocytes, MLNs and PLNs by

661 depleting B cells, monocytes/macrophages, NK cells, dendritic cells, erythrocytes, and  
662 granulocytes using Dynabeads Untouched Mouse T Cells kit (Invitrogen). These cells were  
663 injected i.v. ( $4 \times 10^6$  cells/mouse) into CD45.1 NOD females and analyzed several days later  
664 as indicated.

665

666 **MAIT cell ligands in intestinal content.** WT3-MR1+ cell line, provided by O. Lantz, was used  
667 to detect 5-OP-RU MAIT cells ligand from intestinal contents. Two hours before the  
668 experiment, WT3-MR1+ cells were coated onto a 96-flat-well plate at final concentration of  
669  $0.5 \times 10^6$  cells/mL in media containing DMEM Glutamax 10% FBS, HEPES 1M, sodium  
670 pyruvate 100 mM, NeAA 1% and 1% penicillin/streptomycin. Intestinal content from colons  
671 were recovered, weighed and resuspended in PBS. Fecal supernatants were centrifuged at  
672 14,000 rpm, 5 min, at 4°C and passed through a 0.22  $\mu$ M filter. Supernatants were tested at  
673 final concentration of 0.7 mg intestinal content/mL. Serial dilutions of 5-OP-RU were used as  
674 positive controls and acetyl-6-FP was added to determine MR1-specific activation. MAIT cells  
675 from the spleen of V $\alpha$ 19 transgenic C $\alpha$ -/- mice were purified by negative isolation with  
676 Dynabeads Untouched Mouse T Cells kit (Invitrogen) and co-cultured O.N. with WT3-MR1+  
677 cells at final concentration of  $5 \times 10^5$  MAIT cells/mL. One day after, MAIT cells were stained  
678 with appropriate antibodies and activation was analyzed by flow cytometry.

679

680 **Anti-islet T cell response.** Cells from mouse pancreatic islets were recovered and then  
681 cultured for 7 days in RPMI medium containing 10% FCS, 1% penicillin/ streptomycin and 25  
682 U/mL of human rIL-2. To evaluate peptide-specific reactive T cells, single cell suspensions  
683 from islet culture were stimulated for 5 h with IGRP206-214 peptide (the last 4 h in presence  
684 of Brefeldin A) and stained with CD45, CD19, TCR $\beta$ , CD8 and IFN- $\gamma$  mAbs.

685

686 **In vivo analysis of intestinal permeability.** Intestinal permeability was determined in MR1-/-  
687 and MR1+/- NOD mice by measuring the level of FITC-dextran in the blood. Briefly, mice  
688 were water-starved overnight and in the morning, FITC-dextran (40 kDa; Sigma) was  
689 administered by oral gavages (44 mg/100 g body weight). Blood was collected 4 h later and

690 centrifuged at 3000 g for 20 min at 4°C. Plasma (50 µL) was diluted volume to volume with  
691 PBS to determine fluorescence using a SPARK 10M (TECAN). The concentration was  
692 determined using FITC-dextran dilutions.

693

694 **Alcian blue staining and analysis.** Intestinal sections (1 cm in length) of ileum from mice  
695 were collected and immediately fixed in 4% paraformaldehyde PBS. Paraffin-embedded  
696 sections from ileum were cut into 4 µm slices and stained with Alcian blue periodic acid  
697 using standard techniques. Slices were visualized under a light microscope (Leica) and mean  
698 intensity of staining were evaluated with the software inForm 2.0.2 and image J.

699

700 **DNA extraction and 16S rRNA gene.** PLN were recovered from 15-20 week-old MR1<sup>-/-</sup> and  
701 MR1<sup>+/-</sup> NOD mice and DNA extraction was performed using DNeasy Blood and tissue Kit  
702 (QIAGEN) following the manufacturer's instructions. Quantitative-PCR analysis was  
703 performed with SYBR Green (Roche) and analyzed with a LightCycler 480 (Roche). The  
704 relative expression was calculated using the  $2^{-\Delta\Delta C_t}$  methods and normalized to the expression  
705 of the housekeeping gene GAPDH. The primers used for qPCR are described in  
706 **Supplementary Table 5.** The PCR products were sequenced to confirm their bacterial origins.

707

708 **Statistical analysis.** For human studies, statistical tests between two groups were performed  
709 using two-tailed Mann-Whitney test and signed-rank Wilcoxon test with Graph Pad Prism.  
710 The Kruskal-Wallis test followed by the Wilcoxon rank sum and the Spearman correlation  
711 test was applied for all the correlation analysis with Software R. Corrections for multiple  
712 testing had been performed on the human results when we compared flow cytometry data  
713 between the three groups (the children with recent onset T1D, children with established T1D  
714 and control children); Correction have well been applied after using the signed-rank  
715 Wilcoxon test with an adjustment of the p-value with the Holm method to take into account  
716 the fact that multiple testing have been performed. A factorial discriminant analysis was  
717 performed using the XLSTAT 2016 Software. A logistic regression model was fitted with the  
718 PROC CANDISC software (SAS version p.3) then a backward elimination procedure was  
719 applied. Prognostic validity of the model was evaluated by the receiver operating

720 characteristic (ROC) curve analysis and measured using the area under the ROC curve (AUC).  
721 Statistical analyses were performed using the GraphPad Prism software version 5.00.288 and  
722 the R software version 3.2.3. For mouse studies, statistical analysis was performed with  
723 Prism software (Graph Pad) using nonparametric tests two-tailed Mann-Whitney or  
724 Student's t-test, as appropriate. Diabetes incidence was plotted according to the Kaplan–  
725 Meier method and statistical differences between groups were analyzed using the Gehan–  
726 Breslow-Wilcoxon test or the log-rank test. P values were considered statistically significant  
727 (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001).

**Figure 1. Frequency and phenotype alterations of blood MAIT cells from T1D children.**

MAIT cells were analyzed in blood from children with recent onset T1D (n=41), children with established T1D (n=23), as compared with control children (n=22). **(a)** Representative staining of MAIT cells and MAIT cell frequency among T lymphocytes. **(b)** Representative staining and frequency of MAIT cells expressing different cell surface molecules (CCR6, CD56, CD69, CD25, PD1) and intracellular Bcl-2. P values were determined by Kruskal-Wallis test followed by the Wilcoxon rank sum test adjusted with the Holm method. **(c)** Multi-parametric analysis of the frequency of MAIT cells expressing the different combination of the five surface molecules: CCR6, CD56, CD25, CD69 and PD1. Threshold for representation is cell frequency >0.36% of MAIT cells.

**Figure 2. Functional alterations of blood MAIT cells from T1D children.**

MAIT cells were analyzed in blood from children with recent onset T1D (n=25) and children with established T1D (n=18), as compared with control children (n=18). **(a)** Representative intracellular staining of MAIT cells for cytokines and GzB after PMA/ionomycin stimulation and graphs showing the frequency of MAIT cells producing cytokines and GzB. P values were determined by Kruskal-Wallis test followed by the Wilcoxon rank sum test adjusted with the Holm method. **(b)** Multi-parametric analysis of the frequency of MAIT cells expressing the different combination of the five intracellular molecules: GzB, IFN- $\gamma$ , TNF- $\alpha$ , IL-17 and IL-4. Threshold for representation is cell frequency >0.36% of MAIT cells. Note that data for IL-4 are not shown because IL-4 is expressed with other cytokines and the frequency of each MAIT cell population expressing IL-4 is >0.36%. **(c)** Frequencies of CD69<sup>+</sup> and CD25<sup>+</sup>CD69<sup>+</sup> MAIT cells from children controls (n=6) and children with recent onset T1D (n=7) after ON stimulation with 5-OP-RU at various concentrations (0 to 5 nmol/L). Blocking MR1 mAb was added when indicated. P values were determined by Mann-Whitney test.

**Figure 3. Cytotoxic function of MAIT cells on human  $\beta$  cell line.**

**(a)** Correlation between the age of children with recent onset T1D (n=25) at diagnosis, HbA1c level and the frequency of blood MAIT cells expressing GzB after PMA/Ionomycin stimulation. P values were determined by Spearman test. **(b)** EndoC- $\beta$ H1 cells were cultured O.N. with or without cytokines. Cells were stained to analyze MR1 cell surface expression by flow cytometry (n=4) or directly lysated to analyze the relative quantity of MR1 mRNA by RT q-PCR (n=7). For MR1

cell surface expression MFI  $\pm$  SEM was indicated for each groups and for q-PCR, statistical analysis was performed by two-tailed Mann Whitney test, P-value was indicated on graph and bar represent mean  $\pm$  SEM. **(c-e)** Induction of EndoC- $\beta$ H1 cell apoptosis by MAIT cells in co-culture in presence of MAIT cell ligand (5-OP-RU). MAIT cells from healthy donors were sorted and cultured with IL-7 during 48h. EndoC- $\beta$ H1 cells were cultured with or without cytokines during 24h. Then, MAIT cells and EndoC- $\beta$ H1 cells were co-cultured for 24h at 1:1 ratio with different doses of 5-OP-RU. **(c)** Representative FACS plots of **(d)** EndoC- $\beta$ H1 apoptosis by MAIT cells at different doses of 5-OP-RU [0-1000 nmol/L] (n=4). Blocking MR1 mAb was added when indicated. Statistical analysis were performed by two-tailed Mann Whitney test, P-values were indicated on each graph and bar represent mean  $\pm$  SEM. **(e)** Activation and degranulation of MAIT cells in presence or not of ligand and anti-MR1 (n=2).

**Figure 4. MAIT cell characteristics in patients at different disease stages.** **(a)** MAIT cell staining of surface and intracellular molecules performed with PBMC from fifteen children with recent onset T1D at the time of diagnosis (on the left) and around one year after diabetes onset (on the right). P values were determined by Signed-rank Wilcoxon Test. **(b)** Factorial discriminant analysis based on the expression of surface and intracellular molecules by MAIT cells from the children with recent onset T1D (n=20), the children with established T1D (n=15) and the children controls (n=18). **(c)** ROC Curve of the predictive model defining the T1D phenotype. **(d)** FACS Analysis of MAIT cell frequency among T lymphocytes and frequency of MAIT cells expressing different cell surface molecules from frozen PBMC of adult controls (n=11) and adult at risk T1D donors with at least two autoantibodies (n=11). P values were determined by Mann-Whitney test.

**Figure 5. Characterization of MAIT cells in NOD and C57BL/6 mice.** MAIT cells were identified using mouse MR1-5-OP-RU tetramer and MR1-Ac-6-FP tetramer was used as control. MAIT cells are defined as positive MR1 tetramer (5-OP-RU) and low TCR $\beta$  staining. Flow cytometric analysis of spleen, pancreatic lymph nodes (PLN), pancreatic islets and ileum from C57BL/6 and NOD mice at 9-12 weeks of age. **(a)** Representative dot plots of MAIT cell staining and **(b)** frequencies and absolute numbers of MAIT cells among TCR $\beta$ <sup>+</sup> leucocytes. P values were determined by two-tailed Mann-Whitney test (statistical analysis between tissues from NOD mice; C57BL/6 analyses are not shown). **(c)** Representative dot

plots of CD4 and CD8 expression by MAIT cells in NOD mice and **(d)** graphs showing the proportion of MAIT cell subsets in tissues from C57BL/6 and NOD mice. Data correspond to 8 mice from three independent experiments. Bar represent mean  $\pm$  SEM. **(e)** Representative histograms and **(f)** graphs showing CD69, CD25 and CD44 expression on MAIT cells from NOD mice (white) and C57BL/6 (black). P values were determined by two-tailed Mann-Whitney test (statistical analysis between tissues from NOD mice; C57BL/6 analyses are not shown). **(g)** Representative dot plots and **(h)** graphs showing intracellular cytokine (IFN- $\gamma$ , TNF- $\alpha$ , IL-17A and IL-22) staining of MAIT cells in 10 week-old NOD mice (white) and C57BL/6 mice (black), after PMA/ionomycin stimulation.

**Figure 6. MAIT cell frequencies and function during diabetes development.** **(a)** MAIT cell frequency in different tissues from NOD mice at three stages of disease development (6 weeks, 16 weeks, and diabetic). For each tissue at least 6 mice per group from five independent experiments were analyzed. Statistics were performed with two tailed Mann-Whitney test. **(b)** Purified MAIT cells from CD45.2 V $\alpha$ 19 transgenic C $\alpha$ -/- NOD mice were transferred (i.v.) into CD45.1 NOD mice at 8-10 weeks of age or at 22-25 weeks of age (4.5  $10^6$  cells/mice). At day 5 after transfer, mice were sacrificed and islets and ileum were analyzed. Graphs and representative plots of the frequency and the absolute number of transferred MAIT cells are shown. **(c-d)** Graphs showing the relative quantity of (c) GzB, IFN- $\gamma$  transcripts in MAIT cells from pancreatic islets and (d) IL-22 and IL-17A transcripts in MAIT cells from ileum at three stages of disease development. Each symbol represents a pool of two mice and data were obtained in two independent experiments. Bars represent mean  $\pm$  SEM. **(e-f)** Cytokine intracellular staining after in vitro PMA/ionomycin stimulation (e) or in vivo flagellin stimulation (f) of MAIT cells and conventional  $\alpha\beta$ T cells from pre-diabetic (17 weeks) and diabetic mice; (e) GzB and IFN- $\gamma$  for islets and (f) IL-17A and IL-22 for ileum. Dot plots are representative of two independent experiments, with a total of 6 mice per group.

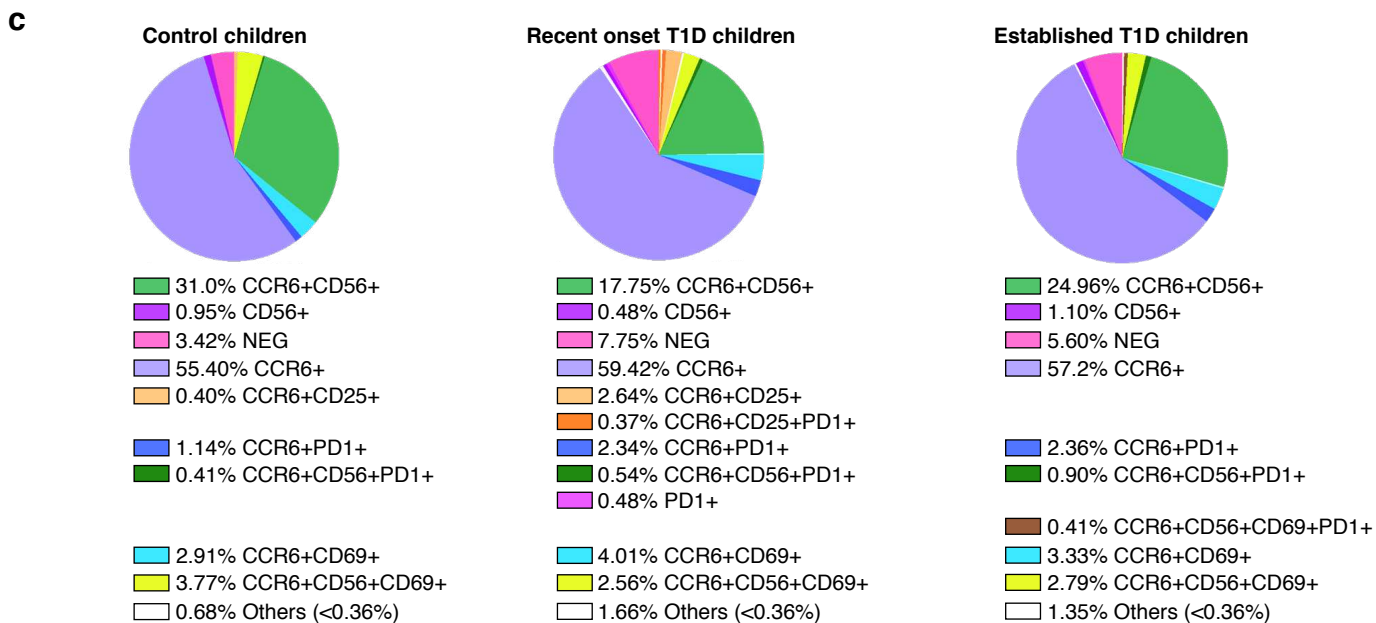
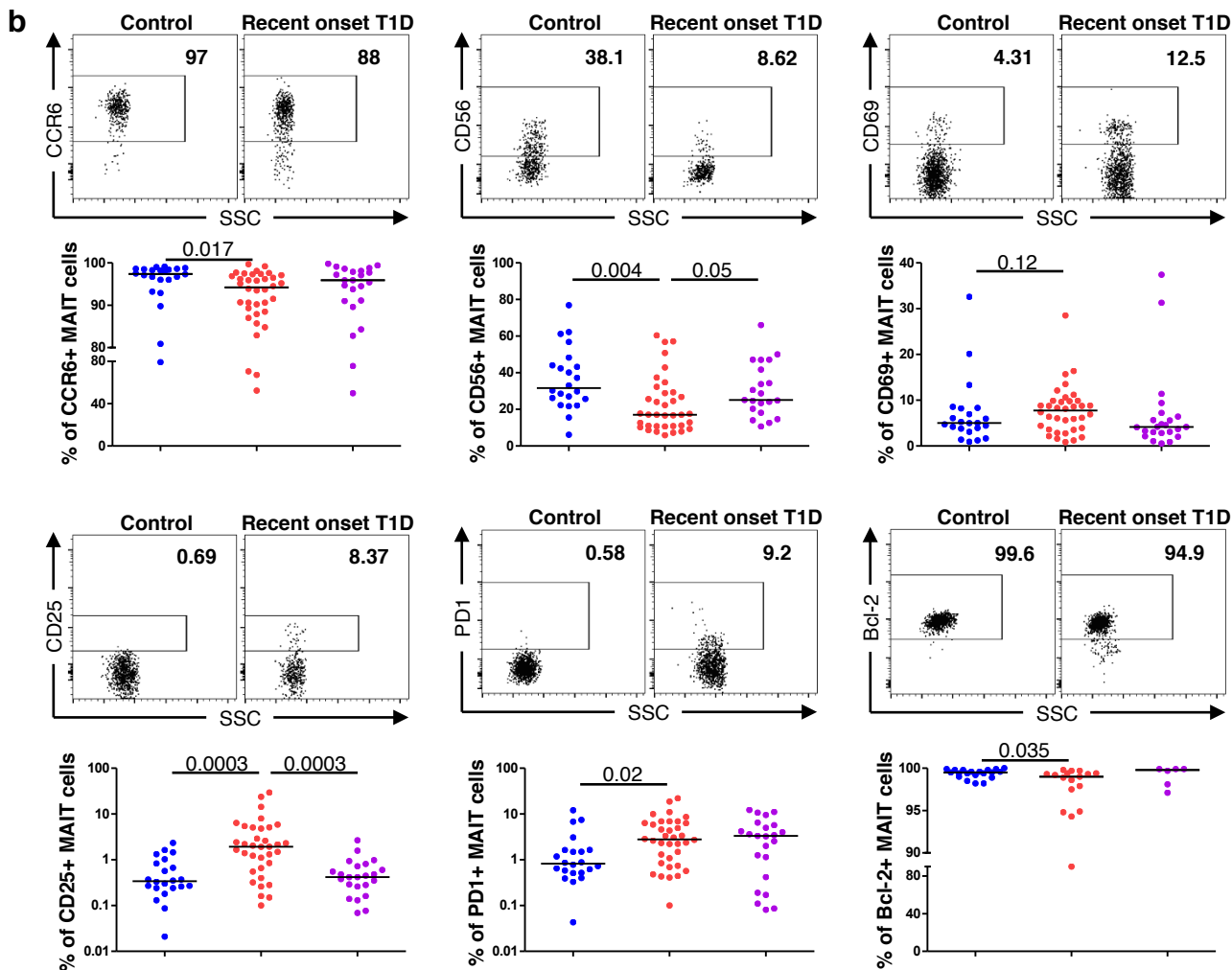
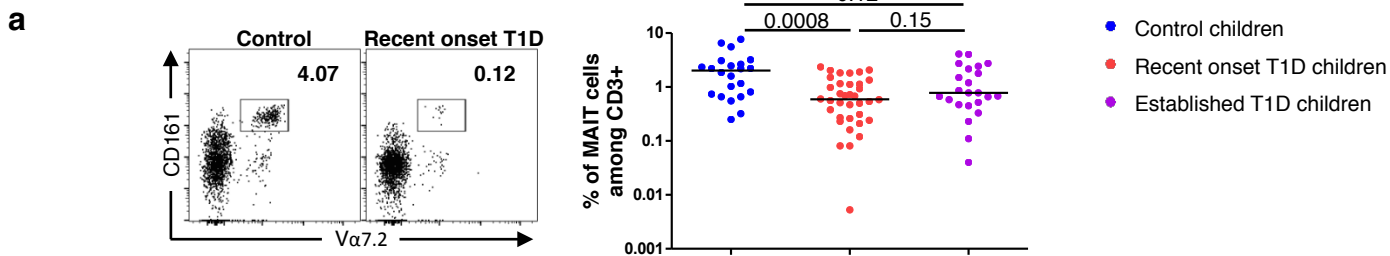
**Figure 7. Gut mucosa alterations during T1D progression in NOD mice.** **(a)** Representative dot plots of ROR $\gamma$ t-GFP expression by MAIT cells; representative histogram and graph showing MFI of ROR $\gamma$ t-GFP<sup>+</sup> MAIT cells at three stages of disease development. Data were pooled from two independent experiments. **(b)** Cells of ileal tissue from NOD mice were obtained and analyzed by RT-qPCR at two stages of the disease (10 weeks and diabetic

mice); the relative quantity of IL-23 transcript is shown. Data are pooled from two independent experiments. **(c)** Intestinal gut permeability was measured by in vivo FITC-dextran assay in NOD mice at two stages of the disease (10 weeks and diabetic). Data are representative of three independent experiments. **(d-e)** Representative FACS plots and dose response of MAIT cell activation after in presence of WT3- MR1, with different doses of 5-OP-RU ligand and in presence or absence of acetyl-6-FP inhibitor ligand at 10 $\mu$ M. **(d-f)** MAIT cells were activated by fecal supernatants in an MR1-dependent manner. The dose response of MAIT cells activation with different concentrations of fecal supernatants in presence or absence of acetyl-6-FP inhibitor ligand (n=16) is shown. **(g)** Activation of purified MAIT cells by fecal supernatant from NOD mice at different stages of disease development. Data were pooled from three independent experiments. All statistical analyses were performed by two-tailed Mann Whitney test, P-value were indicated on each graph and bar represent mean  $\pm$  SEM.

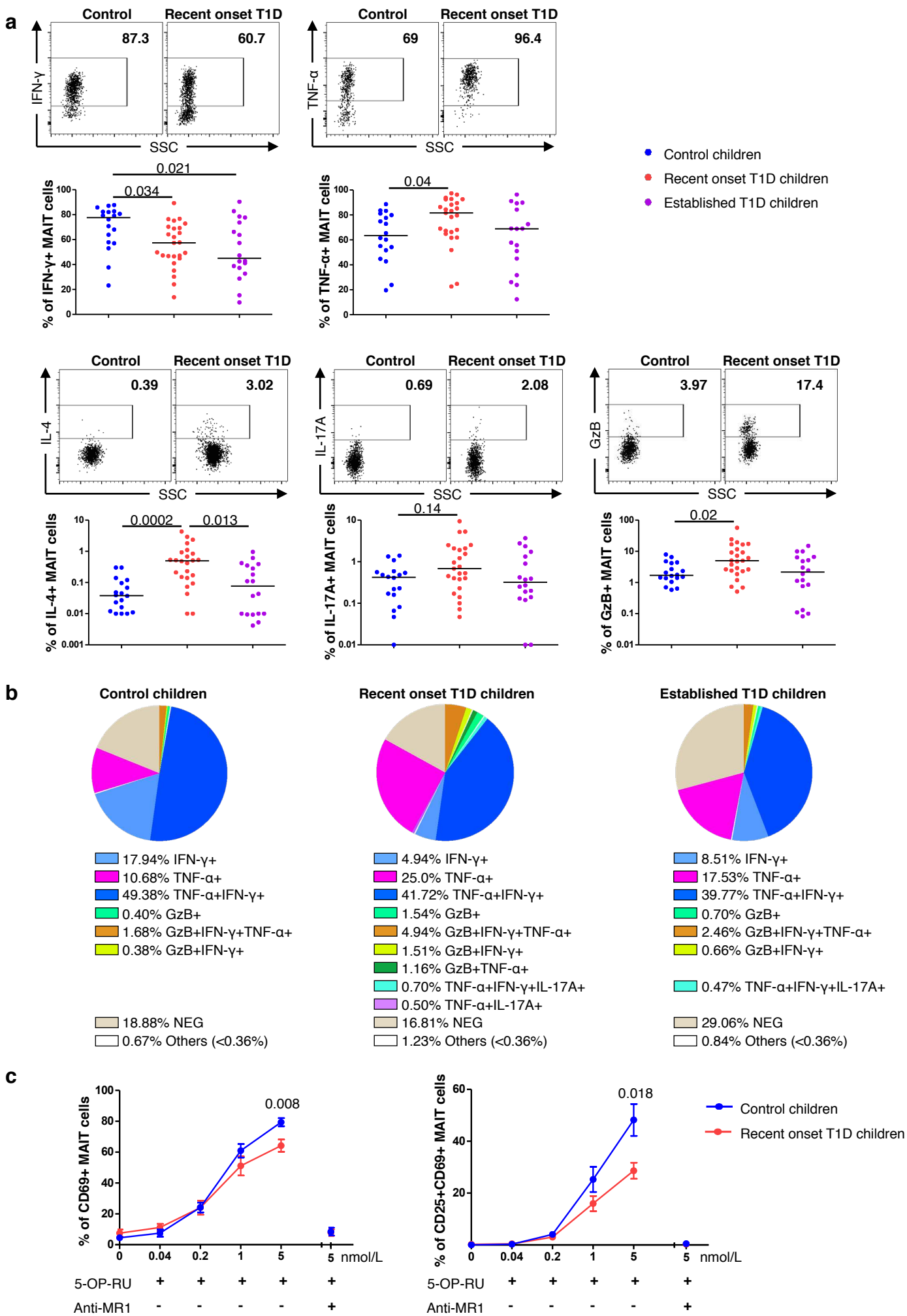
**Figure 8. MAIT cell deficiency exacerbates diabetes development and alters gut mucosa integrity.** All analyses were performed on co-housed MR1<sup>-/-</sup> and MR1<sup>+/-</sup> NOD female littermates. **(a)** Representative dot plots of T cell staining with MR1-5-OP-RU and CD1d- $\alpha$ -Galactosyl Ceramide tetramers of splenic cells, gated on  $\alpha\beta$ T cells. Numbers indicate the percentage of MAIT and iNKT cells among TCR $\beta$  cells. **(b)** Diabetes incidence of MR1<sup>-/-</sup> and MR1<sup>+/-</sup> mice (n=30 and 32 respectively); P values were determined by Gehan-Breslow-Wilcoxon test. **(c)** Representative dot plots and graphs showing percentage of IGRP<sub>206-214</sub> tetramer positive cells among TCR $\beta$ <sup>+</sup> and CD8<sup>+</sup> T cells. **(d)** Frequency of IFN- $\gamma$  IGRP<sub>206-214</sub> specific CD8 T cells from pancreatic islets after culture. **(e)** Analysis of CD11c<sup>+</sup>CD11b<sup>+</sup>CD103<sup>+</sup> dendritic cells from ileum, mesenteric (MLN) and pancreatic lymph nodes (PLN). Representative flow cytometry dots showing the gating strategy, MHC class II staining, and graphs showing the absolute numbers of this DC subset, the frequencies and absolute numbers of this DC subset expressing CD86 and MHC class II. **(f)** Intestinal permeability measured by FITC-dextran assay. **(g)** Occludin, mucin-2 and claudin-4 mRNA levels in epithelial cells from ileum. **(h)** Representative photographs of Alcian blue staining of ileum section and graph showing the mean intensity of the staining in Goblet cells. **(i)** Absolute numbers of lymphocytes infiltrating lamina propria. **(j)** DNA from PLN was extracted and then the relative quantity of 16S bacterial DNA was analyzed by qPCR. For graphs a and c-j,

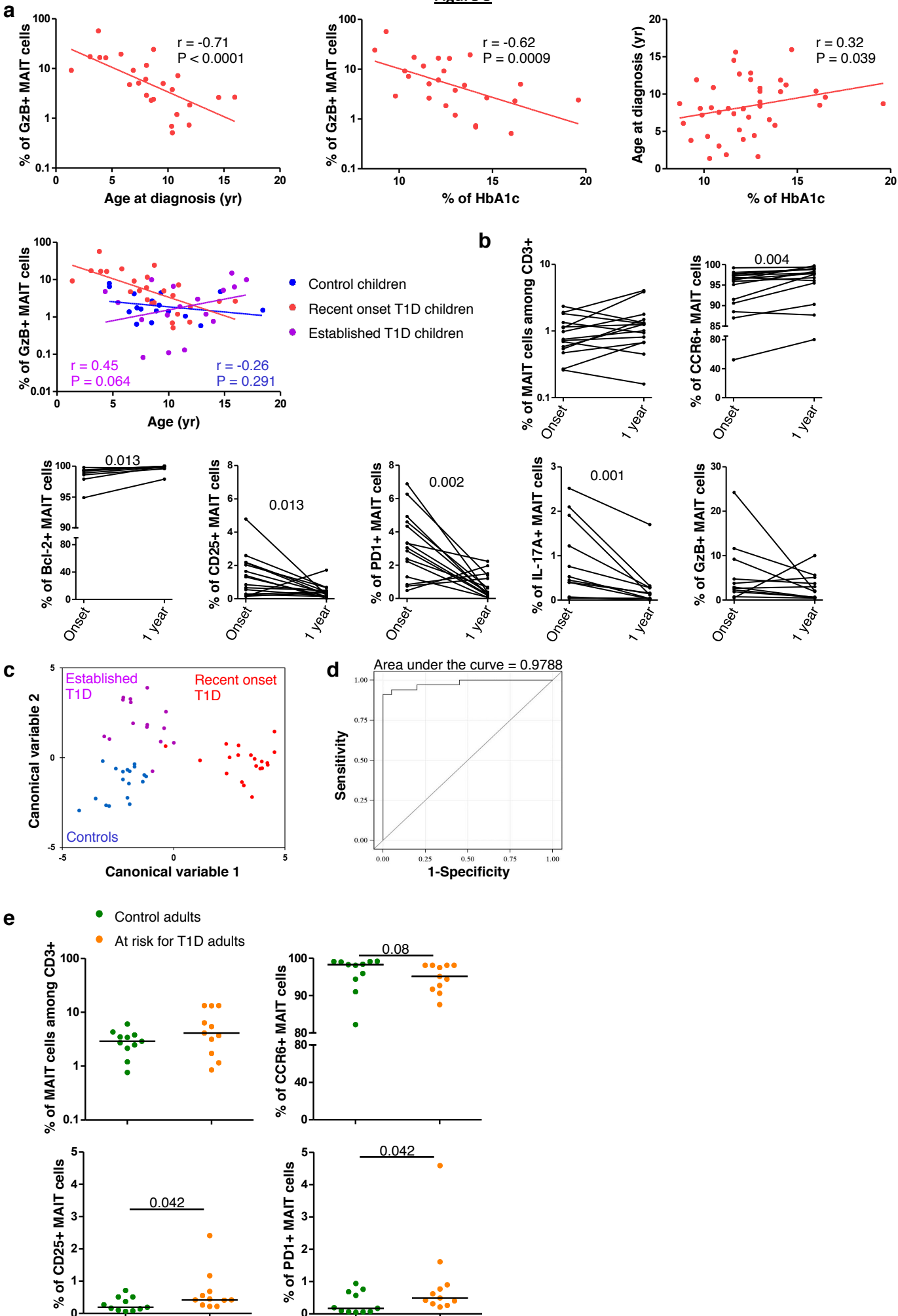


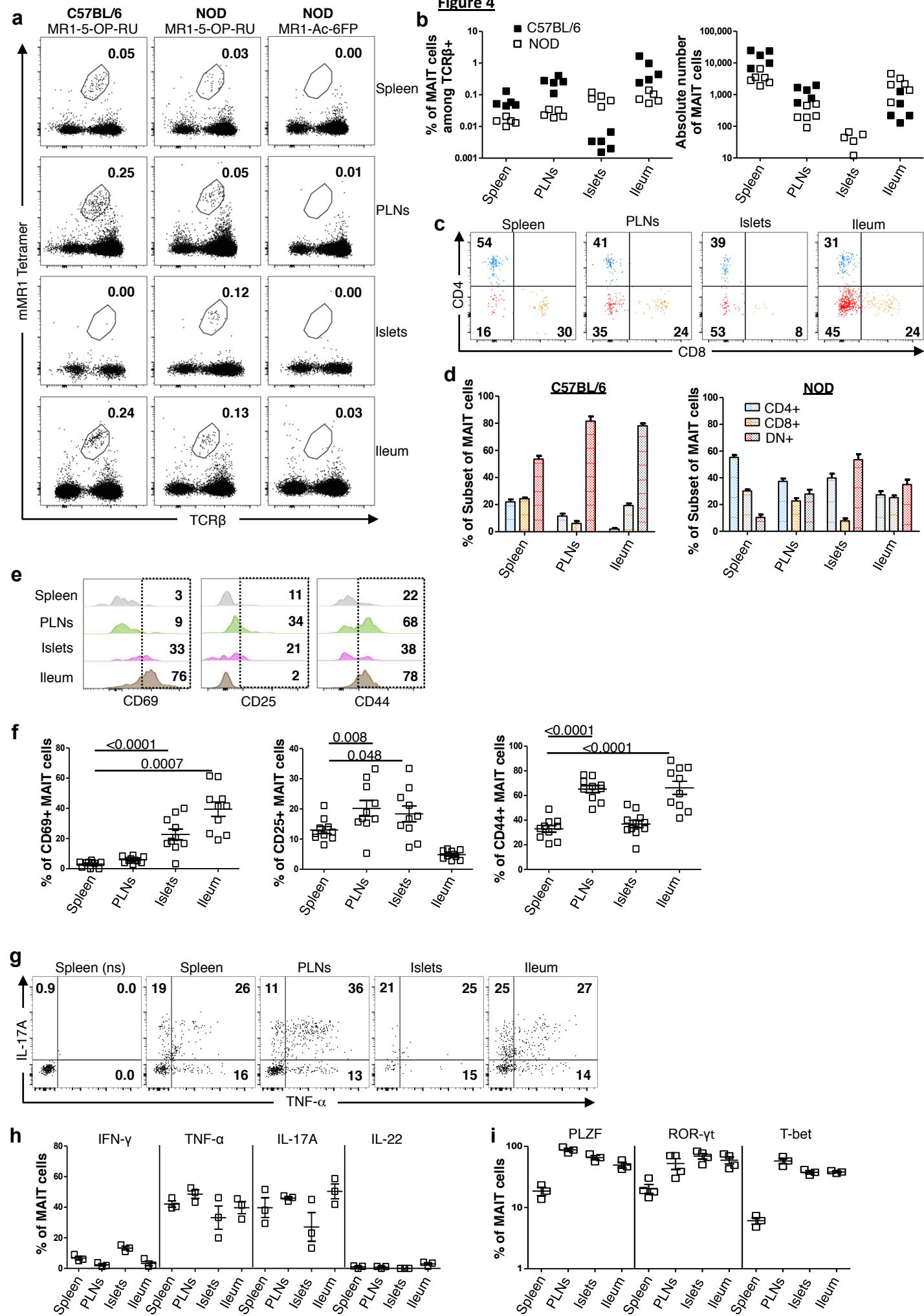
analyses were performed on 15 week-old mice, each point represents an individual mouse, data were pooled from two or three independent experiments, P values were determined by Mann-Whitney test. Bars represent mean  $\pm$  SEM.

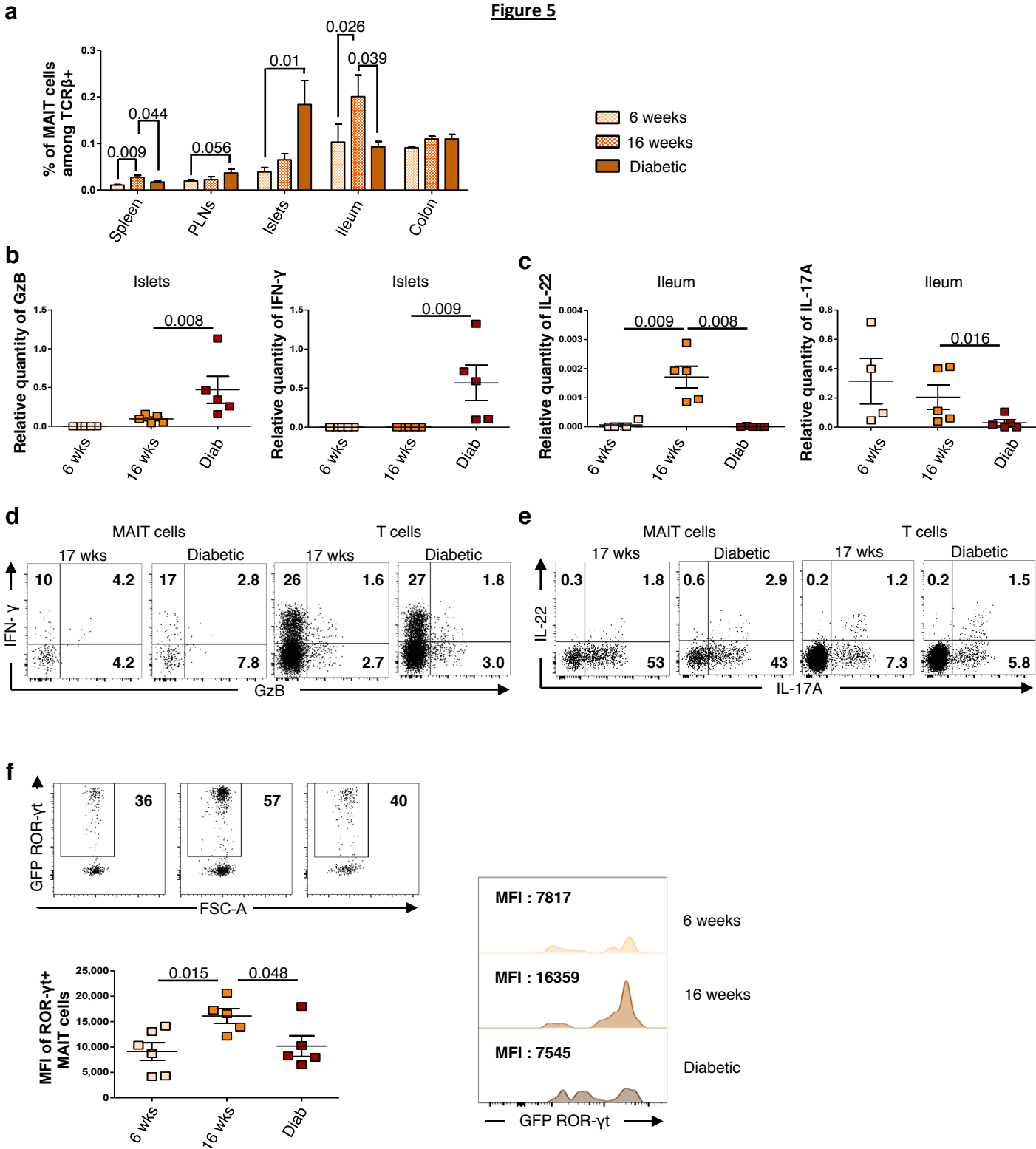
**Figure 1**

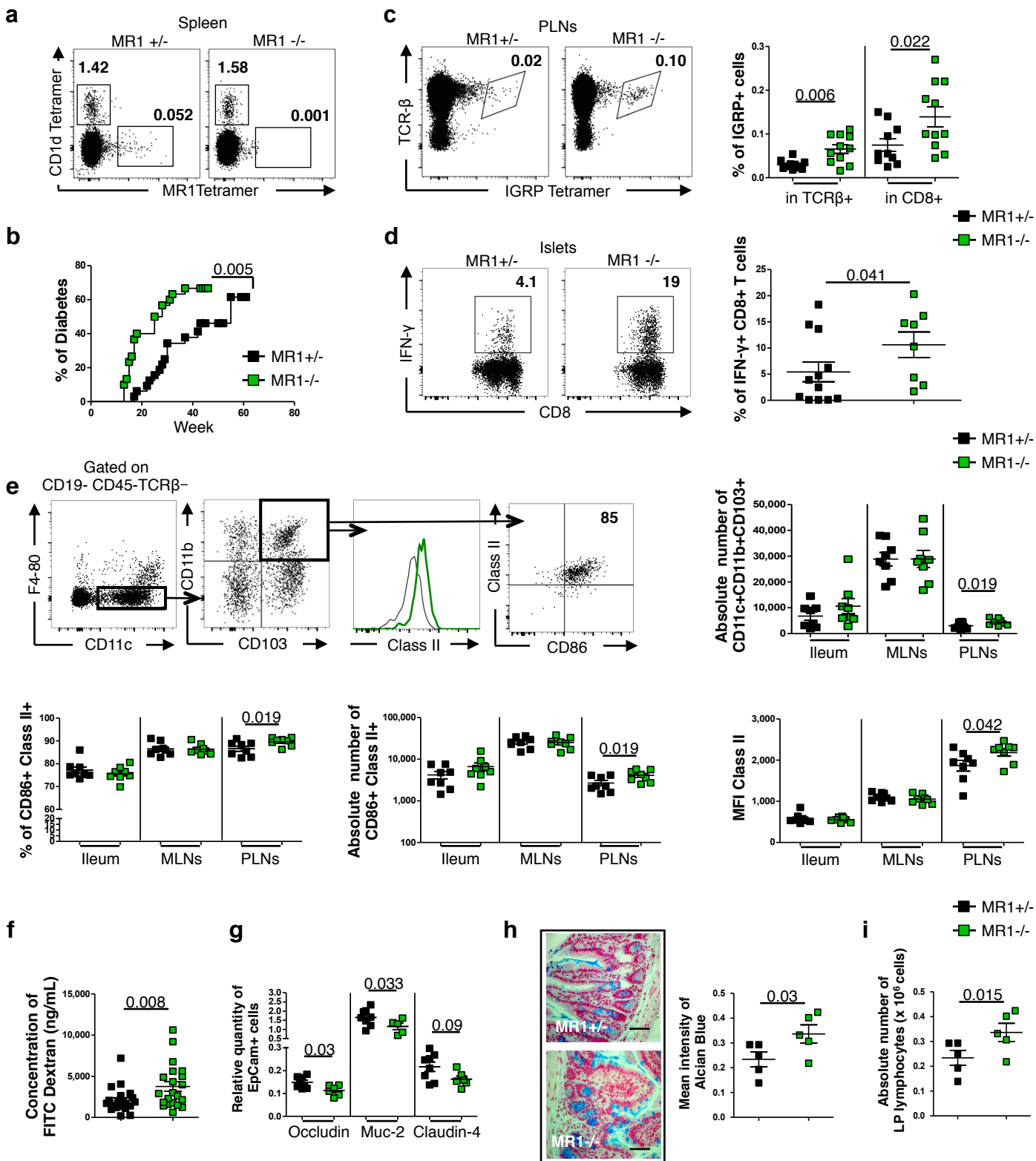
**Figure 2**

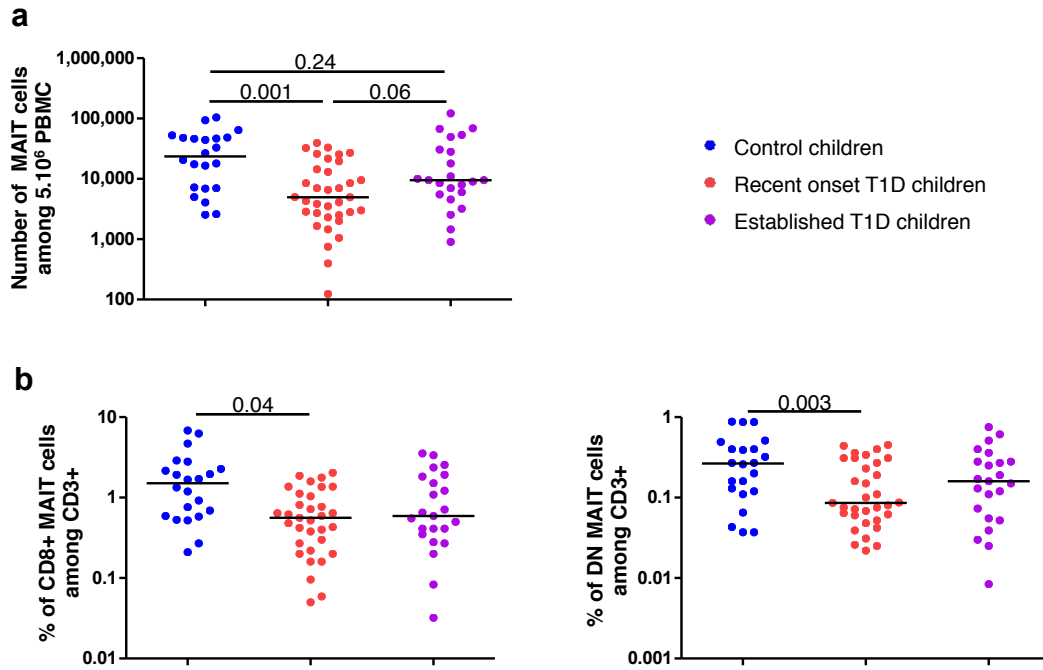


**Figure 3**

**Figure 4**

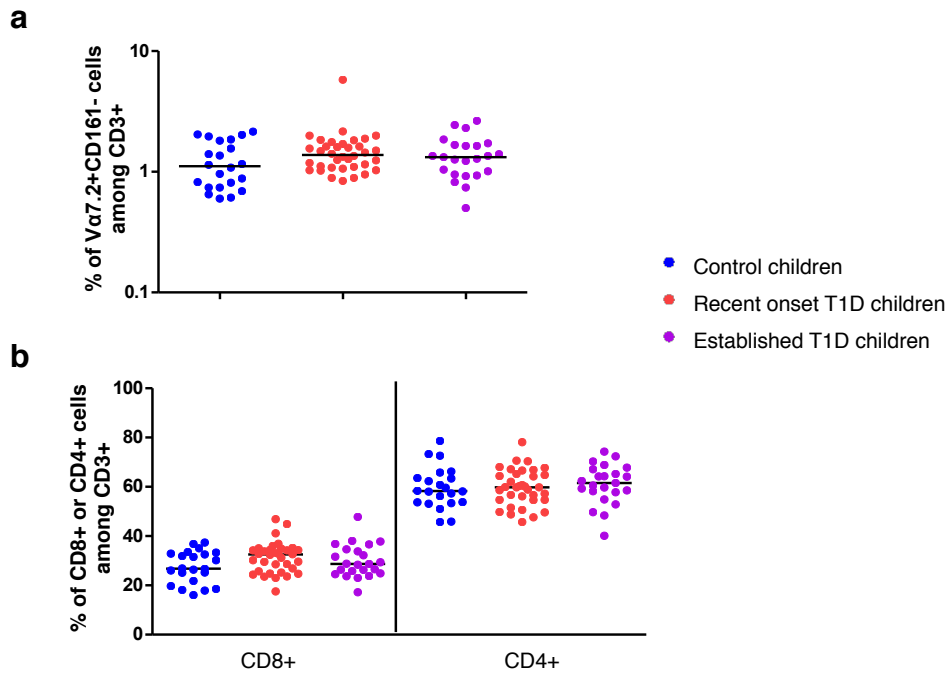
**Figure 5**

**Figure 6**

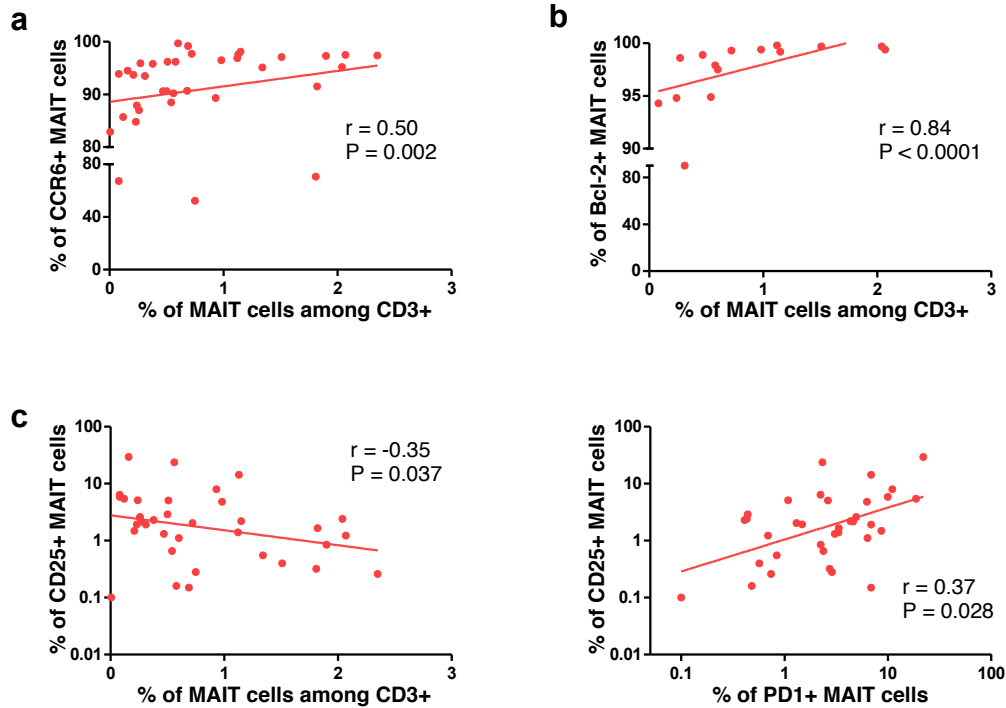


**Supplementary Figure 1 (a)** Number of blood MAIT cells among five millions of PBMC from children with recent onset T1D (n=35), children with established T1D (n=23) as compared with control children (n=22). **(b)** Frequency of the subpopulation CD8+ and DN MAIT cells among T lymphocytes from children with recent onset T1D (n=41), children with established T1D (n=23) as compared with control children (n=22). P values were determined by Kruskal-Wallis test followed by the Wilcoxon rank sum test adjusted with the Holm method.

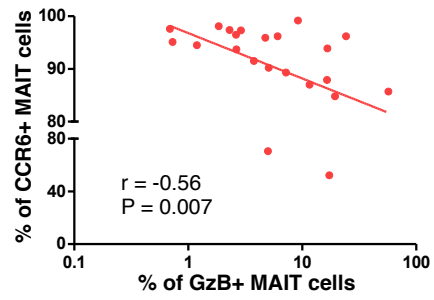
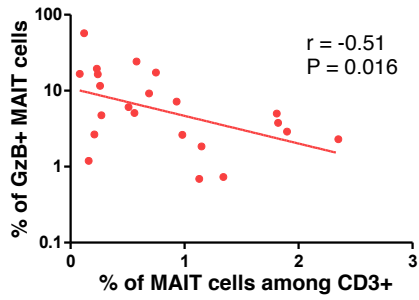




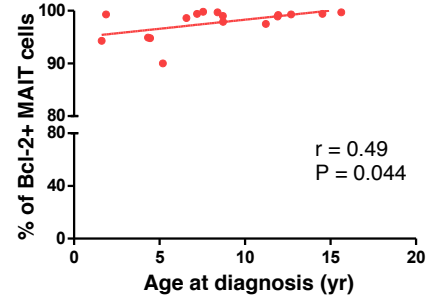
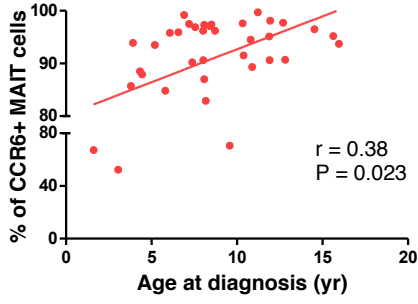
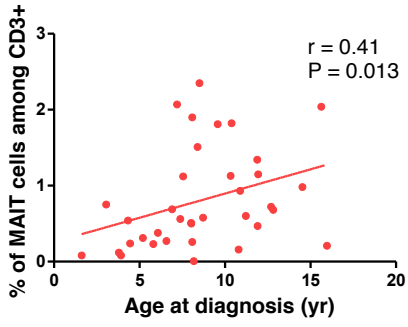
**Supplementary Figure 2 (a)** Frequency of Va7.2+CD161<sup>-</sup> cells among T lymphocytes and **(b)** frequency of the CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes from children with recent onset T1D (n=41), children with established T1D (n=23) as compared with control children (n=22). No significant differences as determined by Kruskal-Wallis test followed by the Wilcoxon rank sum test adjusted with the Holm method.



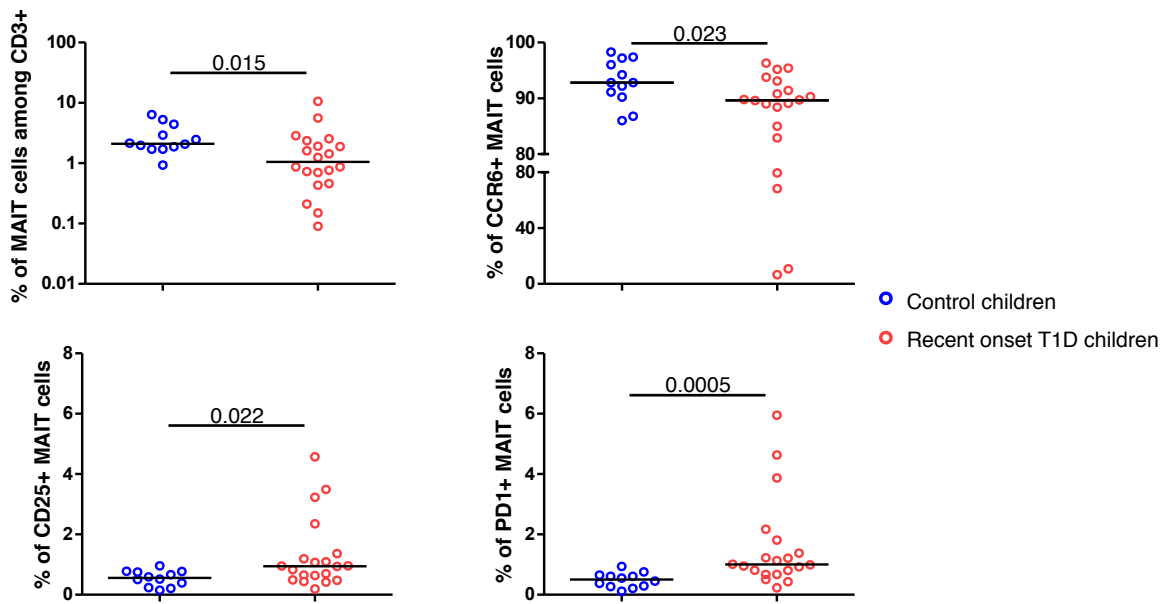
**Supplementary Figure 3 (a)** Correlation between frequency of MAIT cells expressing CCR6 and frequency of MAIT cells among T lymphocytes from children with recent onset T1D (n=41). **(b)** Correlation between frequency of MAIT cells expressing Bcl-2 and frequency of MAIT cells among T lymphocytes from children with recent onset T1D (n=15). Less samples were analyzed for Bcl-2 staining due to limited number of PBMC. **(c)** Correlation between frequency of MAIT cells expressing CD25 and frequency of MAIT cells among T lymphocytes from children with recent onset T1D (n=35). Correlation between frequency of MAIT cells expressing CD25 and frequency of MAIT cells expressing PD1 from children with recent onset T1D (n=35). P values were determined by Spearman test.



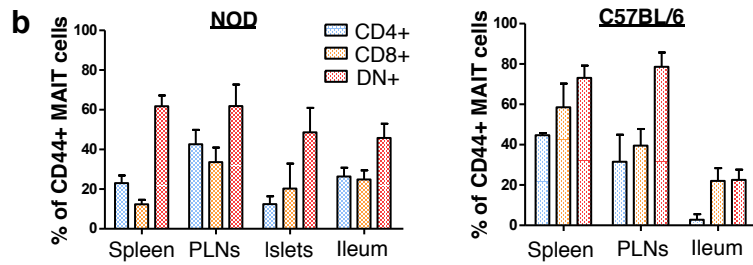
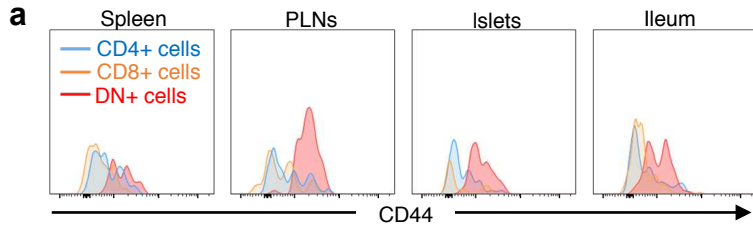
**Supplementary Figure 4** Correlation between frequency of MAIT cells expressing GzB and frequency of MAIT cells among T lymphocytes from children with recent onset T1D (n=22). Correlation between frequency of MAIT cells expressing CCR6 and frequency of MAIT cells expressing GzB (n=22). P values were determined by Spearman test.



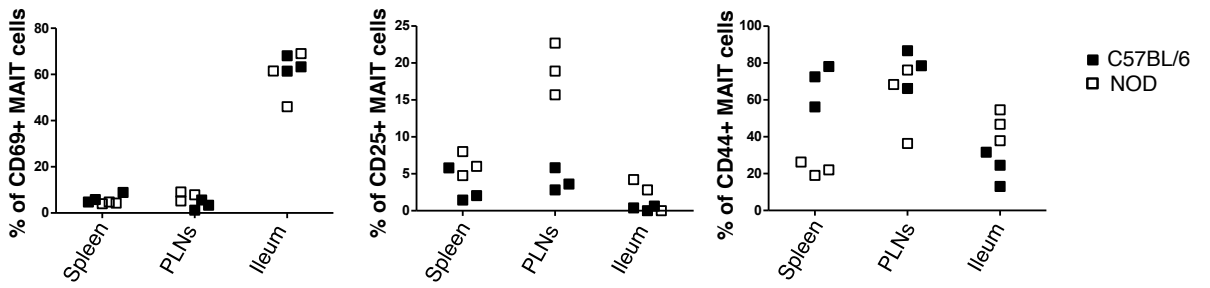
**Supplementary Figure 5** Correlation between frequency of MAIT cells expressing different surface and intracellular molecules and the age of the children with recent onset T1D at the diagnosis. For surface staining  $n=41$  and Bcl-2 analysis  $n=16$ . P values were determined by Spearman test.



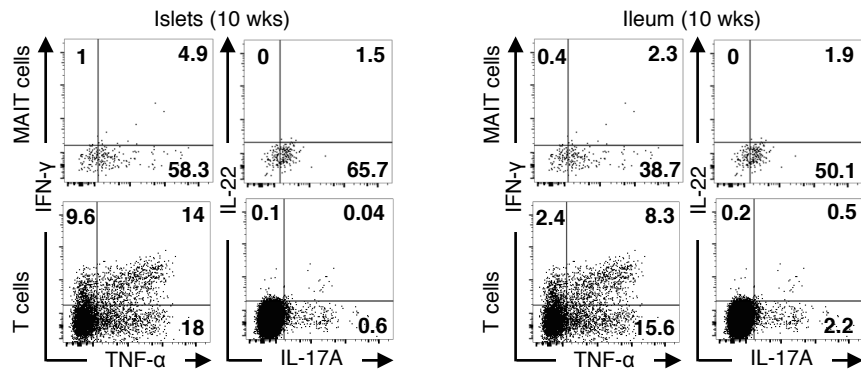
**Supplementary Figure 5** Frequency of MAIT cells among T lymphocytes and frequency of MAIT cells expressing surface molecules after staining on frozen PBMC from children with recent onset T1D (n=20) and control children (n=12). P values were determined by Mann-Whitney Test.



**Supplementary Figure 6.** CD44 expression on MAIT cell subsets in NOD and C57BL/6 mice. **(a)** Representative histogram of CD44 expression on CD4 (blue), CD8 (orange) and DN (red) subsets of MAIT cells from spleen, PLNs, islets and ileum from NOD mice. **(b)** Percentage of CD44+ MAIT cells in each subset of different tissues from 10 week-old NOD and C57BL/6 mice. Bars graphs depict mean  $\pm$  SEM from at least 8 mice, analyzed in four (NOD mice) or two (C57BL/6) separate experiments.

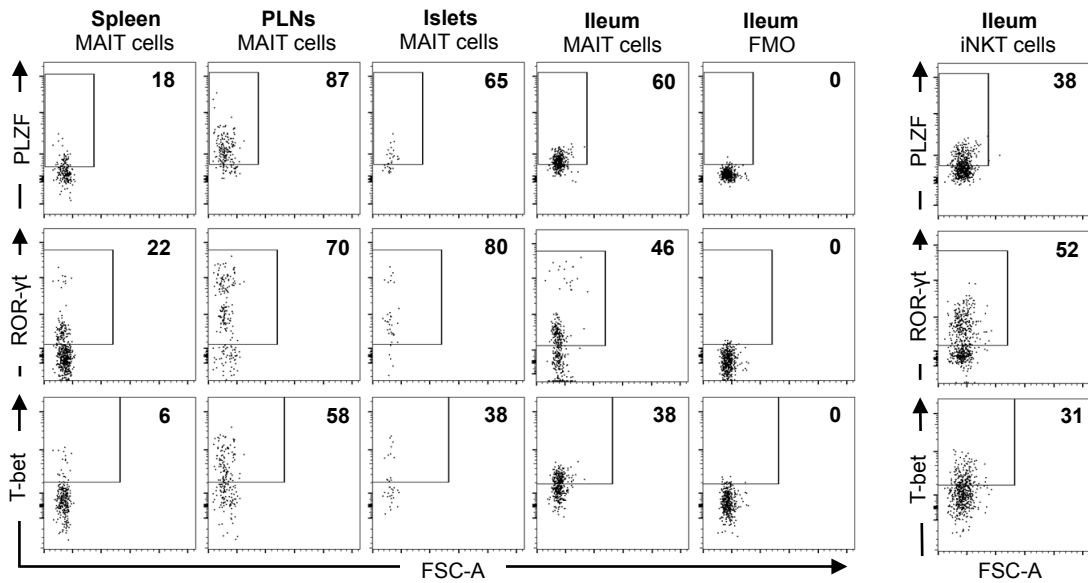


**Supplementary Figure 7.** Comparison of activation markers on MAIT cells between NOD and C56BL/6 mice. Frequency of CD69+, CD25+ and CD44+ MAIT cells from the spleen, PLNs and ileum from 10 week-old NOD (white) and C57BL/6 (black) mice. All data are representative of, at least, two separate experiments and each point represents a pull of two mice.

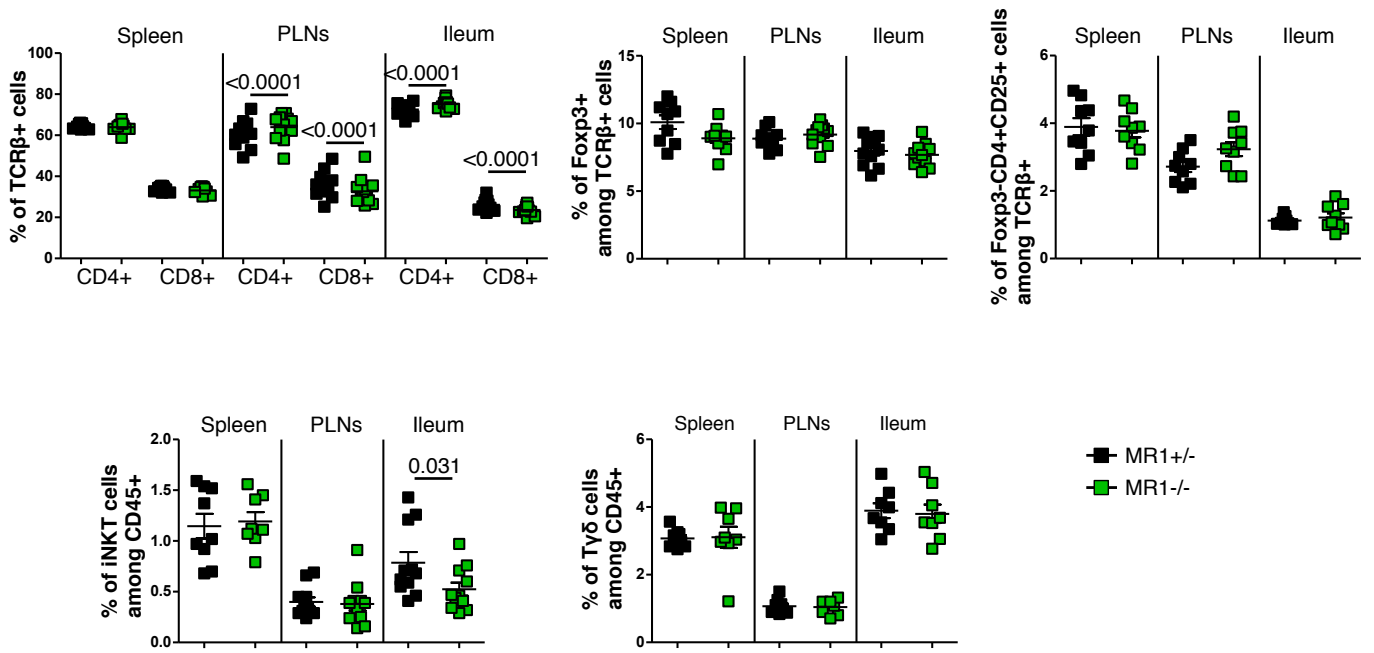


**Supplementary Figure 8.** Cytokine production by MAIT cells in NOD mice. Representative dot plots showing intracellular cytokine staining of MAIT cells from islets and ileum of 10 week-old NOD mice. MAIT cells are analyzed after PMA/ionomycin stimulation and labeled with MR1-5-OP-RU tetramer and then fixed and permeabilized before staining with IL-17, IL-22, TNF- $\alpha$ , IFN- $\gamma$  mAbs. Conventional  $\alpha\beta$ T cells are also analyzed as control. Data are representative of three experiments with combined total of 9 mice.

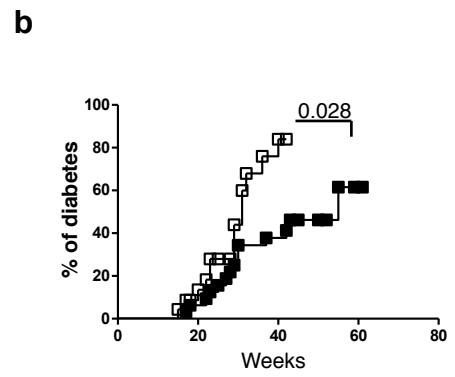
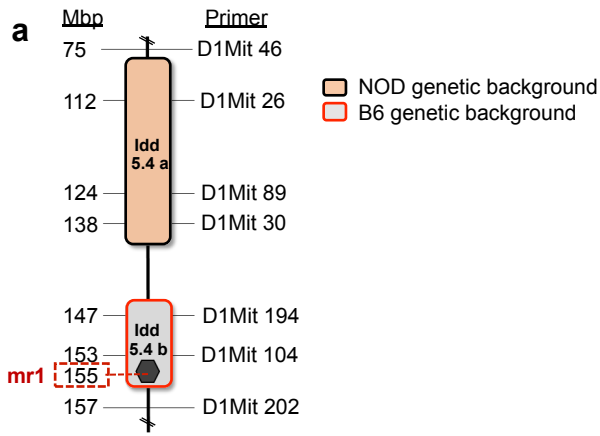




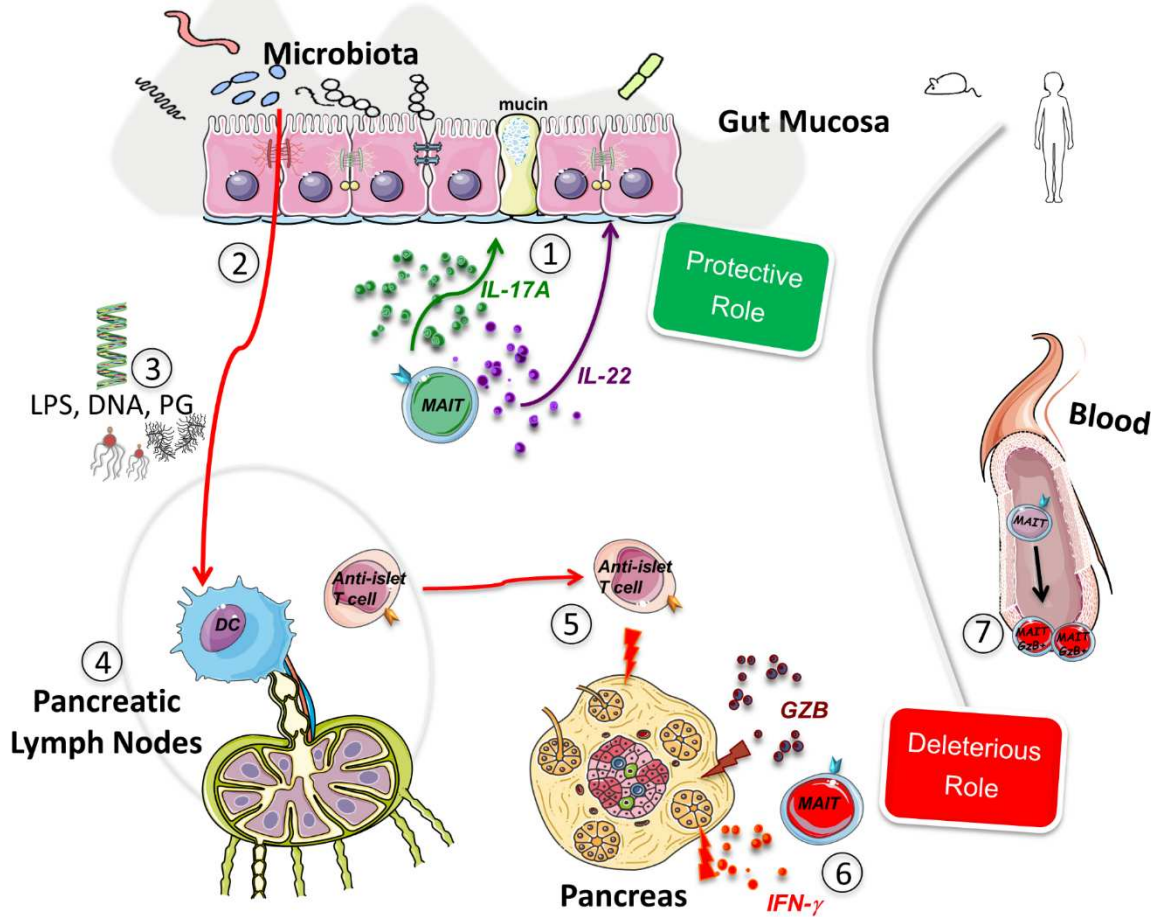
**Supplementary Figure 9.** MAIT cell expression of PLZF, ROR- $\gamma$ t and T-bet. Expression of the transcription factors PLZF, ROR- $\gamma$ t and T-bet by MAIT cells from the spleen, PLNs, pancreatic islets and ileum from 10 week-old NOD mice. Representative dot plots with indicated percentage of PLZF, ROR- $\gamma$ t and T-bet expression in MAIT and iNKT cells from different tissues. Dot plots for each marker show data concatenated from two or three individual mouse tissue samples from one experiment. Data are representative of two similar experiments. FMO, fluorescence minus one.



**Supplementary Figure 10.** Analysis of T cell populations in MR1<sup>-/-</sup> and MR1<sup>+/-</sup> NOD mice. Graphs showing the frequency of conventional CD4 and CD8 T cells, Foxp3 Treg cells, Foxp3<sup>-</sup> CD25<sup>+</sup> CD4<sup>+</sup> T cells, iNKT cells and γδT cells in PLNs and ileum as determined by flow cytometry. Analysis was performed on 8-12 mice per groups, in two independent experiments. P values were determined with Mann-Whitney test and bar represent mean ± s.e.m).



**Supplementary Figure 11.** Generation of MR1<sup>-/+</sup> NOD mice. **(a)** Schematic diagram of mouse chromosome 1, mr1 gene and insulin dependent diabetes (Idd) 5.4a and b loci. **(b)** Cumulative incidence of diabetes of NOD (n=23) and MR1<sup>+/-</sup> NOD (n=32) mice. Statistical analysis was performed with Log rank-test.



**Supplementary Figure 12.** Schematic view of the dual role of MAIT cells in the physiopathology of type 1 diabetes. MAIT cells recognize bacterial metabolites and changes of gut microbiota are associated with diabetes development. In the intestine of 10 week-old NOD females, MAIT cells produce IL-17A and IL-22, two key cytokines involved in the maintenance of gut mucosa integrity. (1) During disease progression, the production of both cytokines by ileal MAIT cell decreases, thereby dampening their protective role. (2) Moreover in MR1<sup>-/-</sup> NOD mice lacking MAIT cells several abnormalities are observed in the gut mucosa: increased gut permeability, decreased tight junction protein expression, alteration of mucus production and distribution, and increased lymphocyte infiltration in the lamina propria. (3) This defective intestinal barrier could favor the translocation of bacterial ligands such as lipopolysaccharide (LPS), DNA, peptidoglycan (PG) that could promote dendritic cell activation as evidenced by their increase MHC class II and CD86 upregulation in pancreatic lymph nodes. (4) Subsequently, these DC can activate autoreactive T cells recognizing beta-cell antigen. (5) These anti-islet T cells migrate to the pancreas where they produce IFN- $\gamma$  and thus destroy beta cells. This mechanism highlights the protective role of MAIT cells against T1D development. (6) However, MAIT cells can also participate to beta-cell death through their production of IFN- $\gamma$  and granzyme B (GzB). (7) Interestingly, in T1D patients there is an increase production of GzB by blood MAIT cells. While MAIT cells exert dual function, analysis of mouse models reveal the dominant protective role of MAIT cells in T1D.