IFNγ regulates MR1 transcription and antigen presentation

Huber ME[1,3], Larson EA[2], Lust TN[3], Heisler CM[3], Harriff MJ[1,3,4].

[1]Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Portland, OR.

[2]Portland Veterans Affairs Research Foundation, Portland, OR.

[3]Division of Pulmonary, Allergy, and Critical Care Medicine, Oregon Health & Science University, Portland, OR

[4]Veterans Affairs Portland Health Care System, Portland, OR.

Author contributions: MEH, EAL, TNL, and CMH performed experiments. MEH and MJH designed the experiments and wrote the manuscript. MEH conducted data analysis and prepared the figures. All authors reviewed the manuscript.

Word count (excluding abstract, figure legends, and bibliography references): 5667

Figures: 12 total (8 figures and 4 supplemental figures)

Tables: 5 total (all supplemental)

Abstract

Antigen presentation molecules play key roles in activating T cell immunity. Multiple complementary pathways are known to regulate classical MHC-I molecules at transcriptional, translational, and post-translational levels. Intracellular trafficking mechanisms dictating post-transcriptional regulation of MR1, the MHC Class I-like molecule which restricts MAIT cells, have been an area of focus; however, little is known about *MR1* transcriptional regulation. We demonstrate that, similar to classical MHC-I, interferons regulate *MR1* transcription. Treatment of airway epithelial cells (AEC) with recombinant IFNβ or IFNγ variably increased *MR1* transcripts, while only IFNγ significantly increased surface MR1 expression and enhanced antigen presentation to MAIT cells. The MR1 promoter contains binding motifs for interferon regulatory factor 1 (IRF1), an important MHC-I transcription factor. IRF1 knockout reduced IFNγ-stimulated MR1 transcription, surface expression, and antigen presentation. Conversely, knockout of Nod-like Receptor family CARD domain containing 5 (NLRC5), a critical component of IFNγ-induced MHC-I transcription, did not significantly impact MR1 expression. These findings were corroborated in primary human AEC treated with IFNγ. In co-culture experiments, MAIT cells incubated with *Streptococcus pneumoniae*-infected primary AEC produced sufficient IFNγ to stimulate upregulation of MR1 expression. Our data support a model where IFNγ from activated MAIT cells or another source stimulates IRF1-dependent MR1 expression and antigen presentation, leading to greater MAIT cell activation. A robust MR1-dependent MAIT cell response may be beneficial for early infection responses, allowing minimal antigen stimulus to generate greater proinflammatory activity.

38 # Introduction

39 Mucosal-associated invariant T (MAIT) cells, an innate-like subset of T lymphocytes that
40 comprise a relatively large proportion of the total $CD8^+$ T cell population in human blood and
41 lungs, play key roles in clearing respiratory bacterial, fungal, and viral infections[1–3]. Upon antigen
42 presentation, MAIT cells are capable of immediate effector function and release inflammatory
43 cytokines like interferon-γ (IFNγ) and tumor necrosis factor (TNFα)[2–5]. This rapid activation
44 primes MAIT cells to coordinate early infection response, but also necessitates tight regulation of
45 antigen presentation to prevent inappropriate MAIT cell activation to inappropriate stimuli.

46 MAIT cells are restricted by the MHC class I-related molecule MR1, which presents small
47 molecule metabolite antigens such as those generated during bacterial riboflavin biosynthesis[2,3,6,7].
48 There is a large pool of potential MR1 ligands produced by commensal airway flora in addition to
49 pathogenic respiratory microbes. *MR1* mRNA is expressed across cell types and tissues, and MR1
50 proteins primarily reside in intracellular compartments like the ER and endosomal compartments[7–
51 11]. The basal intracellular location of MR1 and ligand-induced translocation to the cell surface play
52 critical parts in regulation of MAIT activation (as reviewed in [12–14]).

53 The intracellular trafficking mechanisms dictating post-transcriptional regulation of MR1 have
54 been an area of research focus; however, little is known about *MR1* transcriptional regulation.
55 Multiple complementary pathways regulate classical MHC-Ia molecules at transcriptional,
56 translational, and post-translational levels[15,16]. Interferons (IFNs) like IFNβ and IFNγ drive
57 transcription of MHC-Ia through expression of downstream transcription factors like Interferon
58 Regulatory Factor 1 (IRF1) and Nod-like receptor family CARD domain containing 5 (NLRC5),
59 which in turn bind to the *HLA* promoter to induce transcription[15–19]. Although the *MR1* gene resides

60 on human chromosome 1, outside the chromosome 6 *HLA* locus[7], these pathways may provide
61 insight into transcriptional regulation of MR1. Recent research links *MR1* expression with disease
62 pathology (e.g. meningeal tuberculosis[20], glioma[21], and COPD[22–24]), although specific mechanisms
63 controlling *MR1* transcription remain unclear.

64 Here, we investigated the role of IFNγ in stimulating MR1 expression in human airway epithelial
65 cells (AEC). We found IFNγ promotes MR1 transcription, antigen presentation, and MAIT cell
66 responses. While NLRC5 and IRF1 were both important for IFNγ-induced *HLA-A* transcription,
67 NLRC5 was largely dispensable for *MR1* transcription. Finally, we demonstrate that MAIT cells,
68 activated in co-culture with infected AEC, produce sufficient IFNγ to stimulate *MR1* transcription.
69 Taken together, our data support a model in which IFNγ from activated immune cells induces MR1
70 expression and antigen presentation, leading to greater MAIT cell activation. These results
71 establish an additional level of MR1 regulation, informing our understanding of MAIT cell
72 activation and dysregulation in infection and disease.

73 # Results

74 **MR1 expression increases in infected AECs co-cultured with MAIT cells.**

75 Previously, our lab examined the expression of MR1 in primary human AEC from donors with
76 chronic obstructive pulmonary disease (COPD) or history of tobacco use[22]. MAIT cell responses
77 to these primary AEC infected with *Streptococcus pneumoniae* (*Sp*) were reduced in the contexts
78 of COPD and smoking. These altered MAIT cell responses were MR1-dependent, but we observed
79 minimal differences in the baseline *MR1* mRNA expression of these primary AEC alone. We
80 therefore hypothesized that co-culture with MAIT cells could impact MR1 expression and

81 function. To address this, we examined *MR1* mRNA expression of primary AEC co-cultured with
82 MAIT cells alone or in the context of *Sp* infection.

83 We noticed significantly increased *MR1* mRNA expression in AEC from healthy donors when
84 infected with *Sp* and cultured with MAIT cells (Figure 1A, Supplemental Figure 1A, Table 1).
85 Infection with *Sp* or co-culture with MAIT cells alone did not stimulate a significant response. We
86 replicated this system using a model bronchial epithelial cell line (BEAS-2B cells) infected with
87 *Mycobacterium smegmatis* (*Ms*). Similarly, we observed increased *MR1* expression in the *Ms*-
88 infected BEAS-2B cells co-cultured with MAIT cells, with no impact of either condition alone
89 (Figure 1B, Supplemental Figure 1B, Table 1). Using flow cytometry to quantify surface MR1
90 protein expression, we likewise found increased MR1 expression with both *Ms* infection and
91 MAIT cell co-culture compared to either condition alone (Figure 1C, Supplemental Figure 1C).
92 These data suggest that MAIT cells, when activated by presentation of bacterial antigens, could
93 lead to increased *MR1* mRNA or surface protein expression in the infected cell.

94 We next asked whether microbial infection is required for this transcriptional increase or if the
95 presence of MR1 ligand alone is sufficient. We treated BEAS-2B cells with either the stimulatory
96 antigen 5-(2-oxopropylideneamino)-6-d-ribitylaminouracil (5-OP-RU) or the non-stimulatory
97 ligand 6-formylpterin (6-FP) and measured expression of MR1. Neither 5-OP-RU nor 6-FP
98 increased *MR1* expression alone (Figure 1D-E). In co-culture with MAIT cells, however, *MR1*
99 mRNA expression increased when BEAS-2B cells were treated with 5-OP-RU, while treatment
100 with 6-FP had no impact on *MR1* expression (Figure 1D-E, Supplemental Figure 1D-E). These
101 data demonstrate that the upregulation of *MR1* mRNA expression requires activation of MAIT
102 cells, and this may be stimulated by antigen presentation alone or bacterial infection.

103 **IFNγ stimulates MR1 expression and antigen presentation.**

104 Among the effector molecules produced by activated MAIT cells, IFNγ is well known to stimulate
105 transcription of MHC Class I molecules[15–17]. We hypothesized *MR1* expression could be regulated
106 through similar mechanisms, despite the differences in chromosomal location and gene
107 arrangement from classical *HLA* genes. To test if IFNγ alone is sufficient to stimulate *MR1*
108 expression, we treated primary AEC with recombinant human IFNγ. IFNγ treatment significantly
109 increased *MR1* transcription (Figure 2A, left). As expected, IFNγ also increased expression of
110 positive control *HLA-A* (Figure 2A, right). In BEAS-2B cells treated with IFNγ, we also observed
111 significant increases in both *MR1* and *HLA-A* mRNA expression (Figure 2B).

112 To quantify MR1 protein expression, we measured surface MR1 expression by flow cytometry.
113 We found that IFNγ treatment also significantly increased surface MR1 expression and control
114 MHC-I expression in primary AEC and BEAS-2B cells (Figure 2C-D. This approach does not
115 distinguish between 1) increased surface expression of MR1 proteins due to increased *MR1*
116 transcription and translation or 2) increased translocation of existing MR1 molecules and
117 stabilization on the cell surface. To determine if IFNγ signaling impacts post-transcriptional
118 protein stability of MR1, we utilized BEAS-2B cells expressing MR1-GFP under a doxycycline-
119 inducible promoter[11]. IFNγ treatment did not increase expression of *MR1* mRNA, total MR1-GFP
120 gMFI, or surface MR1 in these doxycycline-treated cells (Figure 3A-C). As expected, IFNγ
121 treatment increased MHC-Ia surface expression and 6-FP treatment induced significant
122 stabilization of total MR1-GFP protein expression and surface translocation (Figure 3C-D).
123 Together, these data indicate that increased surface MR1 in IFNγ-treated wildtype cells resulted
124 from stimulation of *MR1* transcription rather than protein-level impacts.

125 MR1 antigen presentation and MAIT cell responses are increased in MR1 over-expression
126 systems[10,11,25]. We next investigated if the IFNγ-dependent increase in MR1 expression similarly
127 enhanced MAIT cell responses to wildtype cells. BEAS-2B cells were treated with IFNγ for 12
128 hours, thoroughly washed to remove excess soluble IFNγ, then used as antigen-presenting cells in
129 an IFNγ ELISPOT assay to quantify MAIT cell activation. Filtered *M. smegmatis* supernatant was
130 used as the antigen source to avoid potential confounding impacts of IFNγ treatment on bacterial
131 infection. MAIT cell responses to IFNγ pre-treated BEAS-2B cells were significantly increased
132 compared to UT controls (Figure 3E). Therefore, IFNγ treatment is sufficient to stimulate *MR1*
133 transcription, leading to increased protein expression and antigen presentation to MAIT cells.

134 **IFNγ stimulates MR1 transcription via transcription factor IRF1, not NLRC5.**

135 Using MHC-Ia transcription pathways as a starting point, we queried the JASPAR CORE 2018
136 Vertebrates database to determine if the *MR1* promoter contained binding motifs for known IFNγ-
137 induced transcription factors[26–28]. We highlighted notable predicted elements on this region,
138 including those common with IFNγ-mediated *HLA* transcription factor sites (Figure 3F). For
139 example, we found putative binding motifs for IRF1 and members of the NLRC5 enhanceosome
140 complex[18,19,29–31]. This targeted search suggested that IRF1 and NLRC5 could be of interest in
141 IFNγ-mediated *MR1* transcription.

142 We first validated that IFNγ signaling induces *IRF1* and *NLRC5* mRNA expression in our cells.
143 Transcripts of both these genes were significantly increased by IFNγ treatment of both primary
144 human AEC and BEAS-2B cells (Figure 3G-H).

145 To determine if IRF1 or NLRC5 are required for the IFNγ-mediated increase in *MR1* transcription,
146 we first used siRNA to knock down the genes separately or together in BEAS-2B cells. IRF1 KD

147 alone significantly decreased IFNγ-stimulated *MR1* mRNA expression compared to missense
148 controls (Figure 4A, Table 2). Although IRF1 siRNA significantly reduced *IRF1* expression, we
149 were unable to sufficiently knock down *NLRC5* expression by siRNA (Supplemental Figure 2A-
150 B). Therefore, we generated monoclonal NLRC5$^{-/-}$ BEAS-2B cell lines by CRISPR/Cas9. Loss of
151 NLRC5 did not lead to any significant impact to IFNγ-induced *MR1* mRNA expression (Figure
152 4B). We did observe a decrease in *HLA-A* expression with NLRC5 knockout, although not
153 significant (Figure 4C).

154 We used siRNA to silence IRF1 expression in the Cas9^{+} and NLRC5$^{-/-}$ cells (Supplemental Figure
155 2C). *MR1* expression was significantly impacted by IRF1 knockdown in Cas9^{+} control cells
156 (Figure 4B), agreeing with the previous siRNA results in wildtype BEAS-2b cells. A trend in
157 reduced *MR1* expression was also observed in two clones of NLRC5$^{-/-}$ cells treated with IRF1
158 siRNA (Figure 4B, Supplemental Figure 2D). Expression of surface MR1 proteins in IFNγ-treated
159 cells was similarly decreased with IRF1 silencing and unaffected by NLRC5 knockout
160 (Supplemental Figure 2E). Finally, we used ELISPOT assays to quantify if loss of NLRC5 and/or
161 IRF1 would impact the IFNγ-stimulated boost in MR1 antigen presentation to MAIT cells. IRF1
162 siRNA knockdown significantly reduced MAIT cell responses to both Cas9^{+} cells and NLRC5$^{-/-}$
163 cells (Figure 4D). The missense-treated NLRC5$^{-/-}$ cells stimulated similar MAIT cell activity as
164 the Cas9^{+} control cells in response to IFNγ (Figure 4D, Table 2). Together, these results indicate
165 that IRF1 expression is required for IFNγ stimulation of MR1 expression and antigen presentation
166 function, while NLRC5 does not appear to impact this pathway.

167 We next used CRISPR/Cas9 knockout to generate monoclonal IRF1$^{-/-}$ BEAS-2B cell lines to
168 confirm this finding. IFNγ-mediated stimulation of *MR1* mRNA and MR1 surface expression was
169 impaired in IRF1$^{-/-}$ cells compared to Cas9^{+} cells (Figure 4E,G, Supplemental Figure 2F-G).

170 Expression of *HLA-A* mRNA or MHC-Ia surface proteins were likewise impaired in the IRF1$^{-/-}$
171 cells following IFNγ treatment (Figure 4F,H; Supplemental Figure 2G, right). Together the siRNA
172 knockdown and CRISPR knockout results indicate that IRF1 mediates the IFNγ signaling pathway
173 leading to MR1 transcription, surface expression, and antigen presentation, likely through
174 mechanisms independent of the NLRC5 enhanceosome.

175 **MAIT cells produce IFNγ sufficient to induce MR1 transcription pathways.**

176 To validate this mechanism in a physiologically relevant system, we returned to our co-culture
177 experiments. We first demonstrated that co-culture of infected AEC with MAIT cells led to
178 upregulation of IFNγ-stimulated pathways by staining phosphorylated STAT1 (pSTAT1).
179 Ligation of the IFNGR activates Janus kinase (JAK) dimer 1/2, which in turn phosphorylates
180 STAT1[17,32]. As expected, pSTAT1 staining was significantly increased in AEC infected with *S.*
181 *pneumoniae* and co-cultured with MAIT cells, along with AEC treated with recombinant IFNγ
182 (Figure 5A-B, Supplemental Figure 3A, Table 3).

183 To confirm that activation of MAIT cells in co-culture is sufficient to drive IFNγ signaling, we
184 next assessed expression of IFNγ-stimulated genes. Co-culture of *Sp*-infected primary AEC with
185 MAIT cells significantly induced expression of *HLA-A, B2m, IRF1,* and *NLRC5* (Figure 5C-F;
186 Supplemental Figure 3B-D,G; Table 3). We observed increased expression of these genes in AEC
187 incubated with either *Sp* or MAIT cells alone, which may indicate the contribution of other
188 inflammatory signaling pathways in AEC. However, the combined co-culture induced
189 significantly greater expression for almost all genes, pointing to the role of Ag-induced MAIT cell
190 activation in driving inflammatory gene expression.

191 BEAS-2B infected with *Ms* significantly induced expression of *HLA-A* and *IRF1* only in
192 combination with MAIT cell co-culture (Figure 6A,C; Supplemental Figure 3E,H; Table 3). We
193 next used 5-OP-RU as the antigen source to confirm that MR1 antigen presentation alone is
194 sufficient to stimulate MAIT cell IFNγ production and subsequent inflammatory gene expression,
195 absent other microbial stimuli. As expected, *HLA-A* and *IRF1* expression were significantly
196 increased in 5-OP-RU-treated BEAS-2B cells when MAIT cells were present, but not stimulated
197 by treatment with 5-OP-RU alone or MAIT cell co-culture with untreated BEAS-2B cells (Figure
198 6B,D; Supplemental Figure 3F,I; Table 3). Non-stimulatory presentation of 6-FP ligands failed to
199 significantly induce either gene alone or in combination with MAIT cells. Together, these results
200 indicate that activated MAIT cells produce sufficient IFNγ to stimulate expression of downstream
201 genes.

202 Finally, we used our IRF1$^{-/-}$ cells in this co-culture setting to demonstrate the role of IFNγ in
203 mediating MR1 expression, antigen presentation, and MAIT cell activation. IRF1$^{-/-}$ cells infected
204 with *Ms* and co-cultured with MAIT cells failed to exhibit the increase in *MR1* expression seen in
205 the Cas9^{+} control cells (Figure 6E, Table 4). We then used exogenous 5-OP-RU treatment to
206 directly test the role of IRF1 following MR1-dependent MAIT cell activation. *MR1* transcription
207 was enhanced in Cas9^{+} cells with 5-OP-RU and MAIT cell co-culture, confirming that TCR-
208 stimulated MAIT cells directly led to increased *MR1* transcription (Figure 6F, Table 4). In contrast,
209 the IRF1$^{-/-}$ cells treated with 5-OP-RU did not express greater *MR1* transcripts in co-culture,
210 confirming the importance of IRF1 in this pathway.

211 **IFNγ and IFNβ stimulate MR1 transcription by distinct mechanisms.**

212 Our data have shown thus far that IFNγ stimulates *MR1* transcription; however other inflammatory
213 cytokines can also induce transcription. Type I interferons like IFNβ stimulate IRF1 and NLRC5
214 to induce MHC-Ia transcription[15–19,33,34]. Activated MAIT cells may also produce TNFα and IL-
215 17, which have been demonstrated to stimulate transcription of inflammatory genes including *IRF1*
216 and *HLA-A*[35–37]. To assess whether the IFNγ-induced increase in *MR1* transcription is
217 representative of general inflammatory signaling mechanisms or specific to IFNγ stimulus, we
218 treated BEAS-2B cells with recombinant human inflammatory cytokines IFNβ, IFNγ, IFNλ,
219 TNFα, and IL-17. Of these cytokines, only IFNβ and IFNγ elicited a significant increase in *MR1*
220 transcription compared to untreated controls (Figure 7A-B, Table 5).

221 Interestingly, the IFNγ-mediated increase in MR1 expression was significantly greater than the
222 increase due to IFNβ treatment, while expression of *HLA-A* and *B2m* were similarly induced by
223 both IFNγ and IFNβ treatment (Figure 7A-C, Supplemental Figure 4, Table 5). We further
224 investigated the role of type I and II IFNs in mediating expression of MR1 and MHC-Ia. While
225 both IFNβ and IFNγ increased surface expression of MHC-Ia, only IFNγ led to a significant
226 increase in surface MR1 protein expression (Figure 7D). BEAS-2B cells pre-treated with IFNγ
227 induced significantly greater MAIT cell responses to *Ms* infection than control UT cells, while
228 IFNβ pre-treatment did not generate a significantly different dose-response curve (Figure 7E).
229 These data indicate that IFNγ plays the largest role in stimulating MR1 expression and function.

230 We quantified *IRF1* and *NLRC5* transcripts to further explore how MR1 and MHC-I expression
231 are differentially stimulated by IFNβ and IFNγ. Although both IFNγ and IFNβ increased *IRF1*
232 expression, the relative fold change was significantly greater with IFNγ than IFNβ (Figure 7F,

233 Supplemental Figure 4, Table 5). Both interferons induced significant increases in *NLRC5* and
234 *B2m* expression (Figure 7G-H, Supplemental Figure 4, Table 5). In light of our finding that IFNγ
235 stimulates *MR1* transcription through IRF1 and not NLRC5, this magnitude of *IRF1* induction may
236 relate to the specific induction of MR1 surface expression and antigen presentation by IFNγ and
237 not IFNβ. Further, these results indicate that transcription of *MR1* and *HLAA* occur via distinct
238 IFNγ-stimulated mechanisms.

239 Together, our data support a feed-forward model of inflammatory signaling (Figure 8). MR1
240 antigen presentation by an infected cell activates a MAIT cell to release IFNγ, which then acts on
241 the airway epithelial cell to stimulate *IRF1* expression through a pSTAT1 pathway. IRF1 then
242 binds to the *MR1* promoter to induce *MR1* transcription, leading to more MR1 protein available
243 for antigen presentation and subsequent MAIT cell activation.

244 # Discussion

245 MAIT cells are key components of early infection responses. The variety of pathogens producing
246 MR1 antigens and rapid MAIT cell effector function poise MAIT cells to bridge innate and
247 adaptive immune responses. Strict regulation of MAIT cell activation is required to prevent
248 inflammatory damage. Research over the past decade has defined many complementary pathways
249 regulating MR1 intracellular localization, antigen binding, surface translocation, and protein
250 recycling[12–14]. These studies affirm that defining the mechanisms that regulate MR1 is critical to
251 understanding regulation of MAIT cells themselves. Only recently have we begun to appreciate
252 the role of epigenetic regulation in controlling MR1 expression and antigen presentation. Studies
253 of *MR1* DNA methylation and RNA expression suggest that *MR1* transcription is increased during
254 infection[38–41], although effector molecules from herpes simplex viruses were shown to degrade
255 *MR1* transcripts[42–44]. Altered epigenetic regulation of *MR1* in respiratory inflammation[22–24,45] and
256 cancer[21,46] illustrate the complex interplay of activation and repression signals in these diseases.

257 Here, we demonstrate for the first time that IFNγ stimulates *MR1* transcription. We investigated
258 the role of two IFNγ-stimulated transcription factors, IRF1 and NLRC5, in regulating *MR1*
259 transcription. *In-silico* analysis of the *MR1* promoter revealed potential binding sites for IRF1 and
260 components of the NLRC5 enhanceosome[18,19]. Using both siRNA knockdown and CRISPR/Cas9
261 knockout systems, we observed that IFNγ-induced *MR1* transcription was dependent on IRF1, but
262 not NLRC5. Treatment with IFNγ failed to increase MR1 surface expression and antigen
263 presentation in IRF1$^{-/-}$ cells. These results pointed to IRF1 as the primary driver of IFNγ-induced
264 *MR1* transcription in our experimental conditions. The rapid increase in *IRF1* expression after
265 IFNγ treatment is consistent with established models of IRF1 kinetics[18,47]. Recently, Rosain and

266 colleagues performed a comprehensive characterization of two young patients with IRF1-
267 inactivating mutations[48]. *MR1* was among the genes upregulated in primary fibroblasts from
268 healthy controls after 8 hours of IFNγ treatment, while *MR1* was not upregulated in IFNγ-treated
269 fibroblasts with IRF1-inactivation, STAT1-deficiency, or loss of IFNGR1/2[48]. Our co-culture
270 experiments showed that antigen-activated MAIT cells produced sufficient IFNγ to induce *IRF1*
271 expression in both primary AEC and BEAS-2B cells. In similar conditions with *Ms* infection or
272 5-OP-RU treatment and MAIT cell co-culture, IRF1$^{-/-}$ cells failed to replicate the increase in *MR1*
273 mRNA expression seen with control Cas9^{+} cells. We therefore concluded that MAIT cell activation
274 acts through IRF1 to promote *MR1* transcription. The case study of IRF1-deficient patients
275 observed only slightly lower blood MAIT cell frequencies in one individual[48]. However, both
276 experienced persistent early childhood infections from weakly-infectious *Mycobacterium avium*
277 and/or breakthrough infection from the BCG vaccine, consistent with impaired IFNγ immunity[48].
278 Although many factors may contribute to reduced immune function in these individuals, MAIT
279 cells have been tightly linked to anti-mycobacterial immunity[49,50]. A case study of a T-bet-deficient
280 individual with very low MAIT cells underscored the specific importance of IFNγ production by
281 innate-like lymphocytes in controlling *Mtb* infection[51]. It is therefore possible that loss of IRF1
282 function could foster mycobacterial susceptibility through weaker induction of *MR1* transcription
283 and delayed MAIT cell responses. Directly characterizing the dynamics of *MR1* expression in
284 these individuals would shed light on this hypothesis.

285 We were surprised to observe that NLRC5 was not required for the IFNγ-induced increase in MR1
286 expression or antigen presentation, given the importance of NLRC5 in mediating *HLA*
287 transcription[15,16,19]. Although MR1 is an MHC-I-like molecule and they share broad structural
288 homology, the genes reside on different chromosomes and have distinct promoter features[7]. We

289 confirmed that *HLA-A* expression was reduced in NLRC5$^{-/-}$ cells. Since IRF1 can also induce
290 *NLRC5* transcription, we considered whether IRF1 and NLRC5 play a synergistic role in
291 regulating MR1 expression. While the combined loss of IRF1 and NLRC5 reduces Class I mRNA
292 expression, we saw no further impact to MR1 expression or antigen presentation. These results
293 prompted us to explore how MHC-Ia and MR1 transcription signaling pathways diverge. Both
294 type I and type II IFNs are known to stimulate expression of IRF1 and MHC-Ia[15–18,33,34]. We
295 explored whether IFNβ might induce *MR1* transcription similarly to IFNγ. Although both
296 interferons increased *MR1* mRNA expression, IFNγ stimulated a significantly greater increase in
297 *MR1* transcription than IFNβ. Furthermore, only IFNγ induced MR1 surface protein expression
298 and antigen presentation to MAIT cells. Previously, Ussher *et al.* demonstrated that MAIT cell
299 responses to fixed intact *E. coli* were significantly increased when THP1 cells were incubated with
300 either IFNα or IFNγ overnight[52]. This type I IFN increase does not match our results. However,
301 several other groups observed that directly stimulating MAIT cells with IFNβ or IFNα led to
302 increased TCR-dependent and -independent MAIT cell responses to influenza virus and *Klebsiella*
303 *pneumoniae* infection[53–55]. Therefore, type I interferon-induced signaling within MAIT cells may
304 be the primary driver of the observed increase in MAIT cell responses[52]. Type III interferons signal
305 through similar pathways as type I interferons and are critical to some mucosal inflammatory
306 responses[56]. We failed to measure any notable increase in *MR1* or *HLA-A* expression following
307 IFNλ treatment. We observed significant increases in *IRF1* and *β2m* expression with IFNλ;
308 however, these increases were significantly weaker than any IFNγ- or IFNβ-induced *IRF1* or *β2m*
309 expression. This result is consistent with research from Forero *et al.*, who found that IFNλ
310 primarily induces tissue repair pathways and fails to stimulate *IRF1* expression[57,58].

311 Others have shown that IFNγ, not IFNβ, is the primary driver of *IRF1* expression[48]. We quantified
312 the relative increase in *IRF1* transcripts following stimulation by IFNγ or IFNβ and likewise
313 observed the increase in *IRF1* expression was significantly higher with IFNγ compared to IFNβ.
314 In contrast, both interferons led to similar ranges of *NLRC5* expression. One possible model of this
315 data suggests that IFNγ signaling induces sufficient IRF1 expression to promote *MR1*
316 transcription. IFNβ induces less *IRF1* transcription, leading to expression of NLRC5, MHC-Ia,
317 and β_2m, but not enough to stimulate MR1 expression and function. It is tempting to speculate that
318 the specificity of MR1 stimulation by IFNγ may help to compartmentalize immune responses to
319 innate signaling and prevent simultaneous overstimulation of both MHC-Ia and MR1.

320 We also observed a slight increase in *IRF1* expression following TNFα treatment, yet no increase
321 in *HLAA, β2m,* or *MR1* expression. It was surprising that TNFα did not stimulate any significant
322 increase in IRF1-induced genes. The TNF receptor-associated factor 6 (TRAF6) works with
323 cellular inhibitor of apoptosis 2 (cIAP2) to K63 ubiquitinate IRF1, leading to increased function
324 and blocking K48 ubiquitin-mediated IRF1 proteasomal degradation[18,47,59–63]. This process,
325 however, functions in concert with Src-family kinases following TLR4, TLR7/9, or IL-1
326 signaling[18,47,61–65]. It is possible that this signaling occurred in our experimental condition with
327 primary AEC infected with TLR4 ligand-producing *S. pneumoniae*, since we observed increases
328 in *IRF1* and *HLA-A* expression with or without MAIT cells. However, we also observed IRF1-
329 dependent *MR1* expression following 5-OP-RU-induced MAIT cell activation, in which
330 circumstance TLR signaling was likely inactive. Exploring the role of innate sensors and non-
331 interferon cytokines in modulating IRF1 activity may reveal additional factors that can induce or
332 repress *MR1* expression.

333 In co-culture settings, IFNγ-stimulated increases in *MR1* expression resulted in greater MR1
334 antigen presentation and subsequent MAIT cell activation. MAIT cells activated by MR1 antigen
335 presentation produced sufficient IFNγ to promote *MR1* transcription and increased MR1 surface
336 expression. This feed-forward signaling model would support the function of MAIT cells in
337 immune surveillance and early infection response. Robust stimulation of MR1-dependent MAIT
338 cell activation could be beneficial during infection onset, allowing minimal antigen stimulus to
339 generate expansive and rapid proinflammatory activity. MAIT cell effector functions are well-
340 established in priming myeloid cells and recruiting $CD4^+$ and $CD8^+$ T cells[66,67]. In addition to
341 MAIT cells, a number of other cells could produce IFNγ and initiate this feed-forward loop. Local
342 IFNγ production by professional antigen-presenting cells has been observed in infection contexts;
343 for example, alveolar macrophages produce IFNγ during *M. tuberculosis* infection[68,69]. NK cells,
344 ILC, airway-resident lymphocytes, and circulating lymphocytes are also known to make IFNγ in
345 response to a variety of inflammatory stimuli as well[70,71]. Beyond cytokine signaling, toll-like
346 receptors and C-type lectin receptors can also stimulate expression of *IRF1* and IRF1-inducible
347 genes, suggesting that IRF1 inflammation could be mediated in response to non-interferon
348 stimuli[72]. IFNγ and IRF1 signaling through any of these sources could then stimulate MR1
349 expression and activate MAIT cells, leading to enhanced inflammatory responses.

350 However, dysregulation of this feed-forward loop could also lead to MAIT cell-caused pathology.
351 Inappropriate MAIT cell activation is implicated in autoimmune diseases and chronic
352 inflammation[73,74]. Overproduction of IFNγ contributes to inflammatory lung damage and can
353 stimulate further IFNγ production by alveolar macrophages[68,75–77]. $CD8^+$ T cell infiltration is
354 associated with increased disease severity in chronic obstructive pulmonary disease (COPD), and
355 IFNγ signaling is increased in the lungs of COPD patients[77–80]. We previously characterized

356 expression of MR1 and activation of MAIT cells in the context of COPD and cigarette smoke[22].
357 We found that primary AEC from COPD donors induced greater MAIT cell activation in the
358 absence of antigen than healthy AEC. *MR1* transcriptional expression was increased in AEC[22] and
359 PBMC[24] from infected COPD donors. It is possible that the increased IFNγ present in the COPD
360 airway environment could stimulate *MR1* expression and lead to increased MAIT cell activity via
361 the proposed feed-forward signaling loop. IFNγ signaling in response to inappropriate stimuli (e.g.
362 antigens from commensal microbes) could also induce *MR1* transcription and promote ligand-
363 driven MAIT cell inflammatory pathology.

364 Given the potential for inflammatory damage due to this feed-forward loop, we hypothesize that a
365 dampening mechanism exists to turn off this pathway. Constantin *et al.* revealed a potential role
366 for ERK1/2 kinases in suppressing *MR1* expression in melanoma, indicating repression
367 mechanisms can modulate *MR1* transcription[46]. Specifically, they found the transcription factor
368 ELF1 binds to the *MR1* promoter to stimulate *MR1* expression. ERK1/2, members of the
369 MAPK/MEK signaling cascade, inhibited ELF1 function and subsequent *MR1* transcription. The
370 authors suggested ELF1 inhibition may occur through post-translational modification performed
371 by a downstream intermediary protein[46]. Signaling through MEK/ERK was recently demonstrated
372 to inhibit IRF1 expression and activity in TLR-stimulated macrophages; however, ELF1 mediates
373 antiviral activity in airway epithelial cells independent of interferon and IRF1 transcriptional
374 activity[81,82]. Multiple distinct mechanisms of *MR1* transcriptional activation could serve several
375 functions: flexible induction of *MR1* expression and function in the context of distinct stimuli,
376 complementary activation to rapidly enhance MAIT cell responses, and/or as a checkpoint
377 requiring a secondary signal to prevent overactivation. It is well-documented that pathogens target
378 IFNγ signaling and MHC transcription mechanisms to evade immune recognition[34,83,84]. Several

379 MHC-Ia post-transcriptional repression mechanisms have been identified, including through IRF1
380 degradation or downregulation of NLRC5 expression[16,85,86]. In human fibroblasts, *MR1* transcripts
381 were degraded by an RNase protein from herpes simplex virus types 1 and 2, although this
382 mechanism was not specific to *MR1*[42,43]. A greater understanding of how *MR1* transcription is
383 regulated could shed light on these host-pathogen dynamics.

384 Put together, this work demonstrates that IFNγ signaling stimulates *MR1* transcription, surface
385 expression, and antigen presentation. Understanding the mechanisms of *MR1* transcriptional
386 regulation may provide insights into broader immune signaling networks and better inform our
387 knowledge of the roles MR1 and MAIT cells play in infection and inflammatory diseases.

388 # Materials & Methods

389 **Human subjects**

390 This study was conducted according to the principles expressed in the Declaration of Helsinki.
391 Study participants, protocols and consent forms were approved by Oregon Health & Science
392 University Institutional Review Board (IRB00000186). Written and informed consent was
393 obtained from all donors. Human participants are not directly involved in the study. Healthy adults
394 were recruited from among employees at Oregon Health & Science University as previously
395 described to obtain human serum[87].

396 **Cells and bacteria**

397 Primary airway epithelial cells (AEC) were purchased from Lonza Biosciences or harvested from
398 deceased human donor lung tissue through the Cascade Alliance (formerly Pacific Northwest
399 Transplant Bank) as previously described[22,88]. The healthy donor AEC from [22] were likewise
400 grown in Bronchial Epithelial Growth Media ("BEGM", CC-3170) and harvested using
401 ReagentPack Subculture reagents (CC-5034) per manufacturer's protocols (Lonza).

402 The BEAS-2B bronchial epithelial cell line (CRL-9609, American Type Culture Collection) was
403 grown in DMEM medium (Gibco) supplemented with L-glutamine (25030164, Life Technologies)
404 and 10% heat-inactivated fetal bovine serum ("DMEM-FBS"). BEAS-2B cells overexpressing
405 MR1-GFP under a tetracycline-inducible promoter ("BEAS-2B:doxMR1-GFP")[11] were similarly
406 cultured in DMEM-FBS. Expression of MR1-GFP was induced with doxycycline for 16 hours
407 prior to harvest. BEAS-2B cells stably expressing Cas9[89] were grown in DMEM-FBS and used to
408 generate CRISPR knockouts.

409 The MR1-restricted T cell clone (D426G11) was generated and expanded in RPMI medium
410 (Gibco) supplemented with L-glutamine and 10% heat-inactivated human serum ("RPMI-HuS")
411 as previously described[2,87].

412 *Streptococcus pneumoniae*[90] and *Mycobacterium smegmatis* Mc^{2}155 (ATCC) were grown as
413 described in the supplement of [22] and used from frozen stocks. At late log phase, *M. smegmatis*
414 were pelleted and the supernatant was passed through a syringe-driven 0.22 µm filter and frozen
415 for use as antigen in ELISPOT assays.

416 **Generation of stable CRISPR/Cas9 IRF1 or NLRC5 knockout BEAS-2B cells**

417 We generated IRF1$^{-/-}$ and NLRC5$^{-/-}$ CRISPR knockout BEAS-2B cells as previously described[89].
418 Early passage Cas9^{+} BEAS-2B cells were transduced with sgRNA constructs targeting IRF1
419 (CRISPR845545_LV, ThermoFisher) or NLRC5 (CRISPR1120312_LV, ThermoFisher) in the
420 presence of 200 µg Polybrene (Sigma). Following puromycin selection, monoclonal populations
421 were produced by limiting dilution and screened by Western blot or ELISPOT. We validated
422 genomic editing by Sanger sequencing. DNA was isolated from control Cas9^{+}, IRF1$^{-/-}$, and
423 NLRC5$^{-/-}$ BEAS-2B clones using the QIAamp DNA Micro Kit (Qiagen) and amplified by PCR.
424 The OHSU Vollum Institute DNA Sequencing Core performed Sanger sequencing and the
425 resulting sequences were analyzed by TIDE[91] and ICE[92].

426 **Reagents and antibodies**

427 6-formylpterin (6-FP, Schirck's Laboratories) was suspended in 0.01 M NaOH and used at a final
428 concentration of 100 µM. 5-(2-oxopropylideneamino)-6-d-ribitylaminouracil (5-OP-RU) was
429 freshly prepared from equal volumes of 32 mM 5-amino-6-d-ribitylaminouracil (5-A-RU)*HCl
430 (OHSU Medicinal Chemistry Core)[93] and 650 mM methylglyoxal (Sigma) exactly following the

431 second method described in [89] and used at a final concentration of 500 pM. Phytohemagglutinin
432 PHA-L (L4144 Sigma) was suspended in RPMI-HuS and used at 1 μg/well. Doxycycline (Sigma)
433 was suspended in sterile water and used at 2 μg/ml.

434 Recombinant human cytokines were reconstituted in sterile water and supplemented with bovine
435 serum albumin as per manufacturer recommendations. Final concentrations used were: 66 ng/ml
436 IFNγ (R&D Systems 285-IF-100), 66 ng/ml IFNβ (R&D Systems 8199-IF-010), 132 ng/ml IFNλ
437 (PeproTech 300-02K), 66 ng/ml TNFα (R&D Systems 10291-TA-050), and 66 ng/ml IL-17
438 (PeproTech 200-17). Cells were treated with cytokines for 12 hours unless otherwise noted.

439 Antibodies used for ELISPOT assays: α-IFNγ (1-D1K, Mabtech) and alkaline phosphatase-
440 conjugated secondary antibody (7-B6-1-ALP, Mabtech). Antibodies used for Western blot:
441 α-IRF1 (D5E4, Cell Signaling Technology), α-Vinculin (V284, Bio-Rad). Antibodies used for
442 flow cytometry: α-MR1 (26.5, conjugated to APC, Biolegend), α-HLA-A,B,C (W6/32, conjugated
443 to APC, Biolegend), IgG2a isotype (MOPC-173, conjugated to APC, Biolegend),
444 α-phospho-STAT1 (KIKSI0803, conjugated to PE, eBioscience).

445 **Co-culture experiments**

446 Primary AEC were infected with *S. pneumoniae* (20 MOI) in antibiotic-free BEGM. After 1 hour,
447 AEC were washed with PBS to remove non-adhered bacteria, then MAIT cells were added at a
448 1:1 ratio in BEGM complete with gentamycin-amphotericin (GA-1000, Lonza). BEAS-2B cells in
449 antibiotic-free DMEM-FBS were infected with *M. smegmatis* or treated with 6-FP or 5-OP-RU for
450 1 hour, washed with PBS, then MAIT cells were added at a 1:1 ratio in DMEM-FBS with
451 gentamycin. Following overnight co-culture, wells were extensively washed with PBS to remove
452 MAIT cells before harvesting AEC or BEAS-2B cells.

453 **Real-time quantitative PCR (RT-qPCR)**

454 Cell pellets washed with PBS were either used fresh or stored dry at -80°C before thawing in 37°C
455 water bath. RNA was isolated using the RNEasy Plus kit (Qiagen) and cDNA was synthesized
456 using the High Capacity cDNA Reverse Transcription Kit (Life Technologies) as per the
457 manufacturers' protocols. RT-qPCR was performed using TaqMan (Applied Biosystems) gene
458 expression assays: *HPRT1* (Hs02800695_m1), *MR1* (Hs01042278_m1), *HLA-A,H*
459 (Hs01058806_g1), *IRF1* (Hs00971965_m1), *NLRC5* (Hs01072123_m1), and *β2m*
460 (Hs00187842_m1). Gene expression data were normalized to internal control *HPRT1* and relative
461 expression levels for each target gene were determined using the $2^{-\Delta\Delta Ct}$ method[94]. Some uninfected
462 AEC *HPRT1* and *MR1* data were used as controls in [22].

463 **Flow cytometry**

464 To quantify surface expression of MR1 and MHC-I, AEC and BEAS-2B cells were treated as
465 indicated and harvested. Samples were blocked in FACS buffer containing 2% heat-inactivated
466 human serum, 2% heat-inactivated goat serum, and 0.5% heat-inactivated FBS for 30 minutes on
467 ice, then stained with APC-conjugated IgG2a, α-MR1, or α-HLA-A,B,C antibody for 40 minutes.
468 For pSTAT1 staining, cells were permeabilized with 0.2% saponin during the blocking step. Cells
469 were washed with PBS and fixed with 1% paraformaldehyde, then analyzed with a Beckman
470 Coulter CytoflexS. All analyses were performed using FlowJo10 (TreeStar).

471 **Enzyme-linked immunospot (ELISPOT) assays**

472 IFNγ ELISPOT assays were performed as previously described[95] with the following modifications:
473 ELISPOT plates (MSHAS4510, MilliporeSigma) were coated overnight with α-IFNγ antibody,
474 then washed and blocked for 1 hour in RPMI-HuS. BEAS-2B cells were seeded in duplicate ($1x10^5$

475 cells/well) and infected with *M. smegmatis*, treated with a titration of *M. smegmatis* supernatant,
476 or incubated with control PHA or RPMI-HuS medium for 1 hour at 37°C. D426G11 MAIT cell
477 clones were added at a 1:1 ratio in RPMI-HuS with gentamycin for overnight incubation at 37°C.
478 Following extensive washing with PBS-0.05% Tween 20, plates were incubated with ALP
479 secondary antibody for 2 hours before additional washing and colorimetric development. IFNγ
480 spot-forming units (SFU) were quantified by AID ELISPOT reader. For experiments with cytokine
481 pre-treatment, BEAS-2B cells were seeded in 6-well plates and treated with cytokines for 12 hours,
482 then washed 3 times with PBS to remove any excess cytokine before harvesting and seeding into
483 ELISPOT plate.

484 **siRNA gene silencing**

485 Gene silencing in wildtype, $Cas9^{+}$, or $NLRC5^{-/-}$ BEAS-2B cells was performed through
486 nucleofection as in [96] and following the Amaxa Cell Line Nucleofector Kit T (Lonza) protocols.
487 In brief, 2 µg total of Missense (4390843, ThermoFisher), IRF1 (s7501, ThermoFisher), and/or
488 NLRC5 (s38591, ThermoFisher) siRNA were added to $1x10^6$ cells and transfected by the Amaxa
489 Nucleofector 2b machine (Lonza) using program G-016. Cells were incubated for 48 hour before
490 use in assays. Efficiency of gene silencing was validated by RT-qPCR.

491 **Transcription factor binding sites**

492 Putative transcription factor binding sites were acquired through the Eukaryotic Promoter
493 Database browser using the Search Motif Tool to perform on-the-fly scanning for transcription
494 factor motifs using the FindM tool from the Signal Search Analysis (SSA) Server toolkit[28,97–99].

495 **Data analysis**

496 All data were analyzed using Prism (GraphPad) and plots were generated using R 4.4.0 and
497 packages such as tidyverse, ggprism, and rstatix. Statistical significance was determined as
498 indicated by two-tailed unpaired or pairwise t tests, using $\alpha=0.05$.

499 # Acknowledgements

500 We thank the staff at Cascade Alliance (formerly Pacific Northwest Transplant Bank) for
501 procuring the human lung and airway tissue samples used in this study. The D426G11 MAIT cell
502 clone was a kind gift from Dr. David Lewinsohn. We thank the OHSU Vollum Institute DNA
503 Sequencing Core and the OHSU Flow Cytometry Core for their support. We also thank Dr. Laurisa
504 Ankley and Dr. Andew Olive for their technical advice along with Savannah McBride and Dr.
505 Fikadu Tafesse for their aid in generating $Cas9^{+}$ BEAS-2B cells. We are grateful to Dr. Georgiana
506 Purdy, Dr. Elly Karamooz, and Dr. Corinna Kulicke for their scientific guidance.

507 # Figure Legends

508 **Figure 1. Increased MR1 expression following MAIT cell activation.**

509 **(A)** RT-qPCR of RNA isolated from primary human AECs (n=5) infected with *S. pneumoniae*
510 (*Sp*) for one hour and incubated overnight with MAIT cell clone. *MR1* expression was calculated
511 relative to *HPRT1* expression and uninfected no-MAIT (UI-) controls, paired by individual donor.

512 MR1 **(B)** mRNA and **(C)** surface expression of BEAS-2B cells infected with *M. smegmatis* (*Ms*)
513 for one hour and incubated overnight with MAIT cell clone. **(B)** RT-qPCR of *MR1* expression was
514 calculated relative to *HPRT1* expression and UI- control, paired by experimental replicate. **(C)**
515 Geometric mean fluorescence intensity (gMFI) of surface MR1 stained with α-MR1 26.5 Ab,
516 paired by experimental replicate.

517 MR1 **(D)** mRNA and **(E)** surface expression of BEAS-2B cells treated with 5-OP-RU (left,
518 "5-OP") or 6-FP (right) for one hour and incubated overnight with MAIT cell clone. **(D)** RT-qPCR
519 of *MR1* expression was calculated relative to *HPRT1* expression and UT- control, paired by
520 experimental replicate. **(E)** gMFI of surface MR1 stained with α-26.5 Ab, paired by experimental
521 replicate.

522 Pairwise statistical analyses are in Table 1.

523 **Figure 2. IFNγ induces MR1 expression and function.**

524 RT-qPCR of **(A)** primary human AECs or **(B)** BEAS-2B cells treated with media control (UT) or
525 recombinant IFNγ for 12 hours. *MR1* (left) and *HLA-A* (right) expression were calculated relative
526 to *HPRT1* expression and UT control, paired by individual donor or experimental replicate.

527 Flow cytometry of **(C)** primary AECs or **(D)** BEAS-2B cells treated with recombinant IFNγ for
528 12 hours. gMFI of surface MR1 (left, α-26.5 Ab) and MHC-Ia (right, α-W6/32 Ab) are paired by
529 individual donor or experimental replicate.

530 Pairwise T tests were performed by donor **(A** and **C)** or experiment **(B** and **D)**.

531 **Figure 3. Transcriptional stimulation of MR1 by IFNγ.**

532 **(A-D)** BEAS-2B:doxMR1-GFP cells were treated with doxycycline, IFNγ, and/or 6-FP overnight.
533 **(A)** *MR1* expression was calculated relative to *HPRT1* expression and UT control, paired by
534 experimental replicate. gMFI of **(B)** MR1-GFP, **(C)** surface MR1 α-26.5 stain, and **(D)** surface
535 MHC-Ia α-W6/32 stain. Data are experimental replicates.

536 **(E)** ELISPOT of BEAS-2B cells treated with filtered *M. smegmatis* supernatant and MAIT cells.
537 Data points are experimental replicates of no-antigen background-subtracted IFNγ spot-forming
538 units (SFU).

539 **(F)** Putative transcription factor binding sites were acquired through the Eukaryotic Promoter
540 Database browser using the Search Motif Tool to perform on-the-fly scanning for transcription
541 factor motifs using the FindM tool from the Signal Search Analysis (SSA) Server toolkit[28,97–99].
542 Highlighted proteins are involved in IRF1- (green) or NLRC5 enhanceosome- (blue) mediated
543 *HLA* transcription.

544 RT-qPCR of **(G)** primary human AECs or **(H)** BEAS-2B cells treated with recombinant IFNγ for
545 12 hours. *IRF1* (left) and *NLRC5* (right) expression were calculated relative to *HPRT1* expression
546 and UT control, paired by individual donor or experimental replicate.

547 Pairwise T tests were performed by experiment (A-E, H) or donor (G).

548 **Figure 4. IRF1 mediates IFNγ-induced MR1 transcription.**

549 **(A)** RT-qPCR of BEAS-2B cells treated with IRF1, NLRC5, and/or missense siRNA as labeled
550 for 36 hours, then incubated with IFNγ for 12 hours. *MR1* expression was calculated relative to
551 *HPRT1* expression and missense UT control, paired by experimental replicate.

552 **(B-C)** RT-qPCR of $Cas9^{+}$ or $NLRC5^{-/-}$ clone #1 BEAS-2B cells treated with IRF1 or missense
553 siRNA for 36 hours, then incubated with IFNγ for 12 hours. **(B)** *MR1* and **(C)** *HLA-A* expression
554 were calculated relative to *HPRT1* expression and $Cas9^{+}$ or $NLRC5^{-/-}$ clone #1 missense UT
555 controls, paired by experimental replicate.

556 **(D)** Cells in (B-C) were used as antigen-presenting cells in ELISPOT assay, with filtered *M.*
557 *smegmatis* supernatant as the antigen source. Data points are experimental replicates of $Cas9^{+}$ or
558 $NLRC5^{-/-}$ clone #1 missense control no-antigen background-subtracted IFNγ SFU.

559 **(E-F)** RT-qPCR of $Cas9^{+}$ or $IRF1^{-/-}$ clone #2 BEAS-2B cells treated with IFNγ for 12 hours. **(E)**
560 *MR1* and **(F)** *HLA-A* expression were calculated relative to *HPRT1* expression and $Cas9^{+}$ or $IRF1^{-}$
561 $^{/-}$ clone #2 UT controls, paired by experimental replicate.

562 **(G-H)** Flow cytometry of $Cas9^{+}$ or $IRF1^{-/-}$ clone #1 BEAS-2B cells treated with IFNγ for 12 hours.
563 gMFI of **(G)** surface MR1 (α-26.5 Ab) and **(H)** MHC-Ia (α-W6/32 Ab) are paired by experimental
564 replicate.

565 Statistical analyses are in Table 2.

566 **Figure 5. Reciprocal IFNγ signaling in *Sp*-infected AEC co-cultured with MAIT cells.**

567 **(A-B)** Flow cytometry of **(A)** primary human AECs infected with *S. pneumoniae* (*Sp*) for one hour
568 and incubated overnight with MAIT cell clone (n=3), or **(B)** AECs treated with IFNγ for 12 hours
569 (n=3). gMFI of stained pSTAT1 expression is paired by individual donor.

570 **(C-F)** RT-qPCR of primary human AECs infected with *S. pneumoniae* (*Sp*) for one hour and
571 incubated overnight with MAIT cell clone. Expression of **(C)** *β2m,* **(D)** *HLA-A* **(E)** *IRF1*, and **(F)**
572 *NLRC5* were calculated relative to *HPRT1* expression and UI- control, paired by individual donor
573 (n=4 (C) or n=5 (D-F) donors).

574 Pairwise statistical analyses are in Table 3.

575 **Figure 6. IFNγ produced by activated MAIT cells drives IRF1-dependent *MR1* transcription.**

576 **(A-D)** RT-qPCR of wildtype BEAS-2B cells treated as indicated below. Gene expression was
577 calculated relative to *HPRT1* expression and UI- or UT- controls, paired by experiment.

578 **(A)** *HLA-A* and **(C)** *IRF1* expression of BEAS-2B cells infected with *M. smegmatis* (*Ms*) for one
579 hour and incubated overnight with MAIT cell clone.

580 **(B)** *HLA-A* and **(D)** *IRF1* expression of BEAS-2B cells treated with 5-OP-RU (left, "5-OP") or
581 6-FP (right) for one hour and incubated overnight with MAIT cell clone.

582 **(E-F)** RT-qPCR of $Cas9^{+}$ or $IRF1^{-/-}$ clone #1 BEAS-2B cells **(E)** infected with *M. smegmatis* or
583 **(F)** treated with 5-OP-RU for one hour, then incubated overnight with MAIT cell clone. *MR1*
584 expression was calculated relative to *HPRT1* expression and $Cas9^{+}$ or $IRF1^{-/-}$ UT- controls, paired
585 by experimental replicate.

586 Pairwise statistical analyses are in Table 4.

587 **Figure 7. Interferons stimulate MR1 and MHC-Ia transcription through different pathways.**

588 **(A)** RT-qPCR of wildtype BEAS-2B cells treated with recombinant human cytokines for 12 hours.
589 *MR1* expression was calculated relative to *HPRT1* expression and UT controls.

590 **(B-C, F-H)** RT-qPCR of BEAS-2B cells treated with IFNγ or IFNβ for 12 hours. Expression of
591 **(B)** *MR1,* **(C)** *HLA-A* **(F)** *IRF1*, **(G)** *NLRC5*, and **(H)** *β2m* were calculated relative to *HPRT1* and
592 UT control, paired by experiment.

593 **(D)** Flow cytometry of BEAS-2B cells treated with IFNγ or IFNβ for 12 hours. gMFI of surface
594 MR1 (left, α-26.5 Ab) and MHC-Ia (right, α-W6/32 Ab) are paired by experimental replicate.

595 **(E)** ELISPOT of BEAS-2B cells treated with IFNγ or IFNβ for 12 hours, infected with a titration
596 of *M. smegmatis* for one hour, then incubated with MAIT cells overnight. Data points are average
597 SFU of no-antigen background-subtracted IFNγ SFU. Nonlinear regression agonist response
598 curves were computed in GraphPad Prism 10.4.0 and analyzed with extra sum-of-squares F test to
599 compare with UT control.

600 Statistical analyses are in Table 5.

601 **Figure 8. IFNγ signaling induces MR1 expression and MAIT cell activation.**

602 IFNγ from MAIT cells or other cellular sources induces *IRF1* expression and subsequent increase
603 in *MR1* transcription. Increased MR1 expression and antigen presentation enhances MAIT cell
604 responses to antigens from exogenous sources or pathogens like *S. pneumoniae* or *M. smegmatis.*

605 # Supplemental Figure Legends

606 **Supplemental Figure 1. Associated with Figure 1.**

607 **(A)** RT-qPCR of RNA isolated from primary human AECs (n=5) infected with *S. pneumoniae*
608 (*Sp*) for one hour and incubated overnight with MAIT cell clone. *MR1* expression was calculated
609 relative to *HPRT1* expression and uninfected no-MAIT (UI-) controls, paired by individual donor.

610 MR1 **(B)** mRNA and **(C)** surface expression of BEAS-2B cells infected with *M. smegmatis* (*Ms*)
611 for one hour and incubated overnight with MAIT cell clone. **(B)** RT-qPCR of *MR1* expression was
612 calculated relative to *HPRT1* expression and UI- control, paired by experimental replicate. **(C)**
613 gMFI of surface MR1 stained with α-MR1 26.5 Ab, paired by experimental replicate.

614 MR1 **(D)** mRNA and **(E)** surface expression of BEAS-2B cells treated with 5-OP-RU (left, "5-
615 OP") or 6-FP (right) for one hour and incubated overnight with MAIT cell clone. **(D)** RT-qPCR
616 of *MR1* expression was calculated relative to *HPRT1* expression and media no-MAIT (UT-)
617 control, paired by experimental replicate. **(E)** gMFI of surface MR1 stained with α-26.5 Ab, paired
618 by experimental replicate.

619 Pairwise statistical analyses are in Table 1.

620 **Supplemental Figure 2. Associated with Figure 4.**

621 **(A-B)** RT-qPCR of BEAS-2B cells treated with IRF1, NLRC5, and/or missense siRNA as
622 indicated for 36 hours, then incubated with IFNγ for 12 hours. **(A)** *IRF1* and **(B)** *NLRC5* expression
623 were calculated relative to *HPRT1* expression and missense UT control, paired by experimental
624 replicate.

625 **(C-D)** RT-qPCR of Cas9^{+} or NLRC5$^{-/-}$ BEAS-2B cells treated with IRF1 or missense siRNA for
626 36 hours, then incubated with IFNγ for 12 hours. Gene expression of **(C)** NLRC5$^{-/-}$ clone #1 or
627 **(D)** NLRC5$^{-/-}$ clone #2 were calculated relative to *HPRT1* expression and Cas9^{+} or NLRC5$^{-/-}$ clone
628 missense UT controls, paired by experimental replicate.

629 **(E)** Flow cytometry of cells from (C-D). gMFI of surface MR1 (α-26.5 Ab) are from IFNγ-treated
630 Cas9^{+} (left), NLRC5$^{-/-}$ clone #1 (middle), and NLRC5$^{-/-}$ clone #2 (right).

631 **(F)** RT-qPCR of Cas9^{+} or IRF1$^{-/-}$ clone #1 BEAS-2B cells treated with IFNγ for 12 hours. *MR1*
632 expression was calculated relative to *HPRT1* expression and Cas9^{+} or IRF1$^{-/-}$ clone #1 UT controls,
633 paired by experimental replicate.

634 **(G)** Flow cytometry of Cas9^{+} or IRF1$^{-/-}$ clone #2 BEAS-2B cells treated with IFNγ for 12 hours.
635 gMFI of surface MR1 (left, α-26.5 Ab) and MHC-Ia (left, α-W6/32 Ab) are paired by experimental
636 replicate.

637 Statistical analyses are in Table 2.

638 **Supplemental Figure 3. Associated with Figure 5 and Figure 6.**

639 **(A)** Flow cytometry of primary human AECs infected with *S. pneumoniae* (*Sp*) for one hour and
640 incubated overnight with MAIT cell clone. gMFI of stained pSTAT1 expression is paired by
641 individual donor (n=3).

642 **(B-D, G)** RT-qPCR of primary human AECs infected with *S. pneumoniae* (*Sp*) for one hour and
643 incubated overnight with MAIT cell clone. Expression of **(B)** *β2m,* **(C)** *NLRC5* **(D)** *HLA-A*, and
644 **(G)** *IRF1* were calculated relative to *HPRT1* expression and UI- control, paired by individual donor
645 (n=4 (B) or n=5 (C, D, G) donors).

646 **(E-F, H-I)** RT-qPCR of wildtype BEAS-2B cells treated as indicated below. Gene expression was
647 calculated relative to *HPRT1* expression and UI- or UT- controls, paired by experiment.

648 **(E)** *HLA-A* and **(H)** *IRF1* expression of BEAS-2B cells infected with *M. smegmatis* (*Ms*) for one
649 hour and incubated overnight with MAIT cell clone.

650 **(F)** *HLA-A* and **(I)** *IRF1* expression of BEAS-2B cells treated with 5-OP-RU (left) or 6-FP (right)
651 for one hour and incubated overnight with MAIT cell clone.

652 Pairwise statistical analyses are in Table 3.

653 **Supplemental Figure 4. Associated with Figure 7.**

654 **(A-C)** RT-qPCR of wildtype BEAS-2B cells treated with recombinant human cytokines for 12
655 hours. Gene expression was calculated relative to *HPRT1* expression and UT controls.

656 Statistical analyses are in Table 5.

657 # Tables

658 **Table 1 Statistics associated with Figure 1 and Supplemental Figure 1.**

659

Figure	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		Cell	Ag	T cell	Cell	Ag	T cell						
1A, SF1A	*MR1* mRNA	AEC	UI	NT	AEC	*Sp*	NT	5	5	4	1.913	0.1283	ns
1A, SF1A	*MR1* mRNA	AEC	UI	NT	AEC	UI	MAIT	5	5	4	1.702	0.1639	ns
1A, SF1A	*MR1* mRNA	AEC	UI	NT	AEC	*Sp*	MAIT	5	5	4	8.332	0.0011	**
1A, SF1A	*MR1* mRNA	AEC	*Sp*	NT	AEC	UI	MAIT	5	5	4	0.391	0.7157	ns
1A, SF1A	*MR1* mRNA	AEC	*Sp*	NT	AEC	*Sp*	MAIT	5	5	4	3.679	0.0212	*
1A, SF1A	*MR1* mRNA	AEC	UI	MAIT	AEC	*Sp*	MAIT	5	5	4	2.846	0.0466	*
1B, SF1B	*MR1* mRNA	B2B	UI	NT	B2B	*Ms*	NT	3	3	2	1.387	0.2998	ns
1B, SF1B	*MR1* mRNA	B2B	UI	NT	B2B	UI	MAIT	3	3	2	1.653	0.24	ns
1B, SF1B	*MR1* mRNA	B2B	UI	NT	B2B	*Ms*	MAIT	3	3	2	3.689	0.0663	ns
1B, SF1B	*MR1* mRNA	B2B	*Ms*	NT	B2B	UI	MAIT	3	3	2	2.253	0.153	ns
1B, SF1B	*MR1* mRNA	B2B	*Ms*	NT	B2B	*Ms*	MAIT	3	3	2	3.702	0.0658	ns
1B, SF1B	*MR1* mRNA	B2B	UI	MAIT	B2B	*Ms*	MAIT	3	3	2	3.095	0.0905	ns
1C, SF1C	αMR1 gMFI	B2B	UI	NT	B2B	*Ms*	NT	3	3	2	1.31	0.3205	ns
1C, SF1C	αMR1 gMFI	B2B	UI	NT	B2B	UI	MAIT	3	3	2	0.8576	0.4815	ns
1C, SF1C	αMR1 gMFI	B2B	UI	NT	B2B	*Ms*	MAIT	3	3	2	15.02	0.0044	**
1C, SF1C	αMR1 gMFI	B2B	*Ms*	NT	B2B	UI	MAIT	3	3	2	0.7913	0.5117	ns
1C, SF1C	αMR1 gMFI	B2B	*Ms*	NT	B2B	*Ms*	MAIT	3	3	2	12.17	0.0067	**
1C, SF1C	αMR1 gMFI	B2B	UI	MAIT	B2B	*Ms*	MAIT	3	3	2	5.032	0.0373	*
1D, SF1D	MR1 mRNA	B2B	UT	NT	B2B	5-OP	NT	3	3	2	0.8515	0.4842	ns
1D, SF1D	MR1 mRNA	B2B	UT	NT	B2B	UT	MAIT	3	3	2	1.653	0.24	ns
1D, SF1D	MR1 mRNA	B2B	UT	NT	B2B	5-OP	MAIT	3	3	2	4.931	0.0388	*
1D, SF1D	MR1 mRNA	B2B	5-OP	NT	B2B	UT	MAIT	3	3	2	1.635	0.2437	ns
1D, SF1D	MR1 mRNA	B2B	5-OP	NT	B2B	5-OP	MAIT	3	3	2	4.784	0.041	*
1D, SF1D	MR1 mRNA	B2B	UT	MAIT	B2B	5-OP	MAIT	3	3	2	5.377	0.0329	*
1D, SF1D	MR1 mRNA	B2B	UT	NT	B2B	6-FP	NT	3	3	2	0.7058	0.5535	ns
1D, SF1D	MR1 mRNA	B2B	UT	NT	B2B	6-FP	MAIT	3	3	2	1.585	0.2539	ns
1D, SF1D	MR1 mRNA	B2B	6-FP	NT	B2B	UT	MAIT	3	3	2	1.318	0.3182	ns
1D, SF1D	MR1 mRNA	B2B	6-FP	NT	B2B	6-FP	MAIT	3	3	2	1.466	0.2802	ns
1D, SF1D	MR1 mRNA	B2B	UT	MAIT	B2B	6-FP	MAIT	3	3	2	0.8054	0.5051	ns
1D, SF1D	MR1 mRNA	B2B	5-OP	NT	B2B	6-FP	NT	3	3	2	0.5185	0.6557	ns
1D, SF1D	MR1 mRNA	B2B	5-OP	MAIT	B2B	6-FP	MAIT	3	3	2	5.239	0.0346	*
1E, SF1E	αMR1 gMFI	B2B	UT	NT	B2B	5-OP	NT	4	4	3	1.312	0.2808	ns
1E, SF1E	αMR1 gMFI	B2B	UT	NT	B2B	UT	MAIT	4	4	3	0.9286	0.4216	ns
1E, SF1E	αMR1 gMFI	B2B	UT	NT	B2B	5-OP	MAIT	4	4	3	2.826	0.0664	ns
1E, SF1E	αMR1 gMFI	B2B	5-OP	NT	B2B	UT	MAIT	4	4	3	2.042	0.1338	ns
1E, SF1E	αMR1 gMFI	B2B	5-OP	NT	B2B	5-OP	MAIT	4	4	3	3.166	0.0506	ns
1E, SF1E	αMR1 gMFI	B2B	UT	MAIT	B2B	5-OP	MAIT	4	4	3	3.218	0.0487	*
1E, SF1E	αMR1 gMFI	B2B	UT	NT	B2B	6-FP	NT	4	4	3	0.2705	0.8043	ns
1E, SF1E	αMR1 gMFI	B2B	UT	NT	B2B	6-FP	MAIT	4	4	3	0.5864	0.5988	ns

Figure	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		Cell	Ag	T cell	Cell	Ag	T cell						
1E, SF1E	αMR1 gMFI	B2B	6-FP	NT	B2B	UT	MAIT	4	4	3	1.558	0.2171	ns
1E, SF1E	αMR1 gMFI	B2B	6-FP	NT	B2B	6-FP	MAIT	4	4	3	1.349	0.2703	ns
1E, SF1E	αMR1 gMFI	B2B	UT	MAIT	B2B	6-FP	MAIT	4	4	3	0.03772	0.9723	ns
1E, SF1E	αMR1 gMFI	B2B	5-OP	NT	B2B	6-FP	NT	4	4	3	2.235	0.1114	ns
1E, SF1E	αMR1 gMFI	B2B	5-OP	MAIT	B2B	6-FP	MAIT	4	4	3	2.559	0.0833	ns

Definition of abbreviations:
Ag = antigen; df = degrees of freedom; statistic = absolute value of T statistic; AEC = airway epithelial cells; B2B = BEAS-2B cells; UI = uninfected control; *Sp* = *Streptococcus pneumoniae*; *Ms* = *Mycobacterium smegmatis*; UT = media treated control; 5-OP = 5-OP-RU; 6-FP = 6-formylpterin; NT = no T cell control; gMFI = geometric mean fluorescence intensity

667 **Table 2 Statistics associated with Figure 4 and Supplemental Figure 2.**

668

Fig.	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		NLRC5	IRF1	IFNγ	NLRC5	IRF1	IFNγ						
4A	*MR1* mRNA	Mis	Mis	UT	Mis	KD	UT	3	3	2	0.6683	0.5727	ns
4A	*MR1* mRNA	Mis	Mis	UT	KD	Mis	UT	3	3	2	0.5922	0.6138	ns
4A	*MR1* mRNA	Mis	Mis	UT	KD	KD	UT	3	3	2	1.195	0.3545	ns
4A	*MR1* mRNA	Mis	Mis	UT	Mis	Mis	IFNγ	3	3	2	36.62	0.0007	***
4A	*MR1* mRNA	Mis	KD	UT	KD	Mis	UT	3	3	2	1.517	0.2684	ns
4A	*MR1* mRNA	Mis	KD	UT	KD	KD	UT	3	3	2	2.281	0.1501	ns
4A	*MR1* mRNA	Mis	KD	UT	Mis	KD	IFNγ	3	3	2	5.223	0.0348	*
4A	*MR1* mRNA	KD	Mis	UT	KD	KD	UT	3	3	2	3.215	0.0846	ns
4A	*MR1* mRNA	KD	Mis	UT	KD	Mis	IFNγ	3	3	2	12.33	0.0065	**
4A	*MR1* mRNA	KD	KD	UT	KD	KD	IFNγ	3	3	2	14.32	0.0048	**
4A	*MR1* mRNA	Mis	Mis	IFNγ	Mis	KD	IFNγ	3	3	2	11.71	0.0072	**
4A	*MR1* mRNA	Mis	Mis	IFNγ	KD	Mis	IFNγ	3	3	2	1.156	0.3671	ns
4A	*MR1* mRNA	Mis	Mis	IFNγ	KD	KD	IFNγ	3	3	2	17.41	0.0033	**
4A	*MR1* mRNA	Mis	KD	IFNγ	KD	Mis	IFNγ	3	3	2	6.393	0.0236	*
4A	*MR1* mRNA	Mis	KD	IFNγ	KD	KD	IFNγ	3	3	2	3.15	0.0877	ns
4A	*MR1* mRNA	KD	Mis	IFNγ	KD	KD	IFNγ	3	3	2	9.035	0.012	*
SF2A	*IRF1* mRNA	Mis	Mis	UT	Mis	KD	UT	3	3	2	8.304	0.0142	*
SF2A	*IRF1* mRNA	Mis	Mis	UT	KD	Mis	UT	3	3	2	0.7416	0.5356	ns
SF2A	*IRF1* mRNA	Mis	Mis	UT	KD	KD	UT	3	3	2	16.04	0.0039	**
SF2A	*IRF1* mRNA	Mis	Mis	UT	Mis	Mis	IFNγ	3	3	2	10.94	0.0083	**
SF2A	*IRF1* mRNA	Mis	KD	UT	KD	Mis	UT	3	3	2	5.412	0.0325	*
SF2A	*IRF1* mRNA	Mis	KD	UT	KD	KD	UT	3	3	2	0.8864	0.4689	ns
SF2A	*IRF1* mRNA	Mis	KD	UT	Mis	KD	IFNγ	3	3	2	3.729	0.065	ns
SF2A	*IRF1* mRNA	KD	Mis	UT	KD	KD	UT	3	3	2	8.973	0.0122	*
SF2A	*IRF1* mRNA	KD	Mis	UT	KD	Mis	IFNγ	3	3	2	4.914	0.039	*
SF2A	*IRF1* mRNA	KD	KD	UT	KD	KD	IFNγ	3	3	2	3.78	0.0634	ns
SF2A	*IRF1* mRNA	Mis	Mis	IFNγ	Mis	KD	IFNγ	3	3	2	15.06	0.0044	**
SF2A	*IRF1* mRNA	Mis	Mis	IFNγ	KD	Mis	IFNγ	3	3	2	0.8739	0.4743	ns
SF2A	*IRF1* mRNA	Mis	Mis	IFNγ	KD	KD	IFNγ	3	3	2	17.23	0.0034	**
SF2A	*IRF1* mRNA	Mis	KD	IFNγ	KD	Mis	IFNγ	3	3	2	4.394	0.0481	*
SF2A	*IRF1* mRNA	Mis	KD	IFNγ	KD	KD	IFNγ	3	3	2	0.9636	0.4369	ns
SF2A	*IRF1* mRNA	KD	Mis	IFNγ	KD	KD	IFNγ	3	3	2	5.308	0.0337	*
SF2B	*NLRC5* mRNA	Mis	Mis	UT	Mis	KD	UT	3	3	2	14.19	0.0049	**
SF2B	*NLRC5* mRNA	Mis	Mis	UT	KD	Mis	UT	3	3	2	1.149	0.3696	ns
SF2B	*NLRC5* mRNA	Mis	Mis	UT	KD	KD	UT	3	3	2	2.041	0.178	ns
SF2B	*NLRC5* mRNA	Mis	Mis	UT	Mis	Mis	IFNγ	3	3	2	8.078	0.015	*
SF2B	*NLRC5* mRNA	Mis	KD	UT	KD	Mis	UT	3	3	2	0.04497	0.9682	ns
SF2B	*NLRC5* mRNA	Mis	KD	UT	KD	KD	UT	3	3	2	0.7383	0.5372	ns

Fig.	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		NLRC5	IRF1	IFNγ	NLRC5	IRF1	IFNγ						
SF2B	*NLRC5* mRNA	Mis	KD	UT	Mis	KD	IFNγ	3	3	2	5.053	0.037	*
SF2B	*NLRC5* mRNA	KD	Mis	UT	KD	KD	UT	3	3	2	1.568	0.2575	ns
SF2B	*NLRC5* mRNA	KD	Mis	UT	KD	Mis	IFNγ	3	3	2	4.443	0.0471	*
SF2B	*NLRC5* mRNA	KD	KD	UT	KD	KD	IFNγ	3	3	2	15.64	0.0041	**
SF2B	*NLRC5* mRNA	Mis	Mis	IFNγ	Mis	KD	IFNγ	3	3	2	1.171	0.3621	ns
SF2B	*NLRC5* mRNA	Mis	Mis	IFNγ	KD	Mis	IFNγ	3	3	2	2.113	0.1689	ns
SF2B	*NLRC5* mRNA	Mis	Mis	IFNγ	KD	KD	IFNγ	3	3	2	2.631	0.1192	ns
SF2B	*NLRC5* mRNA	Mis	KD	IFNγ	KD	Mis	IFNγ	3	3	2	5.198	0.0351	*
SF2B	*NLRC5* mRNA	Mis	KD	IFNγ	KD	KD	IFNγ	3	3	2	1.353	0.3087	ns
SF2B	*NLRC5* mRNA	KD	Mis	IFNγ	KD	KD	IFNγ	3	3	2	0.1013	0.9286	ns
4B	*MR1* mRNA	Cas9	Mis	UT	Cas9	KD	UT	3	3	2	1.281	0.3286	ns
4B	*MR1* mRNA	Cas9	Mis	UT	KO #1	Mis	UT	3	3	2	N/A	N/A	N/A
4B	*MR1* mRNA	Cas9	Mis	UT	KO #1	KD	UT	3	3	2	4.524	0.0456	*
4B	*MR1* mRNA	Cas9	Mis	UT	Cas9	Mis	IFNγ	3	3	2	8.226	0.0145	*
4B	*MR1* mRNA	Cas9	KD	UT	KO #1	Mis	UT	3	3	2	1.281	0.3286	ns
4B	*MR1* mRNA	Cas9	KD	UT	KO #1	KD	UT	3	3	2	1.352	0.3089	ns
4B	*MR1* mRNA	Cas9	KD	UT	Cas9	KD	IFNγ	3	3	2	4.923	0.0389	*
4B	*MR1* mRNA	KO #1	Mis	UT	KO #1	KD	UT	3	3	2	4.524	0.0456	*
4B	*MR1* mRNA	KO #1	Mis	UT	KO #1	Mis	IFNγ	3	3	2	42.26	0.0006	***
4B	*MR1* mRNA	KO #1	KD	UT	KO #1	KD	IFNγ	3	3	2	1.525	0.2667	ns
4B	*MR1* mRNA	Cas9	Mis	IFNγ	Cas9	KD	IFNγ	3	3	2	4.489	0.0462	*
4B	*MR1* mRNA	Cas9	Mis	IFNγ	KO #1	Mis	IFNγ	3	3	2	1.778	0.2173	ns
4B	*MR1* mRNA	Cas9	Mis	IFNγ	KO #1	KD	IFNγ	3	3	2	2.469	0.1323	ns
4B	*MR1* mRNA	Cas9	KD	IFNγ	KO #1	Mis	IFNγ	3	3	2	6.989	0.0199	*
4B	*MR1* mRNA	Cas9	KD	IFNγ	KO #1	KD	IFNγ	3	3	2	0.01689	0.9881	ns
4B	*MR1* mRNA	KO #1	Mis	IFNγ	KO #1	KD	IFNγ	3	3	2	2.643	0.1183	ns
4C	*HLA-A* mRNA	Cas9	Mis	UT	Cas9	KD	UT	3	3	2	0.5836	0.6186	ns
4C	*HLA-A* mRNA	Cas9	Mis	UT	KO #1	Mis	UT	3	3	2	N/A	N/A	N/A
4C	*HLA-A* mRNA	Cas9	Mis	UT	KO #1	KD	UT	3	3	2	4.16	0.0532	ns
4C	*HLA-A* mRNA	Cas9	Mis	UT	Cas9	Mis	IFNγ	3	3	2	4.282	0.0504	ns
4C	*HLA-A* mRNA	Cas9	KD	UT	KO #1	Mis	UT	3	3	2	0.5836	0.6186	ns
4C	*HLA-A* mRNA	Cas9	KD	UT	KO #1	KD	UT	3	3	2	2.37	0.1412	ns
4C	*HLA-A* mRNA	Cas9	KD	UT	Cas9	KD	IFNγ	3	3	2	6.746	0.0213	*
4C	*HLA-A* mRNA	KO #1	Mis	UT	KO #1	KD	UT	3	3	2	4.16	0.0532	ns
4C	*HLA-A* mRNA	KO #1	Mis	UT	KO #1	Mis	IFNγ	3	3	2	6.47	0.0231	*
4C	*HLA-A* mRNA	KO #1	KD	UT	KO #1	KD	IFNγ	3	3	2	3.794	0.063	ns
4C	*HLA-A* mRNA	Cas9	Mis	IFNγ	Cas9	KD	IFNγ	3	3	2	3.145	0.088	ns
4C	*HLA-A* mRNA	Cas9	Mis	IFNγ	KO #1	Mis	IFNγ	3	3	2	3.393	0.077	ns
4C	*HLA-A* mRNA	Cas9	Mis	IFNγ	KO #1	KD	IFNγ	3	3	2	3.575	0.0701	ns
4C	*HLA-A* mRNA	Cas9	KD	IFNγ	KO #1	Mis	IFNγ	3	3	2	0.2266	0.8418	ns
4C	*HLA-A* mRNA	Cas9	KD	IFNγ	KO #1	KD	IFNγ	3	3	2	4.039	0.0562	ns

Fig.	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		NLRC5	IRF1	IFNγ	NLRC5	IRF1	IFNγ						
4C	*HLA-A* mRNA	KO #1	Mis	IFNγ	KO #1	KD	IFNγ	3	3	2	3.875	0.0606	ns
SF2C	*IRF1* mRNA	Cas9	Mis	UT	Cas9	KD	UT	3	3	2	5.042	0.0372	*
SF2C	*IRF1* mRNA	Cas9	Mis	UT	KO #1	Mis	UT	3	3	2	N/A	N/A	N/A
SF2C	*IRF1* mRNA	Cas9	Mis	UT	KO #1	KD	UT	3	3	2	6.233	0.0248	*
SF2C	*IRF1* mRNA	Cas9	Mis	UT	Cas9	Mis	IFNγ	3	3	2	16.02	0.0039	**
SF2C	*IRF1* mRNA	Cas9	KD	UT	KO #1	Mis	UT	3	3	2	5.042	0.0372	*
SF2C	*IRF1* mRNA	Cas9	KD	UT	KO #1	KD	UT	3	3	2	0.1761	0.8764	ns
SF2C	*IRF1* mRNA	Cas9	KD	UT	Cas9	KD	IFNγ	3	3	2	2.58	0.1231	ns
SF2C	*IRF1* mRNA	KO #1	Mis	UT	KO #1	KD	UT	3	3	2	6.233	0.0248	*
SF2C	*IRF1* mRNA	KO #1	Mis	UT	KO #1	Mis	IFNγ	3	3	2	15.4	0.0042	**
SF2C	*IRF1* mRNA	KO #1	KD	UT	KO #1	KD	IFNγ	3	3	2	2.362	0.1420	ns
SF2C	*IRF1* mRNA	Cas9	Mis	IFNγ	Cas9	KD	IFNγ	3	3	2	6.101	0.0258	*
SF2C	*IRF1* mRNA	Cas9	Mis	IFNγ	KO #1	Mis	IFNγ	3	3	2	2.222	0.1564	ns
SF2C	*IRF1* mRNA	Cas9	Mis	IFNγ	KO #1	KD	IFNγ	3	3	2	5.922	0.0273	*
SF2C	*IRF1* mRNA	Cas9	KD	IFNγ	KO #1	Mis	IFNγ	3	3	2	7.389	0.0178	*
SF2C	*IRF1* mRNA	Cas9	KD	IFNγ	KO #1	KD	IFNγ	3	3	2	1.52	0.2679	ns
SF2C	*IRF1* mRNA	KO #1	Mis	IFNγ	KO #1	KD	IFNγ	3	3	2	7.82	0.0160	*
4D	IFNγ SFU	Cas9	Mis	IFNγ	Cas9	KD	IFNγ	3	3	2.653	5.207	0.0186	*
4D	IFNγ SFU	Cas9	Mis	IFNγ	KO #1	Mis	IFNγ	3	3	3.03	0.08493	0.9376	ns
4D	IFNγ SFU	Cas9	Mis	IFNγ	KO #1	KD	IFNγ	3	3	3.95	9.49725	0.0007	***
4D	IFNγ SFU	Cas9	KD	IFNγ	KO #1	Mis	IFNγ	3	3	3.772	4.5	0.0124	*
4D	IFNγ SFU	Cas9	KD	IFNγ	KO #1	KD	IFNγ	3	3	2.525	0.3875	0.7287	ns
4D	IFNγ SFU	KO #1	Mis	IFNγ	KO #1	KD	IFNγ	3	3	2.843	6.153	0.0101	*
4E	*MR1* mRNA	N/A	Cas9	UT	N/A	Cas9	IFNγ	6	6	5	7.089	0.0009	***
4E	*MR1* mRNA	N/A	KO #2	UT	N/A	KO #2	IFNγ	6	6	5	1.049	0.3424	ns
4E	*MR1* mRNA	N/A	Cas9	UT	N/A	KO #2	UT	6	6	5	N/A	N/A	N/A
4E	*MR1* mRNA	N/A	Cas9	IFNγ	N/A	KO #2	IFNγ	6	6	5.718	5.664	0.0024	**
4F	*HLA-A* mRNA	N/A	Cas9	UT	N/A	Cas9	IFNγ	6	6	5	7.715	0.0006	***
4F	*HLA-A* mRNA	N/A	KO #2	UT	N/A	KO #2	IFNγ	6	6	5	1.094	0.3237	ns
4F	*HLA-A* mRNA	N/A	Cas9	UT	N/A	KO #2	UT	6	6	5	N/A	N/A	N/A
4F	*HLA-A* mRNA	N/A	Cas9	IFNγ	N/A	KO #2	IFNγ	6	6	7.874	10.31	0.0001	***
SF2F	*MR1* mRNA	N/A	Cas9	UT	N/A	Cas9	IFNγ	4	4	3	12.35	0.0011	**
SF2F	*MR1* mRNA	N/A	KO #1	UT	N/A	KO #1	IFNγ	4	4	3	12.78	0.001	**
SF2F	*MR1* mRNA	N/A	Cas9	UT	N/A	KO #1	UT	4	4	3	N/A	N/A	N/A
SF2F	*MR1* mRNA	N/A	Cas9	IFNγ	N/A	KO #1	IFNγ	4	4	3.226	8.578	0.0033	**
4G	αMR1 gMFI	N/A	Cas9	UT	N/A	Cas9	IFNγ	4	4	3	5.082	0.0147	*
4G	αMR1 gMFI	N/A	KO #1	UT	N/A	KO #1	IFNγ	4	4	3	0.5987	0.5916	ns
4G	αMR1 gMFI	N/A	Cas9	UT	N/A	KO #1	UT	4	4	5.949	0.4949	0.6384	ns
4G	αMR1 gMFI	N/A	Cas9	IFNγ	N/A	KO #1	IFNγ	4	4	5.97	0.7191	0.4992	ns
4H	αMHC-Ia gMFI	N/A	Cas9	UT	N/A	Cas9	IFNγ	4	4	3	4.887	0.0164	*

Fig.	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		NLRC5	IRF1	IFNγ	NLRC5	IRF1	IFNγ						
4H	αMHC-Ia gMFI	N/A	KO #1	UT	N/A	KO #1	IFNγ	4	4	3	1.184	0.3216	ns
4H	αMHC-Ia gMFI	N/A	Cas9	UT	N/A	KO #1	UT	4	4	5.827	0.7061	0.5073	ns
4H	αMHC-Ia gMFI	N/A	Cas9	IFNγ	N/A	KO #1	IFNγ	4	4	3.507	3.38	0.034	*
SF2G	αMR1 gMFI	N/A	Cas9	UT	N/A	Cas9	IFNγ	3	3	2	5.068	0.0368	*
SF2G	αMR1 gMFI	N/A	KO #2	UT	N/A	KO #2	IFNγ	3	3	2	1.736	0.2246	ns
SF2G	αMR1 gMFI	N/A	Cas9	UT	N/A	KO #2	UT	3	3	3.48	0.7566	0.4973	ns
SF2G	αMR1 gMFI	N/A	Cas9	IFNγ	N/A	KO #2	IFNγ	3	3	3.933	1.387	0.2389	ns
SF2H	αMHC-Ia gMFI	N/A	Cas9	UT	N/A	Cas9	IFNγ	3	3	2	4.391	0.0481	*
SF2H	αMHC-Ia gMFI	N/A	KO #2	UT	N/A	KO #2	IFNγ	3	3	2	0.04702	0.9668	ns
SF2H	αMHC-Ia gMFI	N/A	Cas9	UT	N/A	KO #2	UT	3	3	3.029	0.4856	0.6602	ns
SF2H	αMHC-Ia gMFI	N/A	Cas9	IFNγ	N/A	KO #2	IFNγ	3	3	2.597	2.543	0.0975	ns

Definition of abbreviations:
df = degrees of freedom; statistic = absolute value of T statistic; Mis = missense siRNA control; KD = siRNA gene knockdown; UT = media control; Cas9 = $Cas9^+$ BEAS-2B control cells; KO = CRISPR gene knockout cell line; N/A = not applicable; gMFI = geometric mean fluorescence intensity

675 **Table 3 Statistics associated with Figure 5, Figure 6, and Supplemental Figure 3.**

676

Figure	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		Cell	Ag	T cell	Cell	Ag	T cell						
5A, SF3A	pSTAT1 gMFI	AEC	UI	NT	AEC	*Sp*	NT	3	3	2	1.018	0.4159	ns
5A, SF3A	pSTAT1 gMFI	AEC	UI	NT	AEC	UI	MAIT	3	3	2	1.472	0.2788	ns
5A, SF3A	pSTAT1 gMFI	AEC	UI	NT	AEC	*Sp*	MAIT	3	3	2	9.043	0.012	*
5A, SF3A	pSTAT1 gMFI	AEC	*Sp*	NT	AEC	UI	MAIT	3	3	2	0.5673	0.6277	ns
5A, SF3A	pSTAT1 gMFI	AEC	*Sp*	NT	AEC	*Sp*	MAIT	3	3	2	4.031	0.0564	ns
5A, SF3A	pSTAT1 gMFI	AEC	UI	MAIT	AEC	*Sp*	MAIT	3	3	2	6.541	0.0226	*
5C, SF3B	*B2m* mRNA	AEC	UI	NT	AEC	*Sp*	NT	4	4	3	3.014	0.057	ns
5C, SF3B	*B2m* mRNA	AEC	UI	NT	AEC	UI	MAIT	4	4	3	4.013	0.0278	*
5C, SF3B	*B2m* mRNA	AEC	UI	NT	AEC	*Sp*	MAIT	4	4	3	4.361	0.0223	*
5C, SF3B	*B2m* mRNA	AEC	*Sp*	NT	AEC	UI	MAIT	4	4	3	0.9405	0.4164	ns
5C, SF3B	*B2m* mRNA	AEC	*Sp*	NT	AEC	*Sp*	MAIT	4	4	3	4.302	0.0231	*
5C, SF3B	*B2m* mRNA	AEC	UI	MAIT	AEC	*Sp*	MAIT	4	4	3	3.741	0.0333	*
5D, SF3D	*HLA-A* mRNA	AEC	UI	NT	AEC	*Sp*	NT	5	5	4	2.918	0.0433	*
5D, SF3D	*HLA-A* mRNA	AEC	UI	NT	AEC	UI	MAIT	5	5	4	2.629	0.0582	ns
5D, SF3D	*HLA-A* mRNA	AEC	UI	NT	AEC	*Sp*	MAIT	5	5	4	2.925	0.043	*
5D, SF3D	*HLA-A* mRNA	AEC	*Sp*	NT	AEC	UI	MAIT	5	5	4	1.346	0.2496	ns
5D, SF3D	*HLA-A* mRNA	AEC	*Sp*	NT	AEC	*Sp*	MAIT	5	5	4	2.268	0.0859	ns
5D, SF3D	*HLA-A* mRNA	AEC	UI	MAIT	AEC	*Sp*	MAIT	5	5	4	3.072	0.0372	*
5E, SF3G	*IRF1* mRNA	AEC	UI	NT	AEC	*Sp*	NT	5	5	4	2.977	0.0409	*
5E, SF3G	*IRF1* mRNA	AEC	UI	NT	AEC	UI	MAIT	5	5	4	4.702	0.0093	**
5E, SF3G	*IRF1* mRNA	AEC	UI	NT	AEC	*Sp*	MAIT	5	5	4	4.822	0.0085	**
5E, SF3G	*IRF1* mRNA	AEC	*Sp*	NT	AEC	UI	MAIT	5	5	4	3.392	0.0275	*
5E, SF3G	*IRF1* mRNA	AEC	*Sp*	NT	AEC	*Sp*	MAIT	5	5	4	4.703	0.0093	**
5E, SF3G	*IRF1* mRNA	AEC	UI	MAIT	AEC	*Sp*	MAIT	5	5	4	3.813	0.0189	*
5F, SF3C	*NLRC5* mRNA	AEC	UI	NT	AEC	*Sp*	NT	5	5	4	2.084	0.1055	ns
5F, SF3C	*NLRC5* mRNA	AEC	UI	NT	AEC	UI	MAIT	5	5	4	3.605	0.0227	*
5F, SF3C	*NLRC5* mRNA	AEC	UI	NT	AEC	*Sp*	MAIT	5	5	4	4.933	0.0079	**
5F, SF3C	*NLRC5* mRNA	AEC	*Sp*	NT	AEC	UI	MAIT	5	5	4	0.6506	0.5508	ns
5F, SF3C	*NLRC5* mRNA	AEC	*Sp*	NT	AEC	*Sp*	MAIT	5	5	4	3.829	0.0186	*
5F, SF3C	*NLRC5* mRNA	AEC	UI	MAIT	AEC	*Sp*	MAIT	5	5	4	5.008	0.0074	**
6A, SF3E	*HLA-A* mRNA	B2B	UI	NT	B2B	*Ms*	NT	3	3	2	0.09115	0.9357	ns
6A, SF3E	*HLA-A* mRNA	B2B	UI	NT	B2B	UI	MAIT	3	3	2	2.963	0.0975	ns
6A, SF3E	*HLA-A* mRNA	B2B	UI	NT	B2B	*Ms*	MAIT	3	3	2	5.839	0.0281	*
6A, SF3E	*HLA-A* mRNA	B2B	*Ms*	NT	B2B	UI	MAIT	3	3	2	3.015	0.0946	ns
6A, SF3E	*HLA-A* mRNA	B2B	*Ms*	NT	B2B	*Ms*	MAIT	3	3	2	5.658	0.0298	*
6A, SF3E	*HLA-A* mRNA	B2B	UI	MAIT	B2B	*Ms*	MAIT	3	3	2	4.79	0.0409	*
6B, SF3F	*HLA-A* mRNA	B2B	UT	NT	B2B	5-OP	NT	3	3	2	0.3951	0.7309	ns

Figure	Data	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
		Cell	Ag	T cell	Cell	Ag	T cell						
6B, SF3F	*HLA-A* mRNA	B2B	UT	NT	B2B	UT	MAIT	3	3	2	2.963	0.0975	ns
6B, SF3F	*HLA-A* mRNA	B2B	UT	NT	B2B	5-OP	MAIT	3	3	2	11.72	0.0072	**
6B, SF3F	*HLA-A* mRNA	B2B	5-OP	NT	B2B	UT	MAIT	3	3	2	3.413	0.0762	ns
6B, SF3F	*HLA-A* mRNA	B2B	5-OP	NT	B2B	5-OP	MAIT	3	3	2	10.2	0.0095	**
6B, SF3F	*HLA-A* mRNA	B2B	UT	MAIT	B2B	5-OP	MAIT	3	3	2	10.94	0.0083	**
6B, SF3F	*HLA-A* mRNA	B2B	UT	NT	B2B	6-FP	NT	3	3	2	0.1102	0.9223	ns
6B, SF3F	*HLA-A* mRNA	B2B	UT	NT	B2B	6-FP	MAIT	3	3	2	2.58	0.1231	ns
6B, SF3F	*HLA-A* mRNA	B2B	6-FP	NT	B2B	UT	MAIT	3	3	2	2.7	0.1142	ns
6B, SF3F	*HLA-A* mRNA	B2B	6-FP	NT	B2B	6-FP	MAIT	3	3	2	2.416	0.1370	ns
6B, SF3F	*HLA-A* mRNA	B2B	UT	MAIT	B2B	6-FP	MAIT	3	3	2	0.3708	0.7464	ns
6B, SF3F	*HLA-A* mRNA	B2B	5-OP	NT	B2B	6-FP	NT	3	3	2	0.4784	0.6796	ns
6C, SF3H	*IRF1* mRNA	B2B	UI	NT	B2B	*Ms*	NT	3	3	2	0.5695	0.6264	ns
6C, SF3H	*IRF1* mRNA	B2B	UI	NT	B2B	UI	MAIT	3	3	2	2.556	0.125	ns
6C, SF3H	*IRF1* mRNA	B2B	UI	NT	B2B	*Ms*	MAIT	3	3	2	7.917	0.0156	*
6C, SF3H	*IRF1* mRNA	B2B	*Ms*	NT	B2B	UI	MAIT	3	3	2	2.662	0.1169	ns
6C, SF3H	*IRF1* mRNA	B2B	*Ms*	NT	B2B	*Ms*	MAIT	3	3	2	7.927	0.0155	*
6C, SF3H	*IRF1* mRNA	B2B	UI	MAIT	B2B	*Ms*	MAIT	3	3	2	7.303	0.0182	*
6D, SF3I	*IRF1* mRNA	B2B	UT	NT	B2B	5-OP	NT	3	3	2	1.442	0.2860	ns
6D, SF3I	*IRF1* mRNA	B2B	UT	NT	B2B	UT	MAIT	3	3	2	2.556	0.1250	ns
6D, SF3I	*IRF1* mRNA	B2B	UT	NT	B2B	5-OP	MAIT	3	3	2	7.652	0.0167	*
6D, SF3I	*IRF1* mRNA	B2B	5-OP	NT	B2B	UT	MAIT	3	3	2	2.693	0.1147	ns
6D, SF3I	*IRF1* mRNA	B2B	5-OP	NT	B2B	5-OP	MAIT	3	3	2	7.67	0.0166	*
6D, SF3I	*IRF1* mRNA	B2B	UT	MAIT	B2B	5-OP	MAIT	3	3	2	8.056	0.0151	*
6D, SF3I	*IRF1* mRNA	B2B	UT	NT	B2B	6-FP	NT	3	3	2	0.212	0.8518	ns
6D, SF3I	*IRF1* mRNA	B2B	UT	NT	B2B	6-FP	MAIT	3	3	2	2.399	0.1385	ns
6D, SF3I	*IRF1* mRNA	B2B	6-FP	NT	B2B	UT	MAIT	3	3	2	2.539	0.1263	ns
6D, SF3I	*IRF1* mRNA	B2B	6-FP	NT	B2B	6-FP	MAIT	3	3	2	2.392	0.1392	ns
6D, SF3I	*IRF1* mRNA	B2B	UT	MAIT	B2B	6-FP	MAIT	3	3	2	3.075	0.0915	ns
6D, SF3I	*IRF1* mRNA	B2B	5-OP	NT	B2B	6-FP	NT	3	3	2	1.361	0.3065	ns

Definition of abbreviations:
Ag = antigen; df = degrees of freedom; statistic = absolute value of T statistic; AEC = airway epithelial cells; B2B = BEAS-2B cells; UI = uninfected control; *Sp* = *Streptococcus pneumoniae*; *Ms* = *Mycobacterium smegmatis*; UT = media treated control; 5-OP = 5-OP-RU; 6-FP = 6-formylpterin; NT = no T cell control

685 **Table 4 Statistics associated with Figure 6E-F.**

686

Figure	Sample 1			Sample 2			n1	n2	df	statistic	p-value	sig.
	Cell	Ag	T cell	Cell	Ag	T cell						
6E	Cas9	UI	NT	Cas9	*Ms*	NT	3	3	2	1.2016	0.3525	ns
6E	Cas9	UI	NT	Cas9	UI	MAIT	3	3	2	2.6842	0.1153	ns
6E	Cas9	UI	NT	Cas9	*Ms*	MAIT	3	3	2	17.538	0.0032	**
6E	Cas9	*Ms*	NT	Cas9	UI	MAIT	3	3	2	0.9623	0.4374	ns
6E	Cas9	*Ms*	NT	Cas9	*Ms*	MAIT	3	3	2	16.509	0.0037	**
6E	Cas9	UI	MAIT	Cas9	*Ms*	MAIT	3	3	2	21.563	0.0021	**
6E	IRF1 KO #1	UI	NT	IRF1 KO #1	*Ms*	NT	3	3	2	1.6008	0.2506	ns
6E	IRF1 KO #1	UI	NT	IRF1 KO #1	UI	MAIT	3	3	2	5.4716	0.0318	*
6E	IRF1 KO #1	UI	NT	IRF1 KO #1	*Ms*	MAIT	3	3	2	13.345	0.0056	**
6E	IRF1 KO #1	*Ms*	NT	IRF1 KO #1	UI	MAIT	3	3	2	5.1824	0.0353	*
6E	IRF1 KO #1	*Ms*	NT	IRF1 KO #1	*Ms*	MAIT	3	3	2	11.169	0.0079	**
6E	IRF1 KO #1	UI	MAIT	IRF1 KO #1	*Ms*	MAIT	3	3	2	3.9011	0.0599	ns
6F	Cas9	UT	NT	Cas9	5-OP	NT	3	3	2	1.3995	0.2966	ns
6F	Cas9	UT	NT	Cas9	UT	MAIT	3	3	2	1.3026	0.3225	ns
6F	Cas9	UT	NT	Cas9	5-OP	MAIT	3	3	2	2.967	0.0973	ns
6F	Cas9	5-OP	NT	Cas9	UT	MAIT	3	3	2	1.29093	0.3258	ns
6F	Cas9	5-OP	NT	Cas9	5-OP	MAIT	3	3	2	3.4192	0.0759	ns
6F	Cas9	UT	MAIT	Cas9	5-OP	MAIT	3	3	2	3.0747	0.0915	ns
6F	IRF1 KO #1	UT	NT	IRF1 KO #1	5-OP	NT	3	3	2	1.7513	0.2220	ns
6F	IRF1 KO #1	UT	NT	IRF1 KO #1	UT	MAIT	3	3	2	2.4937	0.1302	ns
6F	IRF1 KO #1	UT	NT	IRF1 KO #1	5-OP	MAIT	3	3	2	3.0603	0.0922	ns
6F	IRF1 KO #1	5-OP	NT	IRF1 KO #1	UT	MAIT	3	3	2	2.0063	0.1827	ns
6F	IRF1 KO #1	5-OP	NT	IRF1 KO #1	5-OP	MAIT	3	3	2	2.3991	0.1385	ns
6F	IRF1 KO #1	UT	MAIT	IRF1 KO #1	5-OP	MAIT	3	3	2	1.9399	0.1919	ns

687

688 *Definition of abbreviations:*

689 Ag = antigen; df = degrees of freedom; statistic = absolute value of T statistic; Cas9 = Cas9^{+} BEAS-2B control cells;
690 IRF1 KO = CRISPR gene knockout cell line; UI = uninfected control; *Ms* = *Mycobacterium smegmatis*; UT = media
691 treated control; 5-OP = 5-OP-RU; NT = no T cell control

692

693 **Table 5 Statistics associated with Figure 7 and Supplemental Figure 4.**

694

Fig.	Data	Sample 1	Sample 2	n1	n2	df	statistic	p-value	sig.	test
7A	*MR1* mRNA	UT	IFNβ	8	5	4	6.185	0.003472	**	meANOVA
7A	*MR1* mRNA	UT	IFNγ	8	5	4	21.57	0.000027	****	meANOVA
7A	*MR1* mRNA	UT	IFNλ	8	5	4	0.4827	0.654558	ns	meANOVA
7A	*MR1* mRNA	UT	TNFα	8	3	2	3.477	0.073706	ns	meANOVA
7A	*MR1* mRNA	UT	IL-17	8	4	3	0.5318	0.631732	ns	meANOVA
7A	*MR1* mRNA	IFNβ	IFNγ	5	5	5.826	3.964	0.007872	**	meANOVA
7A	*MR1* mRNA	IFNβ	IFNλ	5	5	6.178	5.202	0.001839	**	meANOVA
7A	*MR1* mRNA	IFNβ	TNFα	5	3	4.141	6.591	0.002428	**	meANOVA
7A	*MR1* mRNA	IFNβ	IL-17	5	4	5.213	5.938	0.001675	**	meANOVA
7A	*MR1* mRNA	IFNγ	IFNλ	5	5	7.918	14.1	0.000001	****	meANOVA
7A	*MR1* mRNA	IFNγ	TNFα	5	3	4.562	21.73	0.000009	****	meANOVA
7A	*MR1* mRNA	IFNγ	IL-17	5	4	6.977	17.04	0.000001	****	meANOVA
7A	*MR1* mRNA	IFNλ	TNFα	5	3	4.463	1.297	0.257655	ns	meANOVA
7A	*MR1* mRNA	IFNλ	IL-17	5	4	6.838	0.7041	0.504628	ns	meANOVA
7A	*MR1* mRNA	TNFα	IL-17	3	4	3.633	0.5922	0.588597	ns	meANOVA
SF4A	*HLA-A* mRNA	UT	IFNβ	6	5	4	6.849	0.002378	**	meANOVA
SF4A	*HLA-A* mRNA	UT	IFNγ	6	5	4	9.193	0.000778	***	meANOVA
SF4A	*HLA-A* mRNA	UT	IFNλ	6	4	3	2.529	0.085468	ns	meANOVA
SF4A	*HLA-A* mRNA	UT	TNFα	6	3	2	3.306	0.080582	ns	meANOVA
SF4A	*HLA-A* mRNA	UT	IL-17	6	3	2	3.613	0.068792	ns	meANOVA
SF4A	*HLA-A* mRNA	IFNβ	IFNγ	5	5	7.344	0.07737	0.940398	ns	meANOVA
SF4A	*HLA-A* mRNA	IFNβ	IFNλ	5	4	4.763	5.78	0.002559	**	meANOVA
SF4A	*HLA-A* mRNA	IFNβ	TNFα	5	3	5.873	3.196	0.01925	*	meANOVA
SF4A	*HLA-A* mRNA	IFNβ	IL-17	5	3	4.151	6.29	0.002878	**	meANOVA
SF4A	*HLA-A* mRNA	IFNγ	IFNλ	5	4	5.352	7.464	0.000505	***	meANOVA
SF4A	*HLA-A* mRNA	IFNγ	TNFα	5	3	4.911	3.667	0.014962	*	meANOVA
SF4A	*HLA-A* mRNA	IFNγ	IL-17	5	3	4.278	8.366	0.000829	***	meANOVA
SF4A	*HLA-A* mRNA	IFNλ	TNFα	4	3	2.589	2.187	0.130748	ns	meANOVA
SF4A	*HLA-A* mRNA	IFNλ	IL-17	4	3	4.051	0.8558	0.43978	ns	meANOVA
SF4A	*HLA-A* mRNA	TNFα	IL-17	3	3	2.113	2.66	0.110489	ns	meANOVA
SF4B	*IRF1* mRNA	UT	IFNβ	7	5	4	16.03	0.000089	****	meANOVA
SF4B	*IRF1* mRNA	UT	IFNγ	7	5	4	17.41	0.000064	****	meANOVA
SF4B	*IRF1* mRNA	UT	IFNλ	7	5	4	5.514	0.005281	**	meANOVA
SF4B	*IRF1* mRNA	UT	TNFα	7	3	2	17.05	0.003424	**	meANOVA
SF4B	*IRF1* mRNA	UT	IL-17	7	4	3	0.5277	0.634268	ns	meANOVA
SF4B	*IRF1* mRNA	IFNβ	IFNγ	5	5	4.144	15.12	0.000088	****	meANOVA
SF4B	*IRF1* mRNA	IFNβ	IFNλ	5	5	5.551	12.36	0.00003	****	meANOVA
SF4B	*IRF1* mRNA	IFNβ	TNFα	5	3	4.824	9.799	0.00023	***	meANOVA
SF4B	*IRF1* mRNA	IFNβ	IL-17	5	4	4.044	15.95	0.000084	****	meANOVA
SF4B	*IRF1* mRNA	IFNγ	IFNλ	5	5	4.029	17.05	0.000066	****	meANOVA

Fig.	Data	Sample 1	Sample 2	n1	n2	df	statistic	p-value	sig.	test
SF4B	*IRF1* mRNA	IFNγ	TNFα	5	3	4.016	16.63	0.000074	****	meANOVA
SF4B	*IRF1* mRNA	IFNγ	IL-17	5	4	4.001	17.41	0.000064	****	meANOVA
SF4B	*IRF1* mRNA	IFNλ	TNFα	5	3	5.986	5.755	0.001209	**	meANOVA
SF4B	*IRF1* mRNA	IFNλ	IL-17	5	4	4.217	5.354	0.005049	**	meANOVA
SF4B	*IRF1* mRNA	TNFα	IL-17	3	4	2.198	16.53	0.002389	**	meANOVA
SF4C	*B2m* mRNA	UT	IFNβ	6	5	4	6.381	0.003094	**	meANOVA
SF4C	*B2m* mRNA	UT	IFNγ	6	4	3	12.02	0.001238	**	meANOVA
SF4C	*B2m* mRNA	UT	IFNλ	6	5	4	7.142	0.002033	**	meANOVA
SF4C	*B2m* mRNA	UT	TNFα	6	3	2	2.596	0.121832	ns	meANOVA
SF4C	*B2m* mRNA	UT	IL-17	6	4	3	0.437	0.691667	ns	meANOVA
SF4C	*B2m* mRNA	IFNβ	IFNγ	5	4	5.131	1.619	0.164846	ns	meANOVA
SF4C	*B2m* mRNA	IFNβ	IFNλ	5	5	4.273	4.976	0.006405	**	meANOVA
SF4C	*B2m* mRNA	IFNβ	TNFα	5	3	4.912	5.148	0.003809	**	meANOVA
SF4C	*B2m* mRNA	IFNβ	IL-17	5	4	4.046	6.33	0.003067	**	meANOVA
SF4C	*B2m* mRNA	IFNγ	IFNλ	4	5	4.361	7.76	0.001037	**	meANOVA
SF4C	*B2m* mRNA	IFNγ	TNFα	4	3	4.934	7.108	0.000902	***	meANOVA
SF4C	*B2m* mRNA	IFNγ	IL-17	4	4	3.232	11.71	0.000934	***	meANOVA
SF4C	*B2m* mRNA	IFNλ	TNFα	5	3	3.124	1.005	0.386337	ns	meANOVA
SF4C	*B2m* mRNA	IFNλ	IL-17	5	4	5.267	6.439	0.001104	**	meANOVA
SF4C	*B2m* mRNA	TNFα	IL-17	3	4	2.186	2.447	0.123586	ns	meANOVA
7B	*MR1* mRNA	UT	IFNβ	5	5	4	6.185	0.003472	**	paired t test
7B	*MR1* mRNA	UT	IFNγ	5	5	4	21.57	0.000027	****	paired t test
7B	*MR1* mRNA	IFNγ	IFNβ	3	3	2	3.964	0.007872	**	paired t test
7C	*HLA-A* mRNA	UT	IFNβ	5	5	4	6.849	0.002378	**	paired t test
7C	*HLA-A* mRNA	UT	IFNγ	5	5	4	9.193	0.000778	***	paired t test
7C	*HLA-A* mRNA	IFNγ	IFNβ	4	4	3	0.07737	0.940398	ns	paired t test
7D	αMR1 gMFI	UT	IFNβ	5	5	4	6.403	0.003056	**	paired t test
7D	αMR1 gMFI	UT	IFNγ	5	5	4	2.032	0.111905	ns	paired t test
7D	αMR1 gMFI	IFNγ	IFNβ	5	5	4	10.22	0.000517	***	paired t test
7D	αMHC-Ia gMFI	UT	IFNβ	5	5	4	31.44	0.000006	****	paired t test
7D	αMHC-Ia gMFI	UT	IFNγ	5	5	4	13.56	0.000171	***	paired t test
7D	αMHC-Ia gMFI	IFNγ	IFNβ	5	5	4	3.681	0.021184	*	paired t test
7F	*IRF1* mRNA	UT	IFNβ	5	5	4	16.03	0.000089	****	paired t test
7F	*IRF1* mRNA	UT	IFNγ	5	5	4	17.41	0.000064	****	paired t test
7F	*IRF1* mRNA	IFNγ	IFNβ	4	4	3	15.12	0.000088	****	paired t test
7G	*NLRC5* mRNA	UT	IFNβ	3	3	2	5.159	0.035586	*	paired t test
7G	*NLRC5* mRNA	UT	IFNγ	4	4	3	9.844	0.002229	**	paired t test
7G	*NLRC5* mRNA	IFNγ	IFNβ	3	3	2	2.37	0.124521	ns	paired t test
7H	*B2m* mRNA	UT	IFNβ	5	5	4	6.381	0.003094	**	paired t test
7H	*B2m* mRNA	UT	IFNγ	4	4	4	12.02	0.001238	**	paired t test
7H	*B2m* mRNA	IFNγ	IFNβ	4	4	3	1.619	0.164846	ns	paired t test

Definition of abbreviations:

df = degrees of freedom; statistic = absolute value of statistic; UT = media treated control; meANOVA = Mixed-effects Analysis of Variance

700 # References

701 1. Treiner, E., Duban, L., Bahram, S., Radosavljevic, M., Wanner, V., Tilloy, F., Affaticati, P.,
702 Gilfillan, S. & Lantz, O. Selection of evolutionarily conserved mucosal-associated invariant
703 T cells by MR1. *Nature* **422**, 164–9 (2003).
704 2. Gold, M. C., Cerri, S., Smyk-Pearson, S., Cansler, M. E., Vogt, T. M., Delepine, J., Winata,
705 E., Swarbrick, G. M., Chua, W. J., Yu, Y. Y., Lantz, O., Cook, M. S., Null, M. D., Jacoby,
706 D. B., Harriff, M. J., Lewinsohn, D. A., Hansen, T. H. & Lewinsohn, D. M. Human
707 mucosal associated invariant T cells detect bacterially infected cells. *PLoS Biol.* **8**,
708 e1000407 (2010).
709 3. Le Bourhis, L., Martin, E., Peguillet, I., Guihot, A., Froux, N., Core, M., Levy, E.,
710 Dusseaux, M., Meyssonnier, V., Premel, V., Ngo, C., Riteau, B., Duban, L., Robert, D.,
711 Huang, S., Rottman, M., Soudais, C. & Lantz, O. Antimicrobial activity of mucosal-
712 associated invariant T cells. *Nat. Immunol.* **11**, 701–8 (2010).
713 4. Meermeier, E. W., Harriff, M. J., Karamooz, E. & Lewinsohn, D. M. MAIT cells and
714 microbial immunity. *Immunol Cell Biol* **96**, 607–617 (2018).
715 5. Hinks, T. S. C. & Zhang, X.-W. MAIT Cell Activation and Functions. *Front. Immunol.* **0**,
716 (2020).
717 6. Kjer-Nielsen, L., Patel, O., Corbett, A. J., Le Nours, J., Meehan, B., Liu, L., Bhati, M.,
718 Chen, Z., Kostenko, L., Reantragoon, R., Williamson, N. A., Purcell, A. W., Dudek, N. L.,
719 McConville, M. J., O'Hair, R. A., Khairallah, G. N., Godfrey, D. I., Fairlie, D. P.,
720 Rossjohn, J. & McCluskey, J. MR1 presents microbial vitamin B metabolites to MAIT
721 cells. *Nature* **491**, 717–23 (2012).
722 7. Riegert, P., Wanner, V. & Bahram, S. Genomics, isoforms, expression, and phylogeny of
723 the MHC class I-related MR1 gene. *J. Immunol.* **161**, 4066–4077 (1998).
724 8. Harriff, M. J., Karamooz, E., Burr, A., Grant, W. F., Canfield, E. T., Sorensen, M. L.,
725 Moita, L. F. & Lewinsohn, D. M. Endosomal MR1 Trafficking Plays a Key Role in
726 Presentation of *Mycobacterium tuberculosis* Ligands to MAIT Cells. *PLoS Pathog* **12**,
727 e1005524 (2016).
728 9. McWilliam, H. E., Eckle, S. B., Theodossis, A., Liu, L., Chen, Z., Wubben, J. M., Fairlie,
729 D. P., Strugnell, R. A., Mintern, J. D., McCluskey, J., Rossjohn, J. & Villadangos, J. A. The
730 intracellular pathway for the presentation of vitamin B-related antigens by the antigen-
731 presenting molecule MR1. *Nat. Immunol.* **17**, 531–7 (2016).
732 10. Karamooz, E., Harriff, M. J., Narayanan, G. A., Worley, A. H. & Lewinsohn, D. M. MR1
733 recycling and blockade of endosomal trafficking reveal distinguishable antigen presentation
734 pathways between *Mycobacterium tuberculosis* infection and exogenously delivered
735 antigens. *Sci. Rep.* **9**, 4797 (2019).
736 11. Huber, M. E., Kurapova, R., Heisler, C. M., Karamooz, E., Tafesse, F. G. & Harriff, M. J.
737 Rab6 regulates recycling and retrograde trafficking of MR1 molecules. *Sci. Rep.* **10**, (2020).
738 12. Kulicke, C., Karamooz, E., Lewinsohn, D. & Harriff, M. Covering All the Bases:
739 Complementary MR1 Antigen Presentation Pathways Sample Diverse Antigens and
740 Intracellular Compartments. *Front Immunol* **11**, 2034 (2020).
741 13. Lamichhane, R. & Ussher, J. E. Expression and trafficking of MR1. *Immunology* **151**, 270–
742 279 (2017).

743 14. McWilliam, H. E. G. & Villadangos, J. A. How MR1 Presents a Pathogen Metabolic
744 Signature to Mucosal-Associated Invariant T (MAIT) Cells. *Trends Immunol.* **38**, 679–689
745 (2017).
746 15. van dan Elsen, P. J. Expression Regulation of Major Histocompatibility Complex Class I
747 and Class II Encoding Genes. *Front. Immunol.* **2**, (2011).
748 16. Jongsma, M. L. M., Guarda, G. & Spaapen, R. M. The regulatory network behind MHC
749 class I expression. *Mol. Immunol.* **113**, 16–21 (2019).
750 17. Kalvakolanu, D. V., Nallar, S. C. & Kalakonda, S. Interferons: Cellular and Molecular
751 Biology of Their Actions. in *Encyclopedia of Cancer (Third Edition)* (eds. Boffetta, P. &
752 Hainaut, P.) 286–312 (Academic Press, 2019). doi:10.1016/B978-0-12-801238-3.96116-6.
753 18. Wang, L., Zhu, Y., Zhang, N., Xian, Y., Tang, Y., Ye, J., Reza, F., He, G., Wen, X. &
754 Jiang, X. The multiple roles of interferon regulatory factor family in health and disease. *Sig*
755 *Transduct Target Ther* **9**, 1–48 (2024).
756 19. Cho, S. X., Vijayan, S., Yoo, J.-S., Watanabe, T., Ouda, R., An, N. & Kobayashi, K. S.
757 MHC class I transactivator NLRC5 in host immunity, cancer and beyond. *Immunology* **162**,
758 252–261 (2021).
759 20. Seshadri, C., Thuong, N. T., Mai, N. T., Bang, N. D., Chau, T. T., Lewinsohn, D. M.,
760 Thwaites, G. E., Dunstan, S. J. & Hawn, T. R. A polymorphism in human MR1 is
761 associated with mRNA expression and susceptibility to tuberculosis. *Genes Immun* **18**, 8–
762 14 (2017).
763 21. Kubica, P., Lara-Velazquez, M., Bam, M., Siraj, S., Ong, I., Liu, P., Priya, R., Salamat, S.,
764 Brutkiewicz, R. R. & Dey, M. MR1 overexpression correlates with poor clinical prognosis
765 in glioma patients. *Neuro-Oncology Advances* **3**, vdab034 (2021).
766 22. Huber, M. E., Larson, E., Lust, T. N., Heisler, C. M. & Harriff, M. J. Chronic Obstructive
767 Pulmonary Disease and Cigarette Smoke Lead to Dysregulated Mucosal-associated
768 Invariant T-Cell Activation. *Am J Respir Cell Mol Biol* **68**, 90–102 (2023).
769 23. Sundar, I. K., Yin, Q., Baier, B. S., Yan, L., Mazur, W., Li, D., Susiarjo, M. & Rahman, I.
770 DNA methylation profiling in peripheral lung tissues of smokers and patients with COPD.
771 *Clin Epigenetics* **9**, 38 (2017).
772 24. Szabo, M., Sarosi, V., Baliko, Z., Bodo, K., Farkas, N., Berki, T. & Engelmann, P.
773 Deficiency of innate-like T lymphocytes in chronic obstructive pulmonary disease. *Respir.*
774 *Res.* **18**, 197 (2017).
775 25. McWilliam, H. E., Birkinshaw, R. W., Villadangos, J. A., McCluskey, J. & Rossjohn, J.
776 MR1 presentation of vitamin B-based metabolite ligands. *Curr. Opin. Immunol.* **34**, 28–34
777 (2015).
778 26. Dreos, R., Ambrosini, G., Périer, R. C. & Bucher, P. The Eukaryotic Promoter Database:
779 expansion of EPDnew and new promoter analysis tools. *Nucleic Acids Research* **43**, D92–
780 D96 (2015).
781 27. Meylan, P., Dreos, R., Ambrosini, G., Groux, R. & Bucher, P. EPD in 2020: enhanced data
782 visualization and extension to ncRNA promoters. *Nucleic Acids Res* **48**, D65–D69 (2020).
783 28. Rauluseviciute, I., Riudavets-Puig, R., Blanc-Mathieu, R., Castro-Mondragon, J. A.,
784 Ferenc, K., Kumar, V., Lemma, R. B., Lucas, J., Chèneby, J., Baranasic, D., Khan, A.,
785 Fornes, O., Gundersen, S., Johansen, M., Hovig, E., Lenhard, B., Sandelin, A., Wasserman,
786 W. W., Parcy, F. & Mathelier, A. JASPAR 2024: 20th anniversary of the open-access
787 database of transcription factor binding profiles. *Nucleic Acids Research* **52**, D174–D182
788 (2024).

789 29. Sachini, N. & Papamatheakis, J. NF-Y and the immune response: Dissecting the complex
790 regulation of *MHC* genes. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory*
791 *Mechanisms* **1860**, 537–542 (2017).
792 30. Ning, S., Pagano, J. S. & Barber, G. N. IRF7: activation, regulation, modification and
793 function. *Genes Immun* **12**, 399–414 (2011).
794 31. Sari, G., Dhatchinamoorthy, K., Orellano-Ariza, L., Ferreira, L. M., Brehm, M. A. & Rock,
795 K. IRF2 loss is associated with reduced MHC I pathway transcripts in subsets of most
796 human cancers and causes resistance to checkpoint immunotherapy in human and mouse
797 melanomas. *Journal of Experimental & Clinical Cancer Research : CR* **43**, 276 (2024).
798 32. Barrat, F. J., Crow, M. K. & Ivashkiv, L. B. Interferon target-gene expression and
799 epigenomic signatures in health and disease. *Nat Immunol* **20**, 1574–1583 (2019).
800 33. McNab, F., Mayer-Barber, K., Sher, A., Wack, A. & O'Garra, A. Type I interferons in
801 infectious disease. *Nat Rev Immunol* **15**, 87–103 (2015).
802 34. Lee, A. J. & Ashkar, A. A. The Dual Nature of Type I and Type II Interferons. *Frontiers in*
803 *Immunology* **9**, 2061 (2018).
804 35. Han, L.-T., Hu, J.-Q., Ma, B., Wen, D., Zhang, T.-T., Lu, Z.-W., Wei, W.-J., Wang, Y.-L.,
805 Wang, Y., Liao, T. & Ji, Q.-H. IL-17A increases MHC class I expression and promotes T
806 cell activation in papillary thyroid cancer patients with coexistent Hashimoto's thyroiditis.
807 *Diagnostic Pathology* **14**, 52 (2019).
808 36. Bonelli, M., Dalwigk, K., Platzer, A., Olmos Calvo, I., Hayer, S., Niederreiter, B., Holinka,
809 J., Sevelda, F., Pap, T., Steiner, G., Superti-Furga, G., Smolen, J. S., Kiener, H. P. &
810 Karonitsch, T. IRF1 is critical for the TNF-driven interferon response in rheumatoid
811 fibroblast-like synoviocytes. *Exp Mol Med* **51**, 1–11 (2019).
812 37. Liu, Z. Molecular mechanism of TNF signaling and beyond. *Cell Res* **15**, 24–27 (2005).
813 38. Looney, M., Lorenc, R., Halushka, M. K. & Karakousis, P. C. Key macrophage responses
814 to infection with *Mycobacterium tuberculosis* are co-regulated by microRNAs and DNA
815 methylation. *Front. Immunol.* **12**, (2021).
816 39. Pacis, A., Tailleux, L., Morin, A. M., Lambourne, J., MacIsaac, J. L., Yotova, V., Dumaine,
817 A., Danckaert, A., Luca, F., Grenier, J.-C., Hansen, K. D., Gicquel, B., Yu, M., Pai, A., He,
818 C., Tung, J., Pastinen, T., Kobor, M. S., Pique-Regi, R., Gilad, Y. & Barreiro, L. B.
819 Bacterial infection remodels the DNA methylation landscape of human dendritic cells.
820 *Genome Res.* **25**, 1801–1811 (2015).
821 40. Cizmeci, D., Dempster, E. L., Champion, O. L., Wagley, S., Akman, O. E., Prior, J. L.,
822 Soyer, O. S., Mill, J. & Titball, R. W. Mapping epigenetic changes to the host cell genome
823 induced by Burkholderia pseudomallei reveals pathogen-specific and pathogen-generic
824 signatures of infection. *Sci. Rep.* **6**, 30861 (2016).
825 41. Liu, Y., Zhu, P., Wang, W., Tan, X., Liu, C., Chen, Y., Pei, R., Cheng, X., Wu, M., Guo,
826 Q., Liang, H., Liang, Z., Liu, J., Xu, Y., Wu, X. & Weng, X. Mucosal-Associated Invariant
827 T Cell Dysregulation Correlates With Conjugated Bilirubin Level in Chronic HBV
828 Infection. *Hepatology* **73**, 1671 (2021).
829 42. McSharry, B. P., Samer, C., McWilliam, H. E. G., Ashley, C. L., Yee, M. B., Steain, M.,
830 Liu, L., Fairlie, D. P., Kinchington, P. R., McCluskey, J., Abendroth, A., Villadangos, J. A.,
831 Rossjohn, J. & Slobedman, B. Virus-Mediated Suppression of the Antigen Presentation
832 Molecule MR1. *Cell reports* **30**, 2948 (2020).
833 43. Samer, C., McWilliam, H. E. G., McSharry, B. P., Velusamy, T., Burchfield, J. G., Stanton,
834 R. J., Tscharke, D. C., Rossjohn, J., Villadangos, J. A., Abendroth, A. & Slobedman, B.

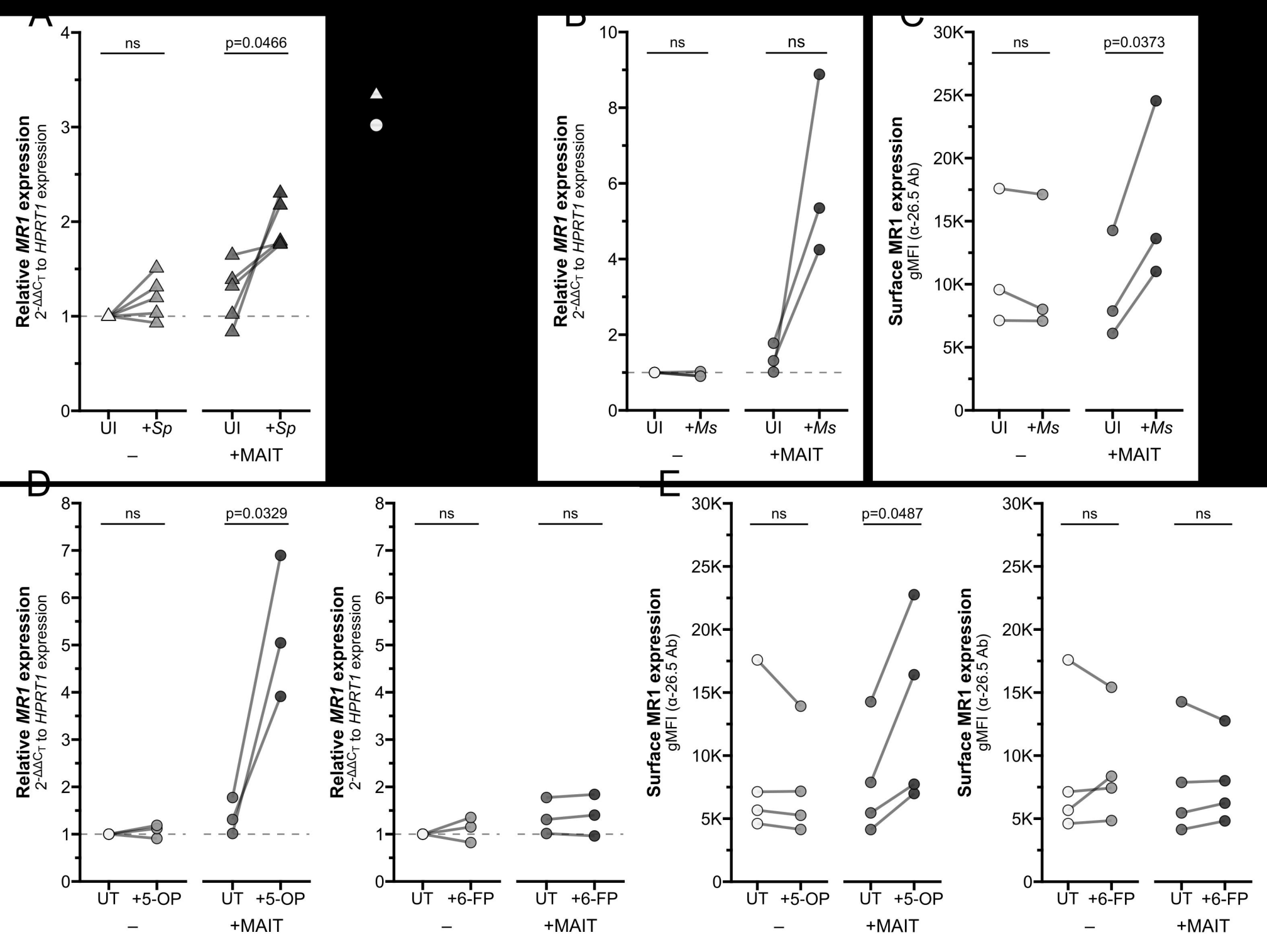
835 Multi-targeted loss of the antigen presentation molecule MR1 during HSV-1 and HSV-2
836 infection. *iScience* **27**, 108801 (2024).
837 44. Tormanen, K., Matundan, H. H., Wang, S., Jaggi, U., Mott, K. R. & Ghiasi, H. Small
838 Noncoding RNA (sncRNA1) within the Latency-Associated Transcript Modulates Herpes
839 Simplex Virus 1 Virulence and the Host Immune Response during Acute but Not Latent
840 Infection. *Journal of Virology* **96**, e00054-22 (2022).
841 45. Rebuli, M. E., Glista-Baker, E., Hoffman, J. R., Duffney, P. F., Robinette, C., Speen, A. M.,
842 Pawlak, E. A., Dhingra, R., Noah, T. L. & Jaspers, I. Electronic-Cigarette Use Alters Nasal
843 Mucosal Immune Response to Live-attenuated Influenza Virus. A Clinical Trial. *Am. J.*
844 *Respir. Cell Mol. Biol.* **64**, 126–137 (2021).
845 46. Constantin, D., Nosi, V., Kehrer, N., Vacchini, A., Chancellor, A., Contassot, E.,
846 Beshirova, A., Prota, G., Navarini, A., Mori, L. & De Libero, G. MR1 Gene and Protein
847 Expression Are Enhanced by Inhibition of the Extracellular Signal-Regulated Kinase ERK.
848 *Cancer Immunology Research* OF1–OF16 (2024) doi:10.1158/2326-6066.CIR-24-0110.
849 47. Narayan, V., Pion, E., Landré, V., Müller, P. & Ball, K. L. Docking-dependent
850 ubiquitination of the interferon regulatory factor-1 tumor suppressor protein by the
851 ubiquitin ligase CHIP. *J Biol Chem* **286**, 607–619 (2011).
852 48. Rosain, J., Neehus, A.-L., Manry, J., Yang, R., Le Pen, J., Daher, W., Liu, Z., Chan, Y.-H.,
853 Tahuil, N., Türel, Ö., Bourgey, M., Ogishi, M., Doisne, J.-M., Izquierdo, H. M., Shirasaki,
854 T., Le Voyer, T., Guérin, A., Bastard, P., Moncada-Vélez, M., Han, J. E., Khan, T.,
855 Rapaport, F., Hong, S.-H., Cheung, A., Haake, K., Mindt, B. C., Pérez, L., Philippot, Q.,
856 Lee, D., Zhang, P., Rinchai, D., Al Ali, F., Ahmad Ata, M. M., Rahman, M., Peel, J. N.,
857 Heissel, S., Molina, H., Kendir-Demirkol, Y., Bailey, R., Zhao, S., Bohlen, J., Mancini, M.,
858 Seeleuthner, Y., Roelens, M., Lorenzo, L., Soudée, C., Paz, M. E. J., González, M. L.,
859 Jeljeli, M., Soulier, J., Romana, S., L'Honneur, A.-S., Materna, M., Martínez-Barricarte, R.,
860 Pochon, M., Oleaga-Quintas, C., Michev, A., Migaud, M., Lévy, R., Alyanakian, M.-A.,
861 Rozenberg, F., Croft, C. A., Vogt, G., Emile, J.-F., Kremer, L., Ma, C. S., Fritz, J. H.,
862 Lemon, S. M., Spaan, A. N., Manel, N., Abel, L., MacDonald, M. R., Boisson-Dupuis, S.,
863 Marr, N., Tangye, S. G., Di Santo, J. P., Zhang, Q., Zhang, S.-Y., Rice, C. M., Béziat, V.,
864 Lachmann, N., Langlais, D., Casanova, J.-L., Gros, P. & Bustamante, J. Human IRF1
865 governs macrophagic IFN-γ immunity to mycobacteria. *Cell* **186**, 621-645.e33 (2023).
866 49. Kim, S.-J. & Karamooz, E. MR1- and HLA-E-Dependent Antigen Presentation of
867 Mycobacterium tuberculosis. *International Journal of Molecular Sciences* **23**, 14412
868 (2022).
869 50. Cross, D. L., Layton, E. D., Yu, K. K. Q., Smith, M. T., Aguilar, M. S., Li, S., Wilcox, E.
870 C., Chapuis, A. G., Mayanja-Kizza, H., Stein, C. M., Boom, W. H., Hawn, T. R., Bradley,
871 P., Newell, E. W. & Seshadri, C. MR1-restricted T cell clonotypes are associated with
872 "resistance" to *Mycobacterium tuberculosis* infection. *JCI Insight* **9**, (2024).
873 51. Yang, R., Mele, F., Worley, L., Langlais, D., Rosain, J., Benhsaien, I., Elarabi, H., Croft, C.
874 A., Doisne, J.-M., Zhang, P., Weisshaar, M., Jarrossay, D., Latorre, D., Shen, Y., Han, J.,
875 Ogishi, M., Gruber, C., Markle, J., Ali, F. A., Rahman, M., Khan, T., Seeleuthner, Y.,
876 Kerner, G., Husquin, L. T., Maclsaac, J. L., Jeljeli, M., Errami, A., Ailal, F., Kobor, M. S.,
877 Oleaga-Quintas, C., Roynard, M., Bourgey, M., Baghdadi, J. E., Boisson-Dupuis, S., Puel,
878 A., Batteux, F., Rozenberg, F., Marr, N., Pan-Hammarström, Q., Bogunovic, D., Quintana-
879 Murci, L., Carroll, T., Ma, C. S., Abel, L., Bousfiha, A., Santo, J. P. D., Glimcher, L. H.,
880 Gros, P., Tangye, S. G., Sallusto, F., Bustamante, J. & Casanova, J.-L. Human T-bet

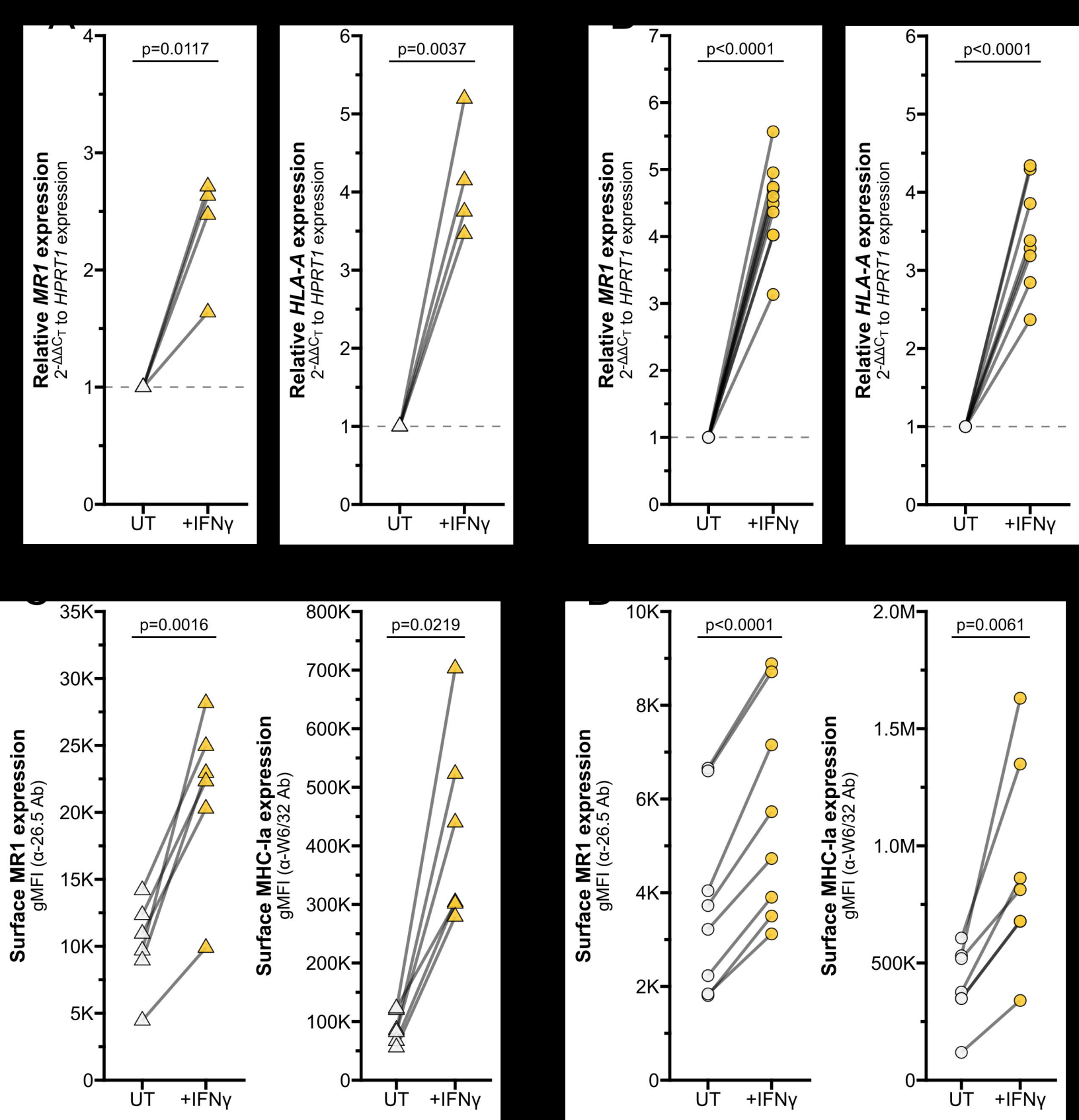
881 governs innate and innate-like adaptive IFN-γ immunity against mycobacteria. *Cell* **183**,
882 1826 (2020).
883 52. Ussher, J. E., van Wilgenburg, B., Hannaway, R. F., Ruustal, K., Phalora, P., Kurioka, A.,
884 Hansen, T. H., Willberg, C. B., Phillips, R. E. & Klenerman, P. TLR signaling in human
885 antigen-presenting cells regulates MR1-dependent activation of MAIT cells. *Eur J Immunol*
886 **46**, 1600–1614 (2016).
887 53. Lamichhane, R., Galvin, H., Hannaway, R. F., Harpe, S. M. de la, Munro, F., Tyndall, J. D.,
888 Vernall, A. J., McCall, J. L., Husain, M. & Ussher, J. E. Type I interferons are important
889 co-stimulatory signals during T cell receptor mediated human MAIT cell activation.
890 *European Journal of Immunology* **50**, 178–191 (2020).
891 54. Pavlovic, M., Gross, C., Chili, C., Secher, T. & Treiner, E. MAIT Cells Display a Specific
892 Response to Type 1 IFN Underlying the Adjuvant Effect of TLR7/8 Ligands. *Front*
893 *Immunol* **11**, 2097 (2020).
894 55. López-Rodríguez, J. C., Hancock, S. J., Li, K., Crotta, S., Barrington, C., Suárez-Bonnet,
895 A., Priestnall, S. L., Aubé, J., Wack, A., Klenerman, P., Bengoechea, J. A. & Barral, P.
896 Type I interferons drive MAIT cell functions against bacterial pneumonia. *Journal of*
897 *Experimental Medicine* **220**, e20230037 (2023).
898 56. Lazear, H. M., Schoggins, J. W. & Diamond, M. S. Shared and Distinct Functions of Type I
899 and Type III Interferons. *Immunity* **50**, 907–923 (2019).
900 57. Forero, A., Ozarkar, S., Li, H., Lee, C. H., Hemann, E. A., Nadjsombati, M. S., Hendricks,
901 M. R., So, L., Green, R., Roy, C. N., Sarkar, S. N., von Moltke, J., Anderson, S. K., Gale,
902 M. & Savan, R. Differential activation of the transcription factor IRF1 underlies the distinct
903 immune responses elicited by type I and type III interferons. *Immunity* **51**, 451-464.e6
904 (2019).
905 58. Goel, R. R., Kotenko, S. V. & Kaplan, M. J. Interferon lambda in inflammation and
906 autoimmune rheumatic diseases. *Nat Rev Rheumatol* **17**, 349–362 (2021).
907 59. Lavi, E., Suzumura, A., Murasko, D. M., Murray, E. M., Silberger, D. H. & Weiss, S. R.
908 Tumor necrosis factor induces expression of MHC class I antigens on mouse astrocytes.
909 *Journal of Neuroimmunology* **18**, 245 (2002).
910 60. Venkatesh, D., Ernandez, T., Rosetti, F., Batal, I., Cullere, X., Luscinskas, F. W., Zhang,
911 Y., Stavrakis, G., García-Cardeña, G., Horwitz, B. H. & Mayadas, T. N. Endothelial TNF
912 receptor 2 induces IRF1 transcription factor-dependent interferon-β autocrine signaling to
913 promote monocyte recruitment. *Immunity* **38**, 1025–1037 (2013).
914 61. Tulli, L., Cattaneo, F., Vinot, J., Baldari, C. T. & D'Oro, U. Src Family Kinases Regulate
915 Interferon Regulatory Factor 1 K63 Ubiquitination following Activation by TLR7/8
916 Vaccine Adjuvant in Human Monocytes and B Cells. *Front. Immunol.* **9**, (2018).
917 62. Liu, A., Gong, P., Hyun, S. W., Wang, K. Z. Q., Cates, E. A., Perkins, D., Bannerman, D.
918 D., Puché, A. C., Toshchakov, V. Y., Fang, S., Auron, P. E., Vogel, S. N. & Goldblum, S.
919 E. TRAF6 Protein Couples Toll-like Receptor 4 Signaling to Src Family Kinase Activation
920 and Opening of Paracellular Pathway in Human Lung Microvascular Endothelia*. *Journal*
921 *of Biological Chemistry* **287**, 16132–16145 (2012).
922 63. Harikumar, K. B., Yester, J. W., Surace, M. J., Oyeniran, C., Price, M. M., Huang, W.-C.,
923 Hait, N. C., Allegood, J. C., Yamada, A., Kong, X., Lazear, H. M., Bhardwaj, R., Takabe,
924 K., Diamond, M. S., Luo, C., Milstien, S., Spiegel, S. & Kordula, T. K63-linked
925 polyubiquitination of transcription factor IRF1 is essential for IL-1-induced production of
926 chemokines CXCL10 and CCL5. *Nat Immunol* **15**, 231–238 (2014).

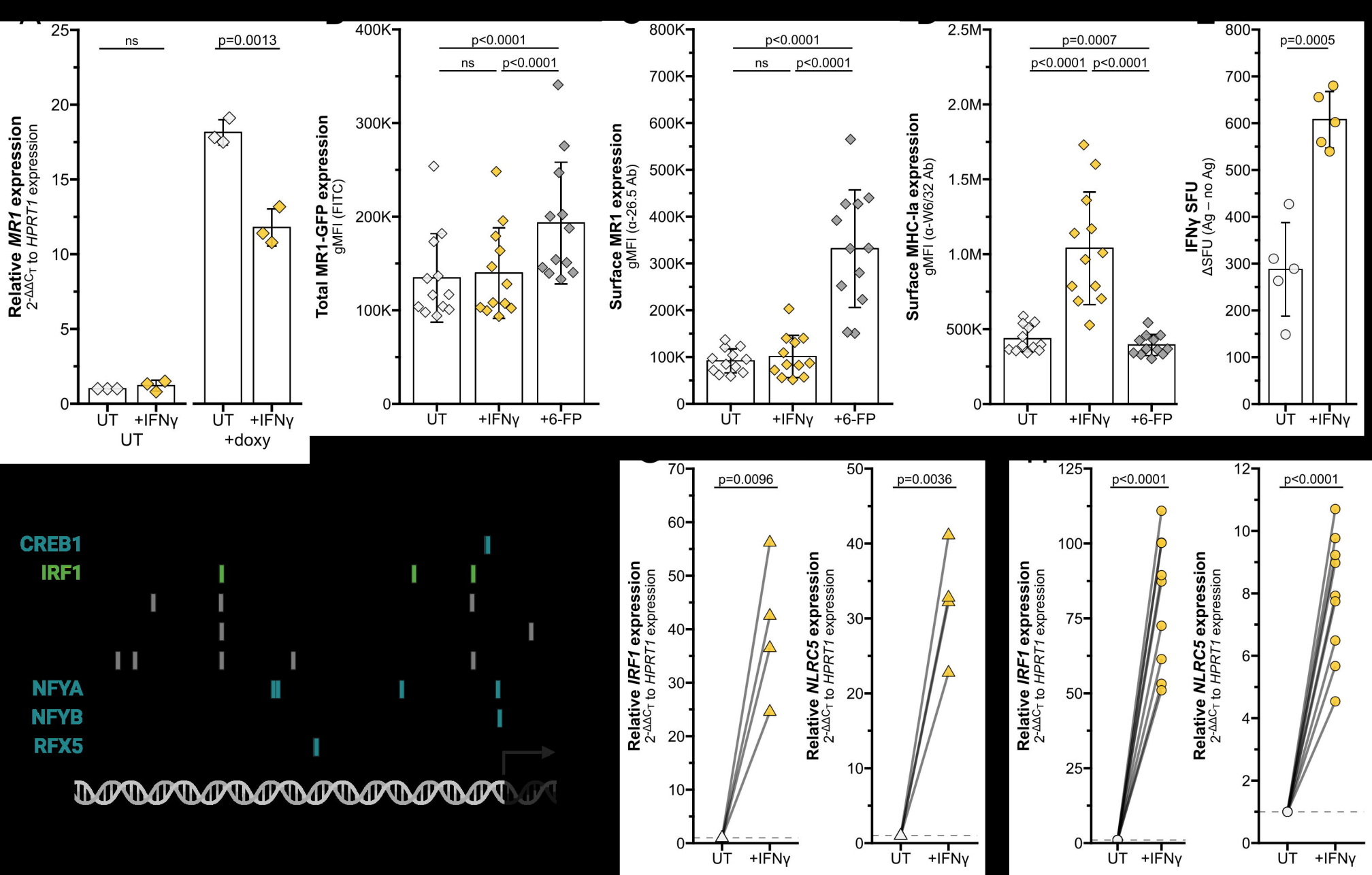
927 64. Hoshino, K., Sasaki, I., Sugiyama, T., Yano, T., Yamazaki, C., Yasui, T., Kikutani, H. &
928 Kaisho, T. Critical role of IkappaB Kinase alpha in TLR7/9-induced type I IFN production
929 by conventional dendritic cells. *J Immunol* **184**, 3341–3345 (2010).
930 65. Sánchez-Tarjuelo, R., Cortegano, I., Manosalva, J., Rodríguez, M., Ruíz, C., Alía, M.,
931 Prado, M. C., Cano, E. M., Ferrándiz, M. J., Campa, A. G. de la, Gaspar, M. L. & Andrés,
932 B. de. The TLR4-MyD88 Signaling Axis Regulates Lung Monocyte Differentiation
933 Pathways in Response to Streptococcus pneumoniae. *Frontiers in Immunology* **11**, 2120
934 (2020).
935 66. Ioannidis, M., Cerundolo, V. & Salio, M. The Immune Modulating Properties of Mucosal-
936 Associated Invariant T Cells. *Front. Immunol.* **0**, (2020).
937 67. Davey, M. S., Morgan, M. P., Liuzzi, A. R., Tyler, C. J., Khan, M. W. A., Szakmany, T.,
938 Hall, J. E., Moser, B. & Eberl, M. Microbe-Specific Unconventional T Cells Induce Human
939 Neutrophil Differentiation into Antigen Cross-Presenting Cells. *The Journal of Immunology*
940 **193**, 3704–3716 (2014).
941 68. Siebeler, R., de Winther, M. P. J. & Hoeksema, M. A. The regulatory landscape of
942 macrophage interferon signaling in inflammation. *Journal of Allergy and Clinical*
943 *Immunology* **152**, 326–337 (2023).
944 69. Frucht, D. M., Fukao, T., Bogdan, C., Schindler, H., O'Shea, J. J. & Koyasu, S. IFN-γ
945 production by antigen-presenting cells: mechanisms emerge. *Trends in Immunology* **22**,
946 556–560 (2001).
947 70. Fenimore, J. & Young, H. A. Regulation of IFN-γ Expression. in *Regulation of Cytokine*
948 *Gene Expression in Immunity and Diseases* (ed. Ma, X.) 1–19 (Springer Netherlands,
949 2016). doi:10.1007/978-94-024-0921-5_1.
950 71. Gomez, J. C., Yamada, M., Martin, J. R., Dang, H., Brickey, W. J., Bergmeier, W.,
951 Dinauer, M. C. & Doerschuk, C. M. Mechanisms of Interferon-γ Production by Neutrophils
952 and Its Function during *Streptococcus pneumoniae* Pneumonia. *Am J Respir Cell Mol Biol*
953 **52**, 349–364 (2015).
954 72. Briard, B., Karki, R., Malireddi, R. K. S., Bhattacharya, A., Place, D. E., Mavuluri, J.,
955 Peters, J. L., Vogel, P., Yamamoto, M. & Kanneganti, T.-D. Fungal ligands released by
956 innate immune effectors promote inflammasome activation during Aspergillus fumigatus
957 infection. *Nat Microbiol* **4**, 316–327 (2019).
958 73. Hinks, T. S. Mucosal-associated invariant T cells in autoimmunity, immune-mediated
959 diseases and airways disease. *Immunology* **148**, 1–12 (2016).
960 74. Pincikova, T., Parrot, T., Hjelte, L., Högman, M., Lisspers, K., Ställberg, B., Janson, C.,
961 Malinovschi, A. & Sandberg, J. K. MAIT cell counts are associated with the risk of
962 hospitalization in COPD. *Respiratory Research* **23**, 127 (2022).
963 75. Mock, J. R., Tune, M. K., Dial, C. F., Torres-Castillo, J., Hagan, R. S. & Doerschuk, C. M.
964 Effects of IFN-γ on immune cell kinetics during the resolution of acute lung injury. *Physiol*
965 *Rep* **8**, e14368 (2020).
966 76. Ramana, C. V., DeBerge, M. P., Kumar, A., Alia, C. S., Durbin, J. E. & Enelow, R. I.
967 Inflammatory impact of IFN-γ in CD8+ T cell-mediated lung injury is mediated by both
968 Stat1-dependent and -independent pathways. *Am J Physiol Lung Cell Mol Physiol* **308**,
969 L650–L657 (2015).
970 77. Southworth, T., Metryka, A., Lea, S., Farrow, S., Plumb, J. & Singh, D. IFN-γ
971 synergistically enhances LPS signalling in alveolar macrophages from COPD patients and

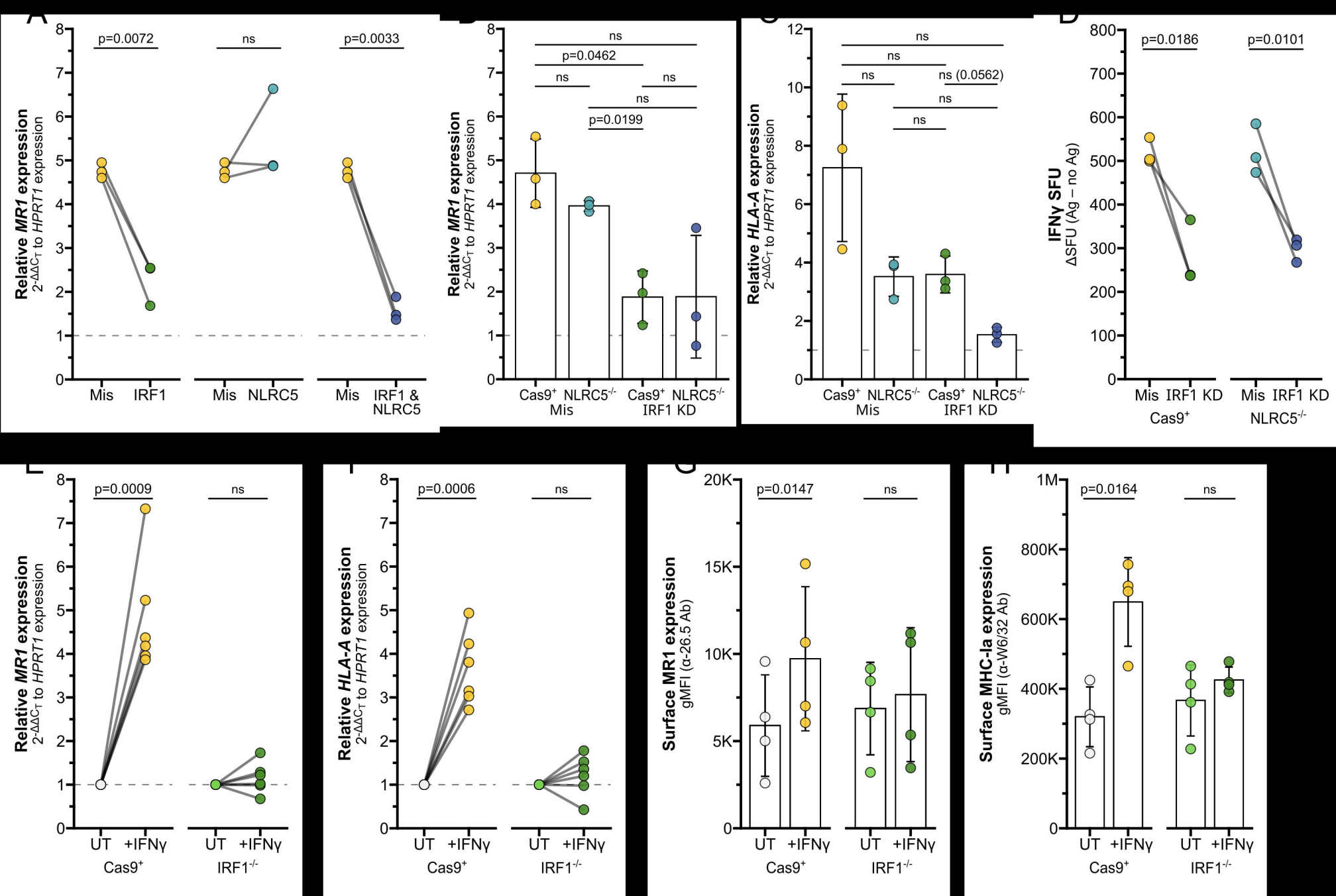
972 controls by corticosteroid-resistant STAT1 activation. *Br J Pharmacol* **166**, 2070–2083
973 (2012).
974 78. Williams, M., Todd, I. & Fairclough, L. C. The role of CD8 + T lymphocytes in chronic
975 obstructive pulmonary disease: a systematic review. *Inflamm. Res.* **70**, 11–18 (2021).
976 79. Zhu, X., Gadgil, A. S., Givelber, R., George, M. P., Stoner, M. W., Sciurba, F. C. &
977 Duncan, S. R. Peripheral T Cell Functions Correlate with the Severity of Chronic
978 Obstructive Pulmonary Disease. *The Journal of Immunology* **182**, 3270–3277 (2009).
979 80. Briend, E., Ferguson, G. J., Mori, M., Damera, G., Stephenson, K., Karp, N. A., Sethi, S.,
980 Ward, C. K., Sleeman, M. A., Erjefält, J. S. & Finch, D. K. IL-18 associated with lung
981 lymphoid aggregates drives IFNγ production in severe COPD. *Respiratory Research* **18**,
982 159 (2017).
983 81. Seifert, L. L., Si, C., Saha, D., Sadic, M., de Vries, M., Ballentine, S., Briley, A., Wang, G.,
984 Valero-Jimenez, A. M., Mohamed, A., Schaefer, U., Moulton, H. M., García-Sastre, A.,
985 Tripathi, S., Rosenberg, B. R. & Dittmann, M. The ETS transcription factor ELF1 regulates
986 a broadly antiviral program distinct from the type I interferon response. *PLoS Pathog* **15**,
987 e1007634 (2019).
988 82. Yang, L. & Ding, J. L. MEK1/2 Inhibitors Unlock the Constrained Interferon Response in
989 Macrophages Through IRF1 Signaling. *Frontiers in Immunology* **10**, 2020 (2019).
990 83. Najjar, I. & Fagard, R. STAT1 and pathogens, not a friendly relationship. *Biochimie* **92**,
991 425–444 (2010).
992 84. Miorin, L. & Sanchez-Aparicio, M. T. SLiMs go viral! One more weapon against
993 interferon. *Cell Host & Microbe* **30**, 286–288 (2022).
994 85. Li, J., Yu, L., Shen, Z., Li, Y., Chen, B., Wei, W., Chen, X., Wang, Q., Tong, F., Lou, H.,
995 Chu, M. & Wei, L. miR-34a and its novel target, NLRC5, are associated with HPV16
996 persistence. *Infection, Genetics and Evolution* **44**, 293–299 (2016).
997 86. Remoli, A. L., Marsili, G., Perrotti, E., Acchioni, C., Sgarbanti, M., Borsetti, A., Hiscott, J.
998 & Battistini, A. HIV-1 Tat Recruits HDM2 E3 Ligase To Target IRF-1 for Ubiquitination
999 and Proteasomal Degradation. *mBio* **7**, 10.1128/mbio.01528-16 (2016).
1000 87. Lewinsohn, D. M., Briden, A. L., Reed, S. G., Grabstein, K. H. & Alderson, M. R.
1001 *Mycobacterium tuberculosis*-reactive CD8+ T lymphocytes: the relative contribution of
1002 classical versus nonclassical HLA restriction. *J. Immunol.* **165**, 925–30 (2000).
1003 88. Wong, J., Korcheva, V., Jacoby, D. B. & Magun, B. E. Proinflammatory responses of
1004 human airway cells to ricin involve stress-activated protein kinases and NF-κB. *American*
1005 *Journal of Physiology-Lung Cellular and Molecular Physiology* **293**, L1385–L1394 (2007).
1006 89. Kulicke, C. A., Swarbrick, G. M., Ladd, N. A., Cansler, M., Null, M., Worley, A., Lemon,
1007 C., Ahmed, T., Bennett, J., Lust, T. N., Heisler, C. M., Huber, M. E., Krawic, J. R., Ankley,
1008 L. M., McBride, S. K., Tafesse, F. G., Olive, A. J., Hildebrand, W. H., Lewinsohn, D. A.,
1009 Adams, E. J., Lewinsohn, D. M. & Harriff, M. J. Delivery of loaded MR1 monomer results
1010 in efficient ligand exchange to host MR1 and subsequent MR1T cell activation. *Commun*
1011 *Biol* **7**, 1–13 (2024).
1012 90. Hartmann, N., McMurtrey, C., Sorensen, M. L., Huber, M. E., Kurapova, R., Coleman, F.
1013 T., Mizgerd, J. P., Hildebrand, W., Kronenberg, M., Lewinsohn, D. M. & Harriff, M. J.
1014 Riboflavin Metabolism Variation among Clinical Isolates of *Streptococcus pneumoniae*
1015 Results in Differential Activation of Mucosal-associated Invariant T Cells. *Am. J. Respir.*
1016 *Cell Mol. Biol.* **58**, 767–776 (2018).

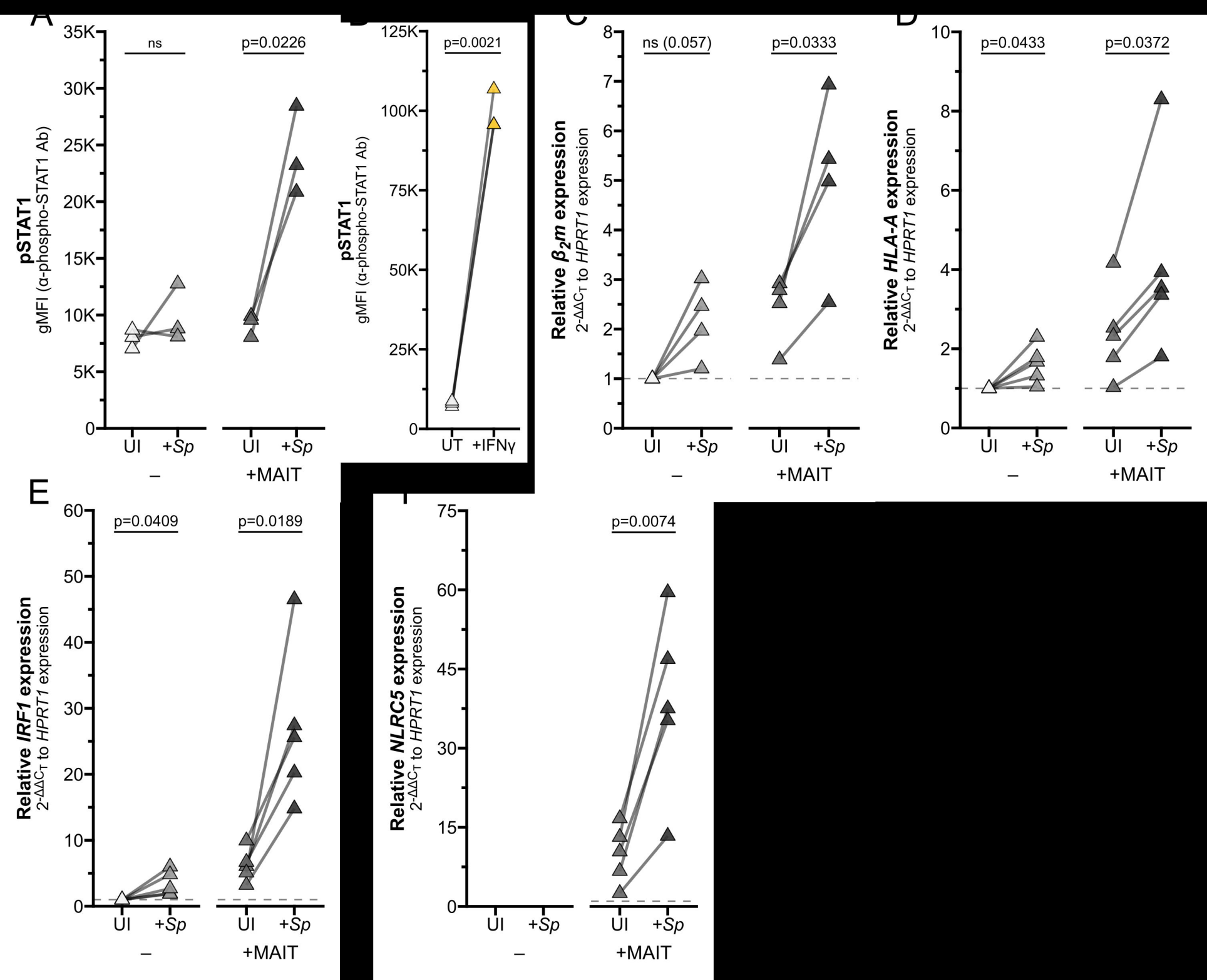
1017 91. Brinkman, E. K., Chen, T., Amendola, M. & van Steensel, B. Easy quantitative assessment
1018 of genome editing by sequence trace decomposition. *Nucleic Acids Research* **42**, e168
1019 (2014).
1020 92. Conant, D., Hsiau, T., Rossi, N., Oki, J., Maures, T., Waite, K., Yang, J., Joshi, S., Kelso,
1021 R., Holden, K., Enzmann, B. L. & Stoner, R. Inference of CRISPR Edits from Sanger Trace
1022 Data. *The CRISPR Journal* **5**, 123–130 (2022).
1023 93. Li, K., Vorkas, C. K., Chaudhry, A., Bell, D. L., Willis, R. A., Rudensky, A., Altman, J. D.,
1024 Glickman, M. S. & Aubé, J. Synthesis, stabilization, and characterization of the MR1 ligand
1025 precursor 5-amino-6-D-ribitylaminouracil (5-A-RU). *PLOS ONE* **13**, e0191837 (2018).
1026 94. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time
1027 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402–8 (2001).
1028 95. Heinzel, A. S., Grotzke, J. E., Lines, R. A., Lewinsohn, D. A., McNabb, A. L., Streblow, D.
1029 N., Braud, V. M., Grieser, H. J., Belisle, J. T. & Lewinsohn, D. M. HLA-E-dependent
1030 presentation of Mtb-derived antigen to human CD8+ T cells. *J. Exp. Med.* **196**, 1473–81
1031 (2002).
1032 96. Muller, R. Y., Hammond, M. C., Rio, D. C. & Lee, Y. J. An Efficient Method for
1033 Electroporation of Small Interfering RNAs into ENCODE Project Tier 1 GM12878 and
1034 K562 Cell Lines. *J Biomol Tech* **26**, 142–149 (2015).
1035 97. Dreos, R., Ambrosini, G., Groux, R., Cavin Périer, R. & Bucher, P. The eukaryotic
1036 promoter database in its 30th year: focus on non-vertebrate organisms. *Nucleic Acids*
1037 *Research* **45**, D51–D55 (2017).
1038 98. Wasserman, W. W. & Sandelin, A. Applied bioinformatics for the identification of
1039 regulatory elements. *Nat Rev Genet* **5**, 276–287 (2004).
1040 99. Ambrosini, G., Praz, V., Jagannathan, V. & Bucher, P. Signal search analysis server.
1041 *Nucleic Acids Res* **31**, 3618–3620 (2003).
1042

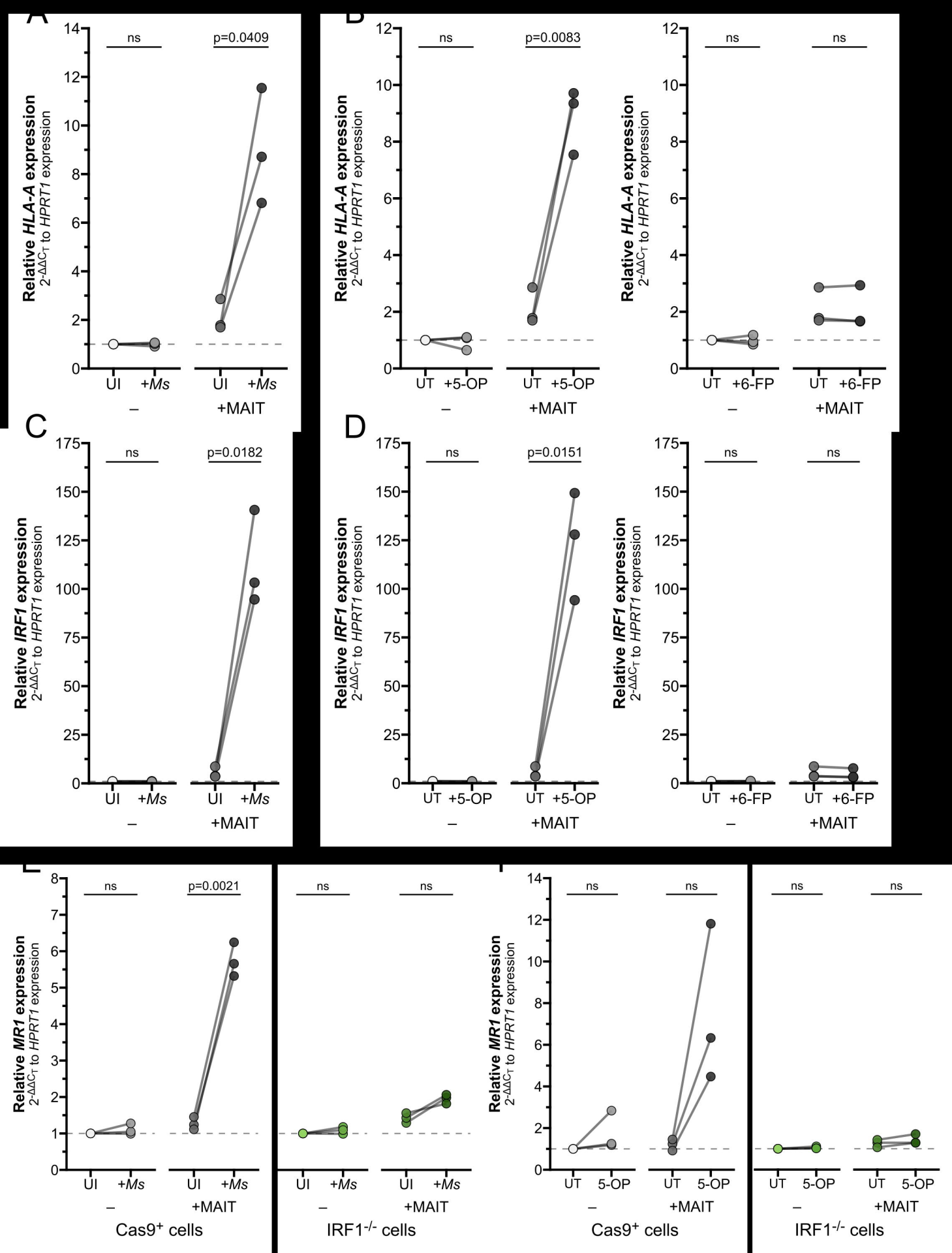

A
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
p=0.0466
UI
+Sp
–
+MAIT
B
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
ns
UI
+Ms
–
+MAIT
C
Surface MR1 expression
gMFI (α-26.5 Ab)
ns
p=0.0373
UI
+Ms
–
+MAIT
D
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
p=0.0329
UT
+5-OP
–
+MAIT
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
ns
UT
+6-FP
–
+MAIT
E
Surface MR1 expression
gMFI (α-26.5 Ab)
ns
p=0.0487
UT
+5-OP
–
+MAIT
Surface MR1 expression
gMFI (α-26.5 Ab)
ns
ns
UT
+6-FP
–
+MAIT

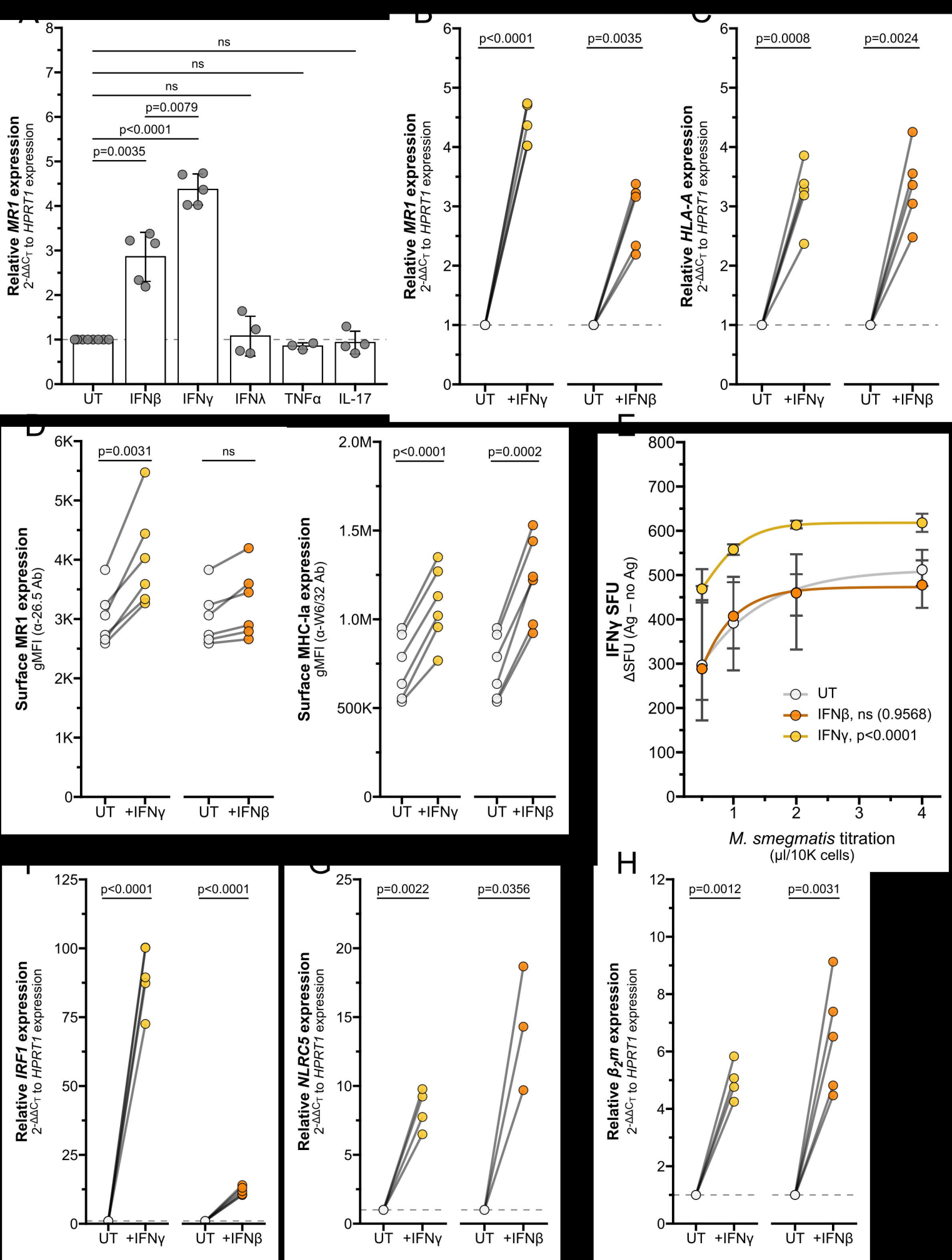

p=0.0117
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
UT
+IFNγ
p=0.0037
Relative HLA-A expression
2-ΔΔCT to HPRT1 expression
UT
+IFNγ
p<0.0001
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
UT
+IFNγ
p<0.0001
Relative HLA-A expression
2-ΔΔCT to HPRT1 expression
UT
+IFNγ
p=0.0016
Surface MR1 expression
gMFI (α-26.5 Ab)
UT
+IFNγ
p=0.0219
Surface MHC-Ia expression
gMFI (α-W6/32 Ab)
UT
+IFNγ
p<0.0001
Surface MR1 expression
gMFI (α-26.5 Ab)
UT
+IFNγ
p=0.0061
Surface MHC-Ia expression
gMFI (α-W6/32 Ab)
UT
+IFNγ

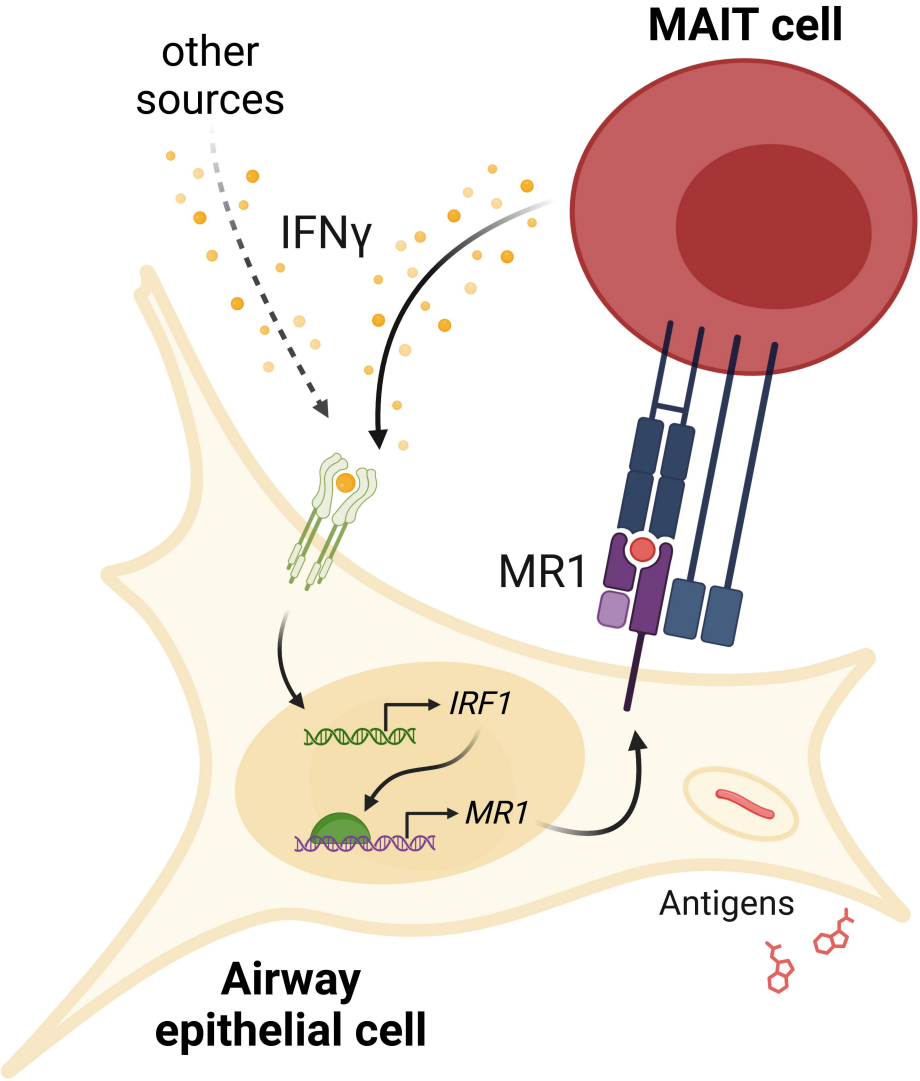

Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
p=0.0013
UT
+IFNγ
UT
+doxy
Total MR1-GFP expression
gMFI (FITC)
p<0.0001
ns
p<0.0001
+6-FP
Surface MR1 expression
gMFI (α-26.5 Ab)
Surface MHC-Ia expression
gMFI (α-W6/32 Ab)
p=0.0007
IFNγ SFU
ΔSFU (Ag – no Ag)
p=0.0005
CREB1
IRF1
NFYA
NFYB
RFX5
Relative IRF1 expression
Relative NLRC5 expression
p=0.0096
p=0.0036
p<0.0001


A
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
p=0.0072
ns
p=0.0033
Mis
IRF1
Mis
NLRC5
Mis
IRF1 & NLRC5
B
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
p=0.0462
ns
ns
ns
p=0.0199
Cas9+ Mis
NLRC5-/- Mis
Cas9+ IRF1 KD
NLRC5-/- IRF1 KD
C
Relative HLA-A expression
2-ΔΔCT to HPRT1 expression
ns
ns
ns
ns (0.0562)
ns
ns
Cas9+ Mis
NLRC5-/- Mis
Cas9+ IRF1 KD
NLRC5-/- IRF1 KD
D
IFNγ SFU
ΔSFU (Ag – no Ag)
p=0.0186
p=0.0101
Mis
IRF1 KD
Cas9+
Mis
IRF1 KD
NLRC5-/-
E
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
p=0.0009
ns
UT
+IFNγ
Cas9+
UT
+IFNγ
IRF1-/-
F
Relative HLA-A expression
2-ΔΔCT to HPRT1 expression
p=0.0006
ns
UT
+IFNγ
Cas9+
UT
+IFNγ
IRF1-/-
G
Surface MR1 expression
gMFI (α-26.5 Ab)
p=0.0147
ns
UT
+IFNγ
Cas9+
UT
+IFNγ
IRF1-/-
H
Surface MHC-Ia expression
gMFI (α-W6/32 Ab)
p=0.0164
ns
UT
+IFNγ
Cas9+
UT
+IFNγ
IRF1-/-


A
pSTAT1
gMFI (α-phospho-STAT1 Ab)
35K
30K
25K
20K
15K
10K
5K
0
ns
p=0.0226
UI
+Sp
UI
+Sp
–
+MAIT
B
pSTAT1
gMFI (α-phospho-STAT1 Ab)
125K
100K
75K
50K
25K
0
p=0.0021
UT
+IFNγ
C
Relative β2m expression
2-ΔΔCT to HPRT1 expression
8
7
6
5
4
3
2
1
0
ns (0.057)
p=0.0333
UI
+Sp
UI
+Sp
–
+MAIT
D
Relative HLA-A expression
2-ΔΔCT to HPRT1 expression
10
8
6
4
2
0
p=0.0433
p=0.0372
UI
+Sp
UI
+Sp
–
+MAIT
E
Relative IRF1 expression
2-ΔΔCT to HPRT1 expression
60
50
40
30
20
10
0
p=0.0409
p=0.0189
UI
+Sp
UI
+Sp
–
+MAIT
F
Relative NLRC5 expression
2-ΔΔCT to HPRT1 expression
75
60
45
30
15
0
p=0.0074
UI
+Sp
UI
+Sp
–
+MAIT


A
B
C
D
E
F
Relative HLA-A expression
2-ΔΔCT to HPRT1 expression
Relative IRF1 expression
2-ΔΔCT to HPRT1 expression
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
p=0.0409
p=0.0083
p=0.0182
p=0.0151
p=0.0021
UI
+Ms
UT
+5-OP
+6-FP
5-OP
–
+MAIT
Cas9+ cells
IRF1-/- cells


A
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
ns
ns
ns
p=0.0079
p<0.0001
p=0.0035
UT
IFNβ
IFNγ
IFNλ
TNFα
IL-17
B
Relative MR1 expression
2-ΔΔCT to HPRT1 expression
p<0.0001
p=0.0035
UT +IFNγ
UT +IFNβ
C
Relative HLA-A expression
2-ΔΔCT to HPRT1 expression
p=0.0008
p=0.0024
UT +IFNγ
UT +IFNβ
D
Surface MR1 expression
gMFI (α-26.5 Ab)
p=0.0031
ns
UT +IFNγ
UT +IFNβ
Surface MHC-Ia expression
gMFI (α-W6/32 Ab)
p<0.0001
p=0.0002
UT +IFNγ
UT +IFNβ
E
IFNγ SFU
ΔSFU (Ag – no Ag)
UT
IFNβ, ns (0.9568)
IFNγ, p<0.0001
M. smegmatis titration
(µl/10K cells)
F
Relative IRF1 expression
2-ΔΔCT to HPRT1 expression
p<0.0001
p<0.0001
UT +IFNγ
UT +IFNβ
G
Relative NLRC5 expression
2-ΔΔCT to HPRT1 expression
p=0.0022
p=0.0356
UT +IFNγ
UT +IFNβ
H
Relative β2m expression
2-ΔΔCT to HPRT1 expression
p=0.0012
p=0.0031
UT +IFNγ
UT +IFNβ


MAIT cell
other sources
IFNγ
MR1
IRF1
MR1
Antigens
Airway epithelial cell