Title: Type I Interferon production in myeloid cells is regulated by factors independent of *Ptpn22*

Running title: *Ptpn22* does not regulate IFN-I production

Jenna R. Barnes, Anam Fatima Shaikh, Alec M. Bevis, Tammy R. Cockerham, Robin C. Orozco

Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045 United States of America

Corresponding Author: Robin C. Orozco, 1200 Sunnyside Dr, University of Kansas, Lawrence, KS, 66045, United States of American, orozco.robin@ku.edu

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ABSTRACT

The immune regulatory gene *PTPN22* is expressed in all immune cells and encodes Lyp in humans and the ortholog PEP in mice. The *PTPN22* alternative allele, 1858C>T, is expressed in 5-15% of the North American population and is strongly associated with the development of autoimmune disease while simultaneously capable of providing protection during virus infection and cancer. In murine models, significant progress has been made in elucidating the molecular mechanisms that PEP and its pro-autoimmune variant (PEP-R619W) modulate T cell function, yet their influence on non-T cell pathways, such as antigen presenting cell cytokine production, remains less defined. Previously, it was reported that PEP promotes type I interferon (IFN-I) production in dendritic cells (DCs) and macrophages following TLR4 stimulus. Here, we show that contrary to previous results, both PEP-WT and the PEP-R619W variant do not promote IFN-I production in DCs and macrophages following exposure to LPS, 3p-hpRNA, or coronavirus MHV A59. We attribute the prior findings to mouse strain-specific differences and conclude that something other than PEP may be regulating IFN-I production in these studies. We further show that PEP and its R619W variant distinctly modulate the production of TNFα, IL-12 and IL-2, in DCs following LPS stimulus. Taken together, our results challenge the current understanding of PEP's role during inflammation while providing new insight into how the PEP-R619W variant may alter myeloid cell function during disease.

36 **INTRODUCTION**

37

38 Allelic variation in immune-regulatory genes can significantly impact disease outcomes [1,
39 2]. One such variant of interest is the 1858C>T allele of the Protein Tyrosine Phosphatase Non-
40 Receptor Type 22 gene (*PTPN22*). *PTPN22* is constitutively expressed in all immune cells and
41 encodes for the phosphatase lymphoid protein Lyp, and its murine ortholog, PEST-domain
42 enriched phosphatase (PEP). The *PTPN22* 1858C>T allele, resulting in an amino acid
43 substitution of arginine (R) to tryptophan (W) at position 620 in human, is expressed in 5-15% of
44 the North American population and is considered the highest non-HLA risk allele for the
45 development of autoimmunity, including type I diabetes, rheumatoid arthritis, and systemic lupus
46 erythematous[3-11].

47

48 To investigate the role of *PTPN22* and its autoimmunity-associated variant during
49 inflammation, researchers have employed mouse models which lack expression of PEP (PEP-
50 null), the murine ortholog of Lyp. Through these studies, it was shown that PEP-null mice have
51 enhanced persistent viral clearance and T cell function[12, 13]. However, because the LYP-R620W,
52 or the murine equivalent PEP-R619W mutation, does not cause a loss of protein expression, but
53 rather altered function[14, 15], using PEP-null models to investigate how the *PTPN22* autoimmunity
54 associated allele drives inflammation is not always appropriate. Furthermore, PEP-null and PEP-
55 R619W mice have been reported to exhibit differing phenotypes [16-20]. As an example, PEP-null
56 mice exhibit an increased frequency and potency of Tregs compared to PEP-WT mice, but this
57 phenotype is not observed in PEP-R619W mice [19, 21, 22]. Using CRISPR-Cas9 generated PEP-
58 R619W mice, we were the first to report that the PEP-R619W variant is similarly protective

59 during chronic virus infection and promotes enhanced T cell function [1]. Through adoptive
60 transfer studies, we and others showed that the enhanced antiviral T cell function in PEP-null and
61 PEP-R619W mice is due to both T cell intrinsic and extrinsic mechanisms [1-3]. However,
62 additional studies are needed to understand the specific antiviral molecular mechanisms that PEP
63 and the PEP-R619W variant may influence. Significant progress has been made in elucidating
64 the molecular mechanisms that PEP and its R619W variant modulate T cell function [12-17, 19, 23-28].
65 However, the role of PEP and its R619W variant on non-T cell pathways that may influence T
66 cell function, such as antigen presenting cell (APC) cytokine production, remains less
67 understood.
68
69 APCs, such as dendritic cells (DCs), produce a variety of cytokines capable of broadly
70 promoting inflammation or directing lymphocyte activation and differentiation [4-6]. Type-I
71 interferons (IFN-I) are potent cytokines produced by DCs which exhibit significant antiviral
72 activity by modulating the activation and effector function of other immune cells, including T
73 cells [29, 30]. Although a short burst of IFN-I secretion is critical for effective clearance of viruses
74 including Lymphocytic Choriomeningitis virus clone 13 (LCMV-cl13)[31-33], prolonged IFN-I
75 production has been shown to contribute to immune dysfunction, such as T cell exhaustion, and
76 persistent viral infection [31, 34]. Thus, tight regulation of IFN-I and other pro-inflammatory
77 cytokines during infection is crucial and mechanisms which alter the production of cytokines
78 have the potential to significantly impact the outcome of infection. Given the versatile role of
79 PEP and its R619W variant during a variety of inflammatory processes, researchers have set out
80 to evaluate the potential impact of PEP and its R619W variant on the production of cytokines by
81 APCs during infection.

82

83 Previously, it was reported that PEP promotes IFN-I production in DCs and macrophages
84 following stimulus with bacterial lipopolysaccharide (LPS) or Poly(I:C)), Toll Like Receptor 4
85 (TLR4) and Toll Like Receptor 3 (TLR3) agonists, respectively [35]. However, these studies were
86 performed using PEP-null mice available at The Jackson Labs which have been found to have
87 numerous SNPs associated with C57BL/6N (B6N) mice [36]. The recommended control for this
88 PEP-null strain are C57BL/6J PEP-WT mice and historically, the majority of *Ptpn22* literature in
89 C57BL/6 mice has also utilized Jackson Labs PEP-null mice with C57BL/6J PEP-WT mice as
90 controls. This poses concern that our current understanding of *Ptpn22* and how it regulates
91 inflammation may have been influenced by mouse strain specific differences driven by genetic
92 variation. To enable a more rigorous investigation into defining how *PTPN22* and its
93 autoimmunity associated allele regulate inflammation, our research team employs C57BL/6J
94 CRISPR-Cas9-generated PEP-null and PEP-R619W mouse models. Using these genetic models,
95 we first set out to determine if previous data regarding the impact of PEP on IFN-I production
96 could be recapitulated. We also further investigated the impact of the PEP-R619W variant on
97 IFN-I production in dendritic cells. In this study, we report that neither PEP nor the PEP-R619W
98 variant regulate IFNβ production in DCs or macrophages following LPS or viral RNA
99 stimulation. However, we found that both PEP and PEP-R619W differentially modulate the
100 production of other pro-inflammatory cytokines by dendritic cells, including IL-2, IL-12, and
101 TNFα.

102

103 **RESULTS**

104

105 **Strain-specific differences impact IFNβ production in PEP-null bone marrow dendritic**
106 **cells (BMDCs)**

107 Previously, it was shown that PEP-null BMDCs and bone marrow macrophages (BMMs)
108 produce reduced levels of IFN-I when stimulated with bacterial lipopolysaccharide (LPS) or
109 Poly(I:C)), TLR4 and TLR3 agonists respectively [35]. Using our C57BL/6J CRISPR-Cas9
110 generated PEP-null model (PEP-null CRISPR) and the commercially available Jackson Labs
111 PEP-null mice (PEP-null JAX), we tested if the B6N SNPs present in the Jackson Labs PEP-null
112 strain were driving this reported phenotype, as opposed to PEP expression. Using qRT-PCR, we
113 confirmed that our CRISPR-Cas9 generated PEP-null mice do not express quantifiable amounts
114 of *Ptpn22* mRNA (Figure 1A). Using FLT3-ligand differentiated BMDCs from C57BL/6J PEP-
115 WT, CRISPR-Cas9 generated PEP-null, and PEP-null Jackson Labs mice we measured for IFNβ
116 production following LPS stimulation. While IFNβ concentration was comparable between PEP-
117 WT and our PEP-null CRISPR mice, BMDCs from the commercial PEP-null JAX strain showed
118 significantly less IFNβ production (Figure 1B). These data successfully recapitulate prior results,
119 but through the use of our CRISPR-Cas9 generated PEP-null model, we observe that PEP does
120 not promote IFNβ production. These strain-specific differences in IFN-I production suggest that
121 other genetic differences associated with the B6N background, rather than *Ptpn22* deficiency
122 alone, may account for the previously reported phenotype.
123

124 In addition to the PEP-null JAX model, studies have also utilized chemical inhibition of
125 PEP to evaluate its role in IFN-I production and activity [28, 35]. However, varying results regarding
126 PEP inhibitors and their specificity have been reported [37]. To evaluate the specificity of the PEP
127 inhibitor LTV-1 during IFN-I production, we stimulated PEP-WT and CRISPR-Cas9 generated

128 PEP-null bone marrow macrophages (BMMs) with LPS in the presence or absence of LTV-1 to
129 inhibit PEP's enzymatic activity. We observed reduced IFNβ production in both PEP-WT and
130 PEP-null BMMs treated with LTV-1, but no significant differences in IFNβ production between
131 the two genotypes (Figure 1C). This data suggests that LTV-1 has off target effects which
132 reduces IFNβ production, rather than the loss of PEP's enzymatic activity.
133
134 **PEP and PEP-R619W do not mediate IFNβ production in myeloid cells**

135 Our initial results showed comparable IFNβ production between PEP-WT and PEP-null
136 CRISPR BMDCs following TLR4 stimulation via LPS. However, beyond TLRs, additional
137 pattern recognition receptors (PRRs) such as RIG-I Like Receptors (RLRs) induce large
138 quantities of IFN-I in response to virus infection [38-40]. To determine if PEP promotes IFN-I
139 production induced by TLR and RLR signaling in macrophages, we cultivated PEP-WT and
140 PEP-null BMMs and then stimulated them with LPS or infected with the murine coronavirus
141 Murine Hepatitis Virus Strain A59 (MHV A59), a MDA5 agonist [40]. There were no differences
142 detected in IFNβ production between PEP-null and PEP-WT BMMs following LPS exposure or
143 MHV A59 infection (Figure 2A). These results suggest that PEP does not promote IFN-I
144 production in BMMs following TLR4 or MDA5 activation.
145

146 Further, it is unknown if the autoimmunity-associated PEP-R619W variant, which alters
147 protein function rather than expression, uniquely affects IFN-I production compared to PEP-WT.
148 To determine if PEP-R619W either phenocopies PEP-null CRISPR DCs or distinctly alters IFN-I
149 production following TLR or RLR activation, we cultivated FLT3-ligand (FLT3-L) BMDCs
150 from C57BL/6J PEP-WT, PEP-null, and PEP-R619W mice and stimulated them with LPS or 5'

151 Triphosphate hairpin ribonucleic acid (3p-hpRNA), a RIG-I agonist. We detected no significant
152 differences in IFNβ production between all three genotypes (PEP-WT, PEP-null, and PEP-
153 R619W) following either LPS or 3p-hpRNA stimulation (Figure 2 B-C). Taken together, these
154 results indicate that neither PEP nor the PEP-R619W variant modulate IFNβ production in
155 dendritic cells mediated by TLR4 or RIG-I activation.

156

157 **PEP and the PEP-R619W variant distinctly modulate the production of pro-inflammatory**
158 **cytokines in dendritic cells**

159

160 Our findings show that PEP nor the PEP-R619W variant impact IFN-I production in DCs
161 and macrophages during TLR4, MDA5, or RIG-I stimulation. However, DCs produce a variety
162 of other cytokines which can promote widespread inflammation or influence T cell activation
163 and differentiation. Considering that the PEP-R619W variant promotes non-T cell mechanisms
164 which enhance T cell function during virus infection, it is possible that PEP regulates the
165 production of other inflammatory cytokines relevant to T cell function. Further, the PEP-R619W
166 variant may distinctly alter these regulatory mechanisms. Previous studies which have
167 investigated the role of PEP and its variant on cytokine production in myeloid cells have reported
168 varied findings[35, 41, 42]. Using PEP-R619W knock-in mouse models, one group reported increased
169 IL-12 production in Granulocyte-macrophage colony stimulating factor (GM-CSF) differentiated
170 PEP-R619W BMDCs post LPS exposure[41]. Another group reported that GM-CSF differentiated
171 PEP-null Jax BMDCs exhibited enhanced secretion of IL-6, IL-8 and TNFα compared to PEP-
172 WT following treatment with muramyl dipeptide (MDP)[42]. They also reported that the loss of
173 PEP reduced while the PEP-R619W variant increased IL-1β secretion by BMDCs following

174 ultra-pure LPS exposure[20]. However, a different group later reported no differences in TNFα, IL-
175 1β, or IL-12 production following treatment with LPS between FLT3-ligand differentiated PEP-
176 WT and PEP-null JAX BMDCs [35]. These contrary results align with previous reports of PEP-null
177 and PEP-R619W mice exhibiting different phenotypes but could also be attributed to the usage
178 of different mouse models, type of stimulus, and selected assays. Using a consistent genetic
179 model and experimental design, we set out to investigate the role of PEP and the PEP-R619W
180 variant in modulating cytokine production. To accomplish this, we again employed FLT3-ligand
181 differentiated BMDCs from PEP-WT, PEP-null CRISPR, and PEP-R619W mice. BMDCs were
182 stimulated with LPS, and the supernatant was collected and analyzed for TNFα, IL-12 (p70), and
183 IL-2 production. We found that compared to PEP-WT, TNFα production was reduced in both
184 PEP-null and PEP-R619W DCs (Figure 3A). Additionally, we observed a reduction in IL-12
185 production by PEP-R619W DCs compared to PEP-WT and PEP-null (Figure 3B). Finally, we
186 observed increased IL-2 production by both PEP-null and PEP-R619W DCs compared to PEP-
187 WT (Figure 3C). Taken together, these findings suggest that PEP and the PEP-R619W variant
188 distinctly modulate cytokine production in dendritic cells.
189
190 **DISCUSSION**
191
192 The *PTPN22* 1858C>T allele is strongly associated with the development of
193 autoimmunity, but the murine equivalent mutation has also been shown to be protective during
194 virus infection and cancer [16, 21]. While the impact of the PEP-R619W variant is well-defined in T
195 cells, the molecular mechanisms by which PEP and its variant impact the function of other
196 immune cells, such as the ability of APCs to produce pro-inflammatory cytokines, remain less

197 understood. Previously, it was suggested that PEP promotes IFN-I production in macrophages
198 and dendritic cells [35]. However, these data were generated using PEP-null mice on a B6N/B6J
199 mixed background. In this paper, we tested if the B6N SNPs present in this PEP-null model were
200 driving this reported phenotype by comparing BMDCs derived from our C57BL/6J CRISPR-
201 Cas9-generated PEP-null model to BMDCs from the B6N/B6J PEP-null mice available at the
202 Jackson labs. While the PEP-null JAX BMDCs had reduced IFN-I production, we did not detect
203 a difference between our PEP-null CRISPR BMDCs and C57BL/6J PEP-WT BMDCs (Figure
204 1B). These strain-specific differences in IFN-I production recapitulate previous findings, while
205 also revealing that unknown genetic variation in the B6N/B6J PEP-null mice may have
206 influenced the previously observed phenotype. This interpretation is further supported by reports
207 of reduced inflammatory responses during virus infection in B6N mice compared to C57BL/6J
208 mice [43, 44]. However, our study results do not address why transgenic PEP-null JAX BMMs
209 which over-express the human protein Lyp have restored IFNβ production [35]. Identifying the
210 mechanism which drives the reduced IFNβ production observed in the B6N/B6J Jackson lab's
211 available PEP-null mice but not in the CRISPR-Cas9 generated PEP-null mice will require
212 further investigation. Regardless, we are not the first to comment on or identify discrepancies
213 regarding previous data reported on PEP's role during IFN-I production in myeloid cells.
214 However, these prior publications have largely focused on differences identified in human
215 samples, as well as in the context of systemic lupus erythematous (SLE) [45-49]. Thus, to the best of
216 our knowledge, we are the first to show that in a mouse model there are strain specific
217 differences affecting IFN-I production by DCs and BMMs.
218

219 Next, when evaluating the specificity of the PEP inhibitor LTV-1 during IFN-I production
220 in BMMs, we found reduced IFNβ production in both PEP-WT and PEP-null macrophages
221 treated with LTV-1 (Figure 1C). These findings suggest that the observed reduction of IFNβ may
222 not be due to the deficiency of PEP nor the inhibition of its enzymatic function, but rather
223 because of non-specific effects of LTV-1. Taken together, our findings reveal additional
224 confounding variables that could impact the results of studies which aim to discern the role of
225 PEP during inflammation through the use of either genetic models or chemical inhibition. Our
226 observations add to the numerous conflicting findings already identified in the field regarding
227 PEP, PEP-R619W, and their effects on cytokine production [20, 35, 41, 42]. These differences likely
228 stem from genetic variation present in both human and animal studies, as well as the utilization
229 of diverse experimental approaches including over-expression, transgenic models, knockdown
230 techniques, and chemical inhibition. Each of these systems offer distinct advantages yet also
231 varying limitations, yielding inconsistent results. To address this lack of clarity, our lab employs
232 CRISPR/Cas9-generated PEP-null and PEP-R619W mouse models on a C57BL/6J background.
233 By using this model, we can precisely evaluate the mechanisms in which PEP and the PEP-
234 R619W variant drive inflammation while minimizing confounding genetic variables.
235
236 Collectively, our results indicate that IFNβ production by DCs and macrophages is not
237 promoted by PEP following TLR4 activation (Figure 1B, 2A, and 2B). Because IFN-I is induced
238 by additional PRRs such as RLRs, we also investigated if PEP regulates IFN-I production
239 induced by RLR signaling but observed no significant differences in IFNβ production between
240 PEP-WT and PEP-null macrophages or dendritic cells following MHV A59 infection or viral
241 RNA stimulus (Figure 2 A,C). Finally, since the PEP-R619W variant still has PEP expression,

242 but causes altered protein binding capabilities, it is possible that PEP-R619W DCs could have
243 distinctive changes in IFN-I production compared to PEP-null cells. Our results suggest that
244 similar to PEP-WT, PEP-R619W does not promote IFNβ production in dendritic cells post LPS
245 or viral RNA stimulus (Figure 2 B-C).
246
247 Although our findings indicate that PEP and PEP-R619W do not promote IFNβ
248 production in myeloid cells, we found that PEP and its variant distinctly modulate the production
249 of other pro-inflammatory cytokines. More specifically, we found that compared to PEP-WT,
250 TNFα production was reduced in both PEP-null and PEP-R619W DCs. Additionally, we
251 observed a reduction in IL-12 production by PEP-R619W DCs compared to PEP-WT and PEP-
252 null (Figure 3). Previously it was reported that PEP-R619W GM-CSF differentiated DCs have
253 increased IL-12 production compared to PEP-WT [41]. These conflicting findings may be due to a
254 variety of factors such as differences in the PEP-R619W mouse models and LPS dosage utilized,
255 as well as functional differences between FLT-ligand differentiated and GM-CSF differentiated
256 BMDCs. Finally, we observed increased IL-2 production by both PEP-null and PEP-R619W
257 DCs compared to PEP-WT. Since DC derived IL-2 can stimulate the growth and activation of T
258 cells, it is possible that the increased IL-2 produced by PEP-null and PEP-R619W DCs is a
259 contributing factor to the enhanced T cell function observed during virus infection.

260 In summary, PEP-R619W DCs have reduced TNFα and IL-12, but increased IL-2
261 production compared to PEP-WT. However, the molecular mechanisms by which PEP and its
262 variant distinctly modulate the production of pro-inflammatory cytokines in dendritic cells
263 remain unknown. Previously, PEP has been shown to bind to the E3 ubiquitin ligase, TNF
264 receptor-associated factor 3 (TRAF3) [35]. TRAF proteins are essential components of cell

265 signaling pathways and TRAF3 is uniquely capable of both positively regulating IFN-I
266 production and negatively regulating the canonical and non-canonical signaling pathways of
267 Nuclear Factor-kappa B (NF-κB) [50]. Based on prior study results [35], the interaction between PEP
268 and TRAF3 is necessary for the induction of IFN-I. However, our data suggests that PEP is not
269 required for the production of IFN-I. Instead, we report that PEP and the PEP-R619W variant
270 distinctly modulate the production of other pro-inflammatory cytokines produced downstream of
271 NF-κB signaling. A possible explanation for these observations is that PEP regulates cytokine
272 production by interacting with TRAF3 or other key signaling proteins involved in the NF-κB
273 signaling cascade, and the R619W mutation potentially alters these interactions. The impact of
274 the PEP-R619W variant on the production of cytokines critical for T cell activation, such as IL-2
275 and IL-12, may begin to further explain how the PEP-R619W variant promotes T cell extrinsic
276 effects which ultimately lead to enhanced immune function and viral clearance. However,
277 additional rigorous studies will be required to tease out the precise mechanisms in which PEP
278 and its PEP-R619W variant regulate these inflammatory processes in DCs and macrophages.
279
280 Altogether, our findings challenge the current understanding of PEP while providing new
281 insight into how the PEP-R619W variant is capable of simultaneously promoting autoimmunity
282 and conferring protection against persistent viral infection and cancer. While our results reveal
283 that changes in IFN-I production are not a key factor in how PEP regulates inflammation as
284 previously regarded, our data indicates that PEP and the PEP-R619W variant have a novel role in
285 modulating the production of other cytokines by dendritic cells which are critical for a variety of
286 inflammatory processes. Future studies will need to evaluate how changes mediated by PEP and

287 the PEP-R619W variant in dendritic cells and other antigen presenting cells are relevant in order
288 to accurately define the broad consequences they have on disease pathogenesis.
289
290 **MATERIAL AND METHODS**
291
292 **Ethics:**
293 All animal studies were reviewed and approved by University of Kansas Animal Care
294 and Use Committee (IACUC) (protocol number: 278-01).
295
296 **Mice:**
297 Mice were housed in general housing conditions and all animal studies were reviewed
298 and approved by the University of Kansas Animal Care and Use Committee (protocol number:
299 278-01). Both males and females ranging from 5-12 weeks of age were used in this study. No
300 sex-based differences were observed in our study results. C57BL/6J WT (PEP-WT) mice were
301 originally purchased from Jackson labs, then bred and maintained in the University of Kansas
302 Animal Care Unit. Ptpn22 $^{-/-}$ (PEP-null CRISPR) mice were generated using CRISPR/Cas9
303 technology on a C57BL/6 background as described previously [7]. Ptpn22 $^{-/-}$ mice (PEP-null
304 JAX) were gifted from Dr. Won Jin Ho (John Hopkins University School of Medicine). These
305 mice are available for purchase from the Jackson Laboratory, and their generation has been
306 previously described [23]. Mice expressing the Ptpn22 pro-autoimmune allele (PEP-R619W) were
307 generated using CRISPR/Cas9 technology on a C57BL/6 background using methods previously
308 reported [16, 21, 51].
309

310 **Bone Marrow Dendritic Cell (BMDC) Culture:**

311 Bone marrow cells were isolated from PEP-WT, PEP-null, and PEP-R619W mouse
312 femurs. Following isolation, bone marrow cells were cultured in Advanced DMEM containing
313 10% FBS, 1% penicillin streptomycin, 1% L-Glutamine, and 100ng/mL FLT3L (Stem Cell
314 Technologies) for 8 days. On day 8, FLT3-ligand differentiated dendritic cells were harvested,
315 counted, and re-plated.

316

317 **BMDC Stimulation:**

318 BMDCs were resuspended and plated at 1 x 10^6 cells/mL in Advanced DMEM (Gibco)
319 containing 10% FBS, 1% penicillin streptomycin and 1% L-Glutamine. The cells were
320 stimulated with either LPS or 3p-hpRNA. Cells stimulated with LPS (500 ng/mL) (Sigma) were
321 incubated overnight (16-18 hours). The supernatant and cells (resuspended in TRIzol
322 (Invitrogen)) were collected and stored frozen at -81 degrees Celsius for future assays. Cells
323 stimulated with 3p-hpRNA (2 ng/mL) complexed with LyoVec (InvivoGen) were incubated
324 overnight (16-18 hours). The supernatant and cells (resuspended in TRIzol (Invitrogen)) were
325 collected and stored frozen at -81 degrees Celsius for future assays.

326

327 **Enzyme-Linked Immunosorbent Assay (ELISA):**

328 Levels of IFNβ, TNFα, IL-12 (p70), and IL-2 in cell culture supernatant were measured
329 using ELISA Kits (IFNβ (PBL Assay Science), TNFα, IL-12 and IL-2 (BioLegend)) according to
330 the manufacturer's instructions, followed by analysis with a BioTek EON microplate reader at
331 450 and/or 570 nm.

332

333 **qRT-PCR:**

334 RNA was extracted using TRIzol according to the manufacturer's instructions
335 (Invitrogen). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit
336 (Applied Biosystems), according to the manufacturer's instructions. Real-time PCR was
337 performed on a Quant Studio 3 system using PowerTrack™ SYBR™ Green Master Mix
338 (Applied Biosystems). Each reaction was measured at least triplicate, and data was normalized to
339 the expression levels of the house keeping gene GAPDH. The designed primers for each selected
340 murine gene are listed:

Gene	**Forward Primer**	**Reverse Primer**
Gapdh	AACGACCCCTTCATTGACCTC	ACTGTGCCGTTGAATTTGCC
Ptpn22	AGCTGATGAAAATGTCCTATTGTGA	GTCCCACTGCATTCTGGTGA

341

342 **Statistical analysis and graphing:**

343 All statistical analysis was performed using GraphPad Prism Software. The type of
344 statistical test is listed in figure legends. Data was considered statistically significant if the p
345 value<0.05. Graphs were made in GraphPad Prism (La Jolla, CA). Figure legends indicate if data
346 shown is pooled from multiple studies or is from representative study.

347

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350 Dr. Linda Sherman, Scripps Research Institute. These mice were gifts from Dr. Sherman. PEP-
351 null JAX mice were a gift from Dr. Won Jin Ho (Johns Hopkins University School of Medicine).

352

CONFLICTS OF INTEREST

None Declared

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545

546

547 **ABBREVIATIONS**

548 IFN-I	Type I Interferon
549 Ptpn22	Protein tyrosine phosphatase non-receptor type 22
550 Lyp	Lymphoid protein
551 PEP	PEST-domain enriched phosphatase
552 DCs	Dendritic Cells
553 TLR4	Toll-Like Receptor 4
554 LPS	Lipopolysaccharide
555 3p-hp-RNA	5’ Triphosphate hairpin ribonucleic acid
556 MHV A59	Mouse Hepatitis virus strain A59
557 TNFα	Tumor Necrosis Factor alpha
558 IL	Interleukin
559 R	Arginine
560 W	Trypotophan
561 HLA	Human Leukocyte Antigen
562 Tregs	T regulatory cell
563 CRISPR-Cas9	Cluster regularly interspaced short palindromic repeats Cas9
564 APC	Antigen Presenting Cell
565 LCMV-cl13	Lymphocytic Choriomeningitis Virus Clone 13
566 TLR3	Toll-like receptor 3
567 SNP	single nucleotide polymorphism
568 RLR	Rig I like receptor
569 MDA5	Melanoma Differentiation Associated protein 5

570	IFNβ	Interferon Beta
571	RNA	Ribonucleic Acid
572	BMDC	Bone Marrow Derived Dendritic cells
573	BMM	Bone Marrow Derived Macrophage
574	JAX	Jackson Labs
575	mRNA	messenger ribonucleic acid
576	FLT3-L	FMS-like tyrosine kinase 3- Ligand
577	GM-CSF	Granulocyte- Macrophage Colony Stimulating factor
578	RIG-I	Retinoic acid inducible gene 1
579	MDP	Muramyl dipeptide
580	ELISA	Enzyme Linked Immunosorbent Assay
581	ANOVA	Analysis of Variance
582	NFκB	Nuclear Factor kappa B
583	TRAF3	TNF receptor-associated factor 3
584	IACUC	Institutional Animal Care and Use Committee
585	DMEM	Dulbecco's Modified Eagle Medium
586	FBS	Fetal Bovine Serum
587	MOI	Multiplicity of Infection
588	Ns	not significant
589	qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction

590 **FIGURE LEGENDS**

591

592 **Figure 1. Strain specific differences impact IFNβ production in PEP-null BMDCs.** Gene
593 expression values of *Ptpn22* relative to *GAPDH* were determined by qRT-PCR for naïve PEP-
594 WT (black bars) and PEP-null (CRISPR) (red) splenocytes, each dot represents a mouse **(A)**.
595 FLT3-L BMDCs were cultivated and differentiated from C57BL/6J PEP-WT (black),
596 CRISPR/Cas9-generated PEP-null (red), and PEP-null mice from the Jackson Laboratory (JAX
597 stock #028977) (gray). Cultures were incubated with LPS (500 ng/mL) for 18 hours. The
598 supernatant was used to determine IFNβ concentration (ELISA) **(B)**. PEP-WT and PEP-null
599 (CRISPR) BMMs were incubated with LPS (500 ng/mL) and 10 uM LTV-1 for 18 hours. The
600 supernatant was used to determine IFNβ concentration (ELISA) **(C)**. ns= no significance;
601 *p<0.01; **p<0.001; ****p<0.0001; Two Way ANOVA with Tukey post hoc analysis. Data is
602 pooled from 2 independent experiments **(B)**. Data is representative of 3 biological replicates **(C)**.

603

604 **Figure 2. PEP and PEP-R619W do not mediate IFNβ production in myeloid cells following**
605 **LPS, MHV A59, or viral RNA stimulation.** C57BL/6J PEP-WT (black, square symbol) and
606 PEP-null (red, circle symbol) BMMs were cultivated and then stimulated with LPS (500 ng/mL)
607 or infected with MHV A59 (MOI 0.1) for 18 hours **(A)**. FLT3-L BMDCs were cultivated from
608 C57BL/6J PEP-WT, PEP-null, and PEP-R619W mice. Cultures were incubated with LPS (500
609 ng/mL) **(B)** or 3p-hpRNA (2 ng/mL) **(C)** for 18 hours. The supernatant was used to determine
610 IFNβ concentration (ELISA). ns= no significance. Two Way ANOVA with Tukey post hoc
611 analysis. Data is representative of 2 biological replicates **(A)**. Data is pooled from 3 independent
612 experiments **(B-C)**.

613

614 **Figure 3. PEP and the PEP-R619W variant distinctly modulate the production of pro-**

615 **inflammatory cytokines in dendritic cells**. FLT3-L BMDCs were cultivated from C57BL/6J

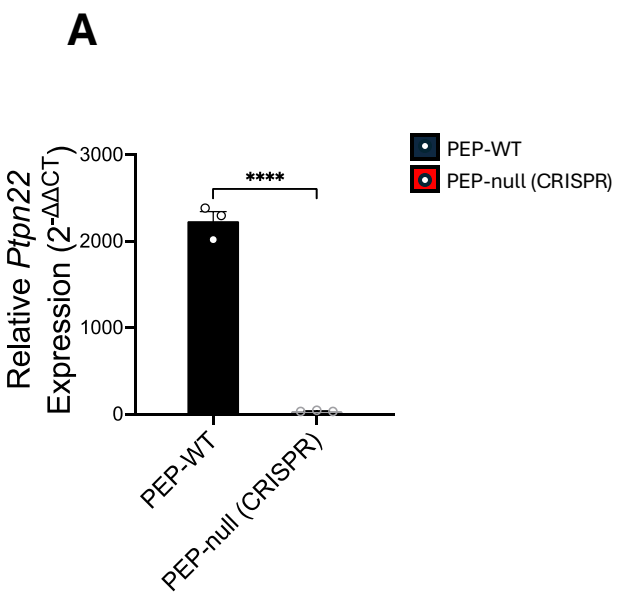
616 PEP-WT (black), PEP-null (red), and PEP-R619W (blue) mice. Cultures were incubated with

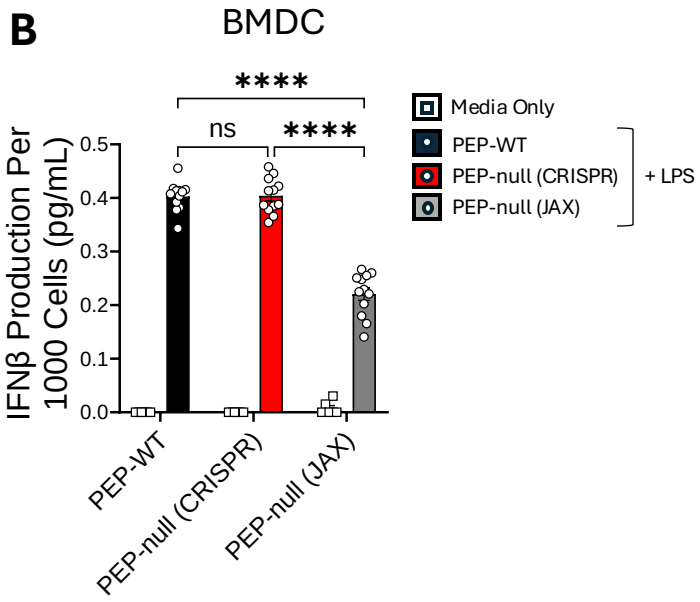
617 LPS (500 ng/mL) for 18 hours. The supernatant was used to determine IL-2 **(A)**, IL-12 **(B)**, and

618 TNFα **(C)** concentration (ELISA). ns= no significance;**$p<0.01$, ****$p<0.0001$; Two Way

619 ANOVA with Tukey post hoc analysis. Data is representative of 2 independent experiments.

620


A
Relative Ptpn22 Expression (2-ΔΔCT)
3000
2000
1000
0
****
PEP-WT
PEP-null (CRISPR)
PEP-WT
PEP-null (CRISPR)


B
BMDC
IFNβ Production Per 1000 Cells (pg/mL)
0.5
0.4
0.3
0.2
0.1
0.0
****
ns
****
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-null (JAX)
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-null (JAX)

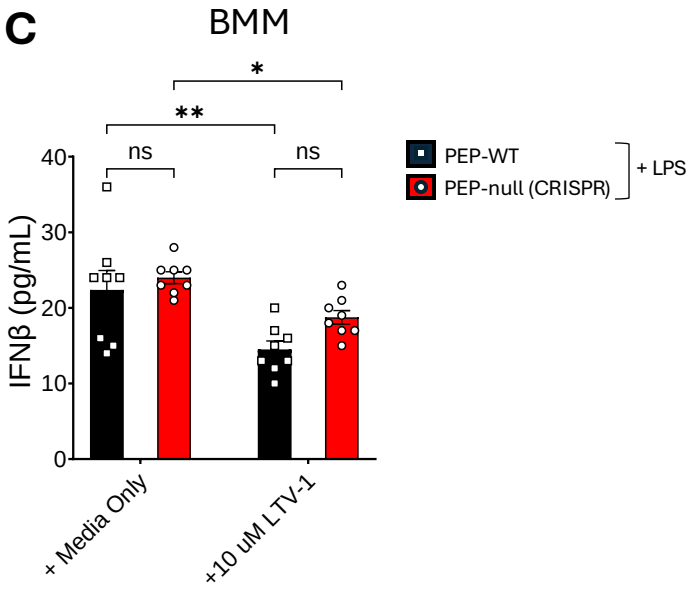
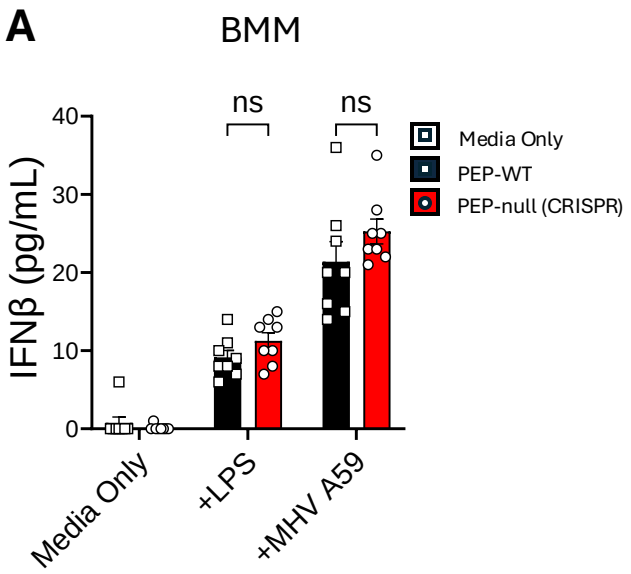
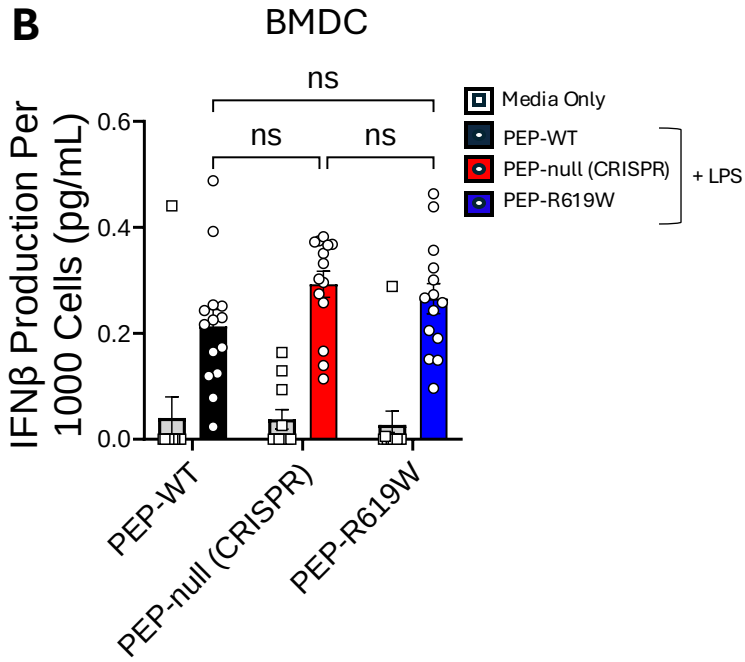

C
BMM
IFNβ (pg/mL)
40
30
20
10
0
*
**
ns
ns
PEP-WT
PEP-null (CRISPR)
+ LPS
+ Media Only
+10 uM LTV-1

Figure 1


A
BMM
IFNβ (pg/mL)
40
30
20
10
0
ns
ns
Media Only
PEP-WT
PEP-null (CRISPR)
Media Only
+LPS
+MHV A59


B
BMDC
IFNβ Production Per 1000 Cells (pg/mL)
0.6
0.4
0.2
0.0
ns
ns
ns
Media Only
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PEP-null (CRISPR)
PEP-R619W
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-R619W

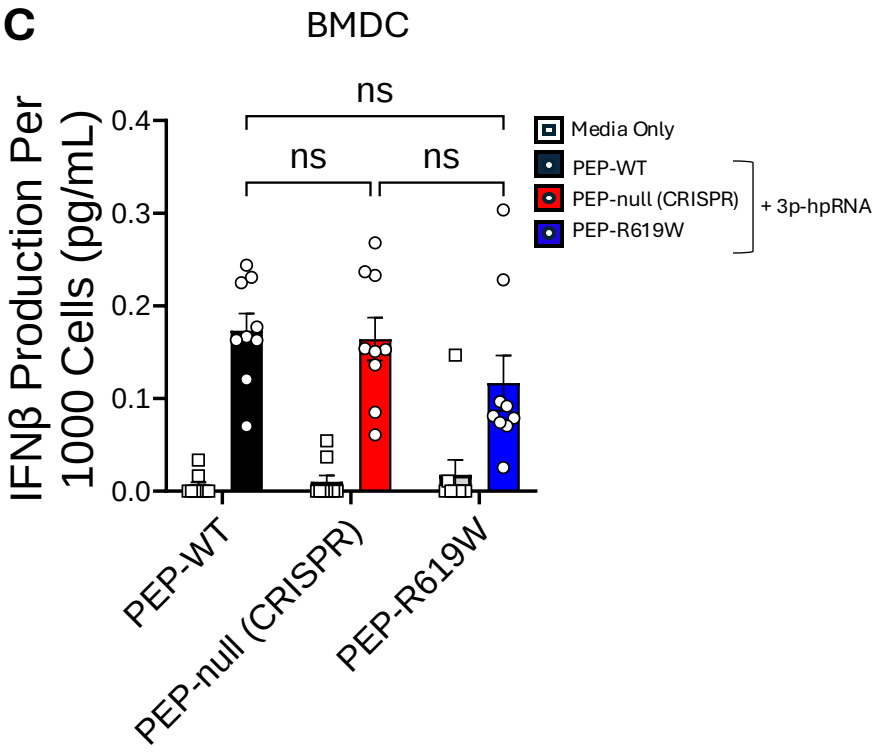
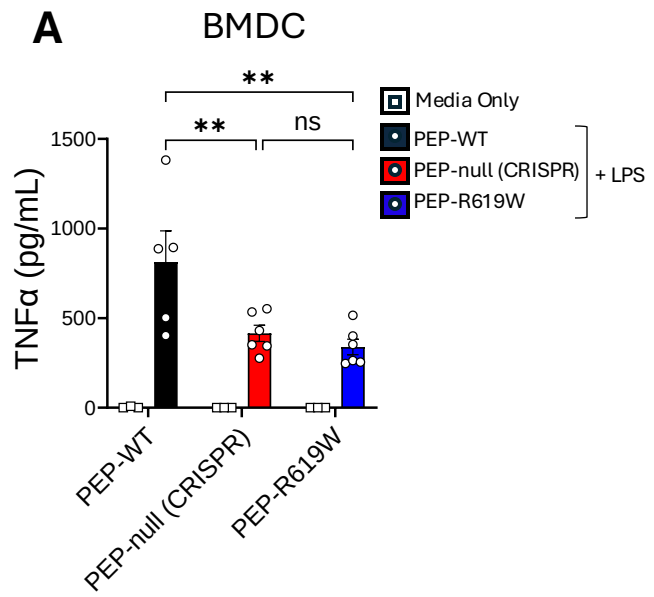

C
BMDC
IFNβ Production Per 1000 Cells (pg/mL)
0.4
0.3
0.2
0.1
0.0
ns
ns
ns
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-R619W
+ 3p-hpRNA
PEP-WT
PEP-null (CRISPR)
PEP-R619W

Figure 2


A
BMDC
TNFα (pg/mL)
1500
1000
500
0
**
**
ns
Media Only
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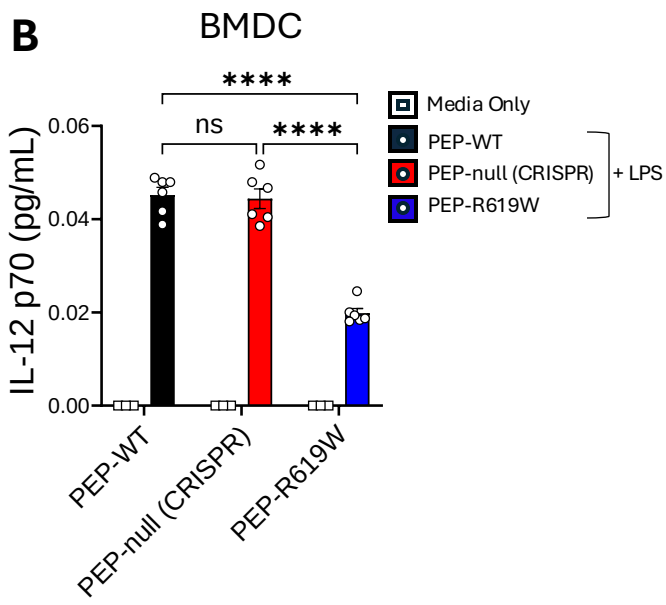
PEP-null (CRISPR)
PEP-R619W
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-R619W


B
BMDC
IL-12 p70 (pg/mL)
0.06
0.04
0.02
0.00
****
ns
****
Media Only
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PEP-null (CRISPR)
PEP-R619W
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-R619W

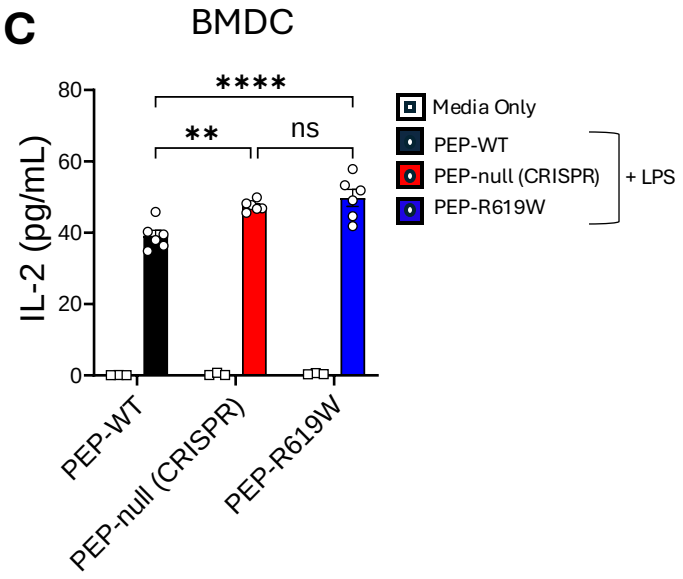
C
BMDC
IL-2 (pg/mL)
80
60
40
20
0
****
**
ns
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-R619W
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-R619W

Figure 3