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

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

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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 factors independent of 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 the role of PEP during inflammation while providing new insight into how the PEP-R619W variant may alter myeloid cell function during disease.

INTRODUCTION

Allelic variation in immune-regulatory genes can significantly impact disease outcomes [1, 2]. One variant of interest is the 1858C>T allele of the Protein Tyrosine Phosphatase Non-Receptor Type 22 gene (*PTPN22*). *PTPN22* is constitutively expressed in all immune cells and encodes for the phosphatase lymphoid protein (Lyp), and its murine ortholog, PEST-domain enriched phosphatase (PEP). The *PTPN22* 1858C>T allele causes an amino acid substitution of arginine (R) to tryptophan (W) at position 620 in humans (Lyp-R620W). It is expressed in 5-15% of the North American population and is considered the highest non-HLA risk allele for the development of autoimmunity, including type I diabetes, rheumatoid arthritis, and systemic lupus erythematous[3-11]. To investigate the role of *PTPN22* and its autoimmunity-associated 1858C>T variant during inflammation, researchers often employ mouse models that lack expression of *Ptpn22* (PEP-null). However, the LYP-R620W variant, or the murine equivalent PEP-R619W variant, does not cause a loss of protein expression, but rather altered function[12, 13]. Thus, using PEP-null models to investigate how the *PTPN22* 1858C>T autoimmunity-associated allele drives inflammation may not always be appropriate as PEP-null and PEP-R619W mice can have differing immune phenotypes at homeostasis and during disease [14-20].

Previously, it was reported that PEP promotes type I interferon (IFN-I) production in dendritic cells (DCs) and macrophages following stimulus with bacterial lipopolysaccharide (LPS) or Poly(I:C)), Toll Like Receptor 4 (TLR4) and Toll Like Receptor 3 (TLR3) agonists, respectively [21]. These studies were performed using PEP-null mice available at The Jackson Laboratory (PEP-null JAX: strain number #028977) compared to C57BL/6J wild-type mice as the recommended controls per The Jackson Laboratory at that time. However, after these studies were published, the Jackson Laboratory discovered that the PEP-null JAX strain contain multiple single nucleotide polymorphisms (SNPs). These SNPs indicate the PEP-null JAX strain is on a mixed C57BL/6J;C57BL/6N background, despite reported backcrossing to the C57BL/6J strain [22]. This is significant because the C57BL/6N SNPs present in this mouse model may impact immune responses such as IFN-I production. This poses concern that our current understanding of *Ptpn22* and how it regulates inflammation may have been confounded by mouse strain-specific genetic variation.

To enable a more rigorous investigation into defining how PEP and the PEP-R619W variant, regulate inflammation and control for potential C57BL/6N-related genetic variation that could confound our results, our research team employs C57BL/6J CRISPR-Cas9-generated PEP-null and PEP-R619W mouse models, which have been maintained on a C57BL/6J background. Here, we employ these genetic models to determine if previous data regarding the impact of PEP on IFN-I production could be recapitulated and define if PEP and the PEP-R619W variant impacted the production of other cytokines in dendritic cells (DCs). We report that neither PEP nor the PEP-R619W variant regulates IFNβ production in DCs or macrophages following LPS or viral RNA stimulation. However, we found that both PEP and PEP-R619W differentially modulate the production of other pro-inflammatory cytokines by dendritic cells, including IL-2, IL-12, and TNFα.

RESULTS

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

Previously, it was shown that PEP-null bone marrow-derived dendritic cells (BMDCs) and bone marrow macrophages (BMMs) produce reduced amounts of IFN-I when stimulated with bacterial lipopolysaccharide (LPS) or Poly(I:C)), TLR4 and TLR3 agonists respectively [21]. However, the PEP-null JAX strain was discovered to have multiple SNPs associated with a mixed genetic background, posing the concern that our current understanding of the role of PEP in regulating IFN-I production may have been influenced by mouse strain-specific differences, such as genetic variation. Using our C57BL/6J CRISPR-Cas9-generated PEP-null model (PEP-null CRISPR), we first set out to determine if previous data regarding the impact of PEP on IFN-I production could be recapitulated. Using qRT-PCR, we confirmed that our PEP-null CRISPR mice do not express quantifiable amounts of *Ptpn22* mRNA (Figure 1A). Then, using FLT3-ligand differentiated BMDCs from C57BL/6J PEP-WT, PEP-null CRISPR, and PEP-null JAX mice we measured for IFNβ production following LPS stimulation. While IFNβ concentration was comparable between PEP-WT and our PEP-null CRISPR mice, BMDCs from the commercial PEP-null JAX strain showed significantly less IFNβ production (Figure 1B). These data successfully recapitulate prior results, but through the use of our CRISPR-Cas9-generated PEP-null model, we observe that PEP does not promote IFNβ production. The differences between the PEP-null JAX and PEP-null CRISPR strains suggest that differences associated with the C57BL/6N background, rather than *Ptpn22* deficiency alone, may account for the previously reported phenotype.

In addition to the PEP-null JAX model, studies have also utilized chemical inhibition of PEP to evaluate its role in IFN-I production and activity [21, 23]. However, varying results regarding PEP inhibitors and their specificity have been reported [24]. To evaluate the specificity of the PEP inhibitor LTV-1 during IFN-I production, we stimulated PEP-WT and PEP-null CRISPR bone marrow macrophages (BMMs) with LPS in the presence or absence of LTV-1 to inhibit PEP's enzymatic activity. We observed reduced IFNβ production in both PEP-WT and PEP-null CRISPR BMMs treated with LTV-1, but no significant differences in IFNβ production between the two genotypes (Figure 1C). These data suggests that the reduction in IFNβ production is due to off-target effects of LTV-1, rather than inhibiting PEP. Given the potential confounding factors associated with the PEP-null JAX mice and off-target effects of LTV-1, we proceeded with our studies to define the impact of PEP and the PEP-R619W variant on inflammation with only our C57BL/6J CRISPR generated mouse strains.

PEP and PEP-R619W do not mediate IFNβ production in myeloid cells

Our initial results showed comparable IFNβ production between PEP-WT and PEP-null CRISPR BMDCs following TLR4 stimulation via LPS. However, beyond TLRs, additional pattern recognition receptors (PRRs) such as RIG-I Like Receptors (RLRs) induce large quantities of IFN-I in response to virus infection [25-27]. Further, it is unknown if the autoimmunity-associated PEP-R619W variant, which alters protein function rather than expression, uniquely affects IFN-I production compared to PEP-WT. To determine if the PEP-R619W variant either phenocopies the complete loss of PEP or distinctly alters IFN-I production following RLR activation, we cultivated PEP-WT, PEP-null CRISPR, and PEP-R619W BMMs and then stimulated them with LPS or infected with the murine coronavirus

127 Murine Hepatitis Virus Strain A59 (MHV A59), a MDA5 agonist [27]. There were no differences
128 detected in IFNβ production between PEP-null CRISPR and PEP-WT BMMs following LPS
129 exposure or MHV A59 infection (Figure 2A). There were also no difference detected in IFNβ
130 production between PEP-WT and PEP-R619W BMMs following MHV A59 infection (Figure
131 2B) or LPS exposure (Figure 2C). These results suggest that PEP does not promote IFN-I
132 production in BMMs following TLR4 or MDA5 activation.
133
134 Next, we generated FLT3-ligand (FLT3-L) BMDCs from C57BL/6J PEP-WT, PEP-null
135 CRISPR, and PEP-R619W mice and stimulated them with LPS or 5' Triphosphate hairpin
136 ribonucleic acid (3p-hpRNA), a RIG-I agonist. We detected no significant differences in IFNβ
137 production between all three genotypes (PEP-WT, PEP-null CRISPR, and PEP-R619W)
138 following either LPS or 3p-hpRNA stimulation (Figure 2 D-E). Taken together, these results
139 indicate that neither PEP nor the PEP-R619W variant modulate IFNβ production in dendritic
140 cells mediated by TLR4 or RIG-I activation.
141
142 **PEP and the PEP-R619W variant distinctly modulate the production of pro-inflammatory**
143 **cytokines in dendritic cells**
144
145 Our findings show that neither PEP nor the PEP-R619W variant impact IFN-I production in DCs
146 and macrophages during TLR4, MDA5, or RIG-I stimulation. However, DCs produce a variety
147 of other cytokines which can promote widespread inflammation or influence T cell activation
148 and differentiation. Prior studies show that PEP-null and the PEP-R619W variant enhances T cell
149 function through both T cell intrinsic and T cell extrinsic mechanisms during virus infection [19 28,
150 29]. Previous studies that have investigated the role of PEP and its variant on cytokine production
151 in myeloid cells have reported varied findings[21, 30, 31]. Using PEP-R619W knock-in mouse
152 models, one group reported increased IL-12 production in Granulocyte-macrophage colony
153 stimulating factor (GM-CSF) differentiated PEP-R619W BMDCs post LPS exposure[30]. Another
154 group reported that GM-CSF differentiated PEP-null JAX BMDCs exhibited enhanced secretion
155 of IL-6, IL-8 and TNFα compared to PEP-WT BMDCs following treatment with muramyl
156 dipeptide (MDP)[31]. They also reported that the loss of PEP reduced while the PEP-R619W
157 variant increased IL-1β secretion by BMDCs following ultra-pure LPS exposure[32]. However, a
158 different group later reported no differences in TNFα, IL-1β, or IL-12 production following
159 treatment with LPS between FLT3-ligand differentiated PEP-WT and PEP-null JAX BMDCs [21].
160 These contrary results align with previous reports of PEP-null and PEP-R619W mice exhibiting
161 different phenotypes but could also be attributed to the usage of different mouse models, type of
162 stimulus, and selected assays. Thus, using our CRISPR-Cas9-generated mouse models, we
163 wanted to know if PEP and the PEP-R619W variant regulated the production of other
164 inflammatory cytokines relevant to modulating T cell function.
165
166 To accomplish this, we again used FLT3-ligand differentiated BMDCs from PEP-WT, PEP-null
167 CRISPR, and PEP-R619W mice. BMDCs were stimulated with LPS, and the supernatant was
168 collected and analyzed for TNFα, IL-12 (p70), and IL-2 production. We found that compared to
169 PEP-WT BMDCs, TNFα production was reduced in both PEP-null CRISPR and PEP-R619W
170 BMDCs (Figure 3A). Additionally, we observed a reduction in IL-12 production by PEP-R619W
171 BMDCs compared to PEP-WT. However, there was no difference detected in IL-12 production
172 between PEP-null CRISPR and PEP-WT BMDCs (Figure 3B). Finally, we observed increased

173 IL-2 production by both PEP-null CRISPR and PEP-R619W BMDCs compared to PEP-WT
174 BMDCs (Figure 3C). Taken together, these findings suggest that PEP and the PEP-R619W
175 variant distinctly modulate cytokine production in dendritic cells.
176
177 **DISCUSSION**
178
179 The *PTPN22* 1858C>T allele is strongly associated with the development of autoimmunity, but
180 the murine equivalent mutation has also been shown to be protective during virus infection and
181 cancer[18, 19, 33]. While the impact of the PEP-R619W variant is well-defined in T cells, the
182 molecular mechanisms by which PEP and its variant impact the function of other immune cells,
183 such as the ability of APCs to produce pro-inflammatory cytokines, remain less understood.
184 Previously, it was suggested that PEP promotes IFN-I production in macrophages and dendritic
185 cells[21]. However, these data were generated using PEP-null mice on a C57BL/6J;C57BL/6N
186 mixed genetic background, posing the concern that our current understanding of PEP's role
187 during IFN-I production may have been influenced by the C57BL/6N SNPs present in the PEP-
188 null JAX mouse model. Using our CRISPR-Cas9-generated PEP-null model, we first set out to
189 determine if previous data regarding the impact of PEP on IFN-I production could be
190 recapitulated. While the PEP-null JAX BMDCs had reduced IFN-I production, we did not detect
191 a difference between our PEP-null CRISPR BMDCs and C57BL/6J PEP-WT BMDCs (Figure
192 1B). These strain-specific differences in IFN-I production recapitulate previous findings, while
193 also revealing that strain specific difference, likely genetic variation between C57LB/6J and
194 C57Bl/6N mice, in the PEP-null JAX mice may have influenced the previously observed
195 phenotype. This interpretation is further supported by reports of reduced inflammatory responses
196 during virus infection in C57BL/6N mice compared to C57BL/6J mice [34, 35]. However, our study
197 results do not address why PEP-null JAX BMMs engineered to overexpress the human protein
198 Lyp have restored IFNβ production [21]. Identifying the mechanism that drives the reduced IFNβ
199 production observed in the C57BL/6J;C57BL/6N PEP-null JAX mice but not in the PEP-null
200 CRISPR mice will require further investigation. Regardless, we are not the first to comment on
201 or identify discrepancies regarding previous data reported on the role of PEP during IFN-I
202 production in myeloid cells. However, these prior publications have largely focused on
203 differences identified in human samples, as well as in the context of systemic lupus erythematous
204 (SLE) [36-40]. Thus, to the best of our knowledge, we are the first to show in a mouse model that
205 there may be strain-specific differences confounding the interpretation of how/if PEP regulates
206 cytokine production in DCs and macrophages.
207
208 Next, when evaluating the specificity of the PEP inhibitor LTV-1 during IFN-I production in
209 BMMs, we found reduced IFNβ production in both PEP-WT and PEP-null CRISPR
210 macrophages treated with LTV-1 (Figure 1C). These findings suggest that the observed reduction
211 of IFNβ may not be due to the deficiency of PEP nor the inhibition of its enzymatic function, but
212 rather because of non-specific effects of LTV-1. Taken together, our findings reveal additional
213 confounding variables that could impact the results of studies that aim to discern the role of PEP
214 during inflammation through the use of either genetic models or chemical inhibition. Our
215 observations add to the numerous conflicting findings already identified in the field regarding
216 PEP, PEP-R619W, and their effects on cytokine production [21, 30-32]. These differences likely stem
217 from genetic variation present in both human and animal studies, as well as the utilization of
218 diverse experimental approaches including over-expression, transgenic models, knockdown

219 techniques, and chemical inhibition. Each of these systems offer distinct advantages yet also
220 varying limitations, yielding inconsistent results. To address this lack of clarity, our lab employs
221 CRISPR/Cas9-generated PEP-null and PEP-R619W mouse models on a C57BL/6J background.
222 By using this model, we can precisely evaluate the mechanisms by which PEP and the PEP-
223 R619W variant drive inflammation while minimizing confounding genetic variables.
224
225 Using our PEP-null CRISPR and PEP-R619W mice, our results indicate that IFNβ production by
226 DCs and macrophages is not promoted by PEP or the PEP-R619W variant following TLR4,
227 MDA-5, or RIG-I stimuli (Figure 1B, Figure 2A-C). Although our findings indicate that PEP and
228 PEP-R619W do not promote IFNβ production in myeloid cells, we found that PEP and the PEP-
229 R619W variant distinctly modulate the production of other pro-inflammatory cytokines. More
230 specifically, we found that compared to PEP-WT, TNFα production was reduced in both PEP-
231 null CRISPR and PEP-R619W DCs (Figure 3A). Additionally, we observed a reduction in IL-12
232 production by PEP-R619W DCs compared to PEP-WT but no significant change in the
233 production of IL-12 was detected in the PEP-null CRISPR BMDCs when compared to PEP-WT
234 BMDCs (Figure 3B). Previously it was reported that PEP-R619W GM-CSF differentiated
235 BMDCs have increased IL-12 production compared to PEP-WT [30]. These conflicting findings
236 may be due to a variety of factors such as differences in the PEP-R619W mouse models, LPS
237 dosage, and/or functional differences between FLT3-ligand differentiated and GM-CSF
238 differentiated BMDCs. Finally, we observed increased IL-2 production by both PEP-null
239 CRISPR and PEP-R619W BMDCs compared to PEP-WT. Since DC derived IL-2 can stimulate
240 the activation of T cells, it is possible that the increased IL-2 produced by PEP-null CRISPR and
241 PEP-R619W DCs may be a contributing factor to the enhanced T cell function observed during
242 virus infection [19, 28, 41].

243
244 Altogether, our findings challenge the current understanding of PEP while providing new insight
245 into how the PEP-R619W variant is capable of simultaneously promoting autoimmunity and
246 conferring protection against persistent viral infection and cancer. While our results reveal that
247 changes in IFN-I production are not a key factor in how PEP regulates inflammation as
248 previously regarded, our data indicates that PEP and the PEP-R619W variant have a novel role in
249 modulating the production of other cytokines by dendritic cells which are critical for a variety of
250 inflammatory processes. Future studies will need to evaluate how changes mediated by PEP and
251 the PEP-R619W variant in dendritic cells and other antigen presenting cells are relevant in order
252 to accurately define the broad consequences they have on disease pathogenesis.
253
254 **MATERIAL AND METHODS**
255
256 **Ethics:**
257 All animal studies were reviewed and approved by University of Kansas Animal Care and Use
258 Committee (IACUC) (protocol number: 278-01).
259
260 **Mice:**
261 Mice were bred and housed in specific pathogen free general housing conditions at University of
262 Kansas (Lawrence, KS). All animal studies were reviewed and approved by the University of
263 Kansas Animal Care and Use Committee (protocol number: 278-01). Both males and females
264 ranging from 5-12 weeks of age were used in this study. C57BL/6J WT (PEP-WT) mice were

265 originally purchased from Jackson labs, then bred and maintained in the University of Kansas
266 Animal Care Unit. Ptpn22 $^{-/-}$ (PEP-null CRISPR) mice were generated using CRISPR/Cas9
267 technology on a C57BL/6J background, as described previously, by Dr. Kerri Mowen and Dr.
268 Linda Sherman (Scripps Research Institute, La Jolla, CA) and were gifted from Dr. Sherman[42].
269 Mice expressing the Ptpn22 pro-autoimmune allele (PEP-R619W) were generated using
270 CRISPR/Cas9 technology on a C57BL/6J background using methods previously reported by Dr.
271 Kerri Mowen and Dr. Linda Sherman (Scripps Research Institute, La Jolla, CA) and were gifted
272 from Dr. Sherman[18, 19, 42]. Approximately every 10 generations, PEP-null CRISPR and PEP-
273 R619W mice are backcrossed to new C57BL/6J wildtype mice to reduce the likelihood strain
274 specific SNPs have arisen and driving a particular phenotype. Mice from the same parental pairs
275 are not used in breeding pairs. B6.Cg-Ptpn22^{tm2Achn}/J Ptpn22 $^{-/-}$ mice (PEP-null JAX) were gifted
276 from Dr. Won Jin Ho (John Hopkins University School of Medicine, Baltimore, MD). These
277 mice are available for purchase from the Jackson Laboratory (strain number #028977), and their
278 generation has been previously described[22].
279
280 **Bone Marrow derived Macrophage (BMM) Culture:**

281 To generate BMMs, bone marrow cells were isolated from PEP-WT, PEP-null JAX, PEP-null
282 CRISPR and PEP-R619W mouse femurs. Following isolation, cells were cultured in Roswell
283 Park Memorial Institute medium (RPMI) supplemented with 10% FBS, 1% L-glutamine, and 1%
284 Penicillin/Streptomycin with 50ng/mL M-CSF (Stem Cell Technologies, Vancouver, CA) for 8
285 days. An additional 10 mL of M-CSF-containing media was added to BMM cultures on day 3,
286 and on day 8, differentiated cells were harvested, counted, and replated for further assays.
287
288 **Bone Marrow Dendritic Cell (BMDC) Culture:**
289 Bone marrow cells were isolated from PEP-WT, PEP-null, and PEP-R619W mouse femurs.
290 Following isolation, bone marrow cells were cultured in Advanced DMEM containing 10% FBS,
291 1% penicillin streptomycin, 1% L-Glutamine, and 100ng/mL FLT3L (Stem Cell Technologies)
292 for 8 days. On day 8, FLT3-ligand differentiated dendritic cells were harvested, counted, and re-
293 plated for further assays.
294
295 **Myeloid cell Stimulation:**
296 *BMM stimulation:*
297 Bone marrow macrophages (BMMs) were plated in a 96-well plate at a density of 5 x 10^4 cells
298 per well. For infection, cells were inoculated with MHV-A59 at a multiplicity of infection (MOI)
299 of 0.1. The plate was incubated for one hour at 37°C with shaking every 10 minutes to ensure
300 even distribution of the virus. Following the one-hour incubation, the plate was centrifuged
301 at 300g for 10 minutes to pellet the cells. The supernatant containing the unadsorbed virus was
302 carefully discarded. The cells were then resuspended in fresh media and incubated at 37°C for 18
303 hours to allow for viral replication. After the 18-hour incubation, the cell culture supernatant was
304 collected and stored at -80°C for subsequent IFNβ ELISA.
305
306 *BMDC stimulation:*
307 BMDCs were resuspended and plated at 1 x 10^6 cells/mL in Advanced DMEM (Gibco)
308 containing 10% FBS, 1% penicillin streptomycin and 1% L-Glutamine. The cells were

309 stimulated with either LPS or 3p-hpRNA. Cells stimulated with LPS (500 ng/mL) (Sigma) were
310 incubated overnight (16-18 hours). The supernatant was collected and stored frozen at -80
311 degrees Celsius for future assays. Cells stimulated with 3p-hpRNA (2 ng/mL) complexed with
312 LyoVec (InvivoGen) were incubated overnight (16-18 hours). The supernatant was collected and
313 stored frozen at -80 degrees Celsius for future assays.
314
315 **Enzyme-Linked Immunosorbent Assay (ELISA):**
316 Levels of IFNβ, TNFα, IL-12 (p70), and IL-2 in cell culture supernatant were measured using
317 ELISA Kits (IFNβ (PBL Assay Science), TNFα, IL-12 and IL-2 (BioLegend)) according to the
318 manufacturer's instructions, followed by analysis with a BioTek EON microplate reader at 450
319 and/or 570 nm.
320
321 **qRT-PCR:**
322 RNA was extracted from 6-8 week old PEP-WT and PEP-R619W mouse splenocytes using
323 TRIzol according to the manufacturer's instructions (Invitrogen). cDNA was synthesized using
324 the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), according to the
325 manufacturer's instructions. Real-time PCR was performed on a Quant Studio 3 system using
326 PowerTrack™ SYBR™ Green Master Mix (Applied Biosystems). Each reaction was measured
327 in duplicate, and data was normalized to the expression levels of the house keeping gene *Gapdh*.
328 Primers for the genes were as follows: *Gapdh* Forward Primer 5'
329 ACGACCCCTTCATTGACCTC 3' and Reverse Primer 5' ACTGTGCCGTTGAATTTGCC 3';
330 *Ptpn22* Forward primer 5' AGCTGATGAAAATGTCCTATTGTGA 3' and Reverse primer 5'
331 GTCCCACTGCATTCTGGTGA 3'.
332
333 **Statistical analysis and graphing:**
334 All statistical analysis was performed using GraphPad Prism Software. The type of statistical test
335 is listed in figure legends. Data was considered statistically significant if the p value<0.05.
336 Graphs were made in GraphPad Prism (La Jolla, CA). Figure legends indicate if data shown is
337 pooled from multiple studies or is from representative study.
338
339 **ACKNOWLEDGEMENTS**

340 The PEP-R619W and PEP-null CRISPR mice were originally generated by Dr. Kerri Mowen and
341 Dr. Linda Sherman, Scripps Research Institute. These mice were gifts from Dr. Sherman. PEP-
342 null JAX mice were a gift from Dr. Won Jin Ho (Johns Hopkins University School of Medicine).
343 MHV A59 was a gift from Dr. Anthony Fehr (University of Kansas).
344

345 **CONFLICTS OF INTEREST**

346 Robin C. Orozco is a paid consultant for the company DesignZyme. DesignZyme had no say in
347 the design, execution, or interpretation of this study. Robin C. Orozco's affiliation with
348 DesignZyme does not impact the integrity of this work.

349
350 **AUTHOR CONTRIBUTIONS**
351

Jenna R. Barnes: Data Curation, Formal Analysis, Investigation, Methodology, validation, Visualization, Writing- original draft, writing- review and editing
Anam Fatima Shaikh: Data Curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing- original draft, writing- review and editing
Alec M. Bevis: Data Curation, Formal Analysis, Investigation, Methodology, Validation, Writing- original draft, writing- review and editing
Tammy R. Cockerham: Methodology, Investigation, Project Administration
Robin C. Orozco: Conceptualization, Formal Analysis, Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing- Original draft, Writing- review and edit

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DATA AVAILABILITY

All data necessary to interpret results is presented in this manuscript. Raw data will be made available upon request.

REFERENCES

1. Conway JR, Kofman E, Mo SS, Elmarakeby H, Van Allen E. Genomics of response to immune checkpoint therapies for cancer: implications for precision medicine. Genome medicine. 2018;10(1):93-. doi: 10.1186/s13073-018-0605-7. PubMed PMID: 30497521.
2. Sivapalan L, Anagnostou V. Genetic variation in antigen presentation and cancer immunotherapy. Immunity. 2022;55(1):3-6. doi: 10.1016/j.immuni.2021.12.010. PubMed PMID: 35021056.
3. Metzler G, Dai X, Thouvenel CD, Khim S, Habib T, Buckner JH, Rawlings DJ. The Autoimmune Risk Variant PTPN22 C1858T Alters B Cell Tolerance at Discrete Checkpoints and Differentially Shapes the Naive Repertoire. Journal of immunology (Baltimore, Md : 1950). 2017;199(7):2249-60. Epub 2017/08/11. doi: 10.4049/jimmunol.1700601. PubMed PMID: 28801357.
4. Cao Y, Liu K, Tian Z, Hogan SL, Yang J, Poulton CJ, Falk RJ, Li W. PTPN22 R620W polymorphism and ANCA disease risk in white populations: a metaanalysis. J Rheumatol. 2015;42(2):292-9. Epub 2014/12/01. doi: 10.3899/jrheum.131430. PubMed PMID: 25448792.
5. Bottini N, Vang T, Cucca F, Mustelin T. Role of PTPN22 in type 1 diabetes and other autoimmune diseases. Seminars in Immunology. 2006;18(4):207-13. doi: 10.1016/j.smim.2006.03.008.
6. Wang X-H, Ma A-G, Han X-X, Chen L, Liang H, Aishan L, Abudumijit A, Xue F. Protein tyrosine phosphatase nonreceptor type 22 (PTPN22) gene single nucleotide polymorphisms and its interaction with T2DM on pulmonary tuberculosis in Chinese Uygur population. Oncotarget. 2017;8(39):65601-8. doi: 10.18632/oncotarget.19274. PubMed PMID: 29029456.
7. Wang H, Wang Z, Rani PL, Fu X, Yu W, Bao F, Yu G, Li J, Li L, Sun L, Yue Z, Zhao Q, Pan Q, Cao J, Wang C, Chi X, Wang Y, Yang Q, Mi Z, ..., Zhang F. Identification of PTPN22, ST6GAL1 and JAZF1 as psoriasis risk genes demonstrates shared pathogenesis between psoriasis and diabetes. Exp Dermatol. 2017;26(11):1112-7. Epub 20170825. doi: 10.1111/exd.13393. PubMed PMID: 28603863.
8. Wang W, Meng X, Liu Y, Ma X, Zhang Q, Li C, Li C, Ren L. Association Between Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22) Polymorphisms and Risk of Ankylosing Spondylitis: A Meta-analysis. Med Sci Monit. 2017;23:2619-24. doi: 10.12659/msm.901083. PubMed PMID: 28555069.
9. Armitage LH, Wallet MA, Mathews CE. Influence of PTPN22 Allotypes on Innate and Adaptive Immune Function in Health and Disease. Front Immunol. 2021;12:636618-. doi: 10.3389/fimmu.2021.636618. PubMed PMID: 33717184.
10. Gomez LM, Anaya JM, Gonzalez CI, Pineda-Tamayo R, Otero W, Arango A, Martín J. PTPN22 C1858T polymorphism in Colombian patients with autoimmune diseases. Genes & Immunity. 2005;6(7):628-31. doi: 10.1038/sj.gene.6364261.
11. Burn GL, Svensson L, Sanchez-Blanco C, Saini M, Cope AP. Why is *PTPN22* a good candidate susceptibility gene for autoimmune disease? FEBS Letters. 2011;585(23):3689-98. doi: 10.1016/j.febslet.2011.04.032.

421 12. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J,
422 Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T. A functional
423 variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nature
424 Genetics. 2004;36(4):337-8. doi: 10.1038/ng1323.
425 13. Begovich AB, Carlton VEH, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander
426 HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang S-YP, Saiki RK,
427 Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffery DA, ..., Gregersen PK. A missense
428 single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase
429 (PTPN22) is associated with rheumatoid arthritis. American journal of human genetics.
430 2004;75(2):330-7. Epub 2004/06/18. doi: 10.1086/422827. PubMed PMID: 15208781.
431 14. Dai X, James RG, Habib T, Singh S, Jackson S, Khim S, Moon RT, Liggitt D, Wolf-Yadlin
432 A, Buckner JH, Rawlings DJ. A disease-associated PTPN22 variant promotes systemic
433 autoimmunity in murine models. J Clin Invest. 2013;123(5):2024-36. Epub 2013/04/24. doi:
434 10.1172/JCI66963. PubMed PMID: 23619366.
435 15. Fousteri G, Jofra T, Debernardis I, Stanford SM, Laurenzi A, Bottini N, Battaglia M.
436 The protein tyrosine phosphatase PTPN22 controls forkhead box protein 3 T regulatory cell
437 induction but is dispensable for T helper type 1 cell polarization. Clin Exp Immunol.
438 2014;178(1):178-89. doi: 10.1111/cei.12393. PubMed PMID: 24905474.
439 16. Knipper JA, Wright D, Cope AP, Malissen B, Zamoyska R. PTPN22 Acts in a Cell
440 Intrinsic Manner to Restrict the Proliferation and Differentiation of T Cells Following
441 Antibody Lymphodepletion. Front Immunol. 2020;11:52-. doi: 10.3389/fimmu.2020.00052.
442 PubMed PMID: 32047502.
443 17. Maine CJ, Marquardt K, Cheung J, Sherman LA. PTPN22 controls the germinal center
444 by influencing the numbers and activity of T follicular helper cells. Journal of immunology
445 (Baltimore, Md : 1950). 2014;192(4):1415-24. Epub 2014/01/22. doi:
446 10.4049/jimmunol.1302418. PubMed PMID: 24453256.
447 18. Orozco RC, Marquardt K, Mowen K, Sherman LA. Proautoimmune Allele of Tyrosine
448 Phosphatase, PTPN22, Enhances Tumor Immunity. J Immunol. 2021;207(6):1662-71. Epub
449 20210820. doi: 10.4049/jimmunol.2100304. PubMed PMID: 34417261; PMCID:
450 PMC8428750.
451 19. Orozco RC, Marquardt K, Pratumchai I, Shaikh AF, Mowen K, Domissy A, Teijaro JR,
452 Sherman LA. Autoimmunity-associated allele of tyrosine phosphatase gene PTPN22
453 enhances anti-viral immunity. PLoS Pathog. 2024;20(3):e1012095-e. doi:
454 10.1371/journal.ppat.1012095. PubMed PMID: 38512979.
455 20. Spalinger MR, Kasper S, Gottier C, Lang S, Atrott K, Vavricka SR, Scharl S, Raselli T,
456 Frey-Wagner I, Gutte PM, Grütter MG, Beer HD, Contassot E, Chan AC, Dai X, Rawlings DJ,
457 Mair F, Becher B, Falk W, Fried M, Rogler G, Scharl M. NLRP3 tyrosine phosphorylation is
458 controlled by protein tyrosine phosphatase PTPN22. J Clin Invest. 2016;126(11):4388. Epub
459 20161101. doi: 10.1172/JCI90897. PubMed PMID: 27801679; PMCID: PMC5096914.
460 21. Wang Y, Shaked I, Stanford SM, Zhou W, Curtsinger JM, Mikulski Z, Shaheen ZR,
461 Cheng G, Sawatzke K, Campbell AM, Auger JL, Bilgic H, Shoyama FM, Schmeling DO,
462 Balfour HH, Jr., Hasegawa K, Chan AC, Corbett JA, Binstadt BA, ..., Peterson EJ. The
463 autoimmunity-associated gene PTPN22 potentiates toll-like receptor-driven, type 1

464 interferon-dependent immunity. Immunity. 2013;39(1):111-22. Epub 2013/07/18. doi:
465 10.1016/j.immuni.2013.06.013. PubMed PMID: 23871208.
466 22. Hasegawa K, Martin F, Huang G, Tumas D, Diehl L, Chan AC. PEST Domain-Enriched
467 Tyrosine Phosphatase (PEP) Regulation of Effector/Memory T Cells. Science.
468 2004;303(5658):685-9. doi: 10.1126/science.1092138.
469 23. Hornick EL, Wallis AM, Bishop GA. TRAF3 enhances type I interferon receptor
470 signaling in T cells by modulating the phosphatase PTPN22. Science signaling.
471 2022;15(753):eabn5507-eabn. Epub 2022/09/27. doi: 10.1126/scisignal.abn5507. PubMed
472 PMID: 36166512.
473 24. Jassim BA, Lin J, Zhang Z-Y. PTPN22: structure, function, and developments in
474 inhibitor discovery with applications for immunotherapy. Expert Opin Drug Discov.
475 2022;17(8):825-37. Epub 2022/06/07. doi: 10.1080/17460441.2022.2084607. PubMed
476 PMID: 35637605.
477 25. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA
478 sensing. Nat Rev Immunol. 2020;20(9):537-51. Epub 2020/03/13. doi: 10.1038/s41577-
479 020-0288-3. PubMed PMID: 32203325.
480 26. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity:
481 update on Toll-like receptors. Nature Immunology. 2010;11(5):373-84. doi:
482 10.1038/ni.1863.
483 27. Zalinger ZB, Elliott R, Rose KM, Weiss SR. MDA5 Is Critical to Host Defense during
484 Infection with Murine Coronavirus. J Virol. 2015;89(24):12330-40. Epub 2015/09/30. doi:
485 10.1128/JVI.01470-15. PubMed PMID: 26423942.
486 28. Maine CJ, Teijaro JR, Marquardt K, Sherman LA. PTPN22 contributes to exhaustion of
487 T lymphocytes during chronic viral infection. Proc Natl Acad Sci U S A. 2016;113(46):E7231-
488 E9. Epub 2016/10/31. doi: 10.1073/pnas.1603738113. PubMed PMID: 27799548.
489 29. Jofra T, Galvani G, Kuka M, Di Fonte R, Mfarrej BG, Iannacone M, Salek-Ardakani S,
490 Battaglia M, Fousteri G. Extrinsic Protein Tyrosine Phosphatase Non-Receptor 22 Signals
491 Contribute to CD8 T Cell Exhaustion and Promote Persistence of Chronic Lymphocytic
492 Choriomeningitis Virus Infection. Front Immunol. 2017;8:811. Epub 20170712. doi:
493 10.3389/fimmu.2017.00811. PubMed PMID: 28747914; PMCID: PMC5506075.
494 30. Zhang J, Zahir N, Jiang Q, Miliotis H, Heyraud S, Meng X, Dong B, Xie G, Qiu F, Hao Z,
495 McCulloch CA, Keystone EC, Peterson AC, Siminovitch KA. The autoimmune disease–
496 associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated
497 with lymphocyte and dendritic cell hyperresponsiveness. Nature Genetics. 2011;43(9):902-
498 7. doi: 10.1038/ng.904.
499 31. Spalinger MR, Lang S, Vavricka SR, Fried M, Rogler G, Scharl M. Protein tyrosine
500 phosphatase non-receptor type 22 modulates NOD2-induced cytokine release and
501 autophagy. PLoS One. 2013;8(8):e72384-e. doi: 10.1371/journal.pone.0072384. PubMed
502 PMID: 23991106.
503 32. Spalinger MR, Kasper S, Gottier C, Lang S, Atrott K, Vavricka SR, Scharl S, Raselli T,
504 Frey-Wagner I, Gutte PM, Grütter MG, Beer HD, Contassot E, Chan AC, Dai X, Rawlings DJ,
505 Mair F, Becher B, Falk W, ..., Scharl M. NLRP3 tyrosine phosphorylation is controlled by
506 protein tyrosine phosphatase PTPN22. J Clin Invest. 2016;126(11):4388. Epub 20161101.
507 doi: 10.1172/JCI90897. PubMed PMID: 27801679; PMCID: PMC5096914.

33. Ho WJ, Croessmann S, Lin J, Phyo ZH, Charmsaz S, Danilova L, Mohan AA, Gross NE, Chen F, Dong J, Aggarwal D, Bai Y, Wang J, He J, Leatherman JM, Yarchoan M, Armstrong TD, Zaidi N, Fertig EJ, Denny JC, Park BH, Zhang ZY, Jaffee EM. Systemic inhibition of PTPN22 augments anticancer immunity. J Clin Invest. 2021;131(17). Epub 20210720. doi: 10.1172/JCI146950. PubMed PMID: 34283806; PMCID: PMC8409589.

34. Eisfeld AJ, Gasper DJ, Suresh M, Kawaoka Y. C57BL/6J and C57BL/6NJ Mice Are Differentially Susceptible to Inflammation-Associated Disease Caused by Influenza A Virus. Front Microbiol. 2019;9:3307-. doi: 10.3389/fmicb.2018.03307. PubMed PMID: 30713529.

35. Malm Tillgren S, Nieto-Fontarigo JJ, Cerps S, Ramu S, Menzel M, Mahmutovic Persson I, Meissner A, Akbarshahi H, Uller L. C57Bl/6N mice have an attenuated lung inflammatory response to dsRNA compared to C57Bl/6J and BALB/c mice. J Inflamm (Lond). 2023;20(1):6-. doi: 10.1186/s12950-023-00331-4. PubMed PMID: 36810092.

36. Wang Y, Ewart D, Crabtree JN, Yamamoto A, Baechler EC, Fazeli P, Peterson EJ. PTPN22 Variant R620W Is Associated With Reduced Toll-like Receptor 7–Induced Type I Interferon in Systemic Lupus Erythematosus. Arthritis & Rheumatology. 2015;67(9):2403-14. doi: https://doi.org/10.1002/art.39211.

37. Ivashkiv LB. PTPN22 in autoimmunity: different cell and different way. Immunity. 2013;39(1):91-3. doi: 10.1016/j.immuni.2013.07.007. PubMed PMID: 23890067; PMCID: PMC3785409.

38. Kariuki SN, Crow MK, Niewold TB. The PTPN22 C1858T polymorphism is associated with skewing of cytokine profiles toward high interferon-alpha activity and low tumor necrosis factor alpha levels in patients with lupus. Arthritis Rheum. 2008;58(9):2818-23. doi: 10.1002/art.23728. PubMed PMID: 18759295; PMCID: PMC2621106.

39. Hamerman JA, Pottle J, Ni M, He Y, Zhang Z-Y, Buckner JH. Negative regulation of TLR signaling in myeloid cells—implications for autoimmune diseases. Immunological Reviews. 2016;269(1):212-27. doi: https://doi.org/10.1111/imr.12381.

40. Berggren O, Rönnblom L, Eloranta M-L. Effect of PTPN22 Gene Variant R620W on Type I Interferon Production Stimulated by Different Toll-like Receptor 7 Agonists: Comment on the Article by Wang et al. Arthritis & Rheumatology. 2016;68(4):1045-. doi: https://doi.org/10.1002/art.39571.

41. Jofra T, Di Fonte R, Hutchinson TE, Dastmalchi F, Galvani G, Battaglia M, Salek‐Ardakani S, Fousteri G. Protein tyrosine phosphatase PTPN22 has dual roles in promoting pathogen versus homeostatic‐driven CD8 T‐cell responses. Immunology & Cell Biology. 2016;95(2):121-8. doi: 10.1038/icb.2016.92.

42. Lin X, Pelletier S, Gingras S, Rigaud S, Maine CJ, Marquardt K, Dai YD, Sauer K, Rodriguez AR, Martin G, Kupriyanov S, Jiang L, Yu L, Green DR, Sherman LA. CRISPR-Cas9–Mediated Modification of the NOD Mouse Genome With Ptpn22R619W Mutation Increases Autoimmune Diabetes. Diabetes. 2016;65(8):2134-8. doi: 10.2337/db16-0061.

549 **ABBREVIATIONS**

550	IFN-I	Type I Interferon
551	Ptpn22	Protein tyrosine phosphatase non-receptor type 22
552	Lyp	Lymphoid protein
553	PEP	PEST-domain enriched phosphatase
554	DCs	Dendritic Cells
555	TLR4	Toll-Like Receptor 4
556	LPS	Lipopolysaccharide
557	3p-hp-RNA	5' Triphosphate hairpin ribonucleic acid
558	MHV A59	Mouse Hepatitis virus strain A59
559	TNFα	Tumor Necrosis Factor alpha
560	IL	Interleukin
561	R	Arginine
562	W	Tryptophan
563	HLA	Human Leukocyte Antigen
564	Tregs	T regulatory cell
565	CRISPR-Cas9	Cluster regularly interspaced short palindromic repeats Cas9
566	APC	Antigen Presenting Cell
567	LCMV-cl13	Lymphocytic Choriomeningitis Virus Clone 13
568	TLR3	Toll-like receptor 3
569	SNP	Single Nucleotide Polymorphism
570	RLR	Rig I like receptor
571	MDA5	Melanoma Differentiation Associated protein 5
572	IFNβ	Interferon Beta
573	RNA	Ribonucleic Acid
574	BMDC	Bone Marrow Derived Dendritic cells
575	BMM	Bone Marrow Derived Macrophage
576	JAX	Jackson Labs
577	mRNA	Messenger Ribonucleic Acid
578	FLT3-L	FMS-like tyrosine kinase 3- Ligand
579	GM-CSF	Granulocyte- Macrophage Colony Stimulating factor
580	RIG-I	Retinoic acid inducible gene 1
581	MDP	Muramyl dipeptide
582	ELISA	Enzyme Linked Immunosorbent Assay
583	ANOVA	Analysis of Variance
584	NFκB	Nuclear Factor kappa B
585	TRAF3	TNF receptor-associated factor 3
586	IACUC	Institutional Animal Care and Use Committee
587	DMEM	Dulbecco's Modified Eagle Medium
588	FBS	Fetal Bovine Serum
589	MOI	Multiplicity of Infection
590	Ns	Not Significant
591	qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction

592 **FIGURE LEGENDS**
593
594 **Figure 1. Strain-specific differences impact IFNβ production in PEP-null BMDCs.** Gene
595 expression values of *Ptpn22* relative to *Gapdh* were determined by qRT-PCR for naïve PEP-WT
596 (black) and PEP-null (CRISPR) (red) splenocytes, each dot represents a mouse **(A)**. FLT3-L
597 BMDCs were cultivated and differentiated from C57BL/6J PEP-WT (black), CRISPR/Cas9-
598 generated PEP-null (red), and PEP-null mice from the Jackson Laboratory (JAX stock #028977)
599 (gray). Cultures were incubated with LPS (500 ng/mL) for 18 hours. The supernatant was used to
600 determine IFNβ concentration (ELISA) **(B)**. PEP-WT and PEP-null (CRISPR) BMMs were
601 incubated with LPS (500 ng/mL) and 10 μM LTV-1 for 18 hours. The supernatant was used to
602 determine IFNβ concentration (ELISA) **(C)**. ns= no significance; *p<0.01; **p<0.001;
603 ****p<0.0001; Unpaired T Test (A), Two Way ANOVA with Tukey post hoc analysis (**B,C**).
604 Data is pooled from 2 independent experiments. Each dot represents a biological replicate.
605
606 **Figure 2. PEP and PEP-R619W do not mediate IFNβ production in myeloid cells following**
607 **LPS, MHV A59, or viral RNA stimulation.** C57BL/6J PEP-WT (black, square symbol),
608 CRISPR-Cas9 generated PEP-null (red, circle symbol), BMMs were cultivated and then
609 stimulated with LPS (500 ng/mL) or infected with MHV A59 (MOI 0.1) for 18 hours
610 **(A)**.C57BL/6J PEP-WT (Black, circle symbol) and CRISPR-Cas9 generated PEP-R619W (Blue,
611 circle symbol) BMMs are cultivated and then stimulated with MHV-A59 (MOI 0.1) **(B)** or with
612 LPS (500 ng/mL) **(C)** for 18 hours. FLT3-L BMDCs were cultivated from C57BL/6J PEP-WT,
613 PEP-null, and PEP-R619W mice. Cultures were incubated with LPS (500 ng/mL) **(D)** or 3p-
614 hpRNA (2 ng/mL) **(E)** for 18 hours. The supernatant was used to determine IFNβ concentration
615 (ELISA). ns= no significance; a Two Way ANOVA with Tukey post hoc analysis was conducted
616 for (A,D,E), a One Way ANOVA was conducted for (B, C). Data is representative of 2
617 independent experiments **(A)** or pooled from 3 independent experiments **(B-E)**. Each dot
618 represent a biological replicate.
619
620 **Figure 3. PEP and the PEP-R619W variant distinctly modulate the production of pro-**
621 **inflammatory cytokines in dendritic cells**. FLT3-L BMDCs were cultivated from C57BL/6J
622 PEP-WT (black), CRISPR-Cas9 generated PEP-null (red), and PEP-R619W (blue) mice.
623 Cultures were incubated with LPS (500 ng/mL) for 18 hours. The supernatant was used to
624 determine TNFα **(A)**, IL-12 **(B)**, and IL-2 **(C)** concentration (ELISA). ns= no
625 significance;**p<0.01, ****p<0.0001; Two Way ANOVA with Tukey post hoc analysis. Data is
626 representative of 2 independent experiments. Each dot represents a biological replicate.
627

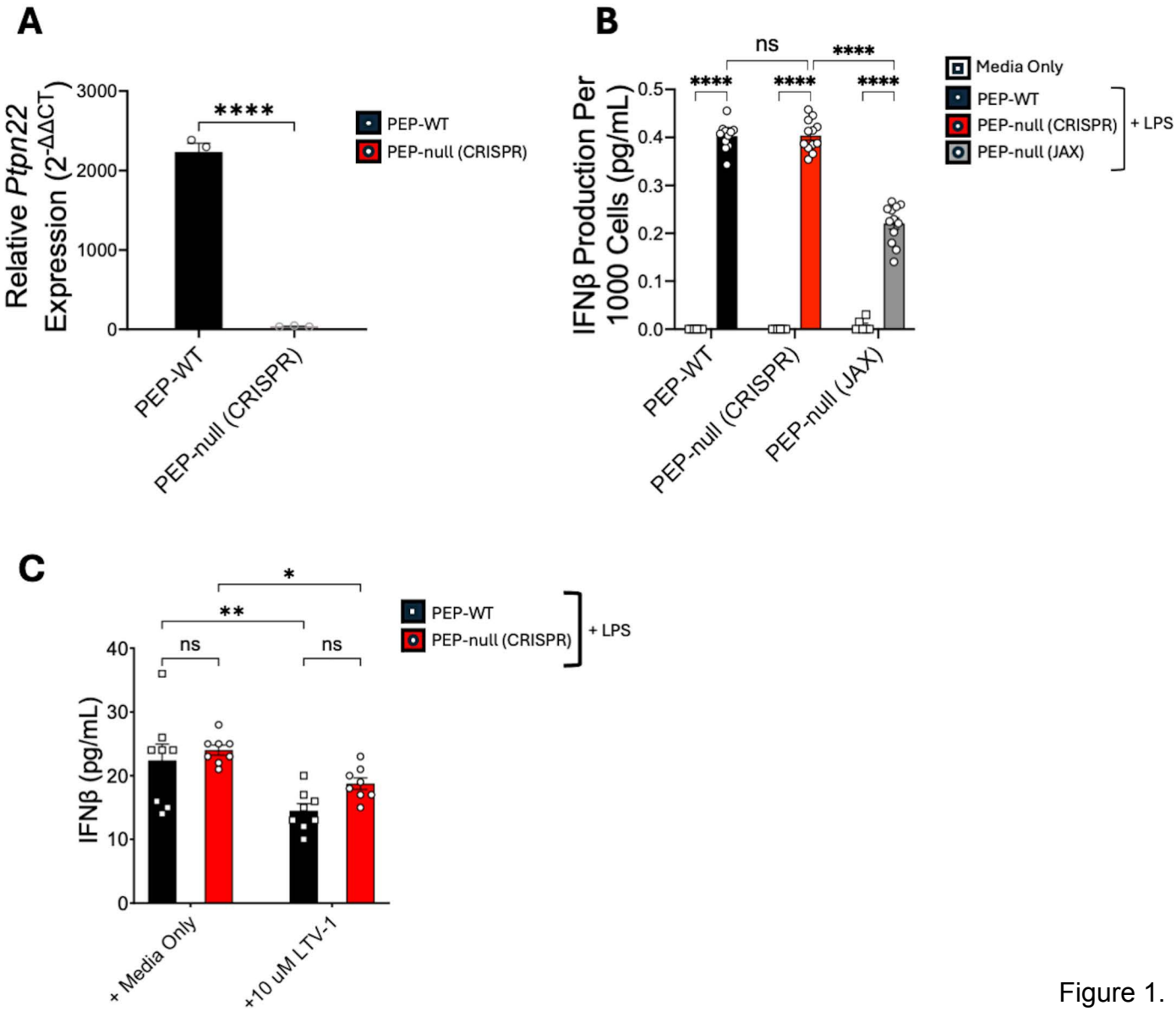
A
Relative Ptpn22 Expression ($2^{-\Delta\Delta CT}$)
3000
2000
1000
0
****
PEP-WT
PEP-null (CRISPR)
B
IFNβ Production Per 1000 Cells (pg/mL)
0.5
0.4
0.3
0.2
0.1
0.0
ns
****
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-null (JAX)
+ LPS
C
IFNβ (pg/mL)
40
30
20
10
0
*
**
ns
+ Media Only
+10 uM LTV-1

Figure 1.

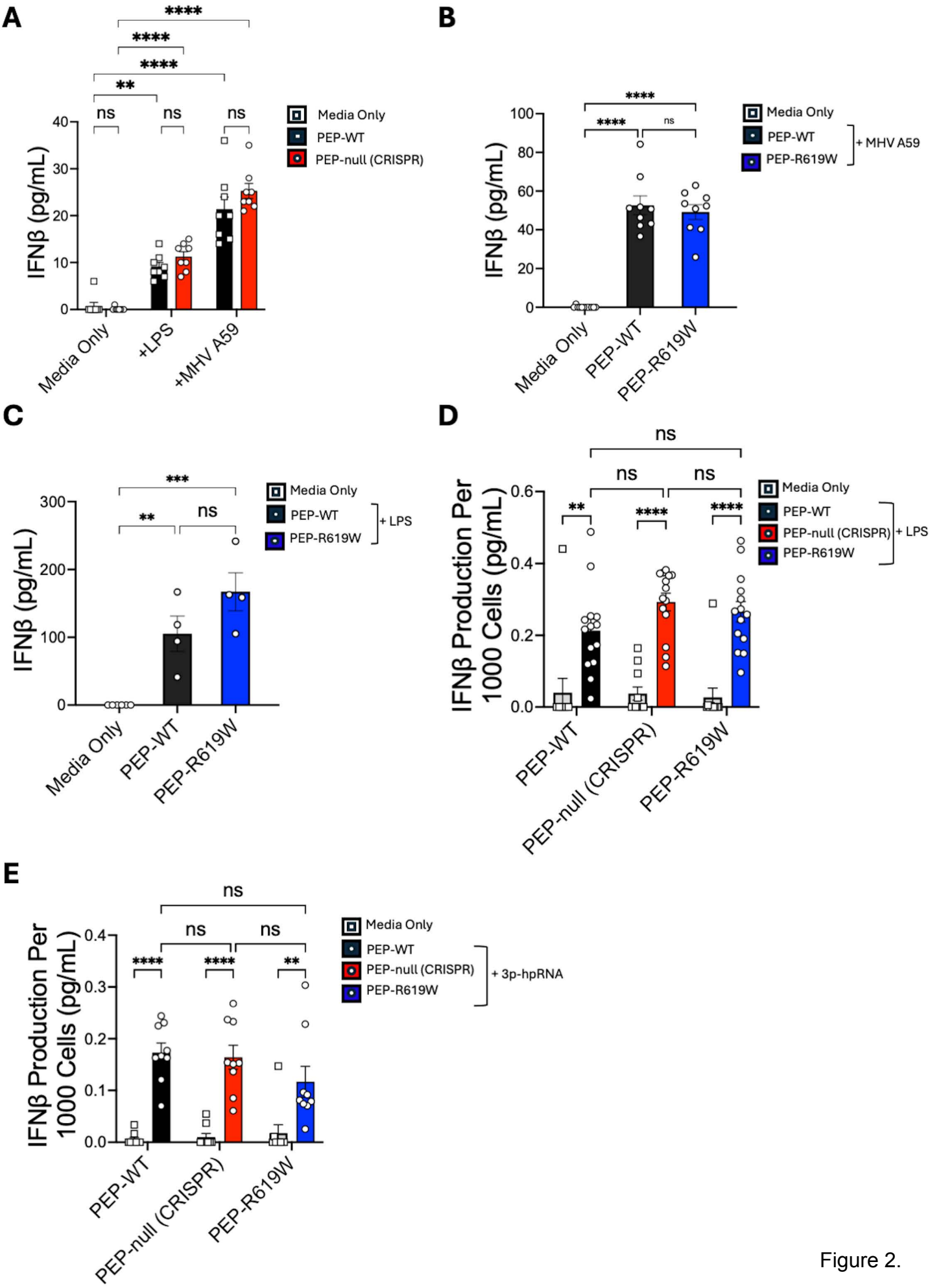
A
****
****
****
**
ns
ns
ns
IFNβ (pg/mL)
40
30
20
10
0
Media Only
+LPS
+MHV A59
Media Only
PEP-WT
PEP-null (CRISPR)
B
****
****
ns
IFNβ (pg/mL)
100
80
60
40
20
0
Media Only
PEP-WT
PEP-R619W
Media Only
PEP-WT
PEP-R619W
+ MHV A59
C
***
**
ns
IFNβ (pg/mL)
300
200
100
0
Media Only
PEP-WT
PEP-R619W
Media Only
PEP-WT
PEP-R619W
+ LPS
D
ns
ns
ns
**
****
****
IFNβ Production Per 1000 Cells (pg/mL)
0.6
0.4
0.2
0.0
PEP-WT
PEP-null (CRISPR)
PEP-R619W
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-R619W
+ LPS
E
ns
ns
ns
****
****
**
IFNβ Production Per 1000 Cells (pg/mL)
0.4
0.3
0.2
0.1
0.0
PEP-WT
PEP-null (CRISPR)
PEP-R619W
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-R619W
+ 3p-hpRNA

Figure 2.

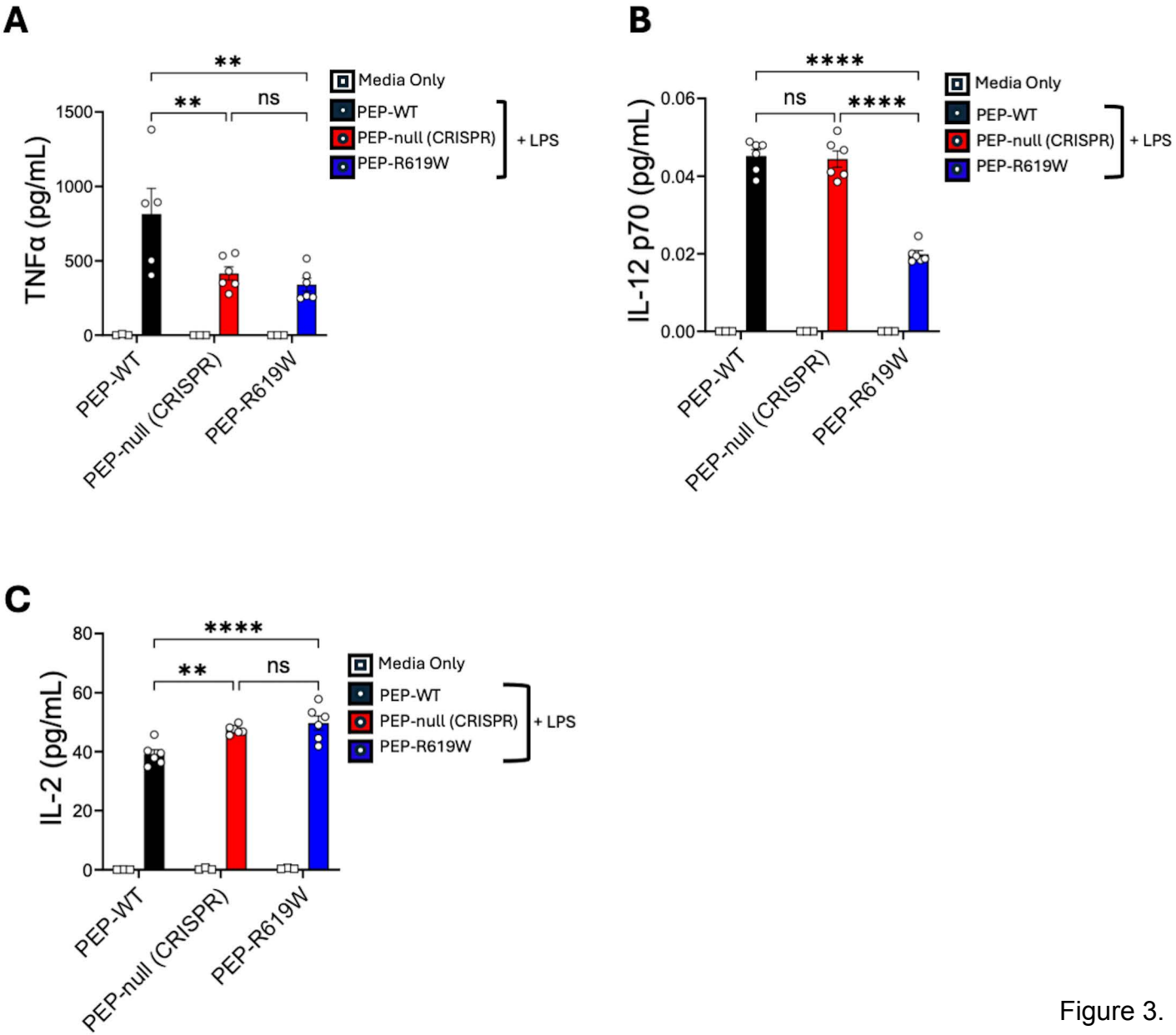
A
TNFα (pg/mL)
1500
1000
500
0
**
**
ns
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-R619W
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-R619W
B
IL-12 p70 (pg/mL)
0.06
0.04
0.02
0.00
****
ns
****
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-R619W
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-R619W
C
IL-2 (pg/mL)
80
60
40
20
0
****
**
ns
Media Only
PEP-WT
PEP-null (CRISPR)
PEP-R619W
+ LPS
PEP-WT
PEP-null (CRISPR)
PEP-R619W

Figure 3.