RESEARCH ARTICLE

C57BL/6 and 129 inbred mouse strains differ in Gbp2 and Gbp2b expression in response to inflammatory stimuli in vivo [version 1; peer review: 2 approved]

Barbara Clough¹, Ryan Finethy²*, Rabia T. Khan¹*, Daniel Fisch¹*, Sarah Jordan, Harshil Patel³, Jörn Coers²,4*, Eva-Maria Frickel¹*

¹Host-Toxoplasma Interaction Laboratory, The Francis Crick Institute, London, NW1 1AT, UK
²Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA
³Bioinformatics and Biostatistics, The Francis Crick Institute, London, NW1 1AT, UK
⁴Department of Immunology, Duke University Medical Center, Durham, North Carolina, USA

* Equal contributors

Abstract

Background: Infections cause the production of inflammatory cytokines such as Interferon gamma (IFNγ). IFNγ in turn prompts the upregulation of a range of host defence proteins including members of the family of guanylate binding proteins (Gbps). In humans and mice alike, GBPs restrict the intracellular replication of invasive microbes and promote inflammation. To study the physiological functions of Gbp family members, the most commonly chosen models are mice harbouring loss-of-function mutations in either individual Gbp genes or the entire Gbp gene cluster on mouse chromosome 3. Individual Gbp deletion strains differ in their design, as some strains exist on a pure C57BL/6 genetic background, while other strains contain a 129-derived genetic interval encompassing the Gbp gene cluster on an otherwise C57BL/6 genetic background.

Methods: To determine whether the presence of 129 alleles of paralogous Gbps could influence the phenotypes of 129-congenic Gbp-deficient strains, we studied the expression of Gbps in both C57BL/6J and 129/Sv mice following in vivo stimulation with adjuvants and after infection with either Toxoplasma gondii or Shigella flexneri.

Results: We show that C57BL/6J relative to 129/Sv mice display moderately elevated expression of Gbp2, but more prominently, are also defective for Gbp2b (formerly Gbp1) mRNA induction upon immune priming. Notably, Toxoplasma infections induce robust Gbp2b protein expression in both strains of mice, suggestive of a Toxoplasma-activated mechanism driving Gbp2b protein translation. We further find that the higher expression of Gbp2b mRNA in 129/Sv mice correlates with a gene duplication event at the Gbp2b locus resulting in two copies of the Gbp2b gene on the haploid genome of the 129/Sv strain.

Conclusions: Our findings demonstrate functional differences between 129 and C57BL/6 Gbp alleles which need to be considered in the design of new mouse models.
and interpretation of studies utilizing mouse models, particularly for phenotypes influenced by Gbp2 or Gbp2b expression.

**Keywords**
Guanylate binding proteins, Toxoplasma gondii, Shigella flexneri, Host-pathogen interaction, innate immune sensing

This article is included in the The Francis Crick Institute gateway.

**Corresponding authors:** Jörn Coers ([jorn.coers@duke.edu](mailto:jorn.coers@duke.edu)), Eva-Maria Frickel ([eva.frickel@crick.ac.uk](mailto:eva.frickel@crick.ac.uk))

**Author roles:** Clough B: Investigation, Writing – Review & Editing; Finethy R: Investigation; Khan RT: Investigation, Writing – Original Draft Preparation; Fisch D: Investigation, Writing – Review & Editing; Jordan S: Investigation; Patel H: Formal Analysis; Coers J: Conceptualization, Funding Acquisition, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Frickel EM: Conceptualization, Funding Acquisition, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

**Grant information:** This work was supported by the Wellcome Trust through a Career Development Fellowship to EMF [091664] and core funding to the Francis Crick Institute. The Francis Crick Institute receives its core funding from Cancer Research UK [FC001076], the UK Medical Research Council [FC001076], and the Wellcome Trust [FC001076]. DF was supported by a Boehringer Ingelheim Fonds PhD fellowship. This work was supported by a National Institute Health grant to JC [AI103197]. JC holds an Investigator in the Pathogenesis of Infectious Disease Awards from the Burroughs Wellcome Fund.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Copyright:** © 2019 Clough B *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**How to cite this article:** Clough B, Finethy R, Khan RT *et al*. C57BL/6 and 129 inbred mouse strains differ in Gbp2 and Gbp2b expression in response to inflammatory stimuli *in vivo* [version 1; peer review: 2 approved] Wellcome Open Research 2019, 4:124 (https://doi.org/10.12688/wellcomeopenres.15329.1)

**First published:** 20 Aug 2019, 4:124 (https://doi.org/10.12688/wellcomeopenres.15329.1)
List of Symbols and Abbreviations

CNV Copy number variation
Gbp Guanylate Binding Protein
PAMP Pathogen-associated molecular pattern
IFNγ interferon gamma
IP intraperitoneally
LPS lipopolysaccharide
CpG unmethylated CpG DNA
Poly(I:C) Polyinosinic-polycytidylic acid

Introduction

Interferon gamma (IFNγ) production during an infection is important to control pathogen replication and mediate an effective host response. IFNγ regulates the expression of a multitude of genes, which includes genes encoding dynamin-like GTPases families: the Mx proteins, the very large interferon-inducible GTPases, the p47 immunity related GTPases (IRGs), and the p65 guanylate binding proteins (Gbps) (Pilla-Moffett et al., 2016). The family of Gbp proteins is highly expressed upon IFNγ stimulation as well as following infections, for example with the protozoan Toxoplasma gondii or the bacterium Listeria monocytogenes (Degrandi et al., 2007). Various Gbps have been shown to control in vivo murine infections with intracellular pathogens, such as BCG Mycobacterium bovis (Kim et al., 2011) Legionella pneumophila (Liu et al., 2018) and Toxoplasma (Selleck et al., 2013). More recently, the family of Gbps has been shown to be involved in rapid activation of murine inflammmasomes (Finethy et al., 2015; Man et al., 2015; Meunier et al., 2014; Meunier et al., 2015; Pilla et al., 2014; Shenoy et al., 2012). Human Gbps have additionally been demonstrated to be important in the control of infection and host cell death. GBP2 and GBP5 inhibit zika virus, measles, influenza A and HIV infectivity (Braun et al., 2019; Krapp et al., 2016), GBP1 acts on dengue virus, vesicular stomatitis virus and encephalomyocarditis virus (Anderson et al., 1999; Pan et al., 2012) and GBP3 on influenza virus (Nordmann et al., 2012). GBP1 impacts Chlamydia trachomatis replication inside macrophages (Al-Zeer et al., 2013), controls intracellular growth of Toxoplasma (Johnston et al., 2016) and blocks intracytosolic actin motility by Shigella flexneri (Piro et al., 2017; Wandel et al., 2017). In terms of host cell death, human GBP5 was reported to promote NLRP3-dependent inflammasome activation in response to bacteria and soluble stimuli (Shenoy et al., 2012) and GBP1 in macrophages promotes pyroptosis during Salmonella infection and apoptosis during Toxoplasma infection (Fisch et al., 2019).

While the cell-intrinsic function of Gbps can be assessed in cell culture models, the interrogation of their physiological functions requires the use of in vivo mouse models, including Gbp gene deletion strains. Recently reported knockouts in Gbp2 (Finethy et al., 2017) and in Gbp5 (Meunier et al., 2014) used homologous recombination in C57BL/6-derived embryonic stem cells or zinc finger nuclease-based gene editing technology in C57BL/6 zygotes, respectively. However, previously reported Gbp2b (formerly Gbp1), Gbp2 and Gbp5 deletion strains were generated in 129-derived embryonic stem cells, and then backcrossed for multiple generations to C57BL/6 mice (Degrandi et al., 2013; Kim et al., 2011; Shenoy et al., 2012), effectively generating congenic mice bearing an interval of 129 DNA surrounding the respective Gbp knockout loci.

Previous work by Staeheli et al., has shown that a number of classical inbred mouse strains do not express Gbp2b protein in the spleen, upon stimulation in vivo with the pathogen-associated molecular pattern (PAMP) poly(I:C) (Staeheli et al., 1984). The work stratified classical inbred mouse strains as “responders” [A/J, BALB/cJ, C3H/HeJ, 129/Ola] or “non-responders” [CBA/J, DBA/2J, C57BL/6]. Further to this, Nguyen et al. have shown that the Gbp2b transcript is not detectable in the lung of C57BL/6J mice following intravenous infection with the PAMP lipopolysaccharide (LPS) (Nguyen et al., 2002). Degrandi et al. confirmed that in vitro stimulation with poly(I:C) and LPS does not upregulate Gbp2b transcripts in C57BL/6J-derived ANA-1 macrophages, yet the study detected induced Gbp2b transcripts following in vitro stimulation with IFNγ (Degrandi et al., 2007). In vivo, the induction of Gbp2b was also observed, both at a transcript and protein level, in C57BL/6J mice following infection with L. monocytogenes or Toxoplasma (Degrandi et al., 2007). IFNγ-stimulated 129xC57BL/6J primary mouse embryonic fibroblasts (MEFs) presented with more Gbp2b protein compared to pure C57BL/6J MEFs as assessed by mass spectrometry (Encheva et al., 2018). Thus, it is apparent that different mouse inbred genetic backgrounds vary in their ability to upregulate Gbp2b in response to different PAMP stimuli. However, despite the reported variation in Gbp2b gene expression, no previous study has systematically analysed the effect of different PAMPs on Gbp2b expression in “responder” and “non-responder” mice nor compared the genetic architecture of the Gbp2b gene across these two categories of mouse strains.

In this study, we examined the Gbp2b and Gbp2 loci in the “non-responder” C57BL/6J and the “responder” 129/Sv mouse strains (Staeheli et al., 1984). We determined the expression profile of Gbp2b and Gbp2 following in vivo stimulation with various PAMPs as well as systemic infections with either the protozoan pathogen Toxoplasma or the bacterial pathogen S. flexneri. We found that PAMP stimulation alone is sufficient to induce Gbp2b expression in 129/Sv but not in C57BL/6J mice, thus confirming and expanding observations made previously by Staeheli and colleagues (Staeheli et al., 1984). Similarly, we found that infections with S. flexneri induced robust Gbp2b expression in 129/Sv but not C57BL/6J mice. In contrast to stimulation with individual purified PAMPs or S. flexneri infections, we unexpectedly found that infection with live Toxoplasma induced robust Gbp2b protein expression without any notable change in Gbp2b mRNA expression in “non-responder” C57BL/6J mice, suggesting that Gbp2b expression is regulated post-transcriptionally by Toxoplasma. Lastly, we also observed notably lower PAMP- or infection-induced expression of Gbp2b in 129/Sv compared to C57BL/6 mice. In conclusion, our studies reveal substantial, mouse strain-dependent variation in Gbp2b and Gbp2 expression. These findings need to be taken into consideration for the design and interpretation of in vivo mouse experiments employed for the study of GBP-related immune functions.
**Results**

*In vivo* administration of various PAMPs leads to robust Gbp2b expression in 129/Sv but not C57BL/6J mice

A previous publication reported a lack of Gbp2b expression following immune stimulation with the TLR3/ RIG-I agonist polyinosinic-polycytidylic acid (poly(I:C)) in C57BL/6J mice *in vivo*, and thus proposed that C57BL/6J mice carry a Gbp2b loss-of-function allele (Staeheli *et al.*, 1984). Given the central role of GBPs in the innate immune response and the broad use of C57BL/6J mice in immunological research, we decided to systematically revisit these observations and to monitor the expression of Gbp2b mRNA and protein both in C57BL/6J and 129/Sv mice in response to various PAMPs.

We initially analysed the expression of Gbp2b, as well as Gbp2 as a control reference transcript, in the spleens of mice 6h after injection with PBS (control), poly(I:C), lipopolysaccharide (LPS), unmethylated CpG DNA (CpG) or the *Toxoplasma* actin-binding protein profilin in order to stimulate RIG-I/TLR3, TLR4, TLR9 or TLR11/12, respectively. In contrast to 129/Sv mice, in which we detected robust induction of Gbp2b mRNA expression in response to poly(I:C), LPS and profilin, we observed no significant differences in Gbp2b expression following PAMP stimulation as compared to PBS in C57BL/6J mice (Figure 1A, underlying data (Clough *et al.*, 2019)). While these findings confirmed previous observations (Staeheli *et al.*, 1984), we also noted diminished expression of Gbp2 mRNA in 129/Sv mice compared with C57BL/6J stimulated with either LPS or profilin (Figure 1B, underlying data (Clough *et al.*, 2019)). These findings prompted us to monitor the expression of seven additional Gbp paralogs (Gbp3 – Gbp9). We observed that in contrast to the remarkable strain-dependent variation in Gbp2b and Gbp2 expression, mRNA

![Figure 1](image-url)

**Figure 1.** Expression of Gbp2b and Gbp2 following various pathogen-associated molecular pattern (PAMP) injections. A and B) mRNA expression of Gbp2b (A) and Gbp2 (B), 6 hours post-intraperitoneal injection of various PAMPs. Analysis of whole spleens of C57BL/6J and 129/Sv mice. Data are represented as fold change over Hprt (2^-ΔCt). Representative experiment with ≥ 3 mice/condition of n=3 experiments. 2-way ANOVA. ****, p<0.0001; ***, p<0.001. C) Immunoblot showing expression of Gbp2b and Gbp2 in protein lysates from spleens of C57BL/6J and 129/Sv mice. Spleens were taken 6 h after IP injection with various PAMPs. Representative immunoblot of n=2 experiments, β-actin as loading control.
expression of other Gbps was comparable in PAMP-stimulated 129/Sv and C57BL/6 mice. The sole exceptions to this were higher expression of Gbp7 and Gbp9 in 129/Sv mice injected with poly(I:C) and higher Gbp5 expression following profilin injection also in 129/Sv (Extended data Figure S1 (Clough et al., 2019)). Analysis of protein levels of Gbp2b and Gbp2 after PAMP stimulation largely reflected the results obtained by transcriptional analysis: we found Gbp2b protein expression in the spleens of C57BL6J mice to be at the threshold or below the level of detection, thus substantially diminished compared to Gbp2b protein expression in 129/Sv mice (Figure 1C, underlying data (Clough et al., 2019)). Also, in agreement with our mRNA expression data (Figure 1B), we observed diminished protein expression of Gbp2 in 129/Sv spleens compared to the same tissue harvested from immune stimulated C57BL/6J mice (Figure 1C, underlying data (Clough et al., 2019)).

In vivo infection with Toxoplasma induces marked Gbp2b protein expression in both mouse strains

We next examined whether strain-dependent variation in Gbp2b and Gbp2 expression also occurred in infected animals. Confirming previous observations by Degrandi et al. (Degrandi et al., 2007), we found the induction of Gbp2b and Gbp2 mRNA expression in the spleens of mice at 8 days post infection to occur only with live Toxoplasma but not with heat-killed (HK) parasites (Figure 2A and B, underlying data (Clough et al., 2019)). We additionally observed significantly

![Graph A: Gbp2b expression](image)

**Figure 2.** Expression of Gbp2b and Gbp2 following Toxoplasma gondii infection. A and B) mRNA expression Gbp2b and Gbp2 was studied on day 8 in spleens of mice IP injected with live or heat killed (HK) Toxoplasma tachyzoites (20,000 for strain Pru, and 100 for strain RH). Data are represented as fold change over Hprt (2^ΔCt). Representative experiment with ≥ 3 mice/condition of n=3 experiments. 2-way ANOVA, ****, p<0.0001; **, p<0.01. C) Immunoblot showing expression of Gbp2b and Gbp2 in protein lysates from spleens of C57BL/6J and 129/Sv mice. Spleens were taken 8 days after mice were injected IP with Toxoplasma as described for A and B. Representative immunoblot of n=2 experiments, β-actin as loading control.
higher expression of Gbp2b mRNA in the spleens of 129/Sv than in the spleens of C57BL/6J mice, infected with either type II (Pru) (p value < 0.0001) or type I (RH) (p value < 0.05) Toxoplasma, thus establishing that both PAMPs and Toxoplasma infections induce significantly more Gbp2b mRNA expression in 129/Sv than in C57BL/6J mice. Similarly, in agreement with our analysis of PAMP-triggered expression (Figure 1), we found that Gbp2 mRNA expression was higher in C57BL/6J compared to 129/Sv mice following infection with either type II (Pru) or type I (RH) Toxoplasma (Figure 2B). All other Gbps were expressed equally between C57BL/6J and 129/Sv with the exception of slightly lower levels of Gbp9 during type I (RH) infection (Extended data Figure S2 (Clough et al., 2019)). We next analysed protein expression in the same animals and observed protein expression levels for Gbp2 as well as Gbp2b were prominently induced in the spleens of both C57BL/6J and 129/Sv mice after infection with live Toxoplasma (Figure 2C, underlying data (Clough et al., 2019)), in spite of an apparent lack of Gbp2b mRNA induction in Toxoplasma-infected C57BL6/J mice.

**In vivo infection with S. flexneri leads to robust Gbp2b expression in 129/Sv but not C57BL/6J mice**

In contrast to the administration of various PAMPs in vivo (Figure 1), we had found that infection with live Toxoplasma induced substantial Gbp2b expression in ‘non-responder’ C57BL/6J mice (Figure 2). This led us to question whether a second infectious agent could similarly provide an induction signal for Gbp2b expression that the PAMP administration alone was lacking. Based on a recent study demonstrating a role for Gbps in resistance to *S. flexneri* infections in mice (Li et al., 2017), we intraperitoneally infected C57BL/6J and 129/Sv mice with *S. flexneri* serotype 2a and monitored mRNA and protein expression at 18 hours-post-infection (hpi). Mirroring our observations with LPS and other PAMPs (Figure 1), we recorded a more than 10-fold induction of Gbp2b mRNA in 129/Sv mice, while expression of Gbp2b was only minimally induced in C57BL6/J mice (Figure 3A, underlying data (Clough et al., 2019)). As seen with injected PAMPs (Figure 1B), we observed an inverse relationship for Gbp2 mRNA expression, which was significantly reduced in 129/Sv compared to C57BL/6J mice (Figure 3B, underlying data (Clough et al., 2019)). All other Gbps were expressed to similar levels during *S. flexneri* infection (Extended data Figure S3 (Clough et al., 2019)). These strain-dependent differences in mRNA expression correlated with corresponding differences in protein expression, where we observed more robust expression of Gbp2b protein in 129/Sv than in C57BL/6J mice, while expression levels of Gbp2 protein were moderately reduced in 129/Sv mice (Figure 3C, underlying data (Clough et al., 2019)). These results demonstrated that both purified PAMPs as well as *S. flexneri* infections lead to high expression of Gbp2b mRNA and protein, but relatively low expression of Gbp2 in 129/Sv compared to C57BL/6J mice.

The 129/Sv genome contains a 25 kb gene duplication spanning Gbp2b and Gbp2

Next, we set out to identify any SNPs or structural variants that could explain the differences in expression of Gbp2b observed in the C57BL/6J and 129/Sv marine strains (Extended data Supplementary Table 1 (Clough et al., 2019)). We compared intronic, exonic and UTR (2kb upstream) sequences of Gbp2b in C57BL/6J and 129/Sv mice. We identified a small number of intronic, but no exonic SNPs that differ between C57BL/6J and 129/Sv (Extended data Supplementary Table 1 (Clough et al., 2019)). Notably, our analysis additionally identified a copy number variation (CNV) in the Gbp2b gene locus (Figure 4A, underlying data (Clough et al., 2019)). This 25kb gene duplication event begins at exon 5 of Gbp2b and continues to exon 4 of Gbp2 in the 129/Sv genome (Figure 4A and Extended data Supplementary Table 2 (Clough et al., 2019)).

Using qPCR we confirmed the presence of an insertion within the 129/Sv strain, that is not present in the C57BL6/J strain (Figure 4B, underlying data (Clough et al., 2019)). The probe located at the intron-exon boundary of exon 1 of Gbp2b confirmed that both C57BL/6J and 129/Sv only carry one copy on the haploid genome, similar to the reference. The CNV probe located in intron 6 confirmed the presence of two Gbp2b copies on the haploid genome of 129/Sv compared to one copy on the C57BL/6J genome (Figure 4). These data demonstrate the presence of a CNV in the Gbp2b locus of 129/Sv beginning after exon 1 and extending into exon 6. Remarkably, the segregation pattern of this CNV (Table 1) correlated with responder and non-responder phenotypes described previously (Stueheli et al., 1984), suggesting a possible causative relationship.

**Discussion**

The GBP family consists of 11 members in mice and 7 in humans and has emerged as a critical regulator of antimicrobial host defence and inflammation (Mitchell & Isberg, 2017; Pilla-Moffett et al., 2016; Saeij & Frickel, 2017; Tretina et al., 2019). Even though the importance of this protein family in antimicrobial immunity is now evident, the molecular function of individual GBP family members remains largely unexplored. To fill this gap in knowledge, several recent studies focused on the function of discrete human GBPs in cell-autonomous immunity (Braun et al., 2019; Krapp et al., 2016; Li et al., 2017; Piro et al., 2017; Wandel et al., 2017). While these cell-based studies have provided several critical insights into the cellular functions of individual GBPs, we depend on animal models to detail the complex organismal responses orchestrated by these important immune proteins. Accordingly, the mouse, as the most widely used animal model for the study of inflammation and immunity, has been applied to dissect Gbp gene function in vivo (Finethy et al., 2017; Kim et al., 2011; Meunier et al., 2014; Shenoy et al., 2012; Yamamoto et al., 2012). Occasionally, results from these mouse studies resulted in contradictory findings, as exemplified for mouse Gbp5 and its potential role in NLRP3 inflammasome activation (Man et al., 2015; Meunier et al., 2014; Shenoy et al., 2012). It is difficult to untangle the exact nature of the reported differences, as macrophages employed in these studies were not treated uniformly. Nevertheless, one potential contributing factor to these discrepancies is the use of mouse strains with individual gene deletions, e.g. in Gbp5, that due to the process by which they were generated bear either C57BL/6 or 129 alleles of neighbouring Gbp paralogs. Thus, proper interpretation of results
obtained with Gbp knockout strains requires an understanding of the differences between C57BL/6 and 129 Gbp alleles and their effects on gene expression and function.

Here, we analysed the expression profile of Gbp2b and Gbp2 following in vivo immune stimulation. We found that Gbp2b expression is vigorously induced in response to systemic immune activation by PAMPs or S. flexneri infection in 129/Sv, but not C57BL6/J mice, while Gbp2 expression was generally higher in C57BL6/J compared to 129/Sv mice. Higher induction of Gbp2b mRNA expression in 129/Sv mice correlated with a Gbp2b gene duplication event in the 129/Sv genome. Previous studies demonstrated an appreciable correlation between CNV and gene expression in the genome of different inbred mouse strains (Chaignat et al., 2011; Henrichsen et al., 2009; Orozco et al., 2009), suggesting that the partial Gbp2b gene duplication extending into the Gbp2 locus could cause the enhanced expression of Gbp2b and the reduced expression of Gbp2 in 129/Sv mice. CNV can impact gene expression through complex mechanisms that go beyond simple gene dosage effects (Weischenfeldt et al., 2013). This is especially true when the CNV only partially overlaps with the complete

Figure 3. Expression of Gbp2b and Gbp2 following Shigella flexneri infection (qPCR and IB). A and B) mRNA expression of Gbp2b and Gbp2 was studied 18 h p.i. in spleens of mice IP injected with Shigella flexneri. Data are represented as fold change over Hprt (2−ΔΔCt). Uninfected mice (n = 3/strain); infected C57BL/6J (n = 15) and 129/Sv (n = 16). C) Immunoblot showing expression of Gbp2b and Gbp2 in protein lysates from spleens of C57BL/6J and 129/Sv mice. Spleens were taken 18h after injection IP with S. flexneri. Representative immunoblot of two mice each of the cohort described in A and B. β-actin as loading control.
gene segment, as is the case for the Gbp2b/ Gbp2 duplication present in the 129/Sv genome. Thus, defining the molecular link between the Gbp2b/ Gbp2 CNV and gene expression may prove to be difficult to ascertain. Nonetheless, considering the association of CNV with phenotypic variation (Weischenfeldt et al., 2013), future studies focused on GBP gene variants need to not only evaluate disease association with GBP SNPs, as successfully demonstrated for the host response to viral infections (Koltes et al., 2015), but also monitor the potential association of GBP CNVs with disease in animals and humans.

Whereas the delivery of PAMPs or infection with S. flexneri resulted in negligible induction of either Gbp2b mRNA or protein expression in C57BL/6J mice, infection with live but not heat-inactivated Toxoplasma led to robust Gbp2b protein expression in the absence of corresponding induction of Gbp2b mRNA expression in the same mouse strain. These data therefore suggest that infections with live Toxoplasma boost Gbp2b translation by an unknown mechanism. Future studies are needed to explore the nature of this mechanism, identify its molecular trigger and determine whether this response is induced.

**Figure 4. Copy number variation within the Gbp2b in inbred mouse strains.** A) Integrative genomics viewer (IGV) screenshot confirming the presence of a copy number gain (chr3:142600561-142625599) in 129S1/SvImJ (top-panel; coverage = 199) relative to C57BL/6NJ (bottom-panel; coverage = 110) overlapping the Gbp1 and Gbp2 loci. Read alignment files were obtained from the Mouse Genomes Project (ftp://ftp-mouse.sanger.ac.uk/current_bams). B) Digital PCR using primers located on two distinct exons was used to assess the presence of CNV on the C57BL/6J and 129/Sv genetic background. Representative of two experiments using 3 mice each. The 129/Sv has only one copy of at exon 1 (two on the diploid genome), but two copies of exon 6 (four on the diploid genome).
by pathogens other than Toxoplasma. Here, we show that S. flexneri infections fail to deliver this putative molecular activator and accordingly S. flexneri infections drive robust Gbp2b expression predominantly or entirely through increased mRNA expression, a response present in 129/Sv, but absent from C57BL/6J mice. Considering the previously reported antimicrobial activities of Gbp2b (Kim et al., 2011), we can therefore expect that the genetic origin of the Gbp2b allele, i.e. 129- versus C57BL/6-derived, will influence the outcome of infection studies with Shigella or related proteobacteria.

Because functional differences between 129 and C57BL/6 Gbp alleles in linkage with a given Gbp knockout allele are expected to affect host immune response and thus confound the interpretation of phenotypes associated with these Gbp knockout lines, future studies need to address these concerns. One strategy would be to generate congenic mouse lines bearing similar 129 congenic DNA elements. While this strategy is admittedly burdensome, ignoring the impact of carrier 129 Gbp alleles on phenotypes associated with these Gbp knockout mice will inevitably lead to data misinterpretation and incorrect assignments of gene functions. The alternative strategy is to resort to the exclusive use of individual Gbp knockout lines that are coisogenic with any given control strain has become remarkably trivial and thus renders the latter strategy highly feasible.

### Methods

**Ethics statement**

All procedures involving mice were approved by the local ethical committee of the Francis Crick Institute Ltd, Mill Hill Laboratory and are part of a project license (PPL 80/2616) approved by the Home Office, UK, under the Animals (Scientific Procedures) Act 1986, or were approved by the Institutional Animal Care and Use Committees at Duke University (protocol registry number A113-16-05). Duke University maintains an animal program that is registered with the United States Department of Agriculture (USDA), assured through the National Institutes of Health/Public Health Service (NIH/PHS), and accredited with Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International (accreditation number 363).

**Parasite culture**

Toxoplasma gondii avirulent type II strain Pru and type I RH strain was used, both a gift from Jeroen Saeij. All strains of Toxoplasma gondii were maintained by serial passage on monolayers of HFF cells, cultured in DMEM with GlutaMAX (Life Technologies) supplemented with 10% FBS (Life Technologies), at 37°C in 5% CO2 (Clough et al., 2016).

**Animal procedures**

In vivo Toxoplasma gondii infection. Mice (Mus musculus) C57BL/6J and 129/S8 were bred at the Francis Crick Institute Ltd. Animals were kept under specific pathogen free (SPF) conditions, housed in rodent facility in individually ventilated cages (GM500 from Tecniplast) (3–4 animals per cage) on standard Aspen bedding (Datesand, UK) with red mouse house enrichment. Animals were housed in light/dark cycle 12:12 (light on at 7am), temperature 19–23°C, Rh 45–65%. Commercial mouse diet (T2018S, Envigo, UK) and water available ad libitum via automated watering system (Edstrom). 6- to 8-week-old male mice (weight between 20–25g) were used. Animals were divided into experimental cohorts. Each cohort was assigned 3 mice and experiment was repeated 2–3 times to achieve statistical significance. In order to study the transcriptional and translational upregulation of murine Gbps after infection, mice were injected IP with live, or heat killed Toxoplasma gondii, with either 20,000 tachyzoites of the type II, avirulent Pru strain or 100 tachyzoites of the virulent type I RH strain. Toxoplasma gondii was heat killed by incubation at 65°C for

### Table 1. qPCR primer sequences (previously published (Yamamoto et al., 2012)).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence 5′ → 3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gbp2b</td>
<td>fwd: ACCCGGAGACTTACTGGCT</td>
</tr>
<tr>
<td></td>
<td>rev: TTTATTGAGCTTGGTCTCTGTATCC</td>
</tr>
<tr>
<td>Gbp2</td>
<td>fwd: CTCAGCATTAGTCGAGGACCTA</td>
</tr>
<tr>
<td></td>
<td>rev: CCGAATGCTCTACCACCCACTC</td>
</tr>
<tr>
<td>Gbp3</td>
<td>fwd: CTGAGCATTAAATTCTGGAAGCCAT</td>
</tr>
<tr>
<td></td>
<td>rev: CCGTCCTGCAAGACGATTC</td>
</tr>
<tr>
<td>Gbp4</td>
<td>fwd: GGAGAGCATTACGAAAGGCAAA</td>
</tr>
<tr>
<td></td>
<td>rev: TCCCAACAGGAGACTCATATT</td>
</tr>
<tr>
<td>Gbp5</td>
<td>fwd: CTGAGCTCAATTTTGTCGAGGA</td>
</tr>
<tr>
<td></td>
<td>rev: CATCGACATAAGTGCAGACCAG</td>
</tr>
<tr>
<td>Gbp6</td>
<td>fwd: AGGACCTGATATGATGCTGA</td>
</tr>
<tr>
<td></td>
<td>rev: GAAATCCATATTTAAGAGGCC</td>
</tr>
<tr>
<td>Gbp7</td>
<td>fwd: TTCCTGTGTCCTAGTGGAAA</td>
</tr>
<tr>
<td></td>
<td>rev: CAAGGGTTCTCATCAAGTAGAT</td>
</tr>
<tr>
<td>Gbp8</td>
<td>fwd: ACATCTGCTCCATGACACATGAG</td>
</tr>
<tr>
<td></td>
<td>rev: AAACCGTGATCTGTGCTGCCC</td>
</tr>
<tr>
<td>Gbp9</td>
<td>fwd: ACCGGGATAGACTGGGTACT</td>
</tr>
<tr>
<td></td>
<td>rev: CGGGCCACACTGCTCATCA</td>
</tr>
<tr>
<td>Gbp10</td>
<td>fwd: AGACCATAACATGATGCTGA</td>
</tr>
<tr>
<td></td>
<td>rev: GAAATCCATATTTAAGAGG</td>
</tr>
<tr>
<td>Gbp11</td>
<td>fwd: GAAGCTGAGGAAATGAGAAGAG</td>
</tr>
<tr>
<td></td>
<td>rev: GCCTTTTCAATCAGTAAAGAGG</td>
</tr>
<tr>
<td>Hprt</td>
<td>fwd: TCAGTCAACGGGGCTACATAA</td>
</tr>
<tr>
<td></td>
<td>rev: GGGCGCTGATCTGCTTAACCA</td>
</tr>
</tbody>
</table>

Wellcome Open Research 2019, 4:124 Last updated: 29 NOV 2019
20 minutes. Mice were euthanised by cervical dislocation in order to analyse the in vivo response to infection by harvesting spleens at 8 days after infection and analysed for Gbp2b and Gbp2 expression. All efforts were made to ameliorate harm to the animals through careful statistical analysis employing the minimum amount of animals to achieve statistical significance.

**In vivo Shigella flexneri infection.** Mice (Mus musculus) C57BL/6J (The Jackson Laboratory, #000664) and 129SvEvTac (Taconic #129SVE) were bred at Duke University Medical Center. Animals were kept under SPF conditions, housed in rodent facility in Allentown IVC140 double sided racks/Jag 75 cages (4 - 5 animals per cage) on 1/8th of an inch standard corncob bedding (The Andersons lab bedding, USA). Animals were housed in light/dark cycle 12:12 (light on at 7am), temperature 20–23°C, Rh 30–70%. Commercial mouse diet (5053 diet, Purina, USA) and bottled tap water was available ad libitum. Wildtype S. flexneri serotype 2a (2457T) was grown overnight in tryptic soy broth (TSB) (Sigma, #22092) at 37°C with aerolization. Saturated cultures were diluted 1:50 in 5 ml fresh TSB and incubated for 2.5 to 4 h at 37°C with shaking until absorbance of samples at a wavelength of 600 nm (OD₆₀⁰) reached 0.8 to 0.9, measured on a Smart-Spec 3000 (Bio-Rad). Bacteria were washed and resuspended in PBS to a final concentration of 1 × 10⁹ colony forming units (CFUs) / mL. Eight male and eight female 129/Sv mice and age- and sex-matched C57BL/6J mice (6 – 12 week of age; weight between 16–27g) were grouped in 4 cohorts of 8 mice and ip. injected with 5 × 10⁶ CFUs. At 18 hpi mice were euthanized and spleens were harvested. Spleens were cut into two roughly equal portions, which were either processed for protein lysates or placed into TRIzol (15596026, Invitrogen) for RNA purification. Organs from uninfected control animals were processed in the same fashion.

**In vivo PAMP stimulation.** Mice were injected IP with Polysaccharide (5.5μg/100μl) (Sigma, SRP8050), Poly(I:C) (100μg/100μl) (Sigma, P1530), CpG (5μg/100μl) (Invivogen, trl-1826), LPS (100μg/100μl) (InvivoGen, trl-3elp/ps) or PBS. Spleens were collected 6 hours after injection and analysed for Gbp2b and Gbp2 expression.

**Quantitative polymerase chain reaction**

Cellular RNA was extracted using the TRIZol (15596026, Invitrogen). RNA quality was determined on a Nanodrop Cellular RNA was extracted using the TRIzol (15596026, Invitrogen) for RNA purification. Organs from uninfected control animals were proc

**Immunoblots**

Protein lysates, prepared from mouse spleens, were run on SDS-PAGE (NuPage 4–12% Bis-Tris, ThermoFisher), immunoblotted and probed for expression of Gbp2b (Rabbit polyclonal antibody 1:5000, (Virreira Winter et al., 2011)) or Gbp2 (goat anti-GBP2 1:200, Santa Cruz #sc-10588). Mouse monoclonal anti-α-actin antibody was used to control for protein loading (1:5000, Sigma #A5411). Secondary HRP-conjugated antibodies (goat anti-rabbit HRP ThermoFisher #G21234 1:20000; donkey anti-goat HRP Abcam #ab97110 1:5000, rabbit antimouse HRP Sigma #SAB3701023 1:20,000) were detected by chemiluminescence (Merck Millipore #WBKLS0500).

**Digital PCR**

Digital PCR was used to assess the presence of copy number variation in the Gbp2b gene using a GeneAMP® PCR system 9700 (Applied Biosystems®) and the QuantStudio™ 3D Digital PCR 20K Chip Kit v2 and Master Mix (Thermo Fisher, A26317). DNA extracted from tails of C57BL/6J and 129/Sv mice and a total concentration of 50ng was used for each chip. The 3D master-mix (Thermo Fisher, A26317), probes and DNA were prepared as per manufacturer’s instructions and 14.5 μL was loaded onto the chip. The two probes were Mm00733848_Cn (intron 6) and Mm00095526_cn (overlaps exon 1 and intron 1). The probe TaqMan® Copy Number Reference Assay (Thermo Fisher, 4458369), Mouse, Tert, which has one copy on the mouse haploid genome was used as the reference. The chips (Thermo Fisher, A26317) were sealed and loaded onto the GeneAMP® PCR system 9700 (Applied Biosystems®) and cycled according to the following parameters: 96°C for 10 minutes, followed by 39 cycles of 60°C for 2 min and 98°C for 30 sec, and a final extension at 60°C for 2 min. After cycling, the end-point fluorescence of the partitions on the chips was measured by transferring the chips to the measurement unit (Thermo Fisher, A26317). The data was analysed using the QuantStudio™ 3D Analysis Suite™ Software v3.0.

**Sequence analysis**

Known SNPs and structural variations that are different between the 129/Sv and C57BL/6J were downloaded from Mouse phenome database (MPD; [http://www.jax.org/phenome](http://www.jax.org/phenome)) and the whole genome sequencing data for the same strains were obtained from the Mouse Genomes Project.

**Statistical analysis**

Graphs were plotted using Prism 8.0.2 (GraphPad Inc.) and presented as means of N = 3 experiments (with usually 3 technical repeats within each experiment) with error bars representing SEM, if not stated otherwise. Data analysis used two-way ANOVA.

**Data availability**

Underlying data

Figshare: Title: C57BL/6 and 129 inbred mouse strains differ in Gbp2 and Gbp2b expression in response to inflammatory stimuli in vivo.
This project contains the following underlying data:

- Fig1+S1_QPCR_PAMPs_GBP1.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP1 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP2.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP2 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP3.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP3 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP4.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP4 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP5.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP5 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP6.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP6 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP7.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP7 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP8.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP8 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP9.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP9 in TAB format.)
- Fig1+S1_QPCR_PAMPs_GBP11.txt (All raw data for Figure 1 and Extended data Supplementary Figure 1 for GBP11 in TAB format.)
- Fig2+S2_QPCRToxo_GBP1.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP1 in TAB format.)
- Fig2+S2_QPCRToxo_GBP2.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP2 in TAB format.)
- Fig2+S2_QPCRToxo_GBP3.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP3 in TAB format.)
- Fig2+S2_QPCRToxo_GBP4.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP4 in TAB format.)
- Fig2+S2_QPCRToxo_GBP5.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP5 in TAB format.)
- Fig2+S2_QPCRToxo_GBP6.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP6 in TAB format.)
- Fig2+S2_QPCRToxo_GBP7.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP7 in TAB format.)
- Fig2+S2_QPCRToxo_GBP8.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP8 in TAB format.)
- Fig2+S2_QPCRToxo_GBP9.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP9 in TAB format.)
- Fig2+S2_QPCRToxo_GBP11.txt (All raw data for Figure 2 and Extended data Supplementary Figure 2 for GBP11 in TAB format.)
- Fig3+S3_Shigella-infection_Gbp1.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP1 in TAB format.)
- Fig3+S3_Shigella-infection_Gbp2.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP2 in TAB format.)
- Fig3+S3_Shigella-infection_Gbp3.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP3 in TAB format.)
- Fig3+S3_Shigella-infection_Gbp4.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP4 in TAB format.)
- Fig3+S3_Shigella-infection_Gbp5.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP5 in TAB format.)
- Fig3+S3_Shigella-infection_Gbp6.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP6 in TAB format.)
Fig3+S3_Shigella-infection_Gbp7.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP7 in TAB format.)

Fig3+S3_Shigella-infection_Gbp8.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP8 in TAB format.)

Fig3+S3_Shigella-infection_Gbp9.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP9 in TAB format.)

Fig3+S3_Shigella-infection_Gbp11.txt (All raw data for Figure 3 and Extended data Supplementary Figure 3 for GBP11 in TAB format.)

Extended data
Figshare: Title: C57BL/6 and 129 inbred mouse strains differ in Gbp2 and Gbp2b expression in response to inflammatory stimuli in vivo.

https://doi.org/10.6084/m9.figshare.8235524.v2 (Clough et al., 2019)

This project contains the following Extended data:

- Gbp2b_ExtendedDataFigureS1.jpg (Extended data Figure S1 as a jpeg File.)
- Gbp2b_ExtendedDataFigureS2.jpg (Extended data Figure S2 as a jpeg File.)
- Gbp2b_ExtendedDataFigureS3.jpg (Extended data Figure S3 as a jpeg File.)
- Gbp2b_ExtendedDataSupplementaryTable 1.xlsx (Extended data Supplementary Table 1 as an Excel File.)
- Gbp2b_ExtendedDataSupplementaryTable 2.xlsx (Extended data Supplementary Table 2 as an Excel File.)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Legends for Extended data:

Extended data Figure S1. Expression of Gbp3-11 in C57BL/6J compared to the 129/Sv following infection. mRNA expression of mouse Gbp3-9 was studied on day 8 in spleens of mice IP injected with live or heat killed (HK) Toxoplasma tachyzoites (20,000 for strain Pru, and 100 for strain RH). Data are represented as fold change over HPRT (2^ΔCt). Data are represented as fold change over HPRT (2^ΔCt). Representative experiment with ≥ 3 mice/condition of n=3 experiments. 2-way ANOVA, *, p<0.05; **, p<0.01.

Extended data Figure S2. Expression of Gbp3-11 in C57BL/6J compared to the 129/Sv following infection. Data are represented as fold change over HPRT (2^ΔCt). Uninfected 6 mice/strain; infected 15 mice/C57BL/6 and 16 mice/129/S8.

Extended data Supplementary Table 1. Single nucleotide polymorphisms of Gbp2b and Gbp2 in 129 versus C57BL/6 mice. Known SNPs and structural variations that are different between the 129/Sv and C57BL/6J were downloaded from Mouse phenome database (MPD; http://www.jax.org/phenome).

Extended data Supplementary Table 2. Genetic variation of Gbp2b and Gbp2 in 129 versus C57BL/6J mice. Table summarizing the location of the gene duplication event within the context of the Gbp2b and Gbp2 genes. The location of the dPCR and qPCR Taqman probes is also marked.

Grant information
This work was supported by the Wellcome Trust through a Career Development Fellowship to EMF [091664] and core funding to the Francis Crick Institute.

The Francis Crick Institute receives its core funding from Cancer Research UK [FC001076], the UK Medical Research Council [FC001076], and the Wellcome Trust [FC001076]. DF was supported by a Boehringer Ingelheim Fonds PhD fellowship. This work was supported by a National Institute Health grant to JC [AI103197]. JC holds an Investigator in the Pathogenesis of Infectious Disease Awards from the Burroughs Wellcome Fund.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
The authors would like to thank Biological Research Facility as well as the Genomics Equipment Park led by Graham Clark both at The Francis Crick Institute.

Author information
Authors Barbara Clough, Ryan Finethy, Rabia T. Khan and Daniel Fisch contributed equally and share authorship amongst themselves. Authors Jörn Coers and Eva-Maria Frickel contributed equally and share authorship amongst themselves.


The study by Clough and co-workers demonstrates variation in the expression of two Gbp genes, Gbp2 and Gbp2b, between inbred mouse strains C57BL/6J and 129/S8. After stimulations with several PAMPs, the commonly used B6 mice expressed higher levels of Gbp2 and lower levels of Gbp2b mRNAs and proteins, as compared to the 129/S8 mice. Importantly, other Gbp genes encoded within the same locus were induced by PAMPs to a similar degree. It is well known that transferring a KO mutation to another background by backcrosses (from 129 to B6 in this case) results in transfer of an approximately 10 cM chromosome segment containing flanking genes. The authors correctly emphasize that the observed differences in Gbp2 and Gbp2b gene expression could influence the phenotypes of Gbp KO mice and lead to inconsistencies among the studies performed using Gbp gene knockouts created in the 129 and B6 backgrounds. To avoid the effect of polymorphisms within the flanking regions of the Gbp locus, the authors propose to use CRISPR for inactivating individual Gbp genes in a single background. However, their findings also suggest that the Gbp2b KO in the B6 mice may have less pronounced phenotype, as compared to 129 background, for example, just because this gene is less prominently expressed in the B6 genetic background and other Gbps may play compensatory roles.

The authors identified a genetic polymorphism within the mouse Gbp locus that is potentially responsible for the observed differences in the Gbp2 and Gbp2b genes expression: a duplication of a DNA segment overlapping these genes. Although it seems likely that this CNV is mechanistically linked to the observed interstrain differences in the Gbp2 and Gbp2b gene expression, this observation does not constitute a definitive proof. The strain distribution pattern (SDP) of the genetic polymorphism and the phenotype would strengthen this link. However, Table 1 does not contain the SDP data, as claimed in the text. This statement needs to be corrected.

Figures 1 and 3 demonstrate differential induction of Gbp2b mRNA 6 h after stimulation with PAMPs or 18 h after acute infection with Shigella flexneri. In both cases there is a good correlation between mRNA and protein levels. However, after 8 days of infection with T. gondii, the levels of the Gbp2b protein in B6 mice is significantly upregulated, while the mRNA levels are not. The authors propose an unknown mechanism of translational upregulation as an explanation, which does not seem convincing, however, since the
mRNA levels in the B6 mice remain low, while the protein levels are clearly upregulated. Although this phenomenon is not central for this paper, it would be helpful to discuss alternative explanations as well.

Overall, this manuscript presents information relevant for studying the roles of Gbp proteins in host immunity using KO mice. This is a nice and clearly presented example of potential sources of inconsistencies between studies using gene knockouts due to background and “passenger gene” effects.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Host resistance to bacterial infections, mouse models of infections, immunogenetics, tuberculosis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 23 August 2019

https://doi.org/10.21956/wellcomeopenres.16734.r36267

© 2019 Staeheli P. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peter Staeheli
Faculty of Medicine, University of Freiburg, Freiburg, Germany

This is a nice study that helps solving some confusion regarding the regulation of the Gbp2 gene by IFN-γ and/or parasite infection in different mouse strains. Work published in 1984 had shown that inbred mouse strains B6 and 129 differ with regard to Gbp2 regulation, but later work with knockout mice yielded conflicting results. The new work finds a convincing explanation for these discrepancies. It turns out that
knockout mice generated from ES cells derived from 129 mice continue to carry a 129-like Gbp2b locus even after extensive backcrossing to B6 mice. Accordingly, the important take-home message of this manuscript is that one should be extremely careful when drawing conclusions from results with Gbp knockout mice which were produced using traditional technology that relied on ES cells from 129 mice. The paper is well written. There are only a few minor issues that should be addressed:

1. The statement in the introduction that IFN-γ induces dynamin-like GTPases, including Mx proteins is not correct. Mx genes are exceptional in that they are induced by type I and type III IFN, but not by IFN-γ.

2. The authors use the term “IFNγ” as short form for interferon-γ. According to current terminology rules, the correct abbreviation is “IFN-γ”.

3. In the last paragraph of the results section it is mentioned that Table 1 shows how markers for exon 1 and exon 6 of the Gbp2 gene segregate in different mouse strains. However, Table 1 is showing qPCR primer sequences. The missing information should be provided.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular virology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.