CD150-dependent hematopoietic stem cells sensing of *Brucella* instructs myeloid commitment

Hysenaj Lisiena^{1,2#}, De Laval Bérengère^{1#}, Arce-Gorvel Vilma¹, Bosilkovski Mile³, Gonzalez Gabriela¹, Debroas Guillaume¹, Sieweke Michael^{1,4§}, Sarrazin Sandrine^{1§*} and Gorvel Jean-Pierre^{1§*}

1 : Aix Marseille University, CNRS, INSERM, CIML, 13009 Marseille, France

2 : Department of Anatomy, University of California, San Francisco, USA

3 : University Clinic for Infectious Diseases and Febrile Conditions, Skopje, Republic of North Macedonia,

4 : Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, 01307 Dresden, Germany

: co-first author

§ : co-last author

*Corresponding authors:

Jean-Pierre Gorvel, gorvel@ciml.univ-mrs.fr, Orcid: <https://orcid.org/0000-0002-2829-9804>
Centre d'Immunologie de Marseille-Luminy, Marseille, France

Sandrine Sarrazin, sarrazin@ciml.univ-mrs.fr, Orcid: <https://orcid.org/0000-0001-6435-0913>
Centre d'Immunologie de Marseille-Luminy, Marseille, France

SUMMARY

This work provides first evidence HSC directly sense *Brucella abortus* via the bacterial outer membrane protein Omp25 and the HSC surface receptor CD150, leading to functional commitment of HSC to myeloid lineage and very early initiation of immune response.

28 **ABSTRACT**

29 So far, hematopoietic stem cells (HSC) are considered the source of mature immune cells, the latter
 30 being the only ones capable of mounting an immune response. Recent evidence shows HSC can
 31 also directly sense cytokines released upon infection/inflammation and pathogen-associated
 32 molecular pattern interaction, while keeping a long-term memory of previous encountered signals.
 33 Direct sensing of danger signals by HSC induces early myeloid commitment, increases myeloid
 34 effector cell numbers and contributes to an efficient immune response. Here, using specific genetic
 35 tools on both host and pathogen sides, we show that HSC can directly sense *B. abortus* pathogenic
 36 bacteria within the bone marrow via the interaction of the cell surface protein CD150 with the
 37 bacterial outer membrane protein Omp25, inducing efficient functional commitment of HSC to the
 38 myeloid lineage. This is the first demonstration of a direct recognition of a live pathogen by HSC
 39 via CD150, which attests of a very early contribution of HSC to immune response.

40

41 **INTRODUCTION**

42 Few pathogens are capable of colonizing the bone marrow (BM) [1-5], the niche of haematopoietic
 43 stems cells (HSCs) responsible for initiating the production of myeloid progenitors and mature
 44 blood-forming cells [6]. *Brucella abortus*, (Gram-negative bacterium responsible for the re-
 45 emerging zoonosis of brucellosis) is able to persist for months in the BM [7]. Human brucellosis
 46 patients suffer from haematological abnormalities suggesting that *Brucella* in the BM may affect
 47 hematopoietic development [8]. HSCs can respond to an infection through pathogen-elicited
 48 cytokines or directly via pathogen recognition receptors (PRR) [9, 10]. CD150 is a key marker of
 49 long term HSC (LT-HSCs) [11] and a microbial sensor in macrophages [12] and dendritic cells
 50 [13]. Here, we show that HSCs within the BM directly sense the outer membrane protein Omp25 of
 51 live *Brucella* through the CD150 receptor. Our *in vivo* and *ex vivo* data demonstrate that *B. abortus*
 52 modulates hematopoiesis by transiently augmenting the production of myeloid cells via CD150.
 53 This is the first demonstration of a direct recognition of a pathogen by HSCs via CD150.

RESULTS

To investigate the consequences of BM infection by a pathogen known to persist for extended periods of time in the hematopoietic niche, we used *Brucella abortus*. We previously demonstrated that this pathogenic bacterium persists for months in murine BM [7]. We began by evaluating if bacteria present in the BM at 8 days post-infection (acute phase of infection) and at 30 days post-infection (chronic phase of infection) are still virulent. For this, we transplanted BM cells of infected mice at those time point into recipient mice (Fig. 1a). Spleen (the natural reservoir for *B. abortus*) and BM cells were harvested eight weeks after transplantation, and bacterial colony forming units (CFU) were enumerated (Fig. 1b). The equivalently high number of bacteria in spleen and bone marrow at 8 weeks post-transplantation shows that the BM hematopoietic environment is permissive for stable infection and replication of *Brucella*.

To further investigate the consequences of BM infection by *B. abortus* on hematopoietic stem cell biology, we analysed the distribution of the hematopoietic stem and progenitor cell compartment (HSPC) by flow cytometry. Absolute numbers of total BM cells or lineage negative progenitors (Lin^-) were not affected by *B. abortus* infection (Supplementary Fig. 1a, b). However, *Brucella* infection induced major phenotypic cell surface marker changes in the HSPC compartment (Fig. 1c, d and Supplementary Fig. 1c for gating strategy), similar to those observed after challenges with PAMPs or attenuated vaccines [10, 14-16]. HSPC (LSK: Lin^- , Sca^+ , cKit^+) expansion was observed at the onset of infection (Day 2 p.i., Fig. 1c, d) and was even more pronounced during the acute phase of infection (Day 8 p.i., Fig. 1c, d). LSK expansion was mainly due to the significant increase of CD48^+ multipotent progenitors (LSK CD48^+) and, to a lesser extent, the increase of short term HSC (ST-HSC: LSK, CD34^- , CD135^- , CD48^- , CD150^-). In addition, the long-term HSC population (LT-HSC: LSK, CD34^- , CD135^- , CD48^- , CD150^+) slightly decreased (Fig. 1c, d). Overall, these data suggest that the presence of *Brucella* in the BM perturbs HSPC homeostasis and leads to an increased output of early multipotent progenitors.

81 Infection and inflammation have been shown to release signals, such as cytokines, that are able to
 82 induce the differentiation of HSC towards the myeloid lineage as evidenced by early up-regulation
 83 of the myeloid master regulator PU.1 [17-20]. To further investigate whether *B. abortus* infection
 84 can induce an early commitment towards the myeloid lineage in HSC, we infected *Pu.1^{+GFP}*
 85 reporter mice harbouring enhanced green fluorescent protein (GFP) knocked into the PU.1 locus
 86 [21, 22] and then analysed GFP expression in HSC (LSK, CD34⁻, CD135⁻, CD48⁻) at 2 days post-
 87 infection. GFP expression in HSC was used as a read-out of early HSC activation/commitment
 88 during *Brucella* infection (Fig. 2a). GFP was upregulated 30 to 40% in BM HSCs of *B. abortus*-
 89 infected WT mice (Fig. 2b, left panel), confirming an induction of PU.1 expression and
 90 consequently a change in HSC fate after *Brucella* infection.

91
 92 We then investigated the molecular mechanisms underlying the early response of HSC during
 93 *Brucella* infection. HSC can directly sense microbial compounds [14, 23, 24]. The CD150 receptor,
 94 known as one of the key markers of HSC [25], is also able to sense bacteria in dendritic cells and
 95 macrophages directly [12]. Indeed, in vitro studies showed that OmpC and OmpF of *E. coli* and
 96 *Salmonella spp.* binds to extracellular domain of CD150 [12]. Moreover, we have recently
 97 demonstrated that the outer-membrane protein 25 (Omp25) of *B. abortus* is a direct ligand of the
 98 extracellular domain of mouse CD150 in dendritic cells [13]. We asked whether HSC could detect
 99 *Brucella* via a direct Omp25/CD150 recognition. For this purpose we generated a new *Pu.1^{+GFP}*
 100 reporter mouse model lacking CD150 (*CD150^{-/-}; Pu.1^{+GFP}*). Infected *CD150^{-/-}* mice showed
 101 equivalent bacterial load in both spleen and BM during the onset and acute phase of infection
 102 (Supplementary Fig. 2). Moreover, infection did not affect either the total number of BM cells or
 103 the number of Lin⁻ cells in *CD150^{-/-}* mice (Supplementary Fig. 1). At 2 days post-infection, BM
 104 HSC from *CD150^{-/-}; Pu.1^{+GFP}* mice did not present any increase of GFP expression (Fig. 2b, right
 105 panel) in contrast to what we observed in *Pu.1^{+GFP}* mice (Fig. 2b, left panel), suggesting that the
 106 induction of PU.1 by *B. abortus* is mediated by CD150.

107

108 To further test whether *B. abortus* directly binds CD150, PU.1 expression in HSC was analysed
 109 during infection with *B. abortus* WT and *B. abortus* lacking Omp25 (*Ba Δomp25*) in *Pu.I^{+/GFP}*
 110 reporter mice (Fig. 2a). The upregulation of PU.1 observed in HSC in response to *B. abortus* WT
 111 infection was abolished when infected with *Ba Δomp25* (Fig. 2b left panel), while bacterial CFU
 112 counts in spleen and BM were similar to those in mice infected with *B. abortus* WT (Supplementary
 113 Fig. 2). These data indicate that PU.1 upregulation in HSC during the onset of infection is
 114 dependent on Omp25/CD150 interaction.

115

116 To further demonstrate that the upregulation of PU.1 in HSC upon *Brucella* infection is due to the
 117 direct recognition of *B. abortus* Omp25 by CD150, we isolated HSC from the BM of *Pu.I^{+/GFP}*
 118 mice and treated them *ex vivo* with outer membrane vesicles (OMV) [26] from either *Brucella* WT
 119 or *Ba Δomp25* (Fig. 2c). At 16 h post-*ex vivo* stimulation of sorted HSC by *B. abortus* WT OMV,
 120 the number of GFP-expressing HSC increased two-fold (Fig. 2d), following the same trend as *in*
 121 *vivo* infection of *Pu.I^{+/GFP}* reporter mice (Fig. 2b). In contrast, upregulation of GFP-expressing
 122 HSC was abolished by either incubation of HSC with *Ba Δomp25* OMV (Fig. 2d) or with *B.*
 123 *abortus* WT OMV in the presence of CD150 blocking peptide (Fig. 2e) or in *CD150^{-/-}* (Fig. 2d).
 124 These data demonstrate that HSC can sense bacteria via a direct interaction of *Brucella* outer
 125 membrane protein Omp25 with CD150. This is the first demonstration of a direct recognition of a
 126 pathogen by HSC via CD150.

127

128 PU.1 upregulation in HSCs is a first sign of commitment towards the myeloid lineage [20, 27]. We
 129 therefore asked if the direct interaction between HSCs and *Brucella* induces a functional
 130 commitment of HSCs towards the myeloid lineage. We analysed the composition of HSC-
 131 downstream progenitors at 8 days post-infection in the BM. As expected, an increase in myeloid-
 132 biased MPP2/3 (Lin⁻, Sca⁺, cKit⁺, CD48⁺, CD135⁻) progenitors was observed in the BM of *wt* mice
 133 infected with *Ba* WT but not in the BM of *wt* mice infected with *Ba Δomp25* or infected mice

134 lacking CD150 (Fig. 3a and Supplementary Fig. 1c for gating strategy). By contrast, the number of
 135 lymphoid-biased MPP4 (Lin⁻, Sca⁺, cKit⁺, CD48⁺, CD135⁺) was similar in infected and non-
 136 infected mice (Fig. 3b). Moreover, analysis of downstream committed progenitors revealed an
 137 increase of GMP and blood myeloid cells (Fig. 3c, d and Supplementary Fig. 1c for gating
 138 strategy). In addition, infection of *wt* mice by the Ba $\Delta omp25c$ complemented strain (Ba $\Delta omp25$
 139 strain complemented with an Omp25-expressing plasmid) was able to rescue the increase of
 140 myeloid MPP2-3 and GMP demonstrating a direct role of Omp25 in controlling the increase of
 141 myeloid commitment via CD150 (Fig. 3a-c). Altogether, these data provide the first evidence that
 142 *B. abortus* induces an increase of myeloid cells production in a Omp25/CD150-dependent manner,
 143 though not at the expense of lymphoid cells.

144
 145 HSCs are known to both self-renew and differentiate in order to replenish the whole hematopoietic
 146 system, properties that can be tested by transplantation in an irradiated host [6, 28]. In order to
 147 functionally confirm that the increased production of myeloid progenitors and mature cells was
 148 initiated by direct stimulation of HSCs by *Brucella*, we co-transplanted *ex vivo* stimulated and
 149 unstimulated HSCs in the same recipient. Towards this end we sorted HSCs (KSL, CD48⁻, CD34⁻,
 150 CD135⁻) from CD45.1 *CD150*^{-/-} or CD45.1 *wt* mice, stimulated them *ex vivo* with *Brucella* for 30
 151 min and treated them with gentamicin to kill extracellular bacteria. We then transplanted the
 152 stimulated HSCs together with non-stimulated competitor HSCs (ratio 2:1) into lethally irradiated
 153 CD45.2 recipient mice (Fig. 3e). Blood analyses at 4 weeks post-transplantation show that HSCs
 154 stimulated with *B. abortus* WT generated more myeloid than lymphoid cells in the peripheral blood,
 155 compared to PBS-treated HSCs (Fig. 3f, upper panel and Supplementary Fig.3 for gating strategy).
 156 Again, the myeloid commitment bias of hematopoietic stem cells was abolished when HSCs were
 157 stimulated *ex vivo* with *B. abortus* $\Delta omp25$ (Fig. 3f, upper panel) or in *CD150*^{-/-} HSCs stimulated
 158 with *B. abortus* WT (Fig. 3f, lower panel). Furthermore, the increased myeloid to lymphoid ratio in
 159 the blood generated by *B. abortus* WT stimulated HSCs was transient and was not observed at 6-
 160 and 8-weeks post-transplantation. This indicates that HSCs re-equilibrate lineage commitment

161 without compromising long-term multi-lineage contribution, as is also observed for direct M-CSF
162 stimulation of HSC [20]. In summary, *in vivo* and *ex vivo* data prove for the first time that HSCs are
163 able to sense directly live bacteria such as *Brucella abortus* via CD150 and transiently increase the
164 production of myeloid cells in response.

165

166 Immune effector cell functions are globally altered in *CD150*^{-/-} mice [29]. We further examined
167 whether, following infection with *Brucella*, the observed lack of HSC myeloid lineage commitment
168 and downstream production of myeloid effectors in *CD150*^{-/-} mice is due to absent CD150-
169 mediated bacterial recognition in HSCs or globally defective innate immune cell effector function.
170 For this, we generated mixed hematopoietic chimera mice by transplanting a 1:1 mix of BM cells
171 from *CD150*^{+/+}; CD45.2 mice and *CD150*^{-/-}; CD45.1 mice into irradiated CD45.2 recipient mice
172 (Fig. 4a). This allowed investigation of both *CD150*^{+/+} HSCs and *CD150*^{-/-} HSCs in the same
173 environment and in the presence of *wt* innate immune cells during infection. At twelve weeks post-
174 transplantation, we infected chimeric mice with *B. abortus*. We then assessed their lineage output in
175 both CD45.2 (*CD150*^{+/+}) and CD45.1 (*CD150*^{-/-}) compartments by analysing the myeloid/lymphoid
176 ratio in mature blood cells and bone marrow progenitors (Fig. 4b-e). At 8 days post-infection with
177 *B. abortus* WT, the blood myeloid/lymphoid ratio was higher in the *wt* compartment compared to
178 the *CD150*^{-/-} compartment (Fig. 4b). The increase of blood myeloid/lymphoid ratio in the *wt*
179 compartment was also abolished upon Ba *Δomp25* infection of chimeric mice (Fig. 4b). In BM
180 progenitor cells, the percentage of GMP (Fig. 4c) and myeloid-biased MPP2-3 cells (Fig. 4d) was
181 also increased in the BM in a *Omp25*/CD150-dependent manner. Interestingly, *Omp25*/CD150
182 interaction did not perturb the number of lymphoid-biased multipotent progenitors MPP4 (Fig. 4e).
183 These results confirm that myeloid commitment induced by *B. abortus* *Omp25*/CD150 interaction
184 is intrinsic to HSC.

185 Infection by several pathogens is sometime associated with reduced red blood cell generation
186 leading to a so called acute arrest of hematopoiesis (AAH) [30]. Interestingly, in the *wt* mice
187 infected with *Brucella* the percentage of erythroid progenitors (MEP) in the BM decreased in a

188 Omp25/CD150 dependant manner (Supplementary Fig.4a). To investigate if the reduced level of
 189 MEP results in anemia, we infected *wt* and *CD150*^{-/-} mice i.p. with *B. abortus*. At D8 p.i. we
 190 measured the hematocrit in the blood of the infected mice (Supplementary Fig.4b). As expected, the
 191 hematocrit of infected mice was reduced compared to non-infected mice in an Omp25/CD150-
 192 dependent manner (Supplementary Fig.4b).

193 These results indicate that in addition to increased myeloid commitment, reduced production of red
 194 cells already at level of progenitors cells is a Omp25/CD150-dependent feature of *Brucella*
 195 infection.

196 Notably, anemia associated with a decrease of red blood cells, hematocrit and haemoglobin was
 197 detected in 302 human patients (31.8% of men and 25% women) (Supplementary Fig.4c). These
 198 clinical data suggest that *Brucella* also alters red cell production in humans as in the mouse model
 199 leading to anemia and lack of body oxygenation. This phenomenea is responsible of fatigue,
 200 changes in metabolism and sometimes organ damage [31].

201 We also observed a splenomegaly in brucellosis patients, corroborating the results we obtained
 202 using the mouse model (Supplementary Fig 3d).

203 In addition, enhanced myeloid commitment has been shown to promote pathogen clearance of *E.*
 204 *coli* and *Salmonella* Typhimurium [17]. Nevertheless, for some pathogens, HSPC expansion is
 205 detrimental to the host and benefits the pathogen [32]. *CD150*^{-/-} mice are protected from
 206 *Trypanosoma cruzi* lethal challenge but are sensitive to *Leishmania major* and to attenuated
 207 *Salmonella* Typhimurium [31]. Bacterial load in the spleen of Ba Δ omp25 *wt* infected mice or
 208 infected *CD150*^{-/-} mice at 4 weeks post-infection decreased compared to *wt* infected mice (Fig 4g).
 209 This suggests that the enhanced transient myeloid commitment induced by Omp25/CD150 benefits
 210 the bacterium. Indeed, *Brucella* infects and replicates in myeloid cells [7, 33]. This may be one of
 211 the strategies established by *Brucella* to promote chronic infection and help bacterial dissemination.
 212 The Omp25/CD150 axis can thus be considered as a new evasion strategy exploited by *B. abortus*
 213 to mediate its dissemination. Here, we present the novel finding demonstrating that CD150 is a

214 bacterial sensor for HSC. How chronic activation of HSC by *B. abortus* could affect long-term
215 function of HSC and the role of CD150 in HSC of patients experiencing a microbial challenge
216 would be worth investigating in the future.

217

218 **METHODS**

219 **Ethics**

220 Animal experimentation was conducted in strict compliance with good animal practice as defined
221 by the French Animal Welfare Bodies (Law 87–848 dated 19 October 1987 modified by Decree
222 2001-464 and Decree 2001-131 relative to European Convention, EEC Directive 86/609). INSERM
223 guidelines have been followed regarding animal experimentation (authorization No. 02875 for
224 mouse experimentation). All animal work was approved by the Direction Départementale Des
225 Services Vétérinaires des Bouches du Rhône and the Regional Ethic Committee (authorization
226 number 13.118). Authorisation of *Brucella* experimentation in BSL3 facility was given under the
227 numbers: AMO-076712016-5, AMO-076712016-6 and AMO-076712016-7. All efforts were made
228 to minimize suffering during animal handling and experimentation.

229 The study in humans was approved by the Ethics Committee of the Medical Faculty in Skopje,
230 Republic of North Macedonia (No 03-7670/2).

231 **Human study:**

232 The values of red blood cells, hematocrit and haemoglobin were retrospectively analyzed in 302
233 patients with human brucellosis before therapy was initiated. The patients were managed at the
234 University clinic of infectious diseases and febrile conditions in Skopje from 2007 to 2018. Males
235 were 217 and females 85 of them with a median age of 39 (range 3-79) years. The diagnosis of
236 brucellosis was based on clinical findings compatible with brucellosis (arthralgia, fever, sweating,
237 malaise, hepatomegaly, splenomegaly, signs of focal disease), confirmed by a qualitative positive
238 Rose Bengal test and a Brucellacapt assay of >1/320. Hemoglobin thresholds used to define anemia
239 were according to World Health Organization [World Health Organization (2008). Worldwide

prevalence of anaemia 1993–2005 (PDF). Geneva: World Health Organization. ISBN 978-92-4-159665-7. Archived (PDF) from the original on 12 March 2009. Retrieved 2009-03-25].

Mice

6-10 week-old female C57BL/6J mice from Charles River, *CD150*^{-/-} mice (kindly provided by Yusuke Yanagi) [29] or Pu.1^{+GFP} mice [21, 22], both on a C57BL/6J background, were used. Animals were housed in cages with water and food *ad libitum* in the CIPHE animal house facility, Marseille. Two weeks before the start of experiments, mice were transferred to the BSL3, CIPHE, Marseille, and kept under strict biosafety containment conditions all along infection with live bacteria.

Bacterial strains

Brucella abortus 2308 (Ba WT), *Brucella abortus* Δ omp25 (kanR) (Ba Δ omp25), or *Brucella abortus* Δ omp25c:pOmp25 (kanR, AmpR) (Ba Δ omp25c) were used for infection. Ba Δ omp25 was a gift from Pr. Ignacio Moriyón, University of Navarra.

***Brucella* infection**

Mice were inoculated intraperitoneally with 1×10^6 CFU in 100 μ l of PBS for each *Brucella* strain. Strains were grown in Tryptic Soy Agar (Sigma Aldrich) for 5 days, then overnight at 37°C for 16 h under shaking in Tryptic Soy Broth (Sigma Aldrich) with kanamycin for Ba Δ omp25 until the OD (OD at 600nm) reached 1.8 and with 25 μ g/mL for the Ba Δ omp25 strain or kanamycin and ampicillin 50 μ g/mL for the Ba Δ omp25pBBR4omp25 strain. All *Brucella* were kept, grown and used under strict biosafety containment conditions all along experiments in the BSL3 facility, Marseille. For Colony Forming Units (CFU) enumeration at different time points post-infection, spleen and bone marrow were collected [7]. Femur and tibia were flushed with 500 μ l of ice-cold

266 PBS to isolate BM cells. BM cell suspension was then plated in TSA plates. Spleens were collected
267 and splenocytes were isolated by mechanical disruption.
268 Organs were harvested at 2, 8 or 30 days post-infection, weighted and then dissociated into sterile
269 endotoxin free PBS. Serial dilutions in sterile 1xPBS were used to count CFU. Serial dilutions were
270 plated in triplicates onto TSB agar to enumerate CFU after 3 days at 37°C.

271

272

273 **Transplantation assay**

274 All donor cells were from *CD150^{+/+}* and *CD150^{-/-}* CD45.1 mice and transplanted into lethally
275 irradiated (5,9 Gy) CD45.2 recipient mice. For competitive assays, mice were transplanted with
276 equal numbers of 1×10^6 total BM cells or 1×10^6 lineage negative cells. For infected HSC
277 transplantation, 1000 Sorted HSC (KSL, CD48⁻, CD135⁻, CD34⁻) were infected with Ba *WT* or Ba
278 *Δomp25* at a MOI of 30:1. Bacteria were centrifuged onto cells at 400 g for 10 min at 15°C and then
279 incubated for 45 min at 37°C under 5% CO₂. Cells were washed twice with medium and then
280 incubated for 1 h in medium containing 100 µg/ml gentamicin (Sigma Aldrich) to kill extracellular
281 bacteria. Cells were then washed 3 times with PBS. Infected cells were mixed in a ratio (2:1) with
282 non-infected HSC before transplantation. Haematopoietic reconstitution and lineage determination
283 were monitored at 4 weeks, 6 weeks and 8 weeks post-transplantation in the peripheral blood. At 8
284 weeks post-transplantation mice were sacrificed and tibia and femurs were harvested. BM cells were
285 flushed from femur and tibia and resuspended in FACS media (PBS, 2%FCS, 5mm EDTA) for
286 Flow Cytometry analyses.

287

288 **Flow Cytometry**

289 For FACS sorting and analysis we used a FACSAriaIII or a LSR-X20 (BD) and the FlowJo
290 software v10 (Treestar). For HSC and progenitor analysis, total BM cells were depleted of mature
291 cells using a direct lineage depletion kit (Miltenyi Biotec) and stained with antibodies anti-CD34-
292 APC or anti-BV421 (BD Bioscience, cloneRAM34), anti-CD135-PE-CF594 or anti-PE (BD

293 Bioscience, clone A2F10.1), anti-CD150-PE-Cy7 or anti-BV711 (BioLegend, clone TC15-
294 12F12.2), anti-CD117-BV605 (BioLegend, clone 2B8), anti-Sca-1-PrpCy5.5 or anti-PE
295 (ThermoFischer Scientist, clone D7), anti-CD48-BV510 or anti-PE-Cy7 (BD Bioscience, clone
296 HM48-1), anti-CD16/32-PE or anti-APC-Cy7 (BD Bioscience, clone 2.4G2). When needed, anti-
297 CD45.1-APC or anti-BV421 (BD Bioscience, clone A20) and anti-CD45.2-FITC or anti-PrpCy5.5
298 (BD Bioscience, clone 104) were added. LIVE/DEAD (UV Fixable Blue Dead Cell Stain,
299 ThermoFischer) was used as viability marker.

300 Blood cells were stained with anti-CD11b FITC (eBioescience, clone M1/70), anti-CD19-PE-Cy7
301 (BioLegend, clone 6D5), anti-CD45.2-PrpCy5.5 (BD Bioscience, clone 104), anti-CD45.1-BV421
302 (BD Bioscience, clone A20), anti-CD3e-APC (BD Bioscience, clone 145-2C11) and anti-Ly6G-PE
303 (BD Bioscience, clone 1A8). Red blood cells were lysed using BD FACS lysing solution (BD) for
304 10 min then fixed for 20 min with Antigen Fix, prior to acquisition.

305

306 **Haematopoietic Stem Cells *ex vivo* challenge with *Brucella abortus* membrane extracts**

307 All cultures were performed at 37°C under 5% CO₂. Sorted HSC from wt or *CD150*^{-/-} mice were
308 cultured in StemSpan SFEMII (Stem Cells) complemented with 50 ng/μL TPO (Peprotech) and 20
309 ng/μL SCF (Peprotech). Cells were stimulated with *Brucella* membrane extracts from Ba WT or
310 with Ba *Δomp25* (10 μg/ml). *Brucella* membrane extracts were a gift from Pr. I. Moriyon,
311 University of Navarra.

312 Sorted HSC were also cultured with blocking CD150 peptide (FCKQLKLYEQVSPPE, Auspep,
313 100 μg/ml) or control peptide (DLSKGSYPDHLEDGY, Auspep, 100 μg/ml) (Thermo Scientific).

314

315 **Statistics**

316 Results were evaluated by GraphPad Prism v8 software (GraphPad Software, San Diego, CA, USA)
317 using. Statistical tests used are indicated in the figure legends. The value of *P < 0.05 was
318 determined as significant.

319

320 **DATA AVAILABILITY**

321 No datasets were generated during the current study.

322

323 REFERENCES

- 324 1. Nebe, C.T., et al., *Detection of Chlamydophila pneumoniae in the bone marrow of two*
325 *patients with unexplained chronic anaemia*. European journal of haematology, 2005.
326 **74**(1): p. 77-83.
- 327 2. Eldin, C., et al., *18F-FDG PET/CT as a central tool in the shift from chronic Q fever to*
328 *Coxiella burnetii persistent focalized infection: A consecutive case series*. Medicine, 2016.
329 **95**(34).
- 330 3. Allen, M.B., et al., *First reported case of Ehrlichia ewingii involving human bone marrow*.
331 J Clin Microbiol, 2014. **52**(11): p. 4102-4.
- 332 4. Hardy, J., P. Chu, and C.H. Contag, *Foci of Listeria monocytogenes persist in the bone*
333 *marrow*. Dis Model Mech, 2009. **2**(1-2): p. 39-46.
- 334 5. Reece, S.T., et al., *Mycobacterium tuberculosis-Infected Hematopoietic Stem and*
335 *Progenitor Cells Unable to Express Inducible Nitric Oxide Synthase Propagate*
336 *Tuberculosis in Mice*. J Infect Dis, 2018. **217**(10): p. 1667-1671.
- 337 6. Weissman, I.L., *Clonal origins of the hematopoietic system: the single most elegant*
338 *experiment*. The Journal of Immunology, 2014. **192**(11): p. 4943-4944.
- 339 7. Gutierrez-Jimenez, C., et al., *Persistence of Brucella abortus in the Bone Marrow of*
340 *Infected Mice*. J Immunol Res, 2018. **2018**: p. 5370414.
- 341 8. Franco, M.P., et al., *Human brucellosis*. The Lancet Infectious Diseases, 2007. **7**(12): p.
342 775-786.
- 343 9. Boettcher, S., et al., *Endothelial cells translate pathogen signals into G-CSF-driven*
344 *emergency granulopoiesis*. Blood, 2014. **124**(9): p. 1393-403.
- 345 10. Boettcher, S. and M.G. Manz, *Regulation of Inflammation- and Infection-Driven*
346 *Hematopoiesis*. Trends Immunol, 2017. **38**(5): p. 345-357.
- 347 11. Kiel, M.J., et al., *SLAM family receptors distinguish hematopoietic stem and progenitor*
348 *cells and reveal endothelial niches for stem cells*. Cell, 2005. **121**(7): p. 1109-21.
- 349 12. Berger, S.B., et al., *SLAM is a microbial sensor that regulates bacterial phagosome*
350 *functions in macrophages*. Nat Immunol, 2010. **11**(10): p. 920-7.
- 351 13. Degos, C., et al., *Omp25 - dependent engagement of SLAMF1 by Brucella abortus in*
352 *dendritic cells limits acute inflammation and favours bacterial persistence in vivo*.
353 Cellular Microbiology, 2020.
- 354 14. Takizawa, H., et al., *Pathogen-Induced TLR4-TRIF Innate Immune Signaling in*
355 *Hematopoietic Stem Cells Promotes Proliferation but Reduces Competitive Fitness*. Cell
356 Stem Cell, 2017. **21**(2): p. 225-240 e5.
- 357 15. Mitroulis, I., et al., *Modulation of Myelopoiesis Progenitors Is an Integral Component of*
358 *Trained Immunity*. Cell, 2018. **172**(1-2): p. 147-161 e12.

- 359 16. Kobayashi, H., et al., *Bacterial c-di-GMP affects hematopoietic stem/progenitors and*
360 *their niches through STING*. Cell Rep, 2015. **11**(1): p. 71-84.
- 361 17. Takizawa, H., S. Boettcher, and M.G. Manz, *Demand-adapted regulation of early*
362 *hematopoiesis in infection and inflammation*. Blood, 2012. **119**(13): p. 2991-3002.
- 363 18. Pronk, C.J., et al., *Tumor necrosis factor restricts hematopoietic stem cell activity in mice:*
364 *involvement of two distinct receptors*. Journal of Experimental Medicine, 2011. **208**(8):
365 p. 1563-1570.
- 366 19. Pietras, E.M., *Inflammation: a key regulator of hematopoietic stem cell fate in health and*
367 *disease*. Blood, 2017. **130**(15): p. 1693-1698.
- 368 20. Mossadegh-Keller, N., et al., *M-CSF instructs myeloid lineage fate in single*
369 *haematopoietic stem cells*. Nature, 2013. **497**(7448): p. 239-43.
- 370 21. Back, J., et al., *PU. 1 determines the self-renewal capacity of erythroid progenitor cells*.
371 Blood, 2004. **103**(10): p. 3615-3623.
- 372 22. Bryder, D., D.J. Rossi, and I.L. Weissman, *Hematopoietic stem cells: the paradigmatic*
373 *tissue-specific stem cell*. The American journal of pathology, 2006. **169**(2): p. 338-346.
- 374 23. Kobayashi, H., et al., *Bacterial c-di-GMP affects hematopoietic stem/progenitors and*
375 *their niches through STING*. Cell reports, 2015. **11**(1): p. 71-84.
- 376 24. Burberry, A., et al., *Infection mobilizes hematopoietic stem cells through cooperative*
377 *NOD-like receptor and Toll-like receptor signaling*. Cell Host Microbe, 2014. **15**(6): p.
378 779-91.
- 379 25. Kiel, M.J., et al., *SLAM Family Receptors Distinguish Hematopoietic Stem and Progenitor*
380 *Cells and Reveal Endothelial Niches for Stem Cells*. Cell, 2005. **121**(7): p. 1109-1121.
- 381 26. Boigegrain, R.A., et al., *Release of periplasmic proteins of Brucella suis upon acidic shock*
382 *involves the outer membrane protein Omp25*. Infect Immun, 2004. **72**(10): p. 5693-703.
- 383 27. Essers, M.A., et al., *IFNalpha activates dormant haematopoietic stem cells in vivo*. Nature,
384 2009. **458**(7240): p. 904-8.
- 385 28. Till, J.E. and E.A. McCulloch, *A direct measurement of the radiation sensitivity of normal*
386 *mouse bone marrow cells*. Radiation research, 1961. **14**(2): p. 213-222.
- 387 29. Davidson, D., et al., *Genetic evidence linking SAP, the X-linked lymphoproliferative gene*
388 *product, to Src-related kinase FynT in T(H)2 cytokine regulation*. Immunity, 2004.
389 **21**(5): p. 707-17.
- 390 30. Bi, L., et al., *Acute arrest of hematopoiesis induced by infection with Staphylococcus*
391 *epidermidis following total knee arthroplasty: A case report and literature review*. Exp
392 Ther Med, 2016. **11**(3): p. 957-960.
- 393 31. Silverberg, D.S., et al., *The pathological consequences of anaemia*. Clin Lab Haematol,
394 2001. **23**(1): p. 1-6.
- 395 32. Abidin, B.M., et al., *Infection-adapted emergency hematopoiesis promotes visceral*
396 *leishmaniasis*. PLoS Pathog, 2017. **13**(8): p. e1006422.

397 33. Salcedo, S.P., et al., *BtpB, a novel Brucella TIR-containing effector protein with immune*
 398 *modulatory functions*. *Frontiers in Cellular and Infection Microbiology*, 2013. **3**.
 399

400 **ACKNOWLEDGEMENTS**

401 We thank all the staff of the CIML and CIPHE mouse houses, Atika Zouine, Marc Barad, and
 402 Sylvain Bigot of the CIML flow cytometry facility, Dr. Hervé Luche, Pierre Grenot of the CIPHE
 403 flow cytometry facility, Claude Napez and Philippe Hoest of the CIPHE BSL-3 facility. We
 404 acknowledge Pr. Ignacio Moriyon (University of Pamplona, France) for generous gift of
 405 mutant *Brucella* strains and Dr. Yusuke Yanagi and Dr. Masato Kubo for providing us with
 406 *CD150^{-/-}* CD45.1 mice. We thank Dr. Taymour Hamoudi from University of California San
 407 Francisco for critical reading of the manuscript and Dr. Sylvie Memet and Dr. Guillaume
 408 Hoeffel for discussions about this work.

409 This study was supported by institutional grants from the Institut National de la Santé et de la
 410 Recherche Médicale, Centre National de la Recherche Scientifique, and Aix-Marseille
 411 University to CIML and grants to JPG from Fondation pour la Recherche médicale (FRM grant
 412 number DQ20170336745), the Agence Nationale de Recherche/Investissements d'Avenir-
 413 Labex INFORM (ANR-11-LABX-0054) and Agence Nationale de Recherche/Investissements
 414 d'Avenir-A*MIDEX (ANR-11-IDEX-0001-02), to SS from ITMO Cancer Aviesan (Alliance
 415 Nationale pour les Sciences de la Vie et de la Santé, National Alliance for Life Science and
 416 Health) within the framework of the Cancer Plan (ISC19032ASA) and to M.H. Sieweke from
 417 the Agence Nationale pour la Recherche (ANR-17-CE15-0007-01 and ANR-18-CE12-0019-03),
 418 Fondation ARC pour la Recherche sur le Cancer (PGA1 RF20170205515), an INSERM-
 419 Helmholtz cooperation and the European Research Council (ERC) under the European Union's
 420 Horizon 2020 research and innovation program (grant agreement number 695093 MacAge).
 421 LH received a fellowship from Investissements d'Avenir-Labex INFORM, BDL received a
 422 fellowship from the Fondation ARC. M.H. Sieweke was supported as a Berlin Institute of

423 Health Einstein visiting fellow at MDC and is an Alexander von Humboldt Professor at TU
424 Dresden.

425

426 **AUTHORS CONTRIBUTION**

427 J.P.G, M.S, S.S conceived and J.P.G, S.S supervised the study. J.P.G , M.S, S.S, B.D.L, L.H, V.A
428 design the experiments. L.H, G.G. and V.A performed all BSL-3 experiments and B.D.L and G.D
429 performed experiments not requiring a BSL-3 facility. M.B was responsible of human studies.
430 J.P.G, M.S, S.S, B.D.L, L.H, V.A interpreted the data. J.P.G , M.S, S.S, B.D.L, L.H, V.A wrote the
431 manuscript

432

433 **DECLARATION OF INTERESTS**

434 The authors declare no competing interests.

435

436 FIGURES LEGENDS

437 **Figure 1: *Brucella abortus* persists in the BM and affects HSPC homeostasis.**

438 **a)** Experimental scheme: Mice were intraperitoneally inoculated with 1×10^6 CFU of wild-type *B.*
 439 *abortus*. BM cells were isolated from femur and tibia of the infected mice, resuspended in PBS and
 440 transplanted into previously lethally irradiated mice. 8 weeks after transplantation CFU per gram of
 441 organ were enumerated from spleens and bone marrow (BM). **b)** Enumeration of CFUs per gram of
 442 spleen and BM at 8 weeks post transplantation (n=7). **c-d)** C57BL/6J wild-type (wt) mice were
 443 intraperitoneally inoculated with 1×10^6 CFU of wild-type *B. abortus*. Two, eight and thirty days
 444 later, FACS analyses were performed for BM cells. Representative FACS profiles (**d**) and frequency
 445 of LSK (lin⁻,Sca⁺,cKit⁺) (from left to right, n=30 ; 15 ; 21), LSK CD48⁺ (lin⁻,Sca⁺,cKit⁺ CD48⁺)
 446 (from left to right, n=28 ; 14 ; 17), LSK CD48⁻ (lin⁻,Sca⁺,cKit⁺ CD48⁻) (from left to right, n=30 ; 17
 447 ; 20), LSK CD48⁻ CD34⁺ (lin⁻,Sca⁺,cKit⁺ CD48⁻, CD34⁺, CD135⁻) (from left to right, n=24 ; 14 ;
 448 19), HSCST (lin⁻,Sca⁺,cKit⁺ CD48⁻, CD135⁻ CD34⁻,CD150⁻) (from left to right, n=25 ; 17 ; 16),
 449 HSC^{LT} (lin⁻,Sca⁺,cKit⁺ CD48⁻, CD135⁻ CD34⁻,CD150⁺) (from left to right, n=22 ; 17 ; 22) in
 450 lineage negative fraction of BM (**c**) for PBS treated (Mock ○) and infected mice (■). Data were
 451 obtained from distinct samples and from
 452 5 independent experiments, each with at least n=3 animals per condition, are shown and mean
 453 ± SEM is represented by horizontal bar. Significant differences from mock are shown. *** $P <$
 454 0.001, ** $P <$ 0.01, * $P <$ 0.05. Absence of P value or ns, non-significant. Since data followed
 455 normal distribution, P-Value were generated using Brown-Forsyth followed by ANOVA Welch test.

457 **Figure 2: *Brucella* induces PU.1 upregulation in a Omp25/CD150 dependent manner**

458 **a)** Experimental scheme *Pu.1*^{+GFP} and *CD150*^{-/-} *Pu.1*^{+GFP} mice were intraperitoneally injected with
 459 PBSx1 (Mock, ○) or inoculated with 1×10^6 CFU of wild-type *B. abortus* (Ba WT, ■), Ba Δ omp25
 460 (■). Two days after, the percentage of GFP expression in HSCs (lin⁻,Sca⁺,cKit⁺ CD48⁻, CD135⁻
 461 CD34⁻) was assessed by Flow Cytometry. **b)** GFP expression in BM HSCs (lin⁻,Sca⁺,cKit⁺ CD48⁻,
 462 CD135⁻ CD34⁻) assessed by Flow Cytometry at D2 p.i.. Data (b) were obtained from distinct
 463 samples (from left to right, n=5 ; 10 ; 5 ; 5 ; 6) from 3 independent experiments. Mean ± SD is
 464 represented by horizontal bar. Significant differences from mock are shown. *** $P <$ 0.001, ** $P <$
 465 0.01, * $P <$ 0.05. Absence of P value or ns, non-significant. Since data did not follow normal
 466 distribution, P-Value were generated using Kruskal-Wallis followed by Dunn's test. **c)** Experimental
 467 scheme: HSC (lin⁻,Sca⁺,cKit⁺ CD48⁻, CD135⁻ CD34⁻) from *Pu.1*^{+GFP} or *CD150*^{-/-} *Pu.1*^{+GFP} mice
 468 were sorted, then stimulated *ex vivo* with PBSx1 or *B. abortus* WT OMVs, *B. abortus* Δ omp25
 469 OMVs. After 16h, the level of GFP in cells was assessed by Flow Cytometry. **d)** Fold change ratio

of GFP expression in HSC 16 hours after *ex vivo* stimulation with *B. abortus* WT OMVs (■), *B. abortus* $\Delta omp25$ OMVs (■) compared to mock (Mock, ○) assessed by Flow Cytometry (from left to right, n=6 ; 6 ; 6 ; 5 ; 5). e) Percentage of GFP⁺ HSC assessed by Flow Cytometry 0h (Mock, ○, n=4) or 16h after *ex vivo* stimulation with *B. abortus* WT (■, n=4), *B. abortus* WT OMVs and the control peptide (100μg/ml) (■, n=2) and blocking peptide of CD150 (100μg/ml) (■, n=2). Data (e, f) were obtained from HSC of a pool of 3-4 mice, the pool of cells have been divided by the number of tested conditions. Each dot is a replicative experiment. Mean ± SD is represented by horizontal bar. Significant differences from mock are shown. ** P< 0.01, * P< 0.05. P-Value were generated using Mann Whitney test.

Figure 3: *B. abortus* induces HSC differentiation towards myeloid lineage.

a-d) C57BL/6J wild-type (*wt*) and *CD150*^{-/-} mice were intraperitoneally inoculated with 1x10⁶ *B. abortus* CFUs. Eight days later, FACS analyses were performed for BM cells. Frequency of (a) MPP2-3 (lin⁻, Sca⁺, cKit⁺ CD48⁺ CD135⁻) (from left to right : n=22 ; 19 ; 14 ; 6 ; 11 ; 9 ; 8), (b) MPP4 (lin⁻, Sca⁺, cKit⁺ CD48⁺ CD135⁺) (from left to right : n=22 ; 21 ; 12 ; 4 ; 9 ; 9 ; 9), (c) GMP (lin⁻, Sca⁻, cKit⁺ CD34⁺ CD16/32⁺) (from left to right : n=16 ; 18 ; 13 ; 4 ; 8 ; 9 ; 9), in Lin⁻ BM cells is shown for (Mock, ○) or inoculated with 1x10⁶ CFU of wild-type *B. abortus* (Ba WT, ■), *B. abortus* $\Delta omp25$ (Ba $\Delta omp25$ ■) or *B. abortus* $\Delta omp25$ complemented with p:Omp25 (Ba $\Delta omp25c$ □) mutants (the latter only for *wt* mice). **d)** Eight days post-infection, myeloid cells (CD45⁺, CD11b⁺) to lymphoid cells (CD3e⁺CD19⁺) ratio in blood is shown for (Mock, ○, n=11) or inoculated with 1x10⁶ CFU of wild-type *B. abortus* (Ba WT, ■, n=9), *B. abortus* $\Delta omp25$ (Ba $\Delta omp25$ ■). **e)** Experimental scheme: HSC from *wt* CD45.1 and *CD150*^{-/-} CD45.1 mice were sorted and then incubated *ex vivo* with *B. abortus* WT and *B. abortus* $\Delta omp25$ for 30 minutes. After 30 minutes, cells were washed and treated for 1 hour with gentamycin to kill extra-cellular bacteria. HSC were then transplanted into lethally irradiated *wt* CD45.2 recipients. FACS analyses of blood samples were performed at 4, 6 and 8 weeks post-transplantation. **f)** myeloid cells (CD45⁺, CD11b⁺) to lymphoid cells (CD3e⁺CD19⁺) ratio in CD45.1⁺ blood cells is shown for hematopoietic cells provided by CD45.1⁺ *wt* mice (upper panel) or *CD150*^{-/-} mice, (lower panel) , non-infected (Mock, ○) or infected with 1x10⁶ CFU of wild-type *B. abortus* (Ba WT, ■), *B. abortus* $\Delta omp25$ (Ba $\Delta omp25$ ■) as described in e (from left to right, for WT n=12 ; 13 ; 10 ; 10 ; 9 ; 9 ; 12 ; 8 ; 8 and for CD150^{-/-} n= 9 ; 4 ; 14 ; 8 ; 11 ; 7). Data were obtained from distinct samples from 4 independent experiments (a-d) or from repetitive sampling from 2 (e-f) independent experiments. Mean ± SEM is represented by horizontal bar. Significant differences from mock are shown. *** P< 0.001, ** P< 0.01, * P<

0.05. Absence of P value or ns, non-significant. Since data did not follow normal distribution, P-Value were generated using Kruskal-Wallis followed by Dunn's test.

Figure 4: HSC myeloid bias induced by *Brucella* Omp25/CD150 interaction is hematopoietic cell autonomous.

a) Experimental scheme: BM cells from *CD150*^{-/-} CD45.1 mice and *wt* CD45.2 mice were isolated from tibia and femur of mice and transplanted into lethally irradiated recipient mice. Twelve weeks after transplantation mice were intraperitoneally injected with PBSx1 (Mock, ○) or inoculated with 1x10⁶ CFU of wild-type *B. abortus* (Ba WT), *B. abortus* Δ omp25 (Ba Δ omp25). Blood and BM were analysed 8 days after. **b)** Myeloid (CD45⁺, CD11b⁺ cells) to lymphoid (CD3e⁺ and CD19⁺ cells) in the blood (from left to right, n=8 ; 8 ; 8 ; 8 ; 7 ; 7); frequency of **c)** GMP (from left to right, n=6 ; 6 ; 7 ; 7 ; 9 ; 9), **d)** MPP2-3 (from left to right, n=6 ; 6 ; 6 ; 6 ; 9 ; 9) and **e)** MPP4 (from left to right, n=6 ; 6 ; 8 ; 8 ; 9 ; 9) in BM Lin⁻ cells of *wt* (circle) and *CD150*^{-/-} (square) compartment is shown for chimeric mice intraperitoneal injected with PBS (Mock, non-filled symbols) or inoculated with 1x10⁶ CFU of wild-type *B. abortus* (symbol filled in black) *B. abortus* Δ omp25 (symbol filled in grey) as described in a. **e-f)** CFU count per gram of organ at Day 30 post infection of WT and *CD150*^{-/-} mice for (e) BM (from left to right n=18 ; 9 ; 8 ; 12 ; 6 ; 6) and (f) spleen (from left to right n= 14 ; 18 ; 7 ; 7 ; 8 ; 6) infected with *B. abortus* WT (Ba WT ■), *B. abortus* Δ omp25 (Ba Δ omp25 ■) or *B. abortus* Δ omp25 complemented with p:Omp25 (Ba Δ omp25c □). Mean $\pm$ SEM is represented by horizontal bar. Significant differences from mock are shown. *** P< 0.001, ** P< 0.01, * P< 0.05. Absence of P value or ns, non-significant. Since data did not follow normal distribution, P-Value were generated using Kruskal-Wallis followed by Dunn's test.

Supplementary 1: *Brucella* infection does not affect the number of lineage negative progenitors and BM cells

a-b) *wt* and *CD150*^{-/-} mice were intraperitoneally injected with PBS or inoculated with 1x10⁶ CFU of *B. abortus*. Eight days later, BM cells were isolated, cells were counted (**a**) and then depleted for mature hematopoietic cells as shown in Methods. Lin⁻ cells (**b**) were also counted for (Mock, ○) or infected *B. abortus* (Ba WT, ■), *B. abortus* Δ omp25 (Ba Δ omp25 ■) or *B. abortus* Δ omp25 complemented with p:Omp25 (Ba Δ omp25c □) mutants (the latter only for *wt* mice). From left to right, for BM, n=11 ; 14 ; 8 ; 5 ; 9 ; 11 ; 9 and for Lin⁻ BM, n=18 ; 16 ; 8 ; 6 ; 6 ; 7 ; 9. Data obtained from distinct samples from 5 independent experiments.

Mean $\pm$ SEM is represented by horizontal bar. Significant differences from mock are shown. *** P<0.001, ** P< 0.01, * P< 0.05. Absence of P value or ns, non-significant. Since data did not follow normal distribution, P-Value were generated using Kruskal-Wallis followed by Dunn's test. c)

Complete FACS gating for analysis of HSC and progenitors from lineage negative fraction of BM. First, cells are gated based on SSC/ FSC and then single cells were selected. Viable cells were gated using UV Fixable Blue Dead stain. Described population are : LSK (lin⁻, Sca⁺, cKit⁺), HSCLT (lin⁻, Sca⁺, cKit⁺ CD48⁻, CD135⁻ CD34⁻, CD150⁺), MPP2-3 (lin⁻, Sca⁺, cKit⁺ CD48⁺, CD135⁻), MPP4 (lin⁻, Sca⁺, cKit⁺ CD48⁺, CD135⁺), GMP (lin⁻, Sca⁻, cKit⁺, CD34⁺, CD16/32⁺) and MEP (lin⁻, Sca⁻, cKit⁺, CD34⁻, CD16/32⁻).

Supplementary 2: Infection burden in *Pu.I*^{+/GFP} mice

a-b) CFU count per gram of organ at Day 2 post infection as described in Fig 2.a. for **(a)** spleen (from left to right n=10 ; 6 ; 6 ; 8) and **(b)** BM (from left to right n=7 ; 6 ; 7 ; 7) of PBS injected (Mock, ○) or infected with *B. abortus* WT (Ba WT, ■), *B. abortus* Δ omp25 (Ba Δ omp25 ■) *Pu.I*^{+/GFP} and *CD150*^{-/-} *Pu.I*^{+/GFP} mice. Data obtained from distinct samples from 3 independent experiments. Mean $\pm$ SEM is represented by horizontal bar. Significant differences from mock are shown. *** P< 0.001, ** P< 0.01, * P< 0.05. Absence of P value or ns, non-significant. Since data did not follow normal distribution, P-Value were generated using Kruskal-Wallis followed by Dunn's test.

Supplementary 3: FACS gating for blood analysis from chimeric mice

Complete FACS gating for blood lineage output from chimeric mice. First, cells are gated based on SSC/ FSC and then single cells were selected. *CD150*^{-/-} and WT cells were separated by gating on respectively CD45.1 and CD45.2 positive cells. In each, lymphoid cells were isolated by gating on CD11b negative cells and then, B cells are CD19 positive cells and T cells are CD3e positive cells. In Cd11b positive cells, granulocytes and monocytes are distinguished by gating onto respectively LY6G positive and negative cells.

Supplementary 4: Brucellosis induces anemia.

a-b) *wt* CD45.1 and *CD150*^{-/-} CD45.1 mice were intraperitoneally inoculated with 1x10⁶ CFU of *B. abortus*. BM cells were isolated from femur and tibia of the infected mice. a) Frequency of **MEP** (lin⁻, Sca⁻, cKit⁺ CD34⁻ CD16/32⁻), in Lin⁻ BM cells is shown for (Mock, ○) or inoculated with 1x10⁶ CFU of wild-type *B. abortus* (Ba WT, ■), *B. abortus* Δ omp25 (Ba Δ omp25 ■) or *B. abortus* Δ omp25 complemented with p:Omp25 (Ba Δ omp25c □) mutants (the latter only for wt mice) (from left to right : n=16 ; 16 ; 8 ; 8 ; 8 ; 7 ; 9). **b)** At eight days post-infection, the percentage of haematocrit measured in blood is shown for (Mock, ○) or inoculated with 1x10⁶ CFU of *wild-type* *B. abortus* (Ba WT, ■), *B. abortus* Δ omp25 (Ba Δ omp25 ■) (from left to right, n= 7 ; 7 ; 6 ; 5 ; 8 ; 8). **c)** Percentage of brucellosis patients that present anemia before antibiotic treatment. Men upper

575 panel and women lower panel. Anemia was characterized by a decrease of hematocrit, hemoglobin
 576 and erythrocytes (hematocrit <40% for men and <35% for women; hemoglobin <14g/dL for men
 577 and <12 g/dL for women, erythrocyte count <4 million for men and <3.8 million/mm³ for women).
 578 Data were obtained from distinct samples from 3 independent experiments (a-b), each with at least
 579 n=4 animals per condition, are shown and mean $\pm$ SEM is represented by horizontal bar. Significant
 580 differences from mock are shown. *** P< 0.001, ** P< 0.01, * P< 0.05. Absence of P value or ns,
 581 non-significant. Since data did not follow normal distribution, P-Value were generated using
 582 Kruskal-Wallis followed by Dunn's test.

Fig.2

Fig.4

a

b

c

Supplementary 4