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## **Sepsis expands a CD39+ plasmablast population that promotes immunosuppression via adenosine-mediated inhibition of macrophage antimicrobial activity**

Daniele Carvalho Nascimento, Paula Ramos Viacava, Raphael Gomes Ferreira, Marina Alves Damaceno, Annie Rocío Piñeros, Paulo Henrique Melo, Paula Barbim Donate, Juliana Escher Toller-Kawahisa, Daniel Zoppi, Flávio Protásio Veras, et al.

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1 **Sepsis expands a CD39+ plasmablast population that promotes**  
2 **immunosuppression via adenosine-mediated inhibition of**  
3 **macrophage antimicrobial activity**

4  
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1 **Abstract**

2 Sepsis results in elevated adenosine in circulation. Extracellular adenosine triggers  
3 immunosuppressive signaling via the A2a receptor (A2aR). Sepsis survivors develop  
4 persistent immunosuppression with increased risk of recurrent infections. We utilized the  
5 cecal ligation and puncture (CLP) model of sepsis and subsequent infection to assess  
6 the role of adenosine in post-sepsis immune suppression. A2aR-deficient mice showed  
7 improved resistance to post-sepsis infections. Sepsis expanded a subset of CD39<sup>hi</sup> B  
8 cells and elevated extracellular adenosine, which was absent in mice lacking CD39-  
9 expressing B cells. Sepsis-surviving B cell-deficient mice were more resistant to  
10 secondary infections. Mechanistically, metabolic reprogramming of septic B cells  
11 increased production of ATP, which was converted into adenosine by CD39 on  
12 plasmablasts. Adenosine signalling via A2aR impaired macrophage bactericidal activity  
13 and enhanced interleukin-10 production. Septic patients exhibited expanded CD39<sup>hi</sup>  
14 plasmablasts and adenosine accumulation. Our study reveals CD39<sup>hi</sup> plasmablasts and  
15 adenosine as important drivers of sepsis-induced immunosuppression, with relevance in  
16 human disease.

## 1 INTRODUCTION

2 Sepsis is a systemic inflammation triggered by pathogens that leads to organ  
3 dysfunction (Singer et al., 2016). Although advances in supportive care have  
4 reduced sepsis mortality over the last decades (Prescott and Angus, 2018),  
5 patients who survive severe sepsis have a high number of re-hospitalizations and  
6 increased mortality mainly due to recurrent infections (Otto et al., 2011; Wang et  
7 al., 2014). Compelling clinical and experimental studies indicate that sepsis may  
8 cause an immunosuppressive state that accounts for increased susceptibility to  
9 secondary, mostly opportunistic, infections (Benjamim et al., 2003; Boomer et al.,  
10 2011; Nascimento et al., 2017; Otto et al., 2011). Potential mechanisms for  
11 sepsis-induced immunosuppression include immune cell apoptosis, expansion of  
12 the regulatory T (Treg) cell population, and impaired microbial killing by  
13 macrophages (M $\phi$ s) (Venet and Monneret, 2018). However, the mechanisms  
14 underlying the persistent dysregulation of the host immune response in sepsis  
15 survivors are not entirely understood.

16 Extracellular adenosine is a signaling molecule that modulates several  
17 immunological processes via specific receptors expressed on immune cells  
18 (Hasko et al., 2008). Adenosine binds four different receptors: adenosine  
19 receptor 1 (A1R), A2aR, A2bR, and A3R (Fredholm et al., 2001). The signaling  
20 mediated by A2aR augments the production of anti-inflammatory cytokines,  
21 inhibits T cell proliferation, suppresses microbial killing by neutrophils and M $\phi$ s,  
22 and enhances differentiation of M2 M $\phi$ s (Hasko et al., 2008; Haskó and  
23 Cronstein, 2013).

1 A major source of extracellular adenosine is ATP hydrolysis by  
2 ectonucleotidases, such as CD39 and CD73 (Allard et al., 2017). CD39  
3 (ectonucleoside triphosphate diphosphohydrolase-1, ENTPD1) catalyzes the  
4 hydrolysis of extracellular ATP and ADP to AMP, while CD73 (ecto-5'  
5 nucleotidase, E5NT) converts AMP into adenosine (Colgan et al., 2006; Dwyer  
6 et al., 2007). During the acute phase of sepsis, patients show elevated adenosine  
7 levels in circulation (Jabs et al., 1998; Martin et al., 2000; Ramakers et al., 2011).  
8 We, therefore, hypothesized that increased extracellular adenosine might  
9 contribute to the development of immune suppression in sepsis survivors via  
10 activation of A2aR in immune cells.

11 Here, we showed that sepsis induced the expansion of splenic CD39<sup>hi</sup>CD138<sup>hi</sup>  
12 plasmablasts, which generated increased extracellular adenosine in sepsis  
13 survivors. Adenosine derived from CD39<sup>hi</sup>CD138<sup>hi</sup> plasmablasts promoted  
14 suppression of the immune response by impairing the microbicidal activity of  
15 macrophages via A2aR activation, rendering sepsis-surviving mice highly  
16 susceptible to secondary infections. Myeloid-specific deletion of A2aR improved  
17 the microbial resistance of sepsis-surviving mice. We found that septic patients  
18 also had an increased frequency of immunosuppressive CD39<sup>hi</sup> plasmablasts  
19 and adenosine accumulation in the blood. Our data, therefore, suggest that  
20 adenosine can be a potential therapeutic target for persistent  
21 immunosuppression in sepsis survivors.

## 1 RESULTS

### 2 Adenosine via A2aR mediates sepsis-induced immunosuppression

3 To investigate whether adenosine contributes to the development of  
4 immunosuppression after sepsis, we utilized cecal ligation and puncture (CLP;  
5 100% lethality), a clinically relevant sepsis model (Nascimento et al., 2010;  
6 Rittirsch et al., 2009). Following CLP, mice received antibiotic treatment  
7 (ertapenem), which increased survival (60%; [Figures 1A and 1B](#)). Mice that  
8 survived were then challenged at day 15 post-CLP with an intranasal (i.n.) sub-  
9 lethal dose of one of two opportunistic human pathogens, *L. pneumophila* or *A.*  
10 *fumigatus*, to examine host susceptibility to secondary infections ([Figure 1A](#)). As  
11 previously described (Nascimento et al., 2017), sepsis-surviving mice showed  
12 impaired microbial clearance and high susceptibility to both infections (100% and  
13 80% lethality, respectively), while all naive or sham-operated immune-competent  
14 mice survived until the end of the study period ([Figures 1C-1F and Figure S1A](#)).  
15 The high susceptibility of sepsis-surviving mice to challenge infection with *L.*  
16 *pneumophila* persisted for at least 75 days post-CLP ([Figure 1C](#)). Mice that  
17 survived moderate CLP model with or without antibiotics (70% and 20% lethality,  
18 respectively) were equally susceptible to the challenge infection with *L.*  
19 *pneumophila* ([Figures S1B and S1C](#)). We then measured adenosine  
20 concentration in the plasma at different time points after sepsis induction ([Figure](#)  
21 [1G](#)). Circulating adenosine increased in sepsis-surviving mice by day 15, which  
22 peaked around day 45 and remained elevated above naïve and sham-operated  
23 mice out to day 75 ([Figures 1H and 1I, Figures S1D and S1E and Figure S2I](#)).  
24

1 To investigate whether the A2aR contributes to immunosuppression, we treated  
2 CLP mice with A2aR antagonist (A2aRi; 8-3-chlorostyryl-caffeine) (Jacobson et  
3 al., 1993) and challenged sepsis-surviving mice with *A. fumigatus* or *L.*  
4 *pneumophila* 15 days post-CLP (Figure 1J). A2aR blockade enhanced host  
5 resistance against both infections (Figure 1K) and reduced microbial loads in the  
6 lungs and spleen of sepsis-surviving mice (Figure 1L). Sepsis-surviving A2aR-  
7 deficient (*Adora2a*<sup>-/-</sup>) mice also showed lower *L. pneumophila* counts in the lung  
8 and spleen than BALB/c WT mice (Figure 1M). The inhibition or deficiency of  
9 A2aR did not alter the survival of antibiotic-treated CLP mice (Figures S2A-S2C).  
10 Thus, adenosine signaling via the A2aR is required for the development of sepsis-  
11 induced immunosuppression.

12

### 13 **Adenosine accumulation in sepsis-surviving mice requires CD39 activity**

14 Because the hydrolysis of extracellular ATP by CD39 is considered the primary  
15 source of extracellular adenosine (Robson et al., 2006), we next asked whether  
16 CD39 contributes to post-sepsis immunosuppression. Flow cytometry analysis  
17 revealed increased CD39 expression in spleen cells from sepsis-surviving mice  
18 15 days post-CLP (Figure 2A). We then incubated spleen cells with ATP and  
19 measured inorganic phosphate (Pi) released in the cell supernatants to analyze  
20 ecto-ATPase activity. Spleen cells from sepsis-surviving mice showed elevated  
21 ecto-ATPase activity 15 days post-CLP compared to naive mice (Figure 2B). This  
22 was reduced by pre-incubation with CD39 inhibitor, ARL 67156 (CD39i)  
23 (Lévesque et al., 2007). These data suggest that CD39 activity is important for  
24 the hydrolysis of ATP by splenic cells from CLP mice.

25



1 To investigate whether CD39 is required for the increased circulating adenosine,  
2 we induced CLP in CD39-deficient (*Entpd1*<sup>-/-</sup>) mice or treated C57BL/6 WT mice  
3 with CD39i (Figure 2C). Both approaches abolished plasma adenosine  
4 accumulation in sepsis-surviving mice (Figures 2D-2F). Moreover, CD39  
5 inhibition improved survival rates of sepsis-surviving mice challenged with *A.*  
6 *fumigatus* or *L. pneumophila* 15 days after CLP (Figure 2G). *L. pneumophila*  
7 counts were reduced in the lungs and spleen of mice treated with CD39i  
8 compared to vehicle-treated mice (Figure 2H). Similarly, sepsis-surviving *Entpd1*<sup>-/-</sup>  
9 mice showed lower fungal burdens in the lungs and a higher survival rate to *A.*  
10 *fumigatus* infection than WT control mice (Figures 2I and 2J). Loss of CD39 did  
11 not alter the survival of antibiotic-treated CLP mice (Figures S2D and S2E).  
12 These results indicate that CD39 is critical for the production of extracellular  
13 adenosine and sepsis-induced immunosuppression development.

14

### 15 **Sepsis promotes the expansion of a CD39-expressing B cell population**

16 CD39 is expressed on many immune cell types (Antonioli et al., 2013; Dwyer et  
17 al., 2007). To gain an unbiased perspective on the CD39 expression pattern in  
18 immune cells from sepsis-surviving mice, we performed high-dimensional flow  
19 cytometric analyses of the splenic CD39<sup>+</sup> cell populations of naïve and sepsis-  
20 surviving mice. We used the t-distributed stochastic neighbor embedding (t-SNE)  
21 algorithm to perform an unsupervised analysis of the entire flow cytometry  
22 dataset (10 samples) generated from naïve and sepsis-surviving mice.  
23 Representative t-SNE maps color-coded according to cluster annotation for  
24 immune cell populations. Expression intensity revealed that most immune cell  
25 populations express CD39 in both naïve and sepsis-surviving mice 15 days after

1 CLP (Figures 3A and 3B and Figures S3A and S3B). However, the frequency of  
2 CD39<sup>+</sup> B cells and the levels of CD39 expression in B cells were particularly  
3 increased in sepsis-surviving mice compared to other immune cell populations  
4 and B cells from naïve mice (Figure 3C and Figure S3B). Moreover, a small  
5 population of CD39<sup>hi</sup> B cells was enriched in the spleens of sepsis survivors  
6 (Figures 3B and 3C and Figure S3B). CD39 expression was also increased in B  
7 cells from the blood but not from peripheral lymph nodes (LNs) of sepsis-surviving  
8 mice (Figures S3C and S3D). Compared with naïve mice, an increased  
9 proportion of CD39<sup>hi</sup> splenic B cells from sepsis-surviving mice co-expressed the  
10 proliferation marker Ki67 (Gerdes et al., 1984), suggesting that sepsis leads to  
11 an expansion of a CD39<sup>hi</sup> B cell population (Figure 3D). Sham and naïve mice ±  
12 antibiotics showed a lower frequency of splenic CD39<sup>+</sup> B cells than sepsis-  
13 surviving mice 15 days after CLP (Figure S1F).

14

15 To characterize B cell subsets expressing CD39, a separate high-dimensional  
16 flow cytometric analysis was performed. We enriched CD45<sup>+</sup>CD19<sup>+</sup> B cell  
17 populations from spleens by negatively selecting against the T and Treg cell  
18 markers CD3 and Foxp3. We then identified various CD19<sup>+</sup> B cell subsets based  
19 on traditional flow cytometry gating strategies, as described (Culton et al., 2006;  
20 Tung et al., 2006): pre-B (CD138<sup>-</sup>IgM<sup>-</sup>IgD<sup>-</sup>), immature B (CD138<sup>-</sup>IgM<sup>+</sup>IgD<sup>-</sup>), early-  
21 (CD138<sup>-</sup>IgM<sup>+</sup>IgD<sup>+</sup>) and late-mature B (CD138<sup>-</sup>IgM<sup>-</sup>IgD<sup>+</sup>) and plasmablast  
22 (CD138<sup>hi</sup>) cells (Figure S3E). Representative t-SNE maps color-coded according  
23 to cluster annotation for distinct CD39<sup>+</sup> B cell subsets showed that CD39 is  
24 expressed in most splenic B cell populations in both naïve and sepsis-surviving  
25 mice 15 days after CLP (Figure 3E). However, t-SNE heatmaps revealed that a

1 differentiated group of cells corresponding to CD138<sup>hi</sup> plasmablasts (orange)  
2 showed the highest CD39 expression in sepsis-surviving mice (Figures 3F and  
3 3G). To extend the analysis of CD39 expression on B cell subsets in sepsis-  
4 surviving mice, we performed a time-course experiment to profile CD39  
5 expression on B1a (CD138<sup>-</sup>CD5<sup>+</sup>CD23<sup>-</sup>), B1b (CD138<sup>-</sup>CD5<sup>-</sup>CD23<sup>-</sup>), and B2  
6 (CD138<sup>-</sup>CD5<sup>-</sup>CD23<sup>+</sup>) and plasmablast (CD138<sup>hi</sup>) cells in the spleen of mice after  
7 CLP induction (Figure 3H). We confirmed that plasmablasts expressed higher  
8 levels of CD39 than other B cell subsets, including B1a, B1b, and B2 cells (Figure  
9 3I). Besides, B1a and B1b cells from sepsis-surviving mice had increased  
10 expression and frequency of CD39, while these parameters were not altered in  
11 B2 cells (Figures 3I-3K). The frequency and the absolute number of  
12 CD39<sup>+</sup>CD138<sup>hi</sup> B cells increased in sepsis-surviving mice, peaking on day 7 and  
13 returning to baseline levels within 75 days after CLP, indicating that sepsis leads  
14 to the expansion of CD39<sup>+</sup>CD138<sup>hi</sup> B cells (Figures 3J-3M). In support of this,  
15 the frequency and number of Ki67<sup>+</sup>CD39<sup>+</sup>CD138<sup>hi</sup> B cells were increased in  
16 sepsis-surviving mice (Figures 3J and 3N-3P). Finally, to address which splenic  
17 B cell compartment CD39<sup>hi</sup> B cells reside, we performed flow cytometry analysis  
18 of splenic cells from sepsis-surviving mice on day 7 after CLP, including additional  
19 surface markers to characterize marginal zone (MZ) (B220<sup>+</sup>IgM<sup>+</sup>CD21<sup>hi</sup>CD23<sup>lo</sup>),  
20 follicular (FO) (B220<sup>+</sup>IgM<sup>+</sup>CD21<sup>int</sup>CD23<sup>hi</sup>), and immature transitional T1 B cells  
21 (B220<sup>+</sup>IgM<sup>+</sup>CD21<sup>-</sup>CD23<sup>-</sup>) (Figure S4A). CD39<sup>hi</sup> B cells were characterized in  
22 immature transitional T1 cells, co-expressing CD138<sup>hi</sup> (Figures S4B and S4C).  
23 Thus, sepsis induces the expansion of a CD39<sup>hi</sup>CD138<sup>hi</sup> plasmablast population.

24

25 **CD39<sup>+</sup> B cells are critical for sepsis-induced immunosuppression**

1 Our results led us to ask whether B cells contribute to extracellular adenosine  
2 production and, consequently, immunosuppression after sepsis. CD19<sup>+</sup> B cells  
3 sorted from the spleens of sepsis-surviving mice at day 15 post-CLP had  
4 increased mRNA levels of *Entpd1* (encoding CD39) (Figure 4A). We then  
5 assessed ATPase activity and the ability of B cells to generate adenosine through  
6 ATP hydrolysis. Upon addition of exogenous ATP, B cells from sepsis-surviving  
7 mice showed higher ecto-ATPase activity and produced more adenosine than  
8 naive B cells, which was abolished by CD39i (Figure 4B and 4C). Next, we  
9 measured plasma adenosine concentrations in *Rag1*<sup>-/-</sup> mice, which lack mature  
10 T- and B-lymphocytes. Sepsis-surviving *Rag1*<sup>-/-</sup> mice showed lower plasma  
11 adenosine concentration than WT mice at 15 days post-CLP. Adoptive transfer  
12 of naive CD19<sup>+</sup> B cells into *Rag1*<sup>-/-</sup> recipient mice before CLP induction increased  
13 the systemic concentration of adenosine to levels comparable to those observed  
14 in septic WT mice (Figures 4D-4F and Figure S2K). Nearly all transferred CD138<sup>hi</sup>  
15 B cells expressed CD39, and the Ki67<sup>+</sup> proportion was comparable between B  
16 cell-recipient *Rag1*<sup>-/-</sup> and WT mice. The absolute number of CD39<sup>+</sup> B cells in  
17 sepsis-surviving *Rag1*<sup>-/-</sup> mice transferred with B cells was lower than WT mice  
18 but significantly higher compared to naive WT mice (Figure 4G). Consistently,  
19 spleen cells from  $\mu$ MT<sup>-/-</sup> mice, which lack mature peripheral B cells, showed low  
20 ecto-ATPase activity, and mice failed to raise plasma concentration of adenosine  
21 15 days after CLP (Figures 4H-4J and Figure S2J). Sepsis-surviving  $\mu$ MT<sup>-/-</sup> mice  
22 were more resistant to *L. pneumophila* infection at day 15 post-CLP, showing  
23 higher survival rate and lower bacterial loads in the lungs and spleen than WT  
24 mice (Figures 4K and 4L). There was no difference in CLP survival between  
25 *Rag1*<sup>-/-</sup>,  $\mu$ MT<sup>-/-</sup>, and WT mice under antibiotic treatment (Figures S2F and S2G).

1  
2 We then investigated whether the absence of CD39 in B cells would reduce  
3 adenosine accumulation in sepsis-surviving mice. To this end, we generated  
4 mixed bone marrow (BM) chimeras whereby irradiated WT mice were  
5 reconstituted with a mixture of 80 %  $\mu$ MT<sup>-/-</sup> BM cells and either 20% WT (WT  
6  $\mu$ MT-chimera) or 20 % *Entpd1*<sup>-/-</sup> (*Entpd1*<sup>-/-</sup>  $\mu$ MT-chimera) BM cells (Figure 4M).  
7 Reconstitution was assessed eight weeks after adoptive transfer (Figures S4D  
8 and S4E). With this experimental approach, reconstituted *Entpd1*<sup>-/-</sup>  $\mu$ MT-chimeric  
9 mice harbored *Entpd1*<sup>-/-</sup> B cells in an environment of mostly WT cells. Splenic B  
10 cells from WT  $\mu$ MT-chimeras showed increased expression of CD39 after CLP  
11 compared to naïve mice. In contrast, CD39 expression was reduced in B cells  
12 from *Entpd1*<sup>-/-</sup>  $\mu$ MT-chimeric mice from both naïve and CLP groups (Figure 4N).  
13 Spleen cells from *Entpd1*<sup>-/-</sup>  $\mu$ MT-chimeric mice showed low ecto-ATPase activity,  
14 and mice failed to increase blood adenosine concentration 15 days after CLP  
15 (Figures 4O and 4P and Figure S2L). Since M $\phi$ s from sepsis-surviving mice have  
16 impaired bactericidal ability (Nascimento et al., 2017), we asked whether the lack  
17 of CD39 on B cells would prevent the reduction of bactericidal ability. To this end,  
18 peritoneal M $\phi$ s isolated from sepsis-surviving WT and *Entpd1*<sup>-/-</sup>  $\mu$ MT-chimeric  
19 mice were cultured and infected with *L. pneumophila*, and the number of viable  
20 intracellular bacteria was assessed (Figure 4Q). The impaired bactericidal activity  
21 found in M $\phi$ s from WT  $\mu$ MT-chimeric mice was not observed in M $\phi$ s from *Entpd1*<sup>-/-</sup>  
22  $\mu$ MT-chimeric mice (Figure 4R). Finally, we investigated whether septic B cells  
23 would directly affect host resistance to infection by transferring sorted CD19<sup>+</sup> B  
24 cells from naïve or sepsis-surviving mice 15 days post-CLP into naïve WT mice.  
25 Seven days later, we challenged mice with *L. pneumophila* i.n. (Figure S5A). Mice

1 receiving septic B cells showed higher bacteria burdens in the lung than those  
2 receiving naive B cells or no B cells, suggesting that septic B cells suppress  
3 infection control (Figure S5B). Altogether, our data suggest that CD39<sup>+</sup> B cells  
4 play a central role in producing adenosine and suppressing the immune response  
5 in sepsis survivors.

6

### 7 **B cell-derived adenosine impairs microbial killing in Mφs *in vitro***

8 To investigate *in vitro* how septic B cells suppress immune responses to infection,  
9 peritoneal Mφs from naive mice were infected with *L. pneumophila* and co-  
10 cultured with B cells isolated from naive or sepsis-surviving mice for 48 h (Figure  
11 5A). Naive B cells did not affect the ability of Mφs to restrict bacterial replication.  
12 However, Mφs co-cultured with septic B cells contained significantly more  
13 bacteria than Mφs co-cultured with naïve B cells or Mφs alone (Figure 5B). Naïve  
14 or septic B cells did not harbor any intracellular bacteria after 48 h of culture.

15

16 The immunomodulatory effects of adenosine include enhanced IL-10 production  
17 (Haskó and Pacher, 2012). IL-10 can compromise host defence by directly  
18 inhibiting microbial killing by Mφs (Couper et al., 2008). IL-10 plays a critical role  
19 in the sepsis-induced impairment of lung host defence to secondary infections  
20 (Nascimento et al., 2017; Steinhauser et al., 1999). IL-10 production by *L.*  
21 *pneumophila*-infected Mφs was enhanced after 48 h of co-culture with septic B  
22 cells, but not naïve B cells (Figure 5C). Naïve or septic B cells alone released  
23 minor levels of IL-10 in response to *L. pneumophila* infection (Figure 5C). *Il10*<sup>-/-</sup>  
24 Mφs exhibited improved killing compared with WT Mφs, and septic B cells failed

1 to suppress bacterial killing in *Il10*<sup>-/-</sup> Mφs (Figure 5D). Thus, IL-10 produced by  
2 Mφs is critical for the suppressive effect of septic B cells.

3  
4 We next sought to explore how septic B cells suppress Mφ bacterial killing. Mφs  
5 treated with CD39i and co-cultured with septic B cells showed enhanced  
6 restriction of *L. pneumophila* replication, similar to levels observed with Mφs co-  
7 cultured with naïve B cells or Mφs alone (Figure 5E). In contrast, the addition of  
8 apyrase, a soluble ATPase, in the culture, impaired the restriction of bacterial  
9 replication by Mφs co-cultured with septic B cells and also with naïve B cells, to  
10 a lesser extent (Figure 5E). Supplementation of cultures with ATPγS, a non-  
11 hydrolyzable ATP analog, did not affect bacterial killing by Mφs alone or in co-  
12 culture with B cells. Conversely, adenosine deaminase (ADA), an adenosine-  
13 degrading enzyme, abrogated the suppressive effect of septic B cells on Mφ  
14 bacterial killing (Figure 5E). Collectively, these data suggest that the hydrolysis  
15 of ATP into adenosine is required for the suppression of Mφ bacterial killing by  
16 septic B cells.

17  
18 Next, *Entpd1*<sup>-/-</sup> Mφs or B cells were co-cultured to examine the relative  
19 contribution of CD39 on these cells to suppress microbial killing. *Entpd1*<sup>-/-</sup> Mφs  
20 co-cultured with septic WT B cells showed bacterial viability similar to that  
21 observed with WT Mφs (Figure S5C). In contrast, reduced bacterial viability was  
22 seen with CD39 deficiency in septic B cells alone or in both Mφs, suggesting  
23 restored bacterial killing (Figure 5F and Figure S5C). The addition of apyrase  
24 impaired bacterial killing in Mφs co-cultured with *Entpd1*<sup>-/-</sup> septic B cells (Figure  
25 5F). CD39<sup>+</sup> B cells sorted from both naive or septic-surviving mice strongly

1 suppressed bacterial killing by Mφs, while CD39<sup>-</sup> septic B cells showed a weak  
2 suppressive effect (Figure 5G). Thus, CD39-expressing septic B cells suppress  
3 Mφ bacterial killing through adenosine production.

4  
5 Next, we investigated the effect of adenosine on Mφ microbial killing. A2aRi  
6 treatment improved bacterial killing, especially in co-culture with septic B cells  
7 (Figure S5D). However, A2bR blockade (A2bRi) did not affect the bactericidal  
8 activity of Mφs alone or in co-culture with B cells (Figure S5E). Peritoneal Mφs  
9 from mice lacking A2aR in myeloid cells (*Adora2a*<sup>ΔLyz2</sup>) limited intracellular  
10 bacterial infection more efficiently than WT (*Adora2a*<sup>fl/fl</sup>) Mφs. Moreover, septic B  
11 cells failed to suppress bacterial killing by *Adora2a*<sup>ΔLyz2</sup> Mφs (Figure 5H). Taken  
12 together, these data support the hypothesis that adenosine derived from CD39<sup>+</sup>  
13 B cells suppresses the immune response after sepsis, at least in part, through  
14 suppression of Mφ microbial killing.

15  
16 We then investigated whether B cell-derived adenosine induces IL-10 production  
17 in Mφs. Although septic B cells enhanced Mφ production of IL-10 in response to  
18 *L. pneumophila* infection, the addition of CD39i or ADA to the co-cultures  
19 completely abrogated this effect, while ATPγS did not alter IL-10 levels (Figure  
20 5I). Moreover, *Entpd1*<sup>-/-</sup> septic B cells failed to increase IL-10 production by *L.*  
21 *pneumophila*-infected Mφs (Figure 5J), suggesting that the hydrolysis of ATP into  
22 adenosine by CD39<sup>+</sup> B cells induces the production of IL-10 by *L. pneumophila*-  
23 infected Mφs. Loss or inhibition of A2aR in *L. pneumophila*-infected Mφs reduced  
24 their production of IL-10 when co-cultured with septic B cells, but also with naïve  
25 B cells or when cultured alone (Figures 5K and 5L). Overall, our findings



1 demonstrate that B cell-derived adenosine suppresses bacterial killing by  
2 inducing the production of IL-10 in Mφs.

3

4 To validate the relevance of our *in vitro* observations *in vivo*, we utilized IL-  
5 10<sup>+/EGFP</sup> reporter mice and found increased IL-10 expression by splenic Mφs from  
6 sepsis-surviving mice 15 days after CLP (Figure 5M). Moreover, sepsis-surviving  
7 mice show robust IL-10 in lung tissue homogenates, which was reduced with  
8 A2aRi or CD39i and in *Adora2a*<sup>-/-</sup> and *µMT*<sup>-/-</sup> mice (Figure S5F). Sham and naive  
9 mice had low levels of IL-10 in lung tissue homogenates (Figures S1G). Finally,  
10 to investigate the role of A2aR on Mφs in developing sepsis-induced  
11 immunosuppression, we induced CLP in *Adora2a*<sup>ΔLyz2</sup> mice. Sepsis-surviving  
12 *Adora2a*<sup>ΔLyz2</sup> mice were resistant to *L. pneumophila* challenge 15 days post-CLP,  
13 showing a lower number of *L. pneumophila* in the lung and spleen and reduced  
14 IL-10 in lung tissue homogenates than control mice (Figures 5N-5P). There was  
15 no difference in CLP survival between *Adora2a*<sup>ΔLyz2</sup> or control mice with antibiotic  
16 treatment (Figure S2H). Together, these data indicate that A2aR signaling on  
17 Mφs is required for IL-10 production and, consequently, for the immune  
18 dysfunction in sepsis-surviving mice.

19

## 20 **Metabolic reprogramming in septic B cells supports their suppressive** 21 **function**

22 Given that septic B cells suppress Mφ-mediated bacterial killing through ATP  
23 hydrolysis into adenosine, we hypothesized a role for B cell metabolic  
24 reprogramming induced by sepsis. To explore this possibility, we initially  
25 assessed the extracellular acidification rate (ECAR) and oxygen consumption

1 rate (OCR) in isolated CD19<sup>+</sup> B cells. Septic B cells showed higher ECAR and  
2 OCR than naive B cells, suggesting that septic B cells have increased glycolytic  
3 flux and mitochondrial activity. We then utilized inhibitors of the metabolic  
4 pathways required for ATP generation (Figure 6A). UK-5099, an inhibitor of the  
5 mitochondrial pyruvate transporter, reduced all respiratory parameters in septic  
6 B cells to similar levels found in naive B cells (Figures 6B-6E), demonstrating a  
7 critical dependence on glycolysis for the increased mitochondrial respiratory  
8 activity of septic B cells. Septic B cells had increased uptake of a fluorescent  
9 glucose analog (2-NBDG) and higher mRNA expression of glycolytic related  
10 genes *Hif1a* (hypoxia-inducible factor-1a), *Hk1* and *Hk2* (hexokinase-1 and 2,  
11 respectively), compared to naive B cells (Figures 6F and 6G). These data suggest  
12 that septic B cells are metabolically reprogrammed toward aerobic glycolysis that  
13 feeds the mitochondrial tricarboxylic acid (TCA) cycle.

14

15 We next investigated the impact of metabolic reprogramming of septic B cells on  
16 ATP production. Consistent with increased glycolytic flux and mitochondrial  
17 activity, unstimulated or IL-4-activated septic B cells contained more intracellular  
18 ATP than naive B cells. The addition of glycolytic inhibitors 2-deoxyglucose (2-  
19 DG), 3PO (PFKFB3 inhibitor), or echinomycin (HIF-1 $\alpha$  inhibitor) reduced ATP  
20 production in septic B cells to similar levels found in naive B cells. Likewise, the  
21 addition of oligomycin (an inhibitor of mitochondrial ATP synthase) or rotenone  
22 (an inhibitor of mitochondrial complex I) decreased ATP production in septic B  
23 cells. However, ATP production in septic B cells was not affected by etomoxir (a  
24 carnitine palmitoyltransferase-1a inhibitor) or BPTES (a glutaminase-1 inhibitor).  
25 These results suggest that the enhanced mitochondrial activity and ATP

1 production in septic B cells require glycolytic activity, but not glutaminolysis or  
2 fatty acid oxidation (Figures 6H). We then investigated if the increased metabolic  
3 affected the extracellular release of ATP and found enhanced ATP release by  
4 septic B cells than naive B cells, which was abrogated by inhibiting the pannexin-  
5 1 ATP-permeable channel (Panx1) with CBX (Figures 6I and 6J).

6  
7 Finally, to indirectly investigate the effect of metabolic reprogramming in  
8 sustaining septic B cells' suppressive activity, peritoneal Mφs from naive mice  
9 were infected with *L. pneumophila* and co-cultured with B cells isolated from naive  
10 or sepsis-surviving mice for 48 h with or without CBX. Panx1 inhibition by CBX  
11 did not directly affect bacterial killing by Mφs alone or in co-culture with naive B  
12 cells. However, the CBX abrogated the inhibitory effect of septic B cells on Mφ  
13 bacterial killing and reduced Mφs production of IL-10 (Figures 6K-6M).  
14 Collectively, our findings suggest that septic B cells have a metabolic  
15 reprogramming toward aerobic glycolysis that feeds mitochondrial TCA cycle,  
16 supporting their suppressive activity by enhancing production and release of  
17 ATP.

18

### 19 **Septic patients have an expansion of CD39<sup>+</sup> plasmablast and adenosine** 20 **accumulation**

21 To investigate the clinical relevance of our findings, we collected peripheral blood  
22 samples from 21 septic patients and 36 age- and sex-matched healthy controls  
23 (HC). All patients presented clinical and laboratory variables that fulfilled sepsis  
24 criteria (Table S1). As observed in septic mice, the frequency of  
25 Ki67<sup>+</sup>CD39<sup>+</sup>CD19<sup>+</sup> B cells was increased in septic patients compared to HC

1 (Figure 7A). Naïve and memory B cells expressed intermediate levels of CD39 at  
2 comparable frequencies between septic patients and HC (Figures 7B and 7C).  
3 However, CD39 was highly expressed in plasmablasts, and septic patients  
4 showed a higher frequency than HC (Figures 7B-7D). Sorted CD19<sup>+</sup> B cells from  
5 septic patients showed increased ecto-ATPase activity compared to HC, which  
6 was inhibited by CD39i (Figure 7E). In line, septic patients had higher plasma  
7 adenosine concentrations than HC (Figure 7F). Dividing septic patients by illness  
8 severity, adenosine levels were higher in patients with septic shock than sepsis  
9 only (Figure 7G), as previously reported (Martin et al., 2000). Moreover, the ecto-  
10 ATPase activity of isolated CD19<sup>+</sup> B cells positively correlated with serum  
11 adenosine concentration in septic patients (Figure 7H), suggesting that the  
12 elevated adenosine concentration was associated with CD39 activity in B cells.

13

14 To investigate whether human septic B cells could suppress monocytes' bacterial  
15 killing capacity, sorted CD19<sup>+</sup> B cells from 3 different septic patients or healthy  
16 volunteers were co-cultured with CD14<sup>+</sup> monocytes isolated from HC blood. We  
17 assessed bacterial load by using an *L. pneumophila* strain stably expressing the  
18 luxCDABE operon. Monocytes co-cultured with B cells from septic patients  
19 contained more bacteria than those co-cultured with B cells from healthy  
20 volunteers or monocytes alone. To address adenosine's contribution to bacterial  
21 killing suppression, we added A2aRi or apyrase in culture. A2aRi improved  
22 bacterial killing by monocytes, whereas apyrase impaired monocyte restriction of  
23 bacterial replication, especially when co-cultured with septic B cells (Figure 7I).  
24 *L. pneumophila*-driven IL-10 production by monocytes was enhanced by co-

1 culture with B cells from septic patients but not healthy volunteers. This was  
2 inhibited by A2aRi and further enhanced with the addition of apyrase ([Figure 7J](#)).  
3  
4 Finally, we analyzed publicly available single-cell RNA-seq data from PBMCs of  
5 septic patients and controls (Reyes et al., 2020). Gene expression analysis  
6 identified one distinct B cell population characterized by both high expression of  
7 CD39 and plasmablast markers (*MZB1*, *JCHAIN*, *FKBP11*, *SEC61G*, *VIMP*,  
8 *ITM2C*, *IGHA1*, and *TXN*). Similar to the CLP model, we found that septic patients  
9 had a greater abundance of CD39<sup>+</sup> plasmablast cells than the controls (no sepsis)  
10 ([Figures 7K and 7L](#)). Furthermore, differential expression analysis of CD39<sup>+</sup>  
11 plasmablast cells against other B cells revealed high expression of mitochondrial  
12 genes and enrichment of genes participating in the metabolic TCA cycle,  
13 respiratory electron transportation, detoxification of reactive oxygen species, and  
14 glycolysis ([Figures S6A and S6B](#)). Overall, these results substantiate our findings  
15 with experimental sepsis, showing an expansion of CD39<sup>+</sup> plasmablasts with  
16 immunosuppressive activity in septic patients.

## 1 **DISCUSSION**

2 Inflammatory environments can induce differentiation and/or expansion of distinct  
3 subsets of regulatory B cells that suppress pathological or protective immune  
4 responses (Rosser and Mauri, 2015). Activated B cells can express CD39 and,  
5 therefore, produce adenosine in the presence of extracellular ATP (Maliszewski  
6 et al., 1994; Saze et al., 2013). Similar to Treg cells, CD39 expression contributes  
7 to B cell suppressive function *in vitro* (Borsellino et al., 2007; Deaglio et al., 2007;  
8 Figueiró et al., 2016; Peres et al., 2015; Saze et al., 2013); however, the  
9 implications of this suppression remain underexplored. In this study, we report  
10 that sepsis induced an expansion of a plasmablast population in mice and human  
11 septic patients with elevated CD39 expression responsible for increasing  
12 circulating extracellular adenosine. Adenosine derived from CD39-expressing B  
13 cells suppressed the host immune response, rendering sepsis-surviving mice  
14 highly susceptible to secondary infections induced by opportunistic human  
15 pathogens, *A. fumigatus* and *L. pneumophila* (Behnsen et al., 2008; Berjeaud et  
16 al., 2016).

17

18 Adenosine signaling mediated by A2aR augments the production of anti-  
19 inflammatory cytokines and suppresses microbial killing by neutrophils and M $\phi$ s  
20 (Hasko et al., 2008; Haskó and Cronstein, 2013). Consistent with this, we found  
21 that septic B cell-derived adenosine efficiently suppressed M $\phi$ s killing ability and  
22 the specific deficiency of A2aR in myeloid cells improved the resistance of sepsis-  
23 surviving mice against secondary infections. These demonstrate the essential  
24 role of adenosine-producing B cells in developing sepsis-induced  
25 immunosuppression by suppressing macrophage function. However, an

1 unexpected finding was that IL-10, which has long been considered the central  
2 suppressive mediator produced by regulatory B cells (Rosser and Mauri, 2015),  
3 did not directly influence the suppressive activity of septic CD39<sup>+</sup> B cells. Instead,  
4 we found that Mφs respond to adenosine from CD39-expressing B cells via A2aR  
5 signaling by producing immunosuppressive IL-10 that impairs their bactericidal  
6 activity. Indeed, it was previously reported that, in the absence of IL-10, a  
7 CD39/CD73 pathway mediates the regulatory function of peritoneal B1 cells via  
8 the production of adenosine (Kaku et al., 2014). This was further supported by  
9 our observations that splenic B1a cells show a slight up-regulation of CD39  
10 expression in sepsis-surviving mice. Thus, we cannot exclude the contribution of  
11 CD39<sup>lo</sup> B1a cells working together with CD39<sup>hi</sup>CD138<sup>hi</sup> plasmablasts to the  
12 immunosuppressive status observed in sepsis-surviving mice.

13

14 We found that septic B cells have a metabolic reprogramming toward aerobic  
15 glycolysis that feeds mitochondrial TCA cycle with pyruvate enhancing production  
16 and release of ATP. These findings are consistent with previous reports of  
17 increased glycolysis and oxygen consumption rate by activated B cells with LPS  
18 or IL-4 (Caro-Maldonado et al., 2014; Waters et al., 2018). We, therefore, propose  
19 that the metabolic reprogramming in septic CD39<sup>+</sup> B cells supports their  
20 suppressive activity by enhancing the production and release of ATP.

21

22 In our study, antibiotic treatment of naive or sham-operated mice did not alter the  
23 frequency of CD39-expressing B cells and plasma adenosine concentration.  
24 Moreover, mice who survived to moderate sepsis receiving or not antibiotic  
25 treatment were equally susceptible to the secondary infection, suggesting that

1 antibiotic treatment does not directly impact sepsis-induced immune  
2 dysregulation. However, it is very complicated to dissociate the consequences of  
3 sepsis itself from the effect of sepsis treatment on the long-term immune  
4 dysregulation found in sepsis survivors since all septic patients inevitably receive  
5 antibiotic treatment. This is the reason why we have established a mouse model  
6 of lethal CLP-induced sepsis followed by a short treatment with ertapenem, a  
7 widely used antibiotic to treat critically ill patients with abdominal sepsis (Solomkin  
8 et al., 2010), intending to reduce infection and mimicking the clinical scenario  
9 (Nascimento et al., 2010). Future studies are required to determine whether the  
10 expansion of CD39<sup>+</sup> B cells reported here is due to inflammation following sepsis  
11 or a combination with changes in the microbiota by antibiotics.

12

13 IL-10 has long been described as a major mediator of sepsis-induced  
14 immunosuppression (Steinhauser et al., 1999). We have previously reported that  
15 IL-33, released during the tissue injury following sepsis, induces activation of  
16 ILC2s and the polarization of IL-10-secreting M2 macrophages that promote the  
17 expansion of the Treg cell population in an IL-10-dependent manner, thereby  
18 contributing to the development of sepsis-induced immunosuppression  
19 (Nascimento et al., 2017). However, our current understanding of the primary IL-  
20 10-secreting immune cells and the signaling that maintains IL-10 production in  
21 sepsis survivors remains limited. This study addressed this gap, revealing that  
22 adenosine-derived from CD39<sup>+</sup> B cells is an essential mediator that triggers IL-  
23 10 production by macrophages in sepsis-surviving mice, thus fitting the pieces of  
24 the intricate mechanism underlying the persistent dysregulation of the host  
25 immune response in sepsis survivors. However, we did not investigate the



1 mechanism by which sepsis induces the expansion of CD39<sup>+</sup> B cells. In this  
2 regard, IL-33 was shown to induce a subset of IL-10-producing regulatory B cells  
3 (Sattler et al., 2014). Based on our previous study (Nascimento et al., 2017), IL-  
4 33 might have a potential involvement, but we cannot exclude the participation of  
5 other cytokines or gut-microbiota-derived metabolites (Rosser et al., 2020;  
6 Yoshizaki et al., 2012). Therefore, future studies are necessary to identify the  
7 mechanism underlying the CD39<sup>+</sup> B cell expansion induced by sepsis.

8

9 In summary, our results revealed a suppressive function of CD39<sup>+</sup> plasmablasts  
10 that is responsible, at least in part, for the long-term immunosuppression  
11 observed in sepsis survivors by generating a high amount of extracellular  
12 adenosine. These findings fill gaps in the current knowledge of the mechanism  
13 underlying the persistent dysregulation of the host immune response in sepsis  
14 survivors.

15

## 16 **LIMITATIONS OF THE STUDY**

17 We demonstrated that the metabolic reprogramming in septic CD39<sup>+</sup>  
18 plasmablasts supports their suppressive activity by enhancing ATP production.  
19 However, extracellular ATP can be released by some bacteria from mouse and  
20 human feces (Mempin et al., 2013). Future studies are needed to determine  
21 whether perturbation of gut microbiota following sepsis might affect the systemic  
22 bioavailability of extracellular ATP and adenosine production by CD39<sup>+</sup> B cells.  
23 In addition, although we provided preliminary evidence showing that  
24 CD39<sup>hi</sup>CD138<sup>hi</sup> B cells are enriched in the transitional 1 population, further

1 studies are needed to better characterize where exactly these cells reside in the  
2 spleen.

3

#### 4 **Acknowledgments**

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12 EX010381).

13

#### 14 **Author Contributions**

15 D.C.N. planned and performed experiments, analysed data and wrote the  
16 manuscript. J.C.A-F planned experiments, analyzed data, wrote the manuscript  
17 and supervised the project. B.R., F.Q.C., V.Q., and T.M.C. planned and analyzed  
18 experiments. P.R.V., R.G.F., A.R.P., P.H.M., P.B.D., F.P.V., R.S.P., J.E.T-K.,  
19 D.C., M.A.D and J.A.R.P performed and analyzed experiments. D.Z. and M.C.B.  
20 provided human samples and analyzed data. L.M-S and D.M.F performed flow  
21 cytometry experiments and analyzed t-SNE data. A.E.R.O., Í.M.S.C., and H.I.N.  
22 re-analyzed public datasets. G.K. provided *Entpd1*<sup>-/-</sup> mice and analyzed data. J.L.  
23 provided *Adora2a*<sup>fl/fl</sup> mice and analyzed data. D.S.Z. provided reagents and  
24 analyzed data.

25

1 **Competing Interests statement**

2 The authors declare no competing interests.

3

4 **Inclusion and diversity**

5 We worked to ensure gender balance in the recruitment of human subjects. One

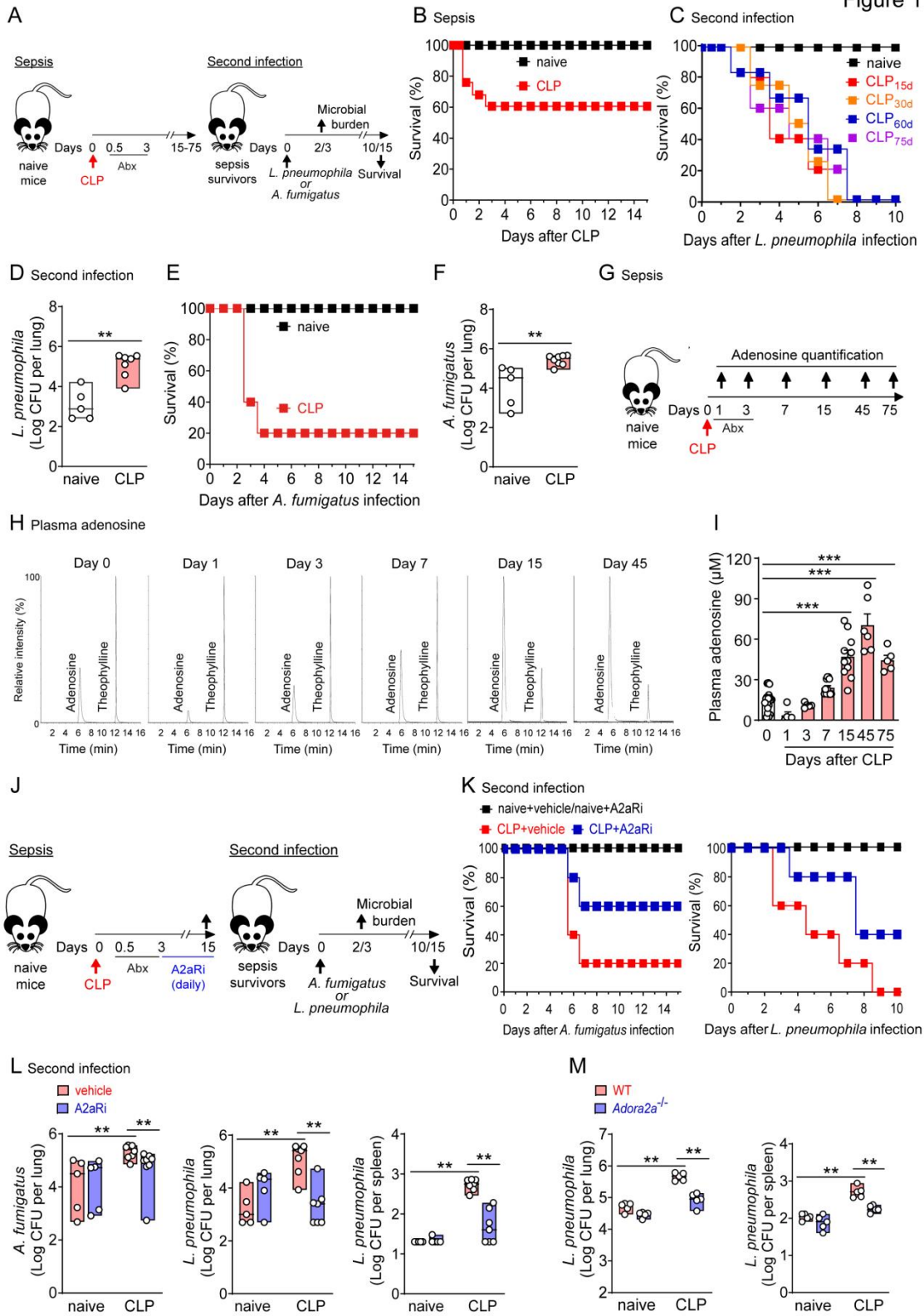
6 or more of the authors of this paper self-identifies as an underrepresented ethnic

7 minority in science. One or more of the authors of this paper self-identifies as a

8 member of the LGBTQ+ community.

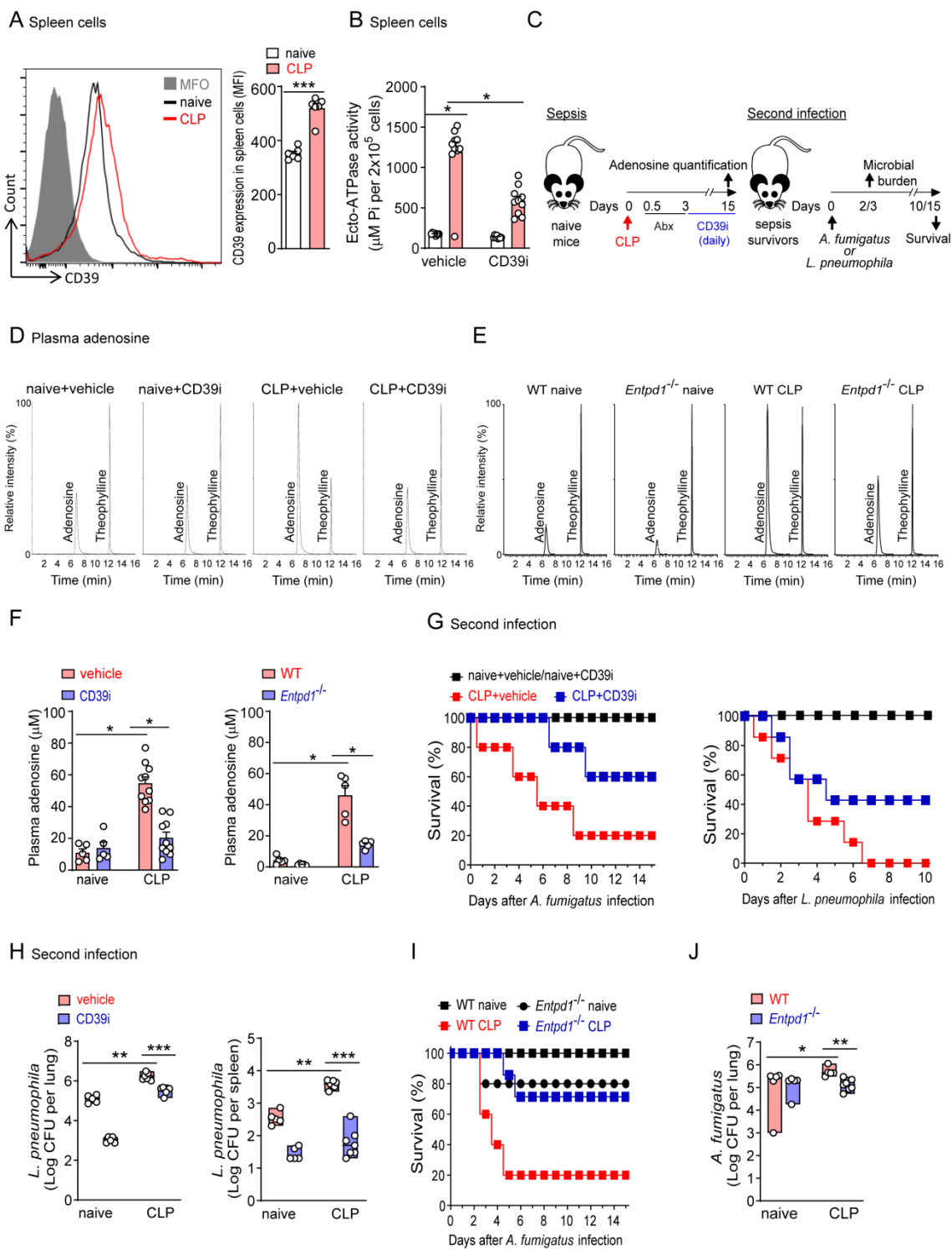
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Figure 1



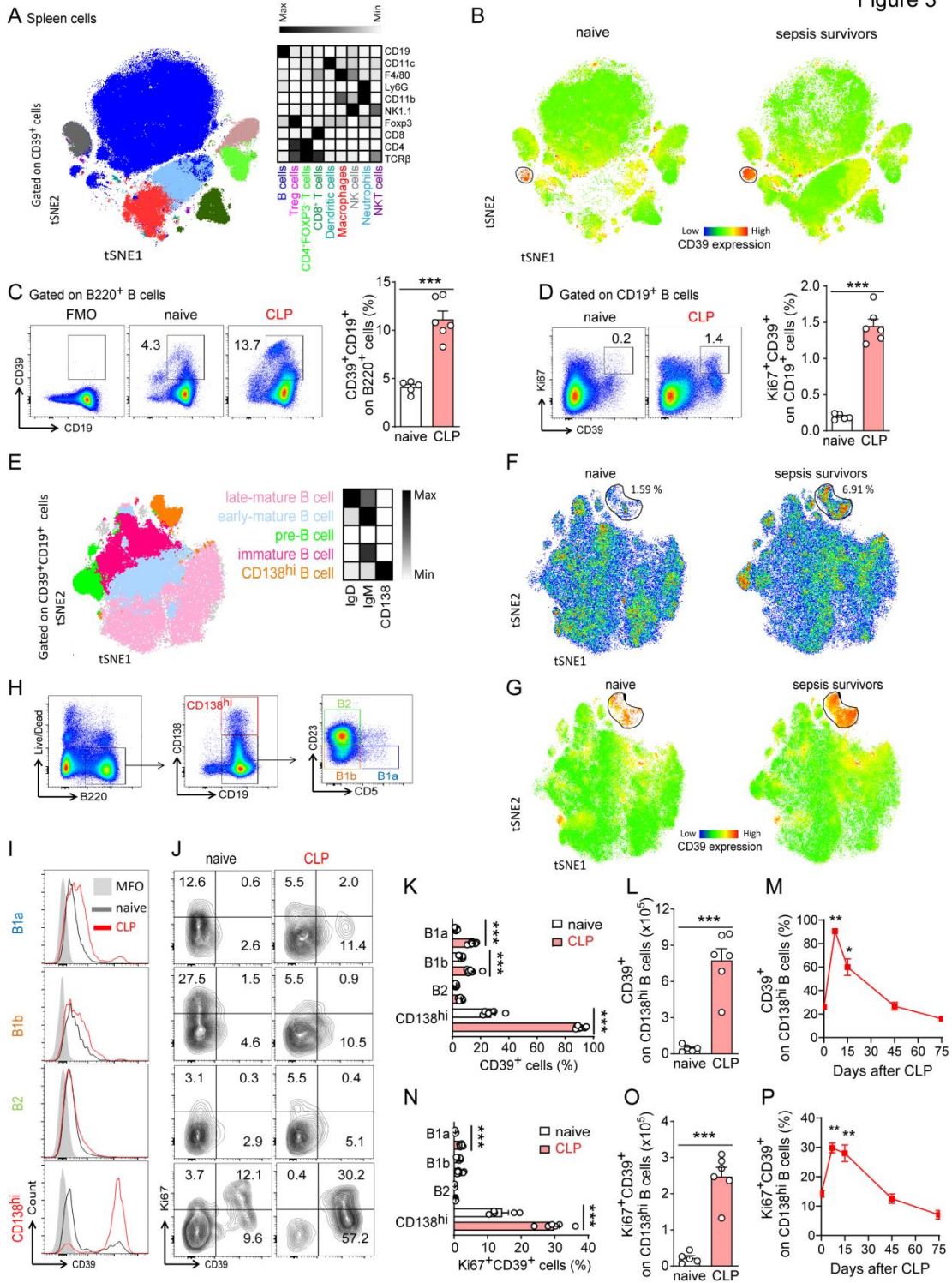
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Figure 2



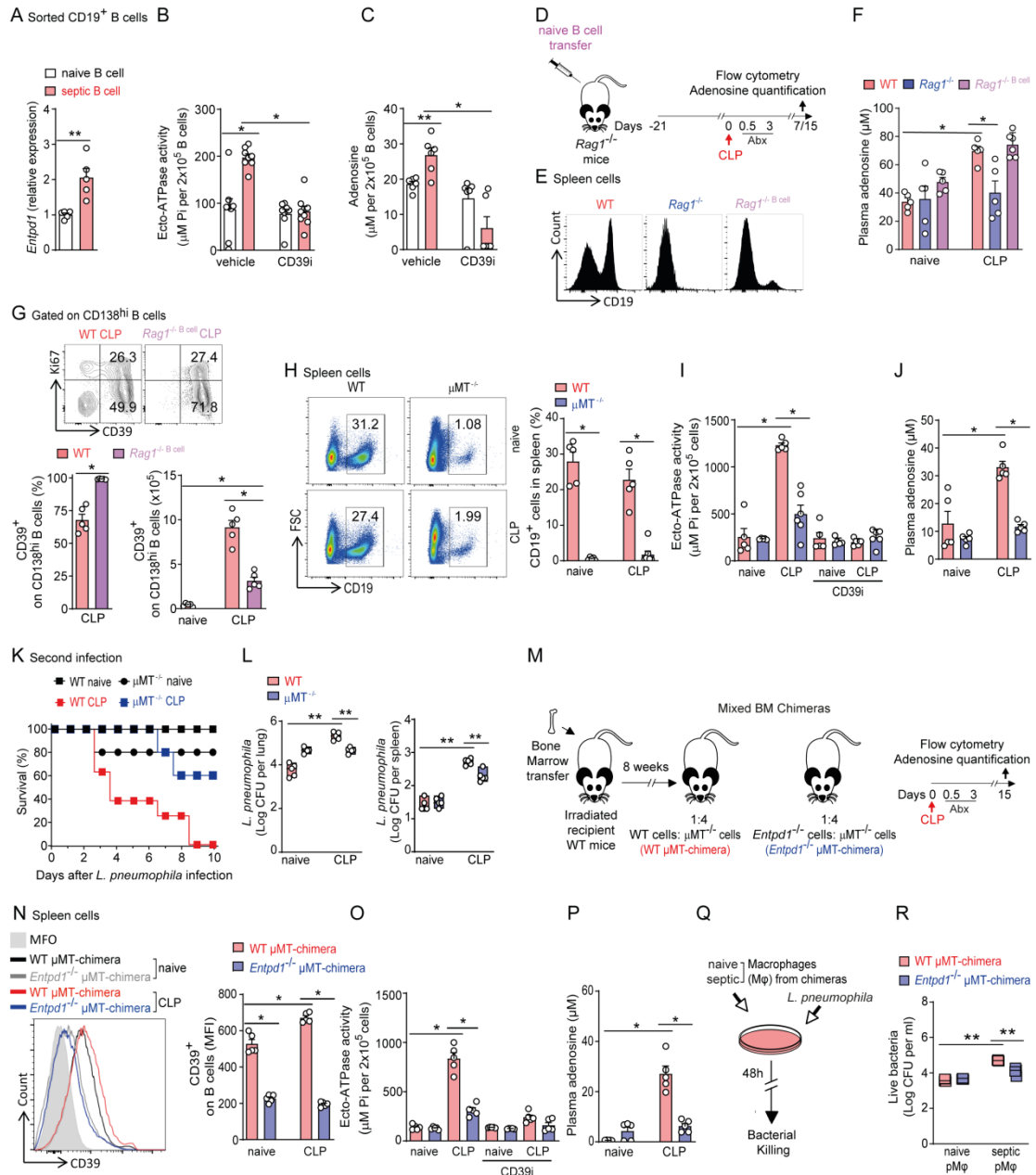
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Figure 3



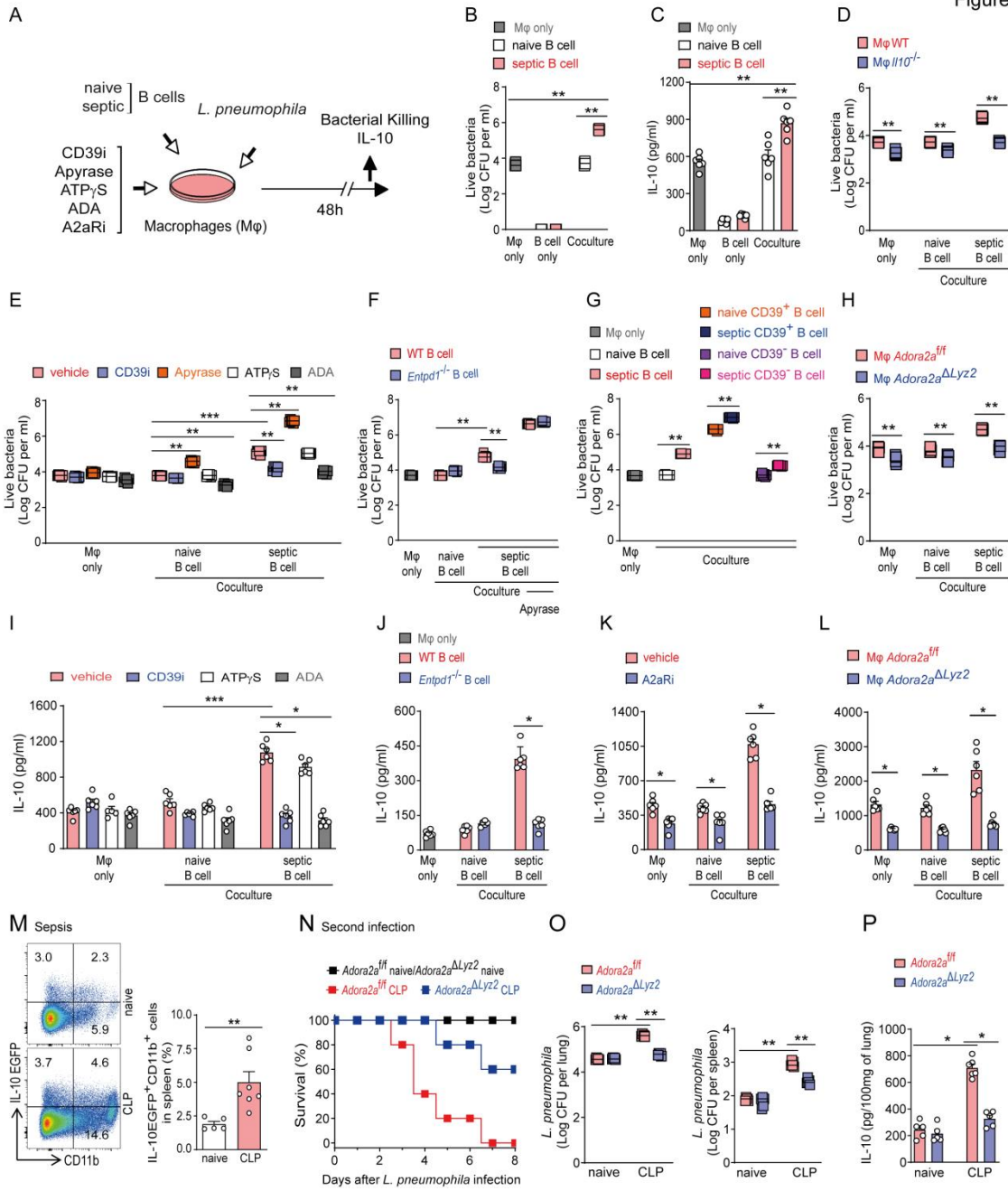
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Figure 4



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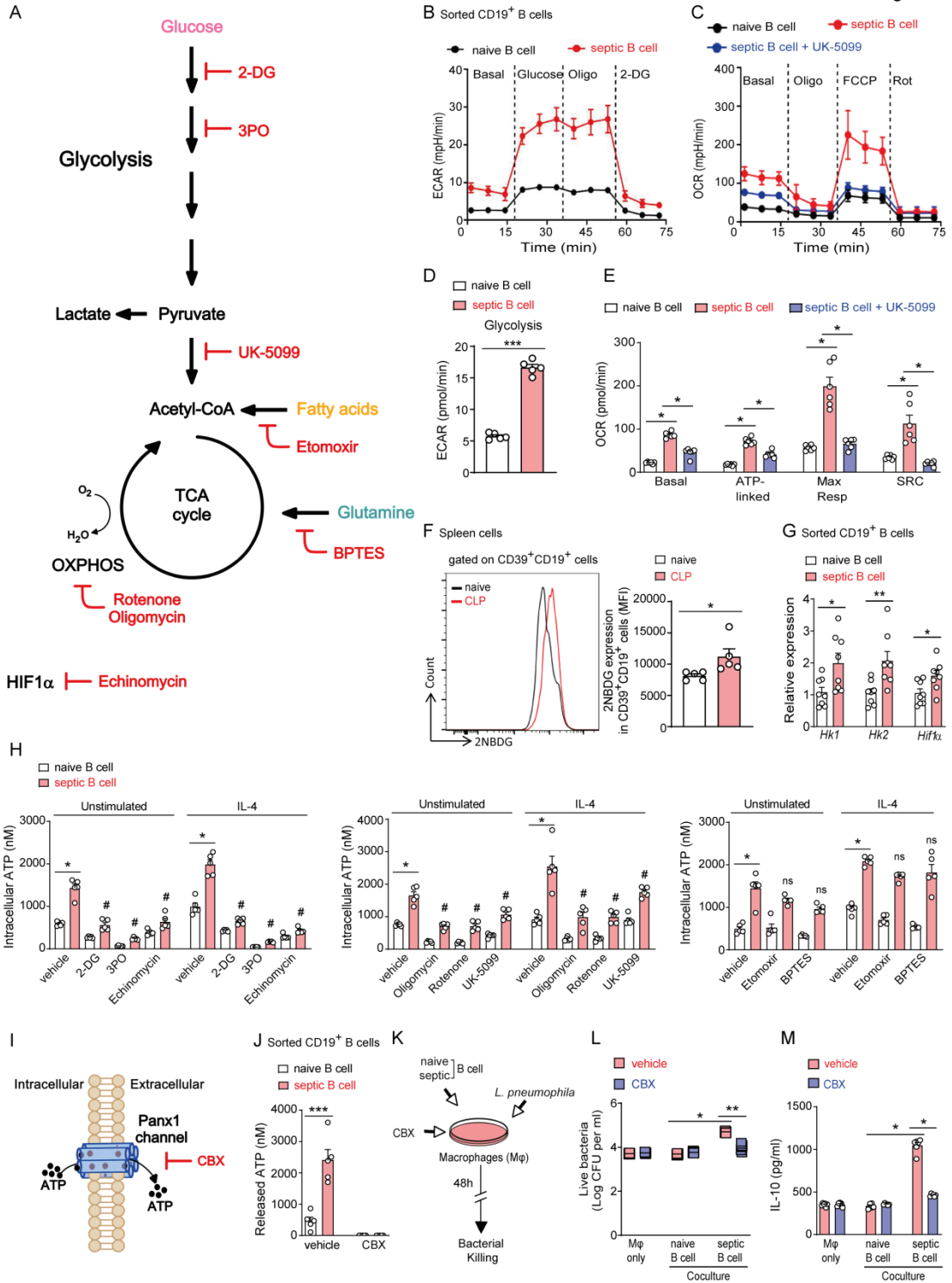
Figure 5



1



Figure 6



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7

1 **FIGURE LEGENDS**

2 **FIGURE 1: Adenosine mediates sepsis-induced immunosuppression**  
3 **through A2aR**

4 (A) Schematic representation of sepsis-induced immunosuppression model (B-  
5 F) (also see STAR Methods).

6 (B) Survival curves after CLP. Naive n=10 and CLP n=30.

7 (C) Survival curves of naive or CLP-surviving mice after *L. pneumophila* infection.  
8 n=5-8.

9 (D) Bacterial load in the lungs of naive or CLP-surviving mice 2 days after *L.*  
10 *pneumophila* infection. n=5-7.

11 (E) Survival curves of naive or CLP-surviving mice after *A. fumigatus* infection.  
12 n=5.

13 (F) Fungal load in the lungs of naive or CLP-surviving mice 3 days after *A.*  
14 *fumigatus* infection. n=5-8.

15 (G) Schematic representation of the experimental protocol for quantification of  
16 plasma adenosine after CLP.

17 (H) Representative HPLC chromatograms and (I) concentrations of plasma  
18 adenosine of naive or CLP-surviving mice. n=5-20.

19 (J) Schematic representation of sepsis-induced immunosuppression model with  
20 A2aR antagonist [A2aRi, 8-(3-Chlorostyryl)-caffeine, 1 mg.kg<sup>-1</sup>] treatment (K-L)  
21 (also see STAR Methods).

22 (K) Survival curves of CLP-surviving mice after *A. fumigatus* or *L. pneumophila*  
23 infections. n=5.

24 (L) Pathogen load in the lungs of CLP-survivors after *A. fumigatus* infection and  
25 in lungs and spleen after *L. pneumophila* infection. n=5-8.

26 (M) Bacterial load in the lungs and spleens of CLP-surviving WT and *Adora2a*<sup>-/-</sup>  
27 mice after *L. pneumophila* infection. n=5.

28 Data are representative of 2-3 independent experiments. \*\*p < 0.01, \*\*\*p < 0.001.  
29 Mantel-Cox log-rank test in B, C, E, K; one-way ANOVA with Dunnett posthoc  
30 tests in I; and Mann–Whitney U test in D, F, L, M.

31

32 **FIGURE 2: CD39 is required for sepsis-induced immunosuppression**

33 (A-B) Splenic cells from CLP-surviving mice were harvested 15 days after CLP.

1 (A) Histogram and mean fluorescence intensity (MFI) of CD39 expression in total  
2 splenic cells. n=6.  
3 (B) Ecto-ATPase activity of splenic cells cultured  $\pm$ CD39 inhibitor (CD39i, ARL  
4 67156, 200 $\mu$ M). n=8-10.  
5 (C) Diagram of sepsis-induced immunosuppression model with CD39i (2 mg.kg<sup>-1</sup>)  
6 treatment (D, F-H) (also see STAR Methods).  
7 (D-E) Representative HPLC chromatograms and (F) concentrations of plasma  
8 adenosine of naïve or CLP-surviving WT, *Entpd1*<sup>-/-</sup> or CD39i-treated mice. n=5-  
9 10.  
10 (G) Survival curves of CLP-surviving WT, *Entpd1*<sup>-/-</sup> or CD39i-treated mice after *A.*  
11 *fumigatus* or *L. pneumophila* infection. n=5-7.  
12 (H) Bacterial load in the lungs and spleens of naïve or CLP-surviving mice after  
13 *L. pneumophila* infection. n=5-7.  
14 (I) Survival curves of CLP-surviving WT or *Entpd1*<sup>-/-</sup> mice after *A. fumigatus*  
15 infection. n=5-7.  
16 (J) Fungal load in the lungs of CLP-surviving WT or *Entpd1*<sup>-/-</sup> mice after *A.*  
17 *fumigatus* infection. n=5-7.  
18 Data are representative of 2-3 independent experiments. \*p < 0.05, \*\*p < 0.01,  
19 \*\*\*p < 0.001. Two-tailed unpaired Student's t-test in A, B, F; Mantel-Cox log-rank  
20 test in G, I; and Mann-Whitney U test in H, J.

21

### 22 **FIGURE 3: Expansion of a CD39<sup>+</sup> B cell subset in sepsis-surviving mice**

23 (A-P) Splenic cells from CLP-surviving mice were harvested 7 or 15 days after  
24 CLP.

25 (A) Unsupervised analysis of single live CD39<sup>+</sup> cells from the flow cytometry  
26 dataset of splenic immune cells of naïve and sepsis-surviving mice 15 days after  
27 CLP, using the t-SNE algorithm as described in STAR Methods. n=10.

28 (B) Heatmap density plots with MFI of CD39 expressed in different cell lineages  
29 with a blue-green-yellow-red continuous color scale. Naïve n=5 and CLP n=5.

30 (C-D) Representative flow cytometry plots and bar graph showing (C) the  
31 frequency of splenic CD39<sup>+</sup>CD19<sup>+</sup> on gated B220<sup>+</sup> B cells or (D) Ki67<sup>+</sup>CD39<sup>+</sup> on  
32 CD19<sup>+</sup> B cells from naïve and sepsis survivors 7 days after CLP. n=5-6.

33 (E-G) Unsupervised analysis of single live CD39<sup>+</sup>CD19<sup>+</sup> B cells from the flow  
34 cytometry dataset of immune cells in the spleen of naïve and sepsis-surviving

1 mice 15 days after CLP, using the t-SNE algorithm as described in STAR  
2 Methods.  
3 (E) t-SNE maps color-coded according to manually gated clusters of distinct  
4 CD39<sup>+</sup> B cell subsets. n=10.  
5 (F) t-SNE maps color-coded according to cell density cluster of distinct CD39<sup>+</sup> B  
6 cell subsets. Naive n=5 and CLP n=5.  
7 (G) t-SNE maps color-coded according to the MFI of CD39 expression intensity  
8 in distinct B cell subsets. Naive n=5 and CLP n=5.  
9 (H-P) B cell subset gating strategy. B1a (CD138<sup>-</sup>CD23<sup>-</sup>CD5<sup>+</sup>), B1b (CD138<sup>-</sup>CD23<sup>-</sup>  
10 CD5<sup>-</sup>), and B2 (CD138<sup>-</sup>CD5<sup>-</sup>CD23<sup>+</sup>) and plasmablast (CD138<sup>hi</sup>) cells among  
11 viable B220<sup>+</sup>CD19<sup>+</sup> cells.  
12 (I) Histogram of CD39 expression in B1a, B1b, B2, and plasmablast cells from  
13 naïve and sepsis survivors 7 days after CLP. n=5-6.  
14 (J, K) Representative flow cytometry plots and graph bars showing the frequency  
15 of CD39 expression in B cell subsets from naive and sepsis survivors 7 days after  
16 CLP. n=5-6.  
17 (L) The absolute number of CD39<sup>+</sup>CD138<sup>hi</sup> cells. n=5-6.  
18 (M) Frequency of CD39<sup>+</sup>CD138<sup>hi</sup> cells. n=5-6.  
19 (J, N) Representative flow cytometry plots and graph bars showing the frequency  
20 of Ki67 and CD39 expression in B cell subsets from naive and sepsis survivors 7  
21 days after CLP. n=5-6.  
22 (O) The absolute number of Ki67<sup>+</sup>CD39<sup>+</sup>CD138<sup>hi</sup> cells. n=5-6.  
23 (P) Frequency of Ki67<sup>+</sup>CD39<sup>+</sup>CD138<sup>hi</sup> cells. n=5-6.  
24 Data are representative of 2-3 independent experiments. \*p < 0.05, \*\*p < 0.01,  
25 \*\*\*p < 0.001. Two-tailed unpaired Student's t-test in C, D, K, N, L, O and one-way  
26 ANOVA with Dunnett posthoc tests in M, P.

27

28 **FIGURE 4: CD39<sup>+</sup> B cells promotes immunosuppression in sepsis-surviving**  
29 **mice**

30 (A-C) Splenic CD19<sup>+</sup> B cells were isolated from CLP-surviving mice 15 days after  
31 CLP.

32 (A) mRNA expression of *Entpd1*. n=5.

33 (B) Ecto-ATPase activity  $\pm$ CD39i. n=8-9.

34 (C) Concentration of adenosine in the cell culture supernatant. n=6.

1 (D) A diagram of B cell transfer into *Rag1*<sup>-/-</sup> mice following by sepsis-induced  
2 immunosuppression model (E-G) (also see STAR Methods).

3 (E) Flow cytometry analysis of CD19<sup>+</sup> splenic cells from WT, *Rag1*<sup>-/-</sup> and *Rag1*<sup>-/-</sup>  
4 B<sup>cell</sup> (B cell transferred) mice 15 days after CLP. n=5-7.

5 (F) Plasma adenosine concentrations from sepsis survivors 15 days after CLP.  
6 n=5-7.

7 (G) Representative flow cytometry plots and graph bars showing the frequency  
8 and the absolute number of splenic CD39<sup>+</sup>CD138<sup>hi</sup> B cells from sepsis survivors  
9 7 days after CLP. n=5.

10 (H-L) Sepsis-surviving WT and  $\mu$ MT<sup>-/-</sup> mice were challenged with *L. pneumophila*  
11 15 days after CLP.

12 (H) Representative flow cytometry plots and graph bars showing the frequency  
13 of splenic CD19<sup>+</sup> B cells from WT and  $\mu$ MT<sup>-/-</sup> mice 15 days after CLP. n=5-7.

14 (I) Ecto-ATPase activity in splenic cells from WT and  $\mu$ MT<sup>-/-</sup> mice cultured  $\pm$ CD39i.  
15 n=5-6.

16 (J) Plasma adenosine concentration in WT and  $\mu$ MT<sup>-/-</sup> mice 15 days after CLP.  
17 n=5.

18 (K) Survival curves of WT and  $\mu$ MT<sup>-/-</sup> mice after *L. pneumophila* infection. n=5-8.

19 (L) Bacterial loads in the lungs and spleens from WT and  $\mu$ MT<sup>-/-</sup> mice after *L.*  
20 *pneumophila* infection. n=5.

21 (M) A diagram of mixed bone marrow (BM) cells reconstitution in irradiated  
22 recipient all mice following by sepsis-induced immunosuppression model (N-P)  
23 (also see STAR Methods).

24 (N) Histogram and MFI of CD39 expression on CD19<sup>+</sup> B cells. n=5.

25 (O) Ecto-ATPase activity of splenic cells cultured in  $\pm$ CD39i. n=5.

26 (P) Plasma adenosine concentration. n=5.

27 (Q) A diagram of peritoneal macrophages (M $\phi$ ) from naive or sepsis-surviving  
28 chimeras exposed to *L. pneumophila in vitro* for analysis of killing assay.

29 (R) The number of viable bacteria recovered from M $\phi$  lysates. n=5.

30 Data are representative of 2-3 independent experiments. \*p < 0.05, \*\*p < 0.01  
31 Two-tailed unpaired Student's t-test in A-C, G-H, J, N-P; one-way ANOVA with  
32 Bonferroni posthoc tests in F, I; Mantel-Cox log-rank test in K; and Mann-Whitney  
33 U test in L, R.

34

1 **FIGURE 5: Septic B cell-derived adenosine impairs macrophage bacterial**

2 (A) Schematic representation of bacterial killing and IL-10 production analysis by  
3 Mφs co-cultured with naive or septic B cells in the presence of *L. pneumophila*  
4 determined after 48h (B-L) (also see STAR Methods). Naive peritoneal  
5 macrophages were pooled from 5 naive mice and B cells were isolated from naïve  
6 or CLP-surviving mice. n=6.

7 (B) The number of viable bacteria recovered from cell lysates.

8 (C) Concentrations of IL-10 in cell supernatant.

9 (D) The number of viable bacteria recovered from lysates of naive WT and *Il10*<sup>-/-</sup>  
10 macrophages and/or naive or septic B cells.

11 (E-F) The number of viable bacteria recovered from lysates of naive  
12 macrophages with naive or septic *Entpd1*<sup>-/-</sup> or WT B cells in the presence of  
13 CD39i, Apyrase (20 U.mL<sup>-1</sup>), ATPγS (100 μM), ADA (4 U.mL<sup>-1</sup>).

14 (G) The number of viable bacteria recovered from lysates of naive macrophages  
15 and/or naive or septic CD39<sup>+</sup> or CD39<sup>-</sup> B cells.

16 (H) The number of viable bacteria recovered from lysates of naive, *Adora2a*<sup>ff</sup> or  
17 *Adora2a*<sup>ΔLyz2</sup> macrophages and/or naive or septic B cells.

18 (I-K) Concentrations of IL-10 in the culture supernatants of naive, *Adora2a*<sup>ff</sup> or  
19 *Adora2a*<sup>ΔLyz2</sup> macrophages with naive or septic *Entpd1*<sup>-/-</sup> B cells in the presence  
20 of CD39i, ATPγS, ADA and A2aRi (100 μM).

21 (M) Representative flow cytometry dot plots and frequency of splenic IL-10-  
22 EGFP<sup>+</sup>CD11b<sup>+</sup> cells 15 days after CLP. n=5-7.

23 (N-P) Sepsis-surviving *Adora2a*<sup>ff</sup> and *Adora2a*<sup>ΔLyz2</sup> mice were challenged with *L.*  
24 *pneumophila* 15 days after CLP, n=5-6.

25 (N) Survival curves after *L. pneumophila* infection.

26 (O) Bacterial loads in the lungs after *L. pneumophila* infection.

27 (P) IL-10 concentrations in the lungs after *L. pneumophila* infection.

28 Data are representative of 2-3 independent experiments. \*p < 0.05, \*\*p < 0.01,  
29 \*\*\*p < 0.001. Mann–Whitney U test in B, D-H, O; two-tailed unpaired Student's t-  
30 test in C, I-M, P; and Mantel-Cox log-rank test in N.

31  
32 **FIGURE 6: Sepsis induces alterations in B cell metabolic reprogramming**

1 (A) Schematic representation detailing pharmacological inhibitors for the  
2 metabolic pathways required for ATP generation.

3 (B-E) CD19<sup>+</sup> B cells were isolated from naive or sepsis-surviving mice and  
4 cultured with IL-4 for 6 h ±UK-5099 (20 μM).

5 (B) Kinetic profile of extracellular acidification rate (ECAR) measured by  
6 Seahorse under basal condition and in response to glucose, oligomycin, and 2-  
7 DG at the indicated time points. n=5.

8 (C) Kinetic profile of oxygen consumption rate (OCR) measured by Seahorse,  
9 under basal condition, and in response to oligomycin, FCCP, and rotenone at the  
10 indicated time points. n=6.

11 (D) Maximal glycolysis calculated from ECAR profile. n=5.

12 (E) Basal respiration, ATP-linked, maximal respiration, and spare respiratory  
13 capacity (SRC) calculated from OCR profile. n=6.

14 (F) Flow cytometry histogram and graph bar showing MFI for 2NBDG in  
15 CD39<sup>+</sup>CD19<sup>+</sup> B cells. n=5.

16 (G) mRNA expression of *Hk1*, *Hk2*, *Hif1α* in CD19<sup>+</sup> B cells determined by qPCR.  
17 n=8.

18 (H) Intracellular ATP quantified in lysates of CD19<sup>+</sup> B cell stimulated with IL-4 in  
19 the presence of 2-DG, 3PO, echinomycin, oligomycin, rotenone, UK-5099,  
20 etomoxir or BPTES. n=5.

21 (I) Illustration showing the simplified representation of the ATP release via the  
22 pannexin-1 channel and inhibition with carbenoxolone (CBX, 100 μM).

23 (J) Extracellular ATP quantified in the cell culture supernatant of CD19<sup>+</sup> B cells  
24 stimulated with IL-4 ±CBX. n=5.

25 (K) Schematic representation of bacterial killing and IL-10 production analysis by  
26 Mφs co-cultured with naive or septic B cells in the presence of *L. pneumophila* ±  
27 CBX determined after 48h (B-L) (also see STAR Methods). Naive peritoneal  
28 macrophages were pooled from 5 naive mice and B cells were isolated from naïve  
29 or CLP-surviving mice. n=6.

30 (L) The number of viable bacteria recovered from cell lysates.

31 (M) IL-10 concentrations in cell culture supernatants.

32 Data are representative of one 2-3 independent experiments. ns, not significant.  
33 \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 between indicated groups and #p<0.05:

1 compared to respective vehicle group. ns, not significant. One-way ANOVA with  
2 Bonferroni posthoc tests in B, C, E; two-tailed unpaired Student's t-test in D, F-  
3 H, J, M; and Mann–Whitney U test in L.

4  
5 **FIGURE 7: Characterization of CD39<sup>+</sup> B cells in septic patients**

6 (A) Representative flow cytometry plots and graph bar showing the frequency of  
7 Ki67<sup>+</sup>CD39<sup>+</sup> in CD19<sup>+</sup> B cells from blood of septic patients (n=18) and healthy  
8 controls (n=16).

9 (B) Gating strategy for the identification of human blood B cell subsets. Naive B  
10 cells (CD38<sup>-</sup>CD27<sup>-</sup>), memory B cells (CD38<sup>-</sup>CD27<sup>+</sup>), and plasmablasts  
11 (CD38<sup>+</sup>CD27<sup>+</sup>) gated in viable CD19<sup>+</sup> B cells from blood of septic patients (n=21)  
12 and healthy controls (n=21).

13 (C) Representative flow cytometry plots of CD39 expression in B cell subsets from  
14 septic patients (n=21) and healthy controls (n=21).

15 (D) Frequency of CD39 high (hi), intermediate (int), and negative (neg)  
16 expression in plasmablast cells from septic patients (n=21) and healthy controls  
17 (n=21).

18 (E) Ecto-ATPase activity of CD19<sup>+</sup> B cells sorted from septic patients (n=20) and  
19 healthy controls (n=21) cultured  $\pm$ CD39i.

20 (F) Plasma adenosine concentrations in septic patients (n=21) and healthy  
21 controls (n=36).

22 (G) Plasma adenosine concentrations in patients with sepsis (n=8) or septic  
23 shock (n=13) and healthy controls (n=36).

24 (H) Correlation between Ecto-ATPase activity of CD19<sup>+</sup> B cells and plasma  
25 adenosine concentration for each septic patient (n=20).

26 (I, J) CD14<sup>+</sup> monocytes (M $\phi$ ) isolated from blood of a healthy donor were co-  
27 cultured with septic or healthy B cells in the presence of *L. pneumophila*  
28 expressing luciferase at a MOI of 0.01 and A2aRi, apyrase or vehicle.

29 (I) Bacterial load determined by measuring the luminescence (RLU) on day 3  
30 after culture.

31 (J) IL-10 concentration in the cell culture supernatants.

32 (K-L) Single-cell RNA-sequencing data of blood cells from individuals with sepsis  
33 (Reyes et al., 2020).



1 (K) UMAP reduction plot of B cells from individuals with no sepsis (left) or patients  
2 with sepsis (right). The cells in black represent the plasmablast cells according to  
3 markers utilized by the original article.  
4 (L) Relative frequency of plasmablast in patients with no sepsis (black) and with  
5 sepsis (orange).  
6 \*,  $p < 0.05$ : compared to vehicle healthy B cell group. #,  $p < 0.05$ : compared to  
7 vehicle group of each individual. Two-tailed unpaired Student's t-test in A, F; one-  
8 way ANOVA with Bonferroni posthoc tests in D, E, G, J;  $p$ -value and correlation  
9 coefficient ( $r$ ) were obtained using the nonparametric Spearman rank correlation  
10 test in H; and Mann–Whitney U test in I.

## 1 STAR METHODS

## 2 RESOURCE AVAILABILITY

### 3 *Lead contact*

4 Further information and requests for reagents may be obtained from the Lead  
5 Contact, José Carlos Alves-Filho ([jcafilho@usp.br](mailto:jcafilho@usp.br)).

### 6 *Materials availability*

7 This study did not generate new or unique reagents.

### 8 *Data and code availability*

9 This paper analyzes existing, publicly available data from (Reyes et al., 2020) at  
10 Broad Institute Single Cell Portal ([https://singlecell.broadinstitute.org/single\\_cell](https://singlecell.broadinstitute.org/single_cell)):  
11 SCP548 (subject PBMCs). These accession numbers for the datasets are listed  
12 in the key resources table. All software and algorithms used in this study are  
13 publicly available and listed in the Key Resource table. All original code has been  
14 deposited and is publicly available at Zenodo  
15 <https://doi.org/10.5281/zenodo.4922037>. DOIs are listed in the key resources  
16 table.

## 17 EXPERIMENTAL MODEL AND SUBJECT DETAILS

### 18 *Animals*

19 C57BL/6 wild-type and BALB/c wild-type mice were purchased from Charles  
20 River.  $\mu$ MT<sup>-/-</sup> (002288), *Adora2a*<sup>-/-</sup> (010685), Foxp3-DTR-EGFP (016958), *Il10*<sup>-/-</sup>  
21 (002251), IL-10<sup>+/-EGFP</sup> (014530), *Lyz2*<sup>Cre</sup> (004781) and *Rag1*<sup>-/-</sup> (002216) mice were  
22 purchased from the Jackson Laboratory. *Entpd1*<sup>-/-</sup> mice were generated as  
23 previously described (Enjoji et al., 1999) and kindly provided by Dr. Gilles  
24 Kauffenstein. *Adora2a*<sup>flox</sup> mice were generated as previously described (Cekic et  
25 al., 2013) and kindly provided by Dr. Joel Linden. Myeloid cell-(*Adora2a*<sup>ΔLyz2</sup>)  
26 specific–*Adora2a*-deficient mice were generated by crossing the *Adora2a*<sup>flox/flox</sup>  
27 mice with *Lyz2*<sup>Cre</sup> mice.  $\mu$ MT<sup>-/-</sup>, *Adora2a*<sup>flox</sup>, *Entpd1*<sup>-/-</sup>, Foxp3-DTR-EGFP, *Il10*<sup>-/-</sup>,  
28 IL-10<sup>+/-EGFP</sup>, *Lyz2*<sup>Cre</sup>, and *Rag1*<sup>-/-</sup> mice used in this study were on a C57BL/6  
29 background. *Adora2a*<sup>-/-</sup> mice used in this study were on a BALB/c J background.  
30 All mice were bred and maintained under specific pathogen-free conditions at the  
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1 Animal Facility of the Ribeirão Preto Medical School, University of São Paulo. All  
2 experiments were carried out with 7-9-week-old male mice according to the  
3 guidelines of the Animal Welfare Committee of the Ribeirão Preto Medical  
4 School, University of São Paulo (protocol number: 070/2012 and 251/2019).

## 6 ***Patients***

7 Adult patients admitted to the Emergency Department of the School of Medicine  
8 of Ribeirão Preto with sepsis or septic shock between October 2020 and  
9 December 2020 were enrolled in the study (Table S1). Besides, age- and sex-  
10 matched healthy control volunteers were also included in the study. All patients  
11 enrolled fulfilled the criteria defined by the Third International Consensus  
12 Definitions for Sepsis and Septic Shock (Sepsis-3) (Singer et al., 2016). The  
13 exclusion criteria included active hematological malignancy or cancer and  
14 transplantation. Informed written consent from all participants was obtained. The  
15 study was approved by the Human Subjects Institutional Committee of the  
16 Ribeirão Preto Medical School, Brazil (Licence number: 30459114.6.0000.5440).

## 18 ***Caecal ligation and puncture (CLP)-induced polymicrobial sepsis model***

19 CLP-induced polymicrobial sepsis model (Rittirsch et al., 2009): Mice were  
20 anesthetized by inhalation administration of isoflurane (1-3 %), and two punctures  
21 were made in the caecum with an 18- or 23-gauge needle to induce lethal or  
22 moderate CLP-induced sepsis, respectively. Sham mice were submitted to the  
23 same procedures without caecal puncture. All mice were given analgesic (12.5  
24 mg.kg<sup>-1</sup>, tramadol, Agener União, subcutaneous) beginning 30 min before CLP  
25 and then every 12 h up to day 3. To increase the survival rates after lethal sepsis  
26 (Nascimento et al., 2010), C57BL/6 background mice received an i.p. injection of  
27 ertapenem sodium (30 mg.kg<sup>-1</sup>, Merck) beginning six h after CLP and continuing  
28 every 12 h for the first three days. In experiments performed on BALB/c  
29 background, mice received antibiotics up to day 4. In some experiments, septic  
30 mice were treated with A2aR antagonist [8-(3-Chlorostyryl)-caffeine, 1 mg.kg<sup>-1</sup> in  
31 DMSO 5 % in PBS], or CD39 inhibitor (ARL 67156 trisodium salt, 2 mg.kg<sup>-1</sup> in  
32 PBS). Mice were injected intraperitoneally with an A2aR antagonist or CD39  
33 inhibitor (200 µL) beginning on day three and continuing for 12 consecutive days  
34 (once a day). Survival was observed for up to 15 days.

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### **Microbial infection**

*Legionella pneumophila* infection (Zamboni et al., 2006): *L. pneumophila* (F2111) was kindly provided by Dr. Paul Edelstein (Edelstein et al., 2003). *L. pneumophila* was grown on charcoal yeast extract agar (10 g.L<sup>-1</sup> 4-morpholinepropanesulfonic acid [MOPS], 10 g.L<sup>-1</sup> Yeast extract, pH6.9, 15 g.L<sup>-1</sup> bacteriological agar, 2 g.L<sup>-1</sup> activated charcoal, supplemented with 0.4 g.L<sup>-1</sup> L-cysteine and 0.135 g.L<sup>-1</sup> Fe(NO<sub>3</sub>)<sub>3</sub>) at 35–37 °C, for 4 days from frozen stocks. Single colonies were streaked on fresh plates and allowed to grow for another 2 days. For *in vivo* infections (Nascimento et al., 2010), mice were intranasally given a single dose of *L. pneumophila* (7 x 10<sup>7</sup> bacteria in 40 µL).

*Aspergillus fumigatus* infection: *A. fumigatus* (strain CBS 144.89) was kindly provided by Dr. Jean-Paul Latge (Beauvais et al., 1997). Conidia were grown on 15 g.L<sup>-1</sup> cristomalt-D diastase malt powder and 15 g.dL<sup>-1</sup> bacteriological agar slants at 35–37 °C for 24 h and allowed to grow at room temperature for another six days. For *in vivo* infections, mice were intratracheally given a single dose of *A. fumigatus* (5 x 10<sup>7</sup> bacteria in 50 µL).

Survival was observed for up to 10 days.

### **Cell purifications**

Primary mouse CD19<sup>+</sup> B cells were purified from the spleen of mice using a FACSAriaII sorter (BD Biosciences). Cell purity was confirmed to be ≥ 95 %. Peritoneal cells of mice were enriched for macrophages by adherence to plastic Petri dishes for 16 h. Peripheral blood mononuclear cells (PBMC) were isolated from human peripheral blood using the ficoll-Paque Plus gradient (GE Healthcare). Primary human CD19<sup>+</sup> B cells were isolated by positive selection from PBMCs using CD19 MACS beads Multisort (Miltenyi Biotec) according to the manufacturer's recommendations. Cell purity was confirmed to be ≥ 95 %. Primary human CD14<sup>+</sup> monocytes were purified by positive selection from PBMCs using CD14 MACS beads Multisort (Miltenyi Biotec) according to the manufacturer's recommendations. Cell purity was confirmed to be ≥ 95 %.

### **B Cell Adoptive transfer**

1 Two protocols were used. First, for reconstitution of *Rag1*<sup>-/-</sup> mice (Guzik et al.,  
2 2007): B cells or vehicle (PBS) were injected i.v. into *Rag1*<sup>-/-</sup> mice (2 x 10<sup>7</sup>  
3 cells/mouse). The recipient mice were then submitted to CLP 3 weeks after  
4 adoptive transfer. Second, to evaluate the role of septic B cells in the control of  
5 bacterial replication (Nascimento et al., 2017): B cells or vehicle (PBS) were  
6 injected i.v. into naïve WT mice (5 x 10<sup>6</sup> cells/mouse). The naïve recipient mice  
7 were then infected i.n. with *L. pneumophila* on day 7 after adoptive transfer.

8

### 9 **Generation of B cell chimeric mice**

10 For the generation of chimeric mice (Tsui et al., 2018), *Entpd1*<sup>-/-</sup>,  $\mu$ MT<sup>-/-</sup>, and  
11 C57BL/6 bone marrow cells were obtained by flushing the femur and tibia.  
12 C57BL/6 mice (12-weeks-old) were irradiated using a Cesium 137 source  
13 irradiator (Mark I model 25) at 7 Gy. On the day after irradiation, the mice were  
14 divided into the following groups: (1) C57BL/6 mice repopulated with a mix of  
15 C57BL/6 BM cells and  $\mu$ MT<sup>-/-</sup> BM cells in proportion 1:4 (i.v.; 5 x 10<sup>6</sup>/mouse),  
16 called WT  $\mu$ MT-chimera; (2) C57BL/6 mice repopulated with a mix of *Entpd1*<sup>-/-</sup>  
17 BM cells and  $\mu$ MT<sup>-/-</sup> BM cells, called *Entpd1*<sup>-/-</sup>  $\mu$ MT-chimera. After bone marrow  
18 transplantation, the mice were treated with the antibiotic Ciprofloxacin  
19 hydrochloride diluted in drinking water (10 mg.mL<sup>-1</sup>, EMS) for 15 days. After 2  
20 months (period required for bone marrow engraftment), mice were submitted to  
21 CLP and were killed on day 15 after CLP.

22

### 23 **Bacterial replication on macrophage in co-culture with B cells**

24 For mouse samples: peritoneal macrophages from naïve mice were added to 48-  
25 well plates at a density of 5 x 10<sup>5</sup> cells per well. Cells were infected by 0.1 MOI  
26 *L. pneumophila* bacteria in the presence of B cells from the spleen of sepsis-  
27 surviving or naive mice or medium alone. In some experiments, we added CD39i  
28 (ARL 67156 200  $\mu$ M, Tocris), Apyrase (20 U.mL<sup>-1</sup>, Sigma), ATP $\gamma$ S (100  $\mu$ M,  
29 Tocris), ADA (4 U.mL<sup>-1</sup>, Sigma), NECA (10  $\mu$ M, Tocris), A2aR antagonist (8-(3-  
30 Chlorostyryl) caffeine, A2aRi, 100  $\mu$ M, Sigma), A2B antagonist (selective inverse  
31 agonist, A2bRi, MRS 1706, 100  $\mu$ M, Tocris), CBX (carbenoxolone disodium salt,  
32 100  $\mu$ M, Sigma) in the cultures. There were no antibiotics in the cell culture  
33 medium used for bacterial infection. On the second day, the supernatants were  
34 collected; cells were lysed with sterile H<sub>2</sub>O and plated for counting of counting of

1 colony-forming units (CFU) after 4 days of incubation at 37 °C (Nascimento et al.,  
2 2010). The results are expressed as log CFU per mL.  
3 For human samples: monocytes from the periphery blood of healthy volunteers  
4 were co-cultured with septic or healthy B cell in 96-well white plates at a density  
5 of  $1 \times 10^5$  for each type of cells per well (triplicate). *L. pneumophila* strains (JR32)  
6 stably expressing the *Photobacterium luminescens luxCDABE operon* was kindly  
7 provided by Dr. Dario Zamboni (Gonçalves et al., 2019). *L. pneumophila* was  
8 grown on charcoal yeast extract agar (10 g.L<sup>-1</sup> 4- orpholinepropanesulfonic acid  
9 [MOPS], 10 g.L<sup>-1</sup> Yeast extract, pH6.9, 15 g.L<sup>-1</sup> bacteriological agar, 2 g.L<sup>-1</sup>  
10 activated charcoal, supplemented with 0.4 g.L<sup>-1</sup> L-cysteine and 0.135 g.L<sup>-1</sup>  
11 Fe(NO<sub>3</sub>)<sub>3</sub>) at 35–37 °C, for 4 days from frozen stocks. Single colonies were  
12 streaked on fresh plates and allowed to grow for another 2 days. Cells were  
13 infected by *L. pneumophila* bacteria expressing luciferase at an MOI of 0.01 in  
14 the presence of A2aRi (8-(3-Chlorostyryl) caffeine, 100 µM, Sigma) or Apyrase  
15 (20 U.mL<sup>-1</sup>, Sigma). There were no antibiotics in the cell culture medium used for  
16 bacterial infection. On the third day, luminescence emission was measured at  
17 470 nm with a Spectra-L plate reader (Molecular Devices, California, USA), and  
18 the supernatants were collected. The results are expressed as live bacteria  
19 (RLU).

20

## 21 **METHODS DETAILS**

22

### 23 ***Adenosine quantification***

24 *Blood collection and sample preparation.* For adenosine quantification in plasma  
25 (Veras et al., 2015), blood was collected in tubes containing 10 µM of pentostatin  
26 (adenosine deaminase inhibitor, Tocris) and heparin (for mouse) or K<sub>3</sub>EDTA (for  
27 human). Blood samples were centrifuged at 10,000 × g for 10 min at 4 °C, and  
28 plasma was stored at –80 °C until the analyses. For adenosine quantification in  
29 the plasma, theophylline (internal standard) and 1 mL of acetonitrile were added  
30 in 200 µL of plasma. The tube was vortexed for 2 min, centrifuged at 10,000 × g  
31 for 10 min at 4 °C, and the supernatants were evaporated by a vacuum  
32 concentrator system (CentriVap, Labcongo Corporation). The dry residue was  
33 resuspended in 100 µL of mobile phase (water with 0.1 % formic acid). A similar  
34 protocol was used to adenosine the curve. The different concentrations of

1 adenosine (Sigma) were added to the plasma (collected without adenosine  
2 deaminase inhibitor) together with theophylline (internal standard). Samples  
3 (100  $\mu$ L) were analyzed by LC-MS/MS using a previously described method  
4 (Veras et al., 2015).

5 For adenosine detection in the supernatant, B cells from sepsis-surviving mice  
6 were stimulated with ATP (100  $\mu$ M, Sigma) by 15 min. At the end of the cultures,  
7 the supernatants were precipitated with 66 % acetonitrile (J. T. Baker) to denature  
8 protein stabilize adenosine and diluted 33 % in water for adenosine analysis.  
9 Different concentrations of adenosine (Sigma) were prepared in methanol to  
10 construct a standard curve.

11

12 *LC-MS/MS equipment and conditions.* For adenosine quantification in plasma,  
13 the analyses were carried out by LC-MS/MS (liquid chromatography-mass  
14 spectrometry). Chromatographic analyzes were performed using high-  
15 performance liquid chromatography equipment (Shimadzu, Kyoto, Japan),  
16 consisted of an LC-10ADVP binary solvent delivery pumps, SLC-10AVP system  
17 controller, SIL-20A Prominence autosampler, and CTO-10ASVP column oven set  
18 at 25 °C. The separations were performed using a 100  $\times$  3.9 mm XTerra MS C18  
19 column with a particle size of 3.5  $\mu$ m (Waters, Milford, MA, USA) and a  
20 20  $\times$  3.9 mm XTerra MS C18 guard column with a particle size of 5  $\mu$ m (Waters,  
21 Milford, MA, USA). The mobile phase was composed of (X) water with 0.1 %  
22 formic acid and (Y) acetonitrile. The binary gradient elution (X: Y proportion, v/v),  
23 at a flow rate of 0.3 mL.min<sup>-1</sup>, was composed by 96:4 from 0 to 5 min; switching  
24 to 50:50 from 5 to 7 min; maintained by 11 min; switching back to the initial  
25 condition from 11 to 13 min, and maintaining on this proportion till 16 min. This  
26 system was coupled to a mass spectrometer composed of mass analyzers of the  
27 triple-quadrupole type (Quattro LC, Micromass, Manchester, UK) with an  
28 electrospray interface, operating in positive mode (ESI +). The temperatures of  
29 the source block were set at 100 °C, and desolvation gas was set at 350 °C.  
30 Argon was used as collision gas, and nitrogen was used as both desolvation  
31 (nearly 360 L.h<sup>-1</sup>) and nebulizer (nearly 40 L.h<sup>-1</sup>) gas. During the analyses, the  
32 voltages employed in the ESI source were 3kV for the capillary, 3V for the  
33 extractor, and 20V for the cone. The ions detection was carried out in the multiple  
34 reaction monitoring (MRM) mode, employing collision energy of 15 eV, monitoring

1 the transitions of the m/z 268 precursor ion to the m/z 136 production for  
2 adenosine (268 > 136) and 181 > 124 for theophylline (internal standard). The  
3 analytical data were calculated using the MassLynx software (Micromass,  
4 Manchester, UK).

5 For adenosine detection in the supernatant, samples or standards (100 µL) were  
6 injected in a Xevo TQ-S system Waters Acuity UPLC HSS with column C18  
7 Acuity UPLC HSS with 1,0x150 mm (liquid chromatography separation). The  
8 temperature of the column oven was set at 40 °C. The solvent system consisted  
9 of 0.1 % acetic acid in water and 0.1 % acetic acid in methanol. The flow rate was  
10 set to 500 µL/min, and an injection volume of 5 µL was used with a total run time  
11 of 4.5 min. The ion positive fragment m/z 268 and 136 were observed. The  
12 analytical data were processed by MassLynx software (Micromass, Manchester,  
13 UK).

14

### 15 **Microbial counts**

16 On day 2 (for *L. pneumophila*) or 3 (for *A. fumigatus*) after infection, lungs and/or  
17 spleen were collected and homogenized for 30 s with a PowerGen 125  
18 homogenizer (Fisher Scientific) in sterile H<sub>2</sub>O (Nascimento et al., 2010). Dilutions  
19 of lung and spleen lysates were plated on charcoal yeast extract agar for the  
20 determination of CFU per organ.

21

### 22 **Flow cytometry**

23 Flow cytometric staining was performed as previously described (Nascimento et  
24 al., 2017). For mouse staining, Foxp3 EGFP, µMT<sup>-/-</sup>, WT cells were stained with  
25 Live/Dead viability dye (Thermo Fisher Scientific) and specific antibodies to CD39  
26 (24DMS1 and Duha59, Thermo Fisher Scientific and BioLegend), CD19 (1D3,  
27 Thermo Fisher Scientific or BD Biosciences; 6D5, BioLegend), CD11b (M1/70,  
28 Thermo Fisher Scientific), CD11c (N418, BioLegend), CD4 (RM4-5, BioLegend),  
29 B220 (CD45R; RA3-6B2, BioLegend or BD Bioscience), CD8 (YTS156.7.7,  
30 BioLegend), TCRβ (H57-597, Thermo Fisher Scientific), NK1.1 (PK136,  
31 BioLegend), Ly6G (1A8, BD Biosciences), CD223 (LAG-3, C9B7W, BioLegend),  
32 IgM (RMM-1, BioLegend), IgD (11-26c.2a, BD Biosciences), CD138 (281-2,  
33 BioLegend), CD3 (17A2, Thermo Fisher Scientific), CD5 (53-7.3, BD  
34 Biosciences), CD23 (B3B4, Thermo Fisher Scientific), CD21/CD35 (CR2/CR1;



1 7E9, BioLegend), Ki67 (B56, BD Biosciences), and CD45 (30-F11, BioLegend)  
2 for 30 min. For human cells staining, PBMC were stained with Live/Dead viability  
3 dye (Thermo Fisher Scientific) and specific antibodies to CD39 (TU66, BD  
4 Biosciences), CD19 (HIB19, BD Biosciences), CD38 (HIT2, BD Biosciences),  
5 CD27 (M-T271, BD Biosciences), Ki67 (20Raj1, Thermo Fisher Scientific) for 30  
6 min. For 2-NBDG uptake detection (Thermo Fisher Scientific), the reaction was  
7 stopped by removing the incubation medium and washing the cells twice with  
8 PBS. Cells were subsequently stained with Live/Dead viability dye (Thermo  
9 Fisher Scientific) and specific antibodies to CD39 and CD19 for 10 min, and flow  
10 cytometry analysis was performed within 30 min. For IL-10 detection, IL-10 EGFP  
11 cells were incubated with phorbol-12-myristate-13-acetate ( $50 \text{ ng.mL}^{-1}$ , Sigma-  
12 Aldrich), ionomycin ( $500 \text{ ng.mL}^{-1}$ , Sigma-Aldrich), and GolgiStop (BD  
13 Biosciences) for 4 h prior to antibody staining. Fresh cells were collected on  
14 Canto, Verse, and Fortessa flow cytometers (BD Biosciences) and analyzed  
15 using FlowJo (TreeStar) software. Cells were sorted using a FACS Aria II.

16

### 17 ***High-dimensional flow cytometric analyses***

18 High-dimensional flow cytometric analysis of the CD39<sup>+</sup> expression on the cell  
19 populations from the spleen of naïve and sepsis-surviving mice were performed  
20 in immune cells based on the following gate strategies, on the live CD45<sup>+</sup> cells:  
21 neutrophils (Ly6G<sup>+</sup>CD11b<sup>+</sup>), Mφ (F4/80<sup>+</sup>CD11b<sup>+</sup>), DCs (CD11c<sup>+</sup>CD11b<sup>+</sup>),  
22 CD4<sup>+</sup>Foxp3<sup>-</sup> T cells (Foxp3<sup>-</sup>CD4<sup>+</sup>TCRβ<sup>+</sup>), CD8<sup>+</sup> T cells (CD8<sup>+</sup>TCRβ<sup>+</sup>),  
23 CD4<sup>+</sup>Foxp3<sup>+</sup> Treg cells (Foxp3<sup>+</sup>CD4<sup>+</sup>TCRβ<sup>+</sup>), B cells (CD19<sup>+</sup> B220<sup>var</sup>), NK cells  
24 (NK1.1<sup>+</sup>) and NKT cells (NK1.1<sup>+</sup>TCRβ<sup>+</sup>). The t-SNE algorithm was performed for  
25 unsupervised analysis of the entire flow cytometry dataset (10 samples per  
26 experiment) generated from naïve and sepsis-surviving mice. The t-SNE  
27 algorithm was run on the DownSample of live CD45<sup>+</sup> CD39<sup>+</sup> populations [100,000  
28 cells, randomly selected from naïve (n=5) and sepsis survivors (n= 5), 50,000  
29 cells each group)]. Flow cytometry-based immune cell populations were overlaid  
30 as a color dimension. For the B cells subpopulation analysis, cells were gated on  
31 the live CD45<sup>+</sup> as follows: total B cells (CD19<sup>+</sup> CD3<sup>neg</sup>), CD39 subset (CD39<sup>+</sup>),  
32 IgD<sup>+</sup> subset (IgD<sup>+</sup> IgM<sup>neg</sup>), IgD<sup>+</sup> IgM<sup>+</sup> subset (IgD<sup>+</sup> IgM<sup>+</sup>), IgDneg IgMneg subset  
33 (IgD<sup>neg</sup> IgM<sup>neg</sup>), and CD138<sup>+</sup> subset (CD138<sup>+</sup>). t-SNE was run on the  
34 DownSample of 150,000 live CD39<sup>+</sup>CD19<sup>+</sup> cells randomly sampled from the

1 spleen of naïve and sepsis-surviving mice (n=5 per group and 75,000 cells from  
2 each group). Gating strategies for flow cytometry analysis are shown in [Figures](#)  
3 [S3A and S3E](#).

#### 4 5 **Ecto-ATPase activity**

6 The ecto-ATPase activity was determined by the malachite green assay, with  
7 some modifications as described (Peres et al., 2015). Splenic or B cells ( $2 \times 10^5$   
8 cells/well for mouse cells and  $1 \times 10^5$  cells/well for human cells) were pre-treated  
9 with CD39i (ARL 67156 trisodium salt, 200  $\mu$ M, Tocris) or vehicle for 30 min and  
10 then stimulated with 1 mM ATP (Sigma) in a 96-well plate at 37 °C for 30 min.  
11 The supernatant cell culture medium was collected, and the malachite green  
12 solution was added (one-part 4.2 g ammonium molybdate dissolved in 100 mL 4  
13 M HCl + three parts 0.045 % malachite green in H<sub>2</sub>O). Next, optical density was  
14 measured at 650 nm. The levels of inorganic phosphate released were calculated  
15 from a parallel prepared phosphate standard curve (K<sub>2</sub>HPO<sub>4</sub>).

#### 16 17 **qPCR**

18 Total RNA from CD19<sup>+</sup> B cells from the spleen was extracted using an RNeasy  
19 Mini Kit (74106, Qiagen), according to the manufacturer's directions. First-strand  
20 cDNA was synthesized from 500  $\mu$ g of RNA using the high-capacity cDNA  
21 Reverse Transcription Kit (4368814, Thermo Fisher Scientific). Quantitative real-  
22 time PCR was performed using Power Syber Green PCR Master Mix TaqMan  
23 (Thermo Fisher Scientific), primers as described in the Key Resources Table and,  
24 the Viiia7 Real-Time PCR system. The data were normalized to *Gapdh* values,  
25 and the fold change was analyzed using the DDCT method. The relative gene  
26 expression was expressed in arbitrary units based on the naive group, which was  
27 assigned a value of 1.

#### 28 29 **ELISA**

30 IL-10 concentration in culture supernatants and lung tissues was determined by  
31 ELISA according to the manufacturer's instructions (R&D Systems).

#### 32 33 **ATP assay**

1 Intracellular or extracellular ATP was detected using the ATPlite Luminescence  
2 ATP Detection Assay (PerkinElmer, Waltham, MA), according to the  
3 manufacturer's protocol. For the detection of intracellular ATP, CD19<sup>+</sup> B cells  
4 were stimulated with IL-4 (10 ng.mL<sup>-1</sup>) at 37°C with 5% CO<sub>2</sub> for 6 h in the presence  
5 obligatory of a pannexin-1 channel inhibitor (carbenoxolone disodium salt, 100  
6 μM, Sigma), and/or with 2-DG (3 mM), 3PO (30 μM), echinomycin (5 nM),  
7 oligomycin (1 μM), rotenone (2.5 μM), UK-5099 (20 μM), etomoxir (3 μM) or  
8 BPTES (10 μM). The cells were lysed to measure ATP levels 6 h later. For the  
9 detection of the extracellular ATP, we performed CD19<sup>+</sup> B cell culture stimulated  
10 with IL-4 (10 ng.mL<sup>-1</sup>) for 6 h in the presence of a CD39 inhibitor (ARL 67156  
11 trisodium salt, 200 μM, Tocris), and culture supernatants were collected and  
12 measured for ATP levels 6 h later. In some experiments, B cells were incubated  
13 with CBX (carbenoxolone disodium salt, 100 μM, Sigma) for measured released  
14 ATP.

15

### 16 ***Glucose uptake assay***

17 Glucose uptake was detected using fluorescent 2-NBDG (Cayman) according to  
18 the manufacturer's protocol. B cells were plated at 1 x 10<sup>6</sup>/well in 12-well plates  
19 and, after 18 h pre-incubation, all culture medium was removed from each well  
20 and replaced with 100 μL of culture medium without glucose in the presence of  
21 fluorescent 2-NBDG (30 μM) compounds. Plates were incubated at 37 °C with 5  
22 % CO<sub>2</sub> for 30 min. The reaction was stopped by removing the incubation medium  
23 and washing the cells twice with PBS.

24

### 25 ***Metabolic Profiling***

26 Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were  
27 measured using the Seahorse XF96 Analyzer (Agilent Technologies) according  
28 to the manufacturer's guidelines (Agilent Seahorse XF24). Isolated CD19<sup>+</sup> B cells  
29 were stimulated with IL-4 (10 ng.mL<sup>-1</sup>) at 37°C with 5% CO<sub>2</sub> for 6 h. Before  
30 analysis, 4 x 10<sup>5</sup> B cells were washed with XF media (for ECAR: non-buffered  
31 RPMI 1640 containing 2 mM L-glutamine and 1 mM sodium pyruvate; for OCR  
32 non-buffered RPMI 1640 containing 1 mM glucose and 1 mM sodium pyruvate)

1 and incubated for 30 min at 37 °C in the absence of CO<sub>2</sub>. ECAR was measured  
2 under basal conditions and, after the addition of the following drugs: 10 mM  
3 glucose, 1 μM oligomycin, 50 mM 2DG (XF Glycolysis Stress test Kit, Agilent  
4 Technologies). OCR was measured under basal conditions and, after the addition  
5 of the following drugs: 1.5 μM Oligomycin, 1 μM FCCP, 5 μM Rotenone (XF Mito  
6 Stress test Kit, Agilent Technologies).

### 7 8 ***Re-analyzing Public Datasets***

9 We re-analyzed single-cell transcriptomic data from the peripheral blood  
10 mononuclear cells (PBMC) of septic patients from a public dataset (Reyes et al.,  
11 2020). Basically, the samples were combined into two major groups for further  
12 comparisons, septic patients (sepsis) and non-septic patients (no sepsis). The  
13 sepsis group included samples from patients with urosepsis and patients with  
14 sepsis admitted to the medical intensive care unit, whereas the no sepsis group  
15 included samples from healthy patients and patients without sepsis admitted to  
16 the medical intensive care unit. B cells were filtered based on the authors'  
17 previous annotation for downstream analysis (Reyes et al., 2020). Specifically,  
18 the data matrices of 7970 B cells were imported to Seurat v3.1 (Stuart et al.,  
19 2019) by filtering genes expressed in at least 10 cells and more than 100 unique  
20 molecular identifiers (UMI) counts per cell. For the pre-processing step, outlier  
21 cells were filtered out based on three metrics (library size < 10000, number of  
22 expressed genes between 200 and 2000, and mitochondrial percentage  
23 expression < 5), resulting in a matrix with 13077 genes and 5104 cells. The  
24 remaining counts were normalized using the 'LogNormalize' method with a scale  
25 factor of 10000. The top 2,000 variable genes were then identified using the 'vst'  
26 method using the FindVariableFeatures function. Percent of mitochondrial genes  
27 was regressed out in the scaling step, and Principal Component Analysis (PCA)  
28 was performed using the top 2,000 variable genes with 50 dimensions.  
29 Additionally, a clustering analysis was performed on the first 7 principal  
30 components using a resolution of 0.6 followed by Uniform Manifold  
31 Approximation and Projection (UMAP), a dimensionality reduction technique for  
32 data visualization. Then, differential gene expression analysis was performed  
33 using FindAllMarkers function with default parameters to obtain a list of significant

1 gene markers for each cluster of cells. The plasmablast cells were identified by  
2 expression of CD39 gene counts > 0 in the RNA assay and BS3 plasmablast  
3 gene markers previously described by Reyes et al. 2020. Relative abundance of  
4 plasmablast in patients with no sepsis and with sepsis was calculated by dividing  
5 the number of plasmablasts in each group by the total number of plasmablasts.  
6 For enrichment analyses, we utilized the EnrichR tool (Chen et al., 2013) with  
7 Reactome 2016 database using plasmablast gene markers as input list.

## 8 9 **QUANTIFICATION AND STATISTICAL ANALYSIS**

10 Prism 8 software (GraphPad) was used for data analysis. We observed normal  
11 distribution. Survival studies were analyzed with the Mantel-Cox log-rank test,  
12 and microbial counts were calculated using the Mann–Whitney U test.  
13 Comparisons for two groups were calculated using unpaired two-tailed Student’s  
14 t-tests and multiple comparisons by one-way ANOVA with Bonferroni’s posthoc  
15 tests. Comparisons for the time course of CLP groups with a control group were  
16 performed using one-way ANOVA results with Dunnett posthoc tests.  
17 Correlations were analyzed with two-tailed nonparametric Spearman rank  
18 correlation tests. Differential gene expression analysis was performed using the  
19 FindAllMarkers function using the Mann–Whitney U test to obtain a list of  
20 significant gene markers for each cluster of cells with default parameters. Data  
21 are represented as means ± SEM or bacterial load as a median. Statistical  
22 significance: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001.

23

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