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Amélie Bonaud, Laetitia Gargowitsch, Simon M Gilbert, Elanchezhian Rajan, Pablo Canales-Herrerias, Daniel Stockholm, Nabila F Rahman, Mark O Collins, Hakan Taskiran, Danika L Hill, et al.

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1 **Sec22b is a critical and non-redundant regulator of plasma cell maintenance.**

2 Amélie Bonaud<sup>1,2,3</sup>, Laetitia Gargowitsch<sup>4</sup>, Simon M. Gilbert<sup>5</sup>, Elanchezhian Rajan<sup>6</sup>, Pablo Canales-  
3 Herrerias<sup>7</sup>, Daniel Stockholm<sup>8,9</sup>, Nabila F. Rahman<sup>10</sup>, Mark O. Collins<sup>6</sup>, Hakan Taskiran<sup>11,12,13</sup>, Danika  
4 L. Hill<sup>14,15</sup>, Andres Alloatti<sup>16,17</sup>, Nagham Alouche<sup>4</sup>, Stéphanie Balor<sup>18</sup>, Vanessa Soldan<sup>18</sup>, Daniel  
5 Gillet<sup>19</sup>, Julien Barbier<sup>19</sup>, Françoise Bachelier<sup>4</sup>, Kenneth G.C. Smith<sup>5,20</sup>, Julia Jellusova<sup>11,12</sup>, Pierre  
6 Bruhns<sup>7</sup>, Sebastian Amigorena<sup>16</sup>, Karl Balabanian<sup>1,2,3</sup>, Michelle A. Linterman<sup>14</sup>, Andrew A. Peden<sup>6</sup> and  
7 Marion Espéli<sup>1,2,3\*</sup>

8

9 *1- Université Paris-Cité, Institut de Recherche Saint-Louis, INSERM U1160, F-75010 Paris, France,*

10 *2- CNRS, GDR3697 “Microenvironment of tumor niches”, Micronit, France,*

11 *3- OPALE Carnot Institute, The Organization for Partnerships in Leukemia, Hôpital Saint-Louis,*  
12 *Paris, France*

13 *4- Université Paris-Saclay, INSERM, Inflammation, Microbiome and Immunosurveillance, Clamart,*  
14 *France.*

15 *5- Department of Medicine, University of Cambridge, Cambridge Biomedical Campus, Addenbrooke’s*  
16 *Hospital, Cambridge, UK.*

17 *6- School of Bioscience, University of Sheffield, Western Bank, Sheffield, S102TN, UK*

18 *7- Institut Pasteur, Université de Paris, Unit of Antibodies in Therapy and Pathology, Inserm*  
19 *UMR1222, F-75015 Paris*

20 *8- PSL Research University, EPHE, Paris, France*

21 *9- Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine, CRSA, F-75012, Paris, France*

22 *10- Dementia Research Institute, University of Cardiff, Hadyn Ellis Building, Maindy Road, Cardiff,*  
23 *CF24 4HQ*

24 *11- Institute of Clinical Chemistry and Pathobiochemistry, School of Medicine, Klinikum rechts der*  
25 *Isar, Technical University of Munich, 81675 Munich, Germany*

26 *12- TranslaTUM, Center for Translational Cancer Research, Technical University of Munich, 81675*  
27 *Munich, Germany*

28 *13- Max Planck Institute of Immunobiology and Epigenetics, 79108 Freiburg im Breisgau, Germany*

29 *14- Lymphocyte Signalling and Development, Babraham Institute, Babraham Research Campus,*  
30 *Cambridge CB22 3AT, UK*

31 *15- Department of Immunology and Pathology, Monash University, Melbourne, Victoria, 3004,*  
32 *Australia*

33 *16- PSL Research University, Institut Curie Research Center, INSERM U932, Paris, France*

34 *17- Facultad de Ciencias Médicas, Instituto de Inmunología Clínica y Experimental de Rosario*  
35 *(IDICER)-CONICET/Universidad Nacional de Rosario, Rosario, Argentina.*

36 *18- METi, Centre de Biologie Intégrative, Université de Toulouse, CNRS, UPS, 31062, Toulouse,*  
37 *France*

38 *19- Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé*  
39 *(DMTS), SIMoS, 91191 Gif-sur-Yvette, France*

40 *20- Cambridge Institute of Therapeutic Immunology & Infectious Disease, Jeffrey Cheah Biomedical*  
41 *Centre Cambridge Biomedical, University of Cambridge, Cambridge, United Kingdom*

42

43 \* Corresponding author: marion.espeli@inserm.fr

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46 *Keywords:* Plasma cell – antibody – SNARE - Endoplasmic reticulum – Mitochondria.

47 **Abstract**

48 Despite the essential role of plasma cells in health and disease, the cellular mechanisms controlling  
49 their survival and secretory capacity are still poorly understood. Here, we identified the Soluble N-  
50 ethylmaleimide-sensitive factor Attachment Protein Receptor (SNARE) Sec22b as a unique and  
51 critical regulator of plasma cell maintenance and function. In absence of Sec22b, plasma cells were  
52 hardly detectable and serum antibody titres were dramatically reduced. Accordingly, *Sec22b* deficient  
53 mice fail to mount a protective immune response. At the mechanistic level, we demonstrated that  
54 Sec22b contributes to efficient antibody secretion and is a central regulator of plasma cell maintenance  
55 through the regulation of their transcriptional identity and of the morphology of the endoplasmic  
56 reticulum and mitochondria. Altogether, our results unveil an essential and non-redundant role for  
57 Sec22b as a regulator of plasma cell fitness and of the humoral immune response.

58

59

60 **Significance Statement:**

61 Despite their central role in health and disease, the cellular mechanisms underlying how plasma cells  
62 persist while producing large quantities of antibodies are still poorly understood. In this paper we  
63 describe that plasma cell survival is under the strict dependency of the Soluble N-ethylmaleimide-  
64 sensitive factor Attachment Protein Receptor (SNARE) molecule Sec22b. We showed that this  
65 molecule is a key regulator of endoplasmic reticulum and mitochondrial structure in plasma cells and  
66 in its absence the humoral immune response is abrogated. Our findings have important implications  
67 for our understanding of antibody mediated immunity but also open avenues for targeting plasma cells  
68 in pathological contexts.

69

70

71 **Introduction:**

72 Plasma cells (PCs) are the cellular source of humoral immunity via the long-term secretion of large  
73 quantities of antibodies that provide protection against reinfection. These cells can also contribute to  
74 diseases including plasmacytomas as well as antibody-mediated autoimmune and inflammatory  
75 pathologies. However, the therapeutic arsenal to target PCs is still very limited. Despite the essential  
76 role of these cells in health and disease, the cellular mechanisms controlling their secretory function  
77 and their survival are poorly understood. Closing this knowledge gap is thus of paramount importance  
78 for designing new approaches to target this cell type.

79 During the transition from B cell to PC, the cell is reprogrammed to produce and secrete around  $10^2$ -  
80  $10^3$  antibodies per second (1). To accommodate this large protein load, PCs expand their endoplasmic  
81 reticulum (ER) and adapt to tolerate the extra stress induced via upregulation of the Ire1 $\alpha$ /Xbp1  
82 branch of the unfolded protein response (UPR) (2–4). Early works from the 70s report that antibody  
83 secretion happens via the conventional constitutive exocytosis pathway meaning that they are not pre-  
84 stocked in granules but secreted as they are produced and trafficked from the ER to the Golgi  
85 apparatus where they are glycosylated (5–7).

86 Here, through a proteomics analysis, we identified the SNARE Sec22b as an interesting molecule  
87 upregulated in mouse and human PCs. Members of the Soluble N-ethylmaleimide-sensitive factor  
88 Attachment Protein Receptor (SNARE) family are essential for the intracellular transport and fusion of  
89 protein cargoes between organelles. They are involved in both regulated and constitutive exocytosis  
90 and form a large family composed of different subtypes (Qa-, Qb-, Qc- and R-SNAREs) that interact  
91 to form a ternary SNARE complex allowing membrane fusion between vesicles and organelles (8).  
92 Distinct sets of SNAREs are expressed on the different organelles and can also be cell type specific  
93 (9). On top of vesicular transport, Sec22b have other non-canonical functions, some of which could be  
94 highly relevant for PC biology, including ER branching, nuclear shuttling of transcription factors as  
95 well as plasma membrane expansion (10–13). Using a conditional KO mouse model, we demonstrated  
96 that Sec22b is essential for PC maintenance and effective humoral response after vaccination and  
97 infection. At the mechanistic level we showed that Sec22b governs expression of genes involved in  
98 cell-cycle but also mitochondrial function and ER structure. Accordingly, Sec22b-deficient PCs

99 displayed a dramatically altered ER network, reduced contact between the ER and the mitochondria  
100 and hyperfused mitochondria associated with poor survival. To date, little is known about how  
101 organelle homeostasis is controlled in PCs and our results highlight Sec22b as a critical regulator of  
102 this process with clear relevance for normal immune response regulation and for PC-mediated  
103 diseases.  
104

105 **Results:**

106 *The Sec22b SNARE and its partner are overexpressed in plasma cells:*

107 To identify new molecular actors that contribute to PC biology we performed a mass-spectrometry  
108 analysis of sorted splenic B cells and *in vitro* generated PCs. All the data generated are available  
109 through a user-friendly interface (<https://plasmacytomics.shinyapps.io/home/>). We first confirmed that  
110 the expression of well-known factors of B cell and PC differentiation behaved as one would predict.  
111 As expected, the B cell transcription factor Pax5 was significantly downregulated in PCs compared to  
112 B cells while the transcription factor Xbp1 was significantly upregulated in PCs (Figure 1A).  
113 Molecules involved in ER to Golgi trafficking, including SNAREs (Syntaxin 5 (Stx5), Sec22b and  
114 Ykt6), the syntaxin regulator Sec1/Munc-18 protein (SCFD1/Sly1) and tethering factors  
115 (USO1/p115), were also upregulated at the protein level in PCs compared to B cells (Figure 1A and  
116 Supplementary Figure 1). We confirmed these results by western blotting and observed that the  
117 SNAREs Stx5, Sec22b and Ykt6 were twice more expressed in PCs compared to B cells (Figure 1B).  
118 Published microarray and RNAseq datasets were also included to the Plasmacytomics interface and in  
119 support of our proteomic data, we also observed a significant increase in the levels of expression of  
120 the genes encoding these three SNAREs in sorted murine PCs compared to B cells (Figure 1C). Data  
121 mining of human RNAseq datasets also showed significantly increased expression of *SEC22B* and  
122 *STX5A* in human PCs compared to B cells (Figure 1D) suggesting that this is a conserved feature in  
123 PCs across species.

124 Previous works have shown that the ER-localised SNARE Sec22b forms a complex with the Golgi-  
125 localised SNARE Stx5 to allow fusion of transport vesicles between these two organelles (9, 14, 15).  
126 In absence of a suitable animal model, we first tested the functional relevance of Stx5 expression in  
127 PC *in vitro* using a shRNA specific for Stx5 together with GFP reporter expression (Supplementary  
128 Figure 2A-D). From day 3, the frequency of total PCs in the *Stx5* knock down (KD) samples  
129 progressively diminished while it was constant in the control samples (Supplementary Figure 2C-D  
130 left panel) and this decrease was due entirely to the loss of GFP<sup>+</sup> PCs in the *Stx5* KD samples  
131 (Supplementary Figure 2D central panel). The frequency of GFP<sup>+</sup> B cells was not modified by *Stx5*  
132 KD (Supplementary Figure 2D, right panel), confirming the specific requirement of *Stx5* expression

133 for PC persistence *in vitro*. We next took advantage of a small molecule, Retro-2, reported to block  
134 Stx5 function by mislocalizing it and blocking its recycling (16, 17). When *in vitro* differentiated WT  
135 PCs were cultured for 5 hours in presence of non-toxic doses of Retro-2 we observed a significant  
136 reduction of antibody secretion (Supplementary Figure 2E). Moreover, injection of a single dose of  
137 Retro-2 intraperitoneally to WT mice led to a reduction of the antibodies secreted by BM PCs *ex vivo*  
138 (Supplementary Figure 2F), supporting a role for Stx5 in antibody secretion on top of PC survival.  
139 Altogether our data suggest that the machinery required for ER to Golgi transport including  
140 Stx5/Sec22b is upregulated during PC differentiation and that disruption of Stx5 leads to defects in  
141 both PC viability and function.

142

143 *Dramatic loss of plasma cells and circulating antibodies in absence of Sec22b:*

144 Taking in consideration the effect of Stx5 on PC maintenance and antibody secretion, we next  
145 explored the implication of its partner Sec22b. We crossed a *Sec22b* floxed mouse model (10) with the  
146 mb1-cre strain (18) to generate a mouse model lacking *Sec22b* expression specifically in the B cell  
147 lineage (*Sec22b*<sup>fllox/fllox</sup> x mb1-cre, hereafter referred to as *Sec22b*<sup>B-KO</sup>) (Supplementary Figure 3A-B).  
148 Data mining of publicly available RNAseq datasets (Immgen) suggest that *Sec22b* is expressed at a  
149 constant low level throughout B cell differentiation (Supplementary Figure 3C) so we investigated  
150 whether *Sec22b* deficiency may alter this process. In absence of *Sec22b*, B cell development was  
151 roughly normal with only a mild reduction of the number of BM mature B cells and splenic follicular  
152 B cells compared to WT mice, whereas BM B cell precursors, splenic immature, marginal zone and  
153 CD93<sup>+</sup>/CD21<sup>+</sup>/CD23<sup>-</sup> B cells were unaffected (Supplementary Figure 3D-E). Despite this roughly  
154 normal B cell development, *Sec22b*<sup>B-KO</sup> mice had almost no circulating antibody at steady state for all  
155 the isotypes tested (Figure 2A). Igκ representing around 90% of secreted antibodies in the mouse were  
156 reduced over 40 times. IgG1 titres were 55 times lower in absence of *Sec22b*. IgM, IgA and IgG3  
157 were 40, 30 and 20 times lower in absence of *Sec22b*, respectively (Figure 2A).

158 This was associated with a ten-fold reduction of the frequency and absolute number of PCs in the  
159 spleen and the BM with the mature CD19<sup>+</sup>/B220<sup>+</sup> PCs being the most affected (Figure 2B and C).  
160 Accordingly, we observed that the few remaining splenic PCs in *Sec22b*<sup>B-KO</sup> mice display a less

161 mature profile than their WT counterparts with reduced expression of the PC markers *Cd93* and  
162 *Tnfrsf13b* and of the PC master regulators *Prdm1*, *Xbp1* and *Irf4*. In contrast enhanced expression of  
163 the B cell master regulators *Pax5* and *Bach2* were detected at the transcriptional level in *Sec22b*<sup>B-KO</sup>  
164 PCs compared to WT (Figure 2D). Thus, our results demonstrate that *Sec22b* plays a critical and non-  
165 redundant role in PC maintenance and in the control of antibody circulating titres at steady state.

166

167 *Sec22b regulates plasma cell maintenance and molecular identity:*

168 To unravel at which step of PC differentiation *Sec22b* is required, we performed *in vitro*  
169 differentiation assays of splenic B cells. In control cultures PC frequency doubles between day 2 and  
170 day 4, whereas it remains constant between these two time points in *Sec22b*<sup>B-KO</sup> cultures (Figure 3A-  
171 B). This was associated with an enhanced apoptosis of *in vitro* derived PCs lacking *Sec22b* as detected  
172 by the frequency of active Caspase3<sup>+</sup> cells (Figure 3C). In addition, cell cycle was impaired in  
173 *Sec22b*<sup>B-KO</sup> PCs generated *in vitro* with a progressive exit from the cell cycle particularly clear at day 4  
174 (Figure 3D). These results indicate that *Sec22b* is not required for the initiation of PC differentiation  
175 but is important for the maintenance and the expansion of this cell subset.

176 To gain further insight into how *Sec22b* controls PC fate we performed RNAseq analysis on *in vitro*  
177 generated PCs. We chose an early time point, 2 days after LPS stimulation, to detect defective  
178 mechanisms before the loss of PCs observed rapidly thereafter. Despite being numerically normal  
179 (Figure 3E-F), *Sec22b* deficient PCs at day 2 post stimulation were transcriptionally very different  
180 from their WT counterparts as shown by unsupervised analyses (Figure 3E-F and Supplementary  
181 Tables 1 and 2). Over 6000 genes were differentially regulated between both genotypes with 3388  
182 genes up-regulated and 3184 genes down-regulated in *Sec22b*<sup>B-KO</sup> PCs compared to WT PCs (14026  
183 genes total, fold change >1.2 and q-value < 0.05) (Figure 3G). Although the cell purity was equivalent  
184 between sorted *Sec22b*<sup>WT</sup> and *Sec22b*<sup>B-KO</sup> PCs (>80%), we cannot formally exclude that the  
185 differences observed result from heterogeneity in the cells generated *in vitro* at this early step in both  
186 conditions. Transcriptional expressions of *Ptprc* (encoding B220) and *Sdc1* (encoding CD138) were  
187 equivalent between samples from *Sec22b*<sup>WT</sup> and *Sec22b*<sup>B-KO</sup> PCs, suggesting that, at least based on  
188 these two markers, the sorted cells were homogeneous between the two groups (Supplementary figure



189 4A-B). Sec22b was recently proposed to contribute to transcription factor nuclear shuttling and this  
190 might account for the important transcriptional differences observed in our datasets(13). Gene set  
191 enrichment analyses (GSEA) revealed that several pathways were significantly different between WT  
192 and Sec22b<sup>B-KO</sup> PCs. In line with our experimental results (Figure 3H), Sec22b seems essential for  
193 promoting cell cycle possibly via control of the Myc/E2F signalling axes. Pathways pertaining to  
194 “Mitochondria” were also significantly downregulated in Sec22b<sup>B-KO</sup> compared to WT PCs, whereas  
195 “UPR”, “ER-Golgi transport” and “protein secretion” pathways were upregulated (Figure 3H and  
196 Supplementary Figures 5 and 6). We thus explored more precisely how *Sec22b* deficiency may affect  
197 these processes. We first observed that the expression of the second R-SNARE normally involved in  
198 ER to Golgi trafficking, Ykt6, was conserved in absence of Sec22b. Moreover, expression of all the  
199 Qa, Qb and Qc SNAREs with whom Sec22b or Ykt6 interact to form a trans-SNARE complex was  
200 significantly increased in Sec22b<sup>B-KO</sup> PCs suggesting the induction of compensatory mechanisms to  
201 deal with cargo transport in absence of Sec22b (Figure 3I).

202 To unravel whether Sec22b intrinsically regulates antibody secretion we took advantage of a droplet  
203 microfluidic-based technique to assess the secretion rate at the single cell level (1). Between 6000 and  
204 10000 cells were individually encapsulated into droplets and analysed for their IgM secretion rate after  
205 2- and 4-days of *in vitro* culture. Up to 12% of encapsulated cells secreted detectable amount of IgM  
206 over the 40 minutes of imaging with less secreting cells in the Sec22b<sup>B-KO</sup> condition. We observed that  
207 *in vitro* generated WT PCs secreted on average 12 IgM/sec at day 2 and 20 IgM/sec at day 4. In  
208 contrast, *Sec22b* deficient PCs secreted on average 2 to 4 times less IgM/sec at day 2 and 4,  
209 respectively (Figure 3J). Hence, *Sec22b* deficiency leads to a significant reduction, albeit not a total  
210 block, in antibody secretion. The massive drop in antibody titre in the serum of *Sec22b* deficient mice  
211 is thus probably caused by the combination of reduced secretion rate together with severely altered PC  
212 maintenance.

213

214 *Sec22b* is essential for ER expansion and structure in PCs:

215 Our transcriptional data indicate that in absence of Sec22b, all three branches of the UPR (i.e. *Ern1*,  
216 *Atf4/Ddit3*, *Perk*, *Atf6*) (Figure 4A-B) were upregulated including the Atf4/Chop pathway normally

217 actively repressed in PCs to protect from ER-stress induced cell death (4). Our results suggest that in  
218 absence of Sec22b this protective mechanism is deficient and could lead to Chop-mediated cell death  
219 and exit from the cell cycle. Exacerbated UPR may be a sign of enhanced ER stress and we thus  
220 assessed whether ER biogenesis might be altered. Using a permeable cell tracker, we showed that the  
221 ER membrane expansion normally observed as PC differentiate was significantly reduced in absence  
222 of Sec22b (Figure 4C). The structure of the ER was also altered in Sec22b<sup>B-KO</sup> PCs. Whereas the  
223 perinuclear envelope was roughly normal, peripheral ER appeared poorly branched with dilated  
224 cisternae (Figure 4D), suggestive of accumulation of hyper-dilated ER and loss of the parallel rough  
225 ER typically seen in WT PCs. Electron microscopy confirmed that the stacked ER sheets characteristic  
226 of PCs were lost in absence of Sec22b with a hyper-dilatation of the ER cisternae and a defective  
227 stacking that was even more evident at day 4 (Figure 4E). Dilated ER was observed in all but one PC  
228 in Sec22b<sup>B-KO</sup> PCs while it was observed in less than 11% of Sec22b<sup>WT</sup> PCs. Sec22b expression is thus  
229 crucial for ER spatial expansion and organisation during PC differentiation.

230

231 *Sec22b deficiency affects PC fitness via altered mitochondrial dynamics:*

232 In addition to protein folding and export, an important function of the ER is the control of organelle  
233 dynamics and in particular of mitochondria. Indeed, ER-mitochondria membrane contact sites are  
234 pivotal for ER-mitochondria exchange but also for mitochondrial fission and thus function (19).  
235 Through our RNAseq analysis we observed a downregulation of several genes involved in  
236 mitochondrial function and dynamics (Figure 3H and Supplementary Figure 5) including *Drp1*, *Inf2*  
237 and *Spire1* that encode proteins involved in mitochondrial fission at the ER-mitochondria contact site  
238 (Figure 5A). We thus quantified ER-mitochondria contact sites by proximity ligation assay (PLA -  
239 measuring the distance between the mitochondrial protein VDAC1 and the ER protein IP3R1) and  
240 revealed that they were significantly reduced in Sec22b<sup>B-KO</sup> PCs (mean 1.233+/-0.06910 for Sec22b<sup>B-</sup>  
241 <sup>WT</sup> vs. 0.8571+/-0.05122 for Sec22b<sup>B-KO</sup>) (Figure 5B and Supplementary Figure 7). Moreover,  
242 mitochondrial content and potential were increased in PCs after 4 days in culture in Sec22b<sup>B-KO</sup> PCs  
243 compared to WT PCs (Figure 5C). Accordingly, we detected more mitochondria, increased total  
244 mitochondrial area but with fewer fragments in Sec22b<sup>B-KO</sup> PCs compared to WT PCs suggesting an

245 hyperfused mitochondrial phenotype (Figure 5D-E). We wondered whether this defective  
246 mitochondrial conformation may contribute to the PC loss observed in absence of Sec22b. Treatment  
247 of WT PCs with M1, a Drp1 antagonist, together with Mdivi, an agonist of mitochondrial fusion,  
248 promoted the generation of a hyperfused mitochondria phenotype comparable to that of Sec22b<sup>B-KO</sup>  
249 PCs (Figure 5F). Moreover, M1+Mdivi-treated WT PCs numbers were significantly reduced in culture  
250 compared to control conditions, confirming that mitochondrial dynamics affect PC fitness (Figure  
251 5G). In addition to this hyperfused phenotype Sec22b<sup>B-KO</sup> PCs presented several abnormalities in  
252 mitochondrial gene expression (Figure 5H) including genes implicated in the respiratory chain (*Atp5*  
253 family), mitochondrial fission (*Mtfp1* and *Mtfr2*), structure (*Timm* and *Tomm* families) and metabolite  
254 transport (*Slc25* family) (Figure 5H) suggesting a global alteration of mitochondrial size and function  
255 in absence of Sec22b. Altogether, these results demonstrate that *Sec22b* expression regulates ER-  
256 mitochondria contact sites and consequently mitochondrial dynamics in PCs with important  
257 consequences for cell fitness and survival.

258

259 *Sec22b* is indispensable for the generation of a protective humoral immune response:

260 Considering the importance of PCs and circulating antibodies for the humoral immune response, we  
261 finally assessed the impact of *Sec22b* deficiency on these processes. Following T-dependent  
262 immunization with sheep red blood cells (SRBC) (Figure 6A-C), the frequency and number of PCs in  
263 the spleen remained extremely low in Sec22b<sup>B-KO</sup> mice, being reduced over 100 times compared to  
264 controls (Figure 6B). A similar observation was made for antibody titres after SRBC immunization  
265 (Figure 6C). We also investigated antigen specific humoral immune response by immunizing ip with  
266 the T-dependent antigen NP-KLH in alum and boosting with NP-KLH only (Figure 6D). Seven days  
267 after the boost, the frequency and numbers of splenic and BM PCs were again dramatically reduced in  
268 absence of Sec22b (Figure 6E). Moreover, we barely detected NP-specific IgM and IgG1 antibodies in  
269 the serum of Sec22b<sup>B-KO</sup> mice throughout the immunization while a potent antibody immune response  
270 with the expected kinetics was observed in control animals (Figure 6F). We next infected WT and  
271 *Sec22b* deficient mice with influenza A virus (Figure 6G) and observed a profound defect in the  
272 frequency and number of PCs in the draining mediastinal lymph nodes (Figure 6H). Flu-specific

273 antibodies were undetectable in the serum of *Sec22b* deficient mice (Figure 6I). These defects were  
274 associated with exacerbated weight loss in *Sec22b*<sup>B-KO</sup> mice compared to their WT littermates  
275 suggesting a poorer control of the infection in absence of *Sec22b* (Figure 6J). Altogether, these results  
276 establish that *Sec22b* is essential and play a non-redundant role for the establishment of a potent and  
277 efficient humoral immune response after both vaccination and infection.

278

279

280 **Discussion**

281 Despite their essential role in health and disease, how PCs maintain a high rate of antibody secretion  
282 while persisting on the long term is unclear. Here we demonstrate that the SNARE Sec22b is essential  
283 for PC maintenance. Accordingly, in absence of *Sec22b* expression in the B cell lineage, the humoral  
284 immune response was severely impaired after both vaccination and infection.

285 The reduced antibody secretion rate observed could be caused by a defective ER to Golgi transport  
286 due to altered formation of the Stx5/Sec22b SNARE complex. Supporting a role for this complex in  
287 this process, PC treatment with Retro-2, a drug known to alter Stx5 cellular localisation (16), also  
288 leads to decreased antibody secretion. However, after Retro-2 treatment but also in absence of Sec22b,  
289 antibody secretion was reduced but not totally abrogated. Like Sec22b, the R-SNARE Ykt6 has been  
290 shown to form a trans SNARE complex with the Qa, Qb and Qc SNAREs Gosr1/2, Stx5 and Bet1 and  
291 could partially compensate for Sec22b loss (20, 21). All these SNAREs are strongly expressed in PCs  
292 and the four Q-SNAREs are twice more expressed in Sec22b<sup>B-KO</sup> PCs thus suggesting that redundant  
293 mechanisms may exist to export antibodies at a suboptimal level via the classical constitutive or via  
294 unconventional secretion pathways (22, 23).

295 Importantly, the altered PC maintenance in absence of Sec22b cannot uniquely be linked to the  
296 defective secretory capacity of the cells as several studies report that PC survival and antibody  
297 secretion are uncoupled. For example, mice lacking Xbp1 or the ligase Rctb, important for Xbp1  
298 activation, display reduced antibody secretion but normal PC numbers (24–27). Moreover, PC  
299 producing pathogenic antibodies often fail to secrete them while the PC compartment is not altered in  
300 these mouse models (28). Another example is PCs generated in the LMP2A model that can survive  
301 without secreting any antibody reinforcing the independence between antibody secretion and PC  
302 survival (29). Additive defects must thus explain the poor persistence of PCs in absence of Sec22b.  
303 We report here that *Sec22b* deficiency is associated with a profound alteration of the PC  
304 transcriptional profile and with deregulation of the UPR as well as morphological alterations of the ER  
305 and mitochondria.

306 In PCs, the Ire1 $\alpha$ /Xbp1 axis is normally highly expressed and favours antibody production and folding  
307 without causing cell death (2, 30) whereas Atf6 is weakly expressed and the Perk-dependent branch of

308 the UPR is repressed (4, 31). Activation of the Perk/Atf4/Chop pathway has been associated with  
309 increased susceptibility to cell death in PCs and is normally suppressed by the Ire1 $\alpha$ /Xbp1-dependent  
310 Ufbp1 protein (32). In absence of Sec22b the upregulation of Perk may contribute to the inhibition of  
311 the cell cycle we observed (33) and eventually to PC cell death (34) hence explaining, in part, the poor  
312 PC maintenance *in vitro* and *in vivo* in absence of Sec22b.

313 An important observation made in absence of Sec22b was the disturbed ER structure with poorly  
314 stacked and hyper-dilated cisternae. This could be the consequence of the reduced transport of  
315 antibodies from the ER to the Golgi apparatus but could also be caused directly by the absence of  
316 Sec22b as was shown in yeast and drosophila (35–37). In yeast, Sec22p interacts with Sey1p to  
317 regulate sterol biosynthesis and with atlastins to support ER homotypic fusion (35, 36). In the  
318 drosophila, Sec22 was identified as an important regulator of ER structure with a dilatation of the ER  
319 when *Sec22* was mutated or knocked-down (37). More recently, *Sec22b* knock-down in HUVECs was  
320 also associated with dilated ER but the mechanism at play remains unclear (38). Interestingly, the long  
321 form of Stx5 was also shown to be important for ER branching via interaction with Climp63 that links  
322 the ER membrane to the microtubules (11). Thus, Sec22b could directly control ER morphology in  
323 PCs either by interacting with the long form of Stx5 or via another partner still to identify.

324 Another striking defect observed in *Sec22b* deficient PCs was the accumulation of hyperfused  
325 mitochondria and the reduction of ER/mitochondria contact sites that regulate mitochondrial fission  
326 (19). Sec22b, through its longin domain, has already been shown to regulate ER/plasma membrane  
327 contact sites and consequently plasma membrane expansion in neurites (12, 39). Whether Sec22b  
328 contributes to ER/mitochondria contact sites directly or indirectly remains to be established. In  
329 addition, we demonstrated that hyperfused mitochondria were associated with poor PC survival hence  
330 suggesting that altered mitochondrial dynamics contribute to the decreased PC number in absence of  
331 Sec22b perhaps through metabolic rewiring.

332 To conclude, we demonstrated that Sec22b is a crucial regulator of PC survival. Altogether, our results  
333 demonstrate that Sec22b-mediated regulation of organelle dynamics is indispensable for PC biology  
334 and for the establishment of a protective humoral immune response (Supplementary Figure 8).  
335 Discovery of pharmacological regulators of SNAREs is an active field of research notably for

336 neurological disorders and toxin antagonisms (40–42). Specifically targeting this molecule in a tissue  
337 and time-controlled manner may thus constitute an attractive avenue for PC-mediated diseases.

338

339

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367



368 **Author Contributions**

369 AB designed and performed experiments, analyzed data, and wrote the manuscript. LG, SMG, ER,  
370 PCH, DS, DLH, NR, MC and MAL performed experiments and analyzed data. NA helped with  
371 experiments. SB and VS performed electron microscopy experiments. AA, DG, JB, PB and SA  
372 provided essential reagents and technologies. J.J. and H.T. planned, performed and analyzed the PLA  
373 assays. KGCS, FB, KB, MAL contributed to the project design and to the manuscript redaction. AAP  
374 contributed to the project design and to the manuscript redaction and set up the PlasmacytOMICs  
375 platform. ME designed the project, analyzed data, and wrote the manuscript. All authors had the  
376 opportunity to review and edit the manuscript.

377

378

379 **Declaration of Interests**

380 The authors declare that no conflict of interest exists.

381

382

383 **Methods**

384 *Mouse model, immunisation, and infection*

385 The Sec22b<sup>fl/fl</sup> mice (C57Bl/6J background) were obtained from Dr. Sebastian Amigorena and crossed  
386 with the mb1-Cre mice (18) (C57Bl/6J background) purchased from The Jackson Laboratory. All  
387 experiments were conducted in compliance with the European Union guide for the care and use of  
388 laboratory animals and has been reviewed and approved by appropriate institutional review  
389 committees (C2EA-26, Animal Care and Use Committee, Villejuif, France and Comité d'éthique  
390 Paris-Nord N°121, Paris, France). In all experiments Sec22b<sup>WT</sup> controls were mb1cre<sup>+</sup> or  
391 Sec22b<sup>fl/fl</sup> / Sec22b<sup>fl/+</sup> mice in equal proportions. Immunizations/infections were performed  
392 intraperitoneally (ip) with 100µg of 4-hydroxy-3-nitrophenylacetyl-keyhole limpet hemocyanine (NP-  
393 KLH) (Biosearch Technologies) adjuvanted with alum (Inject Alum, Thermo Scientific) or with  
394 200µL of Sheep Red Blood Cells (SRBC) (Eurobio) or intranasally with 10<sup>4</sup> plaque-forming units of  
395 influenza A/HK/x31 virus (H3N2) under inhalation anaesthesia with isoflurane. Influenza infections  
396 were performed on chimeric mice. CD45.1 Recipient mice were lethally irradiated with 11gy before  
397 reconstitution with bone marrow cells from Sec22b<sup>B-KO</sup> or Sec22b<sup>B-WT</sup> mice. Chimera were infected 8  
398 to 12 weeks later once reconstitution was complete.

399

400 *Proteomic analysis*

401 Splenic B cells were purified using a mouse B cell isolation kit following the manufacturer's  
402 instructions (Miltenyi Biotec) and cultured for 3 days with 10µg/ml lipopolysaccharide (Sigma). The  
403 differentiated cells were enriched using anti-CD138 antibodies coupled to PE and anti-PE magnetic  
404 beads (Miltenyi Biotec). Purified cells were lysed into FASP buffer. The protein concentration of the  
405 samples was normalised, digested using the FASP method (43) using trypsin and 100µg was  
406 fractionated into 36 fractions by high pH reverse phase chromatography, pooled into 12 fractions and  
407 peptides were analysed using LC-MS/MS on an Orbitrap Elite (Thermo Fisher) hybrid mass  
408 spectrometer equipped with a nanospray source, coupled with an Ultimate RSLCnano LC System  
409 (Dionex). MS data was analysed using MaxQuant (44) version 1.5.8.3. Data was searched against  
410 mouse UniProt sequence databases. Full technical details can be found in the supplementary material.

411

412 *Cell preparation and flow cytometry*

413 Spleen and bone marrow cells were isolated as previously described (45, 46). Briefly, single cell  
414 suspensions were stained with appropriate antibodies (Supplementary table 3) in PBS supplemented  
415 with 2% BSA and 2mM EDTA for cell surface staining. Staining with ER tracker (Invitrogen),  
416 mitoTracker Green (Invitrogen) and mitoTracker Orange CMTMRos (Invitrogen) were performed as  
417 recommended by the supplier. Intracellular staining was performed using the FoxP3/Transcription  
418 factor staining buffer set (eBioscience) according to the provider recommendation. Flow cytometry  
419 analyses were performed on a BD LSR Fortessa cytometer and cell sorting experiments for RNAseq  
420 and Biomark analysis were performed using a BD FACS AriaIII cell sorter. Data were analysed with  
421 the Flowjo software (TreeStar, Ashland, OR).

422

423 *In vitro cell differentiation and infection of primary cells*

424  $1 \times 10^6$  splenocytes were stimulated with  $5 \mu\text{g}/\text{mL}$  of lipopolysaccharide (LPS) (InVivoGen) for 2 or 4  
425 days in complete culture medium composed of RPMI supplemented with 10% foetal calf serum  
426 (Sigma), 0.05mM 2-mercaptoethanol, 100U/mL penicillin-streptomycin, 1mM sodium pyruvate and  
427 0.1mM non-essential amino acids (Gibco). Where indicated, cells were treated with  $20 \mu\text{M}$  M1 and  
428  $10 \mu\text{M}$  Mdivi (Sigma) for 2 days from day 2 to day 4.

429 *Stx5a* specific shRNAs were designed thanks to the RNAi consortium  
430 (<https://portals.broadinstitute.org/gpp/public>) and cloned in the pLKO.3G (addgene #14748) vectors.  
431 Lentiviral particles were produced in the Human Embryonic Kidney 293T (HEK293T) cell line with  
432 the psPAX2 (Addgene #12260) and pMD2.G (Addgene #12259) vectors. For primary cell  
433 transduction, splenocytes were put in culture in complete culture medium in presence of 80ng/ml  
434 CD40L (Thermoscientific) and 1U/mL IL-4 (Miltenyi) for 24 hours to promote B cell entry into cycle.  
435 The cells were then washed and transduced with lentiviral particles together with polybrene. After  
436 24hours cells were washed and differentiated into PCs by addition of LPS as indicated above.

437

438 *Western blot*

439 Cells were resuspended in RIPA lysis buffer supplemented with Protease and Phosphatase Inhibitor  
440 (Thermofisher). Total proteins were quantified with Bradford buffer (Thermofisher). 15µg of proteins  
441 were separated on a NuPAGE™ 4-12% Bis-Tris Gel (Invitrogen) and transferred to a PVDF  
442 membrane. Primary antibodies (Supplementary Table 3) or β-actin (Cell Signaling) were incubated  
443 overnight at 4°C. Secondary antibodies (Supplementary Table 3), specific for the primary antibody  
444 species, conjugated to horseradish peroxidase (HRP) were incubated 2h at room temperature and  
445 detected using Pierce ECL (Thermofisher) and signal was quantified by ChemiDoc™ Touch Gel  
446 Imaging System (BIO RAD).

447

#### 448 *Confocal microscopy*

449 Cells were loaded on poly-Lysine (Sigma) coated slides and fixed in PBS/4% PFA prior to analysis.  
450 Cells were permeabilized with PBS/0.3% Triton, washed in PBS and incubated with primary  
451 antibodies coupled or not overnight at 4°C and then with secondary antibodies 1 hour at room  
452 temperature, when necessary (Supplementary Table 3). Cells were incubated with Hoescht  
453 (Thermofisher) for 1 hour at room temperature. Images were acquired using a LSM800 confocal  
454 microscope equipped with the Airyscan system (Zeiss) using a 63x objective and z-stacks. Images  
455 were analysed with Fiji and Zen.

456

#### 457 *Electron microscopy*

458 Cells were pre-fixed with 2% glutaraldehyde/2% PFA in Sorensen phosphate buffer 0.1M pH7.2 for  
459 15 minutes before being fixed with 2.5% glutaraldehyde/2% PFA in Sorensen phosphate buffer 0.1M  
460 pH7.2 for 2 hours at room temperature. Cells were then washed in Sorensen phosphate buffer,  
461 resuspended in PFA 1% and sent to the METi platform (Toulouse, France). Cells were then post-fixed  
462 with 1% OsO4 in Cacodylate buffer (0.1 M, pH 7.2, EMS, Hatfield, PA), rinsed in the same buffer and  
463 pelleted, concentrated in agarose, and treated for 1 h with 2% aqueous uranyl acetate. The samples  
464 were then dehydrated in a graded ethanol series and embedded in Epon. After 48 h of polymerization  
465 at 60 °C, ultrathin sections (80 nm thick) were mounted on 200 mesh Formvar-carbon-coated copper  
466 grids. Finally, sections were stained with Uranylless and lead citrate. Grids were examined with a TEM

467 (Jeol JEM-1400, JEOL Inc, Peabody, MA, USA) at 80 kV. Images were acquired using a digital  
468 camera (Gatan Orius, Gatan Inc, Pleasanton, CA, USA).

469

#### 470 *ELISA*

471 ELISA assays were performed as previously described (47) for the determination of the different  
472 isotype of Ig or NP-specific antibody titres in sera or culture supernatants. Briefly, plates were pre-  
473 coated with goat anti-mouse IgM (Southern Biotech) or with NP(15)-BSA (Biosearch Technologies).  
474 After a step of saturation in PBS/2%BSA, diluted sera/supernatant were added before incubation with  
475 HRP-conjugated secondary antibody. Enzymatic revelation was performed with the TMB substrate  
476 reagent set (BD OptEIA). All Antibodies used are indicated in Supplementary table 3.

477

#### 478 *Proximity Ligation Assay (PLA)*

479 Anti-VDAC1 (20B12AF2) (abcam) and Anti-IP3R1 (E-8) (Santa Cruz Biotechnology) antibodies  
480 were used to generate PLA probes using Duolink in Situ Probemaker (Sigma). B cells were stimulated  
481 with Lipopolysaccharide (LPS) (InVivoGen) (5µg/ml) for 2 days. Stimulated cells were settled on  
482 PTFE diagnostic slides (Thermo Fisher Scientific) for 30 min at 37°C, fixed with 4 %  
483 paraformaldehyde (Thermo Fisher Scientific) in PBS for 15 min, permeabilized with 0.5% saponin  
484 (quillaja bark, Sigma) in PBS for 30 min and blocked with Duolink Blocking Solution (Sigma) for 1  
485 hour. Cells were incubated with Anti-VDAC1 (1/100) and Anti-IP3R1 (1/100) PLA probes overnight  
486 at 4°C. PLA was performed according to the manufacturer's instructions (Duolink, Sigma). Later,  
487 cells were further incubated with anti-mouse IgM-FITC antibody (1/100) (Jackson Immuno Research)  
488 for 30 min at 4°C. Resulting samples were mounted in Fluoromount-G with DAPI (Invitrogen).  
489 Fluorescence was detected by using an inverted confocal microscope, Leica TCS SP8 (Leica). PCs  
490 were detected based on their high IgM expression. Quantification of detected signal per nucleus in  
491 these cells was performed manually.

492

#### 493 *Hemagglutination inhibition (HAI) assay*

494 Antibody titers pre and post vaccination were determined using the hemagglutination inhibition (HAI)  
495 assay using the standard WHO protocol, as previously described (48). Sera were treated overnight  
496 with receptor-destroying enzyme (Denka Seiken Co.) and were subsequently tested by standard  
497 methods using 4 HA units of virus and a 0.5% suspension of turkey red blood cells. HAI titers were  
498 recorded as the reciprocal of the highest dilution of the serum that completely inhibited agglutination  
499 of erythrocytes by 4 HA units of the virus.

500

#### 501 *Biomark-based transcriptomic analysis*

502 Multiplex qPCR analysis was performed using the Biomark system (Fluidigm). Cells were sorted at  
503 100 cells/well directly into PCR tubes containing 5 $\mu$ L of reverse transcription/pre-amplification mix  
504 as previously described (45). Briefly, the mix contained 2X Reaction mix and SuperscriptIII  
505 (CellDirect One-Step qRT-PCR kit, Invitrogen) and 0,2X Taqman assay (Life technologies)  
506 (Supplementary Table 4). Targeted cDNA pre-amplification was performed for 19 cycles before  
507 processing with Dynamic Array protocol according to the manufacturer's instructions (Fluidigm).  
508 Wells positive for *Gapdh*, *Actb* and control gene (*Prdm1*, *Irf4* and *Xpb1* for PCs and *Pax5* for B cells)  
509 expression and negative for expression of a control gene (*Cd3e*) were considered for further analysis.  
510 Mean expression of *Actb* and *Gapdh* was used for normalization. Unsupervised clustering (Spearman  
511 rank correlation test) and heatmap representation were generated with (<http://www.heatmapper.ca>)  
512 using the Z scores.

513

#### 514 *RNAseq and RNAseq-based transcriptomic analysis*

515 For RNAseq, *in vitro* generated PCs were sorted at day 2 and RNA were extracted with "RNeasy plus  
516 micro kit" (Qiagen) as recommended. RNAs were proceeded by the Integragen company for  
517 sequencing. Libraries were prepared with NEBNext® Ultra™ II Directional RNA Library Prep Kit for  
518 Illumina protocol according supplier recommendations. Briefly the key stages of this protocol were  
519 successively, the purification of PolyA containing mRNA molecules using poly-T oligo attached  
520 magnetic beads from 100ng total RNA (with the Magnetic mRNA Isolation Kit from NEB), a  
521 fragmentation using divalent cations under elevated temperature to obtain approximately 300bp

522 pieces, double strand cDNA synthesis and finally Illumina adapters ligation and cDNA library  
523 amplification by PCR for sequencing. Sequencing was then carried out on Paired End 100b reads of  
524 Illumina NovaSeq. Image analysis and base calling was performed using Illumina Real Time Analysis  
525 (3.4.4) with default parameters.

526 For analysis STAR was used to obtain the number of reads associated to each gene in the Gencode  
527 vM24 annotation (restricted to protein-coding genes, antisense and lincRNAs). Raw counts for each  
528 sample were imported into R statistical software. Extracted count matrix was normalized for library  
529 size and coding length of genes to compute FPKM expression levels. The Bioconductor edgeR  
530 package was used to import raw counts into R statistical software, and compute normalized log2 CPM  
531 (counts per millions of mapped reads) using the TMM (weighted trimmed mean of M-values) as  
532 normalization procedure. The normalized expression matrix from the 500 most variant genes (based  
533 on standard deviation) was used to classify the samples according to their gene expression patterns  
534 using principal component analysis (PCA), hierarchical clustering and consensus clustering. PCA was  
535 performed by FactoMineR::PCA function with “ncp = 10, scale.unit = FALSE” parameters.  
536 Hierarchical clustering was performed by stats::hclust function (with euclidean distance and ward.D  
537 method). Differential expression analysis was performed using the Bioconductor limma package and  
538 the voom transformation. To improve the statistical power of the analysis, only genes expressed in at  
539 least one sample (FPKM  $\geq$  0.1) were considered. A q value threshold of  $\leq$  0.05 and a minimum fold  
540 change of 1.2 were used to define differentially expressed genes. Gene list from the differential  
541 analysis was ordered by decreasing log2 fold change. Gene set enrichment analysis was performed by  
542 clusterProfiler::GSEA function using the fgsea algorithm. Gene sets from MSigDB v7.2 database were  
543 selected among the C2\_curated and Hallmark classes, keeping only gene sets defined by 10-500  
544 genes.

545

#### 546 *DropMap-based analysis of IgM secretion*

547 Dropmap experiment were performed as described(1), modified to detect IgM secretion from single  
548 cell. Cells from *in vitro* cultures at day2 or day 4 after LPS stimulation were centrifuged and  
549 resuspended in DropMap medium (RPMI without phenol red, supplemented with 0.1% Pluronic F68,

550 25 mM HEPES pH 7.4, 10% KO serum replacement (all ThermoFisher) and 0.5% recombinant human  
551 serum albumin (Sigma Aldrich). Microfluidic droplets were generated as water-in-oil emulsions using  
552 a co-flow of aqueous phases, one containing bioassay reagents (bioassay phase) and the other one  
553 containing *in vitro*-generated PCs (cell phase). *Bioassay phase*: Streptavidin-coated paramagnetic  
554 beads (300nm, Ademtech) were washed with PBS using a magnet, and then resuspended in a 1 $\mu$ M  
555 solution of CaptureSelect biotin anti-mouse Ig $\kappa$  conjugate (ThermoFisher) and incubated at room  
556 temperature (RT) for 20 min. The beads were washed with PBS and resuspended in 5% pluronic F127  
557 (ThermoFisher) and incubated for 20 min at room temperature. Following a PBS wash, the beads were  
558 resuspended in DropMap buffer and incubated at RT for 20 min. Beads were washed for the last time  
559 with PBS and resuspended in a solution of 150nM Goat anti-mouse IgM ( $\mu$  chain specific) F(ab')<sub>2</sub>  
560 (Alexa647, Jackson ImmunoResearch) in DropMap buffer. *Cell phase*: To produce single-cell  
561 droplets, cell concentration was adjusted to achieve 0.3 cells per droplet in DropMap buffer. For  
562 calibration purposes with cells, purified monoclonal IgM was diluted in DropMap buffer.  
563 Droplets were produced by hydrodynamic flow-focusing on a custom-made microfluidic device as in  
564 (1), by co-flowing the two aqueous phases. Immediately after generation, droplets were injected into  
565 the 2D observation chamber (1) until filled, which was then closed for image acquisition. The droplet  
566 array in the 2D chamber was imaged using a Nikon Ti-2 Eclipse inverted microscope with motorized  
567 stage and excitation light source (Lumencor Spectra X). Fluorescence was captured using a 10x  
568 objective and a Cy5 filter, and images were recorded by a digital CMOS camera (ORCA-flash 4.0,  
569 Hamamatsu). For each time point, an array of 10x10 images was acquired, and a total of 6 acquisitions  
570 were recorded through 37.5 min. The images were analyzed with a custom Matlab script as described  
571 (1). In brief, the ratio of fluorescent signal between the beadline and the background was estimated for  
572 every droplet at every time point. The fluorescence ratio was then used to estimate the concentration  
573 of IgM in the droplets by using a calibration curve, which was generated by measuring the fluorescent  
574 ratio from different concentrations of purified monoclonal IgM antibody.  
575  
576 *Statistical analysis*



577 The p-values were determined as indicated in the figure legends using the Prism GraphPad software  
578 with the two-tailed unpaired Mann-Whitney non-parametric test for the WT vs Sec22b<sup>B-KO</sup> comparison  
579 ( $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ ;  $****p < 0.0001$ , “ns” = non-significant  $p$ -value), or with the 2way  
580 ANOVA with Sidak correction for multiple comparisons (**££**  $p < 0.01$ , **£££**  $p < 0.001$ ).

581

582

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693 **Figure Legends:**

694 **Figure 1: The Syntaxin-5-Sec22b SNARE complex is overexpressed in plasma cells:**

695 A) Changes in protein abundance between murine Naive B cells and CD138 enriched PCs  
696 generated *in vitro* were measured using label free LC-MS/MS analysis and plotted using the  
697 PlasmacytOMICs interface. The Log<sub>2</sub> Fold change between naïve B cells and the indicated  
698 cell subset is shown for each protein. Error bars show SEM. \* - indicates False Discovery  
699 Adjusted p-value < 0.05. We were unable to consistently measure sufficient peptides for  
700 quantification of CD138 in the PC samples possibly due to its high level of glycosylation. The  
701 fold change shown for XBP1 is an underestimate, as the ratio plotted is calculated using an  
702 imputed value for the B-cell samples as the protein was not detected in these samples (see  
703 Supplementary Figure 1). B) Representative immunoblots for Xbp1, IgM, CD138, Scfd1,  
704 Uso1, Stx5, Ytk6, Sec22b and b-actin (top to bottom respectively) from samples prepared  
705 from splenic B cells (left panel) or CD138 enriched *in vitro* differentiated PCs (right panel).  
706 The band shown for CD138 is the non-glycosylated form of the protein. For Stx5 the two  
707 bands correspond to the short and the long isoforms of the protein. The fold change between  
708 B cells and PCs normalized to b-actin is indicated on the right for each protein. C) The  
709 PlasmacytOMICs interface was used to perform a meta-analysis of gene expression changes  
710 between murine Naive B cells and a range of antibody secreting cell types (mSplPC.Array =  
711 microarray / mouse splenic PCs; mBMPC.Array = microarray / mouse BM PCs; mPB.rSeq =  
712 RNAseq / mouse plasmablasts generated *in vitro*; mSplPC.rSeq = RNAseq / mouse splenic  
713 PCs; mBMPC.rSeq = RNAseq / mouse BM PCs) for the indicated genes. The Log<sub>2</sub> Fold  
714 change between naïve B cells and the indicated cell subset is shown for each gene. Error bars  
715 show SEM . \* - indicates False Discovery Adjusted p-value < 0.05. B) D) Changes in gene  
716 expression between human Naive B cells and selection of human antibody secreting cell types  
717 (hPB.rSeq = RNAseq / human blood PC; hBMPC.rSeq = RNAseq / human BM PC) were

718 calculated for the indicated genes using the PlasmacytOMICcs platform. The Log<sub>2</sub> Fold  
719 change between naïve B cells and the indicated cell subset is shown for each gene. Error bars  
720 show SEM . \* - indicates False Discovery Adjusted p-value < 0.05. Sec22b<sup>WT</sup> controls were  
721 mb1cre<sup>+</sup> and Sec22b<sup>fl/fl</sup> / Sec22b<sup>fl/+</sup> mice.

722

723 **Figure 2: Strong reduction of plasma cells and circulating antibodies in absence of**  
724 **Sec22b:**

725 A) ELISA quantification of Ig titres in sera of unimmunized Sec22b<sup>WT</sup> or Sec22b<sup>B-KO</sup> mice.  
726 B-C) Representative dot plots (top) and quantification (bottom) of absolute number of total  
727 PCs (CD138<sup>+</sup>TACI<sup>+</sup>) and percentage of each PC subsets: PBs (CD138<sup>+</sup>TACI<sup>+</sup>B220<sup>+</sup>CD19<sup>+</sup>),  
728 early PCs (CD138<sup>+</sup>TACI<sup>+</sup>B220<sup>low</sup>CD19<sup>+</sup>) and late PCs (CD138<sup>+</sup>TACI<sup>+</sup>B220<sup>-</sup>CD19<sup>-</sup>) in  
729 Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> mice in spleen (B) and bone marrow (C). Cells were first gated on  
730 their size, structure and viability. Dead cells and doublets were excluded. D) Unsupervised  
731 clustering based on the relative expression of *Prdm1*, *Xbp1*, *Irf4*, *Tnfrsf13b*, *CD93*, *Pax5* and  
732 *bach2* of sorted Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> PCs determined by Biomark multiplex qPCRs at  
733 steady state. The heatmap was generated using the heatmapper.ca website and row Z score  
734 based on (2<sup>-DCt</sup>) values. n= 7-19 mice from 2-5 independent experiments. The p-values were  
735 determined with the two-tailed Mann-Whitney non-parametric test. \**p* < 0.05; \*\**p* < 0.01;  
736 \*\*\**p*<0.001 \*\*\*\**p*<0.0001. “ns” = non-significant *p*-value. Sec22b<sup>WT</sup> controls were mb1cre<sup>+</sup>  
737 and Sec22b<sup>fl/fl</sup> / Sec22b<sup>fl/+</sup> mice.

738

739 **Figure 3: Sec22b is essential for plasma cell maintenance and molecular identity**

740 A-B) Representative dot plots (A) and quantification (B) of PCs (CD138<sup>+</sup>B220<sup>+/-</sup>) generated  
741 *in vitro* from Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> splenocytes after 2 or 4 days of stimulation with LPS.  
742 Cells were gated on their size and structure, on their viability and doublets were excluded. C)

743 Frequency of Caspase 3<sup>+</sup> PCs at day 2 and 4 post LPS stimulation determined by flow  
744 cytometry. D) Flow cytometry analysis of the cell cycle phases based on DAPI and Ki-67  
745 staining in *in vitro* generated Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> PCs at day 2 (left) and day 4 (right)  
746 post LPS stimulation. E-I) RNAseq analysis of Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> PCs generated *in*  
747 *vitro* at day2 post LPS stimulation. E) Principal component analysis of the RNAseq data of  
748 Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> PCs. The proportion of variance is indicated for PC1 and PC2. F)  
749 Unsupervised clustering of Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> PCs based on the 500 most  
750 differentially expressed genes. G) Volcano-plot showing the differentially expressed genes  
751 between Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> PCs. Genes significantly downregulated and upregulated  
752 in Sec22b<sup>B-KO</sup> PCs are shown in green and red, respectively (FC>1.2; q value<0.05). H)  
753 Normalized Enrichment Scores (NES) of representative gene sets significantly enriched in  
754 Sec22b<sup>B-KO</sup> vs Sec22b<sup>WT</sup> PCs and characteristic of selected cellular pathways. Gene sets  
755 significantly downregulated and upregulated in Sec22b<sup>B-KO</sup> PCs are shown in green and red,  
756 respectively. I) Expression of *Ykt6*, *Gosr1*, *Gosr2*, *Stx5* and *Bet1* in fragments per kilobase of  
757 exon per million reads mapped (FPKM) determined by RNAseq in Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup>  
758 PCs. J) Quantification of the IgM secretion rate from *in vitro* generated PCs from Sec22b<sup>WT</sup>  
759 and Sec22b<sup>B-KO</sup> splenocytes after 2 or 4 days of LPS stimulation analysed by DropMap. Each  
760 point represents one cell.

761 (A-B) n=12-16 mice from at least 3 pooled independent experiments. (C-D) n=4 of 1  
762 representative experiment out of 2. (E-I) n=4 in 1 experiment; (J) n=4 in 2 independent  
763 experiments. The p-values were determined with the two-tailed Mann-Whitney non-  
764 parametric test \**p* < 0.05; \*\**p* < 0.01; \*\*\**p*<0.001, “ns” = non-significant *p*-value. Sec22b<sup>WT</sup>  
765 controls were mb1cre<sup>+</sup> and Sec22b<sup>fl/fl</sup> / Sec22b<sup>fl/+</sup> mice.

766

767 **Figure 4: Sec22b is essential for ER expansion and structure in PCs**

768 A) Expression of *Ern1* (encoding Ire1a), *Atf4*, *Atf6*, *Ddit3* (encoding Chop) in fragments per  
769 kilobase of exon per million reads mapped (FPKM) determined by RNAseq in *Sec22b*<sup>WT</sup> and  
770 *Sec22b*<sup>B-KO</sup> PCs. B) Heatmap showing the relative expression of selected ER stress genes  
771 from PCs generated from *Sec22b*<sup>WT</sup> and *Sec22b*<sup>B-KO</sup> splenocytes after 2 days of LPS  
772 stimulation, determined by Biomark multiplex qPCRs at steady state. C) Flow cytometric  
773 quantification of ER-Tracker MFI (geometrical mean) on PCs generated from *Sec22b*<sup>WT</sup> and  
774 *Sec22b*<sup>B-KO</sup> splenocytes after 2 or 4 days of LPS stimulation. n=4, one representative  
775 experiment shown out of 3. D) Confocal microscopy images of *Sec22b*<sup>WT</sup> and *Sec22b*<sup>B-KO</sup>  
776 PCs obtained from splenocytes stimulated with LPS for 2 days. Cells were stained with anti-  
777 IgM to detect antibodies, anti-calnexin to detect the ER and anti-IRF4 antibodies. Nuclei were  
778 counterstained with Hoechst. Images are representative of 60 *Sec22b*<sup>WT</sup> PC and 71 *Sec22b*<sup>B-</sup>  
779 <sup>KO</sup> PC from 2 mice per group. E) Electron microscopy images of *Sec22b*<sup>WT</sup> (left) and  
780 *Sec22b*<sup>B-KO</sup> (right) PCs obtained from splenocytes stimulated with LPS for 2 (top) or 4  
781 (bottom) days. Red arrowheads indicate ER sheets. Scale bar = 1mm or 0.5mm. Images are  
782 representative of 64 WT PCs (9 at day 2 and 55 at day 4), and 39 KO PCs (21 at day 2 and 18  
783 at day 4). Data are from 5 mice per genotype out of 2 experiments.

784 The heatmap were generated using the heatmapper.ca website, row Z score based on ( $2^{-\Delta Ct}$ )  
785 values. The p-values were determined with the two-tailed Mann-Whitney non-parametric test  
786 \* $p < 0.05$ . *Sec22b*<sup>WT</sup> controls were mb1cre<sup>+</sup> and *Sec22b*<sup>fl/fl</sup> / *Sec22b*<sup>fl/+</sup> mice.

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### 788 **Figure 5: Sec22b deficiency affects PC fitness via altered mitochondrial dynamics**

789 A) Expression of *Dnm1l*, *Inf2* and *Spire1* in FPKM determined by RNAseq in *Sec22b*<sup>WT</sup> and  
790 *Sec22b*<sup>B-KO</sup> PCs obtained from splenocytes stimulated with LPS for 2 days. n=4 and data are  
791 representative of 1 experiment. B) PLA performed on splenocytes stimulated 2 days with  
792 LPS. Representative images are shown (left), PC stained with anti-IgM (green), PLA (red dot)



793 and nuclei counterstained with DAPI. Quantification of dot per PC (middle) (one  
794 representative experiment) and mean of dot number per PC in each experiment (right) are  
795 represented. Scale bar = 30 $\mu$ m. n= 1271 cells Sec22b<sup>WT</sup> and 1893 cells Sec22b<sup>B-KO</sup> of 3-4  
796 mice in 3 independent experiment. C) Flow cytometric quantification of Mito-tracker (mitoT)  
797 green (left) and mitoT orange CMTMRos (right) MFI (geometrical mean) on PCs generated  
798 from Sec22<sup>WT</sup> and Sec22b<sup>B-KO</sup> splenocytes after 2 or 4 days of LPS stimulation. n= 4 mice in  
799 1 representative experiment out of 3. D-E) Confocal microscopy images of PCs obtained from  
800 Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> splenocytes stimulated with LPS for 4 days. Cells were stained  
801 with anti-IgM to detect antibodies, anti-cytochrome c to detect mitochondria, and nuclei were  
802 counterstained with Hoechst. Scale bar = 10 $\mu$ m. Representative images (D) and quantification  
803 (E) of the mean area (top), mean intensity (mean per z-stack) (middle) and number of  
804 fragments (over the full cell volume quantified through 10 z-stacks) (bottom) per cell of the  
805 cytochrome c staining in PCs obtained from Sec22<sup>WT</sup> and Sec22b<sup>B-KO</sup> splenocytes stimulated  
806 with LPS for 4 days. n=304 for the Sec22b<sup>WT</sup> and n=231 for the Sec22b<sup>B-KO</sup> from 3  
807 independent mice per genotype. F-G) 3D projection of confocal microscopy images of PCs  
808 obtained from Sec22<sup>WT</sup> and Sec22b<sup>B-KO</sup> splenocytes stimulated with LPS for 4 days and after  
809 2 days of treatment with DMSO or M1/Mdivi for Sec22<sup>WT</sup>. Cells were stained with anti-IgM,  
810 anti-cytochrome c antibodies and nuclei were counterstained with Hoechst. Scale bar = 6mm.  
811 Representative images (F) and quantification (G) of fold change of PC frequency normalized  
812 to control Sec22b<sup>WT</sup> PCs are shown. n=10 from 2 pooled experiments. H) Supervised  
813 heatmap based on RNAseq expression data of 9 mitochondrial gene families differentially  
814 expressed between Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> PCs. For flow cytometry experiment cells were  
815 gated on their size and structure, on their viability and doublets were excluded. The p-values  
816 were determined with the two-tailed unpaired Mann-Whitney non-parametric test \* $p < 0.05$ ;  
817 \*\*\*\* $p < 0.0001$ , “ns” = non-significant  $p$ -value except for PLA experiment, determined with

818 Welch's t test or paired t-test. Sec22b<sup>WT</sup> controls were mb1cre<sup>+</sup> and Sec22b<sup>fl/fl</sup> / Sec22b<sup>fl/+</sup>  
819 mice.

820

821 **Figure 6: Sec22b is indispensable for the generation of a protective humoral immune**  
822 **response:**

823 A) Schematic representation of the SRBC immunisation protocol for Sec22b<sup>WT</sup> and Sec22b<sup>B-</sup>  
824 <sup>KO</sup> mice. B) Representative dot plots (top), frequency (bottom left) and absolute number  
825 (bottom right) of splenic PCs (CD138<sup>+</sup>B220<sup>+/-</sup>) determined by flow cytometry 7 days after  
826 SRBC immunization. C) ELISA quantification of IgM (top) and IgG1 (bottom) serum titres 7  
827 days after SRBC immunized. A-C one representative experiment of 2 is shown. D) Schematic  
828 representation of the NP-KLH immunization/ boost protocol. Sec22b<sup>WT</sup> or Sec22b<sup>B-KO</sup> mice  
829 were immunized, rechallenged 28 days later and analysed 7 days later. Sera were collected at  
830 days 7-, 14-, 21- and 7-days post boost. E) Frequency (left) and absolute number (right) of  
831 splenic PCs (CD138<sup>+</sup>B220<sup>+/-</sup>) (top) and bone marrow (BM) PCs (CD138<sup>+</sup>B220<sup>+/-</sup>) (bottom)  
832 determined by flow cytometry 7 days post boost with NP-KLH (n=9 mice from 2 pooled  
833 independent experiments). F) ELISA quantification of NP-IgM (top) and NP-IgG1 (bottom)  
834 serum titres at days 7-, 14-, 21- post primary immunization and 7-days post boost (n=5 mice.  
835 One experiment representative of 2 is shown). G) Schematic representation of the Flu  
836 infection protocol for Sec22b<sup>WT</sup> and Sec22b<sup>B-KO</sup> mice. Mice were analysed 14 days after  
837 infection with influenza A virus. H) Frequency (left) and absolute number (right) of  
838 mediastinal PCs (CD138<sup>+</sup>B220<sup>+/-</sup>) determined by flow cytometry 14 days after infection with  
839 influenza A virus. I) Quantification of HAI titres 14 days post flu infection. J) Weight change  
840 of Sec22b<sup>WT</sup> or Sec22b<sup>B-KO</sup> mice over 12 days after flu infection. (H-I; n=5, one  
841 representative experiment of 2 is shown, J; n=15 from 2 pooled experiments). For flow  
842 cytometry experiment cells were first gated on their size and structure, then their viability.

843 Dead cells and doublets were excluded. The p-values were determined with the two-tailed  
844 Mann-Whitney non-parametric test  $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ ;  $****p < 0.0001$  or  
845 with the 2way ANOVA with Sidak correction for multiple comparisons (**¶¶**  $p < 0.01$ , **¶¶¶**  
846  $p < 0.001$ ). Sec22b<sup>WT</sup> controls were mb1cre<sup>+</sup> and Sec22b<sup>fl/fl</sup> / Sec22b<sup>fl/+</sup> mice.

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