



HAL
open science

Final step of B-cell differentiation into plasmablasts; the right time to activate plasma cell PIM2 kinase

Marion Haas, Thierry Fest

► To cite this version:

Marion Haas, Thierry Fest. Final step of B-cell differentiation into plasmablasts; the right time to activate plasma cell PIM2 kinase. *Immunology Letters*, 2023, 258, pp.45-50. 10.1016/j.imlet.2023.05.006 . hal-04125746

HAL Id: hal-04125746

<https://hal.science/hal-04125746>

Submitted on 29 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Final step of B-cell differentiation into plasmablasts; the right time to activate**
2 **plasma cell PIM2 kinase**

3

4

5 **Marion Haas^{1,2}, and Thierry Fest^{1,2,*}**

6 ¹ Université de Rennes 1, INSERM, Établissement Français du Sang de Bretagne,
7 Team B_DEVIL, UMR_S1236, Rennes, France.

8 ² Laboratoire d'Hématologie, Centre Hospitalier Universitaire, Rennes, France.

9 *** Correspondence:**

10 Thierry Fest, MD, PhD

11 E-mail: thierry.fest@univ-rennes1.fr

12

13 Abstract word count: 174

14 Main text word count: 3803

15 Reference count: 65

16

17 **Keywords: B cell differentiation, plasma cell, PIM2, ER stress**

18

19 **Abstract**

20 The differentiation of B cells into antibody-secreting plasma cells is a complex process
21 that involves extensive changes in morphology, lifespan, and cellular metabolism to
22 support the high rates of antibody production. During the final stage of differentiation,
23 B cells undergo significant expansion of their endoplasmic reticulum and mitochondria,
24 which induces cellular stress and may lead to cell death in absence of effective
25 inhibition of the apoptotic pathway. These changes are tightly regulated at
26 transcriptional and epigenetic levels, as well as at post-translational level, with protein
27 modifications playing a critical role in the process of cellular modification and
28 adaptation.

29 Our recent research has highlighted the pivotal role of the serine/threonine kinase
30 PIM2 in B cell differentiation, from commitment stage to plasmablast and maintenance
31 of expression in mature plasma cells. PIM2 has been shown to promote cell cycle
32 progression during the final stage of differentiation and to inhibit Caspase 3 activation,
33 raising the threshold for apoptosis. In this review, we examine the key molecular
34 mechanisms controlled by PIM2 that contribute to plasma cell development and
35 maintenance.

37 **1. Introduction**

38 Before becoming CD138-positive non-proliferating plasma cells, B cells first pass
39 through an immature, proliferative plasmablast stage. Plasmablasts emerge after
40 major changes in B cell morphology, epigenome-sustained expression profile and
41 **changes in the proliferation/survival compared to uncommitted B cells**. Using an in vitro
42 model of human naive B cell differentiation, we have shown that the transition from
43 activated B cells to plasmablasts involves an entanglement between cell division and
44 differentiation programs and a cellular machinery largely open to apoptosis ¹. Indeed,
45 the final stage of commitment, when a human B cell becomes a plasmablast, is
46 synchronized with the cell cycle and associated with a dynamic epigenetic process on
47 plasmablast fate-determining genes.

48 In the context of this extensive cell metamorphosis, it is of interest to identify the key
49 protein kinases that control the process by modifying substrate activity. Thus, signal
50 transduction pathways depend on protein phosphorylation to relay information through
51 signaling cascades or effector proteins that elicit the end result of pathway activation.
52 **The cell cycle is a highly regulated process, and protein phosphorylation is a major**
53 **mechanism by which it is controlled. In fact, the cell cycle phosphoproteome is one of**
54 **the largest phosphoproteomes for any cellular process, with up to 70% of proteins**
55 **containing phosphorylation sites. This regulation is particularly important during**
56 **mitosis, where phosphorylation events play a crucial role in ensuring the proper**
57 **segregation of chromosomes and completion of cell division** ². Misregulation of protein
58 kinase signaling has been linked to several human diseases and has been the subject
59 of attention for several decades. Deciphering the cellular roles of any protein kinase
60 requires the elucidation of its downstream effector substrates.

61 In this review we will focus on some of the molecules and pathways that are involved
62 in the changes that take place during the terminal **transition from a non-secreting B cell**
63 **to plasmablasts**. We will then describe the serine/threonine kinase PIM2, its
64 involvement in modifications which, through its action on specific substrates, acts on
65 the control of the cell cycle, the adaptation of the cell to the new constraints of
66 immunoglobulin (Ig) secretion and, finally, the maintenance of plasmablast and plasma
67 cell survival. Curiously to date, this kinase has never been studied during normal and
68 terminal differentiation of the B cell until our recently published article ³. However, PIM2
69 has been extensively studied in malignant plasma cells during multiple myeloma. In
70 this paper, we will present the action of PIM2 in the completion of B cell differentiation
71 into plasmablasts as well as in the survival of plasma cells, thus assuming the role of
72 the pivotal plasma cell kinase.

73

74 **2. Extensive molecular changes during the B cell to plasmablast transition step.**

75 **Transcription factors play a critical role in determining and maintaining cellular identity,**
76 **and their activities are modulated by the underlying epigenetic landscape. As**
77 **differentiating cells evolve, DNA demethylation can contribute to a more permissive**
78 **epigenetic state that allows for greater flexibility in gene expression and cellular**
79 **function**. In activated B cells, plasma cell progenitors may need to adapt transcriptional
80 program to support cellular metamorphosis conducting to an eccentric nucleus and an
81 abundant cytoplasmic endoplasmic reticulum (ER) with abundant secretion of Ig
82 proteins.

83 To study the molecular events that contribute to the cellular changes during the B cell
84 to plasmablast transition, we developed and standardized an in vitro model of human

85 naive B cell differentiation into plasmablasts. Starting from blood donor buffy coat we
86 purify B cells and then culture them with IL-2, CD40L, CpG and anti-IgM Fab'2 to
87 activate them via a transcriptional burst ⁴. **By day 1, B cells are activated with a large**
88 **number of cells in S-phase and significant gene expression modifications as shown**
89 **previously** ^{4,5}. Beyond day 4, culture conditions are modified and cells maintained only
90 with IL-2, IL-4 and IL-10 stimulation for an additional 2-3 days to complete the
91 plasmablast differentiation. A third step of the culture is required with addition of IL-6
92 and interferon α to obtain CD138-positive nonproliferating plasma cells after 3 days ³.
93 We have recently demonstrated that committed B cells, which differentiate into
94 plasmablasts, exhibit an extinction of both IL-4/STAT6 signaling and CBLB ubiquitin
95 ligase expression, coinciding with IRF4 induction ⁶. As a surrogate marker of this
96 commitment, CD23 expression on the membrane surface disappears due to IL-
97 4/STAT6 extinction. **When B cells reach the post-proliferation stage, they undergo a**
98 **transcriptional shift that is supported by a replication-dependent passive demethylation**
99 **of DNA** ^{1,7} and enter the G1 phase of the cell cycle. However, beyond day 4, in the
100 absence of BCR, CpG and CD40L stimuli, and induced by IL-10 (or IL-21), a small
101 fraction of B cells belonging to the CD23-negative subpopulation enters into cell cycle
102 after promoting the G1/S transition ^{1,8}. In contrast to their CD23-positive counterparts
103 that are unable to differentiate, CD23-negative B cell progenitors morphologically
104 change from an activated-B cell phenotype to a plasmablast phenotype. This cellular
105 metamorphosis is accompanied by a dramatic reorganization of the pool of DNA-active
106 enhancers, as evidenced by oxidative enrichment of 5'-methyl-cytosine (5mC) due to
107 the activity of translocation ten-eleven (TET) methylcytosine dioxygenases on selected
108 regions of the genome, including genes encoding plasma cell identity factors, which
109 were previously protected from passive replication-dependent demethylation. TET

110 activity produces stable 5-hydroxymethylcytosine (5hmC) residues along with open
111 chromatin and specific histone methylation marks leading together to active
112 transcription coupled to the S phase of the cell cycle ^{1,9,10}. This TET hemi-
113 hydroxymethylated DNA is misrecognized by DNA methyltransferase 1 (DNMT1),
114 allowing maintenance of DNA methylation.

115 While they become plasmablasts and are fully efficient in Ig secretion, the cells
116 undergo massive changes in their organelles, including membrane amplification,
117 trafficking, endoplasmic reticulum (ER) remodeling and a metabolic switch with a large
118 increase in nutrients to meet the growing need (reviewed in ^{11,12}). At the mature plasma
119 cell stage, to meet cellular needs and adapt to stress conditions, the ER dynamically
120 changes its morphology leading to its peak of protein production; a particular challenge
121 knowing that measurements of their secretion rates - although varying - indicate that
122 these cells release up to ¼-½ of their total weight as secreted glycoprotein each day
123 ¹¹. Ig processing is overwhelmed into the ER lumen, resulting in an increased level of
124 unfolded and misfolded proteins with a high ER stress state, inducing the production
125 of chaperones that attempt to refold the misfolded proteins ¹³. Cells expand their ER
126 primarily through the inositol-requiring enzyme 1 α (IRE1 α) pathway, the most studied
127 unfolded protein response (UPR) signals in plasma cells. IRE1 α leads to the formation
128 of spliced X-box Binding Protein (sXBP1), which in turn promotes the mitochondrial
129 and the ER expansion, both of which are significantly reduced when XBP1 is deleted
130 in B cells ¹⁴⁻¹⁶. In addition to IRE1, UPR in mammals is regulated by two other ER
131 sensors: ATF6, a member of the ATF/CREB transcription factor family, and the R-like
132 ER kinase protein, PERK ^{17,18}. All three UPR response factors are activated after
133 release of the ATPase Ig-binding protein (BiP) (reviewed in ^{5,19}). It is noteworthy that
134 XBP1 expression increases in human activated naïve B cells without the need for

135 BLIMP1 induction ²⁰, which is consistent with mouse activated follicular B cells where
136 the serine/threonine kinase mTORC1 plays a role before Blimp induction and promotes
137 the transcription of various UPR target genes. Thus, during B cell terminal
138 differentiation, mTORC1 expression establishes the secretory program, which
139 gradually transitions to the amplified and sustained Blimp1-IRE1/Xbp1 program ^{21,22}.
140 Nevertheless, BLIMP1 is intimately involved in the UPR, directly regulating IRE1 and
141 ATF6, as well as 38% of the downstream genes of the UPR, which are largely
142 independent of those regulated by XBP1 and are part of the BLIMP1-driven
143 differentiation process ^{23,24}. Finally, plasma cells use autophagy to limit ER expansion
144 and antibody production, and to provide nutrients through the degradation of cellular
145 components (reviewed in ²⁴). It is worth noting that mTORC1 downregulates
146 autophagy, indicating that plasma cells maintain a balance between autophagy and
147 mTORC1 activity to optimize their antibody secretion rates without compromising their
148 survival ²⁵.

149 Unlike IRE1 and ATF6, PERK is a member of the eukaryotic initiation factor 2 (eIF-2 α)
150 kinase and functions to inhibit most cap-dependent protein synthesis at the onset of
151 the UPR. However, paradoxically, it allows ATF4 transcription factors to be translated
152 ^{26,27}. In contrast to other secretory cells such as pancreatic beta cells (**Fest lab,**
153 **unpublished**), the PERK/ATF4 arm of the UPR is selectively inactivated in plasma cells.
154 This has been demonstrated in Perk^{-/-} B cells, which can differentiate and produce Ig
155 synthesis and antibody secretion upon stimulation with LPS ²⁸. Moreover, in our in vitro
156 model of naïve B cell differentiation, PERK and its target genes are immediately
157 downregulated after B cell activation, without an increase in generated plasmablasts ⁵.

158 **3. Plasma cell biology depends of the integrated stress response (ISR).**

159 Chronic ER stress is linked to apoptosis, making it important to understand how plasma
160 cells manage prolonged UPR activation without triggering the terminal proapoptotic
161 aspects of the UPR. Transcriptomic analysis of B cell commitment to plasma cell output
162 revealed that the first enriched gene list was related to cell death and survival. Notably,
163 the proapoptotic *BCL2L11* (encoding BIM) and *CASP3* (encoding Caspase 3) genes
164 were significantly upregulated, alongside the pro-survival genes *BCL2L1* (encoding
165 BCLXL), *MCL1*, and XIAP genes^{1,3,6}. New generated plasmablasts die within days of
166 their generation, and short-lived plasma cells are much more prevalent than long-lived
167 ones (reviewed in¹²). This suggests that plasma cell longevity is only marginally related
168 to reasons intrinsic to the cells themselves – such as an antigen-specific humoral
169 response, as proposed by Amanna et Slifka with their “imprinted lifespan” model of
170 plasma cell longevity²⁹ - but rather depends on their environment. Indeed, individual
171 plasma cells localize among a set of cells that provide localization and survival cues
172 and belong to specialized survival niches^{30 31}. Differentiation and maintenance of
173 plasma cells require an attenuation of the stress response coupled to UPR. The
174 integrated stress response (ISR), a central and evolutionary conserved signaling
175 network, responds to a broad variety of stress conditions and operates by
176 reprogramming translation with reduction of general initiation translation rates and the
177 increase in translation of specific messengers RNAs. Both of these processes
178 reprogram translation towards damage mitigation and recovery by maintaining
179 functional balance of cells throughout their lifespan, allowing them to adapt. ISR does
180 not act like an on-off switch but rather as a molecular rheostat controlling fast and slow
181 changes to support complex cell-wide changes and also local protein synthesis as in
182 the brain at dendrites during memory consolidation and long-term memory formation
183^{32–34}. Pharmacogenetic activation of ISR in rodents impairs long-term memory. Based

184 on these considerations it is tempting to speculate that in plasma cells the ISR may
185 play a role in the process leading to becoming a long-lived cell ^{33,35}. Cellular stress is
186 sensed by four specialized kinases (GCN2, HRI, PKR and PERK as well) that converge
187 on phosphorylation of a single serine on the eukaryotic translation initiation factor eIF2,
188 resulting in blockage of the nucleotide exchange factor eIF2B (reviewed in ³⁶). In
189 plasma cells, as terminal B cell differentiation takes place independently of PERK ²⁸
190 and, GCN2 and HRI respond to nutrient deprivation and heme deficiency, respectively,
191 PKR may be the kinase responsible for ISR-mediated translation regulation in
192 managing ER stress ³⁶. PKR contains a double-stranded RNA binding domain (dsRNA)
193 that binds to dsRNA which induces dimerization of PKR which triggers an activating
194 autophosphorylation of the kinase. Thus activated, PKR phosphorylates eIF2a leading
195 to the inhibition of cellular and viral mRNA translation which represents an efficient way
196 to inhibit viral replication ^{37,38}. However, PKR activation may be detected in absence of
197 dsRNA by a wide range of different cell stresses through the recruitment of PACT
198 (PKR-associated activator) and binding to PKR ³⁹ consistent with several diverse
199 cellular functions where PKR has been implicated such as growth regulation, apoptosis
200 signaling pathway and differentiation (reviewed in ³⁸). For example, in mouse
201 fibroblasts, PKR has been shown to be an important signal in controlling growth arrest
202 and subsequent differentiation to adipocytes ^{40,41}. Interestingly, accumulating evidence
203 suggests that dsRNAs are not limited to virally infected cells, but can be produced from
204 endogenous sources and activate the same receptors that have evolved to detect
205 “danger” signals that alert dysregulated cellular processes leading to an innate immune
206 response ^{42,43}. Interestingly, mitochondrial RNAs, which exist as intermolecular
207 dsRNAs, interact strongly with PKR and regulate downstream activation, especially
208 under stress conditions ⁴⁴. This dsRNA-induced response is consistent with the role of

209 mitochondrial UPR in cytosolic ISR activation ^{42,45}. Taken collectively these data
210 represent a good starting point for further exploration of the PKR-dependent ISR
211 response during terminal B cell differentiation and its possible role in plasma cell
212 survival.

213 **4. PIM2 is strongly induced in B cells engaged in differentiation into**
214 **plasmablasts.**

215 CD23-negative preplasmablasts compared to uncommitted CD23-positive B cells
216 exhibit strong expression of PIM2 - ahead of the PRDM1 gene in our single-cell RNA
217 dataset and detection of the protein by western blotting - which occurs after release of
218 BACH2 repression ³. This upregulation of PIM2 is driven by STAT3 and induced by the
219 cytokines IL-21/IL-10 and IL-6 in plasma cells. Thus, BACH2 repression can be viewed
220 as a gateway to the ultimate commitment of antibody-secreting cells, mediated by an
221 IL2/ERK/ELK1 signaling pathway ^{8,46} and responsible for the release of both PIM2 and
222 BLIMP1 expression, with PIM2 upregulation preceding BLIMP1 expression (Fest lab,
223 unpublished). These findings suggest that PIM2, independent of BLIMP1, plays a
224 critical role in the differentiation of B cells into plasma cells.

225 The kinase domain of PIM2 shares unique structural features with PIM1 and PIM3,
226 making them constitutively active. Characterized by a short half-life, PIM2 expression
227 is tightly regulated and establishes a growth factor-induced, kinase-dependent
228 pathway that promotes cell-autonomous survival ^{47,48}. As previously mentioned, the
229 final commitment of B cells to plasmablasts requires cell cycle entry with the
230 development of an active gene-specific DNA demethylation process targeting plasma
231 cell identity genes, as well as cell survival and proliferation genes ¹. This process may
232 involve an altered balance between DNMT1 and TET enzyme activities. There is some
233 evidence for a relationship between PIM kinases and DNMT1. DNMT1 methylates

234 newly synthesized DNA during S phase with the help of UHRF1 for proper loading
235 within the replication fork ⁴⁹. Several studies have described human DNMT1
236 phosphorylation at multiple serine/threonine sites, including the phosphorylation by
237 Akt/PKC which disrupts the DNMT1/UHRF1/PCNA complex ⁵⁰. On the other hand,
238 PIM1 kinase expression has been implicated in cell senescence through
239 downregulation of DNMT1 via a PIM1-HBP1 axis ⁵¹ and through phosphorylation at
240 Ser³¹¹ of UHRF1, leading to its degradation ⁵². Finally, TET2 is a substrate of AMP-
241 activated kinase (AMPK), which phosphorylates TET2 at serine 99, thereby stabilizing
242 its activity and the production of 5hmC DNA ⁵³. Notably, PIM2 can regulate AMPK
243 activity through a direct Thr467 phosphorylation and/or by suppressing the tumor
244 suppressor LKB1, which activates AMPK after Thr183 phosphorylation ^{54,55}. The
245 information presented in our previous work highlights the role of PIM2 in the G1/S
246 transition of B cells to plasmablasts by activating CDC25A and exporting p27Kip1 out
247 of the nucleus ³. Given this, it is plausible to speculate that the increased cytoplasmic
248 expression of PIM2 may play a role in regulating the balance of TET2 and DNMT1
249 activity, leading to the production of 5hmC prior to complete demethylation beyond S
250 phase. This process may occur on previously protected regions of the genome during
251 B cell maturation, thus contributing to the epigenetic landscape that governs cellular
252 identity ¹.

253 In murine B cells, Pim2 was shown to work in parallel with the Akt/mTOR pathway to
254 promote the survival and resistance to atrophy of resting cells stimulated with the
255 obligate B cell survival factor BLyS ⁵⁶. BLyS-mediated B cell survival in the presence
256 of rapamycin, an Akt/mTOR inhibitor, was dependent on Pim2. When Pim-deficient B
257 cells were cultured with rapamycin, the addition of BLyS did not improve survival,
258 whereas Pim-sufficient B cells were able to utilize BLyS to improve survival. Signaling

259 events downstream of Pim2 facilitated the production of the anti-apoptotic protein Mcl-
260 1 as a mechanism for enhanced survival ⁵⁶. Given the level of PIM2 expression in
261 human plasma cells, it can be estimated that this kinase greatly exceeds the average
262 of 14 substrates per kinase as proposed by human kinome analysis ⁵⁷. Newly
263 generated plasmablasts experience an increase in the expression of BIM and Caspase
264 3 as a result of UPR. PIM2 plays a role in inhibiting apoptosis by promoting the
265 phosphorylation of BAD, which leads to its cytoplasmic sequestration. Additionally,
266 PIM2 prevents the activation of Caspase 3 by promoting the phosphorylation of
267 p21Cip1, which then binds to and inactivates Caspase 3. Furthermore, PIM2 inhibits
268 mitochondrial depolarization, which indirectly contributes to the stabilization of the anti-
269 apoptotic protein XIAP that binds to Caspase 3 and prevents its activation ^{3,6,58}. Finally,
270 short-lived plasma cells retain the requirement of mTORC1 signaling, which is no
271 longer required in long-lived plasma cells ⁵⁹. PIM2 may participate in the maintenance
272 of mTORC1 signaling in nascent plasma cells by downregulating TSC2, a repressor of
273 mTORC1 ⁶⁰.

274 **5. PIM2 in mature plasma cells**

275 Long-lived plasma cells can persist to the end of host's life in the absence of repeated
276 antigen stimulation. Thus, it is critical to understand the maintenance of mature plasma
277 cells and how they overcome pro-apoptotic forces due to ER stress. CD138-positive
278 human plasma cells generated in vitro showed maintenance of high **levels of**
279 cytoplasmic PIM2 expression as well as expression of p-BAD, which **drives BAD**
280 **inactivation and thus stabilizes** mitochondrial membrane polarization ³. In quiescent
281 cells, p21Cip1 is usually nuclear and participates in cell cycle arrest. In mature
282 nonproliferative plasma cells as well as in bone marrow plasma cells, p21Cip1 remains
283 cytoplasmic and maintains binding to Caspase 3, thereby inhibiting Caspase 3

284 activation (³ and Fest lab, unpublished data). It is plausible to hypothesize that the ISR
285 must be tightly regulated to ensure proper functioning and production of a wide range
286 of immunoglobulins. In this context, PIM2 may play a central role in controlling ISR.
287 Our preliminary experiments suggest that PIM2 regulates the activation of PERK and
288 PKR (Fest lab, unpublished data). By modulating the activity of these proteins, PIM2
289 may regulate the threshold at which cells undergo ISR, ensuring a balance between
290 the stress response and efficient Ig production. Further studies are needed to fully
291 understand the regulatory mechanisms involved in this process.

292 Recently, in humans, we found that in addition to high PIM2 expression, bone marrow
293 plasma cells increase PIM1 expression. The latter is essentially nuclear, while PIM2
294 remains present in the cytoplasm (Fest lab, unpublished data). As previous work has
295 shown that PIM1 is essential for the surface expression of CXCR4 and for the migration
296 of cells towards a CXCL12 gradient ⁶¹, we therefore hypothesize that PIMs could be
297 functionally involved in the structuration of a favourable microenvironment in the bone
298 marrow for plasma cell localization and survival.

299 (Insert Figure here)

300 **6. PIM2 and malignant plasma cells**

301 Although the involvement of PIM2 in multiple myeloma (MM) has been recognized for
302 more than a decade, we have further investigated the status of PIM2 in malignant PCs.
303 Data from the DepMap Project (<https://depmap.org/portal/>) involving up to 800 different
304 cancer cell lines, showed that PIM2 (but not PIM1) mRNA expression was particularly
305 high in MM cells compared with other tumor cells. CRISPR and shRNA screening
306 revealed a specific high PIM2 dependency score for malignant plasma cells ³. The
307 control of mitochondrial depolarization by PIM2 is similar in normal and malignant

308 plasma cells (Fest lab, unpublished). The anti-apoptotic effects of PIM2, mediated
309 through BAD phosphorylation, may complement those of MCL1, as BAD only
310 antagonizes BCLXL and BCL2⁶². Therefore, dual inhibition of PIM2 and MCL1 may
311 provide a synergistic effect and offer new treatment options. Additionally, reducing the
312 dose of anti-MCL1 inhibitors, which bind to MCL1 with subnanomolar affinity and can
313 cause cardiotoxicity in some patients, is a potential benefit of this approach⁶³.

314 **7. Conclusions and perspective**

315 In recent years, new factors have emerged that contribute to a better understanding of
316 B cell differentiation into plasmablasts, including specific transcription factors and
317 regulators of genome organization and epigenetic landscape. Protein phosphorylation,
318 a post-translational modification that regulates many biological processes, is of broad
319 interest for identifying therapeutic targets through high specificity drugs. In vitro human
320 B cell differentiation models have identified the serine/threonine kinase PIM2 as
321 specifically linked to the late stage of differentiation by supporting cell cycle entry and
322 inhibiting apoptosis. While several substrates have been studied, the high level of
323 cytoplasmic PIM2 expression in plasma cells suggests that it may act on a broad range
324 of proteins. Immunoprecipitation experiments in MM cell lines and human primary
325 plasmablasts obtained from in vitro culture of B cells revealed an enrichment of various
326 proteins, including several factors acting on the same transcriptional machinery,
327 epigenome, or ISR, indicating that PIM2 binds to protein complexes (Fest lab,
328 unpublished). This link to protein complexes provides a level of regulation through
329 phosphorylation of Ser/Thr residues on a particular peptide motif, out of a total of 38
330 identified motifs, that respond more specifically to PIM2⁶⁴.

331 Future directions for the study of PIM2 in plasma cells may involve rodent models.
332 However, in mouse plasma cells it appears that the expression of Pim1 and Pim2 are

333 at comparable levels, which is not the case in humans (Fest Lab, unpublished). A
334 comparative analysis of these two kinases in the plasma cell seems therefore important
335 by assessing the possibility of redundancy of functions, differential cell
336 compartmentalization and their respective capacity to bind to protein complexes. A
337 second aspect of exploration involves the exact role of PIM2 in the organization of a
338 supportive niche for the long survival of plasma cells including a study of interactions
339 with the microenvironment. IL-6 signals through the STAT3 pathway and is essential
340 for rapid induction of PIM2³. In the bone marrow, apart from stromal cells, other cells
341 such as megakaryocytes produce IL-6 which could correspond to different sites for
342 plasma cell localization with different functional capacities. If PIM2 allows cell cycle
343 entry at plasmablast differentiation, in the long-lived plasma cell it is likely that PIM2
344 participates in the quiescence through factors or functions that need to be identified.
345 Finally, quantitative phosphoproteomics that explore changes in phosphorylation
346 levels of proteins in response to stimuli is a powerful tool for understanding signaling
347 pathways and identifying potential targets. However, it is important to ensure that
348 experimental conditions are reproducible in order to obtain reliable results. This
349 includes carefully preparing the cells and samples, controlling for variability in
350 experimental conditions, and using appropriate statistical methods to analyze the data.
351 In addition, it is important to validate any findings from phosphoproteomic studies using
352 complementary techniques, such as western blotting or functional assays, to confirm
353 the biological relevance of the observed changes in protein phosphorylation⁶⁵.

354

355 AUTHOR CONTRIBUTIONS

356 MH and TF performed the literature search, wrote the manuscript.

357

358 **CONFLICT OF INTEREST**

359 The authors declare that the research was conducted in the absence of any
360 commercial or financial relationships that could be construed as a potential conflict of
361 interest.

362

363 **FUNDING INFORMATION**

364 This study was funded by Programmes labellisés (PGA) 2022 ARC N°
365 ARCPGA2021120004244_4856, Ligue Régionale GO, 2020, and an internal grant
366 from the Hematology Laboratory, CHU de Rennes. M. Haas has received a doctoral
367 fellowship from FHU CAMIn, Ligue Contre le Cancer/Comité d'Ille et Vilaine.

368

369 **REFERENCES**

370

- 371 1. Caron G, Hussein M, Kulis M, et al. Cell-Cycle-Dependent Reconfiguration of the DNA
372 Methylome during Terminal Differentiation of Human B Cells into Plasma Cells. *Cell re-*
373 *ports*. 2015;13(5):1059–1071.
- 374 2. Olsen JV, Vermeulen M, Santamaria A, et al. Quantitative Phosphoproteomics Reveals
375 Widespread Full Phosphorylation Site Occupancy During Mitosis. *Sci Signal*.
376 2010;3(104):ra3.
- 377 3. Haas M, Caron G, Chatonnet F, et al. Pivotal role of PIM2 kinase in plasmablast generation
378 and plasma cell survival, opening new treatment options in myeloma. *Blood*. 2022; 139 (15):
379 2316-2337.
- 380 4. Gallou SL, Caron G, Delaloy C, et al. IL-2 requirement for human plasma cell generation:
381 coupling differentiation and proliferation by enhancing MAPK-ERK signaling. *The Journal of*
382 *Immunology*. 2012;189(1):161–173.
- 383 5. Lemarié M, Chatonnet F, Caron G, Fest T. Early Emergence of Adaptive Mechanisms Sus-
384 taining Ig Production: Application to Antibody Therapy. *Front Immunol*. 2021;12:671998.
- 385 6. Pignarre A, Chatonnet F, Caron G, et al. Plasmablasts derive from CD23– activated B cells
386 after the extinction of IL-4/STAT6 signaling and IRF4 induction. *Blood*. 2021;137(9):1166–
387 1180.
- 388 7. Kulis M, Merkel A, Heath S, et al. Whole-genome fingerprint of the DNA methylome dur-
389 ing human B cell differentiation. *Nature genetics*. 2015;47(7):746–756.

- 390 8. Hipp N, Symington H, Pastoret C, et al. IL-2 imprints human naive B cell fate towards
391 plasma cell through ERK/ELK1-mediated BACH2 repression. *Nature communications*.
392 2017;8(1):1443.
- 393 9. Barwick BG, Scharer CD, Bally APR, Boss JM. Plasma cell differentiation is coupled to
394 division-dependent DNA hypomethylation and gene regulation. *Nat Immunol*.
395 2016;17(10):1216–1225.
- 396 10. Schoeler K, Aufschneider A, Messner S, et al. TET enzymes control antibody production
397 and shape the mutational landscape in germinal centre B cells. *Febs J*. 2019;286(18):3566–
398 3581.
- 399 11. Boothby MR, Brookens SK, Raybuck AL, Cho SH. Supplying the trip to antibody pro-
400 duction—nutrients, signaling, and the programming of cellular metabolism in the mature B
401 lineage. *Cell Mol Immunol*. 2021;1–18.
- 402 12. D’Souza L, Bhattacharya D. Plasma cells: You are what you eat. *Immunological reviews*.
403 2019;288(1):161–177.
- 404 13. Kozutsumi Y, Segal M, Normington K, Gething M-J, Sambrook J. The presence of mal-
405 folded proteins in the endoplasmic reticulum signals the induction of glucose-regulated pro-
406 teins. *Nature*. 1988;332(6163):462–464.
- 407 14. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, et al. XBP1, downstream of Blimp-1, ex-
408 pands the secretory apparatus and other organelles, and increases protein synthesis in plasma
409 cell differentiation. *Immunity*. 2004;21(1):81–93.
- 410 15. Sriburi R, Jackowski S, Mori K, Brewer JW. XBP1. *J Cell Biology*. 2004;167(1):35–41.
- 411 16. Reimold AM, Iwakoshi NN, Manis J, et al. Plasma cell differentiation requires the tran-
412 scription factor XBP-1. *Nature*. 2001;412(6844):300–307.
- 413 17. Brewer JW, Hendershot LM. Building an antibody factory: a job for the unfolded protein
414 response. *Nat Immunol*. 2005;6(1):23–29.
- 415 18. Todd DJ, Lee A-H, Glimcher LH. The endoplasmic reticulum stress response in immunity
416 and autoimmunity. *Nat Rev Immunol*. 2008;8(9):663–674.
- 417 19. Ricci D, Gidalevitz T, Argon Y. The special unfolded protein response in plasma cells.
418 *Immunol Rev*. 2021;303(1):35–51.
- 419 20. Santamaria K, Desmots F, Leonard S, et al. Committed Human CD23-Negative Light-
420 Zone Germinal Center B Cells Delineate Transcriptional Program Supporting Plasma Cell
421 Differentiation. *Front Immunol*. 2021;12:744573.
- 422 21. Gaudette BT, Jones DD, Bortnick A, Argon Y, Allman D. mTORC1 coordinates an im-
423 mediate unfolded protein response-related transcriptome in activated B cells preceding anti-
424 body secretion. *Nature communications*. 2020;1–16.

- 425 22. Gaudette BT, Allman D. Biochemical coordination of plasma cell genesis. *Immunol Rev.*
426 2021;303(1):52–61.
- 427 23. Tellier J, Shi W, Minnich M, et al. Blimp-1 controls plasma cell function through the reg-
428 ulation of immunoglobulin secretion and the unfolded protein response. *Nature immunology.*
429 2016;17(3):323–330.
- 430 24. Trezise S, Nutt SL. The gene regulatory network controlling plasma cell function. *Immu-
431 nol Rev.* 2021;303(1):23–34.
- 432 25. Dossou AS, Basu A. The Emerging Roles of mTORC1 in Macromanaging Autophagy.
433 *Cancers.* 2019;11(10):1422.
- 434 26. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplas-
435 mic-reticulum-resident kinase. *Nature.* 1999;397(6716):271–274.
- 436 27. Harding HP, Novoa I, Zhang Y, et al. Regulated Translation Initiation Controls Stress-In-
437 duced Gene Expression in Mammalian Cells. *Mol Cell.* 2000;6(5):1099–1108.
- 438 28. Gass JN, Jiang H-Y, Wek RC, Brewer JW. The unfolded protein response of B-lympho-
439 cytes: PERK-independent development of antibody-secreting cells. *Mol Immunol.*
440 2008;45(4):1035–1043.
- 441 29. Amanna IJ, Slifka MK. Mechanisms that determine plasma cell lifespan and the duration
442 of humoral immunity. *Immunol Rev.* 2010;236(1):125–138.
- 443 30. Radbruch A, Muehlinghaus G, Luger EO, et al. Competence and competition: the chal-
444 lenge of becoming a long-lived plasma cell. *Nat Rev Immunol.* 2006;6(10):741–750.
- 445 31. Hammarlund E, Thomas A, Amanna IJ, et al. Plasma cell survival in the absence of B cell
446 memory. *Nat Commun.* 2017;8(1):1781.
- 447 32. Sutton MA, Schuman EM. Dendritic Protein Synthesis, Synaptic Plasticity, and Memory.
448 *Cell.* 2006;127(1):49–58.
- 449 33. Costa-Mattioli M, Gobert D, Stern E, et al. eIF2 α Phosphorylation Bidirectionally Regu-
450 lates the Switch from Short- to Long-Term Synaptic Plasticity and Memory. *Cell.*
451 2007;129(1):195–206.
- 452 34. Shrestha P, Klann E. Spatiotemporally resolved protein synthesis as a molecular frame-
453 work for memory consolidation. *Trends Neurosci.* 2022;45(4):297–311.
- 454 35. Shrestha P, Ayata P, Herrero-Vidal P, et al. Cell-type-specific drug-inducible protein syn-
455 thesis inhibition demonstrates that memory consolidation requires rapid neuronal translation.
456 *Nat Neurosci.* 2020;23(2):281–292.
- 457 36. Costa-Mattioli M, Walter P. The integrated stress response: From mechanism to disease.
458 *Science.* 2020;368(6489):.

- 459 37. Dey M, Cao C, Dar AC, et al. Mechanistic Link between PKR Dimerization, Autophos-
460 phosphorylation, and eIF2 α Substrate Recognition. *Cell*. 2005;122(6):901–913.
- 461 38. García MA, Meurs EF, Esteban M. The dsRNA protein kinase PKR: Virus and cell con-
462 trol. *Biochimie*. 2007;89(6–7):799–811.
- 463 39. Patel RC, Sen GC. PACT, a protein activator of the interferon- induced protein kinase,
464 PKR. *Embo J*. 1998;17(15):4379–4390.
- 465 40. Judware R, Petryshyn R. Mechanism of action of a cellular inhibitor of the dsRNA-de-
466 pendent protein kinase from 3T3-F442A cells. *J Biol Chem*. 1992;267(30):21685–21690.
- 467 41. Petryshyn RA, Ferrenz AG, Li J. Characterization and mapping of the double-stranded re-
468 gions involved in activation of PKR within a cellular RNA from 3T3-F442A cells. *Nucleic*
469 *Acids Res*. 1997;25(13):2672–2678.
- 470 42. Chen YG, Hur S. Cellular origins of dsRNA, their recognition and consequences. *Nat Rev*
471 *Mol Cell Bio*. 2022;23(4):286–301.
- 472 43. Dhir A, Dhir S, Borowski LS, et al. Mitochondrial double-stranded RNA triggers antiviral
473 signalling in humans. *Nature*. 2018;560(7717):238–242.
- 474 44. Kim Y, Park J, Kim S, et al. PKR Senses Nuclear and Mitochondrial Signals by Interact-
475 ing with Endogenous Double-Stranded RNAs. *Mol Cell*. 2018;71(6):1051-1063.e6.
- 476 45. Guo X, Aviles G, Liu Y, et al. Mitochondrial stress is relayed to the cytosol by an OMA1–
477 DELE1–HRI pathway. *Nature*. 2020;579(7799):427–432.
- 478 46. Willis SN, Nutt SL. ScienceDirect New players in the gene regulatory network controlling
479 late B cell differentiation. *Current opinion in immunology*. 2019;58:68–74.
- 480 47. Santio NM, Koskinen PJ. PIM kinases_ From survival factors to regulators of cell motil-
481 ity. *International Journal of Biochemistry and Cell Biology*. 2017;93:74–85.
- 482 48. Fox CJ, Thompson CB. The serine/threonine kinase Pim-2 is a transcriptionally regulated
483 apoptotic inhibitor. *Genes & Development*. 2003;17(15):1841–1854.
- 484 49. Sharif J, Muto M, Takebayashi S, et al. The SRA protein Np95 mediates epigenetic inher-
485 itance by recruiting Dnmt1 to methylated DNA. *Nature*. 2007;450(7171):908–912.
- 486 50. Hervouet E, Lalier L, Debien E, et al. Disruption of Dnmt1/PCNA/UHRF1 Interactions
487 Promotes Tumorigenesis from Human and Mice Glial Cells. *Plos One*. 2010;5(6):e11333.
- 488 51. Wang S, Cao Z, Xue J, et al. A positive feedback loop between Pim-1 kinase and HBP1
489 transcription factor contributes to hydrogen peroxide-induced premature senescence and
490 apoptosis. *J Biol Chem*. 2017;292(20):8207–8222.
- 491 52. Yang J, Liu K, Yang J, et al. PIM1 induces cellular senescence through phosphorylation
492 of UHRF1 at Ser311. *Oncogene*. 2017;36:4828–4842.

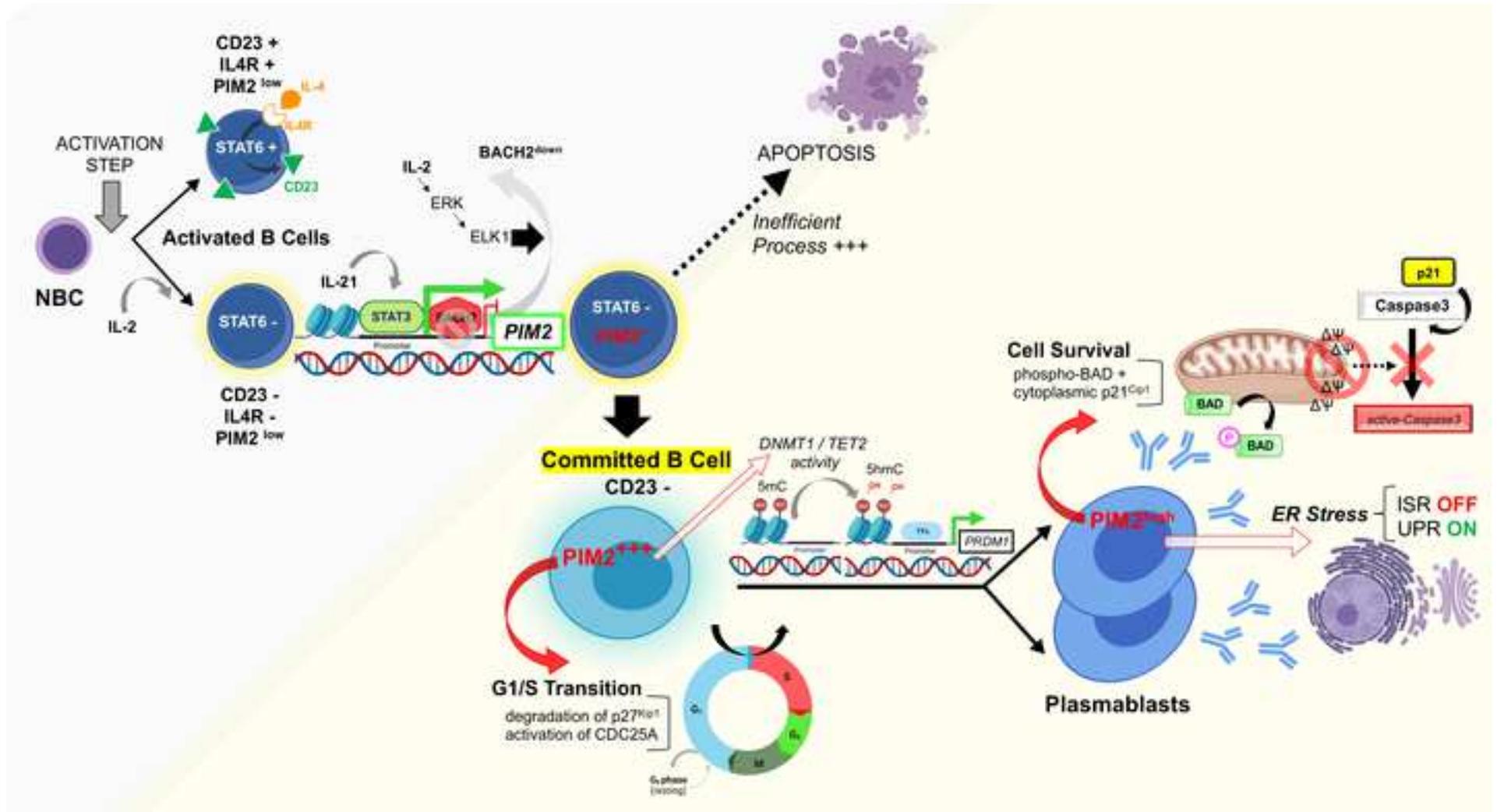
- 493 53. Wu D, Hu D, Chen H, et al. Glucose-regulated phosphorylation of TET2 by AMPK re-
494 veals a pathway linking diabetes to cancer. *Nature*. 2018;559(7715):637–641.
- 495 54. Han X, Ren C, Yang T, et al. Negative regulation of AMPK α 1 by PIM2 promotes aerobic
496 glycolysis and tumorigenesis in endometrial cancer. *Oncogene*. 2019;38(38):6537–6549.
- 497 55. Mung KL, Eccleshall WB, Santio NM, Rivero-Müller A, Koskinen PJ. PIM kinases in-
498 hibit AMPK activation and promote tumorigenicity by phosphorylating LKB1. *Cell Commun*
499 *Signal*. 2021;19(1):68.
- 500 56. Woodland RT, Fox CJ, Schmidt MR, et al. Multiple signaling pathways promote B lym-
501 phocyte stimulator–dependent B-cell growth and survival. *Blood*. 2008;111(2):750–760.
- 502 57. Hu J, Rho H-S, Newman RH, et al. Global analysis of phosphorylation networks in hu-
503 mans. *Biochimica Et Biophysica Acta Bba - Proteins Proteom*. 2014;1844(1):224–231.
- 504 58. Ren K, Gou X, Xiao M, He W, Kang J. Pim-2 Cooperates with Downstream Factor XIAP
505 to Inhibit Apoptosis and Intensify Malignant Grade in Prostate Cancer. *Pathology & Oncol-*
506 *ogy Research*. 2017;25(1):1–8.
- 507 59. Nguyen DC, Garimalla S, Xiao H, et al. Factors of the bone marrow microniche that sup-
508 port human plasma cell survival and immunoglobulin secretion. *Nature communications*.
509 2019;9(1):1–12.
- 510 60. Lu J, Zavorotinskaya T, Dai Y, et al. Pim2 is required for maintaining multiple myeloma
511 cell growth through modulating TSC2 phosphorylation. *Blood*. 2013;122(9):1610–1620.
- 512 61. Grundler R, Brault L, Gasser C, et al. Dissection of PIM serine/threonine kinases in
513 FLT3-ITD–induced leukemogenesis reveals PIM1 as regulator of CXCL12–CXCR4-medi-
514 ated homing and migration. *The Journal of experimental medicine*. 2009;206(9):1957–1970.
- 515 62. Wei AH, Roberts AW, Spencer A, et al. Targeting MCL-1 in hematologic malignancies:
516 Rationale and progress. *Blood Rev*. 2020;44:100672.
- 517 63. Daressy F, Séguy L, Favre L, et al. NA1—115—7, from *Zygogynum pancheri*, is a new
518 selective MCL-1 inhibitor inducing the apoptosis of hematological cancer cells but non-toxic
519 to normal blood cells or cardiomyocytes. *Biomed Pharmacother*. 2022;154:113546.
- 520 64. Johnson JL, Yaron TM, Huntsman EM, et al. An atlas of substrate specificities for the hu-
521 man serine/threonine kinome. *Nature*. 2023;1–8.
- 522 65. Rigbolt KTG, Blagoev B. Quantitative phosphoproteomics to characterize signaling net-
523 works. *Semin Cell Dev Biol*. 2012;23(8):863–871.
- 524

525 **Figure Legend:**

526 **An overview of the demonstrated and few potential effects of PIM2 kinase during**
527 ***in vitro* differentiation of human naive B cells.**

528 To undergo plasma cell differentiation, B cells must respond to various stimuli. Naive
529 B cells (NBC) are initially activated and subsequently selected to commit to the
530 differentiation process. Activated cells are initially screened based on their
531 responsiveness to the cytokine IL-4, which triggers the expression of the CD23 marker
532 through STAT6 activation. Only cells that downregulate the IL-4 receptor expression,
533 and consequently become CD23 negative, can progress in the differentiation process
534 (Pignarre et al., Blood 2021). *In vitro* experiments have shown that minimal IL-2
535 stimulation experienced by recently activated NBCs initiates plasma cell differentiation.
536 Notably, BACH2 has been identified as an early regulator of B cell fate decision, and
537 its timely repression by IL-2-mediated ERK/ELK1 signaling facilitates plasma cell
538 commitment (Hipp et al., Nat Comms 2017). Upon release of BACH2 and in response
539 to the cytokine IL-21, which activates the STAT3 pathway, PIM2 expression is induced.
540 This leads to robust serine/threonine kinase activity, associated with early plasma cell
541 commitment. In fact, the initial high expression of PIM2 enables cell cycle entry by
542 degrading p27Kip1 and activating CDC25A, both of which facilitate the G1/S transition
543 (Haas et al., Blood 2022). Commitment is also accompanied by transcriptional
544 reprogramming of B cells, partially mediated by epigenetic modifications. TET2 activity,
545 but not DNMT1 activity, allows hydroxymethylation of 5-methyl-cytosine residues at
546 the promoters of genes responsible for plasma cell identity, including *PRDM1* (Caron
547 et al., Cell Report 2015). Preliminary findings suggest that PIM2 kinase may play a role
548 in attenuating DNMT1 protein expression. This terminal stage of B cell differentiation
549 gives rise to antibody-secreting plasmablasts, which serve as precursors to plasma
550 cells. These differentiated cells exhibit high levels of PIM2, which plays a crucial role
551 in protecting cells from apoptosis. The kinase accomplishes this by preventing
552 mitochondrial depolarization through phosphorylation of BAD and by stabilizing
553 cytoplasmic p21Cip1 protein, both of which hinder caspase 3 activation (Haas et al.,
554 Blood 2022). Finally, the differentiation process is accompanied by a physical
555 transformation of the cells, characterized by the development of an extensive protein
556 synthesis machinery involving a highly expanded endoplasmic reticulum for
557 immunoglobulin synthesis. This heightened activity induces significant cellular stress,
558 which is counteracted by activating the unfolded protein response (UPR) pathways.

559 Additionally, the Integrated Stress Response (ISR) pathway is potentially inhibited by
560 PIM2, which could inactivate certain ISR kinases, such as PKR and PERK, thereby
561 preventing cell death mediated by the ISR pathway.



Declaration of Interest Statement

This piece of the submission is being sent via mail.