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Transinteractome analysis reveals distinct niche requirements for isotype-based plasma cell subsets in the bone marrow

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Bone marrow (BM) long-lived plasma cells (PCs) are essential for long-term protection against infection, and their persistence within this organ relies on interactions with Cxcl12-expressing stromal cells that are still not clearly identified. Here, using single cell RNAseq and in silico transinteractome analyses, we identified Leptin receptor positive (LepR$^+$) mesenchymal cells as the stromal cell subset most likely to interact with PCs within the BM. Moreover, we demonstrated that depending on the isotype they express, PCs may use different sets of integrins and adhesion molecules to interact with these stromal cells. Altogether, our results constitute an unprecedented characterization of PC subset stromal niches and open new avenues for the specific targeting of BM PCs based on their isotype.

Keywords: Plasma cell · Bone marrow · Niche · Stromal cell · Transinteractome

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

Plasma cells (PCs) correspond to the last stage of B cell differentiation and are key effectors of the humoral immune response through the secretion of large quantities of antibodies (Abs). Following their generation, most PCs are short-lived and rapidly die. Some PCs, however, migrate to the bone marrow (BM) via a mechanism dependent on the chemokine receptor Cxcr4 and its ligand Cxcl12 [1]. Once there, PCs have the capacity to persist for years, possibly even a lifetime as quiescent, long-lived PCs [2–4]. These cells are essential for long-lasting protection against repeat infections and for the protection conferred by vaccination [3, 5, 6].

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Their long-term maintenance depends on extracellular cues from their microenvironment encompassing Cxcl12 and the TNF superfamily, in particular APRIL produced by myeloid cells (including megakaryocytes and eosinophils) and leading to the activation of the prosurvival factor Mcl1 [1, 7, 8, 9–12]. In addition, ligands for the integrins VLA-4 ($\alpha4\beta1$) and LFA-1 ($\alpha L\beta2$) may contribute to BM PC retention and fitness [13–15].

Several studies have highlighted that PCs may form a heterogeneous population. Notably, the expression levels of CD19 and CD45 seem to discriminate short- and long-lived PCs [4, 16, 17]. Moreover, recent reports suggest that upon infection or vaccination, newly generated PCs expressing different isotypes may be transcriptionally distinct [18–21]. In addition, it has now been well documented that IgM^+ and IgA^+ PCs still express a functional BCR at their surface, whereas IgG^+ PCs do not [22, 23].

PCs are scattered throughout the BM and often found in close contact with $Cxcl12^+$ reticular stromal cells associated with the vasculature [13, 24, 25]. Recent single cell RNAseq (scRNAseq) analyses of the BM environment have demonstrated that the stromal cell compartment is highly heterogeneous and composed of diverse cell types, including numerous subsets of fibroblasts, endothelial cells and mesenchymal cells [26–29]. Among these cell populations, Cxcl12 appears most expressed by arteriolar endothelial cells, pericytes, leptin receptor$^+$ ($LepR^+$) mesenchymal cells, a subset of osteo-committed cells and a subset of fibroblasts [27]. Which of these cell types interact with PCs within the BM is still unknown. Other adhesion molecules like VCAM-1 and ICAM-1 interacting with the integrins VLA-4 and LFA-1, respectively, can also be found on different types of stromal cells [15, 25]. Although a lot of work has been performed to decipher the specific cellular components of the HSC niches, equivalent studies are lacking for PCs [30]. Moreover, whether all PCs require equivalent environmental cues within the BM for their maturation and maintenance remains to be investigated.

To address these questions, we performed scRNAseq analyses of mouse BM PCs. Our results confirm the existence of distinct transcriptomic signatures for BM PCs according to the isotype they express. Moreover, through in silico analysis, we identify the stromal cells most likely to interact with PCs in the BM and we showed that IgM^+, IgA^+ and IgG^+ PCs may rely on different interactions with their environment for their maintenance.

Results and discussion

BM plasma cells have a distinct transcriptome based on the isotype they express

As several studies suggest that PCs are a heterogeneous population [20, 22, 23], we performed scRNAseq on fully differentiated $CD138^{high}$ Blimp1-GFP^{high} BM PCs (Fig. 1A). Dimension reduction analysis after exclusion of the Ig transcripts showed a relatively homogenous clustering of PCs that could be explained by our stringent gating strategy (Fig. 1B). However, when we imputed back the isotype of each PC, we observed that IgA^+, IgM^+ and IgG^+ PCs tend to segregate supporting previous reports [18, 19]. We detected 61 differentially expressed genes between BM PC subsets (Fig. 1C). The differentially expressed genes were involved in various pathways, including metabolism (*Bcat2, Ttc19, Inpp4a, Scd2, Far1, Plaat3*), survival/apoptosis (*Pycard, Ikzf3*), protein degradation (*Itch, Nedd4, Slpi, Sel1l*), antigen presentation and cell activation (*H2-Aa, CD74, B2m, Cd69, Ms4a1*) as well as cell adhesion/migration (*Ccr10, Sell, Cxcr4, Cd52, Epcam, Ly6c2*) (Fig. 1C). IgA^+ PCs had higher *Jchain* (critical for dimer formation) expression and were the main cells expressing *Ccr10*, which encodes a chemokine receptor critical for homing to the mucosa (Fig. 1D). IgM^+ cells expressed higher amount of *Ikzf3*, which encodes Aiolos, previously shown to be essential for long-term high-affinity BM PC generation [31]. Some IgM^+ PC also expressed high level of *Ms4a1* that encodes CD20 (Fig. 1E) and could correspond to immature PCs. To focus our study on long-lived PCs, we thus decided to exclude the *Ms4a1* high cells ($n = 9$) from the rest of our study. Interestingly, IgG^+ PCs expressed more *Cxcr4, Epcam, Slpi* and *Ly6c*, encoding proteins that have all been involved in cell adhesion, migration and survival [20, 32–35] (Fig. 1F).

We next validated these transcriptional differences by single cell Q-PCR for selected genes. We observed increased expression of *Ccr10* and *Jchain* in IgA^+ PCs, of *Sell* in IgM^+ PCs and of *Ly6c* in IgG^+ PCs compared to the two other groups (Supporting Information Fig. 1A). We also observed an overrepresentation of the weakly expressed *Wls* and *Clec2i* in IgM^+ PCs, and these transcripts were almost uniquely expressed by IgM^+ PCs (Supporting Information Fig. 1B and C). Wls regulates Wnt protein trafficking that has been shown to contribute to PC resistance to bortezomib [36, 37], whereas *Clec2i* was shown to be up-regulated in $LAG3^+$ Il-10 producing PCs [38]. In addition, *Dock2* and *Plek* implicated in lymphocyte activation/migration [39–41], as well as *Ddit3* encoding the Chop protein [42], were more expressed in IgM^+ PCs than in IgG^+ PCs. Conversely, IgG^+ PCs expressed more *Acp2*, implicated in lysosomal acidification, than IgA^+ PCs [43] (Supporting Information Fig. 1D). Altogether these results provide additional validation that long-lived BM PCs have a specific transcriptional signature based on their isotype and support another recent study highlighting EpCAM as a marker of long-lived PCs [20].

In silico analysis reveals that plasma cells are more likely to form interactions with certain clusters of stromal cells

To unravel the most likely stromal partners of BM PCs, we took advantage of our scRNAseq dataset and of a previously published dataset for BM stromal cells covering broadly bone and marrow non-haematopoietic cells [27] to perform in silico transinteractome analysis using CellPhoneDB [44, 45]. The stroma scRNASeq

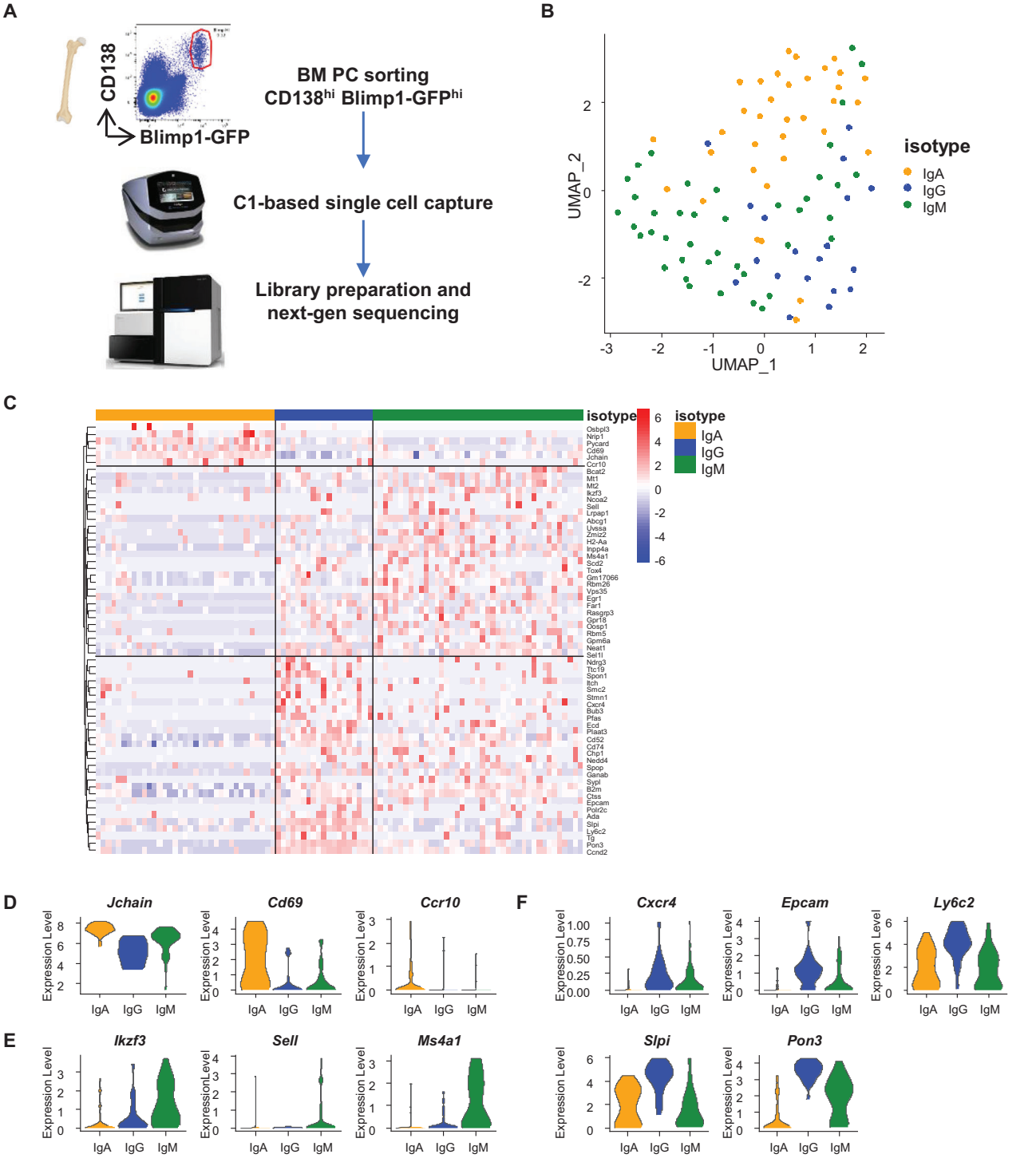

Figure 1. Bone marrow (BM) plasma cells (PCs) segregate based on their isotype. (A) Schematic representation of the single cell RNAseq (scRNAseq) experimental plan. BM PCs (CD138^{hi} Blimp1-GFP^{hi}) were sorted, and single PCs were captured with the C1 technology and imaged for GFP expression. Libraries were then prepared and sequenced. (B) Unsupervised clustering of BM PCs using UMAP. Two independent datasets were pooled. Ig transcripts were excluded from the analysis, and Ig isotypes were subsequently reattributed and colour coded. (C) Supervised heat map of differentially expressed genes between BM PCs based on their isotypes. (D–F) Violin plot showing the expression of selected differentially expressed genes. $n = 95$ cells sorted from six mice in two independent experiments. Differentially expressed genes between isotype-based PC subsets were determined using Wilcox tests with Bonferroni correction.

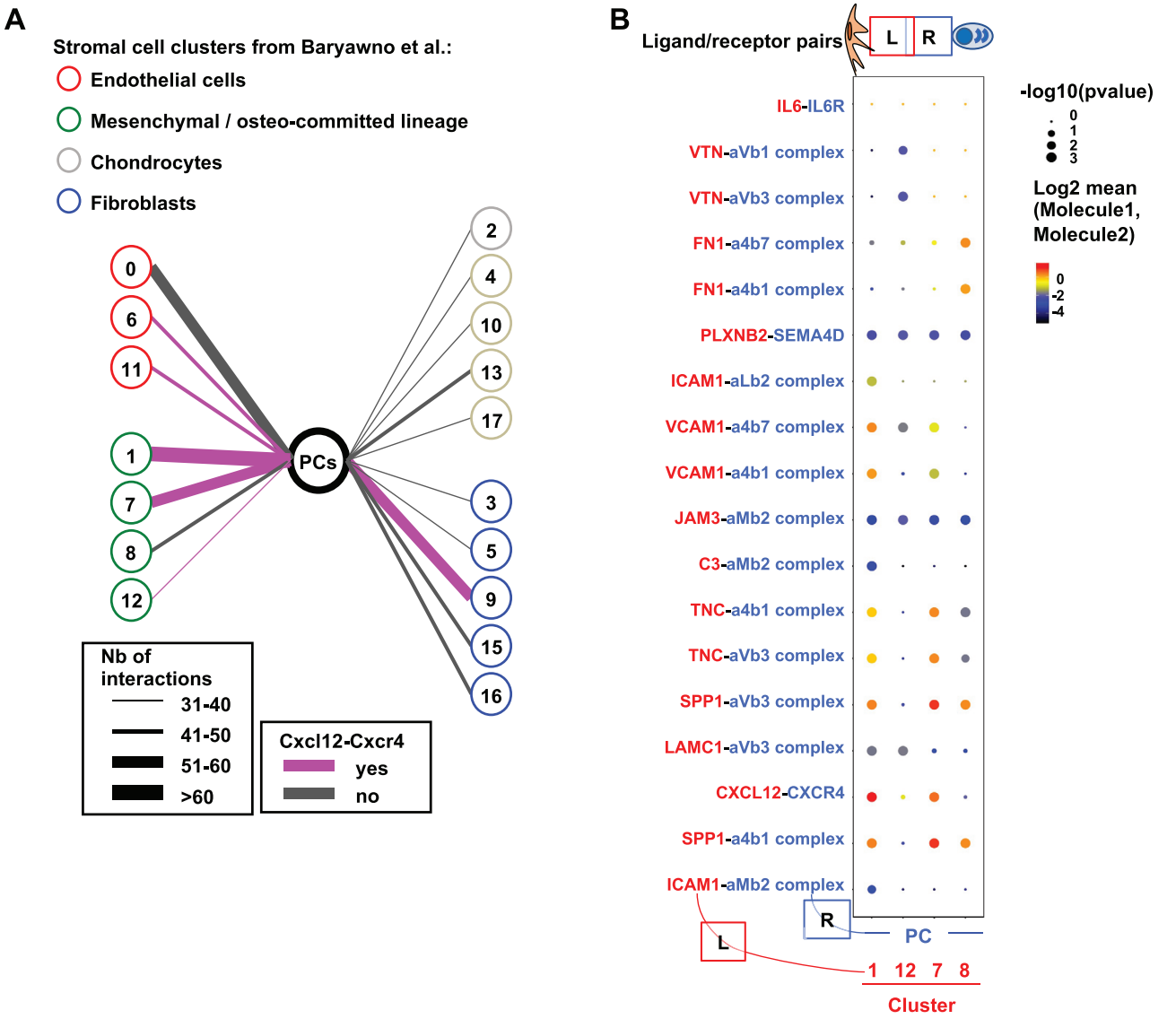

Figure 2. In silico analysis reveals that plasma cells (PCs) are more likely to form interactions with certain clusters of stromal cells. (A) Representation of the number of bi-directional significative interactions identified between PCs and the clusters of stromal cells from Baryawno et al. Stromal cell clusters are numbered and colour-coded. The width of the lines indicates the number of interactions between PCs and a given stromal cell type. The colour of the line indicates whether Cxcl12-Cxcr4 was part of the identified interactions. (B) Balloon plot showing selected interacting ligand–receptor pairs between mesenchymal clusters 1, 12, 7 and 8 and PCs. size = *p*-value, colour = mean expression of the interactants.

dataset used is composed of 17 clusters encompassing 3 subsets of endothelial cells (clusters 0, 6 and 11), 4 clusters of mesenchymal and osteoprogenitor cells (1, 7, 8 and 12), 5 clusters of chondrocytes and their progenitors (2, 4, 10, 13 and 17) and 5 clusters of fibroblasts (3, 5, 9, 15 and 16) (Fig. 2A). Our first analysis revealed a large variation in the number of potential bi-directional interactions between PCs and the different stromal clusters (ranging from 32 to 70). Very few interactions were identified between PCs and chondrocytes and their progenitors, for most of the fibroblast clusters and for Cxcl12-expressing pericytes (cluster 12). The higher numbers of potential interactions were observed between PCs and the LepR$^+$ mesenchymal cells (MSC) (cluster 1), followed by the osteo-committed cells referred to as OLC-1 in [27] (cluster 7), the cluster 9 of fibroblasts and the sinusoidal endothelial cells (cluster 0) (Fig. 2A). Cxcl12-Cxcr4 was one of the identified interactions for clusters 1, 7 and 9 but not for cluster 0 in agreement with the level of expression of *Cxcl12* [27] (Fig. 2A).

We next investigated more precisely the ligand–receptor pairs putatively involved in these cellular interactions and contributing to PC survival within the BM. We first focused on the mesenchymal clusters and observed that most of the interactions identified between PCs and OLC-1 were also observed between PCs and LepR$^+$ MSC, including Cxcl12-Cxcr4 and VCAM1-VLA4 (α4β1 complex) (Fig. 2B). Interestingly, interaction between the integrin LFA1 (αLβ2 complex) and its ligand ICAM1, previously reported to be important for PC maintenance within the BM niches [9], was

only detected with cluster 1. Other potential interactions implicating integrins were observed with cluster 1, including TNC-αVβ3 and ICAM1-αMβ2, suggesting that these integrin pairs may be involved in PC retention in addition to VLA4 and LFA1. Clusters 12 and 8 were forming fewer potential interactions with PCs. However, we observed specific interactions between PCs and these subsets; vitronectin-αVβ1 interaction was specifically found between cluster 12 and PCs, whereas FN1-α4β1 interactions was specific to cluster 8 (Fig. 2B).

Focusing on the interactions between endothelial cells and PCs, we observed a strong implication of PECAM1-CD177, PECAM1-CD38 and Cxcl12-Cxcr4 axes between clusters 6/11 and PCs contrary to the pairs ICAM1-αMβ2, VCAM1-α4β7 and VCAM1-α4β1 that were mainly found between cluster 0 and PCs (Supporting Information Fig. 2A). With fibroblasts, we observed a mostly homogeneous pattern of interactions between the different clusters of fibroblasts and PCs with several interactions involving fibronectin (FN1) and integrins. An exception was cluster 9 that had many more potential interactions than the other fibroblast clusters including Cxcl12-Cxcr4, ICAM1-LFA1, VCAM1-α4β7 and TGFB2-TGFBR1 (Supporting Information Fig. 2B). This cluster of fibroblasts was reported to express several factors involved in HSC and progenitor cell niches, making it functionally similar to the LepR$^+$ mesenchymal cluster 1. Altogether our in silico data provide an unprecedented level of detail in the characterization of PC medullary niches both in terms of the cellular actors potentially involved and of the molecular interactions established.

Plasma cells display an isotype specific interactome with BM stromal cells

Our scRNAseq analysis revealed that several genes associated with cell migration and adhesion were differently expressed depending on PC Ig isotype. As an isotype specific niche was already hinted at for IgG$^+$ PCs [46], we interrogated whether PCs expressing distinct Ig were interacting with different cell types. This new transinteractome analysis revealed that regardless of their isotype, PCs are more likely to form interactions with cluster 1 corresponding to LepR$^+$ MSC (Fig. 3A). For the other potential cellular interactions, however, we detected some Ig isotype specific differences. Fewer potential interactions with sinusoidal endothelial cells (cluster 0), cluster 7 corresponding to OLC-1 mesenchymal cells and fibroblastic cluster 9 were detected for IgA$^+$ PCs compared to IgM$^+$ and IgG$^+$ PCs (Fig. 3A). These results thus suggest that LepR$^+$ mesenchymal cells are likely to constitute the main stromal niche for IgA$^+$, whereas IgG$^+$ and IgM$^+$ PCs could have alternative niches next to OLC-1 and fibroblastic cells.

We next assessed which ligand–receptor pairs were identified among IgA$^+$, IgM$^+$ and IgG$^+$ PCs and the four clusters with whom the most interactions where detected (Clusters 0, 1, 7 and 9). As previously observed when all PCs were analysed together, the set of interactions varies depending on the type of stromal cells (Fig. 2B, Supporting Information Figs. 2 and 3B). Some interactions were equivalent for all PC subsets like, for example ICAM1-LFA1 (αLβ2 complex) (with clusters 0, 1 and 9) or VCAM1-a4b7 complex (with clusters 0, 1 and 7). However, several interactions were different depending on the isotype expressed by the PCs. For example, interactions involving the integrin VLA-5 (α5β1 complex) and its ligands expressed by LepR$^+$ MSC (Cluster 1) and fibroblasts (Cluster 9) or interaction between Plxnb2-Sema4G were statistically significant in IgG$^+$ and IgM$^+$ PCs but not in IgA$^+$ PCs. Interactions involving αMβ2 integrin were statistically significant in IgA$^+$ and IgG$^+$ PCs but not in IgM$^+$ PCs. The Plxnb2-Sema4D interaction was detected with all stromal clusters but was more significant for IgA$^+$ and IgM$^+$ PCs than for IgG$^+$ PCs. The Nrp2-Sema3F and Notch1-Jag1 interactions were only observed for IgG$^+$ PCs with clusters 0, 1 and 7 or cluster 0, respectively. The VCAM1-VLA4 interaction that was previously reported to be important for PC maintenance was identified with clusters 0, 1 and 7 but the mean was always higher for IgM$^+$ PCs (Fig. 3B).

By flow cytometry, we observed differences in the frequency of PCs expressing α4 or β1 chain depending on their isotype (Fig. 3C and Supporting Information Fig. 3). Moreover, VLA4 (α4β1 complex) was more expressed by IgM$^+$ and IgG$^+$ PCs compared to IgA$^+$ PCs (Fig. 3C). VLA4 was previously shown to be critical for survival and retention of PCs within the BM raising the possibility that IgA$^+$ PCs may be less efficiently retained in the BM than IgG$^+$ or IgM$^+$ PCs [8, 14, 15, 47]. Finally, Sema4D was more expressed by IgA$^+$ PCs (Fig. 3D and Supporting Information Fig. 3). Sema4D seems involved in PC generation [48, 49] and was recently shown to be expressed by colon PCs supporting the mucosal origin of these IgA$^+$ PCs [50]. Interestingly, we could not detect Ccr10 interaction with its ligands in our transinteractome analysis despite being included in the list of possible interactions. This may suggest that Ccr10 is not required for IgA$^+$ PC migration and maintenance within the BM and may rather translate the mucosal origin of these cells or be a prerequisite for their migration from the BM to the mucosa. Altogether, these results raise the interesting idea that IgA$^+$ PCs might be more likely than other PC subsets to traffic in and out of the BM towards the mucosa.

Finally, IgG$^+$ PCs seem able to establish more interactions with their microenvironment than the other PCs through interactions via a whole set of integrins, including VLA4 (α4β1), VLA5 (α5β1), LFA1 (αLβ2), Mac1 (αMβ2), the α4β7 integrin and the αVβ3 integrin (Fig. 3E). This observation may support a stronger retention of IgG$^+$ PCs within the BM consistent with their longevity and their critical role for long-term humoral memory.

Concluding remarks

BM long-lived PCs are critical for sustained protection against reinfections and are key for vaccinal protection as for example after tetanus toxin immunization. However, not all vaccine strategies lead to this long-term protection. In this context, improving the quality and longevity of the humoral response, especially in poor responders, by promoting the maintenance of protective PCs of the most relevant isotype would be of major interest. Conversely,

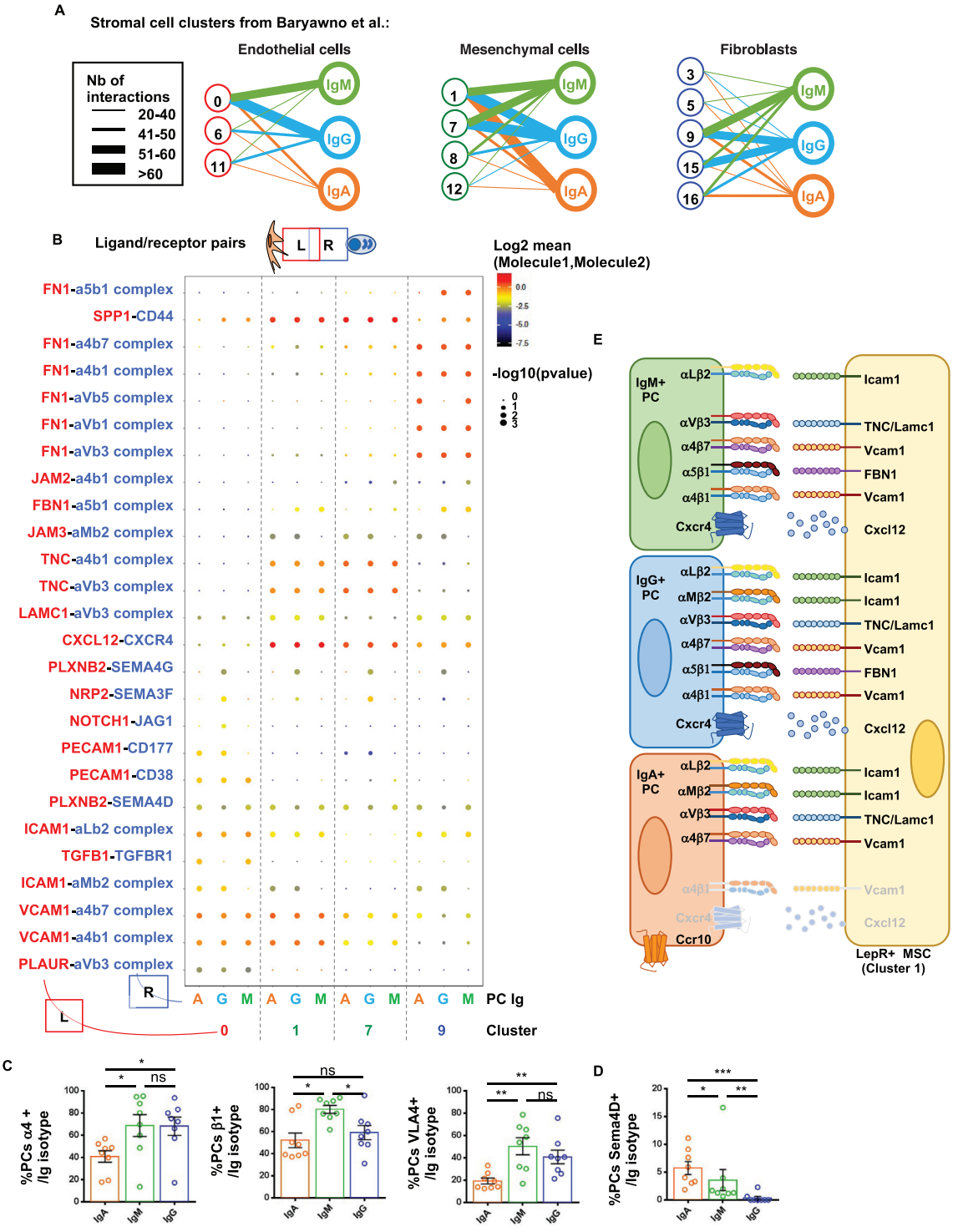

Figure 3. Plasma cells (PCs) display an isotype-specific interactome with bone marrow (BM) stromal cells. (A) Representation of the number of bi-directional significative interactions identified between IgA$^+$, IgG$^+$ and IgM$^+$ and clusters of stromal cells from Baryawno et al. Stromal cell clusters are numbered and colour-coded as in Fig. 2. The width of the lines indicates the number of interactions between PCs and a given stromal cell type. (B) Balloon plot showing selected interacting ligand (L)–receptor (R) pairs between clusters 0, 1, 7 and 9 and PCs segregated on the basis of their isotype. Size = *p*-value; colour = mean expression of the interactants. (C) Flow cytometry-based quantification of α4, β1 and α4β1 complex (VLA4) expressing IgA$^+$, IgM$^+$ and IgG$^+$ PCs. (D) Flow cytometry-based quantification of Sema4D expressing IgA$^+$, IgM$^+$ and IgG$^+$ PCs. (E) Graphical summary of the differential interactions between LepR$^+$ mesenchymal stromal cells (MSC) and PCs segregated based on their isotype. Integrins and chemokine receptors expressed on PC subsets and their ligands expressed by LepR$^+$ MSC are schematically represented. Grey lines and text are used to indicate low expression of a specific receptor/ligand pair. (C and D) $n = 8$ from 3 independent experiments. Mean and SEM are indicated. The *p*-values were determined with the two-tailed Mann–Whitney non-parametric test. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Ns, non-significant *p*-value.

our results open new avenues for the specific targeting of BM PCs based on their isotype. Indeed, therapies oriented on specific interactions depending on the Ig isotype could allow the release of pathogenic PCs from their niches and thus synergize with other treatments in Multiple Myeloma (mostly IgG^+ malignant PCs) or Waldenström macroglobulinemia (mostly IgM^+ malignant PCs). In solid cancers, PC infiltration is associated with good or bad prognostic depending on their isotype [51–54]. Determining whether IgG^+, IgA^+ and IgM^+ PCs depend on distinct niches and interactions in this context as well could prove extremely useful to modulate them specifically.

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Author contributions: Amélie Bonaud designed and performed experiments, analysed data and wrote the manuscript. Pierre Larraufie performed bio-informatics analyses and wrote the manuscript. Mélanie Khamyath performed experiments and analysed data. Ugo Szachnowski, Shaun M. Flint, Claire Toffano-Nioche and Daniel Gautheret performed bio-informatics analyses. Nadège Brunel-Meunier, Annie Munier, François Delhommeau and Tapio Lönnberg assisted with the C1 technology. Karl Balabanian contributed to the project design and to the manuscript redaction. Marion Espéli designed the project, designed and performed experiments, analysed data and wrote the manuscript. All authors had the opportunity to review and edit the manuscript.

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Ethics statement: All mouse experiments were conducted in compliance with the EU guide for the care and use of laboratory animals, which have been reviewed and approved by the relevant institutional review committees (C2EA-26, Animal Care and Use Committee, Villejuif, France and Comité d'éthique Paris Nord No. 121, Paris, France).

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Abbreviations: **Ab**: Antibody · **BM**: Bone marrow · **LepR**: Leptin receptor · **MSC**: Mesenchymal stromal cell · **OLC**: Osteolineage cell · **PC**: plasma cells · **scRNAseq**: single cell RNAseq

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