Review of: "Integrated single-cell (phospho-)protein and RNA detection uncovers phenotypic characteristics of human antibody secreting cells"

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Potential competing interests: I declare no potential competing interests.

In this study, Buijtenen et al. investigated human antibody-secreting cells (ASCs) differentiated from circulating B cells using integrated single-cell RAN-sequencing (scRNA-seq) and single-cell phosphoprotein proteomic analysis. Such a single-cell multi-omic approach taken by the authors' not only provides an important basis to advance our knowledge to differentiated ASCs, but also can facilitate discovery and validation of potential new biomarkers as well as drug targets. The findings are of interest and importance, but there are some concerns to be addressed.

1. The authors performed scRNA-seq on in vitro differentiated IgM/IgG/IgA ASCs, which remain heterogeneous. Therefore, the authors ought to show detailed gene expression profiling, e.g. DEGs, heatmaps, pathway enrichment analysis, etc, of each single cell of IgM, IgG and IgA ASCs for comparison, respectively. The data presented in Figures 2 and 3 are only similar to those of bulk RNA-seq of IgA/IgM/IgG ASCs. Such analyses are important to reveal the differences on the fate programming between IgM, IgG and IgA plasmablasts and plasma cells, respectively, and the potential different layers of plasma cell heterogeneity in gene transcription, and thereby differentiation. In addition, if the memory B cells and/or pre-plasmablasts might exist in lower numbers is also of interest. If this is the case, a hierarchical or pseudotime analysis might help determine fate footprints during differentiation.
2. The proteomic analysis of phosphoproteins in Figure 4 should include all detectable proteins and analyze them at a single-cell level, including PCA, heatmap and pathway analysis between IgM/IgG/IgA plasmablasts/plasma cells. It is then of greater interest to compare the scRNA-seq results with the single-cell proteomic findings between IgM, IgG, and IgA ASCs, respectively, and among three kinds of ASCs, particularly for pathway analysis.
3. Although the authors showed reproducibility of their differentiation methods of ASC production, it might be of interest to compare with other available scRNA-seq analyses of ASCs derived with different differentiation methods to avoid potential intrinsic bias. On the other hand, it is also of importance to compare with circulating IgM/IgG/IgA plasmablasts of vaccinated people at least in discussion.
4. In Figure 5, the authors inferred that mTOR is exclusively activated in IgG plasmablasts in the absence of BCR. The authors might want to discuss the differences in signal transduction of IgM/IgG/IgA plasmablasts/plasma cells in greater detail, and compare with existing knowledge in plasma cell function. For instance, could the authors' findings help resolve the controversial effects of rapamycin on immunization?
5. Did the authors discover potential novel biomarkers or drug targets in this study? If so, please discuss and comment

on them. In addition to analysis and description, new insights are crucial for experimental findings out of cutting-edge technologies.