



**Supplementary figure 4: Defining B cells within fine needle aspirations and B memory enriched PBMC samples, and somatic hypermutation rates of B cell subsets**

- (A) UMAP of all lymph node fine needle aspiration cells (n=70975), with Louvain clusters shown.
- (B) Identification of B cell clusters in (A), based on the expression of *CD19*, *MS4A1* and *CD79A*. Other key lineage markers, as used in Turner et al. (9), are shown: T cells *CD3D*, *CD3E*, *CD3G*, *IL7R*, *CD4*, *CD8A*; NK cells *GZMB*, *GNLY*, *NCAM1*; monocytes *CD14*, *LYZ*, *CST*, *MS4A7*; plasmacytoid dendritic cells *IL3RA*, *CLEC4C* and platelets *PPBP*.
- (C) Identification of the fine needle aspirate B cell sub-clusters in Figure 5A, based on the expression of markers as used in Turner *et al.*: naïve B cells *TCL1A*, *IL4R*, *CCR7*, *IGHM*, *IGHD*; germinal center B cells *BCL6*, *RGS13*, *MEF2B*, *STMN1*, *ELL3*, *SERPINA9*; plasma cells *XBP1*, *IRF4*, *SEC11C*, *FKBP11*, *JCHAIN*, *PRDM1*; resting memory B cells (mem rest) *TNFRSF13B*, *CD27* and *CD24*; activated B cells *TBX21*, *FCRL5*, *ITGAX*, *NKG7*, *ZEB2*, and the lack of *CR2*.
- (D) UMAP of all Bmem enriched (IgD-) PBMC (n=24156 cells), with Louvain clusters shown.
- (E) Annotation of B cell subsets in (C), based on the expression of marker genes as in (B).
- (F) The number of mutations in the CDR or FR regions of B cell receptors for each B cell subset, from circulating B cells at day 28 that share a BCR (Y) with the day 12 germinal center and those that do not (N). The number of cells in each category is shown just above the horizontal axis.
- (G) The replacement:silent ratios for the CDR or FR regions of B cell receptors for each B cell subset, from circulating B cells at day 28 that share a BCR (Y) with the day 12 germinal center and those that do not (N). The number of cells in each category is shown just above the horizontal axis. As in Figure 4, the replacement:silent ratio is calculated as # of replacement mutations / (# of silent mutations + 0.01), to avoid discarding cells with zero silent mutations. The resulting R/S ratio is plotted as a pseudolog. P values from two-tailed Mann-Whitney tests, comparing the indicated cluster against naïve cells, are represented:  $P < 0.0001$ , \*\*\*\*;  $P < 0.001$ , \*\*\*;  $P < 0.01$ , \*\*;  $P < 0.05$ , \*;  $P > 0.05$ , ns). In (C), (D) and (E), the size of the dot represents the percentage of cells within the corresponding cluster expressing the given gene and the color of the dot reflects its scaled normalized expression.

## SessionInfo

```
## R version 3.6.1 (2019-07-05)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: CentOS Linux 7 (Core)
##
## Matrix products: default
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## LAPACK:  /bi/apps/R/3.6.1/lib64/R/lib/libRlapack.so
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## [4] LC_COLLATE=C           LC_MONETARY=C        LC_MESSAGES=C
## [7] LC_PAPER=C              LC_NAME=C            LC_ADDRESS=C
## [10] LC_TELEPHONE=C         LC_MEASUREMENT=C   LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics   grDevices utils     datasets  methods   base
##
## other attached packages:
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## [5] tidyr_1.0.0     tibble_3.0.4    tidyverse_1.3.0 dplyr_1.0.2
## [9] cowplot_1.0.0    ggpubr_0.2.4    magrittr_1.5    ggplot2_3.3.2
## [13] Seurat_3.2.2
##
## loaded via a namespace (and not attached):
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## [7] markdown_1.1          fs_1.3.1          gridtext_0.1.4
## [10] ggtext_0.1.1          rstudioapi_0.10   spatstat.data_1.4-3
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