



Supplementary figure 2: Single cell RNA sequencing quality control

- (A) The number of reads per cell (n=952) cells plotted on a log10 scale for each library, with the 4 experimental conditions (22-36 year old at day 0; 22-36 year old at day 42; 67-86 year old at day 0; 67-86 year old at day 42) shown separately.
- (B) The number of detected transcripts per cell plotted as in (A) plotted on a log10 scale for each library, with the 4 experimental conditions shown separately.
- (C) The percentage of detected mitochondrial transcripts per cell plotted as in (A).
- (D) The percentage of reads per cell that are attributable to the 50 most highly expressed features. Cells with high values represent low complexity libraries.
- (E) UMAP plot of transcriptomes of single HA-binding B cells, after QC, n=789 cells. The first 50 principal components were calculated with features selected by the top 25% of variance, after normalisation for library size using deconvolution. For (F)-(J) UMAP embedding as in (E), with the following:
 - (F) Expression level of *PRDM1*
 - (G) Expression level of *IRF4*
 - (H) Expression level of *XBP1*
 - (I) logicle fluorescence intensity of surface CD38 protein.
 - (J) logicle fluorescence intensity of surface CD20 protein.
- (K) The number of cells, that pass transcriptomic QC, and are not plasma cells (E-J) for 22-36yo and 67-86yo individuals at day 0 and day 42, 771 cells. The median and median absolute deviations, and *P* values from 2 tailed Mann-Whitney tests are shown. Each individual is identifiable by their plotted shape and its colour. There is 1 22-36yo individual (purple circle), who only provides cells only at day 42 and one 67-86yo individual (pink triangle) who only provides cells at day 42.
- (L) The percentage of each condition's cells, after transcriptomic QC, that each individual contributes is plotted.

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
## BLAS: /bi/apps/R/3.6.1/lib64/R/lib/libRblas.so
## LAPACK: /bi/apps/R/3.6.1/lib64/R/lib/libRlapack.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8 LC_NUMERIC=C LC_TIME=C
##  [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] parallel stats4 stats graphics grDevices utils datasets
## [8] methods base
##
## other attached packages:
##  [1] ggpubr_0.2.4 dplyr_1.0.2
##  [3] MultiAssayExperiment_1.12.1 magrittr_1.5
##  [5] scan_1.14.5 scatter_1.14.5
##  [7] ggplot2_3.3.2 SingleCellExperiment_1.8.0
##  [9] SummarizedExperiment_1.16.0 DelayedArray_0.12.0
## [11] BiocParallel_1.20.0 matrixStats_0.55.0
## [13] Biobase_2.46.0 GenomicRanges_1.38.0
## [15] GenomeInfoDb_1.22.0 IRanges_2.20.1
## [17] S4Vectors_0.24.1 BiocGenerics_0.32.0
##
## loaded via a namespace (and not attached):
##  [1] viridis_0.5.1 tidyr_1.0.0 edgeR_3.28.0
##  [4] BiocSingular_1.2.0 viridisLite_0.3.0 DelayedMatrixStats_1.8.0
##  [7] RcppParallel_4.4.4 statmod_1.4.32 dqrng_0.2.1
## [10] GenomeInfoDbData_1.2.2 vipor_0.4.5 yaml_2.2.0
## [13] pillar_1.4.7 lattice_0.20-38 glue_1.4.2
## [16] limma_3.42.0 digest_0.6.23 ggsignif_0.6.0
## [19] RColorBrewer_1.1-2 XVector_0.26.0 colorspace_1.4-1
## [22] cowplot_1.0.0 htmltools_0.4.0 Matrix_1.2-17
## [25] plyr_1.8.5 pkgconfig_2.0.3 zlibbioc_1.32.0
## [28] purrr_0.3.3 scales_1.1.0 RSpectra_0.16-0
## [31] tibble_3.0.4 farver_2.0.1 generics_0.0.2
## [34] ellipsis_0.3.0 withr_2.1.2 crayon_1.3.4
## [37] evaluate_0.14 beeswarm_0.2.3 tools_3.6.1
## [40] lifecycle_0.2.0 stringr_1.4.0 munsell_0.5.0
## [43] locfit_1.5-9.1 irlba_2.3.3 compiler_3.6.1
## [46] rsvd_1.0.2 rlang_0.4.10 grid_3.6.1
## [49] RCurl_1.95-4.12 BiocNeighbors_1.4.1 igraph_1.2.4.2
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## [55] gtable_0.3.0 reshape2_1.4.3 R6_2.4.1
## [58] gridExtra_2.3 knitr_1.26 uwot_0.1.5
## [61] stringi_1.4.3 ggbeeswarm_0.6.0 Rcpp_1.0.3
## [64] vctrs_0.3.6 tidyselect_1.1.0 xfun_0.11
```