SCE assembly for the 2016-17 cohort

EJC

25/06/2020, based on code from May 2019

Setup

The Babraham compute cluster does not contain a global tex installation, so a local tex is added to $PATH to allow knitting to pdf.

```
Sys.setenv(PATH=paste(Sys.getenv("PATH"),
                      "/bi/home/carre/texlive/2017/bin/x86_64-linux/",sep=":"))
```

Load QC-pass SCE

```
load(file = "data/SCE_QC_pass.RData")
```

Normalise by deconvolution, PCA, UMAP, find PB cluster and remove

For convenience PCA + UMAP on the final SCE is re-run (to avoid needing to re-run it for each figure.Rmd).

```
library(SingleCellExperiment)
library(scran)
library(scater)

# Normalisation by deconvolution

set.seed(100)
clust <- quickCluster(sce)
# table(clust)
deconv.sf <- calculateSumFactors(sce, cluster = clust)
# summary(deconv.sf)

sce <- computeSumFactors(sce, cluster = clust, min.mean = 0.1)
sce <- logNormCounts(sce)

# Select 25% of genes with largest variance
dec <- modelGeneVar(sce)
hvg <- getTopHVGs(dec, prop = 0.25)
```

```r
set.seed(10000)
sce <- runPCA(sce, ncomponents = 50, subset_row = hvg)
set.seed(1e+05)
sce <- runUMAP(sce, dimred = "PCA", external_neighbors = TRUE)

# Clustering.
set.seed(1e+06)
g <- buildSNNGraph(sce, use.dimred = "PCA")
set.seed(1e+07)
sce$clusters <- factor(igraph::cluster_louvain(g)$membership)


# So there is always 1 cluster whose row has 9 zeros in Let's
# select that programmatically.
cluster.selector <- table(Cluster = sce$clusters, Batch = sce$library)
cluster.to.discard <- c(1:nlevels(sce$clusters))[apply(cluster.selector,
    1, function(x) sum(x == 0)) == 9]

# Discard the PB cluster, then re-do analysis: The PB cluster
# is so different, that % hvg or # PCs does not influence
# their clustering apart
sce.old <- sce
```

```r
for(p in c(0.1,0.15, 0.2, 0.25, 0.3)){
  load(file = "data/SCE_QC_pass.RData")
sce <- sce[, ! sce.old$clusters == cluster.to.discard]

set.seed(100)
clust <- quickCluster(sce)
# table(clust)
deconv.sf <- calculateSumFactors(sce, cluster=clust)
# summary(deconv.sf)

sce <- computeSumFactors(sce, cluster=clust, min.mean=0.1)
sce <- logNormCounts(sce)

# Select 25% of genes with largest variance
dec <- modelGeneVar(sce)

hvg <- getTopHVGs(dec, prop=p)

for(npcs in c(15, 20, 25, 30, 35, 40, 45, 50, 55, 60)){

set.seed(10000)
  sce <- runPCA(sce, ncomponents=npcs, subset_row=hvg)
set.seed(100000)
sce <- runUMAP(sce, dimred = 'PCA', external_neighbors=TRUE)

# Clustering.
set.seed(1000000)
g <- buildSNNGraph(sce, use.dimred = 'PCA')
set.seed(10000000)
sce$clusters <- factor(igraph::cluster_louvain(g)$membership)
```

```r
library(ggpubr)

plots <- cowplot::plot_grid(
plotUMAP(sce, colour_by="clusters") +
     theme_pubr(legend = "right") +
     guides(fill = guide_legend(title = "Louvain\ncluster",
                                title.position = "top")) +
     theme(legend.key.size = unit(0, 'lines'),
           legend.margin = margin(0,0,0,0, 'lines'),
           aspect.ratio = 1) + rotate_x_text() +
  ggtitle(paste("HVG", p, "%;", npcs, "PCAs")),

cowplot::plot_grid(
  # UMAP both day 0 and day 42
  # Colours from index flow IgD
  plotUMAP(sce, colour_by="IgD.BUV737") +
    viridis::scale_fill_viridis(option="inferno") +
    labs(fill = "Surface\nIgD") +
    theme_pubr() +
    theme(legend.position = "right",
          axis.ticks = element_blank(),
          axis.text = element_blank(),
          legend.key.width = unit(6,"points"),
          legend.key.height = unit(12,"points"),
          legend.margin = margin(0,0,0,0, 'lines'),
          aspect.ratio = 1),
  plotUMAP(sce, colour_by="CD27.BV711") +
    viridis::scale_fill_viridis(option="inferno") +
     labs(fill = "Surface\nCD27") +
     theme_pubr() +
     theme(legend.position = "right",
           axis.ticks = element_blank(),
           axis.text = element_blank(),
           legend.key.width = unit(6,"points"),
           legend.key.height = unit(12,"points"),
           legend.margin = margin(0,0,0,0, 'lines'),
           aspect.ratio = 1),

  # UMAP both day 0 and day 42
  # Colours from index flow CD21
  plotUMAP(sce, colour_by="CD21.PE.cy7") +
    viridis::scale_fill_viridis(option="inferno") +
    labs(fill = "Surface\nCD21") +
    theme_pubr() +
    theme(legend.position = "right",
          axis.ticks = element_blank(),
          axis.text = element_blank(),
          legend.key.width = unit(6,"points"),
          legend.key.height = unit(12,"points"),
          legend.margin = margin(0,0,0,0, 'lines'),
          aspect.ratio = 1),

  # UMAP both day 0 and day 42
```

```r
  # Colours from index flow CD28
  plotUMAP(sce, colour_by="CD38.BV421") +
    viridis::scale_fill_viridis(option="inferno") +
    labs(fill = "Surface\nCD38") +
    theme_pubr() +
    theme(legend.position = "right",
          axis.ticks = element_blank(),
          axis.text = element_blank(),
          legend.key.width = unit(6,"points"),
          legend.key.height = unit(12,"points"),
          legend.margin = margin(0,0,0,0, 'lines'),
          aspect.ratio = 1),

  ncol = 2),
ncol = 2)

print(plots)
}}
```

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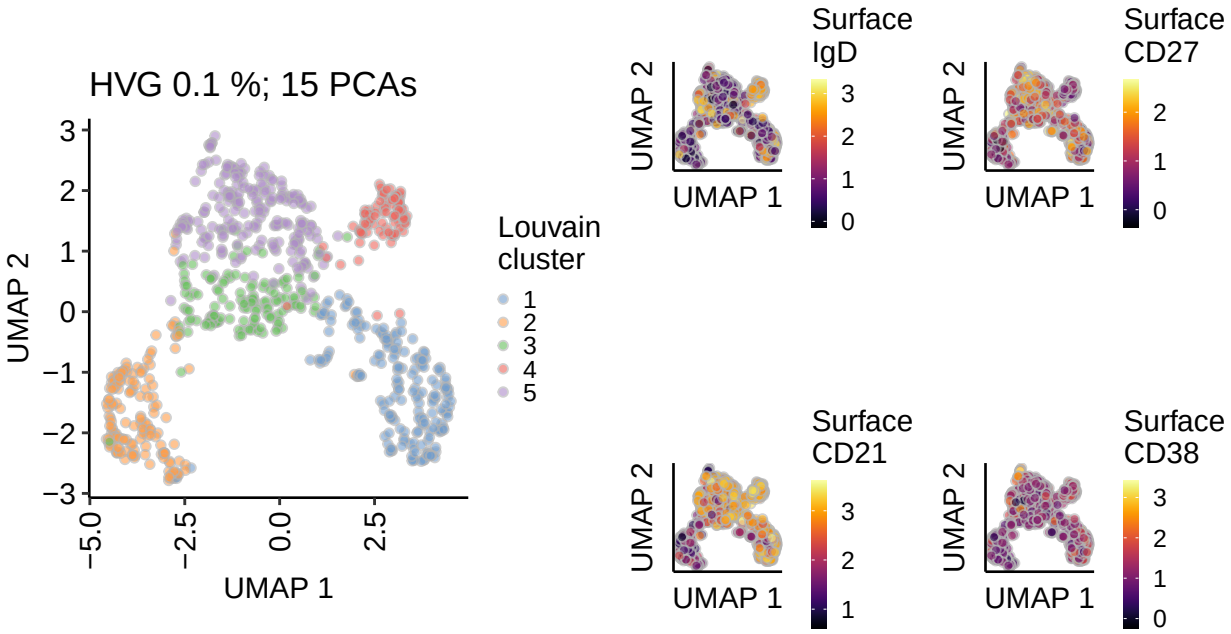


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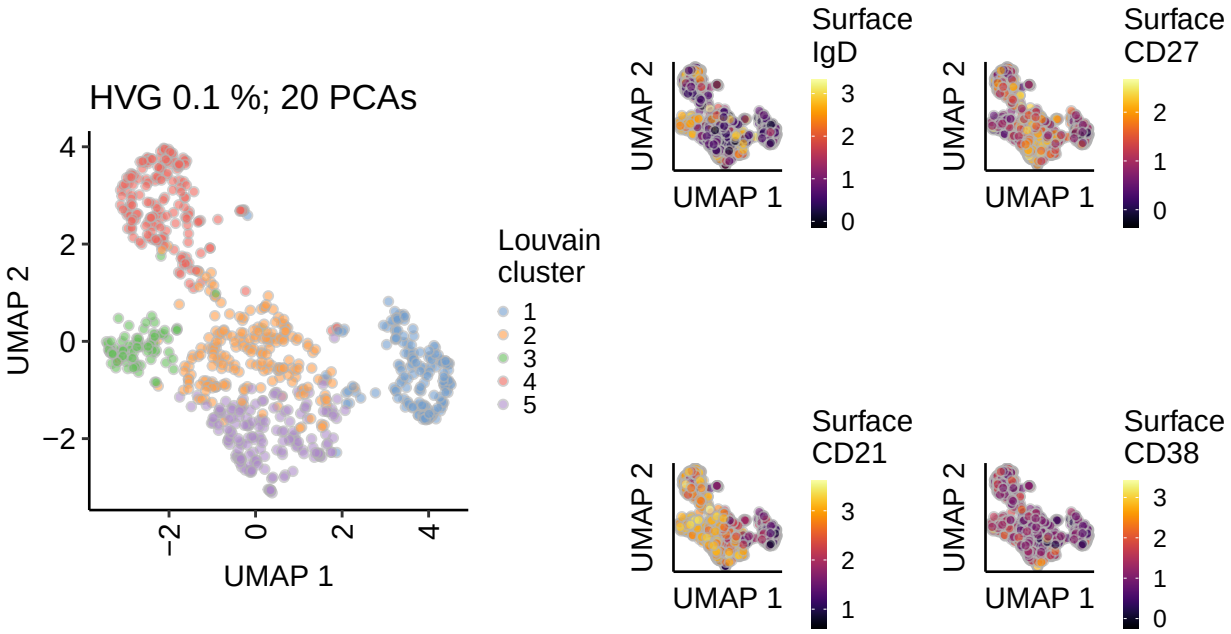


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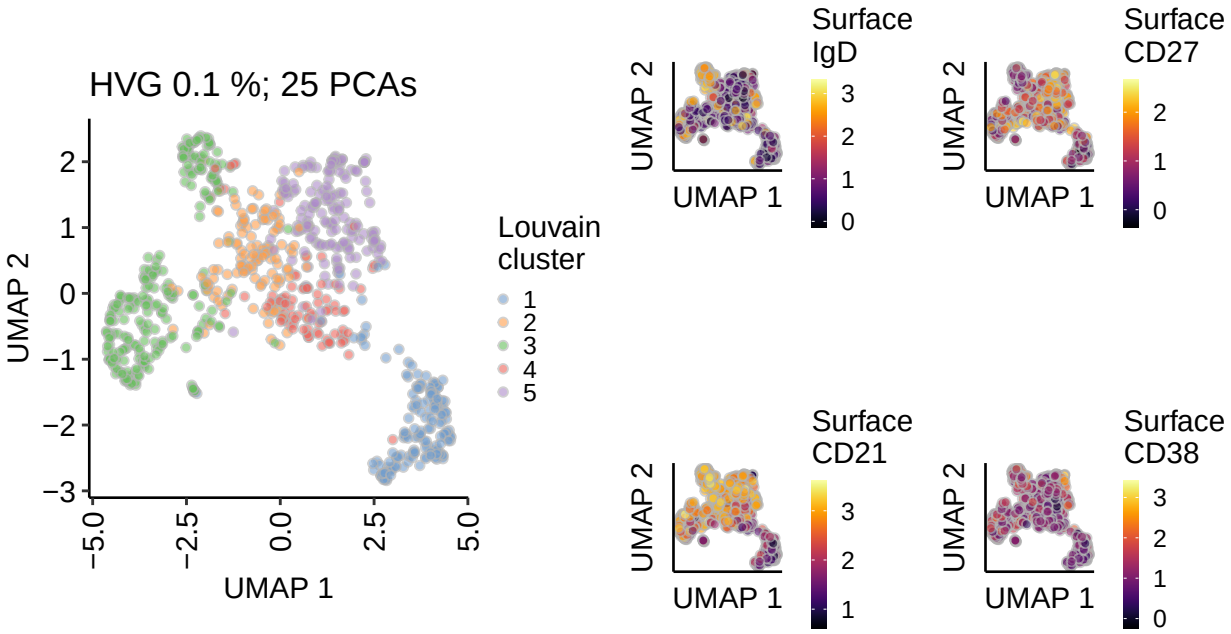


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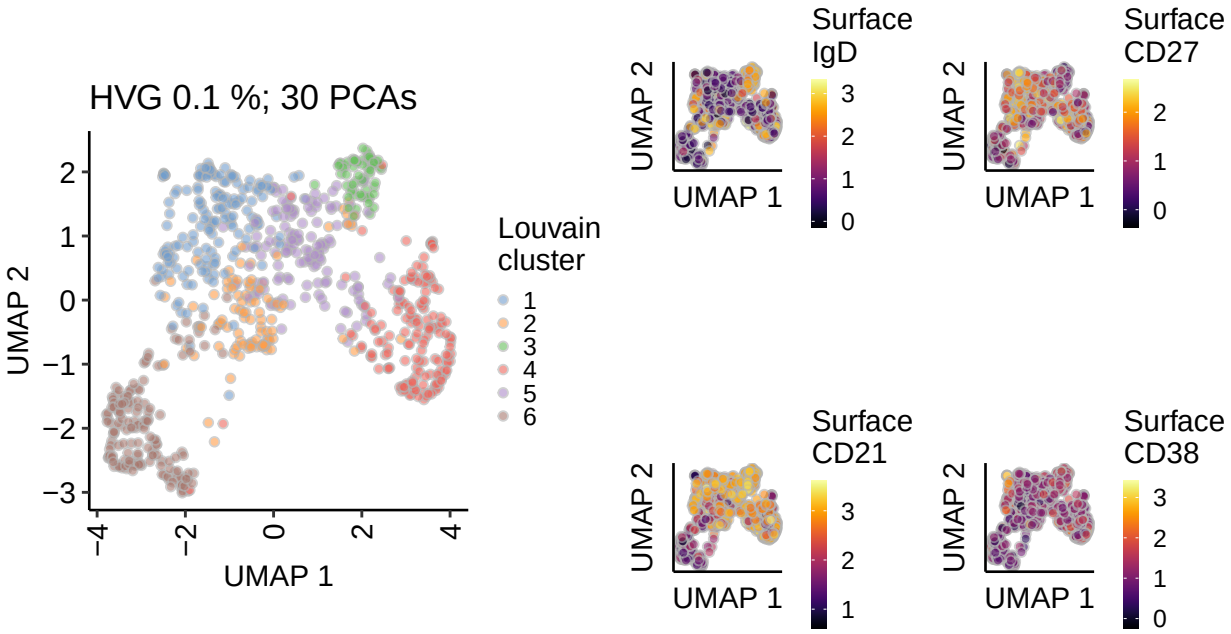


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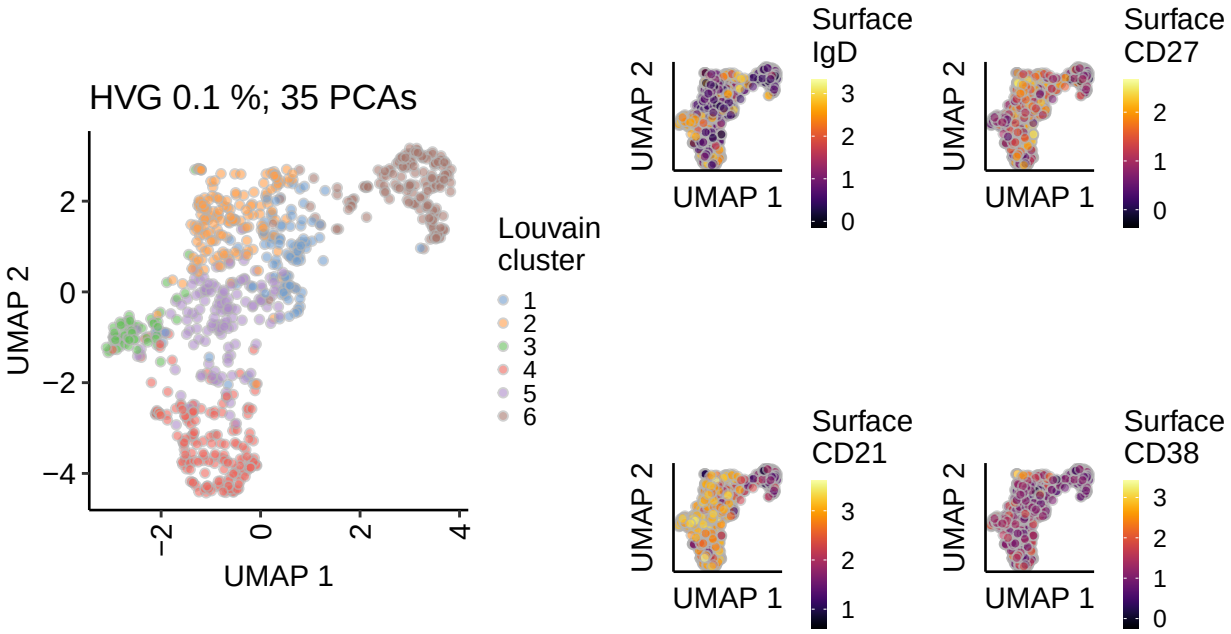


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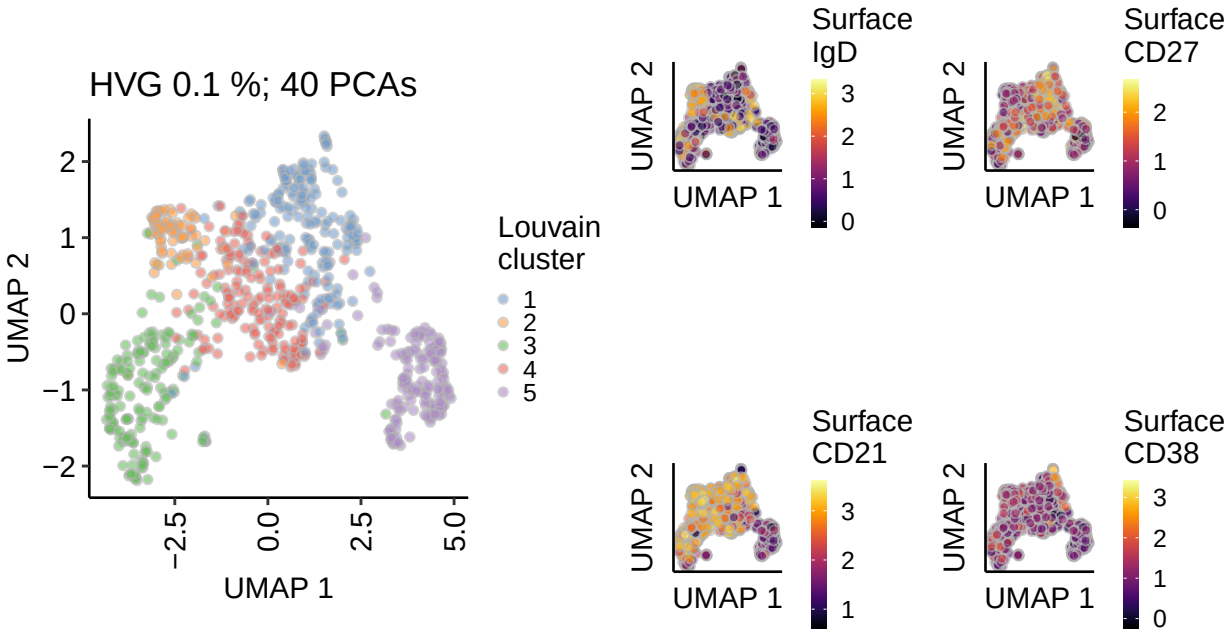


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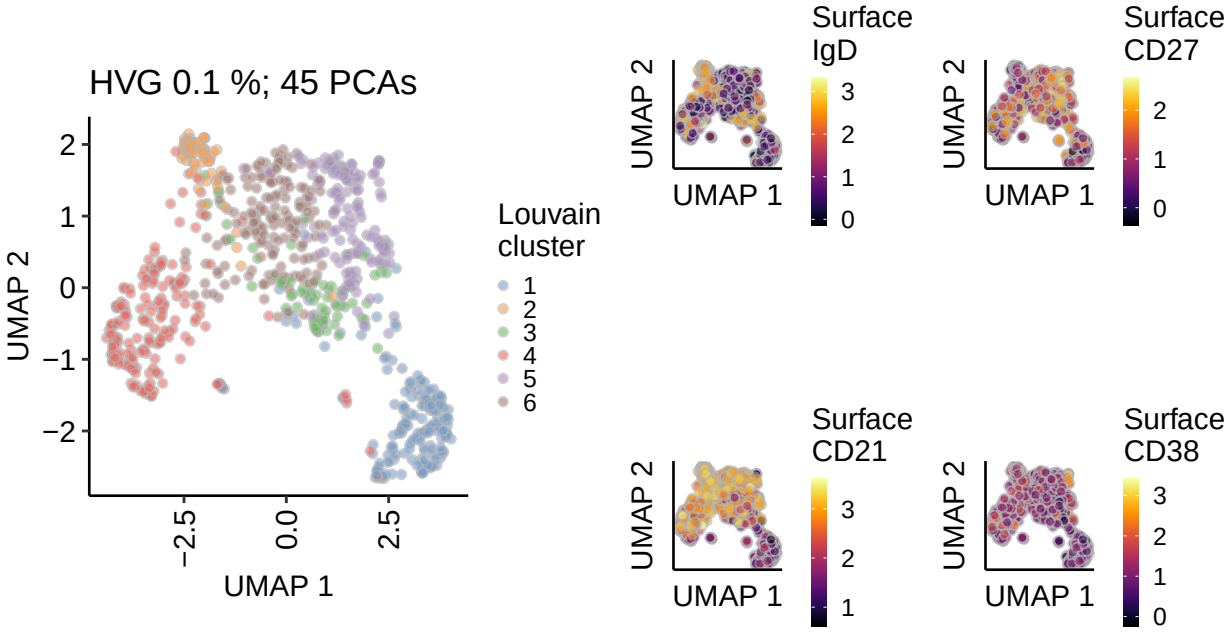


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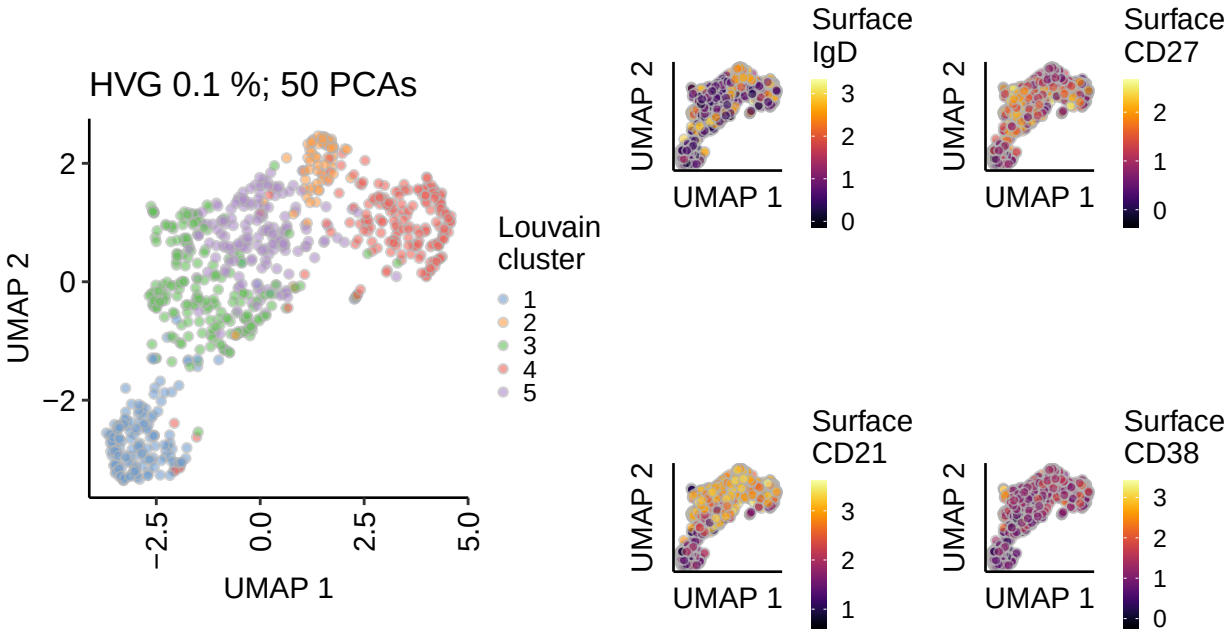


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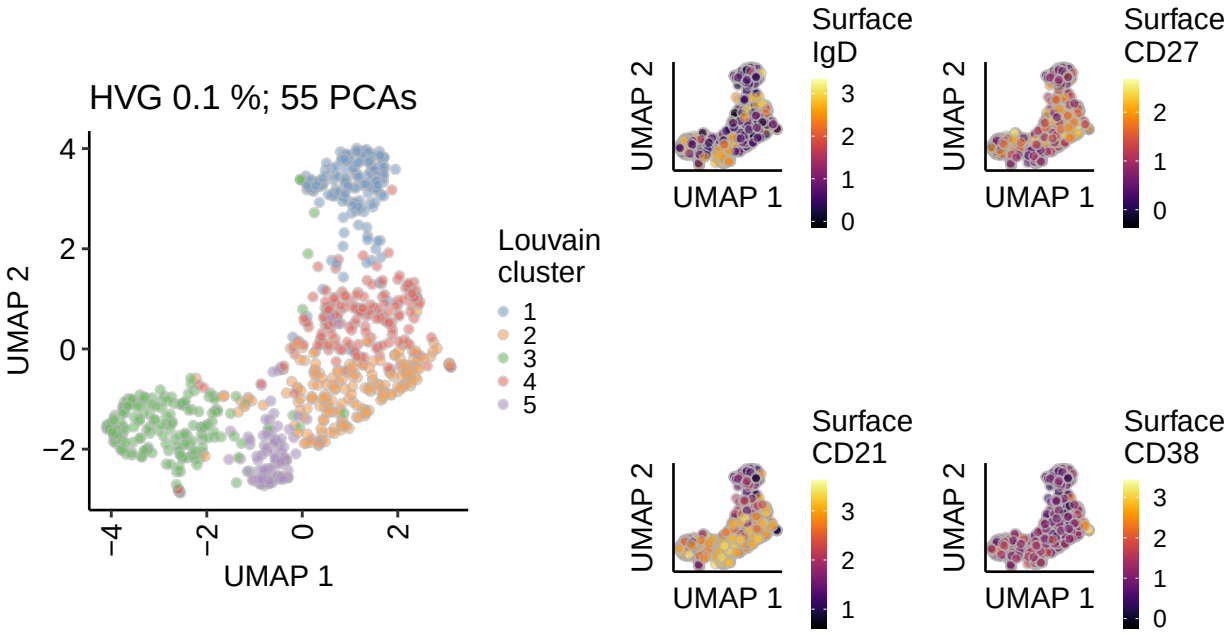


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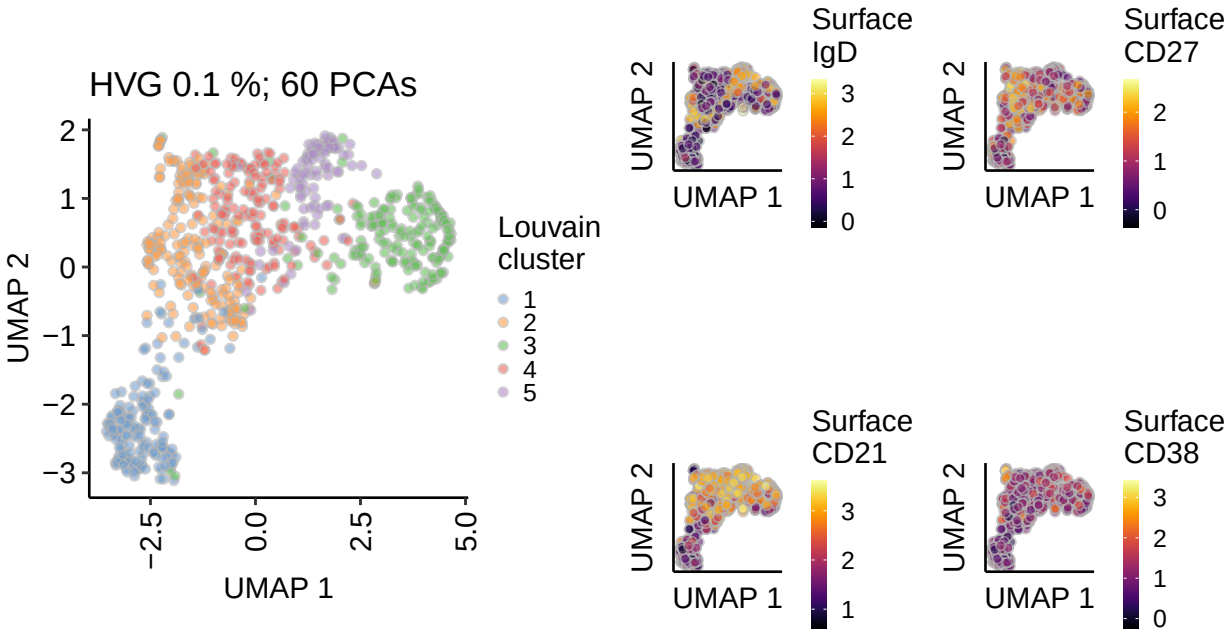


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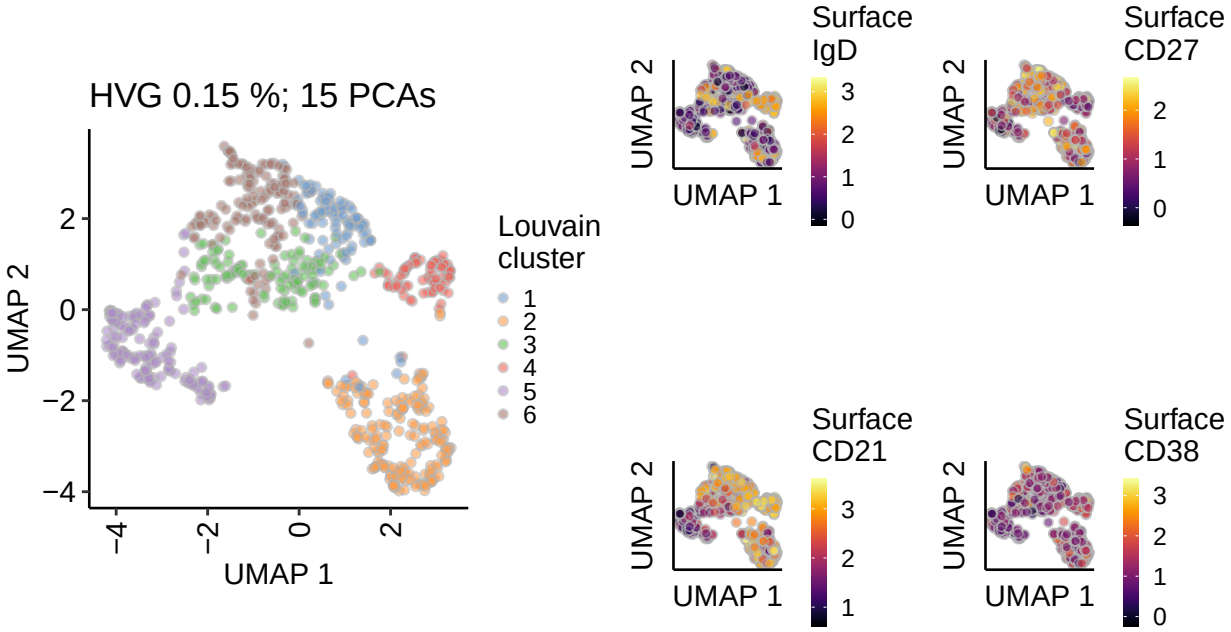


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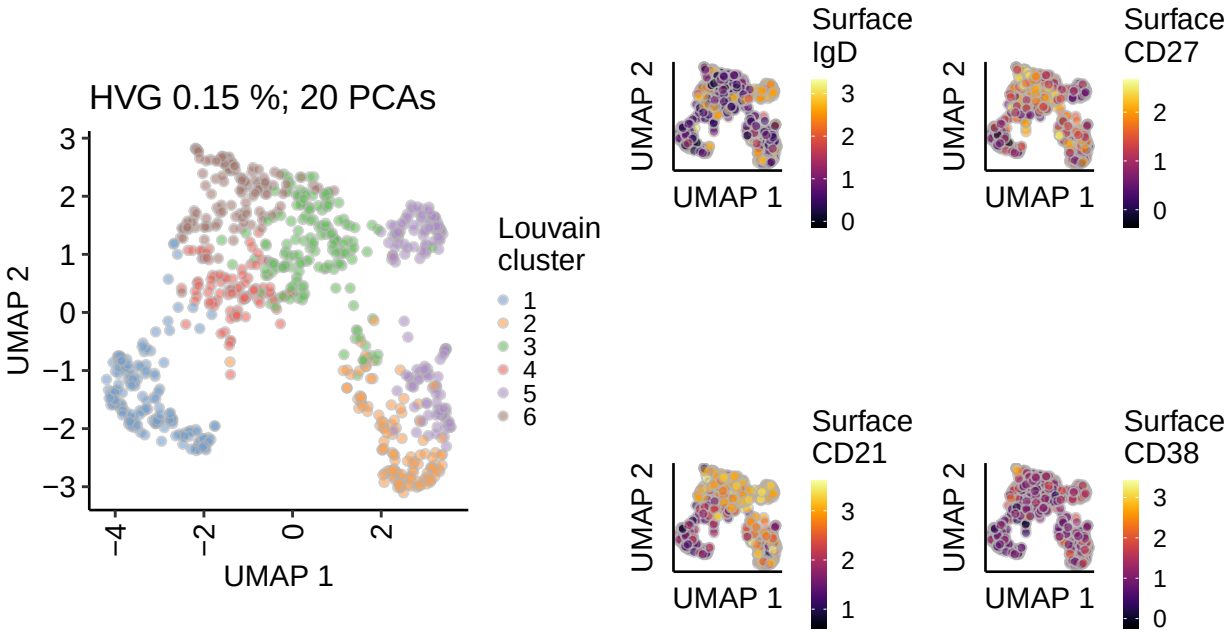


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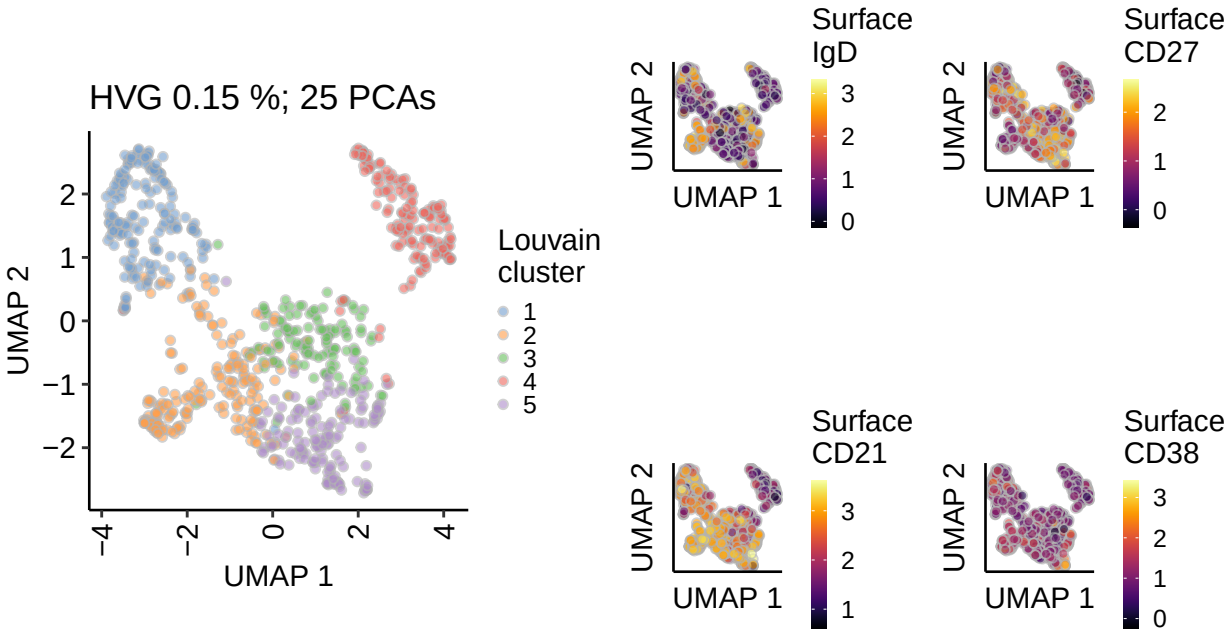


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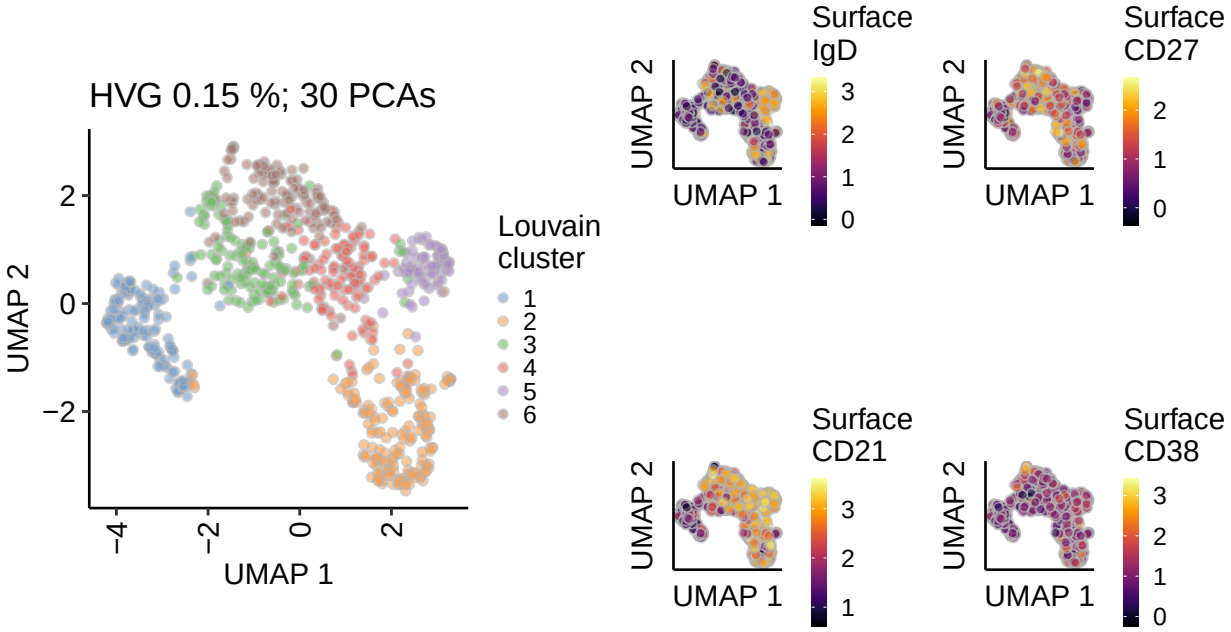


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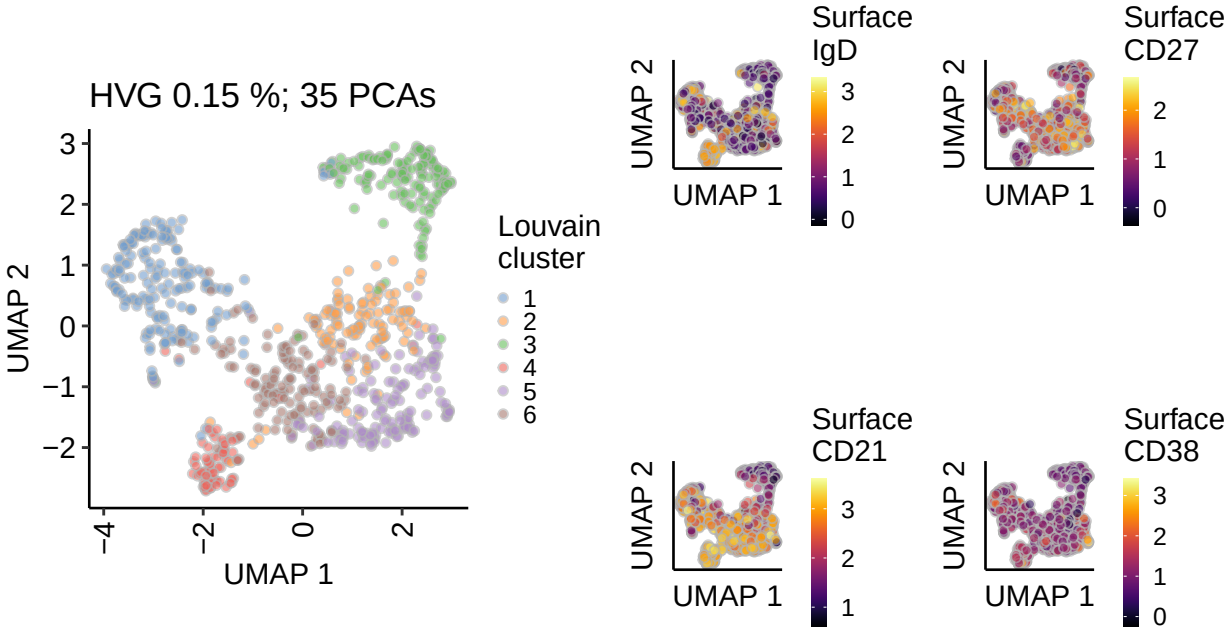


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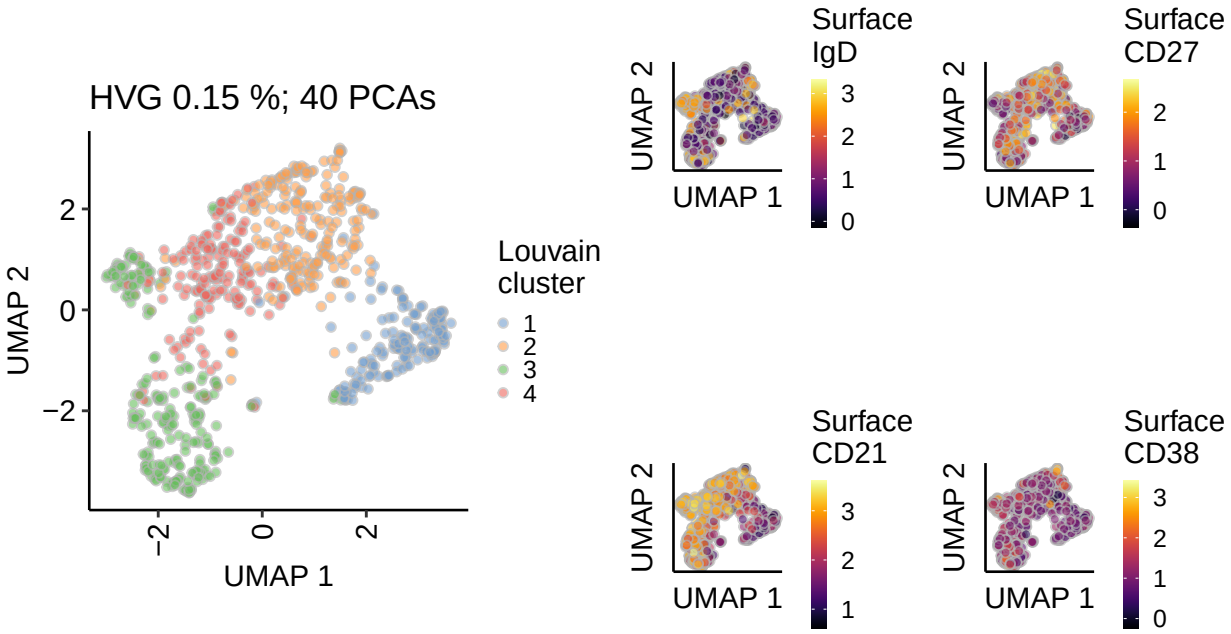


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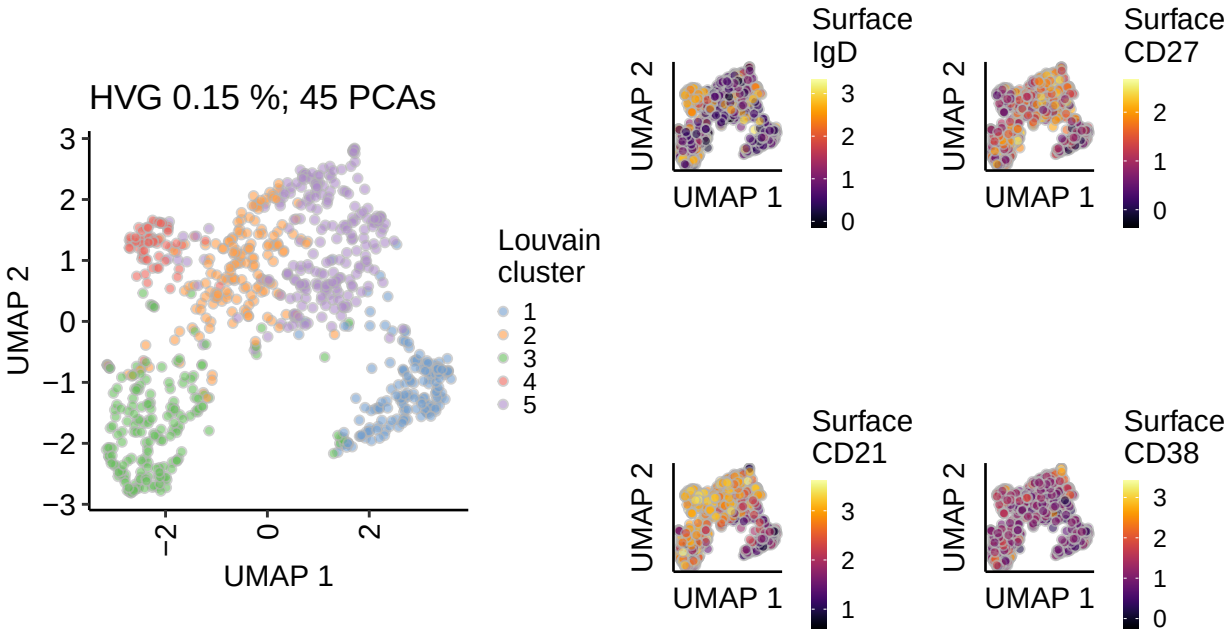


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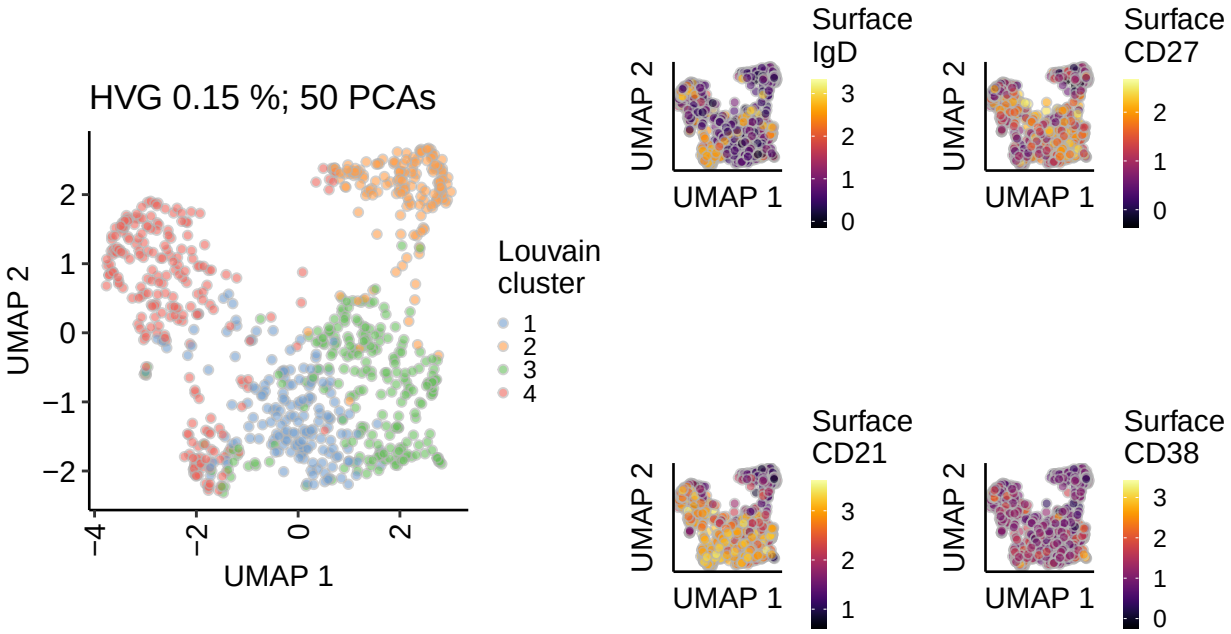


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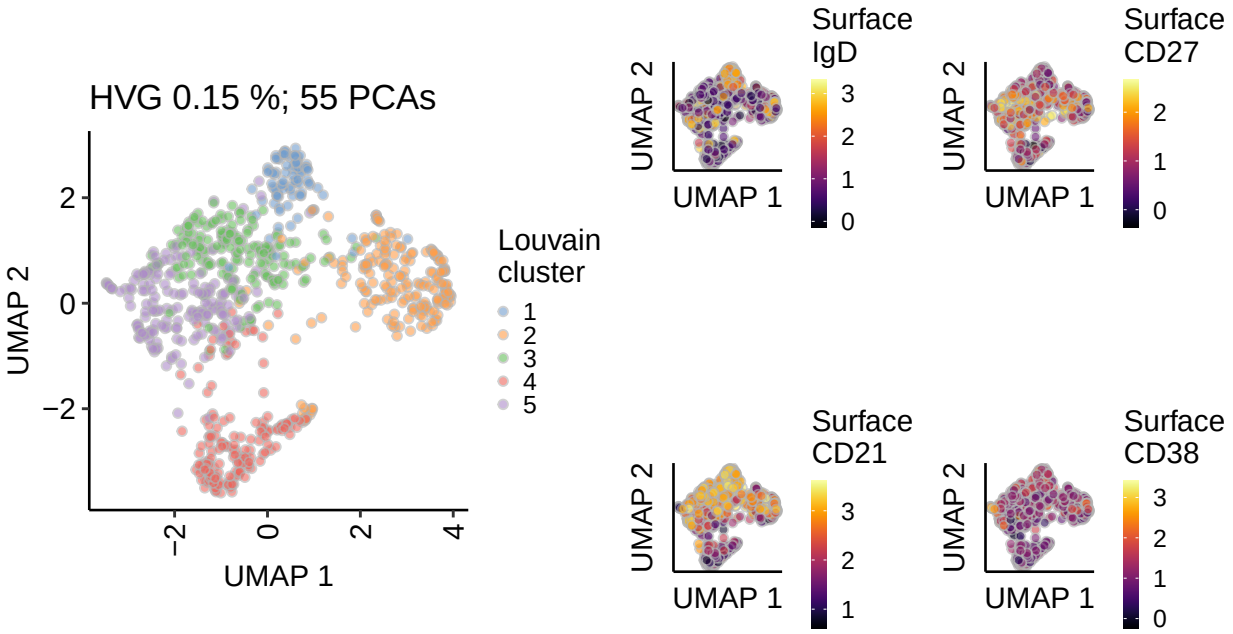


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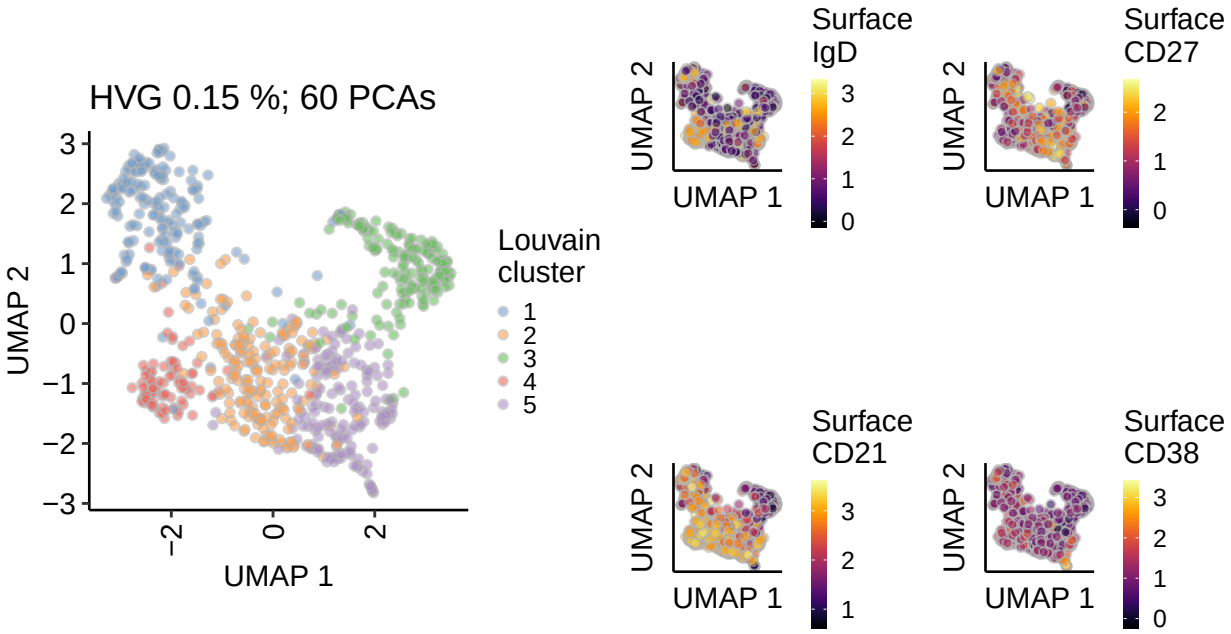


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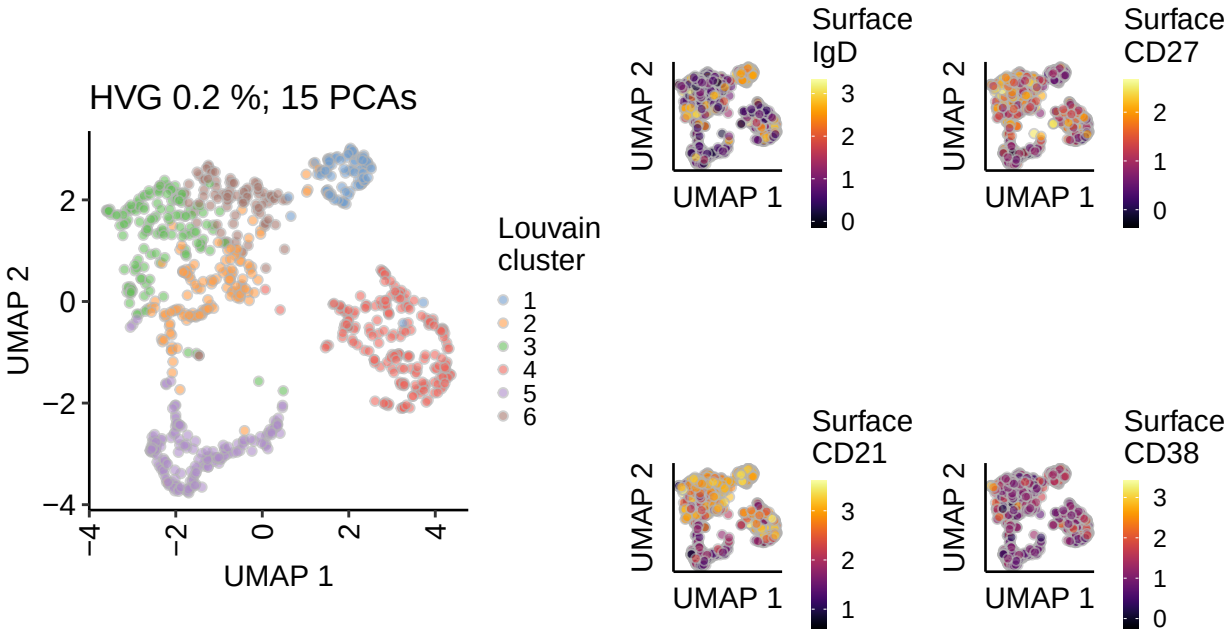


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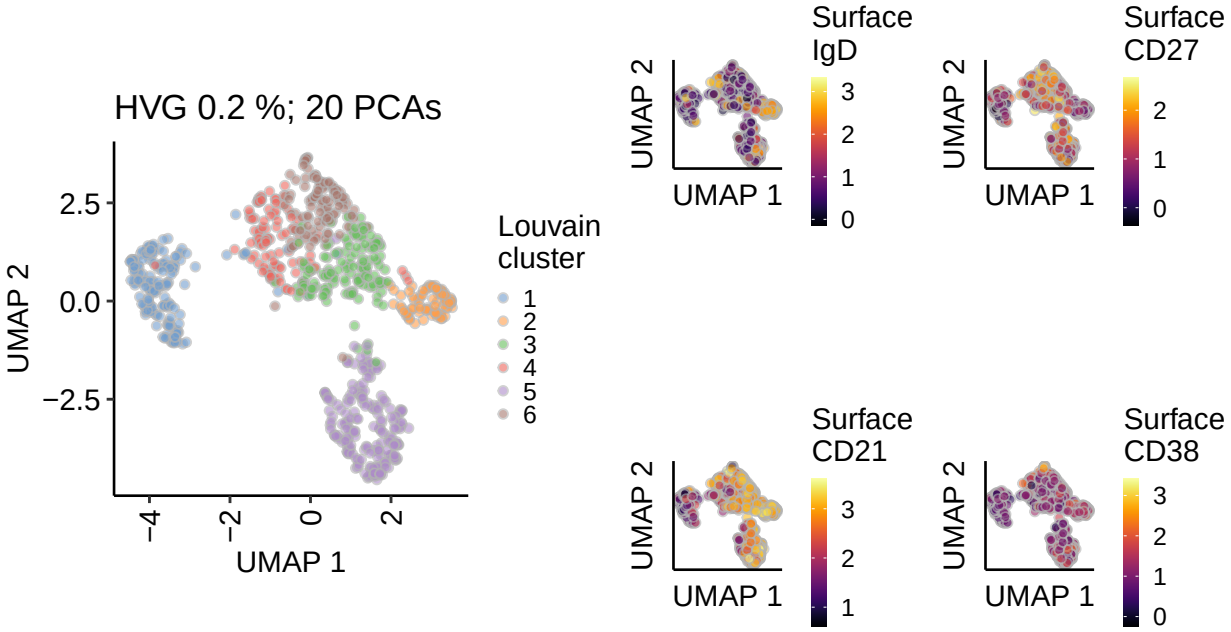


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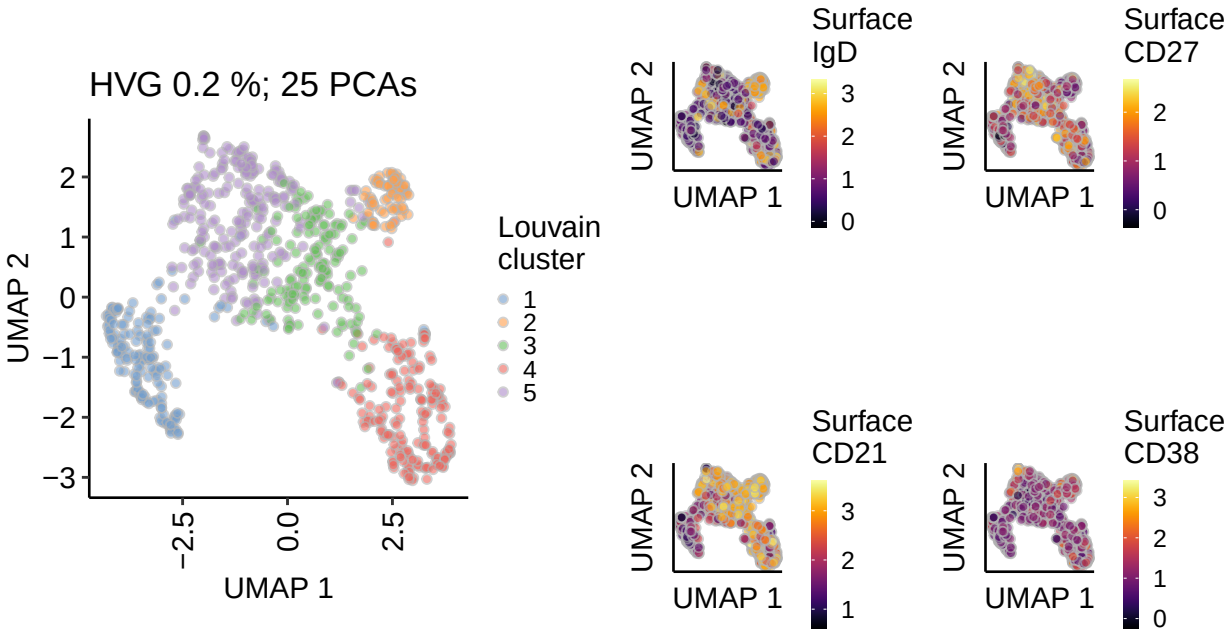


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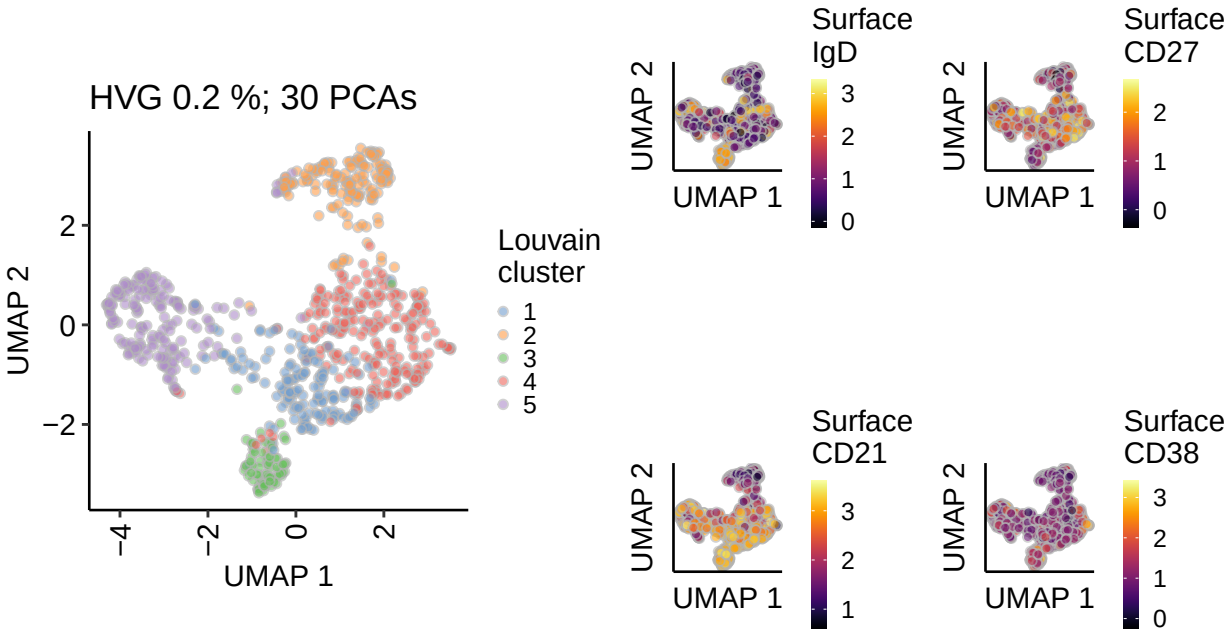


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## Scale for 'fill' is already present. Adding another scale for 'fill', which
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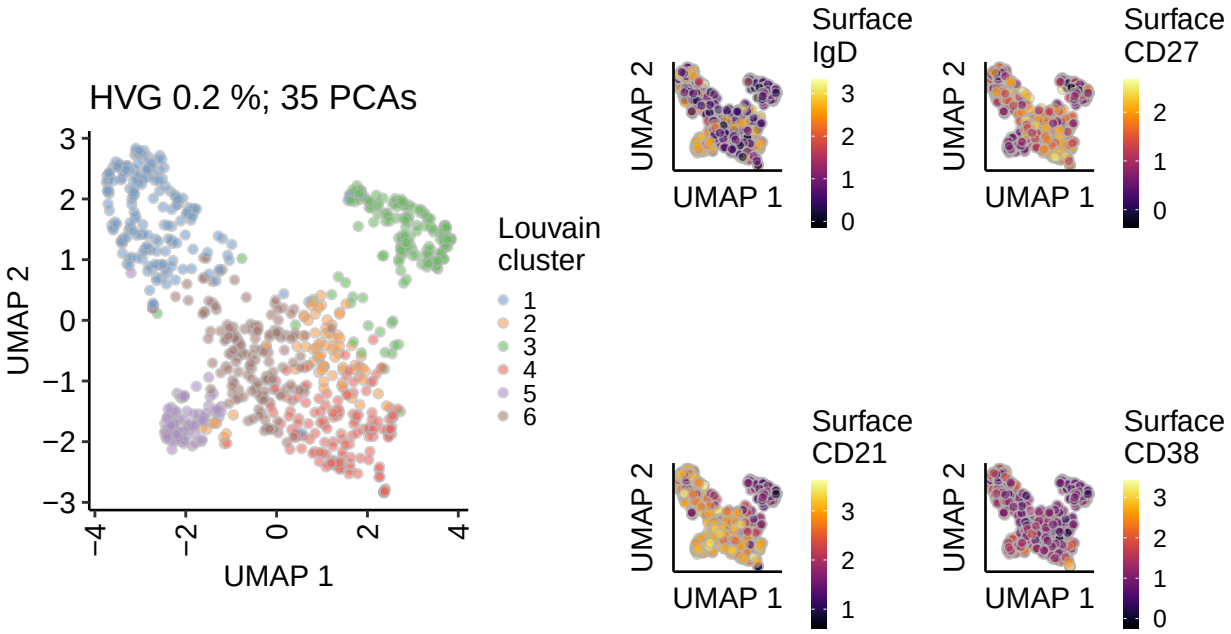


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## Scale for 'fill' is already present. Adding another scale for 'fill', which
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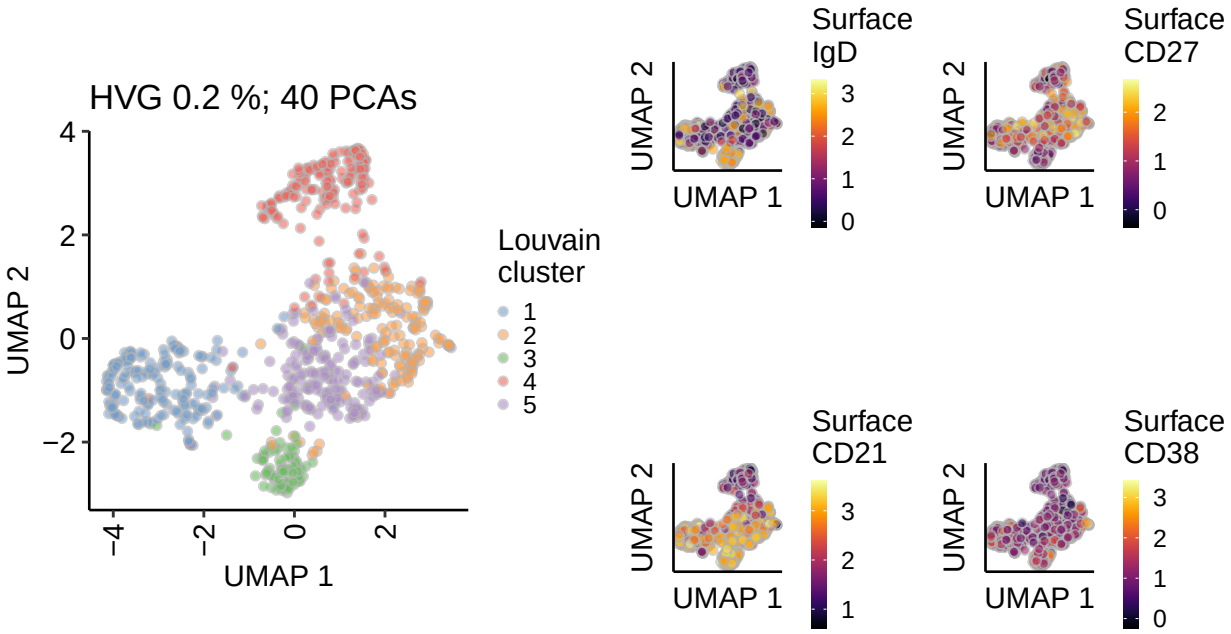


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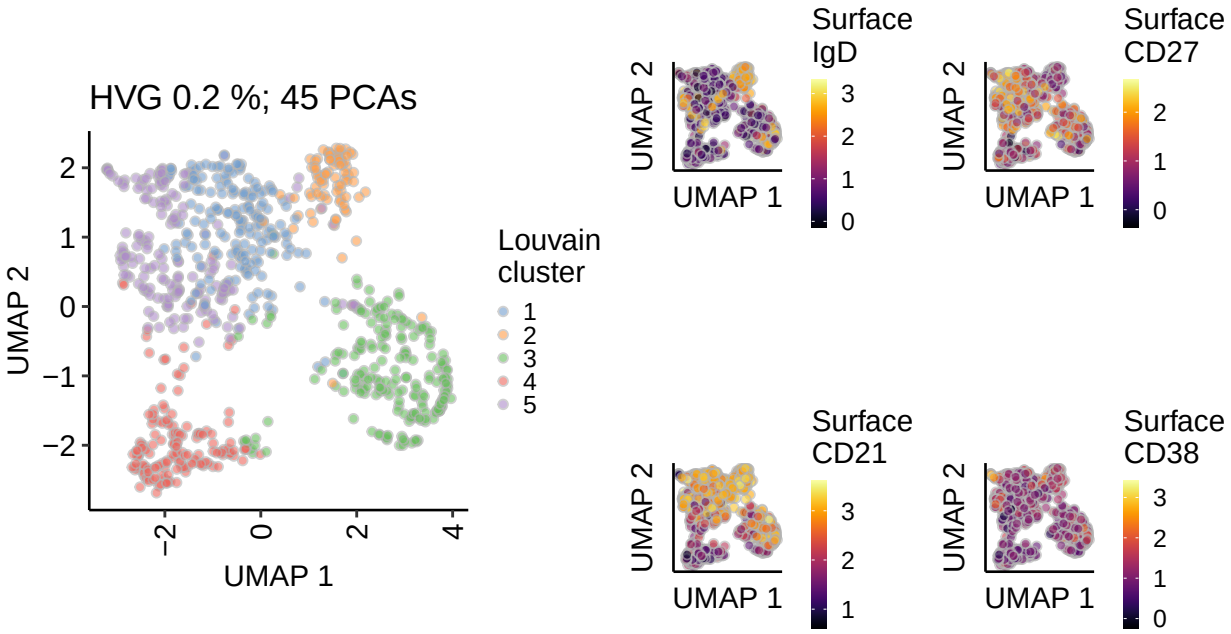


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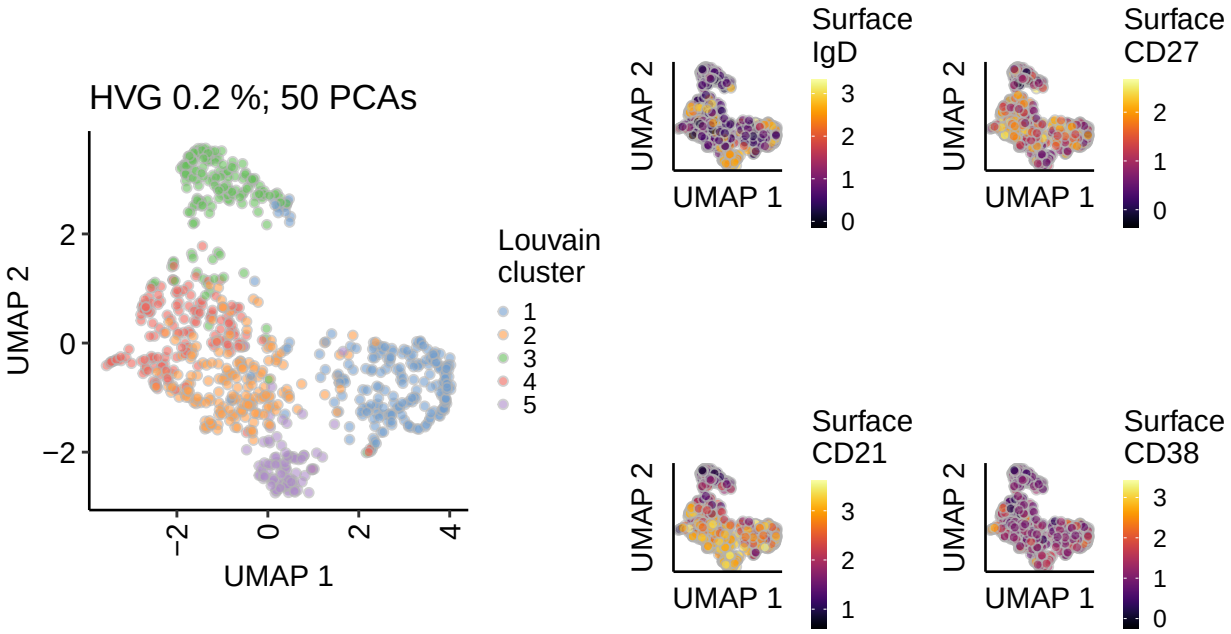


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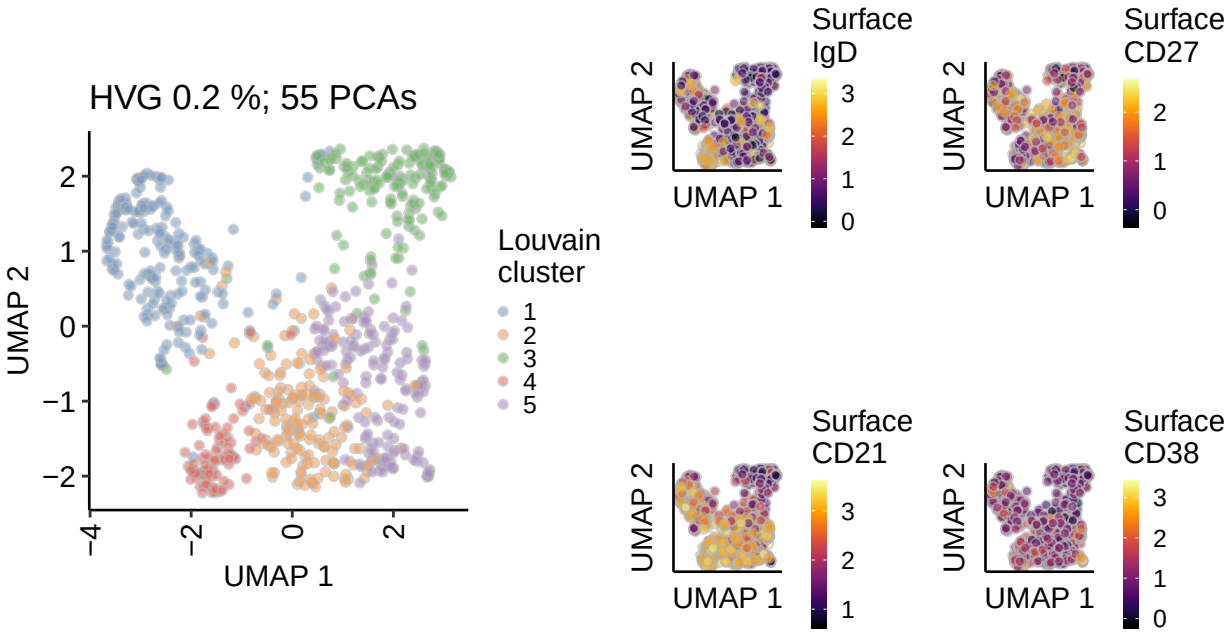


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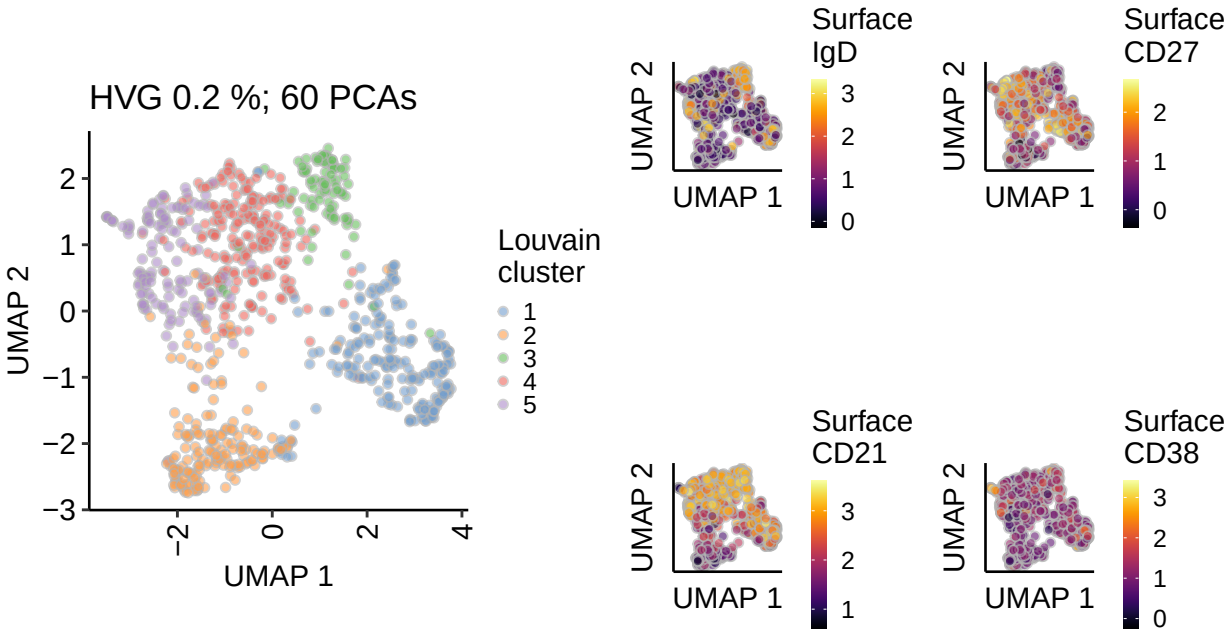


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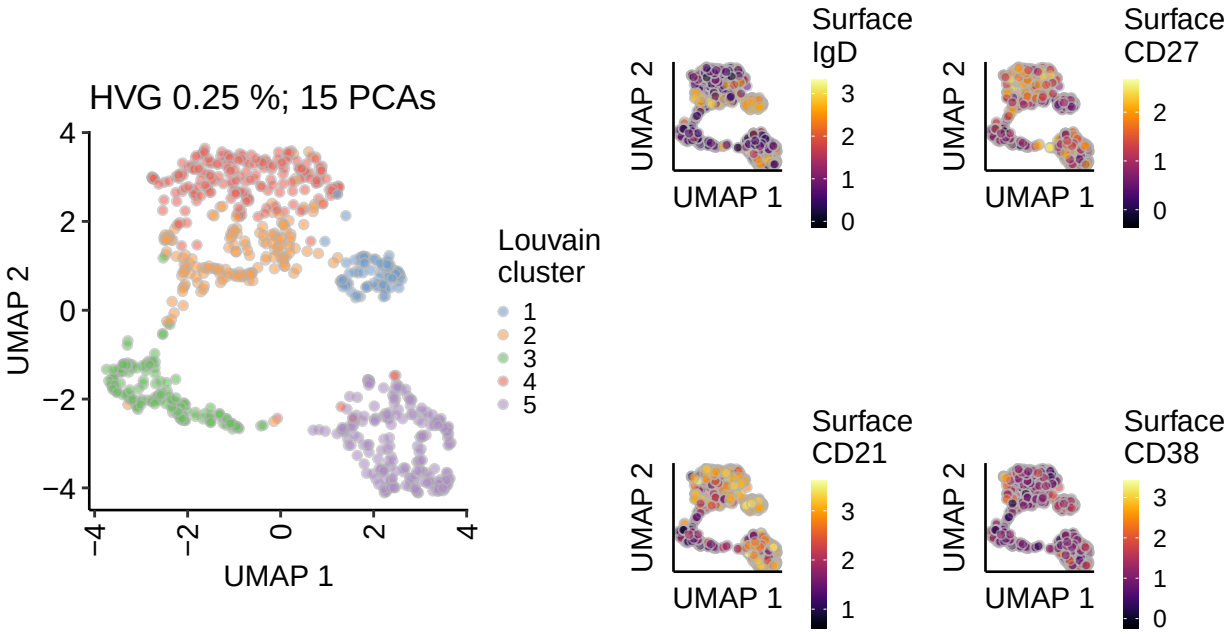


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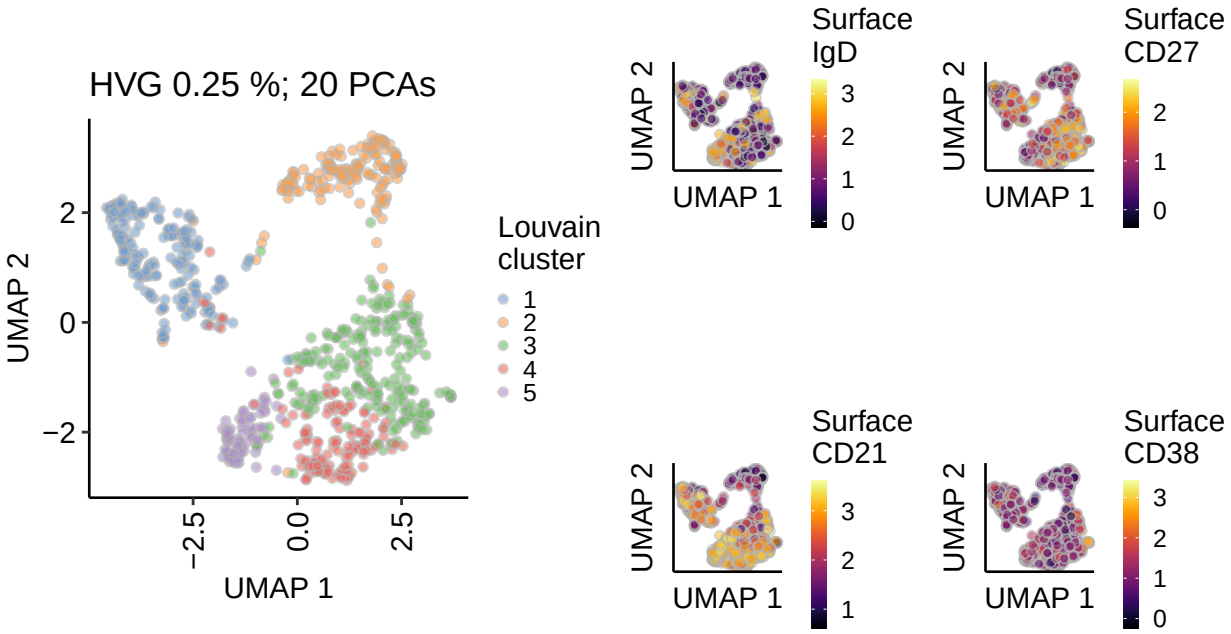


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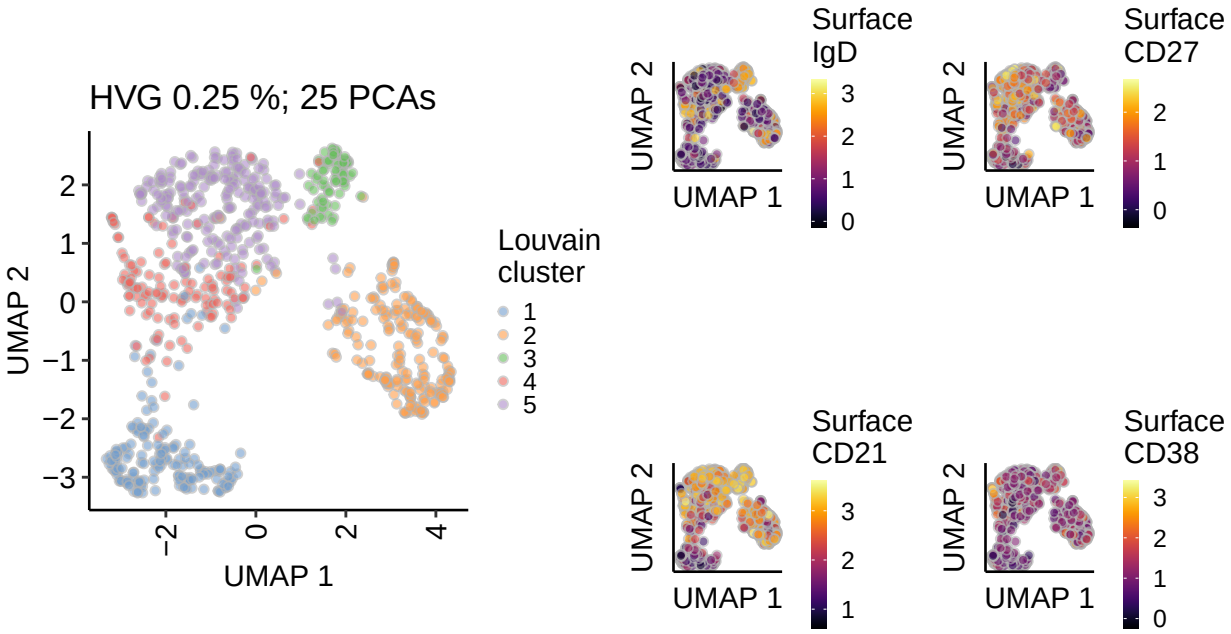


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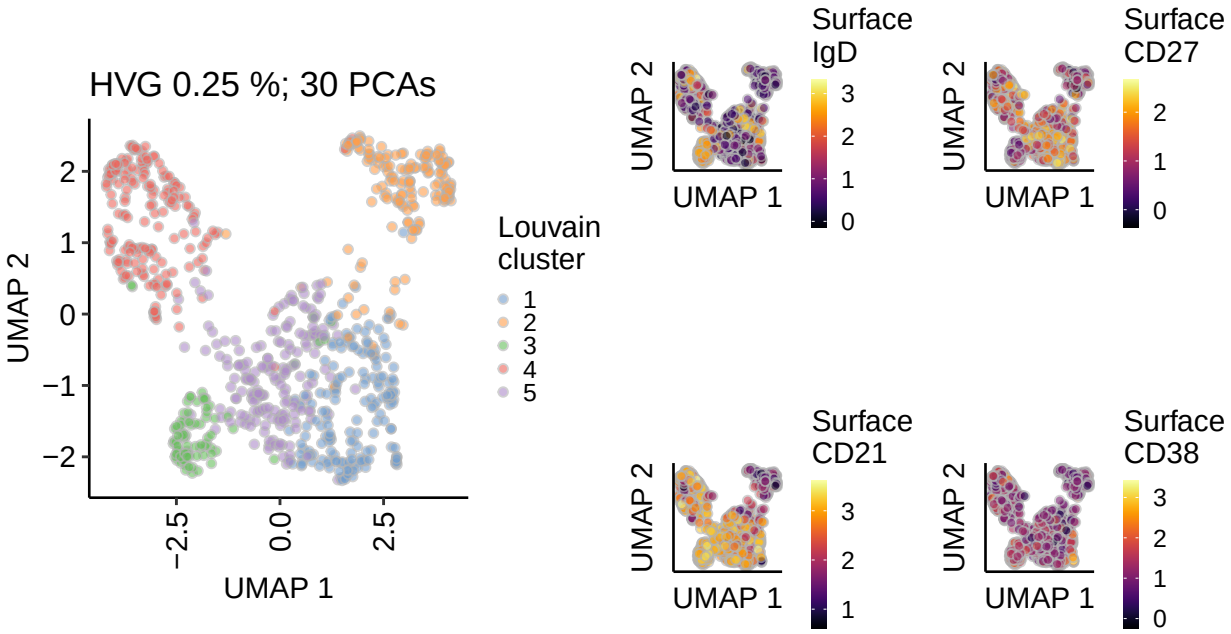


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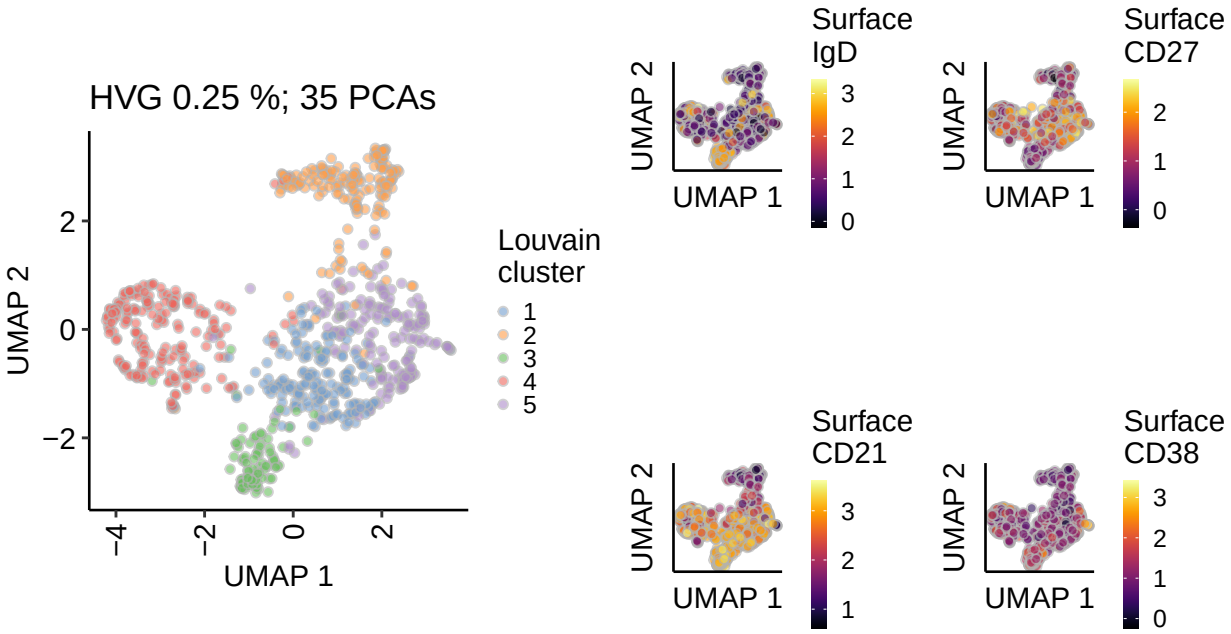


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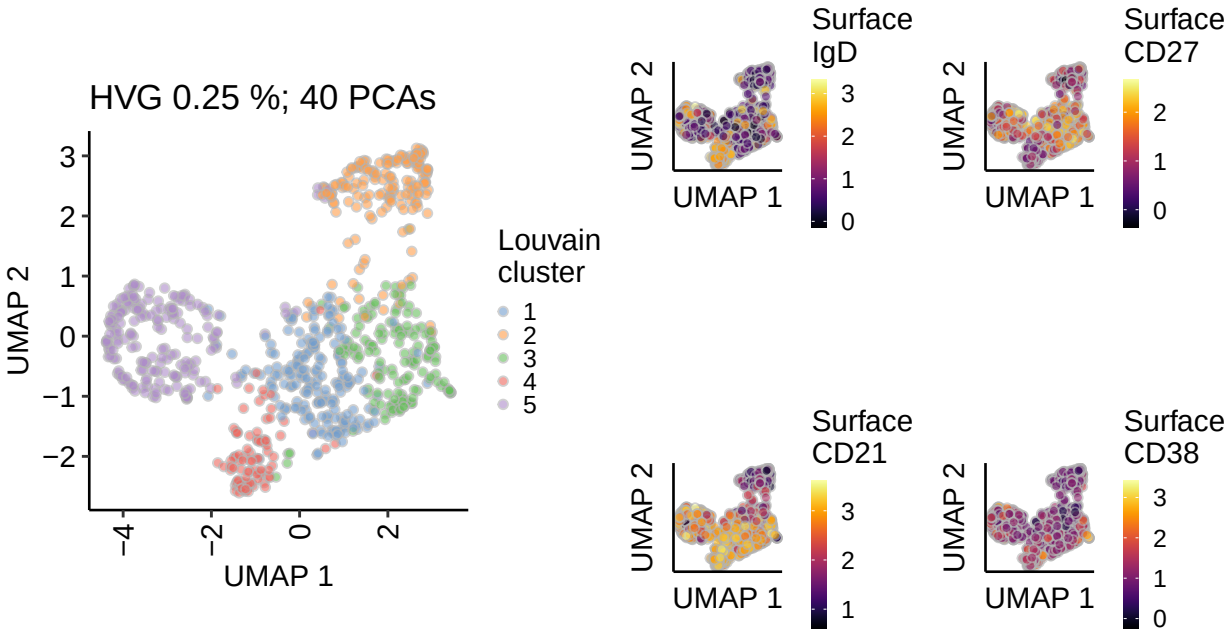


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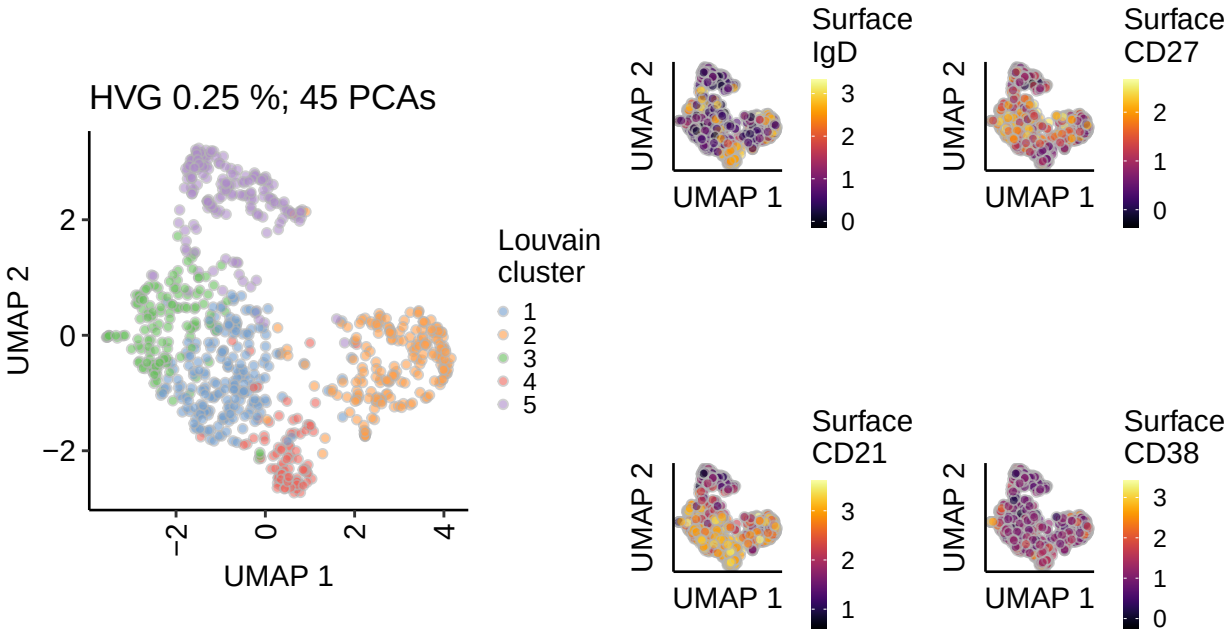


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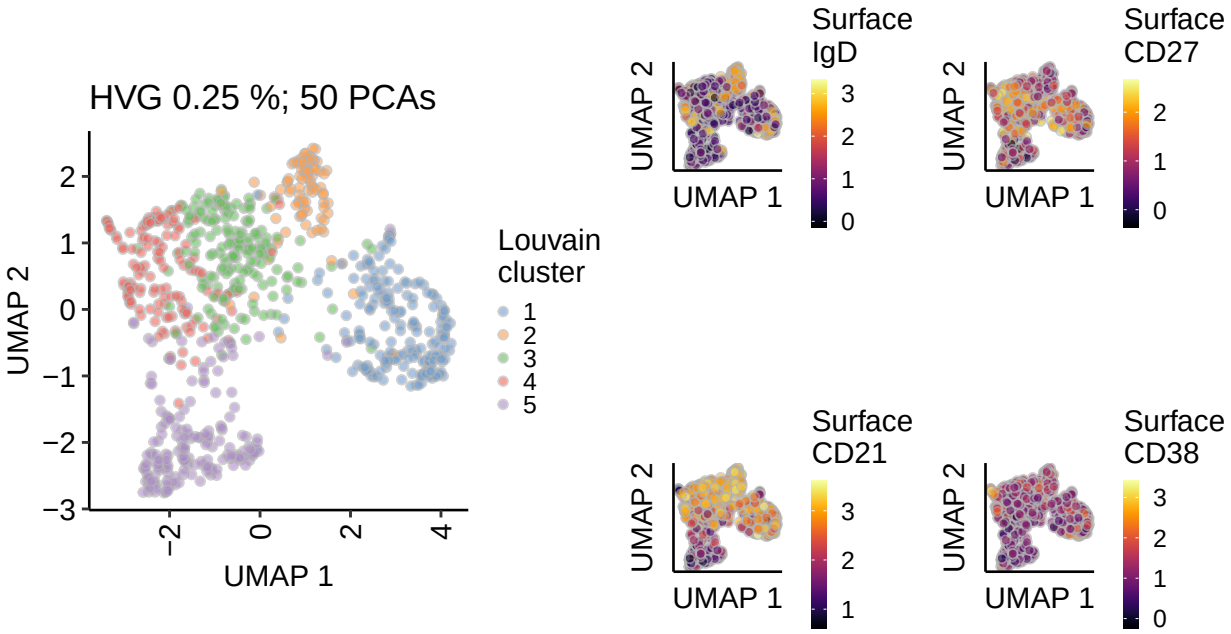


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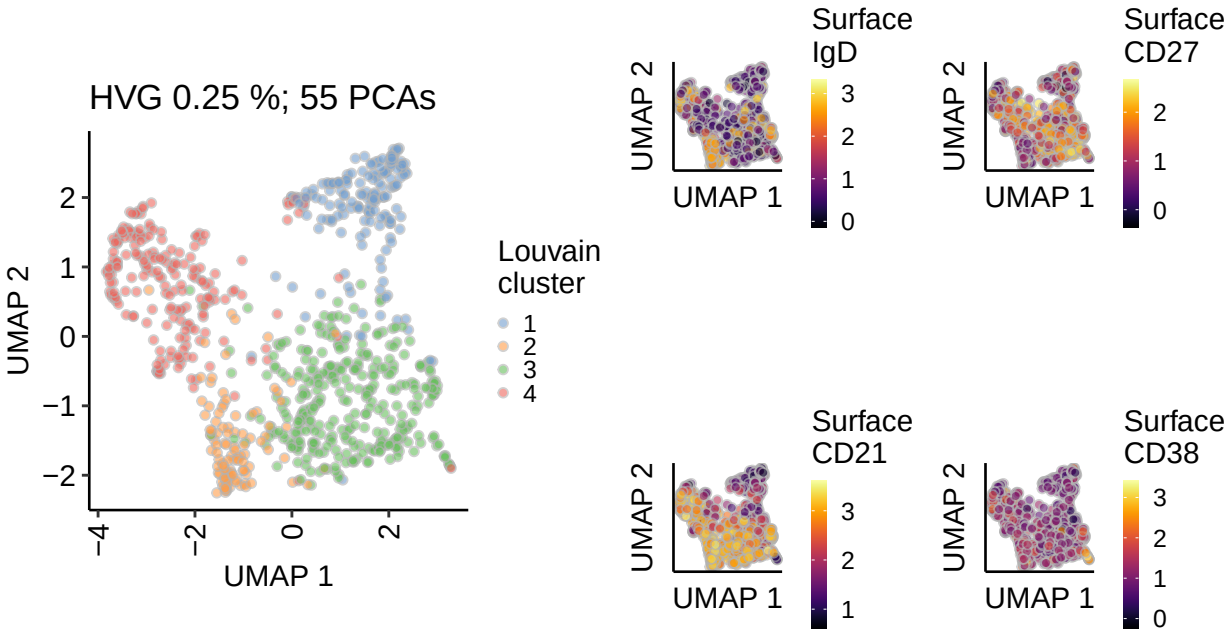


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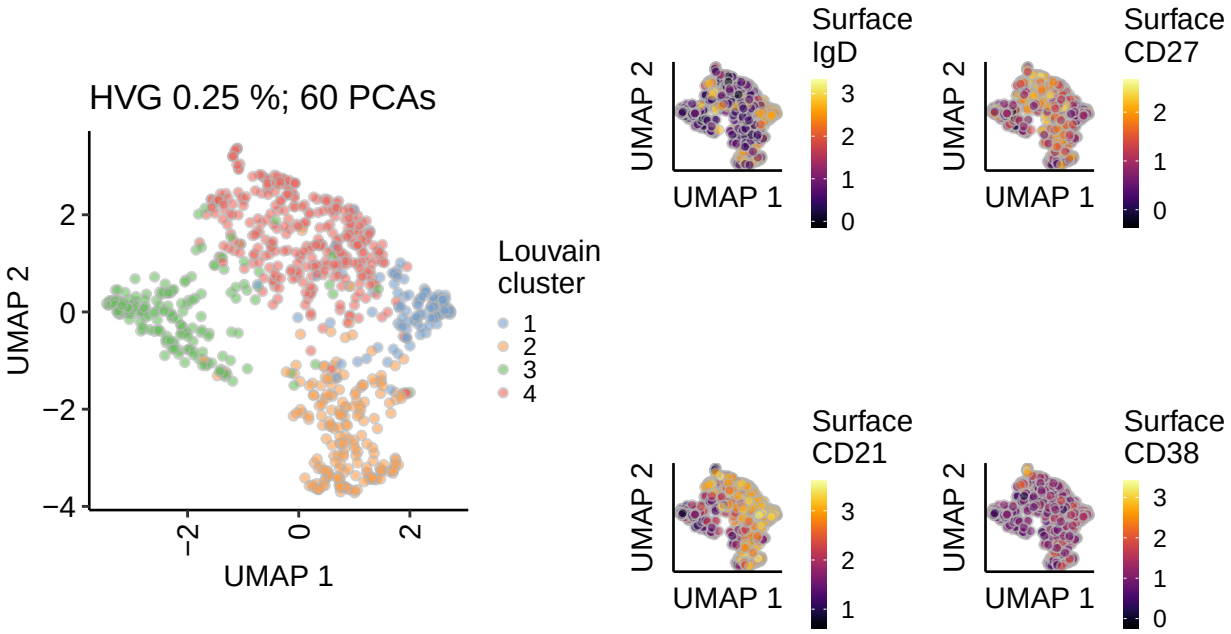


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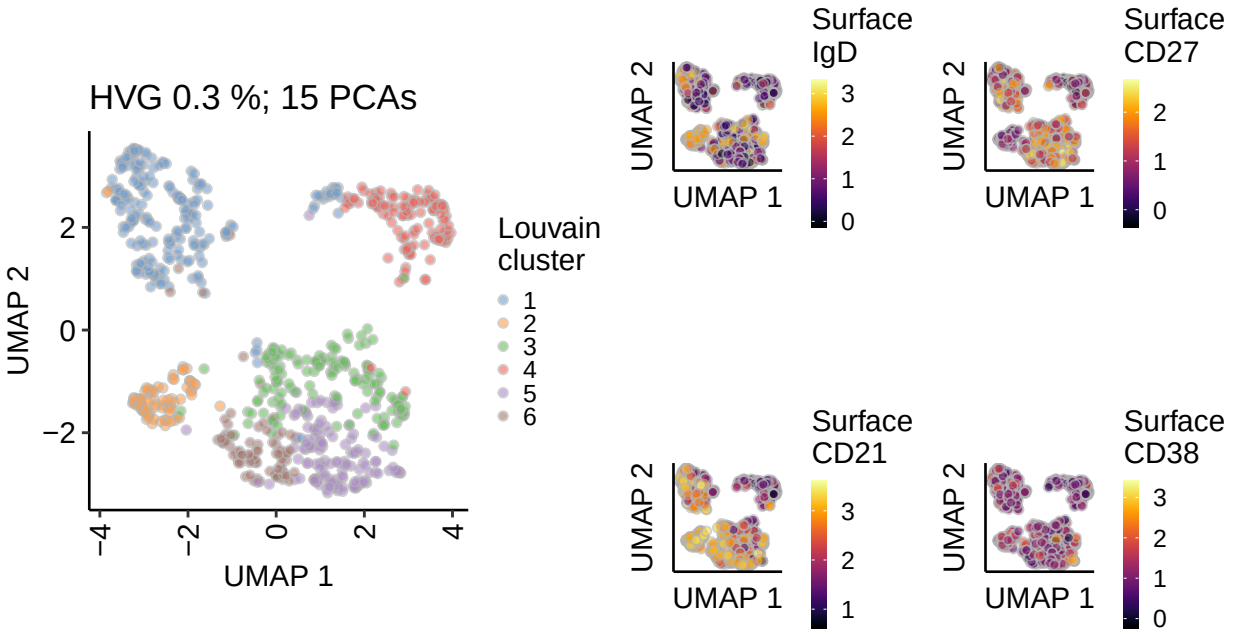


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## Scale for 'fill' is already present. Adding another scale for 'fill', which
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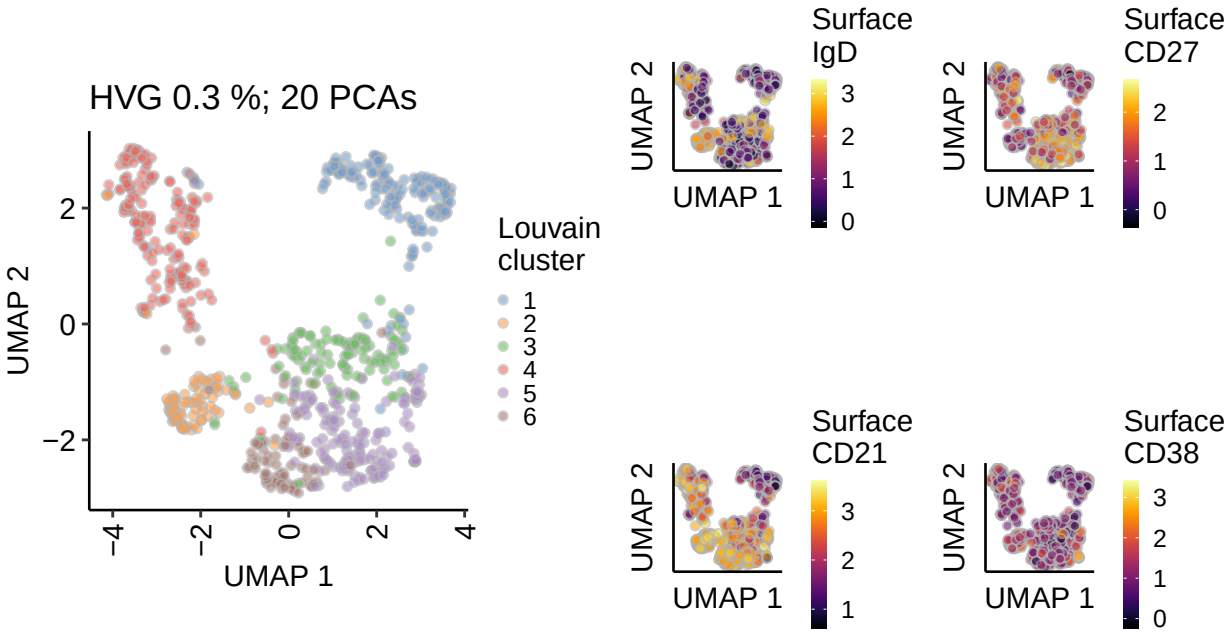


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## Scale for 'fill' is already present. Adding another scale for 'fill', which
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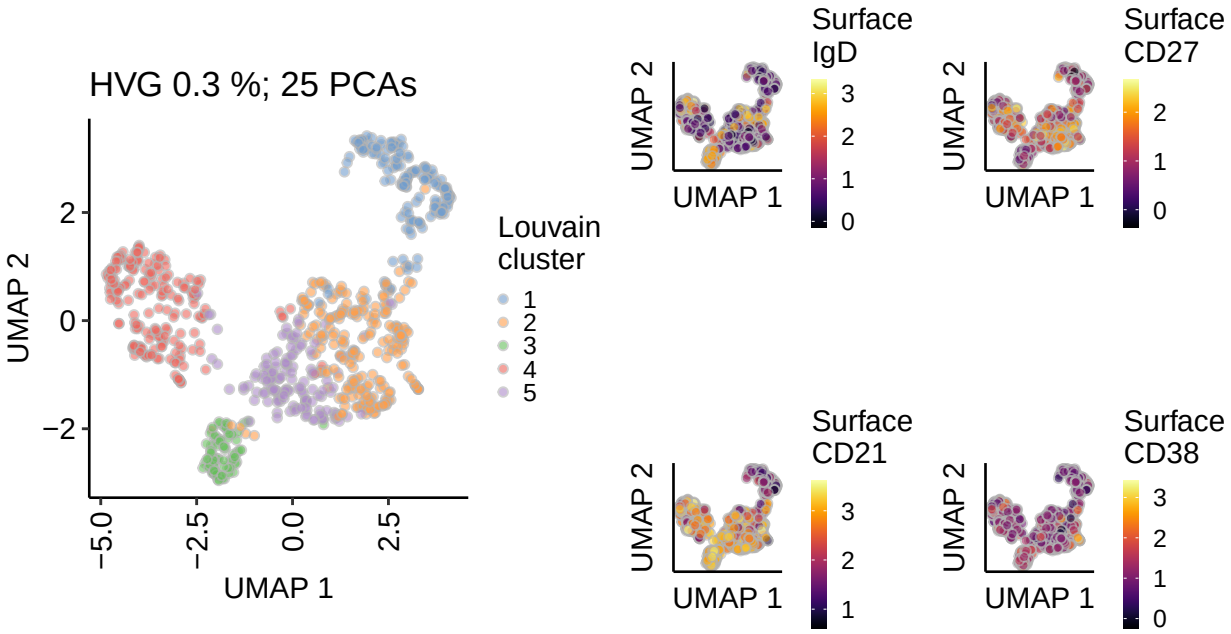


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## Scale for 'fill' is already present. Adding another scale for 'fill', which
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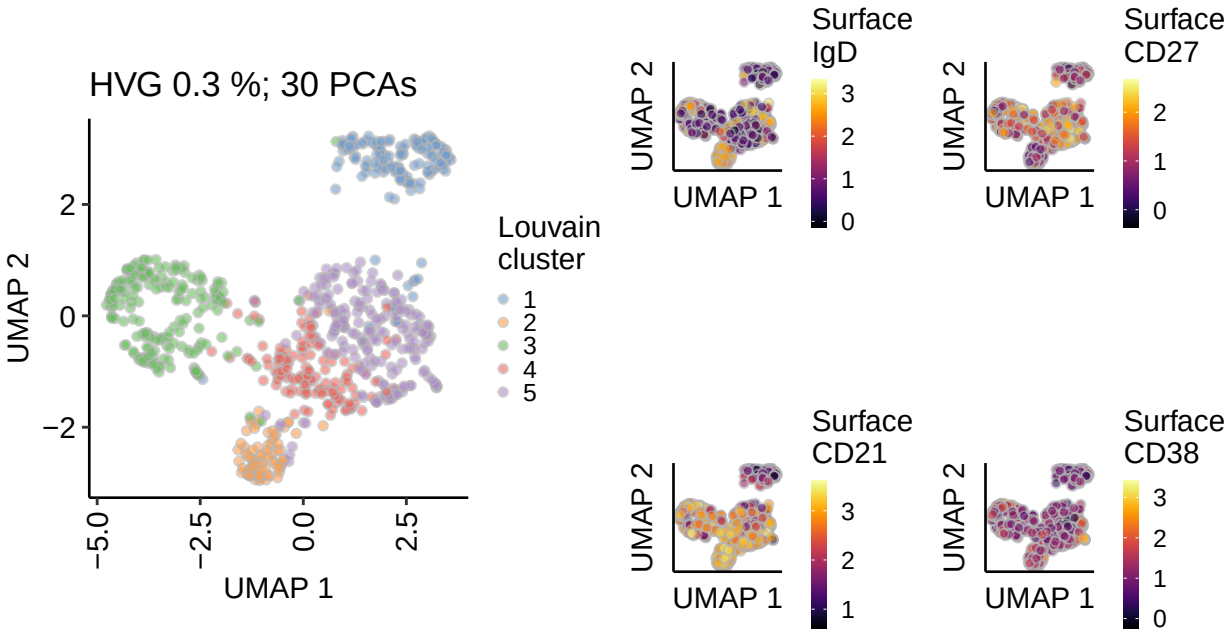


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## Scale for 'fill' is already present. Adding another scale for 'fill', which
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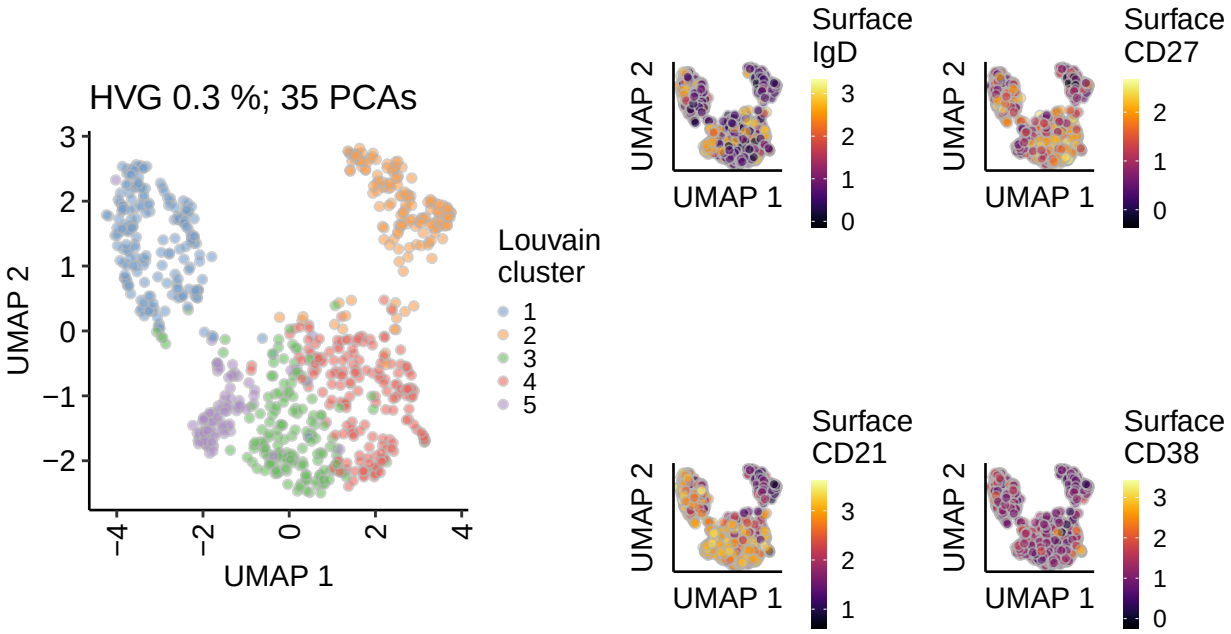


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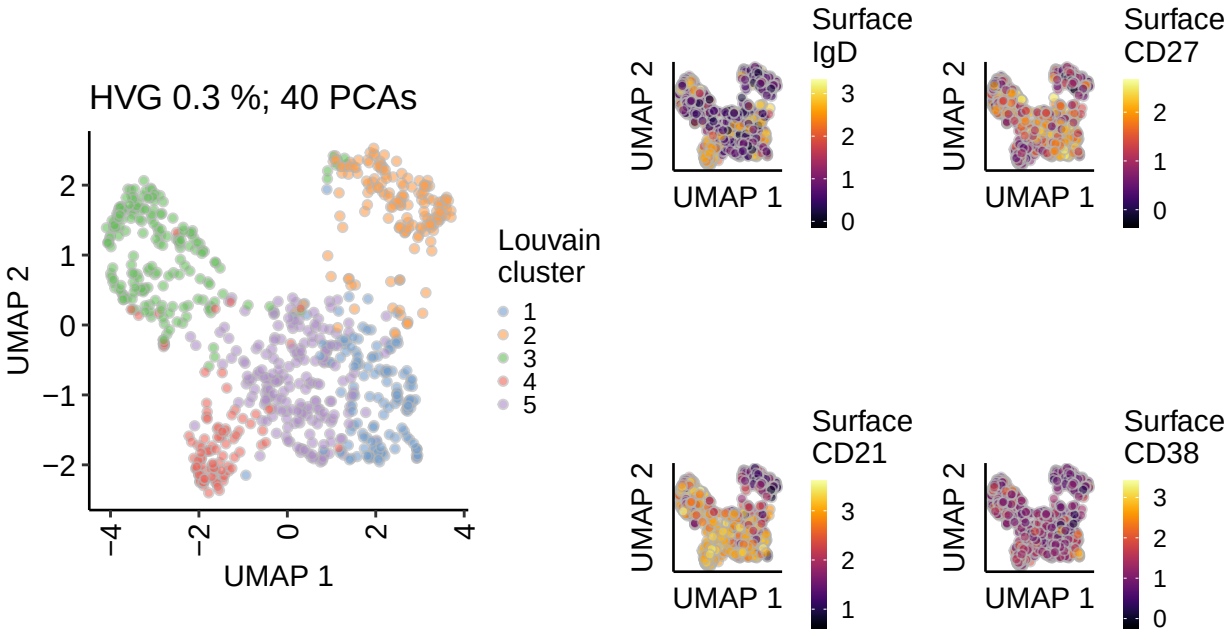


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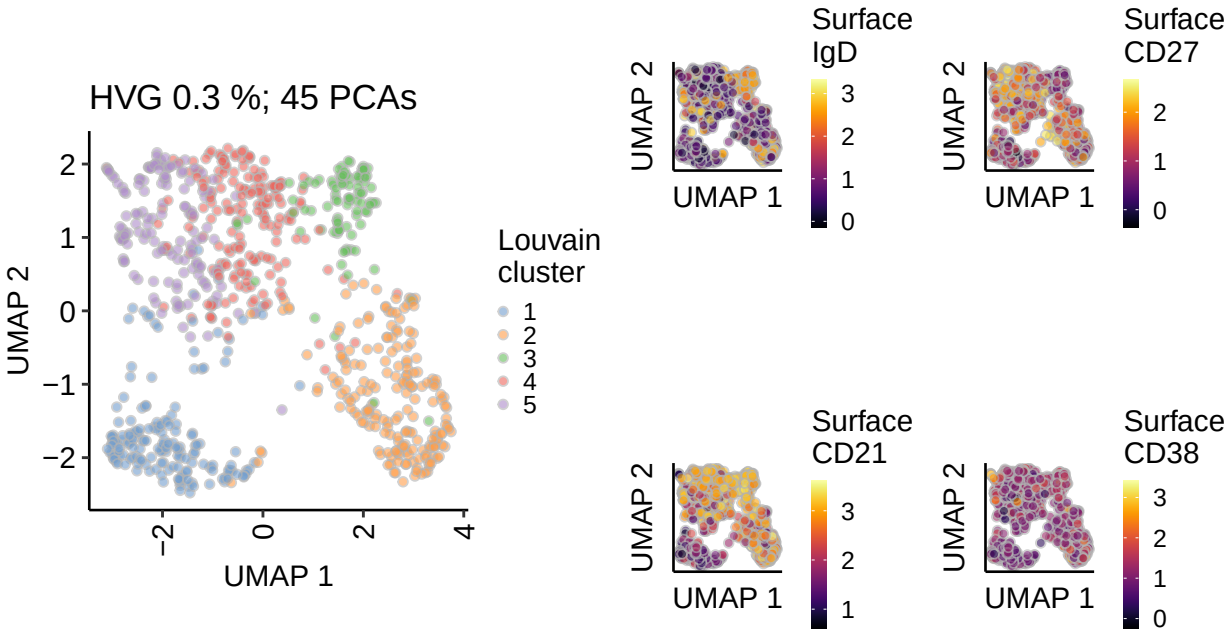


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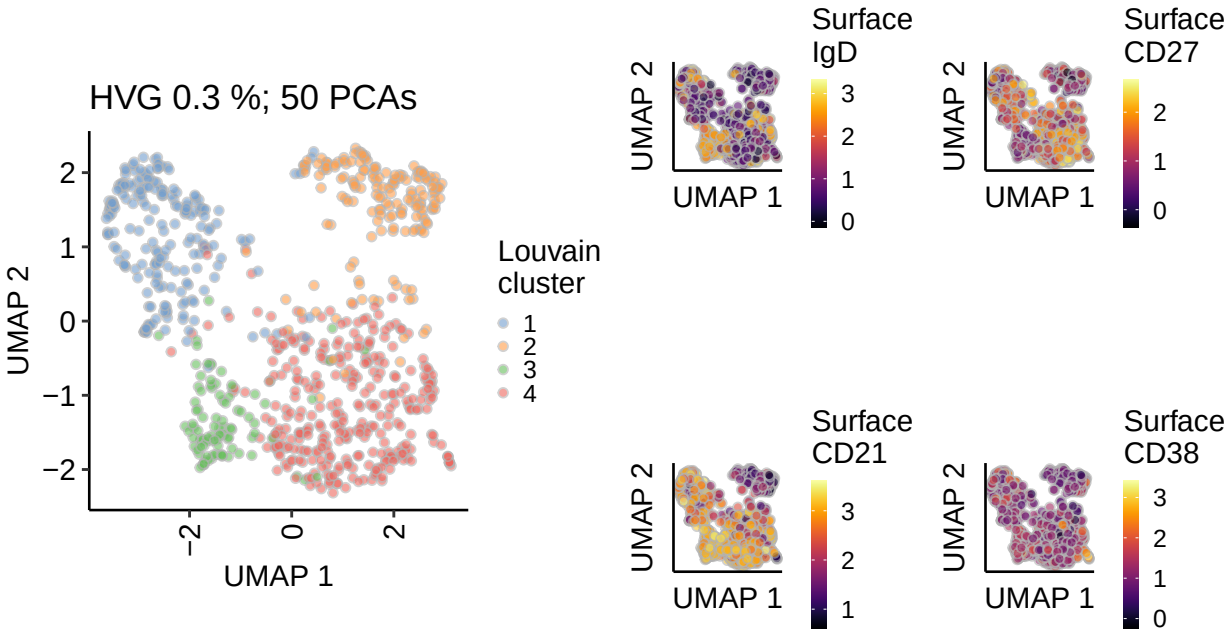


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## Scale for 'fill' is already present. Adding another scale for 'fill', which
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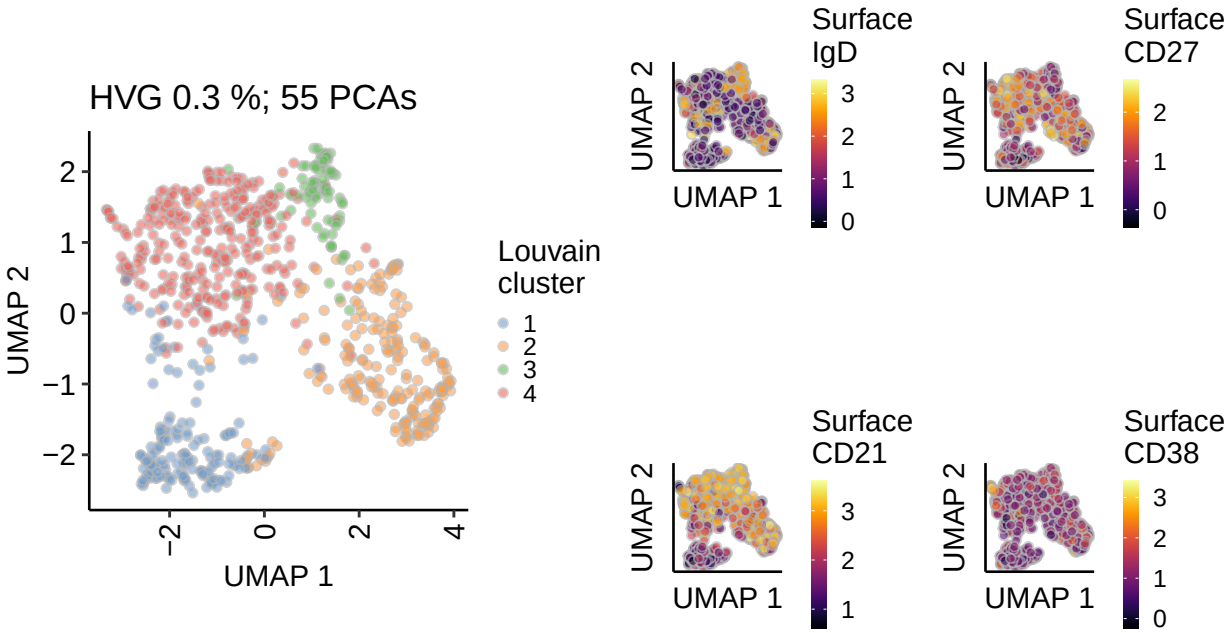
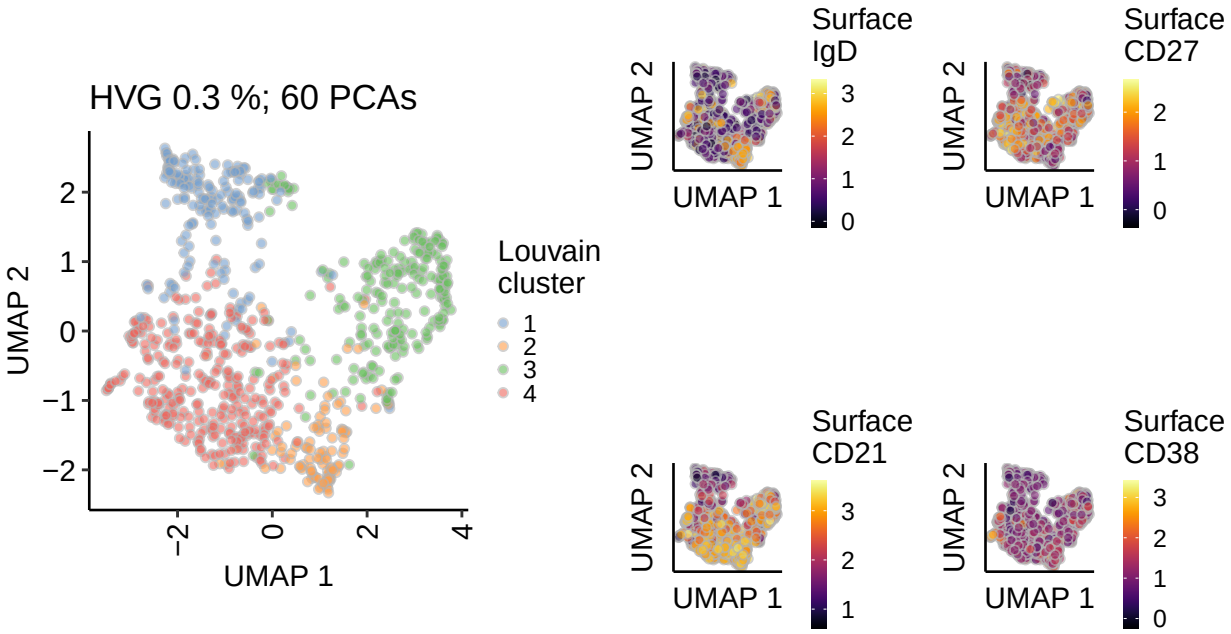

HVG 0.3 %; 55 PCAs
UMAP 2
UMAP 1
Louvain cluster
1
2
3
4
Surface IgD
Surface CD27
Surface CD21
Surface CD38


HVG 0.3 %; 60 PCAs
UMAP 2
UMAP 1
Louvain cluster
1
2
3
4
Surface IgD
Surface CD27
Surface CD21
Surface CD38

Save final SCE: NTC removed, QC passing cells, plasmablasts removed, with dimensionalty reduction pre-calculated

```
# save(sce, file = 'data/SCE_QC_pass_finalised.RData')
```

SessionInfo

```
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_GB.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_GB.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_GB.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
##  [1] grid      parallel  stats4    stats     graphics  grDevices utils
##  [8] datasets  methods   base
##
## other attached packages:
##  [1] Seurat_3.2.2                gtable_0.3.0
##  [3] forcats_0.4.0               stringr_1.4.0
##  [5] purrr_0.3.3                 readr_1.3.1
##  [7] tidyr_1.0.0                 tibble_3.0.4
##  [9] tidyverse_1.3.0             SingleR_1.0.6
## [11] rstatix_0.6.0               ggcyto_1.18.0
## [13] flowWorkspace_3.34.1        ncdfFlow_2.32.0
## [15] BH_1.72.0-2                 RcppArmadillo_0.10.1.2.0
## [17] flowCore_1.52.1             cowplot_1.0.0
## [19] pals_1.6                    MultiAssayExperiment_1.12.1
## [21] destiny_3.0.1               edgeR_3.28.0
## [23] limma_3.42.0                org.Hs.eg.db_3.10.0
## [25] AnnotationDbi_1.48.0        dplyr_1.0.2
## [27] ggpubr_0.2.4                magrittr_1.5
## [29] scater_1.14.5               ggplot2_3.3.2
## [31] scran_1.14.5                SingleCellExperiment_1.8.0
## [33] SummarizedExperiment_1.16.0 DelayedArray_0.12.0
## [35] BiocParallel_1.20.0         matrixStats_0.55.0
## [37] Biobase_2.46.0              GenomicRanges_1.38.0
```

```
## [39] GenomeInfoDb_1.22.0         IRanges_2.20.1
## [41] S4Vectors_0.24.1            BiocGenerics_0.32.0
##
## loaded via a namespace (and not attached):
##   [1] rappdirs_0.3.1                ggthemes_4.2.0
##   [3] bit64_0.9-7                   knitr_1.26
##   [5] irlba_2.3.3                   rpart_4.1-15
##   [7] data.table_1.12.8             RCurl_1.95-4.12
##   [9] generics_0.0.2                RSQLite_2.1.4
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##  [55] crayon_1.3.4                  pkgconfig_2.0.3
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