Endothelial figures ¶

inputs

In [1]:
```
source('jupyterFunctions_perCellType.R')
```

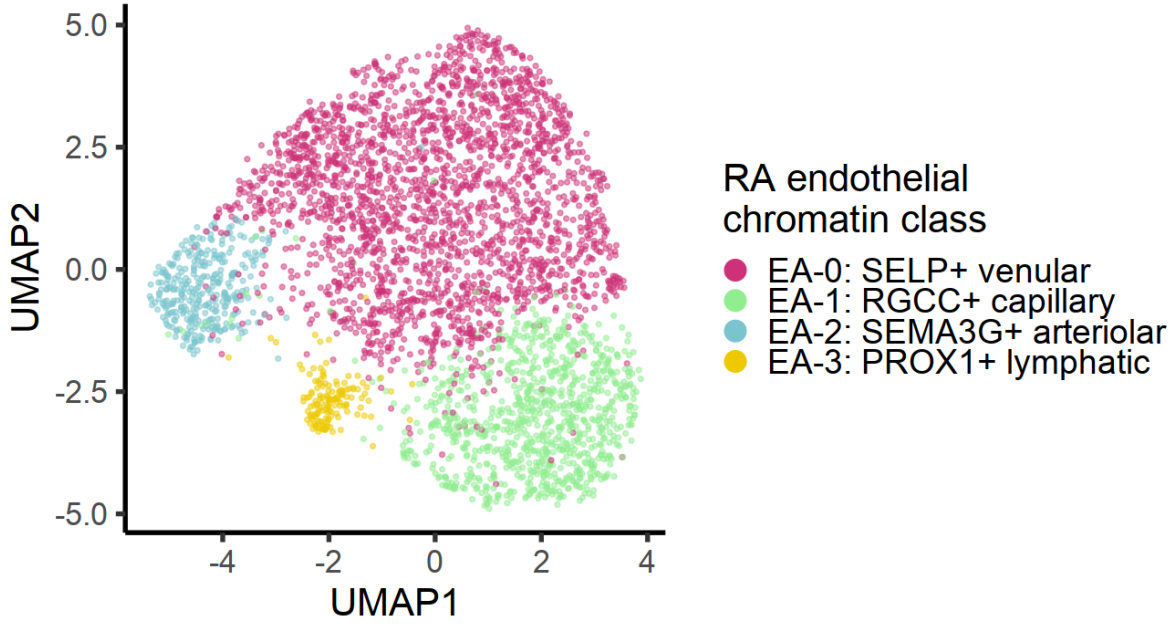
In [2]:
```
CT <- 'endothelial'
CT_label <- 'endothelial'
data_prefix <- paste(sep='','../data/',CT,'/',CT)
ATAC_meta <- readRDS(paste(sep='',data_prefix,'_ATAC_meta.rds'))
chosenPeaks <- readRDS(paste(sep='',data_prefix,'_chosenPeaks.rds'))
snATAC_pxc_norm <- readRDS(paste(sep='',data_prefix,'_snATAC_pxc_norm.rds'))
snRNA_gxc_norm <- readRDS(paste(sep='',data_prefix,'_snRNA_gxc_norm.rds'))
snATAC_pxCT_norm <- readRDS(paste(sep='',data_prefix,'_snATAC_pxCT_norm.rds'))
snRNA_gxCT_norm <- readRDS(paste(sep='',data_prefix,'_snRNA_gxCT_norm.rds'))
chromVARz_mat <- readRDS(paste(sep='',data_prefix,'_ArchR_chromVARz_JASPAR2020.rds'))
ArchR_padj <- readRDS(paste(sep='',data_prefix,'_ArchR_padj_JASPAR2020.rds'))
CITE_meta <- readRDS(paste(sep='',data_prefix,'_CITE_meta.rds'))
class_state_df <- readRDS(paste(sep='',data_prefix,'_class_state_df.rds'))
LDA_res <- readRDS(paste(sep='',data_prefix,'_LDA_stats.rds'))
```

In [3]:
```
ATAC_colors <- readRDS('../data/misc/ATAC_class_colors.rds')
CITE_colors <- readRDS('../data/misc/CITE_state_colors.rds')
ATAC_CITE_conv_df <- readRDS('../data/misc/ATAC_CITE_sample_conversion.rds')
```

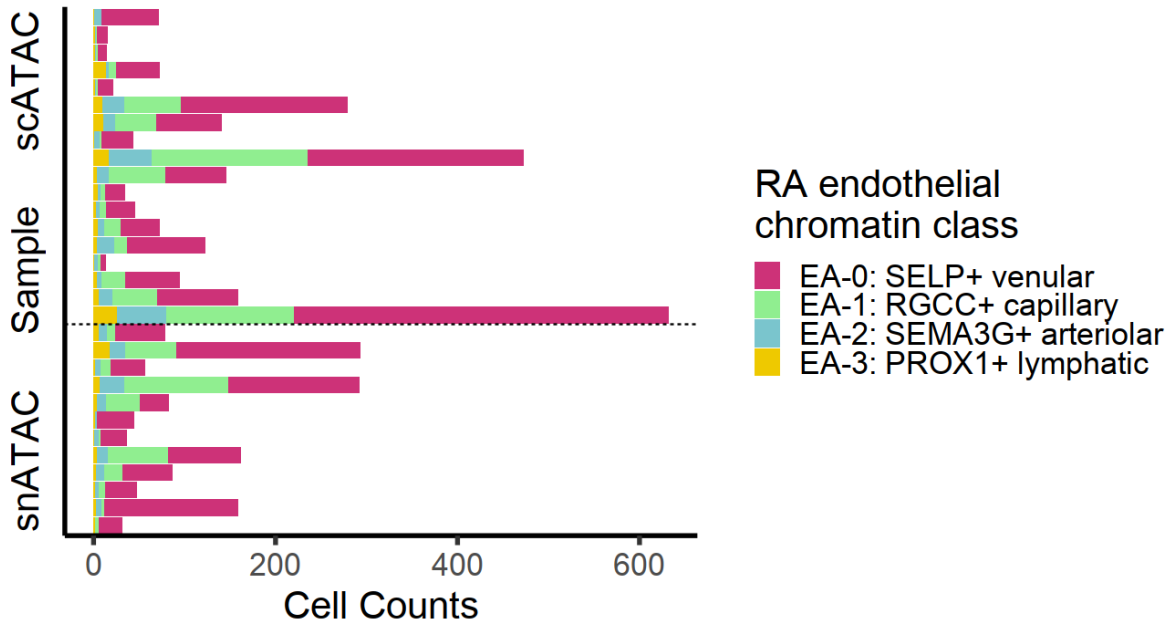
In [22]:
```
save_dir <- NA #'../output/' #or NA if don't want to save
```

ATAC classes

In [5]:
```
#Fig 6a
options(repr.plot.height=6,repr.plot.width=11)
g <- ggplot(ATAC_meta,aes_string(x='UMAP1',y='UMAP2',color='cluster_nam
e')) + geom_point(size=1,alpha=0.5) +
        theme_classic(base_size=25) + scale_color_manual(values=ATAC_col
ors) +
        labs(color=paste(sep='','RA ',CT_label,'\nchromatin class')) +
        theme(legend.text=element_text(size=22)) +
        guides(colour = guide_legend(override.aes = list(size=6,alpha=
1)))
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_ATAC_class_U
MAP.png'),
                            plot=g,units='in',height=6,width=11,dpi=600)
```



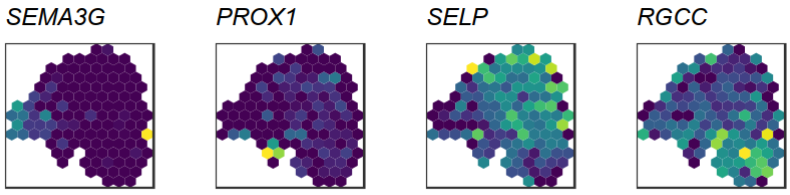
In [6]:
```
#Fig S6a
options(repr.plot.height=6,repr.plot.width=11)
g <- cellCount_bySample_barPlot(ATAC_meta,'sample','cluster_name',paste
(sep='','RA ',CT_label,'\nchromatin class'),
                                ATAC_colors)
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_ATAC_class_c
ellCount.png'),
                            plot=g,units='in',height=6,width=11,dpi=600)
```



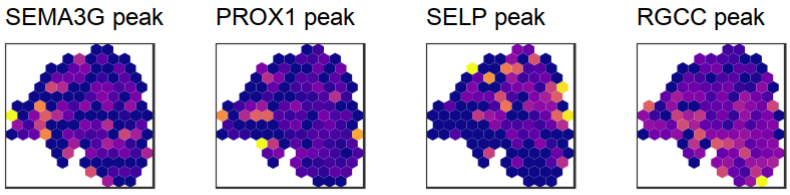
ATAC cluster markers

In [7]:
```
chosenGenes <- names(chosenPeaks)
chosenPeaks <- chosenPeaks[!is.na(chosenPeaks)] #NA means no peak in gen
e's promoter
```

In [8]:
```
#Fig 6b bottom

genes_forUMAPs <- c('SEMA3G','PROX1','SELP','RGCC')
if(!all(genes_forUMAPs %in% names(chosenPeaks))) stop('Genes for UMAP no
t in chosen genes')

multiome_cells <- rownames(ATAC_meta[which(ATAC_meta$assay=='snATAC'),])

options(repr.plot.height=2,repr.plot.width=7)
g <- plot_markerPeaks_norm_hex_v2(ATAC_meta[multiome_cells,],snRNA_gxc_n
orm[genes_forUMAPs,multiome_cells],'UMAP1','UMAP2',
                                  plot_genes=genes_forUMAPs,plotCol=leng
th(genes_forUMAPs),
                                  titleSize=14,hex_bins=12,cutCap=0)
grid.draw(g)
if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_markerGene_U
MAP.png'),
                            plot=g,units='in',height=2,width=7,dpi=600)
```



In [9]:
```
#Fig 6b top

toPlot <- snATAC_pxc_norm[unname(chosenPeaks[genes_forUMAPs]),multiome_c
ells]
rownames(toPlot) <- paste(sep='',names(chosenPeaks[genes_forUMAPs]),' pe
ak')

options(repr.plot.height=2,repr.plot.width=7)
g <- plot_markerPeaks_norm_hex_v2(ATAC_meta[multiome_cells,],toPlot,'UMA
P1','UMAP2',
                                  plot_genes=rownames(toPlot),plotCol=nr
ow(toPlot),titleSize=14,hex_bins=12,cutCap=0,
                                  titleFace='plain',colorOpt='plasma')
grid.draw(g)
if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_markerPeak_U
MAP.png'),
                            plot=g,units='in',height=2,width=7,dpi=600)
```

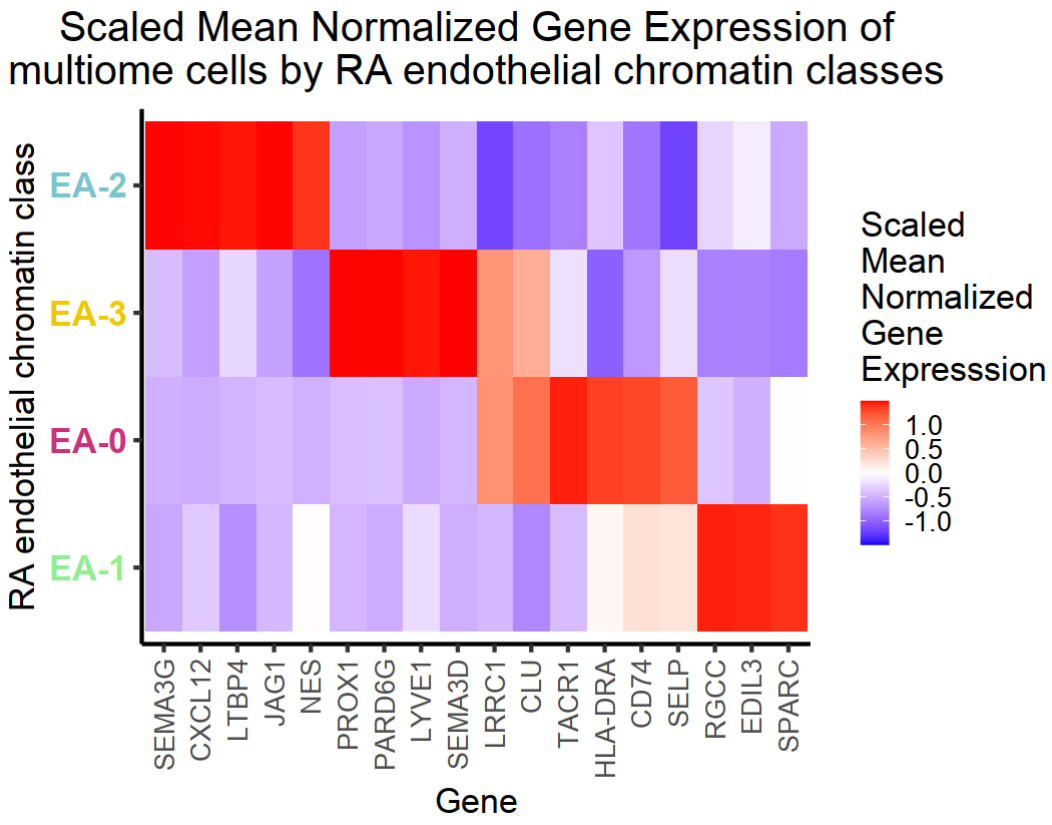


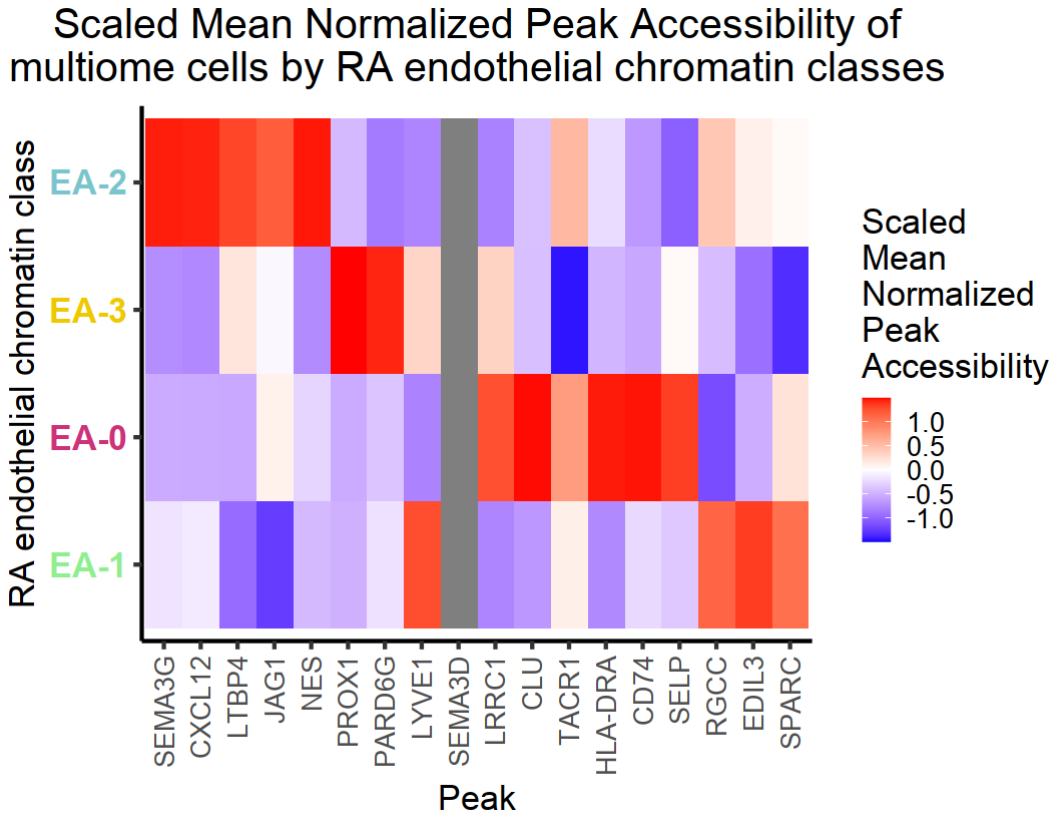
In [10]:
```
class_order <- c('EA-2','EA-3','EA-0','EA-1')
all(class_order %in% ATAC_meta$cluster_abbr)
```

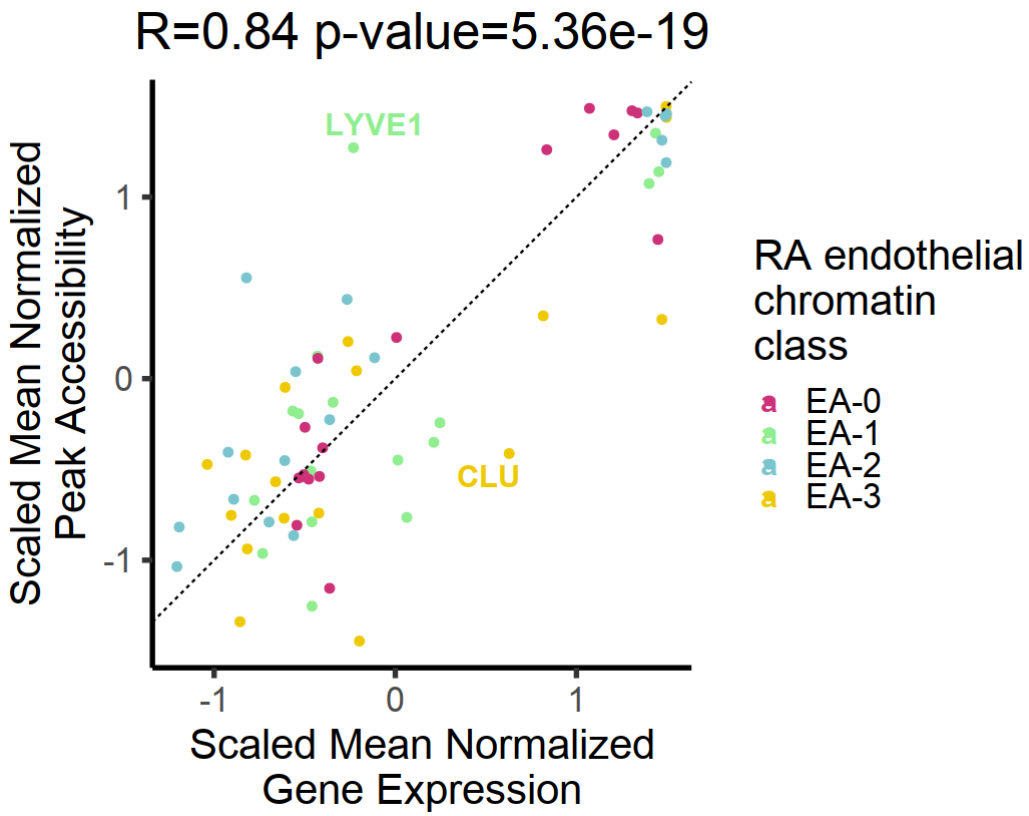
TRUE

In [11]:
```
#Fig S6b

res <- scaleFeat_forHeatmap(chosenGenes,class_order,chosenPeaks,snRNA_gx
CT_norm,snATAC_pxCT_norm)
snRNA_gxCT_norm_subset_scaled <- res$gxCT_norm_subset_scaled
snATAC_pxCT_norm_subset_scaled <- res$pxCT_norm_subset_scaled
fxCT_norm_subset_scaled <- res$fxCT_norm_subset_scaled

scale_lim <- max(abs(snRNA_gxCT_norm_subset_scaled),abs(snATAC_pxCT_norm
_subset_scaled),na.rm=TRUE)

options(repr.plot.height=7,repr.plot.width=9)
g <- pseudobulk_scaled_heatmap(snRNA_gxCT_norm_subset_scaled,'Gene',past
e('RA',CT_label,'chromatin class'),
                               'Scaled\nMean\nNormalized\nGene\nExpresss
ion',
                               plotTit=paste('Scaled Mean Normalized Gen
e Expression of\nmultiome cells by RA',
                                             CT_label,'chromatin classe
s'),
                               scale_lim=scale_lim,clustColors=ATAC_colo
rs)
print(g)
if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_markerGene_h
eatmap.png'),
                            plot=g,units='in',height=7,width=9,dpi=600)

g <- pseudobulk_scaled_heatmap(snATAC_pxCT_norm_subset_scaled,'Peak',pas
te('RA',CT_label,'chromatin class'),
                               'Scaled\nMean\nNormalized\nPeak\nAccessib
ility',
                               plotTit=paste('Scaled Mean Normalized Pea
k Accessibility of\nmultiome cells by RA',
                                             CT_label,'chromatin classe
s'),
                               scale_lim=scale_lim,clustColors=ATAC_colo
rs)
print(g)
if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_markerPeak_h
eatmap.png'),
                            plot=g,units='in',height=7,width=9,dpi=600)

pearR <- cor.test(fxCT_norm_subset_scaled$gene_norm_scale,fxCT_norm_subs
et_scaled$peak_norm_scale,
                  method='pearson')

fxCT_norm_subset_scaled$label <- ''
fxCT_norm_subset_scaled[which(fxCT_norm_subset_scaled$gene=='CLU' & fxCT
_norm_subset_scaled$cluster_abbr=='EA-3'),
                        'label'] <- 'CLU'
fxCT_norm_subset_scaled[which(fxCT_norm_subset_scaled$gene=='LYVE1' & fx
CT_norm_subset_scaled$cluster_abbr=='EA-1'),
                        'label'] <- 'LYVE1'

g <- ggplot(fxCT_norm_subset_scaled,
            aes_string(x='gene_norm_scale',y='peak_norm_scale',color='cl
```

```
uster_abbr',label='label')) +
        geom_point(size=2) + theme_classic(base_size=25) + scale_color_m
anual(values=ATAC_colors) +
        labs(x='Scaled Mean Normalized\nGene Expression',
             y='Scaled Mean Normalized\nPeak Accessibility',
             color=paste(sep='','RA ',CT_label,'\nchromatin\nclass')) +
        geom_abline(slope=1,intercept=0,linetype='dashed') +
        ggtitle(paste(sep='','R=',round(pearR$estimate,2),' p-value=',si
gnif(pearR$p.value,3))) +
        theme(plot.title=element_text(hjust = 0.5)) + geom_text_repel(bo
x.padding = 0.5,size=6.5,fontface='bold',seed=0)
suppressWarnings(print(g)) #points excluded if peak does not exist
if(!is.na(save_dir)) suppressWarnings(ggsave(file=paste(sep='',save_dir,
CT,'_markerGenePeak_scatterplot.png'),
                                             plot=g,units='in',height=7,
width=9,dpi=600))
```

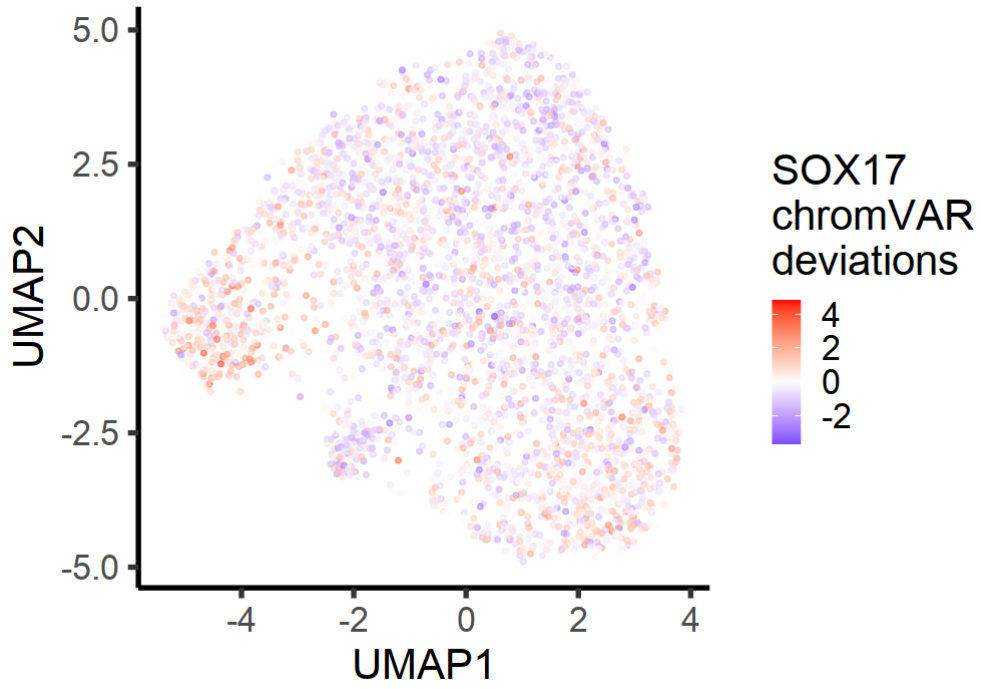

Scaled Mean Normalized Gene Expression of multiome cells by RA endothelial chromatin classes
RA endothelial chromatin class
EA-2
EA-3
EA-0
EA-1
SEMA3G
CXCL12
LTBP4
JAG1
NES
PROX1
PARD6G
LYVE1
SEMA3D
LRRC1
CLU
TACR1
HLA-DRA
CD74
SELP
RGCC
EDIL3
SPARC
Gene
Scaled Mean Normalized Gene Expresssion
1.0
0.5
0.0
-0.5
-1.0


Scaled Mean Normalized Peak Accessibility of multiome cells by RA endothelial chromatin classes
RA endothelial chromatin class
EA-2
EA-3
EA-0
EA-1
SEMA3G
CXCL12
LTBP4
JAG1
NES
PROX1
PARD6G
LYVE1
SEMA3D
LRRC1
CLU
TACR1
HLA-DRA
CD74
SELP
RGCC
EDIL3
SPARC
Peak
Scaled Mean Normalized Peak Accessibility
1.0
0.5
0.0
-0.5
-1.0

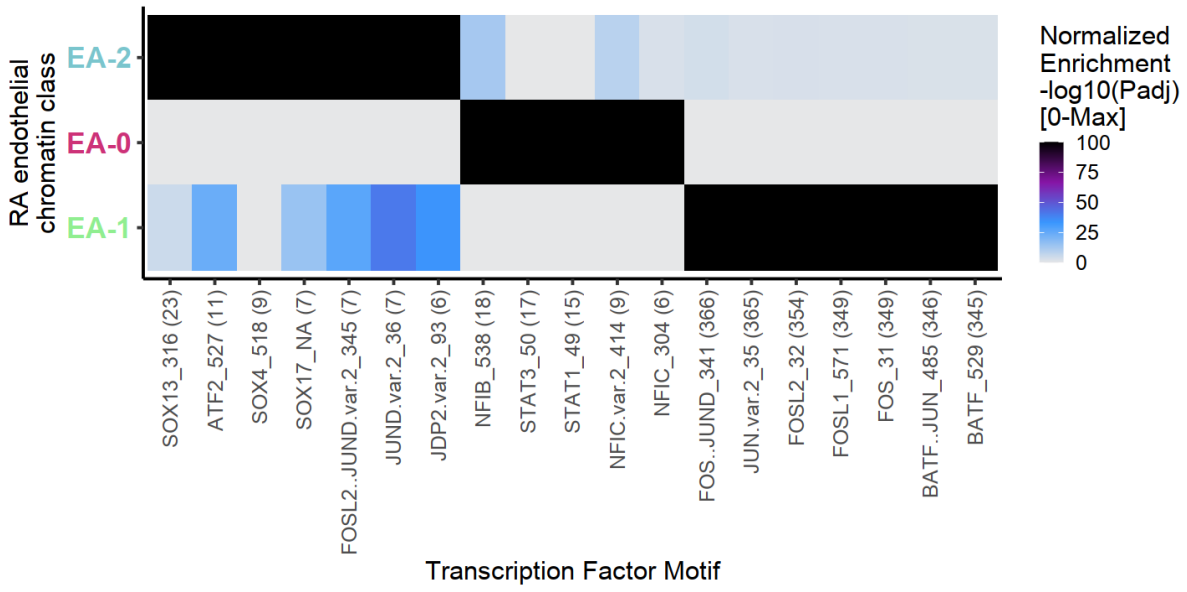

R=0.84 p-value=5.36e-19
LYVE1
CLU
Scaled Mean Normalized Peak Accessibility
Scaled Mean Normalized Gene Expression
1
0
-1
-1
0
1
RA endothelial chromatin class
EA-0
EA-1
EA-2
EA-3

TFs

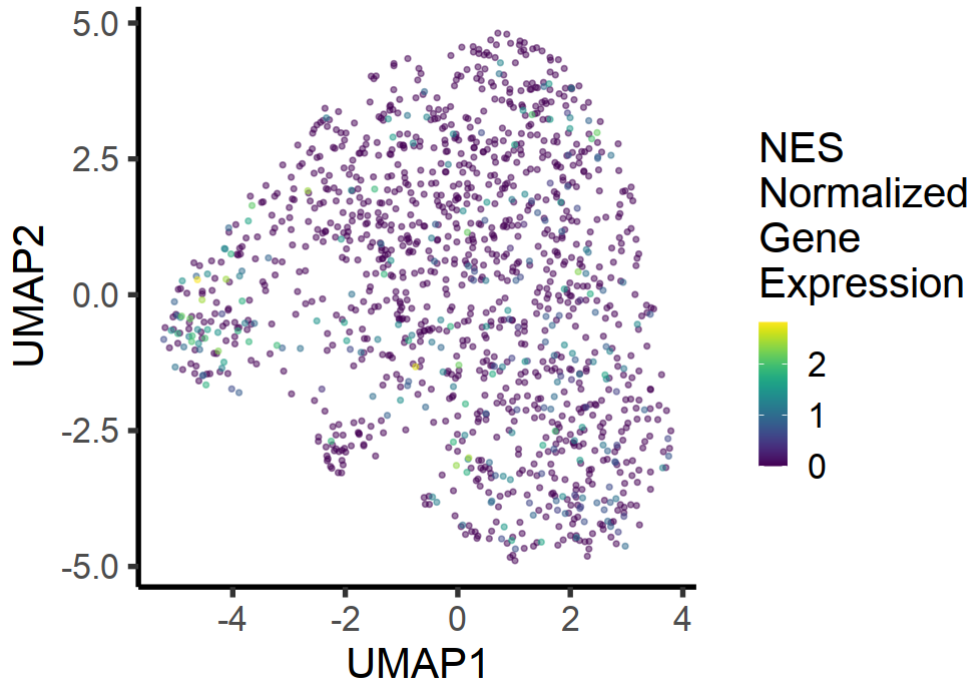
In [12]:
```
#Fig 6c left

#fix cell names
split1 <- str_split_fixed(colnames(chromVARz_mat),'#',2)
new_colnames <- paste(sep='',split1[,1],'_',str_split_fixed(split1[,
2],'-',2)[,1])
if(!identical(sort(new_colnames),sort(rownames(ATAC_meta)))) stop('celln
ames not consistent b/t ATAC_meta and chromVAR')
colnames(chromVARz_mat) <- new_colnames

motif_toPlot <- 'SOX17_NA'
toPlot <- cbind(ATAC_meta,'motif'=chromVARz_mat[motif_toPlot,rownames(AT
AC_meta)])

options(repr.plot.height=6,repr.plot.width=8.5)
g <- ggplot(toPlot,aes_string(x='UMAP1',y='UMAP2',color='motif')) + geom
_point(size=1,alpha=0.5) +
        theme_classic(base_size=25) + scale_color_gradient2(low='blue',m
id='white',high='red',midpoint=0) +
        labs(color=paste(sep='',str_split_fixed(motif_toPlot,'_',2)[,
1],'\nchromVAR\ndeviations'))
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_motif_',moti
f_toPlot,'_UMAP.png'),
                            plot=g,units='in',height=6,width=8.5,dpi=60
0)
```



In [13]:
```
#Fig 6c right

#add hyphen back
if(!identical(sort(colnames(ArchR_padj)),sort(colnames(snRNA_gxCT_nor
m))) &
   all(str_detect(colnames(ArchR_padj),'^[a-zA-Z]{2}[0-9]+$'))){
    colnames(ArchR_padj) <- lapply(colnames(ArchR_padj),FUN=function(s)
{paste(sep='',substr(s,1,2),'-',

substr(s,3,nchar(s)))})
}
if(!identical(sort(colnames(ArchR_padj)),
              sort(colnames(snRNA_gxCT_norm)))) stop('mxCT and gxCT matr
ices do not have same CT.')


options(repr.plot.height=6,repr.plot.width=12)
#only 1 EA-3 marker peak, so no motifs enriched, so excluding here
g <- ArchR_topMotifs_KWspin(ArchR_padj,snRNA_gxCT_norm,cOrd=class_order
[class_order!='EA-3'],cColors=ATAC_colors,
                            minE=5,num_mot=7,minGE=0.05,withinE=0.95,
                            mLab='Transcription Factor Motif',cLab=paste
(sep='','RA ',CT_label,'\nchromatin class'))
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_motif_heatma
p.png'),
                            plot=g,units='in',height=6,width=12,dpi=600)
```

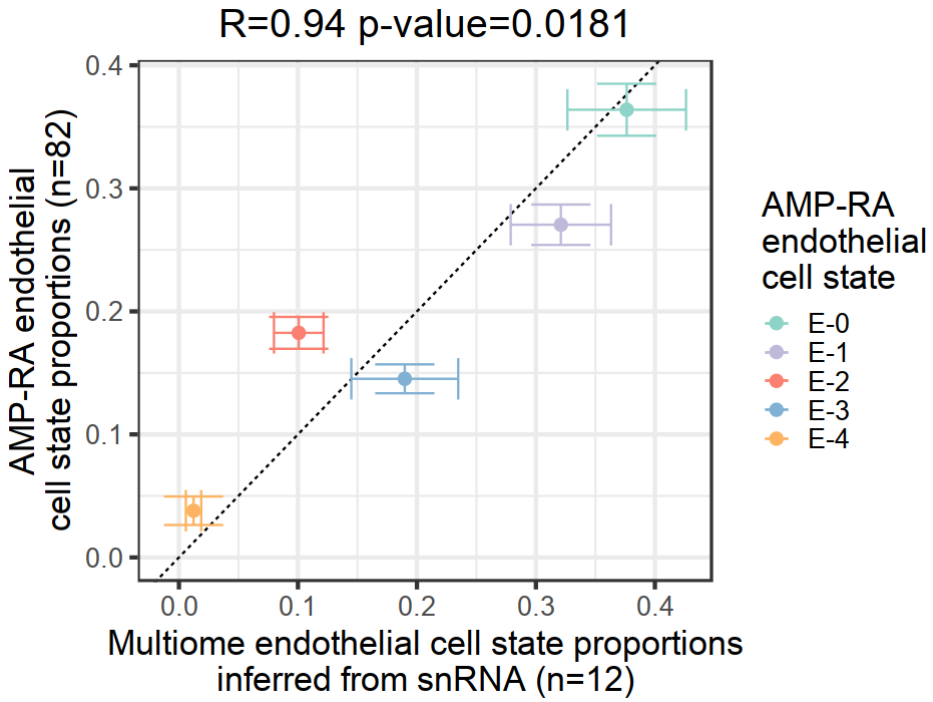


In [14]:
```
#Fig 6d left

gene_toPlot <- 'NES'
toPlot <- cbind(ATAC_meta[multiome_cells,],'gene'=snRNA_gxc_norm[gene_to
Plot,multiome_cells])

options(repr.plot.height=6,repr.plot.width=8.5)
g <- ggplot(toPlot[order(toPlot$gene),],aes_string(x='UMAP1',y='UMAP2',c
olor='gene')) +
                geom_point(size=1,alpha=0.5) +
                theme_classic(base_size=25) + scale_color_viridis(option
= 'viridis') +
                labs(color=paste(sep='',gene_toPlot,'\nNormalized\nGene
\nExpression'))
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_gene_',gene_
toPlot,'_UMAP.png'),
                            plot=g,units='in',height=6,width=8.5,dpi=60
0)
```

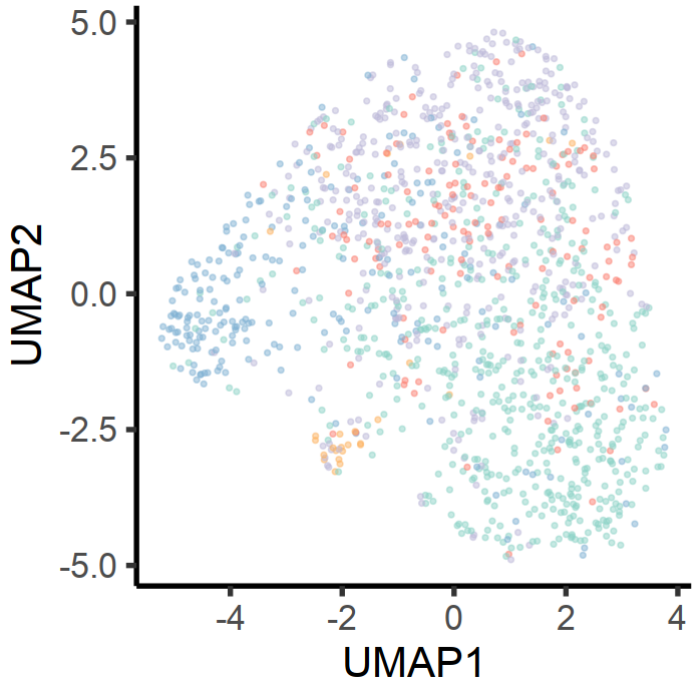


Transcriptional Cell States

In [15]:
```
#Fig S8e

options(repr.plot.height=6,repr.plot.width=8)
g <- symp_prop_df(ATAC_meta[multiome_cells,],CITE_meta,
                  paste(sep='','Multiome ',CT_label,' cell state proport
ions\ninferred from snRNA (n=',
                        length(unique(ATAC_meta[multiome_cells,'sampl
e'])),')'),
                  paste(sep='','AMP-RA ',CT_label,'\ncell state proporti
ons (n=',
                        length(unique(CITE_meta$sample)),')'),
                  paste(sep='','AMP-RA\n',CT_label,'\ncell state'),clust
Colors=CITE_colors)
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_ATAC_CITE_st
ate_prop.png'),
                            plot=g,units='in',height=6,width=8,dpi=600)
```

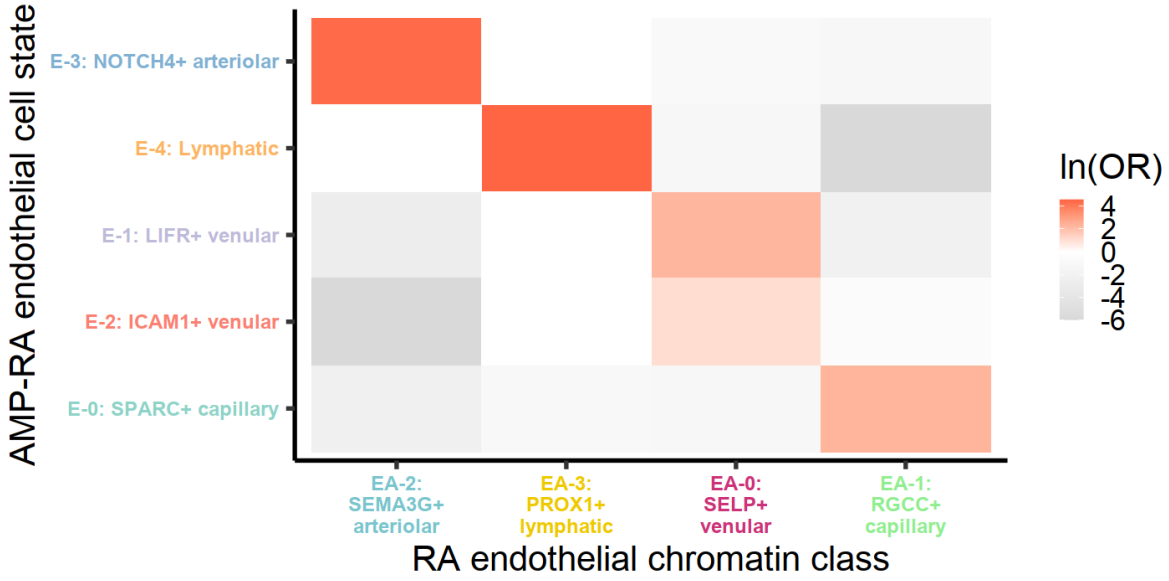


In [16]:
```
#Fig S8h left
options(repr.plot.height=6,repr.plot.width=6)
g <- ggplot(ATAC_meta[multiome_cells,],aes_string(x='UMAP1',y='UMAP2',co
lor='CITE')) +
        geom_point(size=1,alpha=0.5) +
        theme_classic(base_size=25) + scale_color_manual(values=CITE_col
ors) +
        theme(legend.position="none")
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_snATAC_state
_UMAP.png'),
                            plot=g,units='in',height=6,width=6,dpi=600)
```

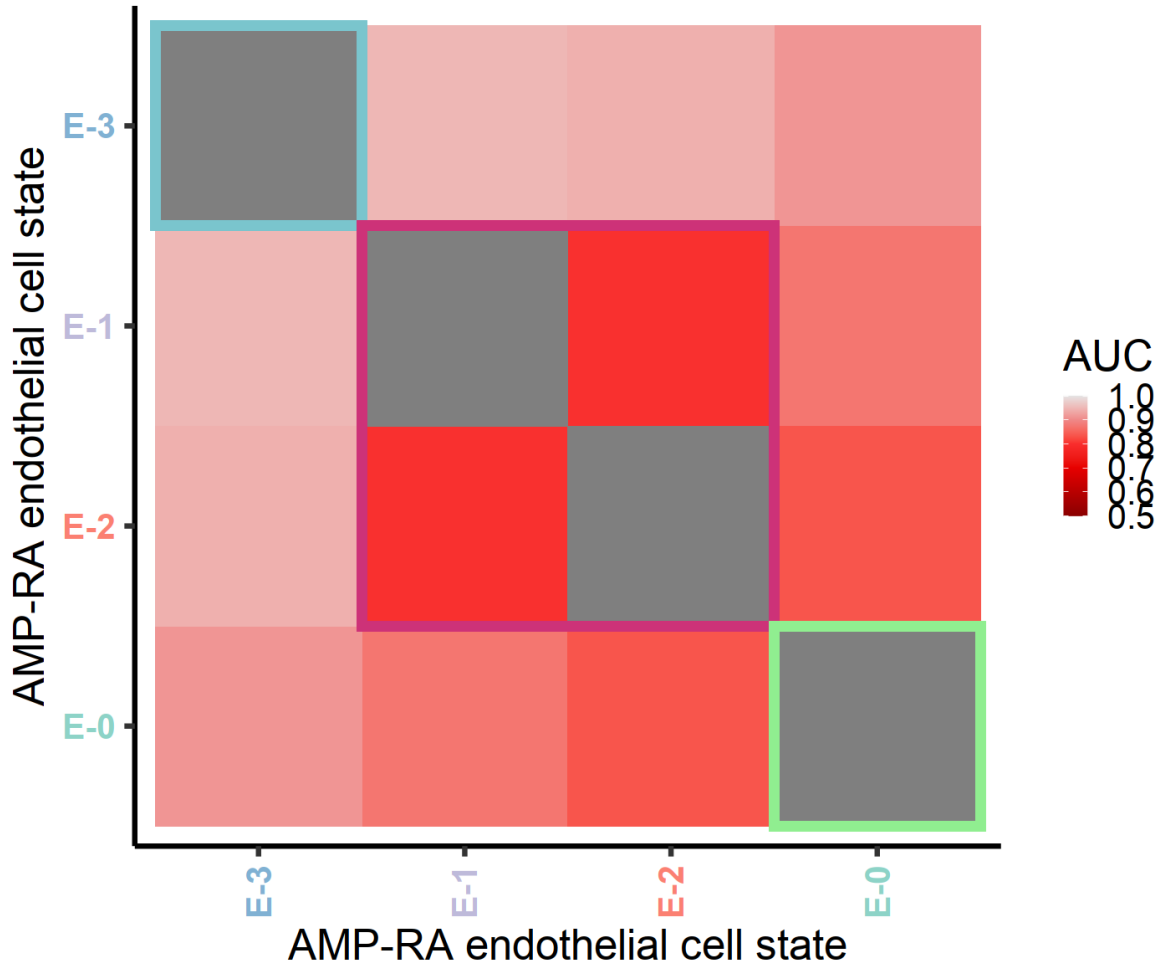


In [17]:
```
#setting order
class_conv_df <- unique(ATAC_meta[,c('cluster_name','cluster_abbr')])
rownames(class_conv_df) <- class_conv_df$cluster_abbr
full_class_order <- class_conv_df[class_order,'cluster_name']

class_state_df$class <- factor(class_state_df$class,levels=class_order)
state_order <- class_state_df[order(class_state_df$class,class_state_df
$intOrd),'state']

state_conv_df <- unique(ATAC_meta[,c('CITE','CITE_abbr')])
rownames(state_conv_df) <- state_conv_df$CITE_abbr
full_state_order <- state_conv_df[state_order,'CITE']
```

In [18]:
```
#Fig S8h right

fisher_df <- calc_OR(ATAC_meta[multiome_cells,], 'cluster_name', 'CITE')
write.table(fisher_df[,c('cluster_name','CITE','OR','pval','padj','CI_low','CI_high')],
            file=paste(sep='',save_dir,CT,'_class_state_OR_table.txt'),quote=FALSE,sep='\t',row.names=FALSE)

g <- plot_OR(fisher_df, 'cluster_name', 'CITE',
             paste('RA',CT_label,'chromatin class'), paste('AMP-RA',CT_label,'cell state'),
             full_class_order, full_state_order,
             clustColors=c(ATAC_colors,CITE_colors))

options(repr.plot.height=6,repr.plot.width=12)
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_class_state_OR_heatmap.png'),
                            plot=g,units='in',height=6,width=12,dpi=600)
```

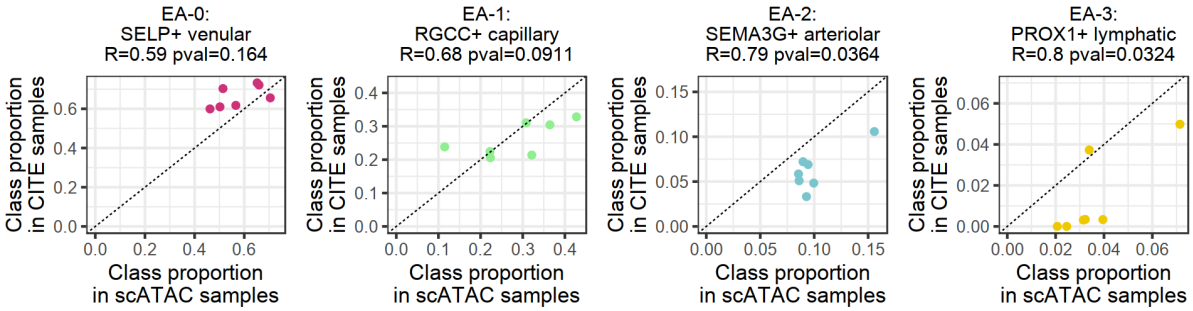


In [19]:
```
#Fig S11e

options(repr.plot.height=10,repr.plot.width=12)
g <- LDA_plots(LDA_res,CT,paste('AMP-RA',CT_label,'cell state'),
               class_state_df=class_state_df,ctOrd_col='intOrd',ctOrd=cl
ass_order,
               clustColors=c(CITE_colors,ATAC_colors))
print(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_LDA_heatmap.
png'),
                            plot=g,units='in',height=10,width=12,dpi=60
0)
```



CITE donor proportions

In [20]:
```
#Fig S12a

tVec <- lapply(sort(unique(ATAC_meta$cluster_name)),replace_space_newlin
e_afterHalf,wiggle=7)
names(tVec) <- sort(unique(ATAC_meta$cluster_name))

options(repr.plot.height=4.25,repr.plot.width=4*length(unique(ATAC_meta
$cluster_abbr)))
g <- donor_prop_comp_plot(ATAC_CITE_conv_df,ATAC_meta[which(ATAC_meta$as
say=='scATAC'),],CITE_meta,
                          clustColors=ATAC_colors,tSize=18,tVec=tVec,cel
lCutoff=100)
grid.draw(g)

if(!is.na(save_dir)) ggsave(file=paste(sep='',save_dir,CT,'_ATAC_CITE_do
nor_prop.png'),
                            plot=g,units='in',height=4.25,width=4*length
(unique(ATAC_meta$cluster_abbr)),dpi=600)
```



Session Info

In [21]:
```
sessionInfo()
```

```
R version 3.6.1 (2019-07-05)
Platform: x86_64-conda_cos6-linux-gnu (64-bit)
Running under: Red Hat Enterprise Linux Server release 6.5 (Santiago)

Matrix products: default
BLAS/LAPACK: /PHShome/kew47/miniconda3/lib/R/lib/libRblas.so

locale:
[1] en_US.UTF-8

attached base packages:
[1] grid      stats     graphics  grDevices utils     datasets  methods
[8] base

other attached packages:
 [1] repr_1.0.1        gridExtra_2.3     scales_1.1.1      viridis_0.5.
1
 [5] viridisLite_0.3.0 ggrepel_0.8.2     ggrastr_0.2.3     ggplot2_3.3.
0
 [9] tidyr_1.0.3       stringr_1.4.0     ROCR_1.0-7        gplots_3.0.
1.1
[13] Rmisc_1.5.1       plyr_1.8.6        lattice_0.20-41   gtools_3.8.2
[17] Matrix_1.2-18

loaded via a namespace (and not attached):
 [1] pbdZMQ_0.3-3        beeswarm_0.2.3      tidyselect_1.1.0
 [4] purrr_0.3.4         colorspace_1.4-1    vctrs_0.3.5
 [7] generics_0.0.2      htmltools_0.4.0     base64enc_0.1-3
[10] rlang_0.4.8         hexbin_1.28.1       pillar_1.4.4
[13] glue_1.4.0          withr_2.2.0         uuid_0.1-2
[16] lifecycle_0.2.0     munsell_0.5.0       gtable_0.3.0
[19] caTools_1.18.0      evaluate_0.14       labeling_0.3
[22] Cairo_1.5-10        vipor_0.4.5         IRdisplay_0.7.0
[25] Rcpp_1.0.4.6        KernSmooth_2.23-15  gdata_2.18.0
[28] IRkernel_1.0.2.9000 jsonlite_1.7.1      farver_2.0.3
[31] digest_0.6.25       stringi_1.4.6       dplyr_1.0.2
[34] tools_3.6.1         bitops_1.0-6        magrittr_1.5
[37] tibble_3.0.1        crayon_1.3.4        pkgconfig_2.0.3
[40] ellipsis_0.3.1      ggbeeswarm_0.6.0    R6_2.4.1
[43] compiler_3.6.1
```

In [ ]:
```

```