```
In [3]:
library('Seurat')
library('data.table')
library('Matrix')
library('tidyverse')
library('ggplot2')
library('ggpubr')
library('biomaRt')
library('cluster')
library('pheatmap')
library('glmGamPoi')
library(org.Hs.eg.db)
library(stringr)
```

```
Warning message:
“Strategy 'multiprocess' is deprecated in future (>= 1.20.0) [2020-10-30]. Instead, explicitly specify either 'multisession' (recommended) or 'multicore'. I
n the current R session, 'multiprocess' equals 'multicore'.”
```

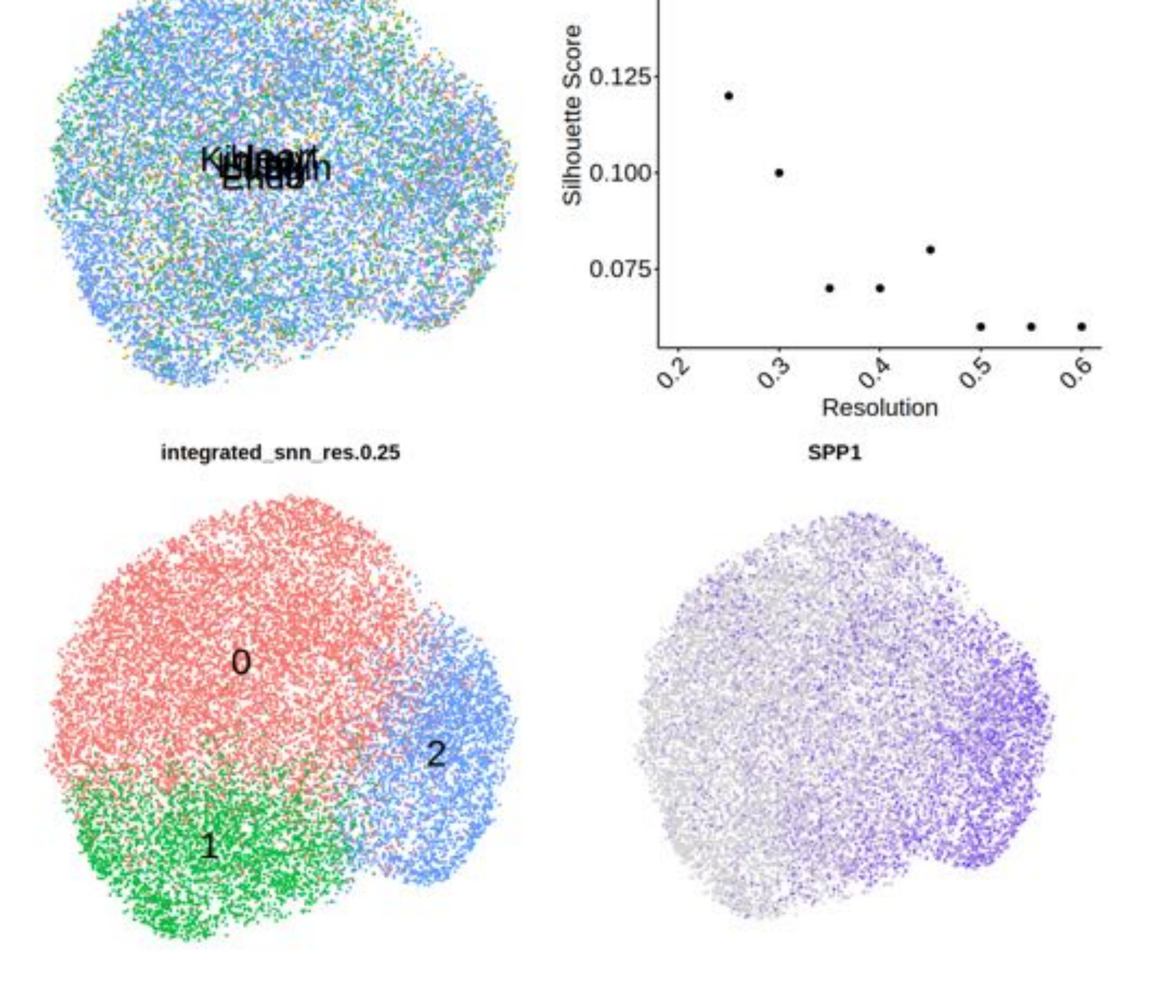
SPP1+ Mac matrix initialization

```
In [2]:
Skin_all <- readRDS('~/Skin_Code_Final.RDS')
Kidney_all <- readRDS( 'Kidney_Code_Final.RDS')
All_endo <- readRDS('~/Endo_Final_Code.RDS')
Lung_all <- readRDS('~/Lung_all_Feb_20.RDS')
All_Heart <- readRDS('~/Heart_Code.RDS')
Liver <- readRDS('~/Liver_cd45-.RDS')

transcriptome_SPP1 <- list(All_Heart, Liver, Lung_all, All_endo, Kidney_all, Skin_all)
organ <- c('Heart', 'Liver', 'Lung', 'Endo', 'Kidney', 'Skin')
extract <- c('Diagnosis', 'orig.ident', 'nCount_RNA', 'nFeature_RNA', 'ribo.ratio', 'heam.ratio', 'mito.ratio', 'cluster_d', 'Organ')
extract_liver <- c('Diagnosis', 'orig.ident', 'nCount_RNA', 'nFeature_RNA', 'ribo.ratio', 'heam.ratio', 'mito.ratio', 'cluster_d', 'Organ', 'Patient')

names(transcriptome_SPP1) <- organ
transcriptome_SPP1$Liver$orig.ident <- transcriptome_SPP1$Liver$Patient
for(i in 1:length(transcriptome_SPP1)) {
    transcriptome_SPP1[[i]]$Organ <- organ[[i]]

    if( i != 2 ) {
        transcriptome_SPP1[[i]]@meta.data <- transcriptome_SPP1[[i]]@meta.data[, extract]
    } else {
        transcriptome_SPP1[[i]]@meta.data <- transcriptome_SPP1[[i]]@meta.data[, extract_liver]
    }
    transcriptome_SPP1[[i]] <- RenameCells(object = transcriptome_SPP1[[i]], add.cell.id = paste0(organ[[i]], '_'))
}

common_g <- rownames(transcriptome_SPP1[[1]]$RNA@counts)
for(i in 2:length(transcriptome_SPP1)) {
    common_g <- intersect( common_g , rownames(transcriptome_SPP1[[i]]$RNA@counts) )
}

SPP1 <- list()
for(i in 1:length(transcriptome_SPP1)) {
    SPP1[[i]] <- transcriptome_SPP1[[i]][, transcriptome_SPP1[[i]]$cluster_d %in%  c('SPP1', 'TREM2')]
    DefaultAssay(SPP1[[i]]) <- 'RNA'
    SPP1[[i]] <- SPP1[[i]][ common_g, ]

}
```

Integrative SPP1+ macophage atlas

```
In [96]:
# for(i in 1:length(SPP1)) {
#     SPP1[[i]] <- SCTransform( SPP1[[i]],  vars.to.regress = c('orig.ident', 'mito.ratio'))
# }

# features <- SelectIntegrationFeatures(object.list = SPP1, nfeatures = 3000)


# SPP1 <- PrepSCTIntegration(object.list = SPP1, anchor.features = features)

# anchors <- FindIntegrationAnchors(object.list = SPP1, normalization.method = "SCT", anchor.features = features,  dims = 1:30)
# SPP1 <- IntegrateData(anchorset = anchors, normalization.method = "SCT")
# SPP1 <- RunPCA(SPP1)

# DefaultAssay( SPP1) <- "integrated"
# SPP1 <- FindNeighbors(SPP1, dims = 1:9, k.param = 60L)
# SPP1 <- FindClusters(SPP1, resolution = seq(from = 0.2, to = .6, by = .05))

# DefaultAssay(SPP1) <- 'integrated'
# resolution <- colnames( SPP1@meta.data )[ startsWith(x =colnames( SPP1@meta.data ), prefix = 'integrated_snn_res')]

# silhouetteee <- list()
# i <- 1
# dist.matrix <- dist(x = Embeddings(object = SPP1[['pca']])[,1:9])
# for(sil in sort(resolution)) {
#     s <- silhouette(x = as.numeric(x = as.factor(x = SPP1@meta.data[, sil])), dist = dist.matrix)
#     silhouetteee[[i]] <- summary(s)
#     i <- i + 1
# }

# sil_score <- c()
# for( sil in silhouetteee) {
#     sil_score <- c(sil_score, mean(sil$clus.avg.widths))
# }

# to_plot <- data.frame('Score' = sil_score[1: length(seq(from = 0.2, to = .6, by = .05))], 'Res' = seq(from = 0.2, to = .6, by = .05))
# to_plot$Score <- round(to_plot$Score,digits = 2)

p1 <- ggpubr::ggscatter(data = to_plot, x = 'Res', y = 'Score', xlab = 'Resolution', ylab = 'Silhouette Score') +
 rotate_x_text(angle = 45) +
   theme(text = element_text(size = 19), plot.title = element_text(hjust = 0.5))

# SPP1 <- RunUMAP(SPP1, dims = 1:9, verbose = F)

p3 <- DimPlot(SPP1, group.by = 'Organ', label = T, label.size = 10) + NoLegend() + NoAxes()
p2 <- DimPlot(SPP1, group.by = 'integrated_snn_res.0.25', label = T, label.size = 10) + NoLegend() + NoAxes()

DefaultAssay(SPP1) <- 'RNA'
p4 <-FeaturePlot(SPP1, 'SPP1') + NoLegend() + NoAxes()

options(repr.plot.width=12, repr.plot.height=12)
CombinePlots(list(p3, p1, p2, p4), ncol = 2)
```

```
Warning message:
“CombinePlots is being deprecated. Plots should now be combined using the patchwork system.”
Warning message:
“Graphs cannot be vertically aligned unless the axis parameter is set. Placing graphs unaligned.”
Warning message:
“Graphs cannot be horizontally aligned unless the axis parameter is set. Placing graphs unaligned.”
```



Signature of matrisome-associated macrophage (MAM)

```
In [21]:
# DefaultAssay(SPP1) <- 'RNA'
# Idents(SPP1) <- SPP1$integrated_snn_res.0.25

# i <- 1
# MAM.marker <- list()
# for( org in organ) {
#     SPP1_tmp <- SPP1[, SPP1$Organ == org]
#     DefaultAssay( SPP1_tmp) <- 'RNA'
#     SPP1_tmp <- NormalizeData(SPP1_tmp)
#     MAM.marker[[i]] <- FindMarkers( SPP1_tmp, ident.1 = 2, logfc.threshold = -Inf, min.pct = -Inf, min.diff.pct = -Inf)
#     i <- i + 1
# }

# MAM_FC_avg <-  MAM.marker[[1]]$avg_log2FC
# MAM_diff <-  MAM.marker[[1]]$pct.1 -  MAM.marker[[1]]$pct.2
# gene <- rownames( MAM.marker[[1]])

# for(i in 2:6) {
#     MAM.marker[[i]] <- MAM.marker[[i]][ gene, ]
#     MAM_FC_avg  <- cbind(MAM_FC_avg, MAM.marker[[i]][, 'avg_log2FC'])
#     MAM_diff  <- cbind(MAM_diff, MAM.marker[[i]]$pct.1 -  MAM.marker[[i]]$pct.2)
# }
# rownames(MAM_FC_avg) <- gene

# MAM_signature <- data.frame( 'log2_FC_avg' = rowMeans( MAM_FC_avg), 'Diff_prop' = rowMeans( MAM_diff))
# MAM_signature$Diff_prop_z <- ( MAM_signature$Diff_prop  - mean(MAM_signature$Diff_prop )) /
#        sd(MAM_signature$Diff_prop )

# MAM_signature_FINAL <- MAM_signature[ MAM_signature$Diff_prop_z > 1.645 & MAM_signature$log2_FC_avg > .25, ]
# MAM_signature_FINAL <- MAM_signature_FINAL[ order(MAM_signature_FINAL$log2_FC_av, decreasing = T),]

# SPP1.m <- FindMarkers( SPP1, ident.1 = 2, logfc.threshold = -Inf, min.pct = -Inf, min.diff.pct = -Inf)
# quantile(SPP1.m$p_val_adj)
```

Top 10 MAM markers by fold change (log2)

```
In [22]:
MAM_signature_FINAL <- MAM_signature_FINAL[ order(MAM_signature_FINAL$log2_FC_avg, decreasing = T),]
MAM_signature_FINAL[1:10, ]
```

A data.frame: 10 × 3

	log2_FC_avg	Diff_prop	Diff_prop_z
	<dbl>	<dbl>	<dbl>
SPP1	2.4085464	0.3800000	8.501538
MMP9	1.4932409	0.2016667	4.204475
LPL	1.2734028	0.3075000	6.754601
FABP5	1.1654772	0.1681667	3.397270
TIMP3	1.1136873	0.1258333	2.377220
MATK	1.0175471	0.3125000	6.875080
IL1RN	1.0167479	0.2603333	5.618088
CD36	0.9195636	0.2443333	5.232558
RGCC	0.9048941	0.1896667	3.915327
FBP1	0.9011744	0.2358333	5.027744

Gene-set pathway enrichment analysis of MAM

```
In [24]:
# GO <- rbind( clusterProfiler::read.gmt('/mnt/storage/projects/huang_macrophage/gene_set/Human/Pathways/c2.cp.v7.5.1.symbols.gmt.txt'),
#   clusterProfiler::read.gmt('/mnt/storage/projects/huang_macrophage/gene_set/Human/Pathways/h.all.v7.5.1.symbols.gmt.txt'))
# GO$prefix <- str_split_fixed(GO$term, pattern = '_', n = 2)[,1]
# GO <- GO[GO$prefix %notin% c( 'WP'), ]
# GO$prefix <- NULL
# Term <- c()
# term <- str_split_fixed(GO$term, pattern = '_', n = 20)
# for( i  in  1:dim(term )[1]) {
#     Term <- c(Term, str_trim(paste0(term[i, 2:20], collapse = ' ')))
# }
# GO$term <- Term


# MAM_signature <- MAM_signature[ order( MAM_signature$log2_FC_avg, decreasing = T), ]
# FC_avg <- MAM_signature$log2_FC_avg
# names(FC_avg) <- rownames(MAM_signature)
# FC_avg <- sort(FC_avg[!startsWith(names(FC_avg), prefix =  'PSM')], decreasing = T)


# set.seed(15)
# mac_1 <- clusterProfiler::GSEA(geneList= FC_avg, TERM2GENE = GO, pAdjustMethod = 'BH',
#                 minGSSize = 5, maxGSSize = 500, pvalueCutoff = 0.1, verbose = TRUE)
# mac_1@result  <- mac_1@result[ order(mac_1@result$NES, decreasing = T), ]
mac_1@result[, c('NES', 'p.adjust')][1:10, ]
```

A data.frame: 10 × 2

	NES	p.adjust
	<dbl>	<dbl>
EPH EPHRIN SIGNALING	2.199151	1.884900e-07
VIBRIO CHOLERAE INFECTION	2.199007	5.139473e-07
OXIDATIVE PHOSPHORYLATION	2.190857	1.009000e-08
DEGRADATION OF THE EXTRACELLULAR MATRIX	2.167248	7.019126e-07
RESPIRATORY ELECTRON TRANSPORT ATP SYNTHESIS BY CHEMIOSMOTIC COUPLING AND HEAT PRODUCTION BY UNCOUPLING PROTEINS	2.157020	9.692996e-08
RESPIRATORY ELECTRON TRANSPORT	2.143049	1.548861e-07
EXTRACELLULAR MATRIX ORGANIZATION	2.121180	1.205780e-08
PARKINSONS DISEASE	2.072704	2.961638e-06
L1CAM INTERACTIONS	2.069807	6.770453e-06
RECYCLING PATHWAY OF L1	2.065577	1.110113e-04

ECM and metabolic signature of MAM

```
In [90]:
# tmp <- mac_1@result[ mac_1@result$p.adjust < .05 &  mac_1@result$NES > 1.6, ][1:100,]
# rownames(tmp) <- 1:100

# met <- c(14,  26, 29, 30, 99, 97, 95, 91, 79, 72, 32, 51)
# fib <- c(1, 4, 7,17, 21, 22, 85,34, 35, 38, 44, 45, 49,  61 )

# met_sig <- unique(c(str_split_fixed( tmp[met, 'core_enrichment'], pattern = '\\/', n = 200)))
# fib_sig <- unique(c(str_split_fixed( tmp[fib, 'core_enrichment'], pattern = '\\/', n = 200)))

saveRDS(list(met_sig, fib_sig, MAM_signature, MAM_signature_FINAL), '~/MAM_sig_Feb_26.RDS')
```

SPP1+ MAM+ enriched matrisome processes (NABA lab, UIC)

```
In [33]:
NABA <- rbind( clusterProfiler::read.gmt('/mnt/storage/projects/huang_macrophage/gene_set/Human/Pathways/c2.cp.v7.5.1.symbols.gmt.txt'),
  clusterProfiler::read.gmt('/mnt/storage/projects/huang_macrophage/gene_set/Human/Pathways/h.all.v7.5.1.symbols.gmt.txt'))
NABA <- NABA[ str_split_fixed( NABA$term , pattern = '_', n = 2)[,1] == 'NABA',]
NABA$term <- as.character(NABA$term)
NABA <- NABA[ NABA$term %notin% c('NABA_MATRISOME', 'NABA_MATRISOME_ASSOCIATED', 'NABA_CORE_MATRISOME'), ]


mam_naba <- rownames( MAM_signature )[ MAM_signature$log2_FC_avg > .25 & MAM_signature$Diff_prop_z > 1.645]
mam_naba <- clusterProfiler::enricher(gene = mam_naba, TERM2GENE = NABA, pAdjustMethod = 'BH',  universe = rownames( MAM_signature),
                pvalueCutoff = 1)

mam_n_naba <- rownames( MAM_signature )[ MAM_signature$log2_FC_avg < -.25 & MAM_signature$Diff_prop_z < -1.975]
mam_n_naba <- clusterProfiler::enricher(gene = mam_n_naba, TERM2GENE = NABA, pAdjustMethod = 'BH',   universe = rownames( MAM_signature),
                pvalueCutoff = 1)

mam_naba@result[, c('qvalue','pvalue', 'geneID')]
```

A data.frame: 4 × 3

	qvalue	pvalue	geneID
	<dbl>	<dbl>	<chr>
NABA_ECM_REGULATORS	0.09265265	0.02934001	MMP9/TIMP3/FAM20C/TGM2/CTSL/MMP19/CD109/ST14/CTSZ/SERPINE1/ADAM9
NABA_ECM_AFFILIATED	0.28605479	0.18116804	SDC2/ANXA2/GPC4/ANXA11/CLEC5A/ANXA4/LGALS9
NABA_ECM_GLYCOPROTEINS	0.78791409	0.75804575	SPP1/EMILIN2/CRELD2
NABA_SECRETED_FACTORS	0.78791409	0.99802452	IL1RN/CCL2

SPP1+ MAM- enriched matrisome processes (NABA lab, UIC)

```
In [34]:
mam_n_naba@result[, c('qvalue','pvalue', 'geneID')]
```

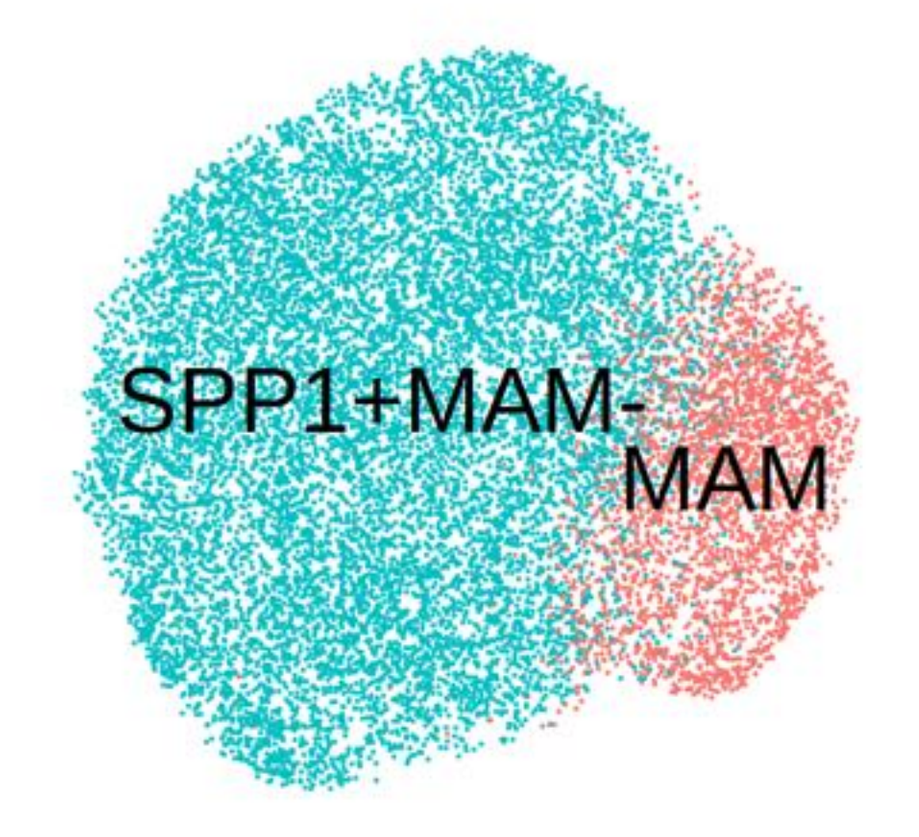
A data.frame: 4 × 3

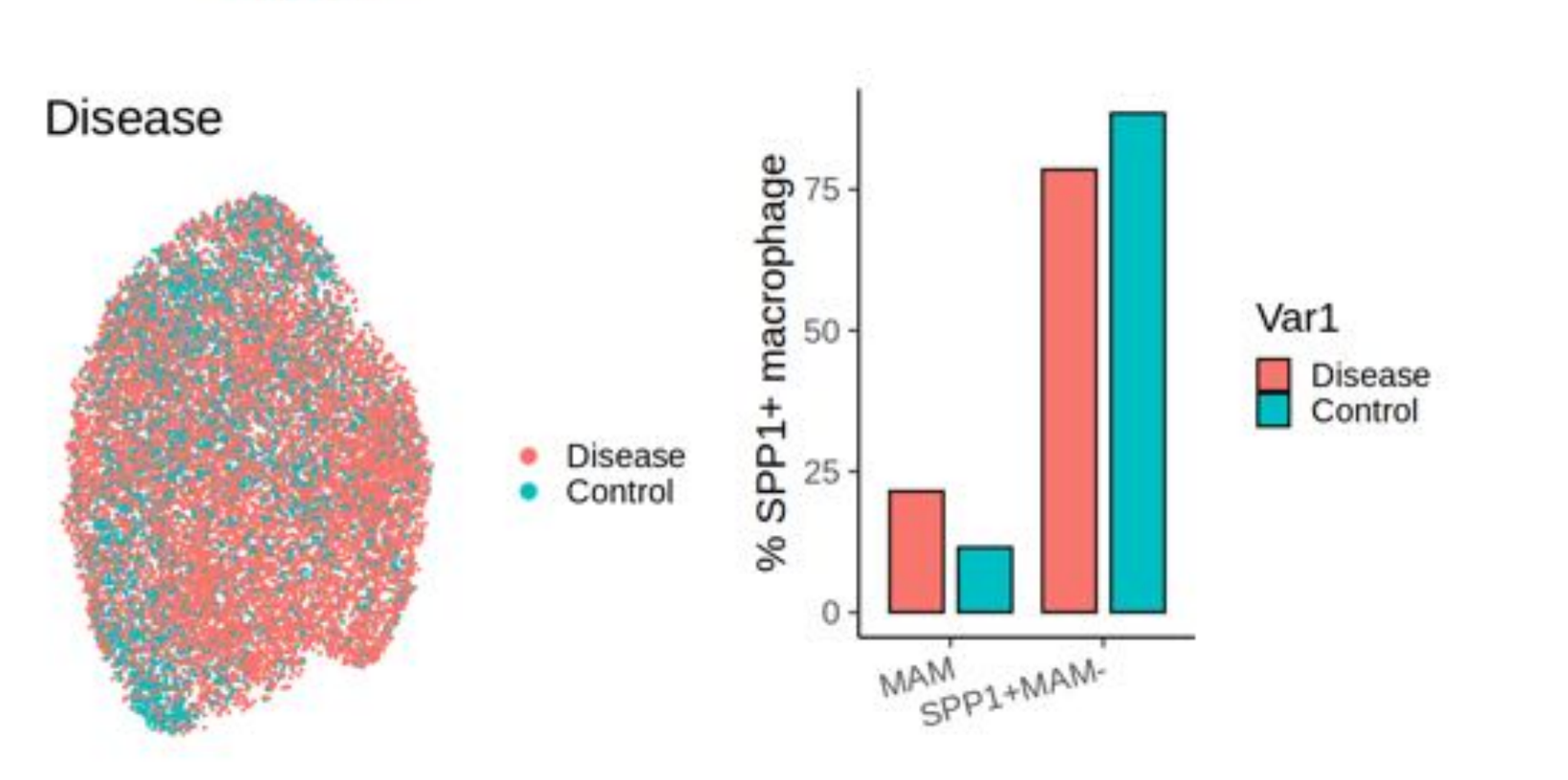
	qvalue	pvalue	geneID
	<dbl>	<dbl>	<chr>
NABA_ECM_AFFILIATED	0.1322600	0.04188233	CLEC12A/CLEC4E/CLEC7A/CLEC10A/C1QC/C1QB/C1QA
NABA_SECRETED_FACTORS	0.3596674	0.22778937	CXCL12/IL18/CXCL2/CXCL3/IL1B/CCL4L2/CXCL8
NABA_ECM_GLYCOPROTEINS	0.7702362	0.95554856	FGL2
NABA_ECM_REGULATORS	0.7702362	0.97563248	SERPINF1/SERPING1

MAM in disease v. control

```
In [35]:
SPP1$Disease <- SPP1$Diagnosis
SPP1$Disease[ SPP1$Disease!= 'Control'] <- 'Disease'
SPP1$Disease <- factor( SPP1$Disease, levels = c('Disease', 'Control'))

SPP1$MAM <- 'MAM'
SPP1$MAM[SPP1$integrated_snn_res.0.25 != 2 ] <- 'SPP1+MAM-'


p1 <- DimPlot(SPP1, group.by = 'Disease') + theme_classic( base_line_size = .5, base_size = 20) +  NoAxes()

to_plot <- melt(table(SPP1$Disease, SPP1$MAM) / rowSums(table(SPP1$Disease, SPP1$MAM)) * 100)
p2 <- ggbarplot(data = to_plot, x= 'Var2', y = 'value', fill = 'Var1', position = position_dodge(0.9)) +
    xlab('') + ylab('% SPP1+ macrophage') + theme_classic( base_line_size = .5, base_size = 20) +
    rotate_x_text(15)

options(repr.plot.width=6, repr.plot.height=6)
DimPlot(SPP1, group.by = 'MAM', label = T, label.size = 15) + NoLegend() + NoAxes()

options(repr.plot.width=10, repr.plot.height=5)
CombinePlots(list( p1, p2), ncol = 2)
```

```
Warning message in melt(table(SPP1$Disease, SPP1$MAM)/rowSums(table(SPP1$Disease, :
“The melt generic in data.table has been passed a table and will attempt to redirect to the relevant reshape2 method; please note that reshape2 is deprecate
d, and this redirection is now deprecated as well. To continue using melt methods from reshape2 while both libraries are attached, e.g. melt.list, you can p
repend the namespace like reshape2::melt(table(SPP1$Disease, SPP1$MAM)/rowSums(table(SPP1$Disease, SPP1$MAM)) *     100). In the next version, this warning
will become an error.”
Warning message:
“CombinePlots is being deprecated. Plots should now be combined using the patchwork system.”
Warning message:
“Graphs cannot be vertically aligned unless the axis parameter is set. Placing graphs unaligned.”
Warning message:
“Graphs cannot be horizontally aligned unless the axis parameter is set. Placing graphs unaligned.”
```





Significance of MAM macrophage in disease by over-representation test:

```
In [36]:
over_t <- phyper( sum( SPP1$Disease == 'Disease' & SPP1$MAM == 'MAM') -1, sum(SPP1$Disease == 'Disease'),
       length(SPP1$MAM ) -sum(SPP1$Disease == 'Disease'), sum(SPP1$MAM == 'MAM'),lower.tail= FALSE)

sprintf('p = %s', over_t)
```

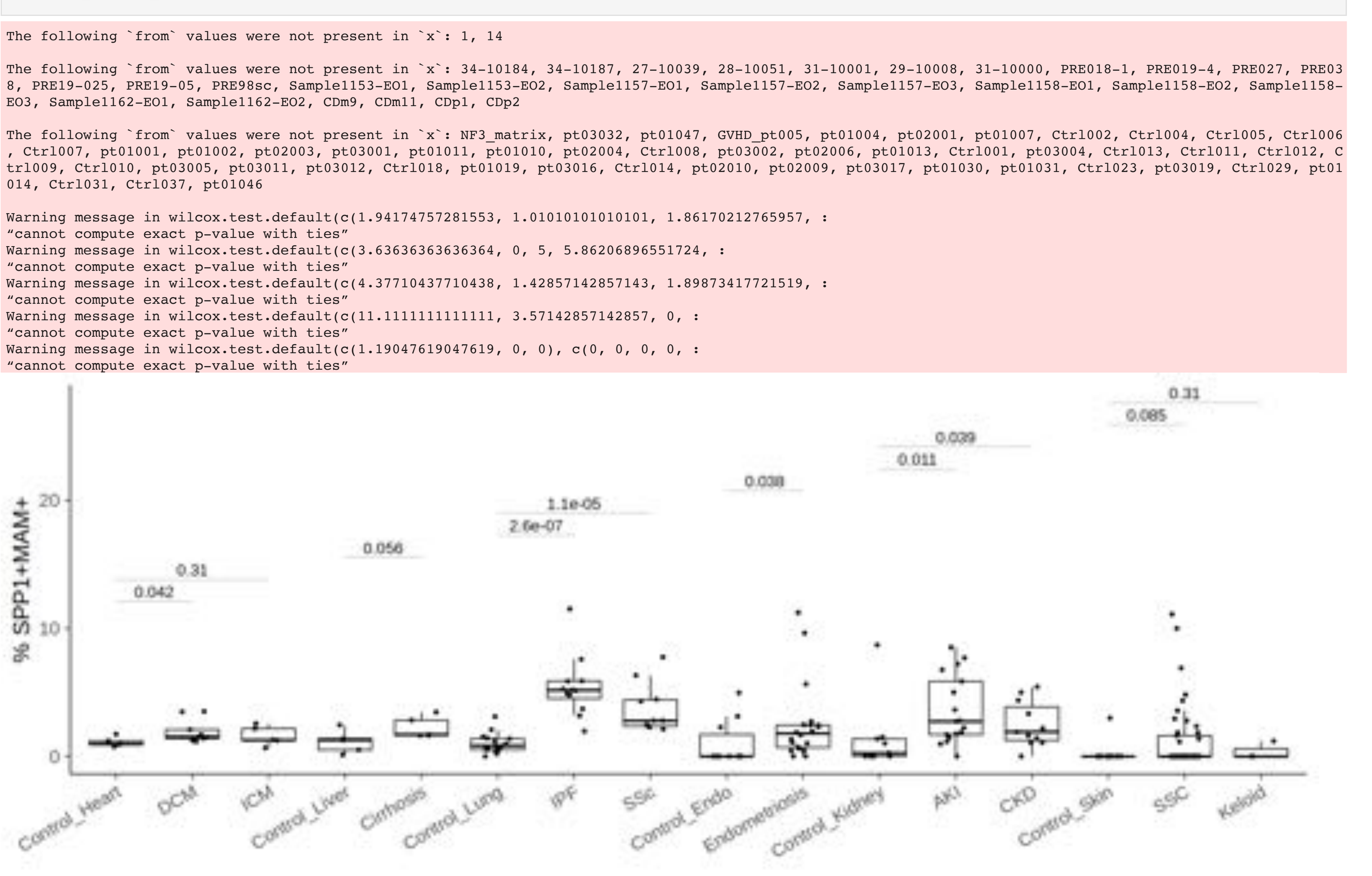
'p = 5.89171901698194e-45'

by Organ

```
In [97]:
SPP1$MAM <- 'MAM'
SPP1$MAM[SPP1$integrated_snn_res.0.25 != 2] <- 'SPP1+MAM-'
```

```
In [98]:
# for(i in 1:length(transcriptome_SPP1)) {
#     transcriptome_SPP1[[i]]$cluster_d <- as.character( transcriptome_SPP1[[i]]$cluster_d)
#     transcriptome_SPP1[[i]]$cluster_d [ colnames(transcriptome_SPP1[[i]]) %in% colnames(SPP1)[ SPP1$MAM == 'MAM']] <- 'MAM'

# }

to_plot <- list()
N <- c(0, 0, 0, 20, 20, 18)
D <- list( c('Control', 'DCM', 'ICM'), c('Control', 'Cirrhosis'), c('Control', 'IPF', 'SSc'),
        c('Control', 'Endometriosis'), c('Control', 'AKI', 'CKD'), c('Control', 'SSC', 'Keloid'))


D <- list( c('Control', 'DCM', 'ICM'), c('Control', 'Cirrhosis'), c('Control', 'IPF', 'SSc'),
        c('Control', 'Endometriosis'), c('Control', 'AKI', 'CKD'), c('Control', 'SSC', 'Keloid'))

for( i in 1: length(transcriptome_SPP1) ) {

    MAM <- (table( transcriptome_SPP1[[i]]$orig.ident, transcriptome_SPP1[[i]]$cluster_d) / rowSums(table(transcriptome_SPP1[[i]]$orig.ident, transcriptome_
    Diagnosis <-  plyr::mapvalues( names(table( transcriptome_SPP1[[i]]$orig.ident ))[ table(transcriptome_SPP1[[i]]$orig.ident) > N[[i]] ],
        from = transcriptome_SPP1[[i]]$orig.ident[ !duplicated( transcriptome_SPP1[[i]]$orig.ident )], to = transcriptome_SPP1[[i]]$Diagnosis[ !duplicated(t
    tmp <- data.frame('MAM' = MAM, 'Diagnosis' = Diagnosis)
    tmp <- tmp[ tmp$Diagnosis %in% D[[i]], ]
    tmp$Diagnosis[ tmp$Diagnosis == 'Control'] <- paste0(tmp$Diagnosis[ tmp$Diagnosis == 'Control'], '_', organ[[i]])

    if(i == 1) {
        all_pat <- tmp
    } else {
        all_pat <- rbind(all_pat, tmp)
    }
}

D <- c('Control_Heart', 'DCM', 'ICM','Control_Liver', 'Cirrhosis', 'Control_Lung', 'IPF', 'SSc',
 'Control_Endo', 'Endometriosis', 'Control_Kidney', 'AKI', 'CKD','Control_Skin', 'SSC', 'Keloid')

Com <- list( c('DCM', 'Control_Heart'), c('ICM', 'Control_Heart'),
            c('Cirrhosis', 'Control_Liver'), c('IPF', 'Control_Lung'), c('SSc', 'Control_Lung'),
            c('Endometriosis', 'Control_Endo'), c('AKI', 'Control_Kidney'), c('CKD', 'Control_Kidney'),
            c('SSC', 'Control_Skin'), c('Keloid', 'Control_Skin'))

all_pat$Diagnosis <- factor( all_pat$Diagnosis, levels = D)
all_pat$MAM <- 100 * all_pat$MAM

options(repr.plot.width=20, repr.plot.height=8)
ggboxplot( data = all_pat, x = 'Diagnosis', y = 'MAM',  add = "jitter" ) +
    theme_classic(base_line_size = .5, base_size = 25) + ylab('% SPP1+MAM+') + rotate_x_text(30) + xlab('')  +
    stat_compare_means( comparisons = Com, method = 'wilcox.test', size = 6, bracket.size = 0.1, tip.length = 0, step.increase = 0.15)
```

```
The following `from` values were not present in `x`: 1, 14

The following `from` values were not present in `x`: 34-10184, 34-10187, 27-10039, 28-10051, 31-10001, 29-10008, 31-10000, PRE018-1, PRE019-4, PRE027, PRE03
8, PRE19-025, PRE19-05, PRE98sc, Sample1153-EO1, Sample1153-EO2, Sample1157-EO1, Sample1157-EO2, Sample1157-EO3, Sample1158-EO1, Sample1158-EO2, Sample1158-
EO3, Sample1162-EO1, Sample1162-EO2, CDm9, CDm11, CDp1, CDp2

The following `from` values were not present in `x`: NF3_matrix, pt03032, pt01047, GVHD_pt005, pt01004, pt02001, pt01007, Ctrl002, Ctrl004, Ctrl005, Ctrl006
, Ctrl007, pt01001, pt01002, pt02003, pt03001, pt01011, pt01010, pt02004, Ctrl008, pt03002, pt02006, pt01013, Ctrl001, pt03004, Ctrl013, Ctrl011, Ctrl012, C
trl009, Ctrl010, pt03005, pt03011, pt03012, Ctrl018, pt01019, pt03016, Ctrl014, pt02010, pt02009, pt03017, pt01030, pt01031, Ctrl023, pt03019, Ctrl029, pt01
014, Ctrl031, Ctrl037, pt01046

Warning message in wilcox.test.default(c(1.94174757281553, 1.01010101010101, 1.86170212765957, :
“cannot compute exact p-value with ties”
Warning message in wilcox.test.default(c(3.63636363636364, 0, 5, 5.86206896551724, :
“cannot compute exact p-value with ties”
Warning message in wilcox.test.default(c(4.37710437710438, 1.42857142857143, 1.89873417721519, :
“cannot compute exact p-value with ties”
Warning message in wilcox.test.default(c(11.1111111111111, 3.57142857142857, 0, :
“cannot compute exact p-value with ties”
Warning message in wilcox.test.default(c(1.19047619047619, 0, 0), c(0, 0, 0, 0, :
“cannot compute exact p-value with ties”
```




```
In [46]:
#saveRDS( SPP1, '~/MAM_FEB_25_2023.RDS')
# saveRDS(transcriptome_SPP1, '~/Transcriptome_SPP1_Feb_26.RDS')
```

```
Error in saveRDS(transcriptome_SPP1, "~/Transcriptome_SPP1_Feb_26.RDS"): error writing to connection
Traceback:

1. saveRDS(transcriptome_SPP1, "~/Transcriptome_SPP1_Feb_26.RDS")
```

```
In [ ]:
```