A multi-omics molecular landscape of 30 tissues in aging rhesus macaques(Macaca mulatta)

Gong-Hua Li

2023-11-02

Contents

1. setwd and env **2**

2. load data **2**

3. quality control **3**

4. set colors **4**

5. Figure 1: overall data distribution **6**
5.1 Figure 1A flowchart . . . 6
5.2 Figure 1B . . . 6
5.3 Figure 1C_1E . . . 8

6 Figure 2: tissue aging DEGs and GO **10**
6.1 protein . . . 12
6.2 mRNA . . . 15
6.3 metabolism . . . 18
6.4 Figure 2A perc changed mols each tissue . . . 21
6.5 Figure 2B GO enrichment . . . 23

7 Figure 3 clustering aging type **25**
7.1 mfuzz promet tissues . . . 25
7.2 Figure 3A whole body mfuzz . . . 27
7.3 Figure 3B Go enrichment . . . 27
7.4 mfuzz for each tissue . . . 29

8 Figure 4 cluster by MAA **35**
8.1 Figure 4A . . . 35
8.2 Figure 4B . . . 38
8.3 Figure 4C . . . 39
8.4 Figure 4D . . . 42

9 Figure 5 experimental validation **43**
9.1 Figure 5B . . . 44
9.2 Figure 5C . . . 46
9.3 Figure 5D . . . 47

10 Figure 6: Translation efficiency **48**
10.1 ratio data construction . . . 48

10.2 Figure 6A design . . . 50
10.3 Figure S9 plot mRNA and protein . . . 50
10.4 Figure S10 predict protein vs measured protein . . . 51
10.5 Figure 6B density plot . . . 51
10.6 Figure 6C ratio plot . . . 53
10.7 Figure 6D correlation . . . 54

11 Figure 7 **56**
11.1 GO data construct . . . 56
11.2 Figure 7A . . . 58
11.3 Figure 7B . . . 61
11.4 Figure 7C . . . 63

1. setwd and env

```
library(ggplot2)
library(reshape2)
#library(Rmisc)
library(Hmisc)
library(EnhancedVolcano)
library(pracma)
library(car)
library(ggrepel)
library(edgeR)
library(grid)
library(gridExtra)
library(Mfuzz)
library(M3C)
library(preprocessCore)
library(rlist)
library(ggsci)
library(scales)
library(data.table)
library(RColorBrewer)
library(readxl)
#setwd("/home/ligh/github/code_forpublication/macaca_multiple_tissue/")
source('./subroutines.R')
source('./subroutines_for_MCMT_aging.R')
```

2. load data

```
#whole body data
load('./data/pro.whole.fdr0.01_v20210108_from_NOVO_remap_solid_tissues.Rdata')
load('./data/mrna.whole_v20210108_solid_tissues.Rdata')
load('./data/met_whole_from_novo_v20210108_solid_tissues.Rdata')

# tissue data
load('./data/pro.tissues_v20210108_solid_tissues.Rdata')
load('./data/mrna.tissues_v20210108_solid_tissues.Rdata')
load('./data/met.tissues_v20210108_solid_tissues_new.Rdata')
```

```
load('./data/met.header.all.hmdb_curated.Rdata')
idx = !is.na(met.header.all.hmdb$hmdbid_highconfidence)
met.header.all.hmdb.v = met.header.all.hmdb[idx,]
```

3. quality control

```
# protein remove outliners
tmp = pro.whole
pp = prcomp(t(tmp),cor=F)
outlinerids = c()
thetissues = unique(pro.whole.info$tissue_en)
for(i in 1:length(thetissues)){
    idx = pro.whole.info$tissue_en == thetissues[i]
    tmpinfo = pro.whole.info[idx,]
    outx = is.outliner(pp$x[idx,1])
    outy = is.outliner(pp$x[idx,2])
    #outy = is.outliner(pp$x[idx,2],coef = 3)
    if(sum(outx | outy) > 0){
        outlinerids = c(outlinerids,rownames(tmpinfo)[outx | outy])
    }
}
outlinerids
```

```
##  [1] "X06080_Skin_of_back"   "X11062_Pituitary"      "X94356_Liver"
##  [4] "X12092_Thyroid_gland"  "X06080_Thyroid_gland"  "X94356_Cecum"
##  [7] "X16002_Adrenal_gland"  "X92338_Adrenal_gland"  "X12390_Fallopian_tube"
## [10] "X16068_Hypothalamus"   "X06070_Pancreas"       "X16086_Uterus"
```

```
vid = !is.element(colnames(pro.whole),outlinerids)
pro.whole = pro.whole[,vid]
pro.whole.info = pro.whole.info[vid,]
```

```
# met remove outliners
tmp = met.whole
pp = prcomp(t(tmp),cor=F)
outlinerids = c()
thetissues = unique(met.whole.info$tissue_en)
for(i in 1:length(thetissues)){
    idx = met.whole.info$tissue_en == thetissues[i]
    tmpinfo = met.whole.info[idx,]
    outx = is.outliner(pp$x[idx,1])
    outy = is.outliner(pp$x[idx,2])
    #outy = is.outliner(pp$x[idx,2],coef = 3)
    if(sum(outx | outy) > 0){
        outlinerids = c(outlinerids,rownames(tmpinfo)[outx | outy])
    }
}
outlinerids
```

```
## NULL
```

```
vid = !is.element(colnames(met.whole),outlinerids)
met.whole = met.whole[,vid]
met.whole.info = met.whole.info[vid,]
```

```
# mrna remove outliners
tmp = mrna.whole
pp = prcomp(t(tmp),cor=F)
outlinerids = c()
thetissues = unique(mrna.whole.info$tissue_en)
for(i in 1:length(thetissues)){
    idx = mrna.whole.info$tissue_en == thetissues[i]
    tmpinfo = mrna.whole.info[idx,]
    outx = is.outliner(pp$x[idx,1])
    outy = is.outliner(pp$x[idx,2])
    #outy = is.outliner(pp$x[idx,2],coef = 3)
    if(sum(outx | outy) > 0){
        outlinerids = c(outlinerids,rownames(tmpinfo)[outx | outy])
    }
}
outlinerids
```

```
## NULL
```

```
vid = !is.element(colnames(mrna.whole),outlinerids)
mrna.whole = mrna.whole[,vid]
mrna.whole.info = mrna.whole.info[vid,]
```

4. set colors

```
alltissues = names(pro.tissues)

tissue.systems = c('Integumentary','Endocrine','Brain','Respiratory','Digestive',
             'Cardiovascular','Cardiovascular','Brain','Digestive','Endocrine',
             'Cardiovascular','Muscle','Reproductive','Digestive','Brain',
             'Immune','Renal','Endocrine','Digestive','Reproductive',
             'Digestive','Brain','Muscle','Immune','Integumentary','Endocrine',
            'Brain','Immune','Cardiovascular','Reproductive')
names(tissue.systems) = alltissues
tissue.color = pal_npg()(10)
names(tissue.color) = levels(factor(tissue.systems))

tissue.systems
```

```
##            Skin_of_back              Pituitary            Frontal_pole
##         "Integumentary"             "Endocrine"                 "Brain"
##                   Lung                   Liver        Arteria_cruralis
##           "Respiratory"             "Digestive"        "Cardiovascular"
##           Femoral_vein             Hippocampus                Ileocecum
##        "Cardiovascular"                 "Brain"              "Digestive"
##           Thyroid_gland         Arteria_carotis                  Muscle
##             "Endocrine"        "Cardiovascular"                "Muscle"
##                   Ovary                   Cecum Superior_temporal_gyrus
##          "Reproductive"             "Digestive"                 "Brain"
```

```
##                  Spleen                   Kidney           Adrenal_gland
##                "Immune"                 "Renal"              "Endocrine"
##                Duodenum           Fallopian_tube                  Stomach
##             "Digestive"           "Reproductive"              "Digestive"
##            Hypothalamus                    Heart                   Thymus
##                 "Brain"                 "Muscle"                 "Immune"
##             Facial_skin                 Pancreas      Supramarginal_gyrus
##          "Integumentary"              "Endocrine"                  "Brain"
##                 Adipose              Aortic_arch                   Uterus
##                "Immune"         "Cardiovascular"           "Reproductive"
```

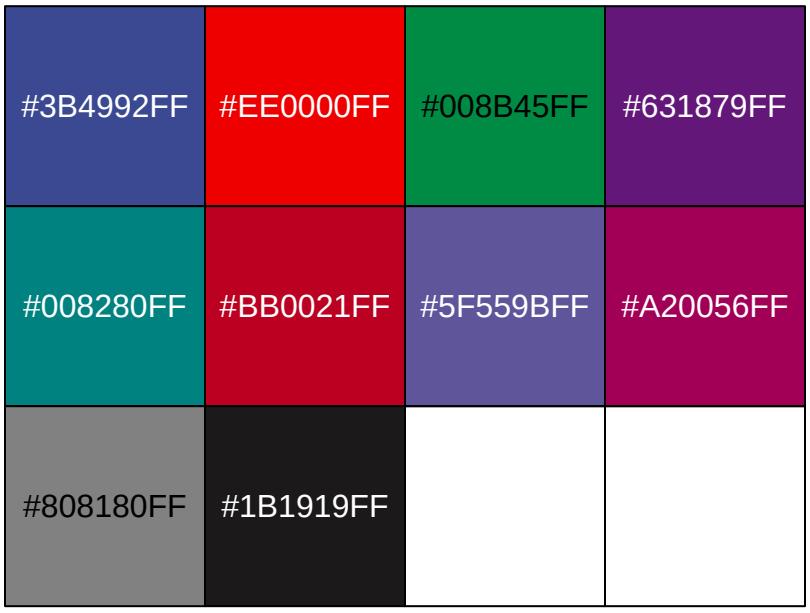
```
tissue.color
```

```
##           Brain Cardiovascular       Digestive       Endocrine          Immune
##     "#E64B35FF"     "#4DBBD5FF"     "#00A087FF"     "#3C5488FF"     "#F39B7FFF"
##  Integumentary          Muscle           Renal    Reproductive     Respiratory
##     "#8491B4FF"     "#91D1C2FF"     "#DC0000FF"     "#7E6148FF"     "#B09C85FF"
```

```
mypal = pal_aaas()(10)
mypal
```

```
##  [1] "#3B4992FF" "#EE0000FF" "#008B45FF" "#631879FF" "#008280FF" "#BB0021FF"
##  [7] "#5F559BFF" "#A20056FF" "#808180FF" "#1B1919FF"
```

```
show_col(mypal)
```



5. Figure 1: overall data distribution

5.1 Figure 1A flowchart

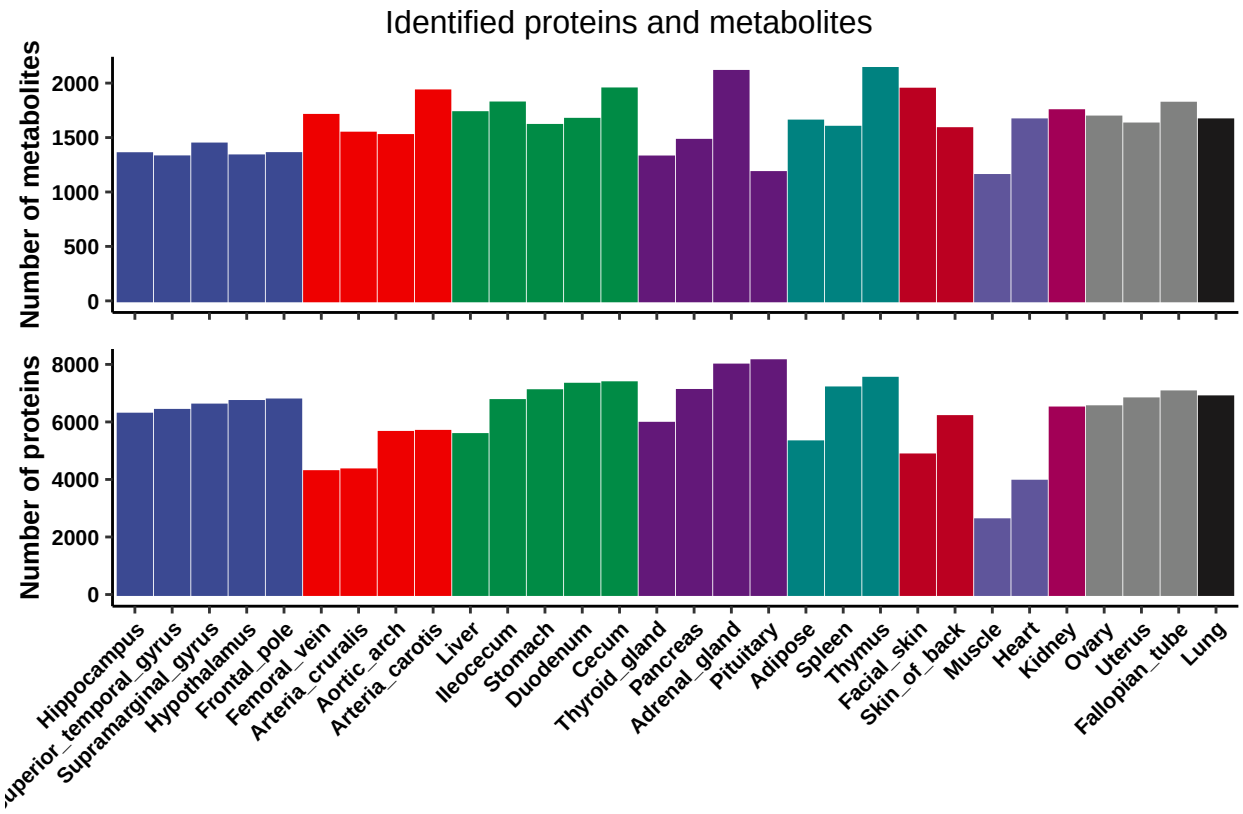
5.2 Figure 1B

```
num_omics = data.frame(num_mrna = rep(0,length(alltissues)),stringsAsFactors = F,
                       tissues = alltissues,
                       tissue_systems = tissue.systems,
                      num_protein = rep(0,length(alltissues)),
                       num_met = rep(0,length(alltissues))
                       )
for(i in 1:length(alltissues)){
    num_omics$num_mrna[i] = nrow(mrna.tissues[[alltissues[i]]])
    num_omics$num_protein[i] = nrow(pro.tissues[[alltissues[i]]])
    num_omics$num_met[i] = nrow(met.tissues[[alltissues[i]]])
}
rownames(num_omics) = alltissues
```

```
idx = sort.int(num_omics$num_protein,decreasing = F,index.return = T)$ix
num_omics.v = num_omics[idx,]
idx = sort.int(num_omics.v$tissue_systems,decreasing = F,index.return = T)$ix
num_omics.v = num_omics.v[idx,]
writetxt(num_omics.v[,c(2,3,1,4,5)],'./out/20230217_aging/Figure1_Overall_data_distribution/Figure_1B_nu
```

```
idx = sort.int(num_omics$num_protein,decreasing = F,index.return = T)$ix
num_omics.v = num_omics[idx,]
idx = sort.int(num_omics.v$tissue_systems,decreasing = F,index.return = T)$ix
num_omics.v = num_omics.v[idx,]

pomics = list()
pomics[[1]] = ggplot(num_omics.v,aes(x = factor(tissues,level = tissues),
                                     y = num_met,
                        color = tissue_systems,fill = tissue_systems))+
  geom_bar(stat="identity")+
theme_classic()+lghplot.addtheme(legend.position = 'none',hjust = 1,size = 10)+
  scale_color_aaas()+scale_fill_aaas()+
 theme(axis.text.x=element_blank(),axis.title.x=element_blank())+
  ylab('Number of metabolites')


pomics[[2]] = ggplot(num_omics.v,aes(x = factor(tissues,level = tissues),
                                     y = num_protein,
                        color = tissue_systems,fill = tissue_systems))+
  geom_bar(stat="identity")+
theme_classic()+lghplot.addtheme(legend.position = 'none',hjust = 1,size = 10)+
  scale_color_aaas()+scale_fill_aaas()+
ylab('Number of proteins')+ xlab('')

#pdf(file = './out/20230217_aging/Figure1_Overall_data_distribution/
#    Figure1B_number_of_omicsV__promet.pdf',width = 7,height = 5)
grid.arrange(arrangeGrob(grobs = pomics,ncol = 1,
                         top = 'Identified proteins and metabolites',
```

```
                    heights = c(3.2,5.8)))
```




```
#dev.off()
```

```
# total identified molecules
vmrna = c()
for (i in 1:length(mrna.tissues)){
    vmrna = unique(c(vmrna,rownames(mrna.tissues[[i]])))
}
length(vmrna)
```

```
## [1] 16614
```

```
vproteins = c()
for (i in 1:length(pro.tissues)){
    vproteins = unique(c(vproteins,pro.tissues.header[[i]]$Gene))
}
length(vproteins)
```

```
## [1] 14295
```

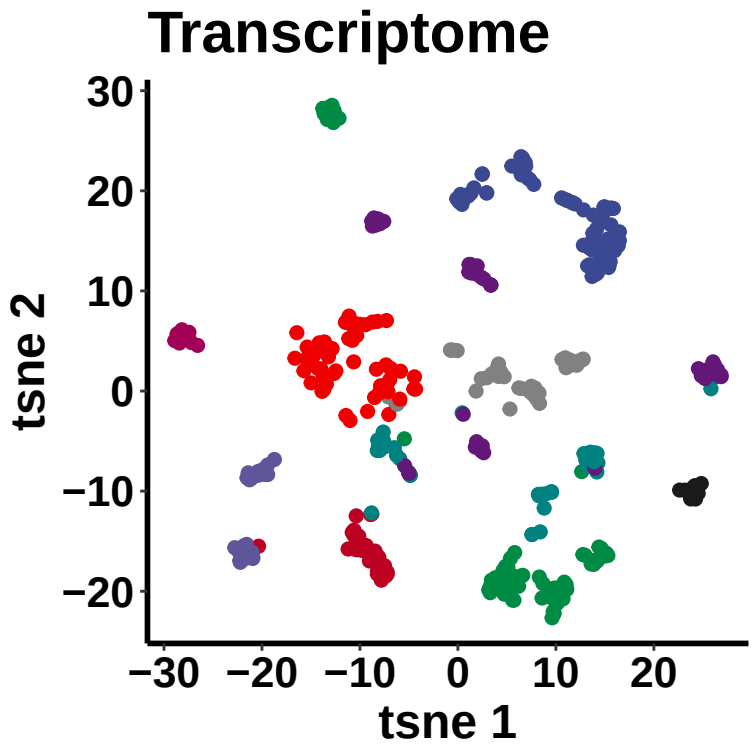
```
vmets = c()
for (i in 1:length(met.tissues)){
    vmets = unique(c(vmets,rownames(met.tissues[[i]])))
}
length(vmets)
```

```
## [1] 5789
```

5.3 Figure 1C_1E

5.3.1 Figure 1C mRNA

```
### for mRNA
mrna.whole.std = standardise_matrix(mrna.whole)

p = tsne(mrna.whole.std,labels=tissue.systems[mrna.whole.info$tissues],
         legendtextsize = 10,dotsize = 2)
p = p + theme_classic()+lghplot.addtheme(legend.position = 'none')+
  scale_color_aaas()+ xlab('tsne 1') + ylab('tsne 2')+
theme(axis.line = element_line(size = 1.0)) + ggtitle('Transcriptome')
#pdf(file ="./out/20230217_aging/Figure1_Overall_data_distribution/
#    Figure1C_tSNE_mrna_using_standardised.pdf",height = 4,width = 4)
print(p)
```




```
#dev.off()
```

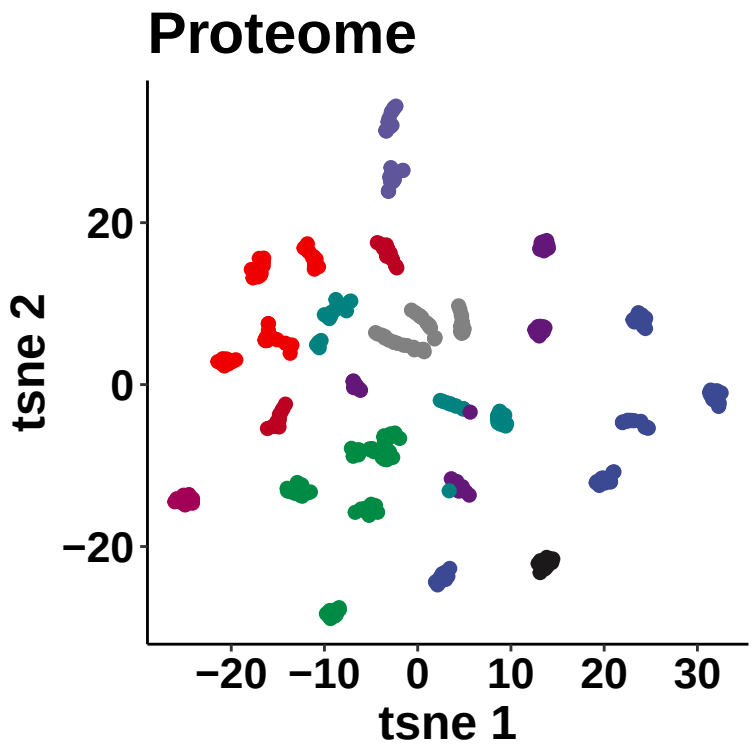
5.3.2 Figure 1D protein

```
ida = rowSums(pro.whole < 0.1) < 0.2 * ncol(pro.whole)
sum(ida)
```

```
## [1] 3087
```

```
pro.whole.cons = pro.whole[ida,]
pro.whole.std = standardise_matrix(pro.whole.cons)

p = tsne(pro.whole.std,labels=tissue.systems[pro.whole.info$tissue_en],
```

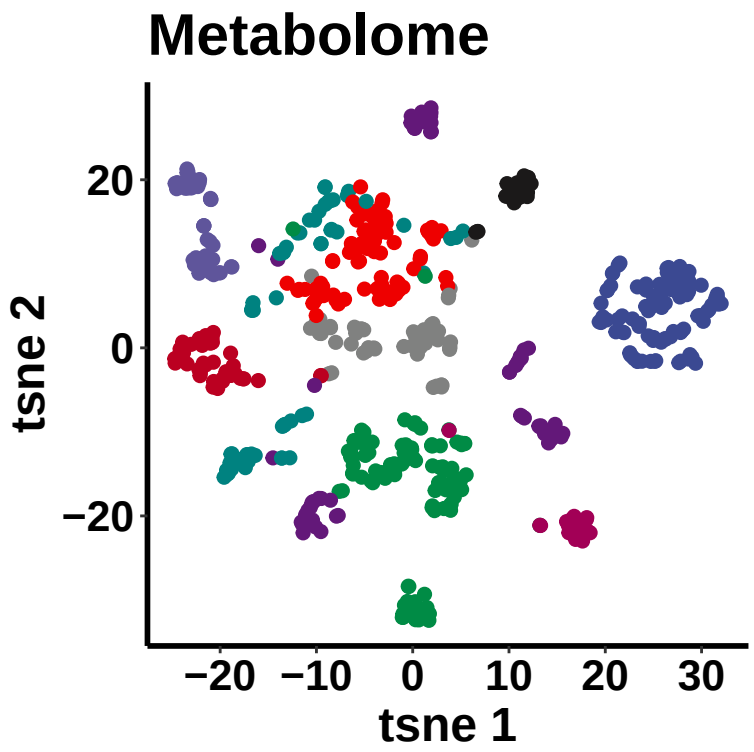
```
        legendtextsize = 10,dotsize = 2)
p = p + theme_classic()+lghplot.addtheme(legend.position = 'none')+
  scale_color_aaas()+ xlab('tsne 1') + ylab('tsne 2') + ggtitle('Proteome')
#pdf(file ="./out/20230217_aging/Figure1_Overall_data_distribution/
#    Figure1D_tSNE_protein_using_standardised.pdf",height = 4,width = 4)
print(p)
```




```
#dev.off()
```

5.3.3 Figure 1E metabolism

```
met.whole.std = standardise_matrix(met.whole)
p = tsne(met.whole.std,labels=tissue.systems[met.whole.info$Tissues],
         legendtextsize = 10,dotsize = 2)
p = p + theme_classic()+lghplot.addtheme(legend.position = 'none')+
  scale_color_aaas()+ xlab('tsne 1') + ylab('tsne 2')+
theme(axis.line = element_line(size = 1.0)) + ggtitle('Metabolome')
#pdf(file ="./out/20230217_aging/Figure1_Overall_data_distribution/
#    Figure1E_tSNE_met_using_std.pdf",height = 4,width = 4)
print(p)
```




```
#dev.off()
```

6 Figure 2: tissue aging DEGs and GO

```
# sub routine plot volcano
plot_Volcano <- function(res2, title){


  tmpup = res2$Pvalue
  tmpup[is.na(tmpup)] = 1
  tmpup[res2$log2FC < 0] = 1
  sortid_up = sort.int(-log10(tmpup),decreasing = T,index.return = T)$ix
  tmpid.up = res2$ID[sortid_up[1:5]]

  tmpdown = res2$Pvalue
  tmpdown[is.na(tmpdown)] = 1
  tmpdown[res2$log2FC > 0] = 1
  sortid_down = sort.int(-log10(tmpdown),decreasing = T,index.return = T)$ix
  tmpid.down = res2$ID[sortid_down[1:5]]

  vid = is.element(res2$ID,c(tmpid.up,tmpid.down))
  tlab = res2$ID
  tlab[!vid] = NA

  keyvals <- rep('gray50', nrow(res2))
  names(keyvals) <- rep('NS', nrow(res2))
```

```
  keyvals[which(res2$log2FC > 0.58 & res2$Pvalue < 0.05)] <- "Brown"
  names(keyvals)[which(res2$log2FC > 0.58 & res2$Pvalue < 0.05)] <- 'High'

  keyvals[which(res2$log2FC < -0.58 & res2$Pvalue < 0.05)] <- "darkblue"
  names(keyvals)[which(res2$log2FC < -0.58 & res2$Pvalue < 0.05)] <- 'Low'
  p = EnhancedVolcano(res2,
                        lab = tlab,
                        x = 'log2FC',
                        y = 'Pvalue',
                        caption = NULL,
                        title = title,
                        border = 'full',
                        titleLabSize = 12,
                        FCcutoff = 0.58,
                        cutoffLineWidth = F,
                        axisLabSize = 12,
                        subtitle = NULL,
                        cutoffLineCol = 'white',
                        gridlines.minor = F,
                        gridlines.major = F,
                        xlab = bquote(~Log[2]~ 'fold change'),
                        pCutoff = 0.05,
                        colCustom = keyvals,
                        colAlpha = 4/5,
                        legendPosition = 'none',
                        legendLabSize = 5,
                        legendIconSize = 3,
                        drawConnectors = TRUE,
                        widthConnectors = 0.5,
                        pointSize = -0.3*log10(res2$Pvalue),labSize = 3,
                        colConnectors = 'black')
    return(p)
}


list_to_matrix <- function(DEproFC,alltissues){
    DEproFC_matrix = list()
    for(i in 1:length(alltissues)){
        tmp = matrix(DEproFC[[i]],1,length(DEproFC[[i]]))
        tmp = as.data.frame(tmp)
        colnames(tmp) = names(DEproFC[[i]])
        DEproFC_matrix[[i]] = tmp
    }
    DEproFC_matrix = t(as.matrix(rbindlist(DEproFC_matrix,fill = T)))
    colnames(DEproFC_matrix) = names(DEproFC)
    #vid = rowSums(is.na(DEproFC_matrix)) < ncol(DEproFC_matrix)/2
    #DEproFC_matrix = DEproFC_matrix[vid,]
    return(DEproFC_matrix)
}

met.class_enrichment <- function(mets,annote){
  require(clusterProfiler)
```

```
  vmet = intersect(mets,rownames(annote))
  fluxgmt = data.frame(ont = annote$sub_class,
                       gene = rownames(annote),stringsAsFactors = F)

  Recon3D <- enricher(gene = vmet,
                TERM2GENE=fluxgmt,
                pAdjustMethod = "BH",
                minGSSize = 1,
                pvalueCutoff =1,
                qvalueCutoff = 1
                )
  Recon3Dout = Recon3D@result
  Recon3Dout$DB = rep('HMDBclass',dim(Recon3Dout)[1])
  n = dim(Recon3Dout)[2]
  Recon3Dout = Recon3Dout[,c(n,1:(n-1))]
  return(Recon3Dout)
}
```

6.1 protein

```
DEproFC = list()
DEproPvalue = list()
DEproAging = list()
DEproFC.develop = list()
DEproPvalue.develop = list()
for(i in 1:length(alltissues)){
    #Mfuzz
    thistissue = alltissues[i]
    thispro = pro.tissues[[thistissue]]
    thispro.header = pro.tissues.header[[thistissue]]
    thispro = delete_dup_genes_forprotein(thispro,pro.tissues.header[[thistissue]])
    thispro.info = pro.tissues.info[[thistissue]]
    thisDEpro = DEGenes.simplified(thispro,catagory = thispro.info$stage == 4,
                    subset = thispro.info$stage == 4 | thispro.info$stage == 1)
    thisDEpro.develop = DEGenes.simplified(thispro,catagory = thispro.info$stage == 2,
                    subset = thispro.info$stage == 2 | thispro.info$stage == 1)
    forsort = thisDEpro$Pvalue
    forsort[is.na(forsort)] = 1
    idx = sort.int(forsort,decreasing = F,index.return = T)$ix
    DEproAging[[i]] = thisDEpro[idx,-5]
    DEproAging[[i]]$log2FC.devControl = thisDEpro.develop$log2FC
    DEproAging[[i]]$Pvalue.devControl = thisDEpro.develop$Pvalue

    DEproFC[[i]] = thisDEpro$log2FC
    names(DEproFC[[i]]) = rownames(thisDEpro)
    DEproPvalue[[i]] = thisDEpro$Pvalue
    names(DEproPvalue[[i]])  = rownames(thisDEpro)

    DEproFC.develop[[i]] = thisDEpro.develop$log2FC
    names(DEproFC.develop[[i]]) = rownames(thisDEpro.develop)
    DEproPvalue.develop[[i]] = thisDEpro.develop$Pvalue
    names(DEproPvalue.develop[[i]])  = rownames(thisDEpro.develop)
```

```
}
names(DEproAging) = alltissues
names(DEproFC) = alltissues
names(DEproPvalue) = alltissues
names(DEproFC.develop) = alltissues
names(DEproPvalue.develop) = alltissues
```

```
proteinVoconoPlot = list()
names.DEproAging = names(DEproAging)
for(i in 1:length(DEproAging)){
    res2 = DEproAging[[i]]

    proteinVoconoPlot[[i]] = plot_Volcano(res2,names.DEproAging[i])
}
```

```
pdf(file = './out/20230217_aging/Figure2_DEG_GO_tissue/
    Figure2A_DEpro_each_tissueV1.pdf',width = 3*5+1,height =3*6)
grid.arrange(arrangeGrob(grobs = proteinVoconoPlot,ncol = 5))
dev.off()
```

```
## pdf
##   2
```

```
#Data S2
openxlsx::write.xlsx(DEproAging, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S2_DE_tissue_Ag

# reducce size Data S2
tpath = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S2_DE_tissue_Aging_pro.xlsx"
sheetNames = openxlsx::getSheetNames(tpath)
xx = list()
for(i in 1:length(sheetNames)){
    tmp = openxlsx::read.xlsx(tpath,sheet = sheetNames[i])
    tmp[c(2,3,4,7,8)] = signif(tmp[c(2,3,4,7,8)],3)
    tmp = tmp[,-c(5,6)]
    xx[[i]] = tmp
}
names(xx) = sheetNames
openxlsx::write.xlsx(xx, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/reduce_Data S2_DE_tissue_Ag
```

```
# num up and down proteins
DEproFC_matrix = list_to_matrix(DEproFC,alltissues)
DEproPvalue_matrix = list_to_matrix(DEproPvalue,alltissues)

DEproFC_matrix.develop = list_to_matrix(DEproFC.develop,alltissues)
DEproPvalue_matrix.develop = list_to_matrix(DEproPvalue.develop,alltissues)

Aging_pro_sigup_matrix = (DEproFC_matrix > 0.58 & DEproPvalue_matrix < 0.05
                          & DEproPvalue_matrix.develop > 0.05) +0
Aging_pro_sigdown_matrix = -((DEproFC_matrix < -0.58 & DEproPvalue_matrix < 0.05
                              & DEproPvalue_matrix.develop > 0.05) +0)
Aging_pro_sigall_matrix = Aging_pro_sigup_matrix + Aging_pro_sigdown_matrix

Aging_pro_updown = data.frame(stringsAsFactors = F,
                       num.up = colSums(Aging_pro_sigup_matrix,na.rm =T)/
                          colSums(!is.na(Aging_pro_sigup_matrix)),
```

```
                        num.down = colSums(Aging_pro_sigdown_matrix,na.rm =T)/
                           colSums(!is.na(Aging_pro_sigdown_matrix)),
                        num.all = colSums(abs(Aging_pro_sigall_matrix),na.rm =T)/
                          colSums(!is.na(Aging_pro_sigall_matrix)),
                         tissues = colnames(Aging_pro_sigup_matrix),
                         tissue_systems = tissue.systems)
rownames(Aging_pro_updown) = Aging_pro_updown$tissues

Aging_pro_updown
```

```
##                              num.up    num.down    num.all
## Skin_of_back            0.036514823 -0.02422270 0.06081448
## Pituitary               0.024935277 -0.02820548 0.05316973
## Frontal_pole            0.022538363 -0.01742606 0.03996803
## Lung                    0.033295982 -0.01888302 0.05220176
## Liver                   0.034220532 -0.01801802 0.05225225
## Arteria_cruralis        0.025117739 -0.04575335 0.07101526
## Femoral_vein            0.041103448 -0.03337010 0.07450331
## Hippocampus             0.013891271 -0.02023320 0.03413379
## Ileocecum               0.021394879 -0.04090909 0.06238408
## Thyroid_gland           0.037166086 -0.01646011 0.05364059
## Arteria_carotis         0.024580336 -0.02637890 0.05096942
## Muscle                  0.062205062 -0.06260720 0.12483912
## Ovary                   0.070639717 -0.08727348 0.15794957
## Cecum                   0.040690691 -0.01725431 0.05795796
## Superior_temporal_gyrus 0.015867713 -0.01687270 0.03274307
## Spleen                  0.019508449 -0.03287250 0.05238900
## Kidney                  0.018391573 -0.01220532 0.03060201
## Adrenal_gland           0.042047532 -0.01391427 0.05604055
## Duodenum                0.030563661 -0.06327753 0.09387003
## Fallopian_tube          0.029572113 -0.04311911 0.07273930
## Stomach                 0.027610674 -0.05751735 0.08519833
## Hypothalamus            0.011605416 -0.00902498 0.02063185
## Heart                   0.043579314 -0.02086957 0.06451613
## Thymus                  0.075391850 -0.32503133 0.40075259
## Facial_skin             0.009045226 -0.01404917 0.02313301
## Pancreas                0.011964948 -0.01736638 0.02933738
## Supramarginal_gyrus     0.017594835 -0.01743904 0.03504522
## Adipose                 0.008937121 -0.08154781 0.09053103
## Aortic_arch             0.066784870 -0.07934633 0.14626454
## Uterus                  0.030058007 -0.04924376 0.07935949
##                                        tissues tissue_systems
## Skin_of_back                      Skin_of_back  Integumentary
## Pituitary                            Pituitary      Endocrine
## Frontal_pole                      Frontal_pole          Brain
## Lung                                      Lung    Respiratory
## Liver                                    Liver      Digestive
## Arteria_cruralis               Arteria_cruralis Cardiovascular
## Femoral_vein                       Femoral_vein Cardiovascular
## Hippocampus                         Hippocampus          Brain
## Ileocecum                             Ileocecum      Digestive
## Thyroid_gland                     Thyroid_gland      Endocrine
## Arteria_carotis                 Arteria_carotis Cardiovascular
## Muscle                                   Muscle         Muscle
```

```
## Ovary                                       Ovary   Reproductive
## Cecum                                       Cecum      Digestive
## Superior_temporal_gyrus Superior_temporal_gyrus          Brain
## Spleen                                     Spleen         Immune
## Kidney                                     Kidney          Renal
## Adrenal_gland                       Adrenal_gland      Endocrine
## Duodenum                                 Duodenum      Digestive
## Fallopian_tube                     Fallopian_tube   Reproductive
## Stomach                                   Stomach      Digestive
## Hypothalamus                         Hypothalamus          Brain
## Heart                                       Heart         Muscle
## Thymus                                     Thymus         Immune
## Facial_skin                         Facial_skin  Integumentary
## Pancreas                               Pancreas      Endocrine
## Supramarginal_gyrus         Supramarginal_gyrus          Brain
## Adipose                                 Adipose         Immune
## Aortic_arch                         Aortic_arch Cardiovascular
## Uterus                                   Uterus   Reproductive
```

```
mean(Aging_pro_updown$num.all)
```

```
## [1] 0.07529843
```

6.2 mRNA

```
DEmrnaFC = list()
DEmrnaPvalue = list()
DEmrnaAging = list()
DEmrnaFC.develop = list()
DEmrnaPvalue.develop = list()
for(i in 1:length(alltissues)){
    #Mfuzz
    thistissue = alltissues[i]
    thismrna = mrna.tissues[[thistissue]]
    #thismrna.header = mrna.tissues.header[[thistissue]]
    thismrna.info = mrna.tissues.info[[thistissue]]
    if (sum(thismrna.info$stage == 1) ==1){
        thismrna = cbind(thismrna[,thismrna.info$stage == 1],thismrna)
        thismrna.info = rbind(thismrna.info[thismrna.info$stage == 1,],thismrna.info)
    }
    if (sum(thismrna.info$stage == 4) ==1){
        thismrna = cbind(thismrna,thismrna[,thismrna.info$stage == 4])
        thismrna.info = rbind(thismrna.info,thismrna.info[thismrna.info$stage == 4,])
    }

    thisDEmrna = DEGenes.simplified(thismrna,catagory = thismrna.info$stage == 4,
                  subset = thismrna.info$stage == 4 | thismrna.info$stage == 1)

    thisDEmrna.develop = DEGenes.simplified(thismrna,catagory = thismrna.info$stage == 2,
                    subset = thismrna.info$stage == 2 | thismrna.info$stage == 1)

    idx = sort.int(thisDEmrna$Pvalue,decreasing = F,index.return = T)$ix
    DEmrnaAging[[i]] = thisDEmrna[idx,-5]
```

```
    DEmrnaAging[[i]]$log2FC.devControl = thisDEmrna.develop$log2FC
    DEmrnaAging[[i]]$Pvalue.devControl = thisDEmrna.develop$Pvalue


    DEmrnaFC[[i]] = thisDEmrna$log2FC
    names(DEmrnaFC[[i]]) = rownames(thisDEmrna)
    DEmrnaPvalue[[i]] = thisDEmrna$Pvalue
    names(DEmrnaPvalue[[i]])  = rownames(thisDEmrna)

    DEmrnaFC.develop[[i]] = thisDEmrna.develop$log2FC
    names(DEmrnaFC.develop[[i]]) = rownames(thisDEmrna.develop)
    DEmrnaPvalue.develop[[i]] = thisDEmrna.develop$Pvalue
    names(DEmrnaPvalue.develop[[i]])  = rownames(thisDEmrna.develop)


}
names(DEmrnaFC) = alltissues
names(DEmrnaPvalue) = alltissues
names(DEmrnaAging) = alltissues
names(DEmrnaFC.develop) = alltissues
names(DEmrnaPvalue.develop) = alltissues



DEmrnaFC_matrix = list_to_matrix(DEmrnaFC,alltissues)
DEmrnaPvalue_matrix = list_to_matrix(DEmrnaPvalue,alltissues)

DEmrnaFC_matrix.develop = list_to_matrix(DEmrnaFC.develop,alltissues)
DEmrnaPvalue_matrix.develop = list_to_matrix(DEmrnaPvalue.develop,alltissues)
```

```
#data S1
openxlsx::write.xlsx(DEmrnaAging, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S1_DE_tissue_A

# reducce size Data S1
tpath = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S1_DE_tissue_Aging_mRNA.xlsx"
sheetNames = openxlsx::getSheetNames(tpath)
xx = list()
for(i in 1:length(sheetNames)){
    tmp = openxlsx::read.xlsx(tpath,sheet = sheetNames[i])
    tmp[c(2,3,4,7,8)] = signif(tmp[c(2,3,4,7,8)],3)
    tmp = tmp[,-c(5,6)]
    xx[[i]] = tmp
}
names(xx) = sheetNames
openxlsx::write.xlsx(xx, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/reduce_Data S1_DE_tissue_Ag
```

```
Aging_mrna_sigup_matrix = (DEmrnaFC_matrix > 0.58 & DEmrnaPvalue_matrix < 0.05 &
                             DEmrnaPvalue_matrix.develop > 0.05) +0
Aging_mrna_sigdown_matrix = -((DEmrnaFC_matrix < -0.58 & DEmrnaPvalue_matrix < 0.05 &
                                DEmrnaPvalue_matrix.develop > 0.05) +0)
Aging_mrna_sigall_matrix = Aging_mrna_sigup_matrix + Aging_mrna_sigdown_matrix

Aging_mrna_updown = data.frame(stringsAsFactors = F,
                               num.up = colSums(Aging_mrna_sigup_matrix,na.rm =T)/
```

```
                                colSums(!is.na(Aging_mrna_sigup_matrix)),
                              num.down = colSums(Aging_mrna_sigdown_matrix,na.rm =T)/
                                colSums(!is.na(Aging_mrna_sigdown_matrix)),
                              num.all = colSums(abs(Aging_mrna_sigall_matrix),na.rm =T)/
                                colSums(!is.na(Aging_mrna_sigall_matrix)),
                             tissues = colnames(Aging_mrna_sigup_matrix),
                             tissue_systems = tissue.systems)
rownames(Aging_mrna_updown) = Aging_mrna_updown$tissues
Aging_mrna_updown
```

```
##                               num.up     num.down     num.all
## Skin_of_back            0.014877204 -0.013853904 0.028731108
## Pituitary               0.016756193 -0.020444159 0.037200353
## Frontal_pole            0.017589731 -0.020362887 0.037952619
## Lung                    0.008547009 -0.015562006 0.024109015
## Liver                   0.015846995 -0.011202186 0.027049180
## Arteria_cruralis        0.016141630 -0.013364575 0.029506205
## Femoral_vein            0.012257843 -0.012772158 0.025030002
## Hippocampus             0.020030204 -0.019791749 0.039821954
## Ileocecum               0.054235073 -0.028506085 0.082741158
## Thyroid_gland           0.026949541 -0.013761468 0.040711009
## Arteria_carotis         0.021807382 -0.029953331 0.051760713
## Muscle                  0.014691302 -0.028301037 0.042992339
## Ovary                   0.025969730 -0.035232818 0.061202547
## Cecum                   0.050902852 -0.040853011 0.091755862
## Superior_temporal_gyrus 0.015590200 -0.023783010 0.039373210
## Spleen                  0.029801597 -0.034164814 0.063966411
## Kidney                  0.020382901 -0.013863674 0.034246575
## Adrenal_gland           0.017783149 -0.026588398 0.044371547
## Duodenum                0.119320039 -0.126266754 0.245586793
## Fallopian_tube          0.049622774 -0.034300381 0.083923155
## Stomach                 0.009763363 -0.008439517 0.018202879
## Hypothalamus            0.001815598 -0.003473319 0.005288917
## Heart                   0.009539101 -0.011616925 0.021156026
## Thymus                  0.006846780 -0.001572909 0.008419689
## Facial_skin             0.039662514 -0.040293329 0.079955843
## Pancreas                0.024070432 -0.024445069 0.048515501
## Supramarginal_gyrus     0.014104710 -0.022870348 0.036975058
## Adipose                 0.014931669 -0.013497554 0.028429222
## Aortic_arch             0.029785810 -0.027024766 0.056810576
## Uterus                  0.020403106 -0.025028911 0.045432017
##                                          tissues tissue_systems
## Skin_of_back                        Skin_of_back  Integumentary
## Pituitary                              Pituitary      Endocrine
## Frontal_pole                        Frontal_pole          Brain
## Lung                                        Lung    Respiratory
## Liver                                      Liver      Digestive
## Arteria_cruralis                Arteria_cruralis Cardiovascular
## Femoral_vein                        Femoral_vein Cardiovascular
## Hippocampus                          Hippocampus          Brain
## Ileocecum                              Ileocecum      Digestive
## Thyroid_gland                      Thyroid_gland      Endocrine
## Arteria_carotis                  Arteria_carotis Cardiovascular
## Muscle                                    Muscle         Muscle
```

```
## Ovary                                     Ovary   Reproductive
## Cecum                                     Cecum      Digestive
## Superior_temporal_gyrus Superior_temporal_gyrus          Brain
## Spleen                                   Spleen         Immune
## Kidney                                   Kidney          Renal
## Adrenal_gland                     Adrenal_gland      Endocrine
## Duodenum                               Duodenum      Digestive
## Fallopian_tube                   Fallopian_tube   Reproductive
## Stomach                                 Stomach      Digestive
## Hypothalamus                       Hypothalamus          Brain
## Heart                                     Heart         Muscle
## Thymus                                   Thymus         Immune
## Facial_skin                         Facial_skin  Integumentary
## Pancreas                               Pancreas      Endocrine
## Supramarginal_gyrus         Supramarginal_gyrus          Brain
## Adipose                                 Adipose         Immune
## Aortic_arch                         Aortic_arch Cardiovascular
## Uterus                                   Uterus   Reproductive
```

```
mean(Aging_mrna_updown$num.all)
```

```
## [1] 0.04937392
```

```
mrnaVoconoPlot = list()
names.DEmrnaAging = names(DEmrnaAging)
for(i in 1:length(DEmrnaAging)){
    res2 = DEmrnaAging[[i]]

    mrnaVoconoPlot[[i]] = plot_Volcano(res2,names.DEmrnaAging[i])
}
#this plot is large, plot to file
pdf(file = './out/20230217_aging/Figure2_DEG_GO_tissue/FigureS2B_DEmrna_each_tissueV1.pdf',
    width = 3*5+1,height =3*6)
grid.arrange(arrangeGrob(grobs = mrnaVoconoPlot,ncol = 5))
dev.off()
```

```
## pdf
##   2
```

6.3 metabolism

```
DEmetFC = list()
DEmetPvalue = list()
DEmetAging = list()
DEmetFC.develop = list()
DEmetPvalue.develop = list()
for(i in 1:length(alltissues)){
    #Mfuzz
    thistissue = alltissues[i]
    thismet = met.tissues[[thistissue]]
    thismet.header = met.tissues.header[[thistissue]]
    thismet.info = met.tissues.info[[thistissue]]
    if (sum(thismet.info$stage == 1) ==1){
        thismet = cbind(thismet[,thismet.info$stage == 1],thismet)
```

```
        thismet.info = rbind(thismet.info[thismet.info$stage == 1,],thismet.info)
    }
    if (sum(thismet.info$stage == 4) ==1){
        thismet = cbind(thismet,thismet[,thismet.info$stage == 4])
        thismet.info = rbind(thismet.info,thismet.info[thismet.info$stage == 4,])
    }

    thisDEmet = DEGenes.simplified(thismet,catagory = thismet.info$stage == 4,
                                subset = thismet.info$stage == 4 | thismet.info$stage == 1)

    thisDEmet.develop = DEGenes.simplified(thismet,catagory = thismet.info$stage == 2,
                                subset = thismet.info$stage == 2 | thismet.info$stage == 1)

    forsort = thisDEmet$Pvalue
    forsort[is.na(forsort)] = 1
    idx = sort.int(forsort,decreasing = F,index.return = T)$ix
    DEmetAging[[i]] = thisDEmet[idx,-5]
    DEmetAging[[i]]$log2FC.devControl = thisDEmet.develop$log2FC
    DEmetAging[[i]]$Pvalue.devControl = thisDEmet.develop$Pvalue

    DEmetFC[[i]] = thisDEmet$log2FC
    names(DEmetFC[[i]]) = rownames(thisDEmet)
    DEmetPvalue[[i]] = thisDEmet$Pvalue
    names(DEmetPvalue[[i]])  = rownames(thisDEmet)

    DEmetFC.develop[[i]] = thisDEmet.develop$log2FC
    names(DEmetFC.develop[[i]]) = rownames(thisDEmet.develop)
    DEmetPvalue.develop[[i]] = thisDEmet.develop$Pvalue
    names(DEmetPvalue.develop[[i]])  = rownames(thisDEmet.develop)


}
names(DEmetAging) = alltissues
names(DEmetFC) = alltissues
names(DEmetPvalue) = alltissues
DEmetFC_matrix = list_to_matrix(DEmetFC,alltissues)
DEmetPvalue_matrix = list_to_matrix(DEmetPvalue,alltissues)

DEmetFC_matrix.develop = list_to_matrix(DEmetFC.develop,alltissues)
DEmetPvalue_matrix.develop = list_to_matrix(DEmetPvalue.develop,alltissues)
```

```
#write Data S3
openxlsx::write.xlsx(DEmetAging, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S3_DE_tissue_Ag

# reducce size Data S2
tpath = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S3_DE_tissue_Aging_metabolite.xlsx"
sheetNames = openxlsx::getSheetNames(tpath)
xx = list()
for(i in 1:length(sheetNames)){
    tmp = openxlsx::read.xlsx(tpath,sheet = sheetNames[i])
    tmp[c(2,3,4,7,8)] = signif(tmp[c(2,3,4,7,8)],3)
    tmp = tmp[,-c(5,6)]
    xx[[i]] = tmp
}
```

```r
names(xx) = sheetNames
openxlsx::write.xlsx(xx, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/reduce_Data S3_DE_tissue_Ag
```

```r
# num DEmets
Aging_met_sigup_matrix = (DEmetFC_matrix > 0.58 & DEmetPvalue_matrix < 0.05 &
                            DEmetPvalue_matrix.develop > 0.05) + 0
Aging_met_sigdown_matrix = -((DEmetFC_matrix < -0.58 & DEmetPvalue_matrix < 0.05  &
                               DEmetPvalue_matrix.develop > 0.05) + 0)
Aging_met_sigall_matrix = Aging_met_sigup_matrix + Aging_met_sigdown_matrix

Aging_met_updown = data.frame(stringsAsFactors = F,
                              num.up = colSums(Aging_met_sigup_matrix,na.rm =T)/
                                colSums(!is.na(Aging_met_sigup_matrix)),
                              num.down = colSums(Aging_met_sigdown_matrix,na.rm =T)/
                                colSums(!is.na(Aging_met_sigdown_matrix)),
                              num.all = colSums(abs(Aging_met_sigall_matrix),na.rm =T)/
                                colSums(!is.na(Aging_met_sigall_matrix)),
                             tissues = colnames(Aging_met_sigup_matrix),
                             tissue_systems = tissue.systems)
rownames(Aging_met_updown) = Aging_met_updown$tissues
Aging_met_updown
```

```
##                               num.up     num.down    num.all
## Skin_of_back            0.036030341 -0.027180784 0.06321113
## Pituitary               0.127226463 -0.078032231 0.20525869
## Frontal_pole            0.013303769 -0.047302291 0.06060606
## Lung                    0.015033073 -0.021046302 0.03607937
## Liver                   0.006362059 -0.029496819 0.03585888
## Arteria_cruralis        0.020116807 -0.020765737 0.04088254
## Femoral_vein            0.000000000 -0.011150235 0.01115023
## Hippocampus             0.014792899 -0.015532544 0.03032544
## Ileocecum               0.012651265 -0.058305831 0.07095710
## Thyroid_gland           0.006051437 -0.084720121 0.09077156
## Arteria_carotis         0.048211509 -0.010886470 0.05909798
## Muscle                  0.011284722 -0.015625000 0.02690972
## Ovary                   0.040852575 -0.080521018 0.12137359
## Cecum                   0.147406266 -0.083204931 0.23061120
## Superior_temporal_gyrus 0.065759637 -0.077853364 0.14361300
## Spleen                  0.053291536 -0.035736677 0.08902821
## Kidney                  0.009730967 -0.062392673 0.07212364
## Adrenal_gland           0.021821632 -0.013757116 0.03557875
## Duodenum                0.034172662 -0.050959233 0.08513189
## Fallopian_tube          0.006607930 -0.042951542 0.04955947
## Stomach                 0.006823821 -0.047766749 0.05459057
## Hypothalamus            0.030780781 -0.024024024 0.05480480
## Heart                   0.028863500 -0.023451594 0.05231509
## Thymus                  0.023887588 -0.136299766 0.16018735
## Facial_skin             0.002056555 -0.011825193 0.01388175
## Pancreas                0.026440678 -0.016949153 0.04338983
## Supramarginal_gyrus     0.126301180 -0.045801527 0.17210271
## Adipose                 0.002421308 -0.007263923 0.00968523
## Aortic_arch             0.015799868 -0.048716261 0.06451613
## Uterus                  0.031384615 -0.035692308 0.06707692
##                                        tissues tissue_systems
```

```
## Skin_of_back                         Skin_of_back  Integumentary
## Pituitary                               Pituitary      Endocrine
## Frontal_pole                         Frontal_pole          Brain
## Lung                                         Lung    Respiratory
## Liver                                       Liver      Digestive
## Arteria_cruralis               Arteria_cruralis Cardiovascular
## Femoral_vein                       Femoral_vein Cardiovascular
## Hippocampus                         Hippocampus          Brain
## Ileocecum                             Ileocecum      Digestive
## Thyroid_gland                     Thyroid_gland      Endocrine
## Arteria_carotis                 Arteria_carotis Cardiovascular
## Muscle                                   Muscle         Muscle
## Ovary                                     Ovary   Reproductive
## Cecum                                     Cecum      Digestive
## Superior_temporal_gyrus Superior_temporal_gyrus          Brain
## Spleen                                   Spleen         Immune
## Kidney                                   Kidney          Renal
## Adrenal_gland                   Adrenal_gland      Endocrine
## Duodenum                             Duodenum      Digestive
## Fallopian_tube                 Fallopian_tube   Reproductive
## Stomach                                 Stomach      Digestive
## Hypothalamus                       Hypothalamus          Brain
## Heart                                     Heart         Muscle
## Thymus                                   Thymus         Immune
## Facial_skin                         Facial_skin  Integumentary
## Pancreas                               Pancreas      Endocrine
## Supramarginal_gyrus         Supramarginal_gyrus          Brain
## Adipose                                 Adipose         Immune
## Aortic_arch                         Aortic_arch Cardiovascular
## Uterus                                   Uterus   Reproductive
```

```
mean(Aging_met_updown$num.all)
```

```
## [1] 0.07502263
```

```
metVoconoPlot = list()
names.DEmetAging = names(DEmetAging)
for(i in 1:length(DEmetAging)){
    res2 = DEmetAging[[i]]

    metVoconoPlot[[i]] = plot_Volcano(res2,names.DEmetAging[i])
}

pdf(file = './out/20230217_aging/Figure2_DEG_GO_tissue/
    FigureS2C_DEmet_each_tissueV1.pdf',width = 3*5+1,height =3*6)
grid.arrange(arrangeGrob(grobs = metVoconoPlot,ncol = 5))
dev.off()
```

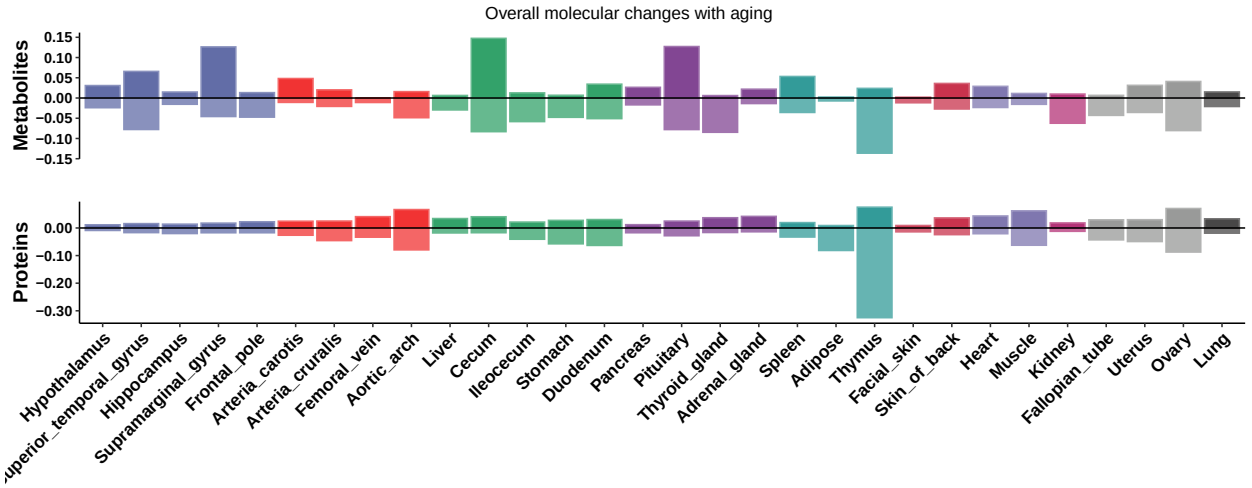
```
## pdf
##   2
```

6.4 Figure 2A perc changed mols each tissue

```
fmt_dcimals <- function(decimals=0){
    function(x) format(x,nsmall = decimals,scientific = FALSE)
}
```

```
plotAgingNum = list()
idx = sort.int(Aging_pro_updown$num.all,decreasing = F,index.return = T)$ix
Aging_pro_updown.v = Aging_pro_updown[idx,]
idx = sort.int(Aging_pro_updown.v$tissue_systems,decreasing = F,index.return = T)$ix
Aging_pro_updown.v = Aging_pro_updown.v[idx,]
tissueindex = rownames(Aging_pro_updown.v)

#metabolites
Aging_met_updown.v = Aging_met_updown[tissueindex,]

p1 = ggplot(Aging_met_updown.v,aes(x = factor(tissues,level = tissues),y = num.up,
                        color = tissue_systems,fill = tissue_systems))+
  geom_bar(stat="identity",alpha = 0.8)

plotAgingNum[[1]] = p1+ geom_bar(stat="identity",aes(y = num.down),alpha = 0.6)+
  geom_hline(yintercept = 0)+
theme_classic()+lghplot.addtheme(legend.position = 'none',hjust = 1,size = 13.5)+
theme(axis.text.y = element_text(size = 9.5, face = "bold", color = "black"))+
  scale_color_aaas(alpha = 0.6)+
scale_fill_aaas(alpha = 0.6)+theme(axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),axis.line.x =element_blank())+
scale_y_continuous(labels = fmt_dcimals(2))+
ylab('Metabolites')+ xlab('')

#protein
Aging_pro_updown.v = Aging_pro_updown[tissueindex,]

p1 = ggplot(Aging_pro_updown.v,aes(x = factor(tissues,level = tissues),y = num.up,
                        color = tissue_systems,fill = tissue_systems))+
  geom_bar(stat="identity",alpha = 0.8)

plotAgingNum[[2]] = p1+ geom_bar(stat="identity",aes(y = num.down),alpha = 0.6)+
  geom_hline(yintercept = 0)+
theme_classic()+lghplot.addtheme(legend.position = 'none',hjust = 1,size = 13.5)+
theme(axis.text.y = element_text(size = 9.5, face = "bold", color = "black"))+
  scale_color_aaas(alpha = 0.6)+
scale_fill_aaas(alpha = 0.6)+
scale_y_continuous(labels = fmt_dcimals(2))+
ylab('Proteins')+ xlab('')

#mrna
Aging_mrna_updown.v = Aging_mrna_updown[tissueindex,]

p1 = ggplot(Aging_mrna_updown.v,aes(x = factor(tissues,level = tissues),y = num.up,
                        color = tissue_systems,fill = tissue_systems))+
  geom_bar(stat="identity",alpha = 0.8)

plotAgingNum[[3]] = p1+ geom_bar(stat="identity",aes(y = num.down),alpha = 0.6)+
  geom_hline(yintercept = 0)+
theme_classic()+lghplot.addtheme(legend.position = 'none',hjust = 1,size = 13.5)+
```

```
theme(axis.text.y = element_text(size = 9.5, face = "bold", color = "black"))+
  scale_color_aaas(alpha = 0.6)+
scale_fill_aaas(alpha = 0.6)+scale_y_continuous(labels = fmt_dcimals(2))+
ylab('mRNAs')+ xlab('')

#pdf(file = './out/20230217_aging/Figure2_DEG_GO_tissue/
#   Figure2A_number_of_Aging_moleculars_prometv1_aaas.pdf',width = 12,height =5)
grid.arrange(arrangeGrob(grobs = plotAgingNum[1:2],
                         top = 'Overall molecular changes with aging',
                         ncol = 1,heights = c(1.7,3.3)))
```




```
#dev.off()
```

6.5 Figure 2B GO enrichment

```
#write for metascape up
inputGOenrich = data.frame(tissues = names(DEproAging),stringsAsFactors = F,
                      genes = rep('c',length(DEproAging)))
#colnames(inputGOenrich) = c('tissue','GeneSymbol')
for(i in 1:length(DEproAging)){
    tmp = DEproAging[[i]]
    tgenes = unique(rownames(tmp)[tmp$Pvalue < 0.05 & tmp$log2FC > 0.58 &
                                      tmp$Pvalue.devControl > 0.05])
    tgenes = tgenes[!is.na(tgenes)]
    tgenes = tgenes[tgenes != '']
    tgenes = paste0(tgenes,collapse =',')
    inputGOenrich$genes[i] = tgenes
}
writetxt(inputGOenrich,'./out/20230217_aging/Figure2_DEG_GO_tissue/protein/_inputGOenrich_age_DEpro_up.t


#write for metascape down
inputGOenrich = data.frame(tissues = names(DEproAging),stringsAsFactors = F,
                      genes = rep('c',length(DEproAging)))
```

```
#colnames(inputGOenrich) = c('tissue','GeneSymbol')
for(i in 1:length(DEproAging)){
    tmp = DEproAging[[i]]
    tgenes = unique(rownames(tmp)[tmp$Pvalue < 0.05 & tmp$log2FC < -0.58 &
                                  tmp$Pvalue.devControl > 0.05])
    tgenes = tgenes[!is.na(tgenes)]
    tgenes = tgenes[tgenes != '']
    tgenes = paste0(tgenes,collapse =',')
    inputGOenrich$genes[i] = tgenes
}
writetxt(inputGOenrich,'./out/20230217_aging/Figure2_DEG_GO_tissue/protein/_inputGOenrich_age_DEpro_dow
```

```
outids = c()
tpath1 = './out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_up/Enrichment_heatmap/Heatm
tpath2 = './out/20230217_aging/Figure2_DEG_GO_tissue/protein//metascape_DEpro_down/Enrichment_heatmap/He
selectGO = readxl::read_xlsx(path = './out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_
                             sheet = 'Enrichment')
go1 = file2frame(tpath1,sep = ',',header = T,row.names =2)
rownames(go1) = paste0(go1$GO,'-',rownames(go1))
go1term = go1$GO
tpath1a = './out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_up/Enrichment_GO/GO_AllLis
go1qval = file2frame(tpath1a,sep = ',',header = T)
rownames(go1qval) = paste0(go1qval$GO,'X_LogP_',go1qval$GeneList)
go1 = abs(as.matrix(go1[,-1]))
cname = colnames(go1)
for(i in 1:nrow(go1)){
    for(j in 1:ncol(go1)){
        tmpname = paste0(go1term[i],cname[j])
        go1[i,j] = abs(go1qval[tmpname,]$Log.q.value.)
    }
}
go1[is.na(go1)] = 0

go1for_write = cbind(data.frame(GO = rownames(go1)),-go1)
outlist = list()
outlist[[1]] = go1for_write;
outlist[[2]] = selectGO;
tmpnames = sort(unique(go1qval$GeneList))
for(i in 1:length(tmpnames)){
    outlist[[i+2]] = go1qval[go1qval$GeneList == tmpnames[[i]],]
}
names(outlist) = c('GOenrichmentALL','selectGO',tmpnames)
openxlsx::write.xlsx(outlist, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S4_GO_tissue_upreg


selectGO = readxl::read_xlsx(path = './out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_
go2 = file2frame(tpath2,sep = ',',header = T,row.names =2)
rownames(go2) = paste0(go2$GO,'-',rownames(go2))
go2term = go2$GO
tpath2a = './out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_down/Enrichment_GO/GO_All
go2qval = file2frame(tpath2a,sep = ',',header = T)
rownames(go2qval) = paste0(go2qval$GO,'X_LogP_',go2qval$GeneList)
go2 = abs(as.matrix(go2[,-1]))
cname = colnames(go2)
```

```
for(i in 1:nrow(go2)){
    for(j in 1:ncol(go2)){
        tmpname = paste0(go2term[i],cname[j])
        go2[i,j] = -abs(go2qval[tmpname,]$Log.q.value.)
    }
}
go2[is.na(go2)] = 0

go1for_write = cbind(data.frame(GO = rownames(go2)),go2)
outlist = list()
outlist[[1]] = go1for_write;
outlist[[2]] = selectGO;
tmpnames = sort(unique(go2qval$GeneList))
for(i in 1:length(tmpnames)){
    outlist[[i+2]] = go2qval[go2qval$GeneList == tmpnames[[i]],]
}
names(outlist) = c('GOenrichmentALL','selectGO',tmpnames)
openxlsx::write.xlsx(outlist, file = "./out/20230217_aging/Figure2_DEG_GO_tissue/Data S5_GO_tissue_downr


thisgo = rbind(go1,go2)
thisgo.matrix = as.matrix(thisgo)
colnames(thisgo.matrix) = capitalize(gsub('X_LogP_','',colnames(thisgo.matrix)))
thisgo.matrix[abs(thisgo.matrix) < -log10(0.05)] = 0
writetxt(thisgo.matrix,'./out/20230217_aging/Figure2_DEG_GO_tissue/Figure2_heatmap_GOenrichment_metascap
thisgo.matrix[thisgo.matrix > 4] = 4
thisgo.matrix[thisgo.matrix < -4] = -4

rownames(thisgo.matrix)[rownames(thisgo.matrix) == 'R-HSA-9716542-Signaling by Rho GTPases, Miro GTPases
rownames(thisgo.matrix)[rownames(thisgo.matrix) == 'R-HSA-1428517-The citric acid (TCA) cycle and respi

graphics.off()
pheatmap::pheatmap(thisgo.matrix,cluster_rows = T,cluster_cols = T,
                   main ='Enrichment of proteome',
                   fontsize_row = 11,fontsize_col = 11,fontsize = 14,
                   treeheight_row = 20,treeheight_col = 20,legend = T,
                  color=colorRampPalette(c('#3B4992','gray99','#BB0021FF'))(50),
                  height = 10,width = 10
                  #file ="./out/20230217_aging/Figure2_DEG_GO_tissue/Figure2_heatmap_GOenrichment_metas
                   )
```

7 Figure 3 clustering aging type

7.1 mfuzz promet tissues

```
tissues = names(pro.tissues)
promet.tissues = list()
promet.tissues.info = list()
promet.tissues.Z = list()
mfuzz.promet.tissues = list()
promet.mstd.eset = list()
```

```
for(i in 1:length(tissues)){
#for(i in 1:1){
    tt = tissues[i]
    thispro = pro.tissues[[tt]]
    thispro = delete_dup_genes_forprotein(thispro,pro.tissues.header[[tt]])
    thispro.header = pro.tissues.header[[tt]]
    thispro = thispro[rowSums(is.na(thispro)) < 1/3*ncol(thispro), ]
    thismet = met.tissues[[tt]]
    rownames(thismet) = paste0('met_',rownames(thismet))
    thispromet = rbind2(thispro,thismet)
    thisinfo = pro.tissues.info[[tt]]
    thisinfo = thisinfo[colnames(thispromet),]
    thispromet.median = t(aggregate(t(thispromet), by=list(thisinfo$stage),
                                    FUN=median, na.rm = T))
    mstd = standardise_matrix(thispromet.median)
    promet.tissues.Z[[i]] = mstd
    promet.tissues[[i]] = thispromet
    promet.tissues.info[[i]] = thisinfo
    mstd.v = mstd[rowSums(is.na(mstd))  == 0,]
    mstd.eset = new("ExpressionSet",exprs = mstd.v)
    promet.mstd.eset[[i]] = mstd.eset
    mfuzz.promet.tissues[[i]] = mfuzz(mstd.eset, c = 8,m = 1.5)

}
names(mfuzz.promet.tissues) = tissues
names(promet.tissues) = tissues
names(promet.tissues.Z) = tissues
names(promet.tissues.info) = tissues
names(promet.mstd.eset) = tissues
```

```
## not run

tissues = names(promet.tissues.mstd.eset)
for(i in 1:length(mfuzz.promet.tissues)){
    tpath = paste0('./out/20210428_aging/promet/tissues/',tissues[i])
    dir.create(tpath)
    pdf(paste0(tpath,'/mfuzz_plot_8A_promet.pdf'),width = 8,height = 4)
    mfuzz.plot(promet.tissues.mstd.eset[[i]],mfuzz.promet.tissues[[i]],
    mfrow=c(2,4),time.labels = c("Juvenile", "Young_adult","Middle_aged",
                                 "Elderly"),new.window = F)
    dev.off()
}
```

```
promet.tissues.mstd.eset = promet.mstd.eset
promet.tissues.Z.t = list()
for(i in 1:length(promet.tissues.Z)){
    tmp = as.data.frame(t(promet.tissues.Z[[i]]))
    promet.tissues.Z.t[[i]] = tmp
}
names(promet.tissues.Z.t) = names(promet.tissues.Z)

promet.whole.Z = t(as.matrix(as.data.frame(rbindlist(promet.tissues.Z.t,fill = T))))
colnames(promet.whole.Z) = paste0(rep(names(promet.tissues.Z),each = 4),'_',rep(1:4,times = 30))
promet.whole.Z.info = data.frame(tissue = rep(names(promet.tissues.Z),each = 4),
```

```
                                  stringsAsFactors = F,
                                 stage = rep(1:4,times = 30))
rownames(promet.whole.Z.info) = colnames(promet.whole.Z)
```

7.2 Figure 3A whole body mfuzz

```
#this is used to reproduce the figures in manuscript
load('./out/promet_outdata.Rdata')
```

```
promet.whole.Z.v = promet.whole.Z[rowSums(is.na(promet.whole.Z)) < 0.5*120,]
promet.whole.Z.mean = t(aggregate(t(promet.whole.Z.v),
                      by=list(promet.whole.Z.info$stage), FUN=mean, na.rm = T))
promet.whole.Z.mean  =promet.whole.Z.mean[-1,]
promet.whole.Z.mean.v = promet.whole.Z.mean[rowSums(is.na(promet.whole.Z.mean))  == 0,]
promet.whole.Z.mean.eset = new("ExpressionSet",exprs = promet.whole.Z.mean.v)
dim(promet.whole.Z.mean.v)
```

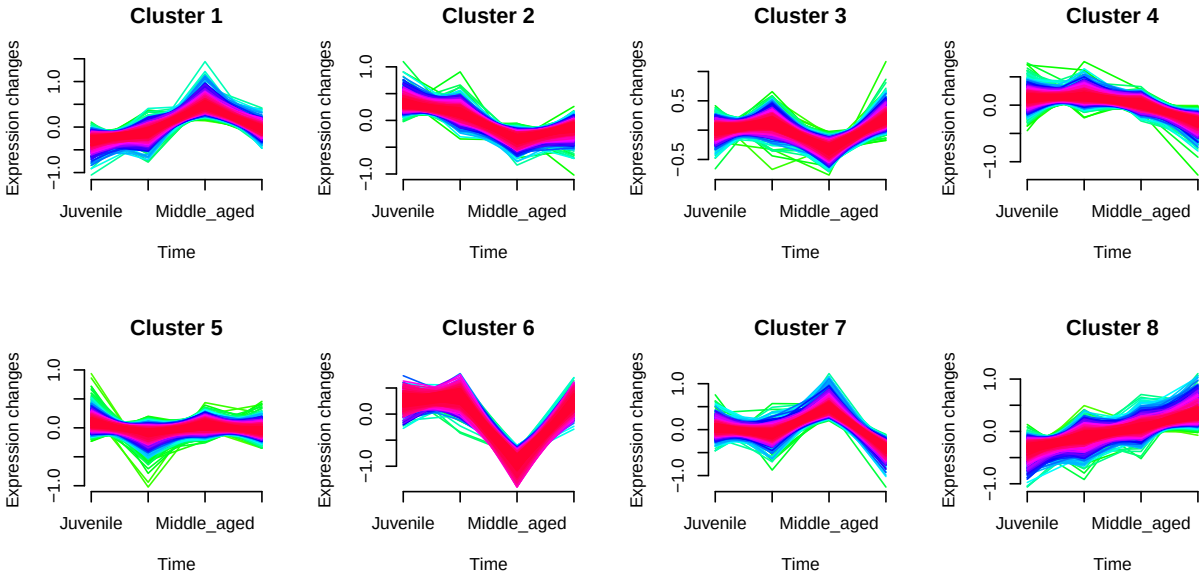
```
## [1] 5331    4
```

```
sum(substr(rownames(promet.whole.Z.mean.v),1,4) == 'met_')
```

```
## [1] 1221
```

```
#mfuzz.promet.whole = mfuzz(promet.norm.eset.stand, c = 8,m = 1.5)
```

```
mfuzz.plot(promet.whole.Z.mean.eset,mfuzz.promet.whole,mfrow=c(2,4),
           time.labels = c("Juvenile", "Young_adult","Middle_aged",
                                  "Elderly"),new.window = F)
```



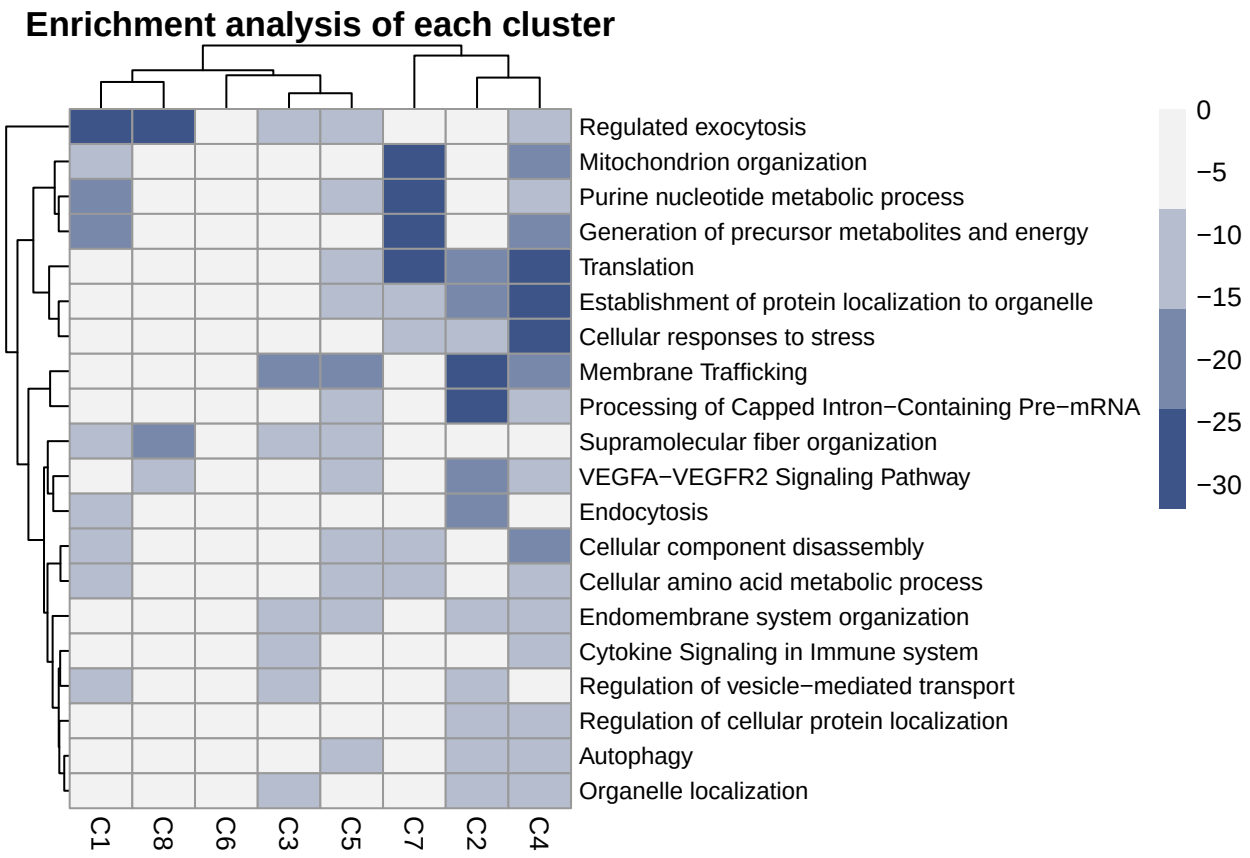
7.3 Figure 3B Go enrichment

```
outids = c()
tpath = paste0('./out/20230217_aging/Figure3_trajactory_analysis/cluster1-8_metascape/metascape_result_
```

```
    my_data <- read_excel(tpath, sheet = "Enrichment")
    idx = regexpr('Summary',my_data$GroupID) >0
    outids = c(outids,my_data$Term[idx])
outids
```

```
##  [1] "R-HSA-2262752" "GO:0006412"    "GO:0045055"    "R-HSA-199991"
##  [5] "GO:0006163"    "GO:0072594"    "GO:0006091"    "R-HSA-72203"
##  [9] "WP3888"        "GO:0022411"    "GO:0007005"    "GO:0006520"
## [13] "GO:0010256"    "GO:0097435"    "R-HSA-1280215" "GO:0051640"
## [17] "GO:0060627"    "GO:1903827"    "GO:0006914"    "ko04144"
```

```
tpath = paste0('./out/20230217_aging/Figure3_trajactory_analysis/cluster1-8_metascape/Enrichment_GO/','G
thisgo = file2frame(tpath,sep = ',')
thisgo= thisgo[!duplicated(thisgo$GO),]
xid = is.element(thisgo$GO,outids)
thisgo = thisgo[xid,]

#rownames(thisgo) = paste0(thisgo$GO,':',thisgo$Description)
rownames(thisgo) = capitalize(paste0(thisgo$Description))
thisgo.matrix = as.matrix(thisgo[,substr(colnames(thisgo),1,6)== 'X_LogP'])
writetxt(thisgo.matrix,'./out/20230217_aging/Figure3_trajactory_analysis/Table S1_GOenrichment_metascape

thisgo.matrix[thisgo.matrix > -3] = 0
colnames(thisgo.matrix) = capitalize(gsub('X_LogP_','',colnames(thisgo.matrix)))
colnames(thisgo.matrix) = gsub('Cluster','C',colnames(thisgo.matrix))

pheatmap::pheatmap(thisgo.matrix,cluster_rows = T,cluster_cols = T,
                   main = 'Enrichment analysis of each cluster',
                   fontsize_row = 9,fontsize_col = 10,fontsize = 10,
                   treeheight_row = 20,treeheight_col = 20,legend = T,
                  color=colorRampPalette(c('#3C5488FF','gray95','gray95'))(7),
                  breaks = c(-32,-24,-16,-8,0),
                  #file ="./out/20230217_aging/Figure3_trajactory_analysis/Figure3B_GOenrichment_metasc
                 height = 3.5,width = 5.5
                  )
```



7.4 mfuzz for each tissue

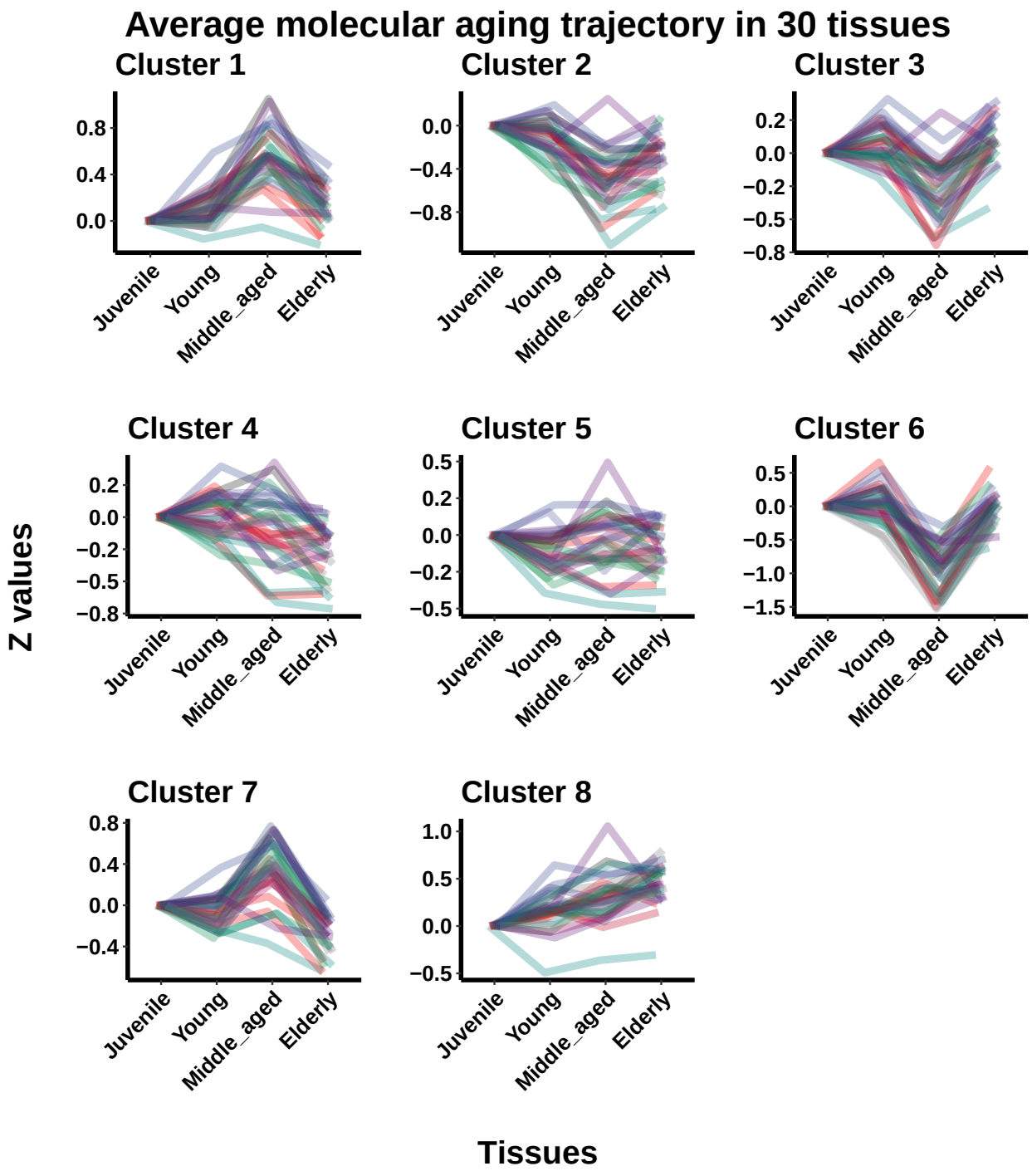
7.4.1 get data

```
# construct data
tissue_trajectory = data.frame()
tissue_names = names(promet.tissues)
for(k in 1:8){
    tmpGeneList = names(mfuzz.promet.whole$cluster)[mfuzz.promet.whole$cluster == k]
    for(i in 1:length(promet.tissues)){

        M1 = promet.tissues[[i]]
        metadata.tissue = promet.tissues.info[[i]]
        idgene1 = intersect(tmpGeneList,rownames(M1))
        cc = repmat(as.matrix(rowMedians(M1[,metadata.tissue$stage == '1'],na.rm = T)),1,ncol(M1))
        tsd = repmat(as.matrix(apply(M1,1,sd,na.rm = T)),1,ncol(M1))

        M1.Z = (M1 - cc)/tsd
        M1.Z.v = M1.Z[idgene1,]
        M1.Z.v.mean = t(aggregate(t(M1.Z.v),
                                  by=list(metadata.tissue$stage), FUN=median, na.rm = T))
        M1.Z.v.mean = M1.Z.v.mean[-1,]
        mean_x = colMeans(M1.Z.v.mean,na.rm  =T)
        mean_x = mean_x - mean_x[1]
```

```
        tmpdata2 = data.frame(expr = mean_x,stringsAsFactors = F,
                              stage = c(1,2,3,4),
                              group = factor(c('Juvenile','Young','Middle_aged','Elderly'),
                                     level = c('Juvenile','Young','Middle_aged','Elderly')),
                              cluster = rep(k,4),
                              tissue = rep(tissue_names[i],4)
                        )
        if (nrow(tissue_trajectory) <1){
            tissue_trajectory = tmpdata2
        }else{
            tissue_trajectory = rbind(tissue_trajectory,tmpdata2)
        }
    }

}
```

```
tissue_trajectory$tissue_system = tissue.systems[tissue_trajectory$tissue]
tissue_trajectory$tissue_system_color = tissue.color[tissue_trajectory$tissue_system]
# to matrix
tissue_trajectory_matrix = matrix(0,nrow(tissue_trajectory)/length(tissue_names),length(tissue_names))
for(i in 1:length(tissue_names)){
    tmptr = tissue_trajectory[tissue_trajectory$tissue == tissue_names[i],]
    tissue_trajectory_matrix[,i] = tmptr$expr
}
rownames(tissue_trajectory_matrix) = paste(tmptr$group,tmptr$cluster,sep = '_C')
colnames(tissue_trajectory_matrix) = tissue_names
```

```
tissue_tr_plot = list()
for (i in 1:8){
    idx = tissue_trajectory$cluster == i
    tissue_tr_plot [[i]] = ggplot(tissue_trajectory[idx,],
                                  aes(x= group,y = expr,group  =tissue)) +
        geom_line(aes(color = tissue_system),alpha = 0.3 ,
                  position = position_dodge(0.2),size = 2)+ scale_color_aaas()+
        lghplot.addtheme(hjust = 1,size = 14)+ ggtitle(paste0('Cluster ',i))+
         theme(axis.line = element_line(size = 1.2))+ xlab('')+ ylab('')+
        scale_y_continuous(labels = scales::comma_format(accuracy =0.1))
}
#pdf(file = "./out/20230217_aging/Figure3_trajactory_analysis/
#    Figure3c_trajectory_for_each_tissue_aaas.pdf",height = 9,width = 8)
grid.arrange(arrangeGrob(grobs = tissue_tr_plot,ncol = 3,heights = c(4,4,4),
            top = textGrob('Average molecular aging trajectory in 30 tissues',
                           gp=gpar(fontface="bold",  fontsize=20)),
            bottom=textGrob('Tissues', gp=gpar(fontface="bold",  fontsize=18)),
            left = textGrob('Z values', gp=gpar(fontface="bold",  fontsize=18),rot=90)))
```

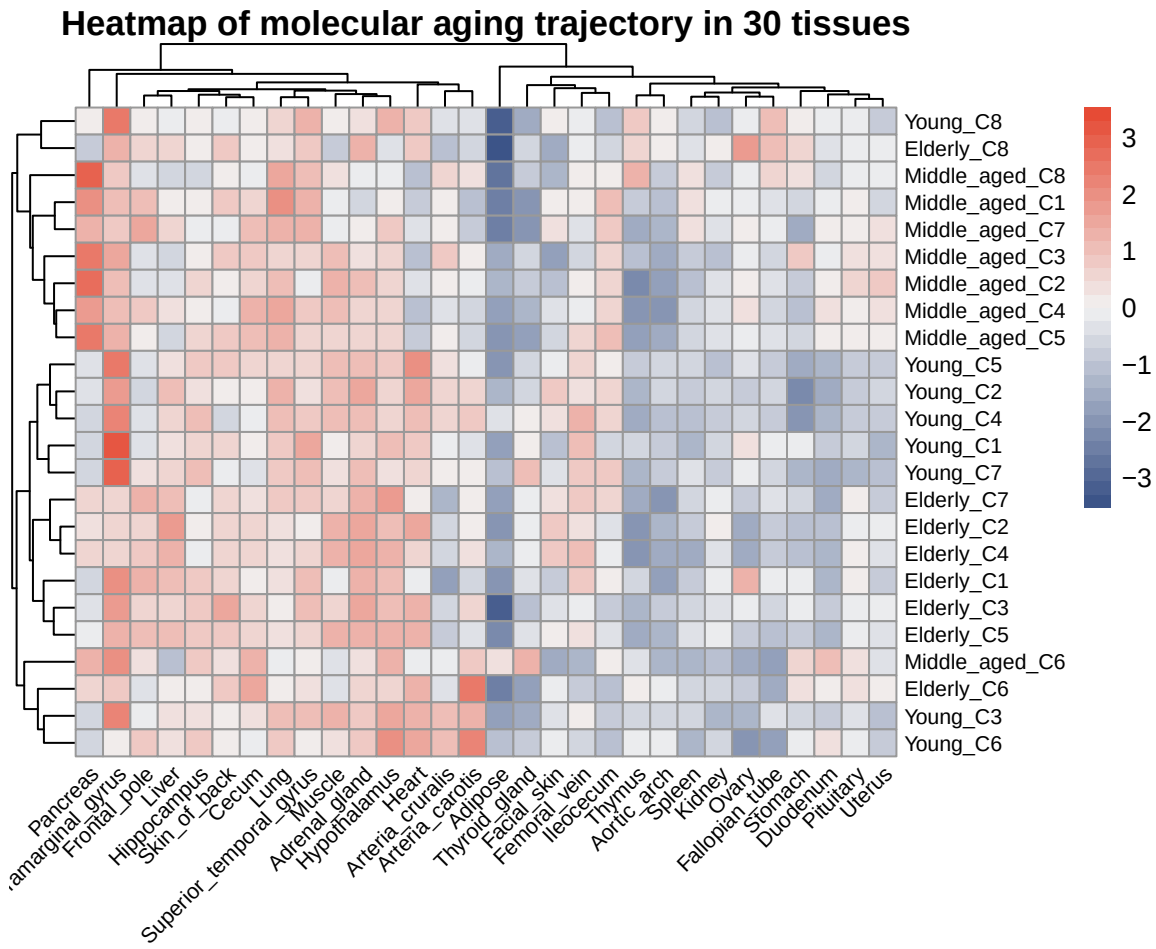



```
#dev.off()
```

7.4.3 Figure 3D heatmap clustering

```
#heatmap cluster '#E64B35FF''#4DBBD5FF'
idx = substr(rownames(tissue_trajectory_matrix),1,3) != 'Juv'
dent = pheatmap::pheatmap(tissue_trajectory_matrix[idx,],scale = 'row',
                    main = 'Heatmap of molecular aging trajectory in 30 tissues',
```

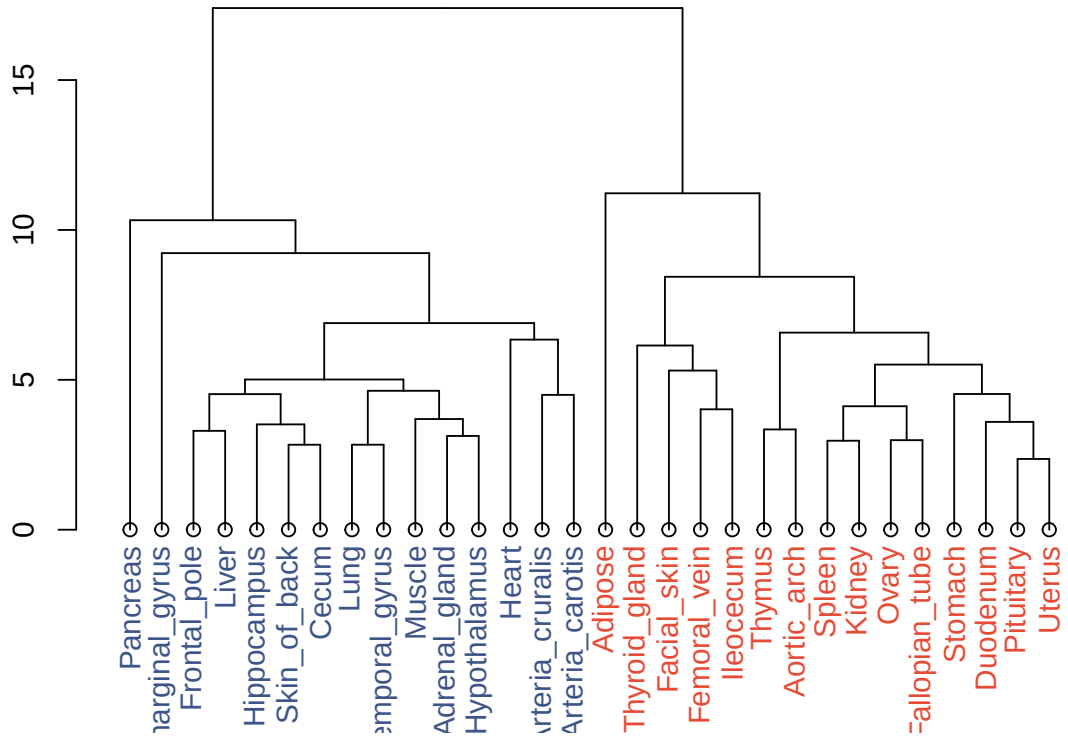
```
                    height = 5,width = 6,angle_col = 45,
                    fontsize_row = 8,fontsize_col = 8,
                    treeheight_row = 20,treeheight_col = 20,
                    #color=colorRampPalette(c('#3B4992','gray95','red'))(30),
                    #file ="./out/20230217_aging/Figure3_trajectory_analysis/Figure3D_heatmap_bas
                    color=colorRampPalette(c('#3C5488FF','gray95','#E64B35FF'))(30))
```



7.4.4 Figure 3E dendgrogam

```
dendcol = as.dendrogram(dent$tree_col)

labelColors = c('#3C5488FF','#E64B35FF')

clusMember = cutree(dent$tree_col,2)

# function to get color labels
colLab <- function(n) {
  if (is.leaf(n)) {
    a <- attributes(n)
    labCol <- labelColors[clusMember[which(names(clusMember) == a$label)]]
    attr(n, "nodePar") <- c(a$nodePar, lab.col = labCol)
```

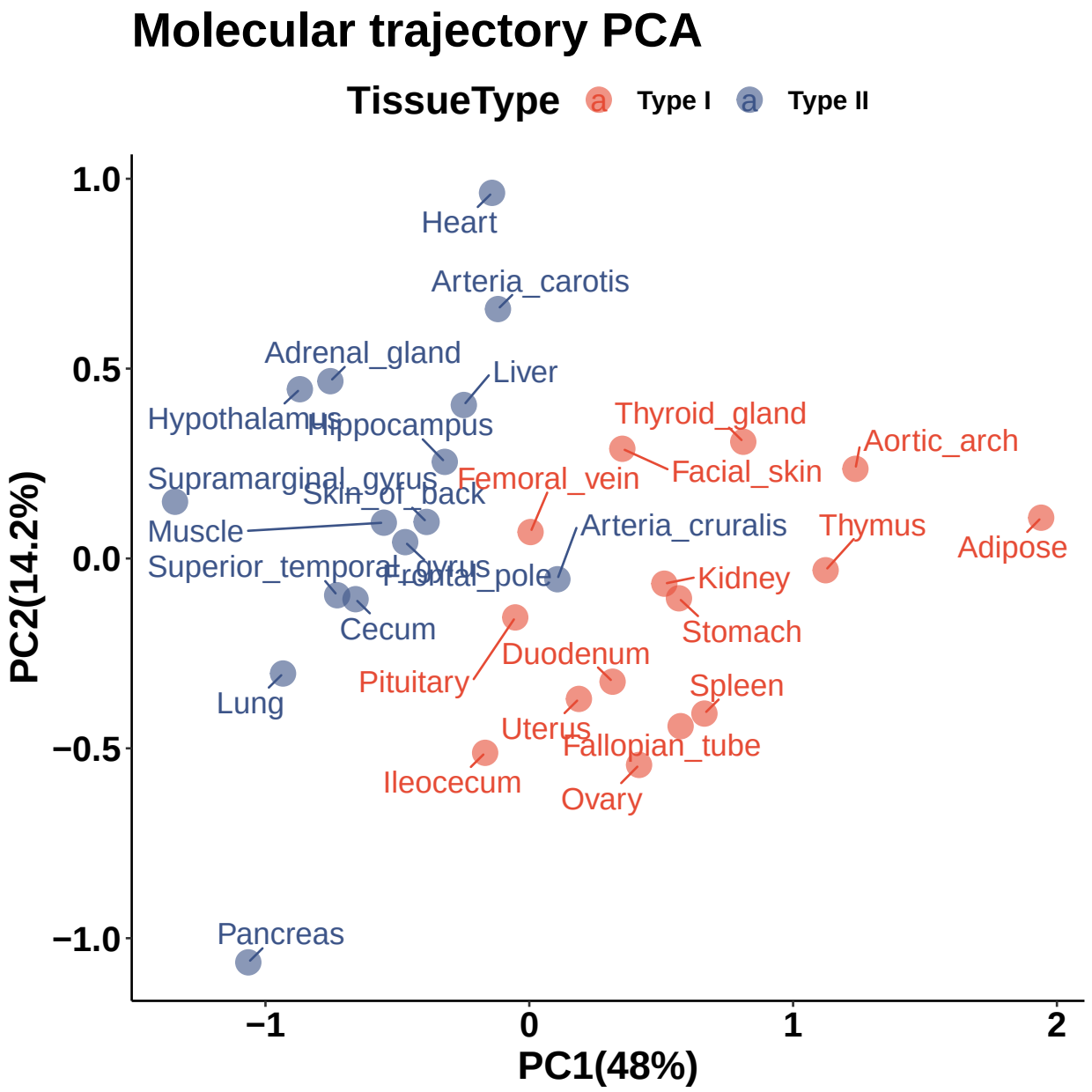
```
  }
  n
}
clusDendro = dendrapply(dendcol, colLab)

dendcolsplit = cut(dendcol,12)
class1 = unlist(cut(dendcol,12)$lower[[1]])
class2 = unlist(cut(dendcol,12)$lower[[2]])
tissueType = data.frame(tissue = c(tissue_names[class1],tissue_names[class2]),
                        stringsAsFactors = F,
                type = c(rep('Type II',length(class1)),rep('Type I',length(class2))))
rownames(tissueType) = tissueType$tissue
tissueType$class = tissueType$type
tissueClass  = tissueType

#pdf('./out/20230217_aging/Figure3_trajectory_analysis/Figure3E_dendrogamV1.pdf',width = 7,height = 5)
plot(clusDendro, main = "Molecular trajectory dendgrogram")
```




```
#dev.off()
#dev.off()
```

7.4.5 Figure 3F trajactory pca

```
# pca
tissue_pca = prcomp(t(tissue_trajectory_matrix),cor=F)
perc_tissue_pca = 100*summary(tissue_pca)$importance

#pdf('./out/20230217_aging/Figure3_trajactory_analysis/Figure3F_PCA.pdf',width = 7,height = 7)
TissueType = tissueType[tissue_names,]$type
ggplot(,aes(tissue_pca$x[,1],tissue_pca$x[,2],color = TissueType)) +
   geom_point(size =5,alpha = 0.6) +
   theme_classic() +lghplot.addtheme(legend.position = 'top')+
   #stat_ellipse(lwd=1,level = 0.95) +
   geom_text_repel(aes(label = tissue_names),size = 5,box.padding = 0.5,face = 'bold')+
   xlab(paste("PC1(",as.character(round(perc_tissue_pca[2,1],1)),'%)',sep = '')) +
   ylab(paste("PC2(",as.character(round(perc_tissue_pca[2,2],1)),'%)',sep = '')) +
   scale_color_manual(values = c('#E64B35FF','#3C5488FF'))+
   theme(legend.text = element_text(size=12,face = 'bold'))+
   labs(title = "Molecular trajectory PCA")
```



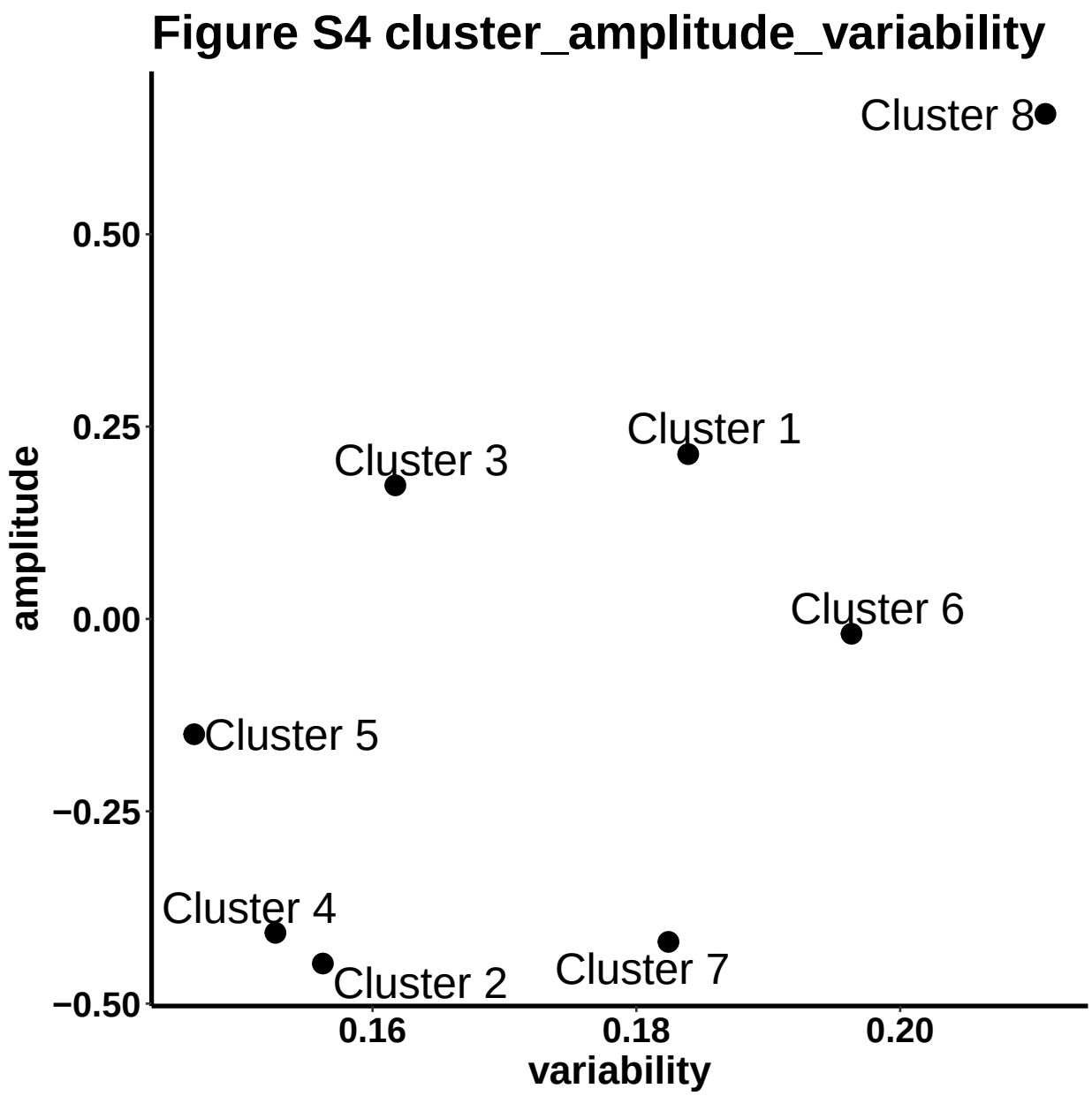

```
#dev.off()
```

8 Figure 4 cluster by MAA

8.1 Figure 4A

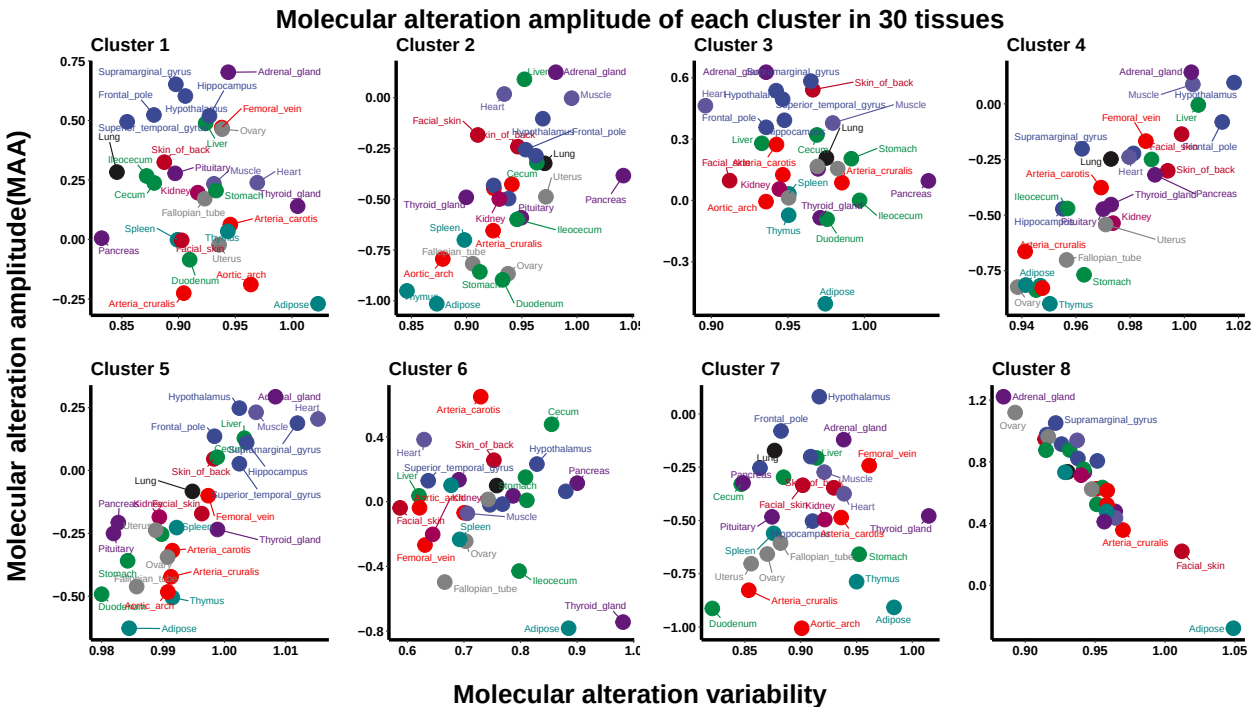
```
promet.norm.eset.stand.matrix = as.matrix(promet.whole.Z.mean.eset)
variability = rep(0,8)
for(j in 1:8){
    tgene = names(mfuzz.promet.whole$cluster)[mfuzz.promet.whole$cluster == j]
    tgene = intersect(tgene,rownames(promet.norm.eset.stand.matrix))
    bx = t(t(promet.norm.eset.stand.matrix[tgene,]) - mfuzz.promet.whole$centers[j,])
```

```
    variability[j] = mean(sqrt(rowSums(bx^2)/(ncol(bx)-1)))
}
tmpdata = data.frame(amplitude = as.vector(mfuzz.promet.whole$centers[,4]-
                                           mfuzz.promet.whole$centers[,1]),
                     stringsAsFactors = F,
                     variability = variability,
                     class = paste0('Cluster ',1:8))
#pdf(file ='./out/20230217_aging/Figure4_MAA_analysis/
#    FigureS4_cluster_amplitude_variability_8_promet.pdf',width = 7,height = 5)
ggplot(tmpdata,aes(variability,amplitude)) + geom_point(size = 4) + lghplot.addtheme(size = 18)+
  geom_text_repel(aes(label = class),size = 7)+theme(axis.line = element_line(size = 1.0))+
  ggtitle('Figure S4 cluster_amplitude_variability')
```



```
#dev.off()
```

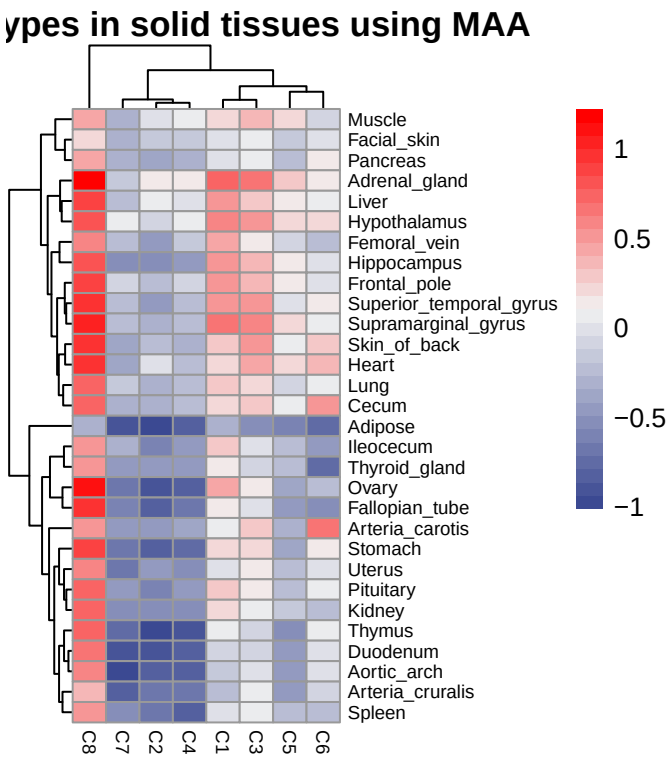
```
tissues = names(promet.tissues.Z)
clusterdist = matrix(0,length(tissues),8)
clusteramplitude = matrix(0,length(tissues),8)
clusteramplitude_xx = matrix(0,length(tissues),8)
for(i in 1:length(tissues)){
    mstd =   promet.tissues.Z[[i]]
    for(j in 1:8){
    tgene = names(mfuzz.promet.whole$cluster)[mfuzz.promet.whole$cluster == j]
    tgene = intersect(tgene,rownames(mstd))
    bx = t(t(mstd[tgene,]) - mfuzz.promet.whole$centers[j,])
    clusterdist[i,j] = mean(sqrt(rowSums(bx^2)/(ncol(bx)-1)),na.rm = T)
    if(length(tgene) < 2){
      clusteramplitude[i,j] = NA
      next;
    }
    tamp = colMeans(mstd[tgene,],na.rm = T)
    clusteramplitude[i,j] = abs(tamp[4]-tamp[1])
    clusteramplitude_xx[i,j] = tamp[4]-tamp[1]
  }
}
rownames(clusteramplitude) = tissues
rownames(clusteramplitude_xx) = tissues
rownames(clusterdist) = tissues

px = list()
for(j in 1:8){
  tmpdata = data.frame(amplitude = clusteramplitude_xx[,j],
                       stringsAsFactors = F,
                       variability = clusterdist[,j],
                       class = tissues,
                      tissue.systems = tissue.systems)
    px[[j]] = ggplot(tmpdata,aes(variability,amplitude,color = tissue.systems)) +
      geom_point(size = 6) + lghplot.addtheme(size = 14)+
    scale_color_aaas()+scale_fill_aaas()+
    geom_text_repel(aes(label = class),size = 3,box.padding = 0.5)+
    theme(axis.line = element_line(size = 1.5))+ ggtitle(paste0('Cluster ',j))+
      xlab('') + ylab('')
}

#pdf(file = "./out/20230217_aging/Figure4_MAA_analysis/
#    FigureXS_cluster8_tissues_amplitude_variability_allV2.pdf",height = 9,width = 16)
grid.arrange(arrangeGrob(grobs = px,ncol = 4,margin = c(1,1,1,1),
                top = textGrob('Molecular alteration amplitude of each cluster in 30 tissues',
                                    gp=gpar(fontface="bold",  fontsize=24)),
                         bottom=textGrob('Molecular alteration variability',
                                    gp=gpar(fontface="bold",  fontsize=24)),
                         #bottom = 'mRNA expression(log2 FPKM)',
                        left = textGrob('Molecular alteration amplitude(MAA)',
                                   gp=gpar(fontface="bold",  fontsize=24),rot=90)))
```




```
#dev.off()
```

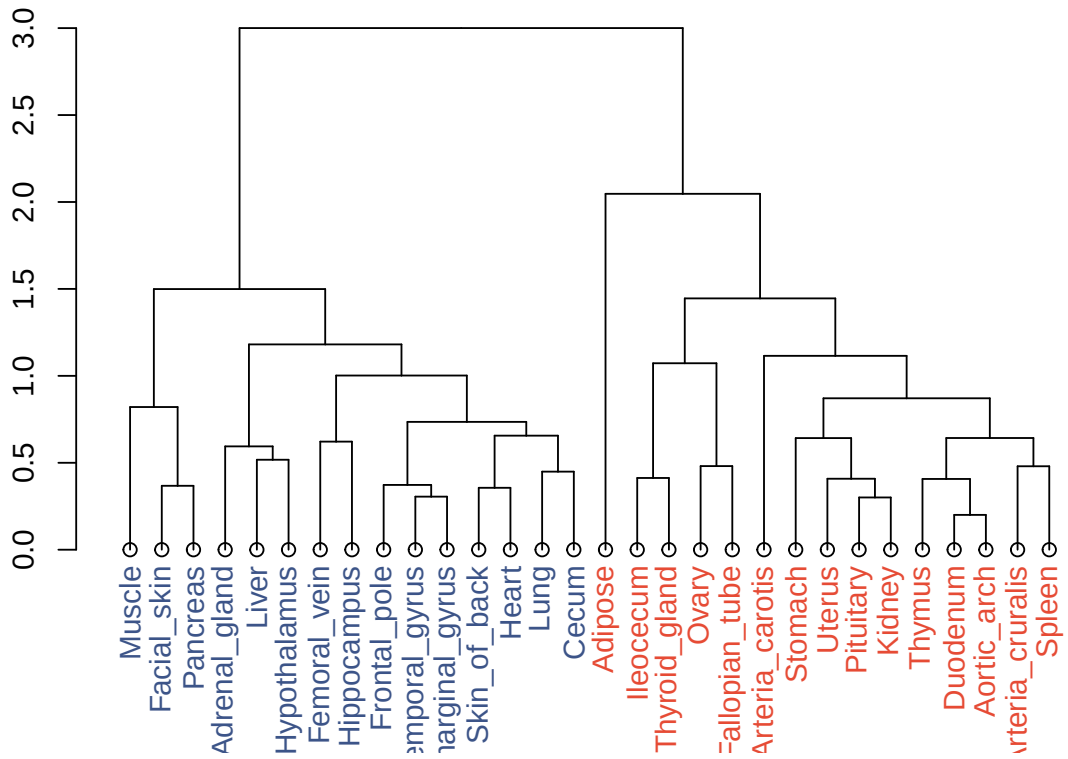
8.2 Figure 4B

```
tmpaa = clusteramplitude_xx
colnames(tmpaa) = paste0("C",1:8)
dent.MAA = pheatmap::pheatmap(tmpaa,scale = 'none',height = 4,width = 3.5,
                              main  = 'Aging types in solid tissues using MAA',
                              fontsize_row = 7,fontsize_col = 7,
                              treeheight_row = 20,treeheight_col = 20,
                              color=colorRampPalette(c('#3B4992','gray95','red'))(30),
             #filename = './out/20230217_aging/Figure4_MAA_analysis                /FigureX_promet_heatmap_
             )
```



8.3 Figure 4C

```
dendrow = as.dendrogram(dent.MAA$tree_row)

labelColors = c('#3C5488FF','#E64B35FF')

clusMember = cutree(dent.MAA$tree_row,2)

# function to get color labels
colLab <- function(n) {
  if (is.leaf(n)) {
    a <- attributes(n)
    labCol <- labelColors[clusMember[which(names(clusMember) == a$label)]]
    attr(n, "nodePar") <- c(a$nodePar, lab.col = labCol)
  }
  n
}
clusDendro = dendrapply(dendrow, colLab)

#pdf('./out/20230217_aging/Figure4_MAA_analysis/
#    FigureX_dendrogam_by_MAA.pdf',width = 7,height = 5)
plot(clusDendro, main = "Molecular trajactory dendgrogram by MAA")
```




```
#dev.off()
```

```
mean_clusteramplitude_xx = rowMeans(clusteramplitude_xx)
names(mean_clusteramplitude_xx) = rownames(clusteramplitude_xx)
tissues_pro = rownames(clusteramplitude_xx)
dendrow = as.dendrogram(dent.MAA$tree_row)
dendrowsplit = cut(dendrow,2.5)
class1 = unlist(cut(dendrow,2.5)$lower[[1]])
class2 = unlist(cut(dendrow,2.5)$lower[[2]])
tissueClass.MAA = data.frame(tissue = c(tissues_pro[class1],
                                        tissues_pro[class2]),stringsAsFactors = F,
                             class = c(rep('Type II',length(class1)),
                                       rep('Type I',length(class2))))
rownames(tissueClass.MAA)  = tissueClass.MAA$tissue
tissueClass.MAA$mean_aging_amplitude=mean_clusteramplitude_xx[rownames(tissueClass.MAA)]


tissueClass$class.MAA = tissueClass.MAA[rownames(tissueClass),]$class
tissueClass$mean_aging_amplitude = tissueClass.MAA[rownames(tissueClass),]$mean_aging_amplitude

tissueClass$type = rep('Undefine',nrow(tissueClass))
tissueClass$type[tissueClass$class == 'Type I' & tissueClass$class.MAA == 'Type I'] = 'Type I'
tissueClass$type[tissueClass$class == 'Type II' & tissueClass$class.MAA == 'Type II'] = 'Type II'
tissueClass
```

```
##                                      tissue     type    class class.MAA
```

```
## Pancreas                                 Pancreas  Type II Type II   Type II
## Supramarginal_gyrus           Supramarginal_gyrus  Type II Type II   Type II
## Frontal_pole                     Frontal_pole  Type II Type II   Type II
## Liver                                   Liver  Type II Type II   Type II
## Hippocampus                       Hippocampus  Type II Type II   Type II
## Skin_of_back                     Skin_of_back  Type II Type II   Type II
## Cecum                                   Cecum  Type II Type II   Type II
## Lung                                     Lung  Type II Type II   Type II
## Superior_temporal_gyrus Superior_temporal_gyrus  Type II Type II   Type II
## Muscle                                 Muscle  Type II Type II   Type II
## Adrenal_gland                 Adrenal_gland  Type II Type II   Type II
## Hypothalamus                   Hypothalamus  Type II Type II   Type II
## Heart                                   Heart  Type II Type II   Type II
## Arteria_cruralis           Arteria_cruralis Undefine Type II    Type I
## Arteria_carotis             Arteria_carotis Undefine Type II    Type I
## Adipose                             Adipose   Type I  Type I    Type I
## Thyroid_gland                 Thyroid_gland   Type I  Type I    Type I
## Facial_skin                     Facial_skin Undefine  Type I   Type II
## Femoral_vein                   Femoral_vein Undefine  Type I   Type II
## Ileocecum                         Ileocecum   Type I  Type I    Type I
## Thymus                               Thymus   Type I  Type I    Type I
## Aortic_arch                     Aortic_arch   Type I  Type I    Type I
## Spleen                               Spleen   Type I  Type I    Type I
## Kidney                               Kidney   Type I  Type I    Type I
## Ovary                                 Ovary   Type I  Type I    Type I
## Fallopian_tube               Fallopian_tube   Type I  Type I    Type I
## Stomach                             Stomach   Type I  Type I    Type I
## Duodenum                           Duodenum   Type I  Type I    Type I
## Pituitary                         Pituitary   Type I  Type I    Type I
## Uterus                               Uterus   Type I  Type I    Type I
##                         mean_aging_amplitude
## Pancreas                         -0.075748775
## Supramarginal_gyrus               0.231195111
## Frontal_pole                      0.186849533
## Liver                             0.210951149
## Hippocampus                       0.044697652
## Skin_of_back                      0.153227025
## Cecum                             0.117593492
## Lung                              0.062388571
## Superior_temporal_gyrus           0.151674043
## Muscle                            0.127531951
## Adrenal_gland                     0.390895560
## Hypothalamus                      0.312324819
## Heart                             0.204160794
## Arteria_cruralis                 -0.302318429
## Arteria_carotis                  -0.015271359
## Adipose                          -0.650050564
## Thyroid_gland                    -0.233352138
## Facial_skin                      -0.068819684
## Femoral_vein                      0.002050297
## Ileocecum                        -0.157174219
## Thymus                           -0.293446352
## Aortic_arch                      -0.341411484
## Spleen                           -0.253614804
```

```
## Kidney                          -0.119119447
## Ovary                           -0.148487802
## Fallopian_tube                  -0.242323566
## Stomach                         -0.151051049
## Duodenum                        -0.334290753
## Pituitary                       -0.074656135
## Uterus                          -0.150139727
```

```
require(scatterplot3d)
#typecolor = '#3B4992','gray99','#EE0000FF',
#pdf('./out/20230217_aging/Figure4_MAA_analysis/Figure4C_3d_scatterplotAA.pdf')
typecolor = tissueClass[rownames(clusteramplitude_xx),]$color


s3d <- scatterplot3d(clusteramplitude_xx[,8], clusteramplitude_xx[,2], clusteramplitude_xx[,4], grid = 
pch=19,
scale.y=.75,

main="Aging amplitude",

xlab='C8',

ylab='C2 ',

zlab='C4')
s3d1 <- scatterplot3d(clusteramplitude_xx[,8], clusteramplitude_xx[,2], clusteramplitude_xx[,4], grid =
              color=typecolor,

pch=19,

scale.y=0.75,
scale.Z=1.25,

main="Aging amplitude",

xlab='C8',

ylab='C2 ',

zlab='C4',


text(s3d$xyz.convert(clusteramplitude_xx[,c(8,2,4)]), labels = rownames(clusteramplitude_xx),
     cex= 1))
#dev.off()
```

8.4 Figure 4D

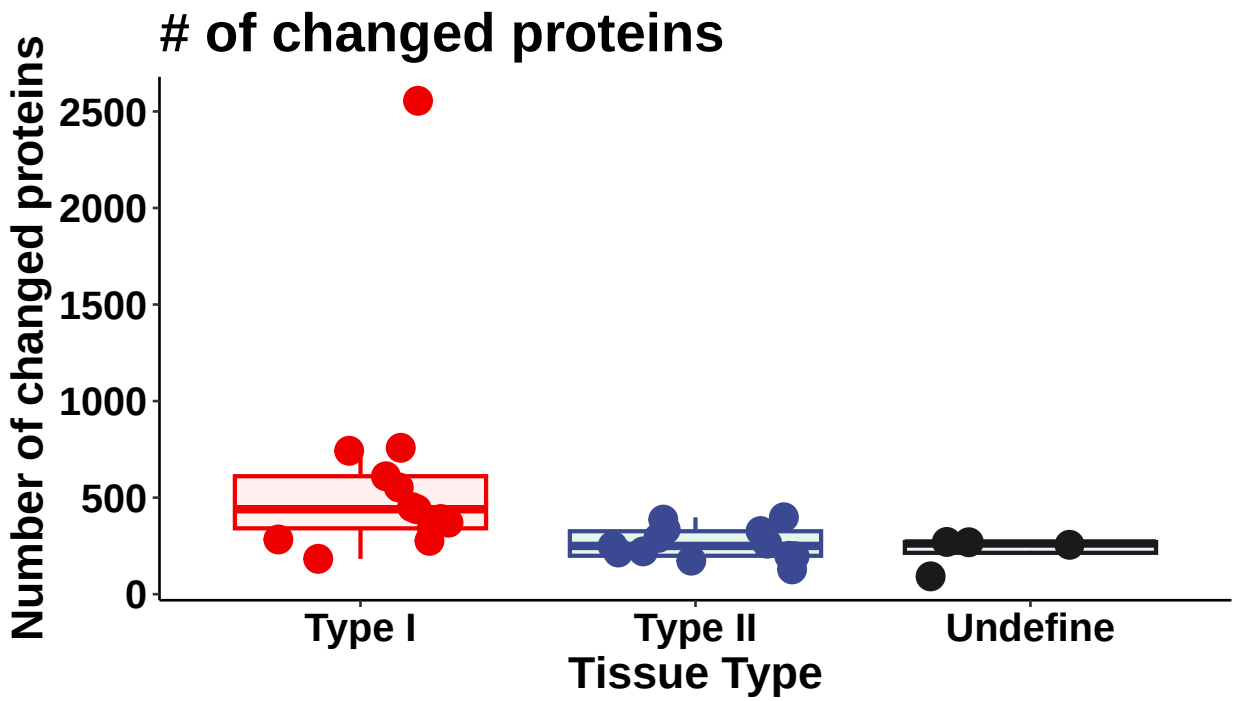
```
require(VennDiagram)
tmpnames = rownames(tissueClass)
Trajactory_Type_I = tmpnames[tissueClass$class == 'Type I']
Trajactory_Type_II = tmpnames[tissueClass$class == 'Type II']
MAA_Type_I = tmpnames[tissueClass$class.MAA == 'Type I']
```

```
MAA_Type_II = tmpnames[tissueClass$class.MAA == 'Type II']
venn.diagram(list(Trajactory_Type_I=Trajactory_Type_I,Trajactory_Type_II = Trajactory_Type_II,
                  MAA_Type_I = MAA_Type_I,MAA_Type_II=MAA_Type_II),
             #fill=c("red","blue",),
             fill = c("cornflowerblue", "green", "yellow", "darkorchid1"),
             alpha=c(0.5,0.5,0.5,0.5), cex=2, cat.fontface=3, cat.cex = 1.3,margin = 0.1,
             filename="./out/20230217_aging/Figure4_MAA_analysis/Venn_trajactory_and_MAA.tiff")
```

```
## [1] 1
```

9 Figure 5 experimental validation

```
# Figure 5A all changed protein
tx = colSums(abs(Aging_pro_sigall_matrix),na.rm = T)
yy1 = tissueClass[names(tx),]
#pdf('./out/20230217_aging/Figure5_markers_HE/Figure_S5_allchanged_proteins.pdf')
ggplot(,aes(x = yy1$type,y = tx,color = yy1$type,fill = yy1$type)) +#geom_violin()+
     geom_boxplot(outlier.size = -1,alpha = 0.1,size = 0.8)+
     geom_jitter(size = 5,width = 0.3)+ theme_classic() +
     ggtitle('# of changed proteins')+
     lghplot.addtheme()+ scale_color_manual(values=c('#EE0000','#3B4992','gray10'))+
     xlab('Tissue Type')+ylab('Number of changed proteins')
```

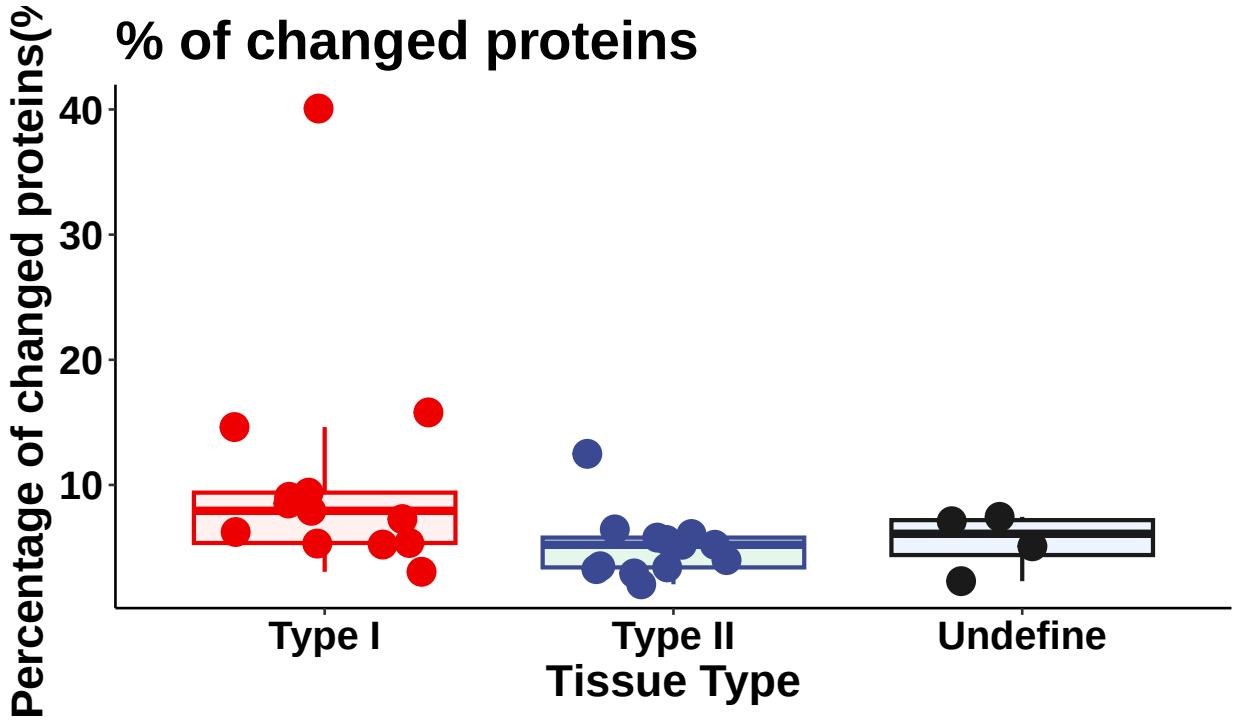



```
#dev.off()
wilcox.test(tx~yy1$type,subset= yy1$type != 'Undefine')
```

```
##
##  Wilcoxon rank sum exact test
##
```

```
## data:  tx by yy1$type
## W = 143, p-value = 0.001914
## alternative hypothesis: true location shift is not equal to 0
```

```
tx = colSums(abs(Aging_pro_sigall_matrix),na.rm = T)/
  colSums(!is.na(Aging_pro_sigall_matrix))
yy1 = tissueClass[names(tx),]
#pdf('./out/20230217_aging/Figure5_markers_HE/Figure4B_allchanged_proteins_percentageV2.pdf')
ggplot(,aes(x = yy1$type,y = tx*100,color = yy1$type,fill = yy1$type)) +#geom_violin()+
     geom_boxplot(outlier.size = -1,alpha = 0.1,size = 0.8)+
     geom_jitter(size = 5,width = 0.3)+ theme_classic() +
     ggtitle('% of changed proteins')+
     lghplot.addtheme()+ scale_color_manual(values=c('#EE0000','#3B4992','gray10'))+
     xlab('Tissue Type')+ylab('Percentage of changed proteins(%)')
```




```
#dev.off()
wilcox.test(tx~yy1$type,subset= yy1$type != 'Undefine')
```

```
##
##  Wilcoxon rank sum exact test
##
## data:  tx by yy1$type
## W = 136, p-value = 0.007244
## alternative hypothesis: true location shift is not equal to 0
```

9.1 Figure 5B

```
p16 = file2frame('./data/P16_tissues_v20230419.txt')
utissue = unique(p16$Tissue)
tpvalues = data.frame(tissue = utissue,
```

```
                    p_Juvenile_vs_Elderly = rep(1,length(utissue)),
                    p_Young_vs_Elderly = rep(1,length(utissue)))
breaks_A = c(0.01,0.08,0.08,0.08,0.08,0.01,0.01,0.01)
breaks_B = c(3,3,3,3,2,2,0.06,3)
for(i in 1:length(utissue)){
    tmpdata = p16[p16$Tissue == utissue[i],]
    tmpdata$Area.Density.log2 = tmpdata$Area.Density*1e3#log2(tmpdata$Area.Density*1e3)
    tmpdata$class = factor(x = tmpdata$class,levels = c('Juvenile','Young','Elderly'))
    tmpp1 = t.test(Area.Density~class,data = tmpdata,subset = tmpdata$class != 'Young')$p.value
    tmpp2 = t.test(Area.Density~class,data = tmpdata,subset = tmpdata$class != 'Juvenile')$p.value
    tpvalues$p_Juvenile_vs_Elderly[i] = tmpp1
    tpvalues$p_Young_vs_Elderly[i] = tmpp2

    df2 = data.frame(tmean = aggregate(tmpdata$Area.Density.log2,
                                       by=list(tmpdata$class), FUN=mean, na.rm = T)$x,
                tsd = aggregate(tmpdata$Area.Density.log2, by=list(tmpdata$class),
                                FUN=sd, na.rm = T)$x,
                    class = factor(x= c('Juvenile','Young','Elderly'),
                                   levels = c('Juvenile','Young','Elderly'))
                 )
    thepath = paste0('./out/20230217_aging/Figure5_markers_HE/Figure5x_p16_stats', utissue[i],'.pdf')
    pdf(thepath)
    p = ggplot(df2, aes(x = class, y = tmean, fill = class)) +
      geom_bar(stat = 'identity',color = 'black',position = position_dodge(),
               alpha = 0.5,size = 1.5)+
      geom_errorbar(aes(ymin = tmean,ymax = tmean+tsd),width = .3,size = 1.5)+
      lghplot.addthemeA(size = 28,sizex = 26,sizey = 26)+
      scale_fill_manual(values = c('#008B45FF','#3B4992FF','#EE0000FF'))+
      theme(axis.line = element_line(size = 1.2))+ xlab('')+
    ylab(bquote('Area Density ' ~ italic(x10) ^italic(3)))+
    #ylab('Log10 Area Density x 1e-7')+
    ggtitle(utissue[i])
    #if(i <=8){
    #    p = p+ scale_y_break(c(breaks_A[i],breaks_B[i]), scales = "free",space  = 0.2)
    #}

    print(p)
    dev.off()
}

tpvalues
```

```
##           tissue p_Juvenile_vs_Elderly p_Young_vs_Elderly
## 1    Aortic_arch          4.546295e-03       4.553667e-03
## 2         Spleen          2.928558e-03       2.941005e-03
## 3         Kidney          3.607333e-04       3.618602e-04
## 4          Ovary          2.399612e-03       2.487708e-03
## 5         Thymus          1.122249e-02       1.151782e-02
## 6         Uterus          1.052903e-04       1.057245e-04
## 7  Thyroid_gland          6.633705e-04       6.751286e-04
## 8        Stomach          3.601685e-05       3.616963e-05
## 9       Pancreas          2.616949e-02       5.453556e-03
## 10  Skin_of_back          2.446174e-02       4.146347e-02
## 11          Lung          4.895734e-03       6.619731e-03
```

```
## 12         Liver          2.051557e-01       2.593903e-01
## 13        Muscle          3.401860e-01       6.380951e-01
```

9.2 Figure 5C

```
p21 = file2frame('./data/P21_tissues_v20230419.txt')
utissue = unique(p21$Tissue)
tpvalues = data.frame(tissue = utissue,
                      p_Juvenile_vs_Elderly = rep(1,length(utissue)),
                      p_Young_vs_Elderly = rep(1,length(utissue)))
breaks_A = c(0.05,0.4,0.4,0.4,0.1,0.4,0.4,0.05)
breaks_B = c(1,4,4,4,2,4,4,1)
for(i in 1:length(utissue)){
    tmpdata = p21[p21$Tissue == utissue[i],]
    tmpdata$Area.Density.log2 = tmpdata$Area.Density*1e3#log2(tmpdata$Area.Density*1e3)
    tmpdata$class = factor(x = tmpdata$class,levels = c('Juvenile','Young','Elderly'))
    tmpp1 = t.test(Area.Density~class,data = tmpdata,subset = tmpdata$class != 'Young')$p.value
    tmpp2 = t.test(Area.Density~class,data = tmpdata,subset = tmpdata$class != 'Juvenile')$p.value
    tpvalues$p_Juvenile_vs_Elderly[i] = tmpp1
    tpvalues$p_Young_vs_Elderly[i] = tmpp2

    df2 = data.frame(tmean = aggregate(tmpdata$Area.Density.log2,
                                       by=list(tmpdata$class), FUN=mean, na.rm = T)$x,
                tsd = aggregate(tmpdata$Area.Density.log2, by=list(tmpdata$class),
                                FUN=sd, na.rm = T)$x,
                    class = factor(x= c('Juvenile','Young','Elderly'),
                                   levels = c('Juvenile','Young','Elderly'))
                 )
    thepath = paste0('./out/20230217_aging/Figure5_markers_HE/Figure4x_p21_stats', utissue[i],'.pdf')
    pdf(thepath)
    p = ggplot(df2, aes(x = class, y = tmean, fill = class)) +
      geom_bar(stat = 'identity',color = 'black',position = position_dodge(),
               alpha = 0.5,size = 1.5)+
      geom_errorbar(aes(ymin = tmean,ymax = tmean+tsd),width = .3,size = 1.5)+
      lghplot.addthemeA(size = 28,sizex = 26,sizey = 26)+
      scale_fill_manual(values = c('#008B45FF','#3B4992FF','#EE0000FF'))+
      theme(axis.line = element_line(size = 1.2))+ xlab('')+
    ylab(bquote('Area Density ' ~ italic(x10) ^italic(3)))+
    #ylab('Log10 Area Density x 1e-7')+
    ggtitle(utissue[i])
    #if(i <=8){
    #    p = p+ scale_y_break(c(breaks_A[i],breaks_B[i]), scales = "free",space  = 0.2)
    #}

    print(p)
    dev.off()
}

tpvalues
```

```
##           tissue p_Juvenile_vs_Elderly p_Young_vs_Elderly
## 1    Aortic_arch          3.371849e-03       3.490217e-03
## 2         Spleen          6.244567e-04       6.370290e-04
```

```
## 3         Kidney          1.052679e-02       1.070538e-02
## 4          Ovary          3.484938e-04       3.559713e-04
## 5         Thymus          4.256548e-02       4.462434e-02
## 6         Uterus          2.571205e-02       2.611076e-02
## 7  Thyroid_gland          1.553183e-02       1.686152e-02
## 8        Stomach          4.794586e-05       4.974984e-05
## 9       Pancreas          2.700608e-01       1.668549e-01
## 10  Skin_of_back          5.553932e-02       2.923295e-01
## 11          Lung          6.940087e-04       6.950478e-04
## 12         Liver          9.815520e-01       9.972549e-01
## 13        Muscle          3.620859e-01       7.890578e-01
```

9.3 Figure 5D

```
Cellcounts = file2frame('./data/cell_counts_v20230407.txt')

utissue = unique(Cellcounts$Tissue)
tpvalues = data.frame(tissue = utissue,
                      p_Juvenile_vs_Elderly = rep(1,length(utissue)),
                      p_Young_vs_Elderly = rep(1,length(utissue)))
for(i in 1:length(utissue)){
    tmpdata = Cellcounts[Cellcounts$Tissue == utissue[i],]
    tmpdata$class = factor(x = tmpdata$class,
                           levels = c('Juvenile','Young','Elderly'))
    tmpp1 = t.test(number~class,data = tmpdata,subset = tmpdata$class != 'Young')$p.value
    tmpp2 = t.test(number~class,data = tmpdata,subset = tmpdata$class != 'Juvenile')$p.value
    tpvalues$p_Juvenile_vs_Elderly[i] = tmpp1
    tpvalues$p_Young_vs_Elderly[i] = tmpp2

    df2 = data.frame(tmean = aggregate(tmpdata$number, by=list(tmpdata$class),
                                       FUN=mean, na.rm = T)$x,
                tsd = aggregate(tmpdata$number, by=list(tmpdata$class), FUN=sd, na.rm = T)$x,
                    class = factor(x= c('Juvenile','Young','Elderly'),
                                   levels = c('Juvenile','Young','Elderly'))
                 )
    thepath = paste0('./out/20230217_aging/Figure5_markers_HE/Figure4x_HE_cellcounts_', utissue[i],'_v1
    pdf(thepath)
    p = ggplot(df2, aes(x = class, y = tmean, fill = class)) +
      geom_bar(stat = 'identity',color = 'black',position = position_dodge(),alpha = 0.5,size = 1.5)+
      geom_errorbar(aes(ymin = tmean,ymax = tmean+tsd),width = .3,size = 1.5)+
      lghplot.addthemeA(size = 28,sizex = 26,sizey = 26)+
      scale_fill_manual(values = c('#008B45FF','#3B4992FF','#EE0000FF'))+
      theme(axis.line = element_line(size = 1.2))+ xlab('')+
      ylab('Number of Parenchymal Cells')+ggtitle(utissue[i])
    print(p)
    dev.off()
}

tpvalues
```

```
##          tissue p_Juvenile_vs_Elderly p_Young_vs_Elderly
## 1        Thymus          3.842297e-07       2.479669e-04
## 2       Stomach          4.735346e-08       4.458945e-06
```

```
## 3   Aortic_arch          8.038428e-07       2.041040e-07
## 4         Ovary          6.576220e-09       6.727973e-05
## 5        Spleen          1.724836e-06       4.997698e-01
## 6 Thyroid_gland          3.814005e-06       9.571183e-02
## 7        Kidney          2.734793e-01       3.090672e-01
```

10 Figure 6: Translation efficiency

10.1 ratio data construction

```
tissues = names(promet.tissues.Z)
clusterdist = matrix(0,length(tissues),8)
clusteramplitude = matrix(0,length(tissues),8)
clusteramplitude_xx = matrix(0,length(tissues),8)
for(i in 1:length(tissues)){
    mstd =   promet.tissues.Z[[i]]
    for(j in 1:8){
    tgene = names(mfuzz.promet.whole$cluster)[mfuzz.promet.whole$cluster == j]
    tgene = intersect(tgene,rownames(mstd))
    bx = t(t(mstd[tgene,]) - mfuzz.promet.whole$centers[j,])
    clusterdist[i,j] = mean(sqrt(rowSums(bx^2)/(ncol(bx)-1)),na.rm = T)
    if(length(tgene) < 2){
      clusteramplitude[i,j] = NA
      next;
    }
    tamp = colMeans(mstd[tgene,],na.rm = T)
    clusteramplitude[i,j] = abs(tamp[4]-tamp[1])
    clusteramplitude_xx[i,j] = tamp[4]-tamp[1]
  }
}
rownames(clusteramplitude) = tissues
rownames(clusteramplitude_xx) = tissues
rownames(clusterdist) = tissues
```

```
ratio.tissues = list()
ratio.tissues.info = list()
pro.tissues.forRatio = list()
mrna.tissues.forRatio = list()
tissues = names(pro.tissues)
overlaptissues = intersect(names(pro.tissues),names(mrna.tissues))
for( i in 1:length(overlaptissues)){
    this_tissue = overlaptissues[i]
    thispro = delete_dup_genes_forprotein(pro.tissues[[this_tissue]],
                                          pro.tissues.header[[this_tissue]])
    thismrna = mrna.tissues[[this_tissue]]
    vcol = intersect(colnames(thispro),colnames(thismrna))
    vrow = intersect(rownames(thispro),rownames(thismrna))
    thispro = thispro[vrow,vcol]
    thismrna  = thismrna[vrow,vcol]
    pro.tissues.forRatio[[i]] = thispro
    mrna.tissues.forRatio[[i]] = thismrna
    ratio.tissues[[i]] = thispro - thismrna
```

```
    ratio.tissues.info[[i]] = pro.tissues.info[[i]][vcol,]
}
names(ratio.tissues) = overlaptissues
names(pro.tissues.forRatio) = overlaptissues
names(mrna.tissues.forRatio) = overlaptissues
names(ratio.tissues.info) = overlaptissues
```

```
ratio_amplitude = list()
pro_amplitude = list()
mrna_amplitude = list()
n = length(ratio.tissues)
ratio_out = data.frame(meanChangeRatio = rep(0,n),stringsAsFactors = F,
                fc_up_down = rep(0,n),
                tissues = names(ratio.tissues),
                meanChangeproZ = rep(0,n),
                fc_up_down_pro = rep(0,n),
                meanChangemrnaZ = rep(0,n),
                fc_up_down_mrna = rep(0,n))
for (i in 1:length(ratio.tissues)){
    bx = ratio.tissues[[i]]
    bx.info = ratio.tissues.info[[i]]
    id = bx.info$stage < 5
    bx = bx[,id]
    bx.info = bx.info[id,]
    cx = t(aggregate(t(bx), by=list(bx.info$stage), FUN=mean, na.rm = T))
    cx = as.matrix(standardise_1(new("ExpressionSet",exprs = cx)))
    cx = cx[-1,]
    ee = cx[,4]-cx[,1]
    ratio_amplitude[[i]] = ee;
    #hist(ee,30)
    ratio_out$meanChangeRatio[i] = mean(ee,na.rm = T)
    ratio_out$fc_up_down[i] = log2(sum(ee > 0,na.rm = T)/sum(ee < -0,na.rm = T))

    bx = pro.tissues.forRatio[[i]]
    bx.info = ratio.tissues.info[[i]]
    id = bx.info$stage < 5
    bx = bx[,id]
    bx.info = bx.info[id,]
    cx = t(aggregate(t(bx), by=list(bx.info$stage), FUN=mean, na.rm = T))
    cx = as.matrix(standardise_1(new("ExpressionSet",exprs = cx)))
    cx = cx[-1,]
    ee = cx[,4]-cx[,1]
    pro_amplitude[[i]] = ee
    #hist(ee,30)
    ratio_out$meanChangeproZ[i] = mean(ee,na.rm = T)
    ratio_out$fc_up_down_pro[i] = log2(sum(ee > 0,na.rm = T)/sum(ee < -0,na.rm = T))

    bx = mrna.tissues.forRatio[[i]]
    bx.info = ratio.tissues.info[[i]]
    id = bx.info$stage < 5
    bx = bx[,id]
    bx.info = bx.info[id,]
    cx = t(aggregate(t(bx), by=list(bx.info$stage), FUN=mean, na.rm = T))
    cx = as.matrix(standardise_1(new("ExpressionSet",exprs = cx)))
```

```
    cx = cx[-1,]
    ee = cx[,4]-cx[,1]
    mrna_amplitude[[i]] = ee
    #hist(ee,30)
    ratio_out$meanChangemrnaZ[i] = mean(ee,na.rm = T)
    ratio_out$fc_up_down_mrna[i] = log2(sum(ee > 0,na.rm = T)/sum(ee < -0,na.rm = T))
}
rownames(ratio_out) = names(ratio.tissues)
names(ratio_amplitude) = names(ratio.tissues)
names(pro_amplitude) = names(ratio.tissues)
names(mrna_amplitude) = names(ratio.tissues)
```

10.2 Figure 6A design

10.3 Figure S9 plot mRNA and protein

```
mRNA.mean = list()
pro.mean = list()
for(i in 1:length(pro.tissues.forRatio)){
    mRNA.mean[[i]] = rowMeans(mrna.tissues.forRatio[[i]],na.rm = T)
    pro.mean[[i]] = rowMeans(pro.tissues.forRatio[[i]],na.rm = T)
}
names(mRNA.mean) = names(mrna.tissues.forRatio)
names(pro.mean) = names(pro.tissues.forRatio)
RNA.v = list_to_matrix(mRNA.mean,names(mRNA.mean))
pro.v = list_to_matrix(pro.mean,names(pro.mean))

pp = list()
for(i in 1:ncol(pro.v)){
    idaa = !is.na(RNA.v[,i]) & !is.na(pro.v[,i])
    tcor = cor.test(RNA.v[idaa,i],pro.v[idaa,i])$estimate
    tmpdata = data.frame(xx = RNA.v[idaa,i],
                         yy = pro.v[idaa,i])
    pp[[i]] = ggplot(tmpdata,aes(x= xx,y = yy))+geom_point(size =1,) + theme_bw()+
        theme(plot.margin = margin(0.1,0.1,0.1,0.1,"cm"))+
        lghplot.addthemeA(size = 16,sizex = 16,sizey = 16)+
        annotate(geom="text", x=4, y=25,
                 label = paste('R=',signif(tcor,3)),
                color="darkblue",size = 8,face = "italic")+
        xlab('')+ylab('')+ggtitle(colnames(pro.v)[i])
}

png(file = "./out/20230217_aging/Figure6_ratio_mrna_pro/Figure S9_mrna_vs_pro.png",
    width = 1250,height = 1500)
grid.arrange(arrangeGrob(grobs = pp, ncol = 5,
                         bottom=textGrob('mRNA expression(log2 CPM)',
                                         gp=gpar(fontface="bold",  fontsize=22)),
                         left = textGrob('Protein abundance(log2 Peak Area)',
                                  gp=gpar(fontface="bold",  fontsize=22),rot=90)))
dev.off()
```

```
## pdf
```

```
##   2
```

10.4 Figure S10 predict protein vs measured protein

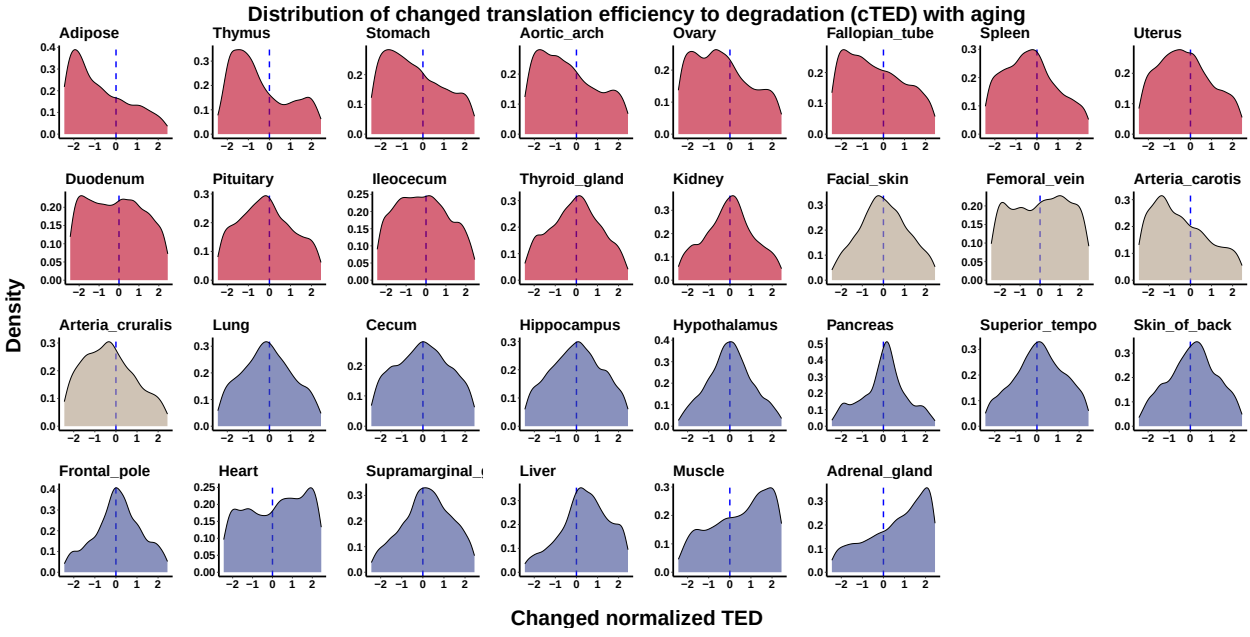
```
ratio = pro.v - RNA.v
ratio = apply(ratio,1,median,na.rm =T)

# remove ratio = 0 and NA
#id = is.na(ratio) | ratio == 0
#ratio = ratio[!id]

pro.prediction = RNA.v + ratio

pp = list()
for(i in 1:ncol(pro.v)){
    idaa = !is.na(pro.prediction[,i]) & !is.na(pro.v[,i])
    tcor = cor.test(pro.prediction[idaa,i],pro.v[idaa,i])$estimate
    tmpdata = data.frame(xx = pro.prediction[idaa,i],
                         yy = pro.v[idaa,i])
    pp[[i]] = ggplot(tmpdata,aes(x= xx,y = yy))+geom_point(size =1,) + theme_bw()+
        theme(plot.margin = margin(0.1,0.1,0.1,0.1,"cm"))+
        lghplot.addthemeA(size = 16,sizex = 16,sizey = 16)+
        annotate(geom="text", x=15, y=25,
                 label = paste('R=',signif(tcor,3)),
                color="darkblue",size = 8,face = "italic")+
        xlab('')+ylab('')+ggtitle(colnames(pro.v)[i])
}

png(file = "./out/20230217_aging/Figure6_ratio_mrna_pro/Figure S10_predPro_vs_pro.png",
    width = 1250,height = 1500)
grid.arrange(arrangeGrob(grobs = pp, ncol = 5,
                         bottom=textGrob('Predicted protein abundance(log2 Peak Area)',
                                         gp=gpar(fontface="bold",  fontsize=22)),
                        left = textGrob('Protein abundance(log2 Peak Area)',
                                        gp=gpar(fontface="bold",  fontsize=22),rot=90)))
dev.off()
```

```
## pdf
##   2
```

10.5 Figure 6B density plot

```
systems.Color = substr(pal_aaas()(10),1,7)
names(systems.Color) = unique(tissue.systems)
systems.Color.fortissue = systems.Color[tissue.systems]
names(systems.Color.fortissue) = names(ratio_amplitude)
tissueClass$color = tissueClass$type
tissueClass$color[tissueClass$color == 'Type I'] = '#BB0021'
tissueClass$color[tissueClass$color == 'Type II'] = '#3B4992'
tissueClass$color[tissueClass$color == 'Undefine'] = '#B09C85'
```

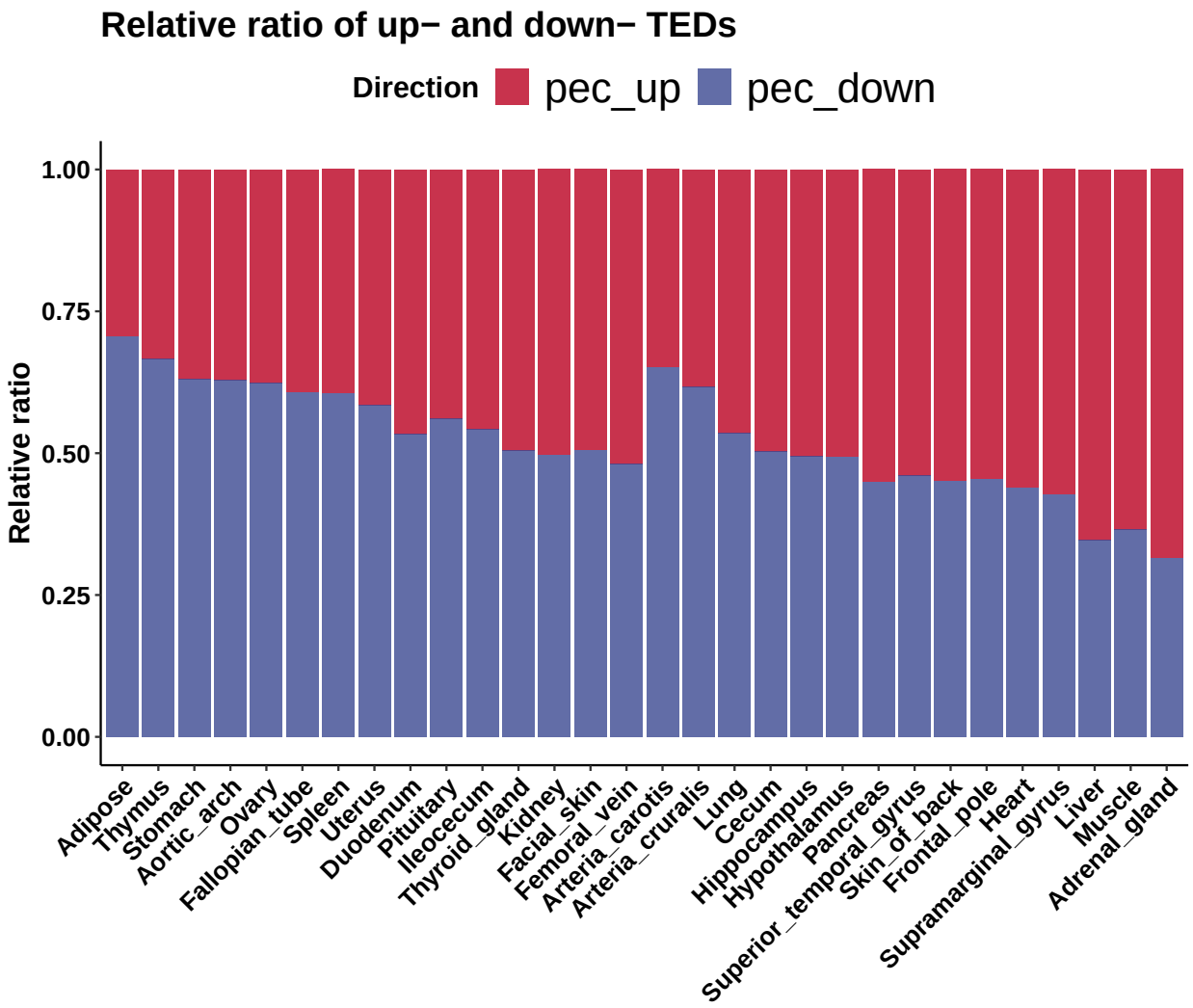
```r
# hist plot
p1 = list()
xplot <- function(ratio_amplitude,xtissue,tissueClass){
    tcolor = tissueClass[xtissue,]$color
    tmp =  ggplot(,aes(x = ratio_amplitude[[xtissue]]))+theme_classic()+
         #geom_histogram(binwidth=0.1,aes(y=..density..), colour="black", fill="white")+
         geom_vline(xintercept=0, linetype="dashed", color = "blue", size=1)+
         geom_density(alpha=.6, fill=tcolor) +lghplot.addtheme()+
      theme(axis.line = element_line(size = 1.2))+
      xlab('')+ylab('')+ggtitle(xtissue)
    return(tmp)
}

index = sort.int(ratio_out$meanChangeRatio,decreasing = F,index.return = T)$ix
vclass = tissueClass[rownames(ratio_out)[index],]
idx = sort.int(vclass$class,decreasing = F,index.return = T)$ix
vclass = vclass[idx,]
for(i in 1:nrow(vclass)){
    xtissue = vclass$tissue[i]
    p1[[i]] = xplot(ratio_amplitude,xtissue,tissueClass)
}
#pdf(file = './out/20230217_aging/Figure6_ratio_mrna_pro/Figure6B_Changed_ratio_distribute_based_on_pro
grid.arrange(arrangeGrob(grobs = p1,ncol = 8,
           top=textGrob('Distribution of changed translation efficiency to degradation (cTED) with aging
                                    gp=gpar(fontface="bold",  fontsize=30)),
            bottom=textGrob('Changed normalized TED',
                                    gp=gpar(fontface="bold",  fontsize=30)),
            left = textGrob('Density',
                                    gp=gpar(fontface="bold",  fontsize=30),rot=90)))
```




```r
#graphics.off()
```

10.6 Figure 6C ratio plot

```
ratio_updown = zeros(length(ratio_amplitude),2)
tissues =names(ratio_amplitude)
rownames(ratio_updown) = tissues
colnames(ratio_updown) = c('pec_up','pec_down')
for(i in 1:length(tissues)){
    tmp = as.vector(ratio_amplitude[[i]])
    tmpN = sum(!is.na(tmp))
    ratio_updown[i,1] = sum(tmp > 0,na.rm =T)/tmpN
    ratio_updown[i,2] = sum(tmp < 0,na.rm =T)/tmpN
}
index = sort.int(ratio_out$meanChangeRatio,decreasing = F,index.return = T)$ix
ratio_updown = ratio_updown[index,]

tmp = melt(ratio_updown,stringsAsFactor =F)
colnames(tmp) = c('tissues','Direction','perctage')
tmp$tissues = factor(tmp$tissues,levels = rownames(vclass))
#pdf('./out/20230217_aging/Figure6_ratio_mrna_pro/
#   Figure6C_tissue_trans_effectiveness_amplitude_ratio_V2.pdf',width = 8,height = 7)
ggplot(data=tmp, aes(x=tissues, y=perctage, fill=Direction))+theme_classic()+
       geom_col(position = "fill",alpha = 0.8)+
      ylab('Relative ratio')+ xlab('')+scale_fill_manual(values=c('#BB0021', '#3B4992'))+
      lghplot.addtheme(legend.position = 'top',hjust = 1,size = 14)+
      ggtitle('Relative ratio of up- and down- TEDs')+
      theme(legend.text=element_text(size=20))
```




```
#graphics.off()
```

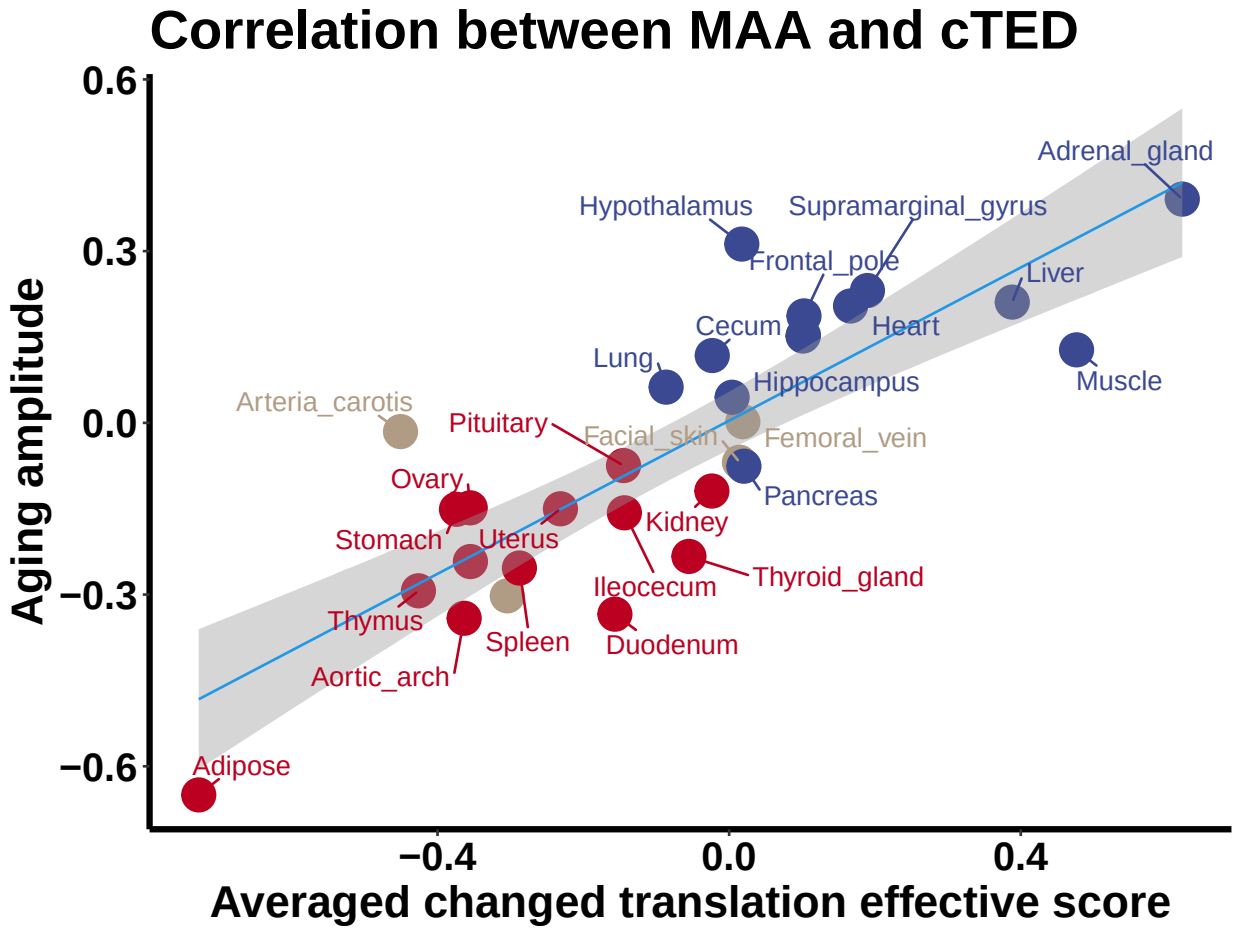
10.7 Figure 6D correlation

```
mean_aging_amplitude = rowMeans(clusteramplitude_xx[,])
mean_aging_amplitude = mean_aging_amplitude[rownames(ratio_out)]
tmpdata = data.frame(ratioChange = ratio_out$meanChangeRatio,stringsAsFactors = F,
                     ratioFC = ratio_out$fc_up_down,
                     amplitude = mean_aging_amplitude,
                    tissues = rownames(ratio_out))
rownames(tmpdata) = tmpdata$tissues
tmpdata$tissueclass = tissueClass[rownames(tmpdata),]$type
tmp1 = tmpdata
cor.test(tmp1$ratioChange,tmp1$amplitude)
```

```
##
##  Pearson's product-moment correlation
##
```

```
## data:  tmp1$ratioChange and tmp1$amplitude
## t = 7.9553, df = 28, p-value = 1.154e-08
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.6747763 0.9176362
## sample estimates:
##       cor
## 0.8326318
```

```r
#pdf('./out/20230217_aging/Figure6_ratio_mrna_pro/Figure6D_overall_translation_effective_score_vs_aging_
p = ggplot(tmp1,aes(ratioChange,amplitude,color = tissueclass)) +
  theme_classic()+
      geom_point(size = 6,aes(color = tissueclass)) +
      lghplot.addtheme()+geom_smooth(color = 4,size = 0.5,method = 'lm')+
      geom_text_repel(aes(label = tissues),size = 4,box.padding = 0.5)+
      #scale_color_aaas(alpha = 0.6)+scale_fill_aaas(alpha = 0.6)+
      scale_color_manual(values=c('#BB0021', '#3B4992','#B09C85'))+
      theme(axis.line = element_line(size = 1.2))+
      xlab('Averaged changed translation effective score')+
      ylab('Aging amplitude')+
      ggtitle('Correlation between MAA and cTED')
print(p)
```



```
#dev.off()
```

11 Figure 7

11.1 GO data construct

```
#outids = c('R-HSA-72766','GO:0006413','hsa03010','ko04142','hsa04120','ko03050')
outids = c('R-HSA-72766','hsa03010','hsa04142','hsa04120','hsa03050')
#tpath = paste0('./out/20210428_aging/promet/tissues/metascape/Aging_up_genes_metascape/Enrichment_GO/'
tpath = paste0('./out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_up/Enrichment_GO/','
thisgo = file2frame(tpath,sep = ',')
thisgo= thisgo[!duplicated(thisgo$GO),]
xid = is.element(thisgo$GO,outids)
thisgo = thisgo[xid,]
#rownames(thisgo) = paste0(thisgo$GO,':',thisgo$Description)
rownames(thisgo) = paste0(thisgo$GO)
thisgo.matrix = -as.matrix(thisgo[,substr(colnames(thisgo),1,6)== 'X_LogP'])

# replace qval
tpath1a = './out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_up/Enrichment_GO/GO_AllLis
go1qval = file2frame(tpath1a,sep = ',',header = T)
rownames(go1qval) = paste0(go1qval$GO,'X_LogP_',go1qval$GeneList)
go1term = rownames(thisgo.matrix)
cname = colnames(thisgo.matrix)
for(i in 1:nrow(thisgo.matrix)){
    for(j in 1:ncol(thisgo.matrix)){
        tmpname = paste0(go1term[i],cname[j])
        thisgo.matrix[i,j] = abs(go1qval[tmpname,]$Log.q.value.)
    }
}
thisgo.matrix[is.na(thisgo.matrix)] = 0


thisgo.matrix[abs(thisgo.matrix) < 3] = 0
colnames(thisgo.matrix) = capitalize(gsub('X_LogP_','',colnames(thisgo.matrix)))
colnames(thisgo.matrix) = gsub('Cluster','C',colnames(thisgo.matrix))
thisgo.matrix.up = thisgo.matrix
tmpname = rownames(thisgo.matrix.up)
#add hsa03050
thisgo.matrix.up = rbind(thisgo.matrix.up,rep(0,30))
rownames(thisgo.matrix.up) = c(tmpname,'hsa03050')
thisgo.matrix.up = thisgo.matrix.up[outids,]
thisgo.matrix.up
```

```
##             Adipose Adrenal_gland Aortic_arch Arteria_carotis Arteria_cruralis
## R-HSA-72766       0             0           0               0                0
## hsa03010          0             0           0               0                0
## hsa04142          0             0           0               0                0
## hsa04120          0             0           0               0                0
## hsa03050          0             0           0               0                0
##             Cecum Duodenum Facial_skin Fallopian_tube Femoral_vein Frontal_pole
```

```
## R-HSA-72766     0        0           0              0          4.3            0
## hsa03010        0        0           0              0          0.0            0
## hsa04142        0        0           0              0          0.0            0
## hsa04120        0        0           0              0          0.0            0
## hsa03050        0        0           0              0          0.0            0
##             Heart Hippocampus Hypothalamus Ileocecum Kidney Liver Lung Muscle
## R-HSA-72766   0.0           0            0         0      0   0.0    0      0
## hsa03010      0.0           0            0         0      0   0.0    0      0
## hsa04142      3.5           0            3         0      0   3.2    0      0
## hsa04120      0.0           0            0         0      0   0.0    0      0
## hsa03050      0.0           0            0         0      0   0.0    0      0
##             Ovary Pancreas Pituitary Skin_of_back Spleen Stomach
## R-HSA-72766     0        0         0            0      0       0
## hsa03010        0        0         0            0      0       0
## hsa04142        0        0         0            0      0       0
## hsa04120        0        0         0            0      0       0
## hsa03050        0        0         0            0      0       0
##             Superior_temporal_gyrus Supramarginal_gyrus Thymus Thyroid_gland
## R-HSA-72766                       0                   0      0             0
## hsa03010                          0                   0      0             0
## hsa04142                          0                   0      0             0
## hsa04120                          0                   0      0             0
## hsa03050                          0                   0      0             0
##             Uterus
## R-HSA-72766      0
## hsa03010         0
## hsa04142         0
## hsa04120         0
## hsa03050         0
```

```r
outids = c('R-HSA-72766','hsa03010','hsa04142','hsa04120','hsa03050')
outids_des = c('Translation','Ribosome','Lysosome',
                            'Ubiquitin mediated proteolysis', 'Proteasome')
tpath = paste0('./out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_down/Enrichment_GO/'
thisgo = file2frame(tpath,sep = ',')
thisgo= thisgo[!duplicated(thisgo$GO),]
xid = is.element(thisgo$GO,outids)
thisgo = thisgo[xid,]
rownames(thisgo) = paste0(thisgo$GO)

# replace qval
tpath1a = './out/20230217_aging/Figure2_DEG_GO_tissue/protein/metascape_DEpro_down/Enrichment_GO/GO_All
go1qval = file2frame(tpath1a,sep = ',',header = T)
rownames(go1qval) = paste0(go1qval$GO,'X_LogP_',go1qval$GeneList)
go1term = rownames(thisgo.matrix)
cname = colnames(thisgo.matrix)
for(i in 1:nrow(thisgo.matrix)){
    for(j in 1:ncol(thisgo.matrix)){
        tmpname = paste0(go1term[i],cname[j])
        thisgo.matrix[i,j] = abs(go1qval[tmpname,]$Log.q.value.)
    }
}
thisgo.matrix[is.na(thisgo.matrix)] = 0
```

```
thisgo.matrix = as.matrix(thisgo[,substr(colnames(thisgo),1,6)== 'X_LogP'])
thisgo.matrix[abs(thisgo.matrix) < 3 ] = 0
colnames(thisgo.matrix) = capitalize(gsub('X_LogP_','',colnames(thisgo.matrix)))
colnames(thisgo.matrix) = gsub('Cluster','C',colnames(thisgo.matrix))
rownames(thisgo.matrix)
```

```
## [1] "R-HSA-72766" "hsa03010"    "hsa03050"    "hsa04142"    "hsa04120"
```

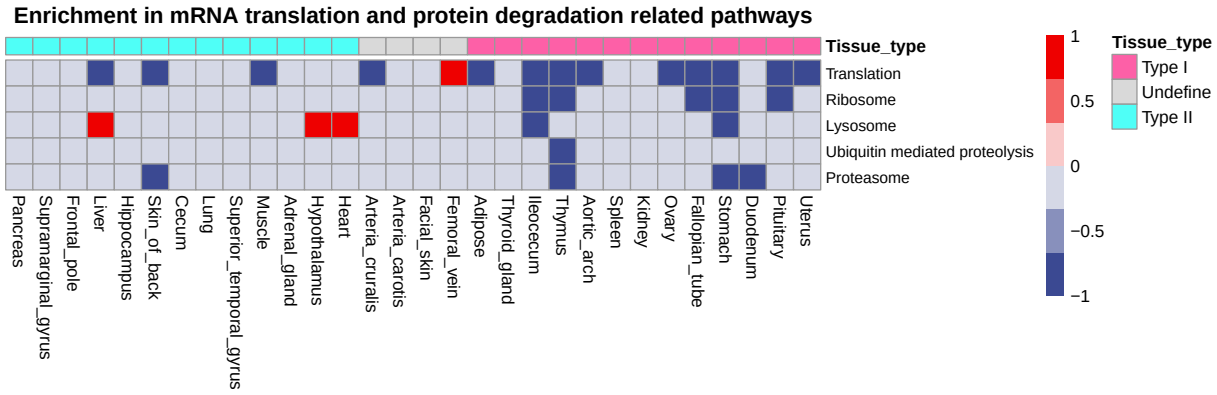
```
thisgo.matrix.down = thisgo.matrix
thisgo.matrix.down = thisgo.matrix.down[outids,]
thisgo.matrix.down
```

```
##             Adipose Adrenal_gland Aortic_arch Arteria_carotis Arteria_cruralis
## R-HSA-72766    -5.2             0        -5.9               0             -4.3
## hsa03010        0.0             0         0.0               0              0.0
## hsa04142        0.0             0         0.0               0              0.0
## hsa04120        0.0             0         0.0               0              0.0
## hsa03050        0.0             0         0.0               0              0.0
##             Cecum Duodenum Facial_skin Fallopian_tube Femoral_vein Frontal_pole
## R-HSA-72766     0      0.0           0           -6.9            0            0
## hsa03010        0      0.0           0           -5.0            0            0
## hsa04142        0      0.0           0            0.0            0            0
## hsa04120        0      0.0           0            0.0            0            0
## hsa03050        0     -4.5           0            0.0            0            0
##             Heart Hippocampus Hypothalamus Ileocecum Kidney Liver Lung Muscle
## R-HSA-72766     0           0            0      -7.2      0    -6    0   -4.6
## hsa03010        0           0            0      -3.9      0     0    0    0.0
## hsa04142        0           0            0      -3.6      0     0    0    0.0
## hsa04120        0           0            0       0.0      0     0    0    0.0
## hsa03050        0           0            0       0.0      0     0    0    0.0
##             Ovary Pancreas Pituitary Skin_of_back Spleen Stomach
## R-HSA-72766  -7.7        0       -11         -3.8      0   -12.0
## hsa03010      0.0        0       -10          0.0      0    -4.7
## hsa04142      0.0        0         0          0.0      0    -5.7
## hsa04120      0.0        0         0          0.0      0     0.0
## hsa03050      0.0        0         0         -3.1      0    -6.7
##             Superior_temporal_gyrus Supramarginal_gyrus Thymus Thyroid_gland
## R-HSA-72766                       0                  0  -34.0             0
## hsa03010                          0                  0   -7.3             0
## hsa04142                          0                  0    0.0             0
## hsa04120                          0                  0   -7.8             0
## hsa03050                          0                  0  -25.0             0
##             Uterus
## R-HSA-72766     -5
## hsa03010         0
## hsa04142         0
## hsa04120         0
## hsa03050         0
```

11.2 Figure 7A

```
outids = c('R-HSA-72766','hsa03010','hsa04142','hsa04120','hsa03050')
outids_des = c('Translation','Ribosome','Lysosome',
```

```
                                'Ubiquitin mediated proteolysis', 'Proteasome')
thisgo.matrix.all = thisgo.matrix.up+ thisgo.matrix.down

tmpnames = rownames(tissueClass)
tmpnames = c(tmpnames[tissueClass$type == 'Type II'],tmpnames[tissueClass$type == 'Undefine'],
             tmpnames[tissueClass$type == 'Type I'])

thisgo.matrix.all = thisgo.matrix.all[,tmpnames]
thisgo.matrix.all[thisgo.matrix.all >= 3] = 1
thisgo.matrix.all[thisgo.matrix.all <= -3] = -1

tclass = data.frame(class = tissueClass[tmpnames,]$type,row.names = tmpnames)
colnames(tclass) <- c("Tissue_type")
ann_colors = list(
   Tissue_type= c('#FD60A7','gray85','#4fffF7')

)
names(ann_colors$Tissue_type) = c('Type I','Undefine', 'Type II')
#sort tissues
tmpname  = colnames(thisgo.matrix.all)
rownames(thisgo.matrix.all) = outids_des
pheatmap::pheatmap(thisgo.matrix.all,cluster_rows = F,annotation_colors = ann_colors,
                   annotation_col = tclass,
                  main = 'Enrichment in mRNA translation and protein degradation related pathways',
                   cluster_cols = F,fontsize_row = 9,fontsize_col = 10,
                  fontsize = 10,treeheight_row = 20,treeheight_col = 20,legend = T,
                   color=colorRampPalette(c('#3B4992','gray99','#EE0000FF'))(6),
                   #file ="./out/20230217_aging/Figure6_heatmap_TED_machanism/Figure 6A_enrichment_metas
                  height = 3,width = 10
                   )
```




```
idx = tclass$Tissue_type != 'Undefine'
tmpaa = thisgo.matrix.all[,idx]
tmpclass = tclass$Tissue_type[idx]
rownames(tmpaa)
```

```
## [1] "Translation"                    "Ribosome"
## [3] "Lysosome"                       "Ubiquitin mediated proteolysis"
## [5] "Proteasome"
```

```r
cor.test(abs(tmpaa[1,]),(tmpclass == 'Type I')+0,method = 'spearman')
```

```
##
##  Spearman's rank correlation rho
##
## data:  abs(tmpaa[1, ]) and (tmpclass == "Type I") + 0
## S = 1571, p-value = 0.01725
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##     rho
## 0.46291
```

```r
cor.test(abs(tmpaa[2,]),(tmpclass == 'Type I')+0,method = 'spearman')
```

```
##
##  Spearman's rank correlation rho
##
## data:  abs(tmpaa[2, ]) and (tmpclass == "Type I") + 0
## S = 1497.7, p-value = 0.01144
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##     rho
## 0.48795
```

```r
cor.test(abs(tmpaa[3,]),(tmpclass == 'Type I')+0,method = 'spearman')
```

```
##
##  Spearman's rank correlation rho
##
## data:  abs(tmpaa[3, ]) and (tmpclass == "Type I") + 0
## S = 3210.5, p-value = 0.6353
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##         rho
## -0.09759001
```

```r
cor.test(abs(tmpaa[4,]),(tmpclass == 'Type I')+0,method = 'spearman')
```

```
##
##  Spearman's rank correlation rho
##
## data:  abs(tmpaa[4, ]) and (tmpclass == "Type I") + 0
## S = 2340, p-value = 0.3273
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
## rho
## 0.2
```

```r
cor.test(abs(tmpaa[5,]),(tmpclass == 'Type I')+0,method = 'spearman')
```

```
##
##  Spearman's rank correlation rho
##
## data:  abs(tmpaa[5, ]) and (tmpclass == "Type I") + 0
## S = 2301.4, p-value = 0.2957
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
```

```
##       rho
## 0.2132007
```

11.3 Figure 7B

```
require(data.table)
amplitude_matrix = list()

for(i in 1:length(pro_amplitude)){
    tmp = matrix(pro_amplitude[[i]],1,length(pro_amplitude[[i]]))
    tmp = as.data.frame(tmp)
    colnames(tmp) = names(pro_amplitude[[i]])
    amplitude_matrix[[i]] = tmp
}
#amplitude_matrix = rbindlist(amplitude_matrix,fill = T)
amplitude_matrix = t(as.matrix(rbindlist(amplitude_matrix,fill = T)))
colnames(amplitude_matrix) = names(pro_amplitude)

vid = rowSums(is.na(amplitude_matrix)) < ncol(amplitude_matrix)/2
amplitude_matrix.v = amplitude_matrix[vid,]
amplitude_matrix.v[is.na(amplitude_matrix.v)] = NA
dim(amplitude_matrix.v)
```

```
## [1] 3902   30
```

```
tcor = cor(t(amplitude_matrix.v),ratio_out$meanChangeRatio,use = "pairwise")
names(tcor) = rownames(amplitude_matrix.v)
gsea_input = sort(tcor,decreasing = T)

calcp <- function(x,y){
    return(cor.test(x,y,use = "pairwise")$p.value)
}
tpvalue = apply(amplitude_matrix.v,1,calcp,ratio_out$meanChangeRatio)
tpvalue = tpvalue[names(gsea_input)]
```

```
xnames = names(tpvalue)
trans_initialA  = xnames[substr(xnames,1,3) == 'EIF']
trans_initialA = sort(trans_initialA)
trans_initialA
```

```
##  [1] "EIF1AX"   "EIF2A"    "EIF2AK2"  "EIF2B1"   "EIF2B2"   "EIF2B3"
##  [7] "EIF2B4"   "EIF2B5"   "EIF2D"    "EIF2S1"   "EIF2S2"   "EIF3A"
## [13] "EIF3B"    "EIF3D"    "EIF3E"    "EIF3F"    "EIF3G"    "EIF3H"
## [19] "EIF3I"    "EIF3J"    "EIF3K"    "EIF3L"    "EIF3M"    "EIF4A1"
## [25] "EIF4A2"   "EIF4A3"   "EIF4B"    "EIF4E"    "EIF4E2"   "EIF4EBP1"
## [31] "EIF4G1"   "EIF4G2"   "EIF4G3"   "EIF4H"    "EIF5A"    "EIF5A2"
## [37] "EIF5B"    "EIF6"
```

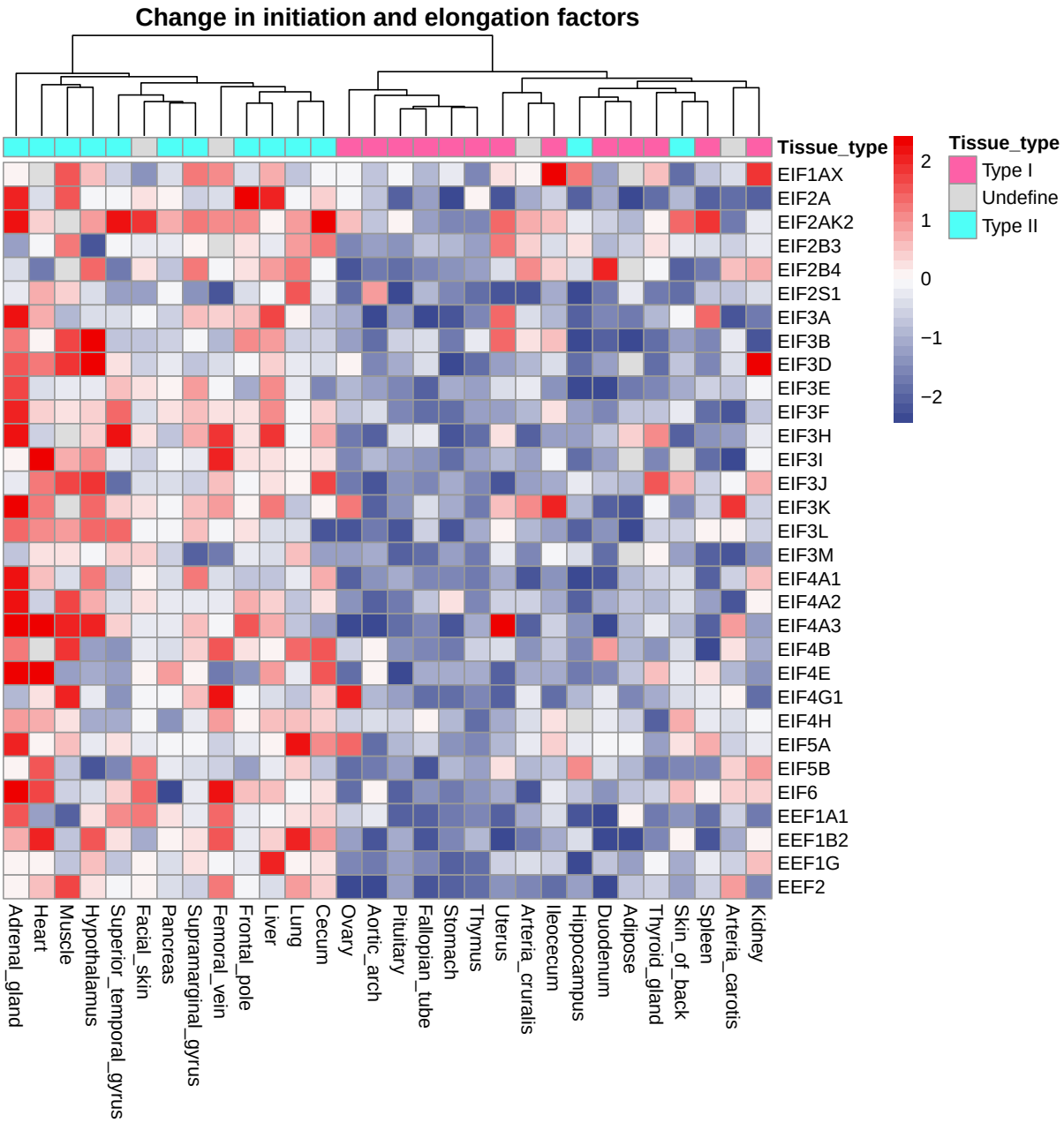
```
trans_initialB  = xnames[substr(xnames,1,3) == 'EEF']
trans_initialB = sort(trans_initialB)
trans_initialB
```

```
## [1] "EEF1A1"    "EEF1A2"    "EEF1AKMT1" "EEF1B2"    "EEF1E1"    "EEF1G"
## [7] "EEF2"
```

```
trans_initialA = c(trans_initialA,trans_initialB)
for(i in 1:length(pro_amplitude)){
    tx = pro_amplitude[[i]][trans_initialA]
    #tx= tx[!is.na(tx)]
    tmp = data.frame(genes = trans_initialA,stringsAsFactors = F,
                     amplitude = as.vector(tx))
    if(i ==1){
        tmpout = tmp;
    }else{
        tmpout = cbind(tmpout,tmp['amplitude'])
    }

}
tmpout = t(tmpout[,-1])

rownames(tmpout) = names(pro_amplitude)
colnames(tmpout) = trans_initialA
idx = colSums(is.na(tmpout)) < 3
sum(idx)
```

```
## [1] 31
```

```
#tmpout = tmpout[rowSums(is.na(tmpout)) < 3,]
tmpout = tmpout[,idx]


metadata <- tissueClass[rownames(tmpout),c('type','type')]
tclass = data.frame(class = metadata$type,row.names = rownames(metadata))
colnames(tclass) <- c("Tissue_type")

ann_colors = list(
   Tissue_type= c('#FD60A7','gray85','#4fffF7')

)
names(ann_colors$Tissue_type) = c('Type I','Undefine', 'Type II')

pheatmap::pheatmap(t(tmpout),annotation_col = tclass,cluster_rows = F,
                   annotation_colors = ann_colors,
                   main = 'Change in initiation and elongation factors',
                   color=colorRampPalette(c('#3B4992','gray99','#EE0000'))(30),
                    #file ="./out/20230217_aging/Figure6_heatmap_TED_machanism/Figure6B_heatmap_transla
                  height = 8,width = 8
                  )
```



11.4 Figure 7C

```
xnames = names(tpvalue)
ribosome = file2frame('./data/ribosome_proteins_from_kegg.txt',header = F)
ribosome = ribosome$V1
trans_initialA = intersect(ribosome,xnames)
trans_initialA
```

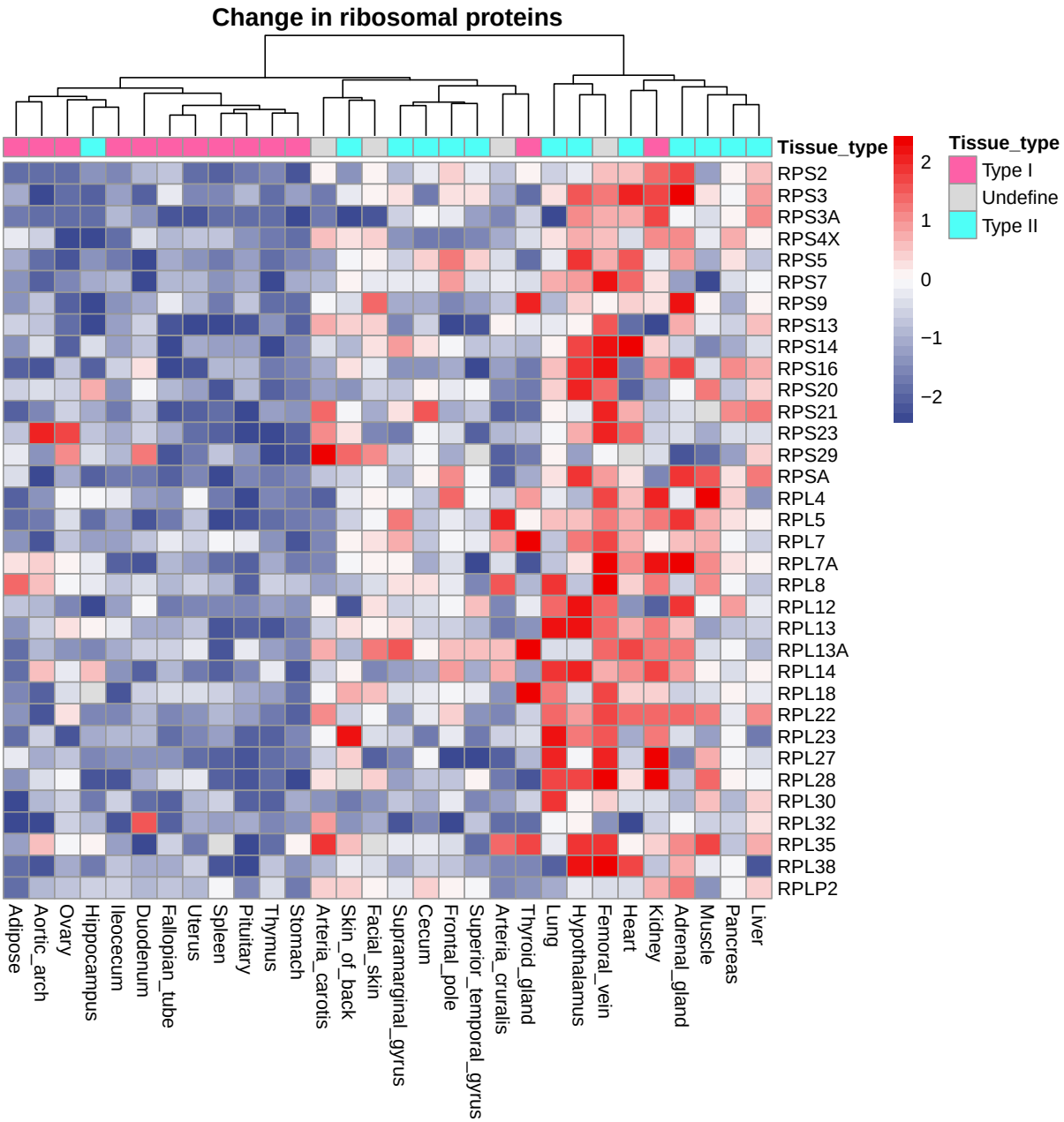
```
##  [1] "RPS2"   "RPS3"   "RPS3A"  "RPS4X"  "RPS5"   "RPS7"   "RPS9"   "RPS13"
##  [9] "RPS14"  "RPS15"  "RPS16"  "RPS20"  "RPS21"  "RPS23"  "RPS24"  "RPS27L"
## [17] "RPS27A" "RPS29"  "FAU"    "RPSA"   "RPL4"   "RPL5"   "RPL6"   "RPL7"
## [25] "RPL7A"  "RPL8"   "RPL12"  "RPL13"  "RPL13A" "RPL14"  "RPL18"  "RPL22"
```

```
## [33] "RPL23"  "RPL23A" "RPL27"  "RPL28"  "RPL30"  "RPL32"  "RPL35"  "RPL38"
## [41] "RPLP1"  "RPLP2"
```

```
for(i in 1:length(pro_amplitude)){
    tx = pro_amplitude[[i]][trans_initialA]
    #tx= tx[!is.na(tx)]
    tmp = data.frame(genes = trans_initialA,stringsAsFactors = F,
                     amplitude = as.vector(tx))
    if(i ==1){
        tmpout = tmp;
    }else{
        tmpout = cbind(tmpout,tmp['amplitude'])
    }

}
tmpout = t(tmpout[,-1])

rownames(tmpout) = names(pro_amplitude)
colnames(tmpout) = trans_initialA
idx = colSums(is.na(tmpout)) < 3
sum(idx)
```

```
## [1] 34
```

```
#tmpout = tmpout[rowSums(is.na(tmpout)) < 5,]
tmpout = tmpout[,idx]
tmpout = tmpout[rownames(tissueClass),]

metadata <- tissueClass#[rownames(tmpout),]
#metadata <- tissueClass[rownames(tmpout),c('class','class')]
tclass = data.frame(class = metadata$type,row.names = rownames(metadata))
colnames(tclass) <- c("Tissue_type")

ann_colors = list(
   Tissue_type= c('#FD60A7','gray85','#4fffF7')

)
names(ann_colors$Tissue_type) = c('Type I','Undefine', 'Type II')

pheatmap::pheatmap(t(tmpout),annotation_col = tclass,cluster_rows = F,
                   annotation_colors = ann_colors,
                   main = 'Change in ribosomal proteins',
                   cluster_cols = T,color=colorRampPalette(c('#3B4992','gray99','#EE0000FF'))(30),
                    #file ="./out/20230217_aging/Figure6_heatmap_TED_machanism/Figure6C_heatmap_ribosom
                  height = 8,width = 8
                  )
```


Change in ribosomal proteins
Tissue_type
RPS2
RPS3
RPS3A
RPS4X
RPS5
RPS7
RPS9
RPS13
RPS14
RPS16
RPS20
RPS21
RPS23
RPS29
RPSA
RPL4
RPL5
RPL7
RPL7A
RPL8
RPL12
RPL13
RPL13A
RPL14
RPL18
RPL22
RPL23
RPL27
RPL28
RPL30
RPL32
RPL35
RPL38
RPLP2
2
1
0
−1
−2
Tissue_type
Type I
Undefine
Type II
Adipose
Aortic_arch
Ovary
Hippocampus
Ileocecum
Duodenum
Fallopian_tube
Uterus
Spleen
Pituitary
Thymus
Stomach
Arteria_carotis
Skin_of_back
Facial_skin
Supramarginal_gyrus
Cecum
Frontal_pole
Superior_temporal_gyrus
Arteria_cruralis
Thyroid_gland
Lung
Hypothalamus
Femoral_vein
Heart
Kidney
Adrenal_gland
Muscle
Pancreas
Liver