

```

sheep<-readRDS("~/Work/tmp/sheep_res0.5.rds")
glist1<-c("PTPRC","FCGR3A","CD3D","CD3E","ACTA2","MYH11","MAP3K7CL","LBH","RERGL",
"PECAM1","VWF","CD24","MT1C","MT1A","BUD23","CITED1","TNNI3","BLVRA","MGP","APO
E","DCN","IGFBP6","AURKA","AURKB","CDK1","TOP2A","HELLS","ZCWPW1","FBXO39","C1QT
NF3","UBQLN3","MORN3","TSSK6","LYZL4","KRT75","TEX30","DDX39A","STK31","NUF2","ME
IOC","IQCA1","DNAH14","SPEM1","TNP1","PRM1")
angrycell::DotPlot2(sheep,features=glist1)
p<-FeaturePlot(sheep,features =
glist1,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=9)
CairoTIFF("refGenes.tiff",width=4500,height=2000)
print(p)
dev.off()
sheep_harmony<-runHarmony(sheep,harmony.by = "orig.ident")
saveRDS(sheep_harmony,file=~ /Work/tmp/sheep_harmony.rds")
sheep_cca<-ccaRun(sheep,by="orig.ident")
saveRDS(sheep_cca,file=~ /Work/tmp/sheep_cca.rds")
#remove batch effect
sheep_cca<-readRDS("~/Work/tmp/sheep_cca.rds")
sheep_cca$Sample<-factor(as.character(sheep_cca$Sample),levels=c("P30","P180","P270","P
360","P540"))
saveRDS(sheep_cca,file=~ /Work/tmp/sheep_cca.rds")
DimPlot(sheep_cca,group.by="integrated_snn_res.0.5",split.by="Sample")
sheep_harmony<-readRDS("~/Work/tmp/sheep_harmony.rds")
sheep_harmony$Sample<-factor(as.character(sheep_harmony$Sample),levels=c("P30","P18
0","P270","P360","P540"))
saveRDS(sheep_harmony,file=~ /Work/tmp/sheep_harmony.rds")
DimPlot(sheep_harmony,group.by="RNA_snn_res.0.5",split.by="Sample")
Idents(sheep_harmony)<-sheep_harmony$RNA_snn_res.0.5
DefaultAssay(sheep_harmony)<-"RNA"
deg<-FindAllMarkers(sheep_harmony)
setorderv(deg,c("avg_log2FC","pct.1","pct.2"),c(-1,-1,1))
write.table(deg,file="sheep_harmony_res0.5_deg.tab",quote=F,sep="\t")
deg %>% split(deg$cluster) %>% write.xlsx(file="sheep_harmony_res0.5.DEG.xlsx")
Idents(sheep_cca)<-sheep_cca$integrated_snn_res.0.5
DefaultAssay(sheep_cca)<-"RNA"
deg<-FindAllMarkers(sheep_cca)
setorderv(deg,c("avg_log2FC","pct.1","pct.2"),c(-1,-1,1))
write.table(deg,file="sheep_cca_res0.5_deg.tab",quote=F,sep="\t")
deg %>% split(deg$cluster) %>% write.xlsx(file="sheep_cca_res0.5.DEG.xlsx")

sheep<-readRDS("~/Work/tmp/sheep_cca.rds")
Idents(sheep)<-sheep$integrated_snn_res.0.5
DefaultAssay(sheep)<-"RNA"
angrycell::DotPlot2(sheep,features=glist1)
p<-FeaturePlot(sheep,features =
glist1,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=9)
CairoTIFF("CCA_refGenes.tiff",width=4500,height=2000)
print(p)
dev.off()

```

```

sheep<-readRDS("~/Work/tmp/sheep_harmony.rds")
Idents(sheep)<-sheep$RNA_snn_res.0.5
DefaultAssay(sheep)<-"RNA"
angrycell::DotPlot2(sheep,features=glist1)
p<-FeaturePlot(sheep,features =
glist1,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=9)
CairoTIFF("harmony_refGenes.tiff",width=4500,height=2000)
print(p)
dev.off()
#####
#####
##-----8 samples-----##
mtglist<-c("AIFM1","AIFM2","AIFM3","ATP6","ATP8","COX1","COX2","COX3","CYTB","ND1","
ND2","ND3","ND4","ND4L","ND5","ND6","CYB")
sheep<-readRDS("~/Work/tmp/Rdata/3-Data_DimR.rds")
mtglist<-mtglist[mtglist %in% rownames(sheep)]
sheep[["percent.mt"]] <- PercentageFeatureSet(object = sheep, features = mtglist)
sheep <- subset(x = sheep, subset = percent.mt < 10 & nFeature_RNA > 200)
VlnPlot(sheep,features=c("nFeature_RNA","nCount_RNA","percent.mt"),pt.size=0)
saveRDS(sheep,file "~/Work/tmp/flt.rds")
sheep$Sample<-factor(as.character(sheep$Sample),levels=c("P0","P30","P90","P180","P270"
,"P360","P540","P720"))
sheep<-FindVariableFeatures(sheep,selection.method = "vst",nfeatures = 2000)
sheep_harmony<-runHarmony(sheep,harmony.by="Sample")
DimPlot(sheep_harmony,group.by="RNA_snn_res.0.5",split.by="Sample",ncol=4)
saveRDS(sheep_harmony,file "~/Work/tmp/harmony.rds")
rm(sheep)
cellDis(sheep_harmony,anno.by="RNA_snn_res.0.5",sample.by = "Sample",save.prefix =
 "~/Work/tmp/tab2.Sample_cellDis_res0.5")
kDEG(sheep_harmony,save.prefix "~/Work/tmp/tab3.clsDEG_res0.5",anno.by =
"RNA_snn_res.0.5")
glist1<-c("PTPRC","FCGR3A","CD3D","CD3E","ACTA2","MYH11","MAP3K7CL","LBH","RERGL",
"PECAM1","VWF","CD24","MT1C","MT1A","BUD23","CITED1","TNNI3","BLVRA","MGP","APO
E","DCN","IGFBP6","AURKA","AURKB","CDK1","TOP2A","HELLS","ZCWPW1","FBXO39","C1QT
NF3","UBQLN3","MORN3","TSSK6","LYZL4","KRT75","TEX30","DDX39A","STK31","NUF2","ME
IOC","IQCA1","DNAH14","SPEM1","TNP1","PRM1")
Idents(sheep_harmony)<-sheep_harmony$RNA_snn_res.0.5
angrycell::DotPlot2(sheep_harmony,features=glist1)
p<-FeaturePlot(sheep_harmony,features =
glist1,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=9)
CairoTIFF("~/Work/tmp/fig1.refGenes.tiff",width=4500,height=2000)
print(p)
dev.off()
clustree(sheep_harmony)+theme(legend.position="bottom")
DimPlot(sheep_harmony,group.by=c("RNA_snn_res0.3","RNA_snn_res.0.5","RNA_snn_res.0.8
"))

```

#QC-stat

```

types<-data.table(table(sheep_harmony$Sample))$V1
cellcount<-data.table(table(sheep_harmony$Sample))$N
meangene<-as.data.frame(tapply(sheep_harmony$nFeature_RNA,
INDEX=sheep_harmony$Sample, FUN=median))[,1]
meancount<-as.data.frame(tapply(sheep_harmony$nCount_RNA,INDEX=sheep_harmony$S
ample,FUN=median))[,1]
meanmt<-as.data.frame(tapply(sheep_harmony$percent.mt,INDEX=sheep_harmony$Sampl
e,FUN=median))[,1]
df<-data.frame(cellcount=cellcount,meangene=meangene,meancount=meancount,meanpc
tMT=meanmt)
rownames(df)<-types
openxlsx::write.xlsx(df,file="~/Work/tmp/tab1.QC_stat.xlsx",rowNames=TRUE)

```

```

#RECODE
library(RECODE)
library(Matrix)
data <- as.matrix(seurat[["RNA"]][@counts])
data_RECODE <- RECODE(data)
seurat[["RECODE"]] <- CreateAssayObject(counts = Matrix(data_RECODE, sparse = TRUE))
DefaultAssay(seurat) <- "RECODE"
saveRDS(seurat,file="~/Work/tmp/dat_recode_seurat.rds")
recode<-readRDS("~/Work/tmp/data_recode_seurat.rds")
ngene_low <- 700
ngene_high <- 6000
mito_high <- 15
numi_low <- 1000
numi_high <- 25000
meta.data <- seurat@meta.data
meta.data$quality <- rep("Good", nrow(meta.data))
meta.data$quality[meta.data$nFeature_RNA<ngene_low|ngene_high<meta.data$nFeature_
RNA] <- "Bad"
meta.data$quality[meta.data$nCount_RNA<numi_low|numi_high<meta.data$nCount_RNA]
<- "Bad"
meta.data$quality[meta.data$percent.mt>mito_high] <- "Bad"
meta.data$quality <- factor(meta.data$quality)
p1 <- ggplot(meta.data, aes(x = nFeature_RNA, y = percent.mt, color = quality))+
  geom_point(size = 0.1, show.legend = F)+
  scale_color_manual(values = c("black", "red"))+
  xlab("nGene")
p2 <- ggplot(meta.data, aes(x = nCount_RNA, y = nFeature_RNA, color = quality))+
  geom_point(size = 0.1)+
  scale_color_manual(values = c("black", "red"))+
  xlab("nUMI")+
  ylab("nGene")+
  guides(color = guide_legend(override.aes = list(size = 5)))
p1+p2
VlnPlot(seurat, features = c("nFeature_RNA", "nCount_RNA", "percent.mt"),ncol = 3,pt.size =
0)

```

```

seurat <- subset(seurat, subset = nFeature_RNA > ngene_low & nFeature_RNA <
ngene_high & percent.mt < mito_high & nCount_RNA > numi_low & nCount_RNA <
numi_high)
dim(seurat)

recode<-autoR(recode)
DefaultAssay(recode)<-"RECODE"
recode <- NormalizeData(recode)
recode <- FindVariableFeatures(recode, selection.method = "vst", nfeatures = 2000)
recode <- ScaleData(recode, features = rownames(recode), do.scale = FALSE, do.center =
TRUE)
DimPlot(recode)
saveRDS(recode,file=~ /Work/tmp/recode_vst2k.rds")
recode<-RunPCA(recode)
#recode_har<-RunHarmony(recode,group.by.vars="Sample",assay.use="RECODE")
recode_har<-RunHarmony(recode,group.by.vars="Sample",assay.use="RECODE",kmeans_ini
t_nstart=20,kmeans_init_iter_max=100)
recode_har<-RunUMAP(recode_har,dims = 1:15,reduction = 'harmony')
recode_har <- FindNeighbors(recode_har,dims = 1:15,reduction = 'harmony')
recode_har <- FindClusters(recode_har,resolution = c(0.3,0.5,0.8,1.2))
#recode_har<-runHarmony(recode_har,harmony.by="Sample",assay.use =
"RECODE",res=c(0.3,0.5,0.8,1,1.2))
saveRDS(recode_har,file=~ /Work/tmp/recode_har.rds")
FeaturePlot(recode_harmony,c("MS4A1","LYZ","NKG7","PPBP","LTF","HBA1","FCER1A","IL7R"
,"FCGR3B")) & scale_colour_gradientn(colours = rev(brewer.pal(n = 11, name = "Spectral")))
Idents(recode_harmony)<-recode_harmony$RECODE_snn_res.0.3
angrycell::DotPlot2(recode_harmony,features=glist1)
p<-FeaturePlot(recode_harmony,features =
glist1,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=9)
CairoTIFF(~ /Work/tmp/fig1_harmony.refGenes.tiff",width=4500,height=2000)
print(p)
dev.off()
clustree(recode_harmony,prefix = "RECODE_snn_res.")+theme(legend.position="bottom")
count_table <- table(recode_harmony@meta.data$RECODE_snn_res.0.3,
recode_harmony@meta.data$Sample)
plot_integrated_clusters(count_table)
Rcpp::sourceCpp("/projects/decpp/fast_de.cpp")
de<-t_test_1vA(recode_harmony@assays$RECODE@data,as.character(recode_harmony@m
eta.data[, "RECODE_snn_res.0.3"]))
setorderv(de,c("avg_logFC","pct.1","pct.2"),c(-1,-1,1))
de %>% filter(p_val<0.05) %>% group_by(cluster) -> de_flt_pSig
write.table(de_flt_pSig,file=~ /Work/tmp/recode_harmony_clusterDEG.tab")
de_flt_pSig %>% split(de_flt_pSig$cluster) %>%
openxlsx::write.xlsx(file=~ /Work/tmp/recode_harmony_clusterDEG.xlsx",rowNames=FALSE)

sheep<-readRDS(~ /Work/tmp/recode_har.rds")
Idents(sheep)<-sheep$RECODE_snn_res.0.3
sheep<-RenameIdents(sheep,"1"="VSMs","6"="T cells","5"="Sperm","3"="Sertoli
cells","18"="Sertoli cells","20"="Sertoli

```

```

cells", "16" = "Plasma", "8" = "Macrophage", "14" = "Leydig cells", "0" = "Late primary
S'cytes", "7" = "Late primary S'cytes", "2" = "Fibroblast", "11" = "Endothelial", "9" = "Elonagated
spermatids", "12" = "Elonagated spermatids", "15" = "Elonagated spermatids", "10" = "Early
primary S'cytes", "19" = "Early primary S'cytes")
angrycell::DotPlot2(sheep, features = rev(unique(c("ACTA2", "MYH11", "DCN", "CD3E", "XCL1", "P
TPRC", "PRM1", "TPPP2", "SPEM1", "GLUL", "SPACA9", "OXT", "BUD23", "DEFB119", "CITED1", "PR
DX6", "PNMT", "HEBP2", "BUD23", "DEFB119", "CITED1", "PRDX6", "PNMT", "HEBP2", "TNNI3", "BL
VRA", "GNB1L", "MZB1", "CD19", "CD79A", "C1QA", "C1QB", "C1QC", "TYROBP", "CST3",
"APOE", "RARRES2", "CLGN", "BCAP29", "CCDC88A", "CCDC39", "ZPBP", "PEBP4", "DNAH12", "CL
GN", "CCDC88A", "DNAH14", "BCAP29", "PIGF", "CRELD2", "SPCS2", "DNAH12", "COL1A1", "COL1
A2", "PCOLCE2", "COL3A1", "RGCC", "CA4", "VIM", "COL4A1", "VWF", "PECAM1", "CFAP97D1", "CC
DC70", "CYLC1", "FBP1", "SPATA7", "HEMGN", "FAM166A", "FBP1", "SPATA7", "FAM166A", "CYLC
1", "SEC23A", "HSPA13", "FEN1", "TOP2A", "STK31", "DDX39A", "ILF2", "SPATA22"))))
DimPlot(sheep, label = TRUE, label.size = 4)
glist <- unique(c("ACTA2", "MYH11", "DCN", "CD3E", "XCL1", "PTPRC", "PRM1", "TPPP2", "SPEM1",
"GLUL", "SPACA9", "OXT", "BUD23", "DEFB119", "CITED1", "PRDX6", "PNMT", "HEBP2", "BUD23",
DEFB119", "CITED1", "PRDX6", "PNMT", "HEBP2", "TNNI3", "BLVRA", "GNB1L", "MZB1", "CD19", "C
D79A", "C1QA", "C1QB", "C1QC", "TYROBP", "CST3",
"APOE", "RARRES2", "CLGN", "BCAP29", "CCDC88A", "CCDC39", "ZPBP", "PEBP4", "DNAH12", "CL
GN", "CCDC88A", "DNAH14", "BCAP29", "PIGF", "CRELD2", "SPCS2", "DNAH12", "COL1A1", "COL1
A2", "PCOLCE2", "COL3A1", "RGCC", "CA4", "VIM", "COL4A1", "VWF", "PECAM1", "CFAP97D1", "CC
DC70", "CYLC1", "FBP1", "SPATA7", "HEMGN", "FAM166A", "FBP1", "SPATA7", "FAM166A", "CYLC
1", "SEC23A", "HSPA13", "FEN1", "TOP2A", "STK31", "DDX39A", "ILF2", "SPATA22"))

```

#体细胞marker: VIM, 生殖细胞marker: DDX4

#Immature leydig cell: INHBA

#leydig cell: IGFBP6, Gli, DLK1, IGF1, IGFBP5, IGFBP3, VIT, CYP11A, INSL3, MGP

#Myoid: ACTA2, MYH11, PTCH11, PTCH2, Gli

#Spermatogonia: UCHL1, HELLS, KIT, UTF1, PIWIL2, GFRA1, FGFR3, TCF3, SSEA4, SOHLH1

#1. 精原干细胞 SSC markers (e.g., UTF1, ID4 and FGFR3)。

#2. differentiating SSC markers(KIT and DMRT1).

#3. Spermatid (SPAG6, ZPBP, CAMK4, CREM) 。

#4. Round S'tids: ECM7, TTC1, SPESP1, WBP11, ECM2, DDX50, DPM1, COX11,

#5. Elongated

S'tids: ART3, LACTB, AK9, SOS2, PCSK7, AK2, MBD1, TBP, TIMM9, WDR66, MLH3, ACYP1, MLK2, THO
P1,

```

glist <- unique(c("VIM", "DDX4", "INHBA", "IGFBP6", "GLI1", "DLK1", "IGF1", "IGFBP5", "IGFBP3", "VI
T", "CPY11A", "INSL3", "MGP", "ACTA2", "MYH11", "PTCH11", "PTCH2", "GLI", "UCHL1", "HELLS", "K
IT", "UTF1", "PIWIL2", "GFRA1", "FGFR3", "TCF3", "SSEA4", "SOHLH1", "UTF1", "ID4", "FGFR3", "KIT",
"DMRT1", "SPAG6", "ZPBP", "CAMK4", "CREM", "ECM7", "TTC1", "SPESP1", "WBP11", "ECM2", "DD
X50", "DPM1", "COX11", "ART3", "LACTB", "AK9", "SOS2", "PCSK7", "AK2", "MBD1", "TBP", "TIMM9",
"WDR66", "MLH3", "ACYP1", "MLK2", "THOP1"))

```

```

glist[glist %in% rownames(sheep) == FALSE]

```

```

rownames(sheep)[grep("PTCH11", rownames(sheep))]

```

```

glist <- glist[glist %in% rownames(sheep)]

```

```

p <- FeaturePlot(sheep, glist, order = TRUE, raster = FALSE, ncol = 9)

```

```

for(gene in glist){

```

```

  of <- paste0(gene, ".pdf")

```

```

pdf(of,width=6,height=5)
p1<-FeaturePlot(sheep,gene,order=TRUE,raster=FALSE)
print(p1)
dev.off()
}

```

```

ref<-read.table("~/Work/tmp/marker_ref.tab",header=T,sep="\t")
ref<-ref[ref$gene.name %in% rownames(sheep),]
for(i in 1:nrow(ref)){
  of<-paste0(ref$cluster[i],"_",ref$gene.name[i],".pdf")
  pdf(of,width=6,height=5)
  p1<-FeaturePlot(sheep,ref[i,"gene.name"],order=TRUE,raster=FALSE)
  print(p1)
  dev.off()
}

```

```

#SSC:
glist<-c("SPIWIL4","TSPAN33","MSL3","EGR4","NANOS3","LPPR3","GFRA1","GFRA1","LIN28"
,"ID4","PAX7","ETV5","ZBTB16","TERT","BMI1")
glist<-glist[glist %in% rownames(sheep)]
for(gene in glist){
  of<-paste0("SSC_",gene,".pdf")
  pdf(of,width=6,height=5)
  p1<-FeaturePlot(sheep,gene,order=TRUE,raster=FALSE)
  print(p1)
  dev.off()
}

```

```

glist<-unique(toupper(c("SYCP3","YBX2","Tex101","Piwil","Spo11","Mei1","Piwil2","Spag6","S
ycp3","Tdrd5","Tbpl1","Hormad1","Mns1","MLH3","TOP2A","SYCP1","SYCP2","PGK2","ACR","
NME8","HSPB9","YBX2","SRD5A1","WNT2B","FZD3","LRP3","TOP2A","MLLT10","LCA5","TME
M107","SPEM1","PRSS37")))
glist<-glist[glist %in% rownames(sheep)]
for(gene in glist){
  of<-paste0("test0822_",gene,".pdf")
  pdf(of,width=6,height=5)
  p1<-FeaturePlot(sheep,gene,order=TRUE,raster=FALSE)
  print(p1)
  dev.off()
}

```

```

glist<-c("ITGA9","GATA4")
glist<-glist[glist %in% rownames(sheep)]
for(gene in glist){
  of<-paste0("test0822_",gene,".pdf")
  pdf(of,width=6,height=5)
  p1<-FeaturePlot(sheep,gene,order=TRUE,raster=FALSE)
  print(p1)
  dev.off()
}

```

```

}
glist<-unique(toupper(c("ZBTB16","GFRA1","NANOS3","EOMES","TSPAN8","SALL4","SDC4","
UCHL1","ECEA3","CRABP1","DMRTB1","TEX101","HSPA5","STRA8","SYCP3","PRDM9","HOR
MAD1","HORMAD2","SYCP1","TEX15","DNMT3L","BAX","RBP4","KIT","DMRT1","STRA8","SO
HLH1","HOHLH2","LMNA","CCNB1IP1","HMGA1","DMC1","CPEB1","TEX11","MEI11","BCL2L2
","EXO1","RARG","ATM1","EGR4","TEX15","REC8","AR","CCNA1","LIPE","KLH10","SLC4A2","L
HB","LMTK2","B4GALNT1","SPAG16","SLC9C1","PACRG","JUND","THEG","CAMK4","TPST2","
NPC1","WIPF3","RASIP1","TAF7L","TALDO1","INPP5B","CYP17A1","PRM1","SPEM1","TSSK6",
"PVRT2","SMCP","GAPDHS","AKAP4","LRP8","CATSPER3","CATSPER1","CATSPER4","TEKT2","
PDGFB"))))
glist<-glist[glist %in% rownames(sheep)]
for(gene in glist){
  of<-paste0("test0822_",gene,".pdf")
  pdf(of,width=6,height=5)
  p1<-FeaturePlot(sheep,gene,order=TRUE,raster=FALSE)
  print(p1)
  dev.off()
}

```

```

sheep<-readRDS("~/Work/tmp/recode_har.rds")

```

```

DefaultAssay(sheep)

```

```

Idents(sheep)<-sheep$RECODE_snn_res.1.2

```

```

sheep<-RenamIdents(sheep,
  "0"="Leydig cells-1",
  "1"="Myoid cells",
  "2"="Late primary S'cytes",
  "3"="Sertoli cells",
  "4"="Late primary S'cytes",
  "5"="T cells",
  "6"="Early primary S'cytes",
  "7"="Myoid cells",
  "8"="Early primary S'cytes",
  "9"="Endothelial",
  "10"="Sperm",
  "11"="Macrophage",
  "12"="SSCs",
  "13"="Sperm",
  "14"="Late primary S'cytes",
  "15"="Round S'tids",
  "16"="Elongated S'tids",
  "18"="Leydig cells-2",
  "19"="Elongated S'tids",
  "20"="Early primary S'cytes",
  "21"="Elongated S'tids",
  "22"="Macrophage",
  "23"="Elongated S'tids",
  "24"="Elongated S'tids",
  "25"="Spermatogonia",
  "26"="Late primary S'cytes",

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"27"="Round S'tids",
"28"="Macrophage",
"29"="Late primary S'cytes",
"30"="Elongated S'tids",
"31"="Late primary S'cytes",
"32"="Sertoli cells",
"33"="Myoid cells",
"34"="T cells",
"35"="Early primary S'cytes",
"36"="Spermatogonia",
"37"="Leydig cells-2",
"38"="Endothelial",
"39"="T cells",
"17"="VSM")
glist<-unique(c("INSL3","IGFBP6","IGFBP5","IGF1","ACTA2","MYH11","DDX39A","ILF2","MISP
3","PIWIL1","S100B","GATM","ESPN","CITED1","DDX39A","CD3E","CD3G","HCST","TEX101","
TDRD5","SYCP3","ACTA2","MYH11","GRIA2","IQCA1","LYZL4","UVRAG","RGCC","CA4","VIM",
"COL4A1","VWF","PECAM1","CA2","CD164L2","SPEM2","WNT3","PTPRC","STRA8","UTF1","KI
T","LIN28","PLZF","BCL2L11","CFAP100","EGR3","IQCA1L","TP53INP2","CFAP97D1","FAM166
A","FEN1","HEMGN","UBXN8","RGS5"))
for(i in glist){
  p1<-VlnPlot(sheep,i,group.by="RECODE_snn_res.1.2",pt.size=0)+NoLegend()
  p2<-VlnPlot(sheep,i,pt.size=0)+NoLegend()
  of<-paste0("vlnplot_",i,".pdf")
  pdf(of,width=8.5,height=4)
  print(p1)
  print(p2) #8,4
  dev.off()
}
sheep$annoV1<-sheep@active.ident
saveRDS(sheep,file="~/Work/tmp/annoV1.rds")
pdf("umap_829.pdf",width=10,height=8)
DimPlot(sheep,label=TRUE,label.size=5,repel=TRUE)
dev.off()
for(i in unique(sheep$RECODE_snn_res.1.2)){
  pdf(paste0("RECODE_res1.2.cellsHighlight",i,".pdf"),width=10,height=8)
  p<-DimPlot(sheep,cells.highlight =
rownames(sheep@meta.data[sheep$RECODE_snn_res.1.2 == i]),pt.size=0.01)
  print(p)
  dev.off()
}

glist<-c("PODXL2","CD99")
for(i in glist){
  p1<-VlnPlot(sheep,i,group.by="RECODE_snn_res.1.2",pt.size=0)+NoLegend()
  p2<-VlnPlot(sheep,i,pt.size=0)+NoLegend()
  of<-paste0("vlnplot_",i,".pdf")
  pdf(of,width=8.5,height=4)
  print(p1)

```

```

print(p2) #8,4
dev.off()
}

color<-c("#E42A3F","#7C9DEC","#F9DF12","#661F62","#F38400",
        "#D396CE","#B79657","#E4ABBE","#4DB85D","#17551A",
        "#10110F","#75c2b3","#7dc2dd","#a81f26")
color[2]<-"chocolate2"
sheep$annoV2<-factor(as.character(sheep$annoV2),levels=c("Spermatogonia","Early
primary S'cytes","Late primary S'cytes","Round S'tids","Elongated S'tids","Sperm","Leydig
cells-1","Leydig cells-2","Sertoli cells","Myoid cells","VSM","T
cells","Endothelial","Macrophage","SSCs"))
color<-c("#7dc2dd","#D396CE","#F9DF12","#10110F","#75c2b3","#E4ABBE","#E42A3F","dar
kolivegreen","#661F62","#7C9DEC","#a81f26","#F38400","#B79657","#4DB85D","#17551A")
names(color)<-c("Spermatogonia","Early primary S'cytes","Late primary S'cytes","Round
S'tids","Elongated S'tids","Sperm","Leydig cells-1","Leydig cells-2","Sertoli cells","Myoid
cells","VSM","T cells","Endothelial","Macrophage","SSCs")

color<-c("#7dc2dd" ,      "chocolate2",      "#F9DF12",
"#10110F" ,      "#75c2b3" ,      "#E4ABBE",
"#E42A3F" ,      "darkolivegreen" ,      "#661F62",
"#7C9DEC" ,      "#a81f26" ,      "#F38400",
"#B79657" ,      "#4DB85D" ,      "#17551A")
names(color)<-c("Spermatogonia", "Early primary S'cytes" , "Late primary S'cytes", "Round
S'tids" , "Elongated S'tids" , "Sperm" , "Leydig cells-1" , "Leydig cells-2" , "Sertoli cells"
, "Myoid cells" , "VSM" , "T cells" , "Endothelial" , "Macrophage" , "SSCs")

pdf("umap_anno.pdf",width=10,height=8)
DimPlot(sheep,cols=color)
dev.off()
pdf("umap_anno_labeled.pdf",width=10,height=8)
DimPlot(sheep,cols=color,label=TRUE,label.size=5,repel=TRUE)
dev.off()
Idents(sheep)<-sheep$Sample
for( sample in unique(sheep$Sample)){
  tmp<-subset(sheep,idents=sample)
  of<-paste0(sample,"_umap_anno.pdf")
  pdf(of,width=10,height=8)
  p1<-DimPlot(tmp,cols=color[names(table(tmp$annoV2))],group.by="annoV2")

p2<-DimPlot(tmp,cols=color[names(table(tmp$annoV2))],label=TRUE,label.size=5,group.by
="annoV2",repel=TRUE)
  print(p1)
  print(p2)
  dev.off()
}
pdf("allSample_umap_anno.pdf",width=10,height=8)
p1<-DimPlot(sheep,cols=color,group.by="annoV2")
p2<-DimPlot(sheep,cols=color,label=TRUE,label.size=5,group.by="annoV2",repel=TRUE)

```

```

print(p1)
print(p2)
dev.off()

pdf("umap_anno_plus.pdf",width=10,height=8)
DimPlot_plus()
dev.off()
sheep$annoV2<-sheep@active.ident
cellDis(sheep,anno.by = "annoV2",sample.by = "Sample",save.prefix="test830")
pdf("umap830.pdf",width=10,height=8)
DimPlot(sheep,label=TRUE,label.size=5)
dev.off()

glist<-c("CCL5","CD34","NKG7")
tmp<-subset(sheep,idents="T cells",invert=TRUE)
cellDis(tmp,anno.by = "annoV2",sample.by = "Sample",save.prefix="excludeT")
pdf("umap_excludeT.pdf",width=10,height=8)
DimPlot(tmp,label=TRUE,label.size=5)
dev.off()
rm(tmp)

Idents(sheep)<-sheep$annoV2
sheep<-subset(sheep,idents=c("T cells","Macrophage"),invert=TRUE)
sheep$annoV2<-droplevels(sheep$annoV2)
saveRDS(sheep,file=~ /Work/tmp/downstream.rds")

glist<-c("HELLS","KIT","UCHL1",
  "CLGN","BCAP29","CCDC88A","CCDC39","ZPBP","PEBP4","DNAH12",
  "TEX101","TDRD5","SYCP3","TOP2A","DDX39","PIWIL1","GRIA2","CCDC173",
  "LHB","BCL2L11","CALCR","CFLF3","EGR3","IQCA1L","IZUMO1","MPIG6B","TP53INP2",
  "FAM166A","FEN1","PPM1L",
  "TNP2","PRM1","WENT3","CD164L2","EFR3B","PRKCD","SPEM2",
  "IGF2","INHBA","DCN","INSL3","MGP","APOE","IGFBP5","IGF1","RBP4",
  "SOX9","AMH","CITED1","CMAS","ESPN","GATM","S100B",
  "ACTA2","MYH11","DLK1","NOTCH3",
  "RGS5",
  "RGCC","CA4","VIM","COL4A1","VWF","PECAM1"
)

slt<-subset(raw,cells=rownames(raw@meta.data[raw$Sample %in% c("P0","P30") &
raw$annoV2 %in% c("Spermatogonia","Early primary S'cytes","Late primary S'cytes","Round
S'tids","Elongated S'tids","Sperm"),]),invert=TRUE)
slt<-subset(slt,cells=rownames(slt@meta.data[slt$Sample == "P90" & slt$annoV2 %in%
c("Round S'tids","Elongated S'tids","Sperm"),]),invert=TRUE)
cellDis(slt,anno.by="annoV2",sample.by="Sample",save.prefix="95_annoV2")
p1<-DimPlot(slt,group.by="annoV2",split.by="Sample",ncol=4,cols=color)
p2<-DimPlot(slt,group.by="annoV2",split.by="Sample",label=TRUE,label.size=5,ncol=4,cols
=color)
pdf("8sample.pdf",width=20,height=10)

```

```

print(p1)
print(p2)
dev.off()
slt<-subset(slt,idents=c("T cells","Macrophage"),invert=TRUE)
slt$annoV2<-droplevels(slt$annoV2)
Idsents(slt)<-slt$Sample
slt<-subset(slt,idents="P720",invert=TRUE)
slt$Sample<-droplevels(slt$Sample)
Idsents(slt)<-slt$annoV2
saveRDS(slt,file=~ /Work/tmp/downstream.rds")
cellDis(slt,anno.by="annoV2",sample.by="Sample",save.prefix="95_annoV2_downstream")
p1<-DimPlot(slt,group.by="annoV2",split.by="Sample",ncol=4,cols=color[names(table(slt$annoV2))])
p2<-DimPlot(slt,group.by="annoV2",split.by="Sample",label=TRUE,label.size=5,ncol=4,cols=
color[names(table(slt$annoV2))])
pdf("7sample_downstream.pdf",width=20,height=10)
print(p1)
print(p2)
dev.off()
pdf("umap_7sample.pdf",width=10,height=8)
p1<-DimPlot(slt,group.by="annoV2",cols=color[names(table(slt$annoV2))])
p2<-DimPlot(slt,group.by="annoV2",cols=color[names(table(slt$annoV2))],label=TRUE,label
.size=5)
print(p1)
print(p2)
dev.off()

```

```

library(AnnotationDbi)
library(clusterProfiler)
ovisdb<-AnnotationDbi::loadDb("~/Work/tmp/ovis.db")
#decombo1<-c("P30","P90","P180","P270","P360","P540")
#decombo2<-c("P0","P30","P90","P180","P270","P360")
decombo1<-c("P180","P540")
decombo2<-c("P0","P180")
Idsents(slt)<-slt$annoV2
#for(ctype in unique(slt$annoV2)[-c(1,2,3,4,5,6,7)]){
for(ctype in unique(slt$annoV2)){
  tmp<-subset(slt,idents=ctype)
  Idsents(tmp)<-tmp$Sample
  for( i in 1:length(decombo1)){ #Round S'tids P30vsP0
    print(ctype)
    print(i)
    if(table(slt$Sample,slt$annoV2)[decombo1[i],ctype] > 3 &
table(slt$Sample,slt$annoV2)[decombo2[i],ctype]>3){
      tmp2<-subset(tmp,idents=c(decombo1[i],decombo2[i]))
      count1<-table(tmp2$Sample)[decombo1[i]]
      count2<-table(tmp2$Sample)[decombo2[i]]
      if( count1 > 3 & count2 > 3){
        deg<-FindMarkers(tmp2,ident.1=decombo1[i],ident.2=decombo2[i],min.cells.group

```

```

=0,min.pct=0,logfc.threshold = -Inf)
  deg$gene<-rownames(deg)
  setorderv(deg,c("avg_log2FC","pct.1","pct.2"),c(-1,-1,1))
  of1 <-paste0(ctype,"_",decombo1[i],"vs",decombo2[i],".xlsx")
  write.xlsx(deg,file=of1)
  enrichOne(markers=deg,OrgDb =
ovisdb,topn=100,kegg.organism="oas",save.prefix=paste0(ctype,"_",decombo1[i],"vs",deco
mbo2[i]))
  }
}
}}

```

```

tmp<-subset(slt,idents=c("SSCs","Early primary S'cytes","Myoid cells"))
groupCPDB(tmp,run.now=FALSE,annotation="annoV2",condition="Sample")

```

```

dat<-read.xlsx("95_annoV2_downstream_distribution.xlsx",rowNames=TRUE)
colnames(dat)<-c("Spermatogonia", "Early primary S'cytes", "Late primary S'cytes", "Round
S'tids", "Elongated S'tids", "Sperm", "Leydig cells-1", "Leydig cells-2", "Sertoli cells"
,"Myoid cells", "VSM", "Endothelial", "SSCs")
#pie plot
for(i in 1:nrow(dat)){
  x <- as.numeric(dat[i,])
  main<-rownames(dat[i,])
  type <- colnames(dat)
  x.flt<-x[x!=0]
  name.flt<-type[x!=0]
  x.pct <- paste(round(100 * x.flt / sum(x.flt),3),"%")
  x.col <- color[x!=0]
  pdf(paste0(main,"_pie.pdf"),width=10,height=6)
  pie(x.flt,labels=x.pct,col= x.col,main=main)
  legend("topright",name.flt,cex=0.85, fill=x.col)
  dev.off()
}

```

```

glist<-unique(c("STRA8","UTF1","FGFR3","GFRA1","PIWIL2","ARHGAP33","DNMT3L","HELLS"
,"KIT","UCHL1","TEX101","TDRD5","SYCP3","TOP2A","DDX39A","PIWIL1","GRIA2","CCDC173"
,"CLGN","BCAP29","CCDC88A","CCDC39","ZBPB","PEBP4","DNAH12","FAM166A","FEN1","PP
M1L","LHB","BCL2L11","CALCR","CFLF3","EGR3","IQCA1L","IZUMO1","MPIG6B","TP53INP2","
TNP2","PRM1","WNT3","CD164L2","EFR3B","PRKCD","SPEM2","SOX9","AMH","CITED1","CM
AS","ESPN","GATM","S100B","IGF2","INHBA","DCN","INSL3","MGP","APOE","IGFBP5","IGF1",
"RBP4","EFEMP1","DLK1","INHA","PDGFRA","RARRES2","ACTA2","MYH11","DLK1","NOTCH3
","RGS5","HCST","CD3G","CD3E","RGCC","CA4","VIM","COL4A1","VWF","PECAM1","C1QA","
C1QB","C1QC","TYROBP","CST3"))
table(glist[glist %in% rownames(slt)==FALSE])
glist<-glist[glist %in% rownames(slt)]
p<-FeaturePlot(slt,glist,order=TRUE,raster=FALSE,ncol=9)
for(gene in glist){

```

```

of<-paste0(gene,".pdf")
pdf(of,width=6,height=5)
p1<-FeaturePlot(slt,gene,order=TRUE,raster=FALSE,cols=c("lightgrey","yellow","red"))
print(p1)
dev.off()
}

```

#910

#1

```

Idsents(slt)<-slt$Sample
p180<-subset(slt,idents="P180")
Idsents(p180)<-p180$annoV2
p180$annoplt<-ifelse(p180$annoV2 %in%
c("SSCs","Spermatogonia"),"SSCs_Spermatogonia",as.character(p180$annoV2))
p180$annoplt<-ifelse(p180$annoplt %in% c("Leydig cells-1","Leydig cells-2"),"Leydig
cells-1_Leydig cells-2",as.character(p180$annoplt))
p180$annoplt<-factor(as.character(p180$annoplt),levels=c("SSCs_Spermatogonia","Early
primary S'cytes","Late primary S'cytes","Round S'tids","Elongated S'tids","Sperm","Sertoli
cells","Myoid cells","Leydig cells-1_Leydig cells-2","VSM","Endothelial"))
saveRDS(p180,file="P180.rds")
group.color<-color[names(table(p180$annoplt))]
group.color[9]<-color[1]
group.color[1]<-color[7]
Rcpp::sourceCpp("/BGFS1/home/kangbx/projects/decpp/fast_de.cpp")
de<-t_test_1vA(p180@assays$RECODE@data,as.character(p180$annoV2))
write.table(de,file="1.de_recode.tab",quote=F,sep="\t")
de$flt<-de$pct.1-de$pct.2
setorderv(de,c("avg_logFC","flt"),c(-1,-1))
top30<-c()
for(i in levels(p180$annoV2)){
  tmp<-de[de$cluster==i,]
  tg<-head(tmp,30)
  top30<-rbind(top30,tg)
}
top30$cluster<-factor(top30$cluster,levels=names(table(top30$cluster)))
pdf("p180_top30_heatmap.pdf",width=18,height=12)
p<-DoHeatmap(p180,features=unique(top30$gene),assay="RNA",group.by
="annoplt",raster = FALSE,group.colors=group.color)+theme(axis.text.y=element_blank())
#p<-doheatmapy(p180,anno_cols_by="annoplt",features=unique(top30$gene))
print(p)
dev.off()
saveRDS(p,file="2p.rds")
rm(p)
#2
#tmp<-subset(slt,idents=c("Early primary S'cytes" , "Late primary S'cytes" , "Round S'tids"
,"Elongated S'tids" , "Sperm"))
#saveRDS(tmp,file="2.rds")
#run_monocle(tmp,prefix="2_trajectory",annotation="annoV2",cores=1,condition="Sample
",order.method="default",useCounts=FALSE)

```

```

run_monocle(slt,prefix="2new_trajectory",annotation="annoV2",select = c("SSCs","Early
primary S'cytes","Late primary S'cytes","Round S'tids","Elongated
S'tids","Sperm"),invert=FALSE,cores=1,condition="Sample",order.method="default",useCou
nts=FALSE)
#monocle2
monocle_cds<-readRDS("/BGFS1/home/yanyl/work/test/my20220914/2_trajectory.Rds")
pdf("monocle2_overview.pdf",width=12,height=8)
p12<-plot_cell_trajectory(monocle_cds,color_by="annoV2")+facet_wrap(~annoV2)+scale_co
lor_manual(values=color[c("Early primary S'cytes","Late primary S'cytes","Round
S'tids","Elongated S'tids","Sperm")])+guides(colour = guide_legend(override.aes =
list(size=5)))+theme(legend.text=element_text(size=10))
p42<-plot_cell_trajectory(monocle_cds,color_by="Sample")+facet_wrap(~Sample)+scale_col
or_manual(values=color_palette)+guides(colour = guide_legend(override.aes =
list(size=5)))+theme(legend.text=element_text(size=10))
p1<-plot_cell_trajectory(monocle_cds,color_by="annoV2")+scale_color_manual(values=colo
r[c("Early primary S'cytes","Late primary S'cytes","Round S'tids","Elongated S'tids","Sperm")])
p2<-plot_cell_trajectory(monocle_cds,color_by="Pseudotime")
p3<-plot_cell_trajectory(monocle_cds,color_by="State")
p4<-plot_cell_trajectory(monocle_cds,color_by="Sample")+scale_color_manual(values=color
_palette)
print(p12)
print(p42)
print(p1)
print(p2)
print(p3)
print(p4)
dev.off()
#3
glist<-c("BOLL","TKTL1","INSL3","TNP2","AFF4","ZBTB16","HSPB9","APOA1","AMH","MYH11
","APOD","INHA")
glist<-"NANOS3"
glist<-glist[glist %in% rownames(slt)]
Idents(slt)<-slt$Sample
for(sample in unique(slt$Sample)){
  tmp<-subset(slt,idents=sample)
  for (gene in glist){
    of<-paste0(gene,"_",sample,".pdf")
    pdf(of,width=6,height=5)
    p1<-FeaturePlot(tmp,gene,order=TRUE,raster=FALSE,cols=c("lightgrey","yellow","red"))
    print(p1)
    dev.off()
  }
}

#GO KEGG enrichment
dat<-read.xlsx("~/Work/tmp/Gokegg.xlsx",sheet=1)
dat$plt<--log10(dat$pvalue)
setorderv(dat,"plt",-1)
#dat$Description<-factor(as.character(dat$Description),levels=unique(dat$Description))

```

```

dat$Group<-factor(as.character(dat$Group),levels=c("P30vsP0","P90vsP30","P180vsP90","P2
70vsP180","P360vsP270","P540vsP360"))
dat$Type<-factor(as.character(dat$Type),levels=c("BP","MF","CC"))
for( group in unique(dat$Group)){
  tmp<-dat[dat$Group==group,]
  setorderv(tmp,"Type",-1)
  #tmp$Description<-droplevels(tmp$Description)
  tmp$Description<-factor(as.character(tmp$Description),levels=unique(tmp$Description))
  of<-paste0(group,"_GO.pdf")
  pdf(of,width=12,height=8)

p<-ggplot(tmp,aes(x=Description,y=plt,fill=Type))+geom_bar(stat="identity",position=posit
ion_dodge(0.8),width=0.5)+scale_fill_manual(values=color_palette)+labs(title=group)

p<-p+theme_pubr()+theme(axis.text.x=element_text(angle=90,hjust=1,vjust=0.5,color="bla
ck",size=12))+ylab(expression(-"log"["10"]*(PValue)))
  print(p)
  dev.off()
}
dat<-read.xlsx("~/Work/tmp/Gokegg.xlsx",sheet=2)
dat$plt<--log10(dat$pvalue)
setorderv(dat,"plt",-1)
#dat$Description<-factor(as.character(dat$Description),levels=unique(dat$Description))
#dat$Group<-factor(as.character(dat$Group),levels=c("P30vsP0","P90vsP30","P180vsP90","
P270vsP180","P360vsP270","P540vsP360"))
for( group in unique(dat$Group)){
  tmp<-dat[dat$Group==group,]
  tmp$Description<-factor(as.character(tmp$Description),levels=unique(tmp$Description))
  of<-paste0(group,"_KEGG_dotplot.pdf")
  pdf(of,width=6,height=4)
  p<-ggplot(tmp,aes(x=Description,y=plt)) +
  geom_point(aes(size=Count,color=plt))+scale_color_gradient(low = "gold2", high =
"red")+coord_flip()+
  labs(color=expression(-log[10](pvalue)),size="Count",y=expression(-"log"["10"]*(PValue)),
x="Description",
title=group)+theme_bw()+theme(axis.text.y=element_text(color="black",size=10))

#p<-ggplot(tmp,aes(x=Description,y=plt))+geom_bar(stat="identity",fill="gold2",position=
position_dodge(0.7),width=0.5)+scale_fill_manual(values=color_palette)+labs(title=group)
  print(p)
  dev.off()
}

slt$annoplt<-factor(as.character(slt$annoV1),levels=c("VSM","Myoid cells","Sertoli
cells","Leydig cells-1","Leydig cells-2","Endothelial","SSCs","Spermatogonia","Early primary
S'cytes","Late primary S'cytes","Round S'tids","Elongated S'tids","Sperm"))
glist<-unique(c("SYNE2",
"CCDC173",

```

```

"CFAP100",
"SPCS2",
"IQCA1L",
"ZPBP",
"SPAM1",
"MAP1B",
"PRSS37",
"GLUL",
"TPPP2",
"PRM1",
"TMEM239",
"CLGN",
"CCDC88A",
"DNAH12",
"DLK1",
"NOTCH3",
"ACTA2",
"MYH11"
))
glist<-c("SOX9","SYCP3","UCHL1")
glist %in% rownames(slt)
for(i in glist){
  p1<-VlnPlot(slt,i,group.by="annoplt",pt.size=0,cols =
color[levels(slt$annoplt)])+NoLegend()
  #p2<-VlnPlot(slt,i,pt.size=0,group.by="annoV2")+NoLegend()
  of<-paste0("vlnplot_",i,".pdf")
  pdf(of,width=8.5,height=4)
  print(p1)
  #print(p2) #8,4
  dev.off()
}
color<-c("#7dc2dd" ,      "chocolate2",      "#F9DF12",
        "#10110F" ,      "#75c2b3" ,      "#E4ABBE",
        "#E42A3F" ,      "darkolivegreen" ,      "#661F62",
        "#7C9DEC" ,      "#a81f26" ,      "#F38400",
        "#B79657" ,      "#4DB85D" ,      "#17551A")
names(color)<-c("Spermatogonia", "Early primary S'cytes" , "Late primary S'cytes", "Round
S'tids" , "Elongated S'tids" , "Sperm" , "Leydig cells-1" , "Leydig cells-2" , "Sertoli cells"
, "Myoid cells" , "VSM" , "T cells" , "Endothelial" , "Macrophage" , "SSCs")
glist<-c("NANOS3","TKTL1","BOLL")
slt2<-subset(slt,idents=c("SSCs","Spermatogonia","Early primary S'cytes","Late primary
S'cytes","Round S'tids","Elongated S'tids","Sperm"))
slt2$annoV2<-factor(as.character(slt2$annoV2),levels=c("SSCs","Spermatogonia","Early
primary S'cytes","Late primary S'cytes","Round S'tids","Elongated S'tids","Sperm"))
for(sample in unique(slt2$Sample)){
  tmp<-subset(slt2,cells=rownames(slt2@meta.data[slt2$Sample==sample,]))
  p<-VlnPlot(tmp,glist,ncol=1,cols=color[c("SSCs","Spermatogonia","Early primary
S'cytes","Late primary S'cytes","Round S'tids","Elongated S'tids","Sperm")],pt.size=0)
  of<-paste0(sample,".pdf")

```

```
pdf(of,width=6,height=10)
print(p)
dev.off()
}
```

```
glist<-c("UCHL1","PRM1","STRA8","SYCP1","SYCP2","PIWIL1","TOP2A","TEX101","IZUMO1","
PGK2","GLUL","NME8","CITED1","TNP1")
slt2@active.ident<-factor(as.character(slt2@active.ident),levels=rev(levels(slt2$annoV2)[c(1,
2,3,4,6,5,7)]))
pdf("20220922_dotplot.pdf",width=8,height=4)
p<-angrycell::DotPlot2(slt2,features=glist)+theme(legend.position="top")
print(p)
dev.off()
```

```
glist<-c("SOX9","SYCP3","UCHL1")
glist %in% rownames(slt)
for(i in glist){
  p1<-VlnPlot(slt,i,group.by="annoV2",pt.size=0,cols =
color[levels(slt$annoV2)])+NoLegend()
  #p2<-VlnPlot(slt,i,pt.size=0,group.by="annoV2")+NoLegend()
  of<-paste0("vlnplot_",i,".pdf")
  pdf(of,width=8.5,height=4)
  print(p1)
  #print(p2) #8,4
  dev.off()
}
```

```
glist<-c("UCHL1","SYCP3","SOX9","FSHR","LHR","AR")
glist<-c("LHCGR","SYCP1","SYCP2","TEX101")
glist<-glist[glist %in% rownames(slt)]
Idents(slt)<-slt$Sample
for(sample in unique(slt$Sample)){
  tmp<-subset(slt,idents=sample)
  for (gene in glist){
    of<-paste0(gene,"_",sample,".pdf")
    pdf(of,width=6,height=5)
    p1<-FeaturePlot(tmp,gene,order=TRUE,raster=FALSE,cols=c("lightgrey","yellow","red"))
    print(p1)
    dev.off()
  }
}
```

```
for(ctype in unique(slt$annoV2)){
  tmp<-subset(slt,idents=ctype)
  Idents(tmp)<-tmp$Sample
  for( i in 1:length(decombo1)){ #Round S'tids P30vsP0
```

```

print(ctype)
print(i)
if(table(slt$Sample,slt$annoV2)[decombo1[i],ctype] > 3 &
table(slt$Sample,slt$annoV2)[decombo2[i],ctype]>3){
  tmp2<-subset(tmp,idents=c(decombo1[i],decombo2[i]))
  count1<-table(tmp2$Sample)[decombo1[i]]
  count2<-table(tmp2$Sample)[decombo2[i]]
  if( count1 >3 & count2 >3){
    deg<-FindMarkers(tmp2,ident.1=decombo1[i],ident.2=decombo2[i],min.cells.group
=0,min.pct=0,logfc.threshold = -Inf)
    deg$gene<-rownames(deg)
    setorderv(deg,c("avg_log2FC","pct.1","pct.2"),c(-1,-1,1))
    of1 <-paste0(ctype,"_",decombo1[i],"vs",decombo2[i],".xlsx")
    write.xlsx(deg,file=of1)
    enrichOne(markers=deg,OrgDb =
ovisdb,topn=100,kegg.organism="oas",save.prefix=paste0(ctype,"_",decombo1[i],"vs",deco
mbo2[i]))
  }
}
}}

```

##part6

```

pdf("retinoic_acid_pathway.pdf",width=9,height=7)
p<-FeaturePlot(dat,c("RBP1","RDH10","ALDH1A1","ALDH1A2","ALDH1A3","RARG","RXRA","
CYP26B1","STRA8"),order=TRUE,cols=c("lightgray","yellow","red"),ncol=3)
print(p)
dev.off()
pdf("acivinInhibin_pathway.pdf",width=9,height=7)
p<-FeaturePlot(dat,c("INHBA","INHBB","INHA","ACVR1B","BMPR1B","ACVR2B","FST","BAMB
I","NOG"),order=TRUE,cols=c("lightgray","yellow","red"),ncol=3)
print(p)
dev.off()
pdf("NOTCH_pathway.pdf",width=9,height=7)
p<-FeaturePlot(dat,c("NOTCH2","NOTCH3","NOTCH4","DLL1","DLL3","DLL4","DLK","HES","
MAML1"),order=TRUE,cols=c("lightgray","yellow","red"),ncol=3)
print(p)
dev.off()
pdf("GDNF_pathway.pdf",width=9,height=7)
p<-FeaturePlot(dat,c("GDNF","GFRA1","GFRA2","RET","KRAS","PIK3CA","AKT2","ETV5","ID4")
,order=TRUE,cols=c("lightgray","yellow","red"),ncol=3)
print(p)
dev.off()
pdf("FGF_pathway.pdf",width=9,height=7)
p<-FeaturePlot(dat,c("FGF9","FGF7","FGFR1","FGFR2","FGFR3","SOS1","FRS2","FRS3","SHOC
2"),order=TRUE,cols=c("lightgray","yellow","red"),ncol=3)
print(p)
dev.off()
pdf("WNT_pathway.pdf",width=9,height=7)

```

```
p<-FeaturePlot(dat,c("WNT3","WNT6","FZD3","ROR2","AES","AXIN2","DACT1","ZBTB33","DVL1"),order=TRUE,cols=c("lightgray","yellow","red"),ncol=3)
print(p)
dev.off()
```

```
monocle_cds<-readRDS("sertoli_annoV2.rds")
genes<-c("AMH","EGR1","BMP2","HES1","AR","EPPIN","DEFB123","TBX22","S100A6","ISG15","MSLN","PRND")
genes %in% monocle_cds@featureData@data[["gene_short_name"]]
monocle_set<-monocle_cds[genes[-11],]
monocle::plot_genes_in_pseudotime(monocle_set)
#monocle_set<-monocle_cds[rowData(monocle_cds)$gene_short_name %in%
genes,colData(monocle_cds)$cell.type %in% c("AFD")]
#plot_genes_in_pseudotime(monocle_set,color_cells_by="pseudotime",min_expr=0.5)
```

```
glist<-c("NR4A1", "MAFB", "ACTA2", "MYH11", "DLK1", "IGF1", "VIT", "HSD17B3",
"CYP26B1", "LHX9", "ETV1", "INSR", "COL1A1", "RPS18", "COL1A2", "DCN", "IGF2", "CYTB",
"COX3", "COX1", "MYL6", "EDNRA", "ATP6", "IGFBP6", "KLK1", "RPL18A", "RPS10", "EEF1A1",
"RPS24", "RPS24", "INSL3")
FeaturePlot(myo,features=glist[glist %in%
rownames(myo)],order=TRUE,cols=c("lightgray","yellow","red"),ncol=6)
Idents(ser)<-ser$RECODE_snn_res.0.3
sertoli<-RenamIdents(ser,"5"="Immature Sertoli-2","8"="Immature
Sertoli-2","1"="Immature Sertoli-1","4"="Immature Sertoli-1","2"="Immature
Sertoli-1","7"="Immature Sertoli-1","0"="Mature Sertoli","3"="Mature Sertoli","6"="Mature
Sertoli")
glist<-c("SOX9", "AR", "ATP5F1E", "LGALS3","NOTCH2", "PDPN", "AMH", "LGALS3",
"AOMM7", "ATP5E", "EGR3","JUN", "NR4A1", "S100A13", "ENO1", "BEX1", "HOPX",
"DEFB119", "CST9L","CYTB", "COX1","CFAP298", "ND4", "RPS18", "SUMO2",
"RPL18A","CNBP", "MGP","APOA1","RPL10", "ATP5F1E", "RPS24", "RPL24", "SEPTIN14",
"CYTB", "COX2", "COX3", "HSPA8")
VlnPlot(sertoli,features=unique(glist[glist %in% rownames(sertoli)]),ncol=6,pt.size=0)
VlnPlot(sertoli,features=unique(glist[glist %in%
rownames(sertoli)]),ncol=6,group.by="Sample",pt.size=0)
monocle_cds<-readRDS("sertoli_annoV2.Rds")
plot_cell_trajectory(monocle_cds,color_by="annotation")+facet_wrap(~Sample)
VlnPlot(sertoli,features=unique(glist[glist %in% rownames(sertoli)]))
```

```
for( type in unique(tmp$annoV2)){
  tmp2<-subset(tmp,annoV2 == type)
  pdf(paste0(type,"_geneVlnplot.pdf"),width=7,height=4)
  p<-VlnPlot(tmp2,features=glist,group.by="Sample",pt.size=0)
  p1<-VlnPlot(tmp2,features=glist,group.by="Sample")
  print(p)
  print(p1)
  dev.off()
}
```

#2023

```

setwd("~/Work/tmp/2023")
dat<-readRDS("../old/sheep_downstream.rds")
Idents(dat)<-dat$annoV2
dat<-RenamelIdents(dat,"Early primary S'cytes"="Spermatocytes",
                    "Late primary S'cytes"="Spermatocytes",
                    "Round S'tids"="Spermatid",
                    "Sperm"="Spermatid",
                    "Elongated S'tids"="Spermatid",
                    "Leydig cells-1"="Leydig & Myoid cells",
                    "Leydig cells-2"="Leydig & Myoid cells",
                    "Myoid cells"="Leydig & Myoid cells")
gn<-subset(dat,idents=c("Spermatogonia"))
saveRDS(gn,file="2_1.rds")
stc<-subset(dat,idents=c("Spermatocytes"))
saveRDS(stc,file="2_2.rds")
matid<-subset(dat,idents="Spermatid")
saveRDS(matid,file="2_3.rds")
fs<-c("UCHL1","UTF1","MKI67","PIWIL4","TSPAN33","GFRA1","KIT","STRA8","SPO11","PRDM
9","CCNA1","GPR85","PRM3")
fs2<-c("AMH","EGR1","BMPR2","HES1","AR","EPPIN","DEFE123","TEX22","S100A6",
"ISG15","MSLN","PRND")
merge<-merge(x=gn,y=c(stc,matid))
merge<-subset(merge,cells=rownames(merge@meta.data[merge$annoV2 %in% c("Round
S'tids","Elongated S'tids","Sperm") & merge$Sample=="P90",]),invert=TRUE)
#DiffusionMap
library(destiny)
library(Seurat)
#data <- FindVariableFeatures(object = data,selection.method = "mvp",verbose = FALSE)
#singlecell <- t(as.matrix(data@assays$RNA@data))
tmp<-subset(merge,features=VariableFeatures(merge))
mtx <- t(as_matrix(tmp@assays$RECODE@data))
#mtx <- mtx[,VariableFeatures(merge)]
dm <- DiffusionMap(mtx,verbose = T)
merge[["dpt"]] <- CreateDimReducObject(embeddings = dm@eigenvectors[,1:2], key =
"DC_", assay = DefaultAssay(merge))
#slingshot
library(slingshot)
library(BUSpaRse)
library(tidyverse)
library(tidymodels)
library(Seurat)
library(scales)
library(viridis)
library(Matrix)

color<-color_palette[1:6]
names(color)<-levels(merge$annoV2)
merge$annoV2<-droplevels(merge$annoV2)
qu5.rd<-merge@reductions$dpt@cell.embeddings[,1:2]

```

```

qu5.label<-as.numeric(merge$annoV2)
qu5.label<-merge$annoV2
names(qu5.label)<-names(merge$annoV2)
lin1<-getLineages(qu5.rd,qu5.label,start.clus="Spermatogonia",end.clus="Sperm")
plot(qu5.rd,col=color[levels(merge$annoV2)][qu5.label],asp=1,pch=10,bty="l")
legend("topright", legend = levels(merge$annoV2),pch = 20,pt.cex=3, col =
color[levels(merge$annoV2)])
lines(SlingshotDataSet(lin1),lwd=3,col="black")
crv1<-getCurves(lin1)
plot(qu5.rd,col=color[levels(merge$annoV2)][qu5.label],asp=1,pch=10,bty="l")
legend("topright", legend = levels(merge$annoV2),pch = 20, pt.cex=3,col =
color[levels(merge$annoV2)])
lines(SlingshotDataSet(crv1),lwd=3,col="black")

nc <- 3
merge$annoV2_num<-as.numeric(merge$annoV2)
table(merge$annoV2,merge$annoV2_num)
sds <- slingshot(Embeddings(merge, "dpt"), clusterLabels = as.numeric(merge$annoV2),
start.clus = "1", stretch = 0,end.clus="6")
sds@metadata$lineages
cell_pal <- function(cell_vars, pal_fun,...) {
  if (is.numeric(cell_vars)) {
    pal <- pal_fun(100, ...)
    return(pal[cut(cell_vars, breaks = 100)])
  } else {
    categories <- sort(unique(cell_vars))
    pal <- setNames(pal_fun(length(categories), ...), categories)
    return(pal[cell_vars])
  }
}
cell_colors <- cell_pal(merge$annoV2, brewer_pal("qual", "Set2"))
cell_colors_clust <- cell_pal(merge$Sample, hue_pal())
#plot(reducedDim(sds), col = cell_colors, pch = 10, cex = 0.5)
plot(Embeddings(merge, "dpt"), col = cell_colors, pch = 10, cex = 0.5)
lines(sds, lwd = 2, type = 'lineages', col = 'black')

plot(qu5.rd,col=color[levels(merge$annoV2)][qu5.label],asp=1,pch=10,bty="l")
lines(sds, linInd = 1) # draws smooth curve for the upper lineage
lines(sds, linInd = 2, type='l') # draws MST edges for the lower lineage
pt <- slingPseudotime(sds)
nms <- colnames(pt)
nr <- ceiling(length(nms)/nc)
pal <- viridisLite::viridis(100, end = 0.95)
par(mfrow = c(nr, nc))
for (i in nms) {
  colors <- pal[cut(pt[,i], breaks = 100)]
  plot(Embeddings(merge, "dpt"), col = colors, pch = 16, cex = 0.5, main = i)
  lines(sds, lwd = 2, col = 'black', type = 'lineages')
}

```

```
#20230303
```

```
#fig1
```

```
dat<-readRDS("~/Work/tmp/old/sheep_downstream.rds")
p<-FeaturePlot(dat,features=c("UTF1","PIWIL1","IZUMO1",
"CCDC173","PPM1L","DLK1","RGS5"),order=TRUE,cols=c("lightgray","yellow","red"),ncol=4)
pdf("fig1.pdf",width=12,height=5)
print(p)
dev.off()
#fig2_1
```

```
#fig2_2
```

```
monocle_cds<-readRDS("~/Work/tmp/2023/deg_monocle_reverse.rds")
color<-c("#7dc2dd", "chocolate2", "#F9DF12",
"#10110F", "#75c2b3", "#E4ABBE",
"#E42A3F", "darkolivegreen", "#661F62",
"#7C9DEC", "#a81f26", "#F38400",
"#B79657", "#4DB85D", "#17551A")
names(color)<-c("Spermatogonia", "Early primary S'cytes", "Late primary S'cytes", "Round
S'tids", "Elongated S'tids", "Sperm", "Leydig cells-1", "Leydig cells-2", "Sertoli cells",
"Myoid cells", "VSM", "T cells", "Endothelial", "Macrophage", "Undifferentiated
spermatogonia")
pData(monocle_cds)$annoV3<-ifelse(pData(monocle_cds)$annoV1=="SSCs","Undifferentiat
ed spermatogonia",as.character(pData(monocle_cds)$annoV2))
pData(monocle_cds)$celltype=factor(as.character(pData(monocle_cds)$annoV3),levels=c("U
ndifferentiated spermatogonia","Spermatogonia","Early primary S'cytes","Late primary
S'cytes","Round S'tids","Elongated S'tids","Sperm"))
p1<-plot_cell_trajectory(monocle_cds,color_by =
"celltype")+scale_color_manual(values=color[levels(pData(monocle_cds)$celltype))]+guides(
color = guide_legend(override.aes = list(linetype = 0
,size=3))) + theme(legend.position="right")
p2<-plot_cell_trajectory(monocle_cds,color_by = "Pseudotime")
p1+facet_wrap(~Sample,ncol=4)
#pdf("fig2_2_sample.pdf",width=12,height=5)
pdf("fig2_2_pesudotime.pdf",width=6,height=5)
#fig2_3
```

```
pData(monocle_cds)$celltype<-ifelse(pData(monocle_cds)$annoV3=="Undifferentiaed
spermatogonia","Undifferentiated spermatogonia",pData(monocle_cds)$annoV3)
pData(monocle_cds)$celltype=factor(as.character(pData(monocle_cds)$annoV3),levels=c("U
ndifferentiated spermatogonia","Spermatogonia","Early primary S'cytes","Late primary
S'cytes","Round S'tids","Elongated S'tids","Sperm"))
monocle_cds<-readRDS("~/Work/tmp/2023/deg_monocle_reverse.rds")
meta<-pData(monocle_cds)[,c("Sample","celltype")]
meta$Sample<-factor(as.character(meta$Sample),levels=rev(levels(meta$Sample)))
angrycell::plot_fraction(meta,color.use = color[levels(pData(monocle_cds)$celltype)])
meta2<-meta
meta2$celltype=ifelse(meta$celltype %in% c("Early primary S'cytes","Late primary
S'cytes"),"Spermatocytes",as.character(meta2$celltype))
meta2$celltype=ifelse(meta$celltype %in% c("Round S'tids","Elongated
```

```

S'tids", "Sperm"), "Spermatid", as.character(meta2$celltype))
meta2$celltype <- factor(as.character(meta2$celltype), levels = c("Undifferentiated
spermatogonia", "Spermatogonia", "Spermatocytes", "Spermatid"))
col9 <- c("#43CD80", "#006401", "#FF3030", "#DAA520", "#000000", "#A020EE", "#1E90FF", "#FF
D500", "#D3DED3")
angrycell::plot_fraction(meta2, color.use = col9) + theme(legend.position = "right")
#fig2_4
meta <- dat@meta.data[, c("Sample", "annoV3")]
p <- plot_fraction(meta, color.use = col16, coord_flip =
FALSE) + theme(legend.position = "right", axis.text.x = element_text(angle = 90, size = 12, hjust = 1, c
olor = "black"))
pdf("fig2_4_cellDis.pdf", width = 6, height = 6)
print(p)
dev.off()

#fig3
object <- readRDS("../old/sertoli_annoV2.rds")
object$annoV3 <- factor(as.character(object@active.ident), levels = c("Immature
Sertoli-1", "Immature Sertoli-2", "Mature Sertoli"))
p <- plotTopH(object, msrt = deg, topn = 30, prefix = "fig3_3", ident.by = "annoV1", annotate_color_
x = scales::hue_pal()(1:3))

#fig3_3
tmp <- read.xlsx("fig3_3_deg.xlsx", sheet = 1)
cluster <- unique(tmp$cluster)
i <- gsub(" ", "_", cluster)
OrgDb = AnnotationDbi::loadDb("~/Work/tmp/ovis.db")
organism = "oas"
ntop = 100
up <- tmp[tmp$avg_log2FC > 0,]
topn <- nrow(up)
teg <- bitr(up$gene, fromType = "SYMBOL", toType = "ENTREZID", OrgDb = OrgDb)
#if(topn >= ntop){t100g <- teg[teg[, 1] %in% up[1:ntop, "gene"]$gene,]}
if(topn >= ntop){t100g <- teg[1:ntop,]}
if(topn < ntop){t100g <- teg}
ego <- enrichGO(t100g$ENTREZID, ont = "ALL", OrgDb = OrgDb, readable = TRUE, pvalueCutoff = 1
, qvalueCutoff = 1)
ek <- enrichKEGG(t100g$ENTREZID, organism = organism, pvalueCutoff = 1, qvalueCutoff = 1)
ek <- setReadable(ek, OrgDb, keyType = "ENTREZID")
of = paste0(i, "_up100_ego.xlsx")
of2 = paste0(i, "_up100_ekegg.xlsx")
openxlsx::write.xlsx(ego, file = of, rowNames = FALSE)
openxlsx::write.xlsx(ek, file = of2, rowNames = FALSE)

tmp <- read.xlsx("fig3_3_deg.xlsx", sheet = 2)
cluster <- unique(tmp$cluster)
i <- gsub(" ", "_", cluster)
OrgDb = AnnotationDbi::loadDb("~/Work/tmp/ovis.db")
organism = "oas"

```

```

ntop=100
up<-tmp[tmp$avg_log2FC>0,]
topn<-nrow(up)
teg<-bitr(up$gene,fromType="SYMBOL",toType="ENTREZID",OrgDb=OrgDb)
#if(topn >=ntop){t100g<-teg[teg[,1] %in% up[1:ntop,"gene"]$gene,]}
if(topn >=ntop){t100g <- teg[1:ntop,]}
if(topn <ntop){t100g<-teg}
ego<-enrichGO(t100g$ENTREZID,ont="ALL",OrgDb=OrgDb,readable=TRUE,pvalueCutoff=1
,qvalueCutoff=1)
ek<-enrichKEGG(t100g$ENTREZID,organism = organism,pvalueCutoff=1,qvalueCutoff=1)
ek<-setReadable(ek,OrgDb,keyType="ENTREZID")
of=paste0(i,"_up100_ego.xlsx")
of2=paste0(i,"_up100_ekegg.xlsx")
openxlsx::write.xlsx(ego,file=of,rowNames=FALSE)
openxlsx::write.xlsx(ek,file=of2,rowNames=FALSE)

tmp<-read.xlsx("fig3_3_deg.xlsx",sheet=3)
cluster<-unique(tmp$cluster)
i<-gsub(" ","_",cluster)
OrgDb=AnnotationDbi::loadDb("~/Work/tmp/ovis.db")
organism = "oas"
ntop=100
up<-tmp[tmp$avg_log2FC>0,]
topn<-nrow(up)
teg<-bitr(up$gene,fromType="SYMBOL",toType="ENTREZID",OrgDb=OrgDb)
#if(topn >=ntop){t100g<-teg[teg[,1] %in% up[1:ntop,"gene"]$gene,]}
if(topn >=ntop){t100g <- teg[1:ntop,]}
if(topn <ntop){t100g<-teg}
ego<-enrichGO(t100g$ENTREZID,ont="ALL",OrgDb=OrgDb,readable=TRUE,pvalueCutoff=1
,qvalueCutoff=1)
ek<-enrichKEGG(t100g$ENTREZID,organism = organism,pvalueCutoff=1,qvalueCutoff=1)
ek<-setReadable(ek,OrgDb,keyType="ENTREZID")
of=paste0(i,"_up100_ego.xlsx")
of2=paste0(i,"_up100_ekegg.xlsx")
openxlsx::write.xlsx(ego,file=of,rowNames=FALSE)
openxlsx::write.xlsx(ek,file=of2,rowNames=FALSE)
#fig4
dat<-readRDS("../old/leydig_tsne.rds")
Idents(dat)<-dat$RECODE_snn_res.0.1
dat<-RenamIdents(dat,"0"="Progenitor",
"5"="Myoid cells","6"="Myoid cells","1"="Myoid cells","7"="Myoid cells",
"3"="Leydig cell-1","4"="Leydig cell-1",
"2"="Leydig cell-2"
)
dat$annoV3<-dat@active.ident
DimPlot(dat,cols=col9)
glist<-c("NR4A1", "MAFB", "ACTA2", "MYH11", "DLK1", "IGF1", "VIT", "HSD17B3",
"CYP26B1", "LHX9", "ETV1", "INSR", "COL1A1", "RPS18", "COL1A2", "DCN", "IGF2", "CYTB",
"COX3", "COX1", "MYL6", "EDNRA", "ATP6", "IGFBP6", "KLK1", "RPL18A", "RPS10", "EEF1A1",

```

```

"RPS24", "RPS24", "INSL3", "INHBA", "FSHR", "LHR")
glist<-glist[glist %in% rownames(dat)]
VlnPlot(dat,glist,group.by="annoV3",ncol=6,pt.size=0) #fig4_2_celltype.pdf 14*14
VlnPlot(dat,glist,group.by="Sample",ncol=6,pt.size=0) #fig4_2_celltype.pdf 16*14
#fig4_3
dat$annoV4<-gsub("-1|-2","",dat$annoV3)
Idents(dat)<-dat$annoV4
deg<-FindAllMarkers(dat,only.pos=TRUE)
setorderv(deg,c("avg_log2FC","pct.1","pct.2"),c(-1,-1,1)) %>% split(deg$cluster) %>%
openxlsx::write.xlsx(file="fig4_3_deg.xlsx",rowNames=TRUE)
dat$annoV4<-factor(as.character(dat$annoV4),levels=c("Myoid cells","Progenitor","Leydig
cell"))
fs<- deg %>% group_by(cluster) %>% top_n(n = topn, wt = avg_log2FC)
fs<-sortMTX(fs,by="cluster",order=levels(dat$annoV4))
dat<-ScaleData(dat,features=unique(fs$gene))
p<-DoHeatmap(dat,features=unique(fs$gene),group.by="annoV4",size=anno_bar_label_size)
+theme(axis.text.y=element_text(color="black",size=0))
CairoTIFF("fig4_3.tiff",dpi=300,width=2000,height=3000)
print(p)
dev.off()
#deg enrichment
system(`/BGFS1/home/yanyl/miniconda3/envs/enrich/bin/R`)
tmp<-read.xlsx("fig4_3_deg.xlsx",sheet=1)
cluster<-unique(tmp$cluster)
i<-gsub(" ","_",cluster)
OrgDb=AnnotationDbi::loadDb("~/Work/tmp/ovis.db")
organism = "oas"
ntop=100
up<-tmp[tmp$avg_log2FC>0,]
topn<-nrow(up)
teg<-bitr(up$gene,fromType="SYMBOL",toType="ENTREZID",OrgDb=OrgDb)
if(topn >= ntop){t100g <- teg[1:ntop,]}
if(topn < ntop){t100g <- teg}
ego<-enrichGO(t100g$ENTREZID,ont="ALL",OrgDb=OrgDb,readable=TRUE,pvalueCutoff=1
,qvalueCutoff=1)
ek<-enrichKEGG(t100g$ENTREZID,organism = organism,pvalueCutoff=1,qvalueCutoff=1)
ek<-setReadable(ek,OrgDb,keyType="ENTREZID")
of=paste0("fig4_3_",i,"_up100_ego.xlsx")
of2=paste0("fig4_3_",i,"_up100_ekegg.xlsx")
openxlsx::write.xlsx(ego,file=of,rowNames=FALSE)
openxlsx::write.xlsx(ek,file=of2,rowNames=FALSE)

tmp<-read.xlsx("fig4_3_deg.xlsx",sheet=2)
cluster<-unique(tmp$cluster)
i<-gsub(" ","_",cluster)
OrgDb=AnnotationDbi::loadDb("~/Work/tmp/ovis.db")
organism = "oas"
ntop=100
up<-tmp[tmp$avg_log2FC>0,]

```

```

topn<-nrow(up)
teg<-bitr(up$gene,fromType="SYMBOL",toType="ENTREZID",OrgDb=OrgDb)
if(topn >= ntop){t100g <- teg[1:ntop,]}
if(topn < ntop){t100g<-teg}
ego<-enrichGO(t100g$ENTREZID,ont="ALL",OrgDb=OrgDb,readable=TRUE,pvalueCutoff=1
,qvalueCutoff=1)
ek<-enrichKEGG(t100g$ENTREZID,organism = organism,pvalueCutoff=1,qvalueCutoff=1)
ek<-setReadable(ek,OrgDb,keyType="ENTREZID")
of=paste0("fig4_3_",i,"_up100_ego.xlsx")
of2=paste0("fig4_3_",i,"_up100_ekegg.xlsx")
openxlsx::write.xlsx(ego,file=of,rowNames=FALSE)
openxlsx::write.xlsx(ek,file=of2,rowNames=FALSE)

```

```

tmp<-read.xlsx("fig4_3_deg.xlsx",sheet=3)
cluster<-unique(tmp$cluster)
i<-gsub(" ","_",cluster)
OrgDb=AnnotationDbi::loadDb("~/Work/tmp/ovis.db")
organism = "oas"
ntop=100
up<-tmp[tmp$avg_log2FC>0,]
topn<-nrow(up)
teg<-bitr(up$gene,fromType="SYMBOL",toType="ENTREZID",OrgDb=OrgDb)
if(topn >= ntop){t100g <- teg[1:ntop,]}
if(topn < ntop){t100g<-teg}
ego<-enrichGO(t100g$ENTREZID,ont="ALL",OrgDb=OrgDb,readable=TRUE,pvalueCutoff=1
,qvalueCutoff=1)
ek<-enrichKEGG(t100g$ENTREZID,organism = organism,pvalueCutoff=1,qvalueCutoff=1)
ek<-setReadable(ek,OrgDb,keyType="ENTREZID")
of=paste0("fig4_3_",i,"_up100_ego.xlsx")
of2=paste0("fig4_3_",i,"_up100_ekegg.xlsx")
openxlsx::write.xlsx(ego,file=of,rowNames=FALSE)
openxlsx::write.xlsx(ek,file=of2,rowNames=FALSE)

```

#fig5

```

glist<-c("STRA8", "REC8", "SPO11", "SYCP1","PRDM9","MSH5", "MSH4","MLH3", "CCNA1",
"TEX101", "SYCP1", "SYCP2","SYCP3", "PIWIL1","ZBPB", "DNAH12")
dat<-readRDS("annoV3_downstream.rds")
dat2<-subset(dat,cells=rownames(dat@meta.data[dat$Sample=="P180" &
dat$annoV3=="Spermatocytes",]))
dat2<-autoR(dat2,resolution=c(0.1,0.2,0.3))
Idents(dat2)<-dat2$RECODE_snn_res.0.2
glist<-glist[glist %in% rownames(dat2)]
FeaturePlot(dat2,glist,order=T,cols=c("lightgrey","yellow","red"),ncol=5)

```

#fig6

```

n1<-"Testosterone pathway"
g1<-c("STAR", "HSD17B3","SHBG", "SRD5A2")
n2<-"Retinoic Acid Pathway"
g2<-c("RBP1", "RDH10", "ALDH1A1", "ALDH1A2", "ALDH1A3", "RARG",
"RXRA","CYP26B1","STRA8")

```

```

n3<-"Activin_Inhibin Pathway"
g3<-c("INHBA", "INHBB", "INHA", "ACVR1B", "BMPR1B", "ACVR2B", "FST", "BAMBI")
n4<-"NOTCH Pathway"
g4<-c("NOTCH2", "NOTCH3", "NOTCH4", "DLL1", "DLL3", "DLL4", "DLK1", "HES1", "MAML1")
n5<-"GDNF Pathway"
g5<-c("GDNF", "GFRA1", "GFRA2", "RET", "KRAS", "PIK3CA", "AKT2", "ETV5", "ID4")
n6<-"FGF Pathway"
g6<-c("FGF9", "FGF7", "FGFR1", "FGFR2", "FGFR3", "SOS1", "FRS2", "FRS3", "SHOC2")
n7<-"WNT Pathway"
g7<-c("WNT3", "WNT6", "WNT2B", "FZD3", "ROR2", "AES", "AXIN2", "DACT1", "ZBTB33",
"DVL1")

```

```

FeaturePlotFmt<-function(x,features=glist,save.prefix="fig2C",ncol=5,width=14){
  len=length(features)
  nc=floor(len/(ncol*ncol))
  reminder=len %% ncol
  if(nc==0){
    tlist<-features
    pdf(paste0(save.prefix,"_all.pdf"),width=width,height=width)

```

```

  p<-FeaturePlot(x,tlist,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=ncol,raster=FALSE
) & NoLegend() & NoAxes()
  print(p)
  dev.off()
}
  if(nc>=1){
    for(i in 1:nc){
      tlist<-features[seq(ncol*ncol*(i-1)+1,ncol*ncol*(i))]
      pdf(paste0(save.prefix,"_",i,".pdf"),width=width,height=width)

```

```

  p<-FeaturePlot(x,tlist,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=ncol,raster=FALSE
) & NoLegend() & NoAxes()
  print(p)
  dev.off()
}}
  if(reminder > 0 ){
    if(len > ncol*ncol){
      rlist<-features[-seq(1,nc*ncol*ncol)]
      cm=width/ncol
      nc2=ceiling(length(rlist)/ncol)
      hei=nc2*cm
      rnc<-i+1
      pdf(paste0(save.prefix,"_",rnc,".pdf"),width=width,height=hei)

```

```

  p<-FeaturePlot(x,rlist,order=TRUE,cols=c("lightgrey","yellow","red"),ncol=ncol,raster=FALSE
) & NoLegend() & NoAxes()
  print(p)
  dev.off()
}}

```

```

}
fig6<-function(dat, gene.plot=g1, title=n1, ncol=5, width=14){
  Idents(dat)<-dat$Sample
  for( sample in unique(dat$Sample)){
    tmp<-subset(dat, ident=sample)
    of<-paste0(title, "_", sample, ".pdf")
    gene.plot=gene.plot[gene.plot %in% rownames(tmp)]
    FeaturePlotFmt(tmp, features=gene.plot, ncol=ncol, save.prefix=of, width=width)
  }
}
fig6(dat, gene.plot=g1, title=n1, ncol=2, width=5)
fig6(dat, gene.plot=g2, title=n2, ncol=3, width=7.5)
fig6(dat, gene.plot=g4, title=n4, ncol=3, width=7.5)
fig6(dat, gene.plot=g5, title=n5, ncol=3, width=7.5)

fig6(dat, gene.plot=g3, title=n3, ncol=3, width=7.5)
fig6(dat, gene.plot=g6, title=n6, ncol=3, width=7.5)
fig6(dat, gene.plot=g7, title=n7, ncol=3, width=7.5)

```

```

#20230330
#fig1
dat<-readRDS("old/raw2_anno.rds")
DimPlot(dat, cols=col16[c(1,3,2,4)])
VlnPlot(dat, features=c("GFRA1", "UCHL1", "KIT", "ZCWPW1", "SYCP3", "CLGN", "CCDC39",
"PRM1", "STRA8"), group.by="Sample", pt.size=0)
VlnPlot(dat, features=c(CCNA2, CCNB1, CCND1, CCNE1, CDK1, CDK4, CDKN1B, KIT, MKI67,
RAD51, BRCA1, CDCA8, TOP2A, HMGB2), group.by="annoV1", pt.size=0)
#fig2
mtx1<-data.frame(goid=c("GO:0006412", "GO:0071560", "GO:0043065", "GO:0048608"),
  Description=c("translation",
    "cellular response to transforming\ngrowth factor beta stimulus",
    "positive regulation of apoptotic process",
    "reproductive structure development"),
  fg=c(18/46,
    6/46,
    7/46,
    6/46),
  bg=c(232/4730,
    48/4730,
    108/4730,
    85/4730),
  pval=c(1.16473E-12, 5.45868E-06, 6.84069E-05, 0.000148528))
mtx1$plt<- -log10(mtx1$pval)
mtx1<-mtx1[order(mtx1$plt, decreasing=FALSE),]
mtx1$Description<-factor(mtx1$Description, levels=mtx1$Description)
pdf("fig2_im1.pdf", width=7, height=3)
p<-ggplot(mtx1, aes(x=Description, y=plt, fill=plt))+geom_bar(stat="identity", position=positi

```

```
on_dodge(0.7),width=0.5,aes(x=Description))+coord_flip() +scale_y_log10(breaks =
c(1,10,100,1000))+theme(panel.background = element_rect(fill = "transparent",colour =
NA))+xlab("Term")
+ylab(expression(-"log"["10"]*" (PValue)"))+theme_bw()+labs(fill=expression(-"log"["10"]*(P
Value)),title="Immatured-1")+scale_fill_gradientn(colours=c("royalblue","yellow","red"))+th
eme(axis.text.y=element_text(size=12,color="black"))
print(p)
dev.off()
```

```
mtx1 <-data.frame(goid=c("GO:0006412",
                        "GO:1903047",
                        "GO:0000819",
                        "GO:0010564"
),
                Description=c("translation",
                              "mitotic cell cycle process",
                              "sister chromatid segregation",
                              "regulation of cell cycle process"
),
                pval=c(4.03141E-15,
                      2.12032E-08,
                      2.89131E-06,
                      8.26998E-06
))
```

```
mtx1$plt<- -log10(mtx1$pval)
mtx1 <-mtx1[order(mtx1$plt,decreasing=FALSE),]
mtx1$Description<-factor(mtx1$Description,levels=mtx1$Description)
pdf("fig2_im2.pdf",width=7,height=3)
p<-ggplot(mtx1,aes(x=Description,y=plt,fill=plt))+geom_bar(stat="identity",position=positi
on_dodge(0.7),width=0.5,aes(x=Description))+coord_flip() +scale_y_log10(breaks =
c(1,10,100,1000))+theme(panel.background = element_rect(fill = "transparent",colour =
NA))+xlab("Term")
+ylab(expression(-"log"["10"]*" (PValue)"))+theme_bw()+labs(fill=expression(-"log"["10"]*(P
Value)),title="Immatured-2")+scale_fill_gradientn(colours=c("royalblue","yellow","red"))+th
eme(axis.text.y=element_text(size=12,color="black"))
print(p)
dev.off()
```

```
mtx1 <-data.frame(goid=c("GO:0019953",
                        "GO:0007283",
                        "GO:0032504",
                        "GO:0003006"
),
                Description=c("sexual reproduction",
                              "spermatogenesis",
                              "multicellular organism reproduction",
                              "developmental process involved\nin reproduction"
```

```

),
pval=c(3.62438E-07,
       1.39972E-06,
       1.8854E-06,
       0.00012028
))
mtx1$plt<- -log10(mtx1$pval)
mtx1<-mtx1[order(mtx1$plt,decreasing=FALSE),]
mtx1$Description<-factor(mtx1$Description,levels=mtx1$Description)
pdf("fig2_m.pdf",width=7,height=3)
p<-ggplot(mtx1,aes(x=Description,y=plt,fill=plt))+geom_bar(stat="identity",position=position_dodge(0.7),width=0.5,aes(x=Description))+coord_flip() +scale_y_log10(breaks =
c(1,10,100,1000))+theme(panel.background = element_rect(fill = "transparent",colour =
NA))+xlab("Term")
+ylab(expression(-"log"["10"]*" (PValue)"))+theme_bw()+labs(fill=expression(-"log"["10"]*" (P
Value)"),title="Matured")+scale_fill_gradientn(colours=c("royalblue","yellow","red"))+theme(
axis.text.y=element_text(size=12,color="black"))
print(p)
dev.off()
#fig3
dat<-readRDS("../old/leydig_tsne.rds")
Idents(dat)<-dat$RECODE_snn_res.0.1
dat<-RenamIdents(dat,"0"="Progenitor",
"5"="Myoid cells","6"="Myoid cells","1"="Myoid cells",
"3"="Leydig cell-1","4"="Leydig cell-1",
"2"="Leydig cell-2")
dat$annoV3<-dat@active.ident
DimPlot(dat,cols=col9)
DimPlotto(dat,cols=col9,split.by="Sample",ncol=4)
#fig4
glist<-c("ACRBP",
"AKAP1",
"ANKRD9",
"BMS1",
"CABS1",
"CALM2",
"CALR3",
"CCT8",
"CRISP3",
"CRLS1",
"DDX39A",
"DKKL1",
"DNAJB7",
"FBP1",
"GSG1",
"GTSF1",
"HSP90B1",
"IQCA1L",

```

"LYAR",
"MEIOB",
"MEIOC",
"OAZ3",
"PIWIL1",
"PRM1",
"PRSS21",
"PRSS37",
"PTPRK",
"RBBP6",
"RNF151",
"SEPTIN2",
"SH3RF2",
"SHCBP1L",
"SMC1B",
"SPACA3",
"SPACA4",
"SPAM1",
"SPATA22",
"SPEM1",
"SPEM2",
"SSNA1",
"STK31",
"SUN5",
"SYCP1",
"SYCP2",
"TEX101",
"TEX37",
"TMEM239",
"TNP2",
"TRAPPC11",
"TSPAN6",
"TSSK3",
"TSSK4",
"TUBA8",
'TULP2',
"ZPBP",
"INHBA",
"INHA",
"KLK1",
"IGFBP3",
"APOA1",
"DLK1",
"VIM",
"COL1A1",
"COL1A2",
"CST3",
"ACTA2",
"RSPH1",

```

"RPL10",
"RPS6",
"PIWIL1",
"APOD",
"HSPB9",
"ROPN1",
"TMEM239",
"TKTL1")
dat<-readRDS("../old/sheep_downstream.rds")
Idents(dat)<-dat$annoV2
dat<-RenamIdents(dat,"Round S'tids"="Spermatid",
                  "Elongated S'tids"="Spermatid")
dat<-subset(dat,idents=c("Early primary S'cytes","Late primary
S'cytes","Spermatid","Sperm"))
glist<-glist[glist %in% rownames(dat)]
dat$annoV3<-factor(as.character(dat@active.ident),levels=c("Early primary S'cytes", "Late
primary S'cytes","Spermatid","Sperm"))
VlnPlot(dat,features=glist,group.by="annoV3",pt.size=0,ncol=9) &
theme(axis.text.x=element_text(angle=90))

#fig5
dir.create("fig5")
setwd("fig5")
dat<-subset(dat,cells=rownames(dat@meta.data[dat$Sample %in%
c("P0","P180","P360"),]))
saveRDS(dat,file="fig5.rds")
Idents(dat)<-dat$Sample
deg1<-FindMarkers(dat,ident.1="P180",ident.2="P0",test.use="wilcox",min.cells.group =
0,min.pct=0,logfc.threshold = -Inf)
write.xlsx(deg1,file="P180vsP0.xlsx",rowNames=TRUE)
deg4<-FindMarkers(dat,ident.1="P0",ident.2="P180",test.use="wilcox",min.cells.group =
0,min.pct=0,logfc.threshold = -Inf)
write.xlsx(deg4,file="P0vsP180.xlsx",rowNames=TRUE)
deg2<-FindMarkers(dat,ident.1="P360",ident.2="P180",test.use="wilcox",min.cells.group =
0,min.pct=0,logfc.threshold = -Inf)
write.xlsx(deg2,file="P360vsP180.xlsx",rowNames=TRUE)
deg3<-FindMarkers(dat,ident.1="P360",ident.2="P0",test.use="wilcox",min.cells.group =
0,min.pct=0,logfc.threshold = -Inf)
write.xlsx(deg3,file="P360vsP0.xlsx",rowNames=TRUE)

deg1<-read.xlsx("P0vsP180.xlsx",rowNames=TRUE)
deg2<-read.xlsx("P360vsP180.xlsx",rowNames=TRUE)
deg3<-read.xlsx("P360vsP0.xlsx",rowNames=TRUE)
deg1$sig<-ifelse((deg1$p_val<0.05 & deg1$avg_log2FC>0.25),"Up","not sig")
deg1$sig<-ifelse(deg1$p_val<0.05 & deg1$avg_log2FC < -0.25,"Down",deg1$sig)
deg2$sig<-ifelse((deg2$p_val<0.05 & deg2$avg_log2FC>0.25),"Up","not sig")
deg2$sig<-ifelse(deg2$p_val<0.05 & deg2$avg_log2FC < -0.25,"Down",deg2$sig)
deg3$sig<-ifelse((deg3$p_val<0.05 & deg3$avg_log2FC>0.25),"Up","not sig")
deg3$sig<-ifelse(deg3$p_val<0.05 & deg3$avg_log2FC < -0.25,"Down",deg3$sig)

```

```

deg1$plt<--log10(deg1$p_val)
deg1$x<-deg1$pct.1-deg1$pct.2
deg2$plt<--log10(deg2$p_val)
deg2$x<-deg2$pct.1-deg2$pct.2
deg3$plt<--log10(deg3$p_val)
deg3$x<-deg3$pct.1-deg3$pct.2
p1<-ggplot(deg1,aes(x=avg_log2FC,y=plt,color=sig))+geom_point()+theme_minimal()+scale_color_manual(values=c("green","royalblue","red"))+labs(color="significant",y=expression(-"log"["10"]*(PValue)),title="P0vsP180")
p2<-ggplot(deg1,aes(x=x,y=avg_log2FC,color=sig))+geom_point()+theme_pubclean()+scale_color_manual(values=c("green","royalblue","red"))+labs(color="significant",x="pct.1-pct.2",title="P0vsP180")+geom_vline(xintercept = 0,linetype=3)
p3<-ggplot(deg2,aes(x=avg_log2FC,y=plt,color=sig))+geom_point()+theme_minimal()+scale_color_manual(values=c("green","royalblue","red"))+labs(color="significant",y=expression(-"log"["10"]*(PValue)),title="P360vsP180")
p4<-ggplot(deg2,aes(x=x,y=avg_log2FC,color=sig))+geom_point()+theme_pubclean()+scale_color_manual(values=c("green","royalblue","red"))+labs(color="significant",x="pct.1-pct.2",title="P360vsP180")+geom_vline(xintercept = 0,linetype=3)
p5<-ggplot(deg3,aes(x=avg_log2FC,y=plt,color=sig))+geom_point()+theme_minimal()+scale_color_manual(values=c("green","royalblue","red"))+labs(color="significant",y=expression(-"log"["10"]*(PValue)),title="P360vsP0")
p6<-ggplot(deg3,aes(x=x,y=avg_log2FC,color=sig))+geom_point()+theme_pubclean()+scale_color_manual(values=c("green","royalblue","red"))+labs(color="significant",x="pct.1-pct.2",title="P360vsP0")+geom_vline(xintercept = 0,linetype=3)
pdf("fig5_dotplot.pdf",width=6,height=6)
print(p1)
print(p2)
print(p3)
print(p4)
print(p5)
print(p6)
dev.off()

```

```

df<-data.frame(group=c("P0vsP180","P0vsP180","P360vsP180","P360vsP180","P360vsP0","P360vsP0"),
               type=c("Up","Down","Up","Down","Up","Down"),

```

```

num=c(table(deg1$sig)[c("Up","Down")],table(deg2$sig)[c("Up","Down")],table(deg3$sig)[c("Up","Down")]))
df$group<-factor(df$group,levels=rev(c("P0vsP180","P360vsP180","P360vsP0")))
ggplot(df,aes(x=group,y=num,fill=type))+geom_bar(position=position_dodge(0.5),stat="identity",width=0.5)+coord_flip()+scale_fill_manual(values=c("green3","chocolate1"))+labs(x="",y="Number of Genes",color="",title="Different Expression Gene Statistics")+theme_minimal()+theme(axis.text = element_text(color="black",size=12))

```

```

up1<-as.vector(rownames(deg1[deg1$sig=="Up",]))
dw1<-as.vector(rownames(deg1[deg1$sig=="Down",]))
up2<-as.vector(rownames(deg2[deg2$sig=="Up",]))
dw2<-as.vector(rownames(deg2[deg2$sig=="Down",]))

```

```

up3<-as.vector(rownames(deg3[deg3$sig=="Up",]))
dw3<-as.vector(rownames(deg3[deg3$sig=="Down",]))
VennDiagram::venn.diagram(list(P0vsP180=up1,P360vsP180=up2,P360vsP0=up3),fill=c("yellow", "blue", "green"),"venn_up.tiff")
VennDiagram::venn.diagram(list(P0vsP180=dw1,P360vsP180=dw2,P360vsP0=dw3),fill=c("yellow", "blue", "green"),"venn_down.tiff")
VennDiagram::venn.diagram(list(P0vsP180=c(up1,dw1),P360vsP180=c(up2,dw2),P360vsP0=c(up3,dw3)),fill=c("yellow", "blue", "green"),"venn_all.tiff")

```

```

g2m_genes<-cc.genes$g2m.genes
g2m_genes<-CaseMatch(search=g2m_genes,match=rownames(obj))
s_genes<-cc.genes$s.genes
s_genes<-CaseMatch(search=s_genes,match=rownames(obj))
obj<-CellCycleScoring(obj,g2m.features=g2m_genes,s.features = s_genes)
obj$Sphase<-PercentageFeatureSet(obj,features=s_genes)
obj$G2Mphase<-PercentageFeatureSet(obj,features=g2m_genes)
p1<-VlnPlot(obj,"Sphase",group.by="Sample",pt.size=0) + labs(title="S phase genes (n=39)")
p2<-VlnPlot(obj,"G2Mphase",group.by="Sample",pt.size=0) + labs(title="G2M phase genes (n=43)")
p3<-VlnPlot(obj,"Sphase",group.by="Sample") + labs(title="S phase genes (n=39)")
p4<-VlnPlot(obj,"G2Mphase",group.by="Sample") + labs(title="G2M phase genes (n=43)")
cowplot::plot_grid(p1,p2,p3,p4,ncol=2)
glist<-read.xlsx("附件2.xlsx",check.names = FALSE)
g1<-glist$canonical.glycolysis[glist$canonical.glycolysis %in% rownames(obj)]
g2<-toupper(glist$oxidative.phosphorylation)[toupper(glist$oxidative.phosphorylation) %in% rownames(obj)]
g3<-toupper(glist$triglyceride.metabolism)[toupper(glist$triglyceride.metabolism) %in% rownames(obj)]
length(g1)
length(g2)
length(g3)
obj$gs1<-PercentageFeatureSet(obj,features=g1)
obj$gs2<-PercentageFeatureSet(obj,features=g2)
obj$gs3<-PercentageFeatureSet(obj,features=g3)
obj$annofinal<-factor(as.character(obj@active.ident),levels=c("Immature Sertoli-1", "Immature Sertoli-2", "Mature Sertoli"))
p1<-VlnPlot(obj,"g1",pt.size=0,group.by="annofinal",assay = "counts") +
labs(title="canonical glycolysis(n=15)",x="")+theme(axis.text.x=element_blank())
p2<-VlnPlot(obj,"g2",pt.size=0,group.by="annofinal",assay="counts") +
labs(title="oxidative phosphorylation(n=57)",x="")+theme(axis.text.x=element_blank())
p3<-VlnPlot(obj,"g3",pt.size=0,group.by="annofinal",assay="counts") +
labs(title="triglyceride.metabolism(n=78)")
cowplot::plot_grid(p1,p2,p3,ncol=1,rel_heights=c(1,1,1.8))

p1<-VlnPlot(obj,"g1",pt.size=0,group.by="annofinal") + labs(title="canonical glycolysis(n=15)",x="")+theme(axis.text.x=element_blank())

```

```

p2<-VlnPlot(obj,"g2",pt.size=0,group.by="annofinal") + labs(title="oxidative
phosphorylation(n=57)",x="")+theme(axis.text.x=element_blank())
p3<-VlnPlot(obj,"g3",pt.size=0,group.by="annofinal") +
labs(title="triglyceride.metabolism(n=78)")
cowplot::plot_grid(p1,p2,p3,ncol=1,rel_heights=c(1,1,1.8))

```

```

#51 fig1
setwd("~/Work/tmp/2023/51")
dat<-readRDS("../old/raw2_anno.rds")
dat<-Renameldents(dat,"Undifferentiated
spermatogonia"="Spermatogonia","Differentiating spermatogonia"="Spermatogonia")
p<-DimPlot(dat,cols=col16)
pdf("fig1.pdf",width=6,height=4.5)
print(p)
dev.off()
#fig2 umi exp
obj<-readRDS("fig2_4_cellcycling.rds")
g2m_genes<-cc.genes$g2m.genes
g2m_genes<-CaseMatch(search=g2m_genes,match=rownames(obj))
s_genes<-cc.genes$s.genes
s_genes<-CaseMatch(search=s_genes,match=rownames(obj))
mtx<-rowSums(as.data.frame.matrix(t(obj@assays$RNA@counts[g2m_genes,])))/length(g2
m_genes)
df<-data.frame(umi=mtx,anno=obj@meta.data[names(mtx),"annofinal"])
#mtx %>% group_by(anno) %>% summarise_all(funs(sum)) -> df
df<-melt(df)
ggplot(df,aes(x=anno,y=value))+geom_violin()

```

```

#fig2_3
for(i in unique(obj$annofinal)){
  tmp<-subset(obj,idents=i)
  Idents(tmp)<-tmp$Sample
  deg<-FindAllMarkers(tmp,only.pos=TRUE)
  write.xlsx(deg,file=paste0(i,"_Fall.xlsx"))
}
deg1<-read.xlsx("Immature Sertoli-1_Fall.xlsx")
deg2<-read.xlsx("Immature Sertoli-2_Fall.xlsx")
deg3<-read.xlsx("Mature Sertoli_Fall.xlsx")
for(i in unique(deg1$cluster)){
  tmp<-deg1[deg1$cluster==i,]
  positive_enrich(tmp,topn=100,OrgDb = ovisdb,fromType =
"SYMBOL",toType="ENTREZID",kegg.organism = NULL,save.prefix = paste0("Immature
Sertoli-1_",i,"_up"),qvalueCutoff=1,pvalueCutoff=1)
}
for(i in unique(deg2$cluster)){
  tmp<-deg1[deg2$cluster==i,]
  positive_enrich(tmp,topn=100,OrgDb = ovisdb,fromType =
"SYMBOL",toType="ENTREZID",kegg.organism = NULL,save.prefix = paste0("Immature

```

```

Sertoli-2_",i,"_up"),qvalueCutoff=1,pvalueCutoff=1)
}
for(i in unique(deg3$cluster)){
  tmp<-deg1[deg3$cluster==i,]
  positive_enrich(tmp,topn=100,OrgDb = ovisdb,fromType =
"SYMBOL",toType="ENTREZID",kegg.organism = NULL,save.prefix = paste0("Mature
Sertoli_",i,"_up"),qvalueCutoff=1,pvalueCutoff=1)
}
top1<-deg1 %>% group_by(cluster) %>% top_n(10,avg_log2FC)
top2<-deg2 %>% group_by(cluster) %>% top_n(10,avg_log2FC)
top3<-deg3 %>% group_by(cluster) %>% top_n(10,avg_log2FC)
top1$group<-"Immature Sertoli-1"
top2$group<-"Immature Sertoli-2"
top3$group<-"Mature Sertoli"
top10<-rbind(top1,top2,top3)
top10<-sortMultiMTX(top10,by="group",by.1="cluster",order=c("Immature
Sertoli-1","Immature Sertoli-2","Mature
Sertoli"),order.1=c("P0","P30","P90","P180","P270","P360","P540"))
glist<-unique(top10$gene)
mtx<-as.data.frame.matrix(t(obj@assays$RNA@data[glist,]))
anno<-data.frame(Celltype=obj@meta.data[rownames(mtx),"annofinal"],Sample=obj@met
a.data[rownames(mtx),"Sample"])
rownames(anno)<-rownames(mtx)
anno<-sortMultiMTX(anno,by="Celltype",by.1="Sample",order=c("Immature
Sertoli-1","Immature Sertoli-2","Mature
Sertoli"),order.1=c("P0","P30","P90","P180","P270","P360","P540"))
mtx_t<-t(mtx[rownames(anno),glist])
mtx_t<-rescale(mtx_t,c(-2,2))
colheat4<-colorRampPalette(c("dodgerblue","seashell","tomato3"))(400)
anno_colors = list(
  Celltype= c("Immature Sertoli-1"=col16[8],"Immature Sertoli-2"=col16[9],"Mature
Sertoli"=col16[10]),
  Sample =
c("P0"=col16[1],"P30"=col16[2],"P90"=col16[3],"P180"=col16[4],"P270"=col16[5],"P360"=col
16[6],"P540"=col16[7])
)
p<-pheatmap::pheatmap(mtx_t,cluster_cols = FALSE,cluster_rows=FALSE,show_colnames =
FALSE,show_rownames = FALSE,annotation= anno,color=colheat4,annotation_colors =
anno_colors)

#fig3
dat<-readRDS("~/Work/tmp/2023/51/fig3_leydig.rds")
dat$annoV3<-dat@active.ident
cellDis(dat,anno.by="annoV3",sample.by="Sample",save.prefix="cellDis_fig3")
meta<-dat@meta.data[,c("Sample","annoV3")]
angrycell::plot_fraction(meta,coord_flip = FALSE)
df<-table(dat@meta.data[, "Sample"],dat@meta.data[, "annoV3"])
fq<-round(prop.table(df,1)*100,3)
df<-melt(fq)

```

```

ggplot(df,aes(x=Var1,y=value,fill=Var2))+geom_bar(stat='identity')+labs(fill="Celltype",x=""
,y="prop(%))+theme_pubr()+scale_fill_manual(values=col16)+theme(axis.text.x=element_te
xt(angle=0,hjust=0.5,vjust=0.5),legend.position="right")
glist<-c("INHA", "PHGDH", "IGFBP7", "TPM2", "TIMP3", "LRRN3", "LHR", "AR",
"CYP17A1", "DKKL1", "HAGH", "ROPN1", "HOATZ", "ND4", "ATP6", "COX1", "CYTB", "PHKG2",
"PRM1", "OAZ3", "SPATA6", "CAPZA3", "TEKT2", "RPS26", "RPL10", "RPL18",
"RPL8", "AMH", "HSP90AB1", "RPS6")
glist<-glist[glist %in% rownames(dat)]
p<-VlnPlot(dat,glist,ncol=10,pt.size=0) & theme(axis.text.x=element_text(angle=90))
p2<-FeaturePlot(dat,glist,ncol=10,cols=c("lightgrey", "yellow", "red"))
pdf("fig3_2-vlnplot.pdf",width=25,height=10)
print(p)
dev.off()
pdf("fig3_2-umap.pdf",width=28,height=8)
print(p2)
dev.off()

```