TRIFID_using_extra_introns

Makes Supplementary Table 5. trifid volcano plot - Figure2F

Required for: Trifid_stats.Rmd - Figure 2E Go_networks_plus_trifid.Rmd - Figure 2G,H

Theres a refseq TRIFID table, so for introns with only a refseq id I use the TRIFID score for refseq transcripts.

```
library(biomaRt)
library(tidyr)
library(dplyr)
library(ggplot2)
library(ggrepel)

library(clusterProfiler)
library(here)
i_am("R/15_TRIFID_using_extra_introns.Rmd")
```

```
lc = read.delim(here("31_leafcutter", "three_database_info_all_junctions.tsv"))
lc = unite(lc, "intron_coords", chr, start, end, strand, sep = ":")
#lc[grep("PEMT",lc$gene),]
head(lc)
```

```
##   annotation            intron_coords  cluster_id      deltapsi      p.adjust
## 1    gencode chr7:43648652:43650493:- clu_35616_- -0.0141028170 2.192287e-123
## 2    gencode chr7:43648652:43650612:- clu_35616_- -0.0408035987 2.192287e-123
## 3    gencode chr7:43648652:43665658:- clu_35616_- -0.0009734716 2.192287e-123
## 4    gencode chr7:43648652:43711400:- clu_35616_- -0.0003668545 2.192287e-123
## 5    gencode chr7:43648652:43729429:- clu_35616_- -0.0681581659 2.192287e-123
## 6    gencode chr7:43650712:43656033:- clu_35616_- -0.0034620531 2.192287e-123
##                                                                                                    transc
## 1                                                                                                 ENST00000
## 2 ENST00000446564.5,ENST00000448704.5,ENST00000451651.5,ENST00000418140.5,ENST00000431651.5,ENST0000
## 3                                                                                                  ENST0000
## 4                                                                                                  ENST0000
## 5                  ENST00000457939.1,ENST00000420441.1,ENST00000415076.6,ENST00000223336.10,ENST0000
## 6                                                                                                  ENST0000
##   min_intron_number mode_intron_number gene
## 1                 2                  2 COA1
## 2                 2                  2 COA1
## 3                 2                  2 COA1
## 4                 2                  2 COA1
## 5                 1                  1 COA1
## 6                 4                  4 COA1
##                                      biotype genes_in_cluster
## 1                             protein_coding             COA1
## 2 nonsense_mediated_decay,protein_coding,lncRNA             COA1
## 3                    nonsense_mediated_decay             COA1
```

```
## 4                                protein_coding             COA1
## 5       nonsense_mediated_decay,protein_coding             COA1
## 6                                protein_coding             COA1
##   is_first_intron
## 1           FALSE
## 2           FALSE
## 3           FALSE
## 4           FALSE
## 5            TRUE
## 6           FALSE
```

```
dim(lc) # some duplications based on gene names/ antisense transcripts.
```

```
## [1] 132587     12
```

```
trifid = read.delim(here("annotations/trifid/gencode37_trifid_predictions.tsv"))
head(trifid)
```

```
##           gene_id gene_name   transcript_id  translation_id          flags
## 1 ENSG00000187010       RHD ENST00000342055 ENSP00000339577 protein_coding
## 2 ENSG00000187010       RHD ENST00000328664 ENSP00000331871 protein_coding
## 3 ENSG00000187010       RHD ENST00000417538 ENSP00000396420 protein_coding
## 4 ENSG00000187010       RHD ENST00000423810 ENSP00000399640 protein_coding
## 5 ENSG00000187010       RHD ENST00000622561 ENSP00000478087 protein_coding
## 6 ENSG00000187010       RHD ENST00000454452 ENSP00000413849 protein_coding
##        ccdsid      appris                ann_type length trifid_score
## 1 CCDS60028.1       MINOR             Alternative    493       0.2105
## 2   CCDS262.1 PRINCIPAL:1               Principal    417       0.4021
## 3 CCDS60031.1       MINOR             Alternative    378       0.0572
## 4 CCDS60027.1       MINOR Alternative Duplication    431       0.0415
## 5 CCDS60027.1       MINOR             Alternative    431       0.0223
## 6 CCDS53285.1       MINOR             Alternative    321       0.0652
##   norm_trifid_score
## 1            0.4210
## 2            0.8043
## 3            0.1145
## 4            0.0830
## 5            0.0447
## 6            0.1303
```

```
length(unique(trifid$transcript_id))#104 688
```

```
## [1] 104688
```

```
trefseq = read.delim(here("annotations/trifid/refseq110_trifid_predictions.tsv"))
nrow(trefseq)
```

```
## [1] 129456
```

```
head(trefseq)
```

```
##   gene_id gene_name transcript_id translation_id flags      ccdsid      appris
## 1    9997      SCO2  NM_001169111   NP_001162582  mRNA CCDS14095.1 PRINCIPAL:1
## 2    9997      SCO2  NM_001169110   NP_001162581  mRNA CCDS14095.1 PRINCIPAL:1
## 3    9997      SCO2  NM_001169109   NP_001162580  mRNA CCDS14095.1 PRINCIPAL:1
## 4    9997      SCO2     NM_005138      NP_005129  mRNA CCDS14095.1 PRINCIPAL:1
## 5    9994  CASP8AP2  NM_001137667   NP_001131139  mRNA           - PRINCIPAL:1
## 6    9994  CASP8AP2     NM_012115      NP_036247  mRNA           - PRINCIPAL:1
##   ann_type length trifid_score norm_trifid_score
## 1        -    266       0.5779            1.0000
## 2        -    266       0.5779            1.0000
## 3        -    266       0.5779            1.0000
## 4        -    266       0.5779            1.0000
## 5        -   1982       0.2909            0.5818
## 6        -   1982       0.2909            0.5818
```

```
trefseq$gene_id = as.character(trefseq$gene_id)
trifid = bind_rows(trifid, trefseq)
nrow(trifid)
```

```
## [1] 234144
```

The reason trifid has so few transcripts is only those with translated sequences are included.

Convert lc introns to transcript table

```
transcripts = separate_longer_delim(lc, transcript_ids, delim=",")
transcripts$transcript_ids = gsub("\\.[0-9]*","",gsub("rna-","",transcripts$transcript_ids))
head(transcripts)
```

```
##   annotation            intron_coords  cluster_id    deltapsi      p.adjust
## 1    gencode chr7:43648652:43650493:- clu_35616_- -0.01410282 2.192287e-123
## 2    gencode chr7:43648652:43650612:- clu_35616_- -0.04080360 2.192287e-123
## 3    gencode chr7:43648652:43650612:- clu_35616_- -0.04080360 2.192287e-123
## 4    gencode chr7:43648652:43650612:- clu_35616_- -0.04080360 2.192287e-123
## 5    gencode chr7:43648652:43650612:- clu_35616_- -0.04080360 2.192287e-123
## 6    gencode chr7:43648652:43650612:- clu_35616_- -0.04080360 2.192287e-123
##    transcript_ids min_intron_number mode_intron_number gene
## 1 ENST00000310564                 2                  2 COA1
## 2 ENST00000446564                 2                  2 COA1
## 3 ENST00000448704                 2                  2 COA1
## 4 ENST00000451651                 2                  2 COA1
## 5 ENST00000418140                 2                  2 COA1
## 6 ENST00000431651                 2                  2 COA1
##                                       biotype genes_in_cluster
## 1                               protein_coding             COA1
## 2 nonsense_mediated_decay,protein_coding,lncRNA             COA1
## 3 nonsense_mediated_decay,protein_coding,lncRNA             COA1
## 4 nonsense_mediated_decay,protein_coding,lncRNA             COA1
```

```
## 5 nonsense_mediated_decay,protein_coding,lncRNA             COA1
## 6 nonsense_mediated_decay,protein_coding,lncRNA             COA1
##   is_first_intron
## 1           FALSE
## 2           FALSE
## 3           FALSE
## 4           FALSE
## 5           FALSE
## 6           FALSE
```

```
dim(transcripts)
```

```
## [1] 379523     12
```

```
#transcripts[grep("PEMT",transcripts$gene),]
```

some numbers on this

```
sig_trans = filter(transcripts, p.adjust < 0.05 & abs(deltapsi) >= 0.1)
sprintf("Leafcutter significant introns (%d) correspond to %d unique transcripts (represented in %d rows
        length(unique(sig_trans$intron_coords)),
        length(unique(sig_trans$transcript_ids)),
        length(sig_trans$transcript_ids))
```

```
## [1] "Leafcutter significant introns (777) correspond to 2094 unique transcripts (represented in 2233
```

```
sprintf("%d of these transcripts are represented in the trifid database.\n",
        sum(unique(sig_trans$transcript_ids) %in% trifid$transcript_id))
```

```
## [1] "1484 of these transcripts are represented in the trifid database.\n"
```

```
transcripts$transcript_ids[grep("PEMT",transcripts$gene,)] %in% trifid$transcript_id
```

```
##  [1]  TRUE  TRUE FALSE  TRUE  TRUE  TRUE  TRUE  TRUE FALSE  TRUE  TRUE FALSE
## [13] FALSE  TRUE  TRUE  TRUE  TRUE FALSE FALSE  TRUE  TRUE  TRUE FALSE FALSE
## [25]  TRUE  TRUE FALSE FALSE  TRUE FALSE FALSE  TRUE  TRUE  TRUE  TRUE FALSE
## [37]  TRUE FALSE  TRUE  TRUE  TRUE  TRUE  TRUE FALSE  TRUE  TRUE  TRUE FALSE
## [49]  TRUE  TRUE  TRUE  TRUE
```

Merge trifid and leafcutter

```
mtrans = merge(transcripts, trifid, by.x ="transcript_ids",by.y="transcript_id")
mtrans = mutate(mtrans, condition = if_else(deltapsi > 0, "beige", if_else(deltapsi < 0, "white", "none
                sig = p.adjust < 0.05 & abs(deltapsi) >= 0.1)

length(unique(mtrans$transcript_ids)) #82 326 transcripts in total
```

```
## [1] 82326
```

```
length(unique(filter(mtrans, sig) %>% pull(transcript_ids)))
```

```
## [1] 1484
```

```
mtrans = arrange(mtrans, desc(trifid_score), desc(norm_trifid_score), p.adjust, desc(abs(deltapsi)))
head(mtrans[c("gene_name", "trifid_score","norm_trifid_score","deltapsi","p.adjust")], n=20)
```

```
##    gene_name trifid_score norm_trifid_score      deltapsi     p.adjust
## 1     LRRC28            1                 1 -0.1277105151 2.653936e-14
## 2      COPS5            1                 1  0.0595450863 1.754366e-08
## 3      STK38            1                 1  0.0312487644 1.250549e-07
## 4        C1D            1                 1  0.0099164702 4.253925e-07
## 5      AAMDC            1                 1 -0.0008870103 9.403490e-07
## 6      AAMDC            1                 1 -0.0006973601 9.403490e-07
## 7      ACTR6            1                 1  0.0581605204 8.495921e-06
## 8     ARFIP1            1                 1  0.0068791997 1.189622e-05
## 9     ARFIP1            1                 1  0.0064822954 1.189622e-05
## 10     GNAI2            1                 1 -0.0279736041 6.917548e-05
## 11      GNB4            1                 1  0.0282956006 2.413151e-04
## 12       DR1            1                 1  0.0045084324 1.478700e-03
## 13      GDI2            1                 1 -0.0327184175 2.460331e-03
## 14      GDI2            1                 1  0.0319582980 2.460331e-03
## 15    ACTR10            1                 1  0.0275113044 2.660836e-03
## 16    ACTR10            1                 1 -0.0265122782 2.660836e-03
## 17  ATP6V1C1            1                 1  0.0274511700 4.226843e-03
## 18  ATP6V1C1            1                 1 -0.0153237824 4.226843e-03
## 19  ATP6V1C1            1                 1 -0.0153237824 4.226843e-03
## 20  ATP6V1C1            1                 1 -0.0036512432 4.226843e-03
```

```
#head(mtrans[mtrans$gene == "PEMT",])
```

Log missing introns

```
missing = transcripts[!transcripts$transcript_ids %in% trifid$transcript_id &
                        !transcripts$intron_coords %in% mtrans$intron_coords,] #annotate later

cat("Unrepresented transcripts include those from genes like: \n")
```

```
## Unrepresented transcripts include those from genes like:
```

```
cat(head(unique(missing$gene[missing$p.adjust < 0.05 & abs(missing$deltapsi)>0.1])))
```

```
## CA5BP1 LYRM4 ALG9 NAV1 LINC01140 SPON2
```

```
table(missing$annotation)
```

```
##
##    cryptic fantom_cat    gencode     refseq
##      21831      18742      26204       2694
```

```
missing[grep( "PEMT", missing$gene),] #Missing PEMT transcripts from non-significant cluster
```

```
##        annotation             intron_coords  cluster_id     deltapsi  p.adjust
## 132066    gencode chr17:17512654:17519027:- clu_19604_- 2.816277e-03 0.1914447
## 132074    gencode chr17:17512654:17576920:- clu_19604_- 3.072487e-05 0.1914447
##         transcript_ids min_intron_number mode_intron_number gene biotype
## 132066 ENST00000490392                 1                  1 PEMT  lncRNA
## 132074 ENST00000472446                 2                  2 PEMT  lncRNA
##        genes_in_cluster is_first_intron
## 132066             PEMT            TRUE
## 132074             PEMT           FALSE
```

Of course fantom and cryptic transcripts cannot be annotated by this trifid (which is based on the ensembl + refseq annotation); but also many transcripts from gencode cannot be found there either - perhaps they're new transcripts, or not protein coding versions. The genocde annotation is always changing.

```
table(mtrans$condition)
```

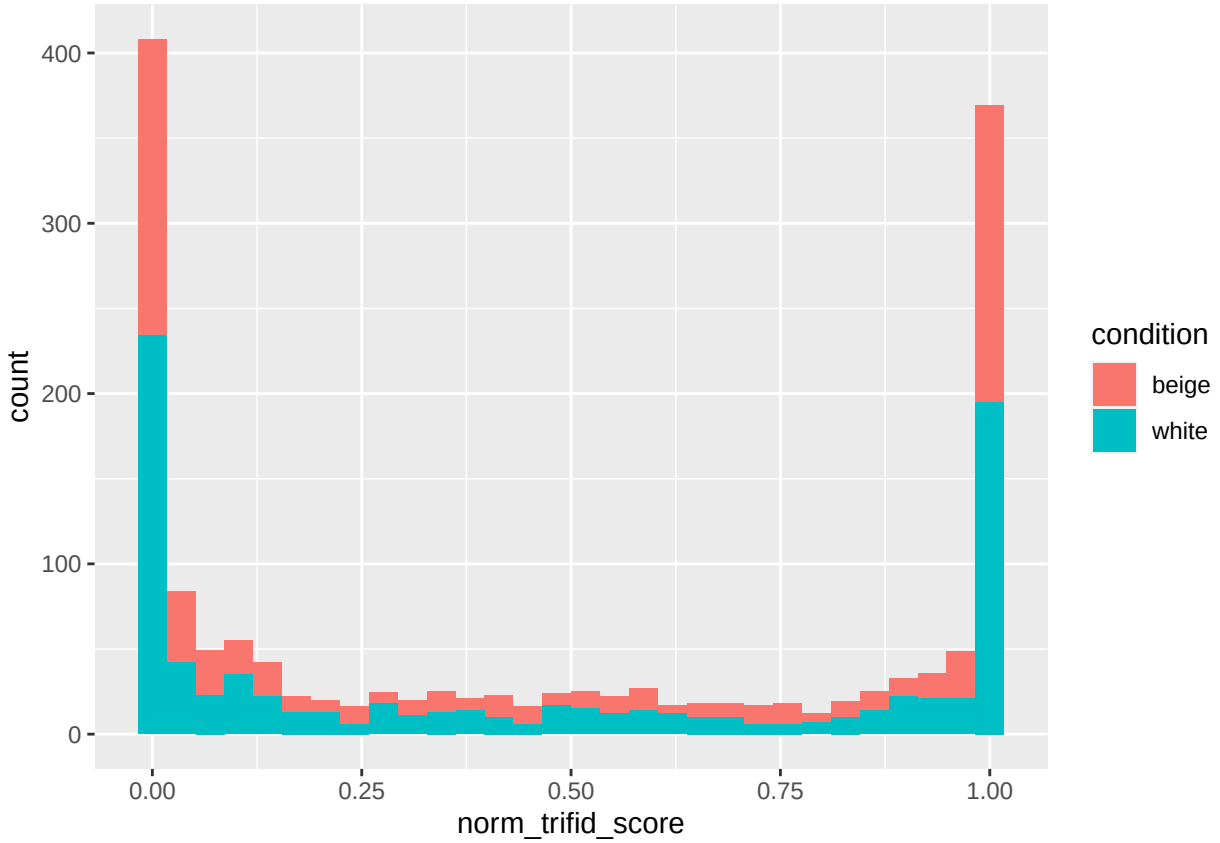
```
##
##  beige  white
## 134112 125512
```

```
table(paste(mtrans$sig, mtrans$condition))
```

```
##
## FALSE beige FALSE white  TRUE beige  TRUE white
##      133409      124660         703         852
```

Histogram

```
ggplot(filter(mtrans, sig)) + geom_histogram(aes(x=norm_trifid_score, fill=condition), bins=30)
```



Flags

```
table(mtrans$flags)
```

```
##
##                       mRNA             non_stop_decay
##                      25475                        146
##    nonsense_mediated_decay nonsense_mediated_decay,RT
##                      41896                       1836
##             protein_coding          protein_coding,RT
##                     189018                       1251
##                  TR_C_gene
##                          2
```

```
table(filter(mtrans, sig) %>% pull(flags))
```

```
##
##                       mRNA             non_stop_decay
##                         79                          2
##    nonsense_mediated_decay nonsense_mediated_decay,RT
##                        197                         13
##             protein_coding          protein_coding,RT
##                       1256                          8
```

Correlation between dPSI and TRIFID score?

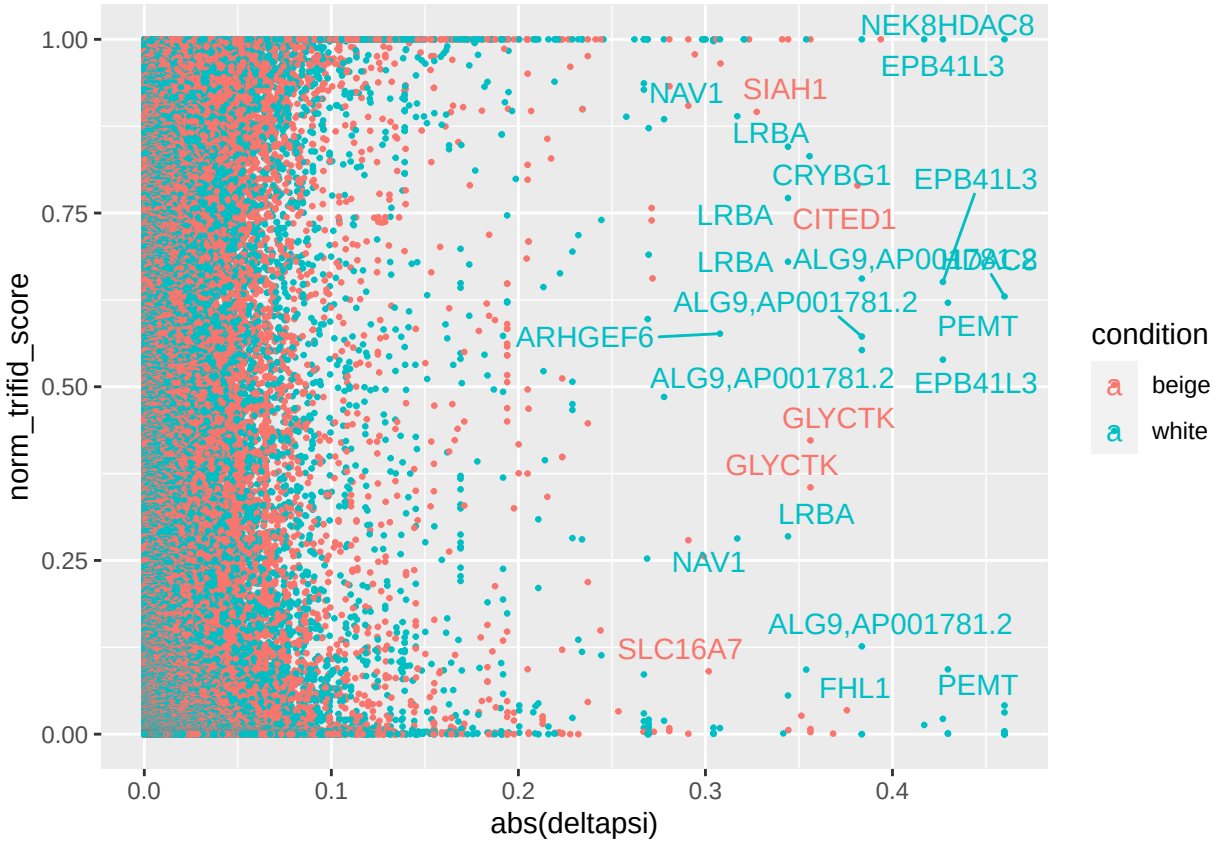
There is actually, if you look within the same cluster if we have a intron with a higher PSI it tends to have higher functionality - or maybe it just has more transcripts?

```
ggplot(mtrans, aes(x=deltapsi, y=norm_trifid_score, colour=condition)) + geom_point(size=0.5) +
    geom_text_repel(data= filter(mtrans, abs(deltapsi) >0.3), aes(label=gene))
```

```
## Warning: ggrepel: 45 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```


```
ggplot(mtrans, aes(x=abs(deltapsi), y=norm_trifid_score, colour=condition)) + geom_point(size=0.5) +
    geom_text_repel(data= filter(mtrans, abs(deltapsi) >0.3), aes(label=gene))
```

```
## Warning: ggrepel: 45 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



Annotate transcript names

```
mart <- useMart(biomart = "ensembl",
  dataset = "hsapiens_gene_ensembl",
   host = "https://sep2019.archive.ensembl.org")

biomaRt::searchAttributes(mart = mart, pattern = "transcript.*name")
```

```
##                               name                  description         page
## 24        external_transcript_name               Transcript name feature_page
## 25 external_transcript_source_name     Source of transcript name feature_page
## 58           entrezgene_trans_name EntrezGene transcript name ID feature_page
## 77              mirbase_trans_name    miRBase transcript name ID feature_page
## 92                 rfam_trans_name       RFAM transcript name ID feature_page
```

```
annot = getBM(c("external_transcript_name","ensembl_gene_id", "ensembl_transcript_id"),
              filters = "ensembl_transcript_id",
              values =  mtrans$transcript_ids,
              mart = mart, useCache = F)

head(annot, n=2); dim(annot)
```

```
##   external_transcript_name ensembl_gene_id ensembl_transcript_id
```

```
## 1                VTI1B-204 ENSG00000100568       ENST00000554659
## 2                VPS36-201 ENSG00000136100       ENST00000378060
```

```
## [1] 69071     3
```

```
#Add gene names to filt_series
mtrans = merge(mtrans,annot, by.x= "transcript_ids",
                        by.y = "ensembl_transcript_id", sort=FALSE,
               all.x = T)
#head(mtrans)
remove(annot)
```

Averaging score across isoforms at each intron

```
summary(mtrans$trifid_score)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.0000  0.0009  0.0785  0.2993  0.6374  1.0000
```

```
head(mtrans)
```

```
##    transcript_ids annotation             intron_coords  cluster_id     deltapsi
## 1 ENST00000301981    gencode chr15:99352471:99361336:+ clu_30900_+  0.007505714
## 2 ENST00000301981    gencode chr15:99276616:99287257:+ clu_30898_+  0.011562718
## 3 ENST00000301981    gencode chr15:99251541:99255898:+ clu_30897_+ -0.127710515
## 4 ENST00000301981    gencode chr15:99256125:99276576:+ clu_30898_+  0.012183427
## 5 ENST00000301981    gencode chr15:99287951:99333923:+ clu_30899_+  0.042272131
## 6 ENST00000301981    gencode chr15:99361511:99363106:+ clu_30901_+  0.004034933
##       p.adjust min_intron_number mode_intron_number   gene
## 1 8.537649e-01                 2                 4 LRRC28
## 2 1.657108e-01                 3                 3 LRRC28
## 3 2.653936e-14                 1                 1 LRRC28
## 4 1.657108e-01                 2                 2 LRRC28
## 5 3.285558e-02                 4                 5 LRRC28
## 6 5.303164e-01                 1                 8 LRRC28
##                                                      biotype
## 1        protein_coding,nonsense_mediated_decay,retained_intron
## 2                                    lncRNA,protein_coding
## 3 nonsense_mediated_decay,lncRNA,protein_coding,retained_intron
## 4                 nonsense_mediated_decay,lncRNA,protein_coding
## 5                       protein_coding,nonsense_mediated_decay
## 6        protein_coding,nonsense_mediated_decay,retained_intron
##   genes_in_cluster is_first_intron         gene_id gene_name  translation_id
## 1           LRRC28           FALSE ENSG00000168904    LRRC28 ENSP00000304923
## 2           LRRC28           FALSE ENSG00000168904    LRRC28 ENSP00000304923
## 3           LRRC28            TRUE ENSG00000168904    LRRC28 ENSP00000304923
## 4           LRRC28           FALSE ENSG00000168904    LRRC28 ENSP00000304923
## 5           LRRC28           FALSE ENSG00000168904    LRRC28 ENSP00000304923
## 6           LRRC28            TRUE ENSG00000168904    LRRC28 ENSP00000304923
##            flags      ccdsid      appris  ann_type length trifid_score
```

```
## 1 protein_coding CCDS10380.1 PRINCIPAL:1 Principal    367            1
## 2 protein_coding CCDS10380.1 PRINCIPAL:1 Principal    367            1
## 3 protein_coding CCDS10380.1 PRINCIPAL:1 Principal    367            1
## 4 protein_coding CCDS10380.1 PRINCIPAL:1 Principal    367            1
## 5 protein_coding CCDS10380.1 PRINCIPAL:1 Principal    367            1
## 6 protein_coding CCDS10380.1 PRINCIPAL:1 Principal    367            1
##   norm_trifid_score condition   sig external_transcript_name ensembl_gene_id
## 1                 1     beige FALSE               LRRC28-201 ENSG00000168904
## 2                 1     beige FALSE               LRRC28-201 ENSG00000168904
## 3                 1     white  TRUE               LRRC28-201 ENSG00000168904
## 4                 1     beige FALSE               LRRC28-201 ENSG00000168904
## 5                 1     beige FALSE               LRRC28-201 ENSG00000168904
## 6                 1     beige FALSE               LRRC28-201 ENSG00000168904
```

```
dim(mtrans)
```

```
## [1] 288536     26
```

```
#mtrans = filter(mtrans, sig)

#unknown gene names get given a ".", change to something more meaningful
mtrans = mutate(mtrans, gene = if_else(gene==".", cluster_id, gene))

#this step  averages the score if multiple transcripts are implicated at an intron
introns = group_by(mtrans, intron_coords,condition, deltapsi, p.adjust, gene, cluster_id, annotation) %
                                                                    mean_norm_score = mean(norm_trifid_sco
                                                                     median_norm_score = median(norm_trifi
                                                                    transcripts = paste(transcript_ids, se
                                                                    transcript_names = paste(external_trans
```

```
## `summarise()` has grouped output by 'intron_coords', 'condition', 'deltapsi',
## 'p.adjust', 'gene', 'cluster_id'. You can override using the `.groups`
## argument.
```

```
introns = arrange(introns, desc(abs(deltapsi)), desc(mean_trifid_score), p.adjust,)
dim(introns) #introns with annotations
```

```
## [1] 87024    12
```

```
head(introns)
```

```
## # A tibble: 6 x 12
## # Groups:   intron_coords, condition, deltapsi, p.adjust, gene, cluster_id [6]
##   intron_coords         condition deltapsi  p.adjust gene  cluster_id annotation
##   <chr>                 <chr>        <dbl>     <dbl> <chr> <chr>      <chr>
## 1 chrX:72330076:723517~ white       -0.460 6.88e- 82 HDAC8 clu_291_-  gencode
## 2 chr17:17577027:17591~ white       -0.430 4.36e-104 PEMT  clu_19605~ gencode
## 3 chr18:5489194:554391~ white       -0.427 4.23e- 13 EPB4~ clu_21093~ gencode
## 4 chr17:28728860:28733~ white       -0.417 5.30e- 40 NEK8  clu_34526~ gencode
## 5 chr2:48569086:485805~ beige        0.394 3.17e- 40 STON~ clu_31216~ gencode
## 6 chr11:111844723:1118~ white       -0.384 2.04e- 62 ALG9~ clu_2011_- gencode
## # i 5 more variables: mean_trifid_score <dbl>, mean_norm_score <dbl>,
## #   median_norm_score <dbl>, transcripts <chr>, transcript_names <chr>
```

```
length(unique(introns$intron_coords))
```

```
## [1] 87024
```

If an intron is not in trifid; report as -0.1

```
sig_clusters = unique(lc$cluster_id[lc$p.adjust < 0.05 & abs(lc$deltapsi) > 0.1 & lc$annotation %in% c(
summary(sig_clusters %in% introns$cluster_id)#420 clusters have at least 1 trifid annotation; 78 do not
```

```
##    Mode   FALSE    TRUE
## logical      58     412
```

```
#must have another annotated intron in the cluster, but not have a score already for that intron
to_add = missing#[missing$cluster_id %in% sig_clusters &
                 #    !missing$intron_coords %in% introns$intron_coords,]
nrow(to_add) #unannotated trifid transcripts, have an annotated cluster and are for an unscored intron
```

```
## [1] 69471
```

```
to_add = mutate(to_add, gene = if_else(gene==".", cluster_id, gene))
to_add = group_by(to_add, intron_coords, deltapsi, p.adjust, gene, cluster_id, annotation) %>%
    summarise(transcripts = paste(transcript_ids,collapse=","),
              )
```

```
## `summarise()` has grouped output by 'intron_coords', 'deltapsi', 'p.adjust',
## 'gene', 'cluster_id'. You can override using the `.groups` argument.
```

```
to_add = mutate(to_add, mean_trifid_score = -0.1,
                        mean_norm_score = -0.1,
                        median_norm_score = -0.1,
                condition=  if_else(deltapsi > 0, "beige", "white"))
head(to_add)
```

```
## # A tibble: 6 x 11
## # Groups:   intron_coords, deltapsi, p.adjust, gene, cluster_id [6]
##   intron_coords        deltapsi p.adjust gene  cluster_id annotation transcripts
##   <chr>                   <dbl>    <dbl> <chr> <chr>      <chr>      <chr>
## 1 chr10:100006342:100~  4.80e-4   0.989  ENSG~ clu_37954~ fantom_cat FTMT237000~
## 2 chr10:1001013:10070~ -2.57e-4   0.0811 <NA>  clu_29373~ cryptic    Unknown
## 3 chr10:100190968:100~  1.20e-2   0.147  CHUK  clu_37959~ gencode    ENST000005~
## 4 chr10:100200780:100~ -8.79e-4   0.920  <NA>  clu_37960~ cryptic    Unknown
## 5 chr10:100246935:100~ -1.12e-2   0.0387 CWF1~ clu_37962~ gencode    ENST000004~
## 6 chr10:100246935:100~  2.18e-4   0.0387 <NA>  clu_37962~ cryptic    Unknown
## # i 4 more variables: mean_trifid_score <dbl>, mean_norm_score <dbl>,
## #   median_norm_score <dbl>, condition <chr>
```

```
nrow(to_add)
```

```
## [1] 45563
```

```
all_introns = bind_rows(in_trifid=introns, not_in_trifid=to_add[to_add$cluster_id %in% introns$cluster_
                        cluster_not_in_trifid= to_add[!to_add$cluster_id %in% introns$cluster_id,],
                        .id = "in_trifid" )
nrow(all_introns)
```

```
## [1] 132587
```

```
length(unique(all_introns$intron_coords))
```

```
## [1] 132587
```

```
table(all_introns$in_trifid)
```

```
##
## cluster_not_in_trifid             in_trifid         not_in_trifid
##                 11293                 87024                 34270
```

```
table(filter(all_introns, p.adjust < 0.05 & abs(deltapsi) > 0.1 & annotation %in% c("gencode","refseq"))
```

```
##
## cluster_not_in_trifid             in_trifid         not_in_trifid
##                    74                   577                    29
```

Gencode + Refseq = 647 + 33 = 680 junctions; of which 577 are in trifid; 29 are not in trifid; and 74 of which none of the junctions in the cluster are in trifid, e.g.

```
filter(all_introns, in_trifid == "cluster_not_in_trifid"& p.adjust < 0.05 & abs(deltapsi) > 0.1) %>%
  arrange(gene) %>% pull(gene) %>% unique()
```

```
##  [1] "AC002074.1"            "AC002467.1"            "AC004889.1"
##  [4] "AC006001.3"            "AC008771.1"            "AC016924.1"
##  [7] "AC021739.2"            "AC022167.2"            "AC025171.1"
## [10] "AC078883.1"            "AC093724.3"            "AC138207.8"
## [13] "AC244154.1"            "AC244669.2"            "AF165147.1"
## [16] "AL451165.2"            "C2orf27A"              "CATG00000105473.1"
## [19] "ENSG00000124003.9"     "ENSG00000174804.3"     "ENSG00000188185.7"
## [22] "ENSG00000188681.7"     "ENSG00000228063.1"     "ENSG00000228782.3"
## [25] "ENSG00000229043.2"     "ENSG00000229180.4"     "ENSG00000246090.2"
## [28] "ENSG00000249042.1"     "ENSG00000272622.1"     "FAHD2CP"
## [31] "FAM66B"                "FRG1HP"                "GABPB1-AS1"
## [34] "GCC2-AS1"              "GPAT2P1"               "HAND2-AS1"
## [37] "HCG18"                 "HSD11B1-AS1"           "ID2-AS1"
## [40] "KCNK15-AS1"            "KCNK15-AS1,AL139352.1" "KTN1-AS1"
## [43] "LINC-PINT"             "LINC00847"             "LINC00886"
## [46] "LINC01119"             "LINC01239"             "LINC01347"
```

```
## [49] "LINC01547"             "LINC02202"             "LINC02607"
## [52] "LINC02749"             "LOC100130027"          "LOC105375587"
## [55] "LOC105379814"          "LOC128966744"          "LYPLAL1-DT"
## [58] "MEG8"                  "MIR31HG"               "MIR99AHG"
## [61] "MRPL45P2"              "NDUFS2"                "PCOTH"
## [64] "PI4KAP2"               "PKD1P5"                "RPARP-AS1"
## [67] "SDCBP2-AS1"            "SNHG10"                "TP73-AS1"
## [70] "WASH8P"                "ZEB1-AS1"              "ZNF583,ZNF582-AS1"
## [73] NA
```

```
filter(all_introns, p.adjust < 0.05 & abs(deltapsi) > 0.1 & annotation %in% c("gencode","refseq")) %>%
  pull(cluster_id) %>% unique() %>% length()
```

```
## [1] 470
```

```
filter(all_introns, p.adjust < 0.05 & abs(deltapsi) > 0.1 & annotation %in% c("gencode","refseq") & in_
  pull(cluster_id) %>% unique() %>% length()
```

```
## [1] 58
```

```
filter(all_introns, p.adjust < 0.05 & abs(deltapsi) > 0.1 & annotation %in% c("gencode","refseq") & in_
  pull(cluster_id) %>% unique() %>% length()
```

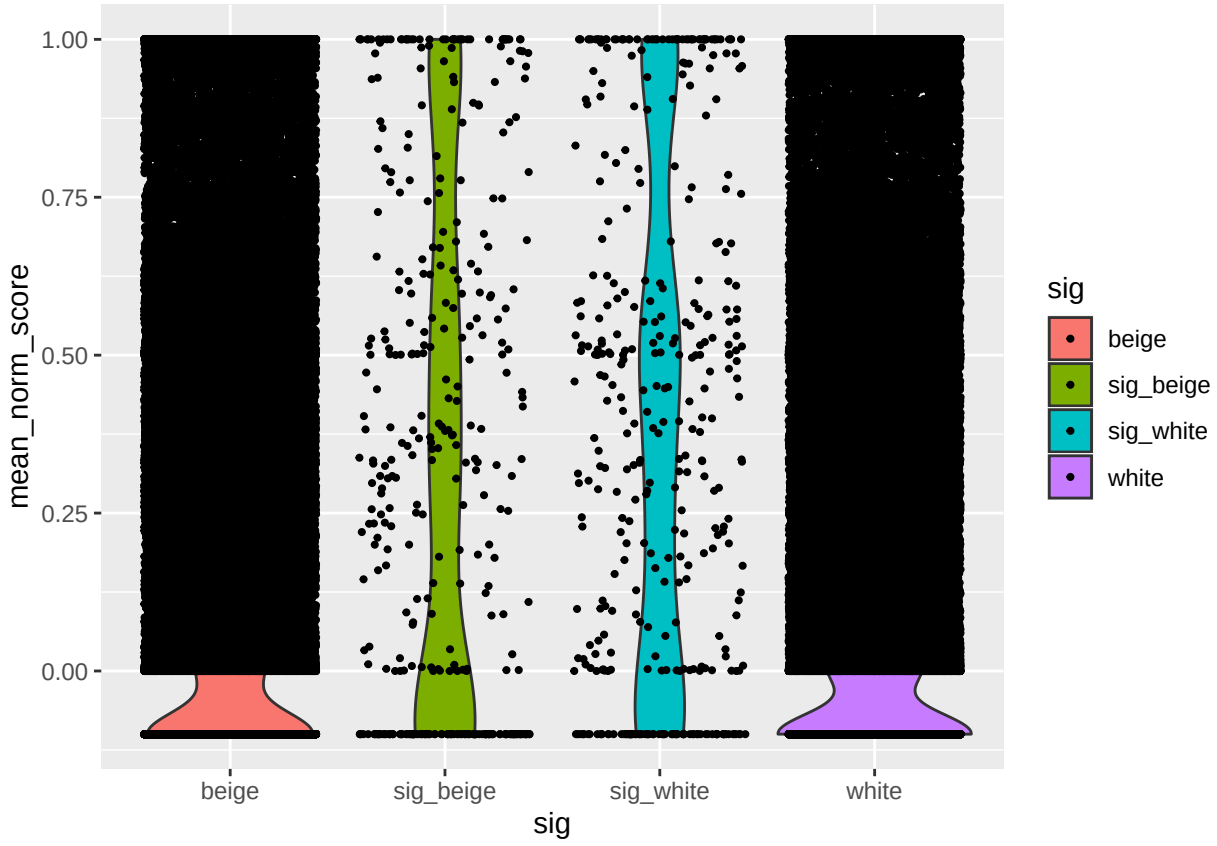
```
## [1] 412
```

Violin plots on *average* TRIFID score

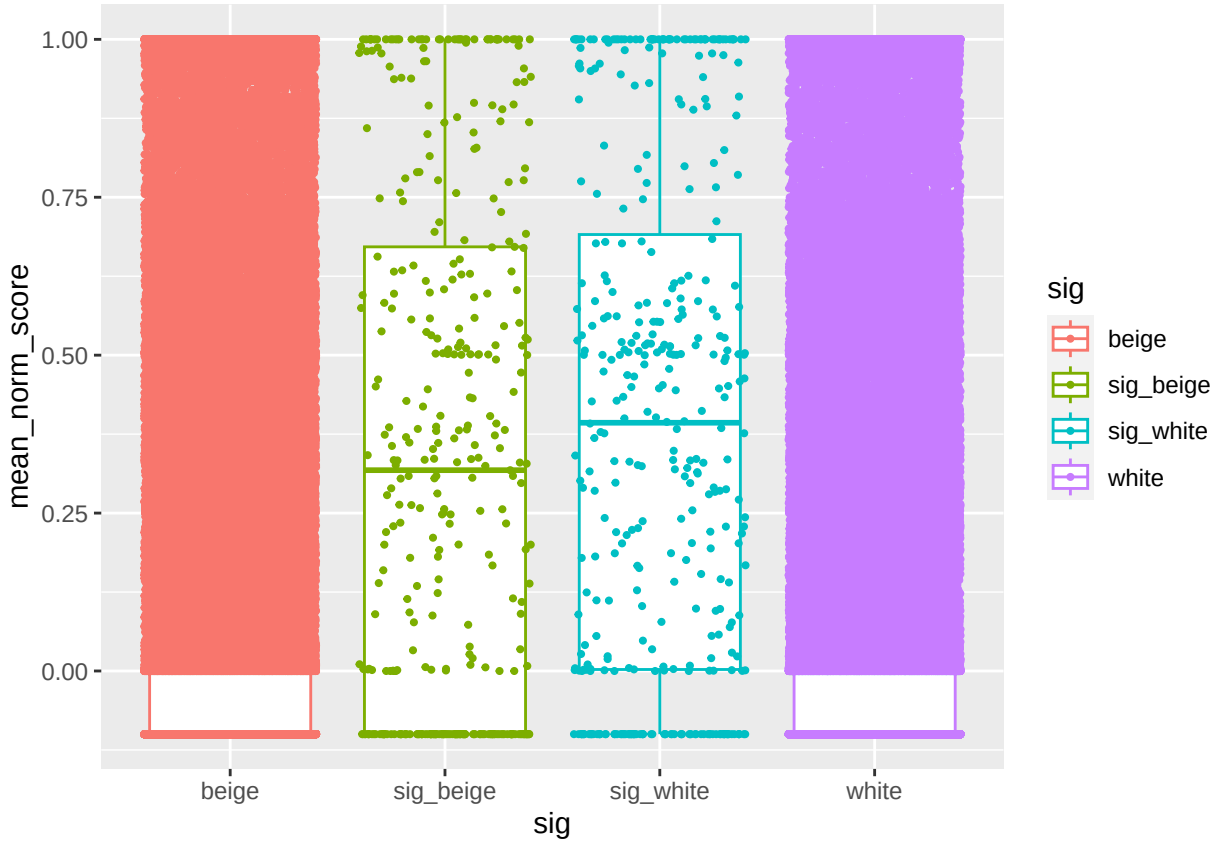
```
all_introns =mutate(all_introns, sig = if_else(p.adjust < 0.05 & deltapsi > 0.1, "sig_beige",
                                    if_else(p.adjust < 0.05 & deltapsi < -0.1, "sig_white",
                                                  if_else(deltapsi > 0, "beige",
                                                          if_else(deltapsi < -0, "white", "neither
table(all_introns$sig)
```

```
##
##     beige sig_beige sig_white     white
##     63426       385       392     68384
```

```
ggplot(all_introns, aes(x=sig, y=mean_norm_score, fill=sig)) + geom_violin() +
    geom_jitter(size=0.75)
```




```
ggplot(all_introns, aes(x= sig,  y=mean_norm_score,colour=sig)) + geom_boxplot() +
    geom_jitter(size=0.75)
```



The non-averaged score would be appropriate if the number of transcripts per intron was similar between the groups. Trifid scores (more appropriate if we're comparing across gene instead of pairwise between within each gene); has the average score HIGHER in white than beige. also a nice validation of the psi threshold.

```
all_introns[grepl("NDUF", all_introns$gene) & all_introns$p.adjust < 0.05 & abs(all_introns$deltapsi)>0
```

```
## # A tibble: 4 x 14
## # Groups:   intron_coords, condition, deltapsi, p.adjust, gene, cluster_id [4]
##   in_trifid           intron_coords condition deltapsi p.adjust gene  cluster_id
##   <chr>               <chr>         <chr>        <dbl>    <dbl> <chr> <chr>
## 1 in_trifid           chr11:475657~ white       -0.103  8.65e-5 NDUF~ clu_38578~
## 2 in_trifid           chr20:138089~ beige        0.103  1.01e-4 NDUF~ clu_6504_+
## 3 cluster_not_in_tri~ chr1:1611971~ beige        0.136  5.55e-8 NDUF~ clu_14507~
## 4 cluster_not_in_tri~ chr1:1611974~ white       -0.136  5.55e-8 NDUF~ clu_14507~
## # i 7 more variables: annotation <chr>, mean_trifid_score <dbl>,
## #   mean_norm_score <dbl>, median_norm_score <dbl>, transcripts <chr>,
## #   transcript_names <chr>, sig <chr>
```

```
write.table(all_introns, here("31_leafcutter/trifid_all_introns.tsv"), sep="\t",quote=F, row.names = F)
```

Select significant introns + alt introns

```
alt_introns = read.delim(here("31_leafcutter/alt_introns_195.tsv"))
alt_introns = unite(alt_introns, "intron_coords", chr, start, end, strand, sep = ":")
sig_junctions = filter(all_introns, (p.adjust < 0.05 & abs(deltapsi) > 0.1 ) |
                                    intron_coords %in% alt_introns$intron_coords) %>% arrange(p.adjust)
nrow(sig_junctions)
```

```
## [1] 1009
```

```
table(sig_junctions$sig)
```

```
##
##     beige sig_beige sig_white     white
##       118       385       392       114
```

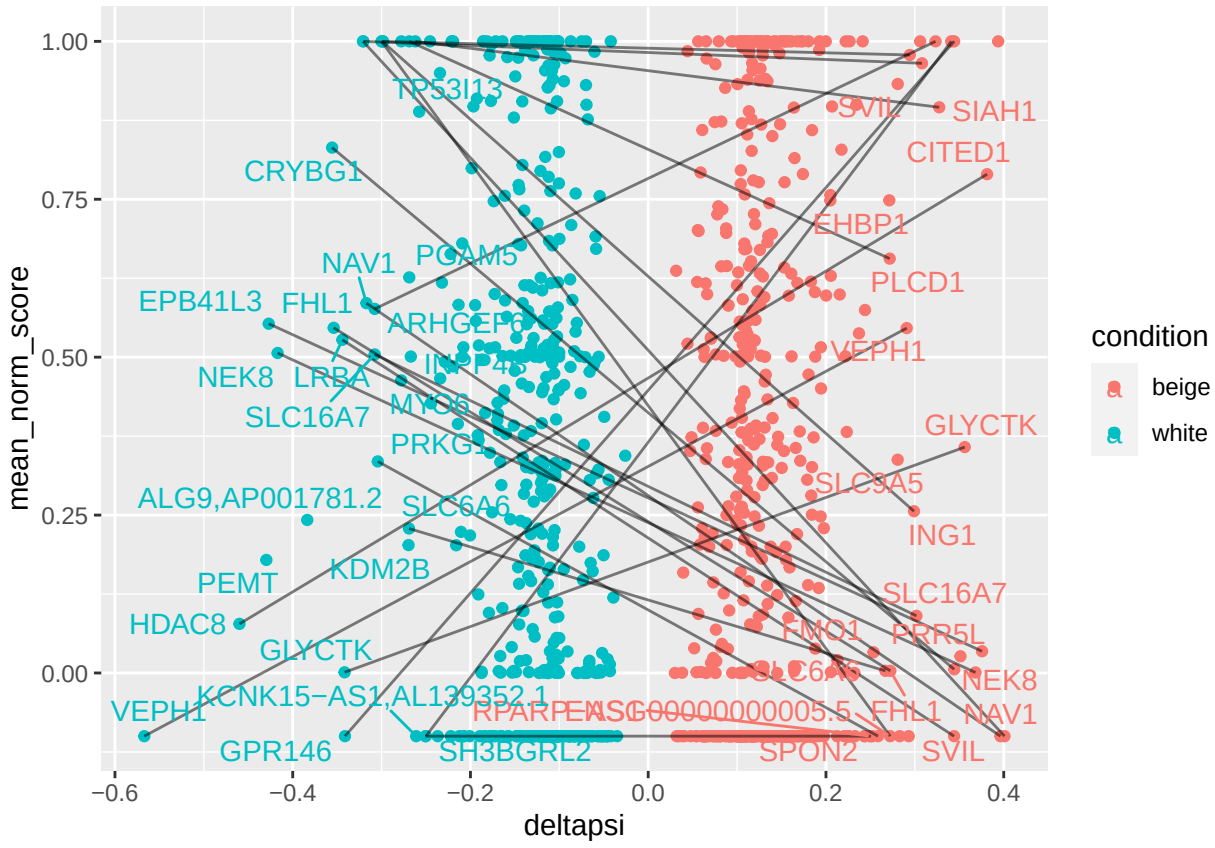
```
write.table(sig_junctions, here("31_leafcutter/trifid_with_alt_introns.tsv"), sep="\t",quote=F, row.nam
```

Plot the TRIFID difference

```
ggplot(sig_junctions, aes(colour=condition, y=mean_norm_score, x=deltapsi)) + geom_jitter() +
    geom_line(data=filter(sig_junctions, abs(deltapsi) > 0.25), aes(group=cluster_id), colour="black", a
    geom_text_repel(data=filter(sig_junctions, abs(deltapsi) > 0.25), aes(label=gene))
```

```
## Warning: Removed 5 rows containing missing values (`geom_text_repel()`).
```

```
## Warning: ggrepel: 18 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



Calculate the TRIFID difference!!

```
#if three introns are signifciant for a cluster
#average TRIFID score between the two introns in the same direction

paste_uq = function(x){
    return = paste(unique(x), collapse=",")
}
nrow(filter(sig_junctions, in_trifid != "cluster_not_in_trifid"))
```

```
## [1] 853
```

```
mean_of_3s = filter(sig_junctions, in_trifid != "cluster_not_in_trifid") %>%
    group_by(cluster_id, condition, p.adjust) %>% summarise(mean_norm_score = mean(mean_norm_score),
                                                            deltapsi = mean(deltapsi),
                                                            gene = paste(unique(gene), collapse=","
                                                            transcript_names= paste(unique(transcri
```

```
## `summarise()` has grouped output by 'cluster_id', 'condition'. You can override
## using the `.groups` argument.
```

```
head(mean_of_3s)
```

```
## # A tibble: 6 x 7
## # Groups:   cluster_id, condition [6]
##   cluster_id  condition p.adjust mean_norm_score deltapsi gene  transcript_names
##   <chr>       <chr>        <dbl>           <dbl>    <dbl> <chr> <chr>
## 1 clu_10104_- beige     1.44e- 2           1        0.0562 DENN~ DENND1A-203
## 2 clu_10104_- white     1.44e- 2           0.316   -0.108  DENN~ DENND1A-202,DEN~
## 3 clu_10181_- beige     8.76e-15           0.513    0.0625 GOLG~ GOLGA2-214,GOLG~
## 4 clu_10181_- white     8.76e-15           0       -0.113  GOLG~ GOLGA2-203
## 5 clu_10209_- beige     2.10e- 8           0.502    0.107  CRAT  CRAT-201,CRAT-2~
## 6 clu_10209_- white     2.10e- 8           0.0049  -0.0449 CRAT  CRAT-207
```

```
nrow(mean_of_3s)#15 clusters must be averaged
```

```
## [1] 838
```

```
#filter(mean_of_3s, is.na(deltapsi))

trifid_diff = pivot_wider(mean_of_3s, names_from = condition, values_from = c(deltapsi, mean_norm_score
                          id_cols=c("cluster_id", "p.adjust"))
head(trifid_diff); nrow(trifid_diff)#420 clusters -
```

```
## # A tibble: 6 x 8
## # Groups:   cluster_id [6]
##   cluster_id  p.adjust deltapsi_beige deltapsi_white mean_norm_score_beige
##   <chr>          <dbl>          <dbl>          <dbl>                 <dbl>
## 1 clu_10104_- 1.44e- 2         0.0562        -0.108                  1
## 2 clu_10181_- 8.76e-15         0.0625        -0.113                  0.513
## 3 clu_10209_- 2.10e- 8         0.107         -0.0449                 0.502
## 4 clu_10638_+ 1.87e- 2         0.106         -0.122                  0.551
## 5 clu_10654_+ 1.08e- 7         0.192         -0.192                 -0.1
## 6 clu_10672_+ 1.39e-62         0.241         -0.246                  1
## # i 3 more variables: mean_norm_score_white <dbl>, gene_beige <chr>,
## #   gene_white <chr>
```

```
## [1] 420
```

```
#should be gencode + refseq - cluster_not_in_trifid
#470  - 58 + alt_genocde/refseq 8 = 420
```

```
## If your other intron is not in trifid speak now
## deltapsi we should have from the other table, just the trifid score we could set to -1
filter(trifid_diff, is.na(deltapsi_beige ) | is.na(deltapsi_white )) # just 2 with an intron pair missi
```

```
## # A tibble: 2 x 8
## # Groups:   cluster_id [2]
##   cluster_id  p.adjust deltapsi_beige deltapsi_white mean_norm_score_beige
##   <chr>          <dbl>          <dbl>          <dbl>                 <dbl>
## 1 clu_30508_+ 2.04e-34          0.149         NA                      -0.1
## 2 clu_3320_-  1.58e-16         NA             -0.104                  NA
## # i 3 more variables: mean_norm_score_white <dbl>, gene_beige <chr>,
## #   gene_white <chr>
```

```
sig_junctions[sig_junctions$cluster_id %in%c("clu_30508_+","clu_3320_-"),] #beacuse the deltapsi goes i
```

```
## # A tibble: 4 x 14
## # Groups:   intron_coords, condition, deltapsi, p.adjust, gene, cluster_id [4]
##   in_trifid     intron_coords       condition deltapsi p.adjust gene  cluster_id
##   <chr>         <chr>               <chr>        <dbl>    <dbl> <chr> <chr>
## 1 not_in_trifid chr15:62570849:625~ beige       0.0540 2.04e-34 <NA>  clu_30508~
## 2 not_in_trifid chr15:62570852:625~ beige       0.243  2.04e-34 <NA>  clu_30508~
## 3 in_trifid     chr3:12941865:1296~ white      -0.147  1.58e-16 IQSE~ clu_3320_-
## 4 in_trifid     chr3:12941865:1302~ white      -0.0618 1.58e-16 IQSE~ clu_3320_-
## # i 7 more variables: annotation <chr>, mean_trifid_score <dbl>,
## #   mean_norm_score <dbl>, median_norm_score <dbl>, transcripts <chr>,
## #   transcript_names <chr>, sig <chr>
```

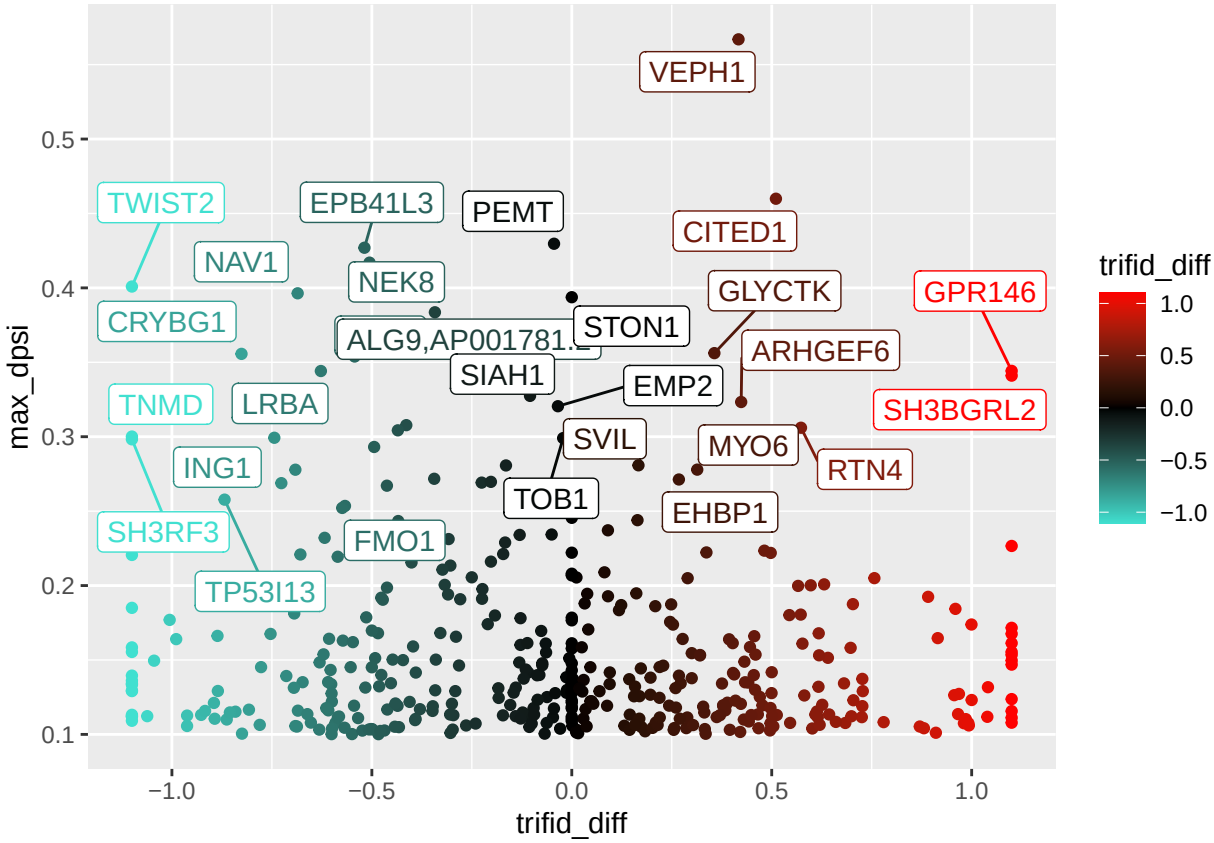
```
trifid_diff = filter(trifid_diff,!is.na(deltapsi_beige ) & !is.na(deltapsi_white ))
```

```
trifid_diff = mutate(trifid_diff, trifid_diff = mean_norm_score_beige-mean_norm_score_white, max_dpsi =
                     gene_to_plot = if_else(trifid_diff > 0, gene_beige, gene_white))
head(trifid_diff)
```

```
## # A tibble: 6 x 11
## # Groups:   cluster_id [6]
##   cluster_id  p.adjust deltapsi_beige deltapsi_white mean_norm_score_beige
##   <chr>          <dbl>          <dbl>          <dbl>                 <dbl>
## 1 clu_10104_- 1.44e- 2         0.0562        -0.108                  1
## 2 clu_10181_- 8.76e-15         0.0625        -0.113                  0.513
## 3 clu_10209_- 2.10e- 8         0.107         -0.0449                 0.502
## 4 clu_10638_+ 1.87e- 2         0.106         -0.122                  0.551
## 5 clu_10654_+ 1.08e- 7         0.192         -0.192                 -0.1
## 6 clu_10672_+ 1.39e-62         0.241         -0.246                  1
## # i 6 more variables: mean_norm_score_white <dbl>, gene_beige <chr>,
## #   gene_white <chr>, trifid_diff <dbl>, max_dpsi <dbl>, gene_to_plot <chr>
```

```
figs = here("R/plots")
ggplot(trifid_diff, aes(y=max_dpsi, x=trifid_diff, colour=trifid_diff)) + geom_point() +
    geom_label_repel(data=filter(trifid_diff, max_dpsi > 0.25), aes(label=gene_to_plot)) +
    scale_color_gradient2(low="turquoise", mid="black", high="red")
```

```
## Warning: ggrepel: 11 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



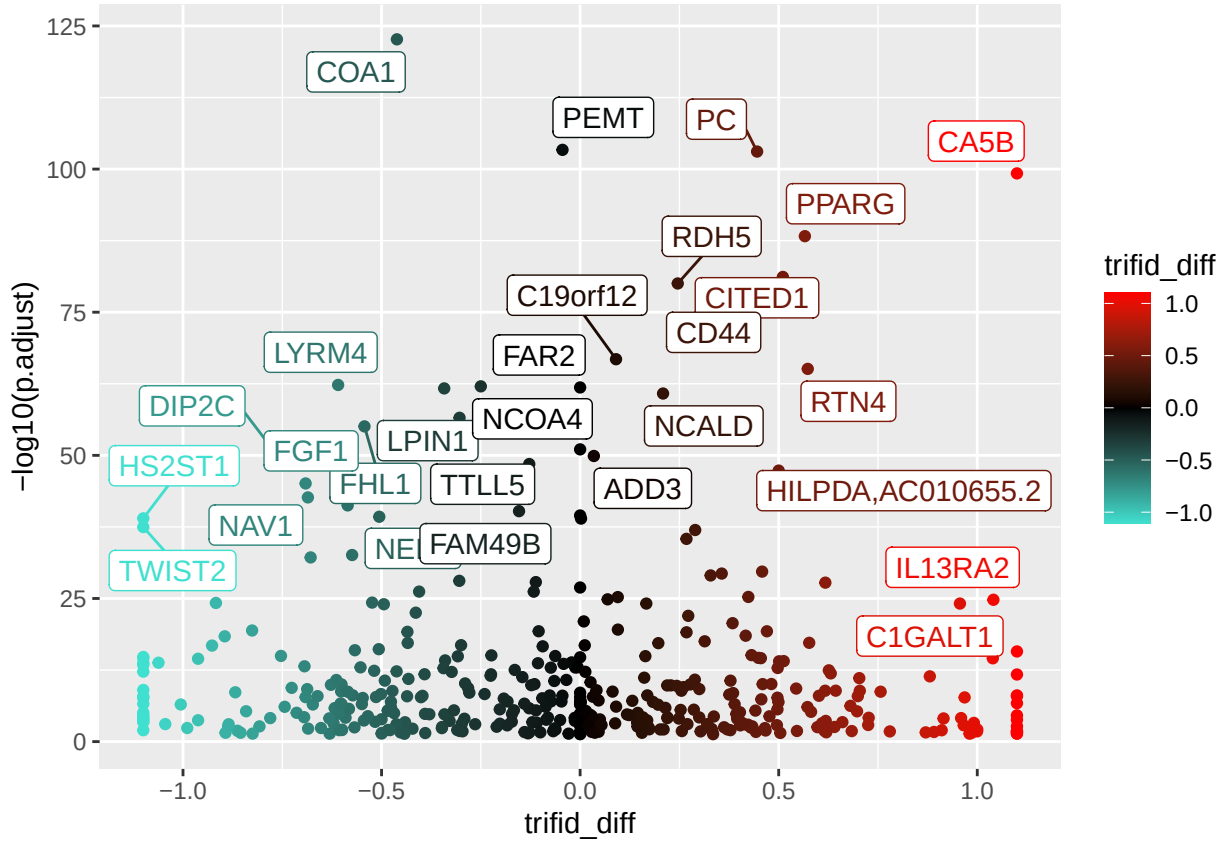

```
ggsave(file.path(figs, "trifid_difference_v_dpsi.pdf"))
```

```
## Saving 6.5 x 4.5 in image
```

```
## Warning: ggrepel: 11 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```

```
ggplot(trifid_diff, aes(y=-log10(p.adjust), x=trifid_diff, colour=trifid_diff)) + geom_point() +
    geom_label_repel(data=filter(trifid_diff, p.adjust < 0.01), aes(label=gene_to_plot)) +
    scale_color_gradient2(low="turquoise", mid="black", high="red")
```

```
## Warning: ggrepel: 336 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```




```
ggsave(file.path(figs, "trifid_difference_v_pvalue.pdf"))
```

```
## Saving 6.5 x 4.5 in image
```

```
## Warning: ggrepel: 336 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```

```
write.table(trifid_diff, here("31_leafcutter/trifid_DIFFERENCE_with_Alt_introns.tsv"), sep="\t",quote=F
```

GSEA

Setup

```
molsig <- clusterProfiler::read.gmt(here("annotations","msigdb.v2023.1.Hs.symbols.gmt"))
head(molsig); nrow(molsig)
```

```
##      term       gene
## 1 chr1p11  LINC02798
## 2 chr1p11    MTIF2P1
## 3 chr1p11    SRGAP2C
## 4 chr1p11 SRGAP2-AS1
## 5 chr1p11  LINC01691
## 6 chr1p11     NBPF26
```

```
## [1] 3961711
```

```
prefixes = c("HALLMARK", "KEGG","REACTOME", "WP", "GOBP", "GOCC","GOMF")
colnames(molsig) = c("term","gene")
some.molsig = molsig[gsub("_.*","", molsig$term) %in% prefixes,]
some.molsig$term = factor(some.molsig$term)
table(gsub("_.*","", some.molsig$term))
```

```
##
##     GOBP     GOCC     GOMF HALLMARK     KEGG REACTOME       WP
##   642656    98915   108833     7322    12796    92769    31635
```

```
rm(molsig)

shorten = function(ont) {
  abbreviate(gsub("_"," ", tolower(ont)),minlength=40, dot=T, named = F)
}
```

```
genelist = trifid_diff$trifid_diff
names(genelist) = trifid_diff$gene_to_plot
genelist = genelist[order(genelist, decreasing = T)]
length(genelist)
```

```
## [1] 418
```

```
summary(genelist)
```

```
##     Min.  1st Qu.   Median     Mean  3rd Qu.     Max.
## -1.10000 -0.46190  0.00000 -0.02781  0.37770  1.10000
```

```
gse = GSEA(genelist, TERM2GENE= some.molsig, pvalueCutoff=1)
```

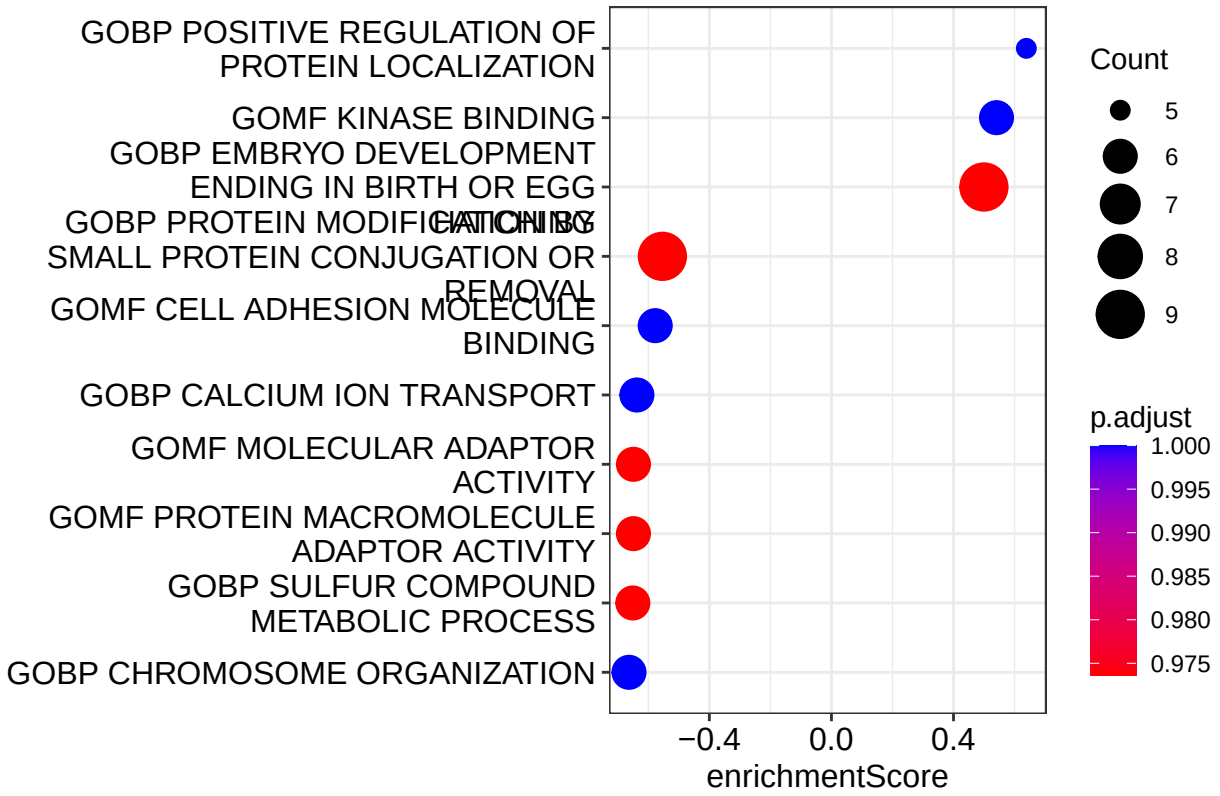
```
## preparing geneSet collections...

## GSEA analysis...

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize,
## gseaParam, : There are duplicate gene names, fgsea may produce unexpected
## results.

## leading edge analysis...

## done...
```

```
head(gse)
```

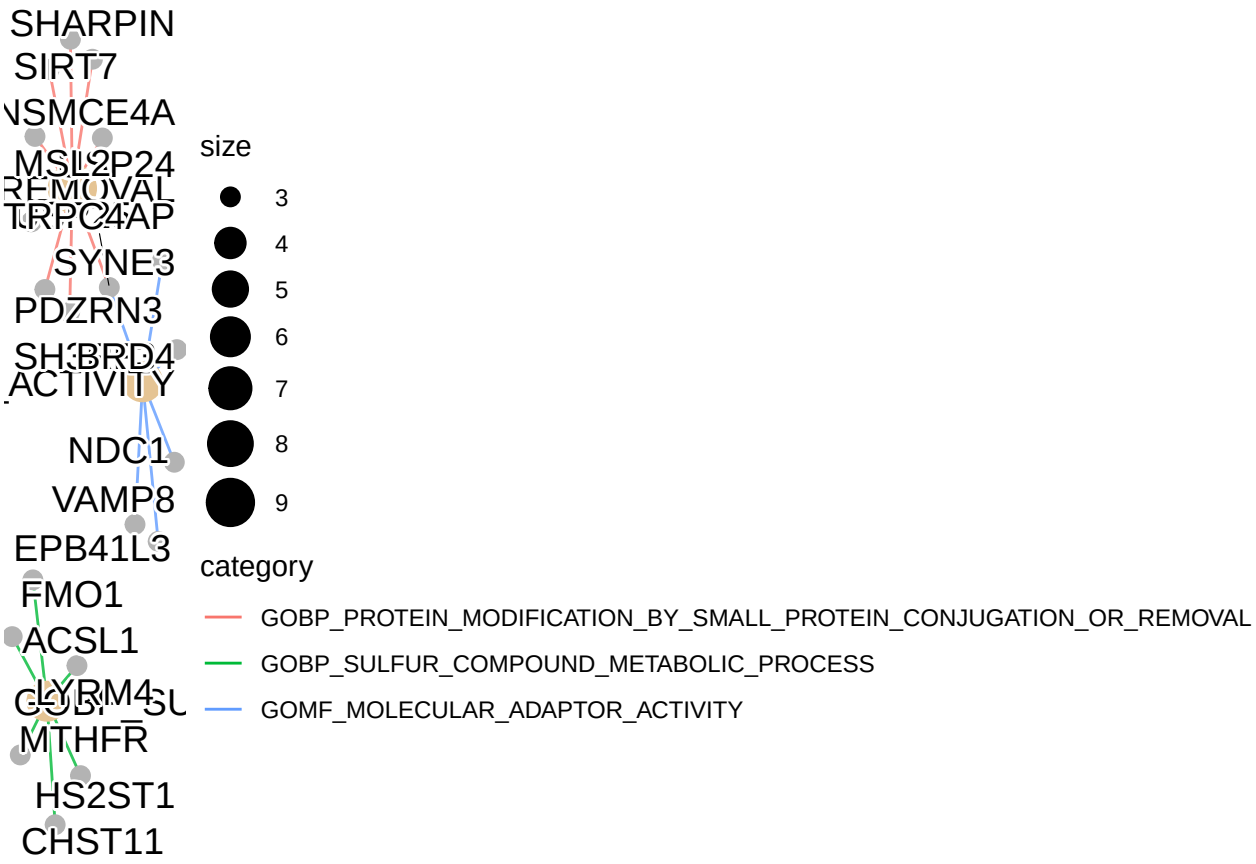
```
##
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL GOBP_PROTEIN_MODIFICATION_BY_SMALL_
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                                                   GOBP_SU
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                                                 G
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                                           GOMF_PROTEIN_
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING                    GOBP_EMBRYO_DEVELOPMENT_I
## GOBP_CHROMOSOME_ORGANIZATION
##
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL GOBP_PROTEIN_MODIFICATION_BY_SMALL_
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                                                   GOBP_SU
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                                                 G
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                                           GOMF_PROTEIN_
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING                    GOBP_EMBRYO_DEVELOPMENT_I
## GOBP_CHROMOSOME_ORGANIZATION
##                                                                  setSize
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL      21
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                                 11
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                        11
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                            11
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING                24
## GOBP_CHROMOSOME_ORGANIZATION                                           10
##                                                                  enrichmentScore
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL      -0.5537734
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                                 -0.6505769
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                        -0.6485722
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                            -0.6485722
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING                 0.4994379
## GOBP_CHROMOSOME_ORGANIZATION                                           -0.6631608
##                                                                         NES
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL -1.693164
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                             -1.663746
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                    -1.658620
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                        -1.658620
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING             1.609796
## GOBP_CHROMOSOME_ORGANIZATION                                       -1.657997
##                                                                       pvalue
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL 0.006894778
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                             0.009356522
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                    0.010188199
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                        0.010188199
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING            0.010794148
## GOBP_CHROMOSOME_ORGANIZATION                                       0.019078419
##                                                                     p.adjust
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL 0.9736322
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                             0.9736322
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                    0.9736322
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                        0.9736322
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING            0.9736322
## GOBP_CHROMOSOME_ORGANIZATION                                       1.0000000
##                                                                        qvalue
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL 0.9736322
```

```
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                              0.9736322
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                     0.9736322
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                         0.9736322
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING             0.9736322
## GOBP_CHROMOSOME_ORGANIZATION                                        1.0000000
##                                                                        rank
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL        73
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                                   85
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                          87
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                              87
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING                  66
## GOBP_CHROMOSOME_ORGANIZATION                                             63
##                                                                                leading_edge
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL tags=43%, list=17%, signal=37%
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                            tags=55%, list=20%, signal=45%
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                   tags=55%, list=21%, signal=44%
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                       tags=55%, list=21%, signal=44%
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING           tags=38%, list=16%, signal=34%
## GOBP_CHROMOSOME_ORGANIZATION                                      tags=60%, list=15%, signal=52%
##
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL USP25/SHARPIN/NSMCE4A/SIRT7/TRPC4AI
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                                                    ACSL1/FMO1/
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                                          VAMP8/EPB41I
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                                              VAMP8/EPB41I
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING                  DEAF1/HOXB4/HOXB5/SMAD3/STK3/I
## GOBP_CHROMOSOME_ORGANIZATION                                                          NSMCE4A/TNKS1BI
```

```
dotplot(gse, showCategory = 10, x="enrichmentScore")
```




```
cnetplot(gse, showCategory=3, categorySize="pvalue", color.params = list(edge=T))
```




```
to_print = gse[,c("ID","setSize", "enrichmentScore"  ,"NES",
                  "pvalue"    ,  "p.adjust" ,   "qvalue"    ,  "core_enrichment"    ,   "rank"  )]
write.table(to_print, here("31_leafcutter","TRIFID_GSEA.txt"))
```

```
custom_ora_to_df = function(res, annot=NULL, other_cols=NULL){
    res_df = res[,c(other_cols,"ID","setSize", "enrichmentScore"  ,"NES",
                  "pvalue"    ,  "p.adjust" ,   "qvalue"    ,  "core_enrichment"    ,   "rank" )]

    print(dim(res_df))
    if (length(annot)>1){
        res_df = merge(res_df, annot, by.x="ID", by.y="term", sort=F)
    }

    res_df = res_df[order(res_df$p.adjust),]
    return(res_df)
}
```

```
sep_go = list()
for (db in prefixes){
    print(db)
    t2g = some.molsig[grep(db, some.molsig$term),]
    ea = GSEA(genelist, TERM2GENE = t2g, pvalueCutoff = 1,minGSSize = 3)
    df = custom_ora_to_df(ea)
    print(head(df[2:8], n=10))
    sep_go[[db]] = ea
```

```
    #print(dotplot(ea, showCategory=20) +ggtitle(db))
    #print(cnetplot(ea, geneSetID = 1:5) + ggtitle(db))
}
```

```
## [1] "HALLMARK"
```

```
## preparing geneSet collections...
```

```
## GSEA analysis...
```

```
## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.
```

```
## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize,
## gseaParam, : There are duplicate gene names, fgsea may produce unexpected
## results.
```

```
## leading edge analysis...
```

```
## done...
```

```
## [1] 29  9
##                                    setSize enrichmentScore       NES     pvalue
## HALLMARK_IL2_STAT5_SIGNALING             5       0.7267555  1.521130 0.06263048
## HALLMARK_COAGULATION                     3       0.8506621  1.455949 0.05150157
## HALLMARK_HEME_METABOLISM                 8       0.5646877  1.382490 0.12240664
## HALLMARK_TGF_BETA_SIGNALING              4       0.7008750  1.349452 0.14669421
## HALLMARK_ANDROGEN_RESPONSE               6      -0.6259678 -1.335228 0.16037736
## HALLMARK_INTERFERON_GAMMA_RESPONSE       3      -0.7579490 -1.287487 0.16165414
## HALLMARK_MTORC1_SIGNALING                3      -0.7318584 -1.243168 0.20864662
## HALLMARK_SPERMATOGENESIS                 4       0.5853886  1.127097 0.34710744
## HALLMARK_ESTROGEN_RESPONSE_LATE          9      -0.4464292 -1.083629 0.36363636
## HALLMARK_UV_RESPONSE_DN                  7       0.4606469  1.077023 0.35073069
##                                     p.adjust    qvalue       core_enrichment
## HALLMARK_IL2_STAT5_SIGNALING       0.7813283 0.7813283        SH3BGRL2/MYO1C
## HALLMARK_COAGULATION               0.7813283 0.7813283                PECAM1
## HALLMARK_HEME_METABOLISM           0.7813283 0.7813283    PC/TNRC6B/SLC25A37
## HALLMARK_TGF_BETA_SIGNALING        0.7813283 0.7813283                 SMAD3
## HALLMARK_ANDROGEN_RESPONSE         0.7813283 0.7813283   TNFAIP8/INPP4B/PGM3
## HALLMARK_INTERFERON_GAMMA_RESPONSE 0.7813283 0.7813283           VAMP8/PTPN1
## HALLMARK_MTORC1_SIGNALING          0.8643931 0.8643931         SLC6A6/SLC1A4
## HALLMARK_SPERMATOGENESIS           0.9918281 0.9918281               IL13RA2
## HALLMARK_ESTROGEN_RESPONSE_LATE    0.9918281 0.9918281     AFF1/LAMC2/SLC1A4
## HALLMARK_UV_RESPONSE_DN            0.9918281 0.9918281 SMAD3/PPARG/PMP22/DBP
## [1] "KEGG"
```

```
## preparing geneSet collections...
```

```
## GSEA analysis...
```

```
## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are duplica

## leading edge analysis...

## done...

## [1] 19  9
##                                          setSize enrichmentScore        NES
## KEGG_AXON_GUIDANCE                             3       0.7769543  1.3135487
## KEGG_WNT_SIGNALING_PATHWAY                     5       0.6065796  1.2333976
## KEGG_PANCREATIC_CANCER                         3       0.6467982  1.0935018
## KEGG_INOSITOL_PHOSPHATE_METABOLISM             4      -0.5676856 -1.0634712
## KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM     4      -0.5676856 -1.0634712
## KEGG_FOCAL_ADHESION                            3      -0.6205295 -1.0418614
## KEGG_CARDIAC_MUSCLE_CONTRACTION                3      -0.6154223 -1.0332865
## KEGG_PPAR_SIGNALING_PATHWAY                    4       0.4922018  0.9235202
## KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS         3      -0.5381482 -0.9035442
## KEGG_PATHWAYS_IN_CANCER                        9       0.3552171  0.8851995
##                                            pvalue  p.adjust    qvalue
## KEGG_AXON_GUIDANCE                       0.1609658 0.9336016 0.9336016
## KEGG_WNT_SIGNALING_PATHWAY               0.2439024 0.9336016 0.9336016
## KEGG_PANCREATIC_CANCER                   0.4205231 0.9336016 0.9336016
## KEGG_INOSITOL_PHOSPHATE_METABOLISM       0.4256619 0.9336016 0.9336016
## KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM 0.4256619 0.9336016 0.9336016
## KEGG_FOCAL_ADHESION                      0.4792899 0.9336016 0.9336016
## KEGG_CARDIAC_MUSCLE_CONTRACTION          0.4970414 0.9336016 0.9336016
## KEGG_PPAR_SIGNALING_PATHWAY              0.5527344 0.9336016 0.9336016
## KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS   0.6469428 0.9336016 0.9336016
## KEGG_PATHWAYS_IN_CANCER                  0.5942982 0.9336016 0.9336016
##                                                core_enrichment
## KEGG_AXON_GUIDANCE                                        RGS3
## KEGG_WNT_SIGNALING_PATHWAY                          SMAD3/WNT5A
## KEGG_PANCREATIC_CANCER                             SMAD3/ARHGEF6
## KEGG_INOSITOL_PHOSPHATE_METABOLISM                PLCD1/INPP4B/PI4KA
## KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM        PLCD1/INPP4B/PI4KA
## KEGG_FOCAL_ADHESION                                     MYLK/LAMC2
## KEGG_CARDIAC_MUSCLE_CONTRACTION                   CACNA2D1/ATP1A2
## KEGG_PPAR_SIGNALING_PATHWAY                          PPARG/ACADL
## KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS              PPP3CC/TOMM40L
## KEGG_PATHWAYS_IN_CANCER                  SMAD3/STK36/PPARG/WNT5A/BID
## [1] "REACTOME"

## preparing geneSet collections...

## GSEA analysis...

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are duplica
```

```
## leading edge analysis...

## done...

## [1] 139   9
##                                                                setSize
## REACTOME_RAB_GEFS_EXCHANGE_GTP_FOR_GDP_ON_RABS                       5
## REACTOME_RAB_REGULATION_OF_TRAFFICKING                               5
## REACTOME_LEISHMANIA_INFECTION                                        4
## REACTOME_ESR_MEDIATED_SIGNALING                                      3
## REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION                          3
## REACTOME_SUMOYLATION_OF_DNA_DAMAGE_RESPONSE_AND_REPAIR_PROTEINS      3
## REACTOME_G_ALPHA_I_SIGNALLING_EVENTS                                 3
## REACTOME_SIGNALING_BY_NOTCH                                          4
## REACTOME_RHO_GTPASE_EFFECTORS                                        5
## REACTOME_INNATE_IMMUNE_SYSTEM                                       12
##                                                               enrichmentScore
## REACTOME_RAB_GEFS_EXCHANGE_GTP_FOR_GDP_ON_RABS                      0.7217667
## REACTOME_RAB_REGULATION_OF_TRAFFICKING                              0.7217667
## REACTOME_LEISHMANIA_INFECTION                                       0.7589379
## REACTOME_ESR_MEDIATED_SIGNALING                                     0.7952007
## REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION                         0.7952007
## REACTOME_SUMOYLATION_OF_DNA_DAMAGE_RESPONSE_AND_REPAIR_PROTEINS    -0.8265060
## REACTOME_G_ALPHA_I_SIGNALLING_EVENTS                                0.7800926
## REACTOME_SIGNALING_BY_NOTCH                                         0.7014041
## REACTOME_RHO_GTPASE_EFFECTORS                                      -0.6680017
## REACTOME_INNATE_IMMUNE_SYSTEM                                       0.5025444
##                                                                        NES
## REACTOME_RAB_GEFS_EXCHANGE_GTP_FOR_GDP_ON_RABS                    1.487766
## REACTOME_RAB_REGULATION_OF_TRAFFICKING                            1.487766
## REACTOME_LEISHMANIA_INFECTION                                     1.466259
## REACTOME_ESR_MEDIATED_SIGNALING                                   1.394049
## REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION                       1.394049
## REACTOME_SUMOYLATION_OF_DNA_DAMAGE_RESPONSE_AND_REPAIR_PROTEINS -1.393343
## REACTOME_G_ALPHA_I_SIGNALLING_EVENTS                              1.367564
## REACTOME_SIGNALING_BY_NOTCH                                       1.355104
## REACTOME_RHO_GTPASE_EFFECTORS                                    -1.344980
## REACTOME_INNATE_IMMUNE_SYSTEM                                     1.335202
##                                                                     pvalue
## REACTOME_RAB_GEFS_EXCHANGE_GTP_FOR_GDP_ON_RABS                  0.06666667
## REACTOME_RAB_REGULATION_OF_TRAFFICKING                          0.06666667
## REACTOME_LEISHMANIA_INFECTION                                   0.05761317
## REACTOME_ESR_MEDIATED_SIGNALING                                 0.09467456
## REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION                     0.09467456
## REACTOME_SUMOYLATION_OF_DNA_DAMAGE_RESPONSE_AND_REPAIR_PROTEINS 0.07815631
## REACTOME_G_ALPHA_I_SIGNALLING_EVENTS                            0.11439842
## REACTOME_SIGNALING_BY_NOTCH                                     0.15020576
## REACTOME_RHO_GTPASE_EFFECTORS                                   0.16412214
## REACTOME_INNATE_IMMUNE_SYSTEM                                   0.12793177
##                                                                  p.adjust
## REACTOME_RAB_GEFS_EXCHANGE_GTP_FOR_GDP_ON_RABS                  0.9922929
## REACTOME_RAB_REGULATION_OF_TRAFFICKING                          0.9922929
## REACTOME_LEISHMANIA_INFECTION                                   0.9922929
## REACTOME_ESR_MEDIATED_SIGNALING                                 0.9922929
```

```
## REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION                     0.9922929
## REACTOME_SUMOYLATION_OF_DNA_DAMAGE_RESPONSE_AND_REPAIR_PROTEINS 0.9922929
## REACTOME_G_ALPHA_I_SIGNALLING_EVENTS                            0.9922929
## REACTOME_SIGNALING_BY_NOTCH                                     0.9922929
## REACTOME_RHO_GTPASE_EFFECTORS                                   0.9922929
## REACTOME_INNATE_IMMUNE_SYSTEM                                   0.9922929
##                                                                    qvalue
## REACTOME_RAB_GEFS_EXCHANGE_GTP_FOR_GDP_ON_RABS                  0.9922929
## REACTOME_RAB_REGULATION_OF_TRAFFICKING                          0.9922929
## REACTOME_LEISHMANIA_INFECTION                                   0.9922929
## REACTOME_ESR_MEDIATED_SIGNALING                                 0.9922929
## REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION                     0.9922929
## REACTOME_SUMOYLATION_OF_DNA_DAMAGE_RESPONSE_AND_REPAIR_PROTEINS 0.9922929
## REACTOME_G_ALPHA_I_SIGNALLING_EVENTS                            0.9922929
## REACTOME_SIGNALING_BY_NOTCH                                     0.9922929
## REACTOME_RHO_GTPASE_EFFECTORS                                   0.9922929
## REACTOME_INNATE_IMMUNE_SYSTEM                                   0.9922929
##                                                                          core_enrichment
## REACTOME_RAB_GEFS_EXCHANGE_GTP_FOR_GDP_ON_RABS                          SBF2/SBF2/DENND1A
## REACTOME_RAB_REGULATION_OF_TRAFFICKING                                  SBF2/SBF2/DENND1A
## REACTOME_LEISHMANIA_INFECTION                                                MYO1C/WNT5A
## REACTOME_ESR_MEDIATED_SIGNALING                                           CITED1/TNRC6B
## REACTOME_ESTROGEN_DEPENDENT_GENE_EXPRESSION                               CITED1/TNRC6B
## REACTOME_SUMOYLATION_OF_DNA_DAMAGE_RESPONSE_AND_REPAIR_PROTEINS            NSMCE4A/NDC1
## REACTOME_G_ALPHA_I_SIGNALLING_EVENTS                                              RGS3
## REACTOME_SIGNALING_BY_NOTCH                                               SMAD3/TNRC6B
## REACTOME_RHO_GTPASE_EFFECTORS                                             CLASP2/FMNL2
## REACTOME_INNATE_IMMUNE_SYSTEM                                 PECAM1/MYO1C/ATAD3B/ATP8B4
## [1] "WP"

## preparing geneSet collections...

## GSEA analysis...

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are duplica

## leading edge analysis...

## done...

## [1] 58  9
##                                                                    setSize
## WP_FOCAL_ADHESION_PI3KAKTMTORSIGNALING_PATHWAY                           3
## WP_THYROID_HORMONES_PRODUCTION_AND_PERIPHERAL_DOWNSTREAM_SIGNALING_EFFECTS       3
## WP_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY                                   3
## WP_TGFBETA_SIGNALING_PATHWAY                                             3
## WP_ALZHEIMERS_DISEASE                                                    4
## WP_ALZHEIMERS_DISEASE_AND_MIRNA_EFFECTS                                  4
## WP_METAPATHWAY_BIOTRANSFORMATION_PHASE_I_AND_II                          4
```

```
## WP_GLYCEROLIPIDS_AND_GLYCEROPHOSPHOLIPIDS                                        3
## WP_MARKERS_OF_KIDNEY_CELL_LINEAGE                                                3
## WP_EXERCISEINDUCED_CIRCADIAN_REGULATION                                          3
##                                                                         enrichmentScore
## WP_FOCAL_ADHESION_PI3KAKTMTORSIGNALING_PATHWAY                               -0.8462425
## WP_THYROID_HORMONES_PRODUCTION_AND_PERIPHERAL_DOWNSTREAM_SIGNALING_EFFECTS    0.8578313
## WP_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY                                       0.8416832
## WP_TGFBETA_SIGNALING_PATHWAY                                                 0.8131015
## WP_ALZHEIMERS_DISEASE                                                        0.7173236
## WP_ALZHEIMERS_DISEASE_AND_MIRNA_EFFECTS                                      0.7173236
## WP_METAPATHWAY_BIOTRANSFORMATION_PHASE_I_AND_II                             -0.6974097
## WP_GLYCEROLIPIDS_AND_GLYCEROPHOSPHOLIPIDS                                    0.7880114
## WP_MARKERS_OF_KIDNEY_CELL_LINEAGE                                            0.7734940
## WP_EXERCISEINDUCED_CIRCADIAN_REGULATION                                      0.7571049
##                                                                                 NES
## WP_FOCAL_ADHESION_PI3KAKTMTORSIGNALING_PATHWAY                           -1.462773
## WP_THYROID_HORMONES_PRODUCTION_AND_PERIPHERAL_DOWNSTREAM_SIGNALING_EFFECTS  1.424712
## WP_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY                                   1.397892
## WP_TGFBETA_SIGNALING_PATHWAY                                             1.350423
## WP_ALZHEIMERS_DISEASE                                                    1.349358
## WP_ALZHEIMERS_DISEASE_AND_MIRNA_EFFECTS                                  1.349358
## WP_METAPATHWAY_BIOTRANSFORMATION_PHASE_I_AND_II                          -1.312544
## WP_GLYCEROLIPIDS_AND_GLYCEROPHOSPHOLIPIDS                                1.308753
## WP_MARKERS_OF_KIDNEY_CELL_LINEAGE                                        1.284642
## WP_EXERCISEINDUCED_CIRCADIAN_REGULATION                                  1.257422
##                                                                             pvalue
## WP_FOCAL_ADHESION_PI3KAKTMTORSIGNALING_PATHWAY                          0.04699248
## WP_THYROID_HORMONES_PRODUCTION_AND_PERIPHERAL_DOWNSTREAM_SIGNALING_EFFECTS 0.05732484
## WP_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY                                  0.07855626
## WP_TGFBETA_SIGNALING_PATHWAY                                            0.11464968
## WP_ALZHEIMERS_DISEASE                                                   0.14957265
## WP_ALZHEIMERS_DISEASE_AND_MIRNA_EFFECTS                                 0.14957265
## WP_METAPATHWAY_BIOTRANSFORMATION_PHASE_I_AND_II                         0.15140187
## WP_GLYCEROLIPIDS_AND_GLYCEROPHOSPHOLIPIDS                               0.15711253
## WP_MARKERS_OF_KIDNEY_CELL_LINEAGE                                       0.17197452
## WP_EXERCISEINDUCED_CIRCADIAN_REGULATION                                 0.20169851
##                                                                            p.adjust
## WP_FOCAL_ADHESION_PI3KAKTMTORSIGNALING_PATHWAY                          0.8939215
## WP_THYROID_HORMONES_PRODUCTION_AND_PERIPHERAL_DOWNSTREAM_SIGNALING_EFFECTS 0.8939215
## WP_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY                                  0.8939215
## WP_TGFBETA_SIGNALING_PATHWAY                                            0.8939215
## WP_ALZHEIMERS_DISEASE                                                   0.8939215
## WP_ALZHEIMERS_DISEASE_AND_MIRNA_EFFECTS                                 0.8939215
## WP_METAPATHWAY_BIOTRANSFORMATION_PHASE_I_AND_II                         0.8939215
## WP_GLYCEROLIPIDS_AND_GLYCEROPHOSPHOLIPIDS                               0.8939215
## WP_MARKERS_OF_KIDNEY_CELL_LINEAGE                                       0.8939215
## WP_EXERCISEINDUCED_CIRCADIAN_REGULATION                                 0.8939215
##                                                                               qvalue
## WP_FOCAL_ADHESION_PI3KAKTMTORSIGNALING_PATHWAY                          0.8939215
## WP_THYROID_HORMONES_PRODUCTION_AND_PERIPHERAL_DOWNSTREAM_SIGNALING_EFFECTS 0.8939215
## WP_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY                                  0.8939215
## WP_TGFBETA_SIGNALING_PATHWAY                                            0.8939215
## WP_ALZHEIMERS_DISEASE                                                   0.8939215
## WP_ALZHEIMERS_DISEASE_AND_MIRNA_EFFECTS                                 0.8939215
```

```
## WP_METAPATHWAY_BIOTRANSFORMATION_PHASE_I_AND_II                                0.8939215
## WP_GLYCEROLIPIDS_AND_GLYCEROPHOSPHOLIPIDS                                      0.8939215
## WP_MARKERS_OF_KIDNEY_CELL_LINEAGE                                              0.8939215
## WP_EXERCISEINDUCED_CIRCADIAN_REGULATION                                        0.8939215
##                                                                                    core_enrichment
## WP_FOCAL_ADHESION_PI3KAKTMTORSIGNALING_PATHWAY                                          FGF1/LAMC2
## WP_THYROID_HORMONES_PRODUCTION_AND_PERIPHERAL_DOWNSTREAM_SIGNALING_EFFECTS   PNPLA2/THRA/PPARG
## WP_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY                                              SMAD3
## WP_TGFBETA_SIGNALING_PATHWAY                                                  SMAD3/CITED1
## WP_ALZHEIMERS_DISEASE                                                        RTN4/WNT5A/BID
## WP_ALZHEIMERS_DISEASE_AND_MIRNA_EFFECTS                                      RTN4/WNT5A/BID
## WP_METAPATHWAY_BIOTRANSFORMATION_PHASE_I_AND_II                          FMO1/CHST11/HS2ST1
## WP_GLYCEROLIPIDS_AND_GLYCEROPHOSPHOLIPIDS                                        PNPLA2
## WP_MARKERS_OF_KIDNEY_CELL_LINEAGE                                       PECAM1/CITED1/WNT5A
## WP_EXERCISEINDUCED_CIRCADIAN_REGULATION                                             QKI
## [1] "GOBP"

## preparing geneSet collections...

## GSEA analysis...

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are duplica

## leading edge analysis...

## done...

## [1] 1245    9
##                                                                 setSize
## GOBP_REGULATION_OF_BINDING                                            8
## GOBP_EMBRYONIC_SKELETAL_SYSTEM_DEVELOPMENT                            8
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL    21
## GOBP_MICROTUBULE_BASED_MOVEMENT                                       6
## GOBP_POLYSACCHARIDE_METABOLIC_PROCESS                                 5
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                               11
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING              24
## GOBP_CHROMOSOME_ORGANIZATION                                         10
## GOBP_POSITIVE_REGULATION_OF_PROTEIN_LOCALIZATION                     10
## GOBP_CELLULAR_CARBOHYDRATE_BIOSYNTHETIC_PROCESS                       4
##                                                                 enrichmentScore
## GOBP_REGULATION_OF_BINDING                                            0.7549030
## GOBP_EMBRYONIC_SKELETAL_SYSTEM_DEVELOPMENT                            0.7156521
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL    -0.5537734
## GOBP_MICROTUBULE_BASED_MOVEMENT                                       0.7797329
## GOBP_POLYSACCHARIDE_METABOLIC_PROCESS                                -0.8425691
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                               -0.6505769
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING               0.4994379
## GOBP_CHROMOSOME_ORGANIZATION                                         -0.6631608
## GOBP_POSITIVE_REGULATION_OF_PROTEIN_LOCALIZATION                      0.6385118
```

```
## GOBP_CELLULAR_CARBOHYDRATE_BIOSYNTHETIC_PROCESS                        -0.8893981
##                                                                             NES
## GOBP_REGULATION_OF_BINDING                                           1.818013
## GOBP_EMBRYONIC_SKELETAL_SYSTEM_DEVELOPMENT                           1.723486
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL -1.710046
## GOBP_MICROTUBULE_BASED_MOVEMENT                                      1.690382
## GOBP_POLYSACCHARIDE_METABOLIC_PROCESS                               -1.676609
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                              -1.673363
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING              1.667341
## GOBP_CHROMOSOME_ORGANIZATION                                        -1.648814
## GOBP_POSITIVE_REGULATION_OF_PROTEIN_LOCALIZATION                     1.643650
## GOBP_CELLULAR_CARBOHYDRATE_BIOSYNTHETIC_PROCESS                     -1.640044
##                                                                         pvalue
## GOBP_REGULATION_OF_BINDING                                          0.007568377
## GOBP_EMBRYONIC_SKELETAL_SYSTEM_DEVELOPMENT                          0.015985173
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL 0.008769033
## GOBP_MICROTUBULE_BASED_MOVEMENT                                     0.011313685
## GOBP_POLYSACCHARIDE_METABOLIC_PROCESS                               0.008744482
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                              0.013029637
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING             0.015604858
## GOBP_CHROMOSOME_ORGANIZATION                                        0.019048574
## GOBP_POSITIVE_REGULATION_OF_PROTEIN_LOCALIZATION                    0.024665895
## GOBP_CELLULAR_CARBOHYDRATE_BIOSYNTHETIC_PROCESS                     0.007778057
##                                                                       p.adjust
## GOBP_REGULATION_OF_BINDING                                          0.9985691
## GOBP_EMBRYONIC_SKELETAL_SYSTEM_DEVELOPMENT                          0.9985691
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL 0.9985691
## GOBP_MICROTUBULE_BASED_MOVEMENT                                     0.9985691
## GOBP_POLYSACCHARIDE_METABOLIC_PROCESS                               0.9985691
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                              0.9985691
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING             0.9985691
## GOBP_CHROMOSOME_ORGANIZATION                                        0.9985691
## GOBP_POSITIVE_REGULATION_OF_PROTEIN_LOCALIZATION                    0.9985691
## GOBP_CELLULAR_CARBOHYDRATE_BIOSYNTHETIC_PROCESS                     0.9985691
##                                                                         qvalue
## GOBP_REGULATION_OF_BINDING                                          0.9985691
## GOBP_EMBRYONIC_SKELETAL_SYSTEM_DEVELOPMENT                          0.9985691
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL 0.9985691
## GOBP_MICROTUBULE_BASED_MOVEMENT                                     0.9985691
## GOBP_POLYSACCHARIDE_METABOLIC_PROCESS                               0.9985691
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                              0.9985691
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING             0.9985691
## GOBP_CHROMOSOME_ORGANIZATION                                        0.9985691
## GOBP_POSITIVE_REGULATION_OF_PROTEIN_LOCALIZATION                    0.9985691
## GOBP_CELLULAR_CARBOHYDRATE_BIOSYNTHETIC_PROCESS                     0.9985691
##
## GOBP_REGULATION_OF_BINDING                                                                                  SMAD3/STK3/I
## GOBP_EMBRYONIC_SKELETAL_SYSTEM_DEVELOPMENT
## GOBP_PROTEIN_MODIFICATION_BY_SMALL_PROTEIN_CONJUGATION_OR_REMOVAL USP25/SHARPIN/NSMCE4A/SIRT7/TRPC4AI
## GOBP_MICROTUBULE_BASED_MOVEMENT
## GOBP_POLYSACCHARIDE_METABOLIC_PROCESS
## GOBP_SULFUR_COMPOUND_METABOLIC_PROCESS                                                                      ACSL1/FMO1/
## GOBP_EMBRYO_DEVELOPMENT_ENDING_IN_BIRTH_OR_EGG_HATCHING                                 DEAF1/HOXB4/HOXB5/SMAD3/STK3/I
## GOBP_CHROMOSOME_ORGANIZATION                                                                                NSMCE4A/TNKS1BI
```

```
## GOBP_POSITIVE_REGULATION_OF_PROTEIN_LOCALIZATION                                                                PEC
## GOBP_CELLULAR_CARBOHYDRATE_BIOSYNTHETIC_PROCESS
## [1] "GOCC"
```

```
## preparing geneSet collections...
```

```
## GSEA analysis...
```

```
## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.
```

```
## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are duplica
```

```
## leading edge analysis...
```

```
## done...
```

```
## [1] 175   9
##                               setSize enrichmentScore       NES     pvalue
## GOCC_TIGHT_JUNCTION                 5       0.8135593  1.606603 0.01921108
## GOCC_NUCLEAR_CHROMOSOME             5      -0.7814929 -1.558495 0.03022995
## GOCC_DISTAL_AXON                    5      -0.7558595 -1.507376 0.04253567
## GOCC_CATALYTIC_COMPLEX             30      -0.4300823 -1.456541 0.04744526
## GOCC_CONTRACTILE_FIBER              7      -0.6570526 -1.456482 0.07871199
## GOCC_RECEPTOR_COMPLEX               4       0.7630071  1.449392 0.09761388
## GOCC_VESICLE_MEMBRANE              31       0.4040671  1.415583 0.06622517
## GOCC_MAIN_AXON                      4      -0.7764133 -1.413963 0.06642066
## GOCC_SPINDLE_POLE                   3       0.8192771  1.411488 0.08786611
## GOCC_APICAL_JUNCTION_COMPLEX        6       0.6591580  1.408337 0.10407240
##                               p.adjust qvalue
## GOCC_TIGHT_JUNCTION                  1      1
## GOCC_NUCLEAR_CHROMOSOME              1      1
## GOCC_DISTAL_AXON                     1      1
## GOCC_CATALYTIC_COMPLEX               1      1
## GOCC_CONTRACTILE_FIBER               1      1
## GOCC_RECEPTOR_COMPLEX                1      1
## GOCC_VESICLE_MEMBRANE                1      1
## GOCC_MAIN_AXON                       1      1
## GOCC_SPINDLE_POLE                    1      1
## GOCC_APICAL_JUNCTION_COMPLEX         1      1
##
## GOCC_TIGHT_JUNCTION
## GOCC_NUCLEAR_CHROMOSOME
## GOCC_DISTAL_AXON
## GOCC_CATALYTIC_COMPLEX                                                                                PHC2/SHARPIN/
## GOCC_CONTRACTILE_FIBER
## GOCC_RECEPTOR_COMPLEX
## GOCC_VESICLE_MEMBRANE        SBF2/PECAM1/FMN2/SBF2/ARHGAP32/MYO1C/DENND1A/ATAD3B/ATP8B4/VPS4A/WNT5A/C
## GOCC_MAIN_AXON
## GOCC_SPINDLE_POLE
## GOCC_APICAL_JUNCTION_COMPLEX
## [1] "GOMF"
```

```
## preparing geneSet collections...

## GSEA analysis...

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.

## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are duplica

## leading edge analysis...

## done...

## [1] 196   9
##                                          setSize enrichmentScore       NES
## GOMF_MOLECULAR_TRANSDUCER_ACTIVITY             9       0.7041565  1.718012
## GOMF_HISTONE_BINDING                           9      -0.7038299 -1.702632
## GOMF_STRUCTURAL_MOLECULE_ACTIVITY              8      -0.7180670 -1.683410
## GOMF_MODIFICATION_DEPENDENT_PROTEIN_BINDING    7      -0.7387111 -1.664323
## GOMF_UBIQUITIN_LIKE_PROTEIN_LIGASE_ACTIVITY    8      -0.7088733 -1.661857
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY               11      -0.6485722 -1.621741
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY   11      -0.6485722 -1.621741
## GOMF_CELL_ADHESION_MOLECULE_BINDING            15      -0.5772856 -1.602119
## GOMF_KINASE_BINDING                            17       0.5408111  1.622002
## GOMF_PROTEIN_DOMAIN_SPECIFIC_BINDING           21       0.4823121  1.557988
##                                              pvalue  p.adjust    qvalue
## GOMF_MOLECULAR_TRANSDUCER_ACTIVITY       0.01081340 0.3819464 0.3733310
## GOMF_HISTONE_BINDING                     0.01020496 0.3819464 0.3733310
## GOMF_STRUCTURAL_MOLECULE_ACTIVITY        0.01134454 0.3819464 0.3733310
## GOMF_MODIFICATION_DEPENDENT_PROTEIN_BINDING 0.01395108 0.3819464 0.3733310
## GOMF_UBIQUITIN_LIKE_PROTEIN_LIGASE_ACTIVITY 0.01480084 0.3819464 0.3733310
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY          0.01449162 0.3819464 0.3733310
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY 0.01449162 0.3819464 0.3733310
## GOMF_CELL_ADHESION_MOLECULE_BINDING      0.01558965 0.3819464 0.3733310
## GOMF_KINASE_BINDING                      0.01870108 0.4072679 0.3980814
## GOMF_PROTEIN_DOMAIN_SPECIFIC_BINDING     0.02946811 0.4442884 0.4342669
##                                                                                                  core_enri
## GOMF_MOLECULAR_TRANSDUCER_ACTIVITY                                                 PECAM1/GPR146/IL13RA2/MCC/MCC/THRA
## GOMF_HISTONE_BINDING                                                                     FAM156B/SBNO2/BRD4/ING1/DEI
## GOMF_STRUCTURAL_MOLECULE_ACTIVITY                                                              EPB41L3/LAMC2/CMTI
## GOMF_MODIFICATION_DEPENDENT_PROTEIN_BINDING                                              SHARPIN/FAM156B/BRD4/ING1
## GOMF_UBIQUITIN_LIKE_PROTEIN_LIGASE_ACTIVITY                                                        SH3RF3/PDZRI
## GOMF_MOLECULAR_ADAPTOR_ACTIVITY                                                  VAMP8/EPB41L3/SYNE3/BRD4/TRPC4A
## GOMF_PROTEIN_MACROMOLECULE_ADAPTOR_ACTIVITY                                      VAMP8/EPB41L3/SYNE3/BRD4/TRPC4A
## GOMF_CELL_ADHESION_MOLECULE_BINDING                                             COBLL1/PI4KA/PTPN1/TNKS1BP1/FGF1
## GOMF_KINASE_BINDING                                                             PRKRIP1/INKA1/SMAD3/STAU2/TTC28/
## GOMF_PROTEIN_DOMAIN_SPECIFIC_BINDING     SH3BGRL2/LNX1/RAPGEF2/THRA/PPARG/CITED1/VPS4A/SLC22A5/QKI
```