

Main Figures

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Figures

This file consists analysis steps and codes of figures in manuscript.

```
mirtarbase_node_perturbations <- readRDS("mirtarbase_node_perturbations.RDS") # data includes results
```

Venn diagrams Figure 4A

```
gene_dist <- mirtarbase_node_perturbations %>% group_by(name) %>%  
  summarise(perturbed = sum(perturbed_count > 0,  
    na.rm = TRUE)) %>% ungroup()
```

```
## 'summarise()' ungrouping output (override with '.groups' argument)
```

```
effective_nodes <- mirtarbase_node_perturbations %>%  
  left_join(gene_dist, by = "name") %>% filter(perturbed >  
  10, perturbed_count > 78) %>% distinct(name)
```

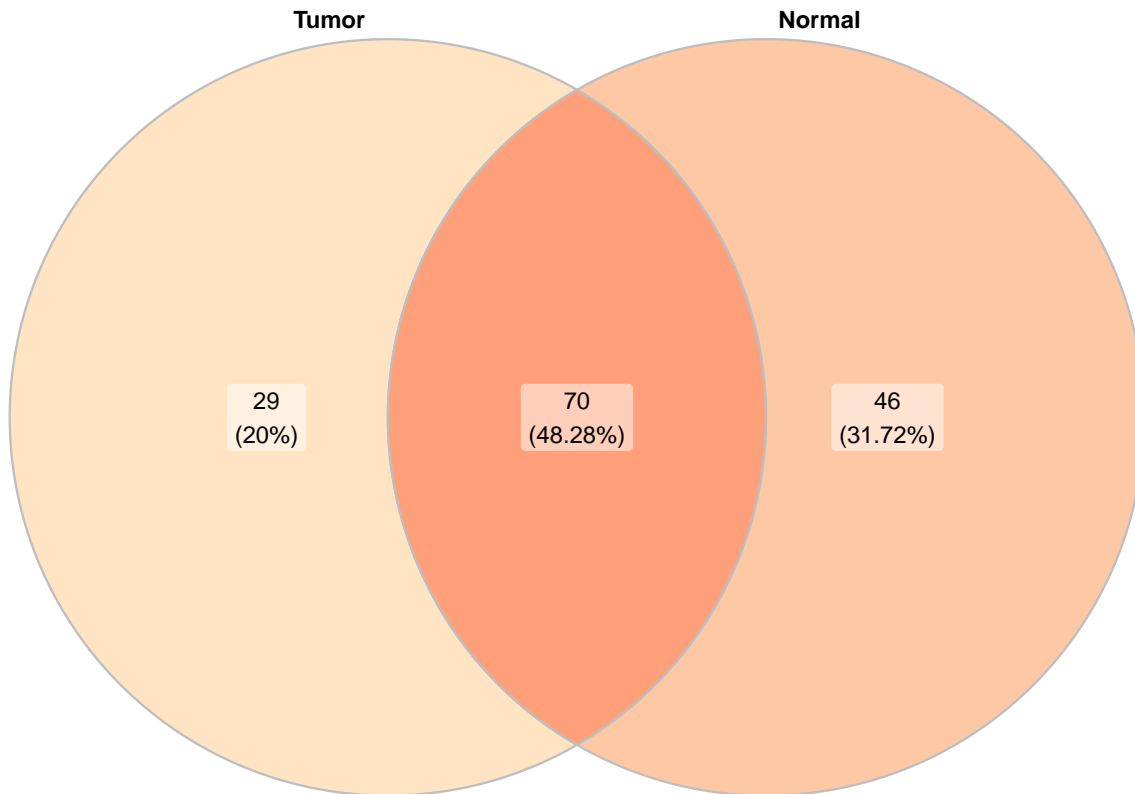
- Detecting highly perturbing gene nodes in tumor tissues

```
lim_1_gene_tumor <- mirtarbase_node_perturbations %>%  
  left_join(gene_dist, by = "name") %>% mutate(tissue_type = ifelse(endsWith(file_name,  
  "01A"), "Tumor", "Normal"), node_type = ifelse(name %in%  
  effective_nodes$name, "effective", "non_effective")) %>%  
  filter(node_type == "effective", perturbed_count >  
    78, perturbed > 10) %>% filter(tissue_type !=  
  "Normal", startsWith(name, "ENS")) %>% distinct(name) %>%  
  pull()
```

- Detecting highly perturbing gene nodes in normal tissues

```
lim_1_gene_normal <- mirtarbase_node_perturbations %>%  
  left_join(gene_dist, by = "name") %>% mutate(tissue_type = ifelse(endsWith(file_name,  
  "01A"), "Tumor", "Normal"), node_type = ifelse(name %in%  
  effective_nodes$name, "effective", "non_effective")) %>%  
  filter(node_type == "effective", perturbed_count >  
    78, perturbed > 10) %>% filter(tissue_type ==  
  "Normal", startsWith(name, "ENS")) %>% distinct(name) %>%  
  pull()
```

Venn diagram of genes



- Detecting highly perturbing mirna nodes in tumor tissues

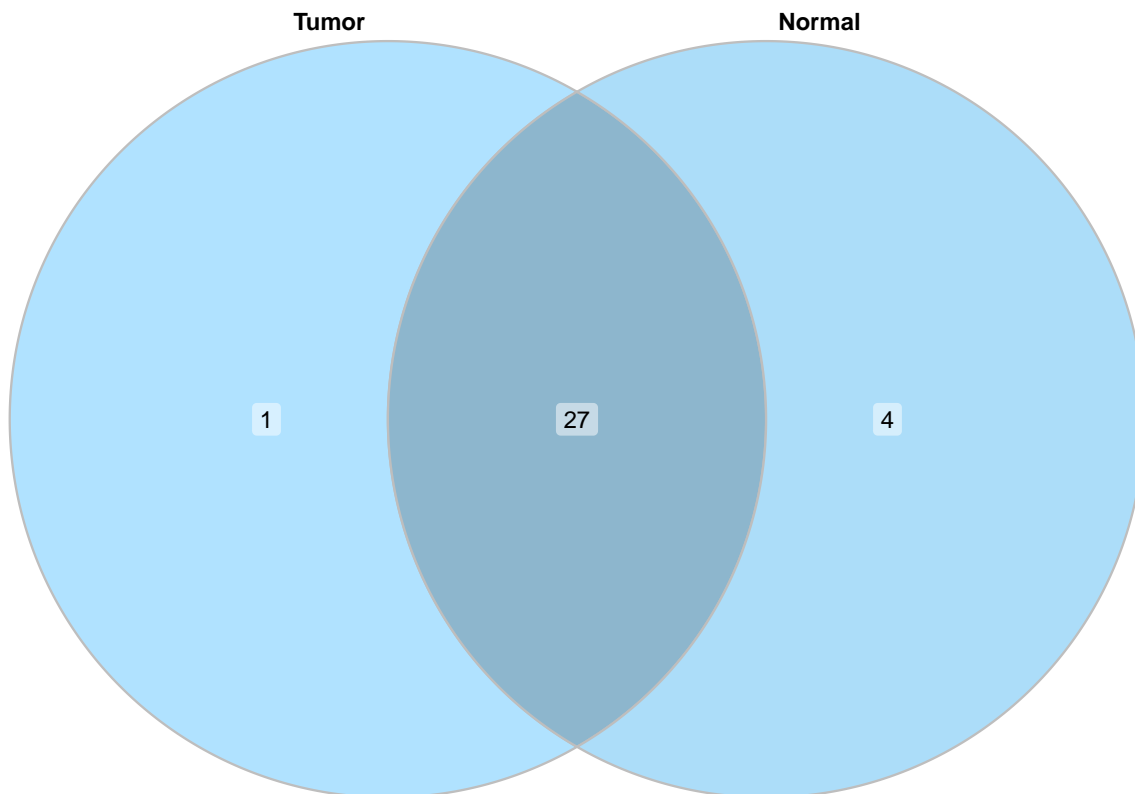
```
lim_1_mirna_tumor <- mirtarbase_node_perturbations %>%  
  left_join(gene_dist, by = "name") %>% mutate(tissue_type = ifelse(endsWith(file_name,  
    "01A"), "Tumor", "Normal"), node_type = ifelse(name %in%  
    effective_nodes$name, "effective", "non_effective")) %>%  
  filter(node_type == "effective", perturbed_count >  
    78, perturbed > 10) %>% filter(tissue_type !=  
    "Normal", startsWith(name, "hsa")) %>% distinct(name) %>%  
  pull()
```

- Detecting highly perturbing mirna nodes in normal tissues

```

lim_1_mirna_normal <- mirtarbase_node_perturbations %>%
  left_join(gene_dist, by = "name") %>% mutate(tissue_type = ifelse(endsWith(file_name,
"01A"), "Tumor", "Normal"), node_type = ifelse(name %in%
effective_nodes$name, "effective", "non_effective")) %>%
  filter(node_type == "effective", perturbed_count >
    78, perturbed > 10) %>% filter(tissue_type ==
  "Normal", startsWith(name, "hsa")) %>% distinct(name) %>%
  pull()

```



```

library(ggpubr)

# pA <- grid.arrange(pa1, pa2)

```

```

pA <- ggpubr::ggarrange(pa1, pa2, nrow = 2)
pA
# ggsave(filename = 'lim1_PE_gene_mirtarbase.svg',
# width = 6, height = 6)

```

Functional Annotation Analysis

```

Anno_tumor_specific <- readRDS("Anno_tumor_specific.RDS") # includes functional annotation of 29 genes

```

#Functional annotation of Tumor specific 29 genes. **Figure 4B**

Tumor only:

```

tumor_only_annotation <- Anno_tumor_specific %>% filter(startsWith(Category,
  c("KEGG", "GO")), !str_detect(Category, "CC_DIRECT")) %>%
  filter(!str_detect(Category, "MF_DIRECT")) %>%
  clean_names() %>% group_by(category) %>% filter(row_number() <
  6)

position <- rev(c("hsa05219:Bladder cancer", "hsa05212:Pancreatic cancer",
  "hsa04510:Focal adhesion", "hsa05206:MicroRNAs in cancer",
  "hsa05200:Pathways in cancer", "GO:0071230~cellular response to amino acid stimulus",
  "GO:0043154~negative regulation of cysteine-type endopeptidase activity involved in apoptotic process",
  "GO:0001701~in utero embryonic development", "GO:0043066~negative regulation of apoptotic process",
  "GO:0000122~negative regulation of transcription from RNA polymerase II promoter"))

tumor_only_annotation %>% group_by(category) %>% arrange(-desc(fold_enrichment))

```

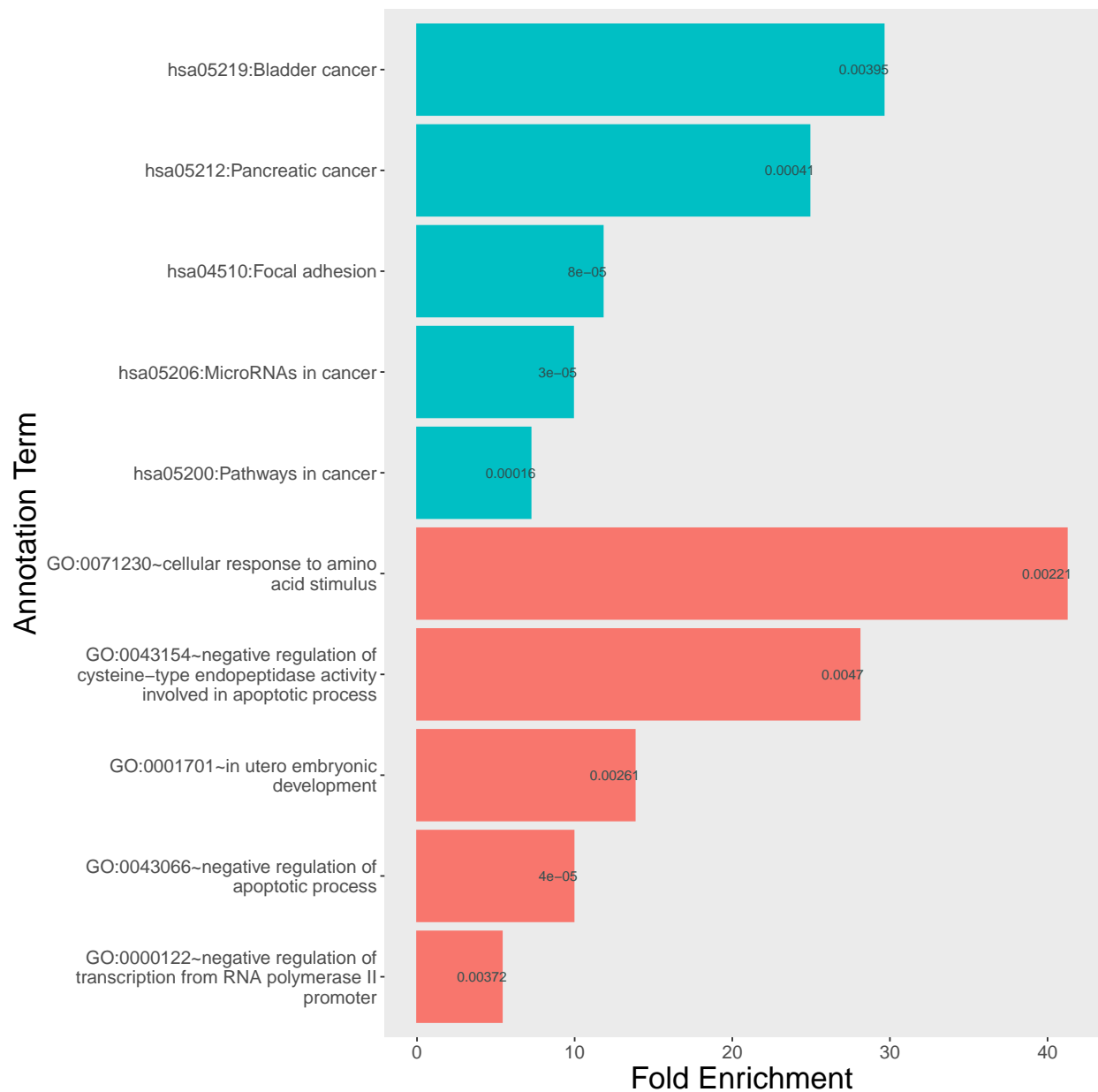
```

## # A tibble: 10 x 13
## # Groups:   category [2]
##   category term count percent p_value genes list_total pop_hits pop_total
##   <chr> <chr> <dbl> <dbl> <dbl> <chr> <dbl> <dbl> <dbl>
## 1 GOTERM_~ GO:0~ 6 20.7 3.72e-3 ENSG~ 26 720 16792
## 2 KEGG_PA~ hsa0~ 7 24.1 1.64e-4 ENSG~ 17 393 6879
## 3 KEGG_PA~ hsa0~ 7 24.1 2.76e-5 ENSG~ 17 286 6879
## 4 GOTERM_~ GO:0~ 7 24.1 4.38e-5 ENSG~ 26 455 16792
## 5 KEGG_PA~ hsa0~ 6 20.7 7.65e-5 ENSG~ 17 206 6879
## 6 GOTERM_~ GO:0~ 4 13.8 2.61e-3 ENSG~ 26 187 16792
## 7 KEGG_PA~ hsa0~ 4 13.8 4.13e-4 ENSG~ 17 65 6879
## 8 GOTERM_~ GO:0~ 3 10.3 4.70e-3 ENSG~ 26 69 16792
## 9 KEGG_PA~ hsa0~ 3 10.3 3.95e-3 ENSG~ 17 41 6879
## 10 GOTERM_~ GO:0~ 3 10.3 2.21e-3 ENSG~ 26 47 16792
## # ... with 4 more variables: fold_enrichment <dbl>, bonferroni <dbl>,
## # benjamini <dbl>, fdr <dbl>

```

```
tumor_only_annotation %>% ggplot(aes(x = fold_enrichment,
  y = term)) + geom_col(aes(color = category, fill = category)) +
  geom_text(aes(label = round(p_value, 5)), hjust = 0.9,
    color = "darkslategrey", size = 3) + xlab("Fold Enrichment") +
  ylab("Annotation Term") + theme(panel.grid = element_blank(),
  axis.title = element_text(size = 20), axis.text = element_text(size = 12),
  legend.position = "none") + scale_y_discrete(labels = scales::wrap_format(40),
  position = "left", limits = position)

ggsave(filename = "mirtarbase_annotation.svg", width = 8,
  height = 6)
```



— This figure was removed from manuscript. ## Network construction with overall functional annotation: Additional figure important genes from functional Annotation

```
significant_node_graph <- readRDS("significant_node_graph.RDS") #consists network of highly perturbing
hg19 <- readRDS("hg19.RDS") #For id matching (obtained via bioMaRt package)
```

Annotation network:

```
Annotation_overall <- readRDS("Annotation_overall.RDS") # consists functional annotation of 145 high p
```

```
gene_top_annotation_graph <- Annotation_overall %>%
  filter(startsWith(Category, c("KEGG", "GO")), !str_detect(Category,
    "CC_DIRECT")) %>% filter(!str_detect(Category,
    "MF_DIRECT")) %>% clean_names() %>% group_by(category) %>%
  filter(row_number() < 6) %>% dplyr::select(term,
    Gene_Count = count, p_value, genes) %>% mutate(genes2 = str_split(genes,
    ", ") %>% unnest() %>% ungroup %>% mutate(genes2 = str_trim(genes2,
    side = "both"), genes2 = ifelse(endsWith(genes2,
    ","), substr(genes2, 0, (nchar(genes2) - 1)), genes2),
    interaction_type = term, Ensembl_Gene_Id = genes2,
    competing_name = genes2) %>% dplyr::select(interaction = term,
    Ensembl_Gene_Id, interaction_type, competing_name)
```

```
## Adding missing grouping variables: 'category'
```

```
## Warning: 'cols' is now required when using unnest().
```

```
## Please use 'cols = c(genes2)'
```

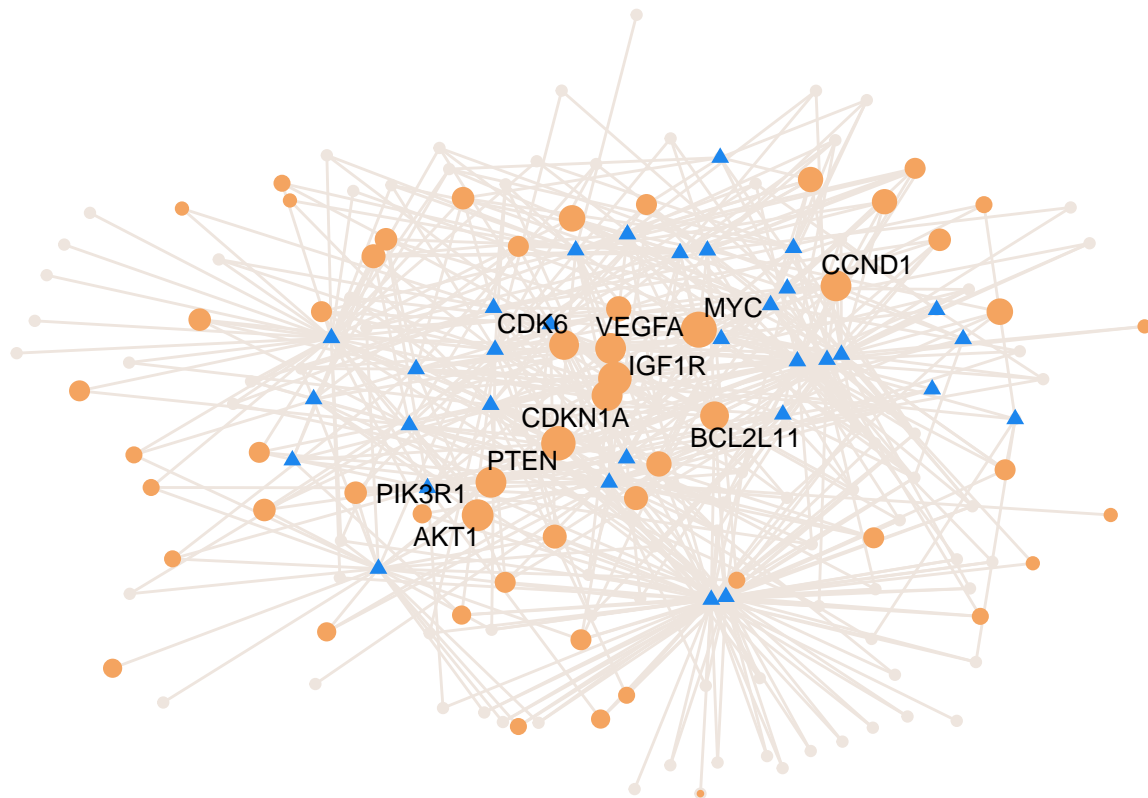
```
top_important <- significant_node_graph %>% bind_rows(gene_top_annotation_graph) %>%
  as_tibble_graph() %>% mutate(type = ifelse(startsWith(name,
    "hsa"), "KEGG", "GO"), type = ifelse(startsWith(name,
    "hsa-"), "miRNA", type), type = ifelse(startsWith(name,
    "ENSG"), "Gene", type)) %>% mutate(centrality = centrality_degree(mode = "all"),
    annotation = ifelse(name %in% gene_top_annotation_graph$Ensembl_Gene_Id,
    "top_annotation", "other")) %>% as_tibble() %>%
  filter(annotation == "top_annotation", centrality >
    10) %>% dplyr::select(name) %>% pull()
```

```
significant_node_graph %>% bind_rows(gene_top_annotation_graph) %>%
  as_tibble_graph() %>% mutate(type = ifelse(startsWith(name,
    "hsa"), "KEGG", "GO"), type = ifelse(startsWith(name,
    "hsa-"), "miRNA", type), type = ifelse(startsWith(name,
    "ENSG"), "Gene", type)) %>% mutate(centrality = centrality_degree(mode = "all"),
    annotation = ifelse(name %in% gene_top_annotation_graph$Ensembl_Gene_Id,
    "Enriched Genes", "other")) %>% left_join(hg19,
    by = c(name = "ensembl_gene_id")) %>% filter(type %in%
    c("miRNA", "Gene")) %>% ggraph(layout = "kk") +
  geom_edge_link(colour = "seashell2") + geom_node_point(color = "seashell2") +
  geom_node_point(aes(filter = annotation == "Enriched Genes",
    size = centrality, color = "Enriched Genes"),
    shape = 16) + geom_node_point(aes(filter = type ==
    "miRNA", color = "miRNA"), shape = 17, size = 2) +
```

```

geom_node_point(aes(filter = type == "GO", size = centrality,
  color = "GO"), shape = 18) + geom_node_point(aes(filter = type ==
  "KEGG", size = centrality, color = "KEGG"), shape = 15) +
theme_graph(base_family = "sans") + geom_node_text(aes(filter = name %in%
top_important, label = hgnc_symbol), size = 3.5,
  repel = TRUE) + theme(plot.margin = margin(0, 0,
  0, 0, "cm"), legend.position = "none") + guides(size = FALSE,
  shape = FALSE) + scale_color_manual(name = "Node types",
  values = c('Enriched Genes' = "sandybrown", miRNA = "dodgerblue2",
  GO = "green", KEGG = "red"))

```



Heat-maps for miRNAs

+Detecting perturbing miRNAs

```

mirna_tumor <- mirtarbase_node_perturbations %>% left_join(gene_dist,
  by = "name") %>% mutate(tissue_type = ifelse(endsWith(file_name,
  "01A"), "Tumor", "Normal"), node_type = ifelse(name %in%
  effective_nodes$name, "effective", "non_effective")) %>%
  filter(node_type == "effective", perturbed_count >
  78, perturbed > 10) %>% filter(tissue_type !=

```

```

    "Normal", startsWith(name, "hsa")) %>% mutate(file_name = str_remove(file_name,
    "-01A")) %>% dplyr::select(file_name, name, tissue_type,
    perturbed_count)

mirna_normal <- mirtarbase_node_perturbations %>% left_join(gene_dist,
  by = "name") %>% mutate(tissue_type = ifelse(endsWith(file_name,
  "01A"), "Tumor", "Normal"), node_type = ifelse(name %in%
  effective_nodes$name, "effective", "non_effective")) %>%
  filter(node_type == "effective", perturbed_count >
    78, perturbed > 10) %>% filter(tissue_type ==
  "Normal", startsWith(name, "hsa")) %>% mutate(file_name = str_remove(file_name,
  "-11A")) %>% dplyr::select(file_name, name, tissue_type,
  perturbed_count)

```

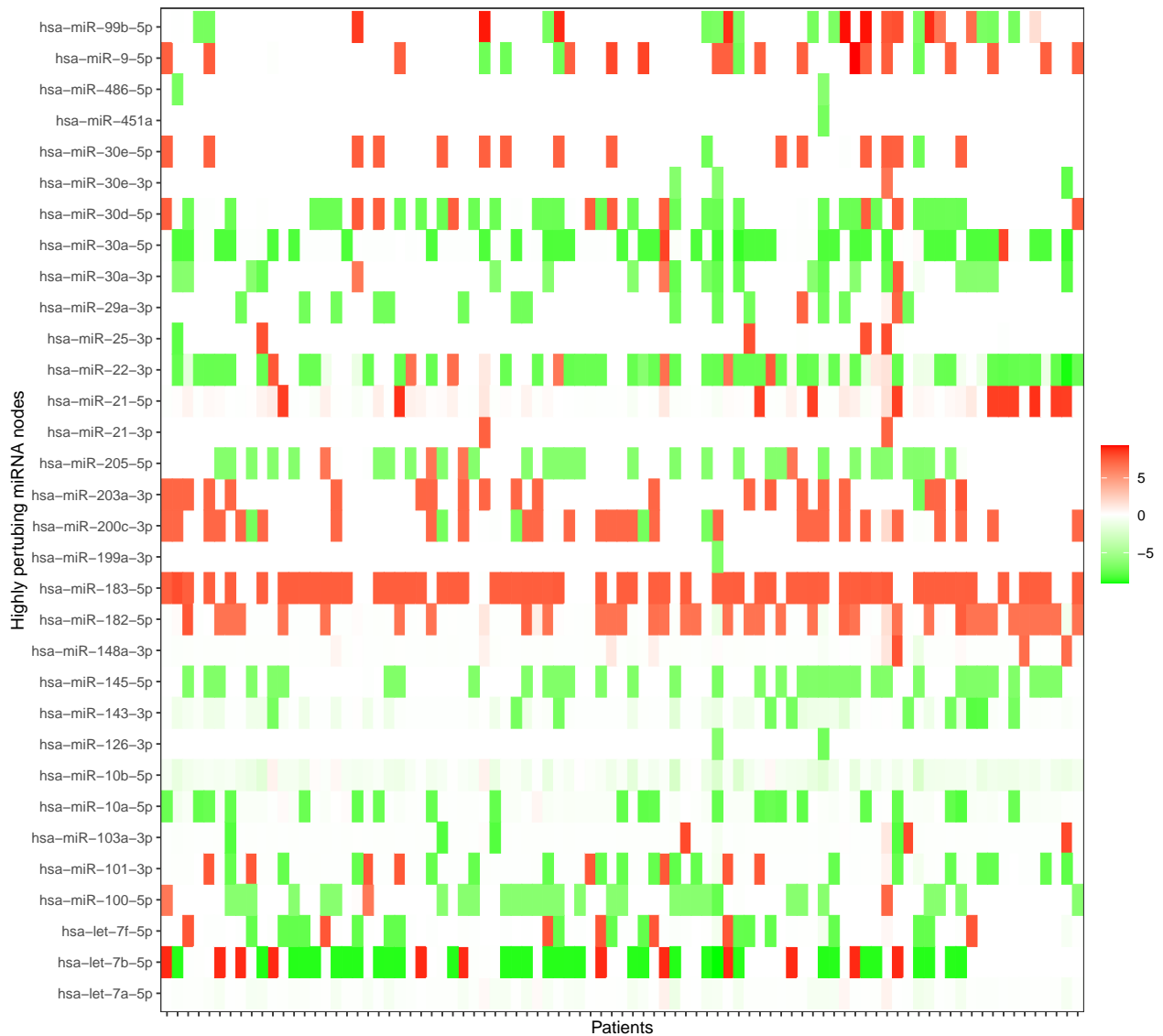
Heat map Figure 4C

```

mirna_tumor%>%
  full_join(mirna_normal, by =c("name", "file_name"), suffix= c("_tumor", "_normal"), fill.na = 0)%>%
  dplyr::select(-3, -5)%>%
  mutate(perturbed_count_tumor = ifelse(is.na(perturbed_count_tumor), 1, perturbed_count_tumor),
    perturbed_count_normal = ifelse(is.na(perturbed_count_normal), 1, perturbed_count_normal),
    log_FC= log2(perturbed_count_tumor/perturbed_count_normal))%>%
  ggplot(aes(x = file_name, y = name, fill = log_FC))+
  geom_tile(aes(colour = log_FC, fill = log_FC))+
  theme_test()+
  theme(axis.text.x = element_blank(), #element_text(angle = 90, vjust = 0, hjust=0)
    plot.title = element_text(hjust = 0.5))+
  scale_colour_gradientn(colours = c("green", "white", "red"), aesthetics = c("colour", "fill"), na.value = "white")
  ylab("Highly perturbing miRNA nodes")+
  xlab("Patients")+
  theme( legend.title = element_blank(), panel.background = element_rect(fill = "white"), plot.margin = margin(10, 10, 10, 10))

ggsave(filename = 'mirna_comparison.svg', width = 8, height = 4)

```

Heat map Figure 4D

```
mirna_tumor%>%
  full_join(mirna_normal, by =c("name", "file_name"), suffix= c("_tumor", "_normal"), fill.na = 0)%>%
  dplyr::select(-3, -5)%>%
  pivot_longer(cols = c("perturbed_count_tumor", "perturbed_count_normal"), names_to = "situ", values_to = "perturbed_count_log")
  mutate(perturbed_count_log = log2(perturbed_count),
         situ = str_replace(situ, "perturbed_count_normal", 'Perturbed count in normal'),
         situ = str_replace(situ, "perturbed_count_tumor", 'Perturbed count in tumor'),
         perturbed_count = as.numeric(str_replace_na(perturbed_count, 0)),
         perturbed_count_log = as.numeric(str_replace_na(perturbed_count_log, 0)))%>%
  ggplot(aes(x = file_name, y = name, fill = perturbed_count_log))+
  geom_tile(aes(colour = perturbed_count_log, fill = perturbed_count_log))+
  theme_test()+
  theme(axis.text.x = element_blank(), #element_text(angle = 90, vjust = 0, hjust=0)
        plot.title = element_text(hjust = 0.5))+
```

```

scale_colour_gradientn(colours = c("green","red", "black"), aesthetics = c("colour", "fill"), na.value = "black",
ylab("Highly perturbing miRNA nodes")+
xlab("Patients")+
facet_grid(rows = "situ")+
theme( legend.title = element_blank(), panel.background = element_rect(fill = "gray93"), plot.margin = margin(5, 5, 5, 5))

ggsave(filename = 'mirna_comparison_normal_tumor.svg', width = 8, height = 7)

```

