

# MEK inhibition activates Wnt signalling and induces stem cell plasticity in colorectal cancer organoids

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2018-04-20

## Abstract

Resistance to Ras pathway inhibition is a major challenge in the treatment of colorectal cancer (CRC), but the underlying mechanisms are incompletely understood. By a large-scale compound screens in CRC, we identified MEK1/2 inhibitors as potent activators of Wnt/beta-catenin signalling. Targeting MEK increased Wnt activity in different CRC cell lines and in murine intestine in vivo. This Wnt response was strongly enhanced by truncating mutations in APC and mediated by downregulation of AXIN1. Using patient-derived CRC organoids, we showed that MEK inhibition leads to increased Wnt activity, elevated LGR5 levels and enrichment of gene signatures associated with stemness and cancer relapse. Our study demonstrates that MEK inhibition affects stem cell plasticity which constitutes an unknown mechanism of drug resistance.

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## 1 About

This document contains computer code to reproduce DigiWest and gene expression analyses presented in the manuscript.

## 2 Dependencies

We load a number of packages whose functions are needed throughout the analysis

```
library(limma)
library(ggrepel)
library(lumi)
library(patchwork)
library(pheatmap)
library(fgsea)
library(tidyverse)
```

## 3 Digital Western (DigiWest) analyses

Antibody specific signalings in the DigiWest experiment were quantified using a specific computer software (Treindl et al. 2016). We load the quantified signal into R.

```
## loads object 'dw_mat'
data('digiwest', package='MEKWnt2018')
```

In order to test for differences in antibody signal between Trametinib-treated and untreated cells using a moderated t-test we set up a design matrix and run limma (Ritchie et al. 2015).

```
## limma setup
treatment <- factor(ifelse(grepl('DMSO', colnames(dw_mat)), 'DMSO', 'TRAM'))

mm <- model.matrix(~treatment)
dw_res <- lmFit(dw_mat, mm) %>%
  eBayes() %>%
  topTable(n=Inf, coef=2) %>%
  rownames_to_column('Gene') %>%tbl_df
```

We generate a volcano plot visualizing the results. We assign a colour to all antibodies with a false discovery rate (FDR) of less 20%. We give different colours to antibodies whose abundance is increase and decreased, respectively, by the Trametinib treatment. We label antibodies targeting selected genes of interest.

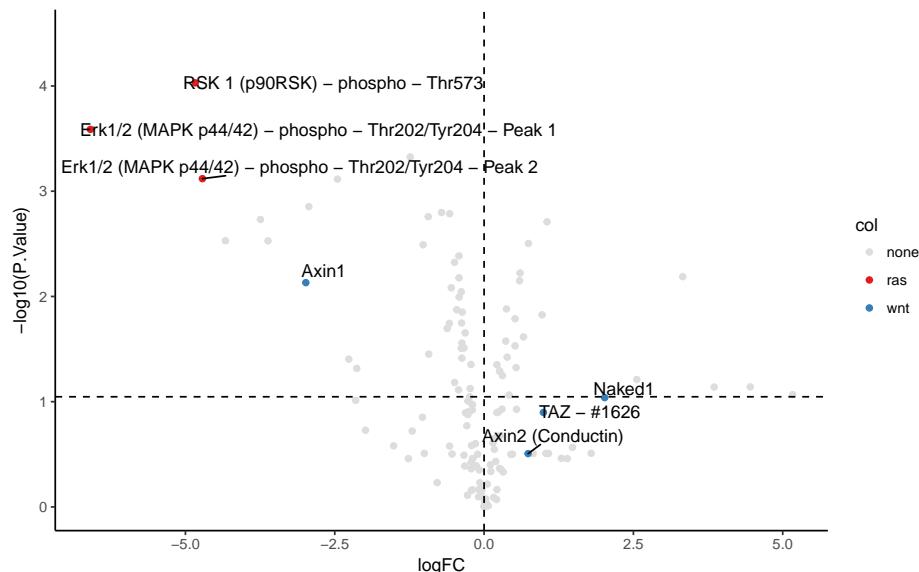
```
## cutoff for 20 % FDR line
fdr20 <- -log10(dw_res %>% filter(adj.P.Val > 0.2) %>%
  .\$P.Value %>% .[1])

wnt_dw <- c('TAZ - #1626', 'Naked1', 'Axin2 (Conductin)', 'Axin1')
ras_dw <- c('Erk1/2 (MAPK p44/42) - phospho - Thr202/Tyr204 - Peak 1',
```

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```
'Erk1/2 (MAPK p44/42) - phospho - Thr202/Tyr204 - Peak 2',  
'RSK 1 (p90RSK) - phospho - Thr573 ')
```

```
dw_res %>% mutate(label = ifelse(Gene %in% c(wnt_dw, ras_dw), Gene, ''),  
                     col = ifelse(Gene %in% wnt_dw, 'wnt',  
                                 ifelse(Gene %in% ras_dw, 'ras', 'none')))) %>%  
ggplot(aes(logFC, -log10(P.Value), label=label)) +  
geom_point(aes(colour=col)) + geom_text_repel() +  
geom_vline(xintercept = 0, linetype='dashed') +  
geom_hline(yintercept = fdr20, linetype='dashed') +  
scale_colour_manual(values=c('#dddddd', '#e41a1c', '#377eb8')) +  
theme_classic() + ylim(c(0, 4.5))
```



## 4 Gene expression analysis of MEK inhibition in patient derived CRC organoids

### 4.1 Illumina BeadChips

In order to examine how MEK treatment affects gene expression in patient derived CRC organoids we performed expression profiling experiments using an Illumina BeadChip platform. The raw data were parsed and normalized using the lumi (Du, Kibbe, and Lin 2008) software and stored as an Rdata file. We next load the normalized expression profiles into working memory. We further load a data frame that maps Illumina probes to gene symbols.

```
data('expr_organoids_norm', package='MEKWnt2018')  
data('probe_mapping_ilmn', package='MEKWnt2018')
```

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### 4.1.1 Reproducibility

In order to make sure that the data are reproducible we can plot MA plots of both replicates corresponding to each sample. As there are many plots we will not display them in the vignette but they can be generated on demand using below code snippet.

```
walk(expr_organoids_norm, ~ plot(., what='MAplot'))
```

### 4.1.2 Differential gene expression

We perform three different gene expression analyses:

1. We compare DGE between Trametinib treatment and DMSO controls for Organoid line 4
2. We compare DGE between Trametinib treatment and DMSO controls for Organoid line 7
3. We compare DGE between Trametinib treatment and DMSO controls as well as Trametinib + PRI-742 treatment (rescue experiment) for Organoid line 4

#### 4.1.2.1 Organoid line 4

We use the limma package for differential gene expression analysis. First we remove unexpressed genes to reduce false positives.

```
## get expression matrix for Organoid 4 samples
o4_expr <- exprs(expr_organoids_norm$o4_norm)

## present probes
o4present <- detectionCall(expr_organoids_norm$o4_norm)
## select probe present in at least one sample
o4_expr_fil <- o4_expr[o4present > 0,]
```

Next we can run limma's moderated t-test to find differentially expressed genes between Trametinib and DMSO treated samples.

```
## vector specifying treatment per sample
treatment <- factor(ifelse(grepl('T10', colnames(o4_expr_fil)), 'TRAM', 'DMSO'))

## model matrix for DGE analysis
mm <- model.matrix(~treatment)
## fit model
fit <- lmFit(o4_expr_fil, mm)
fit <- eBayes(fit)
## make a results table
o4_res <- topTable(fit, coef = 2, n=Inf) %>% as.data.frame() %>%
  rownames_to_column('ProbeID') %>%tbl_df %>%
  left_join(probe_mapping_ilmn)
```

##### 4.1.2.1.1 Visualization

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We would like to visualize the results of the differential expression analysis as a volcano plot in which we want to highlight key target genes of the Ras pathway as well as stem cell and differentiation markers. Hence, we first define a function that we can reuse for later DGE analyses.

```
## we label some genes to specifically highlight them
to_label <- c('CD44', 'EPHB2', 'AXIN2', 'BIRC5', 'ASCL2', 'LGR5',
             'DUSP5', 'FOS', 'FOSL1', 'OLFM4', 'KRT20', 'TFF1')

## load list of canonical Wnt pathway components
data('wnt_genes', package='MEKWnt2018')

## load list of ras pathway members
data('ras_genes', package='MEKWnt2018')

## KRT20 and TFF1 are differentiation markers of CRC cells
kera <- c('KRT20', 'TFF1')

volcano_plot <- function(df, co=0.2){
  ## fdr cutoff
  fdr_co <- df %>% filter(adj.P.Val < co) %>%
    arrange(desc(P.Value)) %>%
    .$P.Value %>% .[1] %>% log10

  ## annotate data for plotting
  df <- df %>% group_by(Symbol) %>% arrange(P.Value) %>%
    dplyr::slice(1) %>% ungroup() %>%
    mutate(target_group = ifelse(Symbol %in% wnt, 'Wnt',
                                 ifelse(Symbol %in% ras, 'Ras',
                                       ifelse(Symbol %in% kera, 'Keratinocyte', 'none'))),
           label = ifelse(Symbol %in% to_label, Symbol, ''))

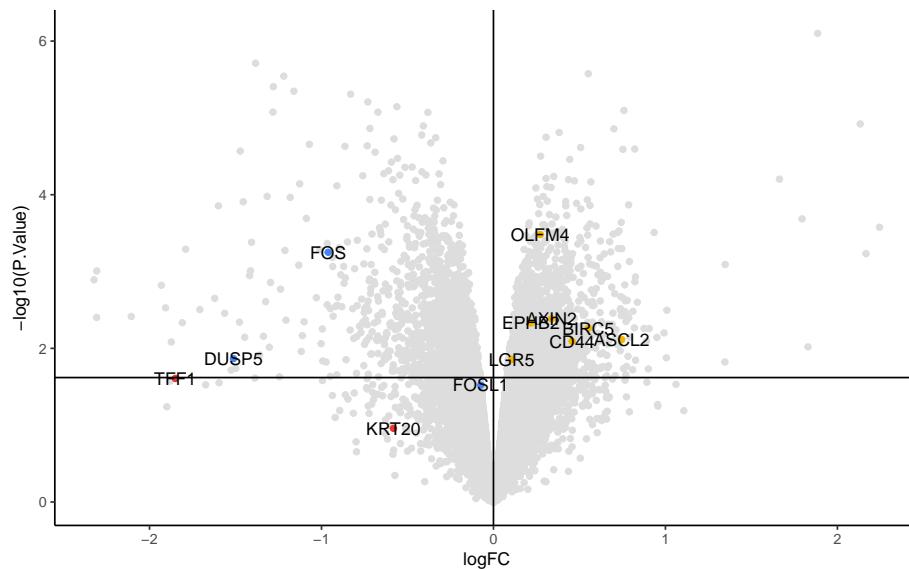
  vp <- ggplot() + geom_point(data=filter(df, target_group == 'none'),
                               aes(x=logFC, y= -log10(P.Value)), colour="#dddddd") +
    geom_point(data=filter(df, target_group == 'Wnt' & label != ''),
               aes(x=logFC, y= -log10(P.Value)), colour = '#f4b400') +
    geom_point(data=filter(df, target_group == 'Ras' & label != ''),
               aes(x=logFC, y= -log10(P.Value)), colour = '#4285f4') +
    geom_point(data=filter(df, target_group == 'Keratinocyte' & label != ''),
               aes(x=logFC, y= -log10(P.Value)), colour = '#f03531') +
    geom_text(data = filter(df, Symbol %in% c(wnt, ras, kera)),
              aes(x=logFC, y= -log10(P.Value), label=label)) +
    geom_vline(xintercept = 0) + geom_hline(yintercept = -1*fdr_co) +
    theme_classic()

  return(vp)
}
```

Now we can use this function to visualize the results of the DGE analysis of the Trametinib treated organoid 4 line.

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```
volcano_plot(o4_res)
```



### 4.1.2.2 Organoid line 7

We repeat the same steps detailed above for the organoid line 7.

```
## get expression matrix for Organoid 4 samples
o7_expr <- exprs(expr_organoids_norm$o7_norm)

## present probes
o7present <- detectionCall(expr_organoids_norm$o7_norm)
## select probe present in at least one sample
o7_expr_fil <- o7_expr[o7present > 0,]
```

Again we run limma's moderated t-test to find differentially expressed genes between Trametinib and DMSO treated samples.

```
## vector specifying treatment per sample
treatment <- factor(ifelse(grepl('TRAM', colnames(o7_expr_fil)), 'TRAM', 'DMSO'))

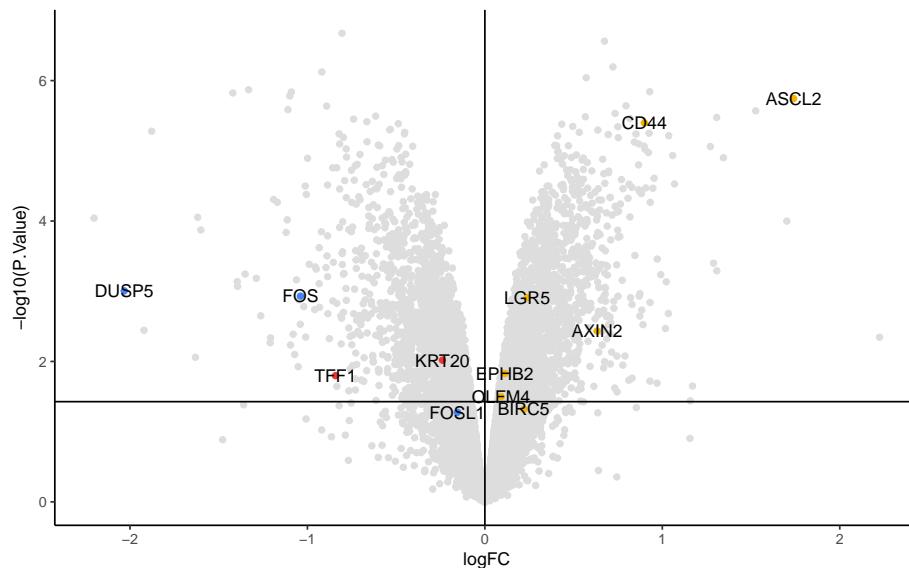
## model matrix for DGE analysis
mm <- model.matrix(~treatment)
## fit model
fit <- lmFit(o7_expr_fil, mm)
fit <- eBayes(fit)
## make a results table
o7_res <- topTable(fit, coef = 2, n=Inf) %>% as.data.frame() %>%
  rownames_to_column('ProbeID') %>% tbl_df %>%
  left_join(probe_mapping_ilmn)
```

#### 4.1.2.2.1 Visualization

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We use the volcano plot function defined above to visualize the results.

```
volcano_plot(o7_res)
```



### 4.1.2.3 Organoid line 15

We repeat the same steps detailed above for the organoid line 7.

```
## get expression matrix for Organoid 4 samples
o15_expr <- exprs(expr_organoids_norm$o15_norm)

## present probes
o15present <- detectionCall(expr_organoids_norm$o15_norm)
## select probe present in at least one sample
o15_expr_fil <- o15_expr[o15present > 0,]
```

Again we run limma's moderated t-test to find differentially expressed genes between Trametinib and DMSO treated samples.

```
## vector specifying treatment per sample
treatment <- factor(ifelse(grepl('TRAM', colnames(o15_expr_fil)), 'TRAM', 'DMSO'))

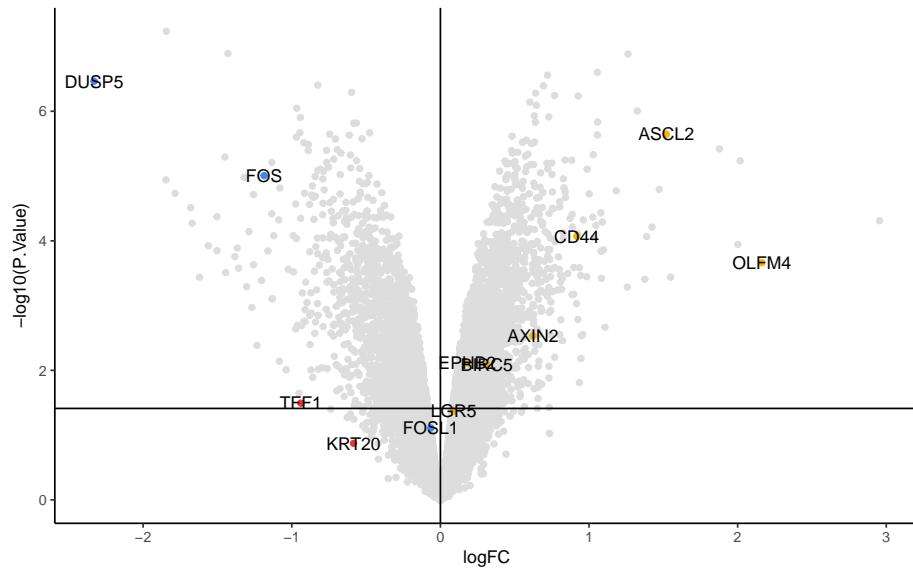
## model matrix for DGE analysis
mm <- model.matrix(~treatment)
## fit model
fit <- lmFit(o15_expr_fil, mm)
fit <- eBayes(fit)
## make a results table
o15_res <- topTable(fit, coef = 2, n=Inf) %>% as.data.frame() %>%
  rownames_to_column('ProbeID') %>%tbl_df %>%
  left_join(probe_mapping_ilmn)
```

#### 4.1.2.3.1 Visualization

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We use the volcano plot function defined above to visualize the results.

```
volcano_plot(o15_res)
```



### 4.1.2.4 Organoid line 19

Here we performed in addition to Trametinib treatment a rescue experiment where we additionally treated the cells with the Wnt signaling inhibitor PRI-724.

```
## get expression matrix for Organoid 4 samples
o19_expr <- exprs(expr_organoids_norm$o19_norm)

## present probes
o19present <- detectionCall(expr_organoids_norm$o19_norm)
## select probe present in at least one sample
o19_expr_fil <- o19_expr[o19present > 0,]
```

We now model contrasts in which we compare Trametinib treatment to DMSO and Trametinib/PRI combination treatment to DMSO.

```
## vector specifying treatment per sample
treatment <- factor(ifelse(grepl('DMSO', colnames(o19_expr_fil)), 'DMSO',
                             ifelse(grepl('P5', colnames(o19_expr_fil)), 'TRAMP5',
                             ifelse(grepl('P10', colnames(o19_expr_fil)), 'TRAMP10',
                             'TRAM'))))

## design matrix
mm <- model.matrix(~0+treatment)

## we define the contrasts
contr <- makeContrasts(treatmentTRAMP10-treatmentDMSO,
                        treatmentTRAM-treatmentDMSO,
                        treatmentTRAMP5-treatmentDMSO,
                        treatmentTRAMP10-treatmentTRAM,
```

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```
levels=c('treatmentDMSO', 'treatmentTRAM',
        'treatmentTRAMP10', 'treatmentTRAMP5'))\n\n## perform DGE analysis\nfit <- lmFit(o19_expr_fil, mm)\nfit <- eBayes(fit)\n\nfit2 <- contrasts.fit(fit, contr)\nfit2 <- eBays(fit2)\n\n## results Trametinib rescue experiments\no19_res <- map(1:length(rownames(contr)), function(i){\n  topTable(fit2, coef = i, n=Inf) %>% as.data.frame() %>%\n  rownames_to_column('ProbeID') %>% tbl_df %>%\n  left_join(probe_mapping_ilmn)\n})
```

### 4.1.2.4.1 Visualization

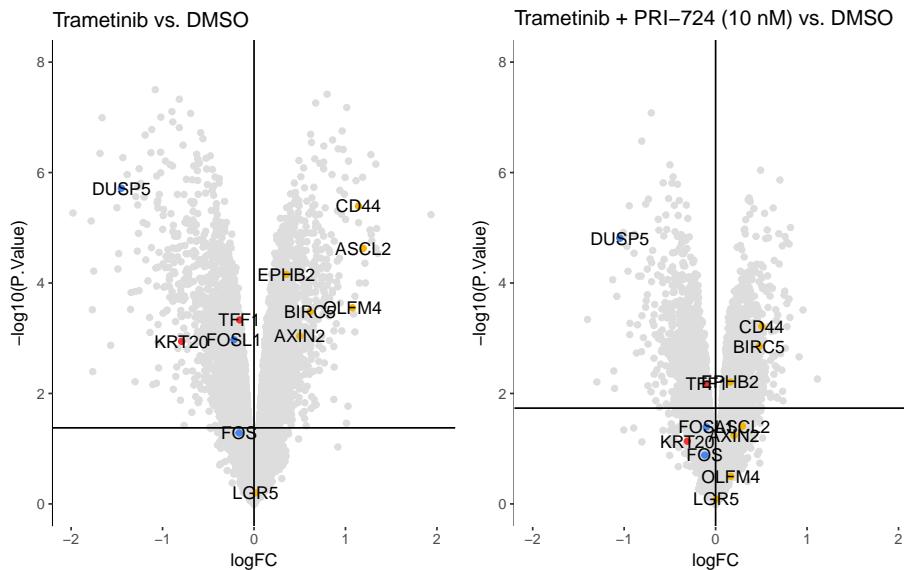
Again we can use the volcano plot function to visualize the results of the different comparisons. We use the same axis limits for all plots to render the results more visually comparable.

```
p1 <- volcano_plot(o19_res[[1]]) + xlim(c(-2, 2)) + ylim(c(0, 8)) +\nggtitle('Trametinib + PRI-724 (10 nM) vs. DMSO')\np2 <- volcano_plot(o19_res[[2]]) + xlim(c(-2, 2)) + ylim(c(0, 8)) +\nggtitle('Trametinib vs. DMSO')\np3 <- volcano_plot(o19_res[[3]]) + xlim(c(-2, 2)) + ylim(c(0, 8)) +\nggtitle('Trametinib + PRI-724 (5 nM) vs. DMSO')\np4 <- volcano_plot(o19_res[[4]]) + xlim(c(-2, 2)) + ylim(c(0, 8)) +\nggtitle('Trametinib + PRI-724 (10 nM) vs. Trametinib')
```

We can plot some of the plots next to each to see the difference well.

```
p2 + p1
```

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## 4.2 Affymetrix chip

To further increase the confidence in our results we repeated the rescue experiments in organoid line 19 using an independent gene expression platform by Affymetrix (U133+2). We repeat the exact same analysis as shown above to examine whether we can find the same results.

### 4.2.1 Loading and normalization

Raw microarray CEL files were parsed and normalized using the RMA and quantile normalization methods implemented in the affy (Gautier et al. 2004) R/Bioconductor (Huber et al. 2015) package. Raw data files can be downloaded from Gene Expression Omnibus (Edgar, Domrachev, and Lash 2002) but cannot be included in the vignette for data size reasons. We load an R data object that contains normalized and annotation expression measurements.

```
data('affy_data_anno', package = 'MEKWnt2018')
```

### 4.2.2 Differentially expressed genes

We perform a differential expression analysis based on the Affymetrix samples similar to what we did for the Illumina data. We first need an expression matrix.

```
## make expression matrix
affy_expr <- affy_data_anno %>% distinct(ProbeID, sample, expr) %>%
  spread(sample, expr) %>% data.frame() %>%
  `rownames<-` (NULL) %>% column_to_rownames('ProbeID')
```

We next define model matrix and contrasts for DGE analysis. After that we perform DGE analysis as described above.

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```
treatment <- affy_data_anno %>% distinct(sample, treatment) %>%
  .$treatment %>% factor()
## design matrix
mm <- model.matrix(~0+treatment)

## first we need to define the contrasts
contr <- makeContrasts(treatmentT10-treatmentDMSO,
                        treatmentT10.P10-treatmentDMSO,
                        treatmentT10-treatmentT10.P10,
                        levels=c('treatmentDMSO', 'treatmentT10',
                                'treatmentT10.P10'))

## perform DGE analysis
fit_affy <- lmFit(affy_expr, mm)
fit_affy <- eBayes(fit_affy)

fit_affy2 <- contrasts.fit(fit_affy, contr)
fit_affy2 <- eBayes(fit_affy2)

## results Trametinib rescue experiments
rescue_res_affy <- map[1:3, function(i){
  topTable(fit_affy2, n=Inf, coef=i) %>%
    rownames_to_column('ProbeID') %>%tbl_df %>%
    inner_join(distinct(affy_data_anno, ProbeID, Symbol)) %>%
    return()
})
names(rescue_res_affy) <- colnames(contr)
```

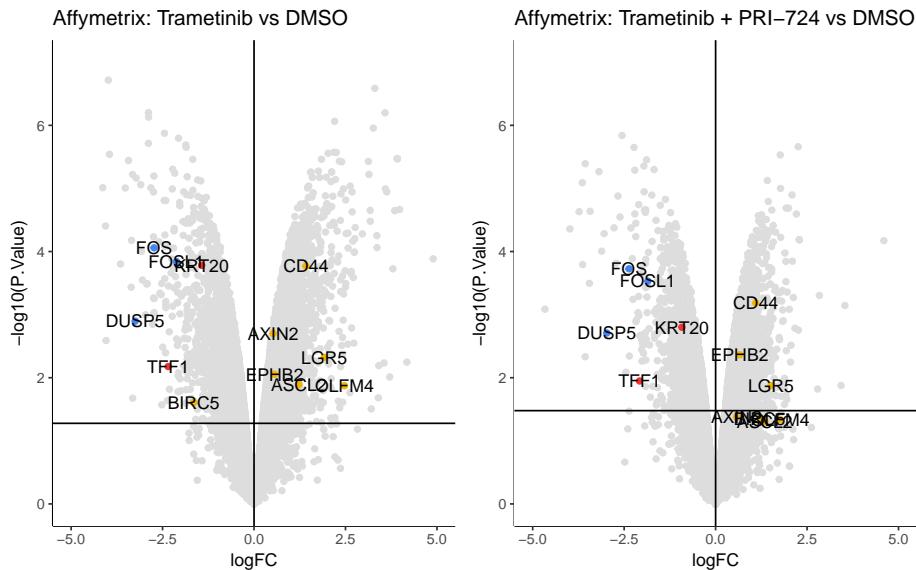
### 4.2.2.1 Visualization

Using the volcano plotting function defined earlier we visualize our results.

```
affy_t10 <- volcano_plot(rescue_res_affy[[1]]) +
  xlim(c(-5, 5)) + ylim(c(0, 7)) +
  ggtitle('Affymetrix: Trametinib vs DMSO')
affy_p10 <- volcano_plot(rescue_res_affy[[2]]) +
  xlim(c(-5, 5)) + ylim(c(0, 7)) +
  ggtitle('Affymetrix: Trametinib + PRI-724 vs DMSO')

## plot to canvas
affy_t10 + affy_p10
```

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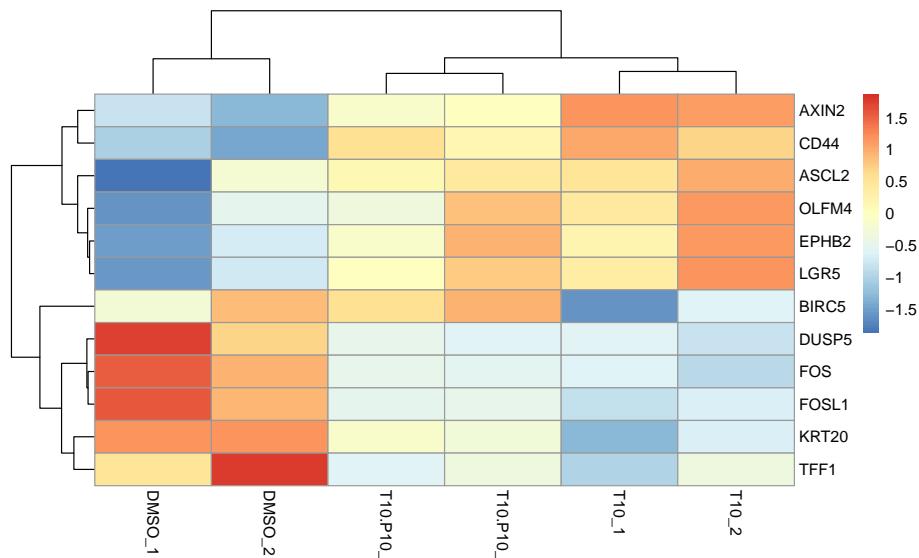
We observe similar results as we did based on the Illumina chips. We see that here there is in fact a difference of LGR5 expression across conditions, suggesting that the Illumina probe(s) might have not worked in the experiment above.

In addition, we plot genes of interest as a heat map with scaled rows.

```
## probes for genes of interest
probes_target_genes <- affy_data_anno %>% filter(Symbol %in% to_label) %>%
  group_by(Symbol, ProbeID) %>% summarise(mad=mad(expr)) %>% ungroup() %>%
  group_by(Symbol) %>% arrange(desc(mad)) %>% dplyr::slice(1) %>% ungroup() %>%
  .\$ProbeID

affy_data_anno %>% filter(ProbeID %in% probes_target_genes) %>%
  unite(experiment, treatment, rep, sep='_') %>%
  distinct(experiment, Symbol, expr) %>%
  spread(experiment, expr) %>% data.frame() %>%
  `rownames<-`(`NULL`) %>% column_to_rownames('Symbol') %>%
  pheatmap(scale='row')
```

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### 4.3 Gene set enrichment analysis

#### 4.3.1 Loading signatures

We want to understand if previously published gene signatures describing stemness and proliferative behaviour of cancer cells change when we treat samples with Trametinib. Specifically we refer to humanized signatures published by Merlos-Suarez et al (Merlos-Suarez et al. 2011). We need to first load these signatures from an R data object generated from the supplemental materials as downloaded from the original publication.

```
## loads an object called 'signatures'  
data('csc_signatures', package='MEKWnt2018')
```

#### 4.3.2 GSEA analysis

We perform gene set enrichment analysis using the Broad Institute's **GSEA** (Subramanian et al. 2007). An R version of the algorithm is implemented in the `fgsea` algorithm (??), which we use for analysis.

We want to visualize the results as a barcode plot. `fgsea` already implements a nice barcode plot, which we cusotomize a bit to adapt it according to our expectations.

```
custom_barcode_plot <- function(df, sig){  
  ## named vector of gene-level stats  
  stat_vector <- setNames(df$t, df$Symbol)  
  ## genes in signature  
  sig_genes <- signatures[[sig]]  
  
  ## generate barcode plot  
  bc_plot <- plotEnrichment(sig_genes, stat_vector)
```

```

## remove unwanted layers
bc_plot$layers <- list()

## add barcode at the bottom
lowest_pos <- min(bc_plot$data[,2])
dash_length <- abs(reduce(range(bc_plot$data[,2]), ` - `)*0.1)
middle <- which.min(abs(sort(df$t, decreasing=T)))

bc_plot_custom <- bc_plot + geom_segment(aes(x=x, xend=x), y=lowest_pos,
                                         yend=lowest_pos-dash_length) +
  geom_line(colour='#4daf4a') +
  geom_hline(yintercept=lowest_pos, colour="#cccccc") +
  geom_hline(yintercept=0, colour="#cccccc") + xlab('') +
  theme_classic() +
  geom_tile(data=tibble(rank=1:length(stat_vector),
                        y=lowest_pos-(1.25*dash_length)),
            aes(x=rank, y=y, fill=rank),
            width=1,
            height=0.5*dash_length) +
  scale_fill_gradient2(low ='#b2182b', high='#2166ac',
                       mid='#f7f7f7', midpoint = middle) +
  scale_x_continuous(expand = c(0, 0)) +
  scale_y_continuous(expand = c(0, 0)) +
  theme(panel.grid=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x = element_blank(),
        legend.position = 'none') +
  ggtitle(paste(sig, 'signature')) +
  ylab('Enrichment score')

return(bc_plot_custom)
}

```

#### 4.3.2.1 Organoid 19T - TRAM vs DMSO

We perform the enrichment analysis of gene signatures based on the Affymetrix data. We analyze the organoid 19 line examining the effects of various treatments on the gene signatures of interest. We first calculate GSEA statistics for each gene set. We then generate barcode plots and annotate them with P-values and normalized enrichment scores.

```

o19stats <- rescue_res_affy[[1]]
o19stats <- setNames(o19stats$t, o19stats$Symbol)

## run GSEA to get p-value and NES for each list
o19_fgsea <- fgsea(signatures,
                     o19stats, nperm=10000)

## generate plots
bc_plots_affy <- map(1:nrow(o19_fgsea), function(j){
  bcp <- custom_barcode_plot(rescue_res_affy[[1]], o19_fgsea$pathway[j]) +

```

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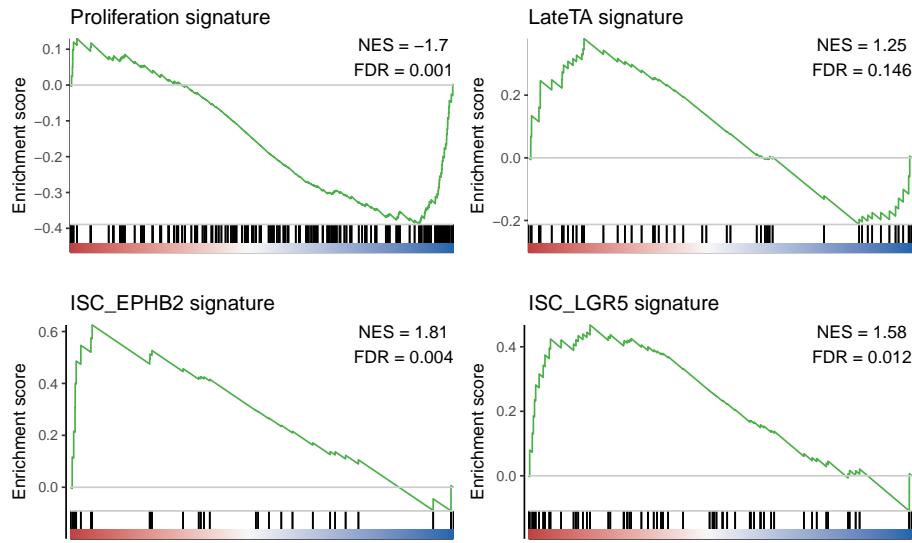
```

    annotate('text', x=Inf , y=Inf, hjust=1, vjust=1,
             label=paste('NES =', round(o19_fgsea$NES[j], 2),
                         '\nFDR =', round(o19_fgsea$padj[j], 3)))

    return(bcp)
  })

## plot to canvas
reduce(rev(bc_plots_affy), `+`) + plot_layout(ncol=2)

```



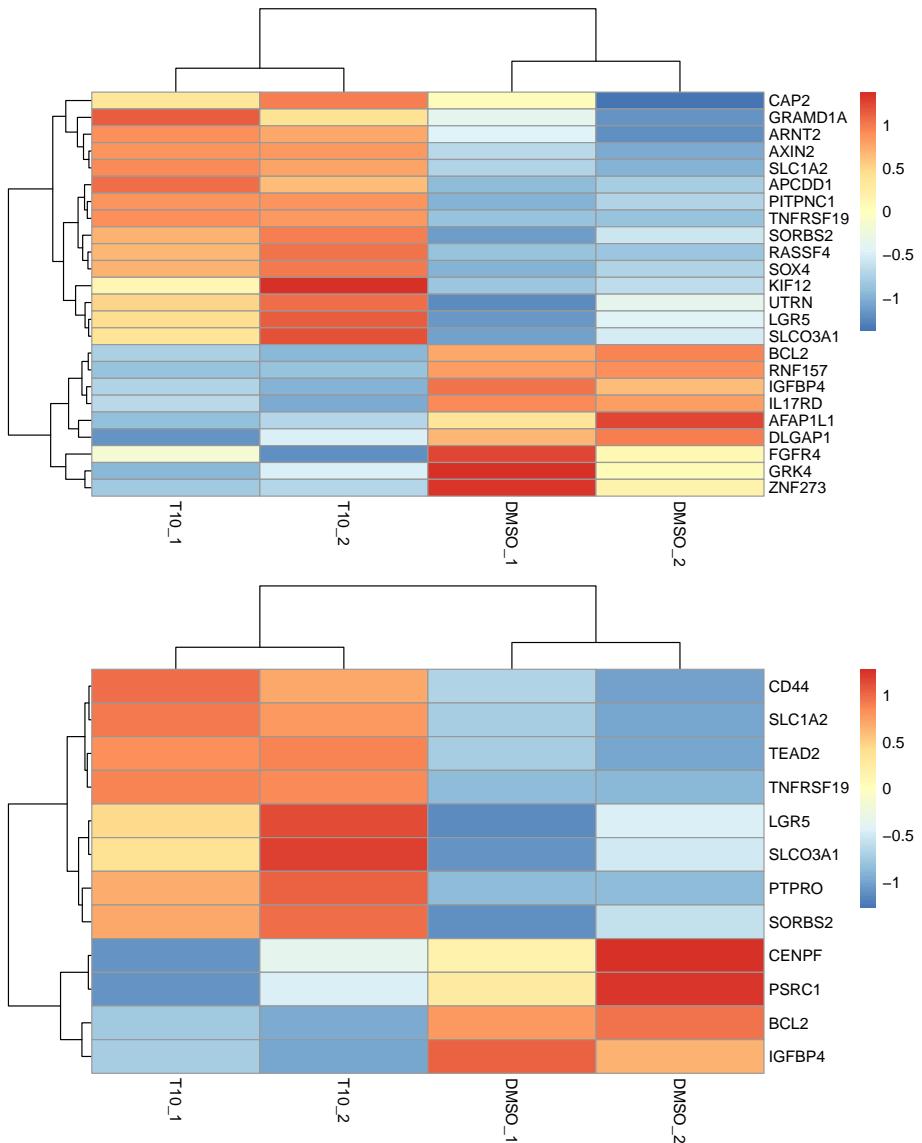
We can further visualize the signatures as heat maps to see information about transcriptomic changes at the gene level.

```

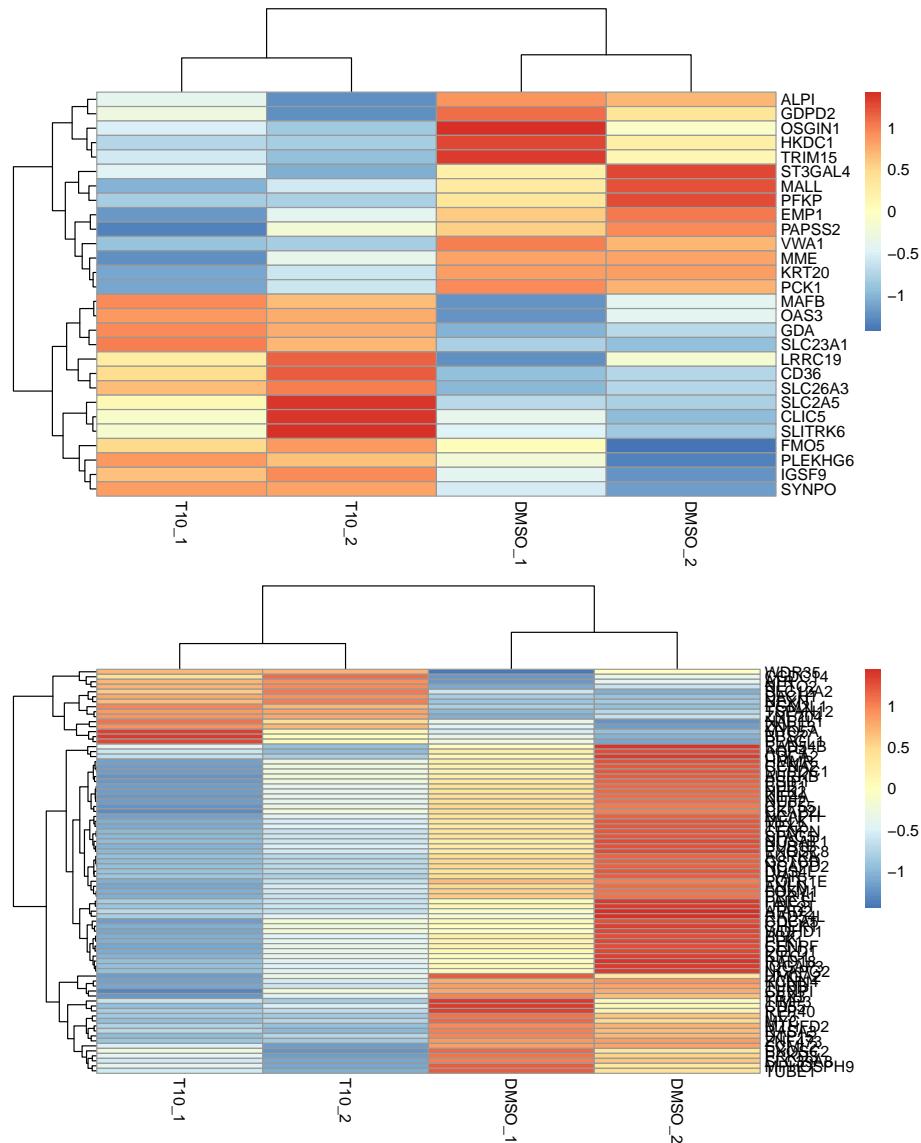
walk(signatures, function(sig){
  affy_data_anno %>% filter(treatment %in% c('DMSO', 'T10')) %>%
    inner_join(rescue_res_affy[[1]] %>% filter(adj.P.Val < 0.2) %>%
      group_by(Symbol) %>% arrange(desc(t)) %>% dplyr::slice(1) %>%
      ungroup() %>% distinct(ProbeID, Symbol)) %>%
    filter(Symbol %in% sig) %>%
    distinct(Symbol, treatment, rep, expr) %>%
    unite(sample, treatment, rep, sep = '_') %>% spread(sample, expr) %>%
    data.frame() %>% `rownames<-` (NULL) %>%
    column_to_rownames('Symbol') %>%
    pheatmap(scale='row')
})

```

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## MEK inhibition activates Wnt signalling and induces stem cell plasticity in colorectal cancer organoids



## 5 Session info

```
sessionInfo()
## R version 3.4.1 (2017-06-30)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Sierra 10.12.6
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.4/Resources/lib/libRlapack.dylib
##
## locale:
```

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```
## [1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] parallel stats      graphics grDevices utils      datasets methods
## [8] base
##
## other attached packages:
## [1] bindrcpp_0.2.2   forcats_0.3.0    stringr_1.3.0
## [4] dplyr_0.7.4       purrr_0.2.4     readr_1.1.1
## [7] tidyverse_1.2.1   tibble_1.4.2     tidyverse_1.2.1
## [10] fgsea_1.4.1      Rcpp_0.12.16   pheatmap_1.0.8
## [13] patchwork_0.0.1   lumi_2.30.0     Biobase_2.38.0
## [16] BiocGenerics_0.24.0 ggrepel_0.7.0   ggplot2_2.2.1.9000
## [19] limma_3.34.9     BiocStyle_2.6.1
##
## loaded via a namespace (and not attached):
## [1] readxl_1.0.0      backports_1.1.2
## [3] fastmatch_1.1-0   plyr_1.8.4
## [5] lazyeval_0.2.1    splines_3.4.1
## [7] BiocParallel_1.12.0 GenomeInfoDb_1.14.0
## [9] digest_0.6.15     foreach_1.4.4
## [11] BiocInstaller_1.28.0 htmltools_0.3.6
## [13] magrittr_1.5       memoise_1.1.0
## [15] Biostrings_2.46.0  annotate_1.56.2
## [17] modelr_0.1.1      matrixStats_0.53.1
## [19] siggenes_1.52.0   prettyunits_1.0.2
## [21] colorspace_1.3-2  rvest_0.3.2
## [23] blob_1.1.1        haven_1.1.1
## [25] xfun_0.1          crayon_1.3.4
## [27] RCurl_1.95-4.10   jsonlite_1.5
## [29] genefilter_1.60.0  bindr_0.1.1
## [31] GEOquery_2.46.15   survival_2.42-3
## [33] iterators_1.0.9   glue_1.2.0
## [35] registry_0.5      gtable_0.2.0
## [37] zlibbioc_1.24.0   XVector_0.18.0
## [39] DelayedArray_0.4.1 scales_0.5.0.9000
## [41] DBI_0.8            rngtools_1.2.4
## [43] xtable_1.8-2      progress_1.1.2
## [45] bumphunter_1.20.0  foreign_0.8-69
## [47] bit_1.1-12         mclust_5.4
## [49] preprocessCore_1.40.0 stats4_3.4.1
## [51] httr_1.3.1         RColorBrewer_1.1-2
## [53] pkgconfig_2.0.1     reshape_0.8.7
## [55] XML_3.98-1.9       locfit_1.5-9.1
## [57] tidyselect_0.2.4    labeling_0.3
## [59] rlang_0.2.0.9001   reshape2_1.4.3
## [61] AnnotationDbi_1.40.0 cellranger_1.1.0
## [63] munsell_0.4.3       tools_3.4.1
## [65] cli_1.0.0           RSQLite_2.1.0
## [67] broom_0.4.4         evaluate_0.10.1
## [69] yaml_2.1.18         knitr_1.20
```

## MEK inhibition activates Wnt signalling and induces stem cell plasticity in colorectal cancer organoids

```
## [71] bit64_0.9-7          beanplot_1.2
## [73] methylumi_2.24.1      nlme_3.1-137
## [75] doRNG_1.6.6           nor1mix_1.2-3
## [77] xml2_1.2.0            biomaRt_2.34.2
## [79] rstudioapi_0.7        compiler_3.4.1
## [81] affyio_1.48.0          stringi_1.1.7
## [83] GenomicFeatures_1.30.3 minfi_1.24.0
## [85] lattice_0.20-35       Matrix_1.2-14
## [87] psych_1.8.3.3         multtest_2.34.0
## [89] pillar_1.2.1          data.table_1.10.4-3
## [91] bitops_1.0-6           rtracklayer_1.38.3
## [93] GenomicRanges_1.30.3  R6_2.2.2
## [95] affy_1.56.0            bookdown_0.7
## [97] RMySQL_0.10.14         KernSmooth_2.23-15
## [99] gridExtra_2.3           IRanges_2.12.0
## [101] nleqslv_3.3.1          codetools_0.2-15
## [103] MASS_7.3-49             assertthat_0.2.0
## [105] SummarizedExperiment_1.8.1 openssl_1.0.1
## [107] pkgmaker_0.22           rprojroot_1.3-2
## [109] withr_2.1.2            GenomicAlignments_1.14.2
## [111] Rsamtools_1.30.0         mnormt_1.5-5
## [113] S4Vectors_0.16.0         GenomeInfoDbData_1.0.0
## [115] mgcv_1.8-23              hms_0.4.2
## [117] quadprog_1.5-5           grid_3.4.1
## [119] base64_2.0                rmarkdown_1.9
## [121] illuminaio_0.20.0          lubridate_1.7.4
```

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