Time-resolved mapping of genetic interactions to model rewiring of signaling pathways

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Abstract

Context-dependent changes in genetic vulnerabilities are important to understand the wiring of cellular pathways and variations in different environmental conditions. However, methodological frameworks to investigate the plasticity of genetic networks over time or in response to external stresses are lacking. To analyze the plasticity of genetic interactions, we performed an arrayed combinatorial RNAi screen in Drosophila cells at multiple time points and after pharmacological inhibition of Ras signaling activity. Using an image-based morphology assay to capture a broad range of phenotypes, we assessed the effect of 12768 pairwise RNAi perturbations in six different conditions. We found that genetic interactions form in different trajectories and developed an algorithm, termed MODIFI, to analyze how genetic interactions rewire over time. Using this framework, we identified more statistically significant interactions compared to end-points assays and further observed several examples of context-dependent crosstalk between signaling pathways such as an interaction between Ras and Rel which is dependent on MEK activity.

Contents

About

This vignette summarizes all source code needed to reproduce all data containing main and supplementary figures. The networks shown in figure 6 can be reproduced using the Cytoscape Session file in the supplements.

1 Analysis of the genome wide chemo-genetic screen.

Data used in this analysis were per-well feature data extracted using the pipeline in "Feature_extraction_genome_wide_screens.Rmd" and the genome-wide HD3 dsRNA library annotation.

Let's define functions that do a B-Score normalization and reduce the chessboard like positional analysis bias.

```
conditional_mean=function(x,y){
  if(!any(is.na(x))){
    if(!any(is.na(y))){
      return((x+y)/2)
    }else{
      return((x))
    }
  }else{
    if(!any(is.na(y))){
      return((y))
    }else{
      return(NA)
    }
  }
}

b_score_norm=function(x){
  if(any(is.numeric(x))){
    testm=matrix(0,16,24)
    dimnames(testm)=list(LETTERS[1:16],1:24)
    for(i in LETTERS[1:16]){
      for(j in 1:24){
        testm[i,j]=x[paste0(i,j)]
      }
    }
    x1=LETTERS[1:16][seq(1,16,by=2)]
    x2=LETTERS[1:16][seq(2,16,by=2)]
    y1=c(1:24)[seq(1,24,by=2)]
    y2=c(1:24)[seq(2,24,by=2)]
    a=mean(c(testm[x1,y1],testm[x2,y2]),na.rm=T)
    b=mean(c(testm[x2,y1],testm[x1,y2]),na.rm=T)
    if(!any(is.na(c(a,b)))){
```

```
      if(a>b){
        testm[x1,y1]=testm[x1,y1]-abs(b-a)
        testm[x2,y2]=testm[x2,y2]-abs(b-a)
      }else{
        testm[x2,y1]=testm[x2,y1]-abs(b-a)
        testm[x1,y2]=testm[x1,y2]-abs(b-a)
      }
    }
    medpol=medpolish(testm,na.rm = T,trace.iter =F)$residuals
    medpol=medpol/(mad(medpol,na.rm = T))
    y=x
    for(i in LETTERS[1:16]){
      for(j in 1:24){
        y[paste0(i,j)]=medpol[i,j]
      }
    }
    return(y)
  }else{
    return(x)
  }
}

## load DMSO and MEK Screens (Geldanamycin and Diap1 co-perturbation screens
#are also loaded but only included for reasons of completeness)
data('genome_wide_gene_drug_feature_data_raw', package='MODIFIdata')

genome_wide_gene_drug_feature_data=genome_wide_gene_drug_feature_data[
  intersect(
    grep("^10*|^20*",
         genome_wide_gene_drug_feature_data$plate),
    grep("^S18$|^S19$|^S17$|^S16$|^S21$|^S20$|^S30$|^S22$|^S23$",
         genome_wide_gene_drug_feature_data$screenID)
  )
  ,]



screenIDs=c("S16","S17","S18","S19","S20","S21","S30","S22","S23")
plates = as.character(c(1001:1047,2001:2041))
wells = sort(unique(genome_wide_gene_drug_feature_data$well))

feature.list=as.list(names(genome_wide_gene_drug_feature_data)[!(
  names(genome_wide_gene_drug_feature_data) %in% c("well","plate","screenID"))])
names(feature.list)=names(genome_wide_gene_drug_feature_data)[!(
  names(genome_wide_gene_drug_feature_data) %in% c("well","plate","screenID"))]

arraylist = lapply(
  feature.list,
  function(feature){
    acast( genome_wide_gene_drug_feature_data,
           well ~ plate ~ screenID,
```

```
            value.var =feature,
            drop=TRUE)
  }
)

myarray=abind(arraylist,along=0)
names(dimnames(myarray))=c("feature","well","plate","screen.id")

myarray=myarray[-grep("dark|fussel|cx|cy|theta",dimnames(myarray)$feature),,,]
```

create a list of control names with associated wells

```
well_array = list(1:8, 1:2, 1:2, 1:2, 1:2, 1:2)
names(well_array) = c("gfp", "rluc", "drk", "dmek", "pten", "gap1")
well_array$gfp = c("A1","C2","E2","G2","I2", "K2", "M2", "O2")
well_array$rluc = c("A2","B1")
well_array$drk = c("B2","J2")
well_array$dmek = c("D2","L2")
well_array$pten = c("F2","N2")
well_array$gap1 = c("H2","P2")

#creates a list of screen.id names
screen_array = names(myarray[1,1,1,])

#two plates entirely failed as assessed by manual inspection ->
#probable contamination
myarray[,,"1013","S30"]=NA
myarray[,,"2028","S19"]=NA
feature_choice=c("actin.b.mad.sd","actin.b.mad.tmean","actin.b.mean.sd",
                 "actin.b.mean.tmean","actin.h.cor.s1.sd",
                 "actin.h.cor.s1.tmean","actin.h.ent.s1.sd",
                 "actin.h.ent.s1.tmean","actin.m.eccentricity.sd",
                 "actin.m.eccentricity.tmean","actin.s.perimeter.sd",
                 "actin.s.perimeter.tmean","actin.s.radius.mean.sd",
                 "actin.s.radius.mean.tmean","actin.s.radius.sd.sd",
                 "actin.s.radius.sd.tmean","cell_number","dist.20.nn.sd",
                 "dist.20.nn.tmean","DNA.b.mad.sd","DNA.b.mad.tmean",
                 "DNA.b.mean.tmean","DNA.h.ent.s1.sd","DNA.h.ent.s1.tmean",
                 "DNA.h.var.s1.tmean","DNA.m.eccentricity.tmean",
                 "DNA.s.perimeter.sd","DNA.s.perimeter.tmean",
                 "DNA.s.radius.mean.sd","DNA.s.radius.mean.tmean",
                 "DNA.s.radius.sd.sd","DNA.s.radius.sd.tmean",
                 "nuclear.displacement.tmean","tubulin.b.mad.sd",
                 "tubulin.b.mad.tmean","tubulin.b.mean.sd",
                 "tubulin.b.mean.tmean","tubulin.b.sd.tmean")
```

2 Data normalization

```
#new_features_data is the data of the reduced number of clusters to
#be used in further analysis
wnorm_array <- myarray[feature_choice,,,]
#Add the minimum of each feature to the respective feature,
#to reduce problems with log()
for(feature in dimnames(wnorm_array)$feature){
  wnorm_array[feature,,,] <-
    wnorm_array[feature,,,] + abs(min(wnorm_array[feature,,,],na.rm=TRUE))
}

#Transform the data with log(x+1)
log_array <- log1p(wnorm_array) #the actual data for later usage

#dividing all well by the plates median, setting the plate to 0, if the median
#is 0 and do nothing if the median is NA
new_features_data <- apply(wnorm_array, c(1,3,4), b_score_norm)

#rearranges the array to the same format as myarray
new_features_data <- filtered_wells <- aperm(new_features_data, c(2,1,3,4))

#Rename the manipulated dimension
names(dimnames(new_features_data))[2] <- "well"
```

3 Normalization for positional biases

```
#we filter out technically failed wells
bad_wells=which(is.na(apply(new_features_data,c(2,3,4),function(x){
  if(max(x,na.rm = T)>50){return(NA)}else{return(1)}})),arr.ind = T)
for(i in 1:nrow(bad_wells)){
  filtered_wells[,bad_wells[i,"well"],bad_wells[i,"plate"]
                 ,bad_wells[i,"screen.id"]]=NA
}

#we calculate average data for each treatment thread is Diap1 knockdown, hsp90
#is Geldanamycin treatment, control is DMSO treatment, mek is MEK inhibitor
#treatment
mean_data <- filtered_wells[,,,c("S16", "S18", "S20","S22")]
dimnames(mean_data)$screen.id <- c("control", "mek", "hsp90","thread")

for(well in dimnames(filtered_wells)$well){
  for(plate in dimnames(filtered_wells)$plate){
    mean_data[,well,plate,"control"]=
      conditional_mean(filtered_wells[,well,plate,"S16"],
                       filtered_wells[,well,plate,"S17"])
```

```
    mean_data[,well,plate,"mek"]=
      conditional_mean(filtered_wells[,well,plate,"S18"],
                       filtered_wells[,well,plate,"S19"])
    mean_data[,well,plate,"hsp90"]=
      conditional_mean(filtered_wells[,well,plate,"S20"],
                       filtered_wells[,well,plate,"S21"])
    mean_data[,well,plate,"thread"]=
      conditional_mean(filtered_wells[,well,plate,"S22"],
                       filtered_wells[,well,plate,"S23"])
  }
}
```

4 Calculation of distance and dsRNA wise quality metrics

Do not execute if you are in a hurry. Takes about 60-90 min to run.

```
#read in the annotations
data('HD3_annotations', package='MODIFIdata')
data('fbgn_knowledgesum', package='MODIFIdata')
data('RNA_expression_values', package='MODIFIdata')

row.names(go)=go[,1]

#creating the list of all controls to be removed from the distance calculation
well_to_gene_mapping=list()
annotation_list=list()
for(i in 1:nrow(annotation)){
  well_to_gene_mapping[[as.character(annotation[i,1])]][[annotation[i,2]]]=
    annotation[i,"gene"]
  annotation_list[[paste(as.character(annotation[i,1]),annotation[i,2],
                         annotation[i,"gene"], sep = ".")]]= annotation[i,]
}


distances_to_gfp=list()
strongest_distances_to_gfp=list()
#Distance between each screen group and the gfp control
for(perturbation in dimnames(mean_data)$screen.id){
  #generating the mean gfp feature vector for the perturbation
  mean_gfp_control <- list()
  for(feature in dimnames(new_features_data)$feature){
    mean_gfp_control[[feature]] <- mean(mean_data[feature,well_array$gfp,,
                                                  perturbation], na.rm = TRUE)
  }
  mean_gfp_control=unlist(mean_gfp_control)
  #calculating the distances of each well in the first screen group
  #to the gfp feature vector:
```

```
  dist_control <- apply(mean_data[,,,perturbation],c(2,3),
                        function(x){sqrt(sum((x-mean_gfp_control)^2))})
  strongest_dist_control <- apply(mean_data[,,,perturbation],c(2,3),
        function(x){names(sort((x-mean_gfp_control)^2,decreasing=T)[c(1,2)])})
  #extracting the features with the biggest distance to the gfp controls
  for(i in rownames(dist_control)){
    for(j in colnames(dist_control)){
      distances_to_gfp[[perturbation]][[
      paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= dist_control[i,j]
      strongest_distances_to_gfp[[perturbation]][[
      paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]=
      paste(strongest_dist_control[1,i,j],strongest_dist_control[2,i,j],sep="_")
    }
  }
}

#Calculating the distances between the control screens and the mek screens
dist_mek <- list()

distmek=apply(mean_data,c(2,3),function(x){
  sqrt(sum((x[,"control"]-x[,"mek"])^2))})
for(i in rownames(distmek)){
  for(j in colnames(distmek)){
    dist_mek[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]=
      distmek[i,j]
  }
}
#Calculating the distances between the control screens and the hsp90 screens
dist_hsp <- list()

disthsp=apply(mean_data,c(2,3),function(x){
  sqrt(sum((x[,"control"]-x[,"hsp90"])^2))})
for(i in rownames(disthsp)){
  for(j in colnames(disthsp)){
    dist_hsp[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]=
      disthsp[i,j]
  }
}
#Calculating the distances between the control screens and the thread screens
dist_thread <- list()

distthread=apply(mean_data,c(2,3),function(x){
  sqrt(sum((x[,"control"]-x[,"thread"])^2))})
for(i in rownames(distthread)){
  for(j in colnames(distthread)){
    dist_thread[[
      paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= distthread[i,j]
  }
}

#Checking for reproducability between 1k and 2k plates via correlation
```

```
fbgn=list()

for(i in 1:nrow(annotation)){
  if(is.null( fbgn[[annotation[i,"fbgn"]]][[1]])){
    fbgn[[annotation[i,"fbgn"]]][[1]]=annotation[i,c("well","plate","gene")]
  }else{
    fbgn[[annotation[i,"fbgn"]]][[2]]=annotation[i,c("well","plate","gene")]
  }
}
for(i in names(fbgn)){
  if(!is.na(i)){
    if(length(fbgn[[i]])<2){
      fbgn[[i]][[2]]=c(NA,NA,NA)
    }
  }
}
designs=list()

for(i in names(fbgn)){
  if(!is.na(i)){
    designs[[paste(fbgn[[i]][[1]][2],
                   fbgn[[i]][[1]][1],
                   fbgn[[i]][[1]][3],sep=".")]]=
      paste(fbgn[[i]][[2]][2],fbgn[[i]][[2]][1],fbgn[[i]][[2]][3],sep=".")
    designs[[paste(fbgn[[i]][[2]][2],
                   fbgn[[i]][[2]][1],
                   fbgn[[i]][[2]][3],sep=".")]]=
      paste(fbgn[[i]][[1]][2],fbgn[[i]][[1]][1],fbgn[[i]][[1]][3],sep=".")
  }
}
x=list()
design1=new_features_data[c("cell_number","actin.s.perimeter.tmean",
                            "actin.m.eccentricity.tmean",
                            "DNA.s.perimeter.tmean","DNA.m.eccentricity.tmean",
                            "tubulin.b.mad.tmean"),,,"S30"]
for(wellplategene in names(designs)){
  if(wellplategene != "NA.NA.NA" & designs[[wellplategene]] != "NA.NA.NA"){
    x[["S30"]][[wellplategene]] <-
      cor(design1[,unlist(strsplit(wellplategene,split = "\\."))[2],
                  unlist(strsplit(wellplategene,split = "\\."))[1]],
          design1[,unlist(strsplit(designs[[wellplategene]],split = "\\."))[2],
                  unlist(strsplit(designs[[wellplategene]],split = "\\."))[1]],
                                      use = "pairwise.complete.obs")
  }
}
design1=new_features_data[c("cell_number","actin.s.perimeter.tmean",
                            "actin.m.eccentricity.tmean",
                            "DNA.s.perimeter.tmean","DNA.m.eccentricity.tmean",
                            "tubulin.b.mad.tmean"),,,"S17"]
for(wellplategene in names(designs)){
  if(wellplategene != "NA.NA.NA" & designs[[wellplategene]] != "NA.NA.NA"){
```

```
      x[["S17"]][[wellplategene]] <- cor(
        design1[,unlist(strsplit(wellplategene,split = "\\."))[2],
                unlist(strsplit(wellplategene,split = "\\."))[1]],
        design1[,unlist(strsplit(designs[[wellplategene]],split = "\\."))[2],
                unlist(strsplit(designs[[wellplategene]],split = "\\."))[1]],
          use = "pairwise.complete.obs")
    }
  }
  #Correlation between screen 16 and 17
  #Calculating the distances between the control screens and the hsp90 screens
  z16_17 <- list()

  z1617=apply(new_features_data,c(2,3),function(x){cor(x[,"S16"],x[,"S17"],
                                                       use="pairwise.complete.obs")})
  for(i in rownames(disthsp)){
    for(j in colnames(disthsp)){
      z16_17[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= z1617[i,j]
    }
  }

  z18_19 <- list()

  z1819=apply(new_features_data,c(2,3),function(x){cor(x[,"S18"],x[,"S19"],
                                                       use="pairwise.complete.obs")})
  for(i in rownames(disthsp)){
    for(j in colnames(disthsp)){
      z18_19[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= z1819[i,j]
    }
  }

  z20_21 <- list()

  z2021=apply(new_features_data,c(2,3),function(x){cor(x[,"S20"],x[,"S21"],
                                                      use="pairwise.complete.obs")})
  for(i in rownames(disthsp)){
    for(j in colnames(disthsp)){
      z20_21[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= z2021[i,j]
    }
  }

  z22_23 <- list()

  z2223=apply(new_features_data,c(2,3),function(x){cor(x[,"S22"],x[,"S23"],
                                                      use="pairwise.complete.obs")})
  for(i in rownames(disthsp)){
    for(j in colnames(disthsp)){
      z22_23[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= z2223[i,j]
    }
  }

  z18_30 <- list()
```

```
z1830=apply(new_features_data,c(2,3),function(x){cor(x[,"S18"],x[,"S30"],
                                                    use="pairwise.complete.obs")})
for(i in rownames(disthsp)){
  for(j in colnames(disthsp)){
    z18_30[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= z1830[i,j]
  }
}

z19_30 <- list()

z1930=apply(new_features_data,c(2,3),function(x){cor(x[,"S19"],x[,"S30"],
                                                   use="pairwise.complete.obs")})
for(i in rownames(disthsp)){
  for(j in colnames(disthsp)){
    z19_30[[paste(j,i,well_to_gene_mapping[[j]][[i]], sep = ".")]]= z1930[i,j]
  }
}

res=list()

for(wellplategene in names(dist_mek)){
  temp=c()
  temp=c(temp,annotation_list[[wellplategene]])
  for(pert in names(distances_to_gfp)){
    temp=c(temp,distances_to_gfp[[pert]][[wellplategene]])
  }
  for(pert in names(distances_to_gfp)){
    temp=c(temp,strongest_distances_to_gfp[[pert]][[wellplategene]])
  }
  if(wellplategene %in% names(x$S17)){
    temp=c(temp,
           dist_mek[[wellplategene]],
           dist_hsp[[wellplategene]],
           dist_thread[[wellplategene]],
           x$S17[[wellplategene]],
           x$S30[[wellplategene]],
           z16_17[[wellplategene]],
           z18_19[[wellplategene]],
           z20_21[[wellplategene]],
           z22_23[[wellplategene]],
           z18_30[[wellplategene]],
           z19_30[[wellplategene]],
           designs[[wellplategene]])

  }else{
    temp=c(temp,
           dist_mek[[wellplategene]],
           dist_hsp[[wellplategene]],
           dist_thread[[wellplategene]],
           NA,
```

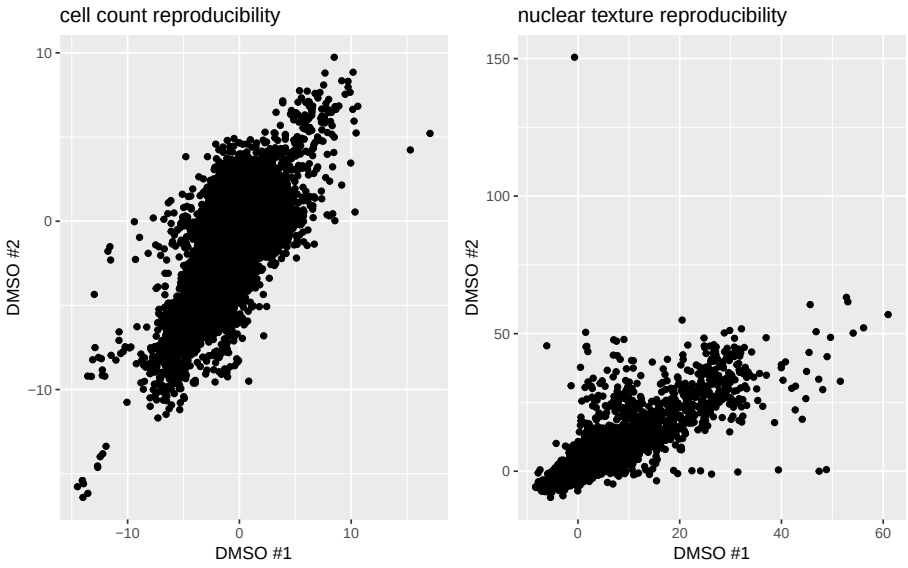
```
            NA,
            z16_17[[wellplategene]],
            z18_19[[wellplategene]],
            z20_21[[wellplategene]],
            z22_23[[wellplategene]],
            z18_30[[wellplategene]],
            z19_30[[wellplategene]],
            NA)
  }
  temp=c(temp,expression_values[as.character(
    annotation_list[[wellplategene]]["fbgn"]),1])
  temp=c(temp,go[as.character(annotation_list[[wellplategene]]["fbgn"]),2])
  #print(length(temp))
  res[[wellplategene]]=temp
}

res=do.call("rbind.data.frame",res)
names(res)=c("plate","well","id","content","query_id","fbgn","gene",
        paste(names(distances_to_gfp),"dist_to_gfp_Q1",sep="_"),
        paste(names(strongest_distances_to_gfp),
              "strongest_dist_to_gfp_Q2",sep="_"),"dist_to_mek_Q3",
        "dist_to_hsp_Q3","dist_to_thread_Q3","design_corr_S17_Q4",
        "design_corr_S30_Q4","biol_corr_DMSO1_DMSO2_Q5",
        "biol_corr_MEK1_MEK2_Q5","biol_corr_HSP1_HSP2_Q5",
        "biol_corr_MEK1_MEK3_Q5","biol_corr_MEK2_MEK3_Q5",
        "biol_corr_THREAD1_THREAD2_Q5","design_pair_position",
        "log2_expression_Q6","go_knowledge_sum_Q7")

#creating the lists for the shiny app / hit identification
#gene_feature contains all genes/wells on all plates with all screens
columns <- list()
i <- 1
setup <- melt(new_features_data[1,,,], value.name = feature,
              varnames = c("well", "plate", "screen"),as.is = T)
for(feature in names(new_features_data[,1,1,1])){
  columns[[feature]] <- melt(new_features_data[feature,,,])[,4]
  i <- i + 1
}
columns <- append(columns, setup[1:3],0)

columns[["merger"]] <- paste(columns[["well"]],columns[["plate"]] ,sep=".")
annotation[["merger"]] <- paste(annotation[["well"]],annotation[["plate"]] ,
                                sep=".")

gene_feature <- merge(as.data.frame(columns),annotation[,c("gene","merger")],
                      by=c("merger"))
gene_feature <- gene_feature[-1]
gene_feature=cbind(gene_feature[,"gene"],gene_feature)
gene_feature <- gene_feature[-ncol(gene_feature)]
names(gene_feature)[1]="gene"
```

```
for(i in names(res)){res[[i]]=as.character(res[[i]])}
for(i in c("control_dist_to_gfp_Q1"  ,
           "mek_dist_to_gfp_Q1"    ,
           "hsp90_dist_to_gfp_Q1"    ,
           "dist_to_mek_Q3"           ,
           "dist_to_hsp_Q3"           ,
           "design_corr_S17_Q4"       ,
           "design_corr_S30_Q4"       ,
           "biol_corr_DMSO1_DMSO2_Q5",
           "biol_corr_MEK1_MEK2_Q5"   ,
           "biol_corr_HSP1_HSP2_Q5"   ,
           "biol_corr_MEK1_MEK3_Q5"   ,
           "biol_corr_MEK2_MEK3_Q5"   ,
           "log2_expression_Q6"      ,
           "go_knowledge_sum_Q7",
           "dist_to_thread_Q3",
           "biol_corr_THREAD1_THREAD2_Q5" ,
           "thread_dist_to_gfp_Q1"
)){
  res[[i]]=as.numeric(res[[i]])
}
res$differnces_in_phenotype_strength_ctrl_mek_Q10=
  res$mek_dist_to_gfp_Q1-res$control_dist_to_gfp_Q1
for(i in c("control_dist_to_gfp_Q1"  ,
           "mek_dist_to_gfp_Q1"    ,
           "hsp90_dist_to_gfp_Q1"    ,
           "dist_to_mek_Q3"           ,
           "dist_to_hsp_Q3"           ,
           "dist_to_thread_Q3",
           "thread_dist_to_gfp_Q1",
           "differnces_in_phenotype_strength_ctrl_mek_Q10"
)){
  res[[i]]=(res[[i]]-mean(res[[i]],na.rm = T))/(sd(res[[i]],na.rm = T))
}

res$biol_corr_max_Q8=apply(res[,c("biol_corr_DMSO1_DMSO2_Q5",
                                  "biol_corr_MEK1_MEK2_Q5"   ,
                                  "biol_corr_HSP1_HSP2_Q5"   ,
                                  "biol_corr_MEK1_MEK3_Q5"   ,
                                  "biol_corr_MEK2_MEK3_Q5")],1,max,na.rm=T)
res$design_corr_max_Q9=apply(res[,c(
  "design_corr_S17_Q4"       ,
  "design_corr_S30_Q4"       )],1,max,na.rm=T)


for(i in names(gene_feature)){gene_feature[[i]]=as.character(gene_feature[[i]])}
for(i in names(gene_feature)[5:length(gene_feature)]){
  gene_feature[[i]]=as.numeric(gene_feature[[i]])
}
```

5 Figure 2-supplemental figure 8A: Plot correlations of screens

Screening sets 16 & S17 are DMSO treated genome wide screens. Screening sets 18 & S19 are MEKi treated genome wide screens.

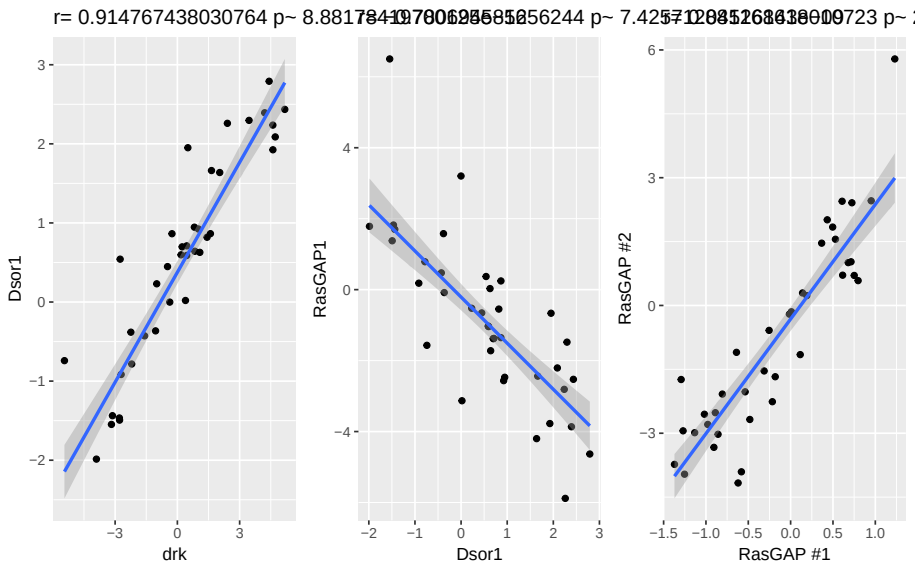
```
a<-gene_feature %>%
  tbl_df() %>%
  filter(screen %in% c("S18","S19","S16","S17")) %>%
  gather(feature,value,-gene,-well,-plate,-screen) %>%
  spread(screen,value) %>%
  filter(feature=="cell_number") %>%
  ggplot(aes(x=S16,y=S17)) +
    labs(x="DMSO #1",y="DMSO #2") +
    ggtitle("cell count reproducibility") +
    geom_point()

b<-gene_feature %>%
  tbl_df() %>%
  filter(screen %in% c("S18","S19","S16","S17")) %>%
  gather(feature,value,-gene,-well,-plate,-screen) %>%
  spread(screen,value) %>%
  filter(feature=="DNA.h.var.s1.tmean") %>%
  ggplot(aes(x=S16,y=S17)) +
    labs(x="DMSO #1",y="DMSO #2") +
    ggtitle("nuclear texture reproducibility") +
    geom_point()

a+b
```



6 Figure 2-supplemental figure 8B: Plot correlations of Ras pathway genes

```
tocor<-gene_feature %>%
  tbl_df() %>%
  filter(screen %in% c("S18"),gene %in% c("Dsor1","drk")) %>%
  group_by(gene) %>%
  summarise_if(is.numeric,mean,na.rm=T) %>%
  gather(feature,value,-gene) %>%
  spread(gene,value) %>%
  dplyr::select(-feature) %>%
  drop_na() %>%
  as.data.frame()

a <- tocor %>%
  ggplot(aes(x=.[[1]],y=.[[2]])) +
    geom_point() +
    geom_smooth(method = "rlm") +
    labs(x="drk",y="Dsor1") +
    ggtitle(paste0("r= ",
                   rcorr(x=tocor[[1]],y=tocor[[2]],
                                type = "pearson")$r[2],
                   " p~ ",rcorr(x=tocor[[1]],y=tocor[[2]],
                                type = "pearson")$P[2] ))

tocor<-gene_feature %>%
  tbl_df() %>%
  filter(screen %in% c("S18"),gene %in% c("Dsor1","RasGAP1")) %>%
  group_by(gene) %>%
  summarise_if(is.numeric,mean,na.rm=T) %>%
  gather(feature,value,-gene) %>%
  spread(gene,value) %>%
  dplyr::select(-feature) %>%
  drop_na() %>%
  as.data.frame()

b <- tocor %>%
  ggplot(aes(x=.[[1]],y=.[[2]])) +
    geom_point() +
    geom_smooth(method = "rlm") +
    labs(x="Dsor1",y="RasGAP1") +
    ggtitle(paste0("r= ",rcorr(x=tocor[[1]],y=tocor[[2]],
                               type = "pearson")$r[2],
                   " p~ ",rcorr(x=tocor[[1]],y=tocor[[2]],
                                type = "pearson")$P[2] ))

tocor <-gene_feature %>%
  tbl_df() %>%
  filter(screen %in% c("S18","S19"),gene %in% c("RasGAP1")) %>%
```

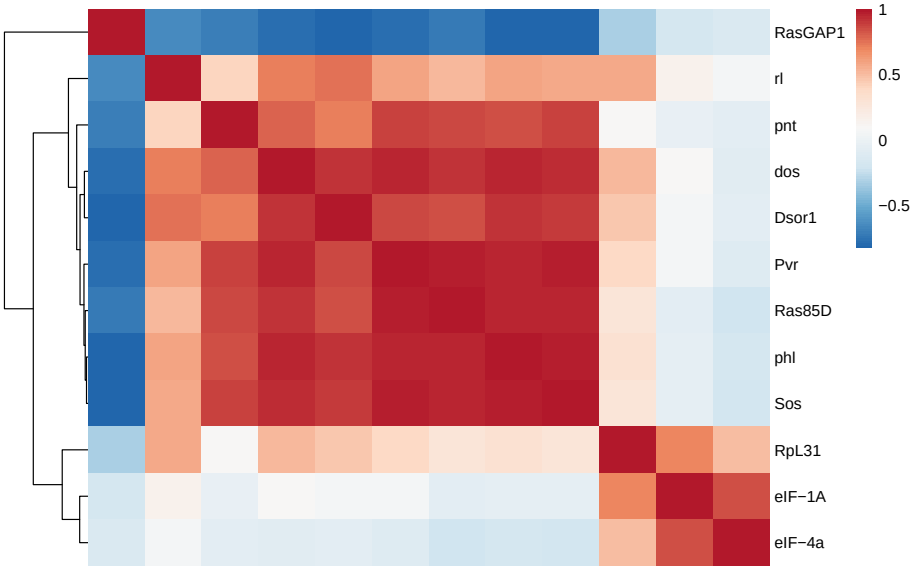
```
  group_by(well,screen) %>%
  filter(n()==2) %>%
  ungroup() %>%
  dplyr::select(-gene,-well) %>%
  gather(feature,value,-plate,-screen) %>%
  group_by(plate,feature) %>%
  summarise(value=mean(value,na.rm=T)) %>%
  spread(plate,value) %>%
  drop_na() %>%
  dplyr::select(-feature)

c <- tocor %>%
  ggplot(aes(x=.[[1]],y=.[[2]])) +
    geom_point() +
    geom_smooth(method = "rlm") +
    labs(x="RasGAP #1",y="RasGAP #2") +
    ggtitle(paste0("r= ",rcorr(x=tocor[[1]],y=tocor[[2]],
                               type = "spearman")$r[2],
                    " p~ ",rcorr(x=tocor[[1]],y=tocor[[2]],
                                 type = "spearman")$P[2] ))

a + b + c
```



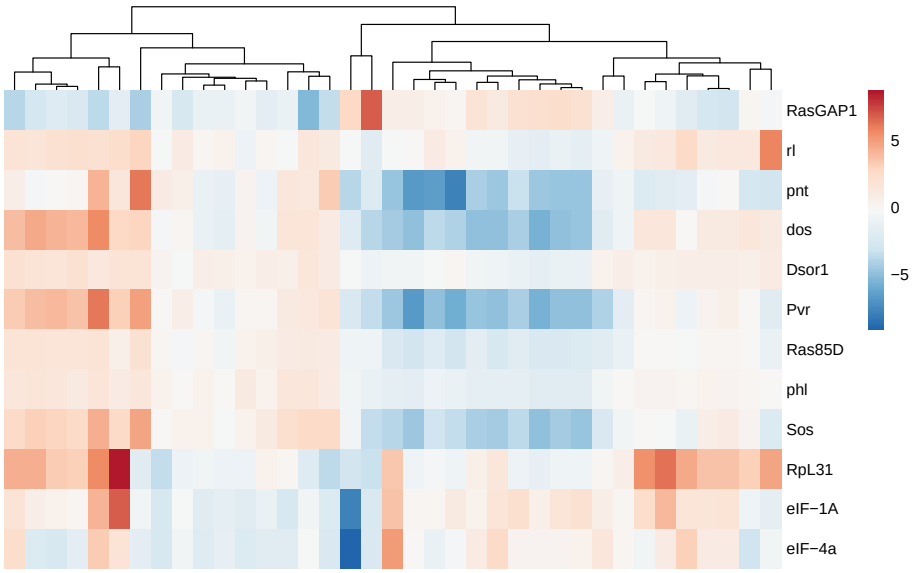
7 Figure 2-supplemental figure 8C&D: Plot heatmap of Ras pathway

```
toplot<-gene_feature %>%
  tbl_df() %>%
  filter(screen %in% c("S18","S19"),gene %in% c("Ras85D","phl","rl","pnt",
```

```
                                                    "Sos","dos","Pvr","RasGAP1",
                                                    "Dsor1","eIF-4a","eIF-1A",
                                                    "RpL31")) %>%
    gather(feature,value,-gene,-well,-plate,-screen) %>%
    filter(feature!="actin.h.cor.s1.sd") %>%
    group_by(gene,feature) %>%
    summarise(value=mean(value,na.rm=T)) %>%
    spread(gene,value) %>%
    as.data.frame()

  row.names(toplot)=toplot$feature

  ordering <- cor(toplot[,-1]) %>% pheatmap::pheatmap( color =
                                colorRampPalette(rev(brewer.pal(n = 7, name =
    "RdBu")))(100),border_color = NA,treeheight_col = 0,show_colnames = F)
```



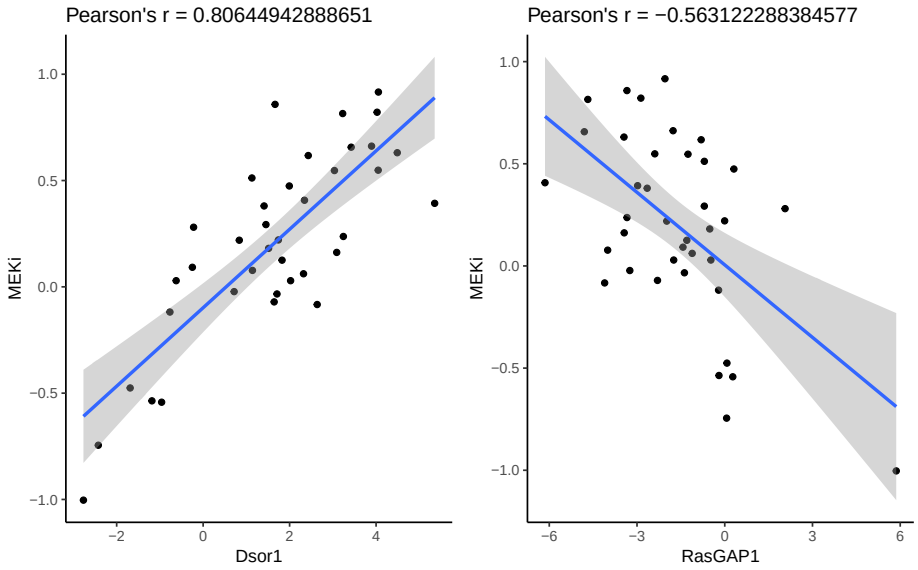

```
  feature_heatmap<-pheatmap::pheatmap(t(toplot[,-1])[ordering$tree_row$order,],#
                    cluster_rows = F,show_colnames = F,color =
                      colorRampPalette(rev(brewer.pal(n = 7, name ="RdBu")
                                       ))(100),border_color = NA)
```



8 Figure 2-supplemental figure 6B: Correlation of biological and chemical perturbation of Ras signaling

```
gene_feature %<>% tbl_df() %>% filter(screen %in% c("S16","S17","S18","S19"))
a<-gene_feature  %>%
  filter(well %in% c("B1","A2"),screen %in% c("S18"),
                            grepl("10*",plate)) %>%
  gather(feature,value,-gene,-well,-screen,-plate) %>%
  group_by(feature) %>%
  summarise(MEKi=median(value,na.rm=T))

b<-gene_feature  %>%
  filter(well %in% c("D2","L2"),screen %in% c("S16","S17"),
                            grepl("10*",plate)) %>%
  gather(feature,value,-gene,-well,-screen,-plate) %>%
  group_by(feature) %>% summarise(Dsor1=median(value,na.rm=T))

c<-left_join(a,b) %>%
  ggplot(aes(x=Dsor1,y=MEKi)) +
  geom_point() +
  geom_smooth(method="lm") +
  ggtitle(paste0("Pearson's r = ",cor(b$Dsor1,a$MEKi))) +
  theme_classic()

b<-gene_feature  %>%
  filter(well %in% c("H2","P2"),screen %in% c("S16","S17"),
         grepl("10*",plate)) %>%
  gather(feature,value,-gene,-well,-screen,-plate) %>%
  group_by(feature) %>%
```

```
  summarise(RasGAP1=median(value,na.rm=T))

d<-left_join(a,b) %>%
  ggplot(aes(x=RasGAP1,y=MEKi)) +
  geom_point() +
  geom_smooth(method="lm") +
  ggtitle(paste0("Pearson's r = ",cor(b$RasGAP1,a$MEKi))) +
  theme_classic()

c+d
```



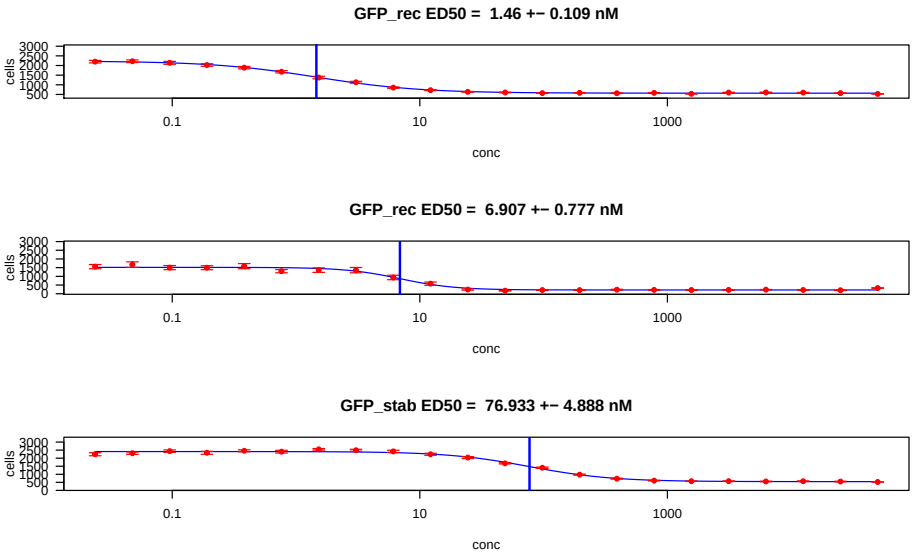
9 Figure 2-supplemental figure 6D-G: Compound characterization on Dmel2 cells

```
data('ED50_feature_data', package='MODIFIdata')

par(mfrow=c(3,1))
################################################################################
# plot dose-response of PD-0325901 treated cells; all conentractions are nM;
#GFP dsRNA is a non-targeting construct against GFP expression plasmid
################################################################################

  feature="cells"
  x=100000
  feature_vector=c()
  for(i in c(2:23)){
    x=x/2
    wells=grep(paste0("^EC50_GFP_..",i,"$"),
```

```
                    paste0(measured_data$mean_per_field_mapping$plate,
                           measured_data$mean_per_field_mapping$well))
      tobind=cbind(rep(x,
                       times=length(
                         measured_data$mean_per_field_data[wells,feature])
      ),
      measured_data$mean_per_field_data[wells,feature]
      )
      row.names(tobind)=paste0(measured_data$mean_per_field_mapping$plate,
                               measured_data$mean_per_field_mapping$well)[wells]
      feature_vector=rbind(feature_vector,tobind)
    }

    feature_vector=as.data.frame(feature_vector)
    print(feature)
  ## [1] "cells"
    colnames(feature_vector)=c("conc",as.character(feature))
    mdl=drm(cells ~ conc, data = as.data.frame(feature_vector),
            fct = LL.4(names=c("Slope","Lower Limit","Upper Limit", "ED50")))
    ed50=ED(mdl,50,interval="delta")
  ##
  ## Estimated effective doses
  ##
  ##        Estimate Std. Error   Lower   Upper
  ## e:1:50  1.45961    0.10887 1.24586 1.67335
    mean=aggregate.data.frame(feature_vector,
                              by = list(feature_vector$conc),mean)[,c(1,3)]
    names(mean)=c("conc","mean")
    plot(mdl,col="blue",type="none",main=paste("GFP_rec","ED50 = ",
              round(ed50[1],digits = 3),"+-",round(ed50[2],digits = 3),"nM"))

    points(mean,pch=19,cex=0.8,col="red",main=feature)


    sem=aggregate.data.frame(feature_vector,by = list(feature_vector$conc),
                             function(x){sd(x)/sqrt(length(x))})[,c(1,3)]#
    names(sem)=c("conc","sem")
    arrows(sem$conc, mean$mean-sem$sem, sem$conc, mean$mean+sem$sem,
           length=0.05, angle=90, code=3,"red")
    abline(  v =  ed50[1],col="blue",lwd=2)

    ##############################################################################
    # plot dose-response of fresh medium on compound treated cells
    #after compound washout
    ##############################################################################

    feature="cells"
    x=100000
    feature_vector=c()
    for(i in c(2:23)){
      x=x/2
```

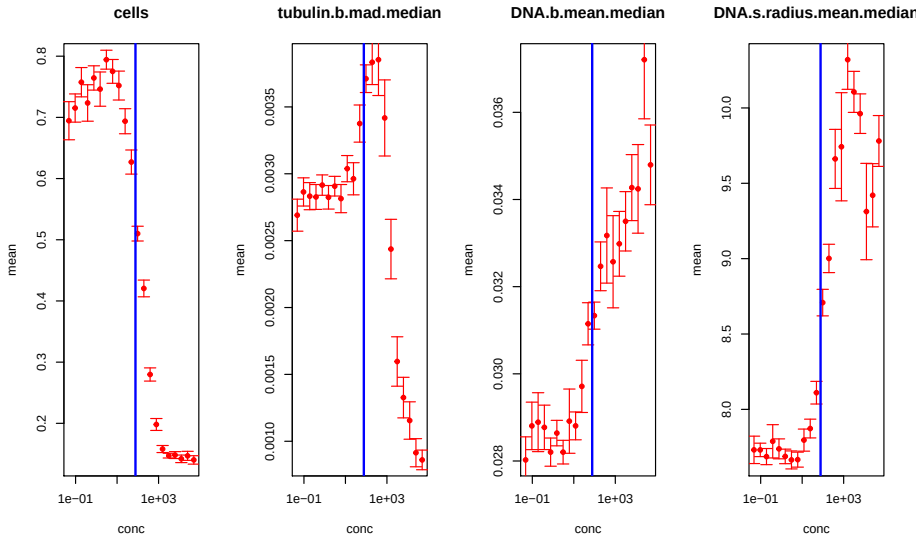
```
    wells=grep(paste0("^EC50_rec.",i,"$"),
               paste0(measured_data$mean_per_field_mapping$plate,
                      measured_data$mean_per_field_mapping$well))
    tobind=cbind(rep(x,
                     times=length(
                       measured_data$mean_per_field_data[wells,feature])
    ),
    measured_data$mean_per_field_data[wells,feature]
    )
    row.names(tobind)=paste0(measured_data$mean_per_field_mapping$plate,
                             measured_data$mean_per_field_mapping$well)[wells]
    feature_vector=rbind(feature_vector,tobind)
  }

  feature_vector=as.data.frame(feature_vector)
  print(feature)
## [1] "cells"
  colnames(feature_vector)=c("conc",as.character(feature))
  mdl=drm(cells ~ conc, data = as.data.frame(feature_vector),
          fct = LL.4(names=c("Slope","Lower Limit","Upper Limit", "ED50")))
  ed50=ED(mdl,50,interval="delta")
##
## Estimated effective doses
##
##        Estimate Std. Error   Lower   Upper
## e:1:50  6.90663    0.77694 5.37855 8.43471
  mean=aggregate.data.frame(feature_vector,
                            by = list(feature_vector$conc),mean)[,c(1,3)]
  names(mean)=c("conc","mean")
  plot(mdl,col="blue",type="none",main=paste("GFP_rec","ED50 = ",
          round(ed50[1],digits = 3),"+-",round(ed50[2],digits = 3),"nM"))

  points(mean,pch=19,cex=0.8,col="red",main=feature)#,log="x",plot.new=F


  sem=aggregate.data.frame(feature_vector,by = list(feature_vector$conc),
                           function(x){sd(x)/sqrt(length(x))})[,c(1,3)]#
  names(sem)=c("conc","sem")
  arrows(sem$conc, mean$mean-sem$sem, sem$conc, mean$mean+sem$sem,
         length=0.05, angle=90, code=3,"red")
  abline(  v =  ed50[1],col="blue",lwd=2)

  ######################################################################
  # plot dose-response of used compound on fresh cells
  ######################################################################

  feature="cells"
  x=100000
  feature_vector=c()
  for(i in c(2:23)){
    x=x/2
```

```
    wells=grep(paste0("^EC50_stab_..",i,"$"),
               paste0(measured_data$mean_per_field_mapping$plate,
                      measured_data$mean_per_field_mapping$well))
    tobind=cbind(rep(x,
        times=length(measured_data$mean_per_field_data[wells,feature])
    ),
    measured_data$mean_per_field_data[wells,feature]
    )
    row.names(tobind)=paste0(measured_data$mean_per_field_mapping$plate,
                             measured_data$mean_per_field_mapping$well)[wells]
    feature_vector=rbind(feature_vector,tobind)
  }

  feature_vector=as.data.frame(feature_vector)
  print(feature)
## [1] "cells"
  colnames(feature_vector)=c("conc",as.character(feature))
  mdl=drm(cells ~ conc, data = as.data.frame(feature_vector),
          fct = LL.4(names=c("Slope","Lower Limit","Upper Limit", "ED50")))
  ed50=ED(mdl,50,interval="delta")
##
## Estimated effective doses
##
##        Estimate Std. Error   Lower   Upper
## e:1:50  76.9334     4.8876 67.3373 86.5296
  mean=aggregate.data.frame(feature_vector,
                            by = list(feature_vector$conc),mean)[,c(1,3)]
  names(mean)=c("conc","mean")
  plot(mdl,col="blue",type="none",main=paste("GFP_stab","ED50 = ",
            round(ed50[1],digits = 3),"+-",round(ed50[2],digits = 3),"nM"))

  points(mean,pch=19,cex=0.8,col="red",main=feature)#,log="x",plot.new=F


  sem=aggregate.data.frame(feature_vector,by = list(feature_vector$conc),
                           function(x){sd(x)/sqrt(length(x))})[,c(1,3)]#
  names(sem)=c("conc","sem")
  arrows(sem$conc, mean$mean-sem$sem, sem$conc, mean$mean+sem$sem,
         length=0.05, angle=90, code=3,"red")
  abline(  v =  ed50[1],col="blue",lwd=2)
```




```
##############################################################################
# plot dose-response of different cellular features
##############################################################################

par(mfrow=c(1,4))

for(feature in c("cells","tubulin.b.mad.median","DNA.b.mean.median",
                 "DNA.s.radius.mean.median")){
  x=100000
  feature_vector=c()
  for(i in c(3:23)){
    x=x/2
    wells=grep(paste0("^EC50_stab_..",i,"$"),
               paste0(measured_data$mean_per_field_mapping$plate,
                      measured_data$mean_per_field_mapping$well))
    tobind=cbind(rep(x,
                     times=length(
                       measured_data$mean_per_field_data[wells,feature])
    ),
    measured_data$mean_per_field_data[wells,feature]
    )
    row.names(tobind)=paste0(measured_data$mean_per_field_mapping$plate,
                             measured_data$mean_per_field_mapping$well)[wells]
    feature_vector=rbind(feature_vector,tobind)
  }

  feature_vector=as.data.frame(feature_vector)
  print(feature)
  colnames(feature_vector)=c("conc",as.character(feature))
  feature_vector$cells=(feature_vector[[feature]]-
                          min(feature_vector[[feature]]))/(
                            max(feature_vector[[feature]])-
                              min(feature_vector[[feature]]))
```

```
    mean=aggregate.data.frame(feature_vector,
                              by = list(feature_vector$conc),mean)[,c(1,3)]
    names(mean)=c("conc","mean")
    plot(mean,pch=19,cex=0.8,col="red",log="x",main=feature)

    sem=aggregate.data.frame(feature_vector,by = list(feature_vector$conc),
                             function(x){sd(x)/sqrt(length(x))})[,c(1,3)]#
    names(sem)=c("conc","sem")
    arrows(sem$conc, mean$mean-sem$sem, sem$conc, mean$mean+sem$sem,
           length=0.05, angle=90, code=3,"red")
    abline(  v =  77,
             col="blue",lwd=2)
  }
## [1] "cells"
## [1] "tubulin.b.mad.median"
## [1] "DNA.b.mean.median"
## [1] "DNA.s.radius.mean.median"
```

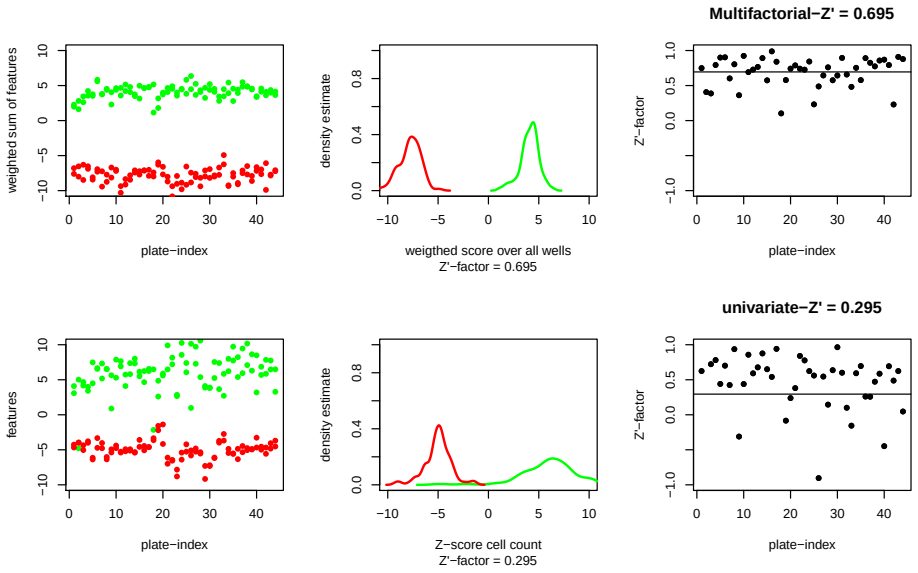


10 Figure 2-supplemental figure 7: Control separation capacity of our morphological assay

```
myarray.norm<-new_features_data

z_kernel=function(x,sigma=1){
  res=0
  for(i in 1:length(x)){
    for(j in 1:length(x)){
      res=res+exp(-(x[i]-x[j])^2/(2*sigma^2))
    }
```

```
  }
  return(res/(length(x)^2))
}

z_factor=function(pos,neg){1-3*(sd(pos)+sd(neg))/(mean(pos)-mean(neg))}

feature<-"cell_number"
plates_that_correlate.idx=c(1:44)
screen<-"S16"

plates=list()
for(i in plates_that_correlate.idx){
  plates[[i]]=t(myarray.norm[,c("B2","J2"),i,screen])
}
cur_neg=do.call("rbind.data.frame",plates)
cur_neg$class="neg"



plates=list()
for(i in plates_that_correlate.idx){
  plates[[i]]=t(myarray.norm[,c("H2","P2"),i,screen])
}
cur_pos=do.call("rbind.data.frame",plates)
cur_pos$class="pos"


dataset=rbind(cur_neg,cur_pos)
lda_anal=lda(class~.,data = dataset)


plates=list()
for(i in plates_that_correlate.idx){
  cur_pos=t(myarray.norm[,c("H2","P2"),i,screen])
  cur_neg=t(myarray.norm[,c("B2","J2"),i,screen])
  neg_P=apply(cur_neg,1,function(x){sum(x*lda_anal$scaling)})
  pos_P=apply(cur_pos,1,function(x){sum(x*lda_anal$scaling)})
  plates[[i]]=cbind(i,pos_P,neg_P,z_factor(pos_P,neg_P))
}
multi=cur_z=do.call("rbind.data.frame",plates)

pos=cur_z[,2]
neg=cur_z[,3]
par(mfrow = c(2,3))
plot(cur_z[,1],cur_z[,2],pch=16,col="green",ylim=c(-10,10),
     ylab="weighted sum of features",xlab="plate-index")
points(cur_z[,1],cur_z[,3],pch=16,col="red")

plot(2,2,xlim=c(-10,10),ylim=c(0,1),
     sub=paste("Z'-factor =",
                      round(mean(cur_z[,4],na.rm = T),digits=3)),
```

```
     ylab="density estimate",
     xlab="weigthed score over all wells",type="n")
lines(density(cur_z[,2],na.rm = T),col="green",lwd=2)
lines(density(cur_z[,3],na.rm = T),col="red",lwd=2)

plot(cur_z[,1],cur_z[,4],
     pch=16,col="black",ylim=c(-1,1),
     main=paste("Multifactorial-Z' =",
                round(mean(cur_z[,4],na.rm = T),digits=3)),
     ylab="Z'-factor",
     xlab="plate-index")
abline(h=mean(cur_z[,4],na.rm = T))

################################################################################

plates=list()
for(i in plates_that_correlate.idx){
  pos_P=cur_pos=myarray.norm["cell_number",c("H2","P2"),i,screen]
  neg_P=cur_neg=myarray.norm["cell_number",c("B2","J2"),i,screen]
  plates[[i]]=cbind(i,pos_P,neg_P,z_factor(pos_P,neg_P))
}
uni=cur_z=do.call("rbind.data.frame",plates)

pos=cur_z[,2]
neg=cur_z[,3]

plot(cur_z[,1],cur_z[,2],pch=16,col="green",ylim=c(-10,10),
     ylab="features",xlab="plate-index")
points(cur_z[,1],cur_z[,3],pch=16,col="red")

plot(2,2,xlim=c(-10,10),ylim=c(0,1),sub=paste("Z'-factor =",
        round(mean(cur_z[,4],na.rm = T),digits=3)),
     ylab="density estimate",
     xlab="Z-score cell count",type="n")
lines(density(cur_z[,2],na.rm = T),col="green",lwd=2)
lines(density(cur_z[,3],na.rm = T),col="red",lwd=2)

plot(cur_z[,1],cur_z[,4],pch=16,col="black",
     ylim=c(-1,1),
     main=paste("univariate-Z' =",round(mean(cur_z[,4],na.rm = T),digits=3)),
     ylab="Z'-factor",
     xlab="plate-index")
abline(h=mean(cur_z[,4],na.rm = T))
```




```
rm(list = ls())
```

11 Analysis of time-resolved genetic interactions

First we collect data, normalize by per-plate negative controls, glog transform each feature's data, scale the data, annotate the wells and reformat to an array with dimensions according to experimental conditions.

We started this analysis on raw feature data produced from fluorescent images using R/EBImage as described in the methods.

```
## load raw per-well level data in the form of a four dimensional array as
#well-set-plate-feature
data('raw_data_array_wCTRL', package='MODIFIdata')

##normalize to negative controls that did not see any query gene
neg=c("A12","A13","C12","C13",
      "E12","E13","G12","G13",
      "I12","I13","K12","K13",
      "M12","M13","O12","O13")

my.data.array.norm=apply(my.data.array,
                         c(2,3,4),
                         function(x){
                           m=median(x[neg],na.rm = T)
                           if(m==0){
                             return(x/1)
                           }else{
                             return(x/m)
                           }
                         }
```

```
                          )

##glog transform all features and and center and scale them by robust Z-Score
for(i in dimnames(my.data.array)[[4]]){
  c=quantile(my.data.array.norm[,,,i],probs = 0.03,na.rm = T)
  my.data.array.norm[,,,i]=glog(x = my.data.array.norm[,,,i] , a = c)
  my.data.array.norm[,,,i]=
    (my.data.array.norm[,,,i]-
       median(my.data.array.norm[,,,i],na.rm = T))/
    mad(my.data.array.norm[,,,i],na.rm = T)
}
my.data.array.norm_zprime<-my.data.array.norm

#extract the cell count data
my.data.array.norm.qc=my.data.array.norm[,,,'cell_number']

#calculate the Z-prime-factor for each
#assay plate between drk and RasGAP1 knockdown
my.data.array.zprime=apply(my.data.array.norm.qc,
                           c(2,3),
                           function(x){
                             negs=x[c("F12","F13")]
                             poss=x[c("H12","H13","J12","J13")]
                             imageHTS::zprime(a = negs,b = poss,method = "maha")
                           }
)

bad_S1S7=which(diag(cor(my.data.array.norm[,"S01",,1],
                        my.data.array.norm[,"S07",,1], use = "p" ))<=0.6)
bad_S2S8=which(diag(cor(my.data.array.norm[,"S02",,1],
                        my.data.array.norm[,"S08",,1], use = "p" ))<=0.6)
bad_S3S9=which(diag(cor(my.data.array.norm[,"S03",,1],
                        my.data.array.norm[,"S09",,1], use = "p" ))<=0.6)
bad_S4S10=which(diag(cor(my.data.array.norm[,"S04",,1],
                         my.data.array.norm[,"S10",,1], use = "p" ))<=0.6)
bad_S5S11=which(diag(cor(my.data.array.norm[,"S05",,1],
                         my.data.array.norm[,"S11",,1], use = "p" ))<=0.6)
bad_S6S12=which(diag(cor(my.data.array.norm[,"S06",,1],
                         my.data.array.norm[,"S12",,1], use = "p" ))<=0.6)

#only the S1S7 and S2S8 had plates which do not correlate <0.6
my.data.array.norm[,"S07",bad_S1S7,]=NA_real_
my.data.array.norm[,"S08",bad_S2S8,]=NA_real_
bad_zprime=which(my.data.array.zprime<0.3,arr.ind = T)

#mask all low quality data from downstream analysis
for(i in 1:nrow(bad_zprime)){
  my.data.array.norm[,bad_zprime[i,1],bad_zprime[i,2],]=NA_real_
}

#load the annotations for each well and each plate and their mapping to HD3-IDs
```

```
data('query_mapping', package='MODIFIdata')
data('target_mapping', package='MODIFIdata')

dimnames(my.data.array.norm)[[1]][
  grep("B02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_1"
dimnames(my.data.array.norm)[[1]][
  grep("D02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_2"
dimnames(my.data.array.norm)[[1]][
  grep("F02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_3"
dimnames(my.data.array.norm)[[1]][
  grep("H02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_4"
dimnames(my.data.array.norm)[[1]][
  grep("J02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_5"
dimnames(my.data.array.norm)[[1]][
  grep("L02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_6"
dimnames(my.data.array.norm)[[1]][
  grep("N02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_7"
dimnames(my.data.array.norm)[[1]][
  grep("P02$",dimnames(my.data.array.norm)[[1]])]="HD31RLUC_8"
dimnames(my.data.array.norm)[[1]][
  grep("B23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_1"
dimnames(my.data.array.norm)[[1]][
  grep("D23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_2"
dimnames(my.data.array.norm)[[1]][
  grep("F23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_3"
dimnames(my.data.array.norm)[[1]][
  grep("H23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_4"
dimnames(my.data.array.norm)[[1]][
  grep("J23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_5"
dimnames(my.data.array.norm)[[1]][
  grep("L23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_6"
dimnames(my.data.array.norm)[[1]][
  grep("N23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_7"
dimnames(my.data.array.norm)[[1]][
  grep("P23$",dimnames(my.data.array.norm)[[1]])]="HD32RLUC_8"

for(i in 1:length(dimnames(my.data.array.norm)[[3]])){
  if(length(grep(paste0("^",
          dimnames(my.data.array.norm)[[3]][i],"$"),query_mappin$V2))!=0){
    dimnames(my.data.array.norm)[[3]][i]=
    query_mappin$V3[grep(paste0("^",
                dimnames(my.data.array.norm)[[3]][i],"$"),query_mappin$V2)]
  }
}

for(i in 1:length(dimnames(my.data.array.norm)[[1]])){
  if(length(grep(paste0("^",
  dimnames(my.data.array.norm)[[1]][i],"$"),target_mappin$V2))!=0){
    dimnames(my.data.array.norm)[[1]][i]=target_mappin$V1[
      grep(paste0("^",
                dimnames(my.data.array.norm)[[1]][i],"$"),target_mappin$V2)]
```

```
  }
}

my.data.array.norm_repl1.1=my.data.array.norm[
  grep("^HD3",dimnames(my.data.array.norm)[[1]]),
  c("S01","S02","S03","S04","S05","S06"),,]
my.data.array.norm_repl1.2=my.data.array.norm[
  grep("^HD3",dimnames(my.data.array.norm)[[1]]),
  c("S07","S08","S09","S10","S11","S12"),,]

my.data.array.norm=abind(my.data.array.norm_repl1.1,
                         my.data.array.norm_repl1.2,rev.along = 0)
dimnames(my.data.array.norm)[[5]]=c("repl1","repl2")

rm(my.data.array.norm_repl1.1,my.data.array.norm_repl1.2)
my.data.array.norm_DMSO_48=my.data.array.norm[,"S07",,,]
my.data.array.norm_DMSO_72=my.data.array.norm[,"S08",,,]
my.data.array.norm_DMSO_96=my.data.array.norm[,"S09",,,]
my.data.array.norm_DMSO=abind(my.data.array.norm_DMSO_48,
                              my.data.array.norm_DMSO_72,
                              my.data.array.norm_DMSO_96,
                              rev.along = 0)
dimnames(my.data.array.norm_DMSO)[[5]]=c("48h","72h","96h")
my.data.array.norm_MEKi_48=my.data.array.norm[,"S10",,,]
my.data.array.norm_MEKi_72=my.data.array.norm[,"S11",,,]
my.data.array.norm_MEKi_96=my.data.array.norm[,"S12",,,]
my.data.array.norm_MEKi=abind(my.data.array.norm_MEKi_48,
                              my.data.array.norm_MEKi_72,
                              my.data.array.norm_MEKi_96,
                              rev.along = 0)
dimnames(my.data.array.norm_MEKi)[[5]]=c("48h","72h","96h")
my.data.array.norm=abind(my.data.array.norm_DMSO,
                         my.data.array.norm_MEKi,
                         rev.along = 0)
dimnames(my.data.array.norm)[[6]]=c("DMSO","MEKi")
names(dimnames(my.data.array.norm))=c("target gene",
                                      "query gene",
                                      "feature",
                                      "replicate",
                                      "time point",
                                      "perturbation")

rm(my.data.array.norm_DMSO_48,
   my.data.array.norm_DMSO_72,
   my.data.array.norm_DMSO_96,
   my.data.array.norm_MEKi_48,
   my.data.array.norm_MEKi_72,
   my.data.array.norm_MEKi_96,
   my.data.array.norm_DMSO,
   my.data.array.norm_MEKi,
   my.data.array)
```

Next we apply the med-polish to call statistic genetic interactions according to methods established by Bernd Fischer. Input is normed and quality controlled data. Output are genetic interactions as pi-scores, main effects and reformatted measurements and p-value associated to interactions in each individual condition.

```
data('id_to_gene_mapping', package='MODIFIdata')
data('query_mapping', package='MODIFIdata')
data('target_mapping', package='MODIFIdata')

id_to_gene=list()
for(i in 1:nrow(id_to_gene_mapping)){
  id_to_gene[[id_to_gene_mapping[i,1]]]=id_to_gene_mapping[i,3]
  id_to_gene[[id_to_gene_mapping[i,2]]]=id_to_gene_mapping[i,3]
}

pb <- txtProgressBar(style = 3,max = 310,initial = 0,min = 1)
counter=1
interaction_scores=my.data.array.norm
mainEffects = list(target = interaction_scores[,1,,,,],
                   query = interaction_scores[1,,,,,],
                   overall = interaction_scores[1,1,,,,]
                   )
#interactions are called using the HD2013SGImaineffects function implemented
#in the R-package whcih supplemented Laufer et al. 2013, Nature methods
#we called interactions indepently for each dsRNA, biological replicates,
#feature, time point and treatment
for(i in 1:155){
  for(j in 1:2){
    for(k in 1:3){
      for(l in 1:2){
        MP=HD2013SGI::HD2013SGImaineffects(x = my.data.array.norm[,,i,j,k,l],
           TargetNeg = c("HD31RLUC_1","HD32RLUC_1","HD31RLUC_2","HD32RLUC_2",
                         "HD31RLUC_3","HD32RLUC_3","HD31RLUC_4","HD32RLUC_4",
                         "HD31RLUC_5","HD32RLUC_5","HD31RLUC_6","HD32RLUC_6",
                         "HD31RLUC_7","HD32RLUC_7","HD31RLUC_8","HD32RLUC_8"),
           na.rm=T
           )
        interaction_scores[,,i,j,k,l]=MP$pi
        mainEffects$query[,i,j,k,l]=MP$queryMainEffect
        mainEffects$target[,i,j,k,l]=MP$targetMainEffect
        mainEffects$overall[i,j,k,l]=MP$neg
      }
    }
    setTxtProgressBar(pb, counter)
    counter=counter+1
  }
}

# annotate genes from dsRNA identifiers
interaction_scores.des1=interaction_scores[
  grep("HD31",dimnames(interaction_scores)[[1]]),,,,,]
```

```
interaction_scores.des2=interaction_scores[
  grep("HD32",dimnames(interaction_scores)[[1]]),,,,,]

for(i in 1:length(dimnames(interaction_scores.des1)[[1]])){
  if(dimnames(interaction_scores.des1)[[1]][i] %in% names(id_to_gene)){
    dimnames(interaction_scores.des1)[[1]][i]=
      id_to_gene[[dimnames(interaction_scores.des1)[[1]][i]]]
  }else{
    dimnames(interaction_scores.des1)[[1]][i]=
      gsub("HD31(.+)","\\1",dimnames(interaction_scores.des1)[[1]][i])
  }
}

for(i in 1:length(dimnames(interaction_scores.des2)[[1]])){
  if(dimnames(interaction_scores.des2)[[1]][i] %in% names(id_to_gene)){
    dimnames(interaction_scores.des2)[[1]][i]=
      id_to_gene[[dimnames(interaction_scores.des2)[[1]][i]]]
  }else{
    dimnames(interaction_scores.des2)[[1]][i]=
      gsub("HD32(.+)","\\1",dimnames(interaction_scores.des2)[[1]][i])
  }
}

for(i in 1:length(dimnames(mainEffects$target)[[1]])){
  if(dimnames(mainEffects$target)[[1]][i] %in% names(id_to_gene)){
    dimnames(mainEffects$target)[[1]][i]=
      id_to_gene[[dimnames(mainEffects$target)[[1]][i]]]
  }
}

# resort interaction, maineffect and measured effect array such that
# dsRNA designs and biological replicates are in one dimension

# main effects, interactions and measured data, do now have the
#dimensions: target.gene-query.gene-feature-time-treatment-replicate
interaction_scores.des2=
  interaction_scores.des2[dimnames(interaction_scores.des1)[[1]],,,,,]
interaction_scores.des1.1=interaction_scores.des1[,,,1,,]
interaction_scores.des1.2=interaction_scores.des1[,,,2,,]
interaction_scores.des2.1=interaction_scores.des2[,,,1,,]
interaction_scores.des2.2=interaction_scores.des2[,,,2,,]
interaction_scores=abind(interaction_scores.des1.1,
                         interaction_scores.des1.2,
                         interaction_scores.des2.1,
                         interaction_scores.des2.2,
                         rev.along = 0)
dimnames(interaction_scores)[[6]]=c("des1repl1","des1repl2",
                                    "des2repl1","des2repl2")

rm(interaction_scores.des1,interaction_scores.des2,
   interaction_scores.des1.1,interaction_scores.des1.2,
```

```
   interaction_scores.des2.1,interaction_scores.des2.2)
my.data.array.norm.des1=my.data.array.norm[grep("HD31",
                        dimnames(my.data.array.norm)[[1]]),,,,,]
my.data.array.norm.des2=my.data.array.norm[grep("HD32",
                        dimnames(my.data.array.norm)[[1]]),,,,,]

for(i in 1:length(dimnames(my.data.array.norm.des1)[[1]])){
  if(dimnames(my.data.array.norm.des1)[[1]][i] %in% names(id_to_gene)){
    dimnames(my.data.array.norm.des1)[[1]][i]=
      id_to_gene[[dimnames(my.data.array.norm.des1)[[1]][i]]]
  }else{
    dimnames(my.data.array.norm.des1)[[1]][i]=
      gsub("HD31(.+)","\\1",dimnames(my.data.array.norm.des1)[[1]][i])
  }
}

for(i in 1:length(dimnames(my.data.array.norm.des2)[[1]])){
  if(dimnames(my.data.array.norm.des2)[[1]][i] %in% names(id_to_gene)){
    dimnames(my.data.array.norm.des2)[[1]][i]=
      id_to_gene[[dimnames(my.data.array.norm.des2)[[1]][i]]]
  }else{
    dimnames(my.data.array.norm.des2)[[1]][i]=
      gsub("HD32(.+)","\\1",dimnames(my.data.array.norm.des2)[[1]][i])
  }
}

for(i in 1:length(dimnames(mainEffects$target)[[1]])){
  if(dimnames(mainEffects$target)[[1]][i] %in% names(id_to_gene)){
    dimnames(mainEffects$target)[[1]][i]=
      id_to_gene[[dimnames(mainEffects$target)[[1]][i]]]
  }
}


my.data.array.norm.des2=
  my.data.array.norm.des2[dimnames(my.data.array.norm.des1)[[1]],,,,,]
my.data.array.norm.des1.1=my.data.array.norm.des1[,,,1,,]
my.data.array.norm.des1.2=my.data.array.norm.des1[,,,2,,]
my.data.array.norm.des2.1=my.data.array.norm.des2[,,,1,,]
my.data.array.norm.des2.2=my.data.array.norm.des2[,,,2,,]
my.data.array.norm.reshaped=abind(my.data.array.norm.des1.1,
                                  my.data.array.norm.des1.2,
                                  my.data.array.norm.des2.1,
                                  my.data.array.norm.des2.2,
                                  rev.along = 0)
dimnames(my.data.array.norm.reshaped)[[6]]=c("des1repl1","des1repl2",
                                             "des2repl1","des2repl2")
rm(my.data.array.norm.des1,
   my.data.array.norm.des2,
   my.data.array.norm.des1.1,
   my.data.array.norm.des1.2,
```

```
    my.data.array.norm.des2.1,
    my.data.array.norm.des2.2)

#statistically analyze the interactions of every condition separately as
#was done in Laufer et al. 2013 or Fischer et al. 2015

D=aperm(interaction_scores,perm = c(1,2,4,5,3,6))
PADJ = D[,,,,,1]
s = rep(NA_real_, dim(D)[5])
i=1
for (i in seq_len(dim(D)[5])) {
    Data = D[,,,,i,]
    d = dim(Data)
    dim(Data) = c(prod(d[1:4]),prod(d[5]))
    s[i] = median(apply(Data,1,sd), na.rm=TRUE)
    padj = rep(NA_real_, nrow(Data))
    K = which(apply(!is.na(Data),1,all))
    fit = limma::eBayes(limma::lmFit(Data[K,]))
    padj[K] = p.adjust(fit$p.value, method="BH")
    PADJ[,,,,i] = padj
    cat(sprintf("i=%2d",i),
                " nr int (1%) = ", sum(padj <= 0.01, na.rm=TRUE)/nrow(Data),
          " nr int (3%) = ", sum(padj <= 0.03, na.rm=TRUE)/nrow(Data), "\n")
}

D=(D[,,,,,1]+D[,,,,,2]+D[,,,,,3]+D[,,,,,4])/4
for(i in 1:dim(D)[5]){
  D[,,,,i]=D[,,,,i]/s[i]
}
Interactions = list(piscore = D,
                    scale = s,
                    padj = PADJ
                )

inter_df=melt(Interactions$piscore,varnames =
                c("target","query",
                  "time","drug","feature"),
              value.name = "piscore")
sig_df=melt(Interactions$padj,varnames =
              c("target","query",
                "time","drug",
                "feature"),value.name = "fdr")
data_df=tbl_df(full_join(inter_df,sig_df))

my.data.array.norm.reshaped_df<-
  melt(my.data.array.norm.reshaped,varnames=
         c("target","query",
           "feature","time",
           "drug","replicate"),
       value.name = "value") %>% tbl_df()
```

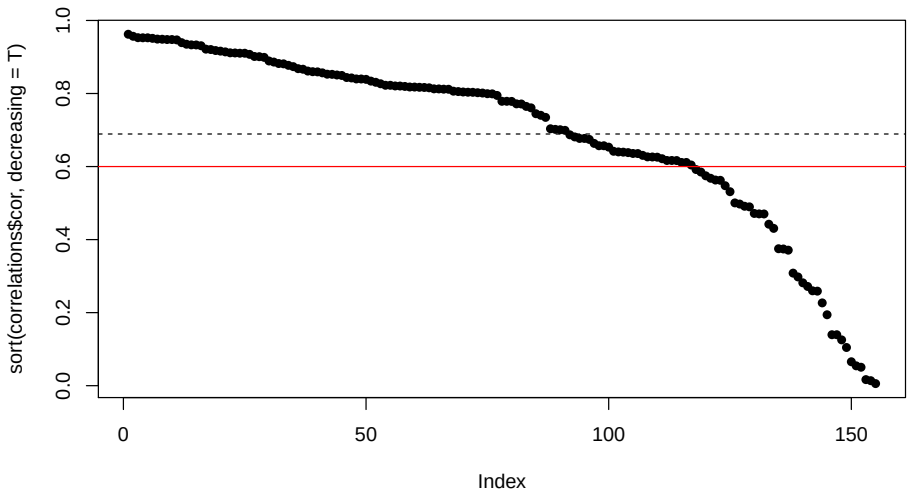
```
rm(sig_df,
  inter_df,
  my.data.array.norm,
  D,
  s,
  PADJ)
```

12 Analyze feature correlation and filter out non-redundant features

Filter non-correlating features

13 Figure 2-supplemental figure 2A: Featurewise biological reproducibility analysis

```
correlations=my.data.array.norm.reshaped_df %>%
  filter(time=="96h",drug=="MEKi") %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  spread(bio,value) %>%
  group_by(feature) %>%
  summarise(cor=cor(repl1,repl2,use = "p",method = "p"))

plot(sort(correlations$cor,decreasing = T),pch=16)
abline(h=0.6, col="red")
abline(h=mean(correlations$cor), col="black",lty=2)
```



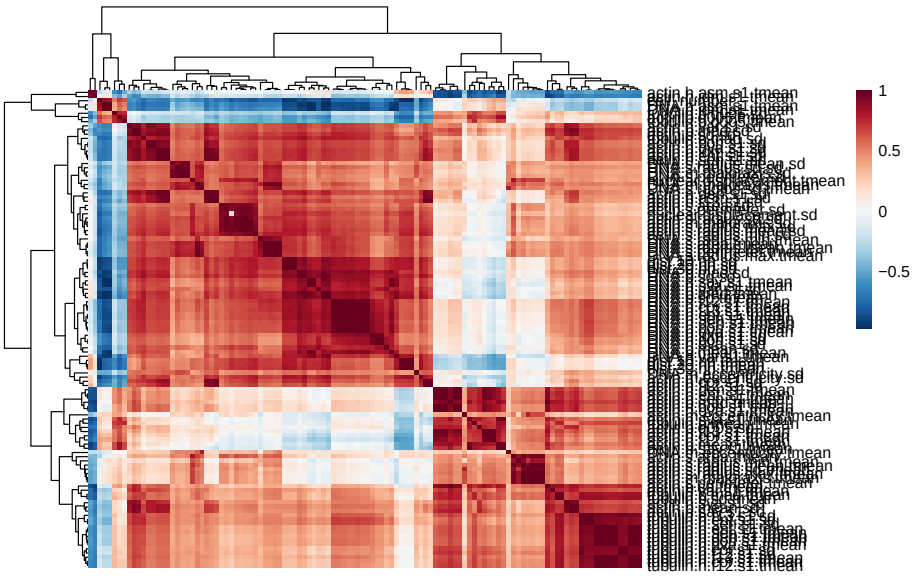

```
mean(correlations$cor)
## [1] 0.6891315
```

```
#Filter features without any reproducible interactions in any condition

goodfeature<-
  correlations %>%
  mutate(feature=factor(feature,levels=feature[order(cor,decreasing=T)])) %>%
  dplyr::filter(cor>=0.6) %>%
  arrange(desc(cor)) %>%
  pull(feature) %>%
  as.character()

allfeature<-
  correlations %>%
  mutate(feature=factor(feature,levels=feature[order(cor,decreasing=T)])) %>%
  arrange(desc(cor)) %>%
  pull(feature) %>%
  as.character()
```

14 Figure 2-supplemental figure 2B: Pairwise feature correlation analysis and redundancy filter

```
require(RColorBrewer)
goodfeature=data_df %>%
  group_by(feature) %>%
  filter(fdr<=0.05 & feature %in% goodfeature) %>%
  mutate(sigIntfeat=length(fdr)) %>%
  pull(feature) %>%
  unique() %>%
  as.character()

##############################################################################
# compute features wise correlations or load them from previous session
#(takes a long time! do not run)
##############################################################################
# subdf=data_df %>% filter(time=="96h",drug=="MEKi",feature %in% goodfeature)
# tmp=combn(as.character(goodfeature),2,simplify = F)
# fun<-function(x){
#   c(cor(filter(subdf,feature %in% x[1])$piscore,
#       filter(subdf,feature %in% x[2])$piscore,
#       method = "p",use = "p"),x)
# }
# multicoreParam <- MulticoreParam(workers = 20,progressbar=TRUE)
# correllations=bplapply(tmp,fun, BPPARAM = multicoreParam)
# cors=do.call("rbind.data.frame",correllations)
# names(cors) = c("corr","feat1","feat2")
# cors %<>% tbl_df() %>% mutate(corr=as.numeric(as.character(corr))) %>%
# arrange(desc(corr))
```

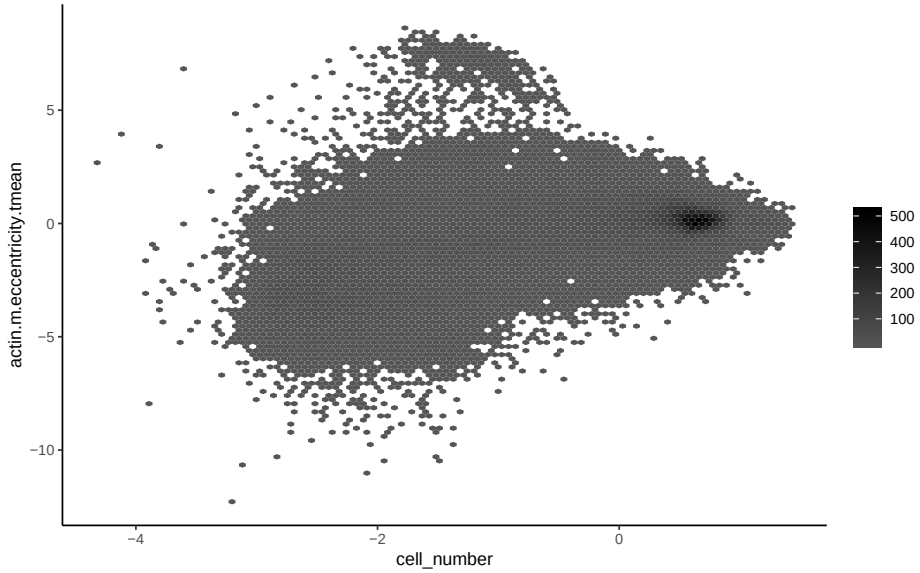
```
data('feature_correlations_interactions', package='MODIFIdata')
upperiag=cors
lowerdiag=upperiag %>% dplyr::select(corr,feat1=feat2,feat2=feat1)
diag=cbind.data.frame("corr"=1,
                      "feat1"=levels(upperiag$feat1),
                      "feat2"=levels(upperiag$feat1))

feature_corrs=bind_rows(upperiag,lowerdiag,diag) %>%
  spread(feat2,corr) %>% dplyr::select(-feat1) %>% t()

x=pheatmap::pheatmap(feature_corrs,color =
          colorRampPalette(rev(brewer.pal(n = 11, name ="RdBu")))(100))
```




```
################################################################################
# filter features such that highest reproducible features are chosen first
# second each feature that shows a pearson correlation higher than 0.7 with
# this feature is disregarded
# iteratively test that for all remaining features
# fix cell_number and tubulin.b.mad.mean
################################################################################

for(i in allfeature){
  if(i %in% goodfeature){
    corrf=c(as.character(filter(cors,feat1==i & abs(corr)>0.7)$feat2),
            as.character(filter(cors,feat2==i & abs(corr)>0.7)$feat1))
    goodfeature=goodfeature[!goodfeature %in% corrf]
  }
}
goodfeature=c("cell_number","tubulin.b.mad.tmean",goodfeature) %>% unique()
```

15 Figure 2-supplemental figure 2C: Scatterplot of uncorrelating features after redundancy checks

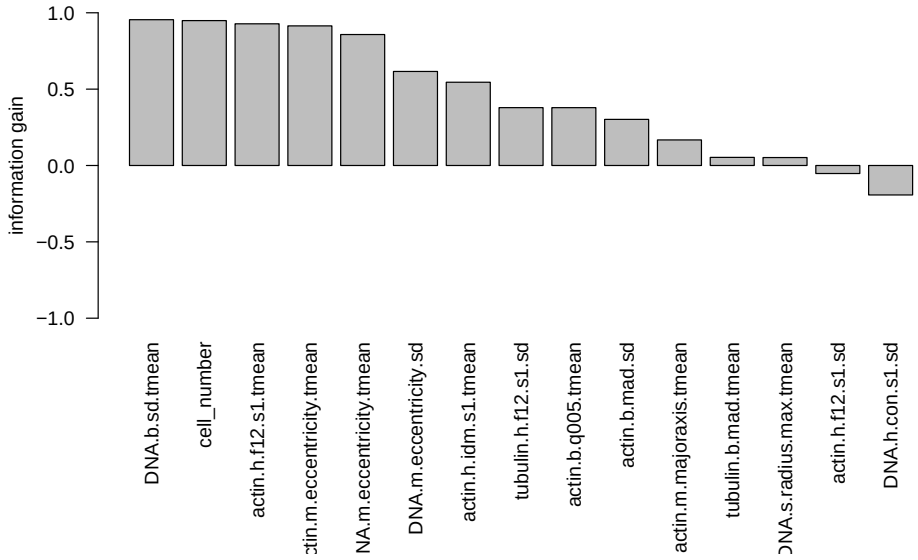
```
#show that they are independent
colfunc <- colorRampPalette(c("#555555", "black"))
colfunc(30)
##  [1] "#555555" "#525252" "#4F4F4F" "#4C4C4C" "#494949" "#464646" "#434343"
##  [8] "#404040" "#3D3D3D" "#3A3A3A" "#373737" "#343434" "#313131" "#2E2E2E"
## [15] "#2B2B2B" "#292929" "#262626" "#232323" "#202020" "#1D1D1D" "#1A1A1A"
## [22] "#171717" "#141414" "#111111" "#0E0E0E" "#0B0B0B" "#080808" "#050505"
## [29] "#020202" "#000000"

my.data.array.norm.reshaped_df %>%
  filter(time=="96h",drug=="MEKi",feature %in% c("cell_number",
                                        "actin.m.eccentricity.tmean")) %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  spread(feature,value) %>%
  ggplot() +
  geom_hex(aes(cell_number, actin.m.eccentricity.tmean), bins = 100) +
  #geom_point(aes(x=cell_number, y=actin.m.eccentricity.tmean)) +
  scale_fill_gradientn("", colours = colfunc(100)) +
  theme_classic()
```



16 Figure 2-supplemental figure 2D: Check if features enrich for non-redundant information content of correlated residuals

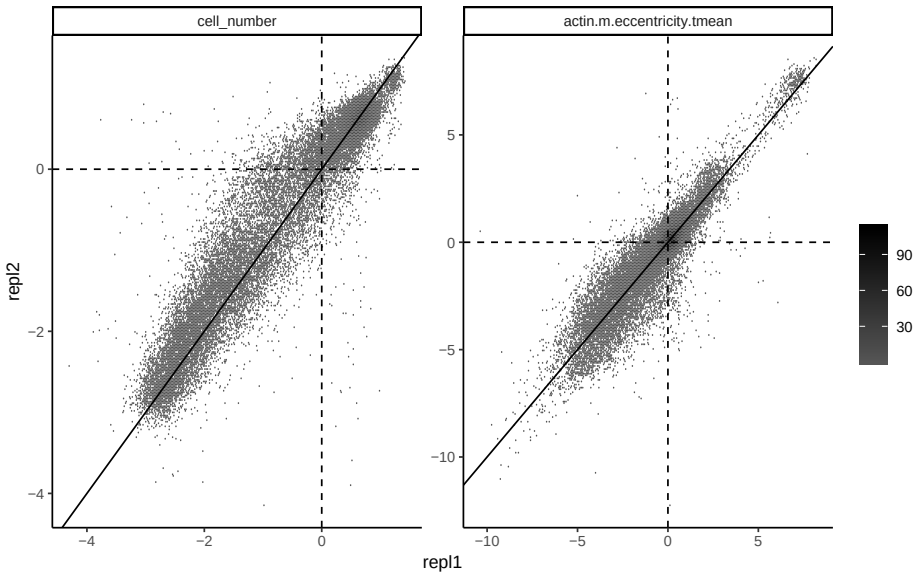
```
data=my.data.array.norm.reshaped_df %>%
  filter(time=="96h",drug=="MEKi",feature %in% c(goodfeature)) %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  group_by(bio,feature,target,query) %>%
  summarise(value=mean(value,na.rm=T))

################################################################################
# reformat data to fit the bernd scheme of target ~ query ~ feature ~ replicate
################################################################################

test1 = drop_na(data)

test1=  acast(data,target ~ query ~ feature ~ bio)

ordered_feat=allfeature[allfeature %in% goodfeature]

test1=test1[,,ordered_feat,]

# !remove nas from the data!
test1[which(is.na(test1))]=0

# set new dimensions to the data frame
test=test1
dim(test) = c(prod(dim(test)[1:2]),dim(test)[3:4])

test=aperm(test,c(1,3,2))

#sub sample the data for quicker evaluation
set.seed(5830458)
sample = sample(seq_len(dim(test)[1]), 6000)
subSampleForStabilitySelection = list(D = test[sample,,],
                                      sample = sample,
                                      phenotype = dimnames(test1)[[3]])
#calculate the information gain per selected feature
stabilitySelection = HD2013SGI::HD2013SGIselectByStability(
                      subSampleForStabilitySelection,
                      verbose = T,preselect = c("cell_number"),Rdim=15)

#plot the information gain
par(mar=c(10, 4, 2, 0))
barplot(stabilitySelection$correlation[
                  order(stabilitySelection$correlation,
                  decreasing = T)],
        names.arg=stabilitySelection$selected[
```

```
                order(stabilitySelection$correlation,
                      decreasing = T)],
              ylim=c(-1,1), las=2, ylab="information gain")
```



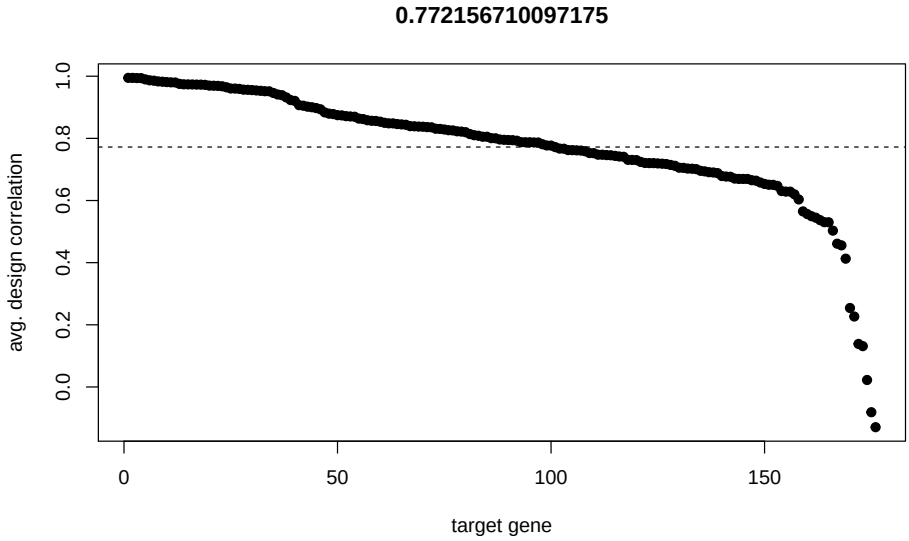
17 Figure 2B Figure 2-supplemental figure 3A,B: Biological replicate correlation

```
my.data.array.norm.reshaped_df %>%
  filter(time=="96h",drug=="MEKi",feature %in% c("cell_number",
                                      "actin.m.eccentricity.tmean")) %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  spread(bio,value) %>%
   ggplot(aes(x=repl1, y=repl2)) +
    geom_hex(aes(repl1, repl2), bins = 250) +
    geom_abline(slope = 1,intercept = c(0,0)) +
    geom_vline(xintercept = 0,lty=2) +
    geom_hline(yintercept = 0,lty=2) +
    scale_fill_gradientn("", colours = colfunc(150)) +
    facet_wrap("feature",scales = "free") +
    theme_classic()
```



18 Figure 2-supplemental figure 3D: dsRNA design replicate correlation

```
data=my.data.array.norm.reshaped_df %>%
   dplyr::filter(time=="96h",drug=="MEKi",feature %in% c(goodfeature)) %>%
   tidyr::extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  tidyr::spread(design,value) %>%
  group_by(target,query,feature) %>%
  summarise(des1=mean(des1),des2=mean(des2))

# calculate the correlation between design replicates for all good
# features separated by queries such that query effects are
# not compromising the correlation
# the target gene design correlations are then averaged over all queries
c_avg_q_all_f <-
  data %>%
  group_by(target,query) %>%
  summarise(correlation=cor(des1,des2,method="p",use="p")) %>%
  group_by(target) %>%
  summarise(correlation2=mean(correlation,na.rm=T)) %>%
  mutate(target=factor(target,levels=target[order(correlation2,decreasing=T)]))

plot(sort(c_avg_q_all_f$correlation2,decreasing = T),
     ylab="avg. design correlation",xlab="target gene",pch=19)
abline(h=mean(c_avg_q_all_f$correlation2),col="black",lty=2)
title(main=mean(c_avg_q_all_f$correlation2))
```

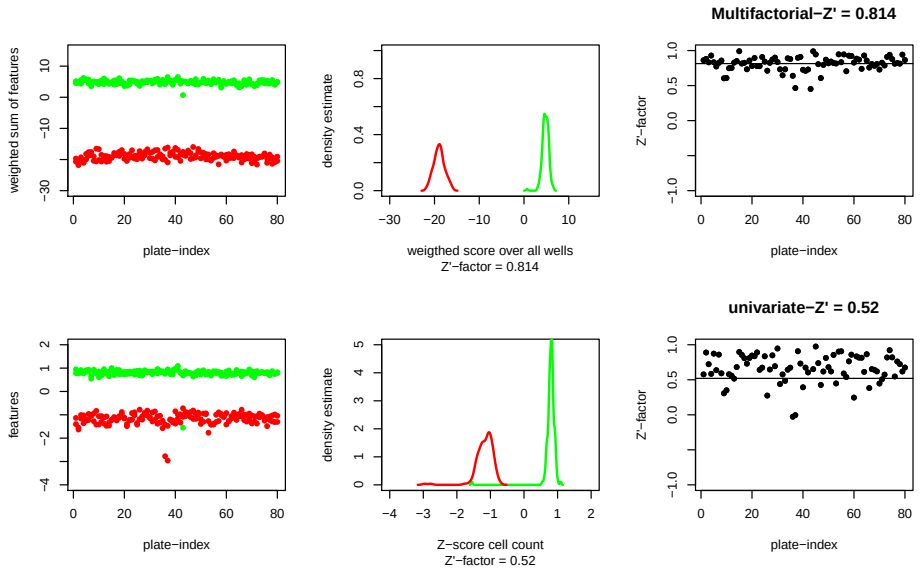


19 Figure 2-supplemental figure 3D: Biological control separation

```
myarray.norm<-my.data.array.norm_zprime[,,,goodfeature]

z_kernel=function(x,sigma=1){
  res=0
  for(i in 1:length(x)){
    for(j in 1:length(x)){
      res=res+exp(-(x[i]-x[j])^2/(2*sigma^2))
    }
  }
  return(res/(length(x)^2))
}

z_factor=function(pos,neg){1-3*(sd(pos)+sd(neg))/(mean(pos)-mean(neg))}

feature<-"cell_number"
plates_that_correlate.idx=c(1:80)
screen<-"S06"

par(mfrow=c(1,2))

plates=list()
for(i in plates_that_correlate.idx){
  plates[[i]]=myarray.norm[c("P12","P13"),screen,i,]
}
cur_neg=do.call("rbind.data.frame",plates)
cur_neg$class="neg"
```

```
plates=list()
for(i in plates_that_correlate.idx){
  plates[[i]]=myarray.norm[c("H12","H13"),screen,i,]
}
cur_pos=do.call("rbind.data.frame",plates)
cur_pos$class="pos"

dataset=rbind(cur_neg,cur_pos) %>% drop_na()
lda_anal=lda(class~.,data = dataset)

plates=list()
for(i in plates_that_correlate.idx){
  cur_pos=myarray.norm[c("H12","H13"),screen,i,]
  cur_neg=myarray.norm[c("P12","P13"),screen,i,]
  neg_P=apply(cur_neg,1,function(x){sum(x*lda_anal$scaling)})
  pos_P=apply(cur_pos,1,function(x){sum(x*lda_anal$scaling)})
  plates[[i]]=cbind(i,pos_P,neg_P,z_factor(pos_P,neg_P))
}
multi=cur_z=do.call("rbind.data.frame",plates)

pos=cur_z[,2]
neg=cur_z[,3]
par(mfrow = c(2,3))
plot(cur_z[,1],cur_z[,2],pch=16,col="green",ylim=c(-30,15),
     ylab="weighted sum of features",xlab="plate-index")
points(cur_z[,1],cur_z[,3],pch=16,col="red")

plot(2,2,xlim=c(-30,15),ylim=c(0,1),sub=paste("Z'-factor =",
    round(mean(cur_z[,4],na.rm = T),digits=3)),
    ylab="density estimate",
    xlab="weigthed score over all wells",type="n")
lines(density(cur_z[,2],na.rm = T),col="green",lwd=2)
lines(density(cur_z[,3],na.rm = T),col="red",lwd=2)

plot(cur_z[,1],cur_z[,4],pch=16,col="black",ylim=c(-1,1),
     main=paste("Multifactorial-Z' =",
          round(mean(cur_z[,4],na.rm = T),digits=3)),
     ylab="Z'-factor",xlab="plate-index")
abline(h=mean(cur_z[,4],na.rm = T))

################################################################################

plates=list()
for(i in plates_that_correlate.idx){
  pos_P=cur_pos=myarray.norm[c("H12","H13"),screen,i,"cell_number"]
  neg_P=cur_neg=myarray.norm[c("P12","P13"),screen,i,"cell_number"]
  plates[[i]]=cbind(i,pos_P,neg_P,z_factor(pos_P,neg_P))
}
uni=cur_z=do.call("rbind.data.frame",plates)

pos=cur_z[,2]
```

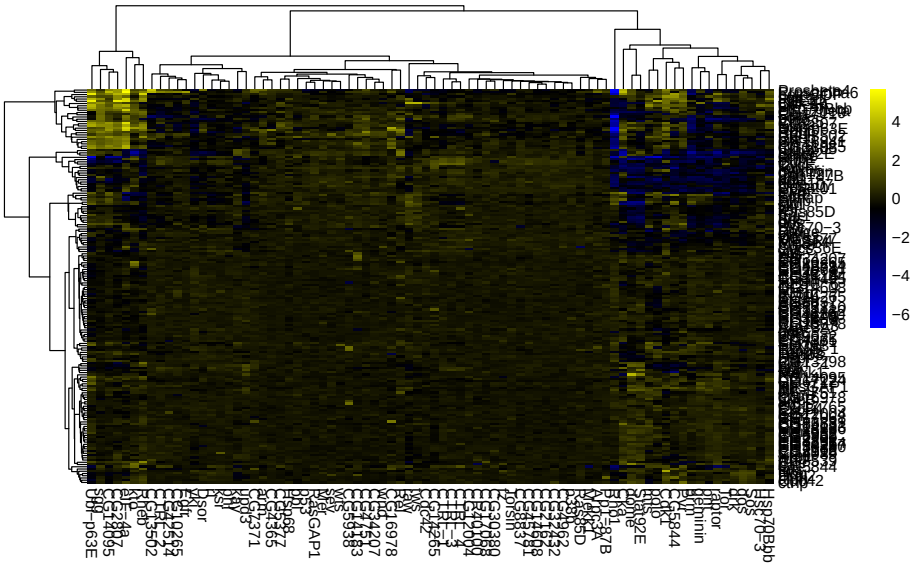
```
neg=cur_z[,3]

plot(cur_z[,1],cur_z[,2],pch=16,col="green",ylim=c(-4,2),ylab="features",
     xlab="plate-index")
points(cur_z[,1],cur_z[,3],pch=16,col="red")

plot(2,2,xlim=c(-4,2),ylim=c(0,5),sub=paste("Z'-factor =",
          round(mean(cur_z[,4],na.rm = T),digits=3)),
     ylab="density estimate",xlab="Z-score cell count",type="n")
lines(density(cur_z[,2],na.rm = T),col="green",lwd=2)
lines(density(cur_z[,3],na.rm = T),col="red",lwd=2)

plot(cur_z[,1],cur_z[,4],pch=16,col="black",ylim=c(-1,1),
     main=paste("univariate-Z' =",round(mean(cur_z[,4],na.rm = T),digits=3)),
     ylab="Z'-factor",xlab="plate-index")
abline(h=mean(cur_z[,4],na.rm = T))
```



20 Figure 2C: Example interaction heatmap

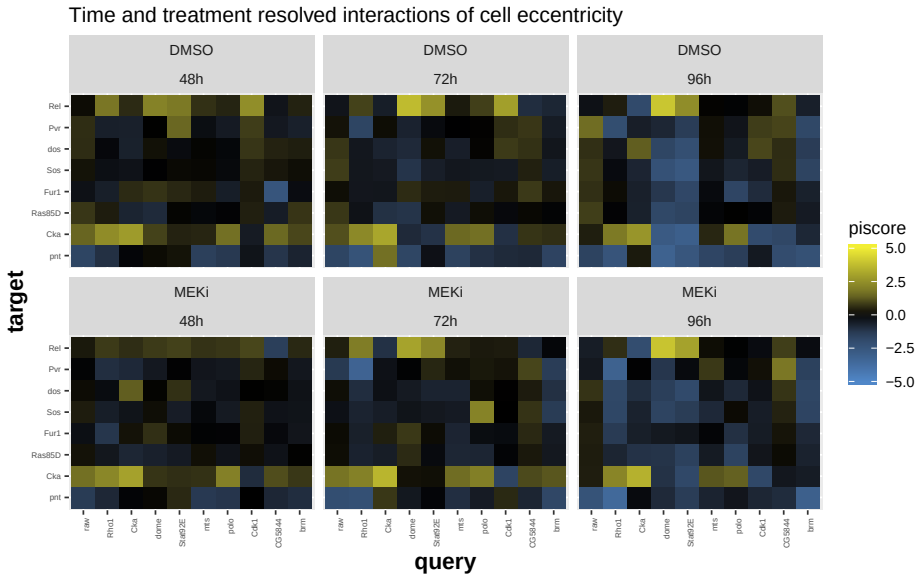
```
test<-melt(interaction_scores) %>% tbl_df()
colnames(test) <- c("target","query","feature","time",
                    "drug","replicate","piscore")
test_sub <- test %>%
  filter(feature %in% goodfeature) %>%
  group_by(target,query,feature,time,drug) %>%
  summarise(piscore=mean(piscore,na.rm=T)) %>%
  ungroup()

eccentr_DMSO_96 <- test_sub %>%
```

```
  filter(time=="96h",
         feature=="actin.m.eccentricity.tmean",
         drug=="DMSO"
         ) %>%
  dplyr::select(target,query,piscore) %>%
  drop_na() %>%
  spread(query,piscore) %>%
  `row.names<-`(.$target) %>%
  dplyr::select_if(is.numeric) %>%
  pheatmap(cluster_rows=TRUE,
           cluster_cols=TRUE,
           color = colorRampPalette(c("blue",
          "black", "yellow"))(1000),clustering_method = 'ward.D2')
```




```
test_sub %>%
  filter(
         feature %in% c("actin.m.eccentricity.tmean"),
         !grepl("CTRL",query),
         !grepl("RLUC",target)
         ) %>%
  dplyr::select(target,query,piscore,drug,time) %>%
  mutate(target=factor(target,levels =
      eccentr_DMSO_96$tree_row$labels[eccentr_DMSO_96$tree_row$order])) %>%
  mutate(query=factor(query,levels =
      eccentr_DMSO_96$tree_col$labels[eccentr_DMSO_96$tree_col$order])) %>%
  filter(query %in% c("raw","Rho1","Cka","dome","Stat92E","mts","polo",
                      "Cdk1","CG5844","brm"),target %in% c("Rel","dos","Sos",
                        "Ras85D","Fur1","Pvr","Cka","pnt")) %>%
  ggplot(aes(x=query,y=target,fill=piscore)) +
  facet_wrap(~drug+time) +
    geom_raster() +
    scale_fill_gradientn(na.value = 'black',
```

```
                          colours = c("#5087C8","black","#F2EE35"),
                          guide = "colorbar",
                          values = rescale(c(-5,-1.5,0,1.5,5)),
                          limits=c(-5,5)) +
      theme(axis.text.x = element_text(angle = 90, hjust = 1) ) +
      theme(axis.text=element_text(size=5),
            axis.title=element_text(size=14,face="bold"))+
      ggtitle("Time and treatment resolved interactions of cell eccentricity")
```




```
rm(test)
rm(test_sub)
rm(test1)
```

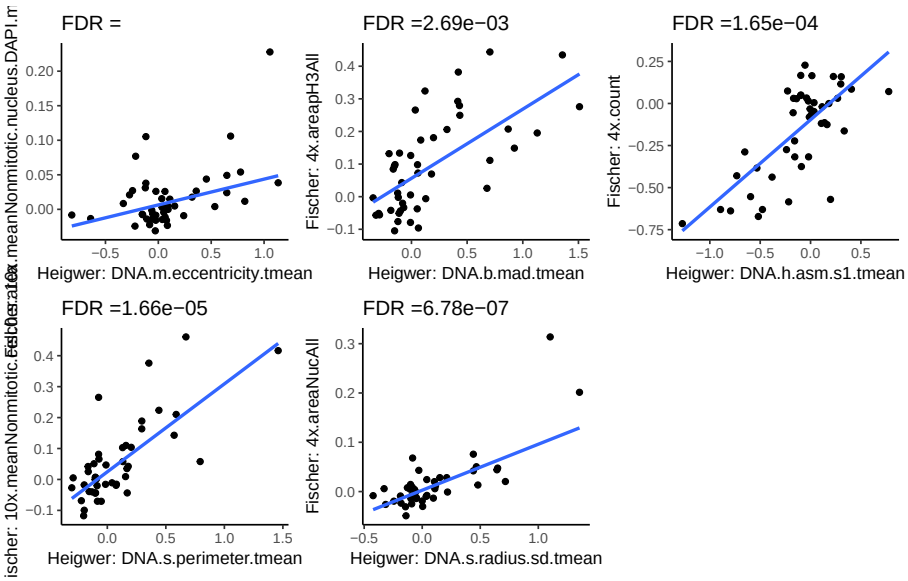
21 Figure 2-supplemental figure 4A-C: Feature comparison to Fischer et al. 2015

```
#load pi score matrix from Fischer et al., Elife 2015
data("pimatrix",package="DmelSGI")
fisheretal=melt(pimatrix$D) %>% tbl_df()

#loadpi-score matrix from Heigwer et al.
heigweretal=melt(interaction_scores) %>% tbl_df()
names(heigweretal)=c("target","query","feature","time",
                     "treatment","replicate","value")

#identify common query genes
commonqueries=levels(heigweretal$query)[levels(heigweretal$query) %in%
                                        levels(fisheretal$query)]
```

```
#it is actually only RasGAP1

#identify common target genes
commontargets=levels(heigweretal$target)[levels(heigweretal$target) %in%
                                           levels(fisheretal$target)]


#filter the data set for the common data points to Fischer et al
heigwercommon=heigweretal %>%
  filter(treatment=="DMSO",
         time=="72h",
         query=="RasGAP1",
         target %in% commontargets
         ) %>%
  dplyr::select(target,query,feature,heigwer=value) %>%
  group_by(target,query,feature) %>%
  summarise(heigwer=mean(heigwer,na.rm=T)) %>%
  ungroup()

#filter the data set for the common data points to Heigwer et al
fishercommon=fisheretal %>%
  filter(query=="RasGAP1",target %in% commontargets) %>%
  dplyr::select(target,query,phenotype,fisher=value) %>%
  group_by(target,query,phenotype) %>%
  summarise(fisher=mean(fisher,na.rm=T))%>%
  ungroup()

#join the commonalities
common=left_join(heigwercommon,fishercommon)

#analyze linear dependency between two features of the different screens
#by linear modeling and anova one needs to note that these interdependencies
#are actually in interaction score space! meaning the a significant
#reproducibility is a very high achievement here

significant_dependencies<-
  common %>%
  group_by(feature,phenotype) %>%
  do(co=lm(heigwer~fisher,data=.)) %>%
  tidy(co) %>%
  ungroup() %>%
  filter(term=="fisher") %>%
  mutate(fdr=p.adjust(p.value)) %>%
  filter(fdr<0.05) %>%
  arrange(fdr)

#we found that the following features can be translated from heigwer et al
#to fisher et al

"DNA.s.perimeter.tmean" = "10x.meanNonmitotic.cell.0.s.area"
"DNA.s.radius.sd.tmean" = "4x.areaNucAll"
```

```
"DNA.m.eccentricity.tmean" = "10x.meanNonmitotic.nucleus.DAPI.m.majoraxis"
"DNA.b.mad.tmean" = "4x.areapH3All"
"DNA.h.asm.s1.tmean" = "4x.count"

#as control we plot these pairwise dependencies
a<-common %>%
  filter(feature=="DNA.m.eccentricity.tmean",
         phenotype=="10x.meanNonmitotic.nucleus.DAPI.m.majoraxis") %>%
  ggplot(aes(x=heigwer,y=fisher)) +
  geom_point() +
  geom_smooth(method="rlm",se=F)+
  theme_classic() +
  xlab("Heigwer: DNA.m.eccentricity.tmean") +
  ylab("Fischer: 10x.meanNonmitotic.nucleus.DAPI.m.majoraxis") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
         filter(feature=="DNA.m.eccentricity.tmean",
                phenotype=="10x.meanNonmitotic.nucleus.DAPI.m.majoraxis") %>%
         .$fdr %>% format(digits =3,scientific=T)))

b<-common %>%
  filter(feature=="DNA.b.mad.tmean",phenotype=="4x.areapH3All") %>%
  ggplot(aes(x=heigwer,y=fisher)) +
  geom_point() +
  geom_smooth(method="rlm",se=F)+
  theme_classic() +
  xlab("Heigwer: DNA.b.mad.tmean") +
  ylab("Fischer: 4x.areapH3All") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
                   filter(feature=="DNA.b.mad.tmean",
                          phenotype=="4x.areapH3All") %>%
                   .$fdr %>% format(digits =3,scientific=T)))

c<-common %>%
  filter(feature=="DNA.h.asm.s1.tmean",phenotype=="4x.count") %>%
  ggplot(aes(x=heigwer,y=fisher)) +
  geom_point() +
  geom_smooth(method="rlm",se=F)+
  theme_classic() +
  xlab("Heigwer: DNA.h.asm.s1.tmean") +
  ylab("Fischer: 4x.count") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
        filter(feature=="DNA.h.asm.s1.tmean",phenotype=="4x.count") %>%
          .$fdr %>% format(digits =3,scientific=T)))

d<-common %>%
  filter(feature=="DNA.s.perimeter.tmean",
         phenotype=="10x.meanNonmitotic.cell.0.s.area") %>%
  ggplot(aes(x=heigwer,y=fisher)) +
  geom_point() +
  geom_smooth(method="rlm",se=F) +
  theme_classic() +
```

```
  xlab("Heigwer: DNA.s.perimeter.tmean") +
  ylab("Fischer: 10x.meanNonmitotic.cell.0.s.area") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
    filter(feature=="DNA.s.perimeter.tmean",
           phenotype=="10x.meanNonmitotic.cell.0.s.area") %>%
      .$fdr %>% format(digits =3,scientific=T)))

e<-common %>%
  filter(feature=="DNA.s.radius.sd.tmean",phenotype=="4x.areaNucAll") %>%
  ggplot(aes(x=heigwer,y=fisher)) +
  geom_point() +
  geom_smooth(method="rlm",se=F)+
  theme_classic() +
  xlab("Heigwer: DNA.s.radius.sd.tmean") +
  ylab("Fischer: 4x.areaNucAll") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
filter(feature=="DNA.s.radius.sd.tmean",phenotype=="4x.areaNucAll") %>%
  .$fdr %>% format(digits =3,scientific=T)))

a+b+c+d+e
```

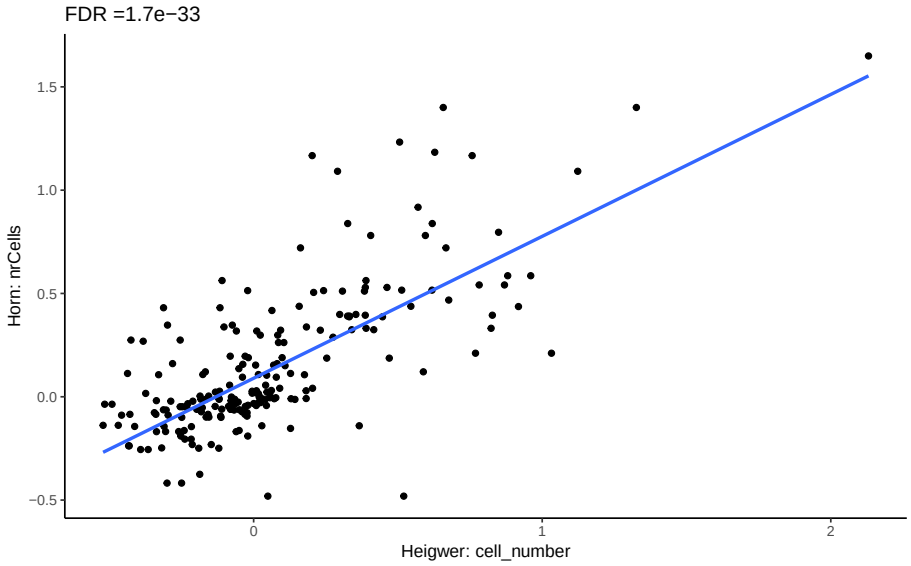


22 Figure 2-supplemental figure 5D-G: Feature comparison to Horn et al. 2011

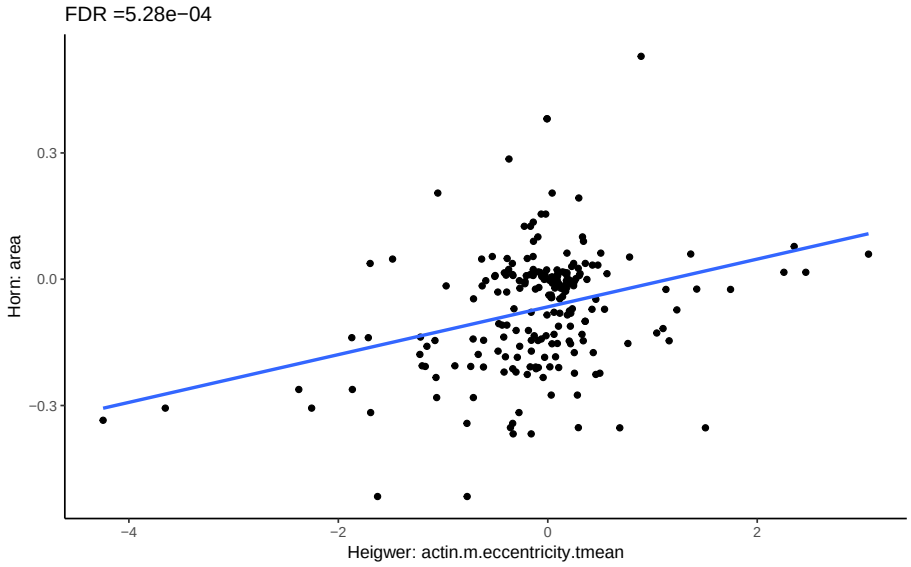
```
#load the pi-score data produced by Horn et al. 2011
#library(RNAinteractMAPK)
data("Dmel2PPMAPK", package="RNAinteractMAPK")
PI <- RNAinteract::getData(Dmel2PPMAPK, type="pi", format="targetMatrix",
                           screen="mean", withoutgroups = c("pos", "neg"))
```

```
hornetal<-melt(PI)
names(hornetal)=c("target","query","repl","phenotype","horn")

hornetal %<>% tbl_df() %>% dplyr::select(-repl)

#identify common query genes
commonqueries=levels(heigweretal$query)[levels(heigweretal$query) %in%
                                          levels(hornetal$query)]
#it is actually only RasGAP1

#identify common target genes
commontargets=levels(heigweretal$target)[levels(heigweretal$target) %in%
                                           levels(hornetal$target)]

#filter the data set for the common data points to Horn et al
heigwercommon=heigweretal %>%
  filter(treatment=="DMSO",time=="72h",query %in% commonqueries,target %in%
           commontargets) %>% #,feature=="DNA.m.majoraxis.tmean"
  dplyr::select(target,query,feature,heigwer=value) %>%
  group_by(target,query,feature) %>%
  summarise(heigwer=mean(heigwer,na.rm=T)) %>%
  ungroup()

#filter the data set for the common data points to Heigwer et al
horncommon=hornetal %>%
  filter(query %in% commonqueries,target %in% commontargets) %>%
  dplyr::select(target,query,phenotype,horn) %>%
  group_by(target,query,phenotype) %>%
  summarise(horn=mean(horn,na.rm=T))%>%
  ungroup()

#join the commonalities
common=left_join(heigwercommon,horncommon)

#analyze linear dependency between two features of the different
#screens by linear modeling and anova
#one needs to note that these interdependencies are actually in interaction
#score space! meaning the a significant reproducibility
#is a very high achievement here
significant_dependencies<-
  common %>%
  group_by(feature,phenotype) %>%
  do(co=lm(heigwer~horn,data=.)) %>%
  tidy(co) %>%
  ungroup() %>%
  filter(term=="horn") %>%
  mutate(fdr=p.adjust(p.value)) %>%
  filter(fdr<0.05) %>%
  arrange(fdr)
```

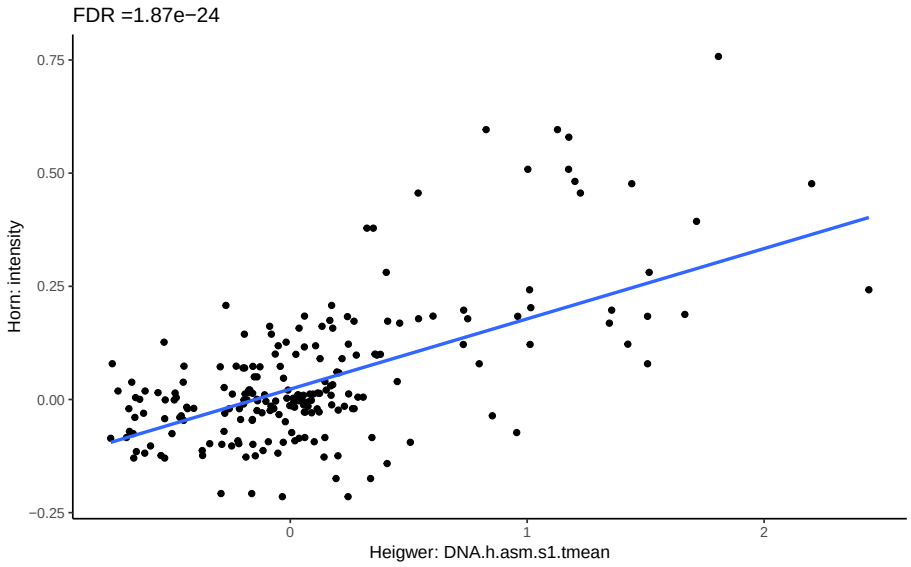
```
"cell_number"="nrCells"
"actin.m.eccentricity.tmean" = "area"
"DNA.b.mean.tmean" = "intensity"

#plot the feature mappings as scatterplot
common %>%
  filter(feature=="cell_number",phenotype=="nrCells") %>%
  ggplot(aes(x=heigwer,y=horn)) +
  geom_point() +
  geom_smooth(method="rlm",se=F)+
  theme_classic() +
  xlab("Heigwer: cell_number") +
  ylab("Horn: nrCells") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
                filter(feature=="cell_number",phenotype=="nrCells") %>%
                  .$fdr %>% format(digits =3,scientific=T)))
```




```
common %>%
  filter(feature=="actin.m.eccentricity.tmean",phenotype=="area") %>%
  ggplot(aes(x=heigwer,y=horn)) +
  geom_point() +
  geom_smooth(method="rlm",se=F)+
  theme_classic() +
  xlab("Heigwer: actin.m.eccentricity.tmean") +
  ylab("Horn: area") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
      filter(feature=="actin.m.eccentricity.tmean",phenotype=="area") %>%
        .$fdr %>% format(digits =3,scientific=T)))
```

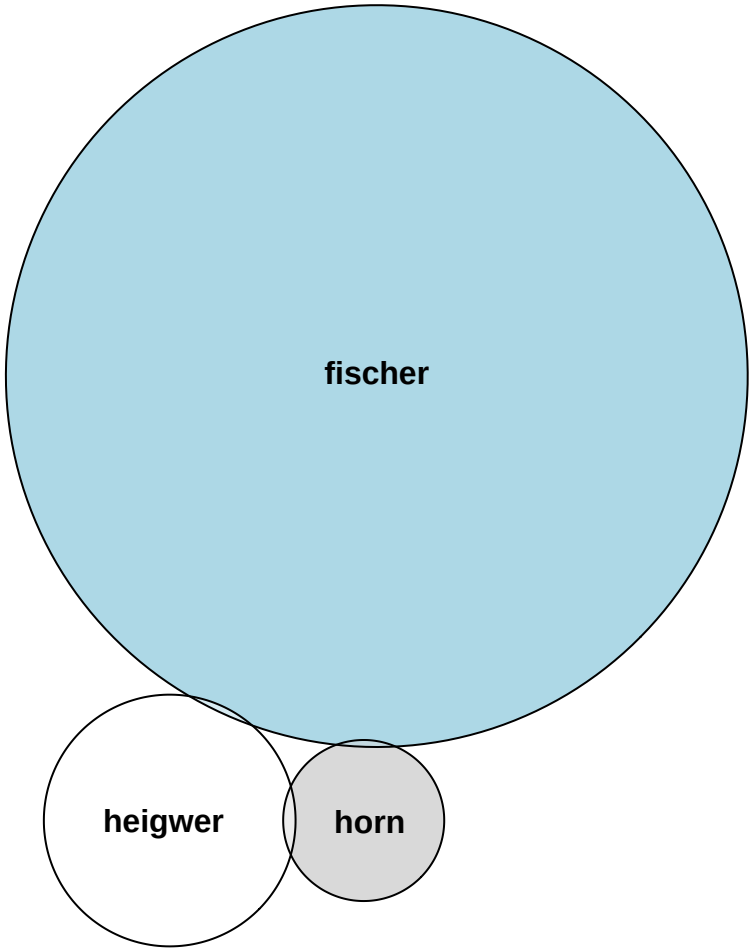



```
common %>%
  filter(feature=="DNA.h.asm.s1.tmean",phenotype=="intensity") %>%
  ggplot(aes(x=heigwer,y=horn)) +
  geom_point() +
  geom_smooth(method="rlm",se=F)+
  theme_classic() +
  xlab("Heigwer: DNA.h.asm.s1.tmean") +
  ylab("Horn: intensity") +
  ggtitle(paste0('FDR =',significant_dependencies %>%
    filter(feature=="DNA.h.asm.s1.tmean",phenotype=="intensity") %>%
      .$fdr %>% format(digits =3,scientific=T)))
```




```
#get all interactions screened by Heigwer et al.
```

```
heigwer_combis<-heigweretal %>%
  dplyr::select(target,query) %>%
  dplyr::distinct() %>%
  filter(!grepl("CTRL",query)) %>%
  mutate(target=as.character(target),query=as.character(query)) %>%
  group_by(target,query) %>%
  filter(target != query) %>%
  mutate(tmp=paste(sort(c(target,query)),collapse="_")) %>%
  .$tmp %>%
  unique()

horn_combis<-hornetal %>%
  dplyr::select(target,query) %>%
  dplyr::distinct() %>%
  filter(!grepl("CTRL",query)) %>%
  mutate(query=gsub(x = query,pattern="Gap1",replacement="RasGAP1"),
         target=gsub(x = target,pattern="Gap1",replacement="RasGAP1")) %>%
  mutate(target=as.character(target),query=as.character(query)) %>%
  group_by(target,query) %>%
  filter(target != query) %>%
  mutate(tmp=paste(sort(c(target,query)),collapse="_")) %>%
  .$tmp %>%
  unique()

fischer_combis<-fisheretal %>%
  dplyr::select(target,query) %>%
  dplyr::distinct() %>%
  filter(!grepl("CTRL",query)) %>%
  mutate(target=as.character(target),query=as.character(query)) %>%
  group_by(target,query) %>%
  filter(target != query) %>%
  mutate(tmp=paste(sort(c(target,query)),collapse="_")) %>%
  .$tmp %>%
  unique()

fit1 <- euler(c("heigwer" = 10273, "horn" = 4107, "fischer" = 90636,
                "heigwer&horn" = 114, "heigwer&fischer" = 57,
                "horn&fischer" = 51,"heigwer&horn&fischer" = 6))

plot(fit1,counts = T)
```

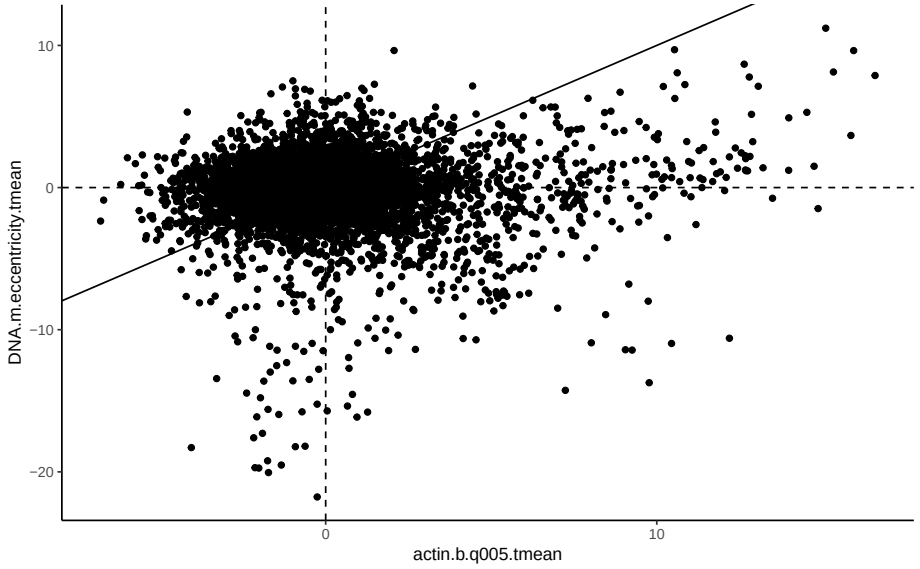



```
rm(heigweretal)
```

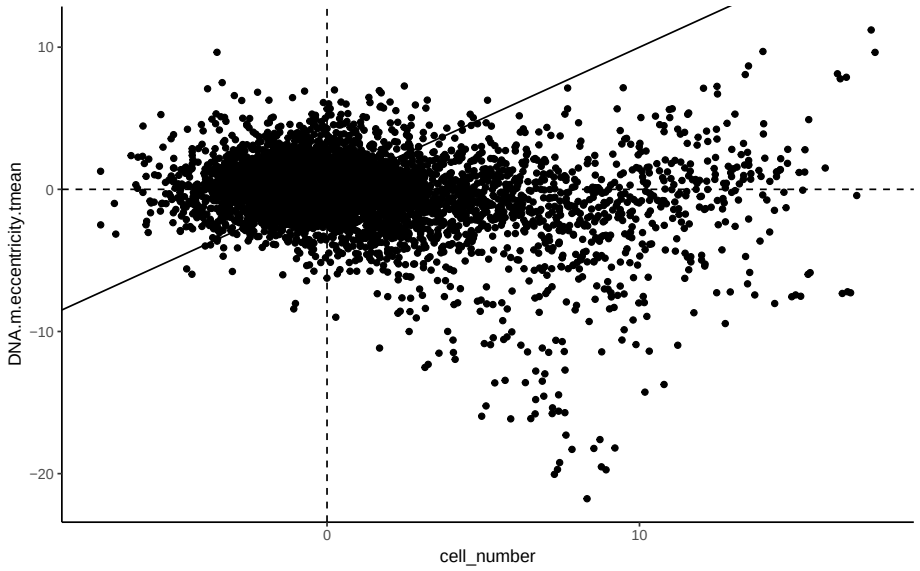
23 Figure 2-supplemental figure 5: Quality control metrics of gene-gene combinatorial screening

```
rm(Interactions)
#plot uncorrelating features pi-scores

colfunc <- colorRampPalette(c("#555555", "black"))

data = data_df %>%
  filter(feature %in% goodfeature, time=="96h",drug=="MEKi") %>%
  dplyr::select(-fdr) %>%
  spread(feature,piscore) %>%
  drop_na()

  data %>%
```

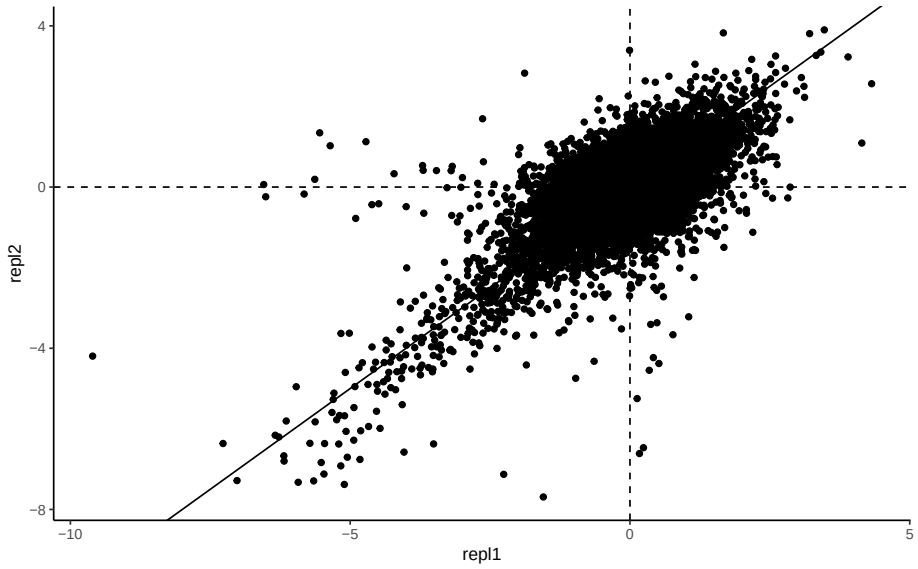
```
ggplot(aes(x=actin.b.q005.tmean, y=DNA.m.eccentricity.tmean)) +
 geom_point() +
  geom_abline(slope = 1,intercept = c(0,0)) +
  geom_vline(xintercept = 0,lty=2) +
  geom_hline(yintercept = 0,lty=2) +
  theme_classic()
```



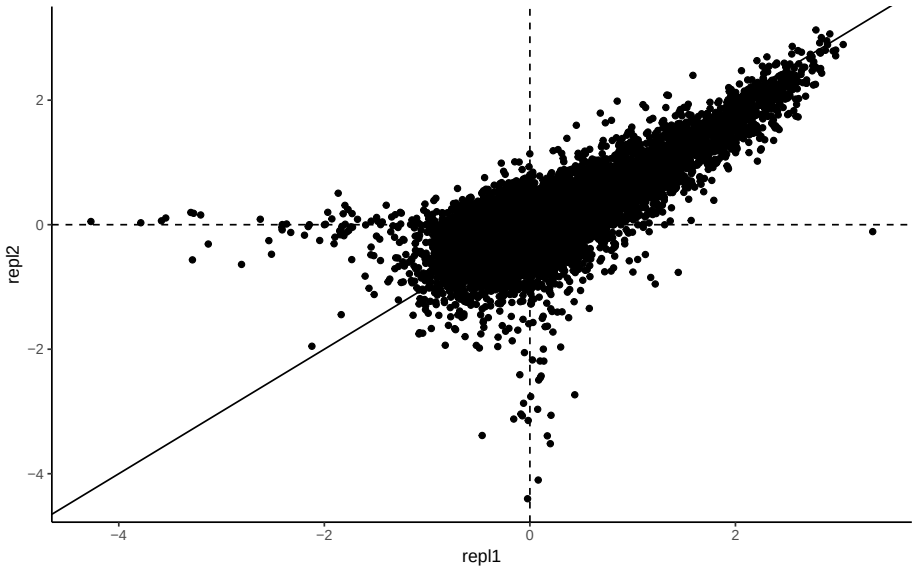

```
  cor(data$actin.b.q005.tmean,data$DNA.m.eccentricity.tmean,use="p",method="p")
## [1] 0.0360771

#plot uncorrelating features pi-scores
#Cell number vs nuclear eccentricity
  data %>%
    ggplot(aes(x=cell_number, y=DNA.m.eccentricity.tmean)) +
      geom_abline(slope = 1,intercept = c(0,0)) +
      geom_vline(xintercept = 0,lty=2) +
      geom_hline(yintercept = 0,lty=2) +
     geom_point() +
      theme_classic()
```




```
  cor(data$cell_number,data$DNA.m.eccentricity.tmean,use="p",method="p")
## [1] -0.2182899

#plot correlating features pi-scores

interaction_scores_df<-melt(interaction_scores,varnames=c("target","query",
    "feature","time","drug","replicate"),value.name = "value") %>% tbl_df()

interaction_scores_df %>%
  filter(feature == "DNA.m.eccentricity.tmean", time=="96h",drug=="MEKi") %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  spread(bio,value) %>%
  ggplot(aes(x=repl1,y=repl2))+
      geom_abline(slope = 1,intercept = c(0,0)) +
      geom_vline(xintercept = 0,lty=2) +
      geom_hline(yintercept = 0,lty=2) +
   geom_point() +
      theme_classic()
```

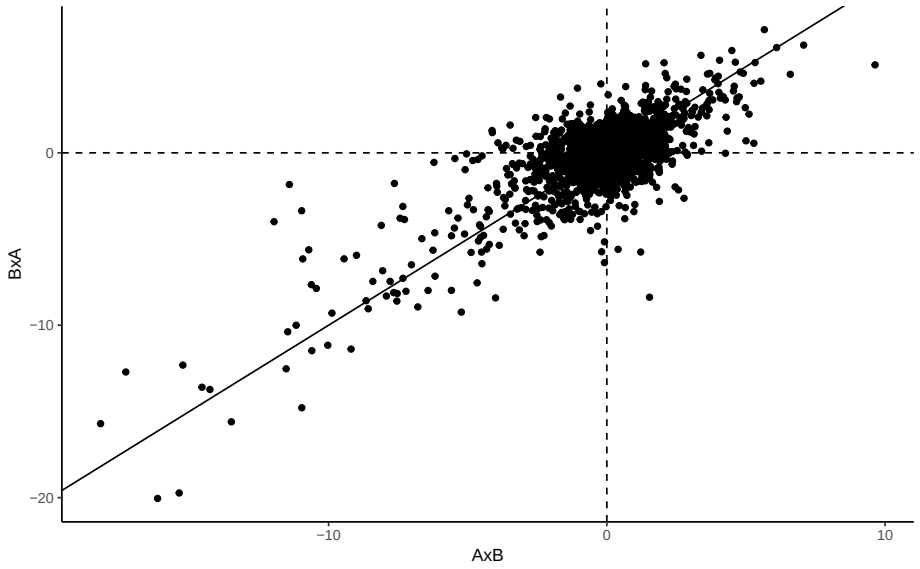



```
d=interaction_scores_df %>%
  filter(feature == "DNA.m.eccentricity.tmean", time=="96h",drug=="MEKi") %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  spread(bio,value)

cor(d$repl1,d$repl2,use="p",method="p")
## [1] 0.6212475

#plot correlating features pi-scores

interaction_scores_df %>%
  filter(feature == "cell_number", time=="96h",drug=="MEKi") %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  spread(bio,value) %>%
  ggplot(aes(x=repl1,y=repl2))+
      geom_abline(slope = 1,intercept = c(0,0)) +
      geom_vline(xintercept = 0,lty=2) +
      geom_hline(yintercept = 0,lty=2) +
      geom_point() +
      theme_classic()
```

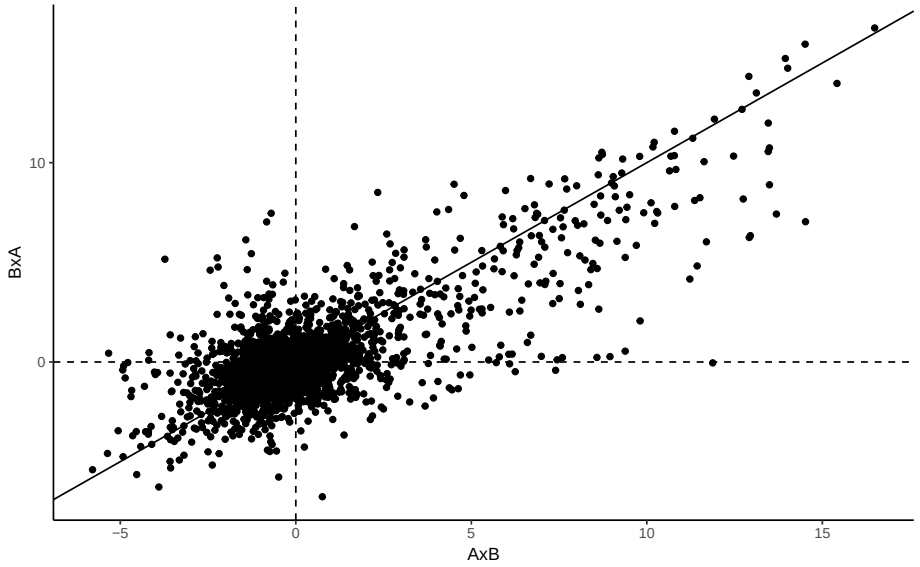



```
d=interaction_scores_df %>%
  filter(feature == "cell_number", time=="96h",drug=="MEKi") %>%
  extract(replicate,c("design","bio"),regex="(des\\d)(repl\\d)") %>%
  spread(bio,value)

cor(d$repl1,d$repl2,use="p",method="p")
## [1] 0.7603566

#plot reversibility of interactions DNA.m.eccentricity.tmean

data=data_df %>%
  dplyr::filter(time=='96h',drug=='MEKi',
                feature=="DNA.m.eccentricity.tmean") %>%
  dplyr::select(target,query,piscore) %>%
  filter(target %in% unique(.$query),query %in% unique(.$target)) %>%
  arrange(target,query) %>%
  mutate(target=as.character(target),query=as.character(query)) %>%
  group_by(target,query) %>%
  filter(target != query) %>%
  mutate(tmp=paste(sort(c(target,query)),collapse="_")) %>%
  group_by(tmp) %>%
  mutate(rep=c("AxB","BxA")) %>%
  dplyr::select(piscore,tmp,rep) %>%
  spread(rep,piscore) %>%
  ungroup()


data %>%
  ggplot(aes(x=AxB,y=BxA))+
       geom_point() +
      geom_abline(slope = 1,intercept = c(0,0)) +
      geom_vline(xintercept = 0,lty=2) +
```

```
        geom_hline(yintercept = 0,lty=2) +
        theme_classic()
```




```
cor(data$AxB,data$BxA,use = "p")
## [1] 0.7479145

#plot reversibility of interactions cell number

data=data_df %>%
  dplyr::filter(time=='96h',drug=='MEKi',feature=="cell_number") %>%
  dplyr::select(target,query,piscore) %>%
  filter(target %in% unique(.$query),query %in% unique(.$target)) %>%
  arrange(target,query) %>%
  mutate(target=as.character(target),query=as.character(query)) %>%
  group_by(target,query) %>%
  filter(target != query) %>%
  mutate(tmp=paste(sort(c(target,query)),collapse="_")) %>%
  group_by(tmp) %>%
  mutate(rep=c("AxB","BxA")) %>%
  dplyr::select(piscore,tmp,rep) %>%
  spread(rep,piscore) %>%
  ungroup()


data %>%
  ggplot(aes(x=AxB,y=BxA))+
    geom_point() +
      geom_abline(slope = 1,intercept = c(0,0)) +
      geom_vline(xintercept = 0,lty=2) +
      geom_hline(yintercept = 0,lty=2) +
      theme_classic()
```




```
cor(data$AxB,data$BxA,use = "p")
## [1] 0.7643091
```

24 Comparison of different statistical measures

First we calculate and save differential interaction estimates for all the different statistical methods.

```
sample_data=interaction_scores[,,goodfeature,,,] %>% melt() %>% tbl_df()
names(sample_data)=c("target","query","feature","time",
                     "treatment","replicate","piscore")

sample_data <- sample_data %>% filter(!grepl("CTRL",query),
                                      !grepl("RLUC",target))


my_rlm<-function(x){
  mdl=rlm(data=x,piscore~time+treatment)
  resi=mdl$residuals %>% sum(.^2)
  vari=x %>% group_by(time,treatment) %>% summarise(v=var(piscore,na.rm=T)) %>%
    .$v %>% sum(na.rm=T)
  timesig=f.robftest(mdl,var = "time")$p.value
  treatsig=f.robftest(mdl,var = "treatmentMEKi")$p.value
  res=cbind(c(timesig,treatsig),mdl$coefficients[-1],resi,vari)
  rownames(res)=c("time","treatmentMEKi")
  colnames(res)=c("pval","estimate","residuals","sumofvariance")
  return(res)
}
```

```
analyzed=sample_data %>%
  group_by(feature,treatment,target,query) %>%
  do(mutate(.,time=sample(time))) %>%
  ungroup() %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  group_by(target,query,feature) %>%
  summarise(pi_mean=mean(piscore,na.rm=T)) %>%
  ungroup()

analyzed2=sample_data %>%
  group_by(feature,treatment,target,query) %>%
  do(mutate(.,time=sample(time))) %>%
  ungroup() %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  group_by(target,query,feature) %>%
  do(mdl=my_rlm(.)) %>%
  tidy(mdl) %>%
  ungroup() %>%
  rename(term=`.rownames`)

analyzed %<>% left_join(analyzed2) %>% group_by(feature) %>%
  mutate(fdr=p.adjust(pval,method = "BH")) %>% ungroup()

save(analyzed,file =
    "time_treatment_rlm_model_analyzed_notscaled!_interactions_shuffled.RData")

analyzed=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  group_by(target,query,feature) %>%
  summarise(pi_mean=mean(piscore,na.rm=T)) %>%
  ungroup()

analyzed2=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  group_by(target,query,feature) %>%
  do(mdl=my_rlm(.)) %>%
  tidy(mdl) %>%
  ungroup() %>%
  rename(term=`.rownames`)

analyzed %<>% left_join(analyzed2) %>% group_by(feature) %>%
  mutate(fdr=p.adjust(pval,method = "BH")) %>% ungroup()

save(analyzed,file =
       "time_treatment_rlm_model_analyzed_notscaled!_interactions.RData")

my_rlm_wotime<-function(x){
  mdl=rlm(data=x,piscore~treatment)
  resi=mdl$residuals %>% sum(.^2)
  vari=x %>% group_by(treatment) %>% summarise(v=var(piscore,na.rm=T)) %>%
    .$v %>% sum(na.rm=T)
```

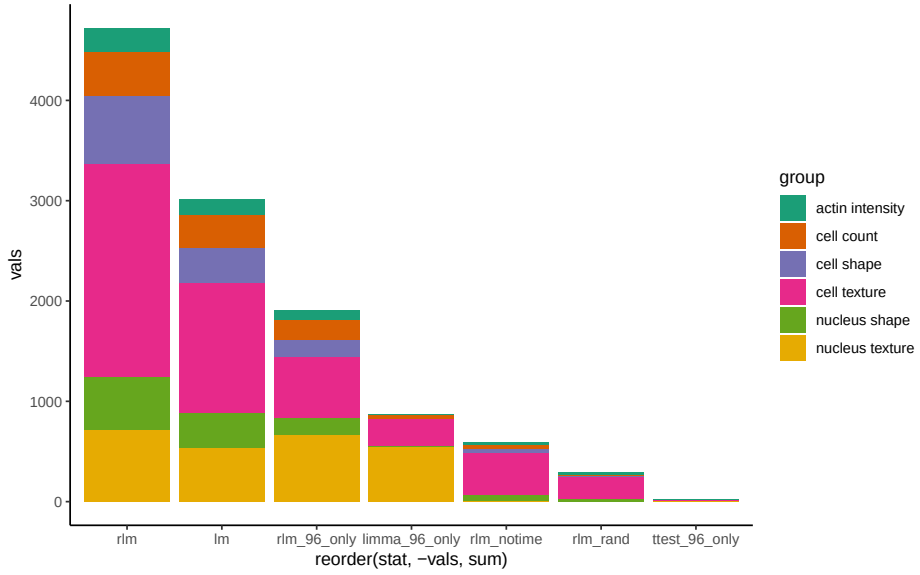
```
  treatsig=f.robftest(mdl,var = "treatmentMEKi")$p.value
  res=cbind(c(treatsig),mdl$coefficients[-1],resi,vari)
  rownames(res)=c("treatmentMEKi")
  colnames(res)=c("pval","estimate","residuals","sumofvariance")
  return(res)
}

analyzed=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(time==96) %>%
  group_by(target,query,feature) %>%
  summarise(pi_mean=mean(piscore,na.rm=T)) %>%
  ungroup()

analyzed2=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(time==96) %>%
  group_by(target,query,feature) %>%
  do(mdl=my_rlm_wotime(.)) %>%
  tidy(mdl) %>%
  ungroup() %>%
  rename(term=`.rownames`)

analyzed %<>% left_join(analyzed2) %>% group_by(feature) %>%
  mutate(fdr=p.adjust(pval,method = "BH")) %>% ungroup()

save(analyzed,file =
       "treatment_rlm_wotime_model_analyzed_time96_interactions.RData")


analyzed2=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(time==96) %>%
  group_by(target,query,feature) %>%
  do(mdl=t.test(piscore~treatment,data = .)) %>%
  tidy(mdl) %>%
  ungroup()

ttested_96h_differentialints<-analyzed2 %>% group_by(feature) %>%
  mutate(fdr=p.adjust(p.value,method = "BH")) %>% ungroup()

save(ttested_96h_differentialints,file =
       "treatment_t_test_analyzed_time96_interactions.RData")

analyzed2=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(time==72) %>%
  group_by(target,query,feature) %>%
  do(mdl=t.test(piscore~treatment,data = .)) %>%
  tidy(mdl) %>%
  ungroup()
```

```
ttested_72h_differentialints<-analyzed2 %>% group_by(feature) %>%
  mutate(fdr=p.adjust(p.value,method = "BH")) %>% ungroup()

save(ttested_72h_differentialints,file =
       "treatment_t_test_analyzed_time72_interactions.RData")


analyzed2=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(time!=48) %>%
  group_by(target,query,feature) %>%
  do(mdl=t.test(piscore~treatment,data = .)) %>%
  tidy(mdl) %>%
  ungroup()

ttested_7296h_differentialints<-analyzed2 %>% group_by(feature) %>%
  mutate(fdr=p.adjust(p.value,method = "BH")) %>% ungroup()

save(ttested_7296h_differentialints,file =
       "treatment_t_test_analyzed_time7296_interactions.RData")


analyzed=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  group_by(target,query,feature) %>%
  summarise(pi_mean=mean(piscore,na.rm=T)) %>%
  ungroup()

analyzed2=sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  group_by(target,query,feature) %>%
  do(
    model=lm(data = .,piscore~time+treatment)
  ) %>%
  tidy(model) %>%
  ungroup()

analyzed=left_join(analyzed,analyzed2)

analyzed <- analyzed %>% group_by(feature) %>%
  mutate(fdr=p.adjust(p.value,method = "BH"))

analyzed <- analyzed %>% filter(!grepl("CTRL",query),
                                !grepl("RLUC",target)) %>% ungroup()

save(analyzed,file =
       "time_treatment_lm_model_analyzed_notscaled!_interactions.RData")
```

25 Figure 3B: comparison of different statistical methods with regards to their power

```
#load various gene annotation like pathway associations or signaling function, plus the grouping of different
data('query_annotation', package='MODIFIdata')
data('target_annotation', package='MODIFIdata')
data('grouping', package='MODIFIdata')

fdrt=0.1


data('time_treatment_rlm_model_analyzed_interactions', package='MODIFIdata')
rlm<-analyzed %>% filter(term=="treatmentMEKi",fdr<fdrt) %>%
  group_by(feature) %>% summarise(n()) %>% dplyr::rename(rlm='n()')
total<-analyzed %>% filter(term=="treatmentMEKi",fdr<1) %>%
  group_by(feature) %>% summarise(n()) %>% dplyr::rename(total='n()')

data('time_treatment_rlm_model_analyzed_interactions_shuffled', package='MODIFIdata')
rlm_rand<-rlm_shuffled %>% filter(term=="treatmentMEKi",fdr<fdrt) %>%
  group_by(feature) %>% summarise(n()) %>% dplyr::rename(rlm_rand='n()')

data('time_treatment_lm_model_analyzed_notscaled!_interactions', package='MODIFIdata')
lm<-lm_analyzed %>% filter(term=="treatmentMEKi",fdr<fdrt) %>%
  group_by(feature) %>% summarise(n()) %>% dplyr::rename(lm='n()')

data('treatment_rlm_wotime_model_analyzed_time487296_interactions', package='MODIFIdata')
rlm_notime<-rlm_notime %>% filter(term=="treatmentMEKi",fdr<fdrt) %>%
  group_by(feature) %>% summarise(n() )%>% dplyr::rename(rlm_notime='n()')

data('treatment_rlm_wotime_model_analyzed_time96_interactions', package='MODIFIdata')
rlm_96_only<-rlm96 %>% filter(term=="treatmentMEKi",fdr<fdrt) %>%
  group_by(feature) %>% summarise(n()) %>% dplyr::rename(rlm_96_only='n()')

data('treatment_t_test_analyzed_time96_interactions', package='MODIFIdata')
ttest_96_only<-ttested_96h_differentialints %>% filter(fdr<fdrt) %>%
  group_by(feature) %>% summarise(n()) %>% dplyr::rename(ttest_96_only='n()')

data('time_treatment_limma_96h_interactions', package='MODIFIdata')
limma_96_only<-limma96h %>% filter(fdr<fdrt) %>% group_by(feature) %>%
  summarise(n()) %>% dplyr::rename(limma_96_only='n()')

joined <- rlm %>% full_join(rlm_rand) %>% full_join(rlm_notime) %>%
  full_join(lm) %>% full_join(rlm_96_only) %>%
  full_join(ttest_96_only) %>% full_join(limma_96_only)
## Joining, by = "feature"
## Joining, by = "feature"
## Joining, by = "feature"
## Joining, by = "feature"
## Joining, by = "feature"
```

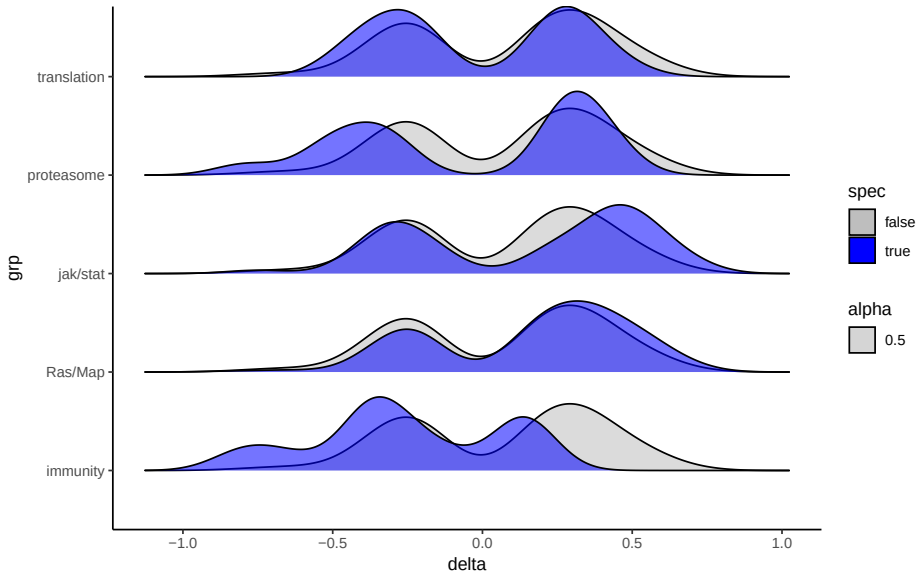
```
## Joining, by = "feature"
## Warning: Column `feature` joining factor and character vector, coercing into
## character vector

joined[is.na(joined)]=0

joined %>%
  gather("stat",value,-feature) %>%
  left_join(grouping) %>%
  group_by(group,stat) %>%
  summarise(vals=sum(value)) %>%
  ggplot(aes(x=reorder(stat,-vals,sum),y=vals,fill=group)) +
    geom_bar(stat = "identity") +
    scale_fill_brewer(palette = "Dark2") +
    theme_classic()
## Joining, by = "feature"
```



26 Figure 3C: Differential interaction scores per pathway

```
data('time_treatment_rlm_model_analyzed_interactions', package='MODIFIdata')

dat <- analyzed %>%
  left_join(target_annotation) %>%
  left_join(query_annotation) %>%
  dplyr::select(target,query,feature,term,fdr,targetpathway,querypathway,
                estimate)
## Joining, by = "target"
```

```
## Warning: Column `target` joining factor and character vector, coercing into
## character vector
## Joining, by = "query"
## Warning: Column `query` joining factor and character vector, coercing into
## character vector

dat_to_plot <- dat %>%
  filter(feature=="cell_number",fdr<0.1,term=="treatmentMEKi") %>% #
  gather(pw,pathway,-estimate,-fdr,-term,-feature,-target,-query) %>%
  dplyr::select(-pw) %>%
  gather(partner,gene,-feature,-term,-fdr,-estimate,-pathway) %>%
  dplyr::select(-partner) %>%
  mutate(delta=estimate) %>%
  bind_rows(mutate(.,pathway="all")) %>%
  filter( pathway %in% c("Ras/Map","jak/stat","immunity","proteasome",
                         "all","translation")) %>%
  mutate(pathway=factor(pathway,levels = c("all","Ras/Map","jak/stat",
                                           "immunity","proteasome",
                                           "translation")))

f<-function(x){
  bind_rows(x,dat_to_plot %>% filter(pathway=="all")) %>%
    mutate(spec=if_else(pathway=="all","false","true"),grp=x$pathway[1])
}

dat_to_plot %>%
  group_by(pathway) %>%
  do(
    f(.)
    ) %>%
  ungroup() %>%
  filter(grp!="all") %>%
  mutate(grp=factor(grp,levels = c("immunity","Ras/Map","jak/stat",
                                   "proteasome","translation"))) %>%
  ggplot(aes(delta, y=grp,fill=spec,height=..density..,alpha=0.5)) +
  geom_joy(scale=0.85) +
  scale_fill_manual(values = c("grey", "blue")) +
  theme_classic()
## Picking joint bandwidth of 0.101
```



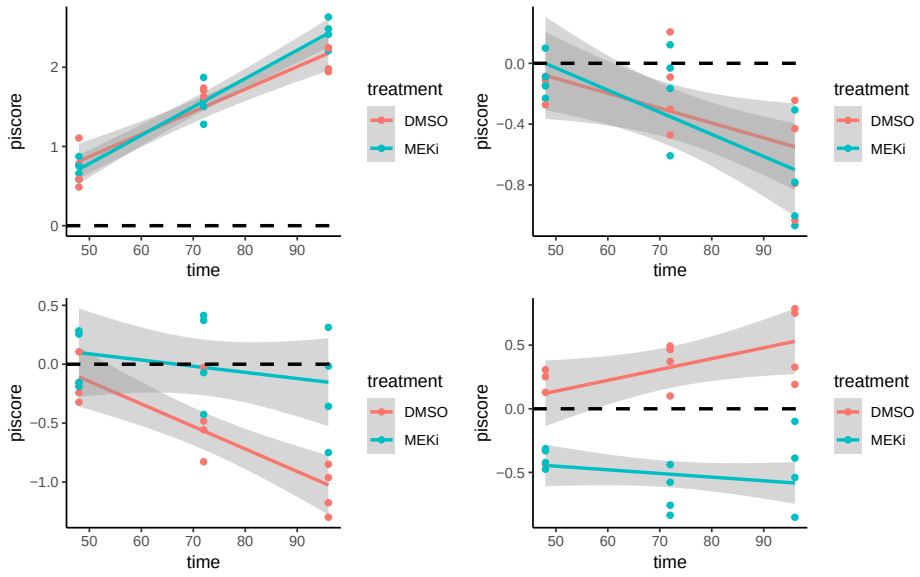

```
dat_to_plot %>%
  group_by(pathway) %>%
  do(
    f(.)
  ) %>%
  filter(grp!="all") %>%
  group_by(grp) %>%
  do(
    mdl=dplyr::select(.,val=delta,grouping=spec) %>%
      ks.test(.$val[.$grouping=="true"],
              .$val[.$grouping=="false"],alternative = "two.sided",data=.)
  ) %>%
  tidy(mdl) %>%
  ungroup() %>%
  mutate(fdr=p.adjust(p.value,method="BH"))
## Warning in ks.test(.$val[.$grouping == "true"], .$val[.$grouping ==
## "false"], : p-value will be approximate in the presence of ties
## Warning in ks.test(.$val[.$grouping == "true"], .$val[.$grouping ==
## "false"], : p-value will be approximate in the presence of ties

## Warning in ks.test(.$val[.$grouping == "true"], .$val[.$grouping ==
## "false"], : p-value will be approximate in the presence of ties

## Warning in ks.test(.$val[.$grouping == "true"], .$val[.$grouping ==
## "false"], : p-value will be approximate in the presence of ties

## Warning in ks.test(.$val[.$grouping == "true"], .$val[.$grouping ==
## "false"], : p-value will be approximate in the presence of ties
## # A tibble: 5 x 6
##   grp       statistic    p.value method                alternative       fdr
##   <fct>         <dbl>      <dbl> <chr>                 <chr>           <dbl>
```

```
## 1 Ras/Map       0.118    1.12e-4 Two-sample Kolmogoro~ two-sided     1.87e-4
## 2 jak/stat      0.282    8.49e-8 Two-sample Kolmogoro~ two-sided     2.12e-7
## 3 immunity      0.519    3.08e-8 Two-sample Kolmogoro~ two-sided     1.54e-7
## 4 proteaso~     0.280    1.07e-2 Two-sample Kolmogoro~ two-sided     1.33e-2
## 5 translat~     0.177    1.22e-1 Two-sample Kolmogoro~ two-sided     1.22e-1


dat_to_plot %>%
  dplyr::select(val=delta,grouping=pathway) %>%
  ks.test(.$val[.$grouping=="Ras/Map"],.$val[.$grouping=="jak/stat"],
          alternative = "two.sided",data=.)
## Warning in ks.test(.$val[.$grouping == "Ras/Map"], .$val[.$grouping == "jak/
## stat"], : p-value will be approximate in the presence of ties
##
##  Two-sample Kolmogorov-Smirnov test
##
## data:  .$val[.$grouping == "Ras/Map"] and .$val[.$grouping == "jak/stat"]
## D = 0.19731, p-value = 0.001747
## alternative hypothesis: two-sided

dat_to_plot %>%
  dplyr::select(val=delta,grouping=pathway) %>%
  ks.test(.$val[.$grouping=="translation"],.$val[.$grouping=="proteasome"],
          alternative = "two.sided",data=.)
## Warning in ks.test(.$val[.$grouping == "translation"], .$val[.$grouping == :
## cannot compute exact p-value with ties
##
##  Two-sample Kolmogorov-Smirnov test
##
## data:  .$val[.$grouping == "translation"] and .$val[.$grouping == "proteasome"]
## D = 0.22506, p-value = 0.2753
## alternative hypothesis: two-sided
```

27 Figure 4A-D: Example stable and differental positive and negative interactions

```
sample_data=interaction_scores[,,goodfeature,,,] %>% melt() %>% tbl_df()
names(sample_data)=c("target","query","feature","time",
                     "treatment","replicate","piscore")

sample_data <- sample_data %>% filter(!grepl("CTRL",query),
                                      !grepl("RLUC",target))

# to illustrate different types of behaviours that can be observed in
#such an analysis we plot representative examples
#condition stable buffering lethal interaction between Proteasome
```

```
#sub unit 4 (Prosbeta4/Prosbeta4) and mikrotubule star (mts/PPP2CA)

a<-sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(target=="Prosbeta4",query=="mts",feature=="cell_number") %>%
  ggplot(aes(x=time,y=piscore,col=treatment)) +
  geom_smooth(formula = y~x,method = "lm") +
  geom_point() +
  geom_hline(yintercept = 0,lty=2,lwd=1) +
  theme_classic()

#condition stable synthetic lethal interaction between kinase supressor of
#ras (ksr) and rolled (rl/ERK)
b<-sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(target=="ksr",query=="rl",feature=="cell_number") %>%
  ggplot(aes(x=time,y=piscore,col=treatment)) +
  geom_smooth(formula = y~x,method = "lm") +
  geom_point() +
  geom_hline(yintercept = 0,lty=2,lwd=1) +
  theme_classic()

#negative dynamic synthetic lethal interaction between skuld (skd/MED13) and
#Dstat  (Stat92E/STAT5B)
c<-sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(target=="skd",query=="Stat92E",feature=="cell_number") %>%
  ggplot(aes(x=time,y=piscore,col=treatment)) +
  geom_smooth(formula = y~x,method = "lm") +
  geom_point() +
  geom_hline(yintercept = 0,lty=2,lwd=1) +
  theme_classic()

#positive dynamic synthetic lethal interaction between Relish (Rel/NFkB) and
#pointed  (pnt/ETS1)
d<-sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(target=="Rel",query=="pnt",feature=="cell_number") %>%
  ggplot(aes(x=time,y=piscore,col=treatment)) +
  geom_smooth(formula = y~x,method = "rlm") +
  geom_point() +
  geom_hline(yintercept = 0,lty=2,lwd=1) +
  theme_classic()

a+b+c+d
```

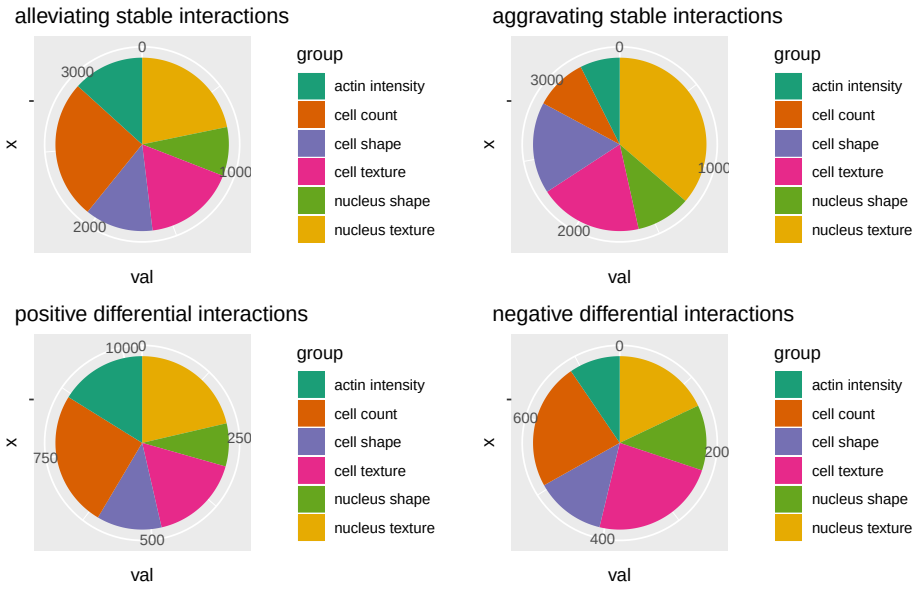


28 Figure 4E: Test that the linear model does predict equal amounts of interactions for every feature independently

```
 p1 <- analyzed %>%
  filter(fdr<0.1,term=="time",estimate>0) %>%
  left_join(grouping) %>%
  group_by(group) %>%
  summarise(count=n(),norm=n[1],val=count/norm) %>%
  ggplot(aes(x="", y=val, fill=group))+
    geom_bar(width = 1, stat = "identity") +
    coord_polar("y") +
    scale_fill_brewer(palette = "Dark2") +
    ggtitle("alleviating stable interactions")
## Joining, by = "feature"
## Warning: Column `feature` joining factor and character vector, coercing into
## character vector

analyzed %>%
  filter(fdr<0.1,term=="time",estimate>0) %>%
  n_distinct()
## [1] 8522

p2 <- analyzed %>%
  filter(fdr<0.1,term=="time",estimate<0) %>%
  left_join(grouping) %>%
  group_by(group) %>%
  summarise(count=n(),norm=n[1],val=count/norm) %>%
```

```
  ggplot(aes(x="", y=val, fill=group))+
    geom_bar(width = 1, stat = "identity") +
    coord_polar("y") +
    scale_fill_brewer(palette = "Dark2") +
    ggtitle("aggravating stable interactions")
## Joining, by = "feature"
## Warning: Column `feature` joining factor and character vector, coercing into
## character vector

analyzed %>%
  filter(fdr<0.1,term=="time",estimate<0) %>%
  n_distinct()
## [1] 9946

p3 <- analyzed %>%
  filter(fdr<0.1,term=="treatmentMEKi",estimate>0) %>%
  left_join(grouping) %>%
  group_by(group) %>%
  summarise(count=n(),norm=n[1],val=count/norm) %>%
  ggplot(aes(x="", y=val, fill=group))+
    geom_bar(width = 1, stat = "identity") +
    coord_polar("y") +
    scale_fill_brewer(palette = "Dark2") +
    ggtitle("positive differential interactions")
## Joining, by = "feature"
## Warning: Column `feature` joining factor and character vector, coercing into
## character vector

analyzed %>%
   filter(fdr<0.1,term=="treatmentMEKi",estimate>0) %>%
  n_distinct()
## [1] 2551

p4 <- analyzed %>%
  filter(fdr<0.1,term=="treatmentMEKi",estimate<0) %>%
  left_join(grouping) %>%
  group_by(group) %>%
  summarise(count=n(),norm=n[1],val=count/norm) %>%
  ggplot(aes(x="", y=val, fill=group))+
    geom_bar(width = 1, stat = "identity") +
    coord_polar("y") +
    scale_fill_brewer(palette = "Dark2") +
    ggtitle("negative differential interactions")
## Joining, by = "feature"
## Warning: Column `feature` joining factor and character vector, coercing into
## character vector

analyzed %>%
   filter(fdr<0.1,term=="treatmentMEKi",estimate<0) %>%
  n_distinct()
## [1] 2172
```

```
p1 + p2 + p3 + p4 + plot_layout(ncol = 2,nrow = 2)
```

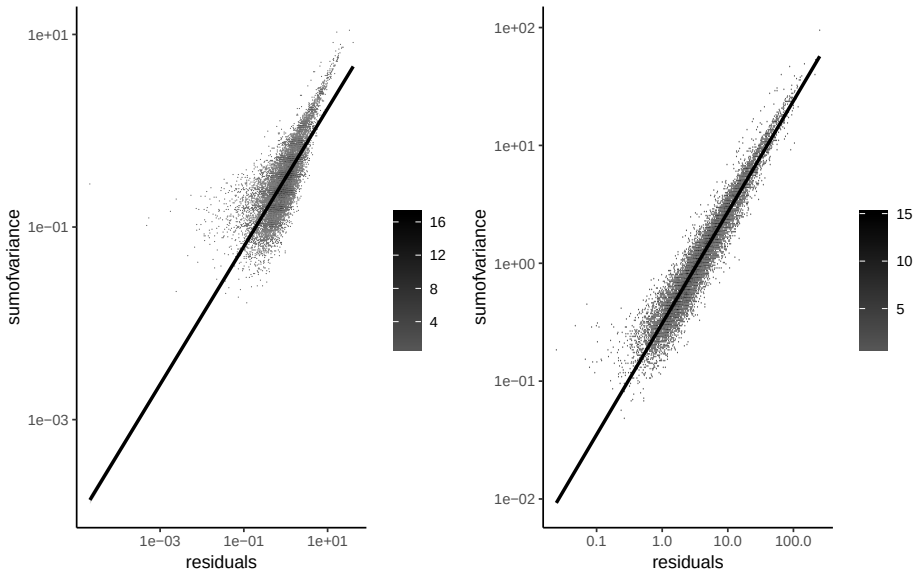


29 Figure 3-supplemental figure 1: Test if all model residuals can be explained by measurement variance

This analysis tests if the residuals of the linear model fit can be explained by the variance in the data. If the variance scales smaller than the residuals it is indicative that the model does not explain the data well. If variance and residuals scale equally we can conclude that the model fits the data well. And if the model fails it is because the data has to high variance rather than the wrong model was chosen.

```
colfunc <- colorRampPalette(c("#555555", "black"))

a<-analyzed %>%
  filter(term=="time",feature=="cell_number") %>%
  ggplot(aes(x=residuals,y=sumofvariance)) +
      geom_hex(aes(residuals, sumofvariance), bins = 250) +
      scale_x_log10() +
      scale_y_log10() +
      geom_smooth(method = "rlm",col="black") +
      scale_fill_gradientn("", colours = colfunc(150)) +
      theme_classic()


analyzed %>%
  filter(term=="time",feature=="cell_number") %>%
  dplyr::select(residuals,sumofvariance) %>% cor(use="p",method="p")
```

```
##                residuals sumofvariance
## residuals      1.0000000     0.9280765
## sumofvariance 0.9280765     1.0000000


b<-analyzed %>%
  filter(term=="time",feature=="actin.m.eccentricity.tmean") %>%
  ggplot(aes(x=residuals,y=sumofvariance)) +
      geom_hex(aes(residuals, sumofvariance), bins = 250) +
      scale_x_log10() +
      scale_y_log10() +
      geom_smooth(method = "rlm",col="black") +
      scale_fill_gradientn("", colours = colfunc(150)) +
      theme_classic()


analyzed %>%
  filter(term=="time",feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(residuals,sumofvariance) %>% cor(use="p",method="p")
##                residuals sumofvariance
## residuals      1.0000000     0.9633149
## sumofvariance 0.9633149     1.0000000

a+b
## Warning in self$trans$transform(x): NaNs produced
## Warning: Transformation introduced infinite values in continuous x-axis
## Warning in self$trans$transform(x): NaNs produced
## Warning: Transformation introduced infinite values in continuous x-axis
## Warning: Removed 143 rows containing non-finite values (stat_binhex).
## Warning: Removed 143 rows containing non-finite values (stat_smooth).
## Warning in self$trans$transform(x): NaNs produced
## Warning: Transformation introduced infinite values in continuous x-axis
## Warning in self$trans$transform(x): NaNs produced
## Warning: Transformation introduced infinite values in continuous x-axis
## Warning: Removed 3 rows containing non-finite values (stat_binhex).
## Warning: Removed 3 rows containing non-finite values (stat_smooth).
```



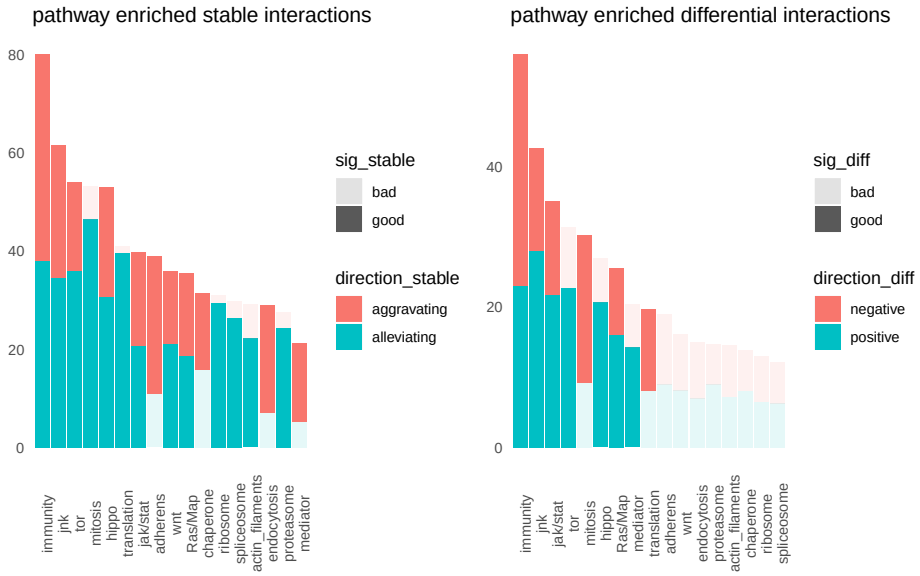
30 Figure 5A-B: pathway specific stable and differential genetic interactions

```
#merge annotations and data
pathwaycoverage <- target_annotation %>% group_by(targetpathway) %>%
  summarise(n=n())

toplot <- analyzed %>%
  filter(term=="time",fdr<=0.3,feature=="cell_number") %>% #
  left_join(target_annotation) %>%
  left_join(query_annotation) %>%
  mutate(direction=ifelse(estimate>0,"alleviating","aggravating")) %>%
  dplyr::select(target,query,targetpathway,querypathway,direction)
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector
## Joining, by = "query"
## Warning: Column `query` joining factor and character vector, coercing into
## character vector


names=toplot %>%
  rowwise() %>%
  do(con=paste(sort(c(as.character(.$target),as.character(.$query))),
               collapse="_")) %>%
  unnest()

toplot<-
  cbind.data.frame(toplot,names) %>%
```

```
  tbl_df() %>%
  dplyr::select(-target,-query) %>%
  dplyr::distinct() %>%
  dplyr::select(targetpathway,querypathway,direction) %>%
  gather(obsolete,pathway,-direction) %>%
  dplyr::select(-obsolete,targetpathway=pathway) %>%
  group_by(targetpathway,direction) %>%
  summarise(count=n()) %>%
  complete(targetpathway,direction,fill = list(0)) %>%
  left_join(pathwaycoverage) %>%
  mutate(n_count=count/n)
## Joining, by = "targetpathway"

sums <- toplot %>% group_by(direction) %>% summarise_if(is.numeric,sum)
tempo <-left_join(toplot,sums %>% dplyr::select(countsum=count,nsum=n,
                                                -n_count,direction))
## Joining, by = "direction"
tempo %<>%
  group_by(targetpathway,direction) %>%
  mutate(p=binom.test(count,countsum,p = n/nsum,alternative = "g")$p.value) %>%
  ungroup() %>%
  mutate(fdr=p.adjust(p,method = "BH"),sig=ifelse(fdr<=0.1,'good','bad'))

tempo_stable <- tempo %>% dplyr::select(pathway=targetpathway,
                                        n_count_stable=n_count,
                                        sig_stable=sig,
                                        direction_stable=direction) %>%
  complete(pathway,direction_stable,fill = list(0))

toplot <- analyzed %>%
  filter(term=="treatmentMEKi",fdr<=0.3,feature=="cell_number") %>%
  left_join(target_annotation) %>%
  left_join(query_annotation) %>%
  mutate(direction=ifelse(estimate>0,"positive","negative")) %>%
  dplyr::select(target,query,targetpathway,querypathway,direction)
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector
## Joining, by = "query"
## Warning: Column `query` joining factor and character vector, coercing into
## character vector


names=toplot %>%
  rowwise() %>%
  do(con=paste(sort(c(as.character(.$target),as.character(.$query))),
               collapse="_")) %>%
  unnest()

toplot<-
  cbind.data.frame(toplot,names) %>%
```

```
  tbl_df() %>%
  dplyr::select(-target,-query) %>%
  dplyr::distinct() %>%
  dplyr::select(targetpathway,querypathway,direction) %>%
  gather(obsolete,pathway,-direction) %>%
  dplyr::select(-obsolete,targetpathway=pathway) %>%
  group_by(targetpathway,direction) %>%
  summarise(count=n()) %>%
  complete(targetpathway,direction,fill = list(0)) %>%
  left_join(pathwaycoverage) %>%
  mutate(n_count=count/n)
## Joining, by = "targetpathway"


sums <- toplot %>% group_by(direction) %>% summarise_if(is.numeric,sum)
tempo <-left_join(toplot,sums %>% dplyr::select(countsum=count,nsum=n,
                                                -n_count,direction))
## Joining, by = "direction"
tempo %<>%
  group_by(targetpathway,direction) %>%
  mutate(p=binom.test(count,countsum,p = n/nsum,alternative = "g")$p.value) %>%
  ungroup() %>%
  mutate(fdr=p.adjust(p,method = "BH"),sig=ifelse(fdr<=0.1,'good','bad'))

tempo_diff <- tempo %>% dplyr::select(pathway=targetpathway,
              n_count_diff=n_count,sig_diff=sig,direction_diff=direction) %>%
  complete(pathway,direction_diff,fill = list(0))

tempo_complete <- full_join(tempo_stable,tempo_diff) %>%
  complete(pathway,direction_stable,direction_diff) %>%
  group_by(pathway) %>%
  mutate(anysig=ifelse(any(c(sig_stable,sig_diff)=="good"),"sig","nonsig")) %>%
  filter(anysig=="sig") %>% ungroup()
## Joining, by = "pathway"

a<-tempo_complete %>%
  dplyr::select(pathway,n_count_stable,direction_stable,sig_stable) %>%
  dplyr::distinct() %>%
  ggplot(aes(x=reorder(pathway,-n_count_stable,sum),y=n_count_stable,
             fill=direction_stable,alpha=sig_stable)) +
  geom_bar(stat="identity") +
  theme(axis.title = element_blank(),
        panel.background = element_blank(),axis.ticks.x = element_blank(),
        axis.ticks.y = element_blank(),axis.text.x = element_text(angle = 90))+
  ggtitle("pathway enriched stable interactions")


b<-tempo_complete %>%
  dplyr::select(pathway,n_count_diff,direction_diff,sig_diff) %>%
  dplyr::distinct() %>%
  ggplot(aes(x=reorder(pathway,-n_count_diff,sum,ma.rm=T),y=n_count_diff,
```

```
              fill=direction_diff,alpha=sig_diff)) +
  geom_bar(stat="identity") +
  theme(axis.title = element_blank(),panel.background = element_blank(),
        axis.ticks.x = element_blank(),axis.ticks.y = element_blank(),
        axis.text.x = element_text(angle = 90))+
  ggtitle("pathway enriched differential interactions")

a+b
## Warning: Using alpha for a discrete variable is not advised.
## Warning: Using alpha for a discrete variable is not advised.
```

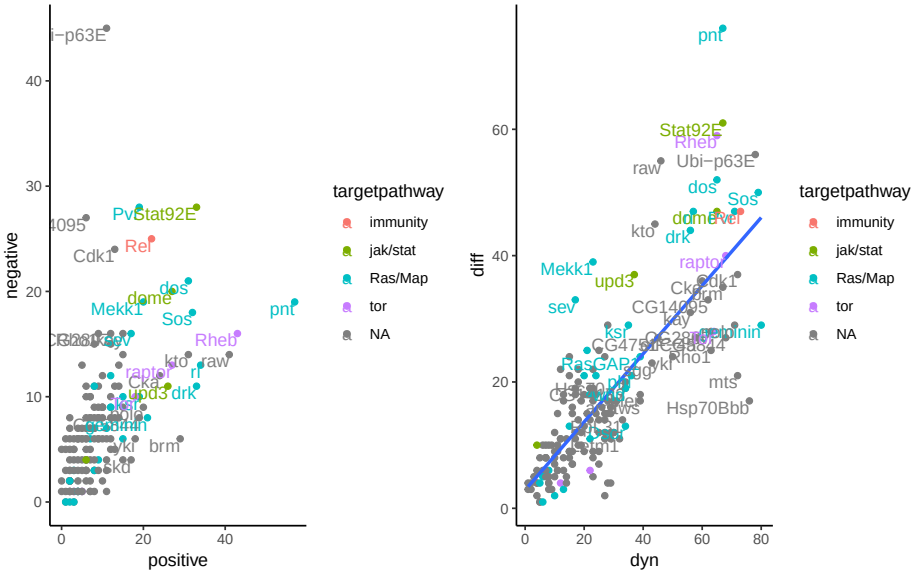


31 Figure 5C-D: gene specific stable and differential genetic interactions

```
#Plot a diagram, that shows which genes have few or a lot of differential
#genetic itneractions
x <- analyzed %>%
     filter(term=="treatmentMEKi",fdr<0.3,feature=="cell_number") %>%
      mutate(sign=ifelse(estimate>0,"positive","negative")) %>%
      dplyr::select(target,query,sign) %>%
      mutate(target=as.character(target),query=as.character(query))


y <- x %>%   rowwise() %>%
  do(con=paste(sort(c(.$target,.$query)),collapse="_")) %>%
  unnest() %>%
  separate(con,into=c('target','query'),sep = "_")
```

```
x$target=y$target
x$query=y$query

x1 <- x %>% dplyr::distinct() %>% group_by(target,sign) %>%
  summarise(n=n()) %>% ungroup()
x2 <- x %>% dplyr::distinct() %>% group_by(query,sign) %>%
  summarise(n=n()) %>% ungroup() %>% dplyr::select(target=query,sign,n)

a<-rbind(x1,x2) %>%
  group_by(target,sign) %>%
  summarise(n=sum(n)) %>%
  spread(sign,n,fill = 0) %>%
  left_join(target_annotation) %>%
  mutate(targetpathway=ifelse(targetpathway %in% c("Ras/Map",
                                                   "jak/stat","immunity","tor"),
                              targetpathway,NA)) %>%
  ggplot(aes(x=positive,y=negative,col=targetpathway,label=target)) +
  geom_point() +
  geom_text(aes(label=ifelse(positive>15|negative>15,as.character(target),'')),
            hjust=1,vjust=1) +
  theme_classic()
## Joining, by = "target"

differential <- analyzed %>%
     filter(term=="treatmentMEKi",fdr<0.3,feature=="cell_number") %>%
      mutate(sign=ifelse(estimate>0,"positive","negative")) %>%
      dplyr::select(target,query,sign) %>%
      mutate(target=as.character(target),query=as.character(query))


y <- differential %>%   rowwise() %>%
  do(con=paste(sort(c(.$target,.$query)),collapse="_")) %>%
  unnest() %>%
  separate(con,into=c('target','query'),sep = "_")

differential$target=y$target
differential$query=y$query
differential=differential %>%
  dplyr::distinct() %>%
  dplyr::select(-sign) %>%
  gather(type,gene) %>%
  dplyr::select(-type,target=gene) %>%
  group_by(target) %>%
  summarise(diff=n())

dynamic <- analyzed %>%
     filter(term=="time",fdr<0.3,feature=="cell_number") %>%
      mutate(sign=ifelse(estimate>0,"positive","negative")) %>%
      dplyr::select(target,query,sign) %>%
      mutate(target=as.character(target),query=as.character(query))
```

```
y <- dynamic %>%   rowwise() %>%
  do(con=paste(sort(c(.$target,.$query)),collapse="_")) %>%
  unnest() %>%
  separate(con,into=c('target','query'),sep = "_")

dynamic$target=y$target
dynamic$query=y$query
dynamic=dynamic %>%
  dplyr::distinct() %>%
  dplyr::select(-sign) %>%
  gather(type,gene) %>%
  dplyr::select(-type,target=gene) %>%
  group_by(target) %>%
  summarise(dyn=n())

b<-dynamic %>%
  left_join(differential) %>%
  left_join(target_annotation) %>%
  mutate(diff=ifelse(is.na(diff),0,diff)) %>%
   mutate(targetpathway=ifelse(targetpathway %in% c("Ras/Map","jak/stat",
                                                    "immunity","tor"),
                               targetpathway,NA)) %>%
  ggplot(aes(x=dyn,y=diff,col=targetpathway,label=target)) +
  geom_point() +
  geom_smooth(aes(x=dyn,y=diff),method="lm",se=F,inherit.aes = F) +
 # geom_abline(slope = 1,intercept = 0) +
  geom_text(aes(label=ifelse(dyn>30|diff>30,as.character(target),'')),
            hjust=1,vjust=1) +
  theme_classic()
## Joining, by = "target"
## Joining, by = "target"

a+b
```



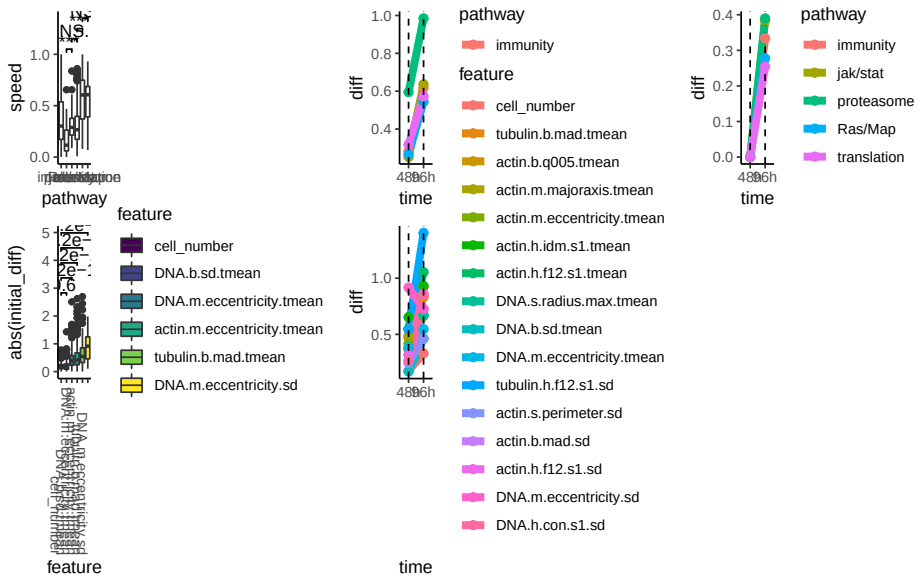
32 Figure 6A-D: gene specific stable and differential genetic interactions

```
#Figure 6A: plot the time dependence of interactions among different core pathways
dat <- analyzed %>%
  left_join(target_annotation) %>%
  left_join(query_annotation) %>%
  dplyr::select(target,query,feature,term,fdr,targetpathway,querypathway,estimate)
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector
## Joining, by = "query"
## Warning: Column `query` joining factor and character vector, coercing into
## character vector

a<-dat %>%
  filter(feature=="cell_number",fdr<0.1,term=="time") %>% #
  gather(pw,pathway,-estimate,-fdr,-term,-feature,-target,-query) %>%
  dplyr::select(-pw) %>%
  mutate(speed=(abs(estimate)-min(abs(estimate)))/
           (max(abs(estimate))-min(abs(estimate)))) %>%
  bind_rows(mutate(.,pathway="all")) %>%
  filter( pathway %in% c("all","Ras/Map","jak/stat",
                         "immunity","proteasome","translation")) %>%
  mutate( pathway=factor(pathway,levels=c("all","immunity",
                                          "jak/stat","Ras/Map",
                                          "proteasome","translation"))) %>%
  ggplot(aes(x=pathway,y=speed)) +
  geom_boxplot() +
```

```
  theme_classic() +
  geom_signif( comparisons = list(c("immunity","jak/stat"),
                                  c("jak/stat","Ras/Map"),
                                  c("proteasome", "Ras/Map"),
                                  c("proteasome", "translation")
  ),
  step_increase=0.1,
  map_signif_level=c("***"=0.001, "**"=0.01, "*"=0.05),
  test = 'wilcox.test',
  test.args = list(alternative="two.sided")
  )

#Figure 6B: plot the time dependence of interaction differences among
#different core pathways

#first filter significant differential interactions:
set<-
  analyzed %>%
  filter(feature %in% goodfeature, term=="treatmentMEKi", fdr<0.1) %>%
  unite(chosen,target,query,feature,sep = "&") %>%
  pull(chosen) %>%
  unique()

interaction_scores_df<-
  melt(interaction_scores) %>%
  tbl_df() %>%
  dplyr::select(target=Var1,query=Var2,feature=Var3,time=Var4,
                treatment=Var5,replicate=Var6,pi=value) %>%
  unite(chosen,target,query,feature,sep = "&",remove = F)

candidates_an <- interaction_scores_df %>%
  filter(chosen %in% set) %>%
  left_join(target_annotation) %>%
  left_join(query_annotation)
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector
## Joining, by = "query"
## Warning: Column `query` joining factor and character vector, coercing into
## character vector

#plot parallel coordinats of the mean differences over time
b<-candidates_an %>%
  filter(chosen %in% set
         ,time!="72h"
         ) %>%
  dplyr::select(target,query,targetpathway,querypathway,pi,
                treatment,time) %>%
  gather(pw,pathway,-target,-query,-pi,-treatment,-time) %>%
  dplyr::select(-pw) %>%
  filter( pathway %in% c("Ras/Map","jak/stat","immunity",
```

```
                          "proteasome","translation")) %>%
  group_by(target,query,pathway,time) %>%
  summarise(diff
            =abs(mean(pi[treatment=="DMSO"],na.rm=T)-
                   mean(pi[treatment=="MEKi"],na.rm=T))) %>%
  group_by(pathway,time) %>%
  summarise(diff=median(diff,na.rm=T)) %>%
  ggplot(aes(y=diff, x=time,group = pathway,label = pathway)) +
    geom_path(aes(color=pathway),alpha = 1,lineend = 'round',
              linejoin = 'round',lwd=2.3) +
    geom_point(aes(color=pathway),alpha = 1,cex=2.3) +
    geom_vline(xintercept =c(1,2),lty=2) +
    #geom_label_repel() +
    theme_classic()

#Figure 6B': plot the time dependence of interaction differences among
#different core pathways, when normalized to the initial difference

c<-candidates_an %>%
  filter(chosen %in% set
         ,time!="72h"
         ) %>%
  dplyr::select(target,query,targetpathway,querypathway,pi,treatment,time) %>%
  gather(pw,pathway,-target,-query,-pi,-treatment,-time) %>%
  dplyr::select(-pw) %>%
  filter( pathway %in% c("Ras/Map","jak/stat","immunity",
                         "proteasome","translation")) %>%
  group_by(target,query,pathway,time) %>%
  summarise(diff=abs(mean(pi[treatment=="DMSO"],na.rm=T)-
                       mean(pi[treatment=="MEKi"],na.rm=T))) %>%
  group_by(pathway,time) %>%
  summarise(diff=median(diff,na.rm=T)) %>%
  group_by(pathway) %>%
  mutate(diff=diff-diff[time=="48h"]) %>%
  ggplot(aes(y=diff, x=time,group = pathway,label = pathway)) +
    geom_path(aes(color=pathway),alpha = 1,lineend = 'round',
              linejoin = 'round',lwd=2.3) +
    geom_vline(xintercept =c(1,2),lty=2) +
    geom_point(aes(color=pathway),alpha = 1,cex=2.3) +
    #geom_label_repel() +
    theme_classic()

#Figure C: initial interaction differences among different example features

#plot examples as ordered boxes
d<-  interaction_scores_df %>%
  filter(chosen %in% set,time=="48h") %>%
  group_by(target,query,feature) %>%
  summarise(initial_diff=mean(pi[treatment=="DMSO"],na.rm=T)-
              mean(pi[treatment=="MEKi"],na.rm=T)) %>%
  ungroup() %>%
```

```
  filter(feature %in% c("cell_number","actin.m.eccentricity.tmean",
                        "DNA.m.eccentricity.tmean","DNA.m.eccentricity.sd",
                        "DNA.b.sd.tmean","tubulin.b.mad.tmean")) %>%
  mutate(feature=factor(feature,levels = c("cell_number","DNA.b.sd.tmean",
                                           "DNA.m.eccentricity.tmean",
                                           "actin.m.eccentricity.tmean",
                                           "tubulin.b.mad.tmean",
                                           "DNA.m.eccentricity.sd"),
                        ordered = T)) %>%
  ggplot(aes(y=abs(initial_diff), x=feature,fill=feature)) +
    geom_boxplot() +
    theme_classic() +
    geom_signif(
      comparisons = list(
        c("cell_number","DNA.b.sd.tmean"),
        c("cell_number","DNA.m.eccentricity.tmean"),
        c("cell_number","actin.m.eccentricity.tmean"),
        c("cell_number","tubulin.b.mad.tmean"),
        c("cell_number","DNA.m.eccentricity.sd")
        ),
      test = "t.test",
      test.args = list(alternative="two.sided"),
      step_increase = 0.2
    ) +
    theme(axis.text.x=element_text(angle=-90,hjust=1))

#Figure 5D: plot parallel coordinats of the mean differences over time
#clearly shows that texture feature mark differential interaction features
#the most time dependent again highlights the different initial interaction
#differences that can be observed depending on the pathway
#shows that DNA exxentricity variation across cells does vary the most
#initially by become surpressed in the course of the experiment
#most feature show a similar rate of differential interaction development

e<-interaction_scores_df %>%
  filter(chosen %in% set
         ,time!="72h"
         ) %>%
  group_by(target,query,feature,time) %>%
  summarise(diff=abs(mean(pi[treatment=="DMSO"],na.rm=T)-
                       mean(pi[treatment=="MEKi"],na.rm=T))) %>%
  group_by(feature,time) %>%
  summarise(diff=median(diff,na.rm=T)) %>%
  ggplot(aes(y=diff, x=time,group = feature,label = feature)) +
    geom_path(aes(color=feature),alpha = 1,lineend = 'round',
              linejoin = 'round',lwd=2.3) +
    geom_point(aes(color=feature),alpha = 1,cex=2.3) +
    geom_vline(xintercept =c(1,2),lty=2) +
    theme_classic()

a+b+c+d+e
```



33 Figure 7A: Small multiple networks

```
nodes_of_interest <- c('hoip','CG2807','eIF-2beta','CG3605','CG11985',
                       'skd','kto','RasGAP1','dome','Rheb','Sos','dos',
                       'drk','Stat92E','swm','Ras85D','Tor','pnt','Pvr',
                       'Cdk12','raptor','Rel','Prosalpha6','Rpn6','Prosbeta4',
                       'RpL31','eIF-4a','eIF-1A','Rpt4')

#compo=  read_csv("./default_node_table.csv") %>%
 #       dplyr::select(target=name,targetpathway)

data("core_genes_and_pathways",package="MODIFIdata")

data_sub <-
  data_df %>%
  filter(feature %in% goodfeature, target %in% nodes_of_interest,
         query %in% nodes_of_interest)

data_sub %>%
  filter(fdr<0.1,feature=="actin.m.eccentricity.tmean",time=="48h",
         drug=="DMSO") %>%
  mutate(sign=sign(piscore)) %>%
  dplyr::select(target,query,piscore,sign) %>%
  left_join(compo) %>%
  write_delim(path = "DMSO_48.tab",delim = "\t")
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector
```

```
data_sub %>%
  filter(fdr<0.1,feature=="actin.m.eccentricity.tmean",
         time=="72h",drug=="DMSO") %>%
  mutate(sign=sign(piscore)) %>%
  dplyr::select(target,query,piscore,sign) %>%
  left_join(compo) %>%
  write_delim(path = "DMSO_72.tab",delim = "\t")
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector

data_sub %>%
  filter(fdr<0.1,feature=="actin.m.eccentricity.tmean",
         time=="96h",drug=="DMSO") %>%
  mutate(sign=sign(piscore)) %>%
  dplyr::select(target,query,piscore,sign) %>%
  left_join(compo) %>%
  write_delim(path = "DMSO_96.tab",delim = "\t")
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector

data_sub %>%
  filter(fdr<0.1,feature=="actin.m.eccentricity.tmean",
         time=="48h",drug=="MEKi") %>%
  mutate(sign=sign(piscore)) %>%
  dplyr::select(target,query,piscore,sign) %>%
  left_join(compo) %>%
  write_delim(path = "MEKi_48.tab",delim = "\t")
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector

data_sub %>%
  filter(fdr<0.1,feature=="actin.m.eccentricity.tmean",
         time=="72h",drug=="MEKi") %>%
  mutate(sign=sign(piscore)) %>%
  dplyr::select(target,query,piscore,sign) %>%
  left_join(compo) %>%
  write_delim(path = "MEKi_72.tab",delim = "\t")
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector

data_sub %>%
  filter(fdr<0.1,feature=="actin.m.eccentricity.tmean",
         time=="96h",drug=="MEKi") %>%
  mutate(sign=sign(piscore)) %>%
  dplyr::select(target,query,piscore,sign) %>%
  left_join(compo) %>%
  write_delim(path = "MEKi_96.tab",delim = "\t")
```

```
## Joining, by = "target"
## Warning: Column `target` joining factor and character vector, coercing into
## character vector

#the node layout was then set in cytoscape as of the correlation network in 7C
#go on to cytoscape using the textfiles that were created
```

34 Figure 7B: Example correlations of differential interactions based on cell eccentricity

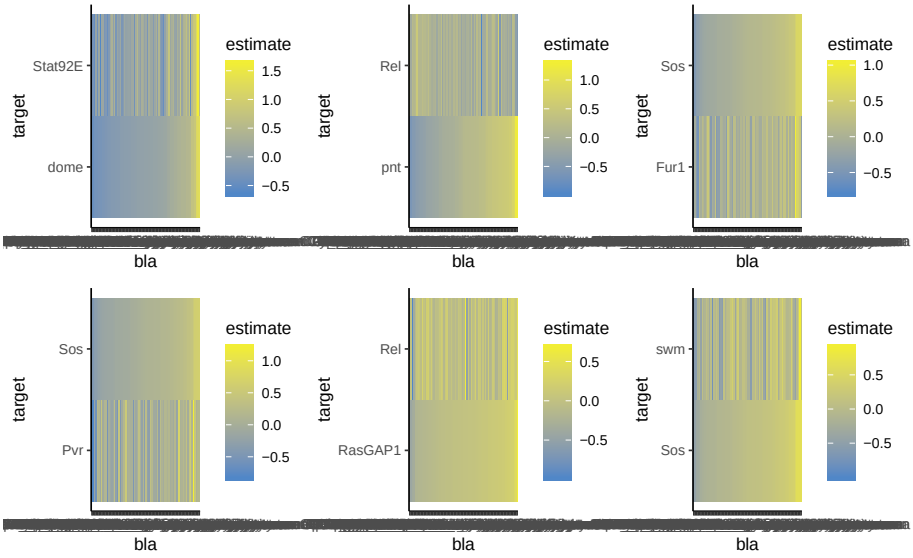
```
a<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("dome","Stat92E"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  mutate(bla=factor(bla,levels = bla[order(dome)])) %>%
  gather(target,estimate,-bla) %>%
  ggplot(aes(x=bla,y=target,fill=estimate)) +
  geom_raster() +
  scale_fill_gradient(low="#5087C8" ,high ="#F2EE35" ) +
  theme_classic()

tocor<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("dome","Stat92E"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  dplyr::select(-bla)# %>%

rcorr(x=tocor[[1]],y=tocor[[2]],type = "pearson")
##      x    y
## x 1.00 0.73
## y 0.73 1.00
##
## n= 76
##
##
## P
##   x  y
## x     0
## y  0


b<-analyzed %>%
```

```
  filter(term=="treatmentMEKi",target %in% c("Pvr","Sos"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  mutate(bla=factor(bla,levels = bla[order(Sos)])) %>%
  gather(target,estimate,-bla) %>%
  ggplot(aes(x=bla,y=target,fill=estimate)) +
  geom_raster() +
  scale_fill_gradient(low="#5087C8" ,high ="#F2EE35") +
  theme_classic()

tocor<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("Pvr","Sos"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  dplyr::select(-bla)# %>%

rcorr(x=tocor[[1]],y=tocor[[2]],type = "pearson")
##      x    y
## x 1.00 0.51
## y 0.51 1.00
##
## n= 76
##
##
## P
##   x  y
## x     0
## y  0


c<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("pnt","Rel"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  mutate(bla=factor(bla,levels = bla[order(pnt)])) %>%
  gather(target,estimate,-bla) %>%
  ggplot(aes(x=bla,y=target,fill=estimate)) +
    geom_raster() +
    scale_fill_gradient(low="#5087C8" ,high ="#F2EE35" ) +
    theme_classic()

tocor<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("pnt","Rel"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
```

```
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  dplyr::select(-bla)# %>%

rcorr(x=tocor[[1]],y=tocor[[2]],type = "pearson")
##       x     y
## x  1.00 -0.37
## y -0.37  1.00
##
## n= 76
##
##
## P
##   x     y
## x       0.001
## y 0.001


d<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("RasGAP1","Rel"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  mutate(bla=factor(bla,levels = bla[order(RasGAP1)])) %>%
  gather(target,estimate,-bla) %>%
  ggplot(aes(x=bla,y=target,fill=estimate)) +
  geom_raster() +
  scale_fill_gradient(low="#5087C8" ,high ="#F2EE35" ) +
  theme_classic()

tocor<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("RasGAP1","Rel"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  dplyr::select(-bla)# %>%

rcorr(x=tocor[[1]],y=tocor[[2]],type = "pearson")
##      x    y
## x 1.00 0.38
## y 0.38 1.00
##
## n= 76
##
##
## P
##   x     y
## x       8e-04
## y 8e-04
```

```
e<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("Sos","swm"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  mutate(bla=factor(bla,levels = bla[order(Sos)])) %>%
  gather(target,estimate,-bla) %>%
  ggplot(aes(x=bla,y=target,fill=estimate)) +
  geom_raster() +
  scale_fill_gradient(low="#5087C8" ,high ="#F2EE35" ) +
  theme_classic()

tocor<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("Sos","swm"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  dplyr::select(-bla)# %>%

rcorr(x=tocor[[1]],y=tocor[[2]],type = "pearson")
##     x   y
## x 1.0 0.5
## y 0.5 1.0
##
## n= 76
##
##
## P
##   x  y
## x     0
## y  0

f<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("Sos","Fur1"),
         feature=="actin.m.eccentricity.tmean") %>%
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  mutate(bla=factor(bla,levels = bla[order(Sos)])) %>%
  gather(target,estimate,-bla) %>%
  ggplot(aes(x=bla,y=target,fill=estimate)) +
  geom_raster() +
  scale_fill_gradient(low="#5087C8" ,high ="#F2EE35" ) +
  theme_classic()

tocor<-analyzed %>%
  filter(term=="treatmentMEKi",target %in% c("Sos","Fur1"),
         feature=="actin.m.eccentricity.tmean") %>%
```

```
  dplyr::select(target,query,feature,estimate) %>%
  spread(target,estimate) %>%
  unite(bla,query,feature) %>%
  dplyr::select(-bla)# %>%

rcorr(x=tocor[[1]],y=tocor[[2]],type = "pearson")
##      x    y
## x 1.00 0.57
## y 0.57 1.00
##
## n= 76
##
##
## P
##   x  y
## x     0
## y  0

a+c+f+b+d+e
```



35 Figure 7C: A correlation network of differential genetic interactions

```
#all differential interaction estimates are being used in this analysis
#all pairwise complete pearson correlation coefficients are calculated
#every positive correlation higher than 0.5 draws an edge

data("target_annotation",package = "MODIFIdata")
data("query_annotation",package = "MODIFIdata")
```

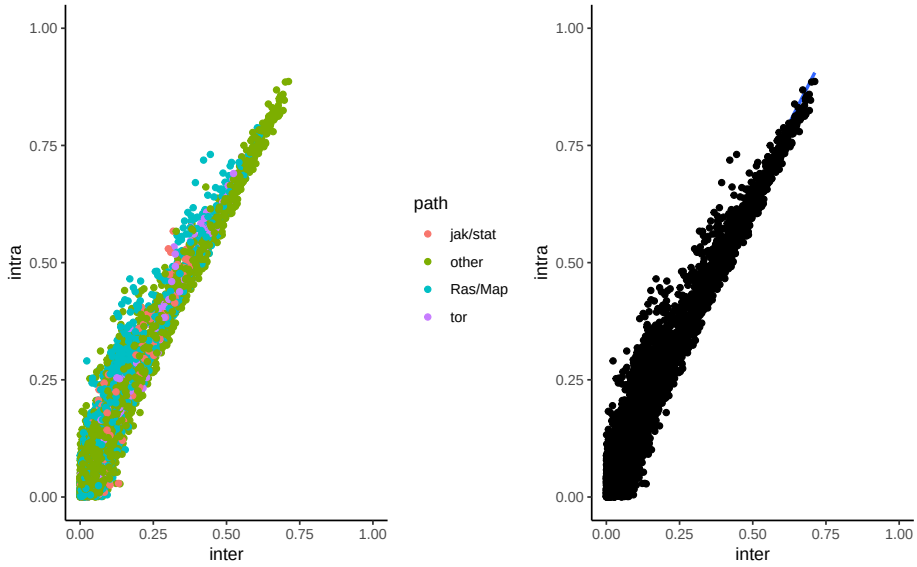
```
data("time_treatment_rlm_model_analyzed_interactions",package = "MODIFIdata")


x=analyzed %>%
  filter(term=="treatmentMEKi") %>%
  dplyr::select(target,query,feature,estimate) %>%
  unite(feat,feature,query) %>%
  spread(target,estimate,fill = 0) %>%
  dplyr::select(-feat) %>%
  cor(use = "p",method = "p") %>%
  melt() %>%
  filter(value!=1) %>%
  dplyr::select(Var1,Var2,Diffcor=value)

names=x %>%
  filter(Diffcor>0.5) %>%
  rowwise() %>%
  do(con=paste(sort(c(as.character(.$Var1),
                      as.character(.$Var2))),collapse="_")) %>%
  unnest() %>%
  separate(con,into=c('target','query'),sep = "_")

df=x %>%
  filter(Diffcor>0.5)

df=cbind.data.frame(names,df) %>%
  dplyr::select(-Var1,-Var2) %>%
  dplyr::distinct() %>%
  left_join(target_annotation) %>%
  left_join(query_annotation) %>%
  dplyr::select(target,query,Diffcor,targetpathway,querypathway)
## Joining, by = "target"
## Joining, by = "query"

write_delim(df,"correlations_of_differential_interactions_v5.tab",delim = "\t")

#go on in cytoscape
```

36 Figure 7-supplemental figure 1: A differential correlation anaylsis according to Billmann et al.

```
x=data_df %>%
  filter(fdr<0.1,!grepl("*RLUC*",target), !grepl("*CTRL*",query)) %>%
  unite(feat,query,feature,time) %>%
   unite(targ,target,drug) %>%
  dplyr::select(-fdr) %>%
  spread(targ,piscore,fill=0) %>%
```

```
  dplyr::select(-feat)

corrs=cor(x,use = "p",method = "p")

corrs[upper.tri(corrs,diag = T)]=NA

corr_df=melt(corrs) %>% separate(Var1,c("gene1","cond1"),sep = "_") %>%
  separate(Var2,c("gene2","cond2"),sep = "_") %>% tbl_df() %>% drop_na()

final_intra_inner_corr <- corr_df %>%
  mutate(comp=if_else(cond1==cond2,"intra","inter")) %>%
  dplyr::select(-cond2) %>%
  spread(comp,value) %>%
  drop_na() %>%
  dplyr::select(-cond1) %>%
  group_by(gene1,gene2) %>%
  summarise(inter=mean(inter,na.rm=T),intra=mean(intra,na.rm=T))

a<-final_intra_inner_corr %>%
  dplyr::select(target=gene1,query=gene2,inter,intra) %>%
  left_join(target_annotation %>%
  dplyr::select(target,targetpathway)) %>%
  mutate(targetpathway=ifelse(targetpathway %in% c("Ras/Map","jak/stat","tor"),
                              targetpathway,"other")) %>%
  left_join(query_annotation %>%
  dplyr::select(query,querypathway)) %>%
  mutate(querypathway=ifelse(querypathway %in% c("Ras/Map","jak/stat","tor"),
                             querypathway,"other")) %>%
  mutate(path=if_else(querypathway %in% c("Ras/Map","jak/stat","tor"),
                      querypathway,if_else(targetpathway %in%
                                             c("Ras/Map","jak/stat","tor"),
                                           targetpathway,"other"))) %>%
  ungroup() %>%
  ggplot(aes(x=inter,y=intra,text=paste0(target,query))) +
    geom_point(aes(col=path)) +
   # geom_text_repel() +
    xlim(0,1) +
    ylim(0,1) +
    theme_classic()
## Joining, by = "target"
## Joining, by = "query"

b<-final_intra_inner_corr %>%
  dplyr::select(target=gene1,query=gene2,inter,intra) %>%
  left_join(target_annotation %>%
  dplyr::select(target,targetpathway)) %>%
  mutate(targetpathway=ifelse(targetpathway %in% c("Ras/Map","jak/stat","tor"),
                              targetpathway,"other")) %>%
  left_join(query_annotation %>%
  dplyr::select(query,querypathway)) %>%
  mutate(querypathway=ifelse(querypathway %in% c("Ras/Map","jak/stat","tor"),
```

```
                                querypathway,"other")) %>%
  mutate(path=if_else(querypathway %in% c("Ras/Map","jak/stat","tor"),
                      querypathway,if_else(targetpathway %in%
                                             c("Ras/Map","jak/stat","tor"),
                                           targetpathway,"other"))) %>%
  ungroup() %>%
  ggplot(aes(x=inter,y=intra)) +
    geom_smooth(mapping = aes(x=inter,y=intra),method="lm") +
    geom_point() +
    xlim(0,1) +
    ylim(0,1) +
    theme_classic()
## Joining, by = "target"
## Joining, by = "query"

a+b
## Warning: Removed 7656 rows containing missing values (geom_point).
## Warning: Removed 7656 rows containing non-finite values (stat_smooth).
## Warning: Removed 7656 rows containing missing values (geom_point).
```

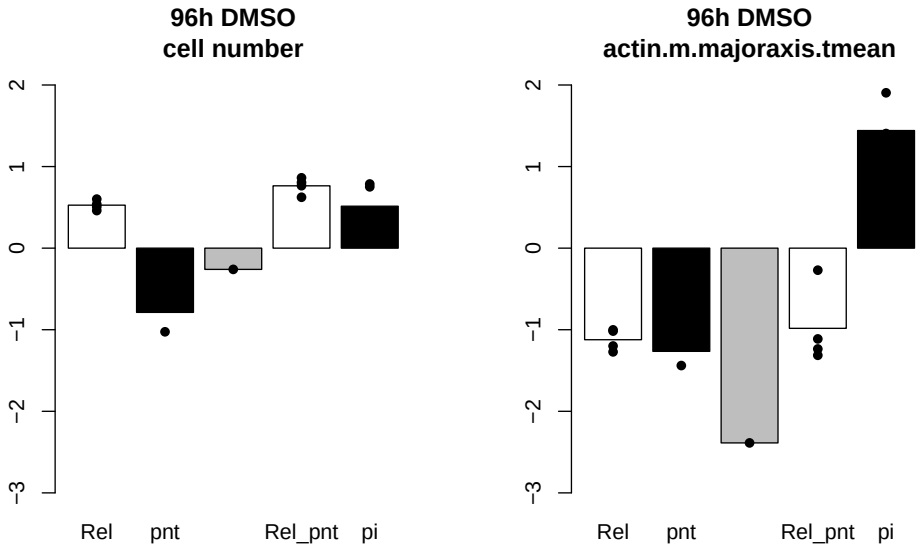


37 Figure 8-supplemental figure 1: Rel-pnt differential interactions

```
plot_effects<-function(target=22,query=68,timepoint="96h",treatment="DMSO",
                       feature="cell_number",ymin,ymax,main="plot"){
  if(is.numeric(target)){
    targetoi=dimnames(Interactions$piscore)[[1]][target]
  }else{
    targetoi=target
```

```
        }
        if(is.numeric(query)){
          queryoi=dimnames(Interactions$piscore)[[2]][query]
        }else{
          queryoi=query
        }

        targetmain=mainEffects$target[grep(
          targetoi,dimnames(mainEffects$target)[[1]]),feature,,timepoint,treatment]
        querymain=mainEffects$query[grep(
          queryoi,dimnames(mainEffects$query)[[1]]),feature,,timepoint,treatment]
        pi=interaction_scores[grep(targetoi,
                                   dimnames(interaction_scores)[[1]]),
                              grep(queryoi,
                                   dimnames(interaction_scores)[[2]]),
                              feature,timepoint,treatment,]

        combieffect=my.data.array.norm.reshaped[
          grep(targetoi,
               dimnames(my.data.array.norm.reshaped)[[1]]),
          grep(queryoi,
               dimnames(my.data.array.norm.reshaped)[[2]])
          ,feature,timepoint,treatment,]
        targetdt=cbind.data.frame(targetoi,c(targetmain))
        querydt=cbind.data.frame(queryoi,c(querymain))
        expecteddt=cbind.data.frame(paste(targetoi,queryoi,"expected",sep="_"),
                                    mean(c(targetmain))+mean(c(querymain)))
        combidt=cbind.data.frame(paste(targetoi,queryoi,sep="_"),c(combieffect))
        pidt=cbind.data.frame("pi",c(pi))
        names(targetdt)=c("gene","data")
        names(querydt)=c("gene","data")
        names(combidt)=c("gene","data")
        names(pidt)=c("gene","data")
        names(expecteddt)=c("gene","data")
        alldt=rbind.data.frame(targetdt,querydt,expecteddt,combidt,pidt)
        mean_Data=dcast(formula = gene~.,data = alldt,fun.aggregate = mean)
        if(missing(ymax)){
          ymax=0
          if(max(alldt$data)>0){
            ymax=max(alldt$data)+0.1
          }
        }
        if(missing(ymin)){
          ymin=0
          if(min(alldt$data)<0){
            ymin=min(alldt$data)-0.1
          }
        }

        x=barplot(mean_Data[,2],names.arg = mean_Data[,1],
                  ylim=c(ymin,ymax)
```

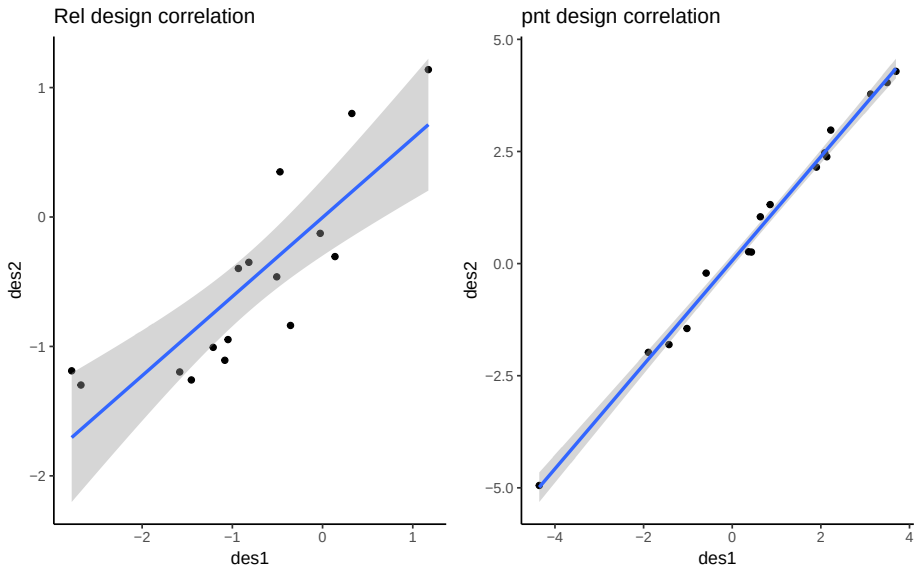
```
              ,col = c("white","black","grey"),
              main=main)
  alldt$gene=as.character(alldt$gene)
  for(k in 1:nrow(mean_Data)){
    alldt$gene[alldt$gene %in% mean_Data[k,1]]=x[k,1]
  }
  points(alldt,pch=16)
}

par(mfrow=c(1,2))
plot_effects(target="Rel",query="pnt",timepoint="96h",treatment="DMSO",
             feature="cell_number",ymin=-3,ymax=2,
             main="96h DMSO\ncell number")
## Using data as value column: use value.var to override.

plot_effects(target="Rel",query="pnt",timepoint="96h",treatment="DMSO",
             feature="actin.m.majoraxis.tmean",ymin=-3,ymax=2,
             main="96h DMSO\nactin.m.majoraxis.tmean")
## Using data as value column: use value.var to override.
```



38 Figure 8-supplemental figure 2: dsRNA correlations

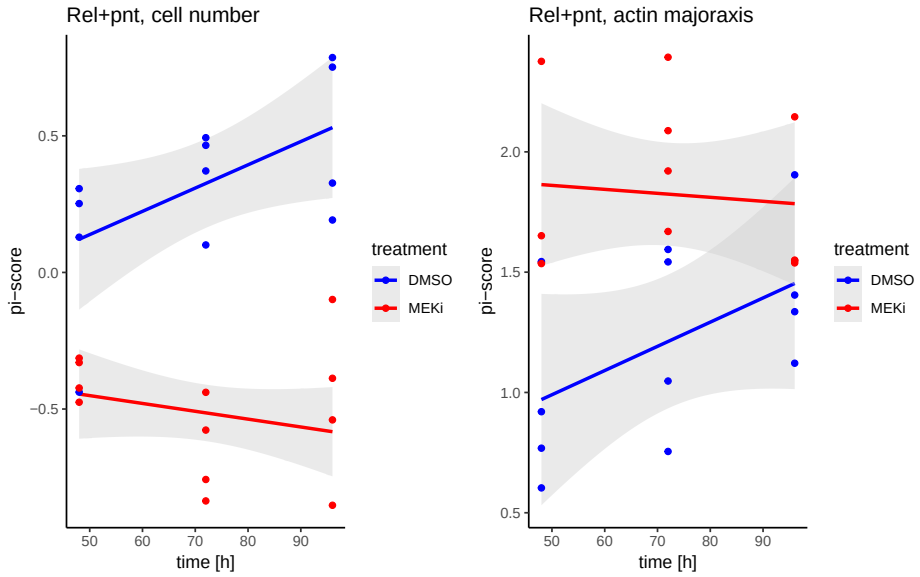
```
sub_df<-my.data.array.norm.reshaped_df %>%
  filter(target=="Rel",
      query %in% c("CTRL_1","CTRL_2","CTRL_3","CTRL_4"),
      time=="96h",drug=="DMSO",feature %in% goodfeature)

a<-sub_df %>%
```

```
  extract(replicate,c("design","rep"),regex = "(des\\d)(repl\\d)") %>%
  group_by(target,feature,design) %>%
  summarise(value=mean(value,na.rm=T)) %>%
  spread(design,value) %>%
  ungroup() %>%
  ggplot(aes(x=des1,y=des2)) +
  geom_point() +
  geom_smooth(method="lm")+
  theme_classic()+
  ggtitle("Rel design correlation")

tocor<-sub_df %>%
  extract(replicate,c("design","rep"),regex = "(des\\d)(repl\\d)") %>%
  group_by(target,feature,design) %>%
  summarise(value=mean(value,na.rm=T)) %>%
  spread(design,value) %>%
  ungroup() %>%
  dplyr::select(x=des1,y=des2)

x<-rcorr(tocor$x,tocor$y,type="p")

sub_df<-my.data.array.norm.reshaped_df %>%
  filter(target=="pnt", query %in%
  c("CTRL_1","CTRL_2","CTRL_3","CTRL_4"),
  time=="96h",drug=="DMSO",feature %in% goodfeature)

b<-sub_df %>%
  extract(replicate,c("design","rep"),regex = "(des\\d)(repl\\d)") %>%
  group_by(target,feature,design) %>%
  summarise(value=mean(value,na.rm=T)) %>%
  spread(design,value) %>%
  ungroup() %>%
  ggplot(aes(x=des1,y=des2)) +
  geom_point() +
  geom_smooth(method="lm") +
  theme_classic()+
  ggtitle("pnt design correlation")

tocor<-sub_df %>%
  extract(replicate,c("design","rep"),regex = "(des\\d)(repl\\d)") %>%
  group_by(target,feature,design) %>%
  summarise(value=mean(value,na.rm=T)) %>%
  spread(design,value) %>%
  ungroup() %>%
  dplyr::select(x=des1,y=des2)

  y<-rcorr(tocor$x,tocor$y,type="p")

  a+b
```



39 Figure 8C-C': Rel-pnt differential interactions

```
a<-sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(target=="Rel",query=="pnt",feature=="cell_number") %>%
  ggplot(aes(x=time,y=piscore,colour=treatment)) +
  geom_smooth(formula = y~x,method = "rlm",aes(fill=treatment)) +
  geom_point() +
  theme_classic() +
  scale_fill_manual(values = c("DMSO"=rgb(204,204,204,0.5,maxColorValue = 255),
                               "MEKi"=rgb(204,204,204,0.5,maxColorValue = 255))) +
  scale_color_manual(values = c("DMSO"="blue","MEKi"="red")) +
  labs(x = "time [h]" , y ="pi-score", title="Rel+pnt, cell number")

b<-sample_data %>%
  mutate(time=ifelse(time=="48h",48,ifelse(time=="72h",72,96))) %>%
  filter(target=="Rel",query=="pnt",feature=="actin.m.majoraxis.tmean") %>%
  ggplot(aes(x=time,y=piscore,colour=treatment)) +
  geom_smooth(formula = y~x,method = "rlm",aes(fill=treatment)) +
  geom_point() +
  theme_classic() +
  scale_fill_manual(values = c("DMSO"=rgb(204,204,204,0.5,maxColorValue = 255),
                               "MEKi"=rgb(204,204,204,0.5,maxColorValue = 255))) +
  scale_color_manual(values = c("DMSO"="blue","MEKi"="red")) +
  labs(x = "time [h]" , y ="pi-score", title="Rel+pnt, actin majoraxis")

a+b
```

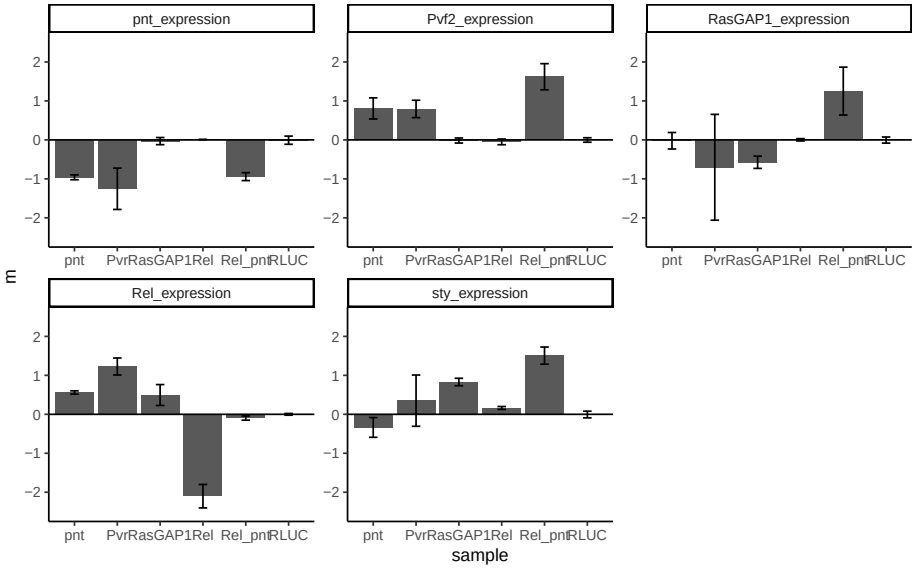


40 Figure 8D,E: Loss of Rel rescues loss of Ras signaling activity by Pvf2 upregulation

```
#read in raw Cp values
data('figure7_qPCR', package='MODIFIdata')

#reformat Cp values
result<-raw_dat %>%
  dplyr::select(Cp,Pos,Name) %>%
  drop_na() %>%
  extract(Pos,c("row","col"),regex = "(\\w)(\\d+)",remove = F) %>%
 mutate(sample=
if_else(col %in% c(1,2,3),"RLUC",
 if_else(col %in% c(4,5,6),"Rel",
         if_else(col %in% c(7,8,9),"pnt",
                 if_else(col %in% c(10,11,12),"Rel_pnt",
                         if_else(col %in% c(13,14,15),"Pvr",
                                 if_else(col %in% c(16,17,18),"RasGAP1","none"
                                 )
                         )
                 )
         )
 )
)
  ) %>%
  mutate(replicate=
           if_else(col %in% c(1,4,7,10,13,16,19,20,21),1,
                   if_else(col %in% c(2,5,8,11,14,17,22,23,24),2,
```

```
                              if_else(col %in% c(3,6,9,12,15,18),3,0
                              )
                      )
              )
    ) %>%
    mutate(primer=
  if_else(row %in% c("A","B","C"),"rps",
     if_else(row %in% c("D","E","F"),"Pvf2",
             if_else(row %in% c("G","H","I"),"sty",
                     if_else(row %in% c("J","K","L"),"Rel",
                             if_else(row %in% c("M","N"),"pnt",
                                     if_else(row %in% c("O","P"),"RasGAP1","none"
                                     )
                             )
                     )
             )
     )
  )
    ) %>%
    mutate(Cp=as.numeric(Cp)) %>%
    filter(primer != "none",replicate!=0,sample!="none") %>%
    dplyr::select(-Pos,-row,-col,-Name) %>%
    group_by(sample,primer,replicate) %>%
    summarise(Cp=mean(Cp)) %>%
    ungroup() %>%
    spread(primer,Cp) %>%
    mutate(pnt_expression=2^-(pnt-rps),
           Rel_expression=2^-(Rel-rps),
           Pvf2_expression=2^-(Pvf2-rps),
           sty_expression=2^-(sty-rps),
           RasGAP1_expression=2^-(RasGAP1-rps))

  #plot the data in bargraphs for the figure
  result %>%
    dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                  sty_expression,RasGAP1_expression,replicate) %>%
    filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
    ungroup() %>% gather(gene,value,-replicate,-sample) %>%
    group_by(gene) %>%
    mutate(value=value/mean(value[sample=="RLUC"])) %>%
    group_by(sample,gene) %>%
    mutate(value=log2(value)) %>%
    summarise(m=mean(value),s=sd(value)/sqrt(length(value)),l=m-s,u=m+s) %>%
    ungroup() %>%
    mutate(sample=factor(c(sample),levels=c("pnt","Pvr","RasGAP1",
                                            "Rel","Rel_pnt","RLUC"))) %>%
    ggplot(aes(x=sample,y=m)) +
    facet_wrap("gene",scales = "free") +
    geom_bar(stat="identity",position=position_dodge()) +
    geom_errorbar(aes(ymin=l, ymax=u),
                  width=.2,                    # Width of the error bars
```

```
                position=position_dodge(.9)) +
  ylim(c(-2.5,2.5)) +
  geom_hline(yintercept = 0) +
  theme_classic()
```




```
#test the statisticss by a two sided t.test
result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="Rel_expression",sample %in% c("RLUC","Rel")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = -6.9639, df = 2.0217, p-value = 0.01944
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -3.3860539 -0.8161683
## sample estimates:
##  mean in group Rel mean in group RLUC
##       -2.101450688       -0.000339577

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
```

```
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="Rel_expression",sample %in% c("RLUC","Pvr")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = 5.6005, df = 2.0412, p-value = 0.02907
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.3025354 2.1530202
## sample estimates:
##  mean in group Pvr mean in group RLUC
##        1.227438201       -0.000339577

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="Rel_expression",sample %in% c("RLUC","pnt")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = 11.708, df = 3.0152, p-value = 0.001307
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.4082181 0.7117819
## sample estimates:
##  mean in group pnt mean in group RLUC
##        0.559660423       -0.000339577

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
```

```
  mutate(value=log2(value)) %>%
  filter(gene=="pnt_expression",sample %in% c("RLUC","Rel_pnt")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = -6.3916, df = 3.9959, p-value = 0.003087
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.3413207 -0.5286793
## sample estimates:
## mean in group Rel_pnt    mean in group RLUC
##          -0.942706259          -0.007706259

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="pnt_expression",sample %in% c("RLUC","pnt")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = -7.7898, df = 3.2603, p-value = 0.003255
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.3250231 -0.5805325
## sample estimates:
##  mean in group pnt mean in group RLUC
##       -0.960484037       -0.007706259

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="sty_expression",sample %in% c("RLUC","Rel_pnt")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
```

```
##
## data:  value by sample
## t = 6.4091, df = 2.5933, p-value = 0.01179
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.6901057 2.3343388
## sample estimates:
## mean in group Rel_pnt    mean in group RLUC
##           1.507242184          -0.004980038

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="sty_expression",sample %in% c("RLUC","RasGAP1")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = 6.511, df = 3.9548, p-value = 0.002987
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.4770095 1.1918794
## sample estimates:
## mean in group RasGAP1    mean in group RLUC
##           0.829464407          -0.004980038

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="RasGAP1_expression",sample %in% c("RLUC","RasGAP1")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = -3.2414, df = 2.9373, p-value = 0.04925
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
```

```
##  -1.136449819 -0.003550181
## sample estimates:
## mean in group RasGAP1    mean in group RLUC
##          -0.574196605          -0.004196605

result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,Pvf2_expression,
                sty_expression,RasGAP1_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","Pvr","RasGAP1","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"],na.rm = T)) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  filter(gene=="Pvf2_expression",sample %in% c("RLUC","Rel_pnt")) %>%
  t.test(data=.,value~sample)
##
##  Welch Two Sample t-test
##
## data:  value by sample
## t = 4.7679, df = 2.118, p-value = 0.03687
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.2338778 3.0127889
## sample estimates:
## mean in group Rel_pnt    mean in group RLUC
##           1.621051716          -0.002281617
```

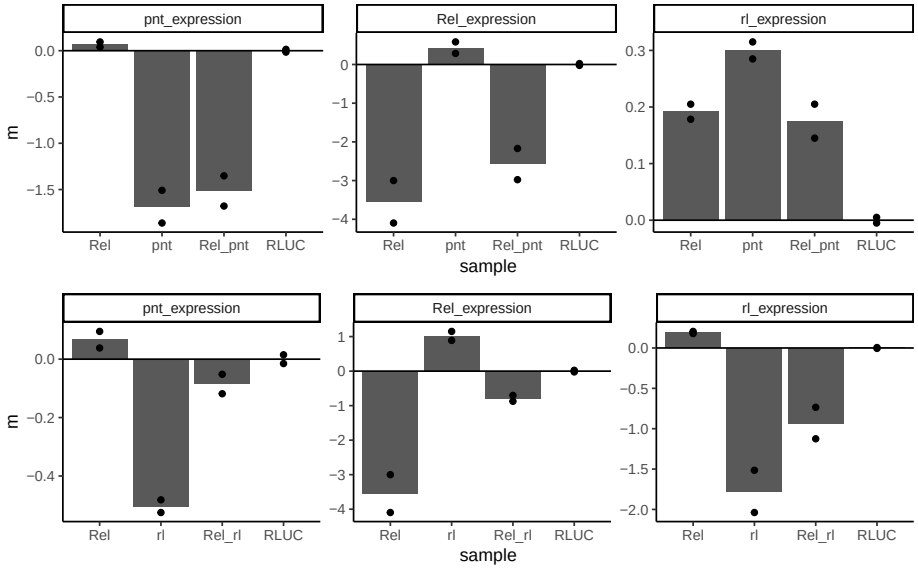
41 Figure 8-supplemental figure 4: Short term expression changes after Rel/Ras signaling co-perturbations

```
#read in raw Cp values
data('figureS10_qPCR', package='MODIFIdata')

#reformat Cp values
result<-raw_data_48h_qpcr %>%
  dplyr::select(Cp,Pos,Name) %>%
  drop_na() %>%
  extract(Pos,c("row","col"),regex = "(\\w)(\\d+)",remove = F) %>%
  mutate(sample=
if_else(col %in% c(1,2),"RLUC",
if_else(col %in% c(3,4),"Rel",
if_else(col %in% c(5,6),"pnt",
       if_else(col %in% c(7,8),"Rel_pnt",
              if_else(col %in% c(9,10),"Sos",
```

```
                        if_else(col %in% c(11,12),"Rel_Sos",
                                if_else(col %in% c(13,14),"rl",
                                        if_else(col %in% c(15,16),"Rel_rl","none"
                                        )
                                )
                        )
                )
        )
)
)
)
  ) %>%
  mutate(replicate=
           if_else(col %in% c(1,3,5,7,9,11,13,15),1,
                   if_else(col %in% c(2,4,6,8,10,12,14,16),2,0
                   )
           )
  ) %>%
  mutate(primer=
           if_else(row %in% c("A","B","C"),"rps",
                   if_else(row %in% c("D","E","F"),"Rel",
                           if_else(row %in% c("G","H","I"),"pnt",
                                   if_else(row %in% c("J","K","L"),"rl","none"
                                   )
                           )
                   )
           )
  ) %>%
  mutate(triplicate=
           if_else(row %in% c("A","D","G","J"),1,
                   if_else(row %in% c("B","E","H","K"),2,
                           if_else(row %in% c("C","F","I","L"),3,0
                           )
                   )
           )
  ) %>%
  mutate(Cp=as.numeric(Cp)) %>%
  filter(primer != "none",replicate!=0,sample!="none") %>%
  dplyr::select(-Pos,-row,-col,-Name) %>%
  group_by(sample,primer,replicate) %>%
  summarise(Cp=mean(Cp)) %>%
  ungroup() %>%
  spread(primer,Cp) %>%
  mutate(pnt_expression=2^-(pnt-rps),
         Rel_expression=2^-(Rel-rps),
         rl_expression=2^-(rl-rps)) %>%
  drop_na()

# Plot relative expression foldchanges normed to non-targeting
#control and housekeeping
```

```
a<-result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,
                rl_expression,replicate) %>%
  filter(sample %in% c("Rel","pnt","Rel_pnt","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"])) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  summarise(m=mean(value),s=sd(value)/sqrt(length(value)),l=m-s,u=m+s) %>%
  ungroup() %>%
  mutate(sample=factor(c(sample),levels=c("Rel","pnt","Rel_pnt","RLUC"))) %>%
  ggplot(aes(x=sample,y=m)) +
  facet_wrap("gene",scales = "free") +
  geom_bar(stat="identity",position=position_dodge()) +
  geom_point(mapping=aes(sample,value),
             data=result %>%
               dplyr::select(sample,pnt_expression,Rel_expression,
                             rl_expression,replicate) %>%
               filter(sample %in% c("Rel","pnt","Rel_pnt","RLUC")) %>%
               ungroup() %>% gather(gene,value,-replicate,-sample) %>%
               group_by(gene) %>%
               mutate(value=value/mean(value[sample=="RLUC"])) %>%
               group_by(sample,gene) %>%
               mutate(value=log2(value))
             ) +
  geom_hline(yintercept = 0) +
  theme_classic()

b<-result %>%
  dplyr::select(sample,pnt_expression,Rel_expression,
                rl_expression,replicate) %>%
  filter(sample %in% c("Rel","rl","Rel_rl","RLUC")) %>%
  ungroup() %>% gather(gene,value,-replicate,-sample) %>%
  group_by(gene) %>%
  mutate(value=value/mean(value[sample=="RLUC"])) %>%
  group_by(sample,gene) %>%
  mutate(value=log2(value)) %>%
  summarise(m=mean(value),s=sd(value)/sqrt(length(value)),l=m-s,u=m+s) %>%
  ungroup() %>%
  mutate(sample=factor(c(sample),levels=c("Rel","rl","Rel_rl","RLUC"))) %>%
  ggplot(aes(x=sample,y=m)) +
  facet_wrap("gene",scales = "free") +
  geom_bar(stat="identity",position=position_dodge()) +
  geom_point(mapping=aes(sample,value),
             data=result %>%
               dplyr::select(sample,pnt_expression,
                             Rel_expression,rl_expression,replicate) %>%
                filter(sample %in% c("Rel","rl","Rel_rl","RLUC")) %>%
               ungroup() %>% gather(gene,value,-replicate,-sample) %>%
               group_by(gene) %>%
```

```
                  mutate(value=value/mean(value[sample=="RLUC"])) %>%
                  group_by(sample,gene) %>%
                  mutate(value=log2(value))
                ) +
  geom_hline(yintercept = 0) +
  theme_classic()

a+b+plot_layout(ncol = 1)
```

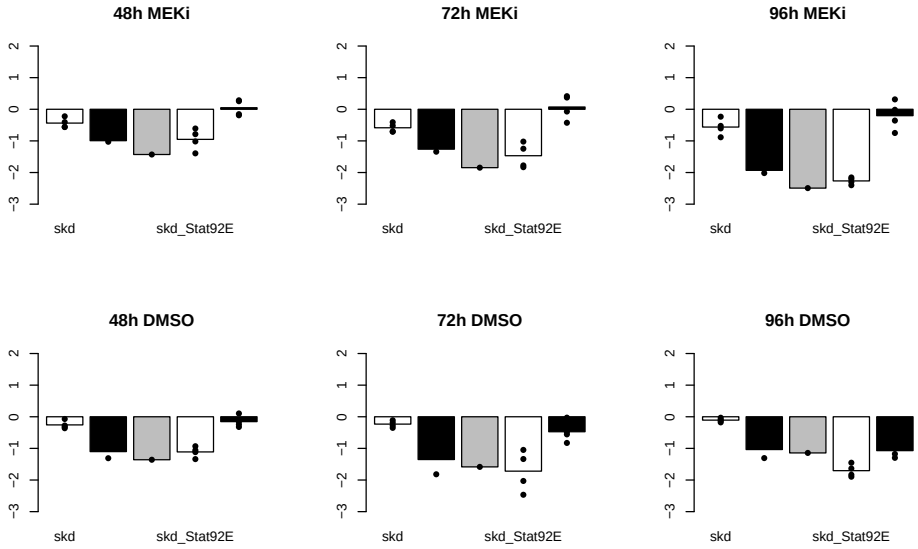


42 Figure 4-supplemental figure 1: Short term expression changes after Rel/Ras signaling co-perturbations

```
plot_effects<-function(target=22,query=68,timepoint="96h",treatment="DMSO",
                       feature="cell_number",ymin,ymax,main="plot"){
  if(is.numeric(target)){
    targetoi=dimnames(Interactions$piscore)[[1]][target]
  }else{
    targetoi=target
  }
  if(is.numeric(query)){
    queryoi=dimnames(Interactions$piscore)[[2]][query]
  }else{
    queryoi=query
  }

  targetmain=mainEffects$target[grep(
```

```
    targetoi,dimnames(mainEffects$target)[[1]]),feature,,timepoint,treatment]
  querymain=mainEffects$query[grep(
    queryoi,dimnames(mainEffects$query)[[1]]),feature,,timepoint,treatment]
  pi=interaction_scores[grep(targetoi,
                             dimnames(interaction_scores)[[1]]),
                        grep(queryoi,
                             dimnames(interaction_scores)[[2]]),
                        feature,timepoint,treatment,]

  combieffect=my.data.array.norm.reshaped[
    grep(targetoi,
         dimnames(my.data.array.norm.reshaped)[[1]]),
    grep(queryoi,
         dimnames(my.data.array.norm.reshaped)[[2]])
    ,feature,timepoint,treatment,]
  targetdt=cbind.data.frame(targetoi,c(targetmain))
  querydt=cbind.data.frame(queryoi,c(querymain))
  expecteddt=cbind.data.frame(paste(targetoi,queryoi,"expected",sep="_"),
                              mean(c(targetmain))+mean(c(querymain)))
  combidt=cbind.data.frame(paste(targetoi,queryoi,sep="_"),c(combieffect))
  pidt=cbind.data.frame("pi",c(pi))
  names(targetdt)=c("gene","data")
  names(querydt)=c("gene","data")
  names(combidt)=c("gene","data")
  names(pidt)=c("gene","data")
  names(expecteddt)=c("gene","data")
  alldt=rbind.data.frame(targetdt,querydt,expecteddt,combidt,pidt)
  mean_Data=dcast(formula = gene~.,data = alldt,fun.aggregate = mean)
  if(missing(ymax)){
    ymax=0
    if(max(alldt$data)>0){
      ymax=max(alldt$data)+0.1
    }
  }
  if(missing(ymin)){
    ymin=0
    if(min(alldt$data)<0){
      ymin=min(alldt$data)-0.1
    }
  }

  x=barplot(mean_Data[,2],names.arg = mean_Data[,1],
            ylim=c(ymin,ymax)
            ,col = c("white","black","grey"),
            main=main)
  alldt$gene=as.character(alldt$gene)
  for(k in 1:nrow(mean_Data)){
    alldt$gene[alldt$gene %in% mean_Data[k,1]]=x[k,1]
  }
  points(alldt,pch=16)
}
```

```
par(mfrow=c(2,3))
plot_effects(target="skd",query="Stat92E",timepoint="48h",treatment="MEKi",
             feature="cell_number",ymin=-3,ymax=2,main="48h MEKi")
## Using data as value column: use value.var to override.
plot_effects(target="skd",query="Stat92E",timepoint="72h",treatment="MEKi",
             feature="cell_number",ymin=-3,ymax=2,main="72h MEKi")
## Using data as value column: use value.var to override.
plot_effects(target="skd",query="Stat92E",timepoint="96h",treatment="MEKi",
             feature="cell_number",ymin=-3,ymax=2,main="96h MEKi")
## Using data as value column: use value.var to override.
plot_effects(target="skd",query="Stat92E",timepoint="48h",treatment="DMSO",
             feature="cell_number",ymin=-3,ymax=2,main="48h DMSO")
## Using data as value column: use value.var to override.
plot_effects(target="skd",query="Stat92E",timepoint="72h",treatment="DMSO",
             feature="cell_number",ymin=-3,ymax=2,main="72h DMSO")
## Using data as value column: use value.var to override.
plot_effects(target="skd",query="Stat92E",timepoint="96h",treatment="DMSO",
             feature="cell_number",ymin=-3,ymax=2,main="96h DMSO")
## Using data as value column: use value.var to override.
```



43 Session info

```
sessionInfo()
## R version 3.5.1 (2018-07-02)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS  10.14.1
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib
```

```
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] hexbin_1.27.2        bindrcpp_0.2.2       ggjoy_0.4.1
##  [4] ggridges_0.5.1       RColorBrewer_1.1-2   MODIFIanalysis_0.1.0
##  [7] drc_3.0-1            ggrepel_0.8.0        eulerr_4.1.0
## [10] scales_1.0.0         FitAR_1.94           bestglm_0.37
## [13] ltsa_1.4.6           leaps_3.0            patchwork_0.0.1
## [16] pheatmap_1.0.10      taRifx_1.0.6.1       abind_1.4-5
## [19] Hmisc_4.1-1          Formula_1.2-3        survival_2.43-1
## [22] lattice_0.20-35      reshape2_1.4.3       ggsignif_0.4.0
## [25] sfsmisc_1.1-2        MASS_7.3-51.1        broom_0.5.0
## [28] forcats_0.3.0        stringr_1.3.1        dplyr_0.7.7
## [31] purrr_0.2.5          readr_1.1.1          tidyr_0.8.2
## [34] tibble_1.4.2         ggplot2_3.1.0        tidyverse_1.2.1
## [37] BiocStyle_2.8.2
##
## loaded via a namespace (and not attached):
##   [1] utf8_1.1.4            tidyselect_0.2.5      RSQLite_2.1.1
##   [4] AnnotationDbi_1.42.1  htmlwidgets_1.3       munsell_0.5.0
##   [7] codetools_0.2-15      preprocessCore_1.42.0 withr_2.1.2
##  [10] colorspace_1.3-2      BiocInstaller_1.30.0  Biobase_2.40.0
##  [13] Category_2.46.0       knitr_1.20            rstudioapi_0.8
##  [16] stats4_3.5.1          robustbase_0.93-3     splots_1.46.0
##  [19] labeling_0.3          hwriter_1.3.2         polyclip_1.9-1
##  [22] bit64_0.9-7           rprojroot_1.3-2       TH.data_1.0-9
##  [25] xfun_0.4              R6_2.3.0              ICSNP_1.1-1
##  [28] locfit_1.5-9.1        bitops_1.0-6          assertthat_0.2.0
##  [31] multcomp_1.4-8        nnet_7.3-12           RNAinteract_1.28.0
##  [34] gtable_0.2.0          affy_1.58.0           cellHTS2_2.44.0
##  [37] sandwich_2.5-0        rlang_0.3.0.1         genefilter_1.62.0
##  [40] splines_3.5.1         lazyeval_0.2.1        acepack_1.4.1
##  [43] checkmate_1.8.5       yaml_2.2.0            modelr_0.1.2
##  [46] backports_1.1.2       RBGL_1.56.0           tools_3.5.1
##  [49] bookdown_0.7          affyio_1.50.0         gplots_3.0.1
##  [52] HD2013SGI_1.20.0      BiocGenerics_0.26.0   Rcpp_0.12.19
##  [55] plyr_1.8.4            base64enc_0.1-3       zlibbioc_1.26.0
##  [58] imageHTS_1.30.0       RCurl_1.95-4.11       rpart_4.1-13
##  [61] S4Vectors_0.18.3      zoo_1.8-4             haven_1.1.2
##  [64] cluster_2.0.7-1       survey_3.34           magrittr_1.5
##  [67] data.table_1.11.8     ICS_1.3-1             openxlsx_4.1.0
##  [70] mvtnorm_1.0-8         hms_0.4.2             evaluate_0.12
##  [73] fftwtools_0.9-8       xtable_1.8-3          XML_3.98-1.16
##  [76] rio_0.5.10            jpeg_0.1-8            readxl_1.1.0
##  [79] IRanges_2.14.12       gridExtra_2.3         compiler_3.5.1
```

```
##  [82] KernSmooth_2.23-15    crayon_1.3.4          htmltools_0.3.6
##  [85] pcaPP_1.9-73          tiff_0.1-5            geneplotter_1.58.0
##  [88] rrcov_1.4-4           lubridate_1.7.4       DBI_1.0.0
##  [91] Matrix_1.2-15         car_3.0-2             cli_1.0.1
##  [94] vsn_3.48.1            gdata_2.18.0          parallel_3.5.1
##  [97] bindr_0.1.1           pkgconfig_2.0.2       foreign_0.8-71
## [100] xml2_1.2.0            foreach_1.4.4         annotate_1.58.0
## [103] prada_1.56.0          rvest_0.3.2           digest_0.6.18
## [106] pls_2.7-0             graph_1.58.2          grpreg_3.2-0
## [109] rmarkdown_1.10        cellranger_1.1.0      htmlTable_1.12
## [112] GSEABase_1.42.0       curl_3.2              EBImage_4.22.1
## [115] gtools_3.8.1          nlme_3.1-137          jsonlite_1.5
## [118] carData_3.0-2         viridisLite_0.3.0     fansi_0.4.0
## [121] limma_3.36.5          pillar_1.3.0          httr_1.3.1
## [124] plotrix_3.7-4         DEoptimR_1.0-8        glue_1.3.0
## [127] zip_1.0.0             png_0.1-7             iterators_1.0.10
## [130] glmnet_2.0-16         bit_1.1-14            class_7.3-14
## [133] stringi_1.2.4         blob_1.1.1            latticeExtra_0.6-28
## [136] caTools_1.17.1.1      memoise_1.1.0         e1071_1.7-0
```