The histone variant H2A.Z: a master regulator of the epithelial-mesenchymal transition - Figures 2, 3 & 4

Sebastian Kurscheid - sebastian.kurscheid@anu.edu.au

Contents

Load libraryd libraries

```
library(BiocGenerics)
library(VennDiagram)
```

```
## Warning: package 'VennDiagram' was built under R version 3.4.1
```

```
## Warning: package 'futile.logger' was built under R version 3.4.1
```

```
library(readr)
library(jsonlite)
library(GenomicRanges)
library(data.table)
library(ggplot2)
library(deepToolsUtils)
library(ggrepel)
library(data.table)
library(zoo)
library(gridExtra)
library(gtools)
library(ade4)
```

Data for analysis has been pre-processed using snakemake. The relevant workflow can be found at [https://github.com/JCSMR-Tremethick-Lab/H2AZ_EMT].

The original R Markdown file used to produce this document can be found at [https://github.com/skurscheid/MDCK_EMT_paper].

Figure 2 - Gene expression data from RNA-Seq experiment.

Data preparation

Load the pre-processed data, including output of kallisto/sleuth and the EMT marker genes defined by Tan et al.

```
baseDir <- "~/Data/Publications/MDCK_EMT_Paper"
setwd(baseDir)
load("data/RNA-Seq/resultsCompressed.rda")
load("data/cfamEnsGenesSigEMTCells.rda")
emtUp <- rtracklayer::import("data/Tan_et_al_EMT_up_genes.bed")
```

```
emtDown <- rtracklayer::import("data/Tan_et_al_EMT_down_genes.bed")
suppressWarnings(emtGenes <- c(emtUp, emtDown))
names(emtGenes) <- emtGenes$name
```

Extract the differential expression data for the two experiments.

```
deTGFbTab <- as.data.table(resultsCompressed[["MDCK"]]$sleuth_results.gene[["conditionMDCKTGFb"]])
deshZTab <- as.data.table(resultsCompressed[["MDCK"]]$sleuth_results.gene[["conditionMDCKshZ"]])
setkey(deshZTab, target_id)
setkey(deTGFbTab, target_id)
```

Load and re-format annotation data.

```
cfamEnsGenesSigEMTCells <- data.table(cfamEnsGenesSigEMTCells)
setkey(cfamEnsGenesSigEMTCells, ensembl_gene_id)
```

Prepare data for plotting

```
m1 <- merge(deTGFbTab[, c("target_id", "qval", "b", "pval")],
    cfamEnsGenesSigEMTCells[, c("ensembl_gene_id", "external_gene_name",
        "epi_mes", "expression")], by.x = "target_id", by.y = "ensembl_gene_id")
m1$experiment <- "TGFb"
m1$logqval <- -log10(m1$qval)
m1$logFC <- log2(exp(m1$b))

# have to manipulate data in order to deal with Inf when
# plotting
m1[which(m1$logqval == Inf), "logqval"] <- 310
m2 <- merge(deshZTab[, c("target_id", "qval", "b", "pval")],
    cfamEnsGenesSigEMTCells[, c("ensembl_gene_id", "external_gene_name",
        "epi_mes", "expression")], by.x = "target_id", by.y = "ensembl_gene_id")
m2$experiment <- "H2A.Z KD"
m2$logqval <- -log10(m2$qval)
m2$logFC <- log2(exp(m2$b))
table(m1$expression)
```

```
##
## down   up
##  113   79
```

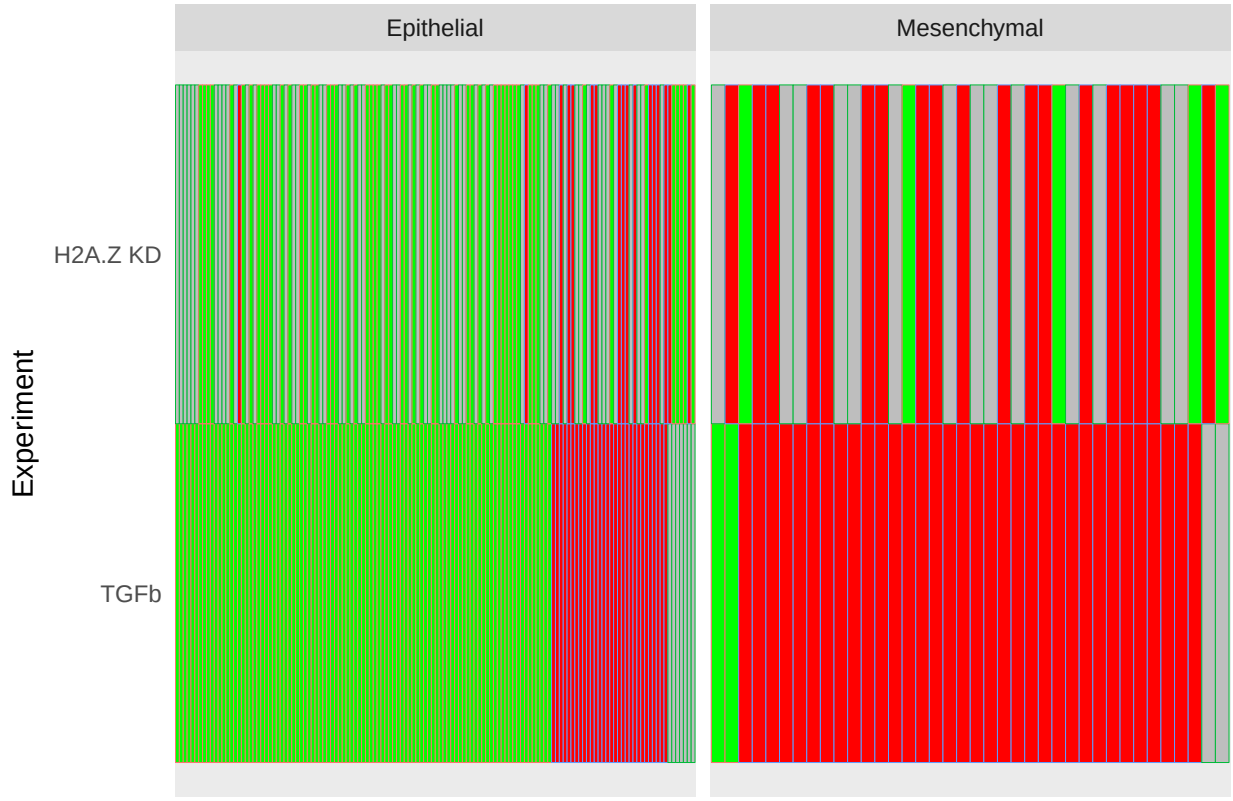
```
setkey(m2, "target_id")
m2[deshZTab[m2$target_id][which(deshZTab[m2$target_id]$b > 0),
    ]$target_id, "expression"] <- "up"
m2[deshZTab[m2$target_id][which(deshZTab[m2$target_id]$b < 0),
    ]$target_id, "expression"] <- "down"

volcanoData <- rbind(m1, m2)
table(volcanoData$experiment, volcanoData$expression)
```

```
##
##            down  up
##   H2A.Z KD  116  76
##   TGFb      113  79
```

```
volcanoData$experiment <- as.factor(volcanoData$experiment)
volcanoData$experiment <- relevel(volcanoData$experiment, "TGFb")
```

“Heatmap”" (Figure 2 A & B)

```
facetTitles <- list(TGFb = expression(paste("TGF-", beta)), `H2A.Z KD` = "H2A.Z KD")
facetLabeller <- function(variable, value) {
    return(facetTitles[value])
}

hmData <- tidyr::spread(volcanoData[volcanoData$qval < 0.1, c("target_id",
    "external_gene_name", "expression", "experiment", "epi_mes")],
    experiment, expression)
hmData <- hmData[order(hmData$TGFb), ]
hmData$target_id <- as.factor(hmData$target_id)
hmData$target_id <- ordered(hmData$target_id, levels = levels(hmData$target_id)[unclass(hmData$target_id
hmDataLong <- melt(hmData, id.vars = c("target_id", "epi_mes"),
    measure.vars = c("TGFb", "H2A.Z KD"))
hmDataLong <- hmDataLong[!hmDataLong$epi_mes %in% c("H2A.Z",
    "TGFB1"), ]
hmDataLong[which(is.na(hmDataLong$value)), ]$value <- "N.S."
hmDataLong$value <- as.factor(hmDataLong$value)
hmDataLong$epi_mes <- as.factor(hmDataLong$epi_mes)
levels(hmDataLong$epi_mes) <- c("Epithelial", "Mesenchymal")
hm1 <- ggplot(hmDataLong, aes(target_id, variable, color = value)) +
    geom_tile(aes(fill = value), show.legend = F) + xlab("") +
    ylab("Experiment") + theme(axis.ticks = element_blank(),
    axis.text.x = element_blank(), plot.background = element_blank(),
    panel.grid = element_blank()) + scale_fill_manual(values = c("green",
    "grey", "red"), breaks = c("1", "2", "3"), labels = c("down",
    "N.S.", "up")) + facet_grid(. ~ epi_mes, scales = "free") +
    guides(fill = guide_legend(title = NULL))

hm1
```




```
ggsave(hm1, filename = "Figure_2A_Heatmap.pdf", width = 114,
    height = 279/3/2, units = "mm", useDingbats = F)
```

Volcano Plots (Figure 2 D & E)

```
# volcano plots
labelSize <- 3
pointSize <- 0.4
highlightPointSize <- 1.6
fontSize <- 12

p1 <- ggplot(volcanoData, aes(x = logFC, y = logqval, color = epi_mes)) +
    geom_point(size = pointSize) + geom_point(data = subset(volcanoData,
    experiment == "TGFb" & logqval > 200), aes(x = logFC, y = logqval,
    color = epi_mes), size = highlightPointSize) + geom_text_repel(data = subset(volcanoData,
    experiment == "TGFb" & logqval > 200), aes(x = logFC, y = logqval,
    label = external_gene_name), show.legend = F, size = labelSize) +
    geom_point(data = subset(volcanoData, experiment == "TGFb" &
        epi_mes %in% c("TGFB1", "H2A.Z")), aes(x = logFC, y = logqval,
        color = epi_mes), size = highlightPointSize) + geom_text_repel(data = subset(volcanoData,
    experiment == "TGFb" & epi_mes %in% c("TGFB1", "H2A.Z")),
    aes(x = logFC, y = logqval, label = external_gene_name),
    show.legend = F, size = labelSize) + geom_point(data = volcanoData[grep("FN1|CDH2",
    volcanoData$external_gene_name)], aes(x = logFC, y = logqval,
    color = epi_mes), size = highlightPointSize) + geom_text_repel(data = volcanoData[grep("FN1|CDH2",
```

```
    volcanoData$external_gene_name)], aes(x = logFC, y = logqval,
    label = external_gene_name), show.legend = F, size = labelSize) +
    geom_point(data = subset(volcanoData, experiment == "H2A.Z KD" &
        logqval > 18), aes(x = logFC, y = logqval, color = epi_mes),
        size = highlightPointSize) + geom_text_repel(data = subset(volcanoData,
    experiment == "H2A.Z KD" & logqval > 18), aes(x = logFC,
    y = logqval, label = external_gene_name), show.legend = F,
    size = labelSize) + geom_point(data = subset(volcanoData,
    experiment == "H2A.Z KD" & epi_mes %in% c("TGFB1")), aes(x = logFC,
    y = logqval, color = epi_mes), size = highlightPointSize) +
    geom_text_repel(data = subset(volcanoData, experiment ==
        "H2A.Z KD" & epi_mes %in% c("TGFB1")), aes(x = logFC,
        y = logqval, label = external_gene_name), show.legend = F,
        size = labelSize) + geom_point(data = subset(volcanoData,
    experiment == "H2A.Z KD" & target_id == "ENSCAFG00000002653"),
    aes(x = logFC, y = logqval, color = epi_mes), size = highlightPointSize) +
    geom_text_repel(data = subset(volcanoData, experiment ==
        "H2A.Z KD" & target_id == "ENSCAFG00000002653"), aes(x = logFC,
        y = logqval, label = external_gene_name), show.legend = F,
        size = labelSize, force = 2.4) + facet_wrap("experiment",
    scales = "free_y", nrow = 2, ncol = 1, labeller = facetLabeller) +
    geom_hline(yintercept = 0) + geom_vline(xintercept = 0) +
    coord_cartesian(xlim = c(-6.2, 6.2), expand = T) + theme(panel.background = element_blank(),
    panel.border = element_blank(), panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(), strip.background = element_blank(),
    strip.text = element_text(size = fontSize), axis.title.x = element_text(size = fontSize),
    axis.title.y = element_text(size = fontSize), axis.text = element_text(size = fontSize),
    legend.text = element_text(size = fontSize), legend.title = element_text(size = fontSize),
    legend.key.size = unit(c(0.5, 0.75), units = "cm")) + xlab("log2 fold-change") +
    ylab("-log10(q-value)") + labs(color = "Gene/Marker type") +
    scale_colour_hue(labels = c("Epithelial", "H2A.Z", "Mesenchymal",
        "TGFB1"))
```

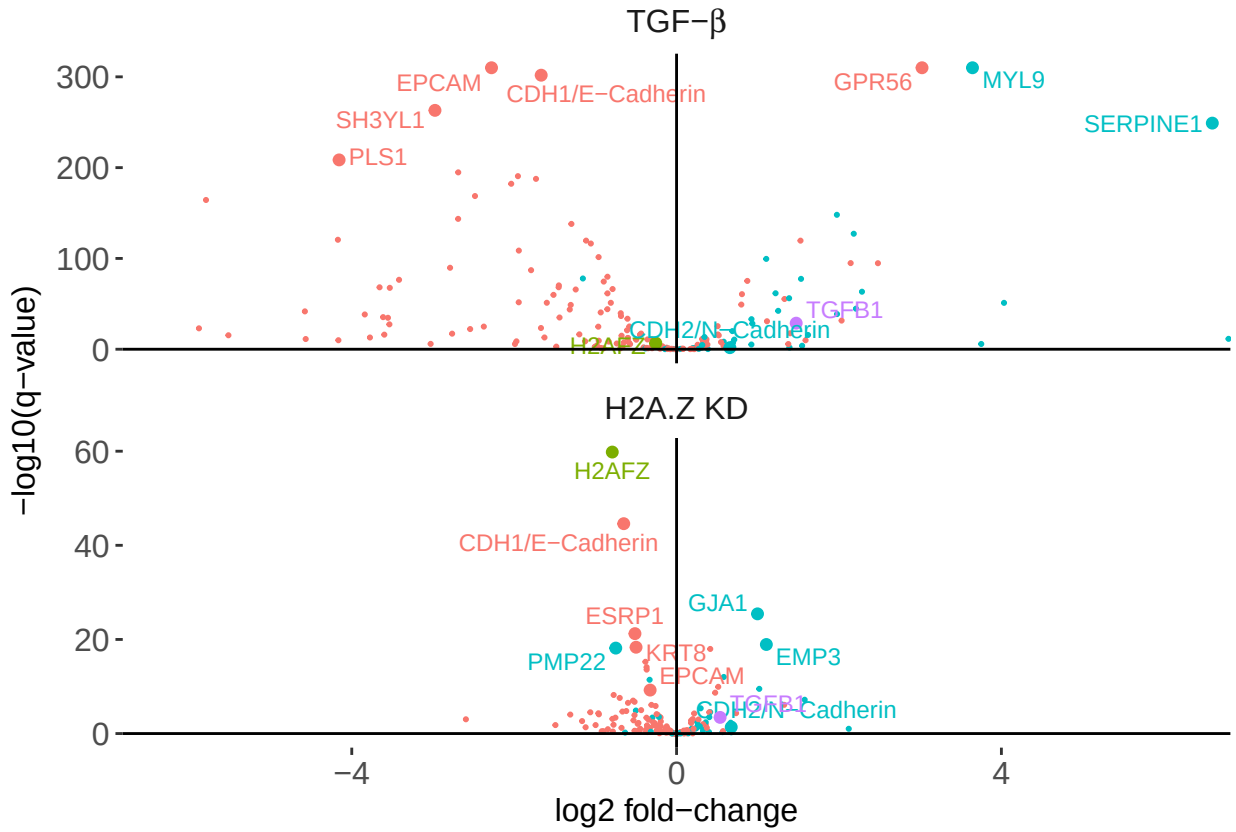
```
## Warning: The labeller API has been updated. Labellers taking ‘variable‘and
## ‘value‘ arguments are now deprecated. See labellers documentation.
```

```
p1 <- p1 + theme(legend.position = "none")
p1
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```

```
## Warning: Removed 2 rows containing missing values (geom_point).
```

```
## Warning: Removed 2 rows containing missing values (geom_text_repel).
```




```
ggsave(p1, filename = "Figure2_panels_D_E_alternative.pdf", width = 114,
    height = 279/3 * 2.2, units = "mm", useDingbats = F)
ggsave(p1, filename = "Figure2_panels_D_E.pdf", width = 8.5,
    height = 5, units = "in", useDingbats = F)
```

Figure 3 - H2A.Z occupancy from ChIP-Seq experiment and integrative analysis.

Data preparation

```
ChIPdataDir <- paste(baseDir, "data", "ChIP-Seq", sep = "/")
list.files(ChIPdataDir)
```

```
## [1] "H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz"
## [2] "H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz"
## [3] "H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz"
## [4] "H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz"
```

Load the coverage data generated by deepTools computeMatrix command

```
files <- list.files(ChIPdataDir, pattern = "Tan")
suppressWarnings(dataList <- lapply(paste(ChIPdataDir, files,
```

```
    sep = "/"), function(x) computeMatrixLoader(x)))
names(dataList) <- files
plotData <- lapply(dataList, function(x) deepToolsUtils::makePlottingData(x))
```

```
## [1] "H2AZ-TGFb_vs_Input-TGFb_normal_subtract_RPKM"
```

```
## Warning in as.data.frame(x[[i]], optional = TRUE): closing unused
## connection 7 (~/Data/Publications/MDCK_EMT_Paper/data/ChIP-Seq/H2AZ-
## WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz)
```

```
## Warning in as.data.frame(x[[i]], optional = TRUE): closing unused
## connection 6 (~/Data/Publications/MDCK_EMT_Paper/data/ChIP-Seq/H2AZ-
## WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz)
```

```
## Warning in as.data.frame(x[[i]], optional = TRUE):
## closing unused connection 5 (~/Data/Publications/
## MDCK_EMT_Paper/data/ChIP-Seq/H2AZ-TGFb_vs_Input-
## TGFb_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz)
```

```
## [1] "H2AZ-TGFb_vs_Input-TGFb_normal_subtract_RPKM"
## [1] "H2AZ-WT_vs_Input-WT_normal_subtract_RPKM"
## [1] "H2AZ-WT_vs_Input-WT_normal_subtract_RPKM"
```

```
names(plotData) <- unlist(lapply(strsplit(files, "\\."), function(x) paste(x[c(1:3)],
    collapse = ".")))
plotData <- lapply(names(plotData), function(x) {
    tab <- plotData[[x]]
    tab$geneset <- x
    return(tab)
})
```

Data formatting

```
names(plotData) <- unlist(lapply(strsplit(files, "\\."), function(x) paste(x[c(1:3)],
    collapse = ".")))
plotData <- do.call("rbind", plotData)
plotDataWT <- plotData[grep("WT", plotData$sample), ]
plotDataTGFb <- plotData[grep("TGFb", plotData$sample), ]
ylimMax <- max(plotData$value + (plotData$sem/2))
ylimMin <- min(plotData$value - plotData$sem)
```

Calculate difference data for the WT and TGF-beta data

```
# make difference data by subtracting WT from TGFb
bin <- 1:300
diff1 <- data.frame(bin = bin, value = plotDataTGFb[plotDataTGFb$geneset ==
    "H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTdown_normal",
    ]$value - plotDataWT[plotDataWT$geneset == "H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanE
    ]$value, sample = "TGFb - WT", group = "down_diff", geneset = "TanEMTdown",
    sem = sqrt((plotDataTGFb[plotDataTGFb$geneset == "H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_
        ]$sem)^2 + (plotDataWT[plotDataWT$geneset == "H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPK
        ]$sem)^2))
diff2 <- data.frame(bin = bin, value = plotDataTGFb[plotDataTGFb$geneset ==
```

```
    "H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTup_normal",
    ]$value - plotDataWT[plotDataWT$geneset == "H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanE
    ]$value, sample = "TGFb - WT", group = "down_diff", geneset = "TanEMTup",
    sem = sqrt((plotDataTGFb[plotDataTGFb$geneset == "H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_
        ]$sem)^2 + (plotDataWT[plotDataWT$geneset == "H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPK
        ]$sem)^2))
diffData <- as.data.table(rbind(diff1, diff2))
```

Set some parameters for plotting

```
diffylimMin <- min(diffData$value - (diffData$sem/2.3))
diffylimMax <- max(diffData$value + (diffData$sem/1.3))
lineSize <- 2
axisLineSize <- 0.75
vlineCol <- "black"
wtCol <- "#1b9e77"
TGFbCol <- "#7570b3"
diffCol <- "darkgrey"
nSplines <- 20
downXAxisMargin <- 3.6
axisTextSize <- 8
```

Figure 3 A & B - ChIP-Seq metagene coverage plots

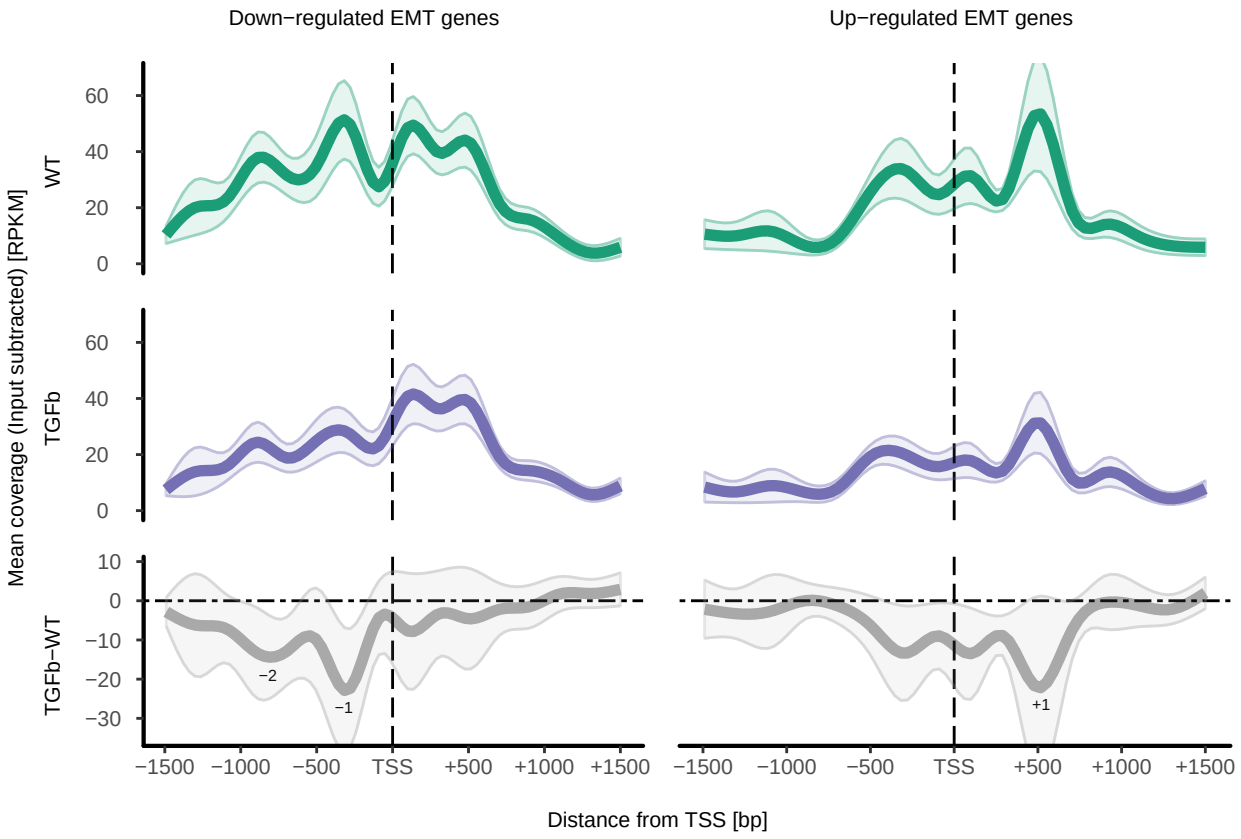
```
nBins <- nrow(plotDataWT[grep("down", plotDataWT$geneset), ])
wtPlotDown <- ggplot(plotDataWT[grep("down", plotDataWT$geneset),
    ], aes(bin, value)) + geom_smooth(method = "lm", formula = y ~
    splines::ns(x, nBins/nSplines), se = F, col = wtCol, size = lineSize) +
    geom_smooth(aes(x = bin, y = value - sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(wtCol, 0.2), size = 0.5, fullrange = F) +
    geom_smooth(aes(x = bin, y = value + sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(wtCol, 0.2), size = 0.5, fullrange = F) +
    facet_wrap(c("geneset"), ncol = 2, labeller = label_both) +
    ylab(NULL) + xlab(NULL) + geom_vline(xintercept = 150, colour = vlineCol,
    linetype = "longdash") + coord_cartesian(ylim = c(0, ylimMax)) +
    theme(axis.title.x = element_blank(), axis.text.x = element_blank(),
        axis.ticks.x = element_blank(), axis.text.y = element_text(margin = margin(0,
            downXAxisMargin, 0, 0, "mm"), size = axisTextSize),
        strip.background = element_blank(), strip.text = element_blank(),
        axis.line.y = element_line(colour = "black", axisLineSize),
        panel.background = element_blank(), panel.border = element_blank(),
        panel.grid.major = element_blank(), panel.grid.minor = element_blank())
wtPlotDownData <- ggplot_build(wtPlotDown)
df1 <- data.frame(x = wtPlotDownData$data[[2]]$x, ymin = wtPlotDownData$data[[2]]$y,
    ymax = wtPlotDownData$data[[3]]$y)
wtPlotDown <- wtPlotDown + geom_ribbon(data = df1, aes(x = x,
    ymin = ymin, ymax = ymax), colour = alpha(wtCol, 0.3), alpha = 0.1,
    inherit.aes = F, fill = wtCol)
```

```
# plot 2 - WT up-regulated
# ------------------------------------------------
wtPlotUp <- ggplot(plotDataWT[grep("up", plotDataWT$geneset),
    ], aes(bin, value)) + geom_smooth(method = "lm", formula = y ~
    splines::ns(x, nBins/nSplines), se = F, col = wtCol, size = lineSize) +
    geom_smooth(aes(x = bin, y = value - sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(wtCol, 0.2), size = 0.5, fullrange = F) +
    geom_smooth(aes(x = bin, y = value + sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(wtCol, 0.2), size = 0.5, fullrange = F) +
    ylab(NULL) + xlab(NULL) + geom_vline(xintercept = 150, colour = vlineCol,
    linetype = "longdash") + coord_cartesian(ylim = c(0, ylimMax)) +
    theme(axis.title.x = element_blank(), axis.text.x = element_blank(),
        axis.ticks.x = element_blank(), axis.text.y = element_blank(),
        axis.line.y = element_blank(), axis.ticks.y = element_blank(),
        strip.background = element_blank(), strip.text = element_blank(),
        panel.background = element_blank(), panel.border = element_blank(),
        panel.grid.major = element_blank(), panel.grid.minor = element_blank())
wtPlotUpData <- ggplot_build(wtPlotUp)
df1 <- data.frame(x = wtPlotUpData$data[[2]]$x, ymin = wtPlotUpData$data[[2]]$y,
    ymax = wtPlotUpData$data[[3]]$y)
wtPlotUp <- wtPlotUp + geom_ribbon(data = df1, aes(x = x, ymin = ymin,
    ymax = ymax), colour = alpha(wtCol, 0.3), alpha = 0.1, inherit.aes = F,
    fill = wtCol)


# plot 3 - TGFb down-regulated
# ------------------------------------------------
TGFbPlotDown <- ggplot(plotDataTGFb[grep("down", plotDataTGFb$geneset),
    ], aes(bin, value)) + geom_smooth(method = "lm", formula = y ~
    splines::ns(x, nBins/nSplines), se = F, col = TGFbCol, size = lineSize) +
    geom_smooth(aes(x = bin, y = value - sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(TGFbCol, 0.2), size = 0.5, fullrange = F) +
    geom_smooth(aes(x = bin, y = value + sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(TGFbCol, 0.2), size = 0.5, fullrange = F) +
    ylab(NULL) + xlab(NULL) + geom_vline(xintercept = 150, colour = vlineCol,
    linetype = "longdash") + coord_cartesian(ylim = c(0, ylimMax)) +
    theme(axis.title.x = element_blank(), axis.text.x = element_blank(),
        axis.ticks.x = element_blank(), axis.text.y = element_text(margin = margin(0,
            downXAxisMargin, 0, 0, "mm"), size = axisTextSize),
        strip.background = element_blank(), strip.text = element_blank(),
        axis.line.y = element_line(colour = "black", axisLineSize),
        panel.background = element_blank(), panel.border = element_blank(),
        panel.grid.major = element_blank(), panel.grid.minor = element_blank())
TGFbPlotDownData <- ggplot_build(TGFbPlotDown)
df1 <- data.frame(x = TGFbPlotDownData$data[[2]]$x, ymin = TGFbPlotDownData$data[[2]]$y,
    ymax = TGFbPlotDownData$data[[3]]$y)
TGFbPlotDown <- TGFbPlotDown + geom_ribbon(data = df1, aes(x = x,
    ymin = ymin, ymax = ymax), colour = alpha(TGFbCol, 0.3),
    alpha = 0.1, inherit.aes = F, fill = TGFbCol)
```

```
# plot 4 - TGFb up-regulated
# ------------------------------------------
TGFbPlotUp <- ggplot(plotDataTGFb[grep("up", plotDataTGFb$geneset),
    ], aes(bin, value)) + geom_smooth(method = "lm", formula = y ~
    splines::ns(x, nBins/nSplines), se = F, col = TGFbCol, size = lineSize) +
    geom_smooth(aes(x = bin, y = value - sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(TGFbCol, 0.2), size = 0.5, fullrange = F) +
    geom_smooth(aes(x = bin, y = value + sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(TGFbCol, 0.2), size = 0.5, fullrange = F) +
    ylab(NULL) + xlab(NULL) + geom_vline(xintercept = 150, colour = vlineCol,
    linetype = "longdash") + coord_cartesian(ylim = c(0, ylimMax)) +
    theme(axis.title.x = element_blank(), axis.text.x = element_blank(),
        axis.ticks.x = element_blank(), axis.text.y = element_blank(),
        axis.line.y = element_blank(), axis.ticks.y = element_blank(),
        strip.background = element_blank(), strip.text = element_blank(),
        panel.background = element_blank(), panel.border = element_blank(),
        panel.grid.major = element_blank(), panel.grid.minor = element_blank())
TGFbPlotUpData <- ggplot_build(TGFbPlotUp)
df1 <- data.frame(x = TGFbPlotUpData$data[[2]]$x, ymin = TGFbPlotUpData$data[[2]]$y,
    ymax = TGFbPlotUpData$data[[3]]$y)
TGFbPlotUp <- TGFbPlotUp + geom_ribbon(data = df1, aes(x = x,
    ymin = ymin, ymax = ymax), colour = alpha(TGFbCol, 0.3),
    alpha = 0.1, inherit.aes = F, fill = TGFbCol)


# plot 5 - diff down-regulated
# ------------------------------------------
annotationSize <- 2
diffPlotDown <- ggplot(diffData[grep("down", diffData$geneset),
    ], aes(bin, value)) + geom_smooth(method = "lm", formula = y ~
    splines::ns(x, nBins/nSplines), se = F, col = diffCol, size = lineSize) +
    geom_smooth(aes(x = bin, y = value - sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(diffCol, 0.2), size = 0.5, fullrange = F) +
    geom_smooth(aes(x = bin, y = value + sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(diffCol, 0.2), size = 0.5, fullrange = F) +
    ylab(NULL) + xlab(NULL) + geom_text_repel(data = subset(diffData,
    geneset == "TanEMTdown")[118], aes(x = bin, y = value, label = "-1"),
    size = annotationSize, nudge_y = -1) + geom_text_repel(data = subset(diffData,
    geneset == "TanEMTdown")[68], aes(x = bin, y = value, label = "-2"),
    size = annotationSize, nudge_y = -1) + geom_vline(xintercept = 150,
    colour = vlineCol, linetype = "longdash") + geom_hline(yintercept = 0,
    linetype = "twodash") + coord_cartesian(ylim = c(diffylimMax,
    diffylimMin)) + scale_x_continuous(breaks = c(0, 50, 100,
    150, 200, 250, 300), labels = c("-1500", "-1000", "-500",
    "TSS", "+500", "+1000", "+1500")) + theme(axis.line.x = element_line(colour = "black",
    axisLineSize), axis.text.x = element_text(size = axisTextSize),
    axis.text.y = element_text(margin = margin(0, 2, 0, 0, "mm"),
        size = axisTextSize), strip.background = element_blank(),
```

```
    strip.text = element_blank(), axis.line.y = element_line(colour = "black",
        axisLineSize), panel.background = element_blank(), panel.border = element_blank(),
    panel.grid.major = element_blank(), panel.grid.minor = element_blank())
diffPlotDownData <- ggplot_build(diffPlotDown)
df1 <- data.frame(x = diffPlotDownData$data[[2]]$x, ymin = diffPlotDownData$data[[2]]$y,
    ymax = diffPlotDownData$data[[3]]$y)
diffPlotDown <- diffPlotDown + geom_ribbon(data = df1, aes(x = x,
    ymin = ymin, ymax = ymax), colour = alpha(diffCol, 0.3),
    alpha = 0.1, inherit.aes = F, fill = diffCol)

# plot 6 - diff up-regulated
# ------------------------------------------
diffPlotUp <- ggplot(diffData[grep("up", diffData$geneset), ],
    aes(bin, value)) + geom_smooth(method = "lm", formula = y ~
    splines::ns(x, nBins/nSplines), se = F, col = diffCol, size = lineSize) +
    geom_smooth(aes(x = bin, y = value - sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(diffCol, 0.2), size = 0.5, fullrange = F) +
    geom_smooth(aes(x = bin, y = value + sem), method = "lm",
        formula = y ~ splines::ns(x, nBins/nSplines), se = F,
        col = alpha(diffCol, 0.2), size = 0.5, fullrange = F) +
    ylab(NULL) + xlab(NULL) + geom_text_repel(data = subset(diffData,
    geneset == "TanEMTup")[202], aes(x = bin, y = value, label = "+1"),
    size = annotationSize, nudge_y = -1) + geom_vline(xintercept = 150,
    colour = vlineCol, linetype = "longdash") + geom_hline(yintercept = 0,
    linetype = "twodash") + scale_x_continuous(breaks = c(0,
    50, 100, 150, 200, 250, 300), labels = c("-1500", "-1000",
    "-500", "TSS", "+500", "+1000", "+1500")) + coord_cartesian(ylim = c(diffylimMax,
    diffylimMin)) + theme(axis.text.y = element_blank(), axis.line.y = element_blank(),
    axis.ticks.y = element_blank(), axis.line.x = element_line(colour = "black",
        axisLineSize), axis.text.x = element_text(size = axisTextSize),
    strip.background = element_blank(), strip.text = element_blank(),
    panel.background = element_blank(), panel.border = element_blank(),
    panel.grid.major = element_blank(), panel.grid.minor = element_blank())
diffPlotUpData <- ggplot_build(diffPlotUp)
df1 <- data.frame(x = diffPlotUpData$data[[2]]$x, ymin = diffPlotUpData$data[[2]]$y,
    ymax = diffPlotUpData$data[[3]]$y)
diffPlotUp <- diffPlotUp + geom_ribbon(data = df1, aes(x = x,
    ymin = ymin, ymax = ymax), colour = alpha(diffCol, 0.3),
    alpha = 0.1, inherit.aes = F, fill = diffCol)

# prepare layout grid for panels
lay <- rbind(c(1, 2, 3, 4), c(1, 5, 6, 7), c(1, 8, 9, 10), c(1,
    11, 12, 13), c(14, 14, 15, 15))

gpFontSize <- gpar(fontsize = 8)
xAx <- grid::textGrob("Distance from TSS [bp]", gp = gpFontSize)
yAx <- grid::textGrob("Mean coverage (Input subtracted) [RPKM]",
    rot = 90, gp = gpFontSize)
topX1 <- grid::textGrob("Down-regulated EMT genes", gp = gpFontSize)
topX2 <- grid::textGrob("Up-regulated EMT genes", gp = gpFontSize)
wtLabel <- grid::textGrob("WT", rot = 90, gp = gpFontSize)
tgfbLabel <- grid::textGrob("TGFb", rot = 90, gp = gpFontSize)
```

```
diffLabel <- grid::textGrob("TGFb-WT", rot = 90, gp = gpFontSize)
emptyBox <- grid::rectGrob(gp = grid::gpar(fill = "white", lty = 0))

gs <- list(yAx, emptyBox, topX1, topX2, wtLabel, wtPlotDown,
    wtPlotUp, tgfbLabel, TGFbPlotDown, TGFbPlotUp, diffLabel,
    diffPlotDown, diffPlotUp, emptyBox, xAx)
grob1 <- gridExtra::grid.arrange(grobs = gs, layout_matrix = lay,
    widths = c(0.5, 0.5, 8, 8), heights = c(1, 4, 4, 4, 1))
```




```
ggsave(grob1, filename = "Figure3_AB.pdf", height = 10.5/2, width = 8.5,
    units = "in", useDingbats = F)
```

Figure 3 C, D & E - Differential coverage and correlation plots

Data preparation

```
g_legend <- function(a.gplot) {
    tmp <- ggplot_gtable(ggplot_build(a.gplot))
    leg <- which(sapply(tmp$grobs, function(x) x$name) == "guide-box")
    legend <- tmp$grobs[[leg]]
    legend
}

cmTGFbUp <- dataList[["H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz
```

```
cmWTUp <- dataList[["H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz"]]$co
diffUp <- cmTGFbUp[, 2:301] - cmWTUp[, 2:301]
rownames(diffUp) <- cmTGFbUp$X4

cmTGFbDown <- dataList[["H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTdown_normal.matri
cmWTDown <- dataList[["H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz"]]
diffDown <- cmTGFbDown[, 2:301] - cmWTDown[, 2:301]
rownames(diffDown) <- diffDown$X4
cmWT <- rbind(cmWTDown, cmWTUp)
cmTGFb <- rbind(cmTGFbDown, cmTGFbUp)

diffUp <- as.data.table(diffUp)
diffUp$geneID <- cmTGFbUp$X4
datUp <- melt(diffUp, id.vars = "geneID", measure.vars <- c(1:300))
datUp <- datUp[order(datUp$geneID), ]
setkey(datUp, "geneID")

diffDown <- data.table(diffDown)
diffDown$geneID <- cmTGFbDown$X4
datDown <- melt(diffDown, id.vars = "geneID", measure.vars <- c(1:300))
datDown <- datDown[order(datDown$geneID), ]
setkey(datDown, "geneID")

cols <- c("#ca0020", "#f7f7f7", "#0571b0")

rollWidth <- 10
rollBy <- 5

datDownSum <- lapply(unique(datDown$geneID), function(x) {
    n <- rollapply(datDown[x]$value, width = rollWidth, by = rollBy,
        FUN = mean)
    df <- data.frame(geneID = x, bin = 1:length(n), value = n)
    return(df)
})
datDownSum <- as.data.table(as.data.frame(do.call("rbind", datDownSum)))
datDownSum$cat <- gtools::quantcut(datDownSum$value, q = 3)
levels(datDownSum$cat) <- c("loss", "no change", "gain")

datUpSum <- lapply(unique(datUp$geneID), function(x) {
    n <- rollapply(datUp[x]$value, width = rollWidth, by = rollBy,
        FUN = mean)
    df <- data.frame(geneID = x, bin = 1:length(n), value = n)
    return(df)
})
datUpSum <- as.data.table(as.data.frame(do.call("rbind", datUpSum)))
datUpSum$cat <- quantcut(datUpSum$value, q = 3)
levels(datUpSum$cat) <- c("loss", "no change", "gain")

TGFbUpb2 <- deTGFbTab[cmTGFbUp$X4][deTGFbTab[cmTGFbUp$X4]$b >
    0]$target_id
TGFbDownb2 <- deTGFbTab[cmTGFbDown$X4][deTGFbTab[cmTGFbDown$X4]$b <
    0]$target_id
minDiff <- 15
```

```
datSum <- rbind(datDownSum, datUpSum)
colnames(datSum)[1] <- "ensembl_gene_id"
setkey(datSum, ensembl_gene_id, bin)
datSum <- datSum[order(datSum$ensembl_gene_id, datSum$bin)]
diffExp <- subset(deTGFbTab, target_id %in% c(TGFbDownb2, TGFbUpb2))
diffExp <- diffExp[order(diffExp$target_id)]
diffExp$logFC <- log2(exp(diffExp$b))
diffH2AZDEcor <- lapply(1:max(datDownSum$bin), function(x) {
    allDat <- merge(subset(datSum, bin == x), diffExp, by.x = "ensembl_gene_id",
        by.y = "target_id")
    allDat <- merge(allDat, cfamEnsGenesSigEMTCells[, c("ensembl_gene_id",
        "external_gene_name", "epi_mes")], all.x = T)
    abLine <- coef(lm(b ~ value, data = allDat))
    allPlot <- ggplot(allDat, aes(x = value, y = b)) + geom_point() +
        geom_abline(slope = abLine[2], intercept = abLine[1],
            colour = "darkgrey", size = 1, linetype = "longdash")
    allCor1 <- suppressWarnings(cor.test(allDat$value, allDat$b,
        method = "spearman"))
    allCor2 <- suppressWarnings(cor.test(allDat[which(abs(allDat$value) >
        minDiff), ]$value, allDat[which(abs(allDat$value) > minDiff),
        ]$b, method = "spearman"))
    return(list(all1 = allCor1, all2 = allCor2, allPlot = allPlot,
        allDat = allDat))
})

allCor <- lapply(diffH2AZDEcor, function(x) {
    data.frame(x$all1$p.value, x$all1$estimate, x$all2$p.value,
        x$all2$estimate)
})
allCor <- do.call("rbind", allCor)
allCor$bin <- 1:nrow(allCor)
save(allCor, file = "allCor.rda")
minCor1 <- which.min(allCor$x.all1.estimate)
allCor[which.min(allCor$x.all1.estimate), ]
```

```
##       x.all1.p.value x.all1.estimate x.all2.p.value x.all2.estimate bin
## rho50     0.01326705      -0.1784557      0.3399112      -0.1487466  51
```

```
maxCor1 <- which.max(allCor$x.all1.estimate)
allCor[which.max(allCor$x.all1.estimate), ]
```

```
##       x.all1.p.value x.all1.estimate x.all2.p.value x.all2.estimate bin
## rho15     0.02251229       0.1646112      0.0295237       0.3750955  16
```

```
minCor2 <- which.min(allCor$x.all2.estimate)
allCor[which.min(allCor$x.all2.estimate), ]
```

```
##       x.all1.p.value x.all1.estimate x.all2.p.value x.all2.estimate bin
## rho40     0.02451926      -0.1622823    0.006332314      -0.3419259  41
```

```
maxCor2 <- which.max(allCor$x.all2.estimate)
allCor[which.max(allCor$x.all2.estimate), ]
```

```
##       x.all1.p.value x.all1.estimate x.all2.p.value x.all2.estimate bin
## rho13     0.09846072       0.1196015    0.001089065       0.4861069  14
```

```
# plot of Spearman correlation between differential
# expression and -------- with genes that have min. +/- 10
# H2A.Z difference in a given bin
corPlot2 <- ggplot(allCor, aes(bin, x.all2.estimate)) + geom_line(col = "darkgrey",
    size = lineSize) + scale_x_continuous(breaks = c(0, 10, 20,
    30, 40, 50, 60), labels = c("-1500", "-1000", "-500", "TSS",
    "+500", "+1000", "+1500")) + geom_text_repel(data = allCor[c(maxCor2,
    minCor2), ], aes(x = bin, y = x.all2.estimate, label = c("-2",
    "+1")), nudge_y = c(-0.15, +0.1), size = 3) + geom_point(data = allCor[c(maxCor2,
    minCor2), ], aes(x = bin, y = x.all2.estimate), colour = "red") +
    geom_hline(yintercept = 0, linetype = "twodash") + theme(panel.background = element_blank(),
    panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
    axis.line = element_line(colour = "black", axisLineSize)) +
    xlab(NULL) + ylab(NULL) + geom_vline(xintercept = 30, colour = vlineCol,
    linetype = "longdash")


datMax <- diffH2AZDEcor[[maxCor2]]$allDat
datMin <- diffH2AZDEcor[[minCor2]]$allDat
abLineMax <- coef(lm(b ~ value, data = datMax))
abLineMin <- coef(lm(b ~ value, data = datMin))

plotMax <- ggplot(datMax, aes(x = value, y = logFC)) + geom_point(aes(colour = epi_mes),
    size = 0.05) + geom_abline(slope = coef(lm(logFC ~ value,
    data = datMax))[2], intercept = coef(lm(logFC ~ value, data = datMax))[1],
    colour = "darkgrey", size = 0.6, linetype = "longdash") +
    geom_point(data = subset(datMax, value < -200 | abs(logFC) >
        3), aes(x = value, y = logFC, colour = epi_mes), size = 1) +
    geom_text_repel(data = subset(datMax, value < -200 | abs(logFC) >
        3), aes(x = value, y = logFC, label = external_gene_name,
        colour = epi_mes), show.legend = F, size = 2) + ylab(NULL) +
    xlab(NULL) + labs(color = "Gene/Marker type") + scale_colour_hue(labels = c("Epithelial",
    "H2A.Z", "Mesenchymal", "TGFB1")) + theme(legend.position = "none",
    panel.background = element_blank(), panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(), axis.line = element_line(colour = "black",
        axisLineSize), axis.text.y = element_text(margin = margin(0,
        3, 0, 0, "mm")))

plotMin <- ggplot(datMin, aes(x = value, y = logFC)) + geom_point(aes(colour = epi_mes),
    size = 0.05) + geom_abline(slope = coef(lm(logFC ~ value,
    data = datMin))[2], intercept = coef(lm(logFC ~ value, data = datMin))[1],
    colour = "darkgrey", size = 0.6, linetype = "longdash") +
    geom_point(data = subset(datMin, value < -200 | abs(logFC) >
        3), aes(x = value, y = logFC, colour = epi_mes), size = 1) +
    geom_text_repel(data = subset(datMin, value < -200 | abs(logFC) >
        3), aes(x = value, y = logFC, label = external_gene_name,
        colour = epi_mes), show.legend = F, size = 2) + ylab(NULL) +
    xlab(NULL) + labs(color = "Gene/Marker type") + scale_colour_hue(labels = c("Epithelial",
    "H2A.Z", "Mesenchymal", "TGFB1")) + theme(legend.position = "none",
    axis.text.y = element_blank(), panel.background = element_blank(),
    panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
    legend.background = element_rect(fill = NA), axis.line = element_line(colour = "black",
        axisLineSize))
```

```
# plotting
# ------------------------------------------------------------------
gpFontSize <- gpar(fontsize = 8)
xAx1 <- grid::textGrob("Distance from TSS [bp]", gp = gpFontSize)
xAx2 <- grid::textGrob("TFGb-WT [RPKM]", gp = gpFontSize)
yAx1 <- grid::textGrob("Spearman\n[rho-statistic]", rot = 90,
    gp = gpFontSize)
yAx2 <- grid::textGrob("Log2 fold-change\n[estimated]", rot = 90,
    gp = gpFontSize)
emptyBox <- grid::rectGrob(gp = grid::gpar(fill = "white", lty = 0))

lay <- rbind(c(1, 2, 2), c(3, 4, 4), c(5, 6, 7), c(8, 9, 9))
# remove legend as we can recycle legend from Figure 2
# (assemble in Illustrator
gs <- list(yAx1, corPlot2, emptyBox, xAx1, yAx2, plotMax, plotMin,
    emptyBox, xAx2)
grob2 <- gridExtra::grid.arrange(grobs = gs, layout_matrix = lay,
    widths = c(1, 10, 10), heights = c(2.5, 0.5, 7.5, 0.5))
```

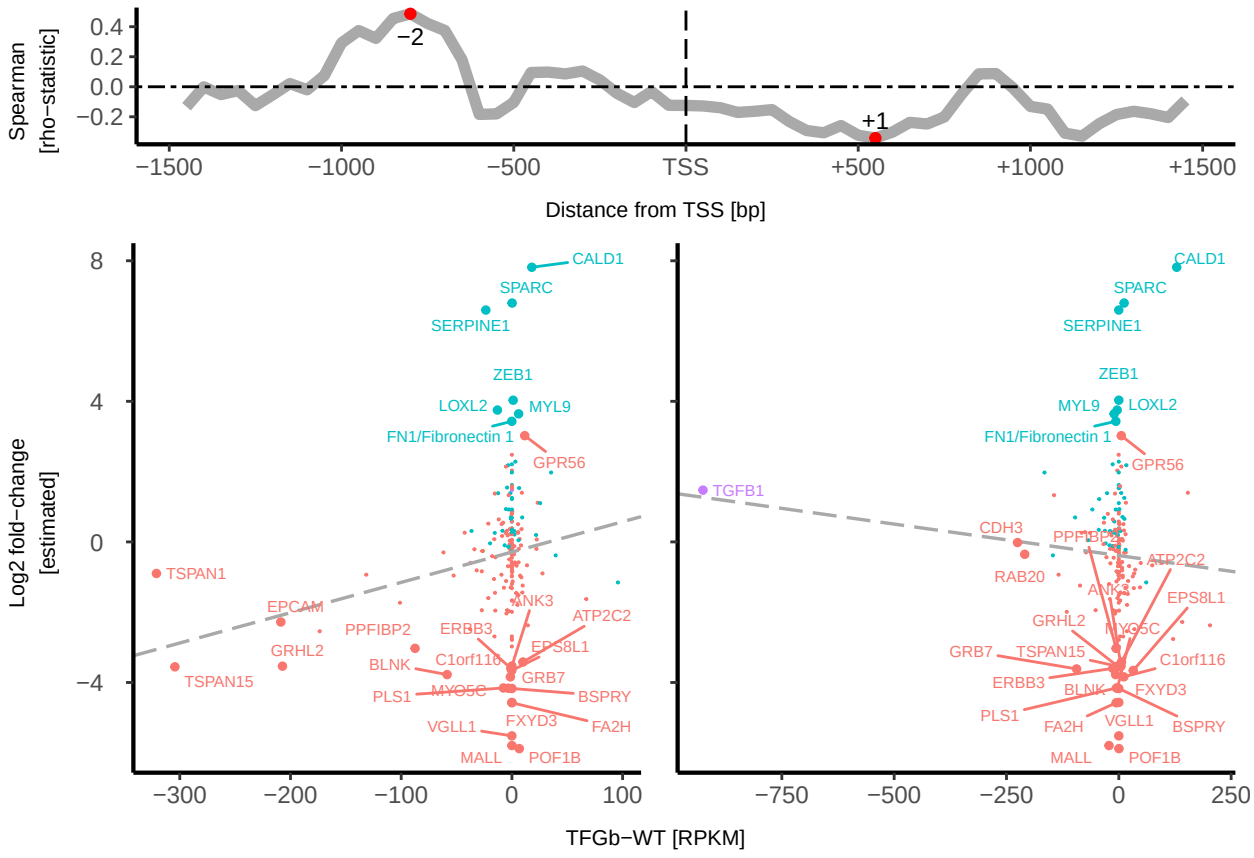


Figure 4 A & B - ChIP-Seq single gene coverage plots

```
# coverage plot for TGFB1
m1 <- melt(dataList[["H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz"]
```

```
    dataList[["H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz"]]$compu
    ])
```

```
## Using X4 as id variables
```

```
m2 <- melt(dataList[["H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz"]]$c
    dataList[["H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTup_normal.matrix.gz"]]$computeMa
    ])
```

```
## Using X4 as id variables
```

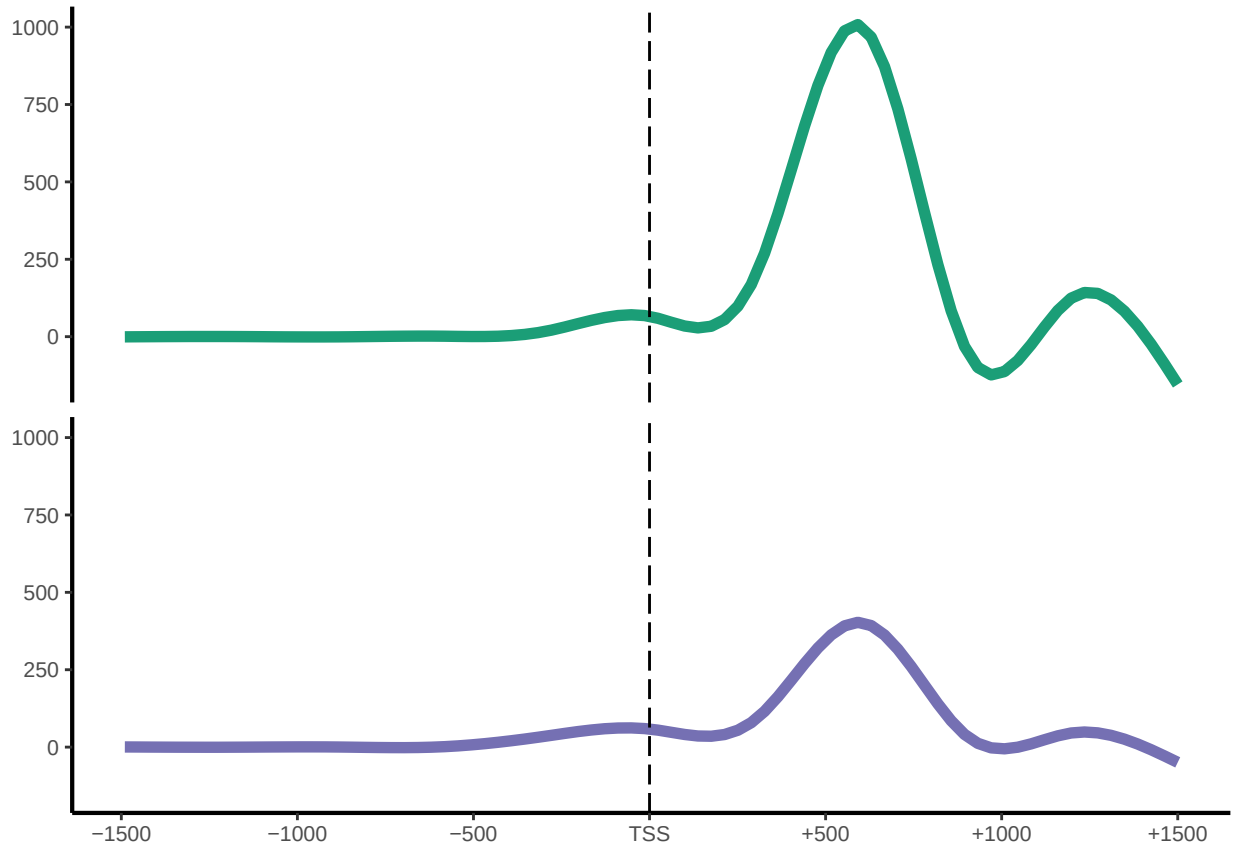
```
m3 <- melt(dataList[["H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz
    dataList[["H2AZ-TGFb_vs_Input-TGFb_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz"]]$co
    ])
```

```
## Using X4 as id variables
```

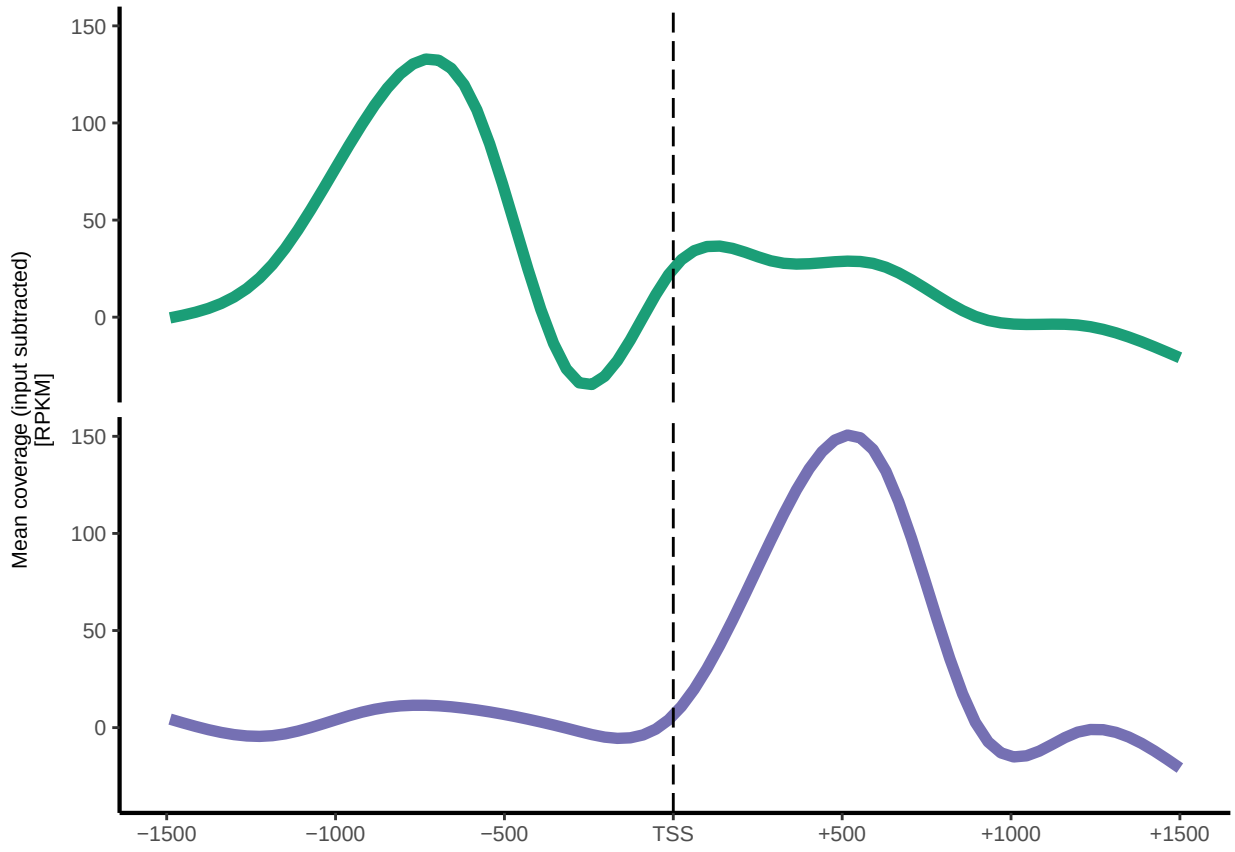
```
m3$set <- "TGFb"
m4 <- melt(dataList[["H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz"]]$
    dataList[["H2AZ-WT_vs_Input-WT_normal.readCount.subtract_RPKM_TanEMTdown_normal.matrix.gz"]]$computeM
    ])
```

```
## Using X4 as id variables
```

```
m4$set <- "WT"
m3 <- rbind(m3, m4)
m3$bin <- rep(1:300, 2)
m3$set <- as.factor(m3$set)
m3$set <- relevel(m3$set, ref = "WT")
m3$gene <- "EPCAM"

m1$set <- "TGFb"
m1$bin <- 1:300
m2$set <- "WT"
m2$bin <- 1:300
m1 <- rbind(m1, m2)
m1$gene <- "TGFB1"
m1$set <- as.factor(m1$set)
m1$set <- relevel(m1$set, ref = "WT")

plotTGFb <- ggplot(m1, aes(bin, value, colour = set)) + geom_smooth(method = "lm",
    formula = y ~ splines::ns(x, 10), se = F, size = lineSize) +
    facet_wrap(c("set"), nrow = 2) + ylab(NULL) + xlab(NULL) +
    geom_vline(xintercept = 150, colour = vlineCol, linetype = "longdash") +
    scale_x_continuous(breaks = c(0, 50, 100, 150, 200, 250,
        300), labels = c("-1500", "-1000", "-500", "TSS", "+500",
        "+1000", "+1500")) + labs(color = "Sample") + theme(axis.line = element_line(colour = "black",
    size = axisLineSize), axis.text = element_text(size = 8),
    strip.background = element_blank(), strip.text = element_blank(),
    panel.background = element_blank(), panel.border = element_blank(),
    panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
    legend.position = "none") + scale_colour_manual(values = c(wtCol,
    TGFbCol))
plotTGFb
```




```
# coverage plot for EPCAM ENSCAFG00000002653
# ------------------------------------------------
plotEPCAM <- ggplot(m3, aes(bin, value, colour = set)) + geom_smooth(method = "lm",
    formula = y ~ splines::ns(x, 10), se = F, size = lineSize) +
    facet_wrap(c("set"), nrow = 2) + ylab("Mean coverage (input subtracted)\n[RPKM]") +
    xlab(NULL) + labs(color = "Sample") + geom_vline(xintercept = 150,
    colour = vlineCol, linetype = "longdash") + scale_x_continuous(breaks = c(0,
    50, 100, 150, 200, 250, 300), labels = c("-1500", "-1000",
    "-500", "TSS", "+500", "+1000", "+1500")) + theme(axis.line = element_line(colour = "black",
    size = axisLineSize), axis.text = element_text(size = 8),
    axis.title = element_text(size = 8), strip.background = element_blank(),
    strip.text = element_blank(), panel.background = element_blank(),
    panel.border = element_blank(), panel.grid.major = element_blank(),
    panel.grid.minor = element_blank()) + scale_colour_manual(values = c(wtCol,
    TGFbCol))
legendPlot <- g_legend(plotEPCAM)
plotEPCAM <- plotEPCAM + theme(legend.position = "none")
plotEPCAM
```



Plotting/writing output

```
ggsave(grob2, filename = "Figure3_CDE.pdf", height = 10.5/2,
    width = 8.5, units = "in", useDingbats = F)
ggsave(plotEPCAM, filename = "Figure4_A.pdf", height = 10.3/3,
    width = 8.4/2, units = "in", useDingbats = F)
ggsave(plotTGFb, filename = "Figure4_B.pdf", height = 10.3/3,
    width = 8.4/2, units = "in", useDingbats = F)
```