Downstream analysis followig the image analysis to quanttify Dvl2_mEos codensates (Figure 4G)

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1 Load dependencies

```
knitr::opts_chunk$set(echo = TRUE)
library(tidyverse)
library(here)
library(cowplot)
library(ggpubr)
theme_set(theme_cowplot())
```

2 Preamble

The image analysis was carried out as follows: In a first step cells were identified by segmentation. Nuclei were identified based on a Hoechst staining (DAPI channel) and cell bodies based on a DyLight Phalloidin staining (Cy5 channel). After segmentation condensates inside cells were identified based on intensity-based thresholding (FITC channel) and counted. Condensate counts and cell counts (number of segmented cells) were saved for each image. An exemplary image analysis can be found with the script images_analysis_condesate_quantification.Rmd in this repository. Two different cell lines and different conditions were compared.
Cell lines: - HEK cells with a DVL1-mEOS tag - HEK cells with a DVL1-mEOS tag and a EVI k.o. (clone 1)

Conditions: - recombinant Wnt3A incubated for 1 h - recombinant Wnt3A incubated for 3 h - recombinant Wnt3A incubated for 16 h - serum-free medium incubated for 1 h - serum-free medium incubated for 3 h - serum-free medium incubated for 16 h

The time course experiment 4 separate xcperiments (i.e. on different days) were performed. Each experiment was carried out on one 384-well plates. Per condition, 15 wells with 4 fields of view per well were analyzed.

3 Load the data after the image analysis

```
raw_data <- read_rds(here("raw_data/raw_data_Fig4G","raw_data_Fig4G.rds"))
```

4 Prepare boxplot

```
HEK293T_Dvl2_mEos_medium <- raw_data %>% filter(plate_id == "Plate_I") %>%
  separate(treatment,c("treatment","time"),sep="_") %>%
   mutate(norm_punct_count = punct_count_adapt/cell_count) %>%
    group_by(barcode,cell_line,plate_id,experiment,treatment,time) %>%
    summarise_if(is.numeric,mean) %>%
    ungroup() %>%
      select(-cell_count,-punct_count_global,-punct_count_adapt) %>%
        filter(cell_line == "HEK293T_Dvl2_mEos" & treatment == "medium") %>%
          rename(norm_punct_count_HEK293T_Dvl2_mEos_medium = norm_punct_count) %>%
            select(norm_punct_count_HEK293T_Dvl2_mEos_medium,
                   experiment,
```

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```
                        time)

boxplot <- raw_data %>% filter(plate_id == "Plate_I") %>%
  filter(cell_line != "HEK293T_wt") %>%
  separate(treatment,c("treatment","time"),sep="_") %>%
   mutate(norm_punct_count = punct_count_adapt/cell_count) %>%
    group_by(barcode,cell_line,plate_id,experiment,treatment,time) %>%
    summarise_if(is.numeric,mean) %>%
    ungroup() %>%
      select(-cell_count,-punct_count_global,-punct_count_adapt) %>%
       left_join(HEK293T_Dvl2_mEos_medium,
                 by = c("experiment","time")) %>%
        mutate(id = paste(cell_line,treatment,sep="_")) %>%
        filter(id != "HEK293T_Dvl2_mEos_medium") %>%
        mutate(norm_punct_count_rel = (
          norm_punct_count/norm_punct_count_HEK293T_Dvl2_mEos_medium)*100) %>%
        unite(condition,cell_line,treatment,time,sep=":") %>%
        mutate(condition = factor(
          condition,
          levels = c("HEK293T_Dvl2_mEos_EVI_KO:recWnt3a:1h",
                     "HEK293T_Dvl2_mEos_EVI_KO:recWnt3a:3h",
                     "HEK293T_Dvl2_mEos_EVI_KO:recWnt3a:16h",
                     "HEK293T_Dvl2_mEos_EVI_KO:medium:1h",
                     "HEK293T_Dvl2_mEos_EVI_KO:medium:3h",
                     "HEK293T_Dvl2_mEos_EVI_KO:medium:16h",
                     "HEK293T_Dvl2_mEos:recWnt3a:1h",
                     "HEK293T_Dvl2_mEos:recWnt3a:3h",
                     "HEK293T_Dvl2_mEos:recWnt3a:16h"),
            labels = c("HEK293T_Dvl2_mEos_EVI_KO:recWnt3a:1h",
                       "HEK293T_Dvl2_mEos_EVI_KO:recWnt3a:3h",
                       "HEK293T_Dvl2_mEos_EVI_KO:recWnt3a:16h",
                       "HEK293T_Dvl2_mEos_EVI_KO:medium:1h",
                       "HEK293T_Dvl2_mEos_EVI_KO:medium:3h",
                       "HEK293T_Dvl2_mEos_EVI_KO:medium:16h",
                       "HEK293T_Dvl2_mEos:recWnt3a:1h",
                       "HEK293T_Dvl2_mEos:recWnt3a:3h",
                       "HEK293T_Dvl2_mEos:recWnt3a:16h"))) %>%
        arrange(condition) %>%
        ggplot(aes(x = condition,y=norm_punct_count_rel)) +
                geom_boxplot(outlier.shape = NA) +
                geom_point(position=position_jitterdodge(
                  jitter.width=0, dodge.width = 0.3, seed = 1234),
                aes(color = experiment), size = 2) +
    ylab(
"% induction relative to medium control of HEK293T_Dvl2_mEos based on normalized condensate counts") +
    xlab("condition") +
    theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1),
          axis.title.y = element_text(size=6))

boxplot
```

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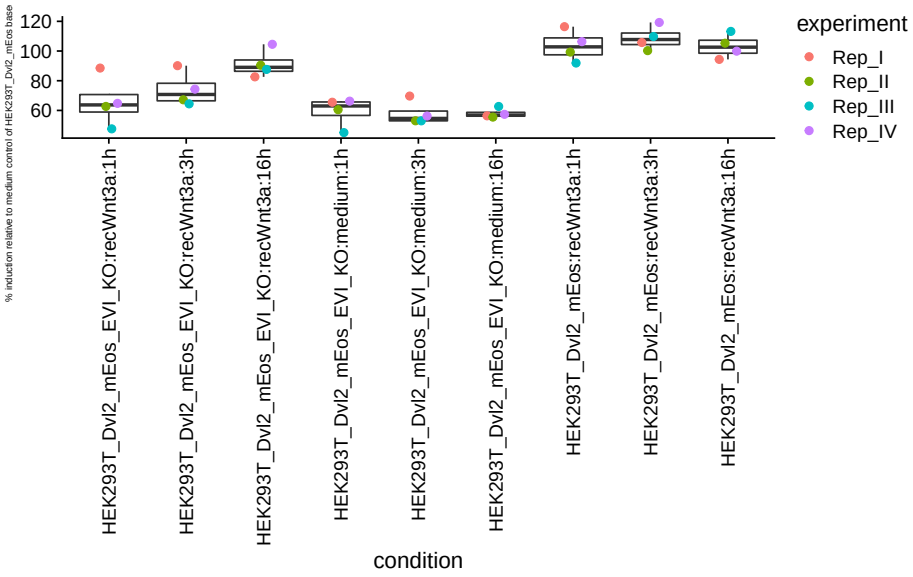


```
ggsave(here("./graphics/Figure4G/","boxplot_figure4g_raw.pdf"),
           plot=boxplot,
           width = 10,height = 10)
```

5 Session info

```
sessionInfo()
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/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] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] ggpubr_0.4.0     cowplot_1.1.1    here_1.0.1       forcats_0.5.1
##  [5] stringr_1.4.0    dplyr_1.0.7      purrr_0.3.4      readr_2.1.1
##  [9] tidyr_1.1.4      tibble_3.1.6     ggplot2_3.3.5    tidyverse_1.3.1
## [13] BiocStyle_2.22.0
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.7          lubridate_1.8.0     assertthat_0.2.1
##  [4] rprojroot_2.0.2     digest_0.6.29       utf8_1.2.2
```

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```
##  [7] R6_2.5.1            cellranger_1.1.0    backports_1.4.0
## [10] reprex_2.0.1        evaluate_0.14       httr_1.4.2
## [13] pillar_1.6.4        rlang_0.4.12        readxl_1.3.1
## [16] rstudioapi_0.13     car_3.0-12          rmarkdown_2.11
## [19] labeling_0.4.2      munsell_0.5.0       broom_0.7.10
## [22] compiler_4.1.2      modelr_0.1.8        xfun_0.28
## [25] pkgconfig_2.0.3     htmltools_0.5.2     tidyselect_1.1.1
## [28] bookdown_0.24       fansi_0.5.0         crayon_1.4.2
## [31] tzdb_0.2.0          dbplyr_2.1.1        withr_2.4.3
## [34] grid_4.1.2          jsonlite_1.7.2      gtable_0.3.0
## [37] lifecycle_1.0.1     DBI_1.1.1           magrittr_2.0.1
## [40] scales_1.1.1        carData_3.0-4       cli_3.1.0
## [43] stringi_1.7.6       farver_2.1.0        ggsignif_0.6.3
## [46] fs_1.5.1            xml2_1.3.3          ellipsis_0.3.2
## [49] generics_0.1.1      vctrs_0.3.8         tools_4.1.2
## [52] glue_1.5.1          hms_1.1.1           abind_1.4-5
## [55] fastmap_1.1.0       yaml_2.2.1          colorspace_2.0-2
## [58] BiocManager_1.30.16 rstatix_0.7.0       rvest_1.0.2
## [61] knitr_1.36          haven_2.4.3
```