Redhai publication 2023 selected plots

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Last modified:

```
print(date())
```

```
## [1] "Tue Dec 19 15:52:34 2023"
```

The following code is used to produce selected plots for the publication: **Rab35 restrains regional Wnt activation by regulating a Cdc42-JNK signalling relay in the intestine**

Import

```
library(tidyverse)
library(plotrix)
library(cowplot)
library(viridis)
library(readxl)
library(ggrepel)
library(padr)
library(gmodels)
library(rstatix)
theme_set(theme_cowplot())
```

Figure 1b

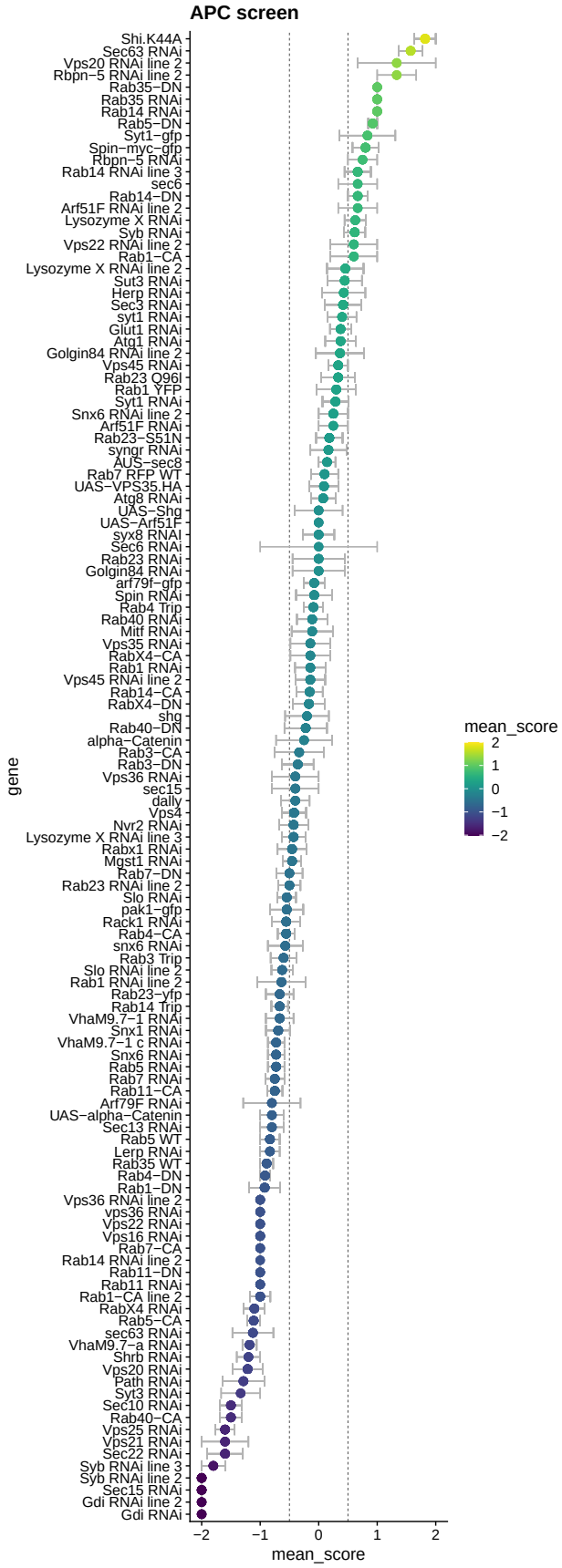
Prepare data

```
screen_result_apc <- read_excel("raw_data/FINAL modifier screen apc .xlsx", range = "A3:T126")

screen_result_apc_long <- screen_result_apc %>%
  rename("gene_name"="...1") %>%
  mutate(GENE = str_replace_all(gene_name, c("rnai"="RNAi", "1DN"="1-DN", "wt"="WT", "-WT"=" WT", "GDI"=
  select(!"gene_name") %>%
  group_by(GENE) %>%
  mutate(duplicate_line = dplyr::row_number()) %>%
  mutate(GENE = ifelse(duplicate_line > 1, paste(GENE, "line", duplicate_line, sep = " "), GENE)) %>%
```

```
  ungroup() %>%
  select(!"duplicate_line") %>%
  pivot_longer(!"GENE", names_to = "column_name", values_to = "score") %>%
  filter(!is.na(score)) %>%
  select(!"column_name")

screen_result_apc_long <- screen_result_apc_long %>%
  left_join(screen_result_apc_long %>%
              group_by(GENE) %>%
              summarise(mean_score = mean(score), sd_score = sd(score), sem_score = std.error(score)))
```

Plot

```
screen_results_apc <- screen_result_apc_long %>%
  ggplot(aes(y=reorder(GENE, mean_score), x=mean_score, color = mean_score)) +
  geom_vline(xintercept = c(0.5, -0.5), size = 0.25, colour = 'gray30', linetype = "dashed") +
  geom_errorbar(aes(xmin = mean_score - sem_score, xmax = mean_score + sem_score), color = "gray70") +
  geom_point(size = 3) +
  scale_color_viridis(limits=c(-2, 2)) +
  theme_cowplot() +
  ylab("gene") +
  xlim(-2, 2) +
  ggtitle("APC screen")

screen_results_apc
```


APC screen
Shi.K44A
Sec63 RNAi
Vps20 RNAi line 2
Rbpn−5 RNAi line 2
Rab35−DN
Rab35 RNAi
Rab14 RNAi
Rab5−DN
Syt1−gfp
Spin−myc−gfp
Rbpn−5 RNAi
Rab14 RNAi line 3
sec6
Rab14−DN
Arf51F RNAi line 2
Lysozyme X RNAi
Syb RNAi
Vps22 RNAi line 2
Rab1−CA
Lysozyme X RNAi line 2
Sut3 RNAi
Herp RNAi
Sec3 RNAi
syt1 RNAi
Glut1 RNAi
Atg1 RNAi
Golgin84 RNAi line 2
Vps45 RNAi
Rab23 Q96l
Rab1 YFP
Syt1 RNAi
Snx6 RNAi line 2
Arf51F RNAi
Rab23−S51N
syngr RNAi
AUS−sec8
Rab7 RFP WT
UAS−VPS35.HA
Atg8 RNAi
UAS−Shg
UAS−Arf51F
syx8 RNAi
Sec6 RNAi
Rab23 RNAi
Golgin84 RNAi
arf79f−gfp
Spin RNAi
Rab4 Trip
Rab40 RNAi
Mitf RNAi
Vps35 RNAi
RabX4−CA
Rab1 RNAi
Vps45 RNAi line 2
Rab14−CA
RabX4−DN
shg
Rab40−DN
alpha−Catenin
Rab3−CA
Rab3−DN
Vps36 RNAi
sec15
dally
Vps4
Nvr2 RNAi
Lysozyme X RNAi line 3
Rabx1 RNAi
Mgst1 RNAi
Rab7−DN
Rab23 RNAi line 2
Slo RNAi
pak1−gfp
Rack1 RNAi
Rab4−CA
snx6 RNAi
Rab3 Trip
Slo RNAi line 2
Rab1 RNAi line 2
Rab23−yfp
Rab14 Trip
VhaM9.7−1 RNAi
Snx1 RNAi
VhaM9.7−1 c RNAi
Snx6 RNAi
Rab5 RNAi
Rab7 RNAi
Rab11−CA
Arf79F RNAi
UAS−alpha−Catenin
Sec13 RNAi
Rab5 WT
Lerp RNAi
Rab35 WT
Rab4−DN
Rab1−DN
Vps36 RNAi line 2
vps36 RNAi
Vps22 RNAi
Vps16 RNAi
Rab7−CA
Rab14 RNAi line 2
Rab11−DN
Rab11 RNAi
Rab1−CA line 2
RabX4 RNAi
Rab5−CA
sec63 RNAi
VhaM9.7−a RNAi
Shrb RNAi
Vps20 RNAi
Path RNAi
Syt3 RNAi
Sec10 RNAi
Rab40−CA
Vps25 RNAi
Vps21 RNAi
Sec22 RNAi
Syb RNAi line 3
Syb RNAi line 2
Sec15 RNAi
Gdi RNAi line 2
Gdi RNAi
gene
−2
−1
0
1
2
mean_score
mean_score
2
1
0
−1
−2

```
ggsave(filename = "Graphics/screen_apc_results.pdf", plot = screen_results_apc, device = pdf, height =
```

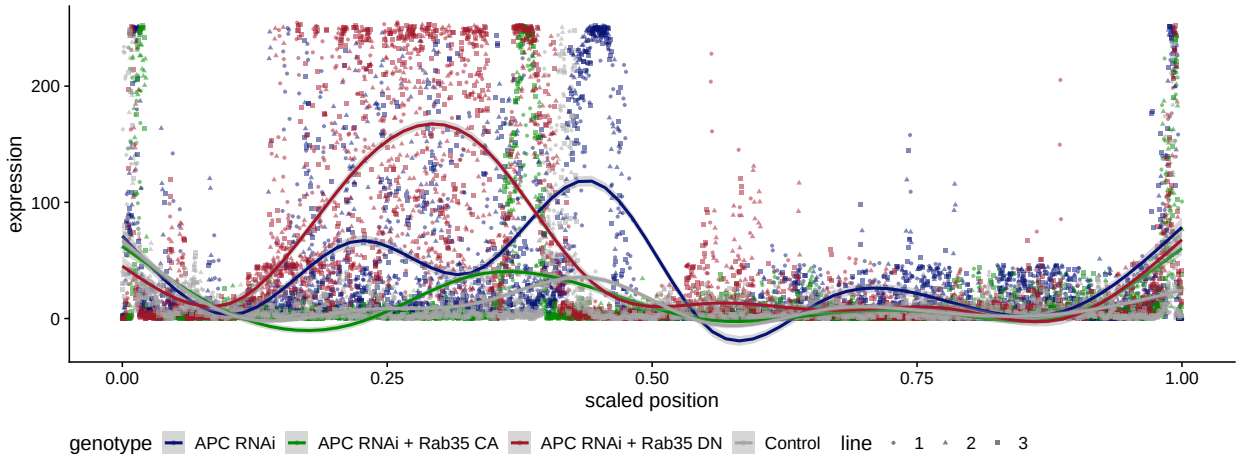
Figure 2b

Prepare data

```
expression_data <- read_excel("raw_data/APC_data.xlsx", col_names = FALSE)
expression_data <- expression_data %>%
  select(where(~!all(is.na(.x)))) %>%
  filter(!row_number() %in% c(1,2,3)) %>%
  split.default(rep(c("Control", "APC RNAi", "APC RNAi + Rab35 CA", "APC RNAi + Rab35 DN"), each = 6)) %
  bind_rows(.id = "genotype")
# Rename:
new_names <- paste(rep(c("position", "expression"), 3), rep(seq(1,3), each = 2), sep = "_")
old_names <- colnames(expression_data)[2:7]
expression_data.wide <- expression_data %>% rename_at(vars(old_names), function(x) new_names)
expression_data.long <- expression_data.wide %>%
  pivot_longer(cols = !genotype, cols_vary = "slowest", names_to = c(".value", "line"), names_pattern =
  mutate(position = as.numeric(position), expression = as.numeric(expression)) %>%
  filter(!is.na(position))
```

Plot

```
line_colors <- c("#A5A5A5", "#A41629", "#008C00", "#061276")

expression_data.long.scaled <-expression_data.long %>%
  group_by(genotype) %>%
  mutate(max_position = max(position)) %>%
  mutate(scaled_position = position/max_position) %>%
  ungroup()

expression_data_APC <- expression_data.long.scaled %>%
  ggplot(aes(x=scaled_position, y=expression, color=genotype)) +
  geom_point(aes(shape = line), size = 1, alpha = 0.5) +
  geom_smooth() +
  labs(x = "scaled position") +
  scale_color_manual(values = rev(line_colors)) +
  theme(legend.position = "bottom")

expression_data_APC
```




```
ggsave(filename = "Graphics/expression_data_APC.pdf", plot = expression_data_APC, device = pdf)
```

Figure 3h

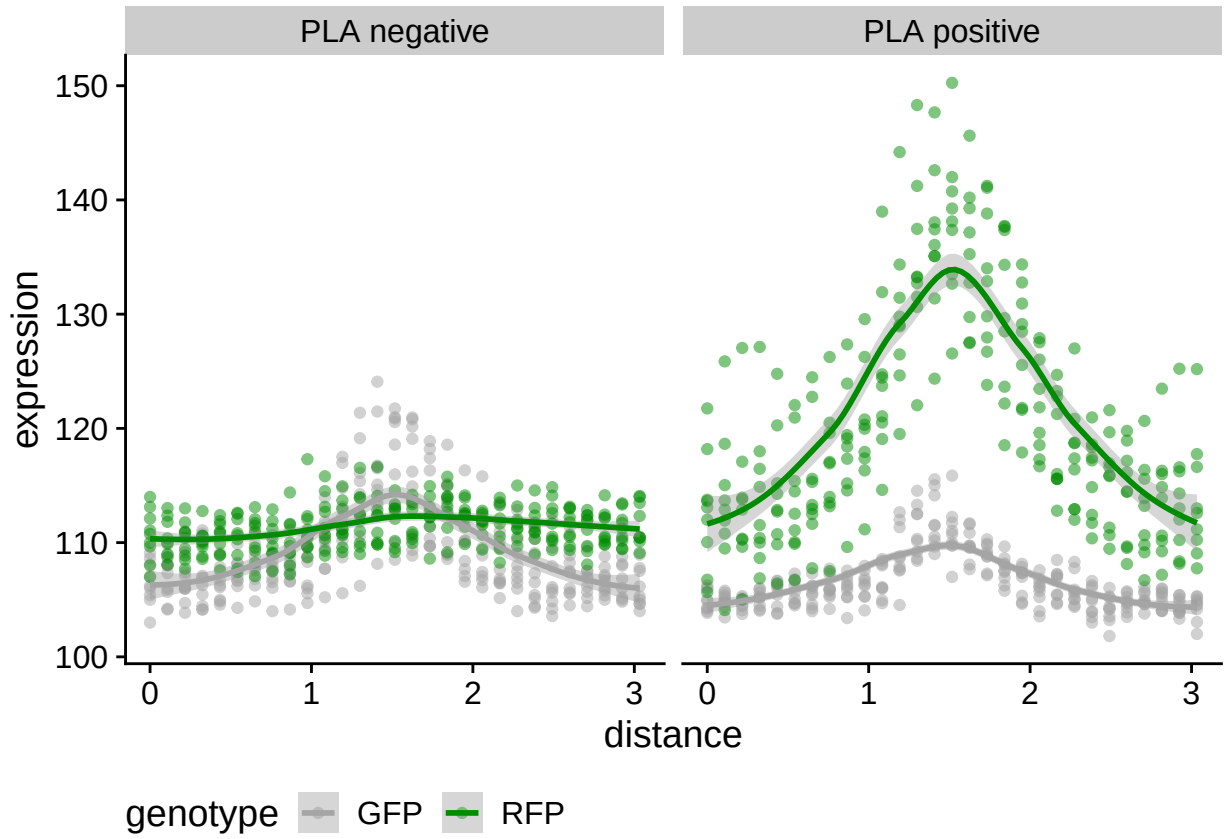
Prepare data

```
pla_negative_gfp <- read_excel("raw_data/PLA quantifications.xlsx", range = "C6:AD35") %>% select(matche

pla_negative_gfp.wide <- pla_negative_gfp %>%
  split.default(rep(paste0("gut", seq(1,ncol(pla_negative_gfp)/6)), each = 6)) %>%
  bind_rows(.id = "gut")

new_names <- paste(rep(c("distance", "expression"), 3), rep(seq(1,3), each = 2), sep = "_")
old_names <- colnames(pla_negative_gfp.wide[2:ncol(pla_negative_gfp.wide)])

pla_negative_gfp.long <- pla_negative_gfp.wide %>%
  rename_at(vars(old_names), function(x) new_names) %>%
  pivot_longer(cols = !gut, cols_vary = "slowest", names_to = c(".value", "cell"), names_pattern = "(.*
  mutate(genotype = "GFP") %>%
  relocate(genotype)

pla_negative_rfp <- read_excel("raw_data/PLA quantifications.xlsx", range = "C39:AD68") %>% select(matcl

pla_negative_rfp.wide <- pla_negative_rfp %>%
  split.default(rep(paste0("gut", seq(1,ncol(pla_negative_rfp)/6)), each = 6)) %>%
  bind_rows(.id = "gut")

old_names <- colnames(pla_negative_rfp.wide[2:ncol(pla_negative_rfp.wide)])

pla_negative_rfp.long <- pla_negative_rfp.wide %>%
  rename_at(vars(old_names), function(x) new_names) %>%
  pivot_longer(cols = !gut, cols_vary = "slowest", names_to = c(".value", "cell"), names_pattern = "(.*
  mutate(genotype = "RFP") %>%
  relocate(genotype)
```

```
pla_negative.long <- bind_rows(pla_negative_gfp.long, pla_negative_rfp.long) %>%
  filter(!is.na(distance))

pla_positive_gfp <- read_excel("raw_data/PLA quantifications.xlsx", range = "C75:AD104") %>% select(mat

pla_positive_gfp.wide <- pla_positive_gfp %>%
  split.default(rep(paste0("gut", seq(1,ncol(pla_positive_gfp)/6)), each = 6)) %>%
  bind_rows(.id = "gut")

old_names <- colnames(pla_positive_gfp.wide[2:ncol(pla_positive_gfp.wide)])

pla_positive_gfp.long <- pla_positive_gfp.wide %>%
  rename_at(vars(old_names), function(x) new_names) %>%
  pivot_longer(cols = !gut, cols_vary = "slowest", names_to = c(".value", "cell"), names_pattern = "(.*
  mutate(genotype = "GFP") %>%
  relocate(genotype)


pla_positive_rfp <- read_excel("raw_data/PLA quantifications.xlsx", range = "C107:AD136") %>% select(ma

pla_positive_rfp.wide <- pla_positive_rfp %>%
  split.default(rep(paste0("gut", seq(1,ncol(pla_positive_rfp)/6)), each = 6)) %>%
  bind_rows(.id = "gut")

old_names <- colnames(pla_positive_rfp.wide[2:ncol(pla_positive_rfp.wide)])

pla_positive_rfp.long <- pla_positive_rfp.wide %>%
  rename_at(vars(old_names), function(x) new_names) %>%
  pivot_longer(cols = !gut, cols_vary = "slowest", names_to = c(".value", "cell"), names_pattern = "(.*
  mutate(genotype = "RFP") %>%
  relocate(genotype)

pla_positive.long <- bind_rows(pla_positive_gfp.long, pla_positive_rfp.long) %>%
  filter(!is.na(distance))
```

Plot

```
pla_plot <- pla_positive.long %>%
  mutate(title = "PLA positive") %>%
  bind_rows(pla_negative.long %>% mutate(title = "PLA negative")) %>%
  ggplot(aes(x=distance, y=expression, color=genotype)) +
  geom_point(alpha=0.5) +
  geom_smooth() +
  scale_color_manual(values = line_colors[c(1,3,2)]) +
  theme(legend.position = "bottom") +
  facet_grid(cols = vars(title))

pla_plot
```




```
ggsave(filename = "Graphics/pla_plot.pdf", plot = pla_plot, device = pdf)
```

Figure 4b

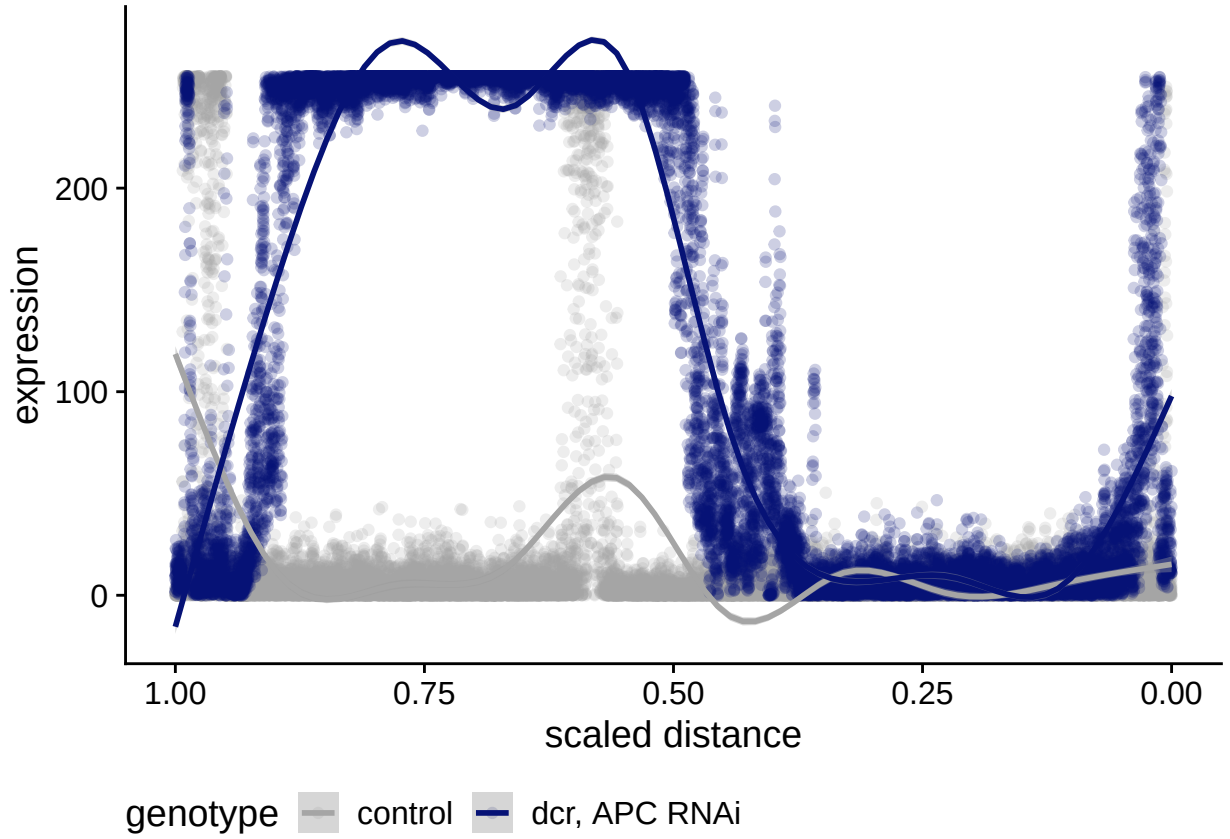
Prepare data

```
fz3_intestine_control <- read_excel("raw_data/Fz3 quantification along intestine myots.xlsx", range = "(

old_names <- colnames(fz3_intestine_control)

fz3_intestine_control.long <- fz3_intestine_control %>%
  rename_at(vars(old_names), function(x) new_names) %>%
  mutate(gut = 1) %>%
  pivot_longer(cols = !gut, cols_vary = "slowest", names_to = c(".value", "cell"), names_pattern = "(.*)
  mutate(genotype = "control") %>%
  relocate(genotype)


fz3_intestine_dcr_apc <- read_excel("raw_data/Fz3 quantification along intestine myots.xlsx", range = "(

old_names <- colnames(fz3_intestine_dcr_apc)

fz3_intestine_dcr_apc.long <- fz3_intestine_dcr_apc %>%
```

```
  rename_at(vars(old_names), function(x) new_names) %>%
  mutate(gut = 1) %>%
  pivot_longer(cols = !gut, cols_vary = "slowest", names_to = c(".value", "cell"), names_pattern = "(.*
  mutate(genotype = "dcr, APC RNAi") %>%
  relocate(genotype)


fz3_intestine.long <- bind_rows(fz3_intestine_control.long , fz3_intestine_dcr_apc.long) %>%
  filter(!is.na(distance))
```

Plot

```
fz3_intestine.long.scaled <- fz3_intestine.long %>%
  group_by(genotype, cell) %>%
  mutate(max_distance = max(distance)) %>%
  mutate(scaled_distance = distance/max_distance) %>%
  ungroup()

fz3_plot <- fz3_intestine.long.scaled %>%
  ggplot(aes(x=scaled_distance, y=expression, color=genotype)) +
  geom_point(alpha=0.2) +
  geom_smooth() +
  scale_color_manual(values = line_colors[c(1,4)]) +
  xlab("scaled distance") +
  theme(legend.position = "bottom") +
  scale_x_reverse()

fz3_plot
```




```
ggsave(filename = "Graphics/fz3_plot.pdf", plot = fz3_plot, device = pdf)
```

Session info

```
sessionInfo()
```

```
## R version 4.2.2 (2022-10-31)
## Platform: aarch64-apple-darwin20 (64-bit)
## Running under: macOS 14.2
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2-arm64/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] rstatix_0.7.2     gmodels_2.18.1.1  padr_0.6.2        ggrepel_0.9.3
```

```
##  [5] readxl_1.4.2      viridis_0.6.3     viridisLite_0.4.2 cowplot_1.1.1
##  [9] plotrix_3.8-2     lubridate_1.9.2   forcats_1.0.0     stringr_1.5.0
## [13] dplyr_1.1.2       purrr_1.0.1       readr_2.1.4       tidyr_1.3.0
## [17] tibble_3.2.1      ggplot2_3.4.2     tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.10       lattice_0.21-8    gtools_3.9.4      digest_0.6.31
##  [5] utf8_1.2.3        R6_2.5.1          cellranger_1.1.0  backports_1.4.1
##  [9] evaluate_0.21     highr_0.10        pillar_1.9.0      rlang_1.1.1
## [13] rematch_1.0.1     rstudioapi_0.14   gdata_2.19.0      car_3.1-2
## [17] Matrix_1.5-4.1    rmarkdown_2.22    labeling_0.4.2    splines_4.2.2
## [21] munsell_0.5.0     broom_1.0.5       compiler_4.2.2    xfun_0.39
## [25] pkgconfig_2.0.3   mgcv_1.8-42       htmltools_0.5.5   tidyselect_1.2.0
## [29] gridExtra_2.3     fansi_1.0.4       tzdb_0.4.0        withr_2.5.0
## [33] MASS_7.3-60       grid_4.2.2        nlme_3.1-162      gtable_0.3.3
## [37] lifecycle_1.0.3   magrittr_2.0.3    scales_1.2.1      cli_3.6.1
## [41] stringi_1.7.12    carData_3.0-5     farver_2.1.1      generics_0.1.3
## [45] vctrs_0.6.2       tools_4.2.2       glue_1.6.2        hms_1.1.3
## [49] abind_1.4-5       fastmap_1.1.1     yaml_2.3.7        timechange_0.2.0
## [53] colorspace_2.1-0 knitr_1.43
```