20200417_Drp1_variant_additional_boxplots.R

kelse

2022-06-01

```
#
# Calculating and plotting Kcat,K0.5, Kcat/K0.5, and vmax for the following:
# Drp1 WT, L230dup, G363D, G401S, and R710G GTPase results
# Experiment initially performed with recombinant L230dup
# However, later MS + SDS-PAGE confirmed protein was truncated and not usable

# Load libraries ----

library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.0 --

## v ggplot2 3.3.3     v purrr   0.3.4
## v tibble  3.1.0     v dplyr   1.0.5
## v tidyr   1.1.3     v stringr 1.4.0
## v readr   1.4.0     v forcats 0.5.1

## Warning: package 'stringr' was built under R version 4.0.5

## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(broom)
library(readxl)
library(minpack.lm)
library(gridExtra)
```

```
##
## Attaching package: 'gridExtra'

## The following object is masked from 'package:dplyr':
##
##     combine
```

```
library(scales)
```

```
##
## Attaching package: 'scales'
```

```
## The following object is masked from 'package:purrr':
##
##     discard

## The following object is masked from 'package:readr':
##
##     col_factor
```

```
library(RColorBrewer)

theme_set(theme_bw() +
            theme(axis.text = element_text(size = 12, color = "black"),
                  panel.grid.major = element_blank(),
                  panel.grid.minor = element_blank())
)

# Import and tidy data ----
pool <- read_csv("Drp1_variant_NADH_depletion_rates.csv")
```

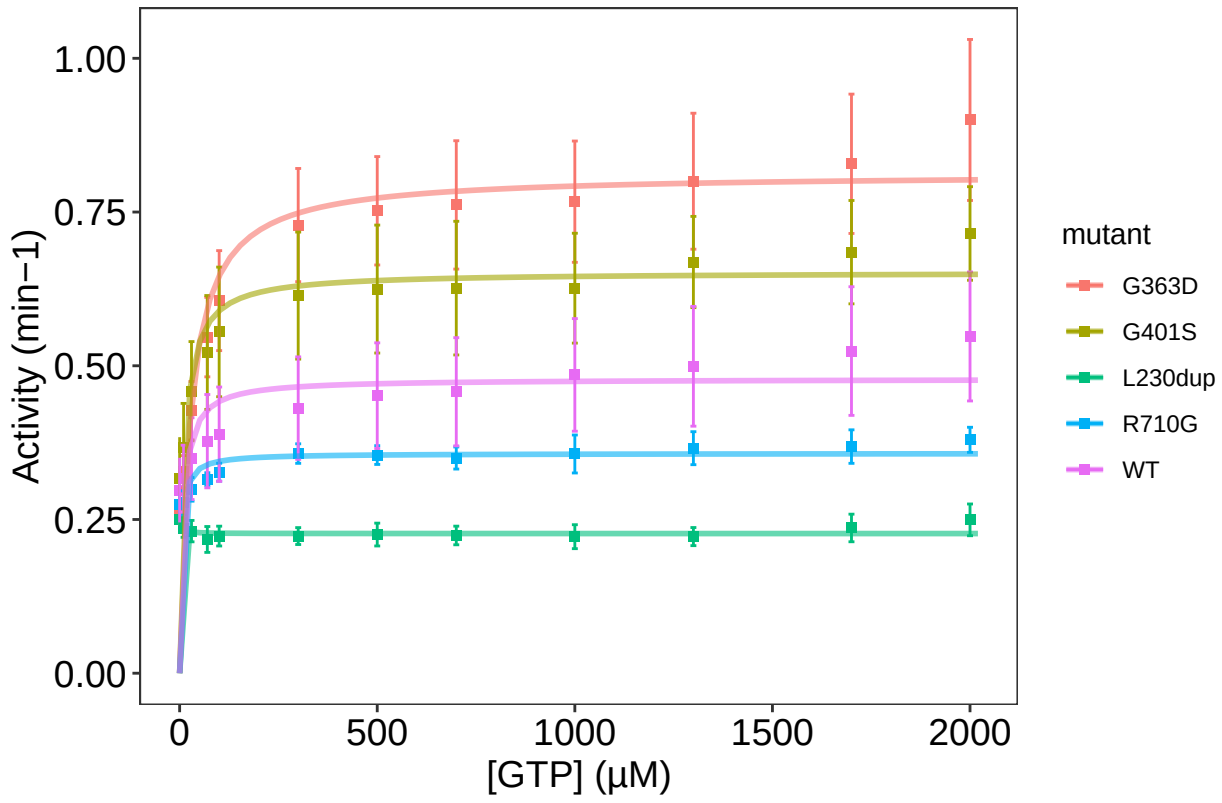
```
##
## -- Column specification --------------------------------------------------------
## cols(
##   mutant = col_character(),
##   gtp = col_double(),
##   tr = col_double(),
##   rep = col_character(),
##   a340_slope = col_double()
## )
```

```
# Determine and plot GTPase activity rate ----

### First, convert NADH oxidation rate into Drp1 activity (converting to min-1)
rates <- pool %>%
  na.omit() %>%
  mutate(., activity = (-a340_slope / (6220 * 0.4649 / 1e6) / 1)) %>%
  group_by(., mutant, gtp) %>%
  summarise(., avg_activity = mean(activity),
            stdev = sd(activity)) %>%
  mutate(., activity = avg_activity - avg_activity[gtp == 0]) %>%
  ungroup()
```

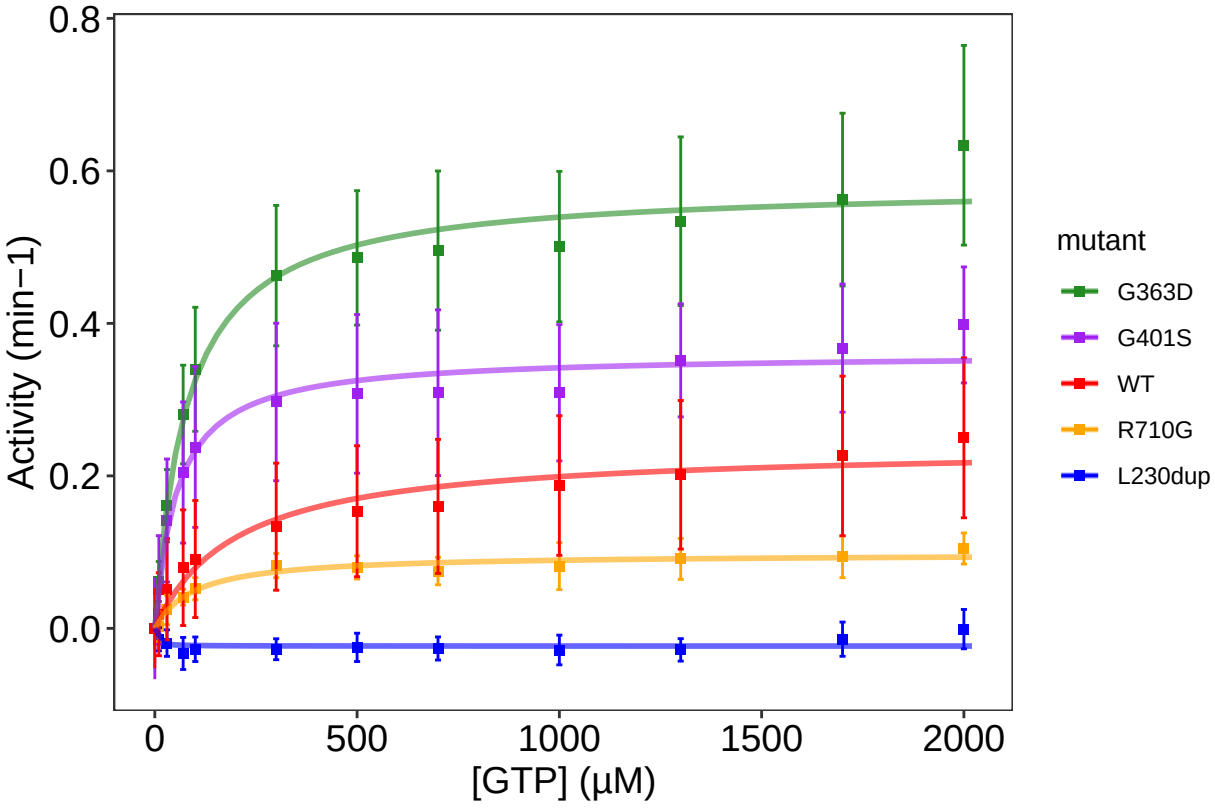
```
## `summarise()` has grouped output by 'mutant'. You can override using the `.groups` argument.
```

```
### activity is now in GTP hydrolyzed (µmol/min)
write_csv(rates, "Drp1_n3_activity_perMIN_KAM.csv")

### Then, plot activity against [GTP] (activity at 0 µM GTP)
kinetic_plot <- rates %>%
  ggplot(., aes(x = gtp, y = avg_activity, color = mutant)) +
  geom_point(shape = 15) +
  geom_errorbar(aes(ymin = avg_activity - stdev,
                    ymax = avg_activity + stdev),
```

```
                width = 15) +
  geom_line(stat = "smooth", method = "nlsLM",
            formula = y ~ (vmax*(x/(x+km))),
            method.args = list(start = c(vmax = 0.6,
                                         km = 200),
                               control = nls.control(maxiter = 100, tol = 1e-6)),
            se = FALSE,
            fullrange = T,
            size = 1,
            alpha = 0.6) +
  scale_x_continuous(limits = c(0, 2020),
                     breaks = c(0, 500, 1000, 1500, 2000)) +
  labs(title = "",
       x = "[GTP] (µM)",
       y = "Activity (min-1)") +
  theme(axis.text = element_text(size = 14, color = "black"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        strip.background = element_blank()
  )

kinetic_plot
```

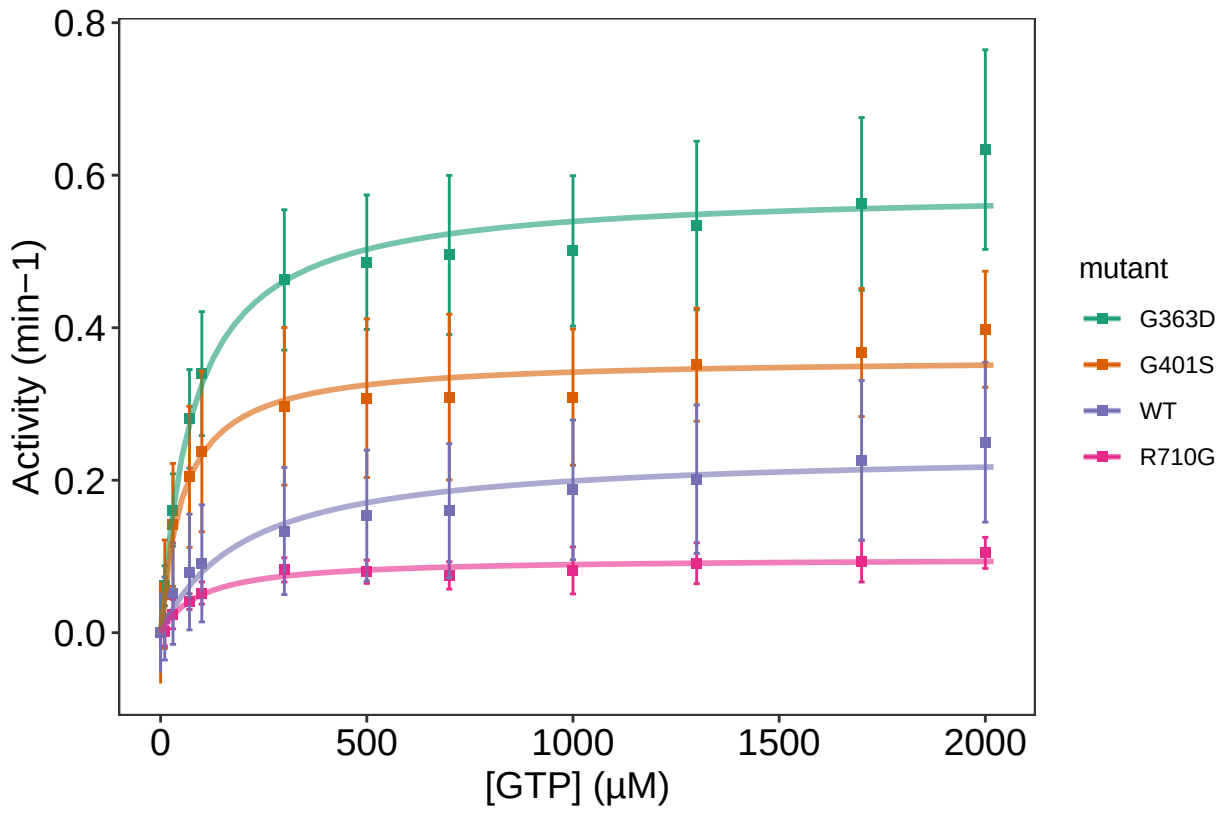


```
ggsave("Drp1_n3_prior_correct_background_KAM.pdf", kinetic_plot,
       width = 10, height = 8, units = "cm", dpi = 300)

kinetic_plot2 <- rates %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("G363D", "G401S", "WT", "R710G", "L230dup"))) %>%
  ggplot(., aes(x = gtp, y = activity, color = mutant)) +
  geom_point(shape = 15) +
  geom_errorbar(aes(ymin = activity - stdev,
                    ymax = activity + stdev),
                width = 15) +
  geom_line(stat = "smooth", method = "nlsLM",
            formula = y ~ (vmax*(x/(x+km))),
            method.args = list(start = c(vmax = 0.6,
                                         km = 200),
                               control = nls.control(maxiter = 100, tol = 1e-6)),
            se = FALSE,
            fullrange = T,
            size = 1,
            alpha = 0.6) +
  scale_x_continuous(limits = c(0, 2020),
                     breaks = c(0, 500, 1000, 1500, 2000)) +
  scale_color_manual(values = c("WT" = "red",
                                "L230dup" = "blue",
                                "G363D" = "forestgreen",
                                "G401S" = "purple",
                                "R710G" = "orange")) +
  labs(title = "",
       x = "[GTP] (µM)",
       y = "Activity (min-1)") +
  theme(axis.text = element_text(size = 14, color = "black"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        strip.background = element_blank()
  )

kinetic_plot2
```




```
ggsave("Drp1_n3_activity_plot_KAM.pdf", kinetic_plot,
       width = 10, height = 8, units = "cm", dpi = 300)

# Kinetic plot - no L230dup due to protein being truncated
# Dark2 color scheme
kinetic_plot2_noL230dup_dark2 <- rates %>%
  filter(., mutant != "L230dup") %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("G363D", "G401S", "WT", "R710G"))) %>%
  ggplot(., aes(x = gtp, y = activity, color = mutant)) +
  geom_point(shape = 15) +
  geom_errorbar(aes(ymin = activity - stdev,
                    ymax = activity + stdev),
                width = 15) +
  geom_line(stat = "smooth", method = "nlsLM",
            formula = y ~ (vmax*(x/(x+km))),
            method.args = list(start = c(vmax = 0.6,
                                         km = 200),
                               control = nls.control(maxiter = 100, tol = 1e-6)),
            se = FALSE,
            fullrange = T,
            size = 1,
            alpha = 0.6) +
  scale_x_continuous(limits = c(0, 2020),
                     breaks = c(0, 500, 1000, 1500, 2000)) +
```

```
  scale_color_brewer(palette = "Dark2") +
  labs(title = "",
       x = "[GTP] (µM)",
       y = "Activity (min-1)") +
  theme(axis.text = element_text(size = 14, color = "black"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        strip.background = element_blank()
    )

kinetic_plot2_noL230dup_dark2
```




```
ggsave("Drp1_n3_activity_plot_noL230dup_dark2_KAM.pdf", kinetic_plot,
       width = 10, height = 8, units = "cm", dpi = 300)

### Calculate Vmax and K0.5
vmax_km <- rates %>%
  group_by(mutant) %>%
  do(tidy(nlsLM(formula = activity ~ (vmax*(gtp/(gtp+km))),
                start = list(vmax = 0.6, km = 200),
                trace = TRUE,
                data = .)))
```

```
## It.    0, RSS =    0.06633, Par. =        0.6        200
## It.    1, RSS =  0.0347267, Par. =   0.614319     161.91
## It.    2, RSS = 0.00968603, Par. =   0.600247    90.4281
## It.    3, RSS = 0.00850219, Par. =   0.581657    78.4188
## It.    4, RSS = 0.00850205, Par. =   0.581673    78.5306
## It.    5, RSS = 0.00850205, Par. =   0.581678     78.535
## It.    6, RSS = 0.00850205, Par. =   0.581678    78.5352
## It.    0, RSS =   0.167063, Par. =        0.6        200
## It.    1, RSS = 0.00736633, Par. =   0.336587    41.5904
## It.    2, RSS = 0.00492244, Par. =    0.35913    52.8343
## It.    3, RSS =  0.0049068, Par. =   0.360299    54.3984
## It.    4, RSS = 0.00490671, Par. =   0.360383    54.5196
## It.    5, RSS = 0.00490671, Par. =   0.360389    54.5276
## It.    6, RSS = 0.00490671, Par. =   0.360389    54.5281
## It.    0, RSS =     1.8792, Par. =        0.6        200
## It.    1, RSS = 0.00593761, Par. = -0.00131521     246.42
## It.    2, RSS = 0.00169771, Par. = -0.0294023    80.6677
## It.    3, RSS = 0.000886447, Par. = -0.0237628    11.5989
## It.    4, RSS = 0.000853498, Par. = -0.0239295    9.22389
## It.    5, RSS = 0.000812471, Par. = -0.0235455    4.46972
## It.    6, RSS = 0.00081081, Par. = -0.0231519    3.14919
## It.    7, RSS = 0.000810531, Par. = -0.0232438    3.60777
## It.    8, RSS = 0.000810514, Par. = -0.0232151    3.48323
## It.    9, RSS = 0.000810512, Par. = -0.0232232    3.52106
## It.   10, RSS = 0.000810512, Par. = -0.0232208    3.50988
## It.   11, RSS = 0.000810512, Par. = -0.0232215    3.51321
## It.   12, RSS = 0.000810512, Par. = -0.0232213    3.51222
## It.    0, RSS =    1.14007, Par. =        0.6        200
## It.    1, RSS = 0.00164511, Par. =  0.0946316    174.034
## It.    2, RSS = 0.000803782, Par. =  0.0958789    59.7287
## It.    3, RSS = 0.000444982, Par. =  0.0972233    88.1554
## It.    4, RSS = 0.000434541, Par. =  0.0979153    95.9725
## It.    5, RSS = 0.000434529, Par. =  0.0979363    96.2567
## It.    6, RSS = 0.000434529, Par. =   0.097936    96.2546
## It.    0, RSS =   0.622683, Par. =        0.6        200
## It.    1, RSS =  0.0033453, Par. =   0.238913    200.307
## It.    2, RSS = 0.00334519, Par. =   0.238937    200.873
## It.    3, RSS = 0.00334518, Par. =   0.238981    201.062
## It.    4, RSS = 0.00334518, Par. =   0.238996    201.125
## It.    5, RSS = 0.00334518, Par. =   0.239001    201.146
## It.    6, RSS = 0.00334518, Par. =   0.239003    201.153
```

```
vmax_km
```

```
## # A tibble: 10 x 6
## # Groups:   mutant [5]
##    mutant  term  estimate std.error statistic  p.value
##    <chr>   <chr>    <dbl>     <dbl>     <dbl>    <dbl>
##  1 G363D   vmax    0.582    0.0156     37.2   4.70e-12
##  2 G363D   km     78.5     11.1         7.09  3.34e- 5
##  3 G401S   vmax    0.360    0.0108     33.3   1.40e-11
##  4 G401S   km     54.5      9.28        5.87  1.56e- 4
##  5 L230dup vmax   -0.0232   0.00321    -7.23  2.84e- 5
##  6 L230dup km      3.51     6.97        0.504 6.25e- 1
```

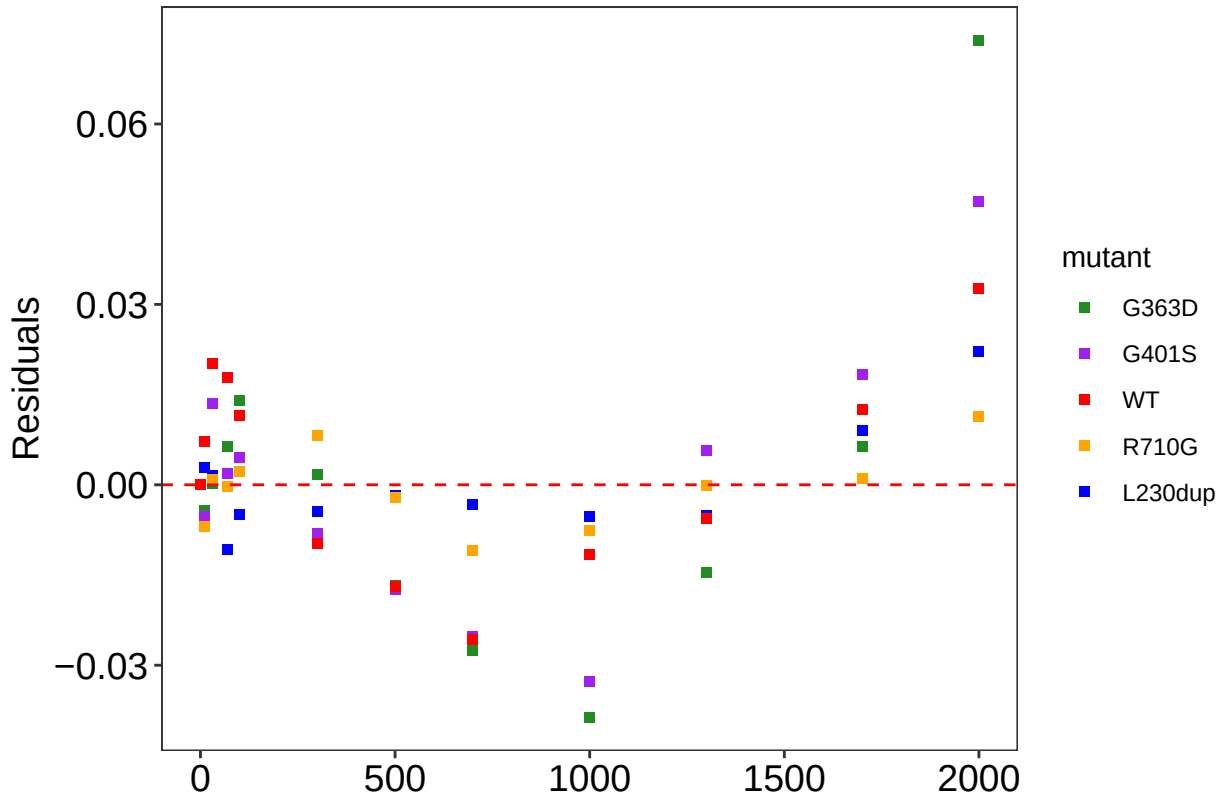
```
##  7 R710G   vmax    0.0979   0.00377    26.0   1.66e-10
##  8 R710G   km     96.3     18.6         5.17  4.18e- 4
##  9 WT      vmax    0.239    0.0146     16.3   1.54e- 8
## 10 WT      km    201.      50.7         3.97  2.66e- 3
```

```
### vmax units = µmol/min
### km units = µM
#### Does the G401S mutation shift G401S to an unfavored ramachandran angle?
#### look at crystal structure and see if this violates it
write_csv(vmax_km, "Drp1_n3_activity_KAM.csv")

### Add residual plot and resume here
vmax_km_residual <- rates %>%
  group_by(mutant) %>%
  do(augment(nlsLM(formula = activity ~ (vmax*(gtp/(gtp+km))),
                   start = list(vmax = 0.6, km = 200),
                   trace = TRUE,
                   data = .))) %>%
  ungroup()
```

```
## It.    0, RSS =    0.06633, Par. =        0.6        200
## It.    1, RSS =  0.0347267, Par. =   0.614319     161.91
## It.    2, RSS = 0.00968603, Par. =   0.600247    90.4281
## It.    3, RSS = 0.00850219, Par. =   0.581657    78.4188
## It.    4, RSS = 0.00850205, Par. =   0.581673    78.5306
## It.    5, RSS = 0.00850205, Par. =   0.581678     78.535
## It.    6, RSS = 0.00850205, Par. =   0.581678    78.5352
## It.    0, RSS =   0.167063, Par. =        0.6        200
## It.    1, RSS = 0.00736633, Par. =   0.336587    41.5904
## It.    2, RSS = 0.00492244, Par. =    0.35913    52.8343
## It.    3, RSS =  0.0049068, Par. =   0.360299    54.3984
## It.    4, RSS = 0.00490671, Par. =   0.360383    54.5196
## It.    5, RSS = 0.00490671, Par. =   0.360389    54.5276
## It.    6, RSS = 0.00490671, Par. =   0.360389    54.5281
## It.    0, RSS =     1.8792, Par. =        0.6        200
## It.    1, RSS = 0.00593761, Par. = -0.00131521     246.42
## It.    2, RSS = 0.00169771, Par. = -0.0294023    80.6677
## It.    3, RSS = 0.000886447, Par. = -0.0237628    11.5989
## It.    4, RSS = 0.000853498, Par. = -0.0239295    9.22389
## It.    5, RSS = 0.000812471, Par. = -0.0235455    4.46972
## It.    6, RSS = 0.00081081, Par. = -0.0231519    3.14919
## It.    7, RSS = 0.000810531, Par. = -0.0232438    3.60777
## It.    8, RSS = 0.000810514, Par. = -0.0232151    3.48323
## It.    9, RSS = 0.000810512, Par. = -0.0232232    3.52106
## It.   10, RSS = 0.000810512, Par. = -0.0232208    3.50988
## It.   11, RSS = 0.000810512, Par. = -0.0232215    3.51321
## It.   12, RSS = 0.000810512, Par. = -0.0232213    3.51222
## It.    0, RSS =    1.14007, Par. =        0.6        200
## It.    1, RSS = 0.00164511, Par. =  0.0946316    174.034
## It.    2, RSS = 0.000803782, Par. =  0.0958789    59.7287
## It.    3, RSS = 0.000444982, Par. =  0.0972233    88.1554
## It.    4, RSS = 0.000434541, Par. =  0.0979153    95.9725
## It.    5, RSS = 0.000434529, Par. =  0.0979363    96.2567
## It.    6, RSS = 0.000434529, Par. =   0.097936    96.2546
```

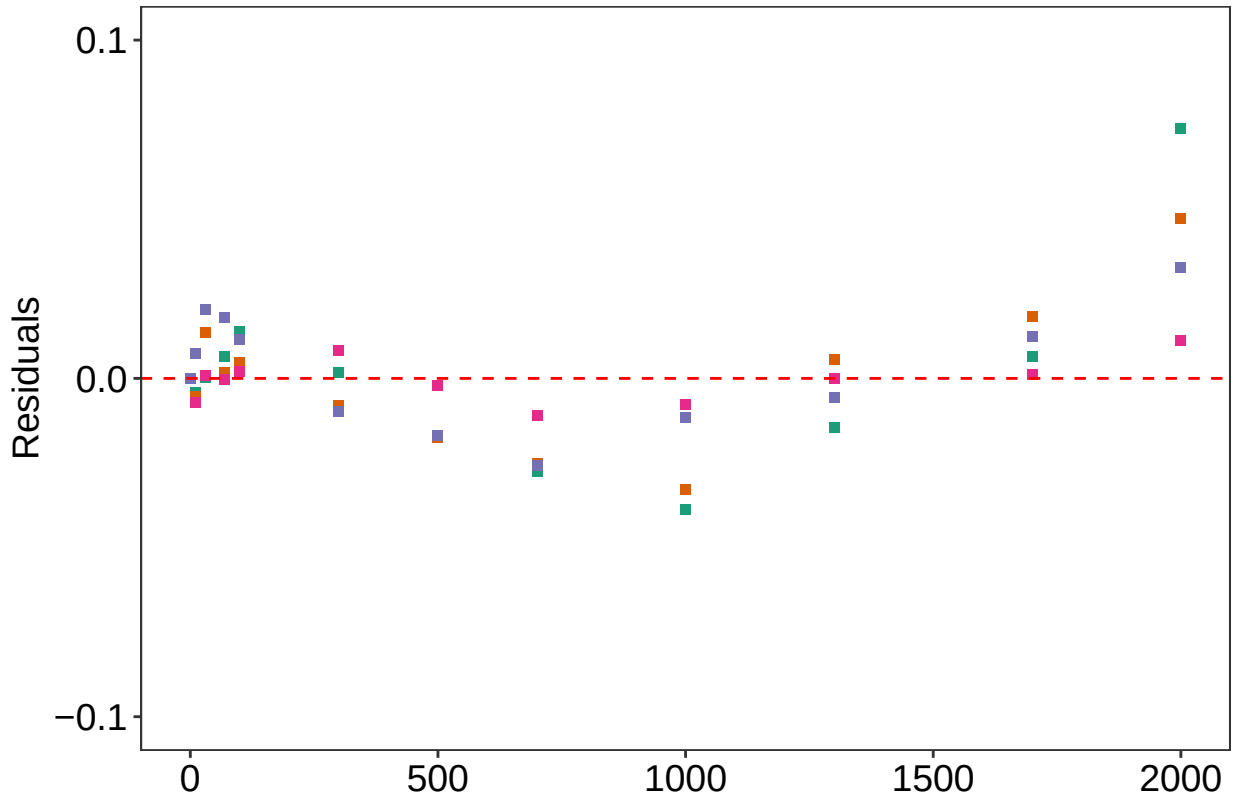
```
## It.    0, RSS =   0.622683, Par. =        0.6        200
## It.    1, RSS =  0.0033453, Par. =   0.238913    200.307
## It.    2, RSS = 0.00334519, Par. =   0.238937    200.873
## It.    3, RSS = 0.00334518, Par. =   0.238981    201.062
## It.    4, RSS = 0.00334518, Par. =   0.238996    201.125
## It.    5, RSS = 0.00334518, Par. =   0.239001    201.146
## It.    6, RSS = 0.00334518, Par. =   0.239003    201.153
```

```
  resid_plot <- vmax_km_residual %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("G363D", "G401S", "WT", "R710G", "L230dup"))) %>%
  ggplot(aes(x = gtp, y = .resid, color = mutant)) +
  geom_point(shape = 15) +
  geom_hline(yintercept = 0, linetype = 2, color = "red") +
  scale_y_continuous(breaks = c(-0.03, 0, 0.03, 0.06, 0.09)) +
  scale_color_manual(values = c("WT" = "red",
                                "L230dup" = "blue",
                                "G363D" = "forestgreen",
                                "G401S" = "purple",
                                "R710G" = "orange")) +
  labs(x = "",
       y = "Residuals") +
  theme_bw() +
  theme(axis.text = element_text(size = 14, color = "black"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        strip.background = element_blank()
  )
resid_plot
```

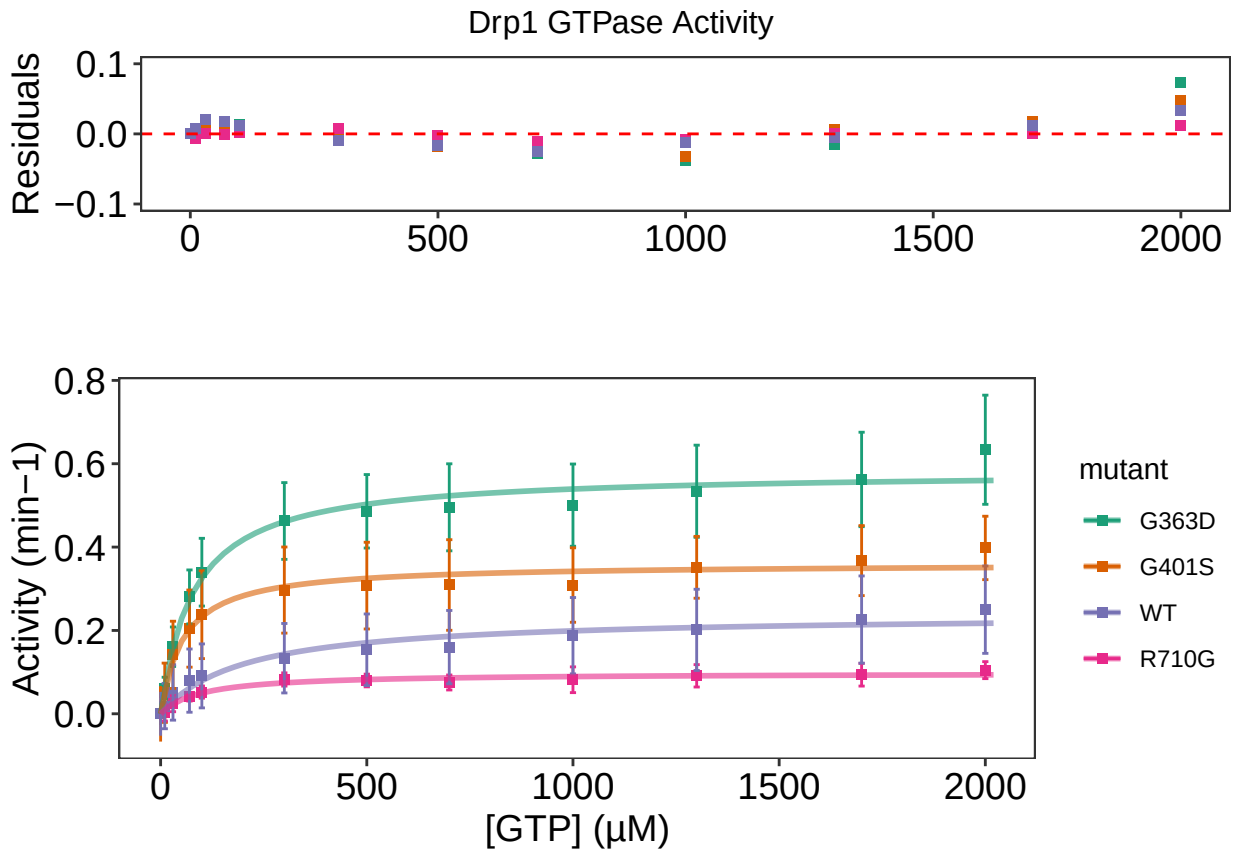



```
### Add residual plot - no L230dup due to protein being truncated
# Dark color scheme
resid_plot_noL230dup <- vmax_km_residual %>%
  filter(., mutant != "L230dup") %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("G363D", "G401S", "WT", "R710G"))) %>%
  ggplot(aes(x = gtp, y = .resid, color = mutant)) +
  geom_point(shape = 15) +
  geom_hline(yintercept = 0, linetype = 2, color = "red") +
  scale_y_continuous(limits =c(-0.1, 0.1),
                     breaks = c(-0.1, 0, 0.1)) +
  scale_color_manual(values = c("WT" = "#7570B3",
                                "G363D" = "#1B9E77",
                                "G401S" = "#D95F02",
                                "R710G" = "#E7298A")) +
  labs(x = "",
       y = "Residuals") +
  theme_bw() +
  theme(axis.text = element_text(size = 14, color = "black"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        strip.background = element_blank(),
        legend.position = "none",
```

```
  )
resid_plot_noL230dup
```




```
# Combine residual plot with activity plot
### generate a layout matrix to plot residuals at 1/3 size of regression plot

lay <- cbind(c(1, 2, 2))

final_plot <- grid.arrange(grobs = list(resid_plot_noL230dup, kinetic_plot2_noL230dup_dark2),
                           layout_matrix = lay,
                           top = "Drp1 GTPase Activity")
```




```
ggsave("Drp1_n3_activity_final_plot__noL230dup_KAM_v2.pdf", final_plot,
       width = 14, height = 10, units = "cm", dpi = 300)

# L230dup appears to be inactive or enzymaticaly dead - makes sense given
# MS confirmed protein to be truncated in GTPase domain
# G363D appears hyperactive
# R710G has decreased Vmax

# Determine if difference between mutants and WT activity is significant

pooled <-  pool %>%
  mutate(., activity = (-a340_slope / (6220 * 0.4649 / 1e6) / 1)) %>%
  group_by(., mutant, gtp, rep) %>%
  summarise(., avg_activity = mean(activity),
            stdev = sd(activity)) %>%
  group_by(mutant, rep) %>%
  mutate(., activity = avg_activity - avg_activity[gtp == 0]) %>%
  ungroup()
```

```
## `summarise()` has grouped output by 'mutant', 'gtp'. You can override using the `.groups` argument.
```

```
pooled
```

```
## # A tibble: 180 x 6
##    mutant   gtp rep   avg_activity    stdev activity
```

```
##    <chr>  <dbl> <chr>        <dbl>    <dbl>    <dbl>
##  1 G363D      0 a            0.238 0.0231     0
##  2 G363D      0 b            0.266 0.00316    0
##  3 G363D      0 c            0.294 0.00277    0
##  4 G363D     10 a            0.296 0.000744   0.0582
##  5 G363D     10 b            0.332 0.00758    0.0663
##  6 G363D     10 c            0.354 0.000539   0.0599
##  7 G363D     30 a            0.369 0.00847    0.130
##  8 G363D     30 b            0.453 0.00910    0.187
##  9 G363D     30 c            0.460 0.0273     0.166
## 10 G363D     70 a            0.464 0.00814    0.226
## # ... with 170 more rows
```

```
# calculate vmax and km for each replicate and then do ANOVA between mutants
vmax_km_reps <- pooled %>%
  group_by(., mutant, rep) %>%
  do(tidy(nlsLM(formula = activity ~ (vmax*(gtp/(gtp+km))),
                start = list(vmax = 0.6, km = 200),
                trace = TRUE,
                data = .))) %>%
  spread(key = term, value = estimate) %>%
  ungroup()
```

```
## It.    0, RSS =  0.0486858, Par. =        0.6        200
## It.    1, RSS =  0.0182182, Par. =   0.437971    36.6901
## It.    2, RSS = 0.00505766, Par. =   0.455104    62.7946
## It.    3, RSS = 0.00412137, Par. =   0.461837    74.0866
## It.    4, RSS = 0.00411141, Par. =   0.462607    75.4468
## It.    5, RSS =  0.0041114, Par. =   0.462641     75.495
## It.    6, RSS =  0.0041114, Par. =   0.462642    75.4963
## It.    0, RSS =   0.131583, Par. =        0.6        200
## It.    1, RSS =   0.103675, Par. =    0.60953    183.686
## It.    2, RSS =  0.0582827, Par. =   0.627313    152.915
## It.    3, RSS =  0.0120307, Par. =   0.643177    86.6515
## It.    4, RSS =  0.0111224, Par. =   0.629554    75.8267
## It.    5, RSS =  0.0111222, Par. =   0.629305    75.7519
## It.    6, RSS =  0.0111222, Par. =   0.629301    75.7475
## It.    7, RSS =  0.0111222, Par. =     0.6293    75.7472
## It.    0, RSS =   0.153078, Par. =        0.6        200
## It.    1, RSS =   0.108755, Par. =   0.614237    176.948
## It.    2, RSS =  0.0434767, Par. =   0.640614    135.023
## It.    3, RSS =  0.0158586, Par. =   0.647404    68.0865
## It.    4, RSS =  0.0128881, Par. =   0.651754    81.5877
## It.    5, RSS =  0.0128553, Par. =   0.653081    83.4951
## It.    6, RSS =  0.0128553, Par. =   0.653151    83.5713
## It.    7, RSS =  0.0128553, Par. =   0.653154    83.5735
## It.    0, RSS =   0.240055, Par. =        0.6        200
## It.    1, RSS =  0.0102026, Par. =   0.306777    63.0258
## It.    2, RSS = 0.00616063, Par. =   0.324095    52.5986
## It.    3, RSS =  0.0061572, Par. =   0.323614    52.8614
## It.    4, RSS =  0.0061572, Par. =   0.323633    52.8907
## It.    5, RSS =  0.0061572, Par. =   0.323635    52.8938
## It.    0, RSS =   0.111987, Par. =        0.6        200
## It.    1, RSS =  0.0854969, Par. =   0.568543    199.662
```

```
## It.    2, RSS =  0.0513612, Par. =   0.511392    160.218
## It.    3, RSS = 0.00560728, Par. =    0.42362    41.5573
## It.    4, RSS = 0.00343431, Par. =    0.40759    42.5923
## It.    5, RSS = 0.00343429, Par. =   0.407531    42.5796
## It.    6, RSS = 0.00343429, Par. =   0.407532    42.5805
## It.    0, RSS =   0.128447, Par. =        0.6        200
## It.    1, RSS =   0.011303, Par. =   0.386585     91.525
## It.    2, RSS =  0.0100259, Par. =   0.392742    80.5274
## It.    3, RSS =  0.0100236, Par. =   0.391973    79.8364
## It.    4, RSS =  0.0100236, Par. =   0.391909    79.7372
## It.    5, RSS =  0.0100236, Par. =     0.3919    79.7227
## It.    6, RSS =  0.0100236, Par. =   0.391899    79.7205
## It.    0, RSS =    1.90232, Par. =        0.6        200
## It.    1, RSS =  0.0016023, Par. = -0.0201155     221.73
## It.    2, RSS = 0.000391597, Par. = -0.0303477    50.7763
## It.    3, RSS = 0.000335459, Par. = -0.0262054    23.1037
## It.    4, RSS = 0.000324476, Par. = -0.0269455      32.96
## It.    5, RSS = 0.00032425, Par. =  -0.026741    31.0905
## It.    6, RSS = 0.000324225, Par. = -0.0267884    31.7195
## It.    7, RSS = 0.000324222, Par. = -0.0267728    31.5184
## It.    8, RSS = 0.000324222, Par. = -0.0267778    31.5841
## It.    9, RSS = 0.000324222, Par. = -0.0267762    31.5628
## It.   10, RSS = 0.000324222, Par. = -0.0267767    31.5697
## It.    0, RSS =    1.94845, Par. =        0.6        200
## It.    1, RSS = 0.00612314, Par. =  -0.014258     243.88
## It.    2, RSS = 0.00191967, Par. = -0.0392797    74.0818
## It.    3, RSS = 0.00152814, Par. = -0.0342205    40.1629
## It.    4, RSS =  0.0014486, Par. = -0.0339998    33.1667
## It.    5, RSS = 0.00127988, Par. = -0.0328675    19.2948
## It.    6, RSS = 0.00116613, Par. = -0.0303677  -0.174056
## It.    7, RSS = 0.00110772, Par. = -0.0298617    1.76499
## It.    8, RSS = 0.00110324, Par. = -0.0300754    2.67573
## It.    9, RSS = 0.00110291, Par. = -0.0301505    2.95068
## It.   10, RSS =  0.0011029, Par. = -0.0301706    3.01979
## It.   11, RSS = 0.00110289, Par. = -0.0301755    3.03622
## It.   12, RSS = 0.00110289, Par. = -0.0301767    3.04006
## It.   13, RSS = 0.00110289, Par. = -0.0301769    3.04096
## It.    0, RSS =    1.83453, Par. =        0.6        200
## It.    1, RSS = 0.00942399, Par. =  0.0145564    260.034
## It.    2, RSS = 0.00583364, Par. = -0.000854959    1875.41
## It.    3, RSS = 0.00438277, Par. = -0.0315246    260.296
## It.    4, RSS =  0.0037569, Par. = -0.0197322    195.354
## It.    5, RSS =   0.002951, Par. = -0.0184746    61.4886
## It.    6, RSS = 0.00264665, Par. =  -0.020051    40.8258
## It.    7, RSS = 0.00183847, Par. =  -0.018925  -0.807377
## It.    8, RSS =  0.0018368, Par. = -0.0192024  0.0248593
## It.    9, RSS = 0.00183659, Par. = -0.0190719  -0.371513
## It.   10, RSS =  0.0018365, Par. = -0.0191368  -0.151941
## It.   11, RSS = 0.00183648, Par. = -0.0191014  -0.266784
## It.   12, RSS = 0.00183647, Par. = -0.0191201   -0.20453
## It.   13, RSS = 0.00183647, Par. =   -0.01911  -0.237687
## It.   14, RSS = 0.00183647, Par. = -0.0191154  -0.219851
## It.   15, RSS = 0.00183647, Par. = -0.0191125  -0.229403
## It.   16, RSS = 0.00183647, Par. = -0.0191141  -0.224261
```

```
## It.   17, RSS = 0.00183647, Par. = -0.0191132  -0.227024
## It.    0, RSS =    1.04179, Par. =        0.6        200
## It.    1, RSS = 0.00511227, Par. =   0.100667    147.137
## It.    2, RSS = 0.000725597, Par. =   0.118533      92.96
## It.    3, RSS = 0.000399688, Par. =   0.110232    49.7269
## It.    4, RSS = 0.000354116, Par. =   0.111328    57.7918
## It.    5, RSS = 0.000353974, Par. =   0.111377    58.2896
## It.    6, RSS = 0.000353974, Par. =   0.111375    58.2815
## It.    7, RSS = 0.000353974, Par. =   0.111375    58.2817
## It.    0, RSS =     1.2253, Par. =        0.6        200
## It.    1, RSS = 0.00153739, Par. =  0.0849249    187.858
## It.    2, RSS = 0.00127532, Par. =   0.085177    120.022
## It.    3, RSS = 0.00126173, Par. =  0.0858739    133.612
## It.    4, RSS = 0.00126172, Par. =  0.0858604    133.806
## It.    5, RSS = 0.00126172, Par. =  0.0858596    133.798
## It.    0, RSS =    1.15885, Par. =        0.6        200
## It.    1, RSS = 0.000829488, Par. =   0.098303    187.106
## It.    2, RSS = 0.000538191, Par. =  0.0985737    126.779
## It.    3, RSS = 0.000530858, Par. =   0.098857    134.796
## It.    4, RSS = 0.000530819, Par. =  0.0989364    135.577
## It.    5, RSS = 0.000530819, Par. =  0.0989419    135.623
## It.    6, RSS = 0.000530819, Par. =  0.0989422    135.626
## It.    0, RSS =   0.969199, Par. =        0.6        200
## It.    1, RSS = 0.00200666, Par. =   0.154416    209.915
## It.    2, RSS = 0.00184114, Par. =   0.155162    244.487
## It.    3, RSS = 0.00182637, Par. =   0.157468    262.458
## It.    4, RSS = 0.00182402, Par. =   0.158505    270.156
## It.    5, RSS = 0.00182367, Par. =   0.158919     273.22
## It.    6, RSS = 0.00182362, Par. =   0.159079    274.403
## It.    7, RSS = 0.00182361, Par. =    0.15914    274.854
## It.    8, RSS = 0.00182361, Par. =   0.159163    275.026
## It.    9, RSS = 0.00182361, Par. =   0.159172    275.091
## It.   10, RSS = 0.00182361, Par. =   0.159175    275.115
## It.    0, RSS =   0.379215, Par. =        0.6        200
## It.    1, RSS = 0.00628758, Par. =   0.303954    173.403
## It.    2, RSS = 0.00571847, Par. =    0.30265    146.551
## It.    3, RSS = 0.00570494, Par. =   0.300639    141.854
## It.    4, RSS = 0.00570402, Par. =   0.300201     140.63
## It.    5, RSS = 0.00570395, Par. =   0.300083    140.298
## It.    6, RSS = 0.00570394, Par. =    0.30005    140.206
## It.    7, RSS = 0.00570394, Par. =   0.300041    140.181
## It.    8, RSS = 0.00570394, Par. =   0.300038    140.174
## It.    0, RSS =   0.451802, Par. =        0.6        200
## It.    1, RSS = 0.00454734, Par. =   0.300615    212.797
## It.    2, RSS = 0.00439775, Par. =   0.302119    231.454
## It.    3, RSS = 0.00438731, Par. =   0.304067     238.81
## It.    4, RSS = 0.00438616, Par. =    0.30476    241.341
## It.    5, RSS = 0.00438603, Par. =    0.30499    242.176
## It.    6, RSS = 0.00438602, Par. =   0.305065    242.448
## It.    7, RSS = 0.00438602, Par. =   0.305089    242.536
## It.    8, RSS = 0.00438602, Par. =   0.305097    242.564
## It.    9, RSS = 0.00438602, Par. =   0.305099    242.573
```

```
vmax_km_reps # results are from a global fit of n = 3 data
```

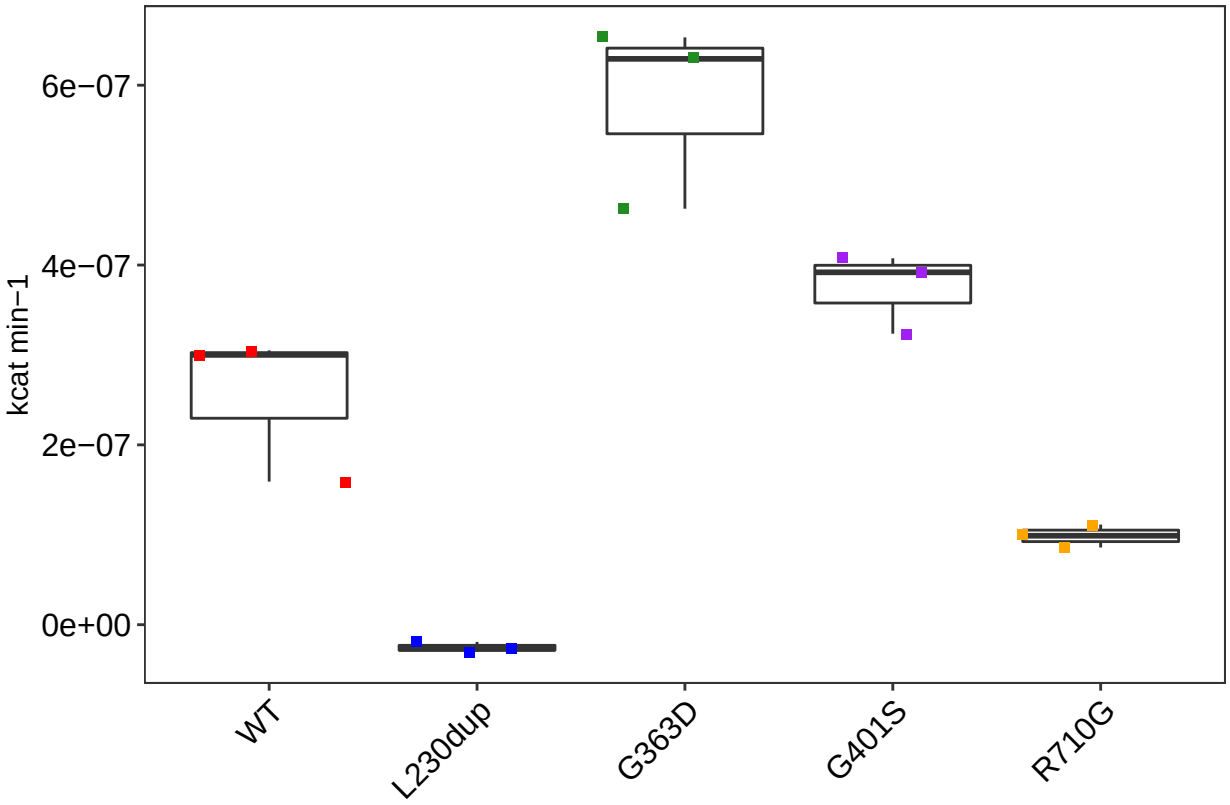
```
## # A tibble: 30 x 7
##    mutant rep   std.error statistic  p.value    km   vmax
##    <chr>  <chr>     <dbl>     <dbl>    <dbl> <dbl>  <dbl>
##  1 G363D  a       0.0108      43.0  1.10e-12  NA    0.463
##  2 G363D  a       9.28         8.13 1.02e- 5  75.5 NA
##  3 G363D  b       0.0177      35.6  7.36e-12  NA    0.629
##  4 G363D  b      11.3          6.72 5.20e- 5  75.7 NA
##  5 G363D  c       0.0196      33.3  1.39e-11  NA    0.653
##  6 G363D  c      13.0          6.44 7.45e- 5  83.6 NA
##  7 G401S  a       0.0120      26.9  1.17e-10  NA    0.324
##  8 G401S  a      11.2          4.71 8.28e- 4  52.9 NA
##  9 G401S  b       0.00860     47.4  4.22e-13  NA    0.408
## 10 G401S  b       5.38         7.91 1.29e- 5  42.6 NA
## # ... with 20 more rows
```

```
# calculate kcat and kcat/K0.5
kcat_reps = subset(vmax_km_reps, select = -c(std.error, statistic, p.value)) %>%
  group_by(., mutant, rep) %>%
  summarise_all(na.omit) %>%
  mutate(kcat = vmax/1000000) %>%
  mutate(kcatkm = kcat/km) %>%
  ungroup()

kcat_reps # kcat and kcat/km values calculated from
```

```
## # A tibble: 15 x 6
##    mutant  rep        km    vmax          kcat    kcatkm
##    <chr>   <chr>   <dbl>   <dbl>         <dbl>     <dbl>
##  1 G363D   a      75.5    0.463   0.000000463   6.13e- 9
##  2 G363D   b      75.7    0.629   0.000000629   8.31e- 9
##  3 G363D   c      83.6    0.653   0.000000653   7.82e- 9
##  4 G401S   a      52.9    0.324   0.000000324   6.12e- 9
##  5 G401S   b      42.6    0.408   0.000000408   9.57e- 9
##  6 G401S   c      79.7    0.392   0.000000392   4.92e- 9
##  7 L230dup a      31.6   -0.0268 -0.0000000268 -8.48e-10
##  8 L230dup b       3.04  -0.0302 -0.0000000302 -9.92e- 9
##  9 L230dup c      -0.227 -0.0191 -0.0000000191  8.42e- 8
## 10 R710G   a      58.3    0.111   0.000000111   1.91e- 9
## 11 R710G   b     134.     0.0859  0.0000000859  6.42e-10
## 12 R710G   c     136.     0.0989  0.0000000989  7.30e-10
## 13 WT      a     275.     0.159   0.000000159   5.79e-10
## 14 WT      b     140.     0.300   0.000000300   2.14e- 9
## 15 WT      c     243.     0.305   0.000000305   1.26e- 9
```

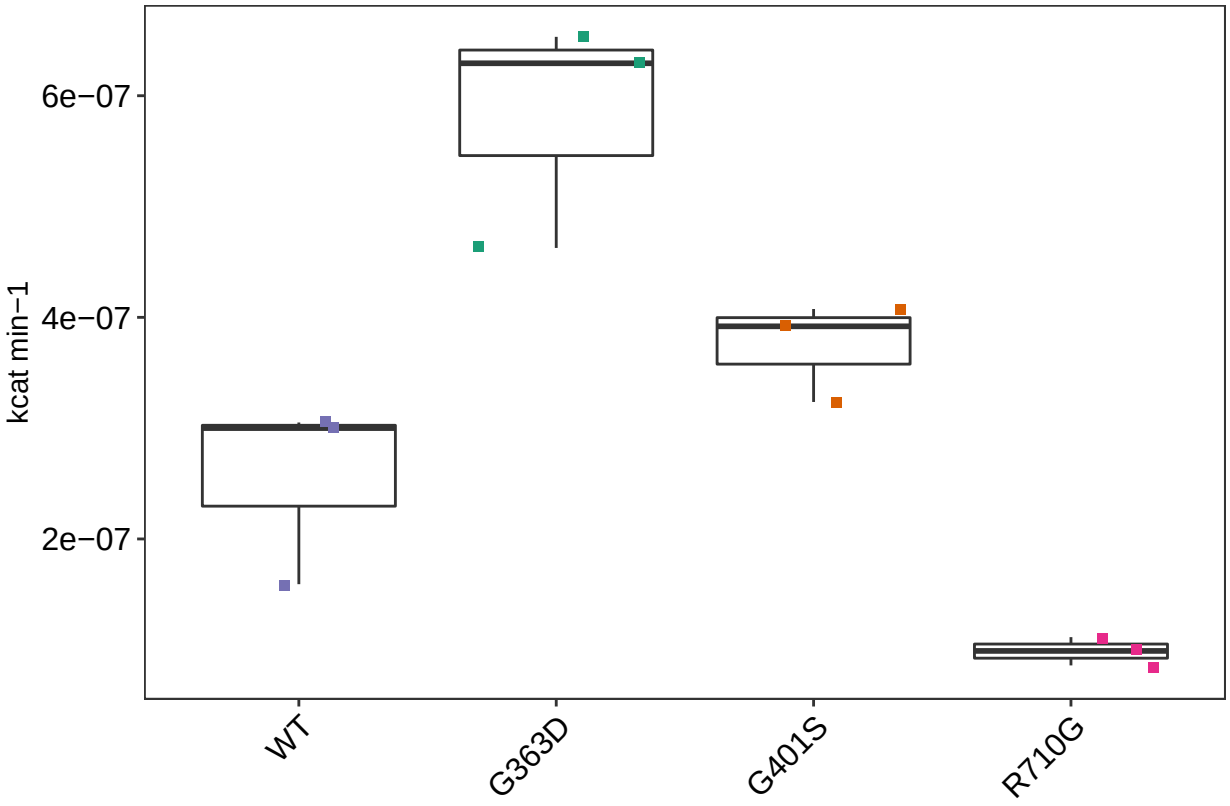
```
          # vmax and km values in vmax_km_reps

# Plot of kcat values
kcat_reps %>%
  mutate(mutant =
           factor(mutant,
```

```
                levels = c("WT", "L230dup", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = kcat)) +
  geom_boxplot() +
  geom_jitter(aes(y = kcat, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "red",
                                "L230dup" = "blue",
                                "G363D" = "forestgreen",
                                "G401S" = "purple",
                                "R710G" = "orange")) +
  labs(x = "",
       y = "kcat min-1") +
    theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```



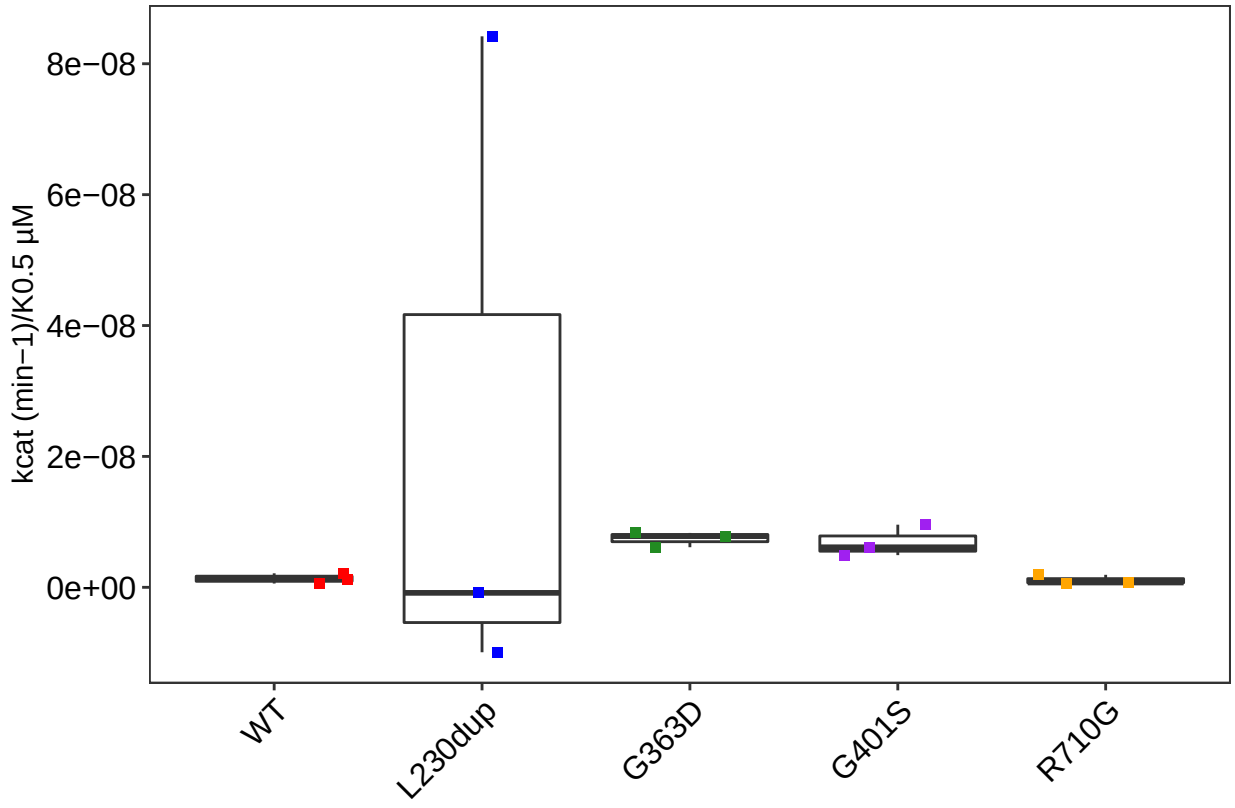

```
ggsave("Drp1_kcat_boxplot_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)

# Plot of kcat values - No L230dup due to truncation
kcat_reps %>%
  filter(., mutant != "L230dup") %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("WT", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = kcat)) +
  geom_boxplot() +
```

```
  geom_jitter(aes(y = kcat, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "#7570B3",
                                "G363D" = "#1B9E77",
                                "G401S" = "#D95F02",
                                "R710G" = "#E7298A")) +
  labs(x = "",
       y = "kcat min-1") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```



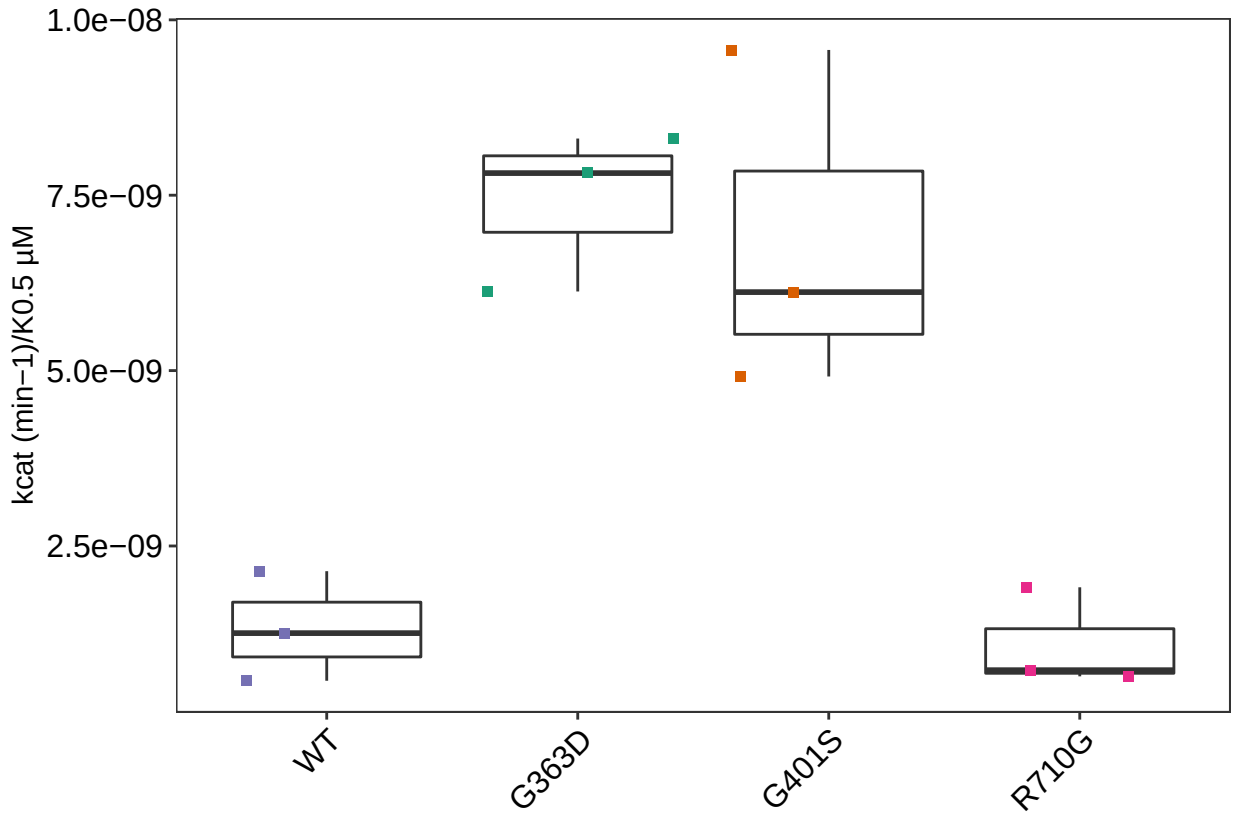

```
ggsave("Drp1_kcat_boxplot_noL230dup_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)


# Plot of kcat/K0.5 values
kcat_reps %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("WT", "L230dup", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = kcatkm)) +
  geom_boxplot() +
  geom_jitter(aes(y = kcatkm, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "red",
                                "L230dup" = "blue",
                                "G363D" = "forestgreen",
```

```
                                "G401S" = "purple",
                                "R710G" = "orange")) +
  labs(x = "",
       y = "kcat (min-1)/K0.5 µM") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```




```
ggsave("Drp1_kcatkm_boxplot_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)

# Plot of kcat/K0.5 values - No L230dup due to protein being truncated
kcat_reps %>%
  filter(., mutant != "L230dup") %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("WT", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = kcatkm)) +
  geom_boxplot() +
  geom_jitter(aes(y = kcatkm, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "#7570B3",
                                "G363D" = "#1B9E77",
                                "G401S" = "#D95F02",
                                "R710G" = "#E7298A")) +
  labs(x = "",
       y = "kcat (min-1)/K0.5 µM") +
```

```
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```




```
ggsave("Drp1_kcatkm_boxplot_noL230dup_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)

# ANOVA on Kcat + post-hoc Tukey
anova_kcat <- kcat_reps %>%
  do(tidy(aov(kcat ~ mutant, data = .)))

anova_kcat %>% print(width = Inf)
```

```
## # A tibble: 2 x 6
##   term         df    sumsq   meansq statistic     p.value
##   <chr>     <dbl>    <dbl>    <dbl>     <dbl>       <dbl>
## 1 mutant        4 6.72e-13 1.68e-13      42.4  0.00000307
## 2 Residuals    10 3.96e-14 3.96e-15      NA   NA
```

```
anova_kcat_tukey <- kcat_reps %>%
  do(tidy(TukeyHSD(aov(kcat ~ mutant, data = .))))

# ANOVA on Kcat/Km + post-hoc Tukey
anova_kcatkm <- kcat_reps %>%
  do(tidy(aov(kcatkm ~ mutant, data = .)))
```

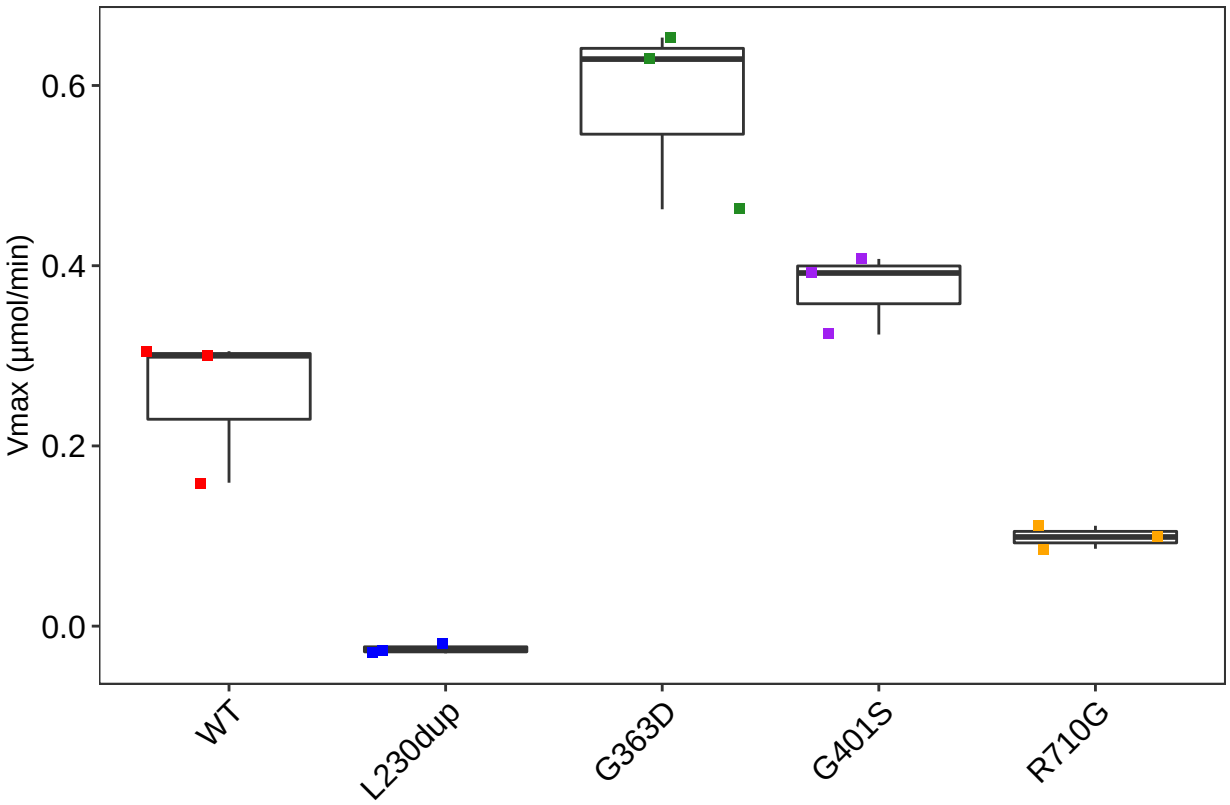
```r
anova_kcatkm %>% print(width = Inf)
```

```
## # A tibble: 2 x 6
##   term         df    sumsq   meansq statistic p.value
##   <chr>     <dbl>    <dbl>    <dbl>     <dbl>   <dbl>
## 1 mutant        4 1.09e-15 2.74e-16     0.506   0.733
## 2 Residuals    10 5.41e-15 5.41e-16    NA      NA
```

```r
anova_kcatkm_tukey <- kcat_reps %>%
  do(tidy(TukeyHSD(aov(kcatkm ~ mutant, data = .))))

# Determine if there any outliers in Vmax values via boxplot
vmax_km_reps %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("WT", "L230dup", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = vmax)) +
  geom_boxplot() +
  geom_jitter(aes(y = vmax, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "red",
                                "L230dup" = "blue",
                                "G363D" = "forestgreen",
                                "G401S" = "purple",
                                "R710G" = "orange")) +
  labs(x = "",
       y = "Vmax (µmol/min)") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```

```
## Warning: Removed 15 rows containing non-finite values (stat_boxplot).
```

```
## Warning: Removed 15 rows containing missing values (geom_point).
```



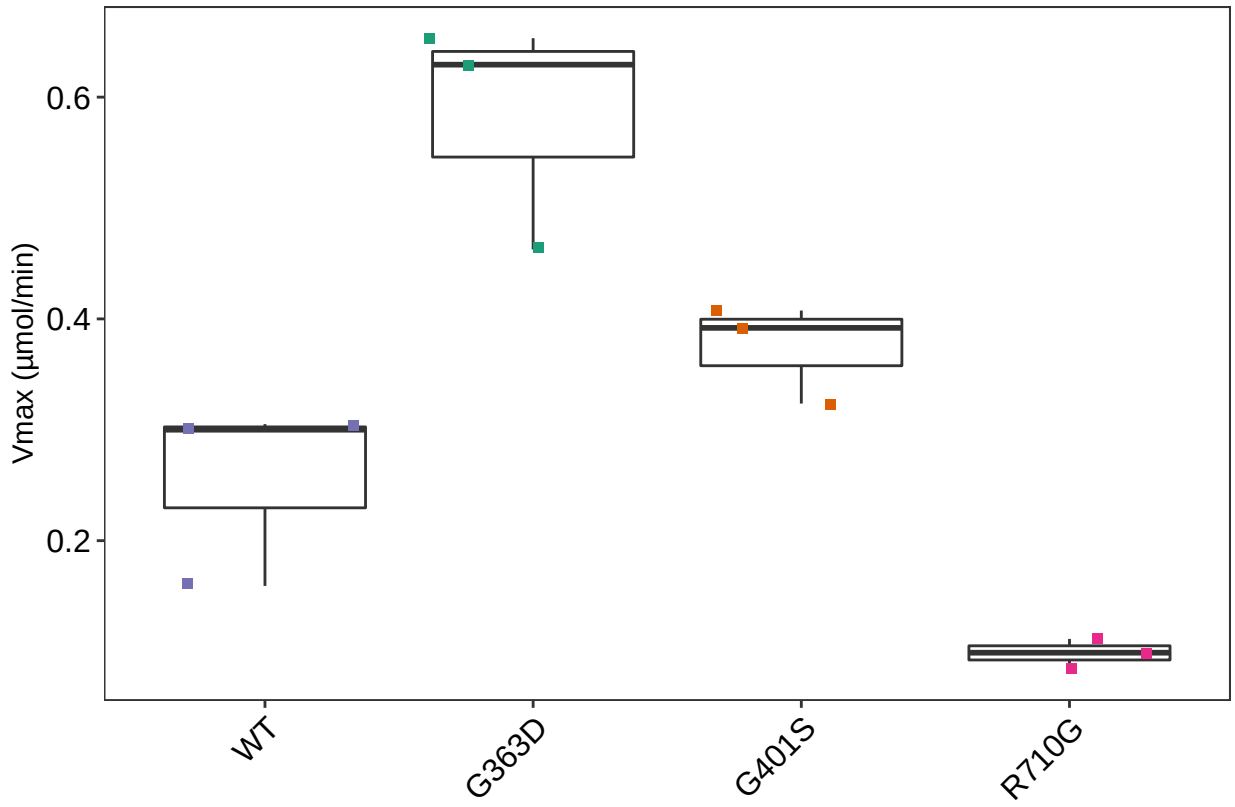

```
ggsave("Drp1_vmax_boxplot_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)
```

```
## Warning: Removed 15 rows containing non-finite values (stat_boxplot).
```

```
## Warning: Removed 15 rows containing missing values (geom_point).
```

```
# Determine if there any outliers in Vmax values via boxplot
# No L230dup due to protein being truncated
vmax_km_reps %>%
  filter(., mutant != "L230dup") %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("WT", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = vmax)) +
  geom_boxplot() +
  geom_jitter(aes(y = vmax, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "#7570B3",
                                "G363D" = "#1B9E77",
                                "G401S" = "#D95F02",
                                "R710G" = "#E7298A")) +
  labs(x = "",
       y = "Vmax (µmol/min)") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```

```
## Warning: Removed 12 rows containing non-finite values (stat_boxplot).

## Warning: Removed 12 rows containing missing values (geom_point).
```




```
ggsave("Drp1_vmax_boxplot_noL230dup_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)
```

```
## Warning: Removed 12 rows containing non-finite values (stat_boxplot).

## Warning: Removed 12 rows containing missing values (geom_point).
```

```
# ANOVA + post-hoc Tukey on Vmax
anova_vmax <- vmax_km_reps %>%
  do(tidy(aov(vmax ~ mutant, data = .)))

anova_vmax %>% print(width = Inf)
```

```
## # A tibble: 2 x 6
##   term         df  sumsq  meansq statistic     p.value
##   <chr>     <dbl>  <dbl>   <dbl>     <dbl>       <dbl>
## 1 mutant        4 0.672  0.168        42.4  0.00000307
## 2 Residuals    10 0.0396 0.00396      NA   NA
```

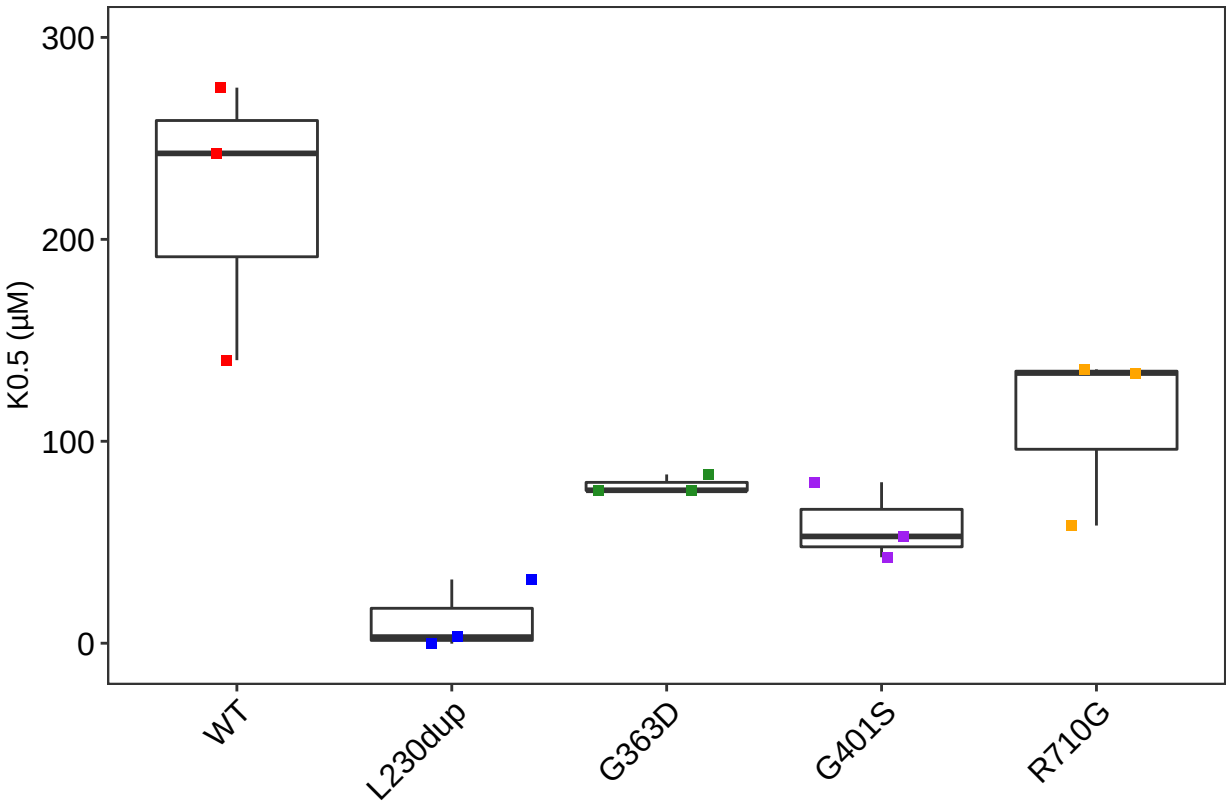
```r
anova_vmax_tukey <- vmax_km_reps %>%
  do(tidy(TukeyHSD(aov(vmax ~ mutant, data = .))))

# significance results of Vmax values between mutants
anova_vmax_tukey %>% print(width = Inf)
```

```
## # A tibble: 10 x 7
##    term   contrast      null.value estimate conf.low conf.high adj.p.value
##    <chr>  <chr>              <dbl>    <dbl>    <dbl>     <dbl>       <dbl>
##  1 mutant G401S-G363D            0   -0.207  -0.377    -0.0382  0.0159
##  2 mutant L230dup-G363D          0   -0.607  -0.776    -0.438   0.00000264
##  3 mutant R710G-G363D            0   -0.483  -0.652    -0.314   0.0000216
##  4 mutant WT-G363D               0   -0.327  -0.496    -0.158   0.000609
##  5 mutant L230dup-G401S          0   -0.400  -0.569    -0.231   0.000114
##  6 mutant R710G-G401S            0   -0.276  -0.445    -0.106   0.00228
##  7 mutant WT-G401S               0   -0.120  -0.289     0.0496  0.214
##  8 mutant R710G-L230dup          0    0.124  -0.0451    0.293   0.189
##  9 mutant WT-L230dup             0    0.280   0.111     0.449   0.00202
## 10 mutant WT-R710G               0    0.156  -0.0131    0.325   0.0742
```

```r
write_csv(anova_vmax_tukey, "anova_tukey_VMAX_results_KAM.csv")

# Determine if there any outliers in Km values via boxplot
vmax_km_reps %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("WT", "L230dup", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = km)) +
  geom_boxplot() +
  geom_jitter(aes(y = km, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "red",
                                "L230dup" = "blue",
                                "G363D" = "forestgreen",
                                "G401S" = "purple",
                                "R710G" = "orange")) +
  scale_y_continuous(limits = c(-5, 300),
                     breaks = c(0, 100, 200, 300)) +
  labs(x = "",
       y = "K0.5 (µM)") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```

```
## Warning: Removed 15 rows containing non-finite values (stat_boxplot).
```

```
## Warning: Removed 15 rows containing missing values (geom_point).
```




```
ggsave("Drp1_km_boxplot_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)
```

```
## Warning: Removed 15 rows containing non-finite values (stat_boxplot).
```

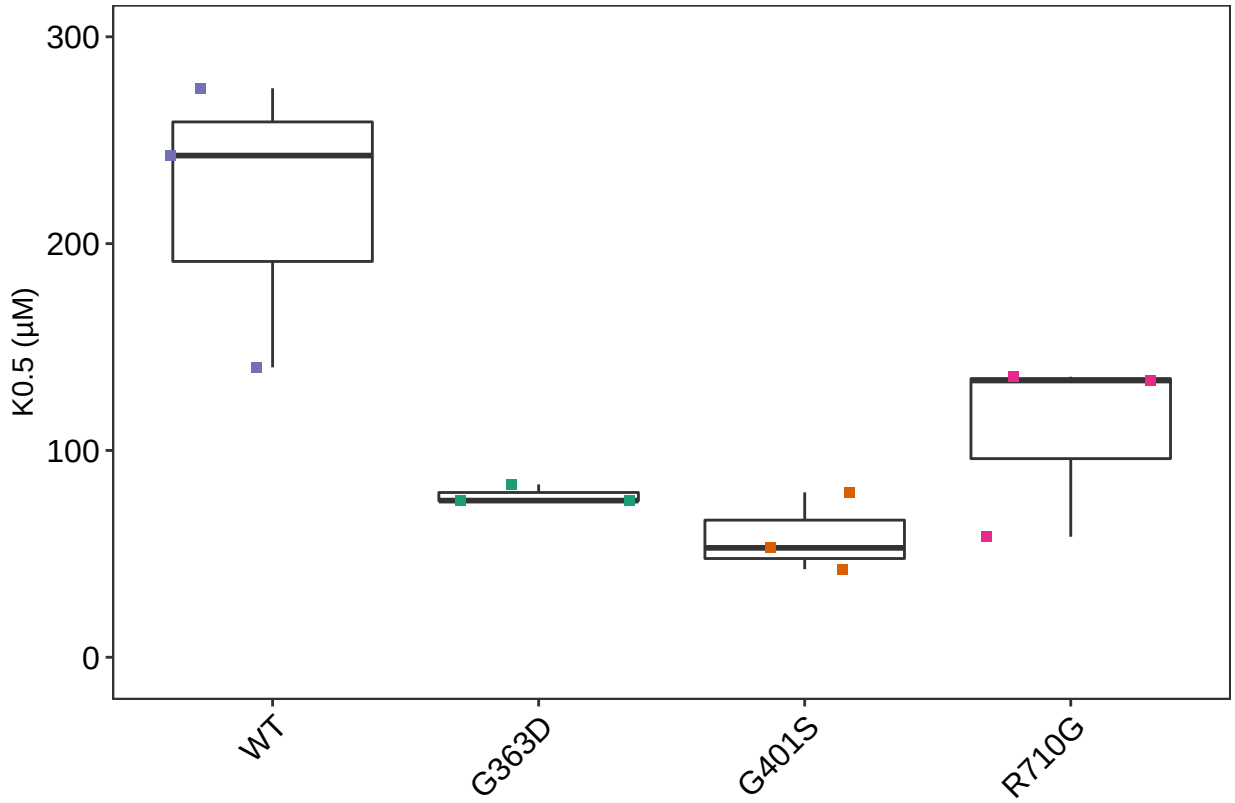
```
## Warning: Removed 15 rows containing missing values (geom_point).
```

```
# Determine if there any outliers in Km values via boxplot
# No L230dup due to protein being truncated
vmax_km_reps %>%
  filter(., mutant != "L230dup") %>%
  mutate(mutant =
           factor(mutant,
                  levels = c("WT", "G363D", "G401S", "R710G"))) %>%
  ggplot(aes(x = mutant, y = km)) +
  geom_boxplot() +
  geom_jitter(aes(y = km, color = mutant), size = 1.5, shape = 15) +
  scale_color_manual(values = c("WT" = "#7570B3",
                                "G363D" = "#1B9E77",
                                "G401S" = "#D95F02",
                                "R710G" = "#E7298A")) +
  scale_y_continuous(limits = c(-5, 300),
                     breaks = c(0, 100, 200, 300)) +
  labs(x = "",
       y = "K0.5 (µM)") +
```

```
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust = 1),
        legend.position = "none")
```

```
## Warning: Removed 12 rows containing non-finite values (stat_boxplot).
```

```
## Warning: Removed 12 rows containing missing values (geom_point).
```




```
ggsave("Drp1_km_boxplot_noL230dup_KAM.pdf",
       width = 8, height = 8, units = "cm", dpi = 300)
```

```
## Warning: Removed 12 rows containing non-finite values (stat_boxplot).
```

```
## Warning: Removed 12 rows containing missing values (geom_point).
```

```
# ANOVA + post-hoc Tukey on Km
anova_km <- vmax_km_reps %>%
  do(tidy(aov(km ~ mutant, data = .)))

anova_km %>% print(width = Inf)
```

```
## # A tibble: 2 x 6
##   term         df  sumsq meansq statistic   p.value
##   <chr>     <dbl>  <dbl>  <dbl>     <dbl>     <dbl>
## 1 mutant        4 72743. 18186.      12.0  0.000793
## 2 Residuals    10 15203.  1520.      NA   NA
```

```r
anova_km_tukey <- vmax_km_reps %>%
  do(tidy(TukeyHSD(aov(km ~ mutant, data = .))))

# significance results of Km values between mutants
anova_km_tukey %>% print(width = Inf)
```

```
## # A tibble: 10 x 7
##    term   contrast      null.value estimate conf.low conf.high adj.p.value
##    <chr>  <chr>              <dbl>    <dbl>    <dbl>     <dbl>       <dbl>
##  1 mutant G401S-G363D            0    -19.9  -125.        84.9    0.968
##  2 mutant L230dup-G363D          0    -66.8  -172.        38.0    0.292
##  3 mutant R710G-G363D            0     31.0   -73.8      136.     0.861
##  4 mutant WT-G363D               0    141.     36.2      246.     0.00873
##  5 mutant L230dup-G401S          0    -46.9  -152.        57.8    0.599
##  6 mutant R710G-G401S            0     50.8   -53.9      156.     0.531
##  7 mutant WT-G401S               0    161.     56.1      266.     0.00352
##  8 mutant R710G-L230dup          0     97.8    -7.00     203.     0.0703
##  9 mutant WT-L230dup             0    208.    103.       313.     0.000494
## 10 mutant WT-R710G               0    110.      5.28     215.     0.0387
```

```r
write_csv(anova_km_tukey, "anova_tukey_Km_results_KAM.csv")

# Perform ANOVA + post-hoc Tukey on activity
# between GTP concentrations and mutant type
activity_aov <- pooled %>%
  group_by(gtp) %>%
  do(tidy(aov(activity ~ mutant, data = .)))

activity_aov
```

```
## # A tibble: 24 x 7
## # Groups:   gtp [12]
##      gtp term         df   sumsq   meansq statistic      p.value
##    <dbl> <chr>     <dbl>   <dbl>    <dbl>     <dbl>        <dbl>
##  1     0 mutant        4 0       0            NaN   NaN
##  2     0 Residuals    10 0       0             NA    NA
##  3    10 mutant        4 0.0124  0.00309       25.6   0.0000311
##  4    10 Residuals    10 0.00121 0.000121      NA    NA
##  5    30 mutant        4 0.0704  0.0176        45.5   0.00000221
##  6    30 Residuals    10 0.00387 0.000387      NA    NA
##  7    70 mutant        4 0.192   0.0480        44.4   0.00000247
##  8    70 Residuals    10 0.0108  0.00108       NA    NA
##  9   100 mutant        4 0.266   0.0665        37.0   0.00000578
## 10   100 Residuals    10 0.0180  0.00180       NA    NA
## # ... with 14 more rows
```

```r
activity_aov_tukey <- pooled %>%
  group_by(gtp) %>%
  do(tidy(TukeyHSD(aov(activity ~ mutant, data = .))))
activity_aov_tukey
```

```
## # A tibble: 120 x 8
```

```
## # Groups:   gtp [12]
##      gtp term   contrast      null.value estimate conf.low conf.high adj.p.value
##    <dbl> <chr>  <chr>              <dbl>    <dbl>    <dbl>     <dbl>       <dbl>
##  1     0 mutant G401S-G363D            0        0        0         0         NaN
##  2     0 mutant L230dup-G363D          0        0        0         0         NaN
##  3     0 mutant R710G-G363D            0        0        0         0         NaN
##  4     0 mutant WT-G363D               0        0        0         0         NaN
##  5     0 mutant L230dup-G401S          0        0        0         0         NaN
##  6     0 mutant R710G-G401S            0        0        0         0         NaN
##  7     0 mutant WT-G401S               0        0        0         0         NaN
##  8     0 mutant R710G-L230dup          0        0        0         0         NaN
##  9     0 mutant WT-L230dup             0        0        0         0         NaN
## 10     0 mutant WT-R710G               0        0        0         0         NaN
## # ... with 110 more rows
```

```
activity_aov_tukey %>%
  arrange(adj.p.value) %>%
  filter(adj.p.value <= 0.05) %>%
  print(width = Inf, n = Inf)
```

```
## # A tibble: 79 x 8
## # Groups:   gtp [11]
##      gtp term   contrast      null.value estimate conf.low conf.high adj.p.value
##    <dbl> <chr>  <chr>              <dbl>    <dbl>    <dbl>     <dbl>       <dbl>
##  1   500 mutant L230dup-G363D          0  -0.513  -0.646    -0.379    0.00000138
##  2   300 mutant L230dup-G363D          0  -0.490  -0.620    -0.360    0.00000166
##  3  1000 mutant L230dup-G363D          0  -0.532  -0.676    -0.389    0.00000192
##  4  1300 mutant L230dup-G363D          0  -0.564  -0.716    -0.411    0.00000201
##  5    70 mutant L230dup-G363D          0  -0.311  -0.399    -0.222    0.00000318
##  6  2000 mutant L230dup-G363D          0  -0.639  -0.824    -0.453    0.00000384
##  7    30 mutant L230dup-G363D          0  -0.178  -0.231    -0.126    0.00000465
##  8   700 mutant L230dup-G363D          0  -0.523  -0.678    -0.368    0.00000469
##  9  1700 mutant L230dup-G363D          0  -0.580  -0.752    -0.407    0.00000478
## 10   100 mutant L230dup-G363D          0  -0.366  -0.480    -0.253    0.00000729
## 11   500 mutant R710G-G363D            0  -0.406  -0.539    -0.273    0.0000121
## 12    30 mutant L230dup-G401S          0  -0.160  -0.213    -0.107    0.0000128
## 13   300 mutant R710G-G363D            0  -0.380  -0.510    -0.250    0.0000174
## 14  1000 mutant R710G-G363D            0  -0.419  -0.562    -0.276    0.0000176
## 15  1300 mutant R710G-G363D            0  -0.443  -0.596    -0.290    0.0000187
## 16  2000 mutant R710G-G363D            0  -0.529  -0.714    -0.344    0.0000216
## 17    70 mutant L230dup-G401S          0  -0.241  -0.329    -0.152    0.0000327
## 18  1700 mutant R710G-G363D            0  -0.469  -0.641    -0.296    0.0000331
## 19    70 mutant R710G-G363D            0  -0.240  -0.328    -0.151    0.0000342
## 20   700 mutant R710G-G363D            0  -0.420  -0.575    -0.265    0.0000345
## 21   500 mutant L230dup-G401S          0  -0.346  -0.480    -0.213    0.0000507
## 22    30 mutant R710G-G363D            0  -0.137  -0.190    -0.0839   0.0000518
## 23   300 mutant L230dup-G401S          0  -0.336  -0.466    -0.206    0.0000524
## 24    10 mutant L230dup-G363D          0  -0.0759 -0.105    -0.0463   0.0000556
## 25  1300 mutant L230dup-G401S          0  -0.387  -0.539    -0.234    0.0000626
## 26   100 mutant R710G-G363D            0  -0.288  -0.402    -0.174    0.0000641
## 27   300 mutant WT-G363D               0  -0.321  -0.451    -0.190    0.0000793
## 28  1000 mutant L230dup-G401S          0  -0.350  -0.494    -0.207    0.0000855
## 29   500 mutant WT-G363D               0  -0.322  -0.456    -0.189    0.0000943
## 30   100 mutant L230dup-G401S          0  -0.274  -0.388    -0.160    0.0000993
```

```
## 31  1700 mutant L230dup-G401S          0  -0.398  -0.570    -0.226    0.000138
## 32   700 mutant L230dup-G401S          0  -0.352  -0.507    -0.197    0.000161
## 33  2000 mutant L230dup-G401S          0  -0.415  -0.601    -0.230    0.000179
## 34    30 mutant R710G-G401S            0  -0.118  -0.171    -0.0653   0.000183
## 35    10 mutant L230dup-G401S          0  -0.0659 -0.0954   -0.0363   0.000188
## 36    70 mutant WT-G363D               0  -0.195  -0.284    -0.107    0.000200
## 37   100 mutant WT-G363D               0  -0.242  -0.356    -0.128    0.000278
## 38  1000 mutant WT-G363D               0  -0.301  -0.445    -0.158    0.000308
## 39   700 mutant WT-G363D               0  -0.325  -0.480    -0.170    0.000315
## 40  1300 mutant WT-G363D               0  -0.320  -0.472    -0.167    0.000315
## 41    30 mutant WT-G363D               0  -0.106  -0.159    -0.0532   0.000449
## 42    10 mutant R710G-G363D            0  -0.0593 -0.0888   -0.0297   0.000451
## 43  2000 mutant WT-G363D               0  -0.370  -0.555    -0.185    0.000466
## 44    70 mutant R710G-G401S            0  -0.170  -0.258    -0.0813   0.000640
## 45  1700 mutant WT-G363D               0  -0.321  -0.493    -0.149    0.000804
## 46   500 mutant R710G-G401S            0  -0.239  -0.373    -0.106    0.00110
## 47  1300 mutant R710G-G401S            0  -0.266  -0.418    -0.113    0.00139
## 48   300 mutant R710G-G401S            0  -0.226  -0.356    -0.0961   0.00140
## 49   100 mutant R710G-G401S            0  -0.195  -0.309    -0.0809   0.00158
## 50    10 mutant R710G-G401S            0  -0.0493 -0.0788   -0.0197   0.00192
## 51  1700 mutant R710G-G401S            0  -0.287  -0.459    -0.115    0.00193
## 52    30 mutant WT-G401S               0  -0.0875 -0.140    -0.0346   0.00203
## 53  1000 mutant R710G-G401S            0  -0.237  -0.381    -0.0938   0.00204
## 54  2000 mutant R710G-G401S            0  -0.305  -0.491    -0.120    0.00209
## 55  1000 mutant WT-L230dup             0   0.231   0.0875    0.374    0.00249
## 56   700 mutant R710G-G401S            0  -0.249  -0.404    -0.0939   0.00255
## 57  1300 mutant WT-L230dup             0   0.244   0.0913    0.397    0.00263
## 58  1700 mutant WT-L230dup             0   0.258   0.0862    0.430    0.00415
## 59  2000 mutant WT-L230dup             0   0.269   0.0833    0.454    0.00528
## 60   500 mutant WT-L230dup             0   0.191   0.0571    0.324    0.00585
## 61    70 mutant WT-G401S               0  -0.126  -0.214    -0.0372   0.00607
## 62    10 mutant WT-G363D               0  -0.0415 -0.0711   -0.0120   0.00656
## 63    30 mutant WT-L230dup             0   0.0724  0.0196    0.125    0.00775
## 64   100 mutant WT-G401S               0  -0.149  -0.263    -0.0355   0.0103
## 65    70 mutant WT-L230dup             0   0.115   0.0270    0.204    0.0107
## 66   300 mutant WT-L230dup             0   0.170   0.0397    0.300    0.0107
## 67   300 mutant WT-G401S               0  -0.166  -0.296    -0.0363   0.0121
## 68   700 mutant WT-L230dup             0   0.198   0.0432    0.353    0.0122
## 69  1000 mutant G401S-G363D            0  -0.182  -0.325    -0.0384   0.0129
## 70   500 mutant G401S-G363D            0  -0.167  -0.300    -0.0333   0.0141
## 71  2000 mutant G401S-G363D            0  -0.223  -0.409    -0.0382   0.0175
## 72   300 mutant G401S-G363D            0  -0.154  -0.284    -0.0241   0.0194
## 73   500 mutant WT-G401S               0  -0.156  -0.289    -0.0222   0.0214
## 74    10 mutant WT-L230dup             0   0.0344  0.00480   0.0639   0.0219
## 75  1300 mutant G401S-G363D            0  -0.177  -0.330    -0.0246   0.0220
## 76   700 mutant G401S-G363D            0  -0.171  -0.326    -0.0162   0.0293
## 77   100 mutant WT-L230dup             0   0.124   0.0103    0.238    0.0315
## 78    10 mutant WT-G401S               0  -0.0315 -0.0611   -0.00196  0.0356
## 79  1700 mutant G401S-G363D            0  -0.182  -0.354    -0.00961  0.0376
```

```
write_csv(activity_aov_tukey, "activity_by_GTP_conc_anova_tukey_KAM.csv")
```