G4

Initialize

load packages

```
library(tidyverse)
library(here)
library(drc)

# custom ggplot colors
gg_color_hue <- function(n) {
  hues = seq(15, 375, length = n + 1)
  hcl(h = hues, l = 65, c = 100)[1:n]
}

#replace first color with black (for dose = 0)
gg_pal = gg_color_hue(10)
gg_pal[1] <- "#000000"
```

import data

```
# number of treatment plates and lines in experiment
n_plates <- 2
n_lines <- 2

# order of cell lines in raw
order <- c("WT", "BRKO")

# initialize raw and metadata, as list
raw <- vector(mode = "list", length = n_lines*n_plates)
metadata <- vector(mode = "list", length = n_plates)

# meta data for plates
metadata[[1]] <- read_csv(here("20220504_DLD1_metadata_1.csv"))
metadata[[2]] <- read_csv(here("20220504_DLD1_metadata_2.csv"))

raw[[1]] <- read_csv(here("20220504_DLD1_NuclightRED_WT_cx5461_pds-hcl.csv")) %>%
  pivot_longer(cols = -(1:2), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata[[1]], by="well") %>%
  mutate(cell_line = order[1])

raw[[2]] <- read_csv(here("20220504_DLD1_NuclightRED_WT_cisplatin.csv")) %>%
  pivot_longer(cols = -(1:2), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata[[2]], by="well") %>%
  mutate(cell_line = order[1])
```

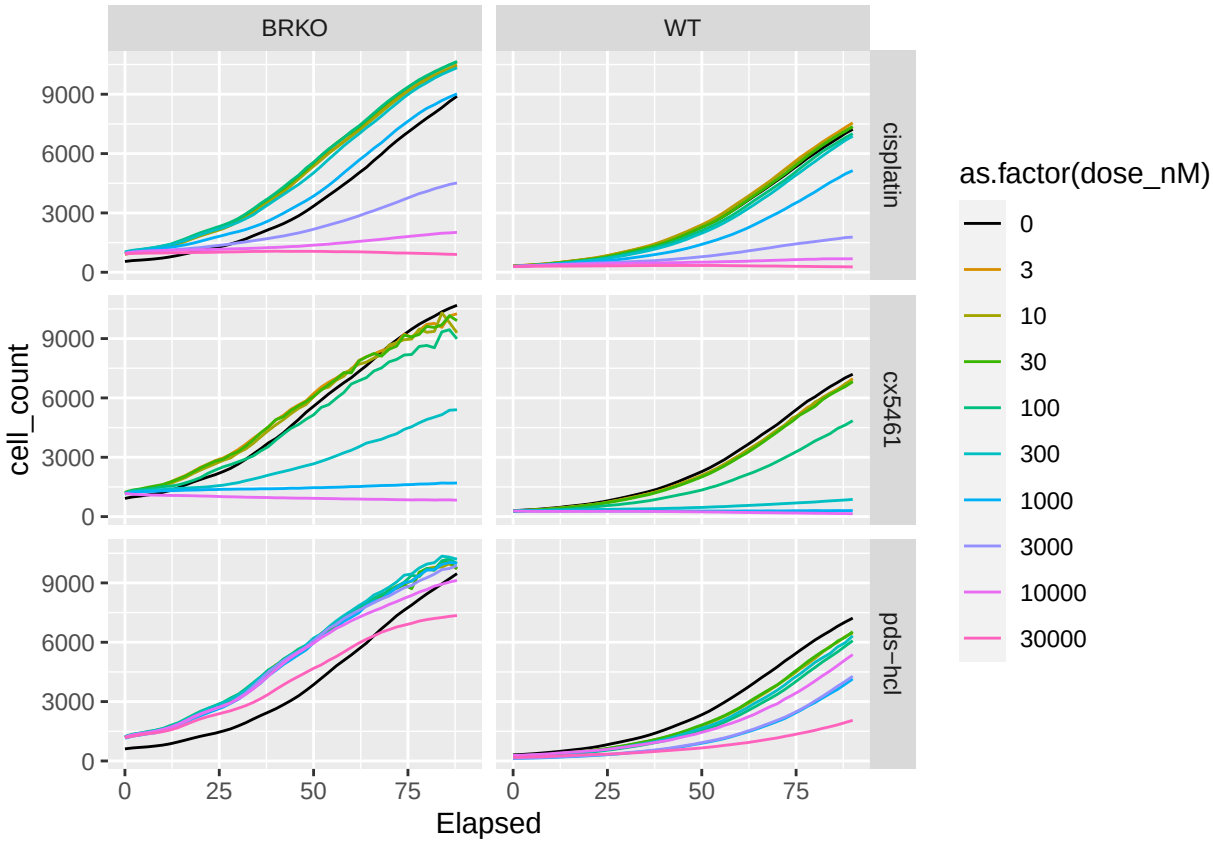
```
raw[[3]] <- read_csv(here("20220504_DLD1_NuclightRED_BracKO_cx5461_pds-hcl.csv")) %>%
  pivot_longer(cols = -(1:2), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata[[1]], by="well") %>%
  mutate(cell_line = order[2])

raw[[4]] <- read_csv(here("20220504_DLD1_NuclightRED_BracKO_cisplatin.csv")) %>%
  pivot_longer(cols = -(1:2), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata[[2]], by="well") %>%
  mutate(cell_line = order[2])

raw <- bind_rows(raw)

raw$compound <- replace(raw$compound, raw$compound == "pbs", "pds-hcl")
raw$compound <- replace(raw$compound, raw$compound == "nahpo4", "cx5461")
raw$compound <- replace(raw$compound, raw$compound == "dmso", "cisplatin")

raw_summary <- raw %>% dplyr::group_by(`Date Time`, Elapsed, compound, dose_nM, cmpd_cat, cell_line) %>%
  summarize(cell_count = mean(cell_count))
```

Plot

growth curves

facet by genotype and drug

```
ggplot(raw_summary, aes(x = Elapsed, y = cell_count, color = as.factor(dose_nM))) +
  geom_line() +
  facet_grid(rows = vars(compound), cols = vars(cell_line)) +
  scale_color_manual(values=gg_pal)
```



logistic modelling

```
raw_temp <- raw %>% mutate(unique = paste(compound,dose_nM,cell_line, sep = ";"))

temp <- map(unique(raw_temp$unique), function (x) drm(subset = unique %in% x, formula = cell_count ~ Ela

# prediction data
elapsed_time <- expand.grid(Elapsed=seq(0,90, length=180))

# predictions and confidence intervals
pm <- map(temp, function(x) predict(x, newdata=elapsed_time))
```

```
## Warning in sqrt(diag(varMat)): NaNs produced

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```
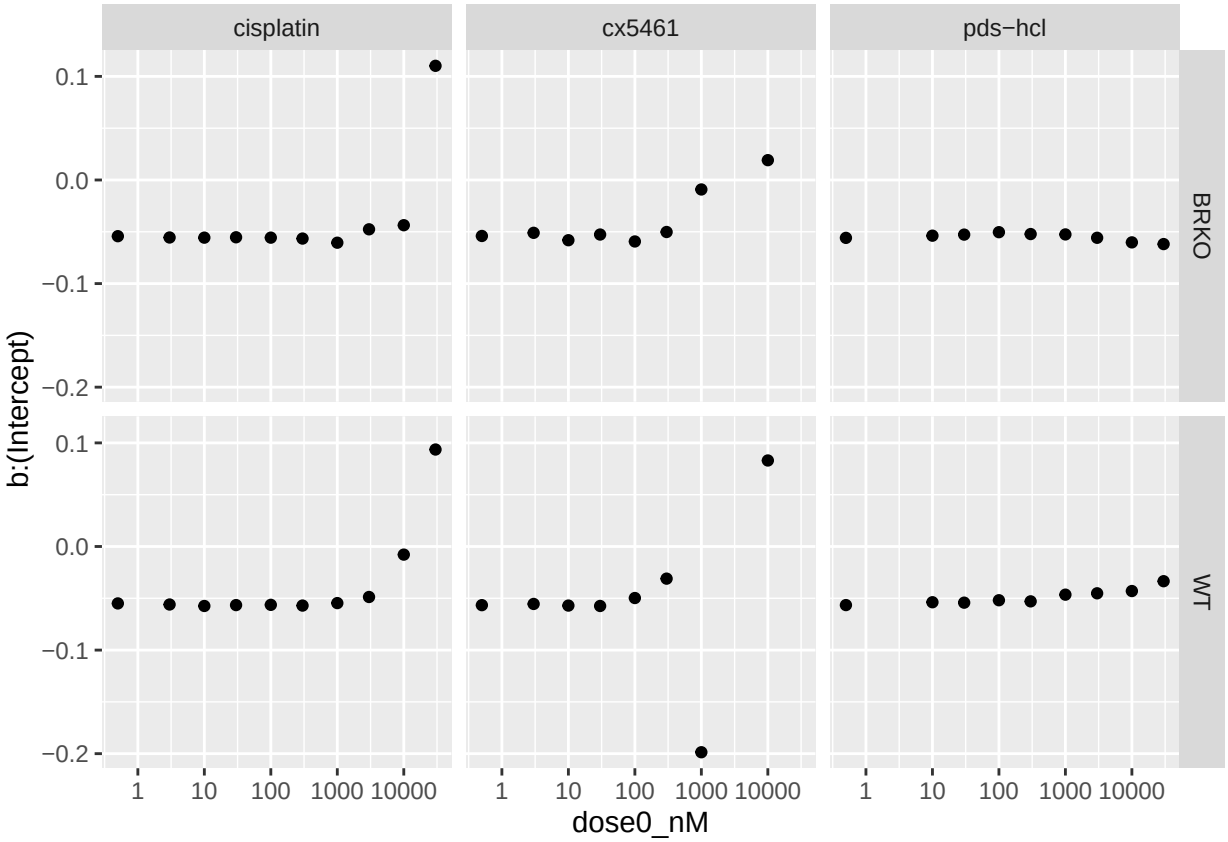

```
# new data with predictions
newdata <- map(pm, function(x) elapsed_time %>% mutate(cell_count = x))
```

```
newdata <- bind_rows(newdata)
newdata$unique <- rep(unique(raw_temp$unique), each=180)

newdata <- newdata %>% separate(unique, into = c("compound", "dose_nM", "cell_line"), sep = ";")

hill_slope <- map(temp, function(x) x$coefficients[1])
hill_slope <- bind_rows(hill_slope)
hill_slope$unique <- unique(raw_temp$unique)
hill_slope <- hill_slope %>% separate(unique, into = c("compound", "dose_nM", "cell_line"), sep = ";") %
  mutate(dose_nM = as.numeric(dose_nM))

hill_slope$dose0_nM <- hill_slope$dose_nM
hill_slope$dose0_nM[hill_slope$dose0_nM == 0] <- 0.5
```

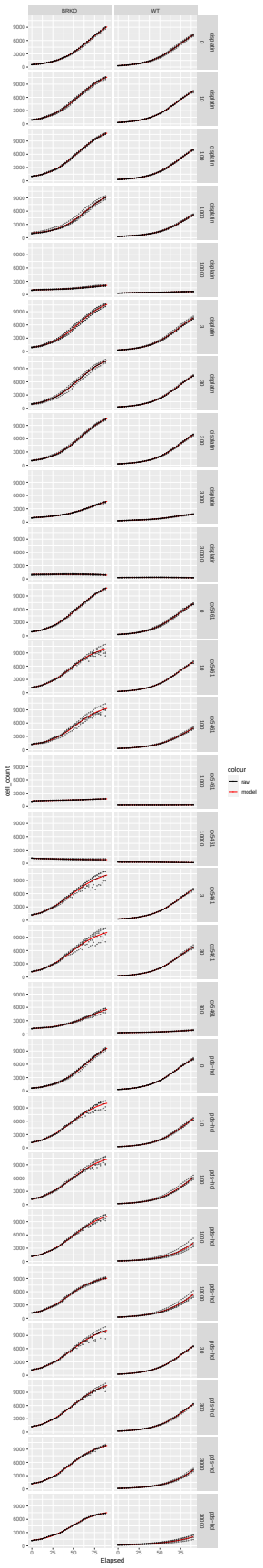
plot slope by concentration for drm modelling

```
hill_slope %>% ggplot(aes(x = dose0_nM, y = `b:(Intercept)`)) +
  geom_point() +
  scale_x_log10() +
  facet_grid(rows = vars(cell_line), cols = vars(compound))
```




```
# plotting the curve
ggplot(data = newdata, aes(x = Elapsed, y = cell_count)) +
  geom_line(aes(color = "red")) +
  geom_point(data = raw, size = 0.01, alpha = 0.5, aes(color = "black")) +
```

```
  scale_color_manual(labels = c("raw", "model"), values = c("black", "red")) +
  facet_grid(rows = vars(compound,as.factor(dose_nM)), cols = vars(cell_line))
```


BRKO
WT
cell_count
Elapsed
colour
raw
model

from DLD1_NuclightRED_20220504_curvefit_slope.R

```
# Import libraries ------------------------------------------------------

library(ggplot2)
library(plotly)
library(patchwork)
library(drc)
library(tidyr)
library(dplyr)

# Import dataset --------------------------------------------------------

wt1 <- read.csv('./20220504_DLD1_NuclightRED_WT_cx5461_pds-hcl.csv')
wt2 <- read.csv('./20220504_DLD1_NuclightRED_WT_cisplatin.csv')
mut1 <- read.csv('./20220504_DLD1_NuclightRED_BracKO_cx5461_pds-hcl.csv')
mut2 <- read.csv('./20220504_DLD1_NuclightRED_BracKO_cisplatin.csv')
metadata1 <- read.csv('./20220504_DLD1_metadata_1.csv')
metadata2 <- read.csv('./20220504_DLD1_metadata_2.csv')



# Process data to long form ---------------------------------------------



##wt and mut not found, need to specify object in the ncol in pivot_longer argument
##Specify wt1, wt2, mut1, mut2
##Ex: changed the wt_long transform in the pivot_longer(cols = 3:ncol(wt1)...)
wt_long1 <- wt1 %>%
  pivot_longer(cols = 3:ncol(wt1), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata1, by="well") %>%
  mutate(cell_line = "WT")

wt_long2 <- wt2 %>%
  pivot_longer(cols = 3:ncol(wt2), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata2, by="well") %>%
  mutate(cell_line = "WT")

mut_long1 <- mut1 %>%
  pivot_longer(cols = 3:ncol(mut1), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata1, by = 'well') %>%
  mutate(cell_line = "BRKO")

mut_long2 <- mut2 %>%
  pivot_longer(cols = 3:ncol(mut1), names_to = "well", values_to = "cell_count") %>%
  left_join(metadata2, by = 'well') %>%
  mutate(cell_line = "BRKO")


## These replacements will only happen on the first assignment
## (All DMSO will be assigned MMAE and not SN38) since the DMSO is not unique
#merged <- rbind(wt_long1, wt_long2, mut_long1, mut_long2) %>%
```

```
#  mutate(compound2 = compound) %>%
#  mutate(compound2 = replace(compound2, compound2 %in% "Vehicle", "CX5461")) %>%
#  mutate(compound2 = replace(compound2, compound2 %in% "Vehicle", "MMAE")) %>%
#  mutate(compound2 = replace(compound2, compound2 %in% "Vehicle", "SN38")) %>%
#  mutate(compound2 = replace(compound2, compound2 %in% "Vehicle", "Cetuximab")) %>%
#  mutate(compound2 = replace(compound2, compound2 %in% "Vehicle", "Cet-CX5461")) %>%
#  mutate(compound2 = replace(compound2, compound2 %in% "Vehicle", "Cet-MMAE")) %>%
#  mutate(compound2 = replace(compound2, compound2 %in% "Vehicle", "Cet-SN38")) %>%
#  mutate(cmpd_dose = paste(compound2, dose_nM, sep = "."))

## Leaving the groups as is
merged <- rbind(wt_long1, wt_long2, mut_long1, mut_long2) %>%
  mutate(compound2 = compound) %>%
  mutate(cmpd_dose = paste(compound2, dose_nM, sep = "."))


# Explore datasets ----------------------------------------------------

#WT line dose response
p1 <- ggplot(wt_long1, aes(x=Elapsed, y = cell_count)) +
  geom_smooth(aes(colour = as.factor(dose_nM)), se=FALSE) +
  scale_color_viridis_d(option="A") +
  facet_wrap(~compound) +
  ggtitle("DLD1 NuclightRED WT")

p2 <- ggplot(wt_long2, aes(x=Elapsed, y = cell_count)) +
  geom_smooth(aes(colour = as.factor(dose_nM)), se=FALSE) +
  scale_color_viridis_d(option="A") +
  facet_wrap(~compound) +
  ggtitle("DLD1 NuclightRED WT")

#Mutant line response
p3 <- ggplot(mut_long1, aes(x=Elapsed, y = cell_count)) +
  geom_smooth(aes(colour = as.factor(dose_nM)), se=FALSE) +
  scale_color_viridis_d(option="A") +
  facet_wrap(~compound) +
  ggtitle("DLD1 NuclightRED BRCA2-/-")

p4 <- ggplot(mut_long2, aes(x=Elapsed, y = cell_count)) +
  geom_smooth(aes(colour = as.factor(dose_nM)), se=FALSE) +
  scale_color_viridis_d(option="A") +
  facet_wrap(~compound) +
  ggtitle("DLD1 NuclightRED BRCA2-/-")

#plot 1
p1+p2+p3+p4


#Compare average effects between cell lines
ggplot(merged, aes(x=Elapsed, y = cell_count)) +
  geom_smooth(aes(colour = cell_line), se=FALSE) +
  scale_color_brewer(palette = "Set1") +
  facet_wrap(~compound) +
```

```
  ggtitle("Average effect per cell line")

#Compare dose response per cell line
ggplot(merged, aes(x=Elapsed, y = cell_count)) +
  geom_smooth(aes(colour = as.factor(dose_nM)), se=FALSE) +
  scale_color_viridis_d(option="A") +
  facet_wrap(~compound2+cell_line) +
  ggtitle("Compound dose effect per cell line")

#Compare compound category per cell line
ggplot(merged, aes(x=Elapsed, y = cell_count)) +
  geom_smooth(aes(colour = as.factor(compound)), se=FALSE) +
  scale_color_viridis_d(option="A") +
  facet_grid(cmpd_cat~cell_line) +
  ggtitle("Average effect per compound category")




# Curve fit ----------------------------------------------------------------

## Case sensitive for compound2 group -- cx needs to be CAPS here
#filt_dat <- merged %>% filter(compound2 %in% c('cx5461'), cell_line %in% "WT")

## Changes cx (lower case) to CX (CAPS/upper case)
filt_dat <- merged %>% filter(compound2 %in% c('Vehicle'),
                              cell_line %in% "WT")

fit2 <- drm(cell_count~Elapsed,
            curveid = cmpd_dose,
            data = filt_dat,
            fct=LL.4(names = c("Slope", "Lower Limit", "Upper Limit", "ED50")))
summary(fit2)
plot(fit2)

## Test all available models using mselect
mselect(fit2, fctList = list(W1.3(),W1.4(), W2.3(), W2.4(),  LL.3()),
        linreg=TRUE)

#Build curve fit coefficient table for WT
fit_coeff <- tibble(
  IC50 = numeric(),
  slope = numeric(),
  cmpd_dose = character(),
  group = character()
)

#Loop through each treatment group and calculate coefficient
for (cmpd_name in unique(merged$compound2)) {

  #Filter data to compound
  filt_dat <- merged %>% filter(compound2 %in% cmpd_name ,
```

```
                                cell_line %in% "WT")

  fit3 <- drm(cell_count~Elapsed,
              curveid = cmpd_dose,
              data = filt_dat,
              fct=LL.4(names = c("Slope", "Lower Limit", "Upper Limit", "IC50")))

  ic50idxs <- grep("IC50:", names(fit3$coefficients))
  ic50s <- fit3$coefficients[ic50idxs]

  slope_idx <- grep("Slope:", names(fit3$coefficients))
  slope_coeff <- fit3$coefficients[slope_idx]

  fit_coeff <- add_row(fit_coeff,
                       IC50 = ic50s,
                       slope = slope_coeff,
                       cmpd_dose = names(ic50s),
                       group = cmpd_name)
}

fit_coeff$cell_line <- "WT"



#Build curve fit coefficient table for B18
fit_coeff_mut <- tibble(
  IC50 = numeric(),
  slope = numeric(),
  cmpd_dose = character(),
  group = character()
)
for (cmpd_name in unique(merged$compound2)) {

  #Filter data to compound
  filt_dat <- merged %>% filter(compound2 %in% cmpd_name , cell_line %in% "B18")

  fit3 <- drm(cell_count~Elapsed,
              curveid = cmpd_dose,
              data = filt_dat,
              fct=LL.4(names = c("Slope", "Lower Limit", "Upper Limit", "IC50")))

  ic50idxs <- grep("IC50:", names(fit3$coefficients))
  ic50s <- fit3$coefficients[ic50idxs]

  slope_idx <- grep("Slope:", names(fit3$coefficients))
  slope_coeff <- fit3$coefficients[slope_idx]

  fit_coeff_mut <- add_row(fit_coeff_mut,
                           IC50 = ic50s,
                           slope = slope_coeff,
                           cmpd_dose = names(ic50s),
                           group = cmpd_name)
}
```

```r
fit_coeff_mut$cell_line <- "B18"


fit_coeff_merge <- rbind(fit_coeff, fit_coeff_mut)


##Since the DMSO and PBS groups are not reassigned, the results have "Intercept" annotated
## Replacing the IC50:(Intercept) annotation in cmpd_dose column to Vehicle with dose of 0
fit_coeff_merge <- fit_coeff_merge %>%
  mutate(cmpd_dose = replace(cmpd_dose, cmpd_dose %in% "IC50:(Intercept)", "IC50:Vehicle.0")) %>%
  separate(cmpd_dose, into = c("var", "compound"), sep = "IC50:") %>%
  separate(compound, into = c("compound", "dose"), sep = "[.]") %>%
  pivot_longer(cols = 1:2, names_to = "feature", values_to = "value")



# Graph both TC50 & SLOPE features ------------------------------------------------
ggplot(fit_coeff_merge %>%
         filter(feature %in% "slope"),
       aes(x = dose, y = value) ) +
  geom_point(aes(colour = cell_line), size =3) +
  facet_wrap(~compound, scales = "free_x")

ggplot(fit_coeff_merge %>%
         filter(feature %in% "IC50"),
       aes(x = dose, y = value) ) +
  geom_point(aes(colour = cell_line), size =3) +
  facet_wrap(~compound)

fit_coeff_merge$dose <- as.numeric(fit_coeff_merge$dose)

ggplot(fit_coeff_merge,
       aes(x = as.factor(dose), y = value) ) +
  geom_point(aes(colour = cell_line), size =3) +
  geom_line(aes(group = cell_line)) +
  facet_grid(feature~compound, scales = "free_y") +
  theme(axis.text.x = element_text(angle = 45, hjust=0.9))

# Graph only SLOPE feature ------------------------------------------------------
# Differential Drug sensitivity on genotype. Growth rate as a drug response metrics, looking at the slo

fit_coeff_merge$dose <- as.numeric(fit_coeff_merge$dose)

# Version1
ggplot(fit_coeff_merge %>%
         filter(feature %in% "slope"),
       aes(x = dose, y = value) ) +
  geom_point(aes(colour = cell_line), size =2.5) +
  scale_colour_manual(name="Isogenics",  values =c("red", "blue")) +
  geom_line(aes(group = cell_line)) +
  facet_wrap(~compound, scales = "free") +
  facet_grid(feature~compound, scales = "free") +
  theme(axis.text.x = element_text(angle = 45, hjust=0.9),
```

```
        axis.text = element_text(size = 10),
        axis.title = element_text(size=14,face="bold"),
        plot.title = element_text(size = 14),
        text = element_text(size = 20),
        legend.title = element_text(size = 15),
        legend.text = element_text(size = 13)) +
  labs(title="Genotype specific drug sensitivity",
       x ="Dose (nM)", y = "Slope")

# Version2
ggplot(fit_coeff_merge %>%
         filter(feature %in% "slope"),
       aes(x = dose, y = value) ) +
  geom_point(aes(colour = cell_line), size =2.5) +
  scale_colour_manual(name="HCT116 Isogenics",  values =c("red", "blue")) +
  geom_line(aes(group = cell_line)) +
  facet_wrap(~compound, scales = "free") +
  facet_grid(feature~compound, scales = "free") +
  theme(axis.text.x = element_text(angle = 45, hjust=0.9),
        axis.text = element_text(size = 17),
        axis.title = element_text(size=23),
        plot.title = element_text(size = 25),
        text = element_text(size = 25),
        legend.title = element_text(size = 20),
        legend.text = element_text(size = 18)) +
  labs(title="Genotype specific drug sensitivity",
       x ="Dose (nM)", y = "Slope")


# Version3 - transform the dose to log
ggplot(fit_coeff_merge %>%
         filter(feature %in% "slope"),
       aes(x = log(dose+0.1), y = value) ) +
  geom_point(aes(colour = cell_line), size =2.5) +
  scale_colour_manual(name="HCT116 Isogenics",  values =c("red", "blue")) +
  geom_line(aes(group = cell_line)) +
  facet_wrap(~compound, scales = "free") +
  facet_grid(feature~compound, scales = "free") +
  theme(axis.text.x = element_text(angle = 45, hjust=0.9),
        axis.text = element_text(size = 17),
        axis.title = element_text(size=23),
        plot.title = element_text(size = 25),
        text = element_text(size = 25),
        legend.title = element_text(size = 20),
        legend.text = element_text(size = 18)) +
  labs(title="Genotype specific drug sensitivity",
       x ="log (nM dose)", y = "Slope")
```