fig2_casp1

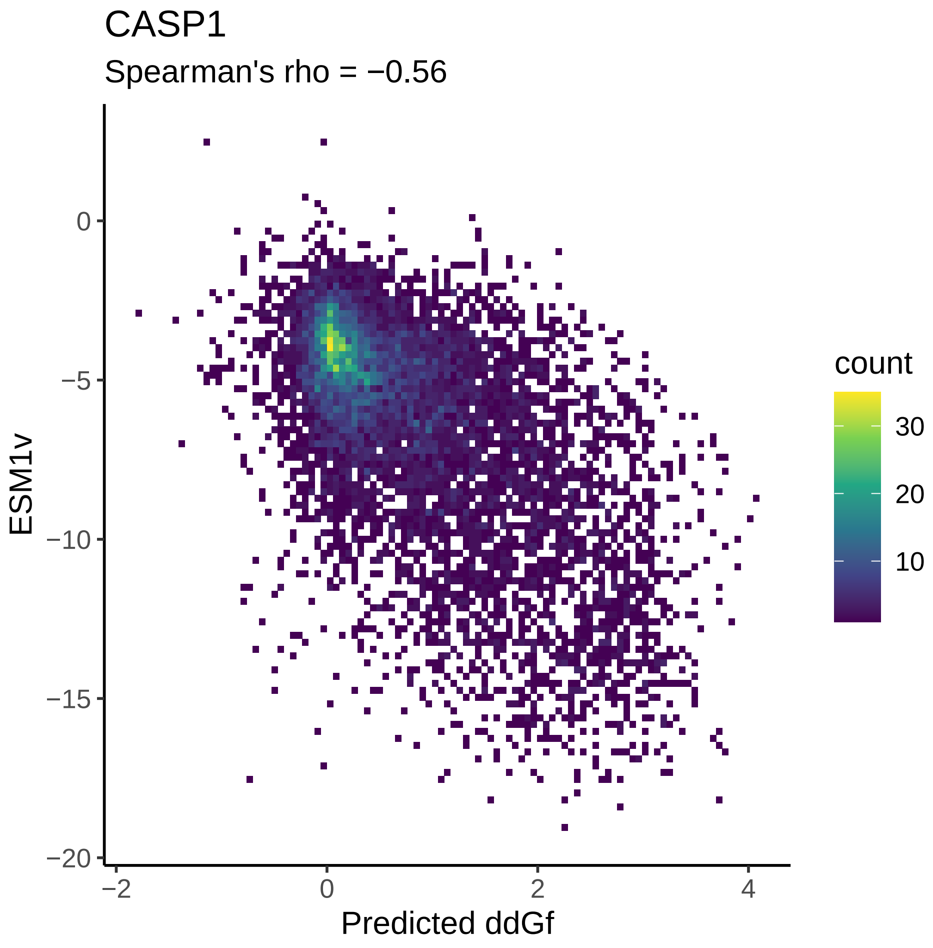
2025-06-12

CASP1

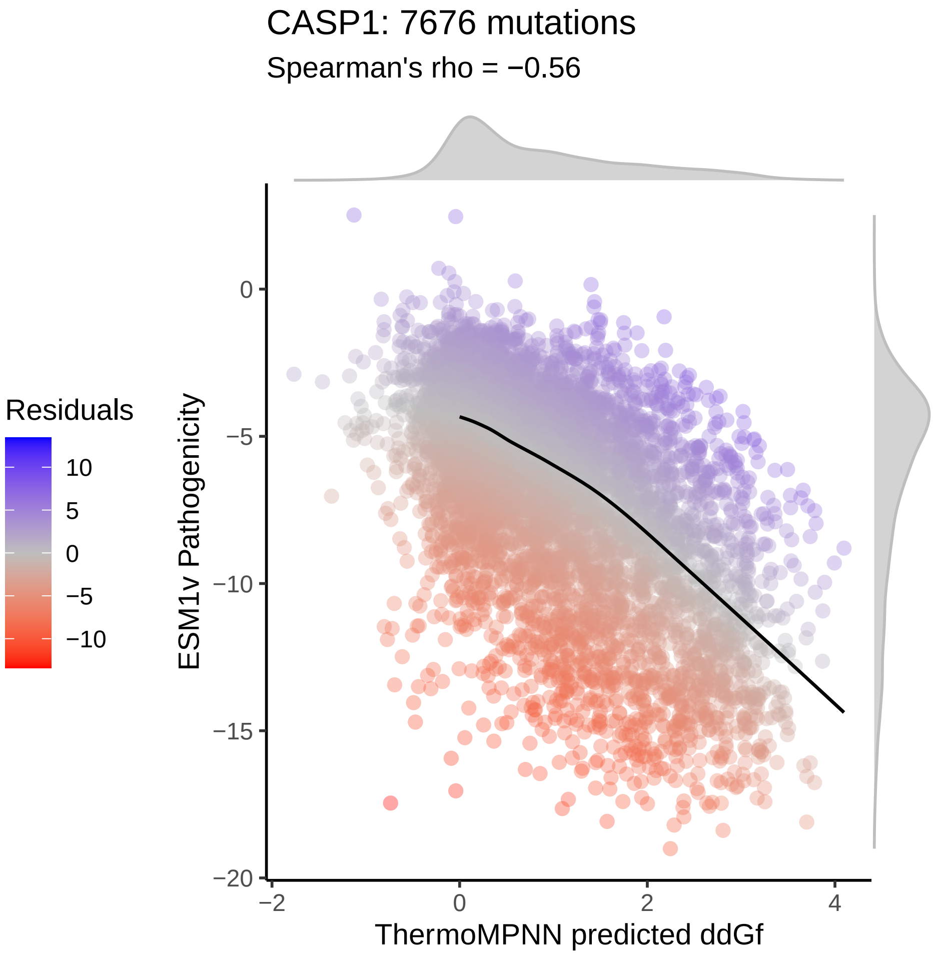
```r
analyze_ddg_vs_esm1v <- function(ddg_file, esm_file, protein_name = "Protein") {
  # Load data
  test_ddg <- fread(ddg_file)
  test_esm <- fread(esm_file)

  # Prepare ESM1v column
  colnames(test_esm)[2] <- "ESM1v"

  # Construct variant column in ddg data
  test_ddg[, new_position := pos + 1]
  test_ddg[, variant := paste0(wtAA, new_position, mutAA)]

  # Merge on variant
  test_df <- merge(test_ddg, test_esm, by = "variant")

  # Rename ddG column
  test_df <- test_df %>% dplyr::rename(ddG_pred = `ddG (kcal/mol)`)

  # Spearman correlation
  spearman_rho <- cor.test(test_df$ddG_pred, test_df$ESM1v, method = "spearman")
  rho_value <- round(spearman_rho$estimate, 2)

  # Plot
  p <- ggplot(test_df, aes(x = ddG_pred, y = ESM1v)) +
    geom_bin2d(bins = 100) +
    scale_fill_continuous(type = "viridis") +
    theme_classic() +
    labs(
      x = "Predicted ddGf",
      y = "ESM1v",
      title = protein_name,
      subtitle = paste("Spearman's rho =", rho_value)
    ) +
    theme(
      text = element_text(size = 12),
      legend.position = "right"
    )

  # Output
  list(
    spearman_rho = spearman_rho,
    plot = p,
    merged_data = test_df
  )
}
casp1_pred <- analyze_ddg_vs_esm1v(
  ddg_file = "/Users/xl7/Documents/0.Projects/01.protein-seq-evo-v1/data/decay_pdb/CASP1/AF-P29466-F1-m
  esm_file = "/Users/xl7/Documents/0.Projects/00.large_supplements/ESM1v_proteome_wide/all_ESM1v/P29466
  protein_name = "CASP1"
)
```

```
## Warning in cor.test.default(test_df$ddG_pred, test_df$ESM1v, method =
## "spearman"): Cannot compute exact p-value with ties
```

```
#result$spearman_rho #-0.5410593
p0 <- casp1_pred$plot
ggsave("/Users/xl7/Documents/0.Projects/01.protein-seq-evo-v1/figs/panels/fig2_casp1_p0.pdf",
       plot = p0, width = 5, height = 5, dpi = 300)
p0
```



```
plot_loess_residuals <- function(test_df, active_site_positions,
                                 span = 0.7, protein_name = "casp1") {

  # Filter out active site positions
  test_df_fil <- test_df %>% filter(!new_position %in% active_site_positions)

  # Fit loess model on filtered data
  loess_fit <- loess(ESM1v ~ ddG_pred, data = test_df_fil, span = span, family = "symmetric")

  # Predict on all data
  test_df$fitted_pred <- predict(loess_fit, newdata = test_df)
  test_df$residuals_pred <- test_df$ESM1v - test_df$fitted_pred

  # Fit line data for the smooth curve
  fit_line_df <- data.frame(
    ddG_pred = seq(0,
                   max(test_df$ddG_pred, na.rm = TRUE),
                   length.out = 200)
  )
  fit_line_df$ESM1v <- predict(loess_fit, newdata = fit_line_df)

  # Spearman correlation
  spearman_result <- suppressWarnings(cor.test(test_df$ddG_pred, test_df$ESM1v, method = "spearman"))
  spearman_rho <- spearman_result$estimate
  spearman_p <- spearman_result$p.value

  # Axis and color scale limits
  xlim_vals <- range(test_df$ddG_pred, na.rm = TRUE)
  ylim_vals <- range(test_df$ESM1v, na.rm = TRUE)
  resid_limit <- max(abs(test_df$residuals_pred), na.rm = TRUE)

  # Main plot
  p <- ggplot(test_df, aes(x = ddG_pred , y = ESM1v, color = residuals_pred)) +
    geom_point(size = 2, alpha = 0.35) +
    geom_line(data = fit_line_df, aes(x = ddG_pred, y = ESM1v),
              inherit.aes = FALSE, color = "black", linewidth = 0.6) +
    labs(
      title = paste0(protein_name, ": ", nrow(test_df), " mutations"),
      subtitle = paste0("Spearman's rho = ", round(spearman_rho, 2)),
      x = "ThermoMPNN predicted ddGf",
      y = "ESM1v Pathogenicity",
      color = "ESM1v-ddGf residuals"
    ) +
    theme_classic() +
    xlim(xlim_vals) +
    ylim(ylim_vals) +
    scale_color_gradient2(
      low = "red", mid = "grey", high = "blue", midpoint = 0,
      limits = c(-resid_limit, resid_limit), name = "Residuals"
    ) +
    theme(legend.position = "left")

  # Add marginal density plots
  p_marginal <- ggMarginal(
    p,
    type = "density",
    margins = "both",
    groupColour = FALSE,
    groupFill = FALSE,
    size = 10,
    colour = "grey",
    fill = "lightgrey"
  )

  return(list(
    plot = p_marginal,
    data = test_df
  ))
}

casp1_pred_residual <- plot_loess_residuals(casp1_pred$merged_data, active_site_positions = c(179, 235,
p1 <- casp1_pred_residual$plot
ggsave("/Users/xl7/Documents/0.Projects/01.protein-seq-evo-v1/figs/panels/fig2_casp1_p1.pdf",
       plot = p1, width = 5, height = 4, dpi = 300)
p1
```



```r
map_loess_residuals_to_pdb <- function(test_df, pdb_path, output_pdb_path) {

  # 1. Compute median residuals per position
  median_residuals <- test_df %>%
    group_by(new_position) %>%
    summarise(median_residuals = median(residuals_pred, na.rm = TRUE), .groups = "drop")

  # 2. Read in PDB
  pdb <- read.pdb(pdb_path)

  # 3. Initialize new B-factor vector
  new_b_factors <- pdb$atom$b

  # 4. Map residuals to matching residue numbers in the PDB
  for (i in seq_len(nrow(median_residuals))) {
    pos <- median_residuals$new_position[i]
    val <- median_residuals$median_residuals[i]
    matching_indices <- which(pdb$atom$resno == pos)
    new_b_factors[matching_indices] <- val
  }

  # 5. Replace non-matching indices with outlier value (e.g., 999)
  matched_positions <- unique(median_residuals$new_position)
  non_matching_indices <- which(!(pdb$atom$resno %in% matched_positions))
  new_b_factors[non_matching_indices] <- 999

  # 6. Assign and save new PDB
  pdb$atom$b <- new_b_factors
  write.pdb(pdb, file = output_pdb_path)

  # Optional: return summary
  return(list(
    min_residual = min(median_residuals$median_residuals, na.rm = TRUE),
    max_residual = max(median_residuals$median_residuals, na.rm = TRUE),
    length(non_matching_indices),
    output_file = output_pdb_path
  ))
}

pdb_residual <- map_loess_residuals_to_pdb(
  test_df = casp1_pred_residual$data,
  pdb_path = "~/Documents/0.Projects/01.protein-seq-evo-v1/data/decay_pdb/CASP1/2hbq.pdb",
  output_pdb_path = "~/Documents/0.Projects/01.protein-seq-evo-v1/data/decay_pdb/CASP1/2hbq_loess_resid
)
```

```
##    PDB has ALT records, taking A only, rm.alt=TRUE
```

```r
print(pdb_residual)
```

```
## $min_residual
## [1] -8.213883
##
## $max_residual
## [1] 5.545446
##
## [[3]]
## [1] 48
##
## $output_file
## [1] "~/Documents/0.Projects/01.protein-seq-evo-v1/data/decay_pdb/CASP1/2hbq_loess_residual.pdb"
```

```
#chimerax
#color byattribute a:bfactor #9 & sel target csab palette -8.3,red:0,white:5.6,blue
```

```
# --- Read PDB and extract protein/ligand atoms ---
pdb <- read.pdb("~/Documents/0.Projects/01.protein-seq-evo-v1/data/decay_pdb/CASP1/2hbq.pdb", rm.alt = 
```

```
##    PDB has ALT records, taking A only, rm.alt=TRUE
```

```
# Select CA atoms from protein chains A and B (exclude ligand and waters)
protein_ca <- pdb$atom[
  pdb$atom$chain %in% c("A", "B") &
  pdb$atom$elety == "CA" &
  pdb$atom$resid != "HOH",
]

# Select all atoms in the ligand (chain C), excluding water
ligand_atoms <- pdb$atom[
  pdb$atom$chain == "C" &
  pdb$atom$resid != "HOH",
]

# Compute minimum distance from each CA atom to any ligand atom
protein_ca$min_dist_to_ligand <- apply(protein_ca, 1, function(atom) {
  dists <- sqrt((as.numeric(atom["x"]) - ligand_atoms$x)^2 +
                (as.numeric(atom["y"]) - ligand_atoms$y)^2 +
                (as.numeric(atom["z"]) - ligand_atoms$z)^2)
  min(dists)
})

# --- Merge with prediction data ---
merged_df <- merge(casp1_pred_residual$data, protein_ca, by.x = "new_position", by.y = "resno") %>%
  filter(residuals_pred <= 0)

# --- Residue-level median residuals ---
merged_df_residue <- merged_df %>%
  group_by(new_position) %>%
  summarise(loess_residual_avg = median(residuals_pred, na.rm = TRUE), .groups = "drop") %>%
  left_join(protein_ca, by = c("new_position" = "resno"))

# --- Exclude orthosteric sites for fitting ---
#orthosteric_sites <- c(179, 237, 283, 285, 339, 340, 341, 342, 343, 383)
orthosteric_sites <- c(179, 235, 236, 237, 238, 239, 283, 284, 285, 338, 339, 340, 341, 342,343, 347 ,34

ortho_cutoff <- max(merged_df_residue %>% filter(new_position %in% orthosteric_sites) %>% pull(min_dist
ortho_cutoff
```

```
## [1] 6.59331
```

```
merged_df_residue %>% filter(min_dist_to_ligand <= ortho_cutoff) %>% pull(new_position)
```

```
##  [1] 179 235 236 237 238 239 283 284 285 338 339 340 341 342 343 347 348 383
```

```
#179, 235, 236, 237, 238, 239, 283, 284, 285, 338, 339, 340, 341, 342,343, 347 ,348, 383

merged_df_residue_fil <- merged_df_residue %>%
  filter(!new_position %in% orthosteric_sites)

# --- Fit exponential model ---
exp_model <- nlsLM(
  abs(loess_residual_avg) ~ a * exp(-b * min_dist_to_ligand),
  data = merged_df_residue,
  start = list(a = 1, b = 0.1)
)
exp_model
```

```
## Nonlinear regression model
##   model: abs(loess_residual_avg) ~ a * exp(-b * min_dist_to_ligand)
##    data: merged_df_residue
##       a       b
## 3.72069 0.03061
##  residual sum-of-squares: 757.6
##
## Number of iterations to convergence: 8
## Achieved convergence tolerance: 1.49e-08
```

```
# --- Prediction grid ---
x_vals <- seq(min(merged_df_residue$min_dist_to_ligand),
              max(merged_df_residue$min_dist_to_ligand), length.out = 200)

# --- Bootstrapping for confidence intervals ---
set.seed(11)
boot_params <- replicate(1000, {
  samp <- merged_df_residue[sample(nrow(merged_df_residue), replace = TRUE), ]
  fit <- try(nlsLM(abs(loess_residual_avg) ~ a * exp(-b * min_dist_to_ligand),
                 data = samp, start = list(a = 1, b = 0.1)), silent = TRUE)
  if (inherits(fit, "try-error")) c(NA, NA) else coef(fit)
})
boot_params <- t(boot_params)[complete.cases(t(boot_params)), ]

boot_preds <- apply(boot_params, 1, function(p) p[1] * exp(-p[2] * x_vals))
fit_df_residue <- data.frame(
  min_dist_to_ligand = x_vals,
  loess_residual_pred = predict(exp_model, newdata = data.frame(min_dist_to_ligand = x_vals)),
  lower = apply(boot_preds, 1, quantile, probs = 0.025),
  upper = apply(boot_preds, 1, quantile, probs = 0.975)
)

# --- Model parameter extraction and derived quantity ---
```

```r
model_summary <- summary(exp_model)
coefs <- coef(exp_model)
se <- model_summary$coefficients[, "Std. Error"]

residue_a <- c(coefs["a"],
               coefs["a"] - 1.96 * se["a"],
               coefs["a"] + 1.96 * se["a"])

residue_b <- c(coefs["b"],
               coefs["b"] - 1.96 * se["b"],
               coefs["b"] + 1.96 * se["b"])

half_d <- log(2) / coefs["b"]
half_d_ci <- quantile(log(2) / boot_params[, "b"], probs = c(0.025, 0.975))
residue_half_d <- c(half_d, half_d_ci)

cat("Parameter a (intercept):", residue_a, "\n")
```

```
## Parameter a (intercept): 3.720693 2.944467 4.496918
```

```r
cat("Parameter b (decay rate):", residue_b, "\n")
```

```
## Parameter b (decay rate): 0.03061466 0.01815429 0.04307504
```

```r
cat("Half-distance (log(2)/b):", residue_half_d, "\n")
```

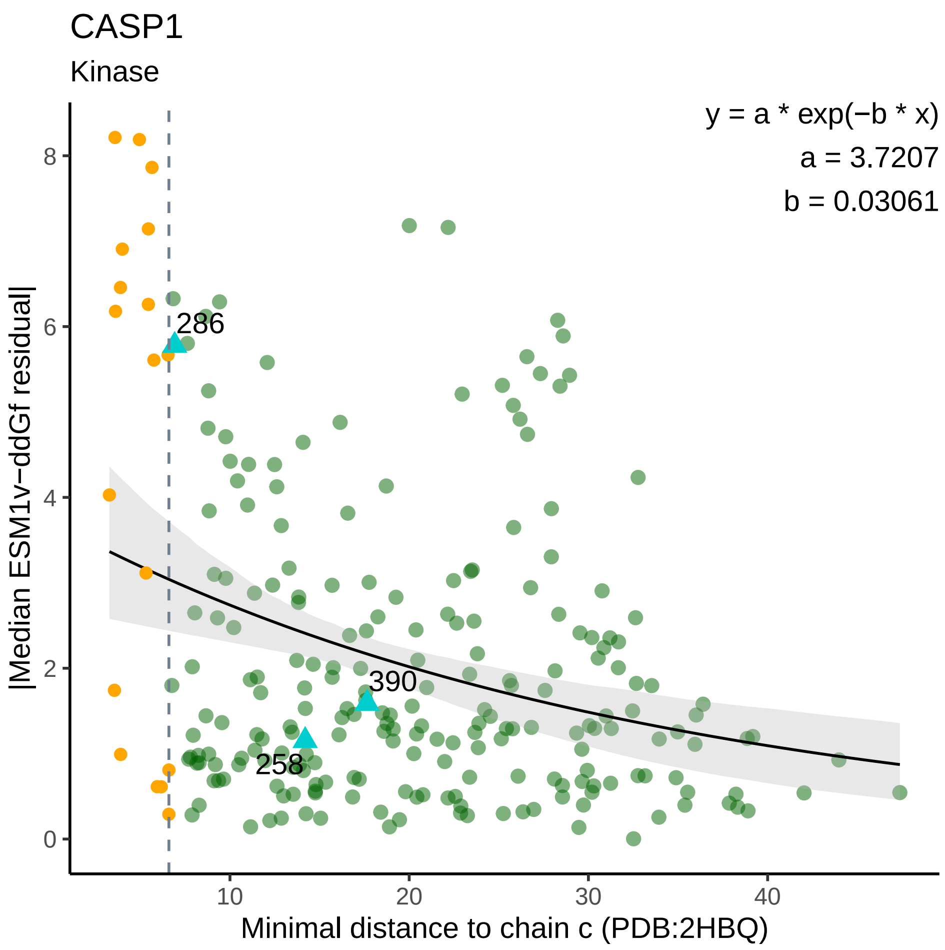
```
## Half-distance (log(2)/b): 22.64102 13.83288 42.30911
```

```r
# Number of iterations to convergence: 8
# Achieved convergence tolerance: 1.49e-08
# Parameter a (intercept): 3.720693 2.944467 4.496918
# Parameter b (decay rate): 0.03061466 0.01815429 0.04307504
# Half-distance (log(2)/b): 22.64102 13.83288 42.30911
```

```r
# --- Annotate site types ---
merged_df_residue <- merged_df_residue %>%
  mutate(site_type = if_else(new_position %in% orthosteric_sites, "orthosteric", "non-orthosteric"))

# --- Plot ---
orange_labs <- orthosteric_sites
cyan_labs <- c(286, 390, 258)
all_labs <- union(orange_labs, cyan_labs)

p2 <- ggplot(merged_df_residue, aes(x = min_dist_to_ligand, y = abs(loess_residual_avg))) +

  # Unlabeled points
  geom_point(data = subset(merged_df_residue, !new_position %in% all_labs),
             aes(color = site_type), size = 2, alpha = 0.5) +

  # CI ribbon
  geom_ribbon(
  data = fit_df_residue,
  aes(x = min_dist_to_ligand, ymin = lower, ymax = upper),
  inherit.aes = FALSE,
  fill = "grey70",
  alpha = 0.3) +

  # Main fit line
  geom_line(
  data = fit_df_residue,
  aes(x = min_dist_to_ligand, y = loess_residual_pred),
  inherit.aes = FALSE,
  color = "black")+


  # Labeled orange points
  geom_point(data = subset(merged_df_residue, new_position %in% orange_labs),
             shape = 16, size = 2, color = "orange") +
  # Labeled cyan points
  geom_point(data = subset(merged_df_residue, new_position %in% cyan_labs),
             shape = 17, size = 3, color = "cyan3") +
  geom_text_repel(data = subset(merged_df_residue, new_position %in% cyan_labs),
                  aes(label = new_position), color = "black") +

  # Reference line
  geom_vline(xintercept = max(merged_df_residue %>% filter(site_type == "orthosteric") %>% pull(min_dis
             linetype = "dashed", color = "slategrey") +

  # Labels and theme
  labs(
    title = "CASP1",
    subtitle = "Kinase",
    x = "Minimal distance to chain c (PDB:2HBQ)",
    y = "|Median ESM1v-ddGf residual|"
  ) +
  theme_classic() +
  theme(legend.position = "none") +
  scale_color_manual(values = c("non-orthosteric" = "darkgreen", "orthosteric" = "orange")) +

  annotate("text", x = Inf, y = Inf, hjust = 1, vjust = 1,
           label = sprintf("y = a * exp(-b * x)\na = %.4f\nb = %.5f", coefs["a"], coefs["b"]),
           size = 4, color = "black", hjust = 0)
```

```
## Warning: Duplicated aesthetics after name standardisation: hjust
```

```
ggsave("/Users/xl7/Documents/0.Projects/01.protein-seq-evo-v1/figs/panels/fig2_casp1_p2.pdf",
       plot = p2, width = 4, height = 4, dpi = 300)
p2
```

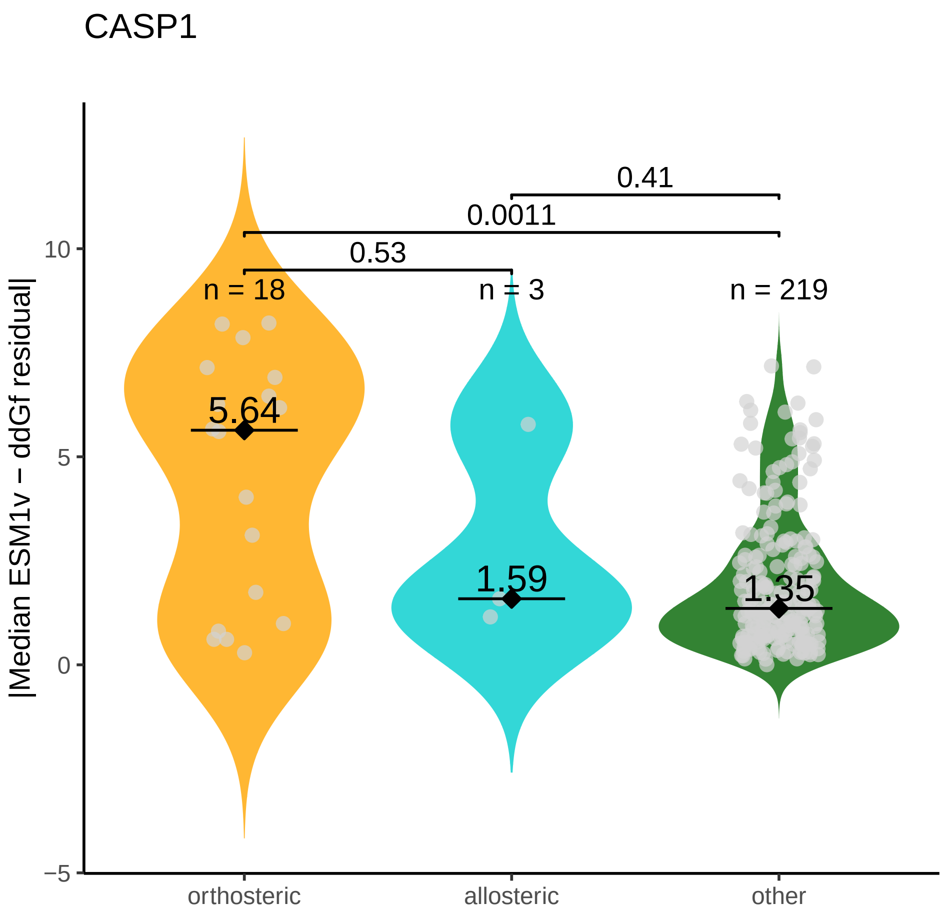


```
lm_model <- lm(log(abs(loess_residual_avg)) ~ min_dist_to_ligand, data = merged_df_residue)
summary(lm_model)
```

```
##
## Call:
## lm(formula = log(abs(loess_residual_avg)) ~ min_dist_to_ligand,
##     data = merged_df_residue)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -5.8410 -0.6348  0.0759  0.7016  1.6726
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         0.844742   0.141996   5.949 9.57e-09 ***
## min_dist_to_ligand -0.024747   0.006522  -3.794 0.000188 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9707 on 238 degrees of freedom
## Multiple R-squared:  0.05704,    Adjusted R-squared:  0.05308
## F-statistic:  14.4 on 1 and 238 DF,  p-value: 0.0001879
```

```
# Call:
# lm(formula = log(abs(loess_residual_avg)) ~ min_dist_to_ligand,
#     data = merged_df_residue)
#
# Residuals:
#     Min      1Q  Median      3Q     Max
# -5.8410 -0.6348  0.0759  0.7016  1.6726
#
# Coefficients:
#                     Estimate Std. Error t value Pr(>|t|)
# (Intercept)         0.844742   0.141996   5.949 9.57e-09 ***
# min_dist_to_ligand -0.024747   0.006522  -3.794 0.000188 ***
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#
# Residual standard error: 0.9707 on 238 degrees of freedom
# Multiple R-squared:  0.05704, Adjusted R-squared:  0.05308
# F-statistic:  14.4 on 1 and 238 DF,  p-value: 0.0001879
```

```
allosteric_sites <- c(286, 390, 258)

merged_df_residue <- merged_df_residue %>%
  mutate(site_class = case_when(
    new_position %in% orthosteric_sites ~ "orthosteric",
    new_position %in% allosteric_sites ~ "allosteric",
    TRUE ~ "other"
  ))

label_df <- merged_df_residue %>%
  group_by(site_class) %>%
  summarise(
    n = n(),
    median_val = median(abs(loess_residual_avg), na.rm = TRUE),
    .groups = "drop"
  )

merged_df_residue$site_class <- factor(merged_df_residue$site_class, levels = c("orthosteric", "alloste

p3 <- ggplot(merged_df_residue, aes(x = site_class, y = abs(loess_residual_avg), fill = site_class)) +
  geom_violin(trim = FALSE, scale = "width", alpha = 0.8, color = NA)+
  geom_jitter(width = 0.15, size = 2, alpha = 0.7, color = "lightgrey") +
  stat_summary(fun = median, geom = "crossbar", width = 0.4, color = "black", fatten = 1) +
  stat_summary(fun = median, geom = "point", shape = 23, size = 2, fill = "black", color = "black", str
  # Add sample size n=xxx above each group
  geom_text(
    data = label_df,
    aes(x = site_class, y = max(abs(merged_df_residue$loess_residual_avg)) * 1.1,
        label = paste0("n = ", n)),
    inherit.aes = FALSE,
    size = 4
  ) +
  geom_text(
  data = label_df,
  aes(x = site_class, y = median_val + 0.5, label = sprintf("%.2f", median_val)),
  inherit.aes = FALSE,
  size = 5
) +
  # Significance bars
  geom_signif(
    comparisons = list(
      c("orthosteric", "allosteric"),
      c("orthosteric", "other"),
      c("allosteric", "other")
    ),
    map_signif_level = FALSE,
    test = "wilcox.test",
    step_increase = 0.1,
    tip_length = 0.01
  ) +

  # Labels and theme
  labs(
    title = "CASP1",
    subtitle = "",
    x = "",
    y = "|Median ESM1v - ddGf residual|"
  ) +
  scale_fill_manual(values = c(
    "orthosteric" = "orange",
    "allosteric" = "cyan3",
    "other" = "darkgreen"
  )) +
  theme_classic() +
  theme(legend.position = "none")
ggsave("/Users/xl7/Documents/0.Projects/01.protein-seq-evo-v1/figs/panels/fig2_casp1_p3.pdf",
       plot = p3, width = 3, height = 4, dpi = 300)
p3
```

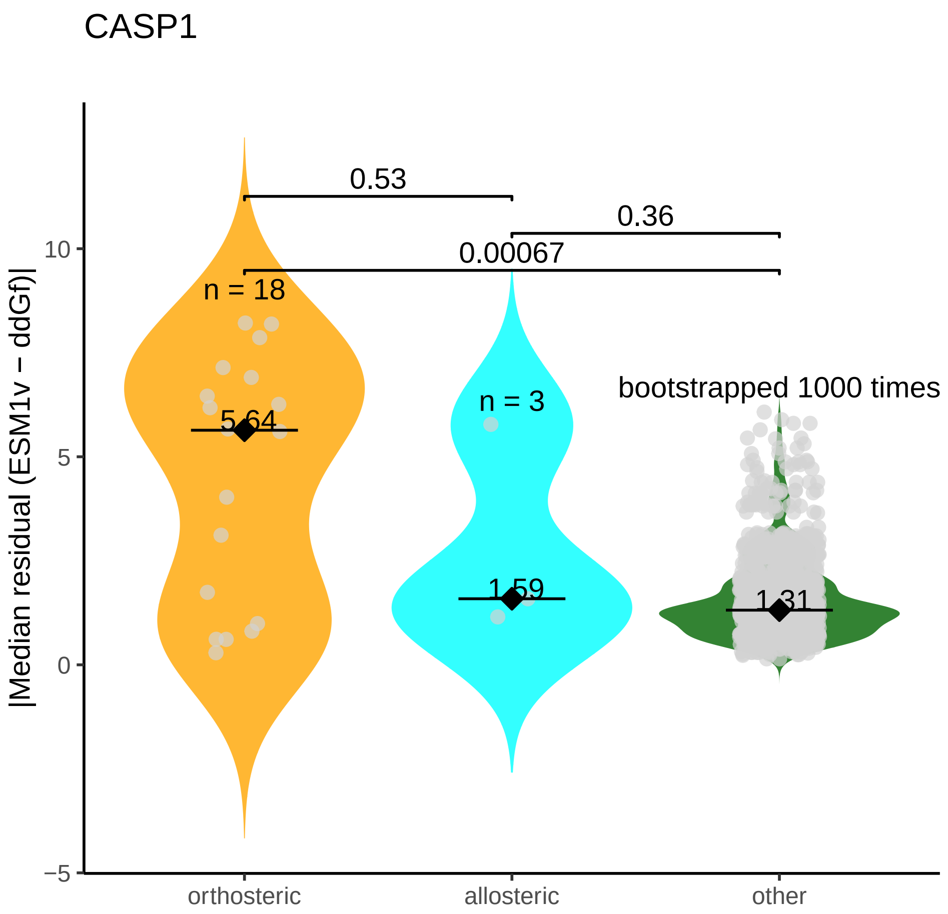


```r
set.seed(11)

# Get fixed per-residue medians
fixed_df <- merged_df_residue %>%
  filter(site_class %in% c("orthosteric", "allosteric")) %>%
  mutate(median_resid = abs(loess_residual_avg)) %>%
  dplyr::select(site_class, median_resid)

# Bootstrap medians for 'other' group
n_sample <- sum(merged_df_residue$site_class == "allosteric")

boot_medians_other <- map_dfr(1:1000, function(i) {
  sampled <- merged_df_residue %>%
    filter(site_class == "other") %>%
    slice_sample(n = n_sample)

  tibble(
    site_class = "other",
    median_resid = median(abs(sampled$loess_residual_avg), na.rm = TRUE),
    replicate = i
  )
})
```

```
# Combine all
plot_df <- bind_rows(
  fixed_df %>% mutate(replicate = NA),
  boot_medians_other
)
head(plot_df)
```

```
## # A tibble: 6 x 3
##   site_class  median_resid replicate
##   <chr>              <dbl>     <int>
## 1 orthosteric         6.26        NA
## 2 orthosteric         5.67        NA
## 3 orthosteric         7.14        NA
## 4 orthosteric         4.03        NA
## 5 orthosteric         6.46        NA
## 6 orthosteric         0.290       NA
```

```
# Labels
label_df <- plot_df %>%
  group_by(site_class) %>%
  summarise(
    n = n(),
    median_val = median(median_resid),
    y_max = max(median_resid),
    .groups = "drop"
  )

label_df <- label_df %>%
  mutate(n_label = case_when(
    site_class == "other" ~ "bootstrapped 1000 times",
    TRUE ~ paste0("n = ", n)
  ))

plot_df$site_class <- factor(plot_df$site_class, levels = c("orthosteric", "allosteric", "other"))

# Plot
p4 <- ggplot(plot_df, aes(x = site_class, y = median_resid, fill = site_class)) +
  geom_violin(data = plot_df,
              trim = FALSE, scale = "width", alpha = 0.8, color = NA) +

  geom_jitter(data = plot_df ,
              width = 0.15, size = 2, alpha = 0.7, color = "lightgrey") +

  stat_summary(fun = median, geom = "crossbar", width = 0.4, color = "black", fatten = 1) +
  stat_summary(fun = median, geom = "point", shape = 23, size = 2.5,
               fill = "black", color = "black", stroke = 0.7) +

 geom_text(
  data = label_df,
  aes(x = site_class, y = y_max * 1.1, label = n_label),
  inherit.aes = FALSE,
  size = 4) +
  geom_text(
    data = label_df,
    aes(x = site_class, y = median_val + 0.25, label = sprintf(" %.2f", median_val)),
    inherit.aes = FALSE,
    size = 4
  ) +

  geom_signif(
    comparisons = list(
      c("orthosteric", "other"),
      c("allosteric", "other"),
       c("allosteric", "orthosteric")
    ),
    test = "wilcox.test",
    map_signif_level = FALSE,
    step_increase = 0.1,
    tip_length = 0.01
  ) +

  labs(
    title = "CASP1",
    subtitle = "",
    x = "",
    y = "|Median residual (ESM1v - ddGf)|"
  ) +
  scale_fill_manual(values = c(
    "orthosteric" = "orange",
    "allosteric" = "cyan",
    "other" = "darkgreen"
  )) +
  theme_classic() +
  theme(legend.position = "none")

ggsave("/Users/xl7/Documents/0.Projects/01.protein-seq-evo-v1/figs/panels/fig2_casp1_p4.pdf",
       plot = p4, width = 3, height = 4, dpi = 300)
p4
```


CASP1
|Median residual (ESM1v − ddGf)|
10
5
0
−5
orthosteric
allosteric
other
0.53
0.36
0.00067
n = 18
n = 3
bootstrapped 1000 times
5.64
1.59
1.31