Analysis and figure plotting for molnupiravir analysis

Analysis of data from mutation annotated tree

```r
CtoTthreshold = 0.2
GtoAthreshold = 0.25
transitionthreshold = 0.9


red <- "#e31919"
blue1 <- "#5450f2"
threshold_branch_length <- 10
library(Biostrings)
```

```
Loading required package: BiocGenerics


Attaching package: 'BiocGenerics'

The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
    table, tapply, union, unique, unsplit, which.max, which.min

Loading required package: S4Vectors

Loading required package: stats4


Attaching package: 'S4Vectors'

The following objects are masked from 'package:base':

    expand.grid, I, unname

Loading required package: IRanges

Loading required package: XVector

Loading required package: GenomeInfoDb


Attaching package: 'Biostrings'

The following object is masked from 'package:base':

    strsplit
```

```r
library(tidyverse)
```

```
-- Attaching packages --------------------------------------- tidyverse 1.3.2 --

v ggplot2 3.4.2     v purrr   1.0.1
v tibble  3.2.1     v dplyr   1.1.2
v tidyr   1.3.0     v stringr 1.5.0
v readr   2.1.4     v forcats 1.0.0
-- Conflicts ------------------------------------------ tidyverse_conflicts() --
x dplyr::collapse()   masks Biostrings::collapse(), IRanges::collapse()
x dplyr::combine()    masks BiocGenerics::combine()
```

```
x purrr::compact()    masks XVector::compact()
x dplyr::desc()       masks IRanges::desc()
x tidyr::expand()     masks S4Vectors::expand()
x dplyr::filter()     masks stats::filter()
x dplyr::first()      masks S4Vectors::first()
x dplyr::lag()        masks stats::lag()
x ggplot2::Position() masks BiocGenerics::Position(), base::Position()
x purrr::reduce()     masks IRanges::reduce()
x dplyr::rename()     masks S4Vectors::rename()
x dplyr::slice()      masks XVector::slice(), IRanges::slice()
```

```
data_nodes <- read_tsv("~/Dropbox/new_mov2/all_nodes.tsv.gz")
```

```
Rows: 17849624 Columns: 19
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr   (4): node_id, consensus_country, consensus_year, age
dbl  (14): num_descendants, date_length, A>C, A>G, A>T, C>A, C>G, C>T, G>A, ...
date  (1): date

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
data_nodes <- data_nodes %>% mutate(total_muts = `A>C` + `A>G` + `A>T` + `C>A` + `C>G` + `


data_muts <- read_tsv("~/Dropbox/new_mov2/all_node_muts.tsv.gz")
```

```
Rows: 17193547 Columns: 11
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (8): node_id, original_nt, alternative_nt, gene, original_aa, alternativ...
dbl (2): nt_index, aa_index
lgl (1): is_synonymous

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
parenthood <- read_tsv("~/Dropbox/new_mov2/parenthood.tsv.gz")
```

```
Rows: 17849623 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (2): child, parent

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
find_children <- function(parenthood, parent) {
  # Find the immediate children of the parent
  children <- parenthood$child[parenthood$parent == parent]

  # Initialize a vector to store all descendants
  all_descendants <- c()

  # Loop through each child and find their descendants
  for (child in children) {
    # Add the child to the list of descendants
    all_descendants <- c(all_descendants, child)

    # Recursively find the descendants of the child
    child_descendants <- find_children(parenthood, child)

    # Add the descendants of the child to the list of all descendants
    all_descendants <- c(all_descendants, child_descendants)
  }

  return(all_descendants)
}

get_parent <- function(parenthood, node) {
  # Find the parent of the node
  parent <- parenthood$parent[parenthood$child == node]

  # If there is no parent (i.e., the node is the root), return NULL
  if (length(parent) == 0) {
    return(NULL)
  }
  return(parent)
}

data_muts <- data_muts %>% filter(gene != "ORF1a")
```

```
library(tidyverse)
library(cowplot)
data2 <- read_tsv("~/Dropbox/metadata_2023-04-29_01-16.tsv.gz", col_select = c("date", "co
```

```
Rows: 15198803 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (2): date, country

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
data3 <- data2 %>%
  select(date, country) %>%
  extract(date, "(\\d{4})", into = "year")
countries_totals <- data3 %>%
  group_by(year, country) %>%
  tally() %>%
  mutate(total_genomes = n)

countries_totals
```

```
# A tibble: 724 x 4
# Groups:   year [10]
   year  country         n total_genomes
   <chr> <chr>       <int>         <int>
 1 2010  Cambodia        2             2
 2 2013  China           1             1
 3 2017  China           7             7
 4 2018  China           1             1
 5 2019  China          34            34
 6 2020  Afghanistan     9             9
 7 2020  Albania         7             7
 8 2020  Algeria       95            95
 9 2020  Andorra        1             1
10 2020  Angola       151           151
# i 714 more rows
```

```r
library(ggrepel)

tallied_big <- data_nodes %>%
  dplyr::rename(country = consensus_country, year = consensus_year) %>%
  filter(flagged, total_muts >= threshold_branch_length) %>%
  group_by(country, year) %>%
  tally() %>%
  dplyr::rename(ga_branches = n) %>%
  full_join(countries_totals) %>%
  replace_na(list("ga_branches" = 0))
```

```
Joining with `by = join_by(country, year)`
```

```r
tallied <- tallied_big %>% filter(year == "2022")


# Define approved and not_approved countries
approved <- c(
  "USA", "United Kingdom", "Germany", "Denmark", "Japan", "India", "Australia", "Israel", 
  "Russia", "South Korea", "New Zealand", "Belgium", "Mauritius", "Vietnam", "Thailand", "
)
not_approved <- c(
  "France", "Canada", "Sweden", "Netherlands", "Finland", "Switzerland", "Norway", "Irelan
)

# Define usage
usage <- c(
  "Australia" = "\n(100 per 10k)",
  "United Kingdom" = "\n(5 per 10k)",
  "Japan" = "(50 per 10k)",
  "Italy" = "\n(10 per 10k)"
)

# List of years
years <- c("2020", "2021", "2022", "2023")
```

```r
lightpurple <- "#c39ecd"
darkpurple <- "#77488c"
darkorange <- "#fe670a"
lightorange <- "#f1ae85"
midorange <- "#ff883c"
year_pal <- c(lightpurple, darkpurple, darkorange, lightorange)
names(year_pal) <- years



# Loop through each year
for (i in 0:length(years)) {
  # Subset data
  data_subset <- data_nodes %>%
    filter(total_muts > 20, consensus_year %in% years[0:i])

  # Define plot
  scatter <- ggplot(data_subset, aes(x = `G>A` / total_muts, y = transitions / total_muts,
    geom_point() +
    theme_bw() +
    labs(x = "G\u00adto\u00adA proportion", y = "Transition proportion", color = "Year") +
    scale_color_manual(values = year_pal) +
    theme(legend.position = "bottom") +
    scale_x_continuous(label = scales::percent) +
    scale_y_continuous(label = scales::percent) +
    coord_cartesian(xlim = c(0, 0.65), ylim = c(0, 1))

  # Save plot
  ggsave(paste0("big_scatter_big_", paste(years[0:i], collapse = "_"), ".pdf"), plot = sca
  ggsave(paste0("scatter_big_", paste(years[0:i], collapse = "_"), ".pdf"), plot = scatter
}


scatter <- scatter +
  annotate("rect", xmin = 0.25, xmax = 0.6, ymin = 0.6, ymax = 1.05, fill = NA, color = "#



tallied$approved <- case_when(
  tallied$country %in% approved ~ "Available",
  tallied$country %in% not_approved ~ "Not available",
  TRUE ~ "Not identified"
```

```
)

country_plot_data = tallied %>% filter(country != "?", total_genomes > 500, year == "2022"

library(knitr)


library(knitr)
library(kableExtra)
```

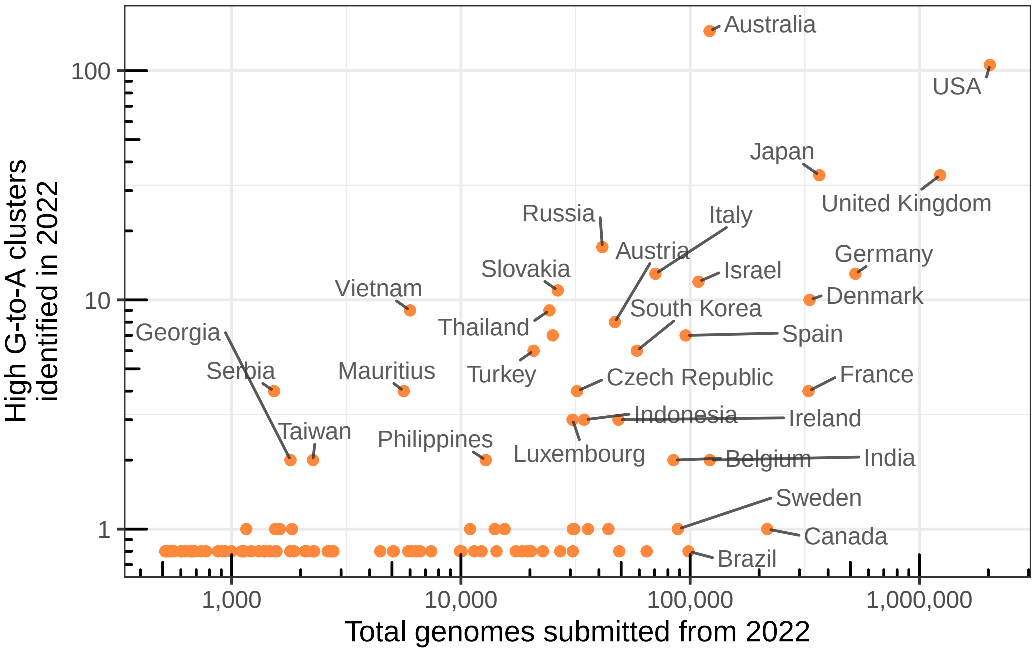
```
Attaching package: 'kableExtra'

The following object is masked from 'package:dplyr':

    group_rows
```

```
forlatex = country_plot_data %>% select(country, ga_branches,total_genomes) %>% arrange(-t

country_plot_data
```

```
# A tibble: 103 x 7
# Groups:   country [103]
   country        year  ga_branches      n total_genomes approved      usage
   <chr>          <chr>       <int>  <int>         <int> <fct>         <chr>
 1 Australia      2022          149 121602        121602 Available     "\n(100 ~
 2 Austria        2022            8  46962         46962 Available     ""
 3 Belgium        2022            2  84600         84600 Available     ""
 4 Cambodia       2022            1   1833          1833 <NA>          ""
 5 Canada         2022            1 217040        217040 Not available ""
 6 Czech Republic 2022            4  32124         32124 Available     ""
 7 Denmark        2022           10 332006        332006 Available     ""
 8 Egypt          2022            1   1621          1621 <NA>          ""
 9 France         2022            4 328527        328527 Not available ""
10 Georgia        2022            2   1805          1805 <NA>          ""
# i 93 more rows
```

```
names(forlatex) <- c("Country", "High G-to-A branches in 2022", "Total genomes in 2022")


latex_table <- kable(forlatex, "latex", booktabs = TRUE,  linesep = "" ,
                     col.names = names(forlatex),
                     align = c('l', 'r', 'r'))
writeLines(latex_table, "~/movmanuscript2/Figures2/countrytable.tex")


country_comp <- ggplot(
  country_plot_data,
  aes( # color = approved,
    x = total_genomes, y = ifelse(ga_branches == 0, 0.8, ga_branches), label = country
  )
) +
  geom_point(alpha = 1, color = midorange) +
  scale_x_log10(labels = scales::comma) +
  scale_y_log10() +
  geom_text_repel(alpha = 0.8, max.overlaps = 7, force = 50, min.segment.length = 0, lineh
  theme_bw() +
  labs(x = "Total genomes submitted from 2022", y = "High G\u00adto\u00adA clusters\nident
  theme(legend.position = "none") +
  annotation_logticks()


country_comp
```

```
Warning: ggrepel: 73 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```

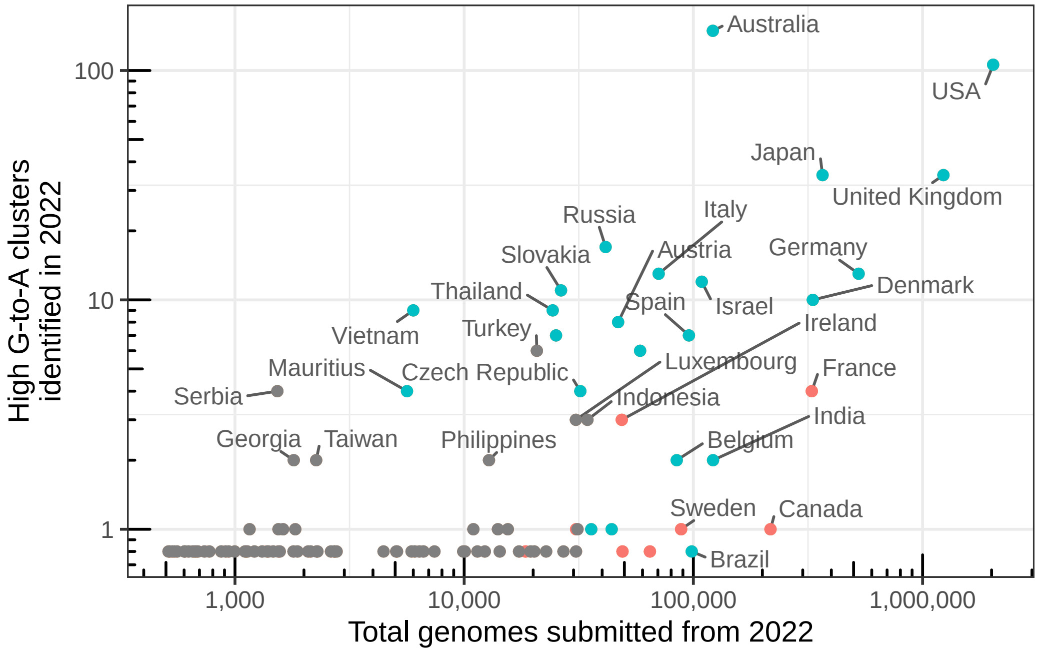


```
ggsave("country_scatter_big.pdf", width = 4, height = 3.5)
```

```
Warning: ggrepel: 76 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```

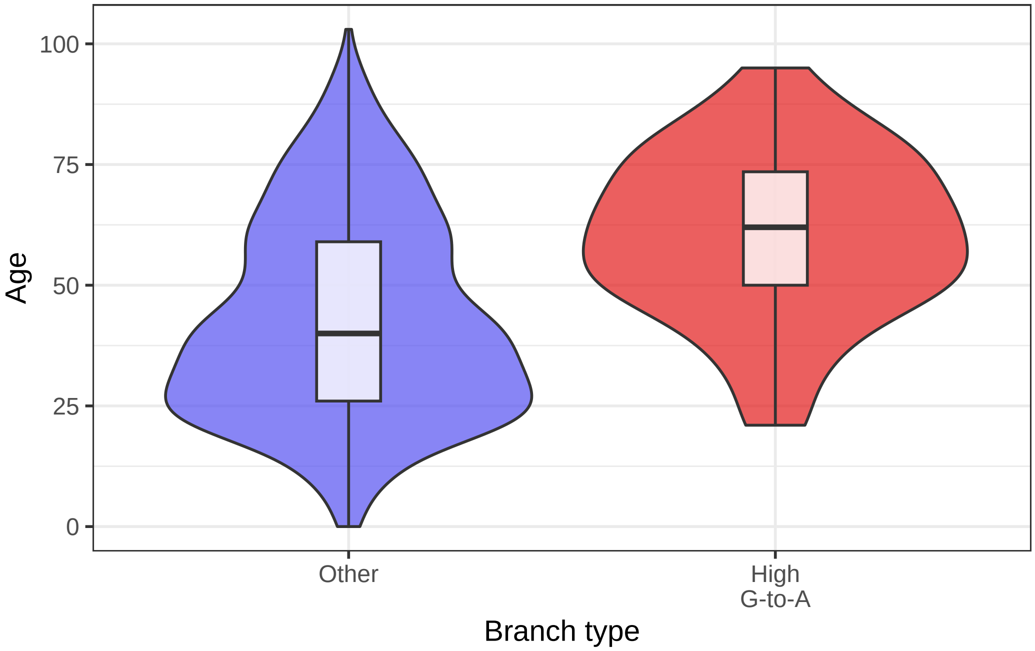
```
country_comp + geom_point(aes(color = approved))
```

```
Warning: ggrepel: 73 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



```
recents <- data_nodes %>% filter(total_muts >= threshold_branch_length, consensus_year ==

recents$branch_type <- ifelse(recents$flagged, "High\nG\u00adto\u00adA", "Other")
recents$branch_type <- fct_relevel(recents$branch_type, "Other")


age_dataset <- recents %>% filter(consensus_country %in% c("USA"), total_muts > 0)
#age_dataset <- age_dataset %>% filter(num_descendants==1)
age <- ggplot(
  age_dataset,
  aes(x = branch_type, y = as.numeric(age), fill = branch_type)
) +
  geom_violin(alpha = 0.7) +
  # geom_jitter(height=0) +
  theme_bw() +
  geom_boxplot(alpha = 0.8, width = 0.15, fill = "white") +
  labs(x = "Branch type", y = "Age") +
  scale_fill_manual(values = c("High\nG\u00adto\u00adA" = "#e31919", "Other" = "#5450f2"))
  theme(legend.position = "none")
age
```

```
Warning in FUN(X[[i]], ...): NAs introduced by coercion
Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning: Removed 1504 rows containing non-finite values (`stat_ydensity()`).

Warning: Removed 1504 rows containing non-finite values (`stat_boxplot()`).
```



```
t.test(as.numeric(age) ~ branch_type, data = age_dataset)
```

```
Warning in eval(predvars, data, env): NAs introduced by coercion


    Welch Two Sample t-test

data:  as.numeric(age) by branch_type
t = -7.6281, df = 74.023, p-value = 6.512e-11
alternative hypothesis: true difference in means between group Other and group High
GtoA is not equal to 0
95 percent confidence interval:
 -21.69284 -12.70724
sample estimates:
      mean in group Other mean in group High\nGtoA
                 43.07774                   60.27778
```

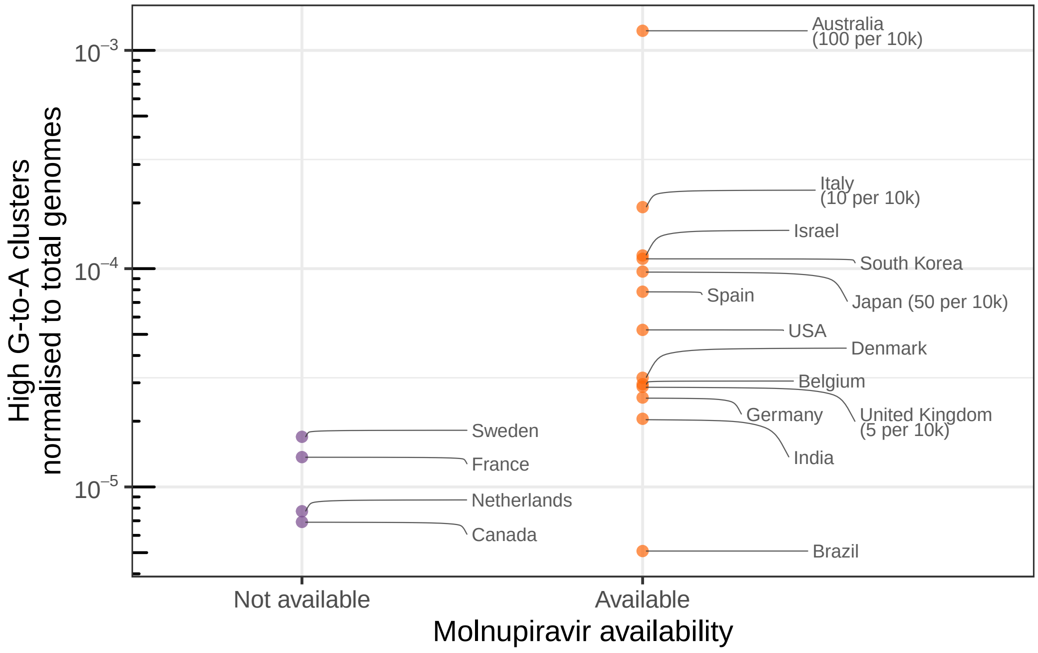
```
ggsave("age_violins.pdf", width = 3, height = 3)
```

```
Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning: Removed 1504 rows containing non-finite values (`stat_ydensity()`).

Warning: Removed 1504 rows containing non-finite values (`stat_boxplot()`).
```

```
set.seed(339)
availability_dataset <- tallied %>%
  filter(country != "?", total_genomes > 50000) %>%
  mutate(usage = usage[country]) %>%
  mutate(usage = ifelse(is.na(usage), "", usage)) %>%
  mutate(approved = factor(as.character(approved), levels = c("Not available", "Available"
availability_plot <- ggplot(availability_dataset, aes(color = approved, x = approved, y =
  geom_point(alpha = 0.7) +
  scale_y_log10() +
  geom_text_repel(
    alpha = 0.8, force = 10, min.segment.length = 0, lineheight = .65, size = 2.5, color =
    # do not pull text toward the point at (0,0)
    max.time = 3,
    segment.square = TRUE,
    segment.size = 0.2,
    segment.curvature = 0.3,
    max.iter = 1e7, nudge_x = 0.5,
    max.overlaps = Inf,
    hjust = 0
  ) +
  theme_bw() +
  labs(x = "Molnupiravir availability", color = "Molnupiravir", y = "High G\u00adto\u00adA
  scale_color_manual(values = c("Not identified" = "gray", "Available" = darkorange, "Not
  theme(legend.position = "none") +
  annotation_logticks(sides = "l") +
  scale_x_discrete(
    expand = expansion(mult = c(0.5, 1.15))
  )
availability_plot <- availability_plot +
  scale_y_log10(labels = function(x) {
    expression_strs <- sapply(x, function(x_val) {
      if(is.na(x_val)){
        return(NA)
      }
      if (x_val == 0) {
        return("0")
      }
      log_val <- log10(x_val)
      paste0("10^", log_val)
    })
    parse(text = expression_strs)
  })
```

```
Scale for y is already present.
Adding another scale for y, which will replace the existing scale.
```

```
  availability_plot
```

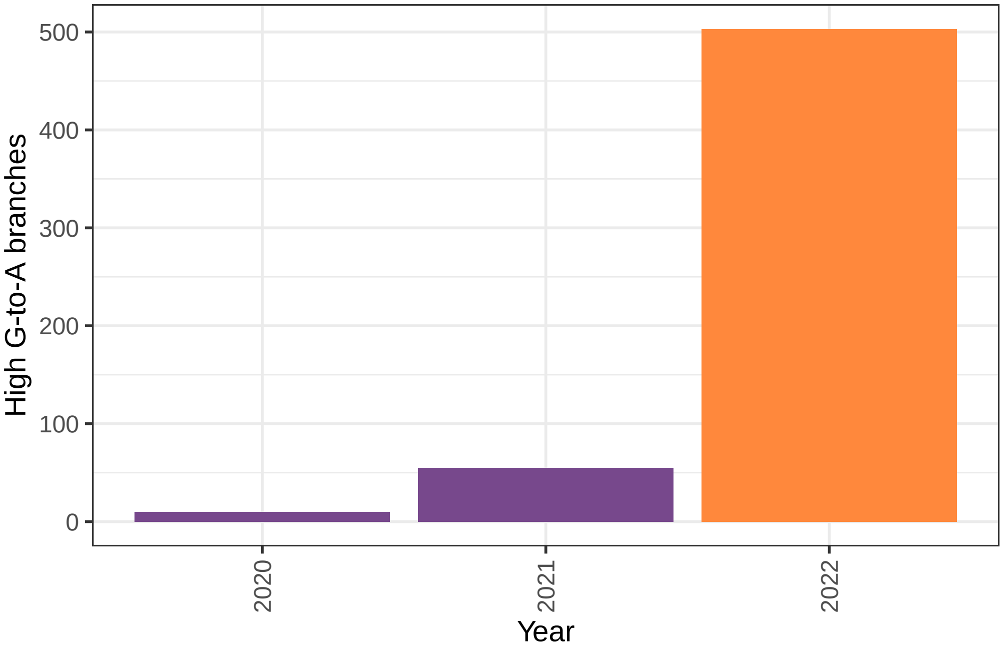


```
t.test(log10(ga_branches + 0.5) / total_genomes ~ approved, data = availability_dataset)
```

```

	Welch Two Sample t-test

data:  log10(ga_branches + 0.5)/total_genomes by approved
t = -2.6847, df = 10.935, p-value = 0.02133
alternative hypothesis: true difference in means between group Not available and group Availa
95 percent confidence interval:
 -1.165237e-05 -1.149542e-06
sample estimates:
mean in group Not available     mean in group Available
               3.307937e-08                6.434033e-06
```

```
ggsave("availability.pdf", width = 3.5, height = 3.5)

by_year <- data_nodes %>%
  filter(flagged, total_muts >= threshold_branch_length) %>%
  group_by(consensus_year) %>%
  tally()
```

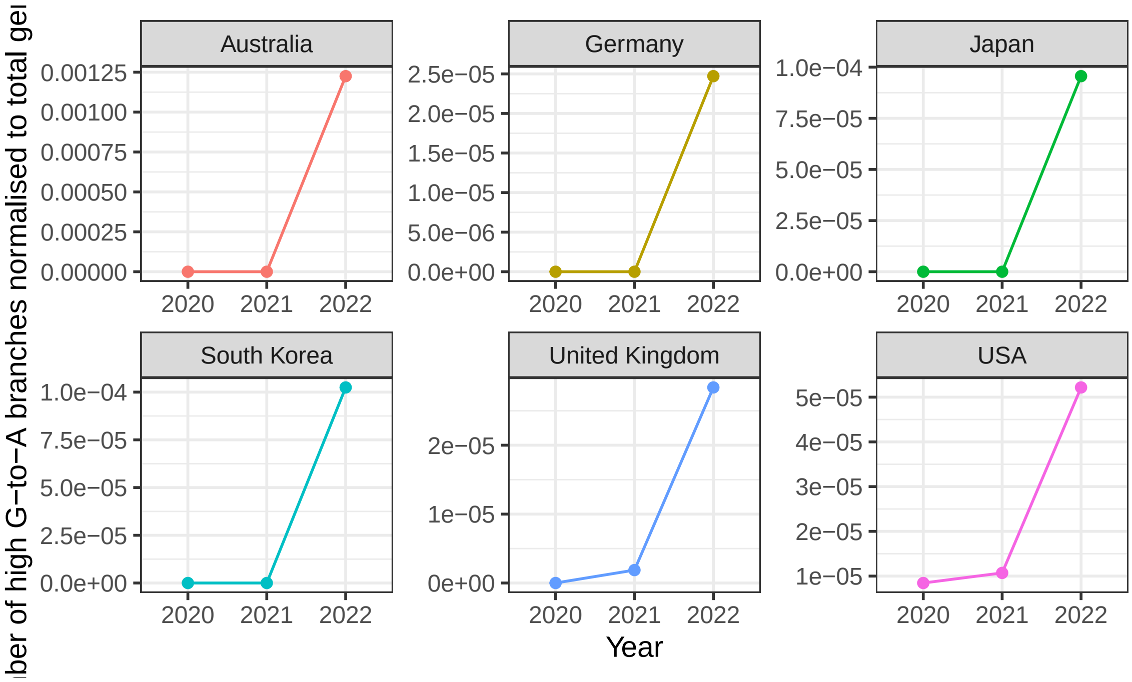
```r
by_year_plot <- ggplot(by_year %>% filter(consensus_year %in% c("2021", "2022", "2020")),
  geom_col() +
  theme_bw() +
  labs(x = "Year", y = "\nHigh G\u00adto\u00adA branches") +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
  scale_fill_manual(values = c(darkpurple, darkpurple, midorange)) +
  theme(legend.position = "none")
by_year_plot
```



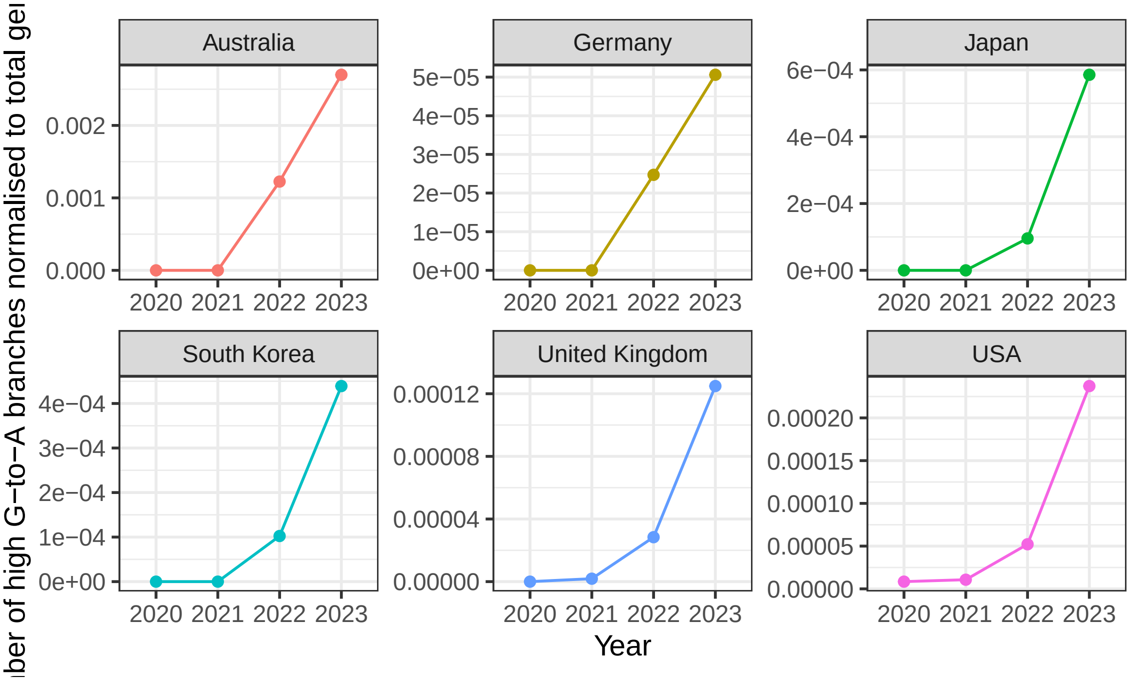
```r
ggsave("byyearplot.pdf", width = 2, height = 3)
```

We also display data on timecourse where we normalise for total genome numbers, use a non log axis.

```r
tallied_big <- tallied_big %>% mutate(p = (ga_branches) / total_genomes)

ggplot(tallied_big %>% filter(country %in% c("Australia", "United Kingdom", "USA", "Japan"
  geom_line() +
  geom_point() +
  theme_bw() +
  facet_wrap(~country, scales = "free") +
  theme(legend.position = "none") +
  labs(y = "Number of high G-to-A branches normalised to total genomes", x = "Year")
```



```
ggsave("~/movmanuscript2/Figures2/supp-countries_timeline.pdf", width = 7.5, height = 4.5)


ggplot(tallied_big %>% filter(country %in% c("Australia", "United Kingdom", "USA", "Japan"
  geom_line() +
  geom_point() +
  theme_bw() +
  facet_wrap(~country, scales = "free") +
  theme(legend.position = "none") +
  labs(y = "Number of high G-to-A branches normalised to total genomes", x = "Year")
```



Processing and analysis of existing genomic datasets

```
library(tidyverse)
tidyverse_conflicts()
```

```
-- Conflicts ------------------------------------------ tidyverse_conflicts() --
x dplyr::collapse()        masks Biostrings::collapse(), IRanges::collapse()
x dplyr::combine()         masks BiocGenerics::combine()
x purrr::compact()         masks XVector::compact()
x dplyr::desc()            masks IRanges::desc()
x tidyr::expand()          masks S4Vectors::expand()
x dplyr::filter()          masks stats::filter()
x dplyr::first()           masks S4Vectors::first()
x kableExtra::group_rows() masks dplyr::group_rows()
x dplyr::lag()             masks stats::lag()
x ggplot2::Position()      masks BiocGenerics::Position(), base::Position()
x purrr::reduce()          masks IRanges::reduce()
x dplyr::rename()          masks S4Vectors::rename()
x dplyr::slice()           masks XVector::slice(), IRanges::slice()
```

```r
nuc_genome_counts <- read_csv("./context_count.csv") %>% dplyr::rename(
  par = residue, context_before = residue_before, context_after = residue_after,
  genome_count = count
)
```

```
Rows: 64 Columns: 4
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (3): residue_before, residue, residue_after
dbl (1): count

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
a <- read_csv("./molnupiravir_rescaled_samples.csv") %>% mutate(trial = "2", treat = "mov"
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
b <- read_csv("./MOV_rescaled_contexts_only.csv") %>% mutate(trial = "2", treat = "mov", c
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
c <- read_csv("./naive_rescaled_contexts_only.csv") %>% mutate(trial = "2", treat = "naive
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
d <- read_csv("./naive_rescaled_samples.csv") %>% mutate(trial = "2", treat = "naive", con
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
e <- read_csv("./agile_placebo_spectrum.csv") %>% mutate(trial = "1", treat = "naive", con
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
f <- read_csv("./agile_molnupiravir_spectrum.csv") %>% mutate(trial = "1", treat = "mov",
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
g <- read_csv("./BA.1_SBS_spectrum_Ruis.csv") %>% mutate(trial = "3", treat = "normal", co
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
long <- read_csv("./long_branch_spectrum_rescaled.csv") %>% mutate(trial = "4", treat = "l
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
specific <- read_csv("./molnupiravir_spectrum_specific.csv") %>% mutate(trial = "5", treat
```

```
Rows: 192 Columns: 2
-- Column specification --------------------------------------------------------
Delimiter: ","
chr (1): Substitution
```

```
dbl (1): Number_of_mutations

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
colors <- c("#3055a2", "#221f20", "#da4837", "#939598", "#3f8347", "#edb9c0", "#4a68af", "
my_levels <- c("C\u00adto\u00adA", "C\u00adto\u00adG", "C\u00adto\u00adT", "T\u00adto\u00a



combo <- bind_rows(a, b, c, d, e, f, g, long, specific) %>%
  filter(!contexts_only) %>%
  separate(Substitution, into = c("context_before", "par", "mut", "context_after"), sep =

data <- combo %>% mutate(mutation_type = factor(paste0(par, "\u00adto\u00ad", mut),
  levels = my_levels
))
```

For convenience to get the total number of each type of mutation we reverse MutTui's normalisations of context numbers.

```
totals <- data %>%
  group_by(trial) %>%
  summarise(total = sum(Number_of_mutations))


normed <- data %>%
  inner_join(totals) %>%
  mutate(Number_of_mutations = Number_of_mutations / total)
```

```
Joining with `by = join_by(trial)`
```

```
multipled <- normed %>%
  inner_join(nuc_genome_counts) %>%
  mutate(Number_of_mutations = Number_of_mutations * genome_count)
```

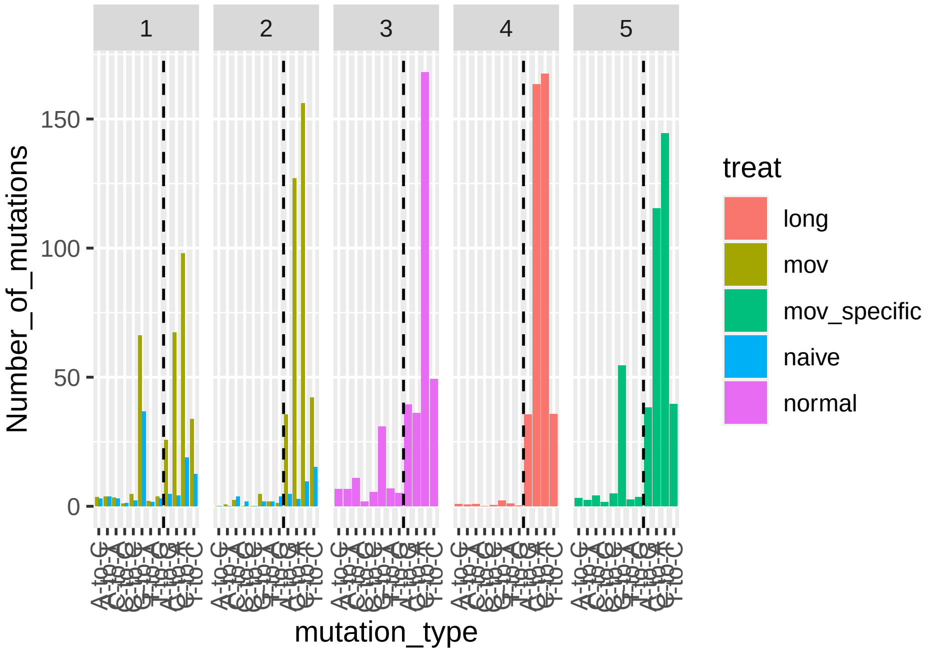
```
Joining with `by = join_by(context_before, par, context_after)`
```

```
just_class <- multipled %>%
  group_by(mutation_type, treat, trial) %>%
  summarise(Number_of_mutations = sum(Number_of_mutations))
```

```
`summarise()` has grouped output by 'mutation_type', 'treat'. You can override
using the `.groups` argument.
```

```
transversions <- c("A\u00adto\u00adC", "A\u00adto\u00adT", "C\u00adto\u00adA", "C\u00adto\

transitions <- c(
  "A\u00adto\u00adG", "G\u00adto\u00adA",
  "C\u00adto\u00adT",
  "T\u00adto\u00adC"
)


just_class <- just_class %>%
  mutate(mutation_type = fct_relevel(mutation_type,
    c(transversions, transitions),
    after = Inf
  ))
```

```
ggplot(just_class %>% filter() %>% arrange(mutation_type), aes(y = Number_of_mutations, x
  geom_col(position = "dodge") +
  facet_grid(. ~ trial) +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
  geom_vline(xintercept = 8.5, linetype = "dashed", color = "black")
```



```
# Directory where your TSV files are
dir <- "./tsv_files"

# List all .tsv files in the directory
files <- list.files(path = dir, pattern = "\\.tsv$", full.names = TRUE)

# Read all files into a list of tibbles, adding the file name as a new column
big_df <- map_dfr(files, ~ read_tsv(.x, col_names = c("index", "par", "A", "C", "G", "T"))
```

```
Rows: 29812 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29694 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T
```

```
i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29617 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29624 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28827 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 25577 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28243 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
Rows: 28934 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28601 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 27536 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29625 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29398 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28785 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
```

```
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 18869 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29494 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29322 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 27603 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29686 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T
```

```
i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29849 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29664 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29348 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29836 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29796 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29638 Columns: 6
```

```
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29668 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29635 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29691 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29662 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28896 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
```

```
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29625 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29761 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29656 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28572 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29602 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
```

```
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29651 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29507 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28393 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 24314 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29243 Columns: 6
-- Column specification ---------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 28345 Columns: 6
-- Column specification ---------------------------------------------------------
```

```
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29482 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29651 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29624 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 29663 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 27976 Columns: 6
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (1): par
dbl (5): index, A, C, G, T
```

```
i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
big_df <- big_df %>% mutate(total_depth = A + C + G + T)

big_df <- big_df %>% separate(file_name, into = c("treat", "patient", "timepoint"), sep =

long_df <- big_df %>%
  pivot_longer(
    cols = c(A, C, G, T),
    names_to = "base",
    values_to = "count"
  ) %>%
  filter(par != base, count > 0) %>%
  filter(count >= total_depth * 0.05, total_depth >= 100) %>%
  mutate(mutation_type = as.factor(paste0(par, "\u00adto\u00ad", base))) %>%
  filter(par != "N") %>%
  group_by(patient, index, par, base) %>%
  filter(row_number() == 1) # ensures we only count each mutation once



burdens <- long_df %>%
  filter(treat != "PAXLOVID") %>%
  group_by(treat, patient) %>%
  tally()

# Split mutation counts into two vectors based on treatment
naive_burden <- burdens %>%
  filter(treat == "NAIVE") %>%
  pull(n)
mov_burden <- burdens %>%
  filter(treat == "MOLNUPIRAVIR") %>%
  pull(n)

length(naive_burden)
```

```
[1] 5
```

```r
sd(naive_burden)
```

```
[1] 3.714835
```

```r
mean(naive_burden)
```

```
[1] 9.6
```

```r
length(mov_burden)
```

```
[1] 8
```

```r
sd(mov_burden)
```

```
[1] 63.19118
```

```r
mean(mov_burden)
```

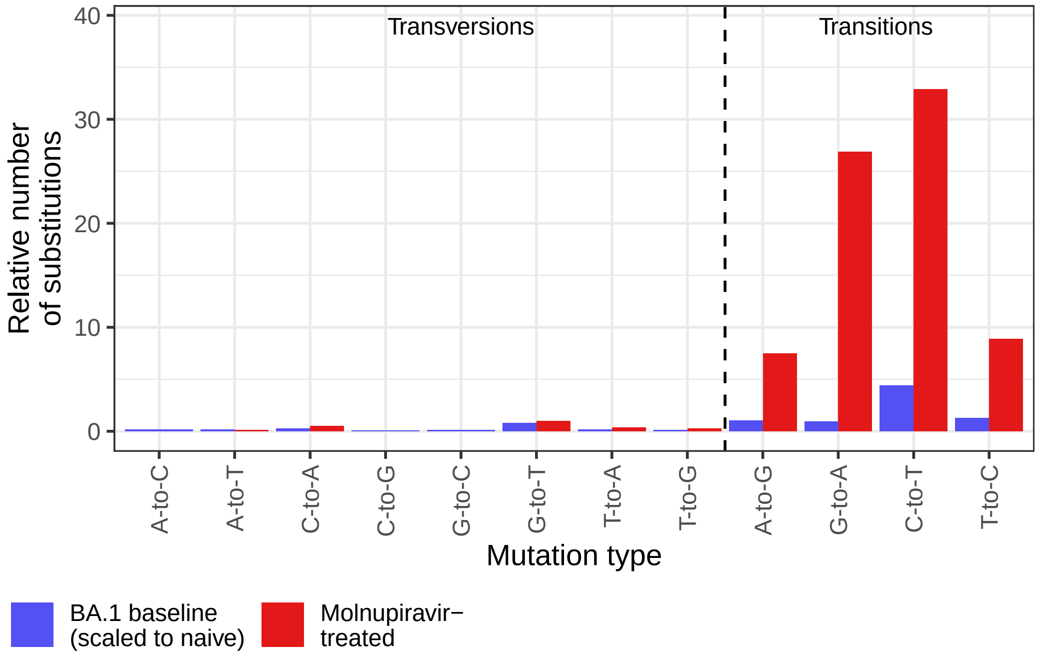
```
[1] 78.375
```

```r
n_patients_naive <- 5
n_patients_mov <- 8

ba1_basic <- just_class %>% filter(trial == 3)
ba1_normed <- ba1_basic %>% mutate(Number_of_mutations = Number_of_mutations * sum(naive_b
```

```r
lookup <- c("MOLNUPIRAVIR" = "mov", "NAIVE" = "normal")

mov_dataset <- long_df %>%
  group_by(mutation_type, treat) %>%
  tally() %>%
  filter(treat == "MOLNUPIRAVIR") %>%
  mutate(treat = "mov") %>%
  mutate(Number_of_mutations = n) %>%
  mutate(mutation_type = fct_relevel(mutation_type, c(transversions, transitions))) %>%
  mutate(Number_of_mutations = Number_of_mutations / n_patients_mov)
naive_dataset <- ba1_normed %>%
  mutate(treat = "normal") %>%
  mutate(mutation_type = fct_relevel(mutation_type, c(transversions, transitions))) %>%
  mutate(Number_of_mutations = Number_of_mutations / n_patients_naive)



relevant_dataset <- bind_rows(mov_dataset, naive_dataset)

relevant_dataset
```

```
# A tibble: 21 x 5
# Groups:   mutation_type [12]
   mutation_type treat      n Number_of_mutations trial
   <fct>         <chr>  <int>               <dbl> <chr>
 1 AtoG          mov       60               7.5   <NA>
 2 AtoT          mov        1               0.125 <NA>
 3 CtoA          mov        4               0.5   <NA>
 4 CtoT          mov      263              32.9   <NA>
 5 GtoA          mov      215              26.9   <NA>
 6 GtoT          mov        8               1     <NA>
 7 TtoA          mov        3               0.375 <NA>
 8 TtoC          mov       71               8.88  <NA>
 9 TtoG          mov        2               0.25  <NA>
10 CtoA          normal    NA               0.285 3
# i 11 more rows
```

```
a <- ggplot(relevant_dataset, aes(y = Number_of_mutations, x = mutation_type, fill = treat
  geom_col(position = "dodge") +
  geom_vline(xintercept = 8.5, linetype = "dashed", color = "black") +
  scale_fill_manual(values = c(blue1, red), labels = c("BA.1 baseline\n(scaled to naive)",
  theme_bw() +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
  labs(fill = "") +
  annotate("text", x = 5, y = 39, label = "Transversions", size = 3) +
  labs(x = "Mutation type", y = "Relative number\nof substitutions") +
  annotate("text", x = 10.5, y = 39, label = "Transitions", size = 3) +
  theme(
    legend.position = "bottom",
    legend.justification = c(0, 1),
    legend.margin = margin(t = 0, r = 0, b = 0, l = -45, unit = "pt")
  )
a
```



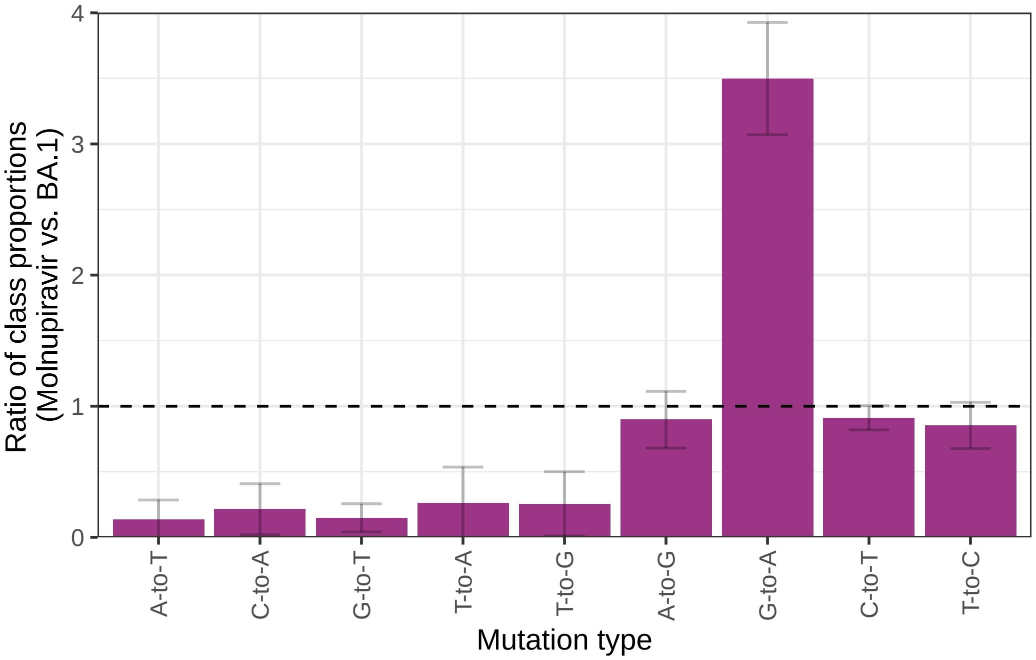
```
naive_props <- naive_dataset %>%
  ungroup() %>%
  mutate(p = Number_of_mutations / sum(Number_of_mutations))
# The BA.1 spectrum props is based on so many mutations (hundreds of thousands) that we ca

naive_props
```

```
# A tibble: 12 x 5
   mutation_type treat  trial Number_of_mutations       p
   <fct>         <chr>  <chr>               <dbl>   <dbl>
 1 CtoA          normal 3                  0.285  0.0297
 2 CtoG          normal 3                  0.0489 0.00509
 3 CtoT          normal 3                  4.39   0.457
 4 TtoA          normal 3                  0.178  0.0185
 5 TtoC          normal 3                  1.29   0.134
 6 TtoG          normal 3                  0.135  0.0141
 7 GtoT          normal 3                  0.806  0.0840
 8 GtoC          normal 3                  0.143  0.0149
 9 GtoA          normal 3                  0.946  0.0985
10 AtoT          normal 3                  0.176  0.0183
11 AtoG          normal 3                  1.03   0.107
12 AtoC          normal 3                  0.172  0.0180
```

```r
mov_for_props <- long_df %>%
  filter(treat == "MOLNUPIRAVIR") %>%
  ungroup()

resample_and_calc_ratios <- function(long_df) {
  resampled <- sample_n(mov_for_props, size = nrow(mov_for_props), replace = TRUE)
  props <- resampled %>%
    group_by(mutation_type) %>%
    tally() %>%
    mutate(p = n / sum(n))
  together <- inner_join(props, naive_props, by = "mutation_type") %>% mutate(ratio = p.x
  return(together %>% select(mutation_type, ratio))
}

bootstrap_count <- 100
bootstrap_ratios <- list()

for (i in 1:bootstrap_count) {
  bootstrap_ratios[[i]] <- resample_and_calc_ratios(long_df)
}

# Convert list to data frame
bootstrap_ratios_df <- bind_rows(bootstrap_ratios)
bootstrap_ratios_df
```

```
# A tibble: 841 x 2
   mutation_type ratio
   <fct>         <dbl>
 1 AtoG          0.891
 2 AtoT          0.174
 3 CtoA          0.107
 4 CtoT          0.816
 5 GtoA          3.84
 6 GtoT          0.285
 7 TtoA          0.259
 8 TtoC          0.843
 9 TtoG          0.340
10 AtoG          0.935
# i 831 more rows
```

```
proportions_wider <- bootstrap_ratios_df %>%
  group_by(mutation_type) %>%
  summarise(sd = sd(ratio), ratio = mean(ratio))



b <- ggplot(proportions_wider %>% mutate(mutation_type = fct_relevel(mutation_type, c(tran
  geom_col(position = "dodge", fill = "#9C3586") +
  scale_y_continuous(expand = c(0, 0)) +
  theme_bw() +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
  labs(x = "Mutation type", y = "Ratio of class proportions   \n(Molnupiravir vs. BA.1)")
  geom_hline(yintercept = 1, linetype = "dashed", color = "black") +
  geom_errorbar(alpha = 0.25, width = 0.4) +
  coord_cartesian(ylim = c(0, 4))

b
```



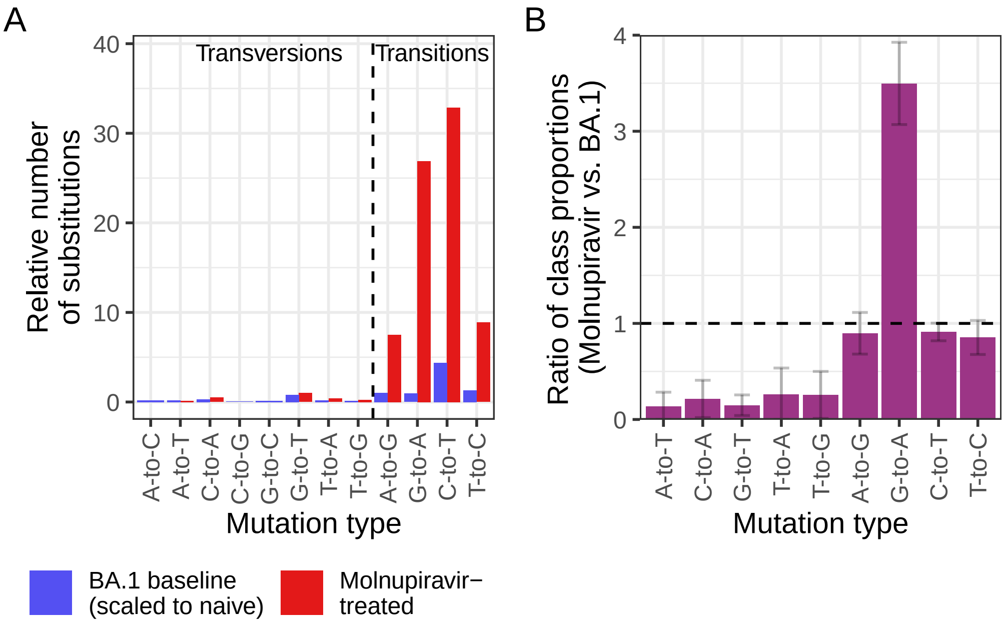
```r
proportions
```

```
function (x, margin = NULL)
{
    if (length(margin))
        sweep(x, margin, marginSums(x, margin), `/`, check.margin = FALSE)
    else x/sum(x)
}
<bytecode: 0x15d7e6be0>
<environment: namespace:base>
```

```r
library(patchwork)
```

```
Attaching package: 'patchwork'

The following object is masked from 'package:cowplot':

    align_plots
```

```r
ab <- a + b + plot_annotation(tag_levels = "A")
ab
```



```r
ggsave("a.pdf", a, width = 3, height = 3.5)
ggsave("b.pdf", b, width = 3, height = 3.5)


mov_props <- mov_for_props %>%
  group_by(mutation_type) %>%
  tally() %>%
  mutate(p = n / sum(n))


perform_sim <- function(n_sample, relevant_props) {
  # Set the number of iterations and the sample size
  n_iterations <- 10000
  15

  # Initialize a vector to hold the result of each iteration
  result <- vector(mode = "logical", length = n_iterations)


  # Run the simulation
  for (i in 1:n_iterations) {
    # Sample mutation types according to their probabilities
    sample_mutation <- sample(relevant_props$mutation_type, size = n_sample, replace = TRU

    # Calculate the proportions of each mutation type in the sample
    sample_prop <- table(sample_mutation) / n_sample

    # Calculate the transition proportion
    transition_prop <- sum(sample_prop[c("C\u00adto\u00adT", "G\u00adto\u00adA", "T\u00adt

    # Check whether the proportions meet the thresholds
    result[i] <- (sample_prop["C\u00adto\u00adT"] > CtoTthreshold & sample_prop["G\u00adto
  }

  # Calculate the proportion of iterations that meet the condition
  proportion <- sum(result) / n_iterations
  proportion
  return(proportion)
}
# Define the mutation counts to consider
mutations <- c(10,11,12,13,14, 15, 20)

# Initialize vectors to hold results
sensitivity <- numeric(length(mutations))
specificity <- numeric(length(mutations))

# Loop over each mutation count
for (i in seq_along(mutations)) {
  # Compute sensitivity and specificity
  sensitivity[i] <- perform_sim(mutations[i], mov_props)
  specificity[i] <- 1 - perform_sim(mutations[i], naive_props)
}

# Create a data frame with the results
results <- data.frame(
  Mutations = mutations,
  Sensitivity = sensitivity,
  Specificity = specificity
)

# Print the results
```

```
print(results)
```

```
  Mutations Sensitivity Specificity
1        10      0.4754      0.9884
2        11      0.6752      0.9655
3        12      0.5606      0.9899
4        13      0.6406      0.9895
5        14      0.6962      0.9860
6        15      0.7091      0.9850
7        20      0.6297      0.9984
```

```
library(ggpmisc)
```

```
Loading required package: ggpp


Attaching package: 'ggpp'

The following object is masked from 'package:ggplot2':

    annotate
```

```
library(ggtext)

normed
```

```
# A tibble: 1,344 x 10
   context_before par   mut   context_after Number_of_mutations trial treat
   <chr>          <chr> <chr> <chr>                       <dbl> <chr> <chr>
 1 A              C     A     A                         0.00147 2     mov
 2 A              C     A     C                         0       2     mov
 3 A              C     A     G                         0       2     mov
 4 A              C     A     T                         0       2     mov
 5 C              C     A     A                         0       2     mov
 6 C              C     A     C                         0       2     mov
 7 C              C     A     G                         0       2     mov
 8 C              C     A     T                         0       2     mov
 9 G              C     A     A                         0       2     mov
10 G              C     A     C                         0.00317 2     mov
# i 1,334 more rows
# i 3 more variables: contexts_only <lgl>, mutation_type <fct>, total <dbl>
```

```r
trial2 <- normed %>%
  filter((treat == "mov" & trial == "2")) %>%
  group_by(mutation_type) %>%
  mutate(Number_of_mutations = Number_of_mutations / sum(Number_of_mutations))
trial1 <- normed %>%
  filter((treat == "mov" & trial == "1")) %>%
  group_by(mutation_type) %>%
  mutate(Number_of_mutations = Number_of_mutations / sum(Number_of_mutations))
long <- normed %>%
  filter((trial == "4")) %>%
  select(-treat, -total, -contexts_only, -trial) %>%
  group_by(mutation_type) %>%
  mutate(Number_of_mutations = Number_of_mutations / sum(Number_of_mutations))
normal <- normed %>%
  filter((trial == "3")) %>%
  select(-treat, -total, -contexts_only, -trial) %>%
  group_by(mutation_type) %>%
  mutate(Number_of_mutations = Number_of_mutations / sum(Number_of_mutations))
normal
```

```
# A tibble: 192 x 6
# Groups:   mutation_type [12]
   context_before par   mut   context_after Number_of_mutations mutation_type
   <chr>          <chr> <chr> <chr>                       <dbl> <fct>
 1 A              C     A     A                          0.0423 CtoA
 2 A              C     A     C                          0.0618 CtoA
 3 A              C     A     G                          0.0655 CtoA
 4 A              C     A     T                          0.0737 CtoA
 5 C              C     A     A                          0.0922 CtoA
 6 C              C     A     C                          0.0506 CtoA
 7 C              C     A     G                          0.125  CtoA
 8 C              C     A     T                          0.0994 CtoA
 9 G              C     A     A                          0.0500 CtoA
10 G              C     A     C                          0.0386 CtoA
# i 182 more rows
```

```
merged <- normed %>%
  group_by(context_before, context_after, par, mut, treat, mutation_type) %>%
  summarise(Number_of_mutations = mean(Number_of_mutations)) %>%
  filter(treat == "mov")
```

```
`summarise()` has grouped output by 'context_before', 'context_after', 'par',
'mut', 'treat'. You can override using the `.groups` argument.
```

```
long_v_merged <- inner_join(long %>% rename(v1 = Number_of_mutations), merged %>% rename(v
```

```
Joining with `by = join_by(context_before, par, mut, context_after,
mutation_type)`
```

```
t1_v_merged <- inner_join(long %>% rename(v1 = Number_of_mutations), trial1 %>% rename(v2
```

```
Joining with `by = join_by(context_before, par, mut, context_after,
mutation_type)`
```

```
t2_v_merged <- inner_join(long %>% rename(v1 = Number_of_mutations), trial2 %>% rename(v2
```

```
Joining with `by = join_by(context_before, par, mut, context_after,
mutation_type)`
```

```
cosine_similarity_compute_fun <- function(data, ...) {
  force(data)

  x <- data$x
  y <- data$y

  similarity <- sum(x * y) / (sqrt(sum(x^2)) * sqrt(sum(y^2)))


  data.frame(x = 0, y = .11, label =paste0("c=",round(similarity, 3) ),color="black",hjust
}
```

```
StatCosineSimilarity <- ggproto(
  "StatCosineSimilarity",
  Stat,
  compute_group = cosine_similarity_compute_fun,
  required_aes = c("x", "y")
)

stat_cosine_similarity <- function(mapping = NULL, data = NULL, geom = "text",
                                   position = "identity", na.rm = FALSE, show.legend = NA,
                                   inherit.aes = TRUE, ...) {
  layer(
    stat = StatCosineSimilarity, data = data, mapping = mapping, geom = geom,
    position = position, show.legend = show.legend, inherit.aes = inherit.aes,
    params = list(na.rm = na.rm, ...)
  )
}


long_v_normal <- inner_join(long %>% rename(v1 = Number_of_mutations), normal %>% rename(v
```

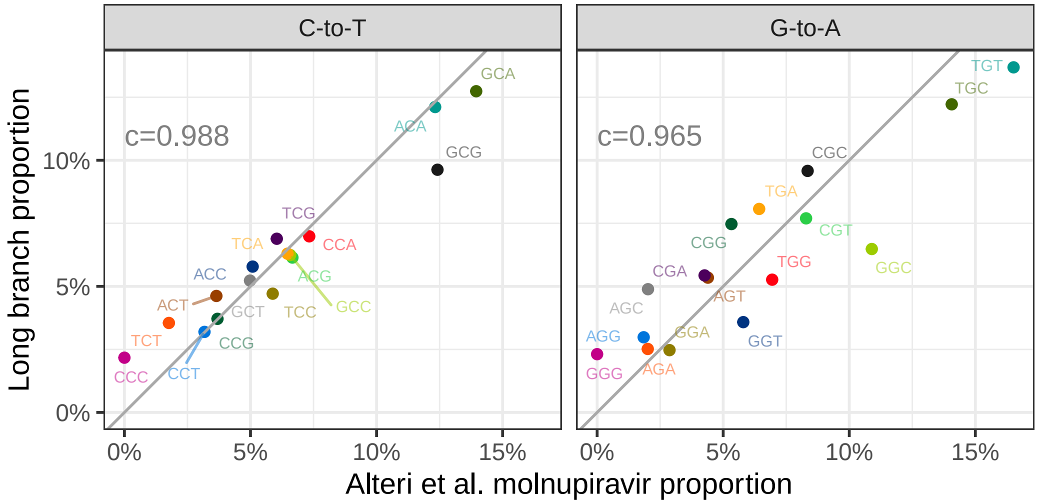
```
Joining with `by = join_by(context_before, par, mut, context_after,
mutation_type)`
```

```
oneset <- unique((t2_v_merged %>% filter(mutation_type %in% c("G\u00adto\u00adA")))$contex


library(pals)
```

```


Attaching package: 'pals'

The following object is masked from 'package:Biostrings':

    alphabet
```

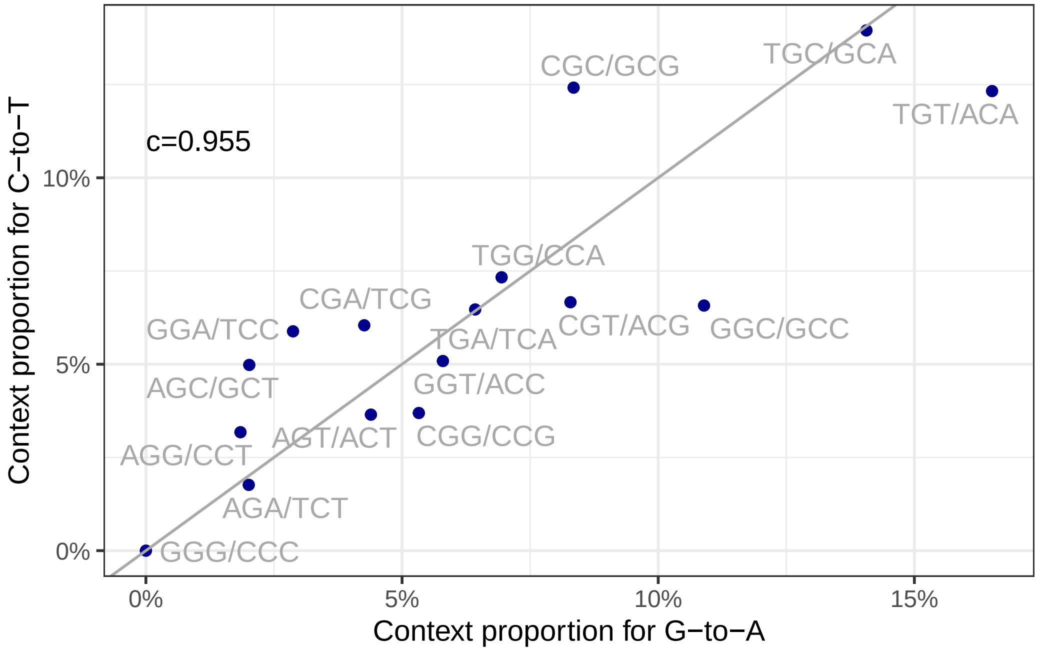
```
colors_16 <- unname(c(alphabet()[26:26], alphabet()[9], alphabet()[2:7], alphabet()[11:15]
reverse_complement <- function(context) {
  rev_nucleotide <- function(x) {
    switch(x,
      "A" = "T",
      "T" = "A",
      "C" = "G",
      "G" = "C",
      x
    )
  }
  rev_context <- sapply(strsplit(context, "")[[1]], rev_nucleotide)
  paste(rev(rev_context), collapse = "")
}

context_colors <- c()
for (i in 1:length(oneset)) {
  context <- oneset[i]
  reverse_context <- reverse_complement(context)

  if (!context %in% names(context_colors)) {
    context_colors[context] <- colors_16[i]
  }

  if (!reverse_context %in% names(context_colors)) {
    context_colors[reverse_context] <- colors_16[i]
  }
}


scatters <- ggplot(t2_v_merged %>% filter(mutation_type %in% c("G\u00adto\u00adA", "C\u00a
  geom_point() +
  labs(x = "Alteri et al. molnupiravir proportion", y = "Long branch proportion") +
  facet_wrap(~mutation_type, ncol = 2) +
  theme_bw() + stat_cosine_similarity()+
  coord_fixed(xlim = c(0, NA), ylim = c(0, NA)) +
  geom_abline(
    intercept = 0, slope = 1, # linetype = "black",
    color = "darkgray"
  ) +
  geom_text_repel(alpha = 0.5, size = 2, max.overlaps = Inf, force = 10) +
  scale_x_continuous(labels = scales::percent) +
  scale_y_continuous(labels = scales::percent) +
  scale_color_manual(values = context_colors) +
  theme(legend.position = "none")


scatters
```

```
Warning: The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```



```
start <- trial2 %>%
  mutate(context_full = paste0(context_before, par, context_after)) %>%
  mutate(rc_context = sapply(context_full, reverse_complement))
GtoA <- start %>% filter(mutation_type == "G\u00adto\u00adA")
CtoT <- start %>% filter(mutation_type == "C\u00adto\u00adT")
joint <- inner_join(GtoA, CtoT, by = c("context_full" = "rc_context"))
```

```
comp <- ggplot(joint, aes(x = Number_of_mutations.x, y = Number_of_mutations.y, label = pa
  geom_point(color = "darkblue") +
  theme_bw() +
  geom_abline(
    intercept = 0, slope = 1, # linetype = "black",
    color = "darkgray"
  ) + stat_cosine_similarity() +
  geom_text_repel(color = "darkgray") +
  scale_x_continuous(labels = scales::percent) +
  scale_y_continuous(labels = scales::percent) +
  labs(x = "Context proportion for G-to-A", y = "Context proportion for C-to-T")

comp
```



```
names(colors) <- my_levels

other_colors <- c("A" = "#111111", "C" = "#555555", "G" = "#999999", "T" = "#cccccc")
all_colors <- c(colors, other_colors)

colors_new <- all_colors
colors_new["A\u00adto\u00adG"] <- "#5c4987"
```

```
colors_new["T\u00adto\u00adC"] <- "#5377ad"

create_scatter_plot <- function(df, x_label, file_name) {
  plot <- ggplot(df %>%
    filter(mutation_type %in% c("G\u00adto\u00adA", "C\u00adto\u00adT", "A\u00adto\u00adG"
    mutate(label = context_full), aes(x = v2, y = v1, label = label, color = mutation_type
    geom_point() +
    labs(x = x_label, y = "Long branch proportion") +
    facet_wrap(~mutation_type, ncol = 2) +
    theme_bw() +
    stat_cosine_similarity() +
    coord_fixed(xlim = c(0, NA), ylim = c(0, NA)) +
    # geom_abline(intercept = 0, slope = 1, color = "darkgray")+
    geom_text_repel(alpha = 0.5, size = 2, max.overlaps = Inf, force = 10) +
    scale_x_continuous(labels = scales::percent) +
    scale_y_continuous(labels = scales::percent) +
    scale_color_manual(values = colors_new) +
    theme(legend.position = "none") +

    geom_smooth(method = "lm", se = FALSE, color = "darkgray", fullrange = F, size = 1)



  return(plot)
}

# Call the function three times with different dataframes and labels
scatters_supplemental <- create_scatter_plot(t2_v_merged, "Alteri et al. molnupiravir prop
```

```
Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
i Please use `linewidth` instead.
```

```
scatters_normal <- create_scatter_plot(long_v_normal, "Ruis et al. BA.1 proportion", "scat
scatters_supplemental2 <- create_scatter_plot(t1_v_merged, "Donovan-Banfield et al. molnup

scatters_supplemental + scatters_supplemental2 + scatters_normal + comp + plot_annotation(
```

```
`geom_smooth()` using formula = 'y ~ x'
```

```
Warning: The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```

```
Warning: The following aesthetics were dropped during statistical transformation: label
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  the data.
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  variable into a factor?
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i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```

```
Warning: Duplicated aesthetics after name standardisation: colour
```

```
`geom_smooth()` using formula = 'y ~ x'
```

```
Warning: The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
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i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
```

```
  variable into a factor?
The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?

Warning: Duplicated aesthetics after name standardisation: colour

`geom_smooth()` using formula = 'y ~ x'

Warning: The following aesthetics were dropped during statistical transformation: label
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The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?

Warning: Duplicated aesthetics after name standardisation: colour
```

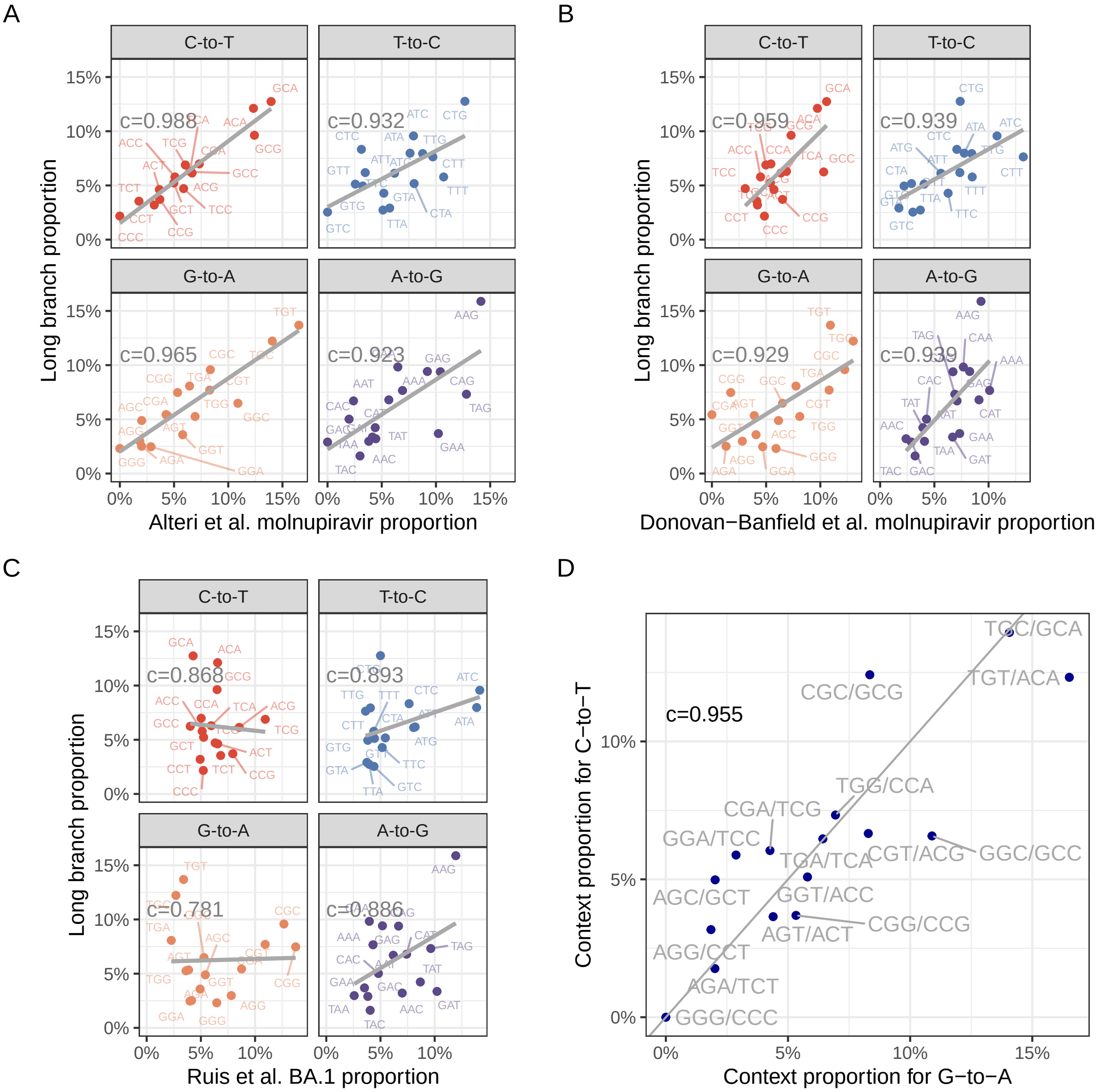

```r
ggsave("supplemental_scatters.pdf")
```

```
Saving 8 x 8 in image
`geom_smooth()` using formula = 'y ~ x'
```

```
Warning: The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
```

```
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?

Warning: The following aesthetics were dropped during statistical transformation: label
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i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?

Warning: Duplicated aesthetics after name standardisation: colour

`geom_smooth()` using formula = 'y ~ x'

Warning: The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
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  variable into a factor?

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  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
The following aesthetics were dropped during statistical transformation: label
```

```
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```

```
Warning: Duplicated aesthetics after name standardisation: colour
```

```
`geom_smooth()` using formula = 'y ~ x'
```

```
Warning: The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```

```
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  the data.
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  the data.
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  variable into a factor?
The following aesthetics were dropped during statistical transformation: label
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```
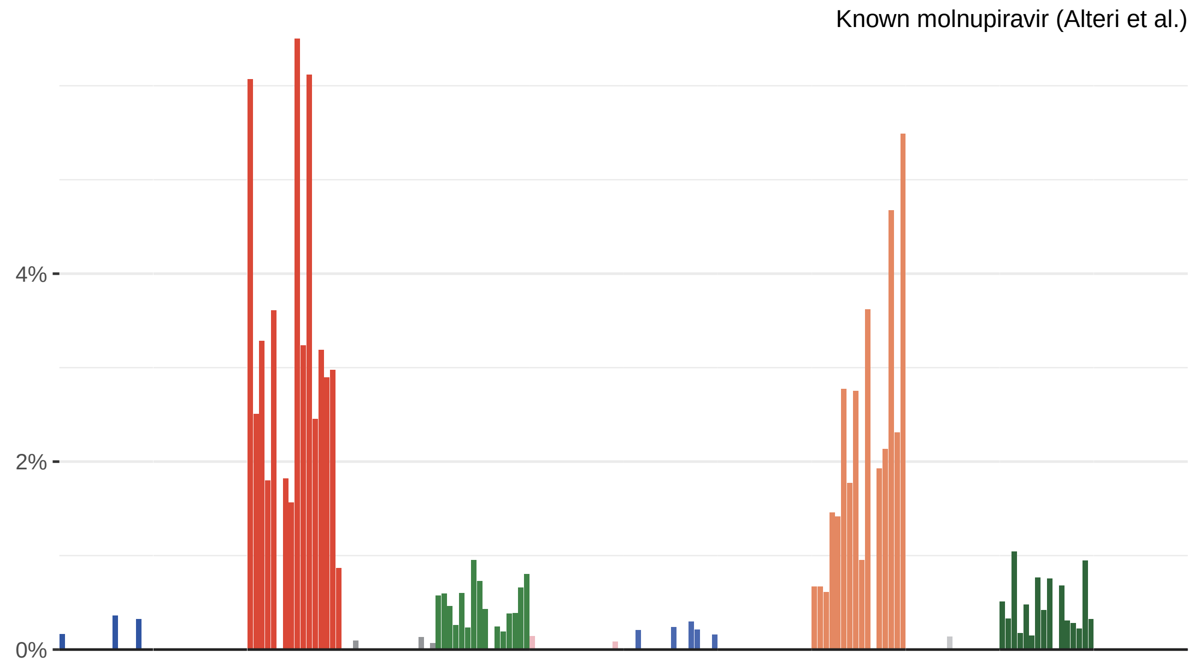

```
Warning: Duplicated aesthetics after name standardisation: colour
```

```r
plot_spectrum <- function(data, globalmax = 0, limit = 0.1, extra_axis = FALSE, title = ""
  if (!globalmax) {
    globalmax <- max(data$Number_of_mutations)
  }
  my_levels <- sort(unique(paste0(data$context_before, data$context_after)))

  data$level <- factor(paste0(data$context_before, data$context_after), levels = my_levels
  data$levelno <- as.numeric(data$level)

  precedings <- data %>%
    group_by(mutation_type, context_before) %>%
    summarise(levelno = mean(levelno))

  offset <- 0.05

  facet_style_labels <- data %>%
    group_by(mutation_type) %>%
    tally() %>%
    mutate(x = mean(data$levelno), y = -0.13 * globalmax - offset * globalmax)


  p <- ggplot(data, aes(x = levelno, y = `Number_of_mutations`, fill = mutation_type)) +
    facet_wrap(~mutation_type, nrow = 1, strip.position = "top") +
    theme_bw() +
    geom_col() +
    theme(panel.spacing = unit(0, "lines"), panel.border = element_blank()) +
    geom_bar(stat = "identity") +
    theme( # remove the vertical grid lines
      panel.grid.major.x = element_blank(),
      panel.grid.minor.x = element_blank()
      # explicitly set the horizontal lines (or they will disappear too)
      # panel.grid.major.y = element_line( size=.2, color="black" )
    ) +
    theme(legend.position = "none") +
    theme(
      axis.title.x = element_blank(),
      axis.text.x = element_blank(),
      axis.ticks.x = element_blank()
    ) +
    scale_x_continuous(expand = c(0, 0)) +
    theme(
      strip.background = element_blank(),
      strip.text.x = element_blank()
    ) +
    scale_fill_manual(values = all_colors) +
    scale_y_continuous(labels = scales::percent, breaks = c(0, 0.02, 0.04), limits = c(NA,
    labs(y = " ", title = title) +
    theme(plot.title = element_text(margin = margin(t = 0, b = -10), size = 10, hjust = 1)
    geom_hline(yintercept = 0, color = "#222222")

  if (extra_axis) {
    p <- p + geom_rect(data = data, aes(xmin = levelno - 0.5, xmax = levelno + 0.5, ymin =

      geom_tile(data = precedings, aes(x = levelno, y = -.09 * .7 * globalmax - globalmax
      geom_text(data = precedings, aes(x = levelno, y = -.09 * .7 * globalmax - globalmax
      geom_tile(data = facet_style_labels, aes(label = mutation_type, fill = mutation_type
      geom_text(data = facet_style_labels, aes(label = mutation_type, label = mutation_typ
  }
  print(p)
  return(p)
}


trial2 <- normed %>%
  filter((treat == "mov" & trial == "2")) %>%
  mutate(Number_of_mutations = Number_of_mutations / sum(`Number_of_mutations`))
ba1 <- normed %>%
  filter((trial == "3")) %>%
  mutate(Number_of_mutations = Number_of_mutations / sum(`Number_of_mutations`))

long <- normed %>%
  filter((trial == "4")) %>%
  mutate(Number_of_mutations = Number_of_mutations / sum(`Number_of_mutations`))


p_t2 <- plot_spectrum(trial2, 0.1, 0.065, FALSE, "Known molnupiravir (Alteri et al.)")
```

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

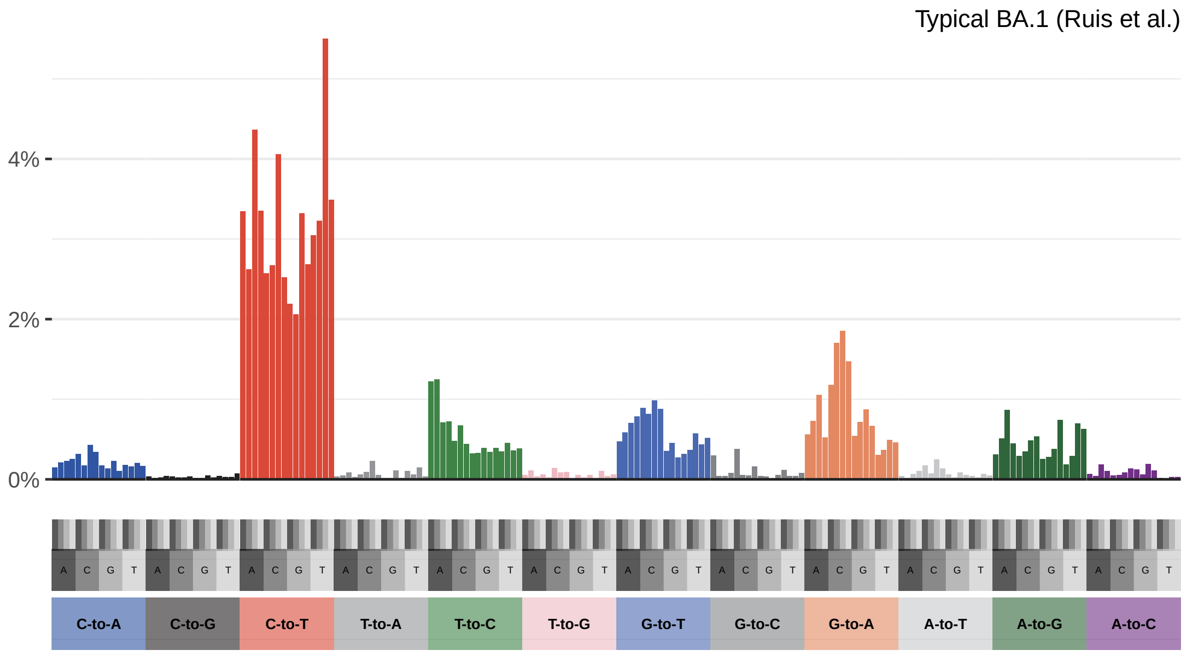


```
p_ba1 <- plot_spectrum(ba1, 0.1, 0.055, TRUE, "Typical BA.1 (Ruis et al.)")
```

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

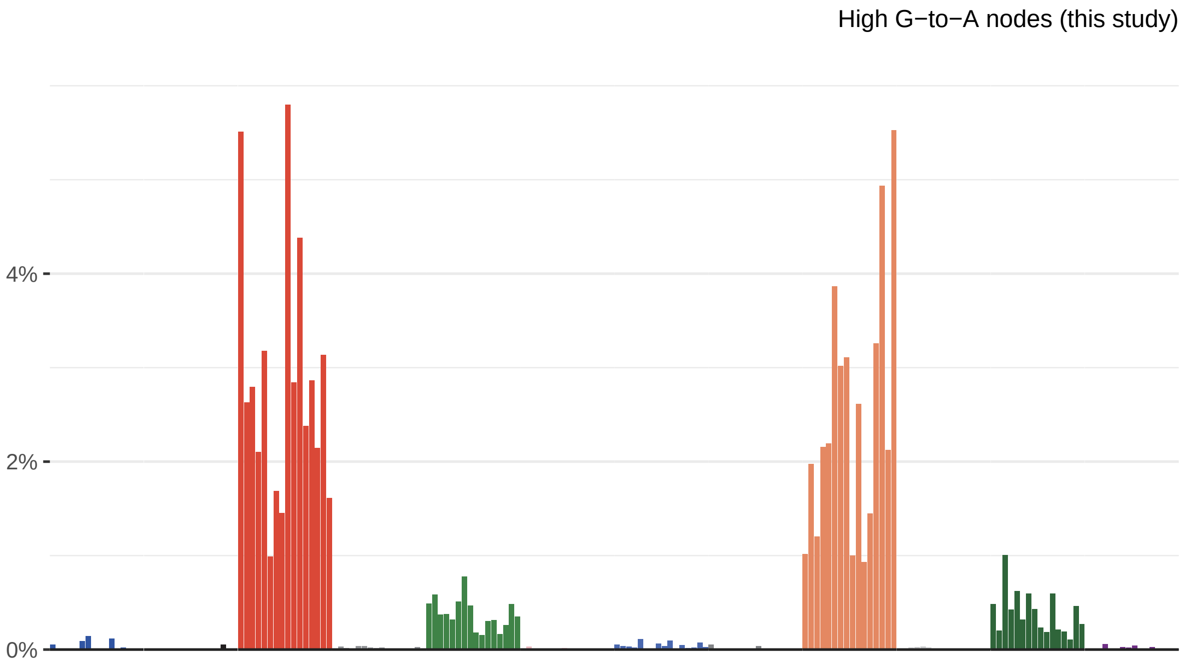
```
Warning in geom_tile(data = facet_style_labels, aes(label = mutation_type, :
Ignoring unknown aesthetics: label
```

```
Warning: Duplicated aesthetics after name standardisation: label
Duplicated aesthetics after name standardisation: label
```

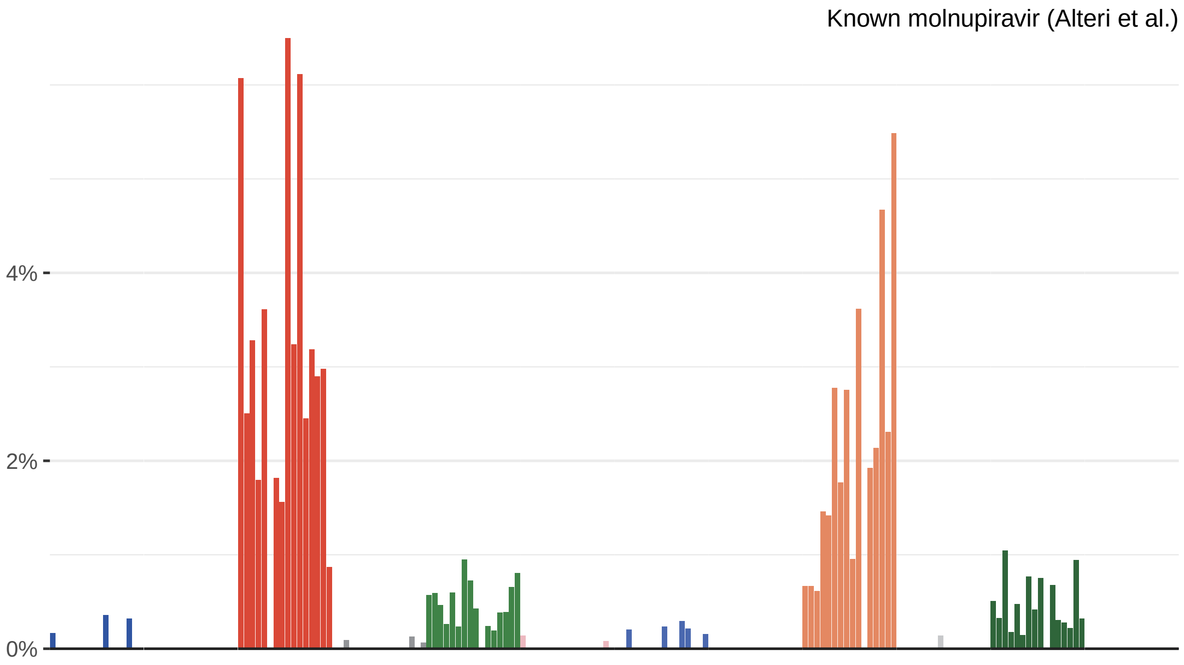


```
p_long <- plot_spectrum(long, 0.1, 0.065, FALSE, "High G-to-A nodes (this study)")
```

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


High G−to−A nodes (this study)
4%
2%
0%

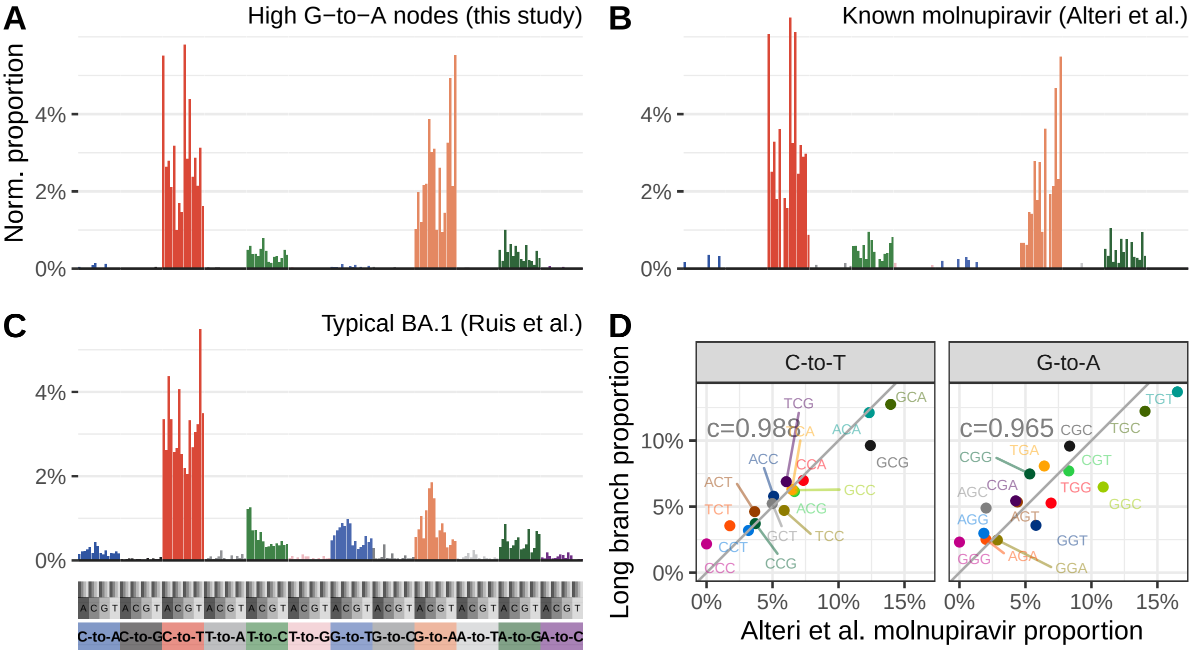
```
p_t2
```


Known molnupiravir (Alteri et al.)
4%
2%
0%

```
stacked <- (p_ba1 / p_t2 / p_long)


plot_grid(p_long + labs(y = "Norm. proportion"), p_t2, p_ba1, (scatters), labels = c("A",
```

```
Warning: Duplicated aesthetics after name standardisation: label
```

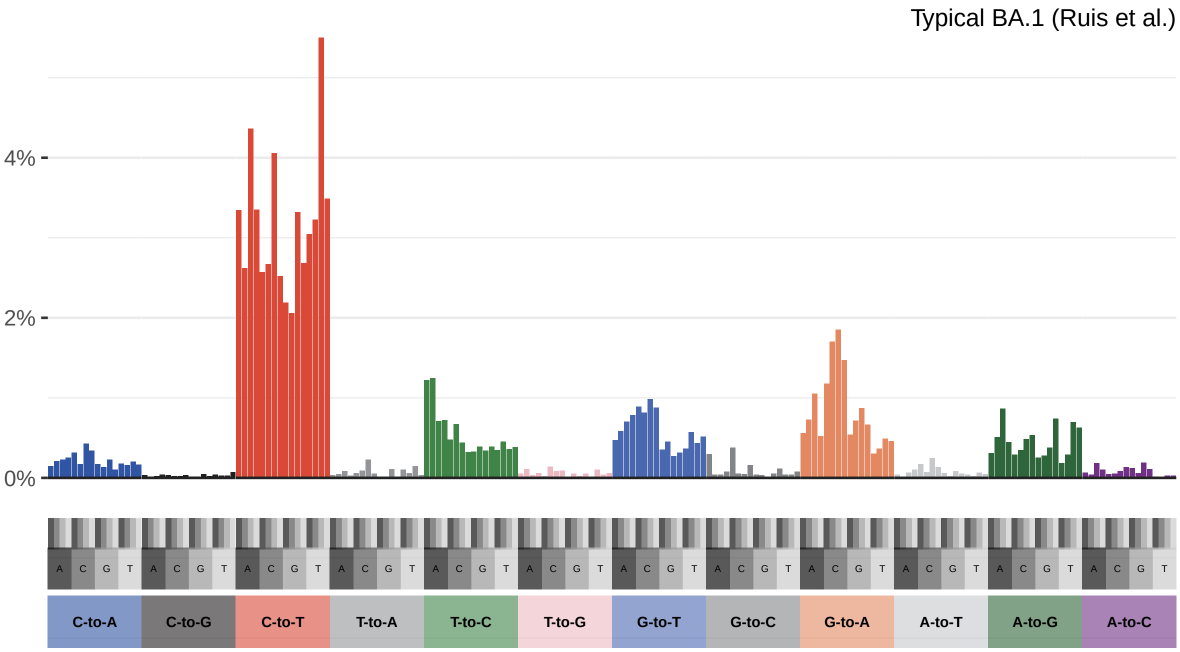
```
Warning: The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```



```
ggsave("t2vlong.pdf", width = 8, height = 4)
```

```
# plot_grid(p_long +labs(y="Norm. proportion") , p_t2  , p_ba1 , (scatters) , rel_heights


ggsave("t2vlong-present.pdf", width = 8, height = 4)

ggsave("~/movmanuscript2/Figures2/spectra.pdf", width = 8, height = 4)


p_ba1
```

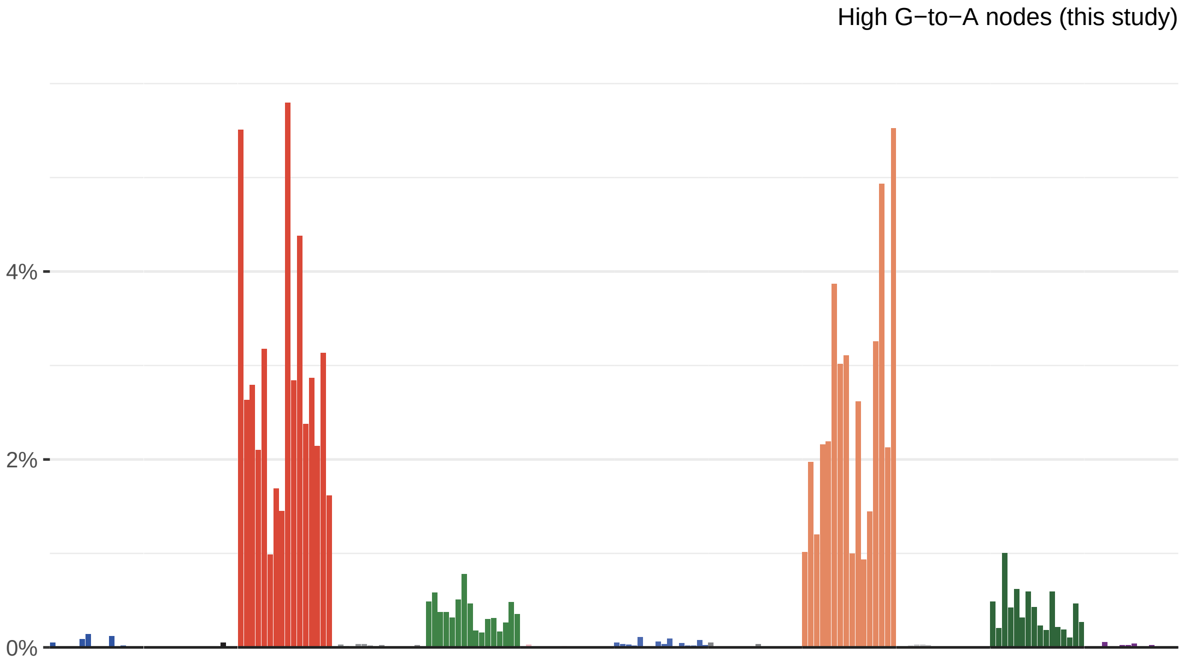
```
Warning: Duplicated aesthetics after name standardisation: label
```



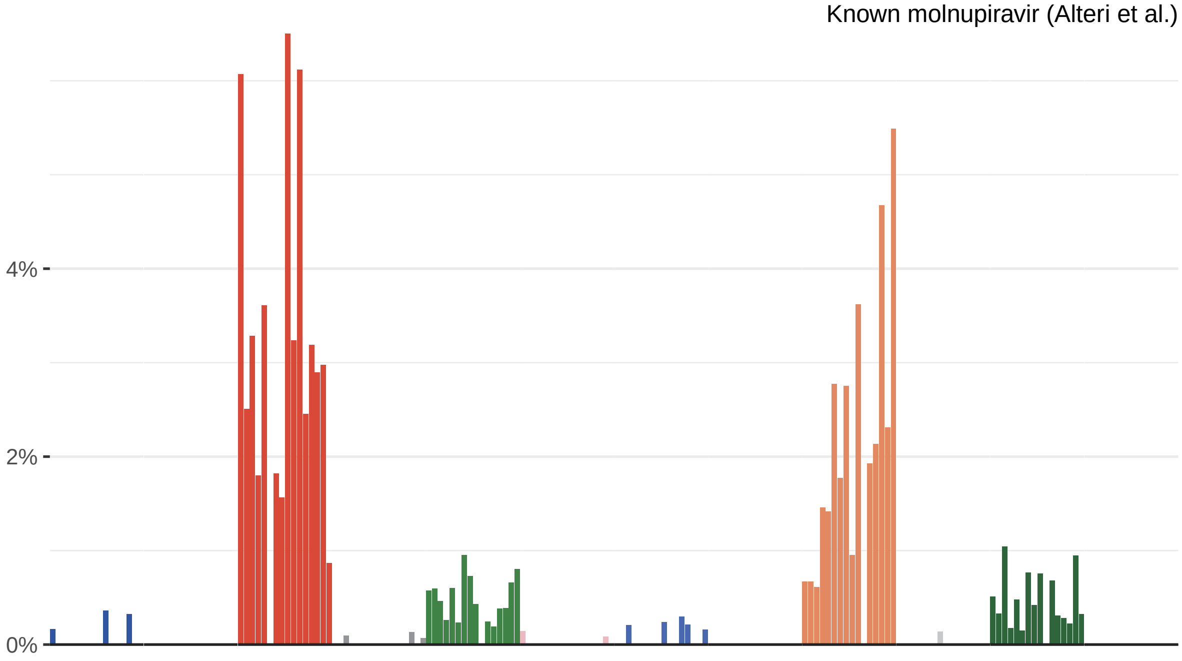
```
ggsave("p_ba1.pdf", width = 0.5 * 10, height = 0.5 * 4.5)
```

```
Warning: Duplicated aesthetics after name standardisation: label
```

```
p_long
```

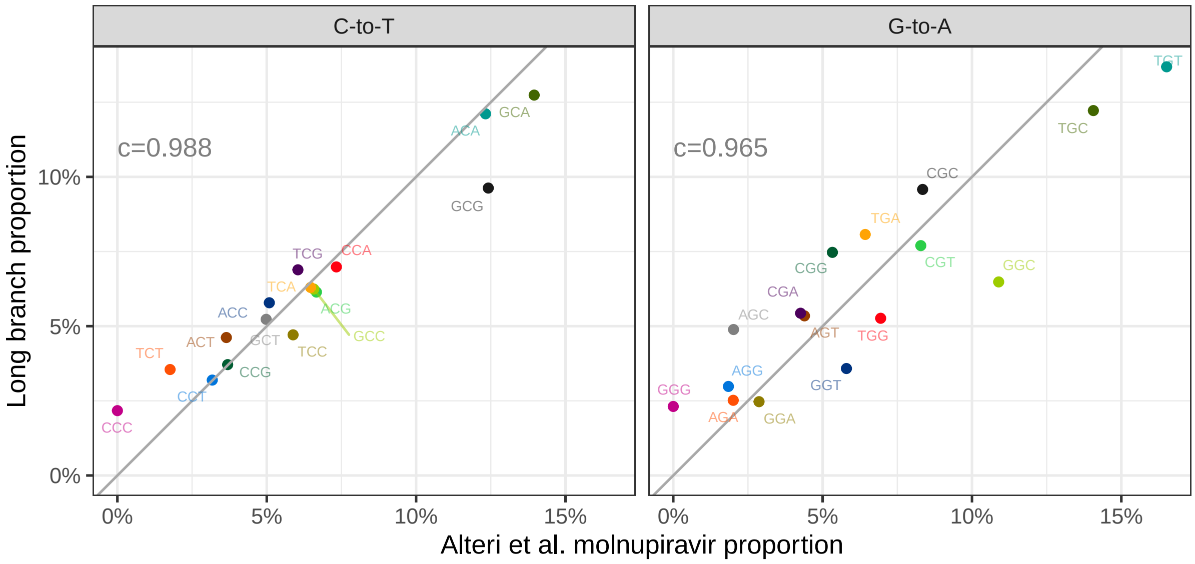


```
ggsave("p_long.pdf", width = 0.5 * 10, height = 0.5 * 4.5)

p_t2
```



```
ggsave("p_t2.pdf", width = 0.5 * 10, height = 0.5 * 4.5)


scatters
```

```
Warning: The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```



```
ggsave("scatters_small.pdf", width = 0.5 * 10, height = 0.5 * 4.5)
```

```
Warning: The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
The following aesthetics were dropped during statistical transformation: colour
i This can happen when ggplot fails to infer the correct grouping structure in
  the data.
i Did you forget to specify a `group` aesthetic or to convert a numerical
  variable into a factor?
```

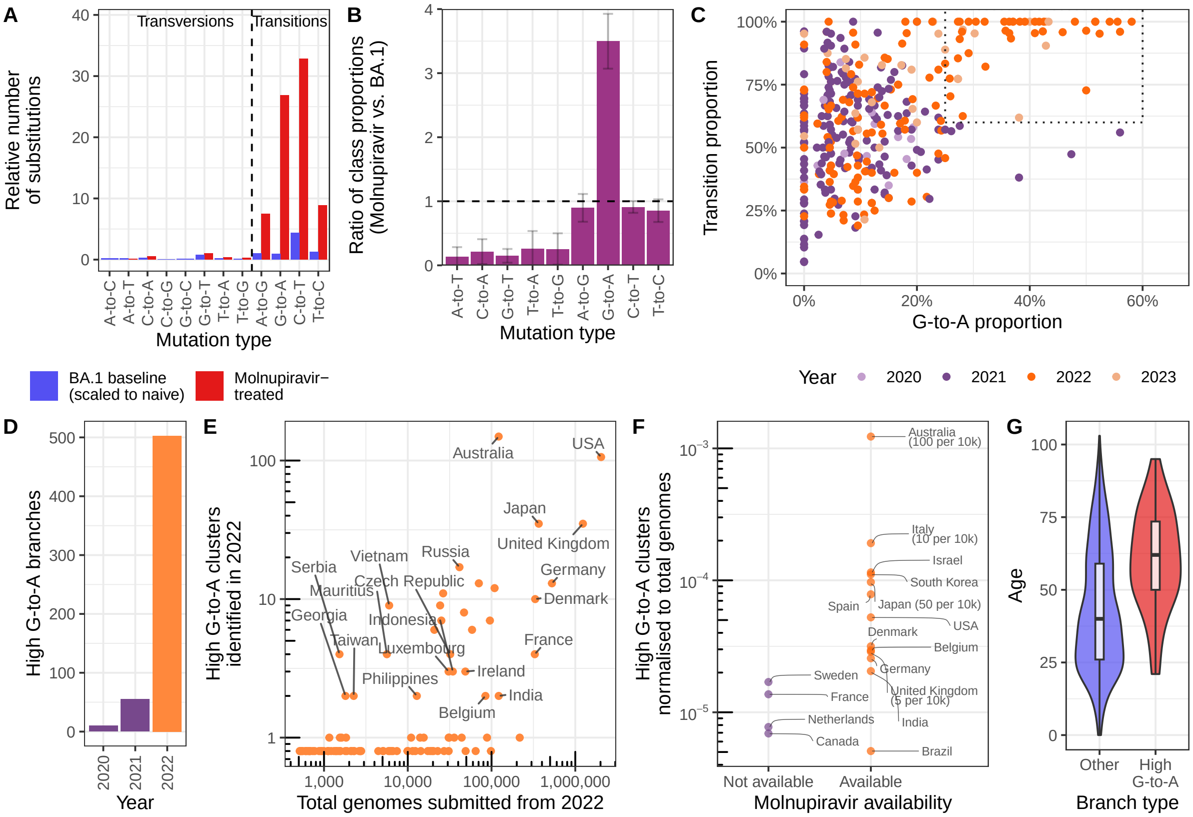
```
toprow <- plot_grid(a, b + labs(caption = "\n\n"), scatter, labels = c("A", "B", "C"), lab
bottomrow <- plot_grid(by_year_plot, country_comp, availability_plot, age, labels = c("D",
```

```
Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning: Removed 1504 rows containing non-finite values (`stat_ydensity()`).
```

```
Warning: Removed 1504 rows containing non-finite values (`stat_boxplot()`).
```

```r
plot_grid(toprow, bottomrow, ncol = 1)
```

```
Warning: ggrepel: 83 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



```r
ggsave("plot.pdf", width = 9.5, height = 6.5)
```

```
Warning: ggrepel: 83 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```

```
library(patchwork)


layout <- "
AAABBBBCCCCCCC
DDEEEEEFFFFFGG
"
a + b + scatter + by_year_plot + country_comp + availability_plot + age + plot_layout(desi
```

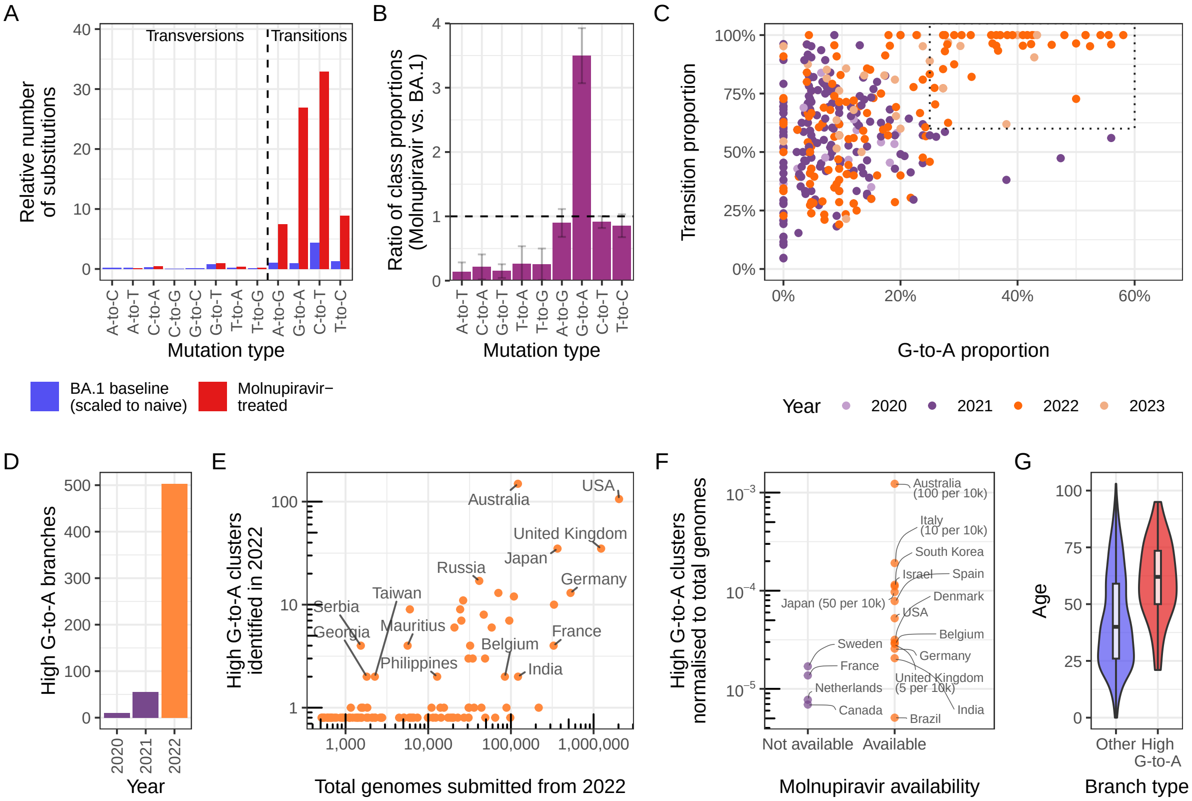
```
Warning in FUN(X[[i]], ...): NAs introduced by coercion
```

```
Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion
```

```
Warning: Removed 1504 rows containing non-finite values (`stat_ydensity()`).
```

```
Warning: Removed 1504 rows containing non-finite values (`stat_boxplot()`).
```

```
Warning: ggrepel: 89 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



```
ggsave("patchwork_version.pdf", width = 9.5, height = 6.5)
```

```
Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning in FUN(X[[i]], ...): NAs introduced by coercion

Warning: Removed 1504 rows containing non-finite values (`stat_ydensity()`).

Warning: Removed 1504 rows containing non-finite values (`stat_boxplot()`).

Warning: ggrepel: 89 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```

```
ggsave("~/movmanuscript2/Figures2/patchworkcombo.pdf", width = 9.5, height = 6.5)
```

```
Warning in FUN(X[[i]], ...): NAs introduced by coercion
```

```
Warning in FUN(X[[i]], ...): NAs introduced by coercion
```

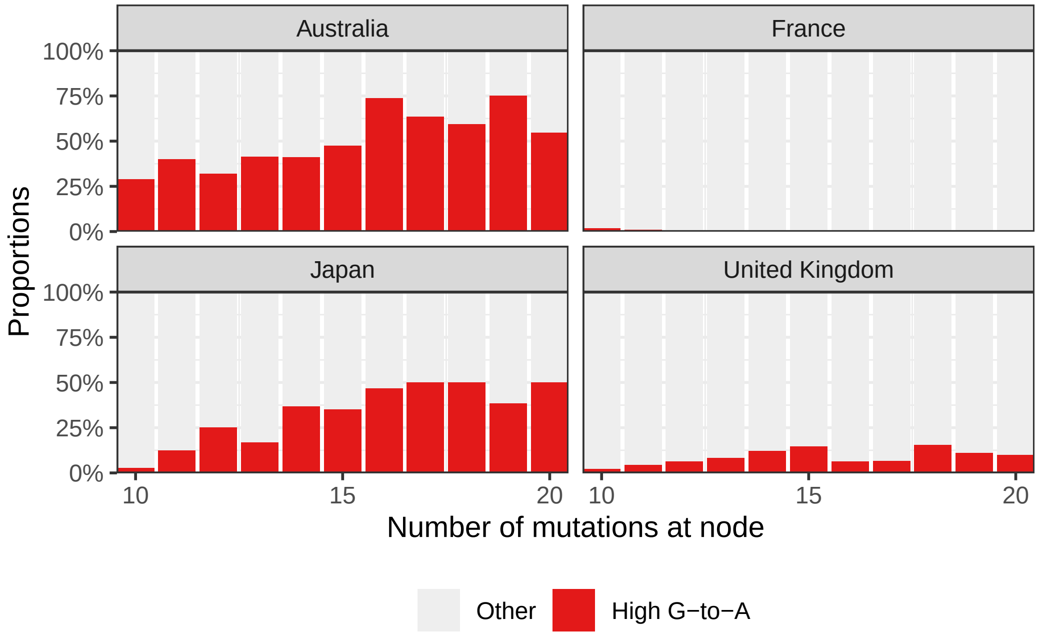
```
Warning in FUN(X[[i]], ...): NAs introduced by coercion
```

```
Warning: Removed 1504 rows containing non-finite values (`stat_ydensity()`).
```

```
Warning: Removed 1504 rows containing non-finite values (`stat_boxplot()`).
```

```
Warning: ggrepel: 89 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```

```
countries <- c("Australia", "United Kingdom", "Japan", "France")
proportions_of_long_branches <- ggplot(data_nodes %>% filter(total_muts > 9, total_muts <
  geom_bar(position = "fill") +
  facet_wrap(~consensus_country) +
  theme_bw() +
  scale_y_continuous(labels = scales::percent, expand = c(0, 0)) +
  labs(x = "Number of mutations at node", y = "Proportions") +
  scale_fill_manual(labels = c("Other", "High G-to-A"), values = c("#eeeeee", red)) +
  scale_x_continuous(expand = c(0, 0), breaks = c(10, 15, 20)) +
  labs(fill = "") +
  theme(legend.position = "bottom")
proportions_of_long_branches
```



```r
ggsave("plotter.pdf", width = 5, height = 4)
```

```r
library(Biostrings)
data("BLOSUM62")
bl62 <- as.data.frame(as.table(BLOSUM62))

colnames(bl62) <- c("original_aa", "alternative_aa", "bl62_score")


all <- inner_join(data_nodes, data_muts %>% right_join(bl62), by = "node_id")
```

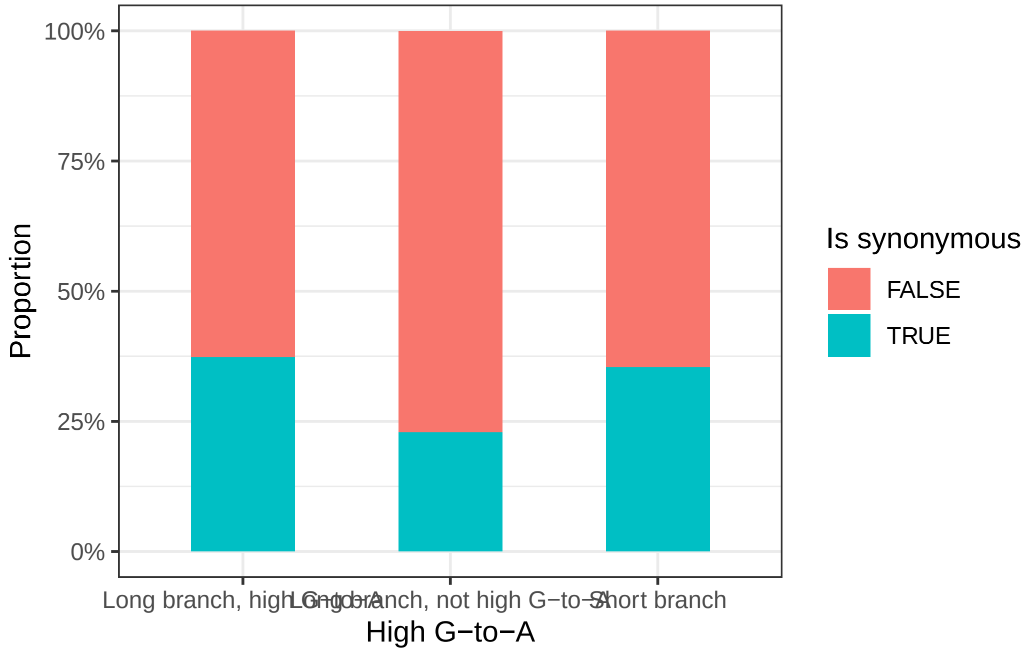
```
Joining with `by = join_by(original_aa, alternative_aa)`
```

```r
adjustment_factor <- 3.24

annotated =  all  %>%
  mutate(branch_type = case_when(
    total_muts >= threshold_branch_length & flagged ~ "Long branch, high G-to-A",
    total_muts >= threshold_branch_length & !flagged ~ "Long branch, not high G-to-A",
    TRUE ~ "Short branch"
  ))
```

```
dnds_stuff <- all %>% filter(gene=="S") %>%
  mutate(branch_type = case_when(
    total_muts >= threshold_branch_length & flagged ~ "Long branch, high G-to-A",
    total_muts >= threshold_branch_length & !flagged ~ "Long branch, not high G-to-A",
    TRUE ~ "Short branch"
  )) %>%
  group_by(branch_type, is_synonymous) %>%
  tally() %>%
  group_by(branch_type) %>%
  mutate(p = n / sum(n), ratio = n / (sum(n) - n), dnds = ratio / adjustment_factor) %>%
  rowwise() %>%
  mutate(confidence_interval = list(binom.test(n, n/p)$conf.int))  %>%
  mutate(
         lower = confidence_interval[1],
         upper = confidence_interval[2])

dnds_stuff
```

```
# A tibble: 6 x 9
# Rowwise:  branch_type
  branch_type  is_synonymous      n     p ratio   dnds confidence_interval lower
  <chr>        <lgl>          <int> <dbl> <dbl>  <dbl> <list>              <dbl>
1 Long branch~ FALSE         9.68e2 0.628 1.69  0.520  <dbl [2]>           0.603
2 Long branch~ TRUE          5.74e2 0.372 0.593 0.183  <dbl [2]>           0.348
3 Long branch~ FALSE         4.63e4 0.772 3.38  1.04   <dbl [2]>           0.768
4 Long branch~ TRUE          1.37e4 0.228 0.296 0.0913 <dbl [2]>           0.225
5 Short branch FALSE         1.07e6 0.646 1.83  0.564  <dbl [2]>           0.646
6 Short branch TRUE          5.86e5 0.354 0.547 0.169  <dbl [2]>           0.353
# i 1 more variable: upper <dbl>
```

```
dnds_stuff %>% ggplot(aes(y = p, fill = is_synonymous, x = branch_type)) +
  geom_col(width = 0.5) +
  scale_y_continuous(label = scales::percent) +
  theme_bw() +
  labs(fill = "Is synonymous", x = "High G-to-A", y = "Proportion")
```



```r
ggsave("plot.png", width = 4, height = 3)

library(gt)
dnds_stuff = dnds_stuff %>% dplyr::filter(!is_synonymous) %>% mutate(total_n = n/p)
# Extract the relevant data for "Long branch, high G-to-A"
long_high <- dnds_stuff %>%
  filter(branch_type == "Long branch, high G-to-A", !is_synonymous)

# Extract the relevant data for "Long branch, not high G-to-A"
long_not_high <- dnds_stuff %>%
  filter(branch_type == "Long branch, not high G-to-A", !is_synonymous)

# Extract the relevant data for "Short branch"
short_branch <- dnds_stuff %>%
  filter(branch_type == "Short branch", !is_synonymous)

# Conduct the proportion test between "Long branch, high G-to-A" and "Long branch, not hig
test1 <- prop.test(x = c(long_high$n, long_not_high$n),
                   n = c(long_high$total_n, long_not_high$total_n),
                   alternative = "two.sided")

# Conduct the proportion test between "Long branch, high G-to-A" and "Short branch"
```

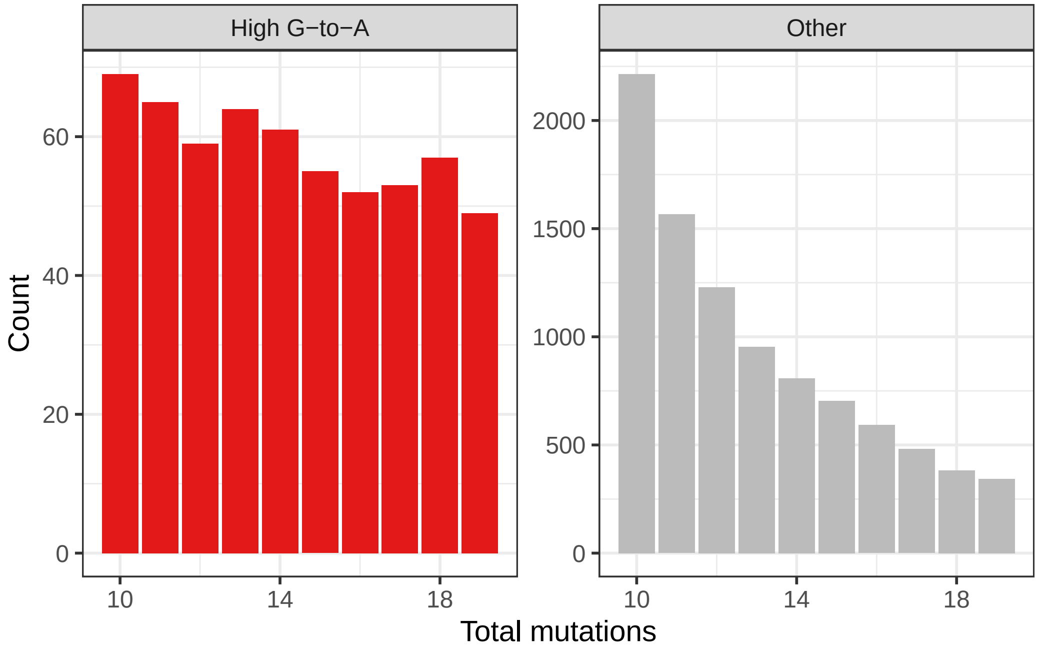
```
test2 <- prop.test(x = c(long_high$n, short_branch$n),
                   n = c(long_high$total_n, short_branch$total_n),
                   alternative = "two.sided")

# Print the results
print(test1)
```

```

	2-sample test for equality of proportions with continuity correction

data:  c(long_high$n, long_not_high$n) out of c(long_high$total_n, long_not_high$total_n)
X-squared = 173.97, df = 1, p-value < 2.2e-16
alternative hypothesis: two.sided
95 percent confidence interval:
 -0.168606 -0.119220
sample estimates:
   prop 1    prop 2 
0.6277562 0.7716692 
```

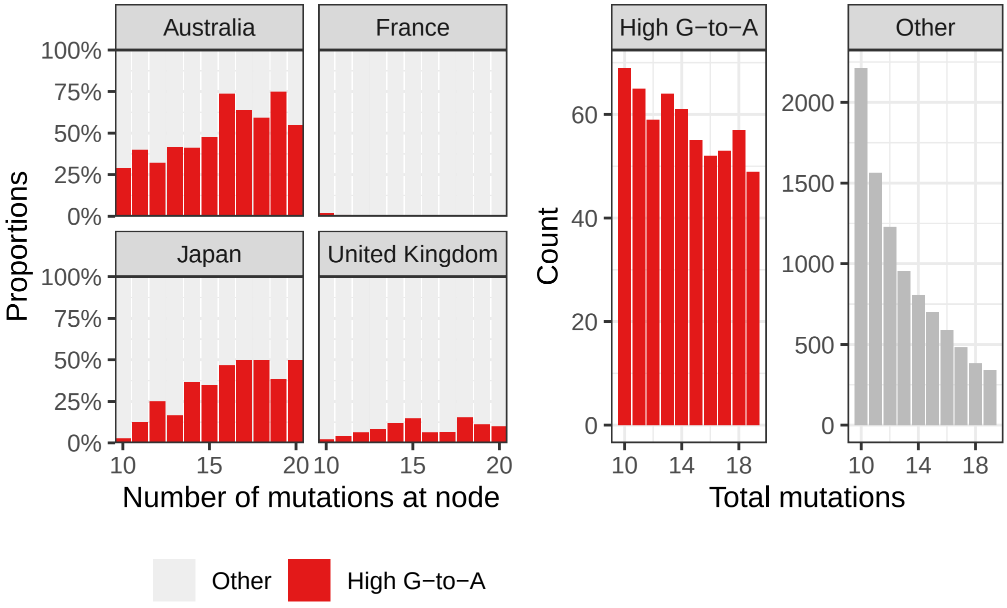
```
print(test2)
```

```

	2-sample test for equality of proportions with continuity correction

data:  c(long_high$n, short_branch$n) out of c(long_high$total_n, short_branch$total_n)
X-squared = 2.2729, df = 1, p-value = 0.1317
alternative hypothesis: two.sided
95 percent confidence interval:
 -0.04315104  0.00577534
sample estimates:
   prop 1    prop 2 
0.6277562 0.6464440 
```

```
for_plot <- data_nodes %>%
  filter(consensus_year %in% c("2022", "2023"), total_muts > 9, total_muts < 20) %>%
  mutate(new = ifelse(flagged, "High G-to-A", "Other"))

distributions <- ggplot(for_plot, aes(x = total_muts, fill = flagged)) +
  geom_bar() +
  facet_wrap(~new, scales = "free_y") +
  theme_bw() +
  scale_x_continuous(breaks = c(10, 14, 18)) +
  scale_fill_manual(values = c("#bbbbbb", red)) +
  labs(x = "Total mutations", y = "Count") +
  theme(legend.position = "none")
distributions
```

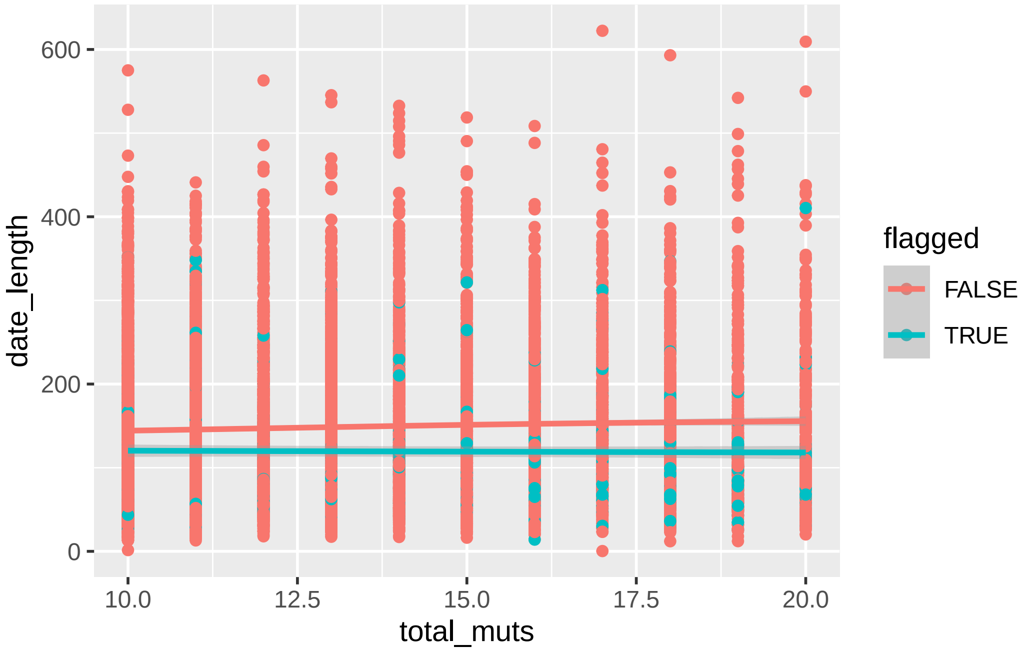


```
proportions_of_long_branches + distributions
```



```r
library(broom)
countries <- c("Australia", "Japan", "United Kingdom", "USA")

dataset <- data_nodes %>% filter(consensus_year == "2022", total_muts >= threshold_branch_

ggplot(dataset, aes(x = total_muts, y = date_length, color = flagged)) +
  geom_point() +
  geom_smooth()
```

```
`geom_smooth()` using method = 'gam' and formula = 'y ~ s(x, bs = "cs")'
```



```
dataset
```

```
# A tibble: 8,388 x 23
   node_id           num_descendants consensus_country consensus_year date
   <chr>                       <dbl> <chr>             <chr>          <date>
 1 USA/TN-ASC-21050~               1 USA               2022           2022-01-04
 2 India/MH-Kasturb~               1 India             2022           2022-01-31
 3 Indonesia/JI-GS-~               1 Indonesia         2022           2022-01-07
 4 USA/NC-CDC-MMB13~               1 USA               2022           2022-01-19
 5 Philippines/PH-R~               1 Philippines       2022           2022-04-11
 6 Philippines/PH-R~               1 Philippines       2022           2022-04-24
 7 Russia/SAR-RII-M~               1 Russia            2022           2022-01-13
 8 node_39341                      2 Indonesia         2022           2021-12-21
 9 node_39384                      2 Indonesia         2022           2021-12-23
10 Indonesia/KI-GS-~               1 Indonesia         2022           2022-01-31
# i 8,378 more rows
# i 18 more variables: date_length <dbl>, age <chr>, `A>C` <dbl>, `A>G` <dbl>,
#   `A>T` <dbl>, `C>A` <dbl>, `C>G` <dbl>, `C>T` <dbl>, `G>A` <dbl>,
#   `G>C` <dbl>, `G>T` <dbl>, `T>A` <dbl>, `T>C` <dbl>, `T>G` <dbl>,
#   total_muts <dbl>, transitions <dbl>, transversions <dbl>, flagged <lgl>
```

```r
t.test(data = dataset, date_length ~ flagged)
```

```

	Welch Two Sample t-test

data:  date_length by flagged
t = 8.7949, df = 552.31, p-value < 2.2e-16
alternative hypothesis: true difference in means between group FALSE and group TRUE is not ec
95 percent confidence interval:
 22.57814 35.56353
sample estimates:
mean in group FALSE  mean in group TRUE 
           148.3975            119.3266 
```

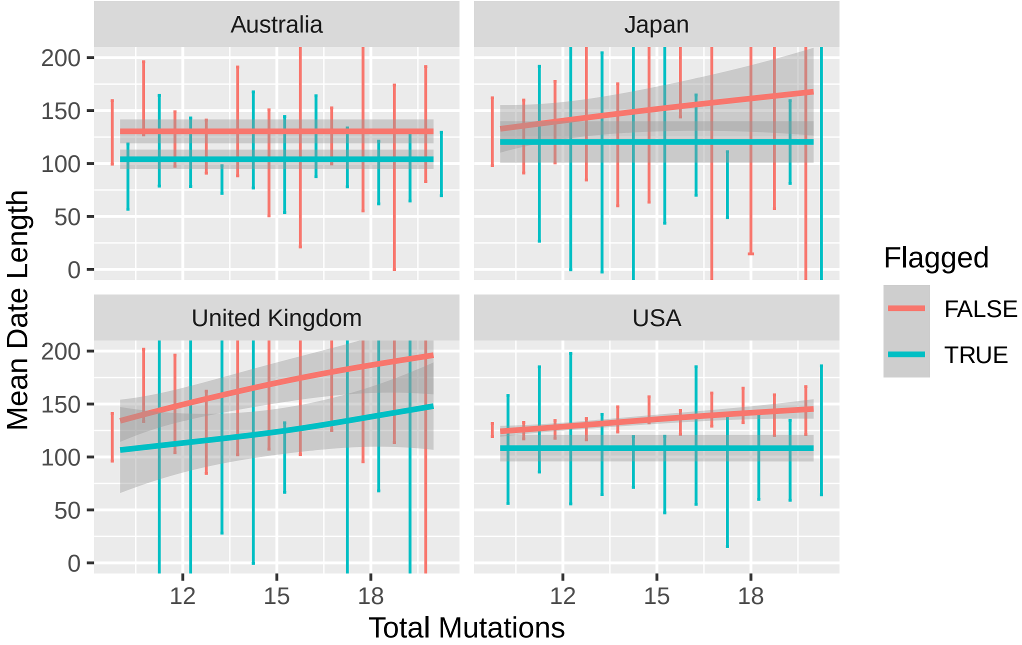
```r
dataset2 <- dataset %>% filter(consensus_country %in% countries)

dataset3 <- dataset2 %>%
  group_by(total_muts, flagged, consensus_country) %>%
  summarize(
    mean_date_length = mean(date_length),
    se_date_length = sd(date_length) / sqrt(n()),
    conf.low = mean_date_length - qt(0.975, df = n() - 1) * se_date_length,
    conf.high = mean_date_length + qt(0.975, df = n() - 1) * se_date_length
  )
```

```
Warning: There were 8 warnings in `summarize()`.
The first warning was:
i In argument: `conf.low = mean_date_length - qt(0.975, df = n() - 1) *
  se_date_length`.
i In group 6: `total_muts = 10`, `flagged = TRUE`, `consensus_country =
  "Japan"`.
Caused by warning in `qt()`:
! NaNs produced
i Run `dplyr::last_dplyr_warnings()` to see the 7 remaining warnings.
```

```
`summarise()` has grouped output by 'total_muts', 'flagged'. You can override
using the `.groups` argument.
```

```r
ggplot(dataset3, aes(x = total_muts, y = mean_date_length, color = flagged)) +
  geom_errorbar(aes(ymin = conf.low, ymax = conf.high), width = 0.2, position = position_d
  facet_wrap(~flagged) +
  labs(x = "Total Mutations", y = "Mean Date Length", color = "Flagged") +
  facet_wrap(~consensus_country) +
  coord_cartesian(ylim = c(0, 200)) +
  geom_smooth(data = dataset2, aes(y = date_length))
```

```
`geom_smooth()` using method = 'gam' and formula = 'y ~ s(x, bs = "cs")'
```



```r
final_df <- tibble()

many_descendants <- data_nodes %>%
  filter(total_muts > 9, flagged, num_descendants > 1)

# Loop through every single_node in many_descendants
for (i in 1:nrow(many_descendants)) {
  single_node <- many_descendants$node_id[i]
  children <- find_children(parenthood, single_node)
  children <- children[!grepl("^node_", children)]
```

```
    # Create a temporary tibble for the current node
    cluster_df <- tibble(node_id = children, cluster = single_node)


    # bind the current tibble with the final one
    final_df <- bind_rows(final_df, cluster_df)
  }

  single_descendants <- data_nodes %>%
    filter(total_muts > 9, flagged, num_descendants == 1)

  single_df <- tibble(node_id = single_descendants$node_id, cluster = single_descendants$nod

  final_df <- bind_rows(final_df, single_df)

  final_df2 <- final_df %>%
    separate_wider_delim(node_id, delim = "|", names = c("name", "epi", "date"), cols_remove
    separate_wider_delim(name, delim = "/", names = c("country", "name2", "year"), cols_remo


  final_df2 %>% filter(country == "England")
```

```
# A tibble: 52 x 8
   country name2         year  name                        epi   date  node_id cluster
   <chr>   <chr>         <chr> <chr>                       <chr> <chr> <chr>   <chr>
 1 England HSLL-1AF5265  2021  England/HSLL-1AF5265/~ EPI_~ 2021~ Englan~ node_1~
 2 England HSLL-1BBA08F  2021  England/HSLL-1BBA08F/~ EPI_~ 2021~ Englan~ node_1~
 3 England PHEP-YYFYNOA  2022  England/PHEP-YYFYNOA/~ EPI_~ 2022~ Englan~ node_1~
 4 England PHEP-YYR9UXC  2022  England/PHEP-YYR9UXC/~ EPI_~ 2022~ Englan~ node_1~
 5 England HSLL-383EB43  2022  England/HSLL-383EB43/~ EPI_~ 2022~ Englan~ node_1~
 6 England MILK-384AB80  2022  England/MILK-384AB80/~ EPI_~ 2022~ Englan~ node_1~
 7 England PHEC-YYDRDI8  2022  England/PHEC-YYDRDI8/~ EPI_~ 2022~ Englan~ node_1~
 8 England PHEC-YYDEK4Q  2022  England/PHEC-YYDEK4Q/~ EPI_~ 2022~ Englan~ node_1~
 9 England DHSC-CYNN473  2022  England/DHSC-CYNN473/~ EPI_~ 2022~ Englan~ node_1~
10 England DHSC-CYD46UZ  2022  England/DHSC-CYD46UZ/~ EPI_~ 2022~ Englan~ node_1~
# i 42 more rows
```

```
  write_csv(final_df2, "associated.csv")
```

```r
library(ggtree)

format_mutation_counts <- function(node_data) {
  # Extract mutation count columns
  mutation_cols <- c("A>C", "A>G", "A>T", "C>A", "C>G", "C>T", "G>A", "G>C", "G>T", "T>A",

  # Create a named vector of mutation counts
  mutation_counts <- sapply(mutation_cols, function(x) node_data[[x]])
  names(mutation_counts) <- mutation_cols

  # Remove zeros
  mutation_counts <- mutation_counts[mutation_counts > 0]

  # Sort in descending order
  mutation_counts <- sort(mutation_counts, decreasing = TRUE)

  # Format as a string
  mutation_str <- paste(names(mutation_counts), mutation_counts, sep = ": ", collapse = ",
  mutation_str <- gsub(">", "\u00adto\u00ad", mutation_str)

  return(mutation_str)
}

prune_and_plot <- function(node_id, parent, node_data) {
  mutation_title <- format_mutation_counts(node_data)
  print(node_id)

  # Create directories if they do not exist
  if (!dir.exists("data")) {
    dir.create("data")
  }

  if (!dir.exists("trees")) {
    dir.create("trees")
  }


  gotree_command <- paste0("~/Dropbox/new_mov_data/gotree_arm64_darwin subtree -i ~/Dropbo

  print(gotree_command)
```

```
# Execute the system call
system(gotree_command)

# Read the newick file
tree <- read.tree(paste0("data/pruned_", node_id, ".nwk"))


get_node_index <- function(tree, node_name) {
  for (i in 1:length(tree$node.label)) {
    if (tree$node.label[i] == node_name) {
      return(i + ape::Ntip(tree)) # Return the index of the node
    }
  }
  return(NULL) # Return NULL if no node with that name was found
}

node_index <- get_node_index(tree, node_id)


# Plot the tree using ggtree
ggtree_plot <- ggtree(tree, aes( # color=node==node_index
)) +
  geom_tiplab(size = 3, aes(label = label)) + # Add tip labels
  geom_point2(aes(subset = !is.na(num_tips)), color = "#4561de") + # Add points to visua
  coord_cartesian(clip = "off") +
  theme_tree2(plot.margin = margin(6, 290, 6, 6)) +
  theme(legend.position = "none") + #+scale_color_manual(values = c("TRUE" = "darkblue",
  geom_text(aes(x = branch, label = ifelse(node == node_index, mutation_title, "")),
    size = 3,
    vjust = -.4, color = "firebrick"
  ) #+ggtitle(node_id)

# Calculate the number of tips
num_tips <- ape::Ntip(tree)

# Calculate a reasonable height for the plot
# Adjust this calculation as needed
plot_height <- max(1.5, num_tips / 5)

# Save the plot to a pdf
# ggsave(filename = paste0("trees/node_", node_id, ".pdf"), plot = ggtree_plot, height =
  return(list(ggtree_plot, plot_height))
}

filtered_nodes <- data_nodes %>%
  filter(total_muts > 9, flagged, num_descendants > 2) %>%
  arrange(desc(num_descendants))

filtered_nodes

library(patchwork)

# Step 1
plots_list <- list()
heights_list <- c()
total_height <- 0
# Introduce plot_number
plot_number <- 1

# In your loop

pdf("trees/combined_plots.pdf", height = 11.7, width = 8.3) # Create a PDF file

for (i in 1:nrow(filtered_nodes)) {
  print(i)

  listed <- prune_and_plot(filtered_nodes$node_id[i], get_parent(parenthood, filtered_node

  ggtree_plot <- listed[[1]]
  plot_height <- listed[[2]]

  # Check if adding the new plot will exceed the page size
  if ((total_height + plot_height) >= 16) { # A4 height in inches
    # Save the existing plots
    if (length(plots_list) > 0) {
      combined_plot <- wrap_plots(plots_list) +
        plot_layout(heights = heights_list / total_height) # Normalize to make it relative
      print(combined_plot)
      ggsave(filename = paste0("trees/combined_", plot_number, ".pdf"), plot = combined_pl

      plot_number <- plot_number + 1 # Increment plot_number
    }
    # Reset the list and total height
    plots_list <- list()
    heights_list <- c()
    total_height <- 0
  }


  if (plot_height < 15 * 5) {
    # Add the new plot
    plots_list[[length(plots_list) + 1]] <- ggtree_plot
    heights_list <- c(heights_list, plot_height)
    total_height <- total_height + plot_height
  }
}

# After the loop, save any remaining plots
if (length(plots_list) > 0) {
  combined_plot <- wrap_plots(plots_list) +
    plot_layout(heights = heights_list / total_height) # Normalize to make it relative
  ggsave(filename = paste0("trees/combined_", plot_number, ".pdf"), plot = combined_plot,
}

dev.off() # Close the PDF device
```

Mutation effects analysis

```
# Function to read FASTA file and convert to a tibble
read_fasta_to_tibble <- function(file_path) {
  # Load the fasta file
  fasta_data <- readDNAStringSet(file_path)

  # Get sequence from the first (and possibly only) sequence in the fasta file
  sequence <- as.character(fasta_data[[1]])
  residues <- strsplit(sequence, "")[[1]]
  # Create a tibble with residue and index
  tibble(
    index = seq_along(residues),
    residue = residues
  )
}
```

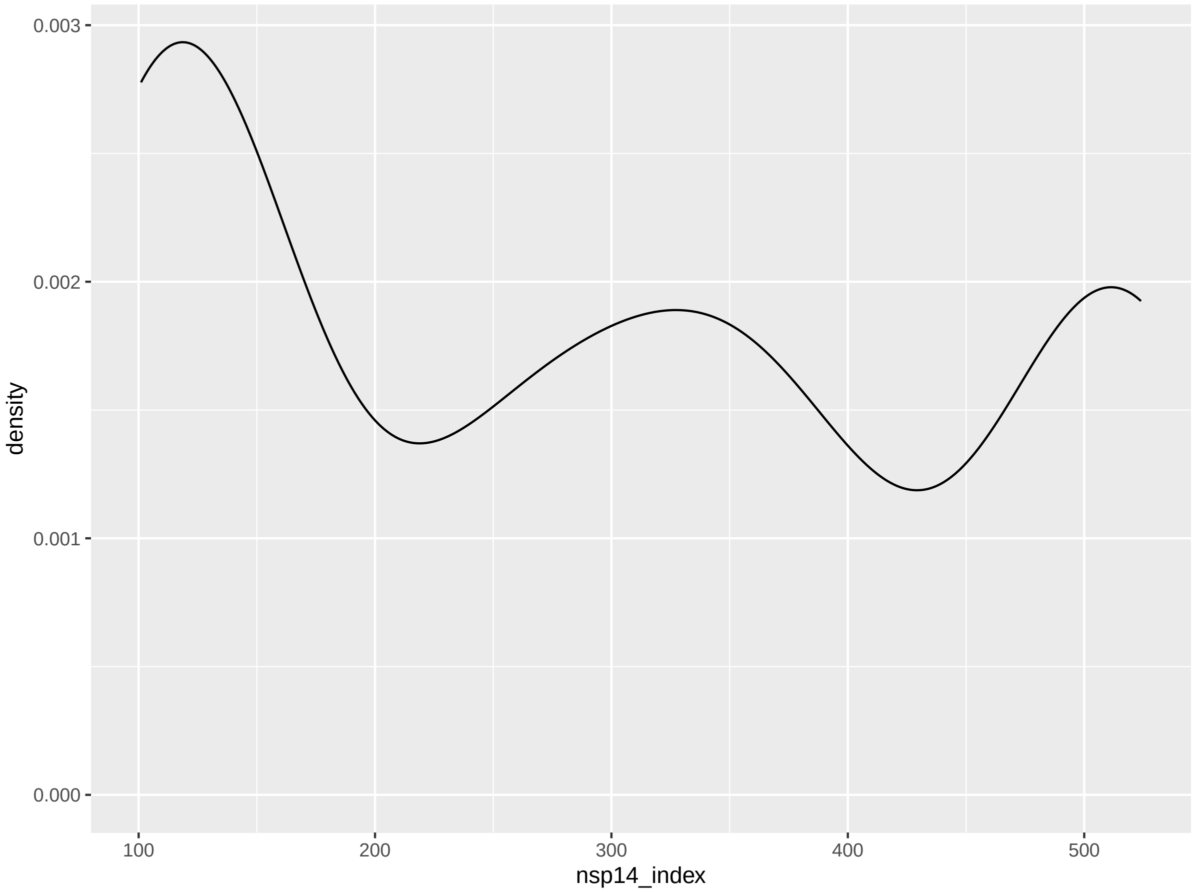
```r
ref_tib <- read_fasta_to_tibble("ref.fa.fasta") %>% mutate(context_before = lag(residue),
ref_tib
```

```
# A tibble: 29,903 x 4
   index residue context_before context_after
   <int> <chr>   <chr>          <chr>
 1     1 A       <NA>           T
 2     2 T       A              T
 3     3 T       T              A
 4     4 A       T              A
 5     5 A       A              A
 6     6 A       A              G
 7     7 G       A              G
 8     8 G       G              T
 9     9 T       G              T
10    10 T       T              T
# i 29,893 more rows
```

```r
library(gggenes)
library(tidyverse)

# Read data
hu1 <- read_tsv("./hu1.tsv")
```

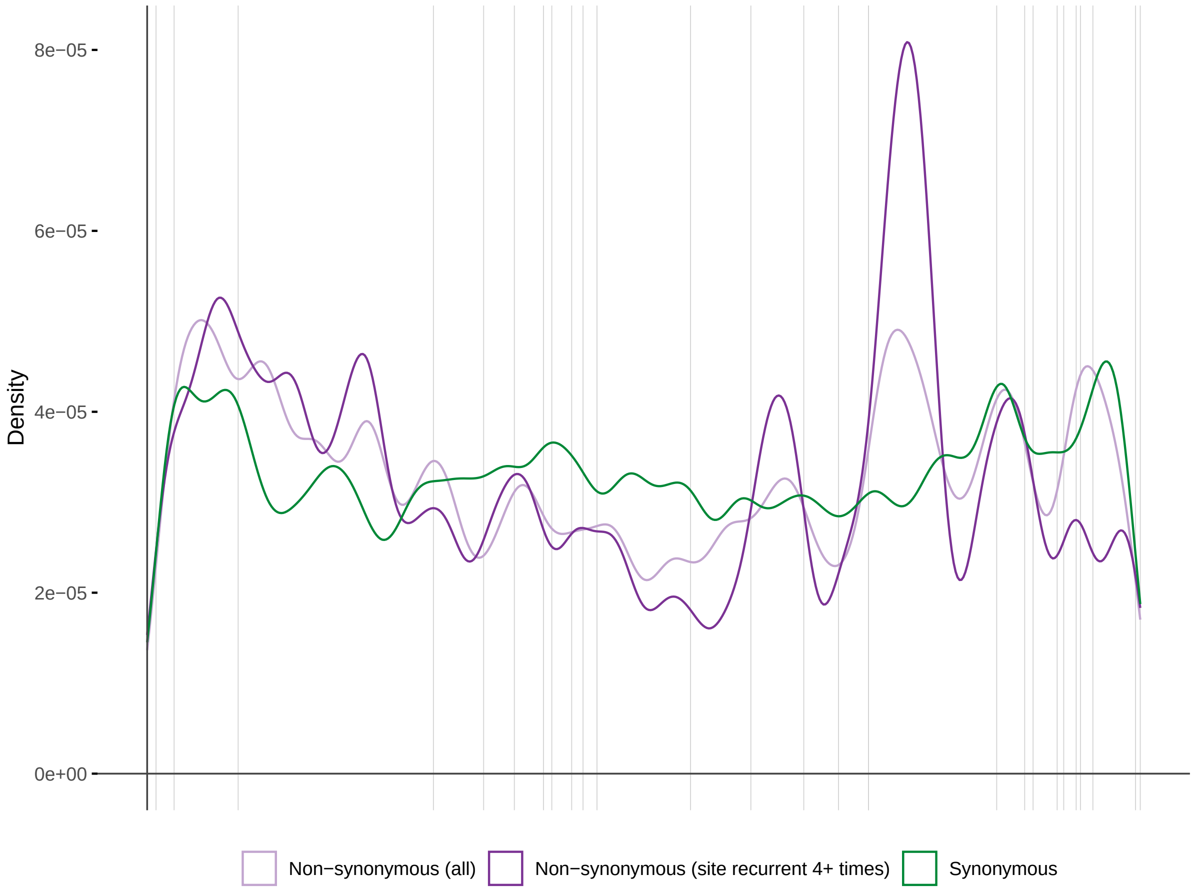
```
Rows: 38 Columns: 4
-- Column specification --------------------------------------------------------
Delimiter: "\t"
chr (2): feature_name, feature_type
dbl (2): start, end

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
# Define unique end_points
end_points <- unique(hu1$end)

# Define a function to generate the vertical line
generate_vline <- function(end_points) {
  geom_vline(
    xintercept = end_points # , linetype = "dashed"
    , color = "lightgray", size = .2
  )
}

# Define common theme
common_theme <- theme(
  axis.ticks = element_line(color = "black"),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank()
)

# Define filtered hu1
filtered_hu1 <- hu1 %>% filter(feature_type %in% c("CDS", "mat_peptide"))

# hu1_plot
hu1_plot <- ggplot(filtered_hu1, aes(xmin = start, xmax = end, y = feature_type, fill = fe
  generate_vline(end_points) +
  scale_fill_manual(values = c("#fbe4bc", "#dff3f8")) +
  labs(x = "Nucleotide position", y = "Feature", fill = "Type") +
  theme_minimal() +
  geom_gene_arrow() +
  geom_gene_label() +
  common_theme +
  labs(y = "") +
  theme(axis.text.y = element_blank(), axis.ticks.y = element_blank()) +
  theme(plot.margin = margin(t = 0, r = 5, l = 5, b = 0)) +
  xlim(c(0, NA))


# Define myset
myset <- all %>%
  mutate(blcut = cut(bl62_score, 3)) %>%
  filter(total_muts > 10, flagged) %>%
  mutate(mut_type = case_when(
    (alternative_aa == "*") & (original_aa != "*") ~ "STOP",
    # bl62_score < -0 ~ "Negative BLOSUM",
    is_synonymous ~ "Synonymous",
    TRUE ~ "Non-synonymous (all)"
  )) %>%
  filter(mut_type != "STOP")

start_nsp14_codon <- 5926
end_nsp14_codon <- 6452


myset <- myset %>%
  mutate(
    is_nsp14 = ifelse(gene == "ORF1ab" & aa_index >= start_nsp14_codon & aa_index <= end_n
    nsp14_index = ifelse(is_nsp14, aa_index - start_nsp14_codon + 1, NA)
  )

nsp14_muts <- myset %>%
  filter(is_nsp14, !is_synonymous) %>%
  group_by(aa_string, nsp14_index) %>%
  tally() %>%
  arrange(-n)

ggplot(nsp14_muts %>% filter(n > 4), aes(x = nsp14_index)) +
  geom_density(bw = 50)
```



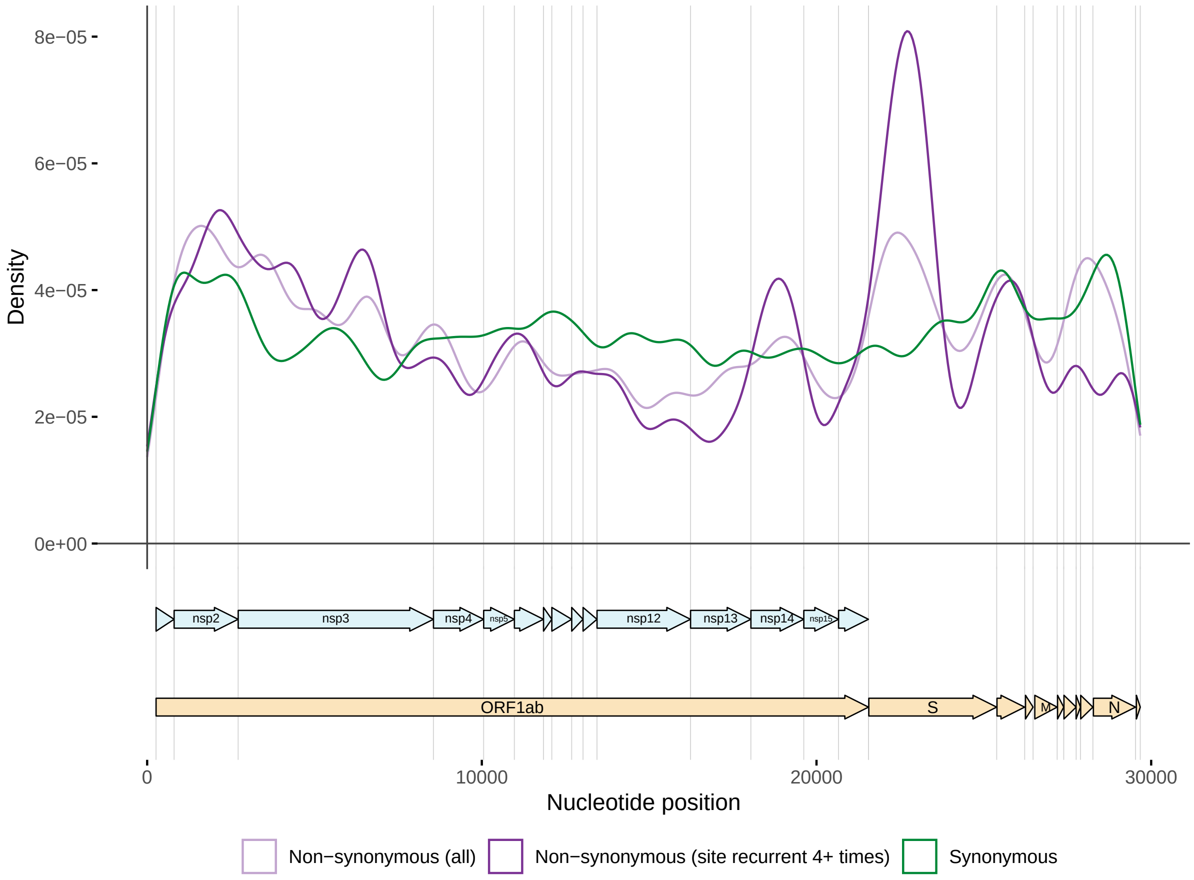
```
multiple <- myset %>%
  filter(!is_synonymous) %>%
  group_by(nt_index) %>%
  tally() %>%
  filter(n > 3) %>%
  mutate(mut_type = "Non-synonymous (site recurrent 4+ times)")

fullmyset <- bind_rows(myset, multiple)

my_colors <- c(
  "STOP" = "#D55E00",
  "Synonymous" = "#008837",
  "Non-synonymous (all)" = "#c2a5cf",
  "Non-synonymous (site recurrent 4+ times)" = "#7b3294"
)
```

```
density_plot <- ggplot(fullmyset, aes(x = nt_index, color = mut_type, group = mut_type)) +
  generate_vline(end_points) +
  geom_density(bw = 500) +
  theme_minimal() +
  common_theme +
  theme(
    axis.title.x = element_blank(),
    axis.text.x = element_blank(),
    axis.ticks.x = element_blank(),
    legend.position = "bottom", # change position to top, bottom, left, right or c(x, y) f
    legend.direction = "horizontal"
  ) +
  geom_hline(yintercept = 0, color = "#444444", size = 0.4) +
  geom_vline(xintercept = 0, color = "#444444", size = 0.4) +
  scale_color_manual(values = my_colors) +
  labs(y = "Density", color = "") +
  theme(plot.margin = margin(t = 5, r = 5, l = 5, b = 0))


density_plot
```



```r
# Legends
legends <- plot_grid(get_legend(density_plot))
legends
```

```r
# Final plot
final_plot <- plot_grid(density_plot + theme(legend.position = "none"), NULL, hu1_plot + t

final_plot
```



```
ggsave("genome.pdf", width = 10, height = 4)
```

```
myset$nt_mut = paste0(myset$original_nt,myset$nt_index, myset$alternative_nt)

fortable <- myset %>%
  filter(!is_synonymous) %>%
  group_by(original_aa, alternative_aa, gene, aa_index, mutation_type,nt_mut) %>%
  tally() %>%
  mutate(mut_types = paste0(mutation_type, ":", n)) %>%
  mutate(nt_muts=paste0(nt_mut, ":", n)) %>%
  group_by(original_aa, alternative_aa, gene, aa_index) %>%
  summarise(n = sum(n), types = paste(mut_types, collapse = ", "),nt_muts =  paste(nt_muts
  arrange(-n) %>%
  filter(gene == "S") %>%
  mutate(mut_format = paste0("S:", original_aa, aa_index, alternative_aa)) %>%
  mutate(type = substr(types, 1, 3)) %>%
  ungroup()
```

```
`summarise()` has grouped output by 'original_aa', 'alternative_aa', 'gene'.
You can override using the `.groups` argument.
```

```
fortable <- fortable %>%
  mutate(index = as.numeric(str_extract(nt_muts, "\\d+"))) %>% inner_join(ref_tib)
```

```
Joining with `by = join_by(index)`
```

```
fortable <- fortable %>%
  mutate(context = paste0(context_before, substr(nt_muts, 1, 1), context_after))


library(gridExtra)
```

```

Attaching package: 'gridExtra'

The following object is masked from 'package:dplyr':

    combine

The following object is masked from 'package:BiocGenerics':

    combine
```

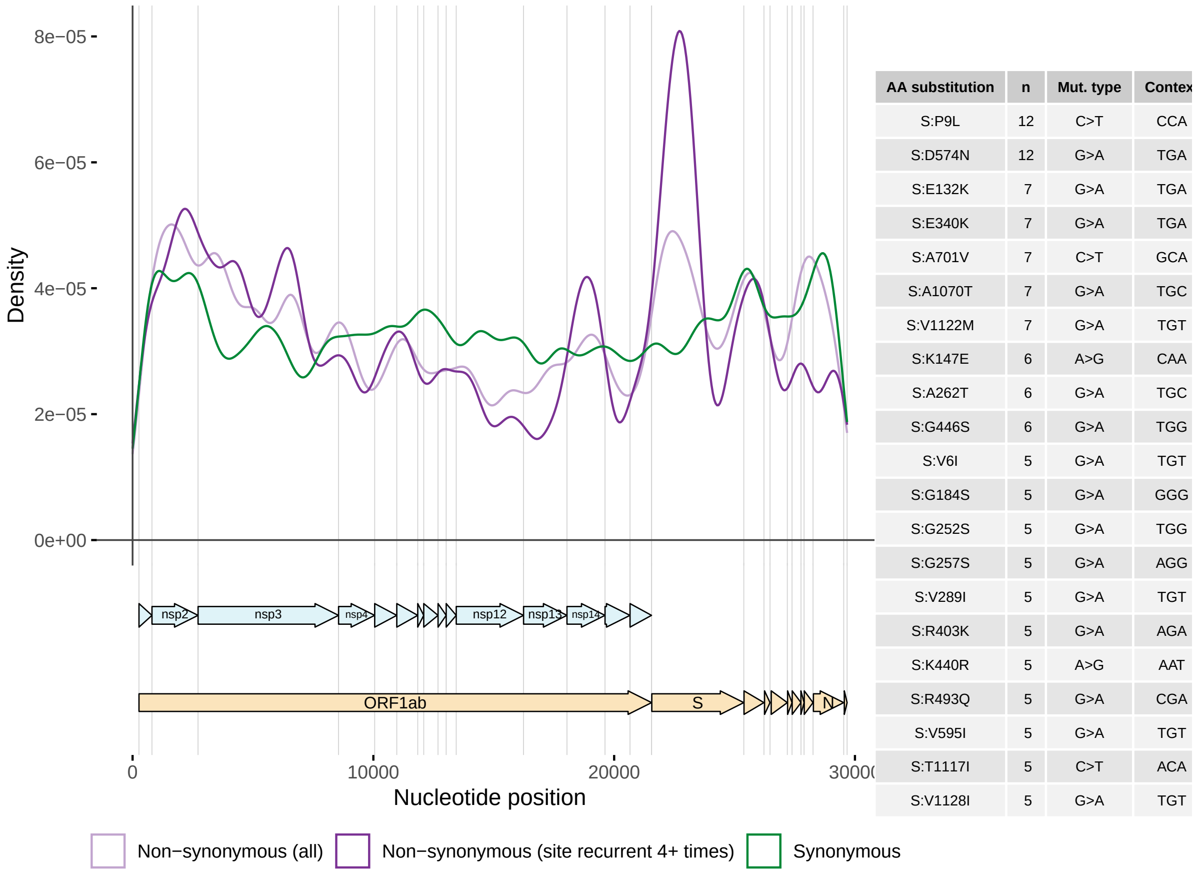
```
table_theme <- ttheme_default(
  core = list(fg_params = list(cex = 0.6)), # font size for table body
  colhead = list(fg_params = list(cex = 0.6)), # font size for column headers
  rowhead = list(fg_params = list(cex = 0.6)) # font size for row headers
)


fortable
```

```
# A tibble: 525 x 14
   original_aa alternative_aa gene  aa_index     n types  nt_muts    mut_format
   <chr>       <chr>          <chr>    <dbl> <int> <chr>  <chr>      <chr>
 1 D           N              S          574    12 G>A:12 G23282A:12 S:D574N
 2 P           L              S            9    12 C>T:12 C21588T:12 S:P9L
 3 A           T              S         1070     7 G>A:7  G24770A:7  S:A1070T
 4 A           V              S          701     7 C>T:7  C23664T:7  S:A701V
 5 E           K              S          132     7 G>A:7  G21956A:7  S:E132K
 6 E           K              S          340     7 G>A:7  G22580A:7  S:E340K
 7 V           M              S         1122     7 G>A:7  G24926A:7  S:V1122M
 8 A           T              S          262     6 G>A:6  G22346A:6  S:A262T
 9 G           S              S          446     6 G>A:6  G22898A:6  S:G446S
10 K           E              S          147     6 A>G:6  A22001G:6  S:K147E
# i 515 more rows
# i 6 more variables: type <chr>, index <dbl>, residue <chr>,
#   context_before <chr>, context_after <chr>, context <chr>
```

```r
# Convert the fortable data frame to a table grob
table_grob <- tableGrob(fortable %>% filter(n > 4) %>% arrange(-n, aa_index) %>% select(mu

fortable %>% filter(n > 4)  %>% group_by(context) %>% tally() %>% arrange(-n)
```

```
# A tibble: 13 x 2
   context     n
   <chr>   <int>
 1 TGT         5
 2 TGA         3
 3 TGC         2
 4 TGG         2
 5 AAT         1
 6 ACA         1
 7 AGA         1
 8 AGG         1
 9 CAA         1
10 CCA         1
11 CGA         1
12 GCA         1
13 GGG         1
```

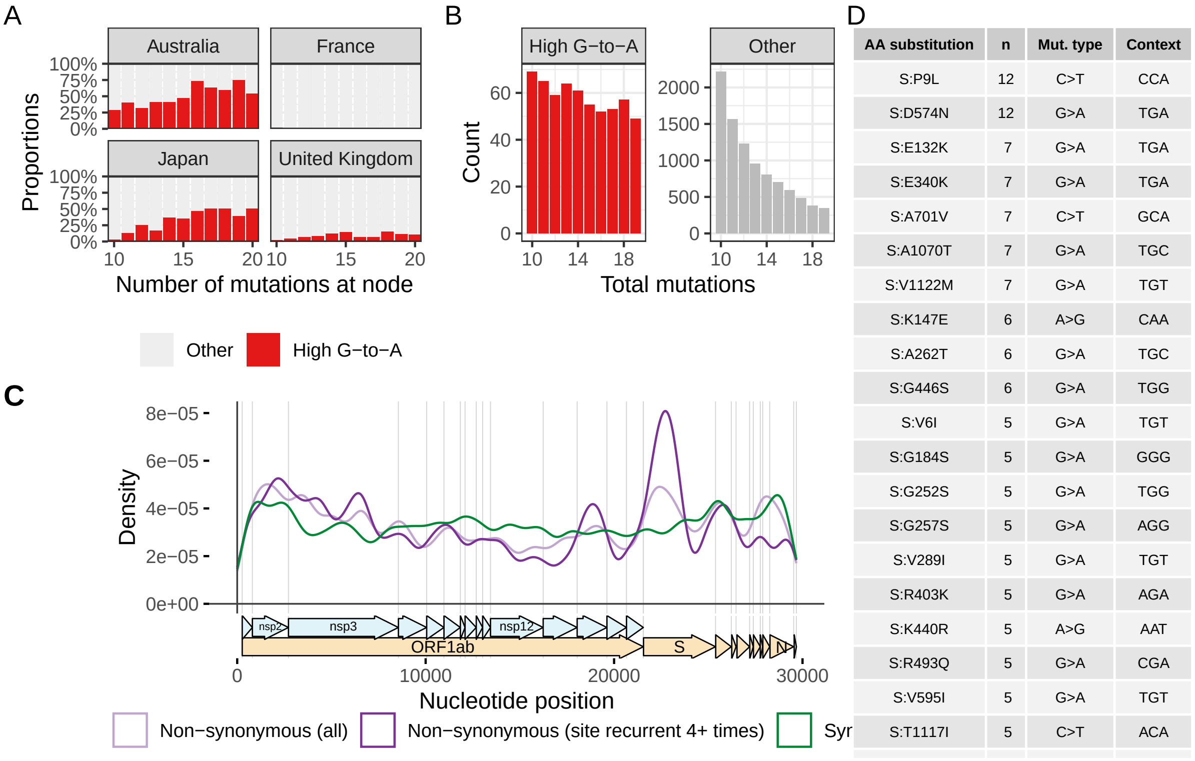
```r
grid.arrange(final_plot, table_grob, ncol = 2, widths = c(3, 1))
```



AA substitution	n	Mut. type	Contex
S:P9L	12	C>T	CCA
S:D574N	12	G>A	TGA
S:E132K	7	G>A	TGA
S:E340K	7	G>A	TGA
S:A701V	7	C>T	GCA
S:A1070T	7	G>A	TGC
S:V1122M	7	G>A	TGT
S:K147E	6	A>G	CAA
S:A262T	6	G>A	TGC
S:G446S	6	G>A	TGG
S:V6I	5	G>A	TGT
S:G184S	5	G>A	GGG
S:G252S	5	G>A	TGG
S:G257S	5	G>A	AGG
S:V289I	5	G>A	TGT
S:R403K	5	G>A	AGA
S:K440R	5	A>G	AAT
S:R493Q	5	G>A	CGA
S:V595I	5	G>A	TGT
S:T1117I	5	C>T	ACA
S:V1128I	5	G>A	TGT

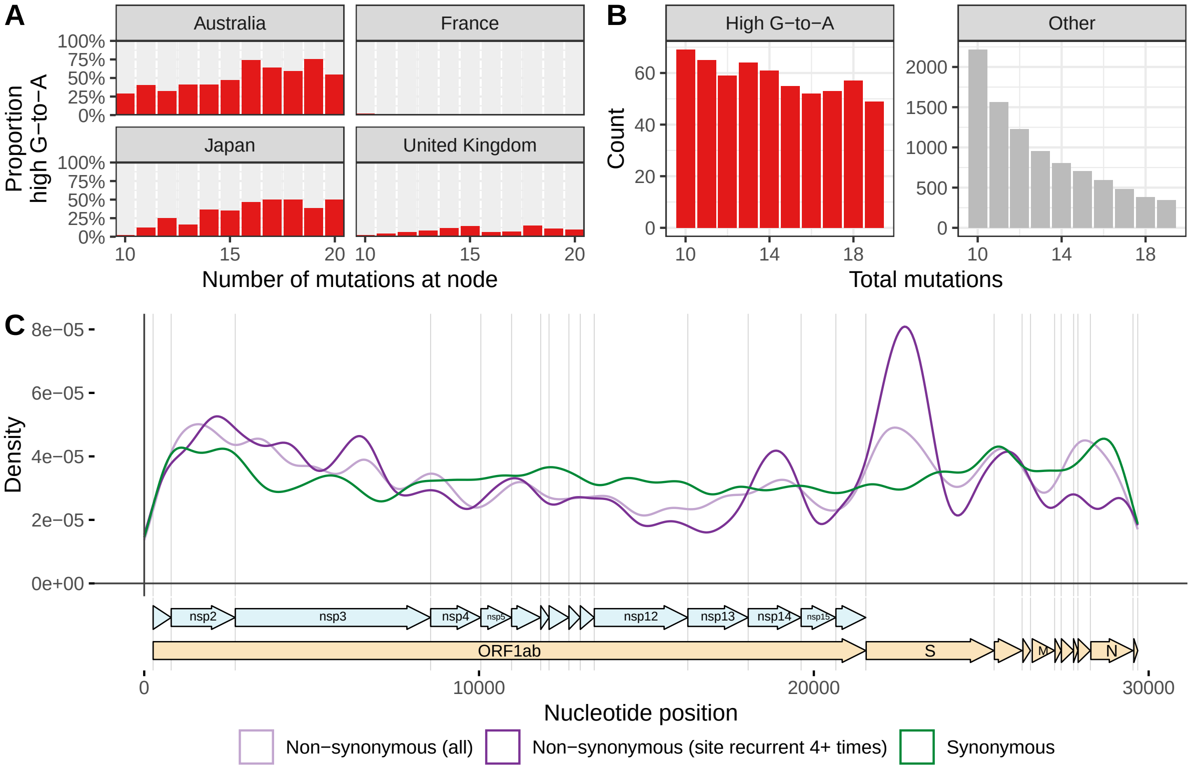
```r
library(ggplotify)
table_plot <- as.ggplot(table_grob)

# Arrange the plot and table using patchwork
final_figure <-
  proportions_of_long_branches + distributions +
  final_plot + table_plot +
  plot_layout(ncol = 2, widths = c(3, 1))
```

```r
layout <- "
AABBDD
CCCCDD
CCCCDD
"
proportions_of_long_branches + distributions +
  final_plot + table_plot +
plot_layout(design = layout) + plot_annotation(tag_levels = "A")
```

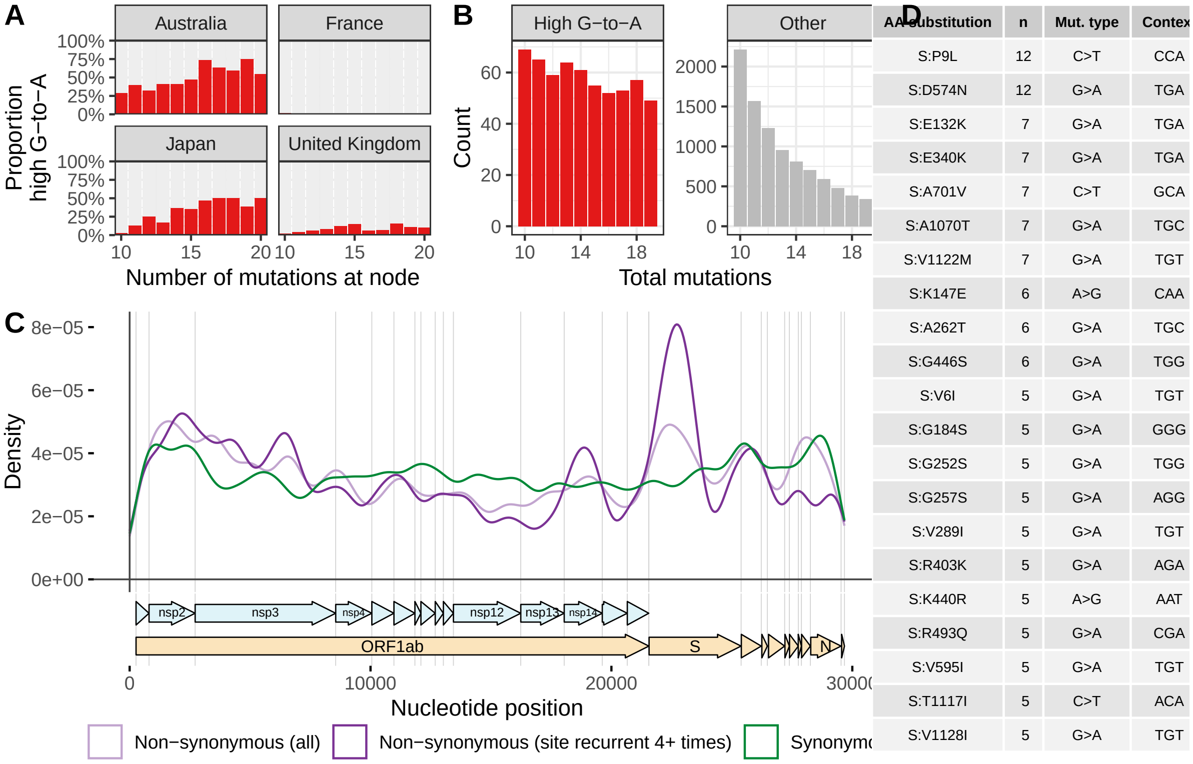


AA substitution	n	Mut. type	Context
S:P9L	12	C>T	CCA
S:D574N	12	G>A	TGA
S:E132K	7	G>A	TGA
S:E340K	7	G>A	TGA
S:A701V	7	C>T	GCA
S:A1070T	7	G>A	TGC
S:V1122M	7	G>A	TGT
S:K147E	6	A>G	CAA
S:A262T	6	G>A	TGC
S:G446S	6	G>A	TGG
S:V6I	5	G>A	TGT
S:G184S	5	G>A	GGG
S:G252S	5	G>A	TGG
S:G257S	5	G>A	AGG
S:V289I	5	G>A	TGT
S:R403K	5	G>A	AGA
S:K440R	5	A>G	AAT
S:R493Q	5	G>A	CGA
S:V595I	5	G>A	TGT
S:T1117I	5	C>T	ACA

```
a <- plot_grid(proportions_of_long_branches + theme(legend.position = "none") + labs(y = "
b <- plot_grid(a, final_plot, ncol = 1, labels = c("", "C"), rel_heights = c(0.4, 0.6))
b
```

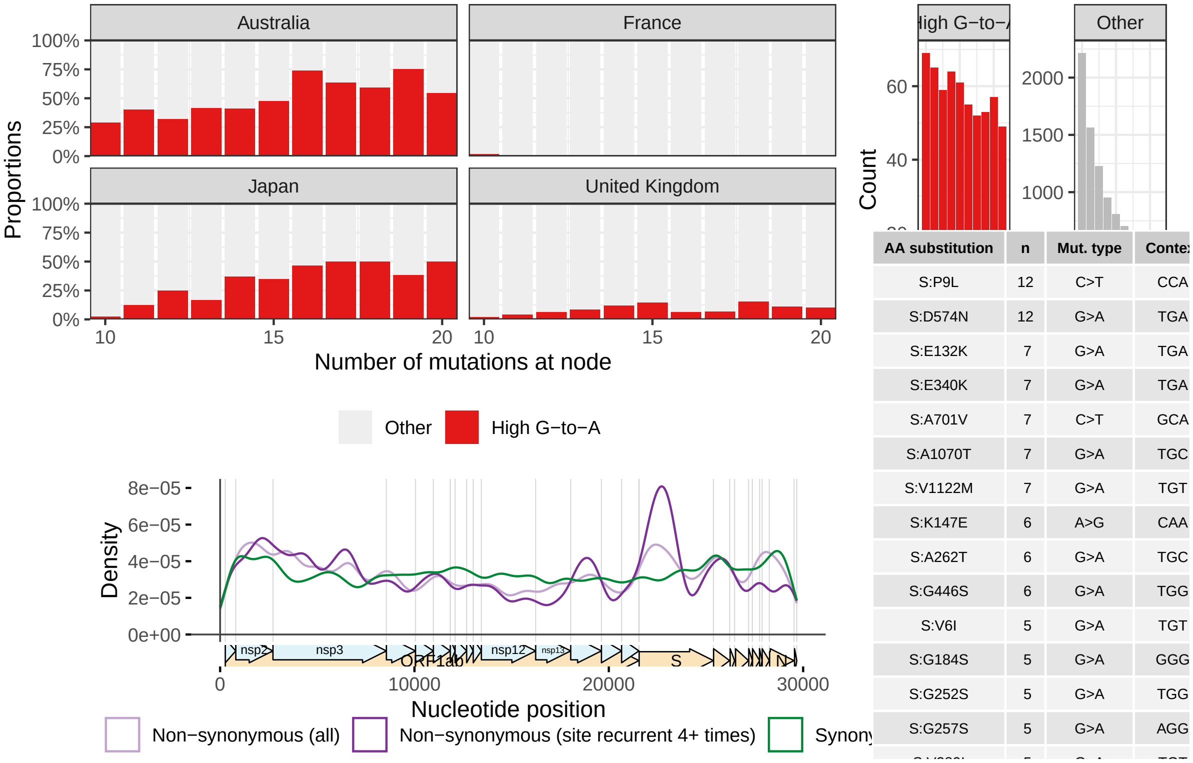


```
c <- plot_grid(b, table_plot, labels = c("", "D"), rel_widths = c(0.75, 0.25))

c
```



AA substitution	n	Mut. type	Contex
S:P9L	12	C>T	CCA
S:D574N	12	G>A	TGA
S:E132K	7	G>A	TGA
S:E340K	7	G>A	TGA
S:A701V	7	C>T	GCA
S:A1070T	7	G>A	TGC
S:V1122M	7	G>A	TGT
S:K147E	6	A>G	CAA
S:A262T	6	G>A	TGC
S:G446S	6	G>A	TGG
S:V6I	5	G>A	TGT
S:G184S	5	G>A	GGG
S:G252S	5	G>A	TGG
S:G257S	5	G>A	AGG
S:V289I	5	G>A	TGT
S:R403K	5	G>A	AGA
S:K440R	5	A>G	AAT
S:R493Q	5	G>A	CGA
S:V595I	5	G>A	TGT
S:T1117I	5	C>T	ACA
S:V1128I	5	G>A	TGT

```r
ggsave("~/movmanuscript2/Figures2/figtt.pdf", width = 9, height = 5.15)

# Print the final figure
print(final_figure)
```



AA substitution	n	Mut. type	Conte
S:P9L	12	C>T	CCA
S:D574N	12	G>A	TGA
S:E132K	7	G>A	TGA
S:E340K	7	G>A	TGA
S:A701V	7	C>T	GCA
S:A1070T	7	G>A	TGC
S:V1122M	7	G>A	TGT
S:K147E	6	A>G	CAA
S:A262T	6	G>A	TGC
S:G446S	6	G>A	TGG
S:V6I	5	G>A	TGT
S:G184S	5	G>A	GGG
S:G252S	5	G>A	TGG
S:G257S	5	G>A	AGG

```
nsp14_muts
```

```
# A tibble: 177 x 3
# Groups:   aa_string [177]
   aa_string      nsp14_index     n
   <chr>                <dbl> <int>
 1 ORF1ab:S6428L          503     6
 2 ORF1ab:A6044T          119     5
 3 ORF1ab:A6245V          320     5
 4 ORF1ab:T6056I          131     5
 5 ORF1ab:T6175I          250     5
 6 ORF1ab:T6303I          378     5
 7 ORF1ab:T6449I          524     5
 8 ORF1ab:V6026I          101     5
 9 ORF1ab:A6296V          371     4
10 ORF1ab:A6319T          394     4
# i 167 more rows
```

```r
nsp14_muts$nsp14_index[1:30]
```

```
 [1] 503 119 320 131 250 378 524 101 371 394 228 113   4 120 317 437 308 471 432
[20] 427 315 461  31 372 287 346 119 187 307 344
```

```r
data_nodes %>%
  filter(flagged) %>%
  filter(total_muts >= 10) %>%
  arrange(-num_descendants)
```

```
# A tibble: 732 x 23
   node_id      num_descendants consensus_country consensus_year date
   <chr>                  <dbl> <chr>             <chr>          <date>
 1 node_2589094              20 Australia         2022           2022-07-12
 2 node_2669086              13 New Zealand       2023           2023-01-11
 3 node_2661929              11 Australia         2022           2022-11-27
 4 node_2324333               9 Australia         2022           2022-09-01
 5 node_1676393               7 Italy             2022           2022-03-30
 6 node_3155949               7 Australia         2022           2022-09-22
 7 node_388695                6 India             2021           2021-10-29
 8 node_2325826               6 Japan             2023           2023-01-31
 9 node_1994127               5 Japan             2022           2022-03-05
10 node_2359282               5 Australia         2022           2022-05-31
# i 722 more rows
# i 18 more variables: date_length <dbl>, age <chr>, `A>C` <dbl>, `A>G` <dbl>,
#   `A>T` <dbl>, `C>A` <dbl>, `C>G` <dbl>, `C>T` <dbl>, `G>A` <dbl>,
#   `G>C` <dbl>, `G>T` <dbl>, `T>A` <dbl>, `T>C` <dbl>, `T>G` <dbl>,
#   total_muts <dbl>, transitions <dbl>, transversions <dbl>, flagged <lgl>
```

```r
myset
```

```
# A tibble: 9,850 x 39
   node_id           num_descendants consensus_country consensus_year date
   <chr>                       <dbl> <chr>             <chr>          <date>
 1 India/HR-NCDC-90~               1 India             2020           2020-12-16
 2 India/HR-NCDC-90~               1 India             2020           2020-12-16
 3 India/HR-NCDC-90~               1 India             2020           2020-12-16
 4 India/HR-NCDC-90~               1 India             2020           2020-12-16
 5 India/HR-NCDC-90~                1 India            2020           2020-12-16
 6 India/HR-NCDC-90~                1 India            2020           2020-12-16
 7 India/HR-NCDC-90~                1 India            2020           2020-12-16
 8 India/HR-NCDC-90~                1 India            2020           2020-12-16
 9 India/HR-NCDC-90~                1 India            2020           2020-12-16
10 India/HR-NCDC-90~                1 India            2020           2020-12-16
# i 9,840 more rows
# i 34 more variables: date_length <dbl>, age <chr>, `A>C` <dbl>, `A>G` <dbl>,
#   `A>T` <dbl>, `C>A` <dbl>, `C>G` <dbl>, `C>T` <dbl>, `G>A` <dbl>,
#   `G>C` <dbl>, `G>T` <dbl>, `T>A` <dbl>, `T>C` <dbl>, `T>G` <dbl>,
#   total_muts <dbl>, transitions <dbl>, transversions <dbl>, flagged <lgl>,
#   nt_index <dbl>, original_nt <chr>, alternative_nt <chr>, gene <chr>,
#   aa_index <dbl>, original_aa <chr>, alternative_aa <chr>, ...
```

Context-specific bayes factors

```
mutations_in_highly_mutated_seq = "A543G, G1068A, G1186A, G1264A, T1370C, G1743A, A2497G,


mutations_in_highly_mutated_seq = str_replace_all(mutations_in_highly_mutated_seq, "nt:",
# Split the string by commas, and then extract the initial nucleotide, index, and final nu
mutations_tibble <- str_split(mutations_in_highly_mutated_seq, ",\\s*") %>%
  unlist() %>%
  tibble(mutation = .)  %>%
  mutate(
    par = str_extract(mutation, "^[A-Z]"),
    index = str_extract(mutation, "[0-9]+"),
    mut = str_extract(mutation, "[A-Z]$")
  ) %>%
  select(-mutation) %>% mutate(index=as.numeric(index)) %>% inner_join(ref_tib)
```

```
Joining with `by = join_by(index)`
```

```
of_interest = mutations_tibble %>% group_by(par,context_before,context_after,mut) %>% tall


unnormalise <- function(df){
```

```
inner_join(df,nuc_genome_counts) %>% mutate(spectrum_value = spectrum_value * genome_count
}

model1 = long %>% mutate(type=paste0(par,mut)) %>% rename(spectrum_value = Number_of_mutat
```

```
Joining with `by = join_by(context_before, par, context_after)`
```

```
model2 = ba1 %>% mutate(type=paste0(par,mut)) %>% rename(spectrum_value = Number_of_mutati
```

```
Joining with `by = join_by(context_before, par, context_after)`
```

```
types_of_interest = c("GA","CT","AG","TC")

library(nnet)

calc_bfs <- function(of_interest){

join_everything = full_join(model1,model2,by=c("par","mut","type","context_before","contex



bfs = c()
# Step 1: Filter data for each type of interest
for (mytype in types_of_interest) {

  filtered = join_everything %>% filter(type==mytype)
  prob1 = dmultinom(filtered$n, size = sum(filtered$n), prob = filtered$spectrum_value_1,
  prob2 = dmultinom(filtered$n, size = sum(filtered$n), prob = filtered$spectrum_value_2,
  bf = prob1/prob2
  bfs[mytype] = bf

}

bfs
}

bfs = calc_bfs(of_interest)
bfs
```

```
          GA           CT           AG           TC 
35017.651240  6068.265541    52.565716     1.227803 
```

```
prod(bfs)
```

```
[1] 13714590434
```