

shm_analysis

Analysis of substitution type and frequency

Load preprocessed data

```
library(dplyr)
library(ggplot2)
library(data.table)
library(scales)

select = dplyr::select
summarise = dplyr::summarise

load("shm_rep12.rda")
df = data.table(shm) %>%
  mutate(region = as.character(region)) %>%
  filter(type == "RNA") %>%
  mutate(region = factor(region, c("FR1", "CDR1", "FR2", "CDR2", "FR3", "CDR3", "FR4"))) %>%
  mutate(mutation.type = ifelse(as.character(from.aa) == as.character(to.aa), "S", "R")) %>%
  group_by(proj, type, sample) %>%
  mutate(weight2 = 1/n())
```

Check if we observe well-documented increase in replacement:synonimic hypermutation ratio in CDR regions:

```
p5=ggplot(df %>% mutate(weight2 = weight2/sum(weight2))) +
  geom_histogram(binwidth = 1, aes(x=pos.aa, weight=weight2, fill=region)) +
  #geom_density(adjust=2, aes(x=pos.aa, weight=weight, fill=region), position = "stack", color = NA) +
  geom_density(binwidth = 1, aes(x=pos.aa, weight=weight2, linetype = mutation.type)) +
  scale_y_continuous("Hypermutation density", expand = c(0,0)) +
  scale_x_continuous("Position in IG, AA", expand = c(0,0)) +
  scale_linetype("SHM type") +
  scale_fill_brewer("Region", palette = "Paired") +
  theme_bw() +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        legend.position = c(0.75, 0.81),
        legend.direction = "horizontal")
```

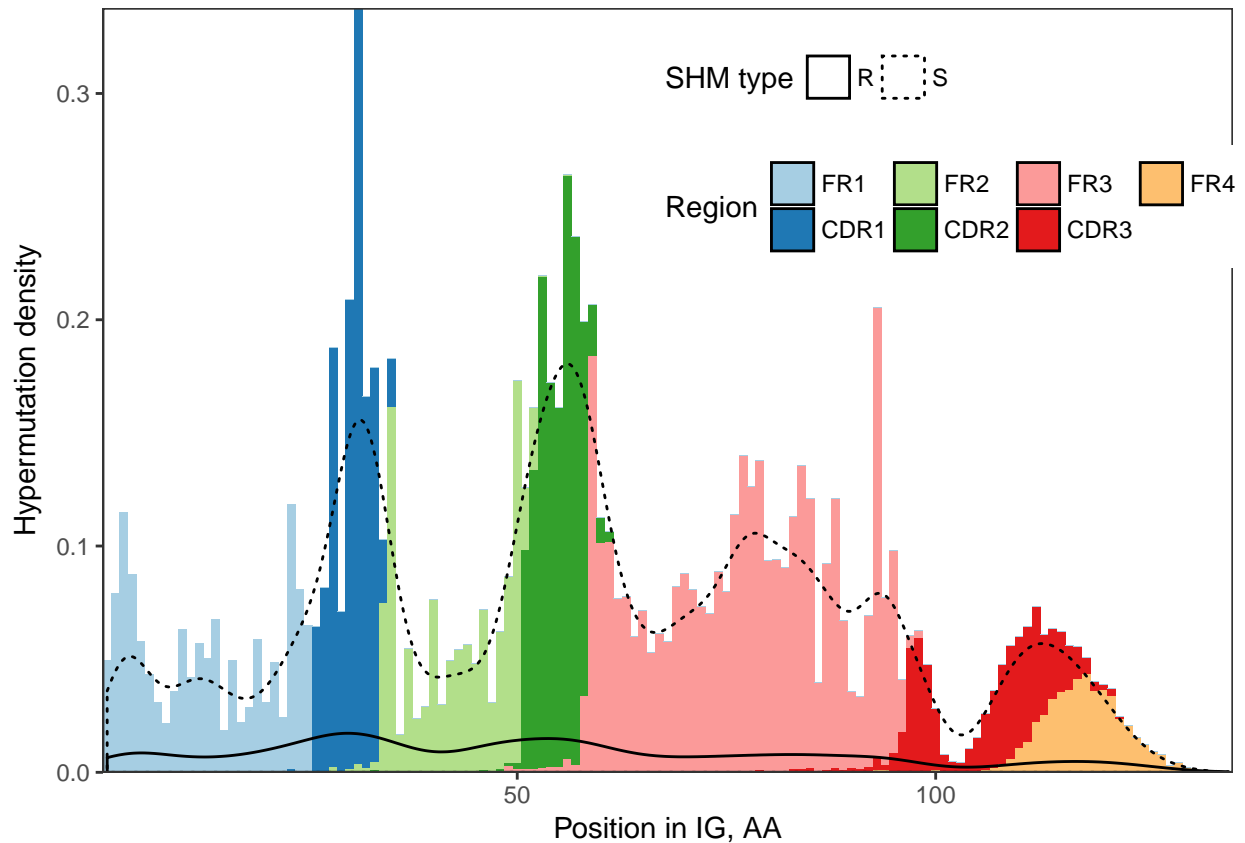
```
## Warning: Ignoring unknown aesthetics: weight
```

```
## Warning: Ignoring unknown parameters: binwidth
```

```
p5
```

```
## Warning in density.default(x, weights = w, bw = bw, adjust = adjust, kernel
## = kernel, : sum(weights) != 1 -- will not get true density
```

```
## Warning in density.default(x, weights = w, bw = bw, adjust = adjust, kernel
## = kernel, : sum(weights) != 1 -- will not get true density
```



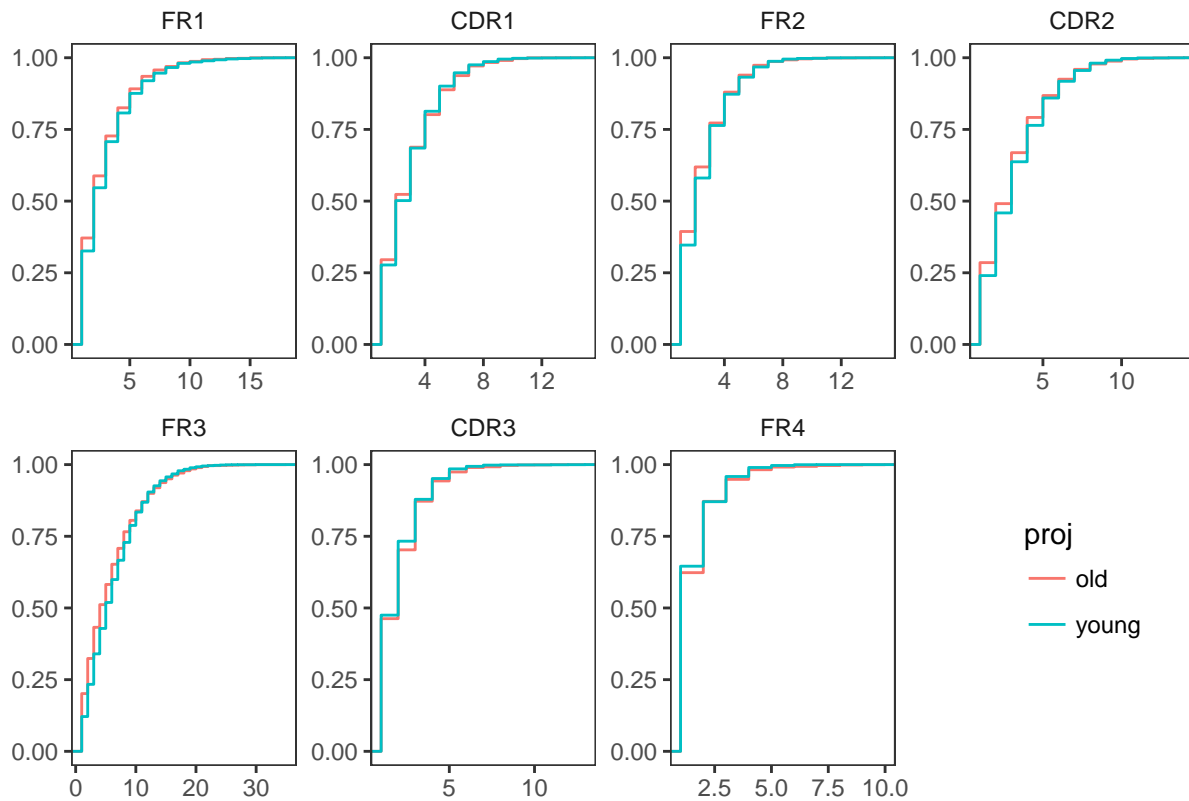
Relative hypermutation burden by region for old and young

```
df <- df %>%
  filter(from.aa != "" | to.aa != "" | is.na(pos.nt))

df.shm.share = df %>%
  group_by(proj, sample, region) %>%
  summarise(count = n()) %>%
  group_by(sample) %>%
  mutate(share = count/sum(count))

df.shm.1 = df %>%
  group_by(proj, sample, region, clone) %>%
  summarise(shms = n())

ggplot(df.shm.1, aes(x = shms, group = proj, color = proj)) +
  stat_ecdf() +
  scale_y_continuous("") +
  xlab("") +
  scale_fill_brewer("Age", palette = "Set1") +
  facet_wrap(~region, scales = "free", ncol=4) +
  theme_bw() +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.position = c(0.90, 0.25),
        strip.background = element_blank())
```

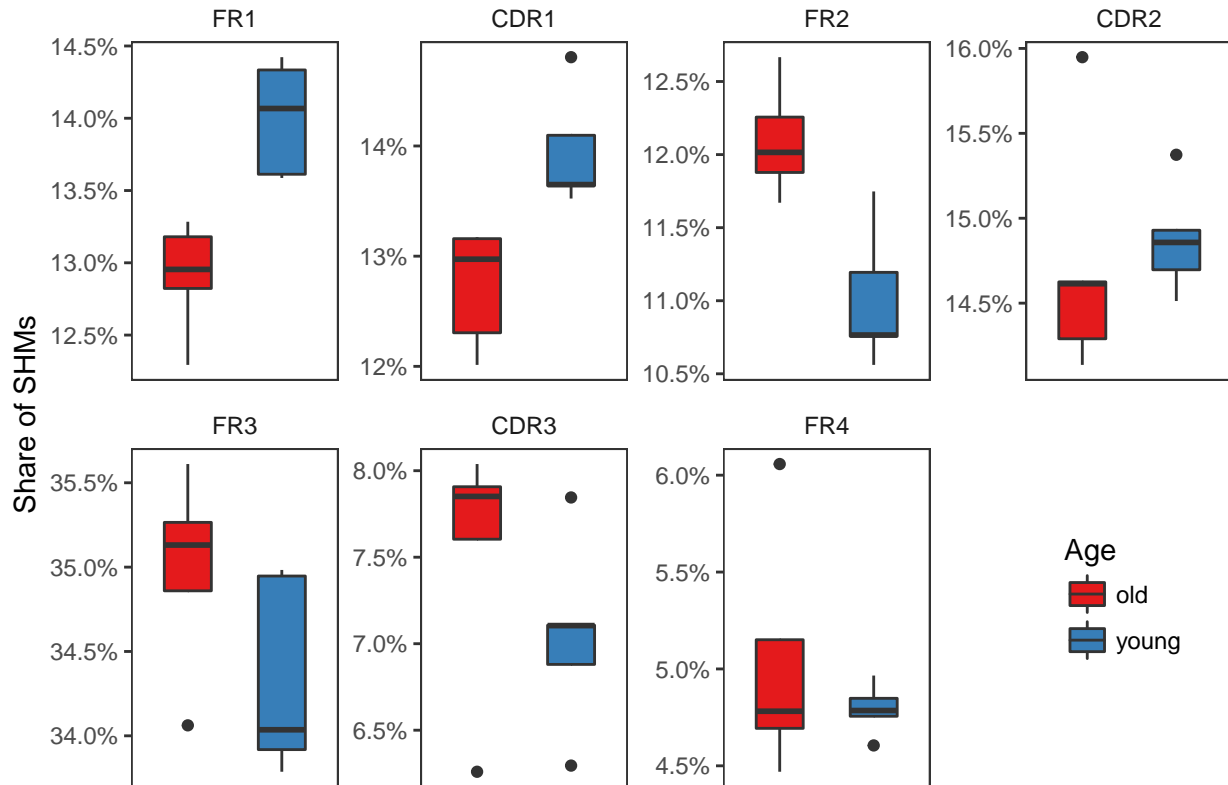


```
dt.p = data.table()
for (r in unique(df.shm.share$region)) {
  tt = t.test(share~proj, df.shm.share %>% filter(region == r))
  p = tt$p.value
  dt.p = rbind(dt.p,
               data.table(region = r, p.adj = p))
}
dt.p$p.adj = p.adjust(dt.p$p.adj, method="BH")
print(dt.p)
```

```
##   region    p.adj
## 1:   FR1 0.0149799
## 2:  CDR1 0.0156139
## 3:   FR2 0.0150996
## 4:  CDR2 0.6833822
## 5:   FR3 0.2015664
## 6:  CDR3 0.3812125
## 7:   FR4 0.5214301
```

```
ggplot(df.shm.share, aes(x = proj, group = proj, y = share, fill = proj)) +
  geom_boxplot(width = 0.5) +
  scale_y_continuous("Share of SHMs", label = percent) +
  xlab("") +
  scale_fill_brewer("Age", palette = "Set1") +
  facet_wrap(~region, scales = "free", ncol=4) +
  theme_bw() +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.position = c(0.90, 0.25),
```

```
axis.text.x = element_blank(), axis.ticks.x = element_blank(),
strip.background = element_blank())
```



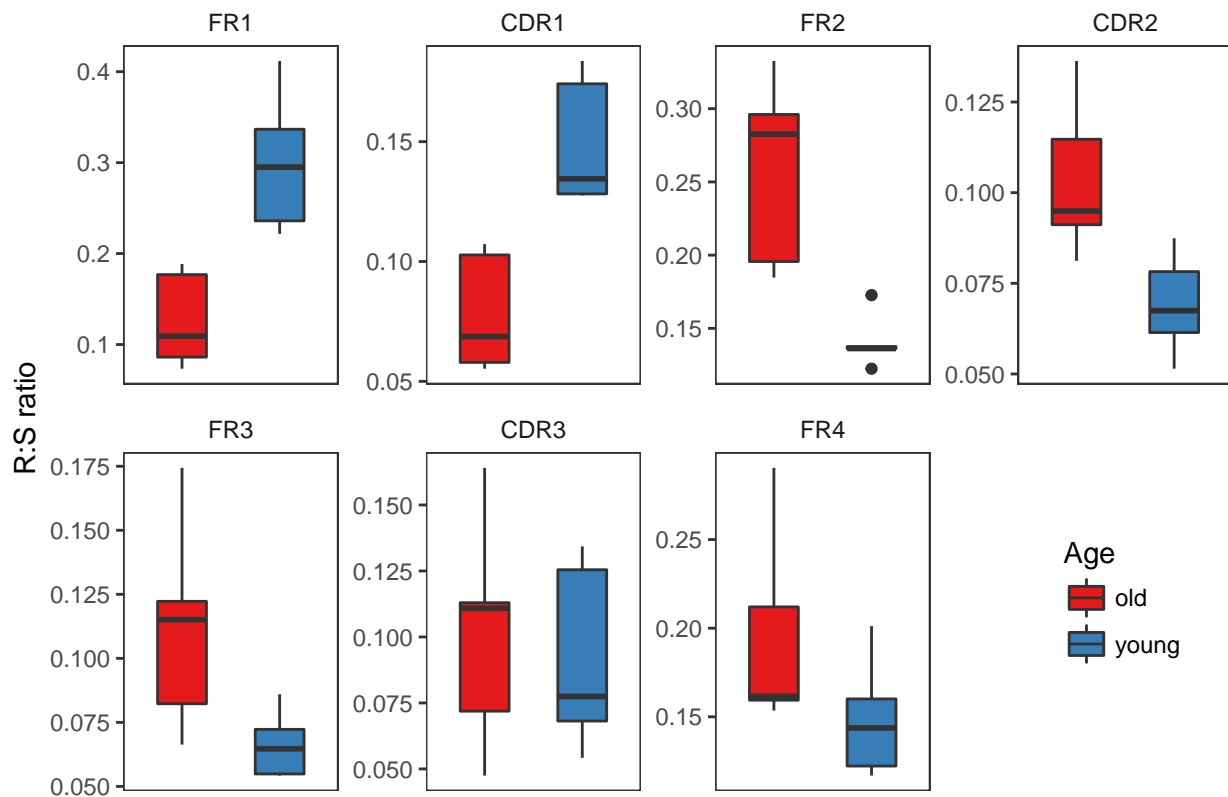
```
dt.p = data.table()
for (r in unique(df.shm.share$region)) {
  tt = t.test(share~proj, df.shm.share %>% filter(region == r))
  p = tt$p.value
  dt.p = rbind(dt.p,
               data.table(region = r, p.adj = p))
}
dt.p$p.adj = p.adjust(dt.p$p.adj, method="BH")
print(dt.p)
```

```
##   region    p.adj
## 1:   FR1 0.01497999
## 2:  CDR1 0.01561392
## 3:   FR2 0.01509968
## 4:  CDR2 0.68338227
## 5:   FR3 0.20156646
## 6:  CDR3 0.38121257
## 7:   FR4 0.52143014
```

Replacement to silent ratio

```
df.shm.rs = df %>%
  group_by(proj, sample, region, mutation.type) %>%
  summarise(count = n()) %>%
  group_by(proj, sample, region) %>%
  summarise(rs = sum(c(0, count[which(mutation.type == "R")])) / sum(c(0, count[which(mutation.type == "S")])))
```

```
ggplot(df.shm.rs, aes(x = proj, group = proj, y = rs, fill = proj)) +
  geom_boxplot(width = 0.5) +
  scale_y_continuous("R:S ratio") +
  xlab("") +
  scale_fill_brewer("Age", palette = "Set1") +
  facet_wrap(~region, scales = "free", ncol=4) +
  theme_bw() +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.position = c(0.90, 0.25),
        axis.text.x = element_blank(), axis.ticks.x = element_blank(),
        strip.background = element_blank())
```



```
dt.p = data.table()
for (r in unique(df.shm.rs$region)) {
  tt = t.test(rs~proj, df.shm.rs %>% filter(region == r))
  p = tt$p.value
  dt.p = rbind(dt.p,
               data.table(region = r, p=p,p.adj = p))
}
dt.p$p.adj = p.adjust(dt.p$p.adj, method="BH")
print(dt.p)
```

```
##   region      p      p.adj
## 1:   FR1 0.004334849 0.01517197
## 2:  CDR1 0.002543057 0.01517197
## 3:   FR2 0.013315718 0.03107001
## 4:  CDR2 0.021882940 0.03829514
## 5:   FR3 0.069422893 0.09719205
```

```
## 6:   CDR3 0.719110691 0.71911069
## 7:   FR4 0.169424088 0.19766144
```

Overall statistics

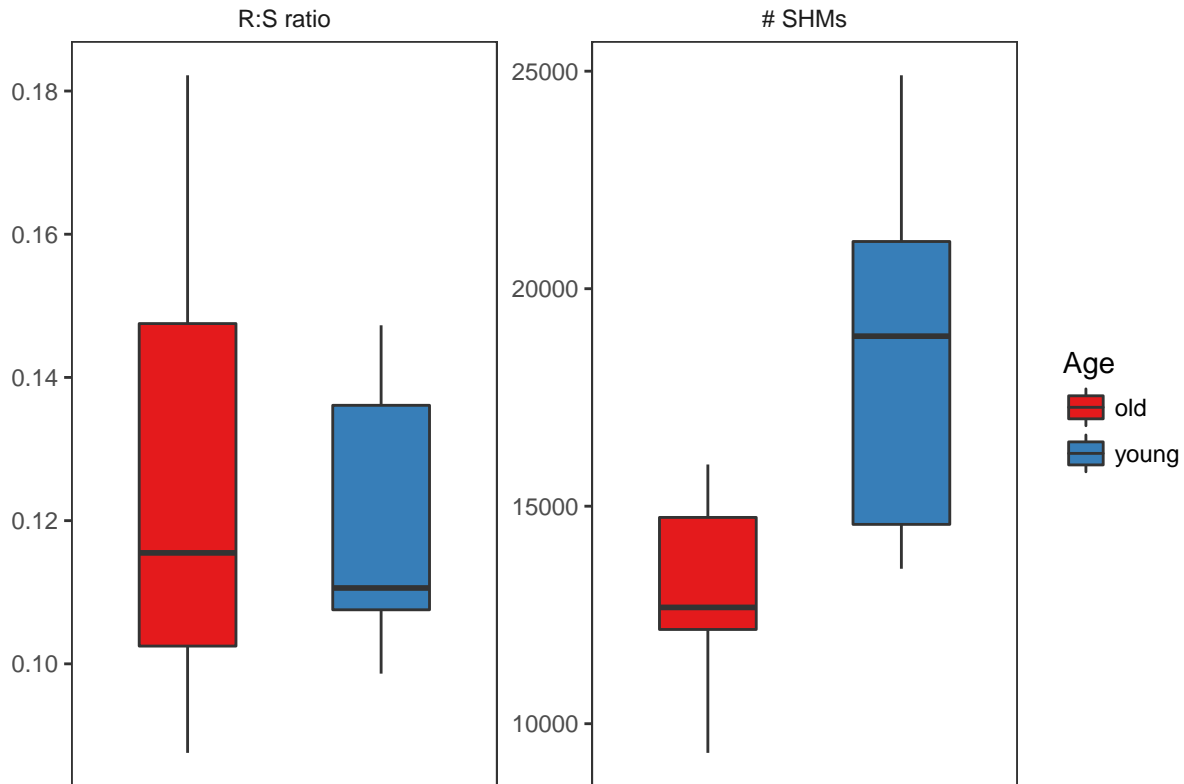
```
df.shm.share.s = df %>%
  group_by(proj, sample, stat="# SHMs") %>%
  summarise(value = n())

df.shm.rs.s = df %>%
  group_by(proj, sample, mutation.type) %>%
  summarise(count = n()) %>%
  group_by(proj, sample, stat="R:S ratio") %>%
  summarise(value = sum(c(0, count[which(mutation.type == "R")])) / sum(c(0, count[which(mutation.type == "S")])))

tmp = rbind(df.shm.share.s,
            df.shm.rs.s)

p6=ggplot(tmp,
  aes(x = proj, y = value, fill = proj))+
  geom_boxplot(width = 0.5) +
  scale_y_continuous("") +
  xlab("") +
  scale_fill_brewer("Age", palette = "Set1") +
  facet_wrap(~stat, scales = "free") +
  theme_bw() +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.text.x = element_blank(), axis.ticks.x = element_blank(),
        strip.background = element_blank())

p6
```



```
dt.p = data.table()
for (ss in unique(tmp$stat)) {
  tt = t.test(value ~ proj, tmp %>% filter(stat == ss))
  p = tt$p.value
  dt.p = rbind(dt.p,
               data.table(stat = ss, p=p))
}
print(dt.p)
```

```
##      stat      p
## 1:    # SHMs 0.05470575
## 2: R:S ratio 0.72838610
```

Substitution patterns

```
df.sign = df %>%
  mutate(mutation.signature = paste(from.nt, to.nt, sep = ">"))
```

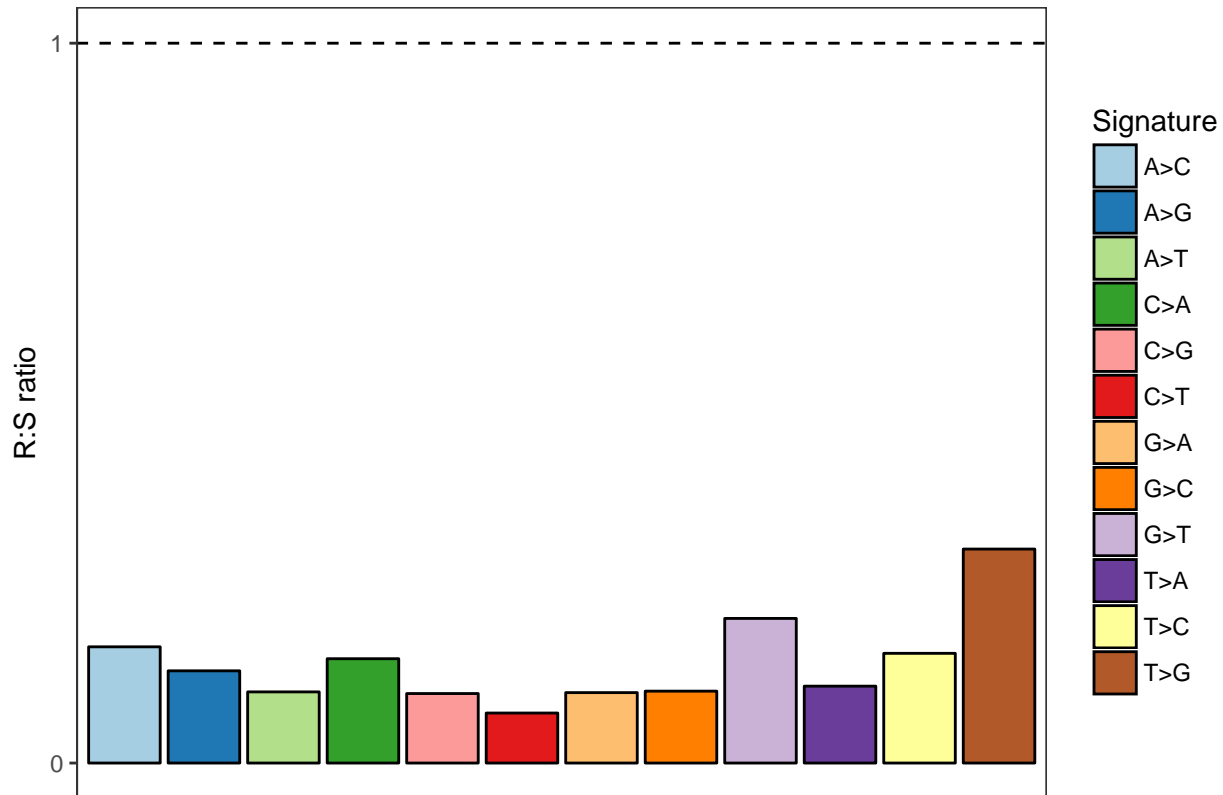
R:S ratio across different substitution patterns at nucleotide level

```
df.sign.rs = df.sign %>%
  group_by(mutation.signature, mutation.type) %>%
  summarise(count = n()) %>%
  dcast(mutation.signature ~ mutation.type, value.var = "count") %>%
  mutate(rs = R/S)

p7=ggplot(df.sign.rs, aes(x = mutation.signature, y = rs, fill = mutation.signature)) +
  geom_bar(stat="identity", color="black") +
  geom_hline(yintercept = 1, linetype="dashed") +
  scale_fill_brewer("Signature", palette = "Paired") +
  scale_y_continuous("R:S ratio", breaks=0:8) +
```

```
xlab("") +
theme_bw() +
theme(panel.grid.major = element_blank(),
      panel.grid.minor = element_blank(),
      axis.text.x = element_blank(), axis.ticks.x = element_blank())
```

p7



Share of different substitution patterns compared between young and old. Age-related difference observed for certain substitution patterns across hypermutations.

```
sign.dict = data.frame(mutation.signature = c("A>C", "A>G", "A>T", "C>A", "C>G", "C>T", "G>A",
                                             "G>C", "G>T", "T>A", "T>C", "T>G"),
                      mutation.signature.rep = c("A>C,T>G", "A>G,T>C", "A>T,T>A", "C>A,G>T",
                                                "C>G,G>C", "C>T,G>A", "C>T,G>A", "C>G,G>C",
                                                "C>A,G>T", "A>T,T>A", "A>G,T>C", "A>C,T>G"))

df.sign.total = df.sign %>%
  group_by(sample) %>%
  dplyr::summarize(total = n())

df.sign.s = df.sign %>%
  group_by(proj, sample, mutation.signature) %>%
  summarise(count = n()) %>%
  merge(df.sign.total) %>%
  mutate(freq = count / total)

df.sign.s$mutation.signature = factor(df.sign.s$mutation.signature,
                                     levels = with(df.sign.s %>% group_by(mutation.signature) %>%
                                                    summarise(freq = sum(freq)),
```



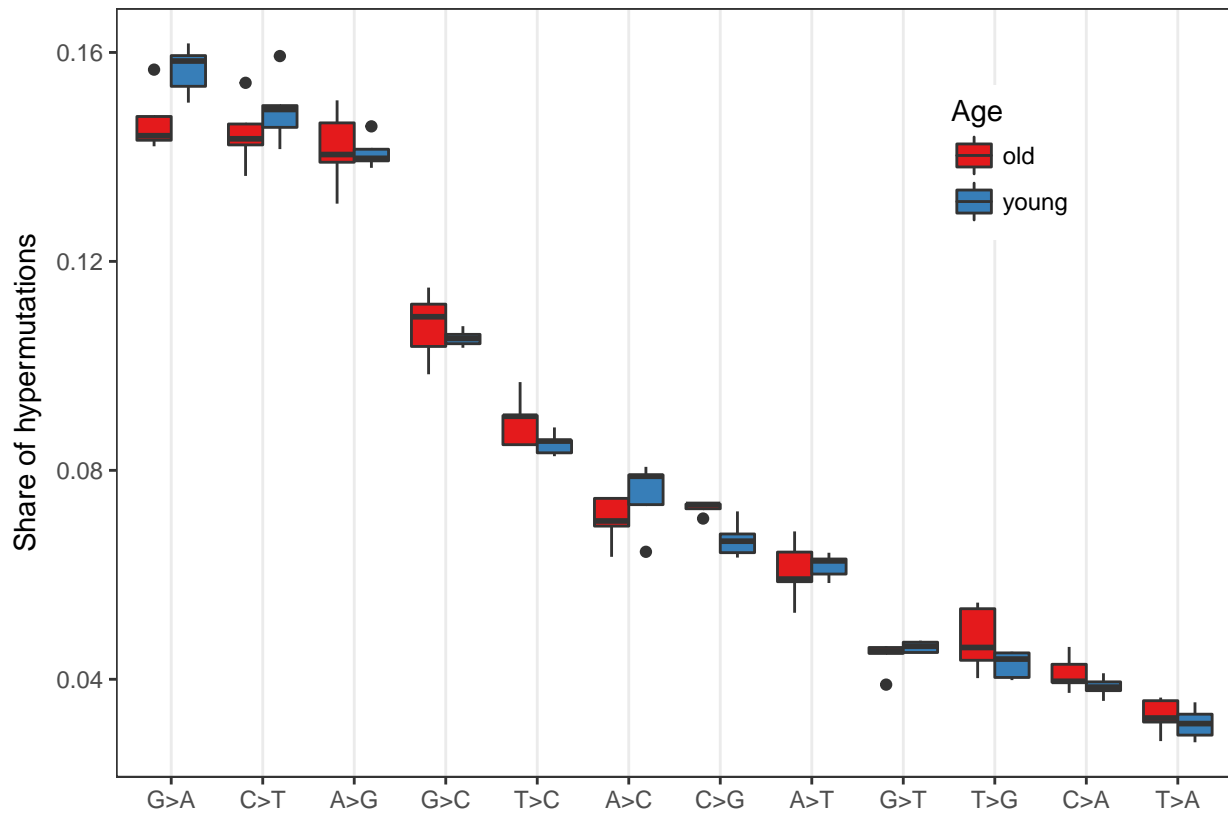
```

mutation.signature[order(-freq)])

p8=ggplot(df.sign.s, aes(x=mutation.signature, y = freq, fill = proj)) +
  geom_boxplot() +
  ylab("") + xlab("") +
  scale_fill_brewer("Age", palette = "Set1") +
  ylab("Share of hypermutations") +
  theme_bw() +
  theme(panel.grid.major.y = element_blank(),
        panel.grid.minor = element_blank(),
        legend.position = c(0.8, 0.8))

```

p8



```

a = aov(freq ~ proj * mutation.signature, df.sign.s)
summary(a)

```

```

##              Df Sum Sq Mean Sq F value Pr(>F)
## proj          1 0.0000 0.000000   0.000 1.00000
## mutation.signature 11 0.2079 0.018897 908.096 < 2e-16 ***
## proj:mutation.signature 11 0.0006 0.000054   2.613 0.00589 **
## Residuals      96 0.0020 0.000021
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```
dt.p = data.table()
```

```

for (ms in unique(df.sign.s$mutation.signature)) {
  tt = t.test(freq ~ proj, df.sign.s %>% filter(mutation.signature == ms))
  p = tt$p.value
}

```

```

dt.p = rbind(dt.p,
             data.table(mutation.signature = ms, p=p))
}

dt.p$p.adj = p.adjust(dt.p$p, method = "BH")
print(dt.p %>% arrange(p.adj))

##      mutation.signature          p      p.adj
## 1          C>G 0.01486223 0.1193861
## 2          G>A 0.01989768 0.1193861
## 3          A>C 0.22858500 0.4337770
## 4          C>A 0.20262026 0.4337770
## 5          G>T 0.25303658 0.4337770
## 6          T>C 0.12999652 0.4337770
## 7          T>G 0.17482864 0.4337770
## 8          C>T 0.30426699 0.4564005
## 9          G>C 0.48021710 0.5942171
## 10         T>A 0.49518091 0.5942171
## 11         A>T 0.72866829 0.7949109
## 12         A>G 0.84966112 0.8496611

aa.classes = data.table(aa = strsplit("I,V,L,F,C,M,A,W,G,T,S,Y,P,H,N,D,Q,E,K,R", ",")[[1]],
                        hydrop = c(rep("hydrophobic", 8), rep("neutral", 6),
                                   rep("hydrophilic", 6)))

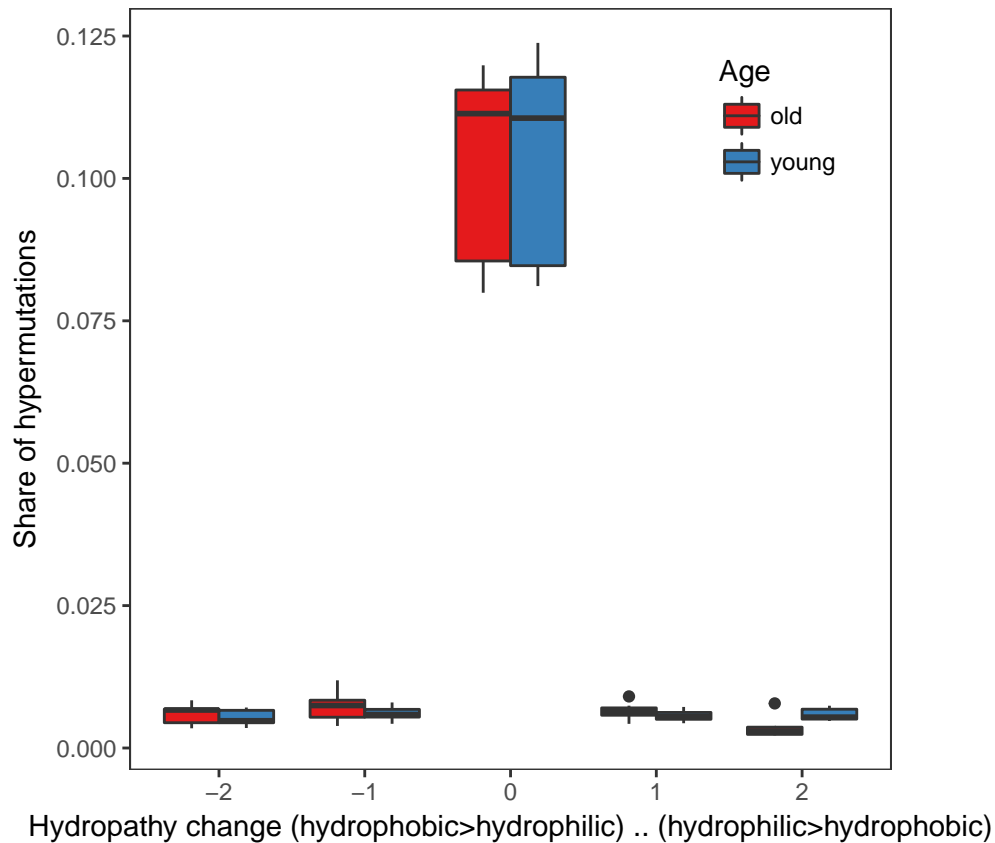
df.aachange = df %>%
  merge(aa.classes %>% mutate(from.aa = aa, from.value = hydrop) %>% select(from.aa, from.value)) %>%
  merge(aa.classes %>% mutate(to.aa = aa, to.value = hydrop) %>% select(to.aa, to.value))

hydrop_toint = function(x) {
  ifelse(x == "hydrophobic", 1, ifelse(x == "neutral", 0, -1))
}

dt.aachange.s = df.aachange %>%
  group_by(sample, proj, from.value, to.value) %>%
  summarise(count = n()) %>%
  group_by(sample, proj) %>%
  mutate(freq = count / sum(count),
         hydrop.change = hydrop_toint(to.value) - hydrop_toint(from.value)) %>%
  group_by(hydrop.change) %>%
  mutate(freq2 = freq / length(unique(paste(from.value, to.value))))

p9=ggplot(dt.aachange.s, aes(x=hydrop.change, group=paste(hydrop.change, proj), fill = proj, y= freq2))
  geom_boxplot() +
  scale_fill_brewer("Age", palette = "Set1") +
  ylab("Share of hypermutations") +
  xlab("Hydropathy change (hydrophobic>hydrophilic) .. (hydrophilic>hydrophobic)") +
  theme_bw() +
  theme(aspect=1,
        legend.position = c(0.85,0.85),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank())
p9

```



```
dt.p = data.table()

for (hc in unique(dt.aachange.s$hydrop.change)) {
  tt = t.test(freq ~ proj, dt.aachange.s %>% filter(hydrop.change == hc))
  p = tt$p.value
  dt.p = rbind(dt.p,
               data.table(hydrop.change = hc, p=p))
}

dt.p$p.adj = p.adjust(dt.p$p, method = "BH")
print(dt.p %>% arrange(p.adj))
```

```
##   hydrop.change      p    p.adj
## 1             2 0.1234031 0.3090164
## 2             1 0.1841250 0.3090164
## 3            -1 0.1854098 0.3090164
## 4            -2 0.5656878 0.7071098
## 5             0 0.9069440 0.9069440
```

```
ggsave("figures/p5.pdf", p5, width = 10, height = 5)
```

```
## Warning in density.default(x, weights = w, bw = bw, adjust = adjust, kernel
## = kernel, : sum(weights) != 1 -- will not get true density
```

```
## Warning in density.default(x, weights = w, bw = bw, adjust = adjust, kernel
## = kernel, : sum(weights) != 1 -- will not get true density
```

```
ggsave("figures/p6.pdf", p6, width = 5, height = 5)
ggsave("figures/p7.pdf", p7, width = 5, height = 5)
ggsave("figures/p8.pdf", p8, width = 8, height = 5)
ggsave("figures/p9.pdf", p9, width = 7, height = 5)
```