

# Integration of Cell Growth and Asymmetric Division During Lateral Root Initiation In *Arabidopsis thaliana*

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This document summarize the analyses done and the generation of the different plots.

The following loads the package used, defines custom colors and sets general parameters:

```
library(tidyverse)
library(ggsignif)
library(ggpubr)

# Custom colors for the plots
colors <- c("#344D90", "#5CC5EF", "#FFB745", "#E7552C")

# General plotting parameters
size_pt <- 4

# Figures folder
save_path = "../Manuscript/Figures/"
```

## Loading the data

The file `divisions_volumes.csv` contains a description of 106 divisions as well as the volumes of daughter and mother cells.

```
div_data <- read_tsv("divisions_volumes.csv")
```

Each line is a daughter cell of a division and each column a specific attribute:

Variable	Description
MovieName	Name of the primordium imaged (A, B, C)
Timepoint	Time point at which the division completed (two daughter cells)
PrimordiaStage	LR stage of development at which the division completed (two daughter cells)
Daughter_UID	MaMuT unique ID of the daughter cell
PlantSeg_ID_daughter	PlantSeg unique ID of the daughter cell
length_daughter	Length of the daughter cell (in um)
Volume_PS_daughter	Volume of the daughter cell (in um <sup>3</sup> )
ratio_Volume_PS_daughter	Ratio of daughter cell volumes (inner/outer for anticlinal divisions, lower/upper for periclinal divisions)
Mother_UID	MaMuT unique ID of the mother cell
PlantSeg_ID_mother	PlantSeg unique ID of the mother cell
MotherTime	Time point at which the mother cell appeared
Volume_PS_Mother	Volume of the mother cell at MotherTime (in um <sup>3</sup> )

Variable	Description
Volume_PS_sum_daughter	Volume of the mother cell upon division (sum of the daughter cells volumes) (in $\mu\text{m}^3$ )
CellFile	Whether the division occurred in master or peripheral cell file
DivisionPlane	Plane of division (anticlinal or periclinal)
DivisionDescription	Description of the division for that daughter cell (build from DivisionDetail and DivisionRound)
DivisionDetail	Type and number of consecutive division (e.g. A1 first anticlinal division, Pf: flanking periclinal, P1: first periclinal)
DivisionRound	Round of division
DivisionFollowed	Type of the following division
DivisionLocation	Relative position of the daughter cell (upper, lower, inner, outer)
DivisionSequence	Sequence of divisions leading to the current divisions
PreviousDivision	Type of the preceding division
DivisionGroup	Classification of divisions in 3 types (A, Pf or P1)

As two daughter cells have the same mother, to avoid double data points when considering ratios of daughter cells volumes or analysing the mother cells, we create a second data frame with only the entries corresponding to a unique mother cell:

```
div_data_unique <- distinct(div_data, Mother_UID, .keep_all = TRUE)
```

## Anticlinal and periclinal divisions are asymmetric and differ in the volume of the mother cell (Figure 3 & S3)

Here we analyse the volumes in mother and daughter cells of anticlinal and periclinal divisions of all anticlinal and periclinal divisions observed in all primordia. Flanking periclinal divisions are excluded from the analysis.

### Figure 3: mother and daughter cells volumes for anticlinal and periclinal divisions

#### Panel A: Volumes among daughters

Distribution of the inner and outer and lower and upper daughter cells for anticlinal and periclinal divisions:

```
vol_daughters <-
  ggplot(data = div_data %>% filter(DivisionDetail != "Pf"),
         aes(x=DivisionLocation, y=Volume_PS_daughter_unit, color = DivisionLocation))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2) +
  scale_color_manual(values=colors[c(1, 3, 2,4)])+
  facet_grid(~DivisionPlane, scales = "free") +
  labs(x= "Daughter cell location",
       y = expression(paste("Daughter cell volume (",mu, m^3, ")")))+
  theme_pubr()+
  theme(legend.position = "none") +
  stat_compare_means(comparisons = list(c(1,2)),
                    method = "wilcox.test",
                    label = "p.values",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

Summary statistics:

```
div_data %>%
  filter(DivisionDetail != "Pf") %>%
  group_by(DivisionPlane, DivisionLocation) %>%
  summarize(n = n(),
            median = median(Volume_PS_daughter_unit),
            mad = mad(Volume_PS_daughter_unit)) %>%
  knitr::kable()
```

DivisionPlane	DivisionLocation	n	median	mad
anticlinal	inner	22	1009.2951	300.2973
anticlinal	outer	21	2122.9098	1647.2936
periclinal	lower	21	762.7092	213.2969
periclinal	upper	25	1144.5258	392.9329

### Panel B: Volumes ratios

Distribution of volume ratios (inner/outer for anticlinal divisions and upper/lower for periclinal divisions):

```
vol_ratio <-
  ggplot(data = div_data_unique %>% filter(DivisionDetail != "Pf"),
         aes(x=DivisionPlane, y=ratio_Volume_PS_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  scale_color_manual(values=colors[c(1, 4)])+
  scale_y_continuous(limits = c(0,1.6), breaks=seq(0,2,0.5))+
  geom_hline(yintercept=1, color="grey", size=1, alpha=0.5) +
  labs(x= "Division type",
       y = "Daughter cell volume ratio (a.u.)")+
  theme_pubr()+
  theme(legend.position = "none") +
  stat_compare_means(comparisons = list(c(1, 2)),
                    label = "p.signif",
                    method="wilcox.test",
                    label.x.npc = "middle",
                    label.y = 1.5,
                    tip.length = 0.05)
```

Summary statistics:

```
div_data_unique %>%
  filter(DivisionDetail != "Pf") %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
            median = median(ratio_Volume_PS_daughter),
            mad = mad(ratio_Volume_PS_daughter)) %>%
  knitr::kable()
```

DivisionPlane	n	median	mad
anticlinal	22	0.5916578	0.3196904
periclinal	27	0.7727448	0.4055331

## Panel C: Volume of mother cells

The distribution of the volumes of mother cells undergoing anticlinal or periclinal divisions:

```
vol_mothers <-  
  ggplot(data = div_data_unique %>% filter(DivisionGroup != "Pf"),  
    aes(x=DivisionGroup, y=Volume_PS_sum_daughter, color = DivisionPlane))+  
  geom_boxplot(outlier.shape=NA)+  
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+  
  scale_x_discrete(name = "Division type", labels = c("anticlinal", "periclinal"))+  
  scale_color_manual(values=colors[c(1, 4)])+  
  labs(y = expression(paste("Mother cell volume (",mu, m^3, ")")))+  
  theme_pubr()+  
  theme(legend.position = "none") +  
  stat_compare_means(comparisons = list(c(1,2)),  
    method = "wilcox.test",  
    label = "p.value",  
    label.x.npc = "middle",  
    tip.length = 0.05,  
    label.y = 6900)
```

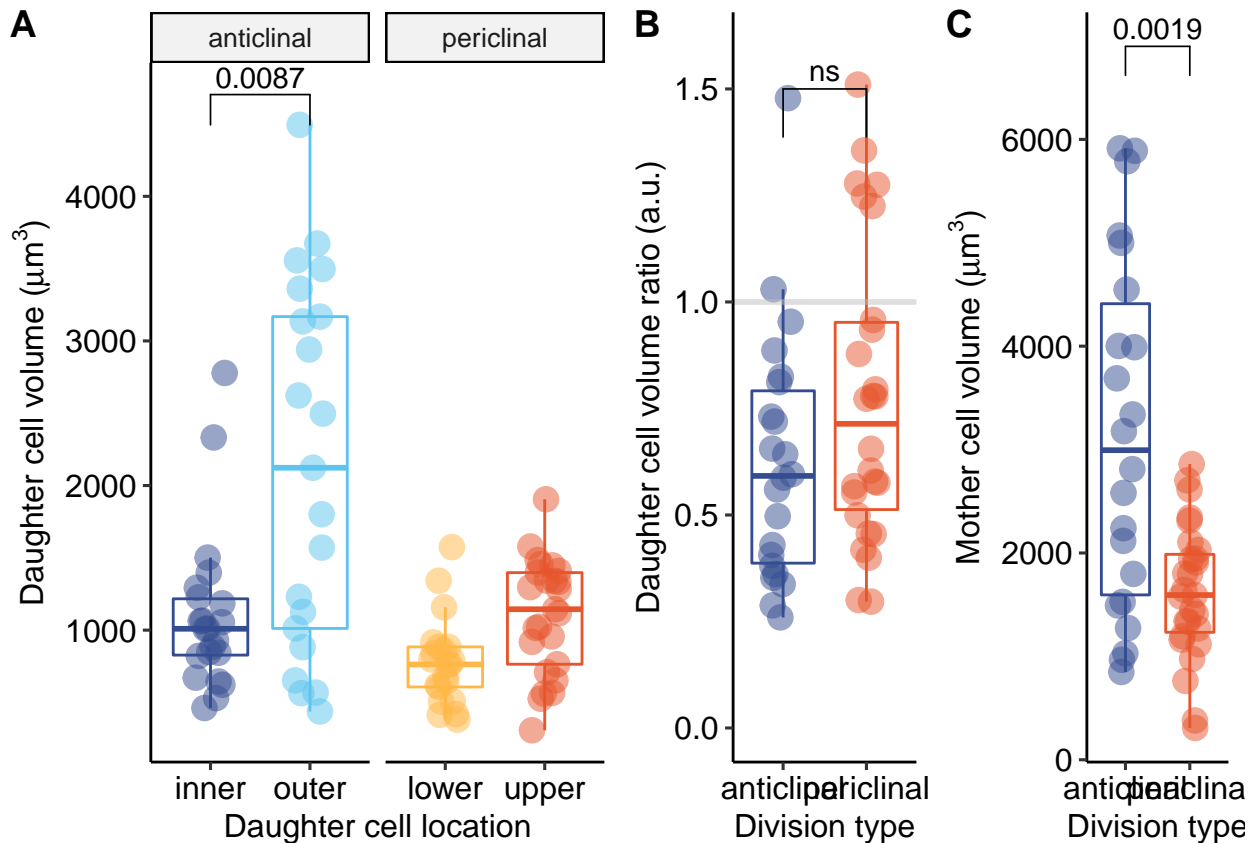
Summary statistics

```
div_data %>%  
  filter(DivisionDetail != "Pf") %>%  
  group_by(DivisionPlane) %>%  
  summarize(n = n(),  
    median = median(Volume_PS_sum_daughter),  
    mad = mad(Volume_PS_sum_daughter),  
    cv = mad/median) %>%  
  knitr::kable()
```

DivisionPlane	n	median	mad	cv
anticlinal	43	3177.841	2046.4879	0.6439870
periclinal	46	1795.345	677.7202	0.3774874

Assembly of figure 3:

```
ggarrange(vol_daughters,  
  ggarrange(vol_ratio, vol_mothers, labels = c("B", "C"), ncol=2, align = "h"),  
  labels= c("A"), ncol=2, align="h")
```



```
ggsave(filename = paste0(save_path, "Figure3.pdf"), device = 'pdf', height = 4.5, width = 12, units = "in
```

**Figure S3: volumes in daughter cells of anticlinal periclinal divisions in each LRP.**

#### Anticlinal divisions

Distribution of daughter cells volumes of anticlinal divisions for each LRP analyzed:

```
vol_ant <- ggplot(data = div_data %>% filter(DivisionPlane == "anticlinal"),
  aes(x=MovieName, y=Volume_PS_daughter_unit, color = DivisionLocation))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionLocation) +
  labs(x = "Primordium",
  y = expression(paste("Daughter cell volume (",mu, m^3, ")")))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,2)])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3), c(1,3)),
  method = "wilcox.test",
  label = "p.signif",
  label.x.npc = "middle",
  tip.length = 0.05)
```

#### Periclinal divisions

Distribution of daughter cells volumes of periclinal divisions for each LRP analyzed:

```

vol_per <- ggplot(data = div_data %>% filter(DivisionPlane == "periclinal"),
  aes(x=MovieName, y=Volume_PS_daughter_unit, color = DivisionLocation))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionLocation) +
  labs(x = "Primordium",
  y = expression(paste("Daughter cell volume (",mu, m^3, ")")))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(3,4)])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3), c(1,3)),
  method = "wilcox.test",
  label = "p.signif",
  label.x.npc = "middle",
  tip.length = 0.05)

```

## Ratios

Distribution of daughter cells volumes ratio for each LRP analyzed:

```

ratios <- ggplot(data = div_data_unique %>% filter(DivisionDetail != "Pf"),
  aes(x=MovieName, y=ratio_Volume_PS_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionPlane) +
  scale_color_manual(values=colors[c(1, 4)])+
  scale_y_continuous(limits = c(0,1.6), breaks=seq(0,2,0.5))+
  geom_hline(yintercept=1, color="grey", size=1, alpha=0.5) +
  labs(x = "Primordium",
  y = "Daughter cell volume ratio (a.u.)")+
  theme_pubr()+
  theme(legend.position = "none")

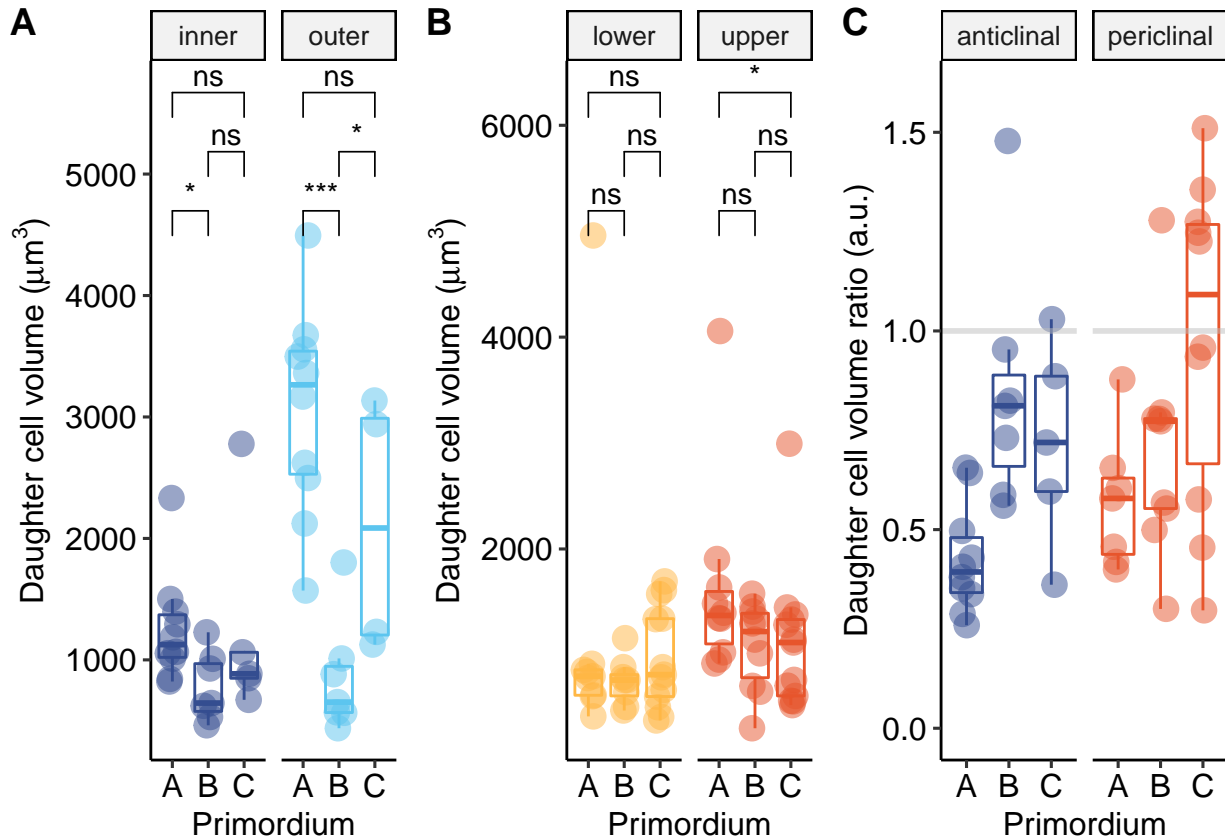
```

Assembly of figure S3:

```

ggarrange(vol_ant, vol_per, ratios, labels = c("A", "B", "C"), ncol = 3, align = "h")

```



```
ggsave(filename = paste0(save_path, "FigureS3.pdf"), device = 'pdf', height = 4.5, width = 12, units = "in")
```

## Daughter cells volume in master or peripheral cell files (Figure 4)

### Panel A: Volume of daughter cells for anticlinal divisions

Distribution of daughter cells volume upon anticlinal for divisions occurring in the central or peripheral cells files:

```
vol_ant <- ggplot(data = div_data %>% filter(DivisionPlane == "anticlinal"),
  aes(x=CellFile, y=Volume_PS_daughter_unit, color = DivisionLocation))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionLocation) +
  labs(x = "Cell file",
  y = expression(paste("Daughter cell volume (", mu, "m^3, ")")))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,2)])+
  stat_compare_means(comparisons = list(c(1,2)),
  method = "wilcox.test",
  label = "p.signif",
  label.x.npc = "middle",
  tip.length = 0.05)
```

Summary statistics:

```
div_data %>%
  filter(DivisionPlane == "anticlinal") %>%
  group_by(DivisionLocation, CellFile) %>%
  summarize(n = n(),
            median = median(Volume_PS_sum_daughter),
            mad = mad(Volume_PS_sum_daughter),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionLocation	CellFile	n	median	mad	cv
inner	master	6	4343.995	1609.008	0.3703983
inner	periphery	16	2409.719	1531.872	0.6357054
outer	master	6	4343.995	1609.008	0.3703983
outer	periphery	15	2580.461	1611.684	0.6245722

## Panel B: Volume of daughter cells for periclinal divisions

Distribution of daughter cells volume upon periclinal for divisions occurring in the central or peripheral cells files:

```
vol_per <- ggplot(data = div_data %>% filter(DivisionPlane == "periclinal"),
                 aes(x=CellFile, y=Volume_PS_daughter_unit, color = DivisionLocation))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionLocation) +
  labs(x = "Cell file",
       y = expression(paste("Daughter cell volume (",mu, m^3, ")")))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(3,4)])+
  stat_compare_means(comparisons = list(c(1,2)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

Summary statistics:

```
div_data %>%
  filter(DivisionPlane == "periclinal") %>%
  group_by(DivisionLocation, CellFile) %>%
  summarize(n = n(),
            median = median(Volume_PS_sum_daughter),
            mad = mad(Volume_PS_sum_daughter),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionLocation	CellFile	n	median	mad	cv
lower	master	18	1950.812	683.8583	0.3505507
lower	periphery	12	1304.051	758.1787	0.5814026
upper	master	21	1940.410	730.3698	0.3763997
upper	periphery	12	1487.726	586.7427	0.3943890



## Panel C: Ratio of daughter cells for periclinal and anticlinal divisions

Distribution of daughter cells volume ratio for divisions occurring in the central or peripheral cells files:

```
vol_ratio <- ggplot(data = div_data_unique,
                    aes(x=CellFile, y=ratio_Volume_PS_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionPlane) +
  geom_hline(yintercept=1, color="grey", size=1, alpha=0.5) +
  labs(x = "Cell file",
       y = "Daughter cell volume ratio (a.u.)")+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,4)])+
  stat_compare_means(comparisons = list(c(1,2)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

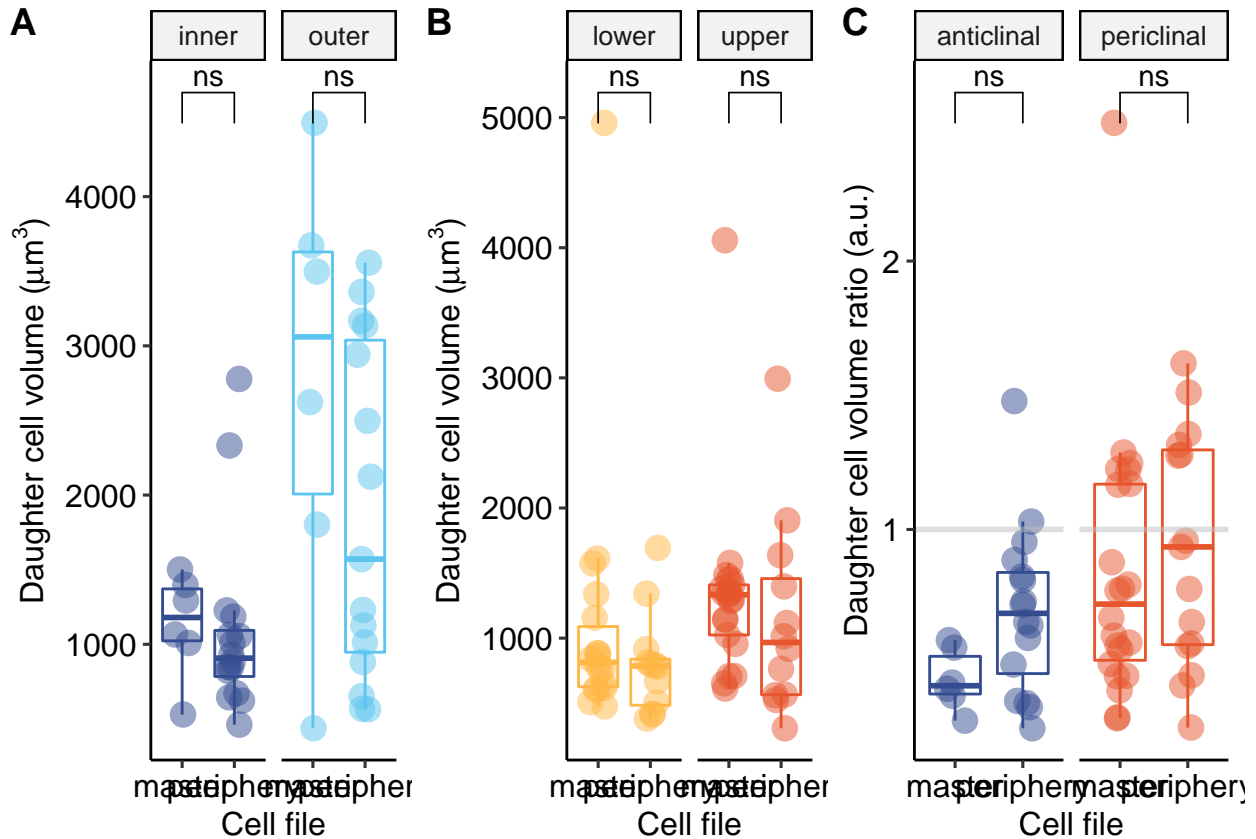
Summary statistics:

```
div_data_unique %>%
  group_by(DivisionPlane, CellFile) %>%
  summarize(n = n(),
            median = median(ratio_Volume_PS_daughter),
            mad = mad(ratio_Volume_PS_daughter),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionPlane	CellFile	n	median	mad	cv
anticlinal	master	6	0.4178670	0.1242020	0.2972285
anticlinal	periphery	16	0.6875407	0.2885638	0.4197042
periclinal	master	22	0.7220644	0.3972036	0.5500945
periclinal	periphery	15	0.9350091	0.5473554	0.5854012

Assembly of figure 4:

```
ggarrange(vol_ant, vol_per, vol_ratio, labels = c("A", "B", "C"), ncol = 3, align = "h")
```



```
ggsave(filename = paste0(save_path, "Figure4.pdf"), device = 'pdf', height = 4.5, width = 12, units = "in")
```

## Volume partition in successive rounds of anticlinal cell division (Figure 5 and S4)

### Volumes

Panel B: Distribution of mother cell volumes:

```
vol_ant_mother <- ggplot(data = div_data %>% filter(DivisionPlane == "anticlinal"),
  aes(x=DivisionDetail, y=Volume_PS_sum_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionPlane) +
  labs(y = expression(paste("Mother cell volume (",mu, m^3, ")")))+
  scale_x_discrete(name = "Division round", labels = c("1st", "2nd", "3rd"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,2)])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3)),
    method = "wilcox.test",
    label = "p.signif",
    label.x.npc = "middle",
    tip.length = 0.05)
```

Summary statistics:

```
div_data %>%
  filter(DivisionPlane == "anticlinal") %>%
  group_by(DivisionPlane, DivisionDetail) %>%
  summarize(n = n(),
            median = median(Volume_PS_sum_daughter),
            mad = mad(Volume_PS_sum_daughter),
            cv = mad/median) %>%
knitr::kable()
```

DivisionPlane	DivisionDetail	n	median	mad	cv
anticlinal	A1	14	2580.461	1936.542	0.7504634
anticlinal	A2	21	3177.841	1391.959	0.4380203
anticlinal	A3	8	3940.807	2300.300	0.5837130

and for all three rounds together:

```
div_data %>%
  filter(DivisionPlane == "anticlinal") %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
            median = median(Volume_PS_sum_daughter),
            mad = mad(Volume_PS_sum_daughter),
            cv = mad/median) %>%
knitr::kable()
```

DivisionPlane	n	median	mad	cv
anticlinal	43	3177.841	2046.488	0.643987

Panel C: Distribution of daughter cell volumes:

```
vol_ant <- ggplot(data = div_data %>% filter(DivisionPlane == "anticlinal"),
                 aes(x=DivisionDetail, y=Volume_PS_daughter_unit, color = DivisionLocation))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionLocation) +
  labs(y = expression(paste("Daughter cell volume (",mu, m^3, ")")))+
  scale_x_discrete(name = "Division round", labels = c("1st", "2nd", "3rd"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,2)])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

Summary statistics:

```
div_data %>%
  filter(DivisionPlane == "anticlinal") %>%
  group_by(DivisionLocation, DivisionDetail) %>%
  summarize(n = n(),
```

```

median = median(Volume_PS_daughter_unit),
mad = mad(Volume_PS_daughter_unit),
cv = mad/median) %>%
knitr::kable()

```

DivisionLocation	DivisionDetail	n	median	mad	cv
inner	A1	7	1009.5228	260.6571	0.2581983
inner	A2	11	848.5884	305.9232	0.3605084
inner	A3	4	1203.2559	643.3449	0.5346701
outer	A1	7	1570.9386	1492.5402	0.9500946
outer	A2	10	2310.1419	1513.4756	0.6551440
outer	A3	4	2468.5584	1387.4036	0.5620299

#### Panel D: Distribution of daughter cell volumes ratios:

```

vol_ratio <- ggplot(data = div_data_unique %>% filter(DivisionPlane == "anticlinal"),
                  aes(x=DivisionDetail, y=ratio_Volume_PS_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionPlane) +
  geom_hline(yintercept=1, color="grey", size=1, alpha=0.5) +
  labs(y = "Daughter cell volume ratio (a.u.)")+
  scale_x_discrete(name = "Division round", labels = c("1st", "2nd", "3rd"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,2)])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)

```

Summary statistics:

```

div_data_unique %>%
  filter(DivisionPlane == "anticlinal") %>%
  group_by(DivisionPlane, DivisionDetail) %>%
  summarize(n = n(),
            median = median(ratio_Volume_PS_daughter),
            mad = mad(ratio_Volume_PS_daughter),
            cv = mad/median) %>%
knitr::kable()

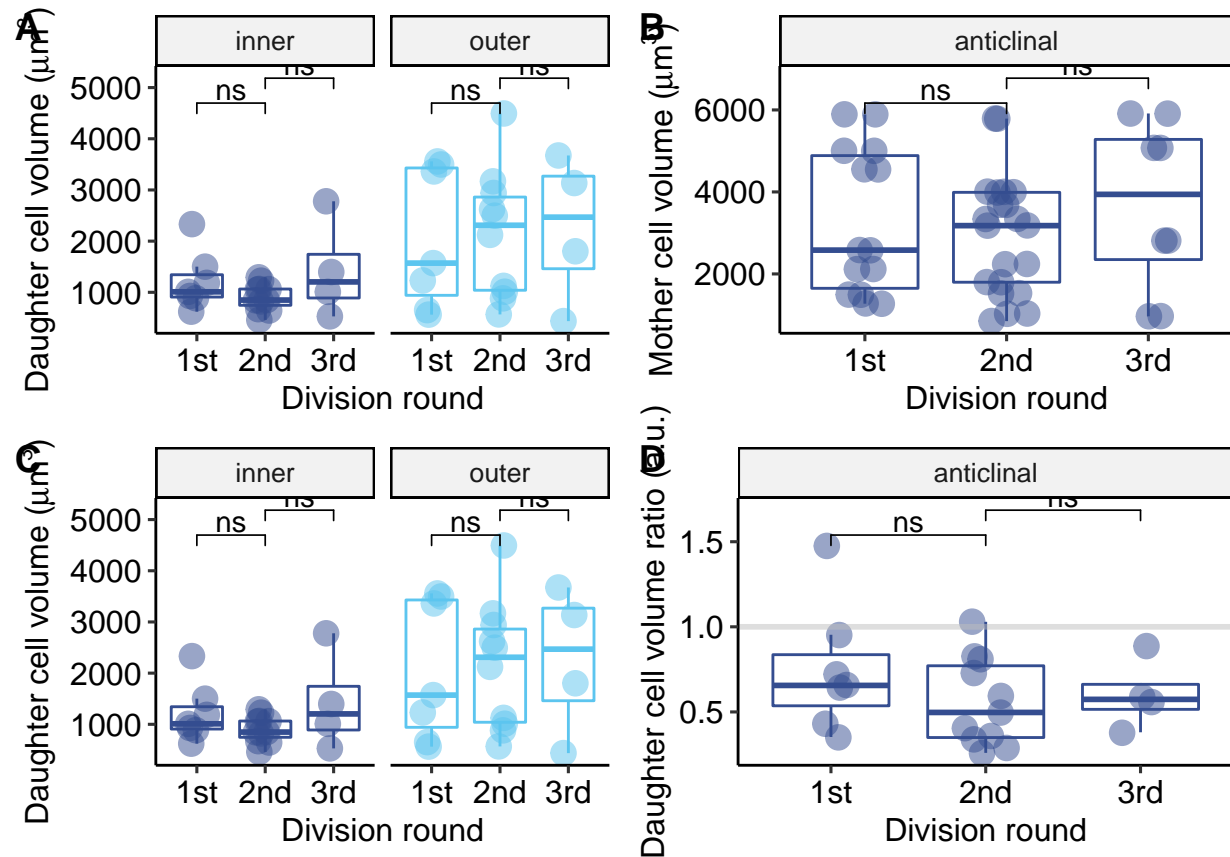
```

DivisionPlane	DivisionDetail	n	median	mad	cv
anticlinal	A1	7	0.6557308	0.3356810	0.5119189
anticlinal	A2	11	0.4969268	0.3101118	0.6240593
anticlinal	A3	4	0.5737705	0.1534989	0.2675267

#### Assembly of figure 5

# Panel A is placeholder for the schematic

```
ggarrange(vol_ant, vol_ant_mother,
          vol_ant, vol_ratio,
          labels = c("A", "B", "C", "D"), ncol = 2, nrow = 2, align = "h")
```

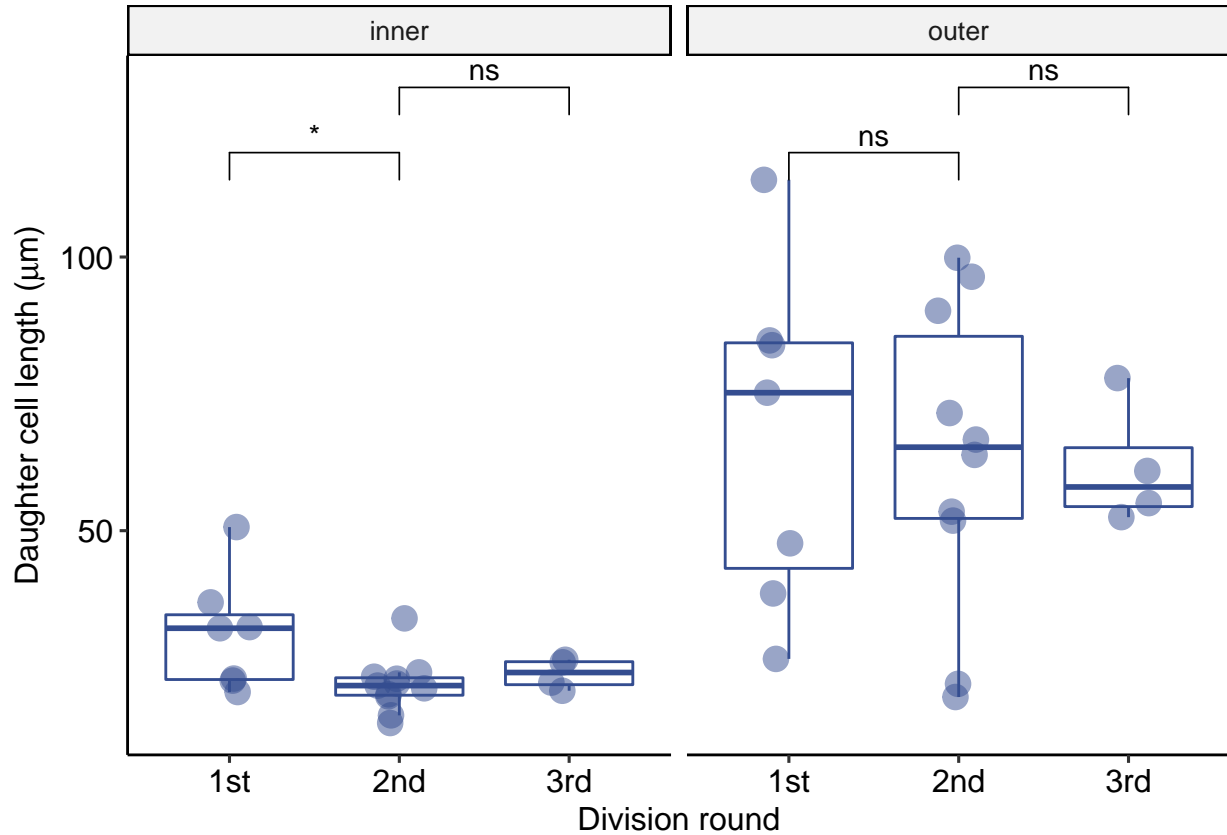


```
ggsave(filename = paste0(save_path, "Figure5_elements.pdf"), device = 'pdf', height = 9, width = 12, unit = "cm")
```

### Cells length during consecutive A divisions (figure S4)

```
length_ant_mother <- ggplot(data = div_data %>% filter(DivisionPlane == "anticlinal"),
                           aes(x=DivisionDetail, y=length_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionLocation) +
  labs(y = expression(paste("Daughter cell length (", mu, m, ")")))+
  scale_x_discrete(name = "Division round", labels = c("1st", "2nd", "3rd"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,2)])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

```
ggarrange(length_ant_mother, ncol = 1, align = "h")
```



```
ggsave(filename = paste0(save_path, "FigureS4.pdf"), device = 'pdf', height = 4.5, width = 9, units = "in")
```

Summary statistics for Figure S4

```
div_data %>%
  filter(DivisionPlane == "anticlinal") %>%
  group_by(DivisionLocation, DivisionDetail) %>%
  summarize(n = n(),
            median = median(length_daughter),
            mad = mad(length_daughter)) %>%
  knitr::kable()
```

DivisionLocation	DivisionDetail	n	median	mad
inner	A1	7	32.17610	13.641725
inner	A2	11	21.69293	2.401853
inner	A3	4	24.08379	3.106043
outer	A1	7	75.21033	40.798483
outer	A2	10	65.25497	28.430370
outer	A3	4	57.98337	6.267164

## Volumes repartition and cell growth preceding periclinal divisions (Figure 6)

### Preparation of the data:

Focus on the consecutive rounds of anticlinal divisions that lead to a periclinal of the inner cells:

```
P1 <- subset(div_data, ((PreviousDivision == "A1" |
                        PreviousDivision == "A2" |
                        PreviousDivision == "A3") &
                        DivisionDetail == "P1")) %>%
distinct(Mother_UID, Timepoint, .keep_all = TRUE)
```

### Panel B: Distribution of volumes of the mother cells

```
vol_ant_mother <- ggplot(data = P1,
                        aes(x=PreviousDivision, y=Volume_PS_sum_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionPlane) +
  labs(y = expression(paste("Mother cell volume (",mu, m^3, ")")))+
  scale_x_discrete(name = "Division round", labels = c("A1 inner", "A2 inner", "A3 inner"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[4])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

Summary statistics:

```
P1 %>%
  group_by(DivisionPlane, PreviousDivision) %>%
  summarize(n = n(),
            median = median(Volume_PS_sum_daughter),
            mad = mad(Volume_PS_sum_daughter),
            cv = mad/median) %>%
knitr::kable()
```

DivisionPlane	PreviousDivision	n	median	mad	cv
periclinal	A1	14	1698.662	509.5035	0.2999440
periclinal	A2	10	1490.580	610.5625	0.4096139
periclinal	A3	2	1553.046	580.9449	0.3740681

For all three rounds together:

```
P1 %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
            median = median(Volume_PS_sum_daughter),
            mad = mad(Volume_PS_sum_daughter),
            cv = mad/median) %>%
```

```
knitr::kable()
```

DivisionPlane	n	median	mad	cv
periclinal	26	1618	562.7054	0.3477783

## Panel C: Distribution of daughter cells volume ratio

```
ratio_P1 <- ggplot(data = P1,
                  aes(x=PreviousDivision, y=ratio_Volume_PS_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  facet_grid(~DivisionPlane) +
  geom_hline(yintercept=1, color="grey", size=1, alpha=0.5) +
  labs(y = "Daughter cell volume ratio (a.u.)")+
  scale_x_discrete(name = "Division round", labels = c("A1 inner", "A2 inner", "A3 inner"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[4])+
  stat_compare_means(comparisons = list(c(1,2), c(2,3)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

Summary statistics:

```
P1 %>%
  group_by(DivisionPlane, PreviousDivision) %>%
  summarize(n = n(),
            median = median(ratio_Volume_PS_daughter),
            mad = mad(ratio_Volume_PS_daughter),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionPlane	PreviousDivision	n	median	mad	cv
periclinal	A1	14	0.6168192	0.3077987	0.4990095
periclinal	A2	10	0.8364693	0.5642828	0.6746007
periclinal	A3	2	0.6392995	0.2076870	0.3248666

For all three rounds together:

```
P1 %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
            median = median(ratio_Volume_PS_daughter),
            mad = mad(ratio_Volume_PS_daughter),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionPlane	n	median	mad	cv
periclinal	26	0.7139403	0.3447475	0.48288



## Panel D: Mother cell growth during interphase preceding periclinal division

The change in volume between two divisions is the volume of the mother cell (as sum of daughter cell volume) minus the volume of this cell right after the previous division divided by this original volume:

$$r_{\text{expansion}} = \frac{V_{\Sigma \text{ daughters}} - V_{\text{last div}}}{V_{\text{last div}}}$$

```
div_data_unique <- div_data_unique %>%
  mutate(vol_change_since_prev_div = abs(Volume_PS_sum_daughter - Volume_PS_Mother_unit)/Volume_PS_Mother_unit)

div_data <- div_data %>%
  mutate(vol_change_since_prev_div = abs(Volume_PS_sum_daughter - Volume_PS_Mother_unit)/Volume_PS_Mother_unit)

vol_change_AP <- ggplot(data = div_data_unique %>% filter(DivisionDetail != "Pf"),
  aes(x=DivisionPlane, y=vol_change_since_prev_div, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  scale_color_manual(values=colors[c(1, 4)])+
  labs(x= "Division type",
    y = "Mother cell expansion ratio (a.u.)")+
  theme_pubr()+
  theme(legend.position = "none") +
  stat_compare_means(comparisons = list(c(1, 2)),
    label = "p.value",
    method="wilcox.test",
    label.x.npc = "middle",
    label.y = 1.5,
    tip.length = 0.05)
```

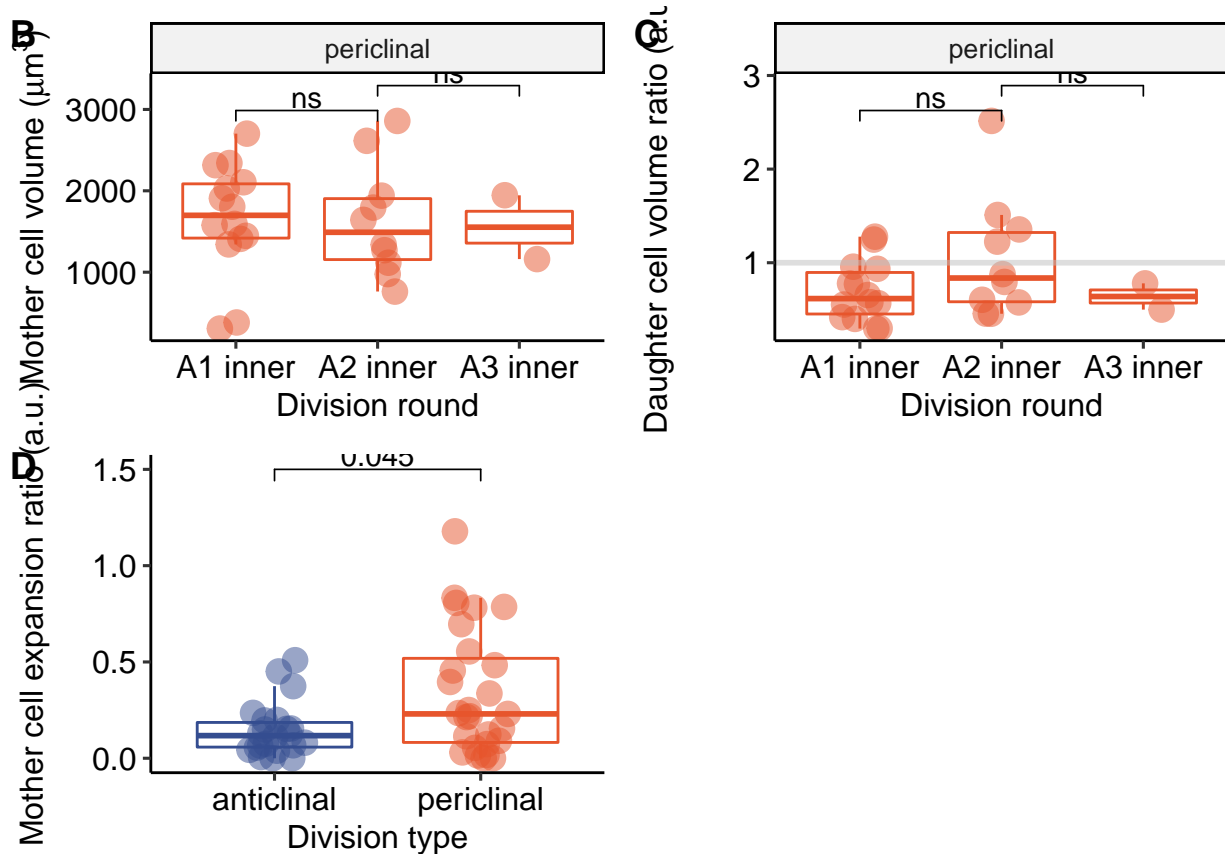
Summary statistics:

```
div_data_unique %>%
  filter(DivisionDetail != "Pf") %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
    median = median(vol_change_since_prev_div),
    mad = mad(vol_change_since_prev_div),
    cv = mad/median) %>%
  knitr::kable()
```

DivisionPlane	n	median	mad	cv
anticlinal	22	0.1175311	0.1008506	0.8580758
periclinal	27	0.2302684	0.2964123	1.2872472

## Assembly of Figure 6

```
# Panel A is for schematic
ggarrange(vol_ant_mother, ratio_P1, vol_change_AP,
  labels = c("B", "C", "D"), ncol = 2, nrow = 2, align = "v")
```



```
ggsave(filename = paste0(save_path, "Figure6_BCD.pdf"), device = 'pdf', height = 9, width = 12, units = "in")
```

## Volumes in daughter cells of flanking periclinal divisions (Figure 7)

Panel A is a schematic

### Data preparation

We isolate the large daughter cells of consecutive anticlinal divisions that undergo a flanking periclinal divisions:

```
Pf <- subset(div_data, (grepl("A1_outer; Pf", DivisionSequence)
| grepl("A2_outer; Pf", DivisionSequence)
| grepl("A3_outer; Pf", DivisionSequence)
| (grepl("A1_outer; A2", DivisionSequence)
| (grepl("A2_outer; A3", DivisionSequence)
| DivisionDescription == "A1_outer")
& DivisionDetail != "P1"))

Pf <- distinct(Pf, Mother_UID, Timepoint, .keep_all= TRUE)
Pf1 <- Pf %>% filter(DivisionDetail == "Pf") %>% mutate(ratio_Volume_PS_daughter = 1/ratio_Volume_PS_daughter)
Pf2 <- Pf %>% filter(DivisionPlane == "anticlinal")
Pf <- rbind(Pf1, Pf2)

# Regularise the ratio, to always be <1 (smaller/bigger)
Pf <- Pf %>%
```

```
mutate(ratio_Volume_PS_daughter = case_when(ratio_Volume_PS_daughter>1 ~ 1/ratio_Volume_PS_daughter,
                                             TRUE ~ ratio_Volume_PS_daughter))
```

## Panel B: mother cells volumes

```
vol_pf_A_mother <- ggplot(data = Pf,
                          aes(x=DivisionPlane, y=Volume_PS_sum_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  labs(y = expression(paste("Mother cell volume (",mu, m^3, ")")))+
  scale_x_discrete(name = "division", labels = c("anticlinal", "flanking periclinal"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,4)])+
  stat_compare_means(comparisons = list(c(1,2)),
                    method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

Summary statistics

```
Pf %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
            median = median(Volume_PS_sum_daughter),
            mad = mad(Volume_PS_sum_daughter),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionPlane	n	median	mad	cv
anticlinal	20	3075.788	2263.572	0.7359323
periclinal	10	1796.979	1236.178	0.6879204

## Panel C: daughter cells volumes

```
ratio_pf_A_mother <- ggplot(data = Pf,
                             aes(x=DivisionPlane, y=ratio_Volume_PS_daughter, color = DivisionPlane))+
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  geom_hline(yintercept=1, color="grey", size=1, alpha=0.5) +
  labs(y = "Daughter cell volume ratio (a.u.)")+
  scale_x_discrete(name = "division", labels = c("anticlinal", "flanking periclinal"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_y_continuous(limits = c(0, 1.5))+
  scale_color_manual(values=colors[c(1,4)])+
  stat_compare_means(comparisons = list(c(1,2))
                    , method = "wilcox.test",
                    label = "p.signif",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

Summary statistics

```
Pf %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
            median = median(ratio_Volume_PS_daughter),
            mad = mad(ratio_Volume_PS_daughter),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionPlane	n	median	mad	cv
anticlinal	20	0.5737705	0.2947860	0.5137699
periclinal	10	0.7160348	0.1787075	0.2495794

## Panel D: Mother cell expansion

```
ratio_exp_pf_A_mother <- ggplot(data = Pf,
                                aes(x=DivisionPlane, y=vol_change_since_prev_div, color = DivisionPlane))
  geom_boxplot(outlier.shape=NA)+
  geom_jitter(size = size_pt, width = 0.15, alpha=1/2)+
  labs(y = "Mother cell expansion ratio (a.u.)")+
  scale_x_discrete(name = "division", labels = c("anticlinal", "flanking periclinal"))+
  theme_pubr()+
  theme(legend.position = "none") +
  scale_color_manual(values=colors[c(1,4)])+
  stat_compare_means(comparisons = list(c(1,2)),
                    method = "wilcox.test",
                    label = "p.value",
                    label.x.npc = "middle",
                    tip.length = 0.05)
```

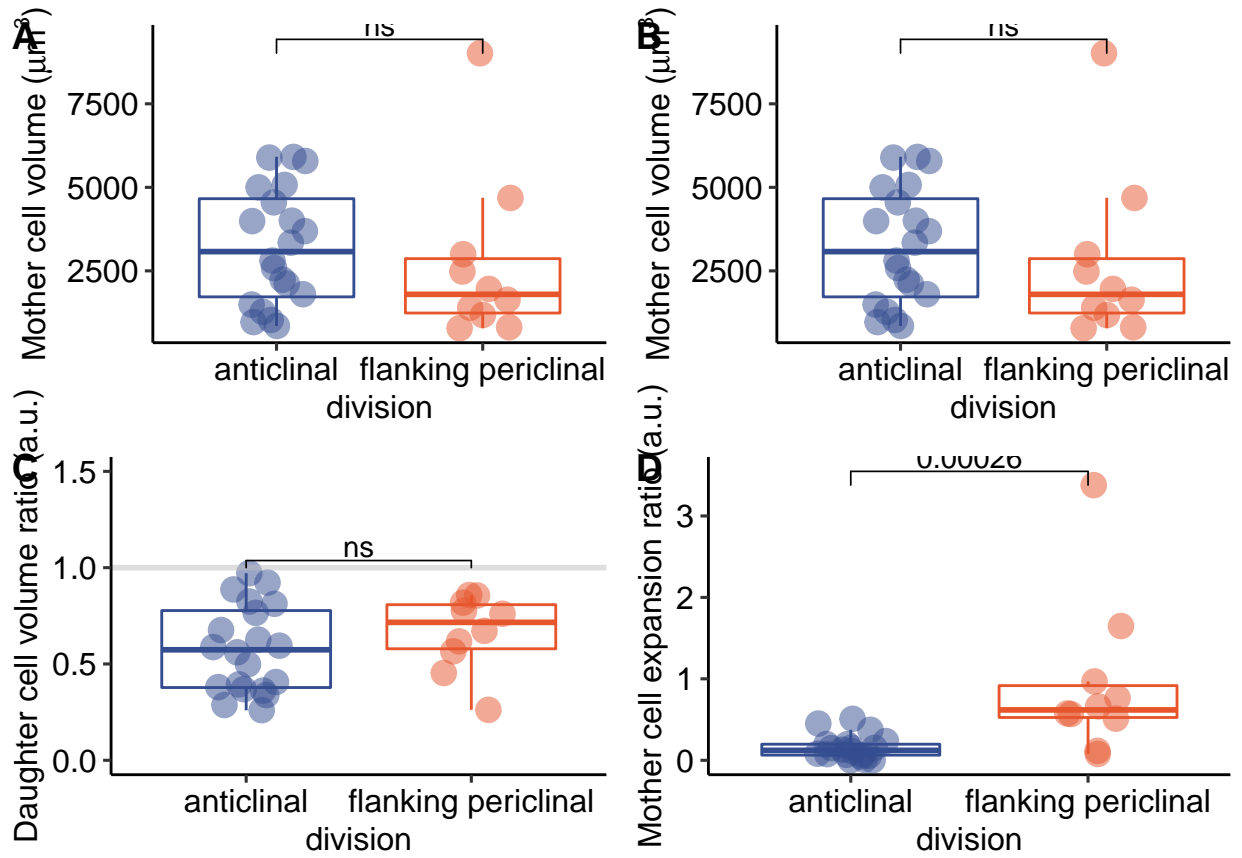
Summary statistics

```
Pf %>%
  group_by(DivisionPlane) %>%
  summarize(n = n(),
            median = median(vol_change_since_prev_div),
            mad = mad(vol_change_since_prev_div),
            cv = mad/median) %>%
  knitr::kable()
```

DivisionPlane	n	median	mad	cv
anticlinal	20	0.1214982	0.1047048	0.861780
periclinal	10	0.6184245	0.3665025	0.592639

## Assembly Figure 7

```
# Duplicated panel A is just a placeholder for schematic.
ggarrange(vol_pf_A_mother, vol_pf_A_mother, ratio_pf_A_mother, ratio_exp_pf_A_mother,
          labels = c("A", "B", "C", "D"), ncol = 2, nrow =2, align = "h")
```



`ggsave(filename = paste0(save_path, "Figure7_BCD.pdf"), device = 'pdf', height = 9, width = 12, units = "in")`