Hevia_et_al_2022: Algorithms and data description

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Contents

Reference

Hevia et al. (2022) The neurogenic fate of the hindbrain boundaries relies on Notch3-dependent asymmetric cell divisions.

Main data structures

Here we load and describe the main data structures used in the rest of the document.

Packages used

```
library(dplyr)
```

```
##
```

```
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
library(tidyr)
# library(rgl)  not used here
# library(Rvcg) not used here
library(zoo)
```

```
##
## Attaching package: 'zoo'

## The following objects are masked from 'package:base':
##
##     as.Date, as.Date.numeric
```

Cell tracking data

Data created by Mamut software was read into R and included in several data structures. We list here the main ones. Original data and processing algorithms are available upon request.

`tracksTable` A data frame (118 by 4), one track per row, columns:
- `trackLabel`: A label ID
- `TrackType`: One of "PN", "PQ", "N", "CN", "PP", "IND1", "NONE"
- `divTime`: Frame of cell division or 9999 if no division
- `plfType`: One of "PLF" "NPLF" " "

`tracks` A list of 118 data frames, one per track, named by trackLabel, each one with columns `POSITION_X POSITION_Y POSITION_Z POSITION_T`, space-time coordinates

`cellsTable` A data frame (264 by 9), one row for each cell, columns:
- `cellLabel`: A label ID, the track ID followed by 00: progenitor, 01, 02: descend
- `track`: its track ID
- `trackType`: as before
- `cellType`: One of "P" "D1" "D2" "21" "22" "11" "12"
- `cellLastFrame`: The last frame where the cell was observed.
- `hasDivision`: TRUE or FALSE
- `plfType`: as before
- `cellFirstFrame`: First appearance of the cell.
- `isShort`: Is it a short lived division? Yes if both descendants live less than 3 hpf.

`cells` A list of (264) data frames, each with 4 columns (x, y, z, t coordinates) and as many rows as frames the cell has been spotted.

```
load("data/tracking.RData")

# frame num to hpf conversion
# t0 is 32hpf, each frame adds 3.5 minutes
frame2hpf <- function(fr) return(32+3.5*fr/60)

# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

```
## Colors
# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~


cellColor <- list(PQ= "black",PN=  "#4C5E85",IND1=  "white",
                  PP="#48125E", CN="darkgrey", N="darkgrey", NONE="white",
                  PD="#5D9B90", ND="#95CC86")
plfColor <- list(NPLF="darkgrey", PLF="#4C5E85")

vio <- rgb(18761,4626,24158,maxColorValue = 2^16)
gri <- rgb(18247,30069,37779,maxColorValue = 2^16)
gri2 <- rgb(28013,50115,35723,maxColorValue = 2^16)
yil <- rgb(63993,59881,30069,maxColorValue = 2^16)
cols <- c(vio, gri, gri2, yil)
paletaCova <- colorRampPalette(cols, space="Lab")

paletaPP <- colorRampPalette(c("white", cellColor$PP), space="Lab")
paletaPN <- colorRampPalette(c("white", cellColor$PN), space="Lab")
```

Ventricular surface modeling data

Starting with the WRL files produced by Imaris (available upon request), one ventricular shape for each one of 45 frames, we used MeshLab to convert them to PLY format, we read it into R using function `readAllPlys()` and package `Rvcg`.

Then some cleaning was done using functions `remBoxPoints()` and `changeZsign()` and package `rgl`.

The meshes produced (also available upon request) were sectioned by vertical plane at y=39. The resulting sections of 45 shapes are stored in `sec39`.

The sections were used to measure the distance from a cell in a given time frame to the ventricular surface. This is stored in

`cellPaths`	A 3 way array, frames by cells by coords, (194 by 264 by 7). `coords` are `x, y, z` (cell position), `nx, ny, nz` (for the nearest point in the ventricular surface), and dist from the cell to the nearest point (see function `fillNearest` below.
`sec39`	List of 45 sections at AP=39 of the ventricular shape every 5 frames.

```
load("data/ventrSurf.RData")
```

Ventricular surface modeling algorithms

```
# functions in this chunk are not run in this document,
# they are listed just as documentation.

# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
## read all ply files and store meshes in VSmeshList
# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

remBoxPoints <- function(msh, clip=0.98){
  # given a mesh3d return it removing
  # all vertices and faces in the extreme values of coords
  minmax = apply(msh$vb, 1, FUN = function(coor){fivenum(coor)[c(1,5)]}) # is 2x4
  cnt = apply(minmax, 2, mean)
```

```
  hwd = apply(minmax, 2, FUN = function(itv){(itv[2]-itv[1])/2}) # half width, 1x4
  mins = (cnt - clip*hwd)[1:3]
  mxs = (cnt + clip*hwd)[1:3]
  # print(mins)
  keepone = function(vtx){ # 1 if vtx is to keep, 0 if not, vtx is 1x3
    if (all(((mins<vtx)&(vtx<mxs)))) return(1)
    else return(0)
  }
  ret = clipMesh3d(msh, function(ar3xv){apply(ar3xv, 1, keepone)},
                   bound = 0.5, greater = TRUE,
                   attribute = "vertices")
  return(ret)
}


readAllPlys <- function(isolate=TRUE){
  # read all ply files and store meshes in VSmeshList
  # When isolate, just the main connex mesh component is kept
  plys <- dir("plys")
  meshList <- lapply(plys,
                     function(ply){
                       message("Preparing mesh ", ply)
                       vM = vcgPlyRead(paste("plys/", ply, sep=''))
                       vM = remBoxPoints(vM)
                       if (isolate)
                         vM = vcgIsolated(changeZsign(vM))
                       else
                         vM = changeZsign(vM)
                       #return(unCapSupVentr(vM))
                       return(vM)
                     }
  )
  # rename frames to match cell data, first frame is 000, last is 217
  seqfr = c(0, seq(4,214, by=5), 217)
  seqfr = paste0("F", formatC(seqfr, width = 3, flag = "0"))
  names(meshList) <- seqfr
  return(meshList)
}
# VSmeshListIsol <- readAllPlys(isolate=TRUE)
# VSmeshListNoIs <- readAllPlys(isolate=FALSE)
# removed! load ("./allWithMeshesAndDists.RData") if needed

changeZsign <- function(amesh){
  # meshes as produced by Imaris have the DV axis in the wrong direction
  amesh$vb[3,] <- (-1)*amesh$vb[3,]
  amesh$normals[3,] <- (-1)*amesh$normals[3,]
  bmesh <- tmesh3d(vertices = amesh$vb, indices = amesh$it, normals = amesh$normals)
  bmesh$remvert <- amesh$remvert
  return(bmesh)
}

## to show the meshes
viewVSmeshes <- function(mshL = VSmeshListIsol, folder=NULL, boxed=TRUE){
```

```
  for (mshnum in seq(1,length(mshL),by=1)){
    msh = mshL[[mshnum]]
    if (boxed)
      plot3d(msh, col = "red",
             xlim = c(0,250), ylim = c(0,50), zlim = c(-120,-60), aspect="iso",
             # forceClipregion = FALSE,
             xlab='L/M', ylab='A/P', zlab='D/V',
             main=names(mshL)[mshnum])
    else
      plot3d(msh, col = "red", type = 'shade',
             xlim = c(0,250), ylim = c(0,50), zlim = c(-120,-60), aspect="iso",
             box = FALSE, axes = FALSE,
             # forceClipregion = FALSE,
             xlab='L/M', ylab='A/P', zlab='D/V',
             main=names(mshL)[mshnum])
    Sys.sleep(1)
    if (!is.null(folder))
      snapshot3d(paste0(folder, "/", names(mshL)[mshnum], ".png"))
  }
}
# viewVSmeshes(mshL = VSmeshListIsol, folder="pngsIsol")
# viewVSmeshes(mshL = VSmeshListNoIs, folder="pngsNoIs")

# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
# extract and plot vertical AP sections of the mesh
# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

sectionMesh <-
  function(aMesh, y0c=30){
    # vertical section at y0c, return a of segments x0,z0,x1,z1
    # aMesh$vb[,n] is 4-vec of coords for vertex n
    # aMesh$it[,1] is a face, 3-vec integers for the vertices num
    message("Compute section at y=", y0c)
    keepit = function(fce){ # true if face cuts plane y0
      # face is a vector of 3 vertice numbers
      ys <- aMesh$vb[2,fce][1:3] # the y values of the 3 vtx
      if ((min(ys)<=y0c)&&(y0c<=max(ys))) return(TRUE)
      else return(FALSE)}
    cutSeg <- function(p0,p1){ # given points p0 p1, return xz cut with y0c or NULL
      if ((min(p0[2],p1[2])<=y0c)&&(y0c<=max(p0[2],p1[2]))){
        if (p0[2]==p1[[2]]) return(p0[c(1,3)])
        else {
          pc = p0 + (p1-p0)*(y0c-p0[2])/(p1[2]-p0[2])
          return(pc[c(1,3)])}}
      else return(NULL)
    }
    cutFace <- function(fce){ # given face that cuts, return x0,z0,x1,z1
      res = rep(0,4)
      if (!is.null(p1 <- cutSeg(aMesh$vb[,fce[1]],aMesh$vb[,fce[2]]))){
        res[1:2] <- p1
        if (!is.null(p2 <- cutSeg(aMesh$vb[,fce[2]],aMesh$vb[,fce[3]])))
          res[3:4] <- p2
        else {
```

```
          p2 <- cutSeg(aMesh$vb[,fce[3]],aMesh$vb[,fce[1]])
          res[3:4] <- p2
        }
      }
      else{
        res[1:2] <- cutSeg(aMesh$vb[,fce[2]],aMesh$vb[,fce[3]])
        res[3:4] <- cutSeg(aMesh$vb[,fce[3]],aMesh$vb[,fce[1]])
      }
      return(res)
    }
    clm = apply(aMesh$it,2,FUN=keepit) # the faces that cut
    lins = apply(aMesh$it[,clm],2,FUN=cutFace)
    return(lins)
  } ## end sectionMesh()


# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
# Distances from cell to ventricular surface
# to plot distances over time
# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

nearestPoint <- function(pnt, amesh){
  # return the nearest mesh vtx to pnt and distance: 1x4
  # brute force approach
  sqdsts <- apply(amesh$vb, 2, function(vtx) sum((vtx[1:3]-pnt)^2))
  minsqdst <- min(sqdsts)
  ind <- match(minsqdst, sqdsts)
  return(c(amesh$vb[,ind][1:3], sqrt(minsqdst)))
}

# Warning: this is very data dependent,
# now we have VSs F000, F004, ..., F214, F217
fillNearest <- function(mshList = VSmeshListIsol, cPth = cellPaths){
  # fill nearest point coords and dist in cellPaths and return it
  # cPth  is 3d array: frame x cell x 3/7coords
#  dimnames(cPth) <- dimnames(cellPaths)
  frNam <- names(mshList[-(1:5)]) # just 24 ... 217
  frNum <- as.character( as.numeric(substr(frNam,2,4)))
  message("Doing frames ", paste(frNam, collapse = ' '))
  for (frI in 1:length(frNam)){
    message("Filling frame ", frNam[frI])
    cellsCoords <- cPth[frNum[frI],,]
    cCN <- apply(cellsCoords, 1,
                 function(crds){
                   if (is.na(crds[1]))
                     return(crds)
                   else {
                     res <- c(crds[1:3], nearestPoint(crds[1:3],
                                                      mshList[[frNam[frI]]]))
                     return(res)
                   }
                 })
    cPth[frNum[frI],,] <- t(cCN)
```

```
  }
  return(cPth)
}
# This was used to put nearest point and distance into cellPaths$coords columns:
#    cellPaths <- fillNearest(cPth = cellPaths)
```

Data on cell differentiation

Data for Figures 2C and Sup 3 A-B

```
diffCounts <- read.csv("data/diffCounts.csv",
                       stringsAsFactors=TRUE)
diffCounts$percHuC <-
  100*diffCounts$Huc / (diffCounts$Huc + diffCounts$noHuC)

percBoundTime <- diffCounts %>% group_by(bound,time) %>%
  summarise(percHuC = mean(percHuC), .groups = 'keep')
percHuC_30 <- percBoundTime[percBoundTime$time=="30hpf", "percHuC"]$percHuC
percHuC_48 <- percBoundTime[percBoundTime$time=="48hpf", "percHuC"]$percHuC

summary(diffCounts)
```

```
##       id     bound       time         Huc              noHuC
##  emb1e1:8   r2r3:10   30hpf:20   Min.   :  0.00   Min.   : 36.0
##  emb2e5:8   r3r4:10   48hpf:20   1st Qu.:  0.00   1st Qu.: 59.0
##  emb5e3:8   r4r5:10              Median : 16.50   Median : 86.5
##  emb5e4:8   r5r6:10              Mean   : 34.23   Mean   : 86.4
##  emb5e5:8                        3rd Qu.: 69.50   3rd Qu.:108.0
##                                  Max.   :119.00   Max.   :171.0
##     percHuC
##  Min.   : 0.00
##  1st Qu.: 0.00
##  Median :17.18
##  Mean   :21.36
##  3rd Qu.:41.84
##  Max.   :48.82
```

```
diffCounts
```

```
##        id bound  time Huc noHuC   percHuC
## 1  emb1e1  r2r3 30hpf   0    44  0.000000
## 2  emb2e5  r2r3 30hpf   0    45  0.000000
## 3  emb5e3  r2r3 30hpf   0    97  0.000000
## 4  emb5e4  r2r3 30hpf   4   149  2.614379
## 5  emb5e5  r2r3 30hpf   0    53  0.000000
## 6  emb1e1  r3r4 30hpf   0    37  0.000000
## 7  emb2e5  r3r4 30hpf   1    36  2.702703
## 8  emb5e3  r3r4 30hpf   0   111  0.000000
## 9  emb5e4  r3r4 30hpf   0    92  0.000000
## 10 emb5e5  r3r4 30hpf   0    39  0.000000
## 11 emb1e1  r4r5 30hpf   0    50  0.000000
## 12 emb2e5  r4r5 30hpf   3    53  5.357143
## 13 emb5e3  r4r5 30hpf   0   107  0.000000
## 14 emb5e4  r4r5 30hpf   0   108  0.000000
## 15 emb5e5  r4r5 30hpf   2    46  4.166667
```

```
## 16 emb1e1  r5r6 30hpf   2    86  2.272727
## 17 emb2e5  r5r6 30hpf   2    76  2.564103
## 18 emb5e3  r5r6 30hpf   0   129  0.000000
## 19 emb5e4  r5r6 30hpf   0   137  0.000000
## 20 emb5e5  r5r6 30hpf   2    63  3.076923
## 21 emb1e1  r2r3 48hpf  33    68 32.673267
## 22 emb2e5  r2r3 48hpf  29    71 29.000000
## 23 emb5e3  r2r3 48hpf  58   100 36.708861
## 24 emb5e4  r2r3 48hpf  71   104 40.571429
## 25 emb5e5  r2r3 48hpf  81    96 45.762712
## 26 emb1e1  r3r4 48hpf  52    61 46.017699
## 27 emb2e5  r3r4 48hpf  40    50 44.444444
## 28 emb5e3  r3r4 48hpf  68   108 38.636364
## 29 emb5e4  r3r4 48hpf  87    92 48.603352
## 30 emb5e5  r3r4 48hpf  60    66 47.619048
## 31 emb1e1  r4r5 48hpf  72    84 46.153846
## 32 emb2e5  r4r5 48hpf  83    87 48.823529
## 33 emb5e3  r4r5 48hpf  74   122 37.755102
## 34 emb5e4  r4r5 48hpf  85   122 41.062802
## 35 emb5e5  r4r5 48hpf  66    74 47.142857
## 36 emb1e1  r5r6 48hpf  47    69 40.517241
## 37 emb2e5  r5r6 48hpf  72    91 44.171779
## 38 emb5e3  r5r6 48hpf  87   171 33.720930
## 39 emb5e4  r5r6 48hpf 119   140 45.945946
## 40 emb5e5  r5r6 48hpf  69   122 36.125654
```

Data for Figures 4C and Sup 4 A-B

```
percNotch <- read.csv("data/percNotch.csv", stringsAsFactors = TRUE)
percBoundTimeNotch <- percNotch %>% group_by(bound,time) %>%
  summarise(percNotch = mean(percNotch, na.rm = TRUE), .groups = 'keep')
summary(percNotch)
```

```
##     id       time     bound      percNotch
##  emb1:8   26hpf:20   r2r3:10   Min.   : 0.000
##  emb2:8   36hpf:20   r3r4:10   1st Qu.: 8.079
##  emb3:8              r4r5:10   Median :15.849
##  emb4:8              r5r6:10   Mean   :31.978
##  emb5:8                        3rd Qu.:57.514
##                                Max.   :73.786
##                                NA's   :4
```

```
percNotch
```

```
##      id  time bound percNotch
## 1  emb1 26hpf  r2r3 14.285714
## 2  emb2 26hpf  r2r3 13.793103
## 3  emb3 26hpf  r2r3 12.280702
## 4  emb4 26hpf  r2r3  2.222222
## 5  emb5 26hpf  r2r3  3.508772
## 6  emb1 26hpf  r3r4 14.285714
## 7  emb2 26hpf  r3r4 15.789474
## 8  emb3 26hpf  r3r4 15.909091
## 9  emb4 26hpf  r3r4  8.333333
## 10 emb5 26hpf  r3r4  8.888889
## 11 emb1 26hpf  r4r5 11.764706
```

```
## 12 emb2 26hpf  r4r5  2.941176
## 13 emb3 26hpf  r4r5 11.627907
## 14 emb4 26hpf  r4r5  0.000000
## 15 emb5 26hpf  r4r5  7.317073
## 16 emb1 26hpf  r5r6 18.181818
## 17 emb2 26hpf  r5r6  2.127660
## 18 emb3 26hpf  r5r6  4.878049
## 19 emb4 26hpf  r5r6  6.250000
## 20 emb5 26hpf  r5r6  6.896552
## 21 emb1 36hpf  r2r3 71.304348
## 22 emb2 36hpf  r2r3 65.263158
## 23 emb3 36hpf  r2r3 72.413793
## 24 emb4 36hpf  r2r3 55.913978
## 25 emb5 36hpf  r2r3        NA
## 26 emb1 36hpf  r3r4 68.965517
## 27 emb2 36hpf  r3r4 56.626506
## 28 emb3 36hpf  r3r4 61.016949
## 29 emb4 36hpf  r3r4 44.318182
## 30 emb5 36hpf  r3r4        NA
## 31 emb1 36hpf  r4r5 49.397590
## 32 emb2 36hpf  r4r5 56.034483
## 33 emb3 36hpf  r4r5 61.157025
## 34 emb4 36hpf  r4r5 52.380952
## 35 emb5 36hpf  r4r5        NA
## 36 emb1 36hpf  r5r6 73.786408
## 37 emb2 36hpf  r5r6 60.176991
## 38 emb3 36hpf  r5r6 53.658537
## 39 emb4 36hpf  r5r6 67.521368
## 40 emb5 36hpf  r5r6        NA
```

Data on differentiated boundary cells for Figure 7C

```
diff4872 <- read.csv("data/diff4872.csv", stringsAsFactors=TRUE)
diff4872
```

```
##       emb  time bound  percHuC nCells
## 1  emb1e6 48hpf  r4r5 29.86111    144
## 2  emb2e3 48hpf  r4r5 48.05195    154
## 3  emb2e1 48hpf  r4r5 41.37931    174
## 4  emb3e7 48hpf  r4r5 35.77236    123
## 5  emb3e1 48hpf  r4r5 30.07519    133
## 6  emb3e6 72hpf  r4r5 86.50000    200
## 7  emb3e5 72hpf  r4r5 81.76101    159
## 8  emb2e1 72hpf  r4r5 75.00000    124
## 9  emb1e8 72hpf  r4r5 87.43169    183
## 10 emb1e2 72hpf  r4r5 74.28571    175
## 11 emb1e6 48hpf  r5r6 18.38235    136
## 12 emb2e3 48hpf  r5r6 30.18868    212
## 13 emb2e1 48hpf  r5r6 39.59732    149
## 14 emb3e7 48hpf  r5r6 47.28682    129
## 15 emb3e1 48hpf  r5r6 28.57143    154
## 16 emb3e6 72hpf  r5r6 79.75709    247
## 17 emb3e5 72hpf  r5r6 85.71429    154
## 18 emb2e1 72hpf  r5r6 82.44275    131
## 19 emb1e8 72hpf  r5r6 85.97561    164
```

```
## 20 emb1e2 72hpf  r5r6 87.09677    248
```

Data on differentiated BCP cells for figure 7F

```
BCPdiff <- data.frame(
Volum.colocalization.BCP.HuC = c(349941.69,674825.048,540146.442,
                                 310148.821,228214.554,206442.811,431405.527),
Volum.HuC.total = c(5885563.63,5911144.78,
                    6345403.54,5908902.65,6034069.95,6127675.38,6114207.62),
perc.colocalization = c(5.945763430647,
                        11.41614819321,8.51240490214748,5.24883957937605,
                        3.7820999075425,3.36902329509498,7.05578799105288)
)
rownames(BCPdiff) = c("E1", "E2", "E3", "E4", "E5", "E6", "E7")

BCPdiff
```

```
##    Volum.colocalization.BCP.HuC Volum.HuC.total perc.colocalization
## E1                     349941.7         5885564            5.945763
## E2                     674825.0         5911145           11.416148
## E3                     540146.4         6345404            8.512405
## E4                     310148.8         5908903            5.248840
## E5                     228214.6         6034070            3.782100
## E6                     206442.8         6127675            3.369023
## E7                     431405.5         6114208            7.055788
```

Data on differentiated BCP cells for figure S6M

```
gad1b <- data.frame(
  volum.colocalization.BCP.plus.gad1b = c(78331.3964,100902.226,156688.351,
                128461.239,140192.32,96225.9263),
  volum.gad1b.total = c(1075545.88,1109593.04,1264493.15,
                1502678.07,1525520.79,1467844.24),
  perc.colocalization = c(7.28294328085753,9.09362463196417,
                12.3913957936427,8.54881970826925,9.18980068439448,6.55559518358706)
)


gad1b
```

```
##   volum.colocalization.BCP.plus.gad1b volum.gad1b.total perc.colocalization
## 1                            78331.40           1075546            7.282943
## 2                           100902.23           1109593            9.093625
## 3                           156688.35           1264493           12.391396
## 4                           128461.24           1502678            8.548820
## 5                           140192.32           1525521            9.189801
## 6                            96225.93           1467844            6.555595
```

```
vglut2a <- data.frame(
  volum.colocalization.BCP.plus.vglut2a = c(197080.231,256436.078,164943.557,
                72228.248,83761.2547,66543.6099),
  volum.vglut2a.total = c(1735948.63,1640904.43,1950472.17,
                1823985.65,1837058.62,1830909.55),
  perc.colocalization = c(11.3528838120054,15.6277278134961,
                8.45659628150449,3.95991317146601,4.55953086026182,3.63445643177731)
)
```

```
vglut2a
```

```
##   volum.colocalization.BCP.plus.vglut2a volum.vglut2a.total perc.colocalization
## 1                             197080.23            1735949           11.352884
## 2                             256436.08            1640904           15.627728
## 3                             164943.56            1950472            8.456596
## 4                              72228.25            1823986            3.959913
## 5                              83761.25            1837059            4.559531
## 6                              66543.61            1830910            3.634456
```

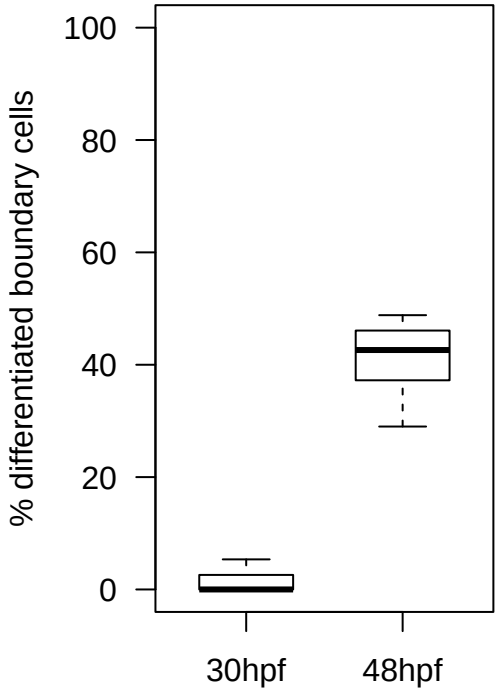
For description of 2 groups

```
  descr1 <- function(gr){
    gr <- na.omit(gr)
    return(list(N=length(gr), M=mean(gr, na.rm=TRUE),
                SD=sd(gr, na.rm = TRUE), SEM=sd(gr, na.rm = TRUE)/sqrt(length(gr))))
  }

descr2groups <- function(gr1, gr2, nams=NULL, tit = NULL, not.paired=TRUE){
  # given array of two rows
  tab <- rbind(unlist(descr1(gr1)), unlist(descr1(gr2)))
  if (!is.null(nams)){
    rownames(tab) <- nams}
  pv <- t.test(gr1,gr2, paired= (!not.paired))$p.value

  if (!is.null(tit))
    print(paste("   ", tit))
  print(round(tab,2))
  print(paste(" t.test p-value:", pv, ifelse(pv<0.001, " *** ",
                                      ifelse(pv<0.01, " ** ",
                                             ifelse(pv<0.05, " * ", "ns")))))
}
descr2cols <- function(df, not.paired=TRUE, nams = NULL, tit = NULL){
  # given a 2 column data.frame or tibble
  descr2groups(unlist(df[,1]), unlist(df[,2]),
               not.paired = not.paired, nams = nams, tit = tit)
}
```

Figures as included in the paper

Figure 2C

```
b1 <- diffCounts[diffCounts$time=="30hpf","percHuC"]
b2 <- diffCounts[diffCounts$time=="48hpf","percHuC"]


boxplot(b1,b2, main = "",
        xlab = "", ylab = "% differentiated boundary cells", las=1,
        ylim=c(0,100), family = "Arial",
        names =  c("30hpf", "48hpf"), col ="white",
        boxwex=0.6)
```




```
descr2groups(b1,b2, nams=c("30hpf", "48hpf"))
```

```
##        N     M   SD  SEM
## 30hpf 20  1.14 1.71 0.38
## 48hpf 20 41.57 5.80 1.30
## [1] " t.test p-value: 1.79453265173272e-19  *** "
```

Figure 2E

```
load("data/tracking.RData")


# cellsTable["c1812_01","hasDivision"] <- TRUE


plotLineageTree <- function(sortBy="", selTrackType=NULL, selPlfType=NULL){
  # plot the lineages tree by track and color
  # sortBy can be "", "divTime", "onlyPs", "plfType"
  # selTrackType, can be a list of track types to plot
  # selPlfType, can be a list of plf types to plot  NO BOTH should be given
  tRange=frame2hpf(c(24, 217)) # range(dataset$POSITION_T))
  #plfColor <- list(NPLF="darkgrey", PLF="blue")
  #plfColor <- list(NPLF=grey(0.2), PLF="blue")

    # what cells to plot
  if (length(selTrackType)==0){
    if (length(selPlfType)==0) selTrackType<-sort(unique(cellsTable$trackType))
    else selTrackType <- sort(unique(cellsTable[cellsTable$plfType!="", "trackType"]))
  }
  if (length(selPlfType)==0){
    cellsToPlot=cellsTable[cellsTable$trackType %in% selTrackType, ]
    colsInPlot = cellColor[cellsToPlot$trackType]
  } else { # deal with plfType
    cellsToPlot=cellsTable[cellsTable$plfType %in% selPlfType, ]
    colsInPlot = plfColor[cellsToPlot$plfType]
  }
  tracksInPlot = tracksTable[(tracksTable[,1] %in% cellsToPlot$track) , ]
  firstFrame = apply(tracksInPlot, 1,
                     function(tr){
                       trL=tr[1]
                       return(min(tracks[[trL]][,4]))
                     })
  if (length(selPlfType)==0){
    ord = order(tracksInPlot[,"TrackType"], tracksInPlot[,"divTime"], firstFrame)
    tit = gsub(",", ":",
               toString(format(t(cbind(selTrackType, cellColor[selTrackType])))))
    titMain = paste("Lineage tree", "for types", toString(selTrackType))
  }
  else{
    ord = order(tracksInPlot[,"plfType"], tracksInPlot[,"divTime"], firstFrame)
    tit = gsub(",", ":", toString(format(t(cbind(selPlfType, plfColor[selPlfType])))))
    titMain = paste("Lineage tree", "for types", toString(selPlfType))
  }
  if (sortBy=="divTime")
    ord = order(tracksInPlot[,"divTime"], firstFrame)

  plot(0, 0,
       xlim = tRange,
       ylim=c(1,dim(tracksInPlot)[1]),
       main = titMain,
       sub = tit,
```

```
      ylab = "Track number", xlab = "Time (hpf)",
      type="n", yaxt="n")
  #axis(2, at = seq(1, length(cells), by = 1), las=2, pos=tRange[1]-0.1)
  axis(2, labels = TRUE, tick = TRUE, at = seq(1, dim(tracksInPlot)[1] , by = 1))
  ## no legend
  for (i in 1:dim(cellsToPlot)[1]){
    # color = ""
    tTy = cellsToPlot[i, "trackType"]
    cTy = cellsToPlot[i, "cellType"]
    cLb = cellsToPlot[i, "cellLabel"]
    cTr = cellsToPlot$track[i]
    # ara ha de ser l'index de cTr
    tNum = match(cTr, tracksInPlot[ord,1])
    #if (tNum==5) print(cLb)
    path = cells[[cLb]][,4]
    xinc = c(0, 0.2, -0.2, 0.3, 0.1, 0.2, 0.4)[
      cTy==c("P", "D1", "D2",  "11", "12", "21", "22")]
    lines(x=frame2hpf( path), y=rep(tNum+xinc,length(path)), type="l",
          col=ifelse(length(selPlfType)==0,
                     cellColor[[ cellsToPlot[i, "trackType"] ]],
                     plfColor[[ cellsToPlot[i, "plfType"] ]]))
    if (cellsToPlot[i,"hasDivision"]){
      points(x=frame2hpf(cellsToPlot[i,"cellLastFrame"]), col=colsInPlot[i][[1]],
             y=tNum+xinc, pch=16,  cex=0.8)

    }
  }
   #return(cellsToPlot)
}
```

```
plotLineageTree(selPlfType = c("PLF", "NPLF"))
```

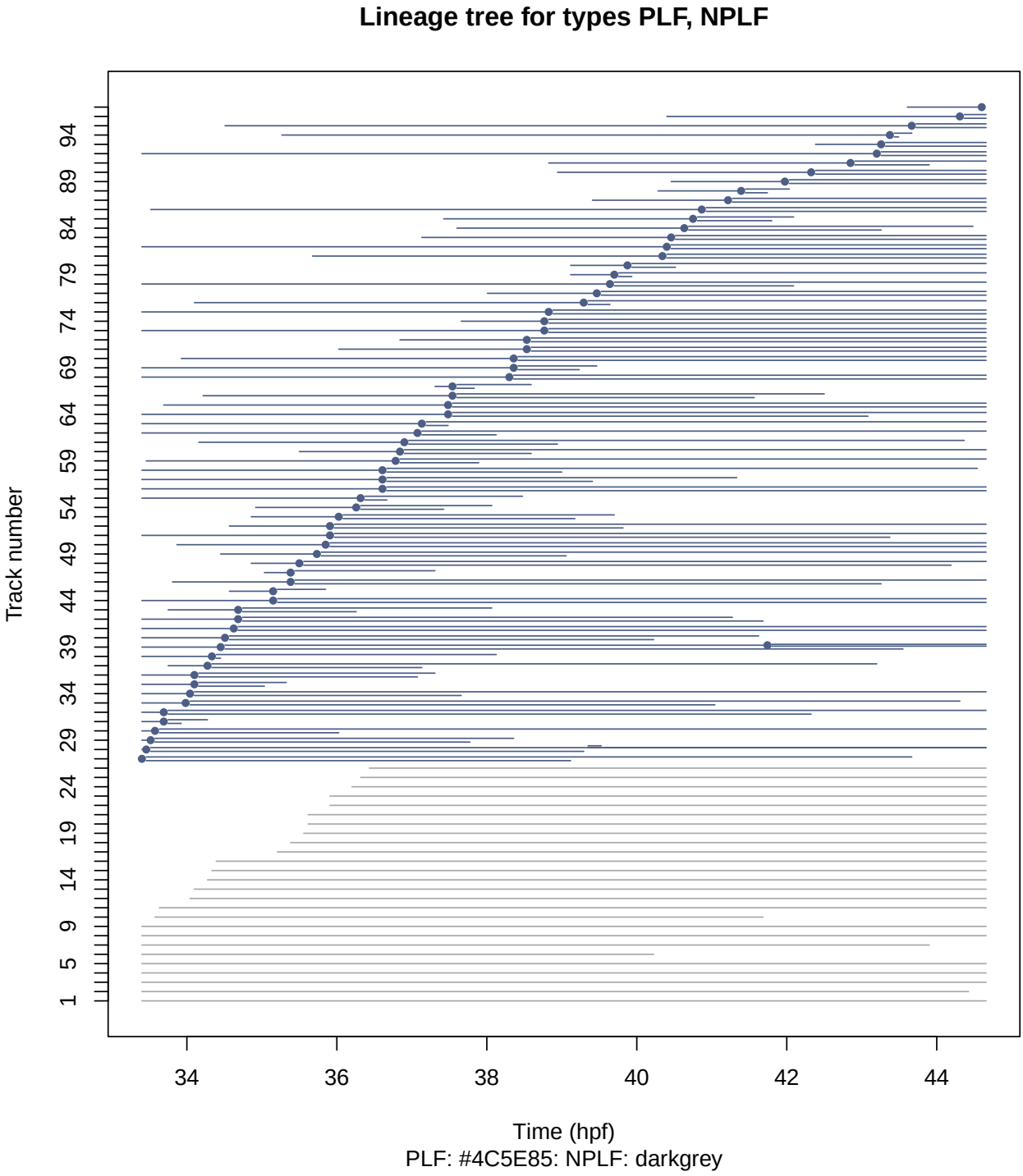

Lineage tree for types PLF, NPLF
Track number
1
5
9
14
19
24
29
34
39
44
49
54
59
64
69
74
79
84
89
94
34
36
38
40
42
44
Time (hpf)
PLF: #4C5E85: NPLF: darkgrey

Figure 3B

```
# some new colors
cellColor <- list(PQ= "black",PN=  "#2A6684",IND1=  "white",
                  PP="#B74C04", CN="darkgrey", N="darkgrey", NONE="white",
                  PD="#5D9B90", ND="#95CC86")
plfColor <- list(NPLF="darkgrey", PLF="#4C5E85")



# plot just the not shortLived tracks
plotLineageTree <- function(sortBy="", selTrackType=NULL){
  # plot the lineages tree by track and color
  # sortBy can be "", "divTime", "onlyPs", "plfType", "LMposition"
  # selTrackType, can be a list of track types to plot
  tRange=frame2hpf(c(24, 217)) #   range(dataset$POSITION_T))

  # what cells to plot
  if (length(selTrackType)==0){
    if (length(selPlfType)==0) selTrackType<-sort(unique(cellsTable$trackType))
    else selTrackType <- sort(unique(cellsTable[cellsTable$plfType!="", "trackType"]))
  }
  cellsToPlot=cellsTable[((cellsTable$trackType %in% selTrackType)&(!cellsTable$isShort)), ]
  colsInPlot = cellColor[cellsToPlot$trackType]

  tracksInPlot = tracksTable[(tracksTable[,1] %in% cellsToPlot$track) , ]
  firstFrame = apply(tracksInPlot, 1,
                     function(tr){
                       trL=tr[1]
                       return(min(tracks[[trL]][,4]))
                     })

    ord = order(tracksInPlot[,"TrackType"], tracksInPlot[,"divTime"], firstFrame)
    tit = gsub(",", ":", toString(format(t(cbind(selTrackType, cellColor[selTrackType])))))
    titMain = paste("Lineage tree", "for types", toString(selTrackType))

  if (sortBy=="divTime")
    ord = order(tracksInPlot[,"divTime"], firstFrame)
  else if (sortBy =="LMposition"){
    divTimes <- tracksInPlot[,"divTime"]
    toSortLM <- apply(tracksInPlot, 1,
                      function(tr){
                        trL <- tr[1]
                        trDivT <- as.numeric(tr[3])
                        trC <- tracks[[trL]]
                        return(trC[trC[,4]==trDivT,1])
                      })
     ord <- order(toSortLM)
  } else {stop("sortBy not implemented")}

  plot(0, 0,
       xlim = tRange,
       ylim=c(1,dim(tracksInPlot)[1]),
       main = titMain,
```

```
      sub = tit,
      ylab = ifelse((sortBy %in% c("LMposition", "TypeLMposition")), "LM position", "Track number") ,
      xlab = "Time (hpf)",
      type="n", yaxt="n")
  #axis(2, at = seq(1, length(cells), by = 1), las=2, pos=tRange[1]-0.1)
  if (sortBy=="LMposition"){
    axis(2, labels = round(toSortLM[ord],0), tick = TRUE,
         at = seq(1, dim(tracksInPlot)[1] , by = 1), las = 1, cex=0.8)
  }
  else if (sortBy=="TypeLMposition"){
    axis(2, labels = round(toSortLM[ord],0), tick = TRUE,
         at = seq(1, dim(tracksInPlot)[1] , by = 1), las = 1, cex=0.8)
  }
  else axis(2, labels = TRUE, tick = TRUE, at = seq(1, dim(tracksInPlot)[1] , by = 1))
  ## no legend
  for (i in 1:dim(cellsToPlot)[1]){
    tTy = cellsToPlot[i, "trackType"]
    cTy = cellsToPlot[i, "cellType"]
    cLb = cellsToPlot[i, "cellLabel"]
    cTr = cellsToPlot$track[i]
    # ara ha de ser l'index de cTr
    tNum = match(cTr, tracksInPlot[ord,1])
    #if (tNum==5) print(cLb)
    path = cells[[cLb]][,4]
    xinc = c(0, 0.2, -0.2, 0.3, 0.1, 0.2, 0.4)[
      cTy==c("P", "D1", "D2",  "11", "12", "21", "22")]
    cCol <- cellColor[[ cellsToPlot[i, "trackType"] ]]

    lines(x=frame2hpf( path), y=rep(tNum+xinc,length(path)), type="l",
          col=cCol)
    if (cellsToPlot[i,"hasDivision"]){
      points(x=frame2hpf(cellsToPlot[i,"cellLastFrame"]), col=cCol,
             y=tNum+xinc, pch=16,  cex=0.8)
    }
  }
} # end plotLineageTree
```

```
plotLineageTree(selTrackType = c("PP", "PN"), sortBy = "divTime")
```

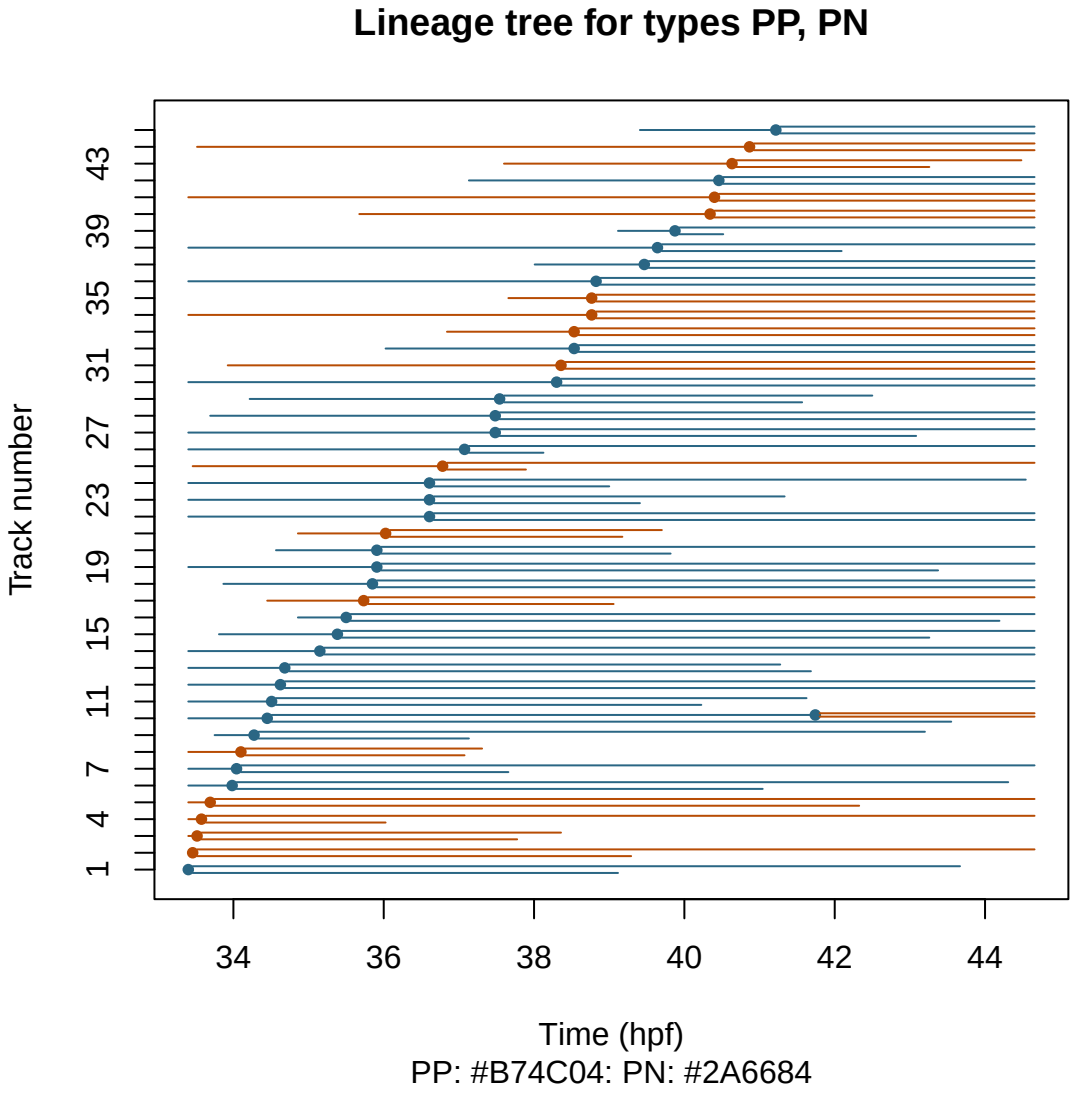

Lineage tree for types PP, PN
Track number
1
4
7
11
15
19
23
27
31
35
39
43
34
36
38
40
42
44
Time (hpf)
PP: #B74C04: PN: #2A6684

Figure 3E

```
load("data/tracking.RData")

progCells <- cellsTable[cellsTable$trackType %in% c("PN","PP", "PQ"),
                        "cellLabel"] # 187 cells
# We build an array cells x frames filled by 0/1
# according if the cell is there on the frame
# So: isthere(c,f) = ifelse("c is in f", 1, 0)
# but: A Dx cell is_in a frame if it already has been seen in a previous frame.
# change x to 0/1 according abscence/presence
isthere <- t(vapply(progCells,
                    FUN = function(cn){
                      cF <- cellsTable[cn,"cellFirstFrame"]
                      cL <- cellsTable[cn, "cellLastFrame"]
                      # print(paste(cn, cF, cL))
                      if (cellsTable[cn,"trackType"]=="PQ")
                        c(rep.int(0, cF-24), # 24 is first frame, 217 is last
                          rep.int(1, 217-cF+1))
                      else if (cellsTable[cn,"cellType"] == "P")
                        c(rep.int(0, cF-24),
                          rep.int(1, cL - cF + 1),
                          rep.int(0, 217-cL))
                      else # it is a xD cell
                        c(rep.int(0, cF-24),
                          rep.int(1, 217-cF+1))
                    }, FUN.VALUE = rep.int(0, 194))) # 187 cells x 194 frames
colnames(isthere) <- as.character(24:217)

# colnames are 24 to 217
xvals <- round(frame2hpf( as.numeric(colnames(isthere))),
               1)
cellsToPlot <- isthere[cellsTable[progCells,"trackType"] %in% c("PP","PN"),]
nP <- apply(cellsToPlot[cellsTable[rownames(cellsToPlot),
                                   "cellType"] %in% c("P", "PD", "D1", "D2"),],
            2, sum)
nN <- apply(cellsToPlot[(cellsTable[rownames(cellsToPlot),"cellType"] %in% c("D1", "D2"))
                        & (cellsTable[rownames(cellsToPlot),"trackType"] == "PN"),],
            2, sum)

cellsToPlot <- isthere[cellsTable[progCells,"trackType"]=="PQ",]
nQ <- apply(cellsToPlot,
            2, sum)

totalNPQ1 <- nN+nP+nQ ## <<< to be used on the new dists graph
```

```
# we split the lines and xvals in two: 1.. 217-51  and 217-51 .. 217
# to not count cell of unknown final fate
ind1 <- ( 24:(217-51)) - 23
ind2 <- ((217-51):217) - 23

xvals1 <- xvals[ind1]
xvals2 <- xvals[ind2]
```

```r
nN1 <- nN[ind1]
nN2 <- nN[ind2]
nP1 <- nP[ind1]
nP2 <- rep(nP[143], length(ind2))

cellColor <- list(PQ= "black",PN= "#2A6684",IND1= "white", PP="#0031BF", ###"#B74C04",
                  CN="darkgrey", N="darkgrey", NONE="white",
                  PD="#5D9B90", ND="#95CC86")


plot(0,0, type="l",
     xlim = frame2hpf(c(20,220)), ylim=range(c(nP,nN, totalNPQ1)),
     xlab = "Time (hpf)", ylab = "Number of cells")
lines(x=xvals1, y=nN1, lwd=2, col = cellColor$ND)
lines(x=xvals2, y=nN2, lwd=2, lty=3, col = cellColor$ND)

lines(x=xvals1, y=nP1, lwd=2, col = cellColor$PP)
lines(x=xvals2, y=nP2, lwd=2, lty=3, col = cellColor$PP)

lines(x=xvals, y=totalNPQ1, lwd=2, col = "black")

legend(x="topleft", legend=c( "P", "N", "Total"),
       text.col = c(unlist(cellColor[c("PP", "ND")]), "black")
       )
```

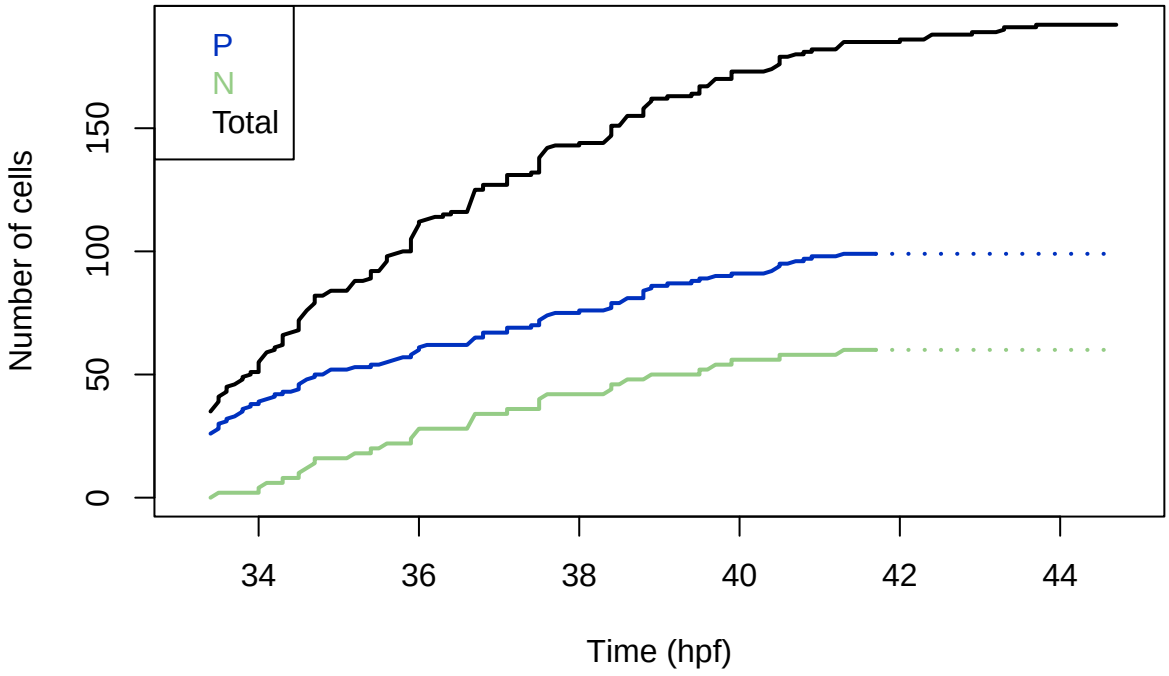


Figure 3H

```
#load("../VentrSurf21/allButMeshes.RData")
load("data/ventrSurf.RData")
# source("../VentrSurf21/processVS.R")

progCells <- ((cellsTable$trackType %in% c("PP","PN","PQ")) & (cellsTable$cellType != "ND"))

# dimnames(cellPaths)

plotAveDistNDvsProg <- function(plotND = TRUE){
  average_wona <- function(frxcl){ # average for each frame skipping NAs
    print(dim(frxcl))
    res <- apply(frxcl, 1,
                 FUN = function(x){ifelse(all(is.na(x)), NA, mean(x,na.rm = TRUE))})
    return(res)}
  distsProgs <- cellPaths[ , progCells, "dist"] # it is a frame x cells array of distances
  distsND <- cellPaths[, cellsTable$cellType == "ND", "dist"] # it is a frame x cells array of distance
  yyProgs <- average_wona(distsProgs)
  yyND    <- average_wona(distsND)
  xx      <- as.numeric(rownames(distsProgs))
  xx      <- frame2hpf(xx)
  plot(0,0, ylim=c(0,30), xlim=range(xx),
       xlab="Time hpf", ylab="Distance to VS (µm)",
       main=paste("Average distance to Ventricular Surface"),
       type="l")
  lines(x = xx[!is.na(yyND)], y = yyND[!is.na(yyND)], col = cellColor$ND, lwd=4)
  lines(x = xx[!is.na(yyProgs)], y = yyProgs[!is.na(yyProgs)], col = "#0031BF", lwd=4)
  # return(distsND)
}
```

```
plotAveDistNDvsProg()
```

```
## [1] 194 157
## [1] 194  30
```

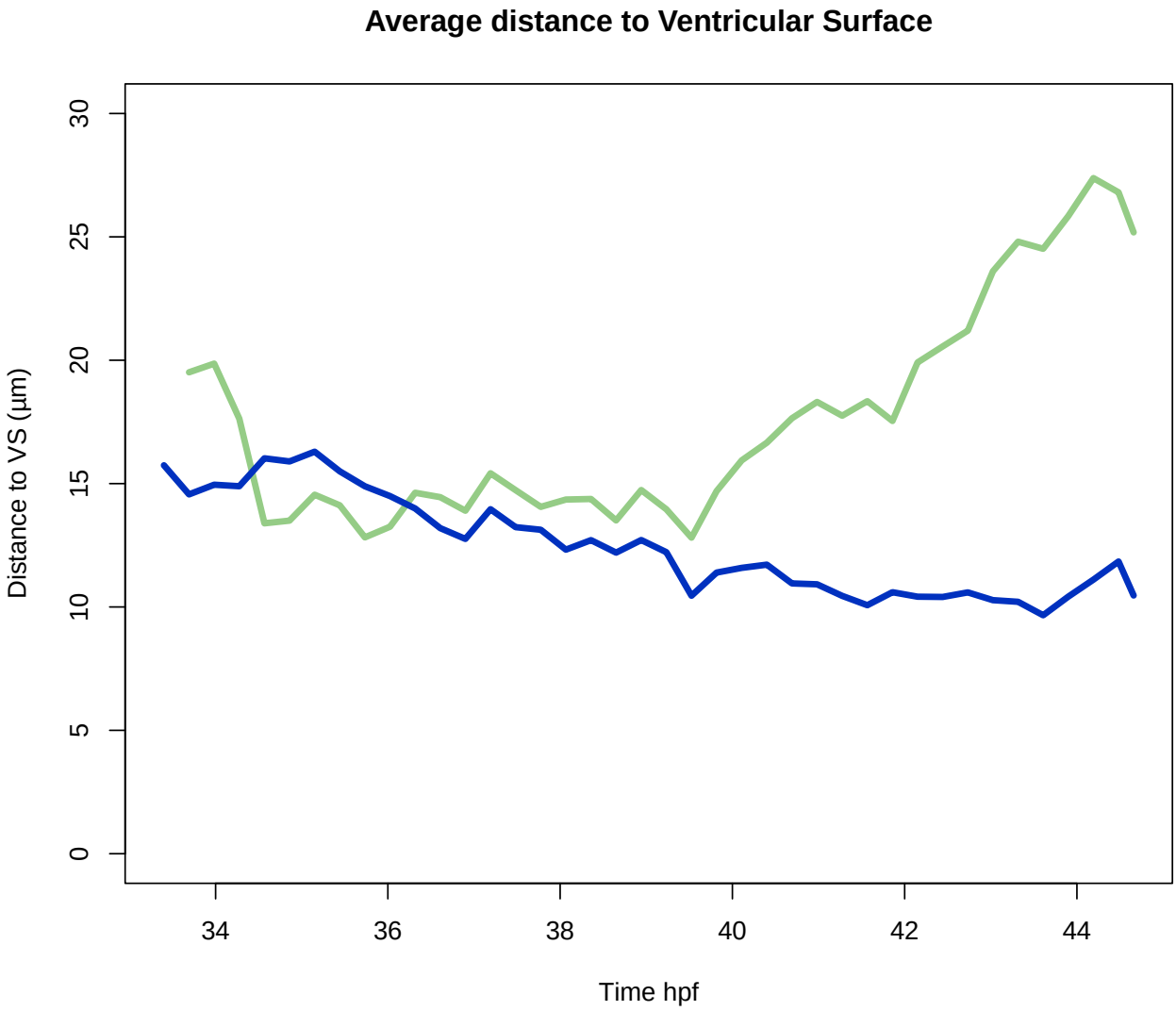
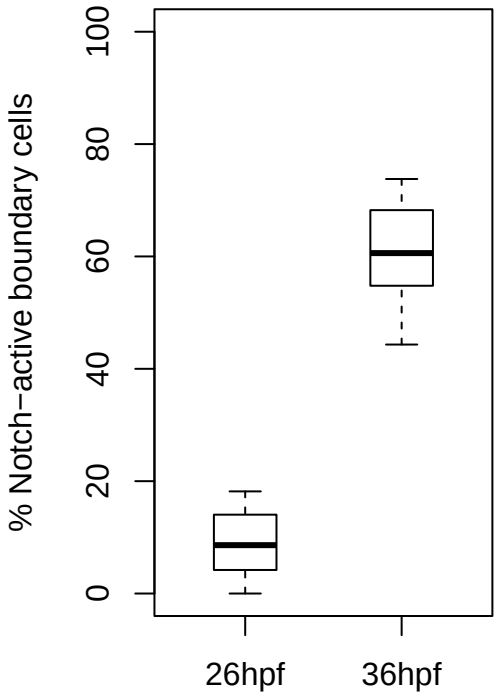

Average distance to Ventricular Surface
Distance to VS (µm)
0
5
10
15
20
25
30
34
36
38
40
42
44
Time hpf

Figure 4C

bp notch active 26-36

```
b1 <- percNotch$percNotch[percNotch$time=="26hpf"]
b2 <- percNotch$percNotch[percNotch$time=="36hpf"]

boxplot(b1, b2,
        ylim=c(0,100),
        main = "", xlab = "",
        ylab = "% Notch-active boundary cells",
        names =  c("26hpf", "36hpf"), col ="white",
        boxwex=0.4, outpch='.', cex=2)
```




```
descr2groups(b1,b2, nams=c("26hpf", "36hpf"))
```

```
##        N     M   SD  SEM
## 26hpf 20  9.06 5.41 1.21
## 36hpf 16 60.62 8.69 2.17
## [1] " t.test p-value: 8.60491568290165e-17  *** "
```

Figure 4 I-J

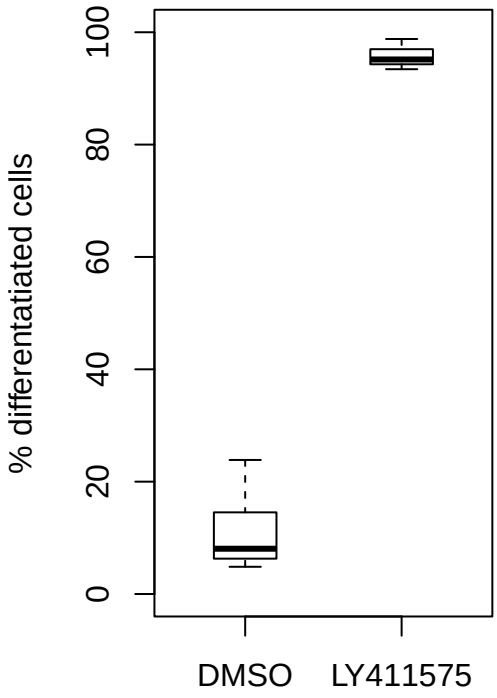
```
DMSO <- data.frame(
  HuC = c(7L, 24L, 13L, 21L, 8L, 10L, 8L, 11L),
  Total = c(113L, 132L, 151L, 88L, 125L, 133L, 165L, 101L))
DMSO$percHuC <- DMSO$HuC/DMSO$Total*100
DMSO
```

```
##   HuC Total   percHuC
## 1   7   113  6.194690
## 2  24   132 18.181818
## 3  13   151  8.609272
## 4  21    88 23.863636
## 5   8   125  6.400000
## 6  10   133  7.518797
## 7   8   165  4.848485
## 8  11   101 10.891089
```

```
LY <- data.frame(
  HuC = c(71L, 122L, 82L, 99L, 79L, 142L, 104L, 112L),
  Total = c(74L, 129L, 83L, 101L, 84L, 152L, 110L, 117L))
LY$percHuC <- LY$HuC/LY$Total*100
LY
```

```
##   HuC Total  percHuC
## 1  71    74 95.94595
## 2 122   129 94.57364
## 3  82    83 98.79518
## 4  99   101 98.01980
## 5  79    84 94.04762
## 6 142   152 93.42105
## 7 104   110 94.54545
## 8 112   117 95.72650
```

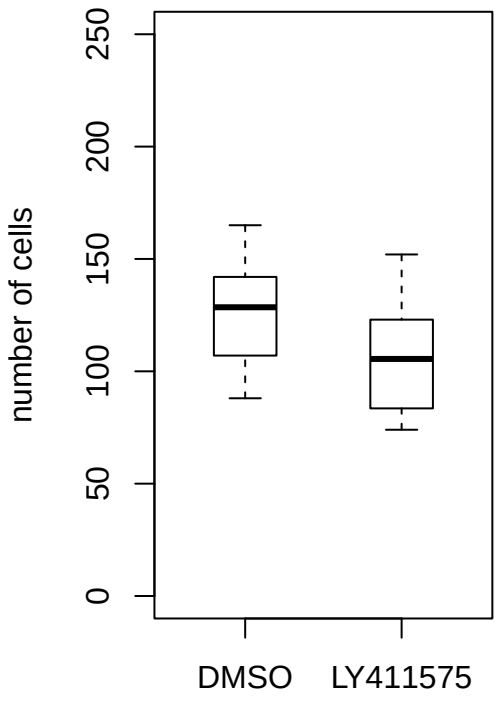
```
b1 <- DMSO$percHuC
b2 <- DMSO$Total
b3 <- LY$percHuC
b4 <- LY$Total

# fill colors
rellcols <- c("white", "white")

boxplot(b1,b3,
        ylim = c(0,100),
        ylab="% differentatiated cells",
        names = c("DMSO", "LY411575"),
        col = rellcols,
        boxwex=0.4, outpch='.', cex=2)
```




```
descr2groups(b1,b3, nams=c("DMSO", "LY411575"), not.paired = TRUE)
```

```
##           N     M   SD  SEM
## DMSO      8 10.81 6.73 2.38
## LY411575 8 95.63 1.91 0.67
## [1] " t.test p-value: 4.44043249694964e-10  *** "
```

```
maxRight <- 250

boxplot(b2, b4,
        ylim = c(0, maxRight),
        ylab="number of cells",
        names = c("DMSO", "LY411575"),
        col = rellcols,
        boxwex=0.4, outpch='.', cex=2)
```

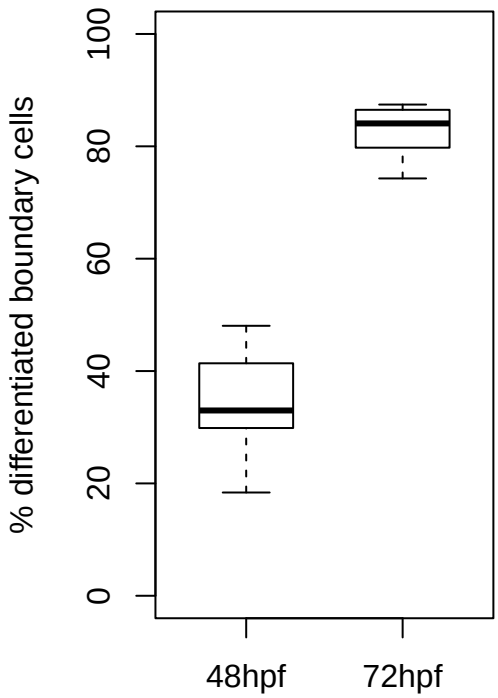



```
descr2groups(b2,b4, nams=c("DMSO", "LY411575"), not.paired = TRUE)
```

```
##           N      M    SD  SEM
## DMSO      8 126.00 25.27 8.93
## LY411575 8 106.25 26.30 9.30
## [1] " t.test p-value: 0.147972179934964 ns"
```

Figure 7C

```
b1 <- diff4872$percHuC[diff4872$time=="48hpf"]
b2 <- diff4872$percHuC[diff4872$time=="72hpf"]

boxplot(b1, b2, ylim=c(0,100),
        main = "", xlab = "",
        ylab = "% differentiated boundary cells",
        names =  c("48hpf", "72hpf"), col ="white",
        boxwex=0.6, outpch='.', cex=2)
```

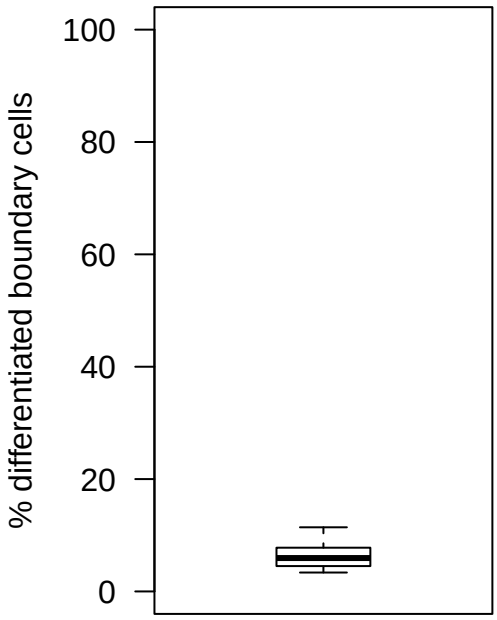



```
descr2groups(b1,b2, nams=c("48hpf", "72hpf"), not.paired = TRUE)
```

```
##         N     M   SD  SEM
## 48hpf  10 34.92 9.28 2.93
## 72hpf  10 82.60 4.89 1.55
## [1] " t.test p-value: 1.25029141236881e-09  *** "
```

Figure 7L

```
boxplot(BCPdiff$perc.colocalization, main = "",
        xlab = "", ylab = "% differentiated boundary cells", las=1,
        ylim=c(0,100), family = "Arial",
        col ="white",
        boxwex=0.6)
```




```
# descr2groups(b1,b2, nams=c("30hpf", "48hpf"))
```

Figure 7O

```
b1 <- gad1b$perc.colocalization
b2 <- vglut2a$perc.colocalization


boxplot(b1,b2, main = "",
        xlab = "", ylab = "% differentiated boundary cells", las=1,
        ylim=c(0,100), family = "Arial",
        names =  c("gad1b", "vglut2a"), col ="white",
        boxwex=0.6,
        outpch='.', cex=2)
```

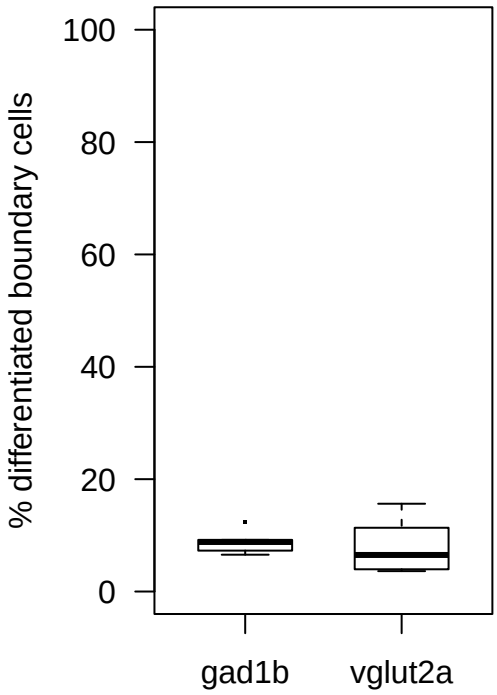
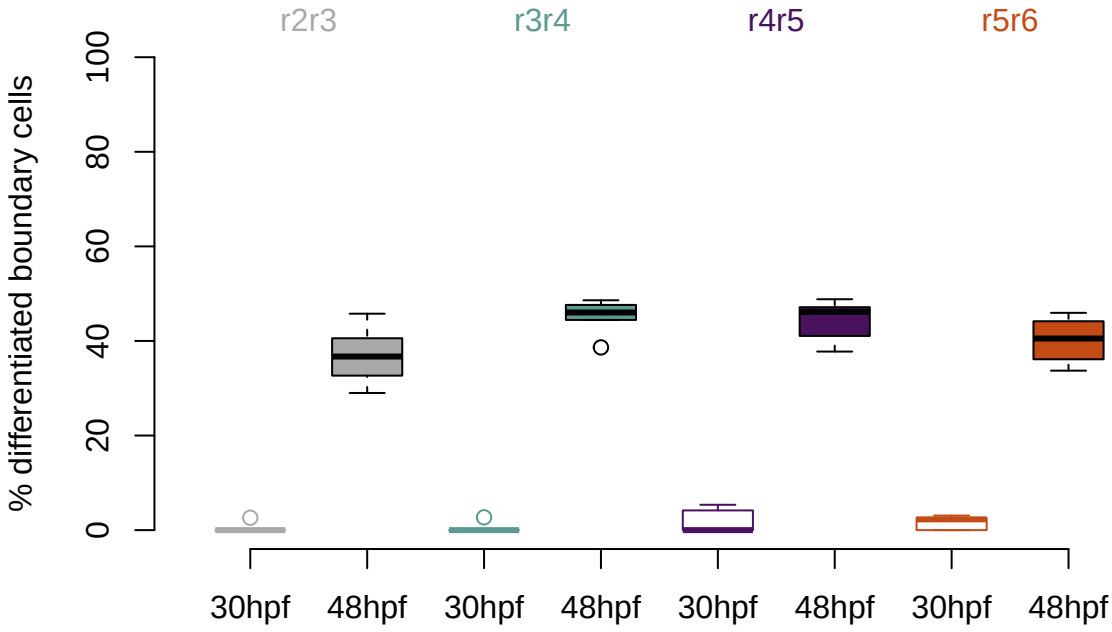


Figure Sup3 A-B

```
diffCounts$percHuC <- 100*diffCounts$Huc/(diffCounts$Huc+diffCounts$noHuC)

b1<- diffCounts %>% filter(time=="30hpf", bound=="r2r3")
b2 <- diffCounts %>% filter(time=="48hpf", bound=="r2r3")
b3<- diffCounts %>% filter(time=="30hpf", bound=="r3r4")
b4 <- diffCounts %>% filter(time=="48hpf", bound=="r3r4")
b5<- diffCounts %>% filter(time=="30hpf", bound=="r4r5")
b6 <- diffCounts %>% filter(time=="48hpf", bound=="r4r5")
b7<- diffCounts %>% filter(time=="30hpf", bound=="r5r6")
b8 <- diffCounts %>% filter(time=="48hpf", bound=="r5r6")

opar<-par(no.readonly = TRUE)
par(bty="n")

boundaryColors = c("darkgray", "#5D9B90", "#48125E", "#c44b16")
altcols <- paste(rbind(boundaryColors, rep("black",4)))

boxplot(b1$percHuC, b2$percHuC, b3$percHuC, b4$percHuC,
        b5$percHuC, b6$percHuC, b7$percHuC, b8$percHuC,
        ylab = "% differentiated boundary cells",
        names =  rep(c("30hpf", "48hpf"),4),
        col = paste(rbind(rep("white",4), boundaryColors)),
        boxcol= altcols, medcol = altcols, staplecol = altcols,
        whiskcol = altcols, outcol = altcols,
        ylim=c(0,100),
        boxwex=0.6
        )
mtext(text=c("r2r3","r3r4", "r4r5", "r5r6"), side=3,
      at=seq(1.5, 7.5, by=2), col=boundaryColors)
```




```
paste(rbind(rep("white",4), boundaryColors))
```

```
## [1] "white"    "darkgray" "white"    "#5D9B90"  "white"    "#48125E"  "white"
## [8] "#c44b16"
```

```
par(opar)

descr2groups(b1$percHuC,b2$percHuC, tit = "r2r3", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r2r3"
##       N     M   SD  SEM
## 30hpf 5  0.52 1.17 0.52
## 48hpf 5 36.94 6.56 2.94
## [1] " t.test p-value: 0.00017966409092005  *** "
```

```
descr2groups(b3$percHuC,b4$percHuC, tit = "r3r4", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r3r4"
##       N     M   SD  SEM
## 30hpf 5  0.54 1.21 0.54
## 48hpf 5 45.06 3.93 1.76
## [1] " t.test p-value: 3.66182536599032e-06  *** "
```

```
descr2groups(b5$percHuC,b6$percHuC, tit = "r4r5", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r4r5"
##       N     M   SD  SEM
## 30hpf 5  1.90 2.64 1.18
## 48hpf 5 44.19 4.62 2.06
## [1] " t.test p-value: 1.1495142048472e-06  *** "
```

```
descr2groups(b7$percHuC,b8$percHuC, tit = "r5r6", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r5r6"
##       N     M   SD  SEM
## 30hpf 5  1.58 1.47 0.66
## 48hpf 5 40.10 5.18 2.32
## [1] " t.test p-value: 3.08634422148325e-05  *** "
```

```
plot(0,0, type="n", xlab="", ylab="",
     xlim=c(0.5,2.5), ylim=c(0,100),
     xaxt="n", yaxt="n")
points(x=rep(1,4), y=percHuC_30, pch=21,cex=2, bg=boundaryColors )
points(x=rep(2,4), y=percHuC_48, pch=21,cex=2, bg=boundaryColors)

axis(1, at=1:2, labels=c("30 hpf", "48 hpf"), cex.axis=1.6)
axis(2, at=seq(0,100,by=20), labels = seq(0,100,by=20), las=2, cex.axis=1.6)

legend(x="topleft",legend = levels(percBoundTime$bound),
       text.col=boundaryColors, cex=1.2, bty="n")
```

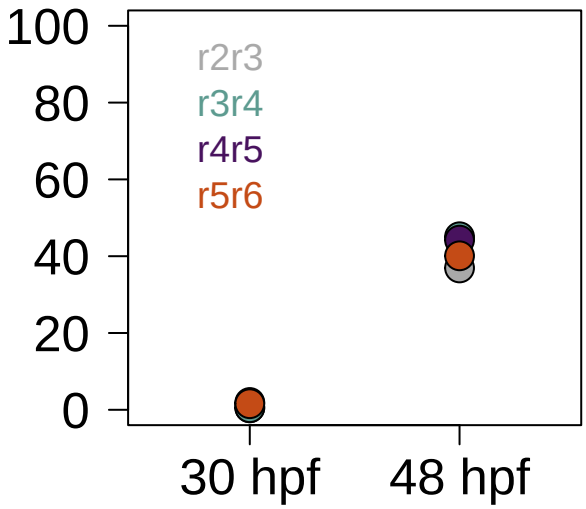
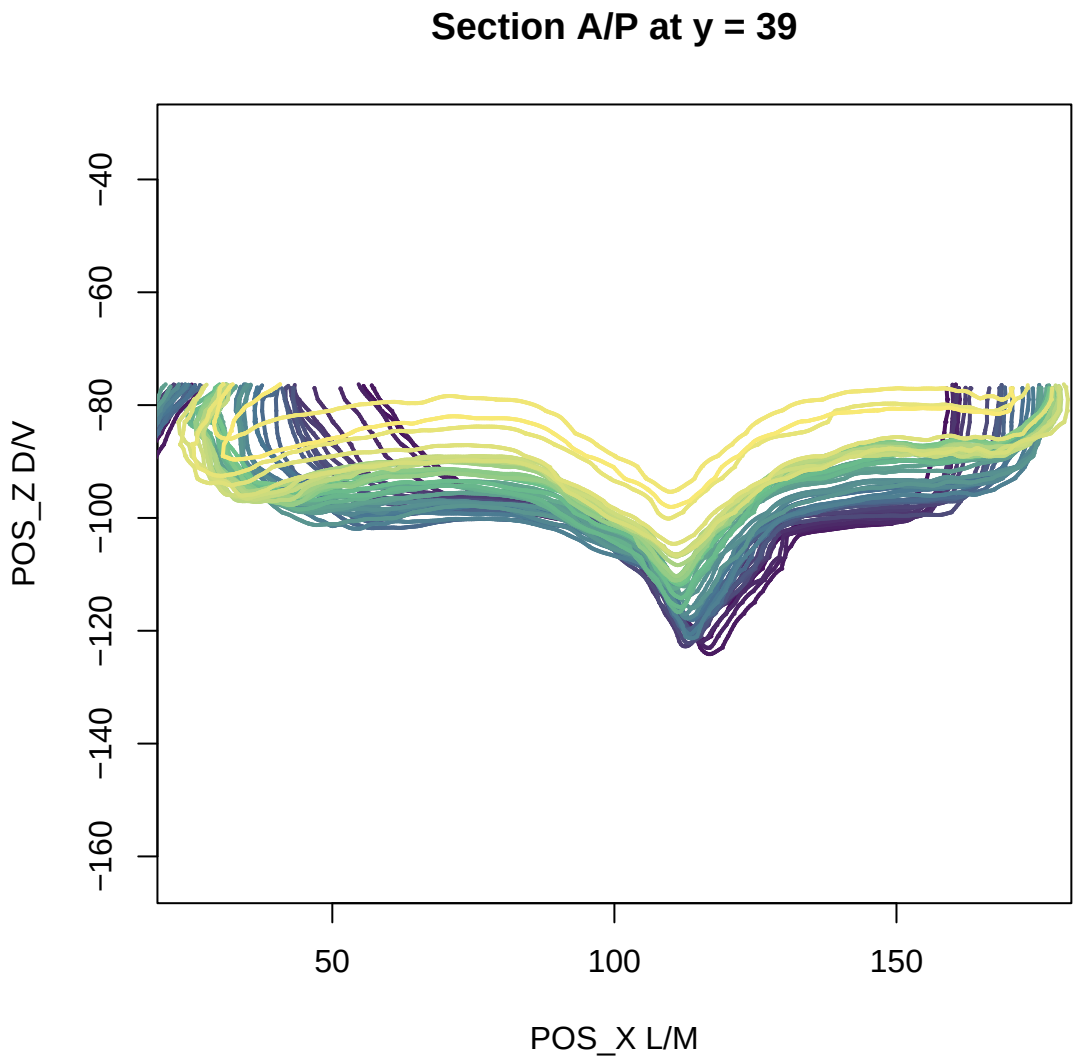

100
80
60
40
20
0
r2r3
r3r4
r4r5
r5r6
30 hpf
48 hpf

Figure Sup3 C-D

```
# load("../VentrSurf21/allButMeshes.RData")
# load("data/ventrSurf.RData")
# source("../VentrSurf21/processVS.R")

#YLIMS <- c(-120,-20)
YLIMS <- c(-120, -75)

plotSectionY <- function(plotOnly=NULL, singleColor=NULL, grayBg=NULL){
  # If plotOnly is a frame name, highlight it with singleColor
  # If grayBg can be NULL. If "gray" or "gradient" all VSs are drawn
  # otherwise, for plotOnly FALSE, paletaCova is used
  # plotArr <- !is.null(addArrows)
  # ylm=YLIMS
  # if (plotArr||plotDivPoints){
  #   ylm[1]<--180
  # }
  ns <- length(secs39)
  #cols=terrain.colors(ns)
  if (is.null(grayBg))
    cols <- paletaCova(ns)
  else if (grayBg=="gray")
    cols <- rep(gray(0.8), ns)
  else
    cols=rev(gray.colors(ns))

  plot(0,0, asp=1,
       xlim = c(25,175), ylim = YLIMS,
       xlab="POS_X L/M", ylab="POS_Z D/V",
       main=paste("Section A/P at y =", 39),
       type="n")
  # legend(x="topleft",
  #        legend=names(secs39)[seq(1,ns,by=5)],
  #        text.col= cols[seq(1,ns,by=5)])
  if (is.null(plotOnly)||(!is.null(grayBg)))
    for (nn in 1:length(secs39)){ # sec is 4 x nsegs, each col is x0,z0,x1,z1
      sec = secs39[[nn]]
      #lines(x=t(sec[c(1,3),]), y=t(sec[c(2,4),]), col=cols[nn])
      ll <- apply(sec, 2,
                  FUN=function(c4)
                    lines(x=c4[c(1,3)], y=c4[c(2,4)],
                          lwd=2,
                          xlim = c(0,175), ylim = c(-120,-20),
                          col=cols[nn]))
    }
  if (!is.null(plotOnly)){
    sec <- secs39[[plotOnly]]
    ll <- apply(sec, 2,
                FUN=function(c4)
                  lines(x=c4[c(1,3)], y=c4[c(2,4)],
                        lwd=4,
                        col=singleColor))
  }
```

```
}



# We take 4 frames as representatives
someSecs39 = c("F025", "F085", "F145", "F218")
someCols = paletaCova(5)[1:4]
```

```
plotSectionY()
```




```
plotSectionY(plotOnly = someSecs39[1], singleColor = someCols[1], grayBg = "gray")
plotSectionY(plotOnly = someSecs39[2], singleColor = someCols[2], grayBg = "gray")
plotSectionY(plotOnly = someSecs39[3], singleColor = someCols[3], grayBg = "gray")
plotSectionY(plotOnly = someSecs39[4], singleColor = someCols[4], grayBg = "gray")
```

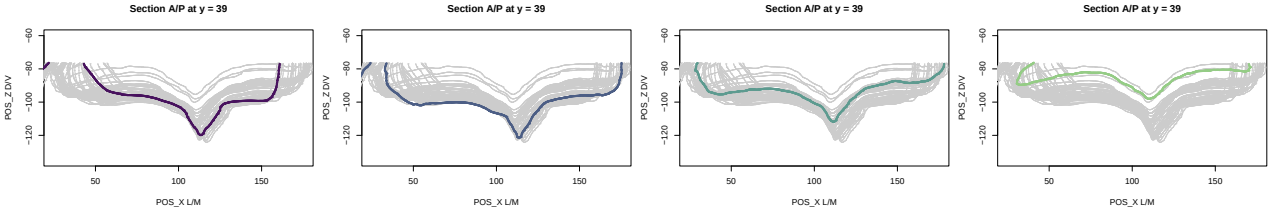
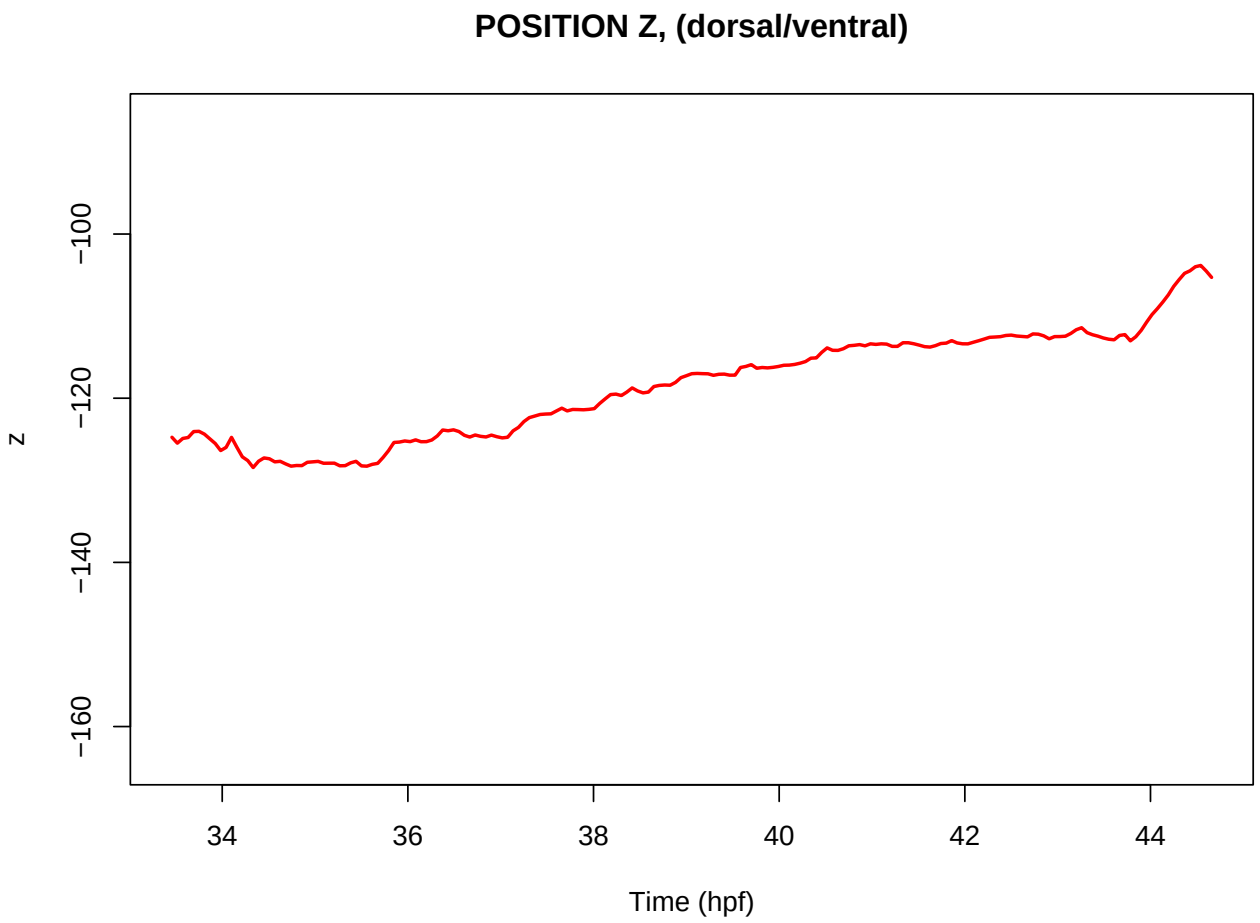

Section A/P at y = 39
POS_Z DV
−60
−80
−100
−120
50
100
150
POS_X L/M
Section A/P at y = 39
POS_Z DV
−60
−80
−100
−120
50
100
150
POS_X L/M
Section A/P at y = 39
POS_Z DV
−60
−80
−100
−120
50
100
150
POS_X L/M
Section A/P at y = 39
POS_Z DV
−60
−80
−100
−120
50
100
150
POS_X L/M

Figure Sup3 E-F

```
plotPosTrack =
  function(data=cellPaths, what="x", main=what,
           ave=FALSE, groupByType = "", remark = NULL, noTracks = FALSE){
    # groupByType may be "", or a list of cellTypes
    # remark may be a list of cells to draw its path thicker and with color
    framesT = frame2hpf( as.numeric(dimnames(data)$frame))
    meanNARM <- function(xx) mean(xx,na.rm = TRUE)
    plot(0, 0,
         xlim = range(framesT),
         ylim= range(data[,,what], na.rm = TRUE),
         main = main,
         sub = "",
         xlab = "Time (hpf)", ylab = what,
         type="n")
    if (any(groupByType==""){
      if (!noTracks)
        apply(data[,,what], 2, # has 194 x 221
              function(yvals){
                lines(x=framesT[!is.na(yvals)], y=yvals[!is.na(yvals)],
                      col=gray(0.6))
              })
      if (ave){
        if (what=="x"){ # average left and right
          pth <- apply(data[,,what], 2,meanNARM)
          #print(pth)
          aveR <- apply(data[,(pth>112),what], 1, meanNARM)
          aveL <- apply(data[,(pth<112),what], 1, meanNARM)
          lines(x=framesT[!is.na(aveR)], y=aveR[!is.na(aveR)], col="red", lwd=2)
          lines(x=framesT[!is.na(aveL)], y=aveL[!is.na(aveL)], col="red", lwd=2)
        } else {
          ave0 = apply(data[,,what], 1, meanNARM)
          lines(x=framesT[!is.na(ave0)], y=ave0[!is.na(ave0)], col="red", lwd=2)
        }
      }
    }
    else{ # deal with groupByType
      cols = rainbow(length(groupByType))
      legend(x="topleft",
             legend=groupByType,
             text.col=cols)
      for (cln in names(cells)){
        cTy = cellsTable[cln,"trackType"]
        if (cTy %in% groupByType){
          yvals=data[,cln,what]
          if (!noTracks)
            lines(x=framesT[!is.na(yvals)], y=yvals[!is.na(yvals)],
                  col=ifelse(ave, gray(0.6), cols[match(cTy, groupByType)]))
        }
      }
      if (ave){
        for (cTy in groupByType){
          wh = cellsTable[,"trackType"]==cTy
```

```
          dd=data[,wh,what]
          mdd = apply(dd, 1, meanNARM)
          lines(x=framesT[!is.na(mdd)], y=mdd[!is.na(mdd)],
              col=cols[match(cTy, groupByType)], lwd=2)
        }
      }
    }
    if (!is.null(remark)){
      for (cl in remark){
        dd <- data[,cl,what]
        lines(x=framesT[!is.na(dd)], y=dd[!is.na(dd)],
              col=cellColor[[cellsTable[cl, "trackType"]]], lwd=3)
      }
    }
  }

shift_array3 <-
  function(arr, shift=1){ # move all shift down based in first dim
    rarr = arr
    nro = dim(arr)[1]
    if (shift>0)
      rarr[1:(nro-shift), , ] = arr[(1+shift):nro, , ]
    else # shift<0
      rarr[(1-shift):nro, , ] = arr[1:(nro+shift), , ]
    return(rarr)
  }

mav_paths <-
  # do a moving average over path coordinates
  function(data=cellPaths, mavSize=5){
    if (!(floor(mavSize/2)+0.5==mavSize/2))
      stop(paste("mavSize", mavSize, "should be odd number"))
    halfSize = floor(mavSize/2)
    res = data[1+halfSize:(dim(data)[1]-halfSize), , ]
    for (shi in 1:halfSize) {
      sa = shift_array3(data,shift = shi)
      res = res + sa[1+halfSize:(dim(data)[1]-halfSize), , ]
      sa = shift_array3(data,shift = -shi)
      res = res + sa[1+halfSize:(dim(data)[1]-halfSize), , ]
    }
    return(res/mavSize)
  }
```

```
mavCellPaths = mav_paths(cellPaths, mavSize = 3)

plotPosTrack(data=mavCellPaths[,progCells,],
             what="z", main="POSITION Z, (dorsal/ventral)", ave = TRUE, noTracks = TRUE)
```




```
plotPosTrack(data=mavCellPaths[,progCells,], what="x",
             main="POSITION X, (lat/med/lat)", ave = TRUE)
```

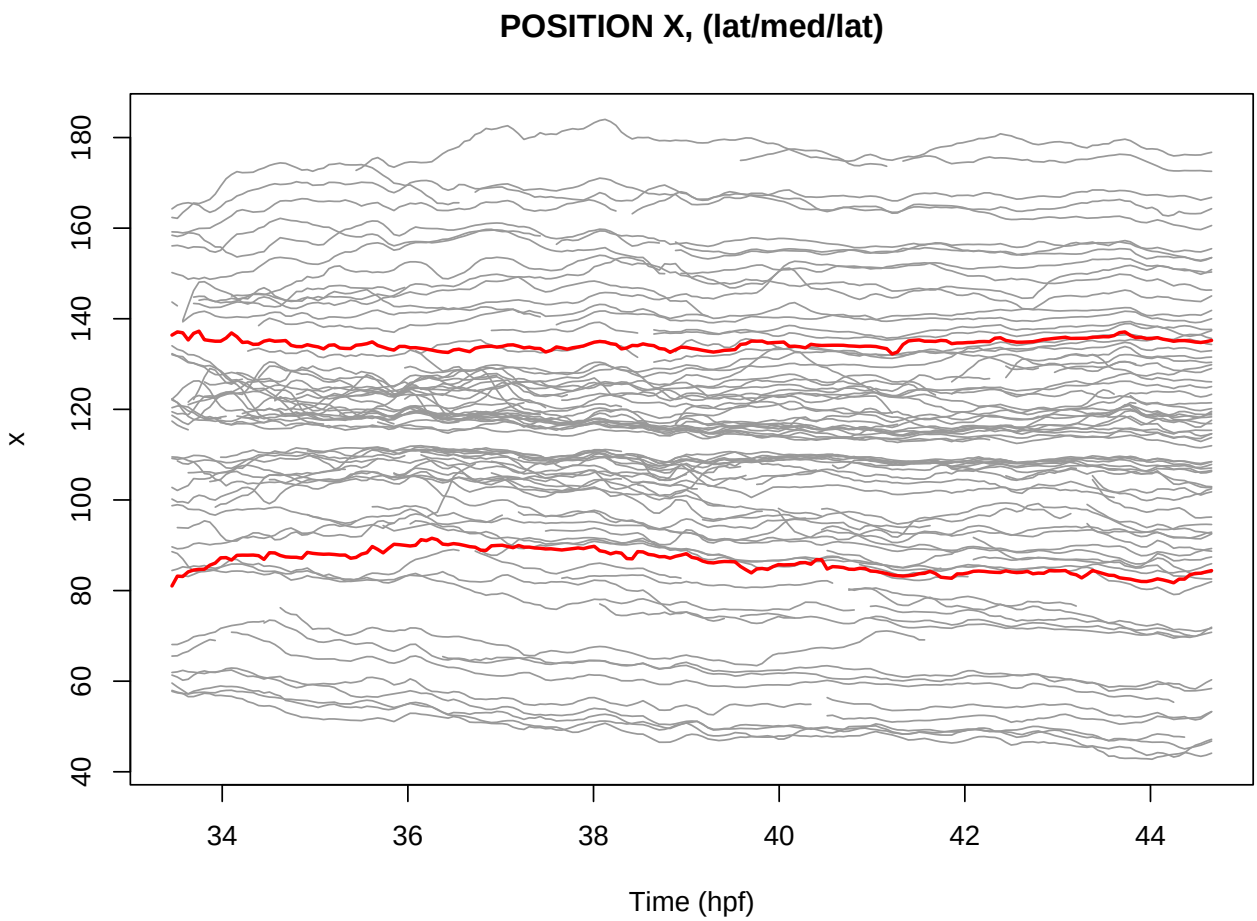

POSITION X, (lat/med/lat)
x
40
60
80
100
120
140
160
180
34
36
38
40
42
44
Time (hpf)

Figure Sup 3 G

```
numdivs <- data.frame(
  hpf = c(30L,31L,32L,33L,34L,35L,36L,
          37L,38L,39L,40L,41L,42L,43L,44L,45L,46L,47L,48L,
          49L,50L,51L,52L,53L,54L,55L,56L,57L,58L,59L,60L),
  PN = c(0L,0L,4L,0L,1L,2L,1L,1L,1L,
         0L,0L,0L,0L,0L,0L,0L,0L,0L,0L,0L,0L,0L,0L,0L,0L,
         0L,0L,0L,0L,0L,0L,0L),
  PP = c(0L,0L,1L,2L,2L,1L,0L,5L,5L,
         0L,0L,1L,0L,1L,0L,0L,0L,0L,0L,0L,0L,0L,0L,1L,0L,
         0L,0L,0L,0L,0L,0L,0L),
  Ind = c(0L,0L,3L,9L,15L,16L,18L,16L,
          16L,8L,3L,2L,6L,5L,2L,2L,0L,0L,0L,0L,3L,0L,
          1L,0L,0L,1L,0L,0L,0L,1L,0L),
  nB = c(5L,5L,5L,5L,5L,5L,5L,5L,5L,
         5L,5L,5L,5L,5L,5L,5L,2L,2L,2L,2L,2L,2L,2L,2L,
         2L,2L,2L,2L,2L,2L,2L)
)
#add totals
numdivs$tot <- apply(numdivs[,2:4], 1, sum)

numdivs
```

Data on number of cell divisions by type and hour pf.

```
##    hpf PN PP Ind nB tot
## 1   30  0  0   0  5   0
## 2   31  0  0   0  5   0
## 3   32  4  1   3  5   8
## 4   33  0  2   9  5  11
## 5   34  1  2  15  5  18
## 6   35  2  1  16  5  19
## 7   36  1  0  18  5  19
## 8   37  1  5  16  5  22
## 9   38  1  5  16  5  22
## 10  39  0  0   8  5   8
## 11  40  0  0   3  5   3
## 12  41  0  1   2  5   3
## 13  42  0  0   6  5   6
## 14  43  0  1   5  5   6
## 15  44  0  0   2  5   2
## 16  45  0  0   2  5   2
## 17  46  0  0   0  2   0
## 18  47  0  0   0  2   0
## 19  48  0  0   0  2   0
## 20  49  0  0   0  2   0
## 21  50  0  0   3  2   3
## 22  51  0  0   0  2   0
## 23  52  0  1   1  2   2
## 24  53  0  0   0  2   0
## 25  54  0  0   0  2   0
## 26  55  0  0   1  2   1
## 27  56  0  0   0  2   0
```

```
## 28  57  0  0   0  2   0
## 29  58  0  0   0  2   0
## 30  59  0  0   1  2   1
## 31  60  0  0   0  2   0
```

```
# library(zoo)
mav5numdivs <- zoo::rollmean(c(0,0,numdivs$tot,0,0),5)

barplot( mav5numdivs,
        main = "", names.arg = 30:60)
```

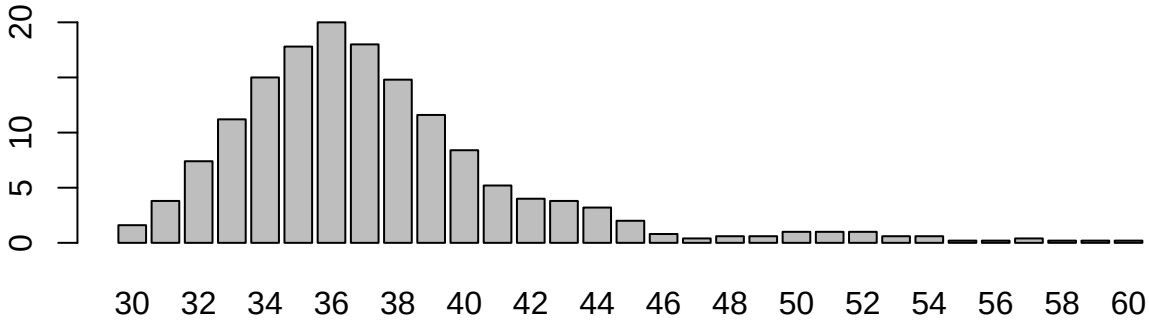
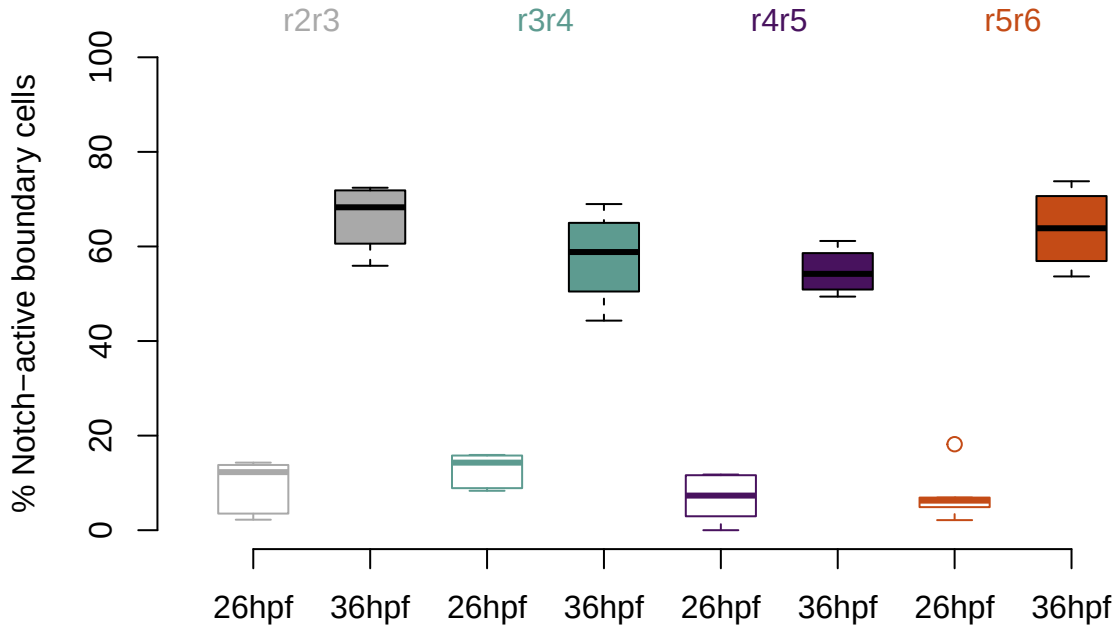


Figure Sup 4 A-B

notch active vs bound and 26-36 hpf

```
pw_percNotch <-pivot_wider(percNotch, names_from = c(time, bound), values_from = percNotch)

opar<-par(no.readonly = TRUE)
par(bty="n")

boxplot(pw_percNotch[,c(2,6,3,7,4,8,5,9)],
        ylab = "% Notch-active boundary cells",
        names =  rep(c("26hpf", "36hpf"),4),
        boxlty=rep(1,8),
        col = c("white", boundaryColors[1], "white", boundaryColors[2],
                "white", boundaryColors[3], "white", boundaryColors[4] ),
        boxcol= altcols, medcol = altcols, staplecol = altcols,
        whiskcol = altcols, outcol = altcols,
        ylim=c(0,100),
        boxwex=0.6
        )

mtext(text=c("r2r3","r3r4", "r4r5", "r5r6"), side=3,
      at=seq(1.5, 7.5, by=2), col=boundaryColors)
```




```
par(opar)

descr2cols(pw_percNotch[,c(2,6)], tit = "r2r3", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r2r3"
##       N     M   SD  SEM
## 30hpf 5  9.22 5.86 2.62
## 48hpf 4 66.22 7.56 3.78
## [1] " t.test p-value: 2.74404565196448e-05  *** "
```

```
descr2cols(pw_percNotch[,c(3,7)], tit = "r3r4", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r3r4"
##       N     M    SD  SEM
## 30hpf 5 12.64  3.74 1.67
## 48hpf 4 57.73 10.30 5.15
## [1] " t.test p-value: 0.00170158398092096  ** "
```

```
descr2cols(pw_percNotch[,c(4,8)], tit = "r4r5", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r4r5"
##       N     M   SD  SEM
## 30hpf 5  6.73 5.23 2.34
## 48hpf 4 54.74 5.06 2.53
## [1] " t.test p-value: 3.56889512735125e-06  *** "
```

```
descr2cols(pw_percNotch[,c(5,9)], tit = "r5r6", nams=c("30hpf", "48hpf"))
```

```
## [1] "    r5r6"
##       N     M   SD  SEM
## 30hpf 5  7.67 6.16 2.75
## 48hpf 4 63.79 8.75 4.37
## [1] " t.test p-value: 8.70923321486027e-05  *** "
```

```
plot(NULL,NULL, type="n",
     xlim=c(0.5,2.5), ylim=c(0,100),
     xaxt="n", yaxt="n",
     main= "",
     xlab="", ylab="") #, ylab="% of Notch-active cells by boundary")
points(x=ifelse(percBoundTimeNotch$time=="26hpf",1,2),
       y=percBoundTimeNotch$percNotch,
       pch=21,cex=1.6,bg=rep(boundaryColors,each=2))

legend(x="topleft",legend = levels(percBoundTime$bound),
       text.col=boundaryColors, cex=1.2, bty="n")
axis(1,at=1:2,labels = c("26hpf","30hpf"), cex.axis=1.6)

axis(2, at=seq(0,100,by=20), labels = seq(0,100,by=20), las=2, cex.axis=1.6)
```

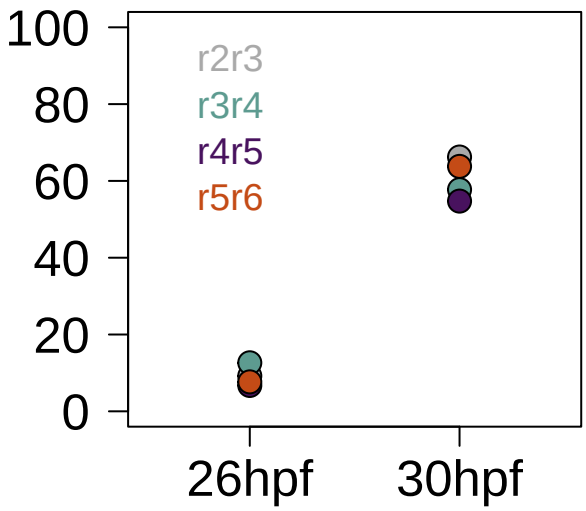
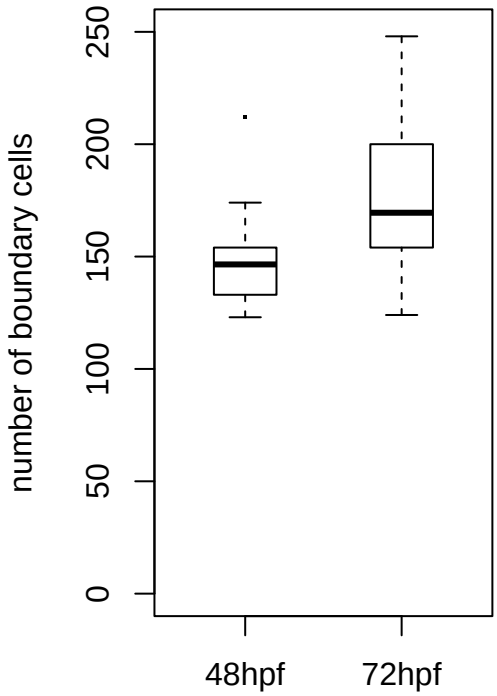


Figure Sup 6A

```
tot48 <- diff4872$nCells[diff4872$time=="48hpf"]
tot72 <- diff4872$nCells[diff4872$time=="72hpf"]

boxplot(tot48, tot72,
        ylim = c(0,maxRight),
        xlab="", ylab = "number of boundary cells",
        names = c("48hpf", "72hpf"),
        col = rep("white",2),
        boxwex=0.4, outpch='.', cex=2)
```




```
# descripcion total number of cells
descr2groups(tot48, tot72, nams = c("48hpf", "72hpf"), not.paired=TRUE)
```

```
##        N     M    SD   SEM
## 48hpf 10 150.8 26.11  8.26
## 72hpf 10 178.5 42.73 13.51
## [1] " t.test p-value: 0.100837106653853 ns"
```