BS_CK_Consolidated

May 23, 2025

[24]:
```
from PIL import Image
import numpy as np
import matplotlib.pyplot as plt
import networkx as nx
import os
from scipy.optimize import curve_fit
import matplotlib as mpl
from PIL import ImageColor
from scipy.spatial.distance import pdist, squareform
from scipy.stats import ks_2samp, gaussian_kde
from scipy import interpolate

import pandas as pd
import matplotlib.patches as mpatches


label_size = 18
mpl.rcParams['xtick.labelsize'] = label_size
mpl.rcParams['ytick.labelsize'] = label_size
markers = ['o','>','s','d','+','*','<']
```

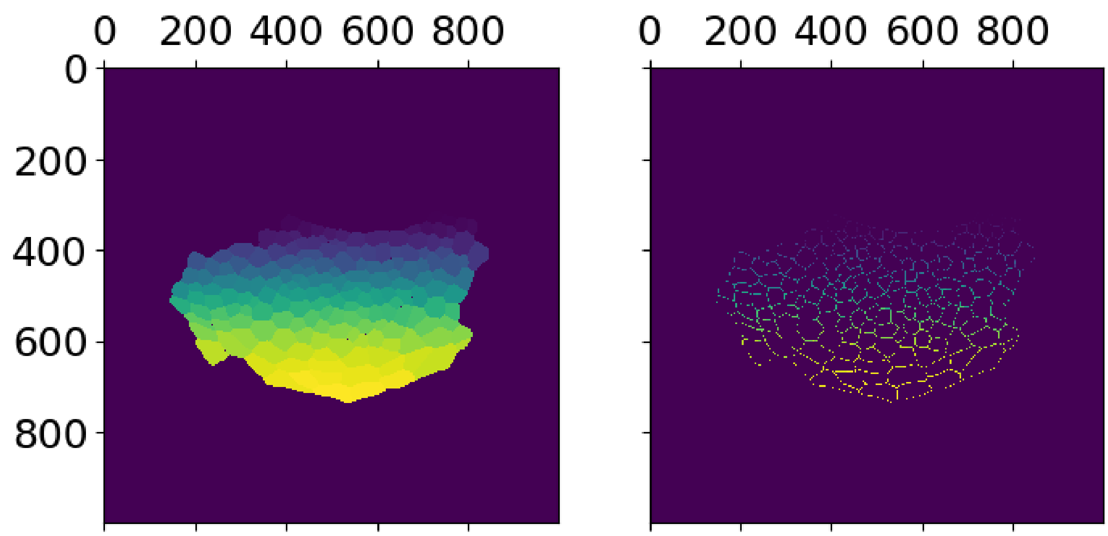
[25]:
```
#CDF : Cumulative Distribution Function
def CDF(X):
    X = np.sort(X)
    Y = np.arange(1,len(X)+1)/len(X)
    return(X,Y)
```

[26]:
```
def load_cellpose_masks(npyfilepath,display=False):
    seg = np.load(npyfilepath,allow_pickle=True)
    M = seg.all()['masks']
    O = seg.all()['outlines']
    if display == True :
        fig,ax = plt.subplots(ncols=2,figsize=(8,4),sharey=True)
        ax[0].matshow(M,cmap='viridis')
        ax[1].matshow(O,cmap='viridis')
        return(M,O)
    else:
```

```
        return(M,O)
```

Demonstration of the function `load_cellpose_masks()`

[27]:
```
M,O = load_cellpose_masks('sample_cellpose_seg.npy',True)
```



Measures of roundness/cell shape

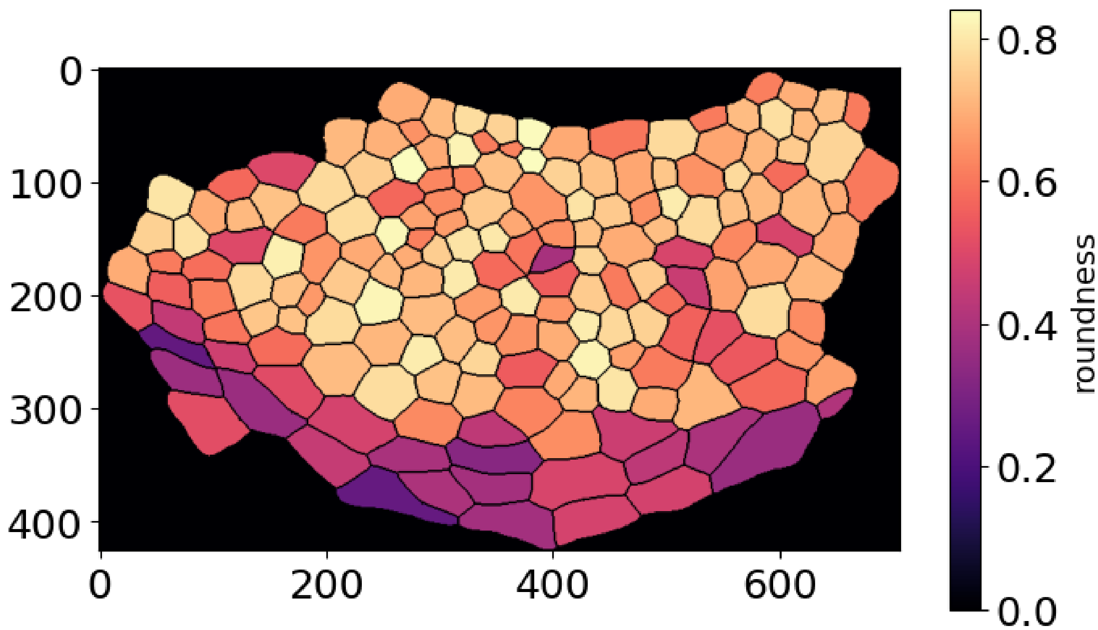
[28]:
```
'''
Computes the ratio of the cell area to the area of the circle whose diameter is equal to the length of the longest
diagonal of the polygon (cell outline). As the cell shape approaches that of a circle the value approaches 1
'''

def compute_roundness(Outlines,Masks):
    Round_Mat = np.zeros(Outlines.shape)
    O_ = np.ones(Outlines.shape)
    O_[O > 0] = 0
    n_cells = np.max(Masks)+1
    Roundness_Values = np.zeros(n_cells)
    for i in range(1,n_cells):
        BI = (Masks == i)
        Z = BI*(Outlines==i)
        y,x = np.nonzero(Z)
        pos = np.array([x,y]).T
        diameter = np.max(pdist(pos,metric='euclidean'))
        Circle_area = np.pi * diameter * diameter * 0.25
        Cell_area = np.sum(BI)
```

```
        roundness = Cell_area/Circle_area
        Roundness_Values[i] = roundness
        Round_Mat[BI] = roundness
        Round_Mat *= O_
    return(Round_Mat,Roundness_Values)

'''
Computes the ratio of square root of the area of a cell to the perimeter of the␣
 ↪cell
- area is the total number of pixels belonging to a cell
- perimeter is the number of pixels that are at the boundary of the cell
'''
def compute_peri_by_area(Outlines,Masks):
    PeriArea = np.zeros(Outlines.shape)
    O_ = np.ones(Outlines.shape)
    O_[O > 0] = 0
    n_cells = np.max(Masks)+1
    peri_area_values = np.zeros(n_cells)

    for i in range(1,n_cells):
        BI = (Masks == i)
        Area = np.sum(BI)
        Peri = np.sum(Outlines == i)
        PeriArea[BI] = np.sqrt(Area)/Peri
        peri_area_values[i] = np.sqrt(Area)/Peri
        PeriArea *= O_
    return(PeriArea,peri_area_values)


def display_tissue_with_roundness_values(npyfilepath,measure_type=1):
    M,O = load_cellpose_masks('sample_cellpose_seg.npy',False)
    if measure_type == 1:
        RM,RV = compute_roundness(O,M)
    elif measure_type == 2:
        RM,RV = compute_peri_by_area(O,M)
    else:
        print('measure type undefined!')
        return None

    #--- compute bounds of the image-----#
    x_ = np.sum(M[1:-1,1:-1],axis=0)
    y_ = np.sum(M[1:-1,1:-1],axis=1)


    x_min = np.min(np.nonzero(x_ > 0)[0])-2
    x_max = np.max(np.nonzero(x_ > 0)[0])+2
    y_min = np.min(np.nonzero(y_ > 0)[0])-2
```

```
    y_max = np.max(np.nonzero(y_ > 0)[0])+2
    #--------------------------------------#
    w = 8
    h = ((y_max-y_min)/(x_max-x_min))* w

    fig,ax = plt.subplots(figsize=(w,h))
    im = ax.imshow(RM[y_min:y_max,x_min:x_max],cmap='magma')
    cb = fig.colorbar(im)
    cb.set_label('roundness',fontsize=14)
    plt.show()
```

Demonstration of the function `display_tissue_with_roundness_values()` which calls `compute_roundness()` or `compute_peri_by_area()` internally

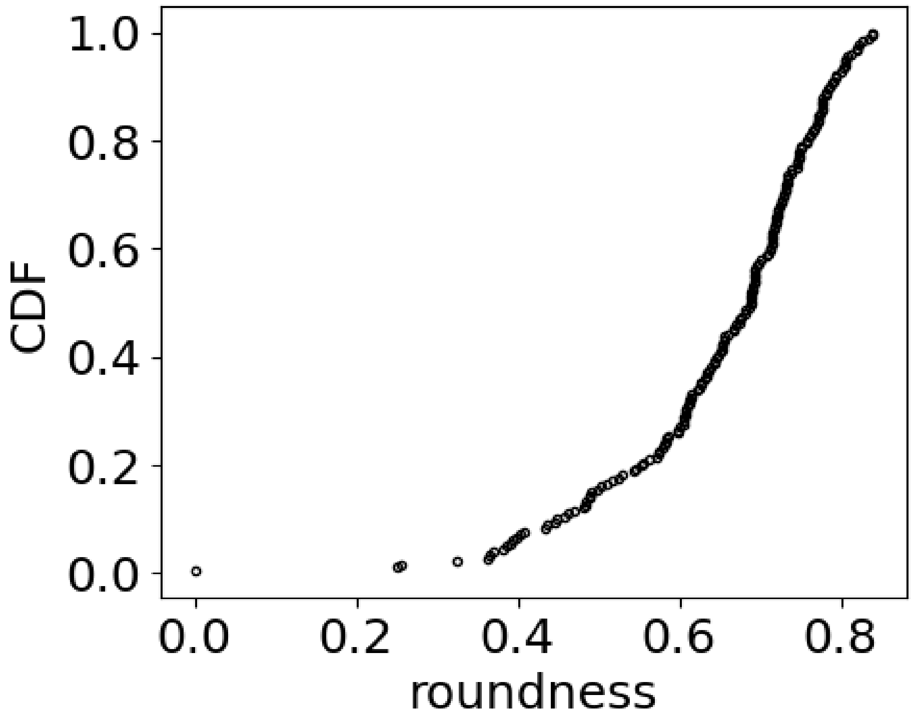
[29]:
```
display_tissue_with_roundness_values('sample_cellpose_seg.npy',1)
```



Plotting the cumulative distribution of the roundness values using `CDF()` function

[30]:
```
M,O = load_cellpose_masks('sample_cellpose_seg.npy',False)
RM,RV = compute_roundness(O,M)

X,Y = CDF(RV)
fig,ax = plt.subplots(figsize=(5,4))
ax.plot(X,Y,'.',color='k',markerfacecolor='none')
ax.set_xlabel('roundness',fontsize=18)
```

```
ax.set_ylabel('CDF',fontsize=18)
plt.show()
```



0.1 Making figures

Plotting the division angles at four time blocks 32hpf, 38hpf, 44hpf and 50hpf

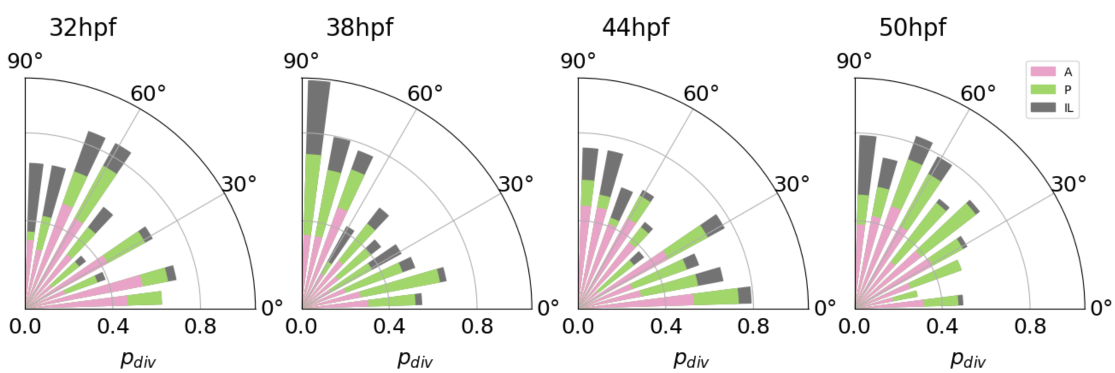
[31]:
```
def plot_empirical_division_angles():
    S = open('20250304_OCD_PLOT.csv','r').read()
    S = S.replace('IL-P','IL').replace('IL-A','IL')
    f = open('tmp.csv','w')
    f.write(S)
    f.close()

    df = pd.read_csv('tmp.csv')

    Stages = [32,38,44,50]

    Groups = ['A','P','IL']

    BSCols = ['#737373','#a1d76a','#e9a3c9']

    patch_3 = mpatches.Patch(color=BSCols[0], label='IL')
```

```
    patch_2 = mpatches.Patch(color=BSCols[1], label='P')
    patch_1 = mpatches.Patch(color=BSCols[2], label='A')

    BINS = np.linspace(0,np.pi/2,11)

    fig, ax = plt.
↪subplots(ncols=4,subplot_kw=dict(projection='polar'),figsize=(16,4))

    Data = []

    for j in range(4):
        stage = Stages[j]
        df_ = df[df.STAGE == stage]
        All = np.array(df_.DEG_IL_CORR) * (np.pi/180)

        A = np.array(df_[df_.REGION == 'A'].DEG_IL_CORR) * (np.pi/180)
        B = np.array(df_[df_.REGION == 'P'].DEG_IL_CORR) * (np.pi/180)
        C = np.array(df_[df_.REGION == 'IL'].DEG_IL_CORR) * (np.pi/180)


        All = np.pi/2 - np.abs((np.pi/2 - All))

        A = np.pi/2 - np.abs((np.pi/2 - A))
        B = np.pi/2 - np.abs((np.pi/2 - B))
        C = np.pi/2 - np.abs((np.pi/2 - C))

        Y0,X = np.histogram(All,bins=BINS,density=True)


        X = 0.5 *(X[:-1] + X[1:])
        Y1,_ = np.histogram(A,bins=BINS,density=False)
        Y2,_ = np.histogram(B,bins=BINS,density=False)
        Y3,_ = np.histogram(C,bins=BINS,density=False)




        Y2 += Y1
        Y3 += Y2

        denom = Y3[0]/Y0[0]

        Y1 = Y1/denom
        Y2 = Y2/denom
        Y3 = Y3/denom

        Data.append([Y3,Y2,Y1])
```

```
    w = 0.9*(X[1] - X[0])

    Labs = ['32hpf','38hpf','44hpf','50hpf']


    for i in range(4):
        bars_3 = ax[i].bar(X,Data[i][0],width = 0.
↪7*w,color=BSCols[0],edgecolor='None') ;
        bars_2 = ax[i].bar(X,Data[i][1],width = 0.
↪7*w,color=BSCols[1],edgecolor='None') ;
        bars_1 = ax[i].bar(X,Data[i][2],width = 0.
↪7*w,color=BSCols[2],edgecolor='None') ;

        ax[i].set_thetamin(0)
        ax[i].set_thetamax(90)
        ax[i].set_xticks(np.array([0,30,60,90])*(np.pi/180))
        ax[i].set_rticks([0,0.4,0.8])
        ax[i].set_rlim(0,1.05)
        ax[i].text(85*(np.pi/180),1.25,Labs[i],fontsize=20)
        ax[i].text(330*(np.pi/180),0.5,r'$p_{div}$',fontsize=18)

    plt.legend(handles=[patch_1,patch_2,patch_3],loc='best', bbox_to_anchor=(1,␣
↪1.1),ncol=1)

    plt.show()
    return(None)
```

Usage of `plot_empirical_division_angles()`

[32]:
```
plot_empirical_division_angles()
```



Figures realted midline ablation study

[33]:
```python
def compute_selectcells_roundness(Outlines,Masks,CID):
    Round_Mat = np.zeros(Outlines.shape)

    n_cells = len(CID)
    Roundness_Values = np.zeros(n_cells)
    for i in range(n_cells):
        cid = CID[i]
        BI = (Masks == cid)
        Z = BI*(Outlines==cid)
        y,x = np.nonzero(Z)
        pos = np.array([x,y]).T
        diameter = np.max(pdist(pos,metric='euclidean'))
        Circle_area = np.pi * diameter * diameter * 0.25
        Cell_area = np.sum(BI)
        roundness = Cell_area/Circle_area
        Roundness_Values[i] = roundness
    return(Roundness_Values)

def compute_roundness_nearest_cells(filename,sp_cid,howmany=200):
    seg = np.load(filename,allow_pickle=True)
    M = seg.all()['masks']
    O = seg.all()['outlines']
    n_cells = np.max(M) + 1
    Centroids = np.zeros((n_cells,2))
    for i in range(1,n_cells):
        ii,jj = np.nonzero(M == i)
        Centroids[i,:] = np.mean(jj),np.mean(ii)
    spc_x,spc_y = Centroids[sp_cid,:]
    IDX = np.nonzero(Centroids[:,1] >= spc_y)[0]
    Centroids_ = Centroids[IDX,:]
    D = (Centroids_[:,0] - spc_x)**2 + (Centroids_[:,1] - spc_y)**2
    idx_ = np.argsort(D)
    CID = IDX[idx_[:howmany]]
    Roundness_Values = compute_selectcells_roundness(O,M,CID)
    return(Roundness_Values,CID)

def␣
↪compute_roundness_nearest_cells_two_halves(filename,sp_cid,ab_side,howmany=100):
↪
    seg = np.load(filename,allow_pickle=True)
    M = seg.all()['masks']
    O = seg.all()['outlines']
    n_cells = np.max(M) + 1
    Centroids = np.zeros((n_cells,2))
    for i in range(1,n_cells):
        ii,jj = np.nonzero(M == i)
        Centroids[i,:] = np.mean(jj),np.mean(ii)
```

```
    spc_x,spc_y = Centroids[sp_cid,:]
    IDX_left  = np.nonzero((Centroids[:,1] >= spc_y)  * (Centroids[:,0] <= spc_x))[0]
    IDX_right = np.nonzero((Centroids[:,1] >= spc_y)  * (Centroids[:,0] > spc_x))[0]


    Centroids_L = Centroids[IDX_left,:]
    Centroids_R = Centroids[IDX_right,:]


    D_L = (Centroids_L[:,0] - spc_x)**2 + (Centroids_L[:,1] - spc_y)**2
    D_R = (Centroids_R[:,0] - spc_x)**2 + (Centroids_R[:,1] - spc_y)**2

    idx_left  = np.argsort(D_L)
    idx_right = np.argsort(D_R)

    CID_L = IDX_left[idx_left[:howmany]]
    CID_R = IDX_right[idx_right[:howmany]]

    RV_L,RV_R = compute_selectcells_roundness(O,M,CID_L),compute_selectcells_roundness(O,M,CID_R)

    if ab_side == 0:
        return(RV_L,RV_R,CID_L,CID_R)
    elif ab_side == 1:
        return(RV_R,RV_L,CID_R,CID_L)

def st_line(x,m,c):
    y = m*x + c
    return(y)


def fit_kde(X):
    kde = gaussian_kde(X)
    x_values = np.linspace(0, 1, 1000)
    kde_values = kde(x_values)
    return(x_values,kde_values)


def fit_CDF(A):
    X = np.sort(A)
    Y = np.arange(1,len(A)+1)/len(A)
    f = interpolate.interp1d(X, Y, fill_value='extrapolate')
    x_fit = np.linspace(0.2,0.9,10000)
    y_fit = f(x_fit)
    y_fit[y_fit > 1] = 1
```

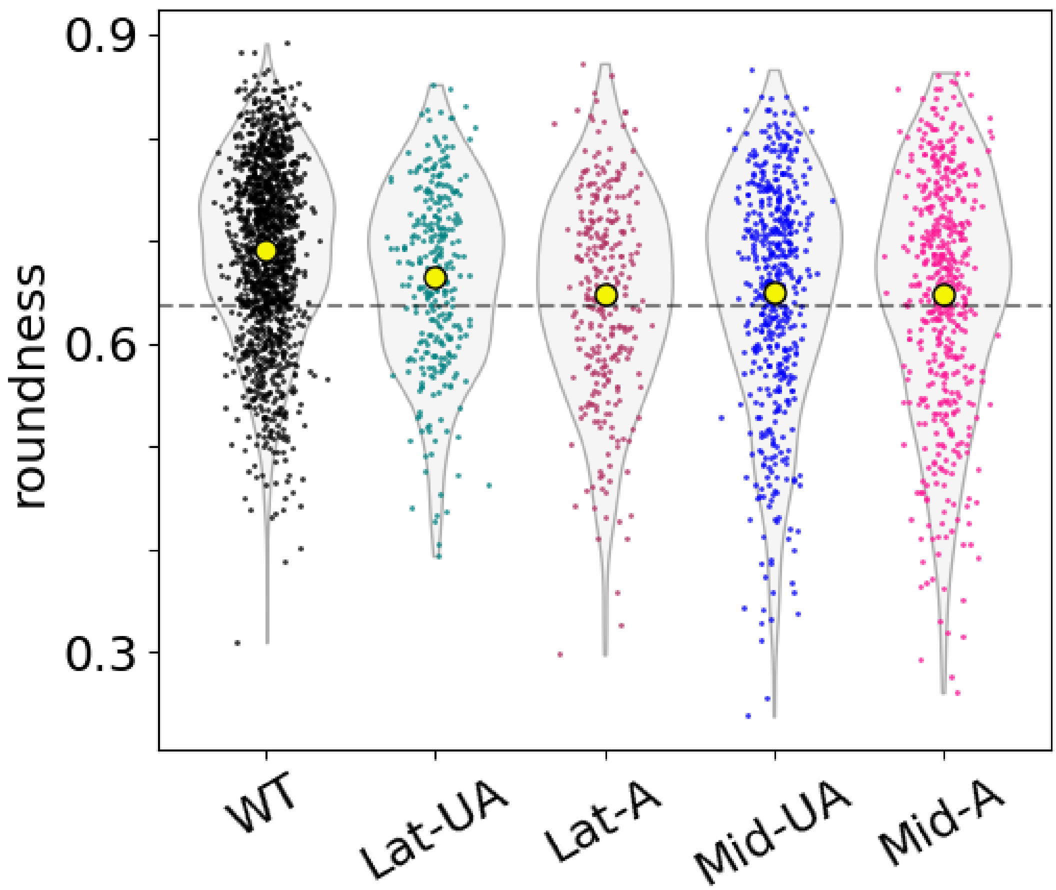
```
    y_fit[y_fit < 0] = 0


    return(x_fit,y_fit)
```

Loading data related to roundness values of different subset of cells computed using functions defined above

[34]:
```
Colors = ['#000000','#008080','#b03060','#0000ff','#ff1493','#ffa500']
COLORS = [ImageColor.getcolor(ele, "RGB") for ele in Colors]

DataDict = dict(np.load('Roundness_Values_Dict.npz',allow_pickle=True))

Keys = ['RV_WT','RV_Ctrl_Ab','RV_Ctrl_Nb','RV_Exp_Ab','RV_Exp_Nb']

Labels = ['WT','Lat-UA','Lat-A','Mid-UA','Mid-A']

Data =␣
↪[DataDict['RV_WT'],DataDict['RV_Ctrl_Nb'],DataDict['RV_Ctrl_Ab'],DataDict['RV_Exp_Nb'],Data
```

Roundness values of the 5 different groups

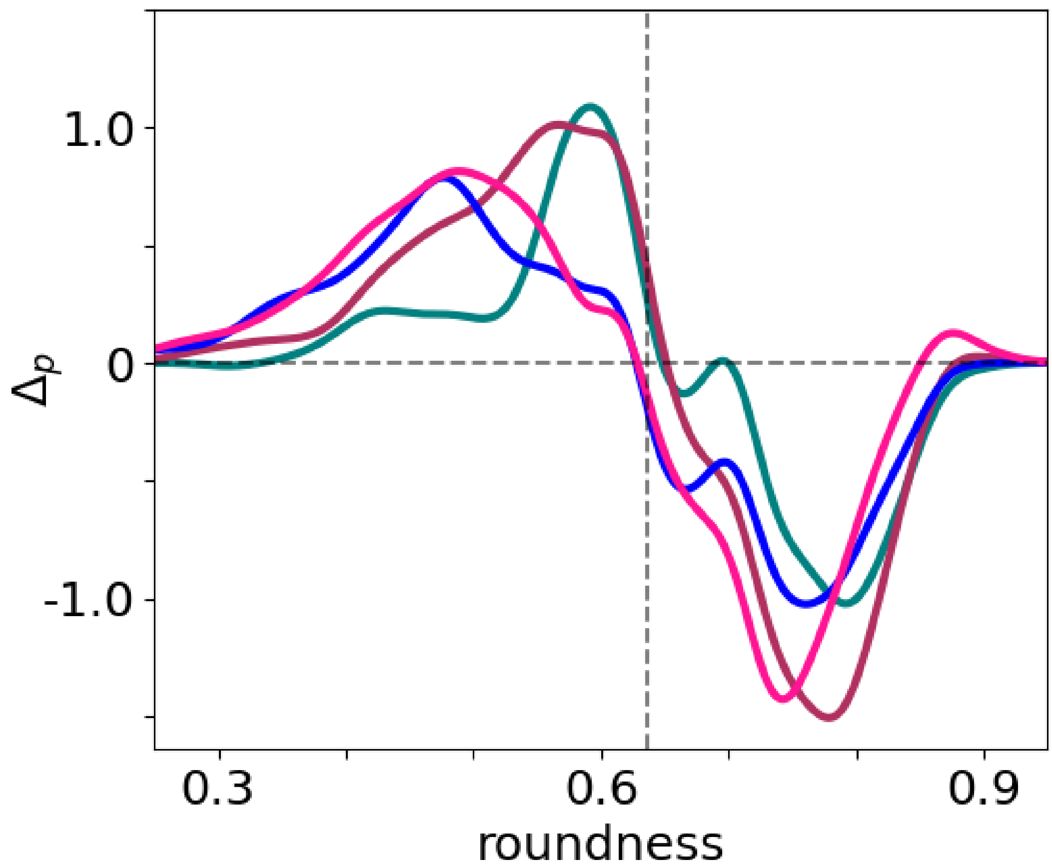
[35]:
```
fig,ax = plt.subplots(figsize=(6,5))
ax.set_yticks([0.3,0.6,0.9])
plots = ax.violinplot(Data,showextrema=False,showmeans=False,widths=0.8)
for pc in plots['bodies'] :
    pc.set_facecolor('#e2dfdf')
    pc.set_edgecolor('k')
for j in range(5):
    y_ = Data[j]
    x_ = np.random.normal(j+1,0.1,len(y_))
    ax.plot(x_,y_,'.',color=Colors[j],markerfacecolor='None',alpha=0.
↪8,markersize=2)
    ax.plot(j+1,np.
↪mean(Data[j]),'o',color='#f4f405',markersize=8,markeredgecolor='k')
ax.axhline(0.6366,linestyle='--',color='k',alpha=0.5)
ax.set_yticks([0.3,0.4,0.5,0.6,0.7,0.8,0.9])
ax.set_yticklabels(['0.3','','','0.6','','','0.9'])
ax.set_xticks([1,2,3,4,5])
ax.set_xticklabels(Labels,rotation=30)
ax.set_ylabel('roundness',fontsize=18)
#plt.savefig('Fig5b.svg',bbox_inches='tight')
plt.show()
```



Difference in PDF(roudness) compared to WT

[36]:
```
px1,py1 = fit_kde(Data[0])

px2,py2 = fit_kde(Data[1])
px3,py3 = fit_kde(Data[2])
px4,py4 = fit_kde(Data[3])
px5,py5 = fit_kde(Data[4])

fig,ax = plt.subplots(figsize=(6,5))
ax.plot(px2,py2-py1,color=Colors[1],label=Labels[1],lw=3)
ax.plot(px3,py3-py1,color=Colors[2],label=Labels[2],lw=3)
ax.plot(px4,py4-py1,color=Colors[3],label=Labels[3],lw=3)
ax.plot(px5,py5-py1,color=Colors[4],label=Labels[4],lw=3)
ax.set_xlim(0.25,0.95)
ax.set_xticks([0.3,0.6,0.9])
ax.set_yticks([-1,0,1])
ax.set_ylabel('$\Delta_p$',fontsize=18,labelpad=-10)
#ax.legend(loc='best',fontsize=14,ncol=2)
```

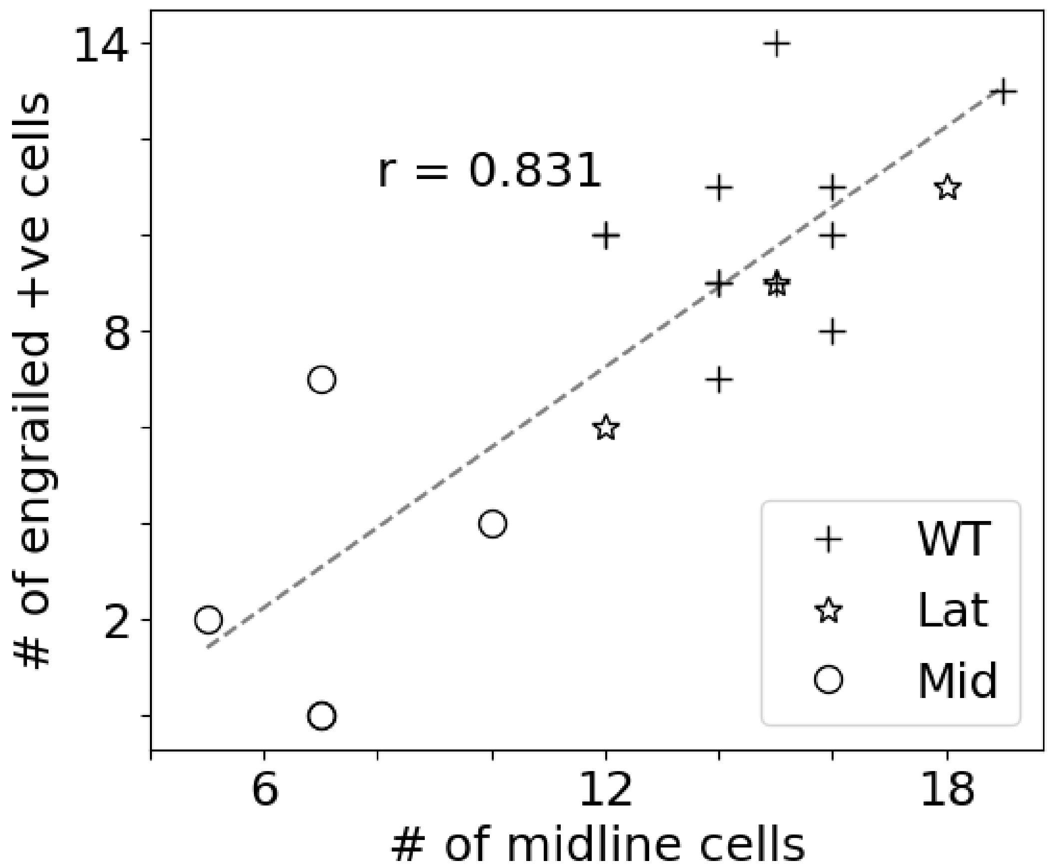
```
ax.set_yticks([-1.5,-1.0,-0.5,0,0.5,1.0,1.5])
ax.set_yticklabels(['','-1.0','','0','','1.0',''])
ax.set_xticks([0.3,0.4,0.5,0.6,0.7,0.8,0.9])
ax.set_xticklabels(['0.3','','','0.6','','','0.9'])
ax.axvline(0.6366,linestyle='--',color='k',alpha=0.5)
ax.axhline(0.0,linestyle='--',color='k',alpha=0.5)
ax.set_xlabel('roundness',fontsize=18)
#plt.savefig('Fig5b_prime.svg',bbox_inches='tight')
plt.show()
```



[ ]:

[37]:
```
fig,ax = plt.subplots(figsize=(6,5))
df = pd.read_csv('MIDLINE_ABL_RESULTS.csv')
TMC = np.array(df.Total_mid_cells)
ENC = np.array(df.Eng_total)
popt,_ = curve_fit(st_line,TMC,ENC)
m,c  = popt
x_fit = np.linspace(np.min(TMC),np.max(TMC),1000)
```

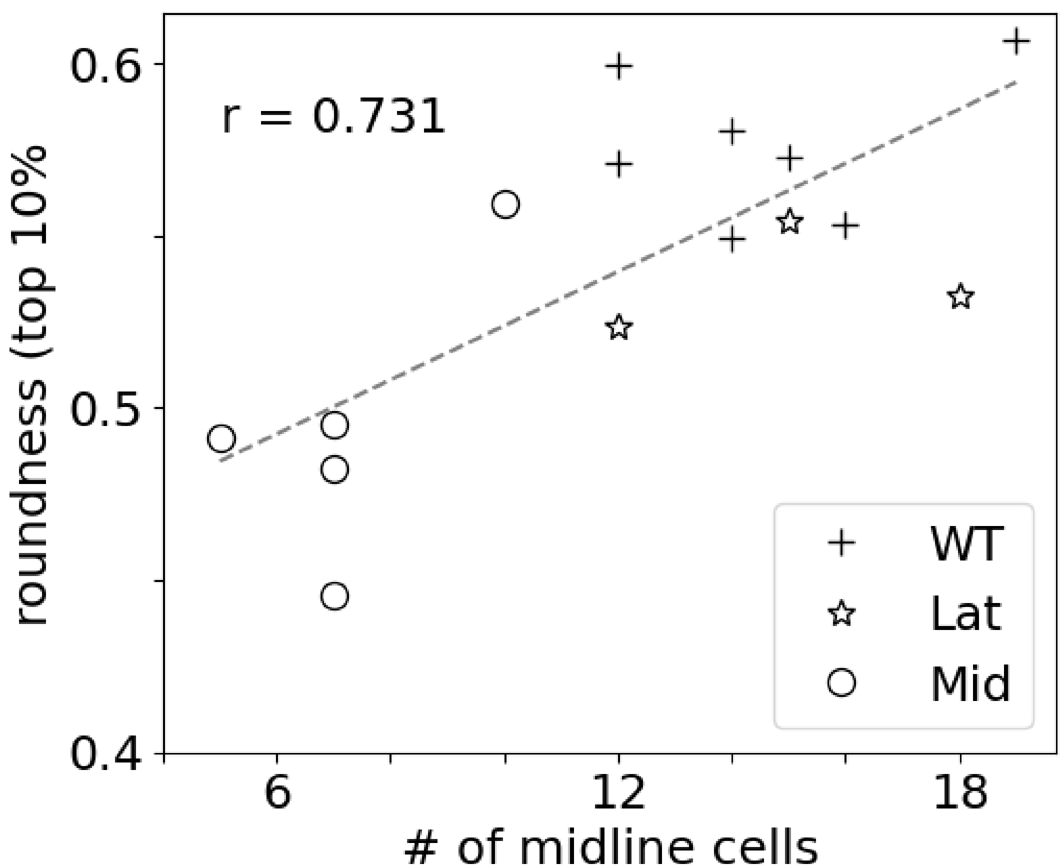
```
y_fit = st_line(x_fit,m,c)

r_cor = np.round(np.corrcoef(TMC,ENC)[0,1],3)

#0-4 Exp 5-7 Cntrl 8-19 WT
ax.plot(TMC[8:],ENC[8:
↪],'+',color='k',markerfacecolor='None',markersize=10,label='WT')
ax.plot(TMC[5:8],ENC[5:
↪8],'*',color='k',markersize=10,markerfacecolor='None',label='Lat')
ax.plot(TMC[0:5],ENC[0:
↪5],'o',color='k',markersize=10,markerfacecolor='None',label='Mid')

ax.plot(x_fit,y_fit,linestyle='--',color='k',alpha=0.5)

ax.text(8,11,'r = '+str(r_cor),fontsize=18)

ax.set_xticks([4,6,8,10,12,14,16,18])
ax.set_xticklabels(['','6','','','12','','','18'])

ax.set_yticks([0,2,4,6,8,10,12,14])
ax.set_yticklabels(['','2','','','8','','','14'])

ax.legend(loc=4,fontsize=18)
ax.set_xlabel('# of midline cells',fontsize=18)
ax.set_ylabel('# of engrailed +ve cells',fontsize=18)
#plt.savefig('Fig5c_prime.svg',bbox_inches='tight')
plt.show()
```



[ ]:

[38]:
```
ptile = 10 # 10 percentile
#7-3-5
TMC_subset = [15,19,16,14,12,14,12,15,18,12,7,7,5,10,7]
RV_Ind = np.load('RV_Ind_Tissues.npy')
RV_ptile = [np.percentile(RV_Ind[i,:],ptile) for i in range(15)]

popt,_ = curve_fit(st_line,TMC_subset,RV_ptile)
m,c  = popt
x_fit = np.linspace(np.min(TMC_subset),np.max(TMC_subset),1000)
y_fit = st_line(x_fit,m,c)
r_cor = np.round(np.corrcoef(TMC_subset,RV_ptile)[0,1],3)

fig,ax = plt.subplots(figsize=(6,5))
ax.plot(TMC_subset[:7],RV_ptile[:
↪7],'+',color='k',markerfacecolor='None',markersize=10,label='WT')
ax.plot(TMC_subset[7:10],RV_ptile[7:
↪10],'*',color='k',markersize=10,label='Lat',markerfacecolor='None')
```

```
ax.plot(TMC_subset[10:],RV_ptile[10:
 ↪],'o',color='k',markersize=10,label='Mid',markerfacecolor='None')
ax.plot(x_fit,y_fit,'--',color='k',alpha=0.5)
ax.text(5,0.58,'r = '+str(r_cor),fontsize=18)
ax.legend(loc=4,fontsize=18)
ax.set_yticks([0.4,0.45,0.5,0.55,0.6])
ax.set_yticklabels(['0.4','','0.5','','0.6'])
ax.set_xticks([4,6,8,10,12,14,16,18])
ax.set_xticklabels(['','6','','','12','','','18'])
#plt.savefig('Fig5c.svg',bbox_inches='tight')
ax.set_xlabel('# of midline cells',fontsize=18)
ax.set_ylabel('roundness (top 10%',fontsize=18)
plt.show()
```




```
[ ]:
```