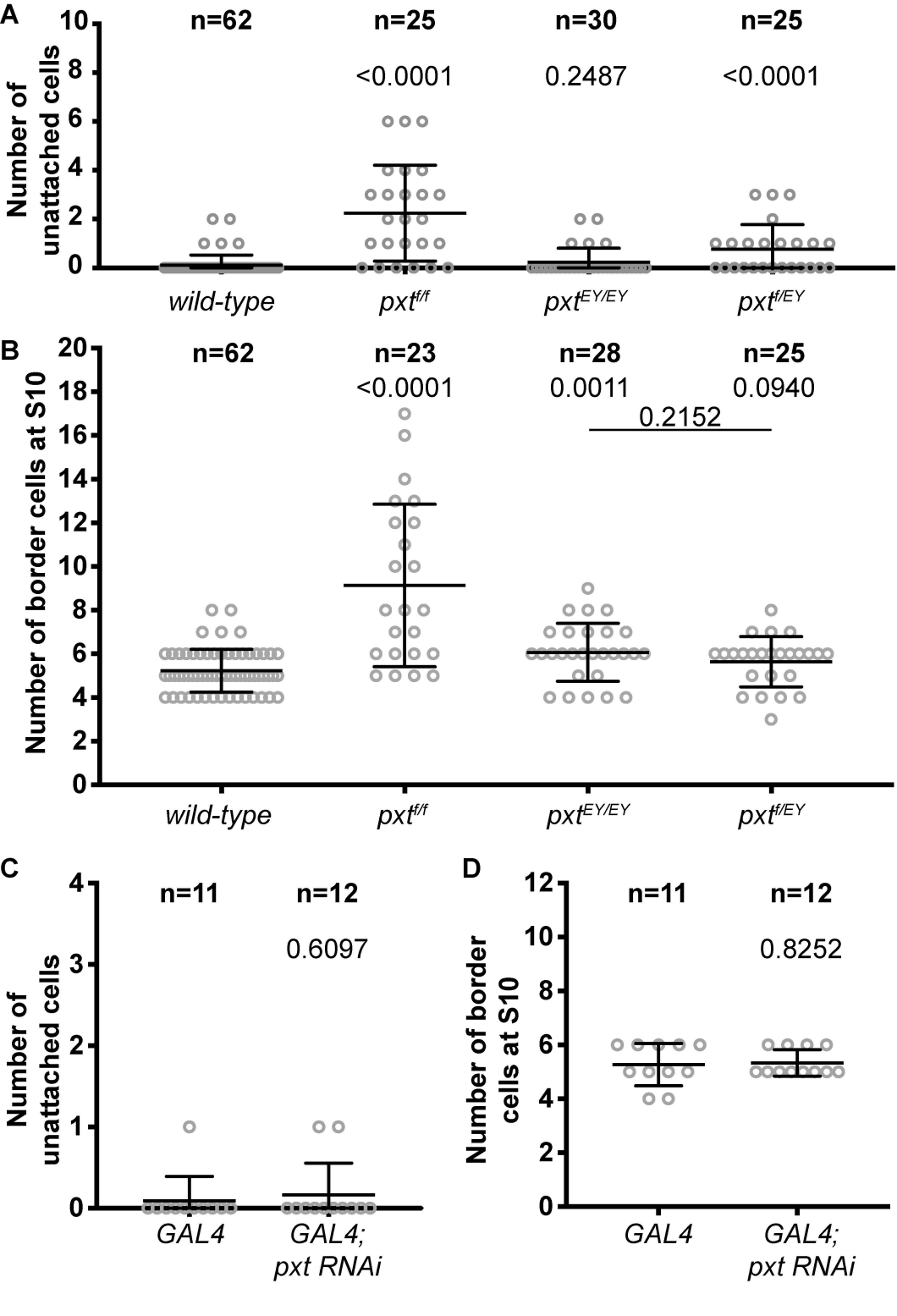
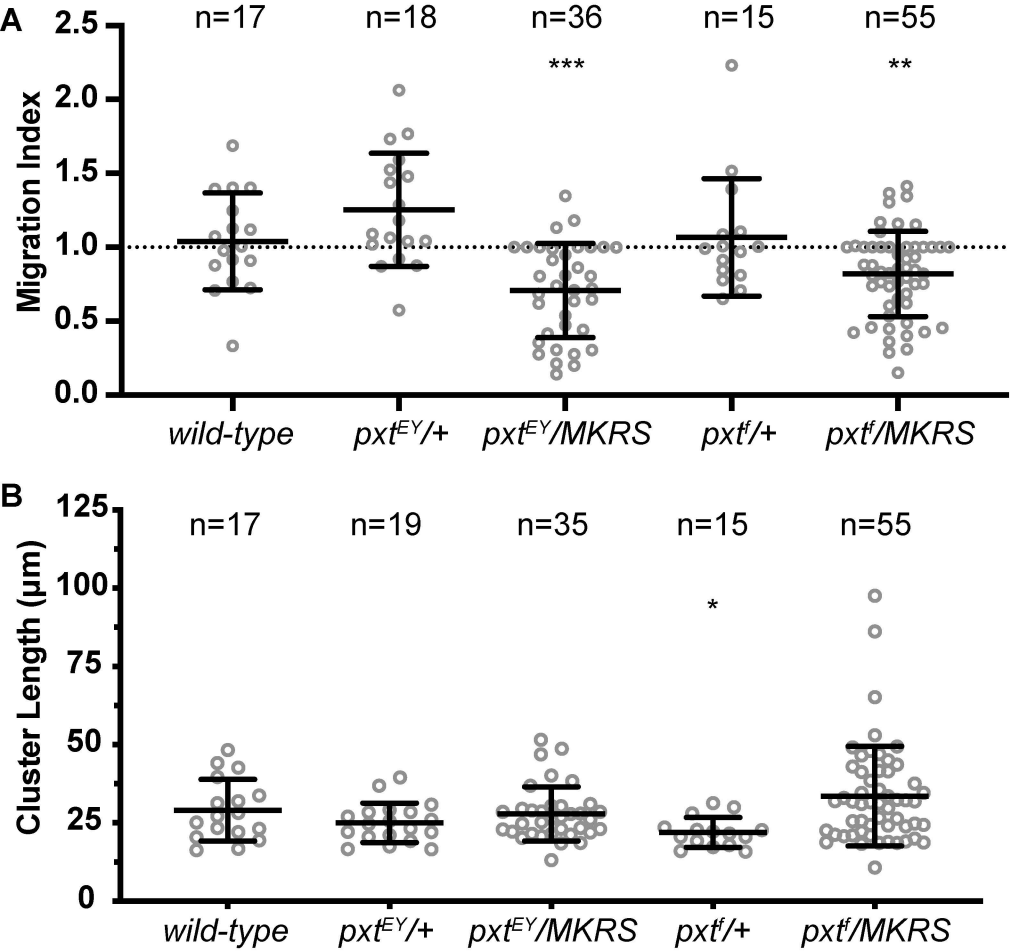
Supplemental Materials

Molecular Biology of the Cell

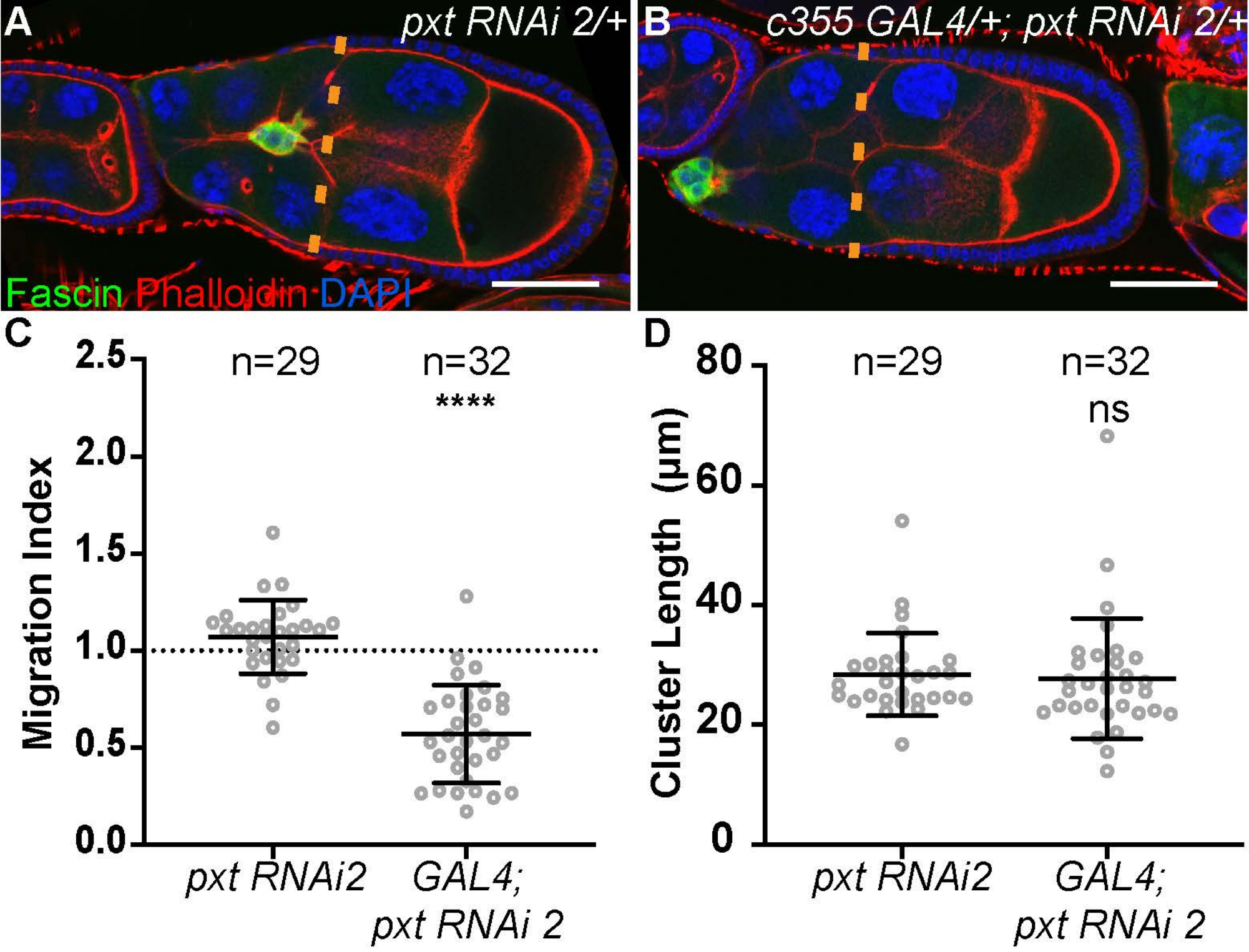
Fox et al.


A
Number of unattached cells
n=62
n=25
n=30
n=25
<0.0001
0.2487
<0.0001
wild-type
pxt^f/f
pxt^EY/EY
pxt^f/EY
B
Number of border cells at S10
n=62
n=23
n=28
n=25
<0.0001
0.0011
0.0940
0.2152
wild-type
pxt^f/f
pxt^EY/EY
pxt^f/EY
C
Number of unattached cells
n=11
n=12
0.6097
GAL4
GAL4; pxt RNAi
D
Number of border cells at S10
n=11
n=12
0.8252
GAL4
GAL4; pxt RNAi

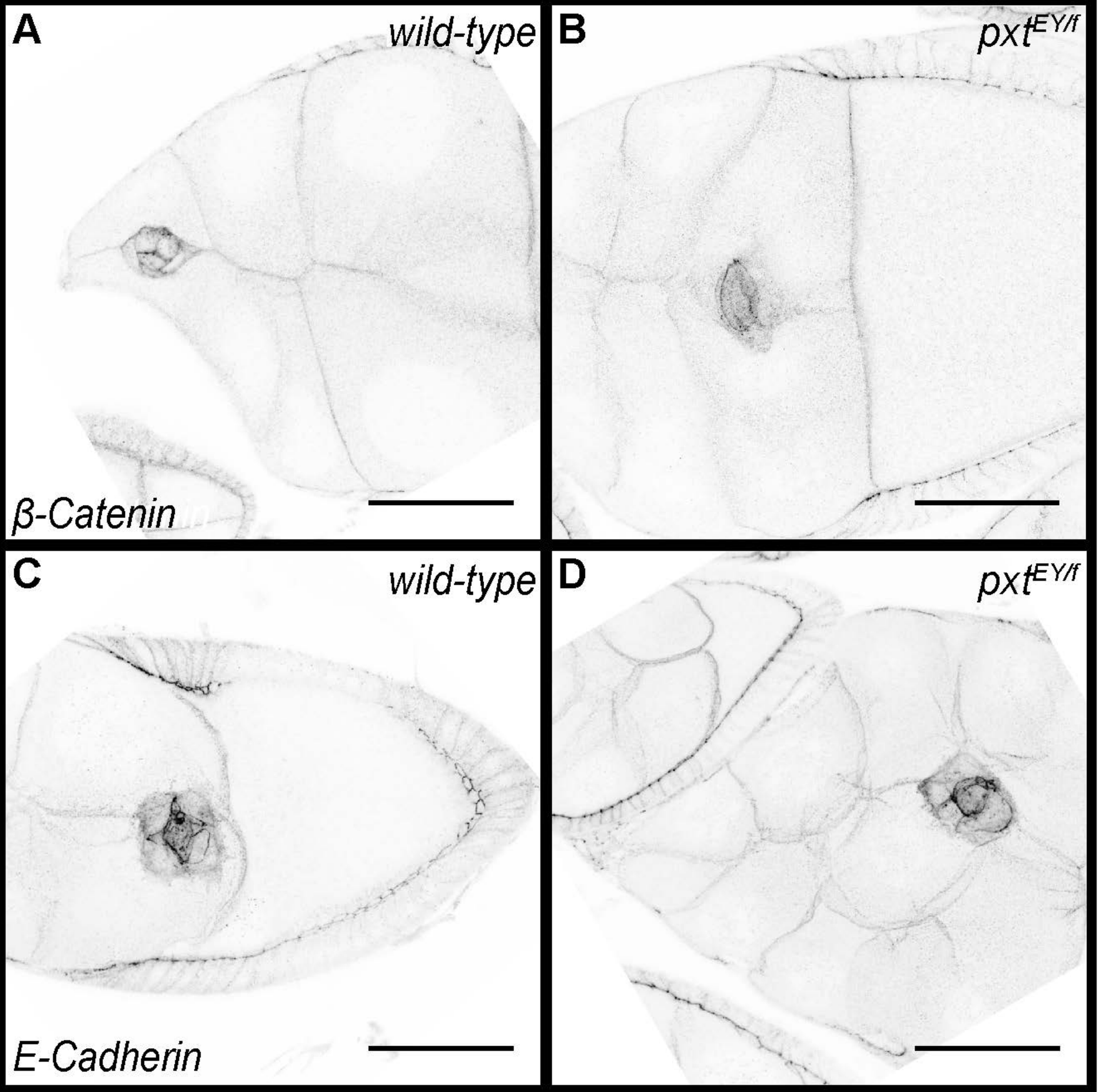
Supplementary Figure 1: Pxt regulates border cell cluster morphology during S10. A, C. Graphs of the quantification of the number of Eya stained somatic cells left between the nurse cells and visible during Stage 10 for the indicated genotypes. B, D. Graphs of the quantification of the number of cells within each border cell cluster, including trailing cells, at S10 for the indicated genotypes using Eya to mark the nuclei of the cells. In C-D, *GAL4 = c355 GAL4/+* and *GAL4; pxt RNAi = c355 GAL4/+; pxt RNAi (V14379)/+*. In A-D, each circle represents a single border cell cluster; n = number of follicles. Each line indicates the average and the whiskers indicate the SD. The p-values (two-tailed unpaired t-tests) are displayed on the graphs and are in comparison to the respective control, unless otherwise indicated. Loss of Pxt, via $pxt^{f/f}$ or $pxt^{EY/f}$, results in border cells detaching and being left along the migration path (A). Additionally, $pxt^{f/f}$ and $pxt^{EY/EY}$ result in an increase in the number of border cells (9.1 and 6.1, respectively, compared to 5.2; B). Conversely, somatic knockdown of Pxt (*c355* GAL4; *pxt RNAi*) does not result in border cells being left behind (C) or increased border cell numbers (D).


A
Migration Index
2.5
2.0
1.5
1.0
0.5
0.0
n=17
n=18
n=36
n=15
n=55
***
**
wild-type
pxt^EY/+
pxt^EY/MKRS
pxt^f/+
pxt^f/MKRS
B
Cluster Length (µm)
125
100
75
50
25
0
n=17
n=19
n=35
n=15
n=55
*
wild-type
pxt^EY/+
pxt^EY/MKRS
pxt^f/+
pxt^f/MKRS

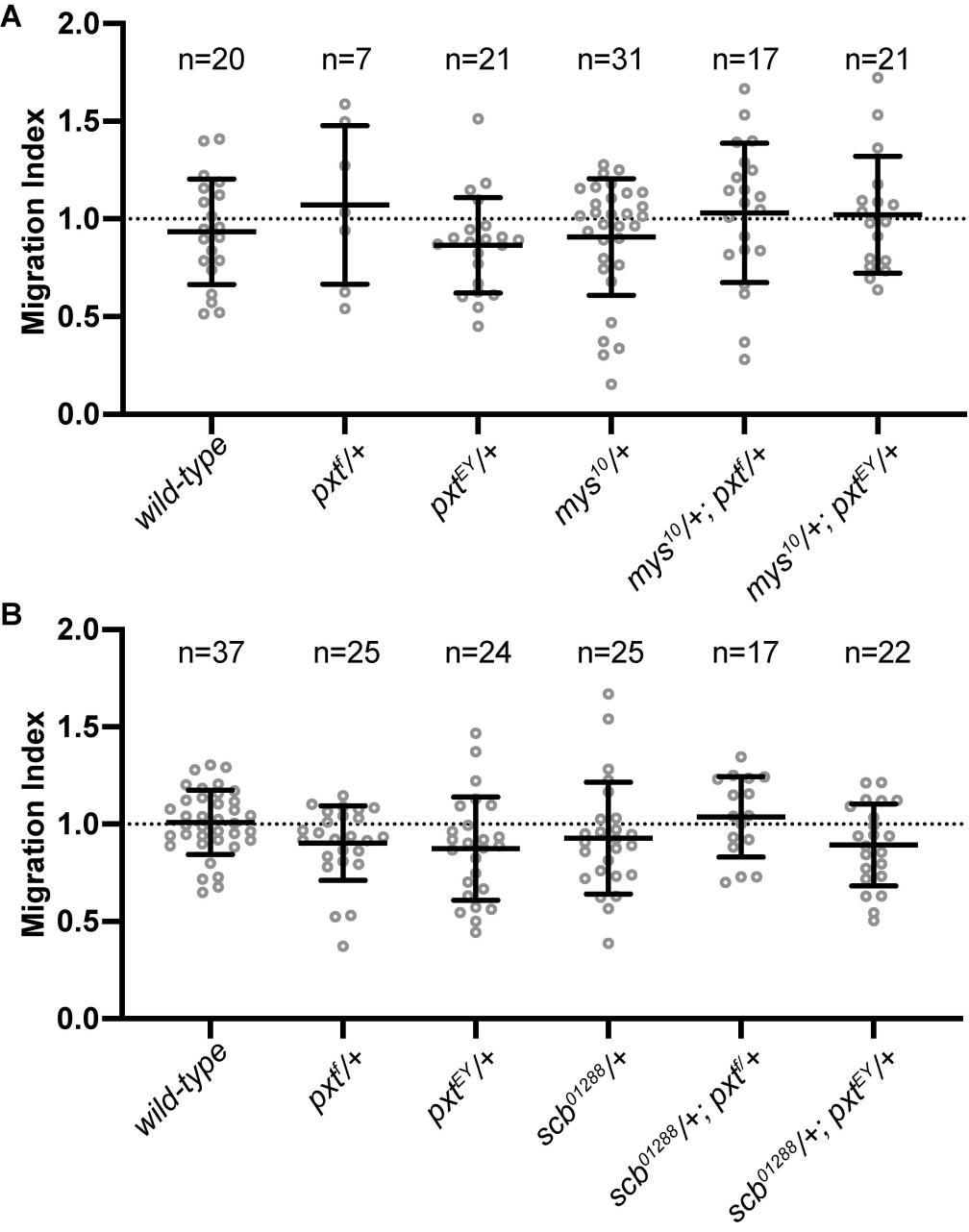
Supplementary Figure 2: MKRS balancer genetically interacts with *pxt* mutants to cause border cell migration defects during S9. A. Graph of the migration index quantification during S9 for the indicated genotypes. Dotted line at 1 indicates an on-time migration. B. Graph of the quantification of primary cluster length for the indicated genotypes; measured as described in Figure 3. In A-B, each circle represents a single border cell cluster; n = number of follicles. Each line indicates the average and the whiskers indicate the SD. ***$p<0.001$, **$p=0.01$, and *$p<0.05$ (two-tailed unpaired t-tests). Heterozygosity for *pxt* mutations over a wild-type chromosome has no effect on border cell migration or cluster length, while heterozygosity for a *pxt* mutation over the MKRS balancer results in delayed border cell migration.


A
pxt RNAi 2/+
B
c355 GAL4/+; pxt RNAi 2/+
Fascin Phalloidin DAPI
C
n=29
n=32
****
Migration Index
2.5
2.0
1.5
1.0
0.5
0.0
pxt RNAi2
GAL4; pxt RNAi 2
D
n=29
n=32
ns
Cluster Length (µm)
80
60
40
20
0
pxt RNAi2
GAL4; pxt RNAi 2

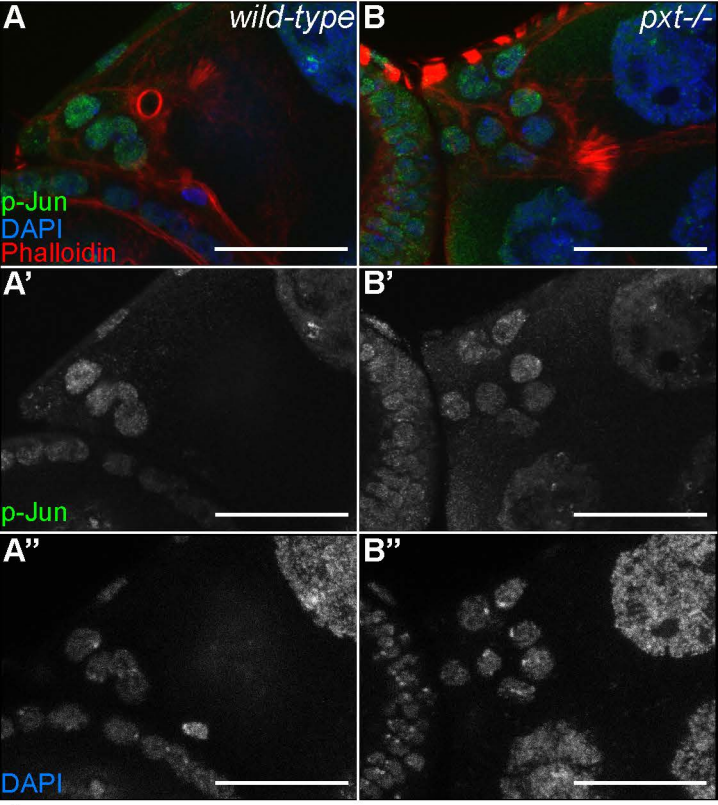
Supplementary Figure 3: Somatic knockdown with a second *pxt* RNAi line results in delayed border cell migration during S9. A-B. Maximum projection of 3 confocal slices of S9 follicles of the indicated genotypes; anterior is to the left. A. RNAi only control (*pxt RNAi 2/+*, V104446). B. Somatic knockdown of *pxt* (c355 GAL4/+; *pxt RNAi 2/+*). Merged images: Fascin, green; Phalloidin (F-actin), red; and DAPI (DNA), blue. Orange dashed lines represent the position of the outer follicle cells. C. Graph of the migration index quantification during S9 for the above indicated genotypes. Dotted line at 1 indicates an on-time migration. D. Graph of the quantification of primary cluster length for the above indicated genotypes; measured as described in Figure 3. In C-D, each circle represents a single follicle; n = number of follicles. Each line indicates the average and the whiskers indicate the SD. ****$p<0.0001$, and ns = $p> 0.05$ (two-tailed unpaired t-tests). Somatic knockdown of Pxt with a second RNAi construct (Vienna 104446) results in delayed border cell migration (B, C), and normal cluster morphology (D). Scale bars= 50μm.

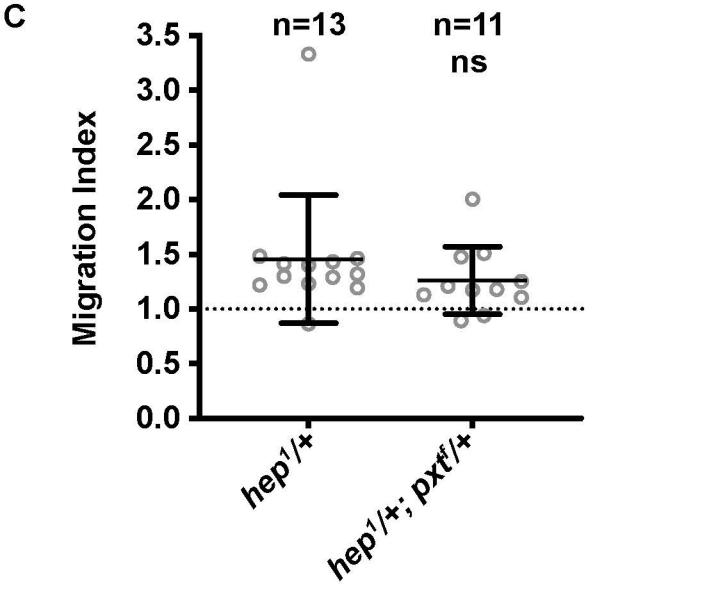

A
wild-type
β-Catenin
B
pxt^EY/f
C
wild-type
E-Cadherin
D
pxt^EY/f

Supplementary Figure 4: Pxt does not regulate β-Catenin and E-cadherin localization. A-D. Inverted maximum projection of 3 confocal slices of S9 follicles of the indicated genotypes stained for β-Catenin (A-B) or *Drosophila* E-Cadherin (C-D); anterior is to the left. A, C. wild-type (*yw*). B, D. $pxt^{EY/f}$. In both wild-type and *pxt* mutant follicles, β-Catenin and E-Cadherin localize to the border cell-border cell contacts. Scale bars= 50μm.


A
Migration Index
2.0
1.5
1.0
0.5
0.0
n=20
n=7
n=21
n=31
n=17
n=21
wild-type
pxt^f/+
pxt^EY/+
mys^10/+
mys^10/+; pxt^f/+
mys^10/+; pxt^EY/+
B
Migration Index
2.0
1.5
1.0
0.5
0.0
n=37
n=25
n=24
n=25
n=17
n=22
wild-type
pxt^f/+
pxt^EY/+
scb^01288/+
scb^01288/+; pxt^f/+
scb^01288/+; pxt^EY/+

Supplementary Figure 5: Dominant genetic interactions between *pxt* and integrin subunits. A-B. Graphs of the migration index quantification during Stage 9 for the indicated genotypes. Dotted line at 1 indicates an on-time migration. Each circle represents a single border cell cluster; n = number of follicles. Each line indicates the average and the whiskers indicate the SD. The migration index of the double heterozygotes is not statistically different ($p>0.05$, two-tailed unpaired t-test) from the single heterozygotes. Heterozygosity for mutations in βPS-integrin (*mys*^{10}/+), αPS3-integrin (*scb*^{01288}/+), or *pxt* (*pxt*^{f}/+ or *pxt*^{EY}/+), and double heterozygotes for *pxt* and an integrin subunit (*mys*^{10}/+; *pxt*/+ and *scb*^{01288}/+; *pxt*/+) exhibit normal border cell migration.


A
wild-type
B
pxt-/-
p-Jun
DAPI
Phalloidin
A'
B'
p-Jun
A''
B''
DAPI


C
n=13
n=11
ns
Migration Index
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
hep1/+
hep1/+; pxtf/+

Supplemental Figure 6: Pxt acts independent of the JNK pathway. A-B. Maximum projection of 3 confocal slices of S9 follicles of the indicated genotypes. Wild-type and *pxt* mutant follicles were stained in the same tube and Pxt antibody staining (not shown) was used to differentiate between the genotypes. A. *wild-type* (*yw*). B. *pxt-/-* (*pxt*$^{f/f}$). A-B. Merged images: p-Jun, green; Phalloidin (F-actin), red; and DAPI (DNA), blue. A'-C'. p-Jun, white. A"-B". DAPI, white. C. Graphs of the migration index quantification during S9 for the indicated genotypes; data represents two independent experiments. Dotted line at 1 indicates an on-time migration. Each circle represents a single border cell cluster; n = number of follicles. Each line indicates the average and the whiskers indicate the standard deviation (SD). ns = $p>0.05$ (two-tailed unpaired t-test). The levels of p-Jun in the border cells appear similar in wild-type and *pxt* mutant follicles (A-B"). Heterozygosity for mutations in the Drosophila JNK Kinase Hemipterous (hep^{1}/+), and double heterozygotes for *pxt* and *hep* (*hep*^{1}*/+; pxt/+*) do not exhibit delayed border cell migration (C). Scale bars= 50μm.