SUPPLEMENTAL MATERIAL

Figure S1. Additional evidence of centriole loss and *fzr RNAi* efficacy.

A) Centrioles are not present in polyploid cells of the L3 Malpighian tubule (Tubule), but are present in the adjacent diploid cells of the hindgut pylorus. **A')** Asl only channel from panel **A). B)** Representative pattern of EdU localization during WT L2 endocycles. **C)** EdU does not incorporate in L2 rectal cells of *fzr RNAi* animals. **D)** Quantitation of EdU incorporation in WT and *fzr* animals. Animals were scored as EdU positive when more than 10 EdU positive cells were present in the rectum. From N=11 animals/genotype. Dashed lines delineate the hindgut ileum (left) and rectum (right). Scale bars=20μm.

Figure S2. Additional evidence of extra centrosomes and division outcomes in WT papillar cells. A) Cnn antibody stain (Green) in a WT papillar cell. Phospho-Histone (Green, Chromosomal) and DAPI (DNA, Purple) label chromosomes. **B)** Representative successive serial EM sections (Z1-5) of mitotic papillar tissue, showing one centriole in cross-section and a second centriole at right angle. 10/10 serially sectioned cells examined exhibited this arrangement of only 2 centriole pairs. **C)** Time-lapse of two adjacent papillar cells, each with three detectable centrosomes, undergoing centrosome clustering and a bipolar division. Arrowheads indicate centrosomes. Transgenes indicated in panels. **D)** Time-lapse of a papillar cell with two detectable centrosomes undergoing a bipolar division with an anaphase bridge (arrow). Transgenes indicated in panels. **E)** Representative 3D-volumetric quantitation of two separate tripolar papillar divisions from the analysis in **Fig3G**. Two independent cell divisions are shown, each with 3 daughters of separate colors, the DNA content of each indicated on the Y axis. **F)** Plot of

the frequency of WT papillar division errors, separated by class and number of centrosomes. Scale bars: 5μm.

Figure S3. Differences in the response to extra centrosomes between papillar cells and diploid cells. A) Diagram of centrosome number and tripolar division frequency in *SAK-OE* 2N neuroblasts and 8N papillar cells. **B)** Metaphase centrosome number in metaphase polyploid papillar cells (N=46) or diploid neuroblasts (N=47) of *SAK-OE* animals, from a minimum of 6 replicates/tissue type. For papillar cells, this number represents the average from three different *SAK-OE* transgenes: either Ubi- or UAS-promoter driven. For the hindgut, the driver was *byn Gal4,* and for neuroblasts, the driver was *daughterless (da) Gal4*. The distribution of centrosome number between these two tissues is not significantly different by T test (P=0.9). **C)** Time-lapse of *SAK-OE* neuroblast undergoing spindle pole clustering and a bipolar division. Jupiter (Jup) GFP labels microtubules. Arrowheads label spindle poles. Time in all panels in this figure is indicated in minutes relative to anaphase onset. **D)** Time-lapse analysis of spindles during a tripolar division of an 8N *Jup GFP, SAK-OE* papillar cell. Arrowheads label spindle poles. **E)** Division outcome in *SAK-OE* animals for polyploid papillar cells, broken down by transgene. Also indicated is the division outcome in *fzr RNAi; SAK-OE* animals (N=8-24 animals/genotype, chr.=chromosome). **F)** Time-lapse analysis of a representative bipolar division of a *fzr SAK-OE* papillar cell. CenpC-Tomato labels kinetochores, Moe GFP labels cell membranes. Note that at 4:00, one can clearly count 8 kinetochores (the diploid number) segregating to the left daughter cell. **G)** Evidence of extra centrosomes

in *fzr SAK-OE* animals at the time of mitosis. Cnn=Centrosomin, GTU=gamma tubulin, PH3=phospho-histone H3. Scale bars: 5μm.

Figure S4. Additional evidence that multipolarity does not impede papillar development. A-F) Tripolar aneuploidy does not alter papillar base cell gene expression. Single adult rectal papillae outlined in white. DNA-purple, Specific Gal4 trap-Green. **A)** WT *itp-r83A*(10H02) Gal4 driven UAS-GFP. **B)** *SAK-OE Itp-r83A*(10H02) Gal4 driven UAS-GFP. **C)** WT *AICR2*(10H05) Gal4 driven UAS-GFP. **D)** *SAK-OE AICR2*(10H05) Gal4 driven UAS-GFP. **E)** WT *Syt4*(11G04) driven UAS-GFP. **F)** *Syt4*(11G04) driven UAS-GFP (Green) pattern in a *SAK-OE* papilla (DNA in Purple). **G)** Schematic of lineage labeling scheme used in Fig4L-N. **H)** Graph of clone size distribution from lineage experiments. Scale bars: 20μm.

Figure S5. *fzr RNAi* does not cause a delay in pupal mitotic proliferation.

A) Representative z-slice from WT rectum aged 24-32 hrs. ppf, labeled for EdU (purple) and Phospho-Histone H3 (PH3, green, nuclear). Moesin (Moe)-GFP (green, membrane) also marks cell membranes. **A')** EdU only channel from **A. A'')** PH3/Moe only channel from **A.** Arrowheads indicate mitotic cells. White box indicates the region shown in the inset. **B)** Representative z-slice from *fzr RNAi* rectum aged 24-32 hrs. ppf, labeled for EdU (purple) and PH3 (green, nuclear). Moe-GFP (green, membrane) also marks cell membranes. **B')** EdU only channel from **B. B'')** PH3/Moe only channel from **B.** Arrowheads indicate mitotic cells. White box indicates the region shown in the inset. **C)** Graph of EdU positive cells from experiments in **A** and **B.** Standard deviation indicated.

D) Graph of PH3 positive cells from experiments in **A** and **B.** Standard deviation indicated. N=5 for WT and N=8 for *fzr*. All animals of both genotypes were found to contain at least 94 EdU positive cells and at least 5 Phospho-Histone H3 positive cells. Scale bars: 20μm.

Figure S6. Additional evidence that *byn-Gal4* driven transient *fzr* and *Notch* knockdown affect papillar development. A) Low magnification of entire WT adult hindgut. **B)** Low magnification of transient *fzr RNAi* adult hindgut. **C)** Low magnification of transient *N RNAi* adult hindgut. In each panel, *UAS-GFP* (green) outlines the hindgut, DNA is in purple, and a white line indicates the rectum. Scale bars: 200μm.

Supplemental Movies.

Supplemental Movie 1. Wild type bipolar division in papillar cell with 2 centrosomes. For Movies 1-3, 1 frame =2 min. Genotype: *his RFP, UAS-cnn GFP ; byn Gal4 , UAS-moe GFP*
Supplemental Movie 2. Wild type bipolar divisions in papillar cells with 3 centrosomes.
Supplemental Movie 3. Wild type tripolar division in papillar cell with 3 centrosomes.
Supplemental Movie 4. *SAK-OE* neuroblast with clustering centrosomes and bipolar division. Frame Rate: 1 frame =1 min. Genotype: *his RFP, UAS-cnn GFP ;; da Gal4*
Supplemental Movie 5. *SAK-OE* neuroblast with clustering spindle poles and bipolar division. Frame Rate: 1 frame =1 min. Genotype: *Jupiter GFP*
Supplemental Movie 6. *SAK-OE* papillar cell with tripolar division. 1 frame =2 min. Genotype: *his RFP, UAS-cnn GFP ; byn Gal4 , UAS-moe GFP*
Supplemental Movie 7. *SAK-OE* papillar cell with tripolar spindle. 1 frame =2 min. Genotype: *Jupiter GFP*
Supplemental Movie 8. *fzr RNAi ; SAK-OE* papillar cell with bipolar division. 1 frame =1 min. Genotype: *CenpC Tomato; byn Gal4 , UAS-moe GFP*

Figure S1

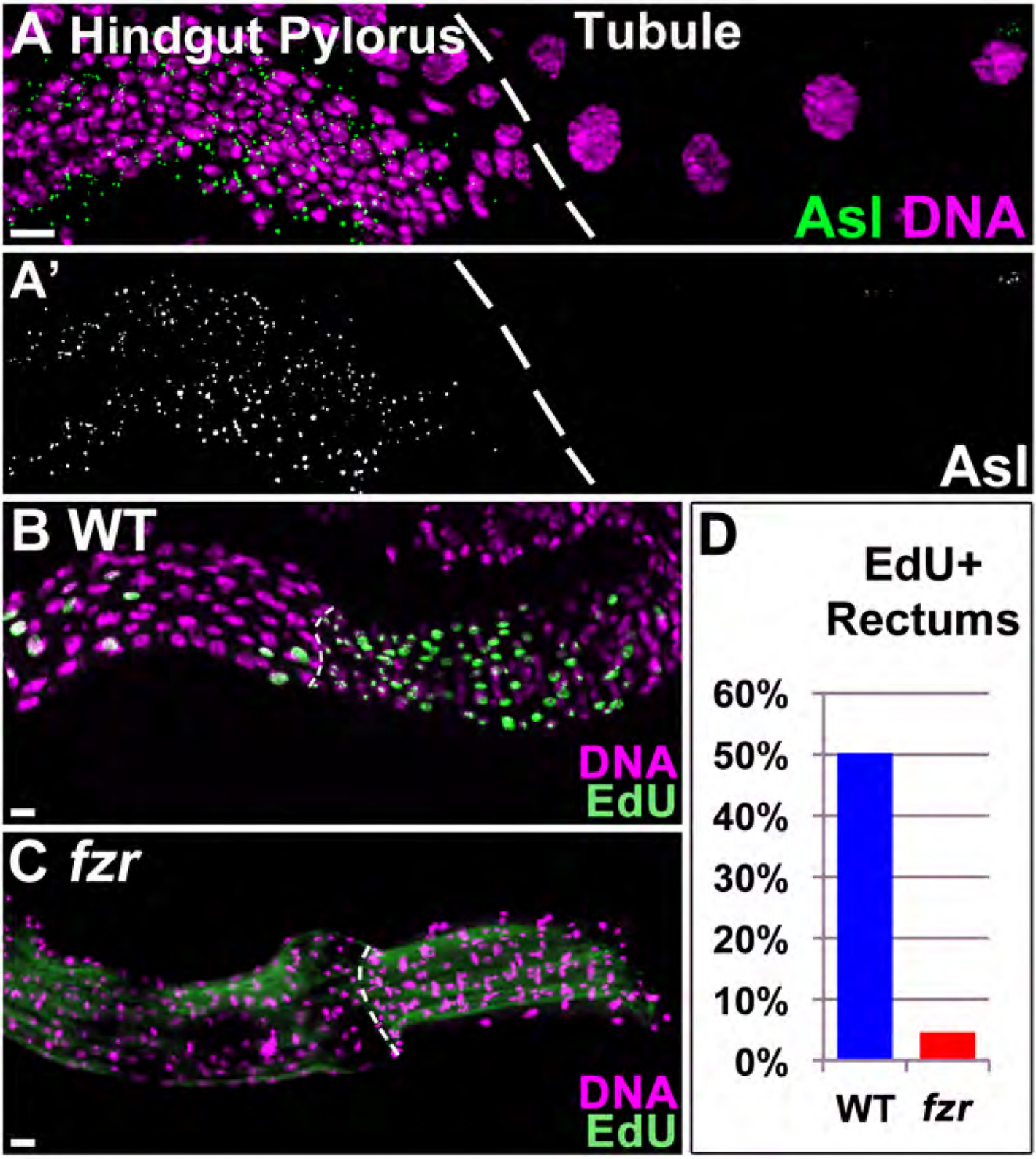

A Hindgut Pylorus
Tubule
Asl DNA
A'
Asl
B WT
DNA
EdU
C fzr
DNA
EdU
D
EdU+
Rectums
60%
50%
40%
30%
20%
10%
0%
WT
fzr

Figure S2

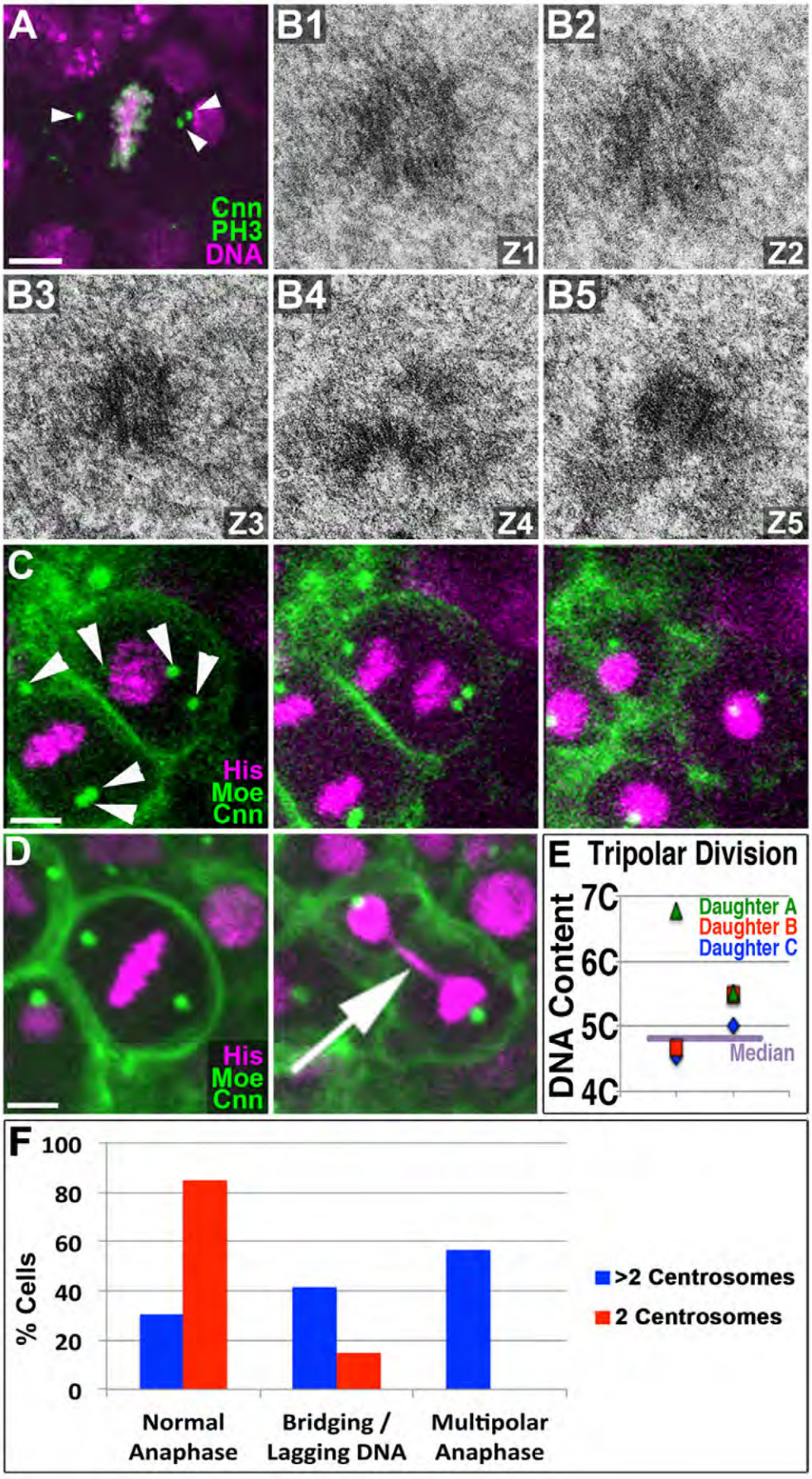

A
Cnn
PH3
DNA
B1
Z1
B2
Z2
B3
Z3
B4
Z4
B5
Z5
C
His
Moe
Cnn
D
His
Moe
Cnn
E Tripolar Division
DNA Content
7C
6C
5C
4C
Daughter A
Daughter B
Daughter C
Median
F
% Cells
100
80
60
40
20
0
Normal Anaphase
Bridging / Lagging DNA
Multipolar Anaphase
>2 Centrosomes
2 Centrosomes

Figure S3

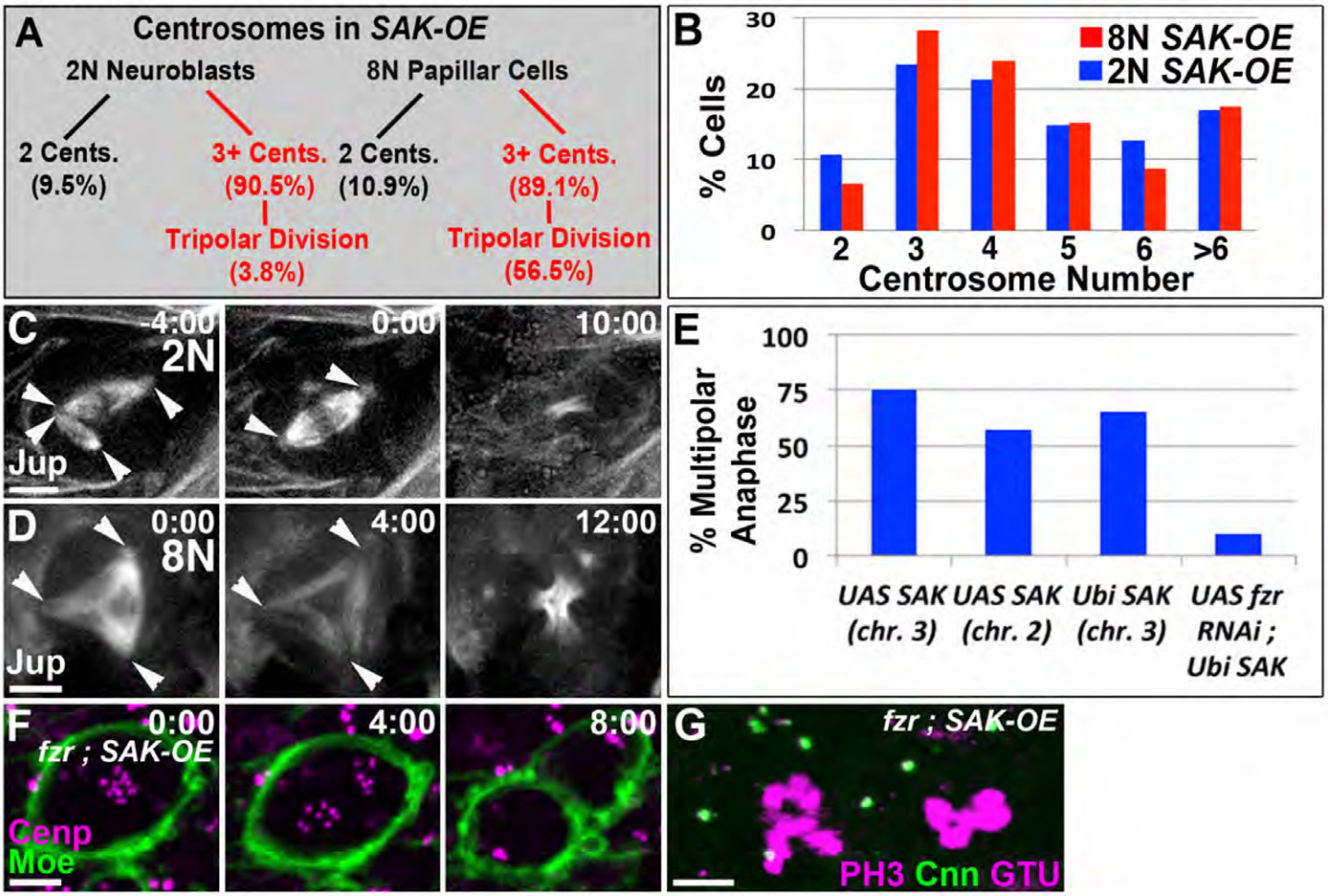

A
Centrosomes in SAK-OE
2N Neuroblasts
8N Papillar Cells
2 Cents. (9.5%)
3+ Cents. (90.5%)
2 Cents. (10.9%)
3+ Cents. (89.1%)
Tripolar Division (3.8%)
Tripolar Division (56.5%)
B
% Cells
30
20
10
0
8N SAK-OE
2N SAK-OE
2
3
4
5
6
>6
Centrosome Number
C
-4:00
2N
Jup
0:00
10:00
D
0:00
8N
Jup
4:00
12:00
E
% Multipolar Anaphase
100
75
50
25
0
UAS SAK (chr. 3)
UAS SAK (chr. 2)
Ubi SAK (chr. 3)
UAS fzr RNAi ; Ubi SAK
F
0:00
fzr ; SAK-OE
Cenp
Moe
4:00
8:00
G
fzr ; SAK-OE
PH3 Cnn GTU

Figure S4

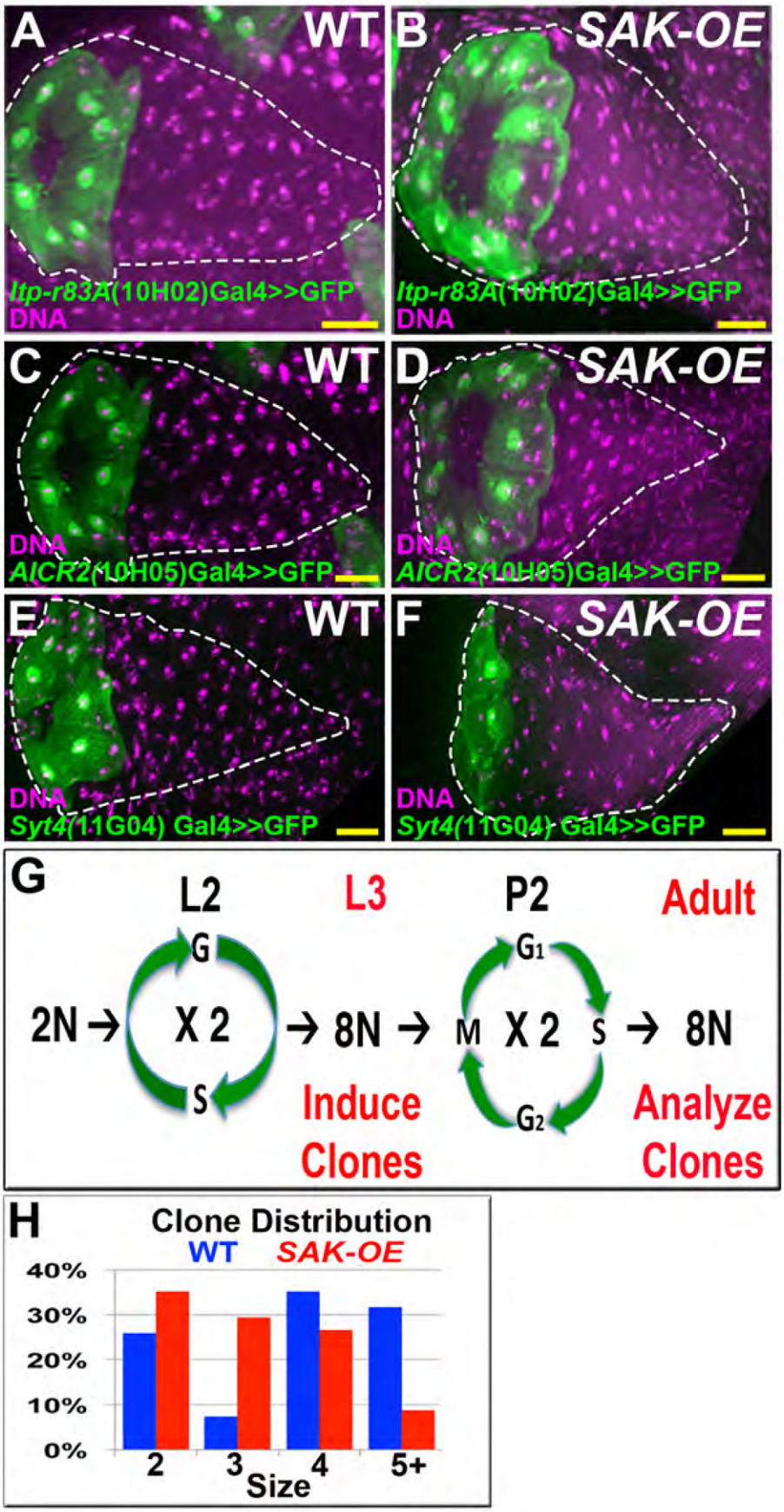

A
WT
Itp-r83A(10H02)Gal4>>GFP
DNA
B
SAK-OE
Itp-r83A(10H02)Gal4>>GFP
DNA
C
WT
DNA
AICR2(10H05)Gal4>>GFP
D
SAK-OE
DNA
AICR2(10H05)Gal4>>GFP
E
WT
DNA
Syt4(11G04) Gal4>>GFP
F
SAK-OE
DNA
Syt4(11G04) Gal4>>GFP
G
L2
L3
P2
Adult
2N →
G
X 2
S
→ 8N →
M
X 2
S
→ 8N
G1
G2
Induce Clones
Analyze Clones
H
Clone Distribution
WT
SAK-OE
40%
30%
20%
10%
0%
2
3
4
5+
Size

Figure S5

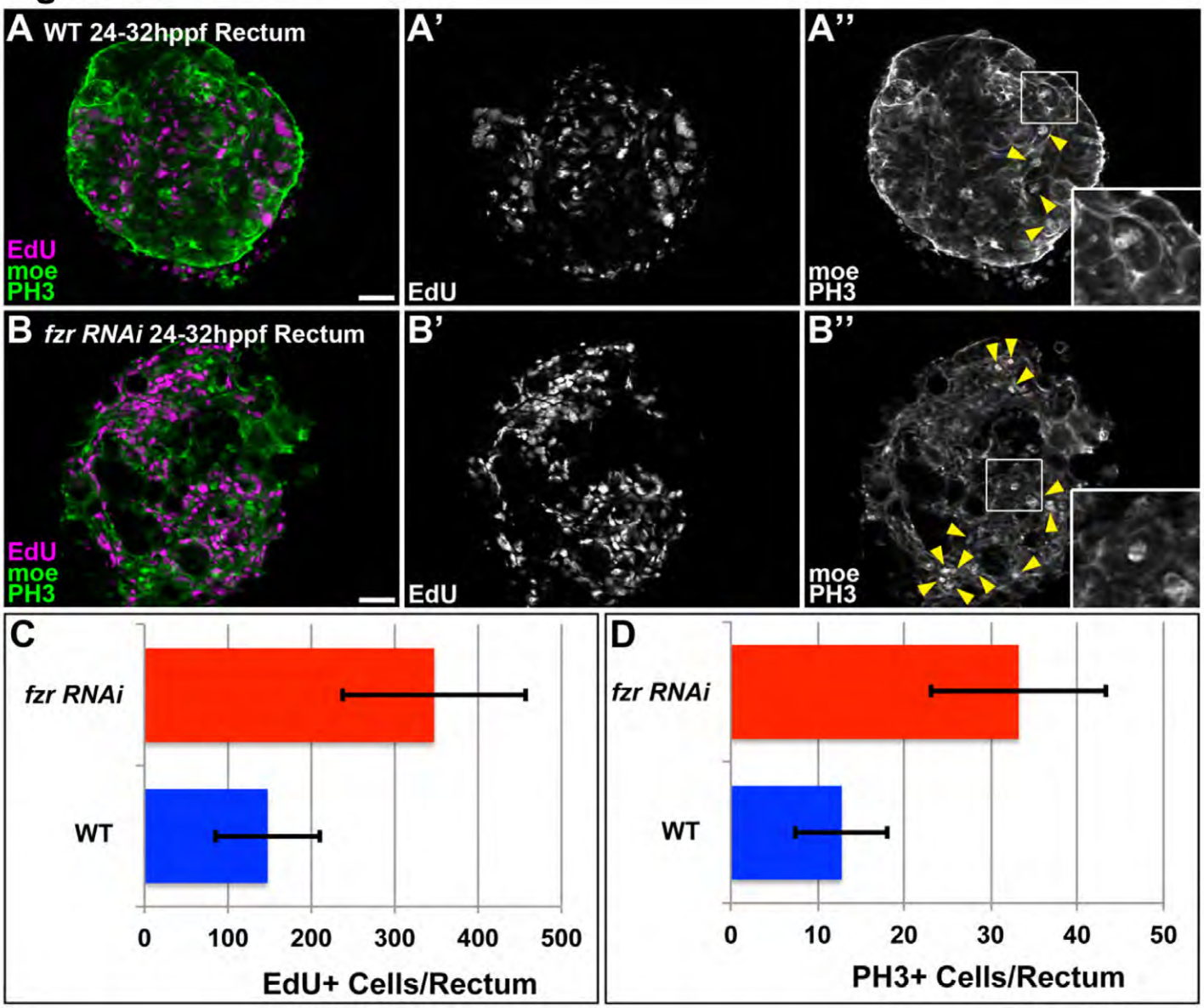
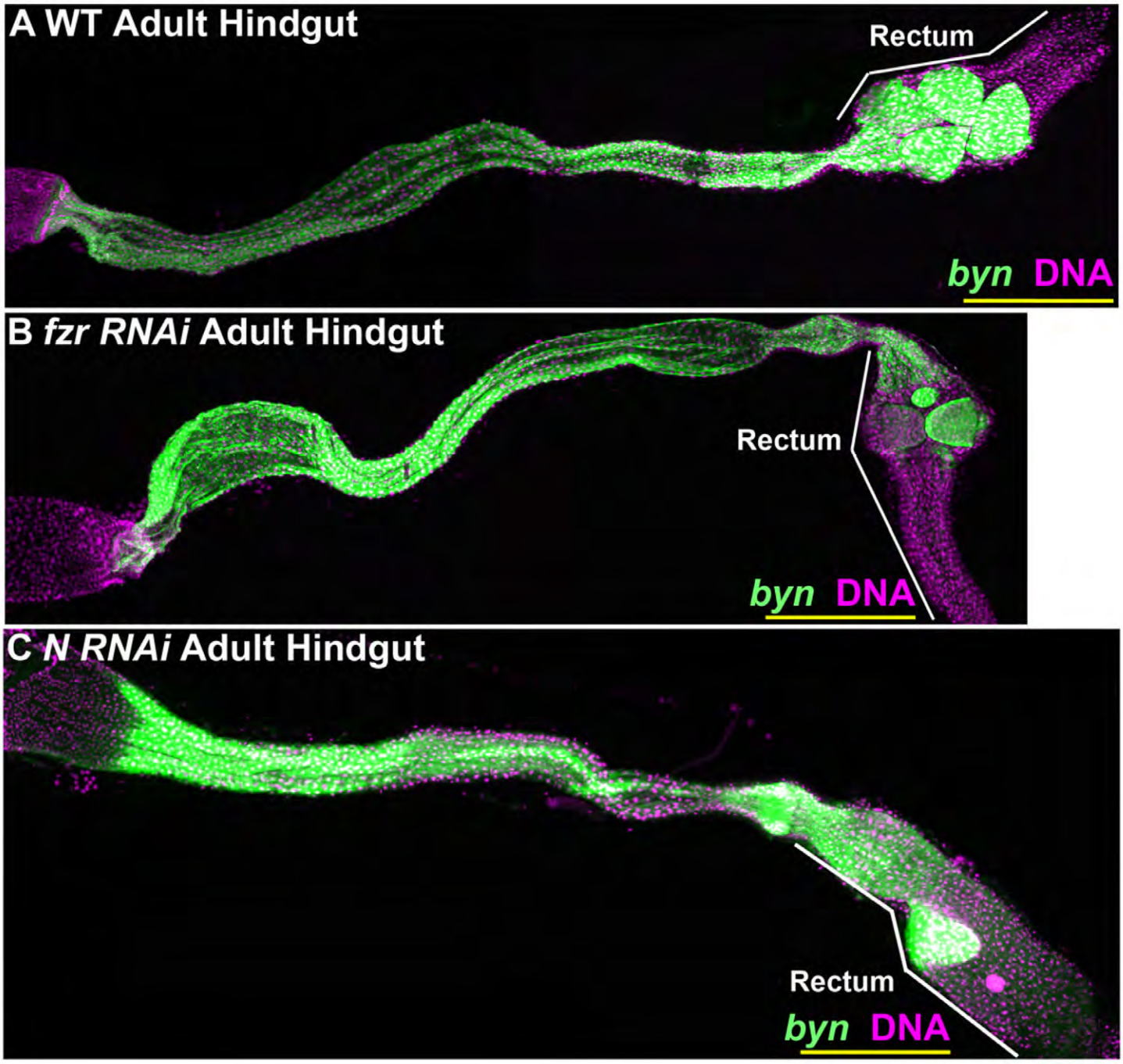
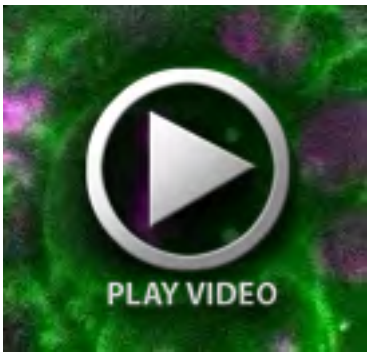

A WT 24-32hppf Rectum
EdU
moe
PH3
A'
EdU
A''
moe
PH3
B fzr RNAi 24-32hppf Rectum
EdU
moe
PH3
B'
EdU
B''
moe
PH3
C
fzr RNAi
WT
0
100
200
300
400
500
EdU+ Cells/Rectum
D
fzr RNAi
WT
0
10
20
30
40
50
PH3+ Cells/Rectum

Figure S6


A WT Adult Hindgut
Rectum
byn DNA
B fzr RNAi Adult Hindgut
Rectum
byn DNA
C N RNAi Adult Hindgut
Rectum
byn DNA

Movie 1.

Movie 2.



Movie 3.

Movie 4.

Movie 5.

Movie 6.

Movie 7.

Movie 8.