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 fzt 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 ipt-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. fzt 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 fzt RNAi rectum aged 24-32 hrs. ppf, labeled for EdU (purple) and PH3 (green, nuclear). Moesin-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 S4

(A) WT

(B) SAK-OE

(C) WT

(D) SAK-OE

(E) WT

(F) SAK-OE

(G) L2 → L3 → P2 → Adult

2N → X 2 → 8N

Induce Clones

(H) Clone Distribution

WT SAK-OE

Size 2 3 4 5+
Figure S5

A WT 24-32hpf Rectum

A' EdU

A' EdU

A' moe PH3

A'" moe PH3

B fze RNAi 24-32hpf Rectum

B' EdU

B' EdU

B' moe PH3

B' moe PH3

C fze RNAi

WT

D fze RNAi

WT

EdU+ Cells/Rectum

PH3+ Cells/Rectum