Cell Reports, Volume *28*

Supplemental Information

Control of Intestinal Cell Fate by Dynamic Mitotic Spindle Repositioning Influences Epithelial Homeostasis and Longevity

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Figure S1

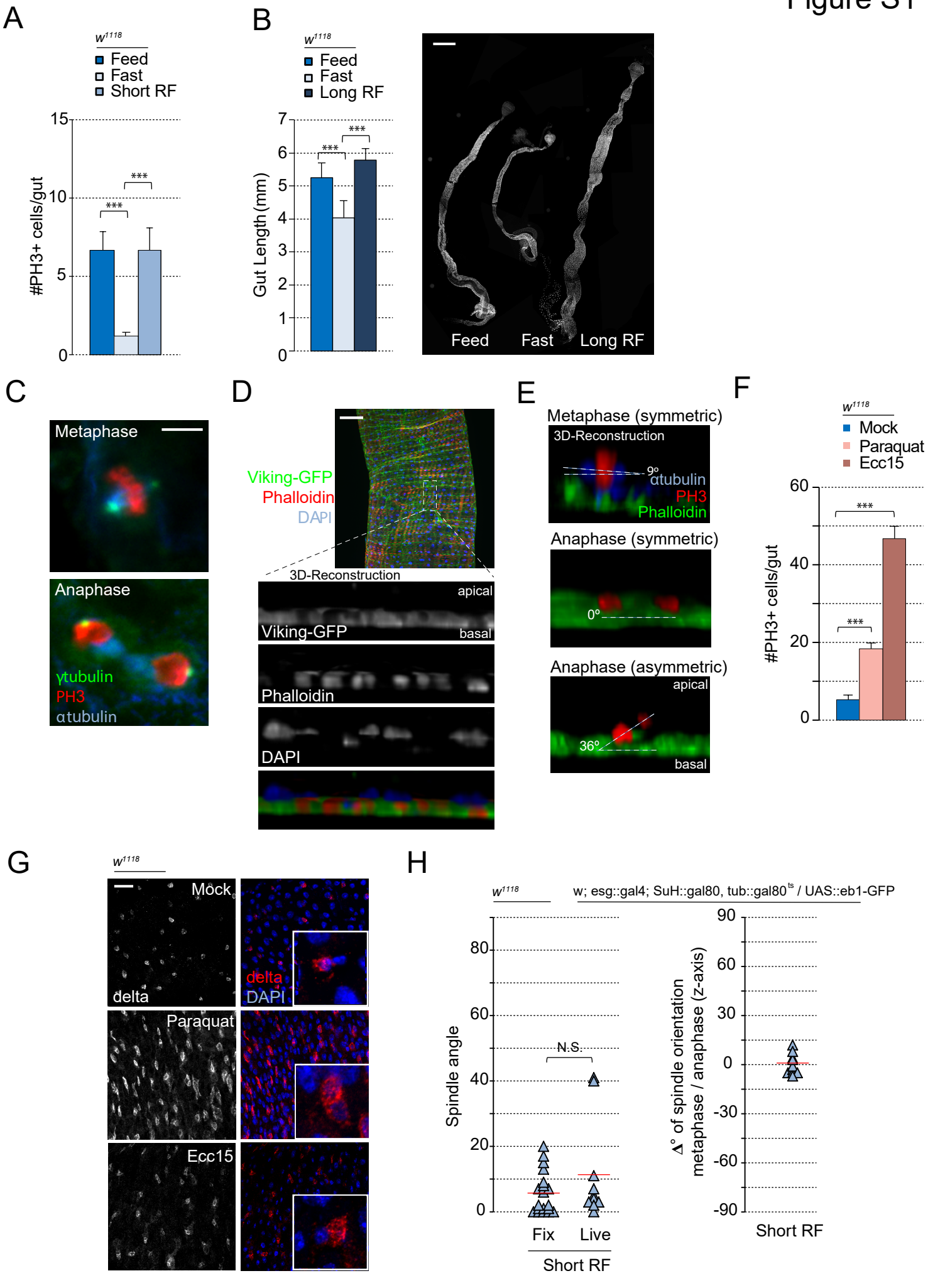
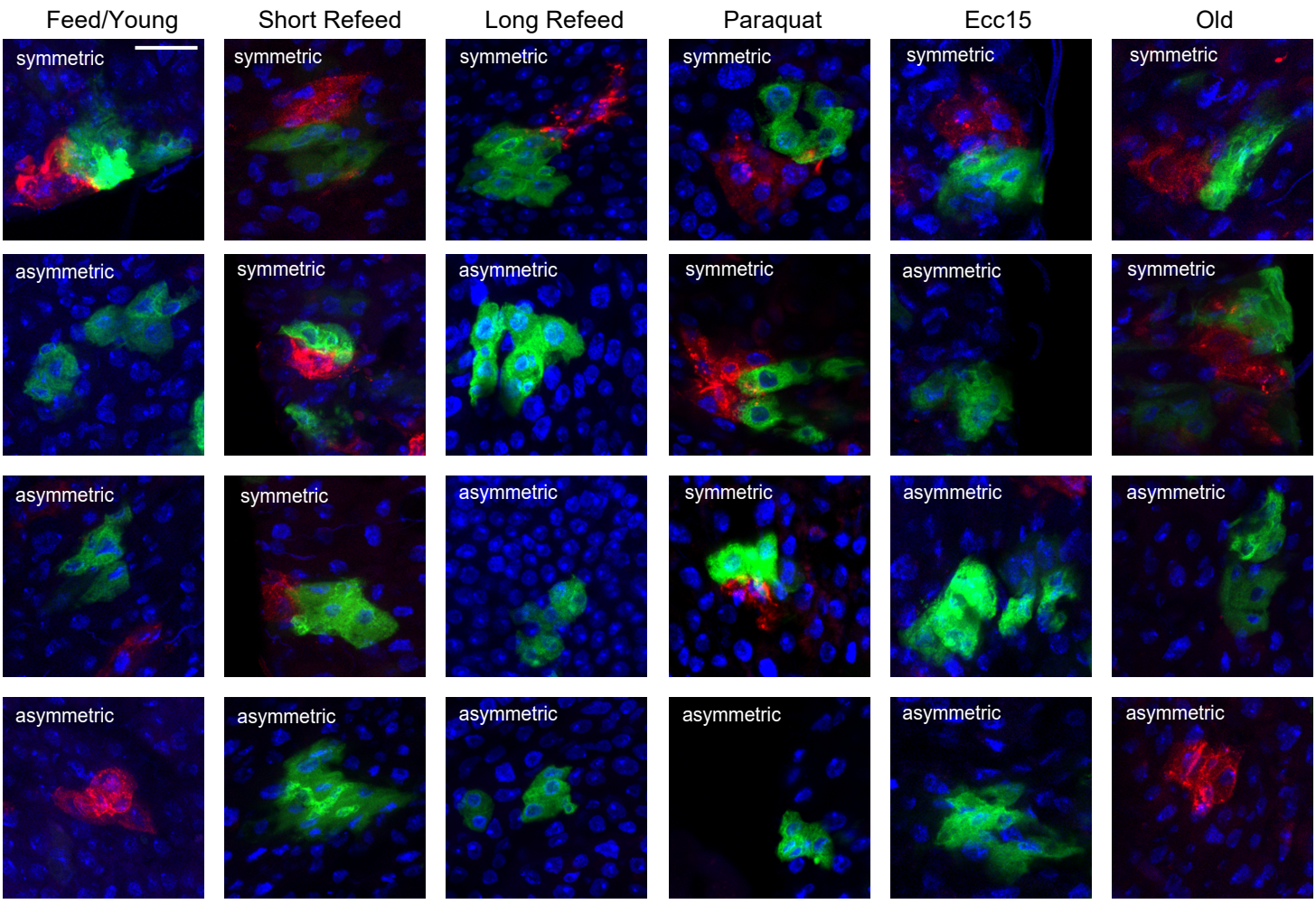

A
w1118
Feed
Fast
Short RF
#PH3+ cells/gut
***
***
B
w1118
Feed
Fast
Long RF
Gut Length (mm)
***
***
Feed
Fast
Long RF
C
Metaphase
Anaphase
γtubulin
PH3
αtubulin
D
Viking-GFP
Phalloidin
DAPI
3D-Reconstruction
apical
Viking-GFP
basal
Phalloidin
DAPI
E
Metaphase (symmetric)
3D-Reconstruction
9°
αtubulin
PH3
Phalloidin
Anaphase (symmetric)
0°
Anaphase (asymmetric)
apical
36°
basal
F
w1118
Mock
Paraquat
Ecc15
#PH3+ cells/gut
***
***
G
w1118
Mock
delta
delta
DAPI
Paraquat
Ecc15
H
w1118
w; esg::gal4; SuH::gal80, tub::gal80ts / UAS::eb1-GFP
Spindle angle
N.S.
Fix
Live
Short RF
Δ° of spindle orientation metaphase / anaphase (z-axis)
Short RF

Figure S1: Visualizing spindle orientation and additional characterization of the *Drosophila* intestine after fasting/refeeding, Paraquat treatment, and *Ecc15* infection, Related to Figure 1.

A. The number of anti-phosphohistone H3+ (PH3) cells in the entire gut decreased after fasting, but recovered after refeeding. B. *Drosophila* intestines substantially decreased in size after fasting, but recover after refeeding. C. Triple staining with anti-γ-tubulin, anti-PH3, and anti-α-tubulin labelling centrosomes and the microtubule network of the mitotic spindle in dividing ISCs. D. 3-D reconstruction of the *Drosophila* intestine to visualize spindle orientation along the apical/basal axis. Fluorescently tagged collagen (Vkg-GFP) or F-actin (Phallodin staining) labeled the basement membrane. E. Spindle orientations were quantified in anaphase ISCs in the posterior midgut by measuring the angle of the line bisecting segregating chromosomes with the basement membrane. F. Mitotic activity of ISCs in the entire gut, as determined by anti-PH3 staining, increased after Paraquat treatment or *Ecc15* infection. G. Representative images of ISCs (as determined by Delta staining) in the posterior midgut after mock or Paraquat treatment, or *Ecc15* infection. Insets depict a likely clone. H. Spindle orientation in live ISCs phenocopied fixed ISCs with a shift to planar spindles. On average, spindle orientation did not change dramatically between metaphase and anaphase. mean ± SD (B) or mean ± SEM (A,F); n≥20 flies (A,F), n≥10 flies (B), spindle orientation was collected from 15 fixed intestines and 6 live intestines (H); N.S. = not significant, ***P<0.001, based on Kruskal-Wallis test (A,B,F), Mann-Whitney test (H). Red bar = mean (H). Scale bar = 500μm (B), 5μm (C), 50μm (D), 20μm (G).

Figure S2

A



B

	Feed/Young		Short Refeed		Long Refeed		Paraquat		Ecc15		Old	
	#GFP+ Cells	#RFP+ Cells	#GFP+ Cells	#RFP+ Cells	#GFP+ Cells	#RFP+ Cells	#GFP+ Cells	#RFP+ Cells	#GFP+ Cells	#RFP+ Cells	#GFP+ Cells	#RFP+ Cells
Clone 1	0	10	5	27	7	12	4	9	12	0	4	3
Clone 2	7	7	7	11	8	0	37	0	10	0	7	5
Clone 3	8	1	6	9	21	23	7	5	11	0	15	15
Clone 4	9	0	9	5	3	0	9	4	8	0	12	9
Clone 5	10	1	0	7	3	0	5	5	10	6	5	0
Clone 6	0	7	12	5	4	0	20	18	19	1	17	16
Clone 7	0	4	6	3	8	0	43	13	6	0	0	5
Clone 8	6	7	11	5	7	0	3	5	11	1	11	11
Clone 9	8	3	14	0	5	0	5	0	9	0	8	3
Clone 10	8	0	0	8	5	0	6	0	9	4	9	0
Clone 11	5	0	7	3	5	0	4	0	7	11	6	0
Clone 12	6	0	7	6	5	0	5	4	3	3	7	9
Clone 13	5	0	5	7	5	0	7	3	0	4	8	5
Clone 14	7	0	0	14	3	9	27	5	0	8	3	0
Clone 15	8	0	0	13	5	0	0	18	5	0	12	12
Clone 16	8	1	5	3	3	8	5	8	4	0	0	16
Clone 17	9	4	0	7	13	4	16	0	10	5	7	0
Clone 18	7	0	4	4	47	21	21	23	0	7	7	11
Clone 19	4	1	0	5	12	0	4	3	7	3	14	6
Clone 20	4	3	5	5	33	0	17	11	4	0	11	0

Figure S2: Additional Twinspot images and quantification, Related to Figure 2.

A. Examples of Twinspot clones deriving from symmetric or asymmetric fate specifications. B. Quantification of the number of RFP+ and GFP+ in randomly selected clones. n = 20 clones from at least five flies (B). Scale bar = 20μm.

Figure S3

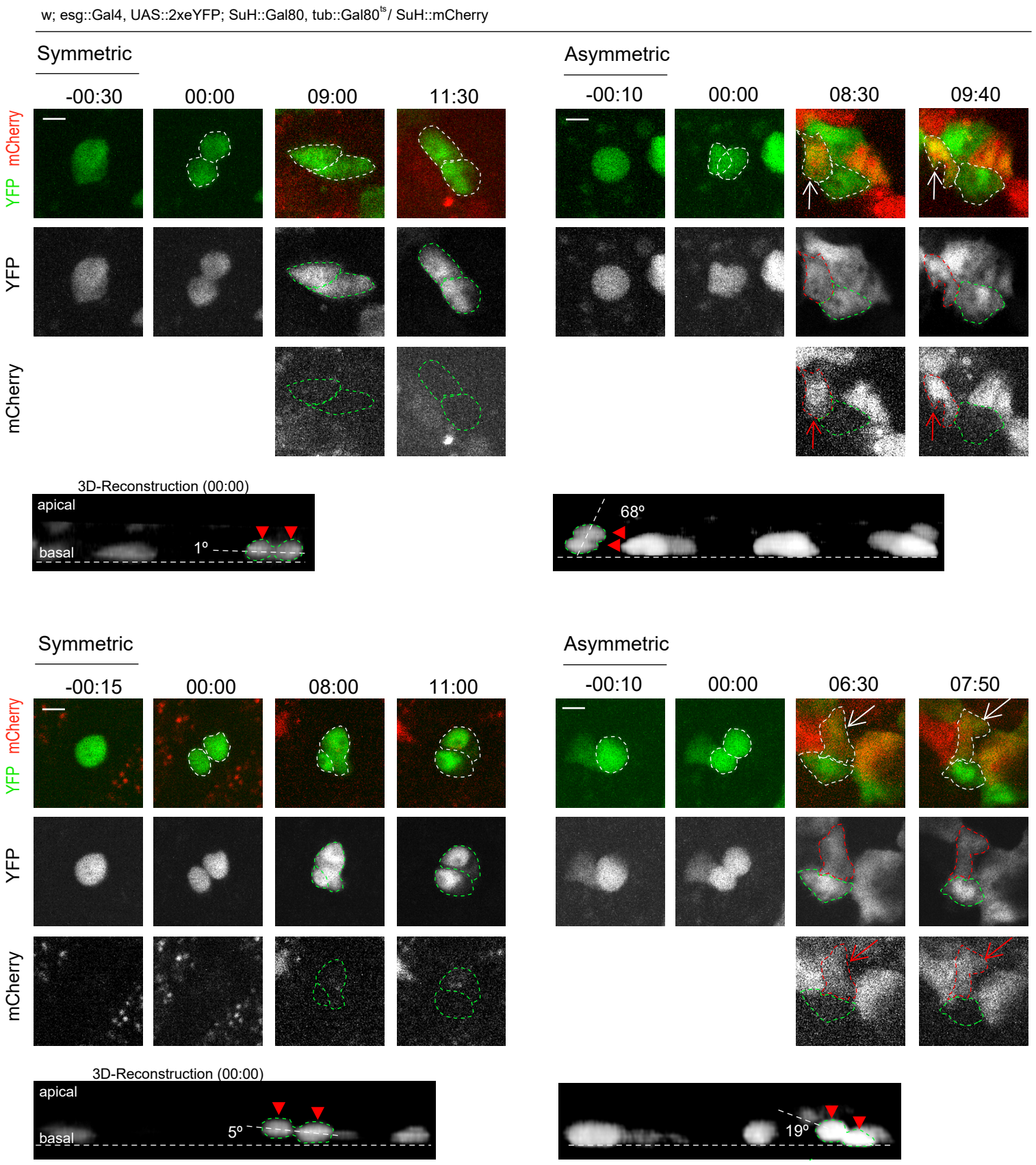
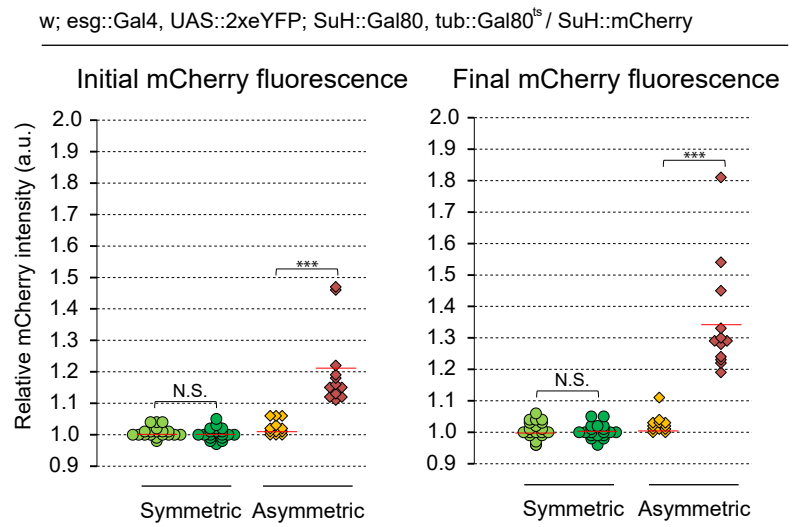

w; esg::Gal4, UAS::2xeYFP; SuH::Gal80, tub::Gal80ts/ SuH::mCherry
Symmetric
-00:30
00:00
09:00
11:30
Asymmetric
-00:10
00:00
08:30
09:40
YFP mCherry
YFP
mCherry
3D-Reconstruction (00:00)
apical
basal
1º
68º
Symmetric
-00:15
00:00
08:00
11:00
Asymmetric
-00:10
00:00
06:30
07:50
YFP mCherry
YFP
mCherry
3D-Reconstruction (00:00)
apical
basal
5º
19º

Figure S3: Additional examples of lineage tracing from long-term live imaging experiments, Related to Figure 2.

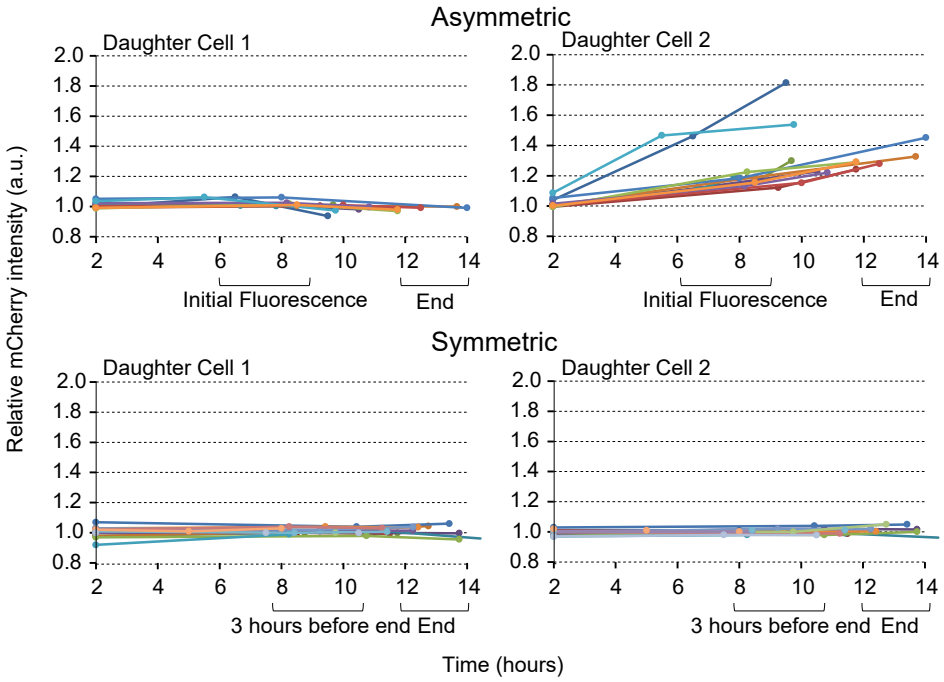
Long-term live imaging was performed on *ex vivo* intestines for 10-15 hours, time (hours:minutes). ISCs expressed eYFP while enteroblasts (EBs) expressed mCherry. mCherry was not imaged for the first two hours to reduce phototoxicity. Telophase/cytokinesis was set at 00:00 (hours:minutes). During a symmetric outcome, the dividing cell (-00:30 for example 1; -00:15 – 00:00 for example2) forms two YFP+ mCherry- daughter cells that remain mCherry- for the duration of the movie (11:30 for example 1; 11:00 for example 2). During an asymmetric outcome ,the dividing cell (-00:10 – 00:00) forms two daughter cells: One of the daughter cells remains mCherry- while the other daughter cell becomes mCherry+ (arrow, 08:30 for example 1; 6:30 for example 2). Cell bodies are outlined with green for mCherry- and red for mCherry+. Insets depict 3D-reconstruction of the two segregating cell bodies during telophase/cytokinesis (arrowhead and outlined). The representative symmetric lineages were generated from a planar spindle (1° and 5°) while the representative asymmetric lineages were generated from a more oblique angle (68° and 19°). Scale bar = 5μm.

Figure S4

A



B



C

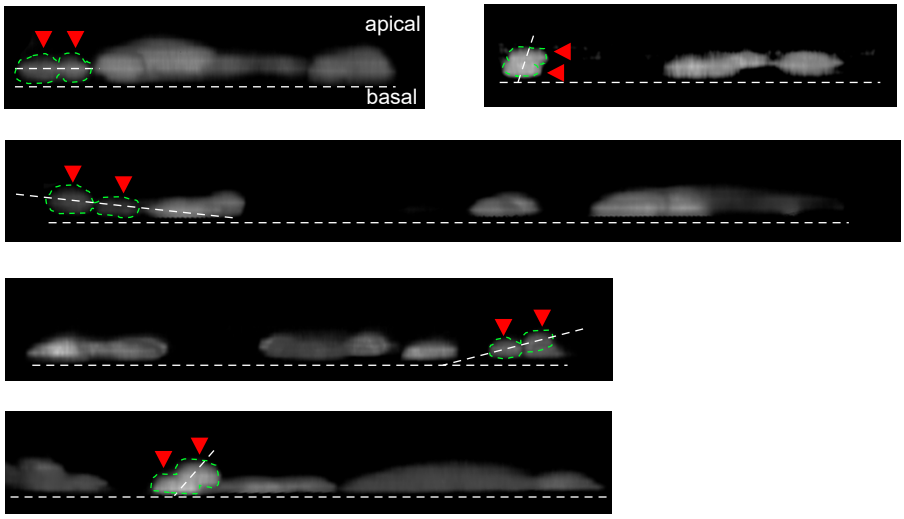


Figure S4: Additional characterization of lineage tracing from long-term live imaging experiments, Related to Figure 2.

Long-term live imaging was performed on *ex vivo* intestines for 10-15 hours to lineage trace cell fates of daughter cells. A. mCherry fluorescence, normalized to background, was measured from both daughter cell pairs (greens and yellow/red) of 31 randomly selected dividing ISCs. During an asymmetric outcome, intensity was measured when fluorescence was first visible (initial mCherry fluorescence; 6-9 hours post mitosis) and at the end of the timelapse (final mCherry fluorescence; generally 11-13 hours post mitosis). High fluorescent intensity was observed in only one of the daughter cell (red). During an outcome scored as symmetric (in which neither cell became mCherry+ at the end of the timelapse), fluorescence was measured at the end of the timelapse (generally 11-14 hours post mitosis) and three hours prior (generally 8-11 hours post mitosis). B. Representation of mCherry fluorescence over time in 31 randomly selected asymmetric versus symmetric outcomes. Each trace represents a daughter cell. C. Additional 3D-reconstruction of dividing ISCs. Spindle angle is measured between the basement membrane estimated by the location of neighboring ISCs and the spindle estimated by the vector bisecting the two segregating cell bodies (green outline and red arrowhead). N.S. = not significant, ***P<0.001, based on Mann-Whitney test (A). Red bar = mean (A).

Figure S5

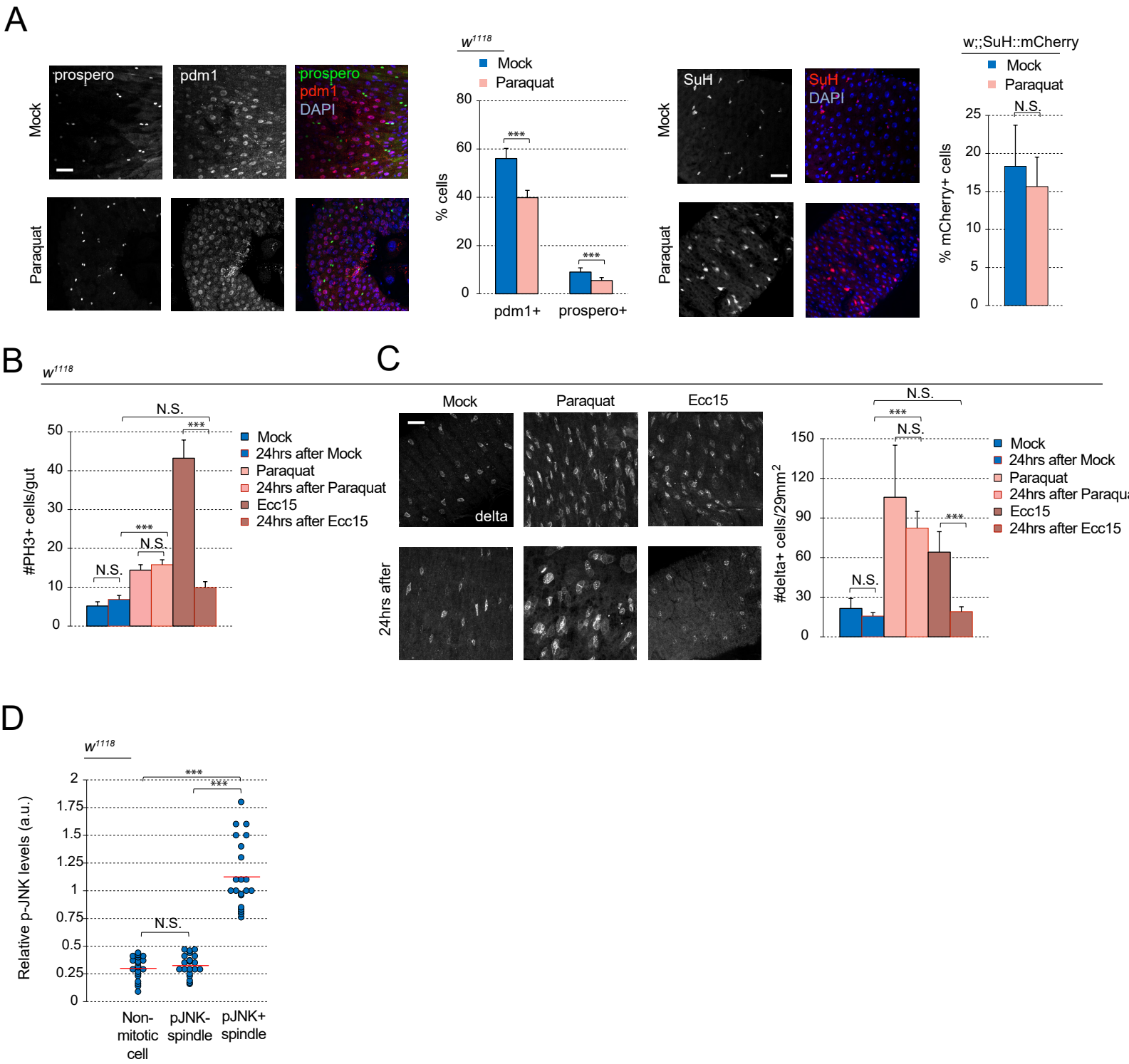

A
prospero
pdm1
prospero
pdm1
DAPI
Mock
Paraquat
w1118
Mock
Paraquat
% cells
pdm1+
prospero+
SuH
SuH
DAPI
w;;SuH::mCherry
% mCherry+ cells
N.S.
B
w1118
#PH3+ cells/gut
Mock
24hrs after Mock
Paraquat
24hrs after Paraquat
Ecc15
24hrs after Ecc15
C
Mock
Paraquat
Ecc15
delta
24hrs after
#delta+ cells/29mm2
D
w1118
Relative p-JNK levels (a.u.)
Non-mitotic cell
pJNK- spindle
pJNK+ spindle

Figure S5: Effects of Paraquat on intestine composition, Related to Figures 3.

A. Paraquat treatment decreased the ratio of enterocytes and enteroendocrine cells (as determined by pdm1 and prospero staining respectively), but did not significantly change the relative number of enteroblasts (as determined by a Su(H)::mCherry reporter). B and C. 8d flies were treated with 5mM Paraquat in 5% sucrose, *Ecc15* in 5% sucrose, or 5% sucrose alone (mock control) for 24 hours, and then transferred to normal food for an additional 24 hours. Flies treated with Paraquat did not recover after 24 hours on normal food, maintaining elevated mitotic activity and ISC number (as determined by PH3 and delta respectively). Flies infected with *Ecc15* recovered completely after 24 hours on normal food, with baseline mitotic activity and ISC numbers. D. Quantification of relative levels of phosphorylated JNK (pJNK) at a randomly selected subset of pJNK- versus pJNK+ spindles. mean ± SD (A,C) or mean ± SEM (B); n=9 flies (A,C), n=15 flies (B), n=21 cells from ≥13 flies (D); N.S. = not significant, ***P<0.001, based on Mann-Whitney test (A,B,C), Kruskal-Wallis test (D). Red bar = mean (D). Scale bar = 20μm.

Figure S6

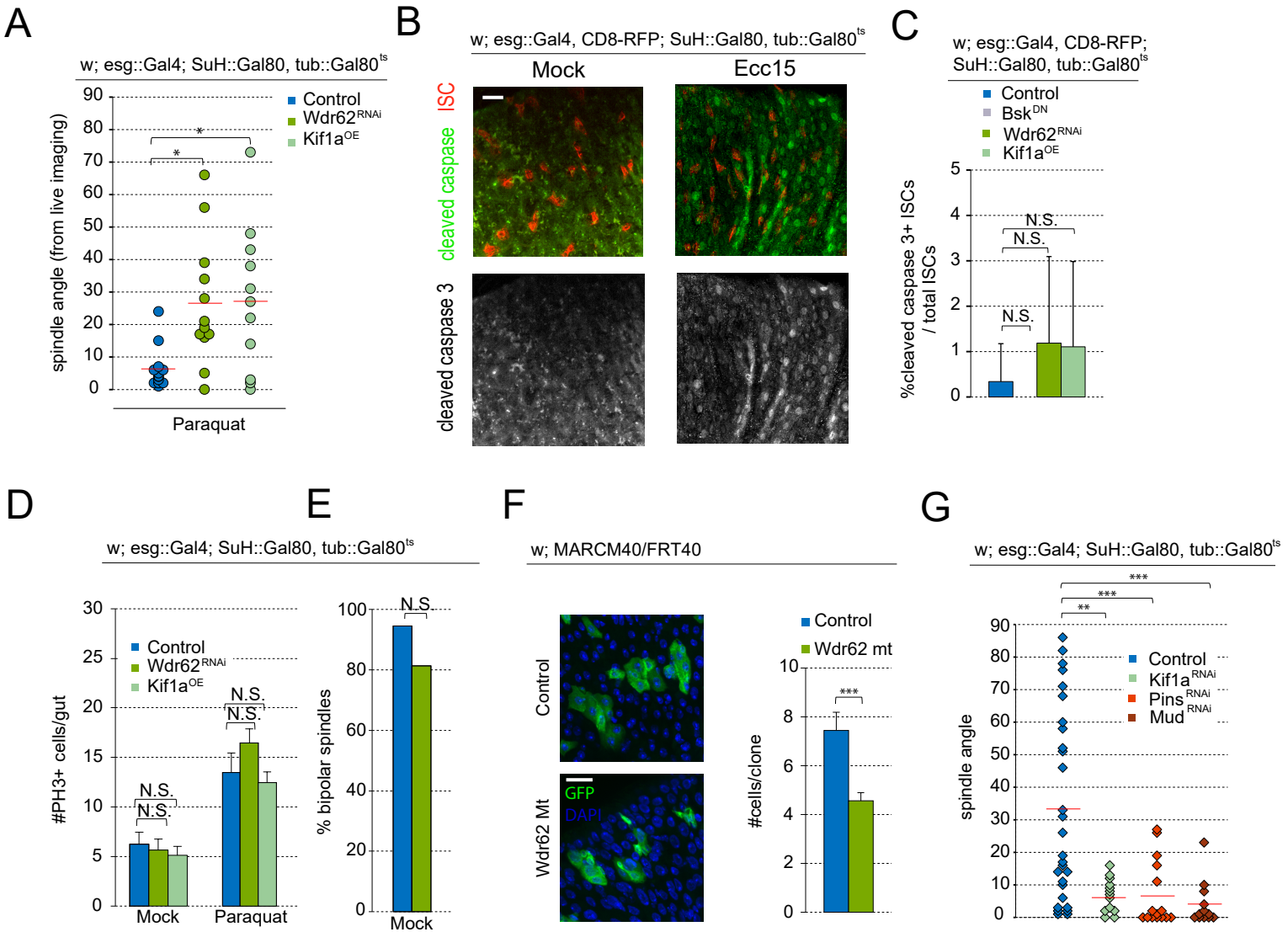
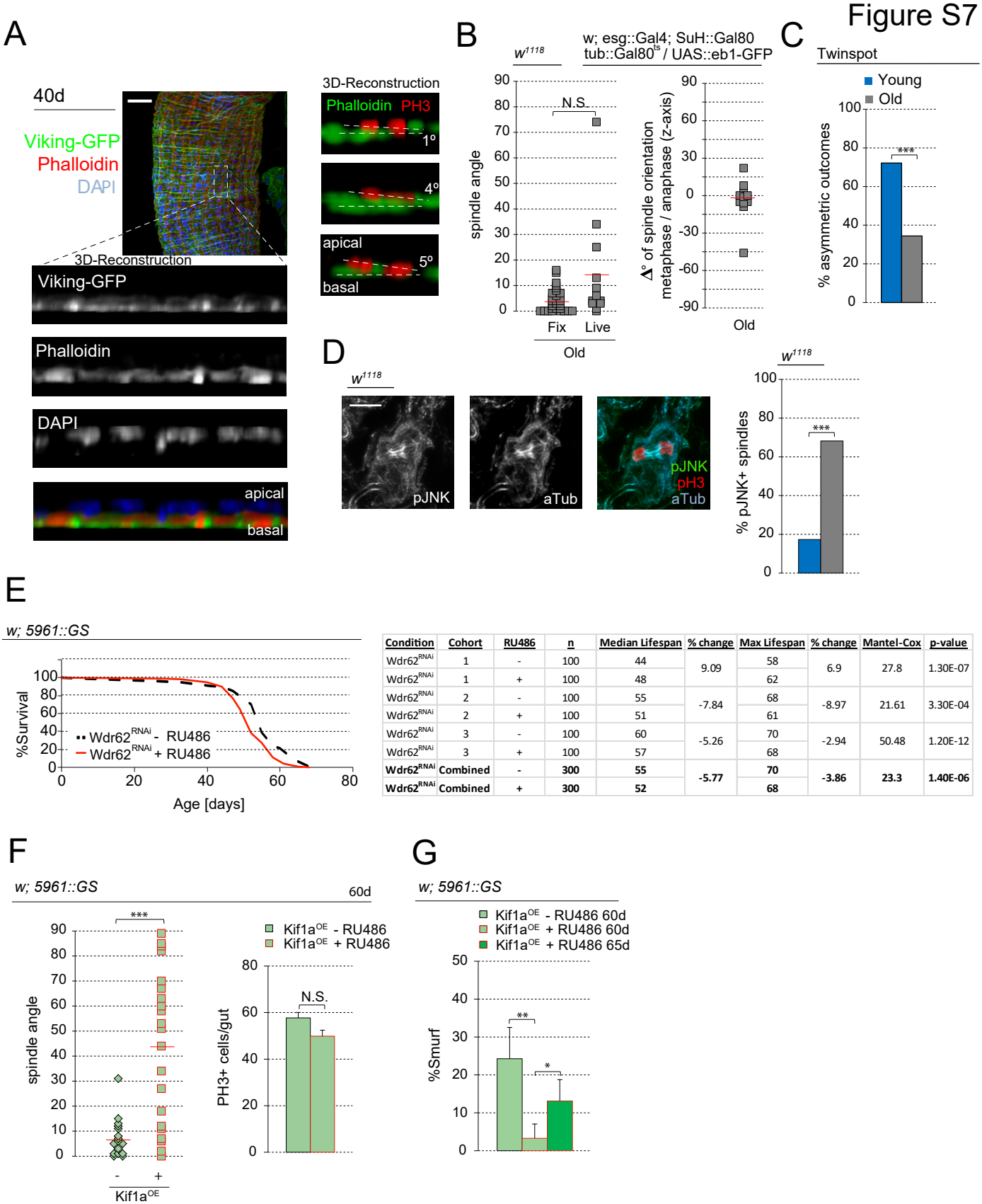


Figure S6: Expression of Wdr62 RNAi and full length Kif1a does not cause apoptosis or block mitosis, Related to Figure 4 and 5.

A. Spindle orientation was quantified by live imaging of *ex vivo* intestines. B. Cleaved caspase 3 staining was validated in *Ecc15*-infected intestine. After 8 hours post infection, many cells were positive for cleaved caspase 3 compared to mock controls. C. Expressing Bsk^{DN}, $Wdr62^{RNAi}$, or full-length Kif1a did not cause apoptosis in ISCs. D. Neither $Wdr62^{RNAi}$ nor $Kif1a^{OE}$ affected the number of PH3+ cells in the whole gut compared to controls. E. $Wdr62^{RNAi}$ did not significantly affect the percentage of bipolar spindles. F. MARCM clones from ISCs homozygous for $wdr62^{\Delta 3\text{-}9}$ are smaller in size than from control ISCs. G. Expression of alternate RNAi against Kif1a, Pins, or Mud also promoted planar spindles. Control data were taken from Figure 3B, justified by the pooling of cells from 25 flies from five independent experiments. mean ± SD (C) or mean ± SEM (D); n=7 flies (C), n≥20 flies (D), n=18 cells from ≥15 flies (E), n≥40 clones from 12 flies (F), spindle orientation was quantified from ≥7 flies (A), ≥10 flies (G); N.S. = not significant, *P<0.05, ***P<0.001, based on Kruskal-Wallis test (A,C,D,G), Chi-Squared test (E), Mann-Whitney test (F). Red bar = mean (D,G). Scale bar = 20μm.

Figure S7



Condition	Cohort	RU486	n	Median Lifespan	% change	Max Lifespan	% change	Mantel-Cox	p-value
Wdr62RNAi	1	-	100	44	9.09	58	6.9	27.8	1.30E-07
Wdr62RNAi	1	+	100	48		62			
Wdr62RNAi	2	-	100	55	-7.84	68	-8.97	21.61	3.30E-04
Wdr62RNAi	2	+	100	51		61			
Wdr62RNAi	3	-	100	60	-5.26	70	-2.94	50.48	1.20E-12
Wdr62RNAi	3	+	100	57		68			
Wdr62RNAi	**Combined**	**-**	**300**	**55**	**-5.77**	**70**	**-3.86**	**23.3**	**1.40E-06**
Wdr62RNAi	**Combined**	**+**	**300**	**52**		**68**			

Figure S7: Additional characterization of spindle orientation in aging ISCs, Related to Figure 7.

A. Spindle orientation is measurable in old flies despite age, with Viking-GFP and Phalloidin still lining the basement membrane. B. Spindle orientation in ISCs from live tissue was not significant different compared to ISCs from fixed tissue. On average, spindle orientation did not change dramatically between metaphase and anaphase. C. Twinspot analysis revealed an increase in symmetric division in old vs young mock-treated ISCs. Old ISCs were from 30d flies as most 40d flies did not survive heat shock. Young flies were taken from Figure 2A as clones were obtained from 10 flies in three independent experiments. D. pJNK localized to the spindle in most old ISCs. E. $Wdr62^{RNAi}$ expression, pooled from three independent experiments (graph), resulted in a modest decrease in lifespan (~6%). Within separate cohorts, $Wdr62^{RNAi}$ expression resulted in substantial increase in lifespan within one cohort, but decreased lifespan within two cohorts (statistical analysis detailed in the chart). RU486 was fed continuously from 10d. F. Spindle orientation remained largely oblique in long-lived survivors (60d) expressing full-length Kif1a. Kif1a expression did not rescue mitotic activity, as determined by PH3 staining. RU486 was fed continuously from 10d. G. Smurf assay was extended to 65d in Kif1a over-expressing flies (RU486 fed to 30d females continuously). %Smurfs increased from 60d to 65d. mean ± SD (C,D,F: spindle angle,G) or mean ± SEM (F: #PH3+ cells); n=133 and 58 clones from 10 young and old flies respectively (C), n=23 cells from 20 flies (D), n= 15 flies (F: #PH3+ cells), n=4 cohorts; ≥ 30 flies per cohort (G), spindle orientation was quantified from 15-18 flies per fixed condition (B, F: spindle angle) and 7 flies for live imaging (B); N.S. = not significant, *P<0.05, **P<0.01, ***P<0.001, based on Mann-Whitney test (B,F), Chi-Squared test (C,D), Kruskal-Wallis test (G). Red bar = mean (B,F: spindle angle). Scale bar = 50μm (A), 5μm (D).