

1 **Text S2: Comparison of isolated crypt analyses with histological sectioning data.**

2 This crypt isolation and 3D imaging has an advantage over histological sectioning in that
3 each crypt can be scored in 3 dimensions instead of one plane. It further improved the speed,
4 efficiency and resolution of scoring the budding crypts when compared to micro-dissection
5 [1,2,3] or serial sectioning [4]. The thickness of paraffin-embedded or frozen tissue sections
6 is not accurate due to rough surfaces, undulations and inclinations with standard errors of up
7 to 10% [5]. This causes inaccuracy in serial reconstruction. The accuracy of reconstruction is
8 greatly improved using confocal sectioning as the slices are continuous. The removal of the
9 mucosa layer from the crypt also enables direct identification of crypt surface and shape.
10 These advantages allow finer details (i.e. stage 1 onset of fission process) of the budding
11 process previously hidden among the sectioning gaps to be identified. From the data
12 presented here, the colonic crypt production is primarily due to asymmetrical budding rather
13 than symmetrical crypt fission [2,6].

14 As the animal ages, the budding frequency decreases and crypt production slows to a rate
15 sufficient for maintenance and turnover (to maintain a stable crypt number). It is still unclear
16 whether new crypt production is required to replace crypts lost through mechanical damage
17 or whether all crypts have a definite lifetime. From our studies, the length of crypts from the
18 proximal regions is shorter than the other regions of the colon. This observation is in
19 agreement with Wright and Alison [7] who documented a smaller number of cells in
20 proximal colon crypt. After birth, fission rate is high to populate the growing colon with
21 sufficient crypts.

22 In early 1980s, Bjerknes and Cheng [8] measured Swiss albino mice colon crypt from
23 perfusion isolated epithelium, estimating the crypt length to be $275 \pm 112 \mu\text{m}$ (SD, $n=36$)
24 while Goodlad and Wright [9] measured the crypts from Balb-c mice using microdissection,
25 estimating an average crypt length of $282.9 \pm 96.0 \mu\text{m}$ (S.D, $n=45$) and basal crypt width of
26 $44 \mu\text{m}$ (calculated from data reported in ref [9]). They measured crypts from the whole colon
27 without distinguishing the regions (proximal, middle and distal). The width reported by
28 Goodlad and Wright is in range with the measurements made in this study (i.e. basal width of
29 $42 \pm 7 \mu\text{m}$ for 40 weeks old C57BL/6) although the average crypt length ($156 \pm 30 \mu\text{m}$, 40 weeks
30 old, all regions) reported here is shorter than both Goodlad and Wright [9] and Bjerknes and
31 Cheng [8].

32 To make sense of the differences in crypt length reported, firstly it should be taken into
33 account the different mouse strains used; Swiss albino mice are significantly larger in size
34 than C57BL/6. Secondly during microdissection, primarily the large prominent crypts are
35 scored (i.e. smaller crypts might be missed out) due to the low sampling numbers.
36 Furthermore, in the 1980s, Goodlad and Wright used a visual measurement technique with a
37 calibrated eyepiece graticule attached to the microscope while Bjerknes and Cheng measured
38 crypt lengths off a 100x magnified image projection on a screen. We believe that the change
39 from manual to digital measuring techniques may account for the differences in crypt length
40 reported then and in this study. With the advancement of optical imaging, accurate digitised
41 measurement is now possible. In this study, over two thousand individual crypts were
42 isolated and scored for a population mean of crypt lengths in the colon using high resolution
43 quantitative confocal microscopy measurement. The measurements obtained have
44 substantially smaller variations than previously described (SD of 30 μ m compared to 96 and
45 112 μ m). We also distinguish between the different regions of the colon (i.e. proximal,
46 middle and distal) which have been shown to have significantly different in crypt lengths
47 (Figure 2C).

48 To further validate the accuracy of the crypt length measurements used in this study, we
49 compared crypt lengths estimated from sectioned samples from the proximal, middle and
50 distal regions of the colon from C57BL/6 mice. Sectioning results were in a similar range to
51 that reported in this study using isolated crypts confocal microscopy technique (Figure S5). It
52 is also clear from Figure S5 that only limited number of whole crypts can be scored in each
53 section due to obscuration from view of portions of crypts due to sectioning (white arrows).
54 This evidence supports the notion that the crypt isolation technique described in this study is
55 a more efficient and accurate method for studying crypt morphology.

56 This isolation method was particularly appropriate for colon as it preserves the whole crypt.
57 In the small intestine, the villi are usually lost along with the top portions of the isolated
58 crypts, making crypt identification more difficult. The crypts from the proximal regions of
59 the colon tended to be more fragile and a different preparation (see material and methods
60 section) is required to isolate intact proximal crypts.

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