

1 **Text S1: Image processing and compartmental analysis.**

2 The image stacks acquired from the confocal microscope are in the Olympus Image Binary  
3 (OIB) format. This was converted to a portable tiff format readable by MATLAB [1] using  
4 Fiji/ImageJ [2] with the Bio-Format plugin. Import of 3D image stacks: Individual region 3D  
5 stacks of the regions defined in Figure S3A were also extracted using the drawing tools in  
6 Fiji/ImageJ. The extracted regional image stacks were saved as separate tiff files. Both the  
7 whole and region tiff files were imported into MATLAB for processing and analysis.

8 Image processing (Import and pre-processing): For every tiff file (both regions and whole  
9 crypt), the intensities for the different fluorescence channels were separated as shown in  
10 Figure S1 (Step A). In this study, DAPI (blue),  $\beta$ -catenin (green) and E-cadherin (red)  
11 channels were separated into their respective mono-colour stacks (Figure S1 Step A, Figure  
12 S2A and Figure 5). For each channel (mono-colour) signal stack, selective filtering and  
13 image intensity threshold were applied and the region of interest isolated. Specifically, DAPI  
14 marked the nuclei compartment, E-cadherin outlined the membrane boundary and  $\beta$ -catenin  
15 marked primarily the membrane and cytosol. 3D binary masks were generated for each signal  
16 (Figure S1, Step C).

17 Image processing (Compartment Masks): To generate the compartment masks, mathematical  
18 operations were applied to the signal intensities tiffs/masks. Three compartments (nuclear,  
19 cytosol and membrane compartments) were defined and used in this study. The nuclear mask  
20 was generated from the DAPI signal (Figure S2B). As observed from the individual signal  
21 images (Figure 5), both E-cadherin and  $\beta$ -catenin were primarily localised to the membrane.  
22 Taking into account the heterogeneity observed in the E-cadherin staining, in order to  
23 enhance the membrane signal's contrast and continuity, an "OR" operation was applied  
24 between the E-cadherin and  $\beta$ -catenin intensities image stack (see Figure S1, Step B and  
25 Figure S2C) and segmented to acquire an enhanced membrane mask. A "NOT" operation  
26 was applied between enhanced membrane mask and the nuclear mask to determine the final  
27 membrane mask (Figure S2C). A whole cell mask was generated using the DAPI nuclear and  
28 E-cadherin membrane masks (Figure S2D) and the cytosol mask was formulated from the  
29 difference between the whole cell mask, the nucleus and membrane masks (Figure S2E).

30 Image processing and Analysis (Signal Compartmentalisation): The 3D binary compartment  
31 masks (membrane, cytosol and nuclear, Figure S1 Step C) were applied over the  $\beta$ -catenin  
32 and E-cadherin 3D signal channels to quantify the expression and localisation of the signal

33 intensities in the respective compartments (Figure S1 Step D, Figure S3B-D). The total 3D  
34 intensities of signal residing in each compartment were quantified (Figure S4) and tabulated,  
35 together with the volume of each compartment (in the form of total pixels/voxels). These  
36 intensity and volume data were consolidated for all region image stacks. The integrated  
37 (relative) intensities per pixel for each crypt, compartment and region of the crypt were  
38 calculated and analysed statistically. These data provided compartment proportion  
39 information for each region as well as the change in intensities both in terms of subcellular  
40 compartments and regions along the length of the crypt.

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42 **References:**

- 43 1. Mathworks (2007) MATLAB. version. 7.4.0.287 (R2007a) ed: The Mathworks, Inc.
- 44 2. Abramoff MD (2004) Image processing with ImageJ. Biophotonics Int 11: 36-42.

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