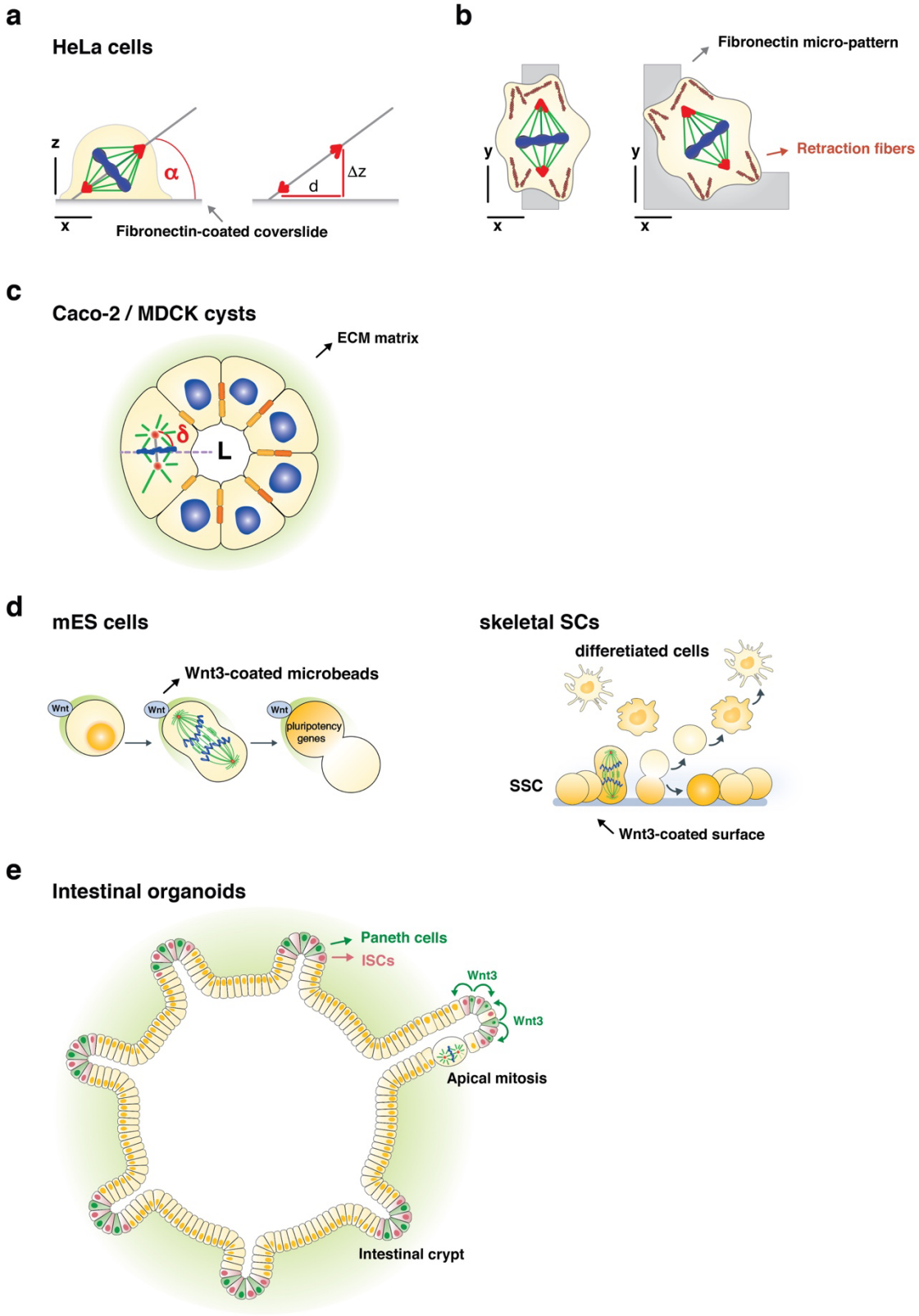

Figure S1

Supplementary Box 1: Experimental assays employed to study spindle orientation mechanisms

Several imaging-based assays can be employed to study mechanisms of spindle orientation *in vitro*, either in metaphase or in anaphase. It is important to stress here that the molecular events underlying spindle orientation during mitotic progression are substantially different, therefore it is important to measure the spindle axis angle in the mitotic phase one wish to analyze, generally metaphase or anaphase. Here we survey the most common ones.

1. Adherent cells on fibronectin-coated coverslips. The easiest assay to assess integrin-mediated division orientation of cultured epithelial cells is to plate them on fibronectin-coated coverslips, and measure the angle between the mitotic spindle axis and the substrate, which is around zero for wild-type cells[1] (see figure part **a**). Technically this can be performed in a number of ways. The easiest approach is to visualize by immunofluorescence or fluorescent probes the spindle poles (with γ-tubulin for instance) and the chromosomes, and acquire a confocal x-z optical section passing through the poles. The orientation angle in metaphase can be directly measured as the angle between the spindle axis and the substratum (figure part **a**, left). Alternatively, it is possible to acquire several confocal x-y stacks of fixed metaphase cells, and calculate the spindle inclination using the formula arctan(Δz/d), with d being the distance between centrosomes in the maximum intensity projection, and Δz being the projection of the centrosome distance in the z axis (figure part **a**, right). Measurements of the spindle alignment to the substratum in anaphase are conducted similarly, oftentimes using the lines connecting separating sister chromatids rather than centrosomes.

2. Division orientation on ECM pattern. Pioneering studies in the Bornens and Thery lab introduced the use of micro-contact printing to generate extra-cellular matrix patterns of defined shapes that ca ben used to evaluate the retraction-fibers-mediated spindle orientation in the x-y plane [2,3]. In the years, the most widely used patterns have

been L-shaped and striped pattern (see figure part **b**). Notably, ECM pattern on stretchable substrates have become instrumental to analyse orientation response to tension at a single cell level or in monolayered epithelia[4].

3. Three-dimensional cysts. Molecular events coupling epithelial polarity to division orientation have been investigated in 3D cysts of human intestinal Caco-2 cells and canine kindey MDCK cells [5]. These cells, when plated in matrigel, undergo planar divisions and grow in monolayered spheres with the apical side facing the lumen. Defects in orientation mechanism results in multi-lumen cysts or disorganized cellular spheres (see figure part **c**). Scoring of the multilumen cysts combined with imaging of the spindle axis alignment to apico-basal polarity in cells provide information on mechanisms of spindle coupling to epithelial polarity.

4. Wnt3-coated surfaces. Filming mitoses of cells in contact with microbeads coated with Wnt3-lipidated ligands is becoming a popular tool to analyse coupling of division orientation to localized Wnt signals, mimicking niche microenvironment [6]. This experimental setting was successfully used to monitor asymmetric division of murine embryonic stem cells (see figure part **d**). An interesting variant developed by the Habib lab are Wnt3a-coated platforms that ca be employed to culture stem cells in matrigel to follow differentiation programs [7].

5. Organoids. The implication and impact of division orientation in the morphogenesis and homeostasis of epithelial tissues can be evaluated in organoid cultures, recapitulating the complexity of tissual architecture and signalling. Intestinal organoids, growing from doublet of intestinal stem cells and niche-acting Paneth cells, are ideal to study spindle orientation mechanisms as they form monolayered crypts enriched in proliferating cells, and undergo Wnt3-dependent oriented divisions promoting migration along the crypt accompanied to differentiation [8] (see figure part **e**). Interestingly, mini-guts grown from intestinal cells isolated from wild-type mice might

also allow the study of reinsertion mechanisms known to correct some type of misoriented divisions in living organisms[9], which do not seem not be active in Caco-2 and MDCK cysts, to the best of our knowledge.