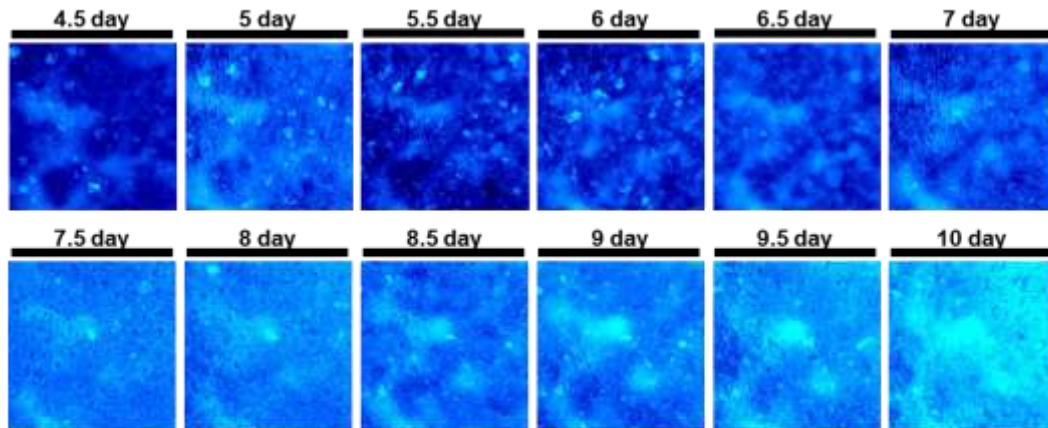
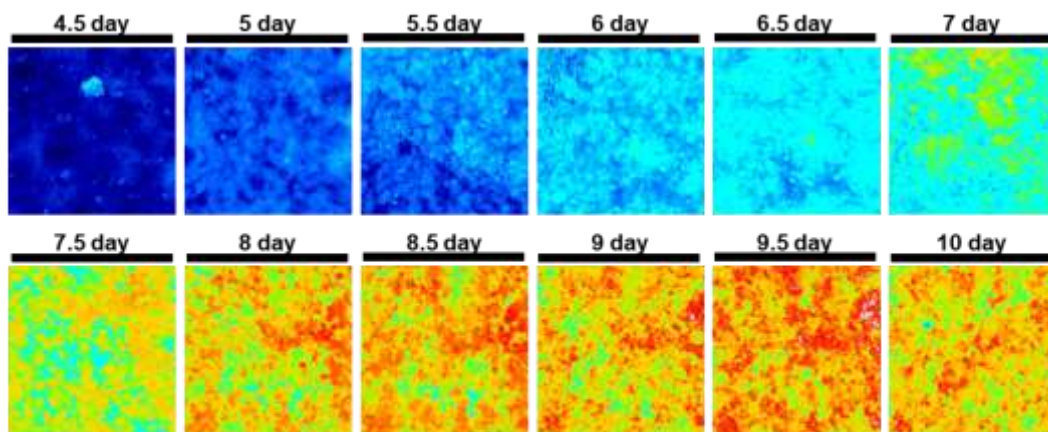


1

A Normal Culture



B ALI Culture



2

3

4

5

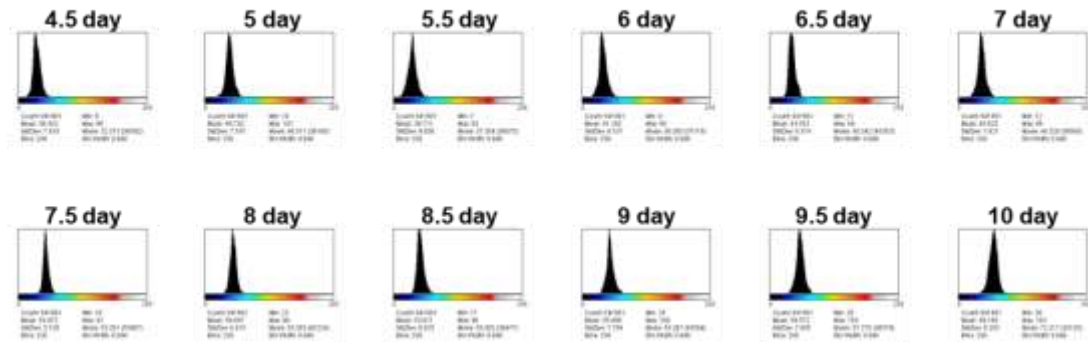
6

7

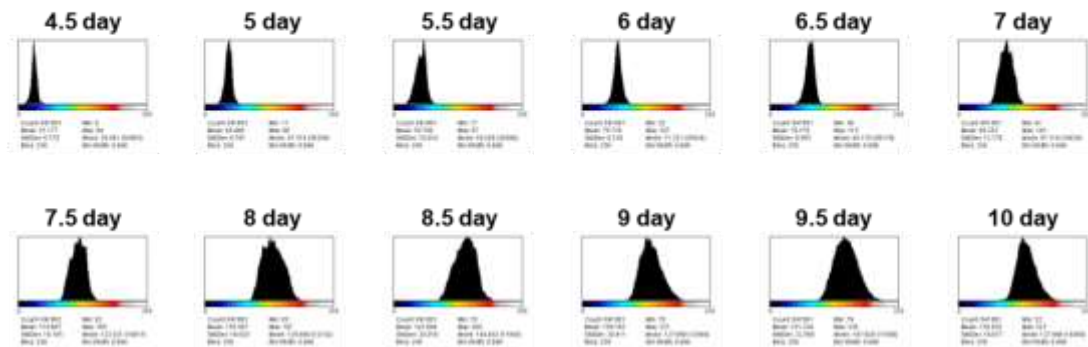
8

Supplementary Figure S1. ALI thickens the cell three-dimensionally. hICs were cultured in Normal and ALI culture methods for 10 days. The same position was imaged every 12 hours using Cell3iMager Estier. The images were processed by ImajeJ to create a heat map to visualize the three-dimensional structure. Before imaging, dead cells accumulated on the hIC were washed away, and 50 μ L of medium was added to the insert to prevent diffuse reflection of light before imaging.

A Normal Culture

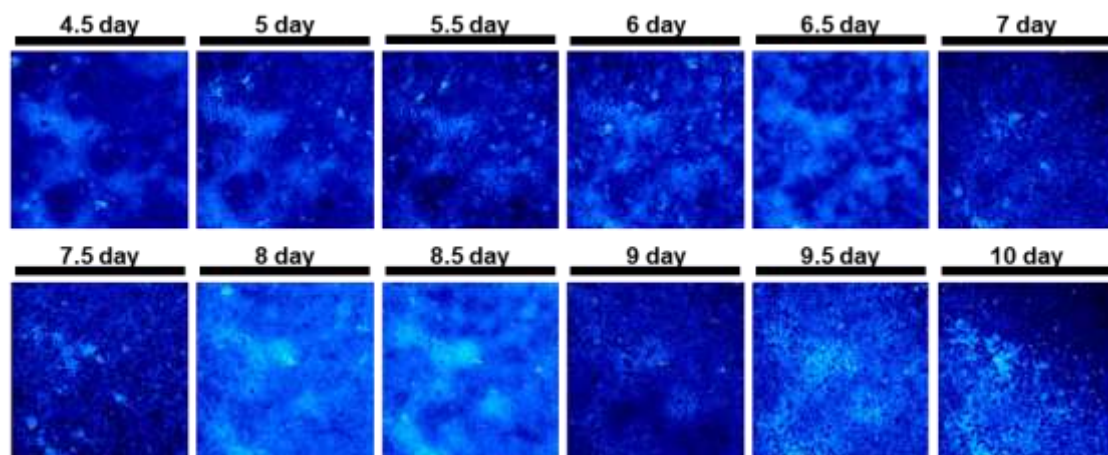


B ALI Culture

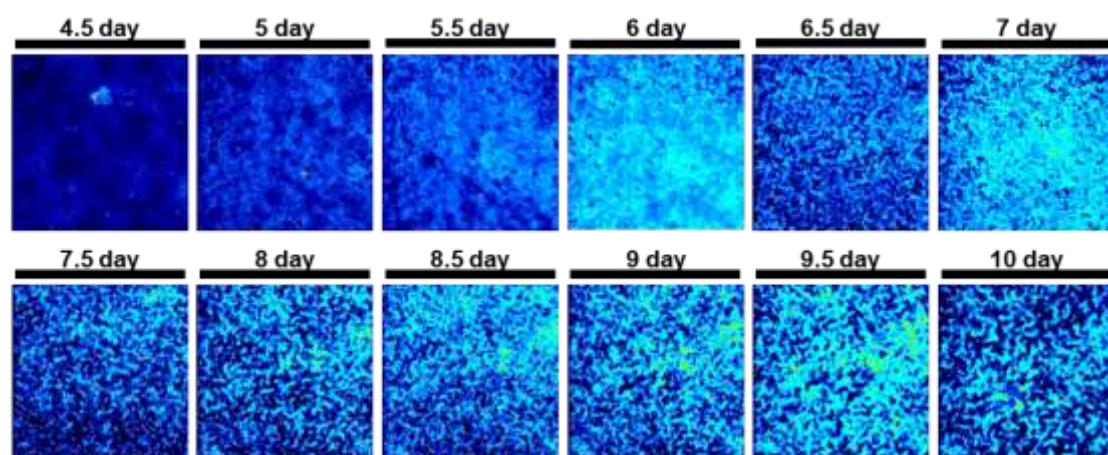


Supplementary Figure S2. ALI causes variation in cell thickness. To make it easier to understand the thickness variation of the hICs, histograms were created. Since the same imaging datas were used as in Figure 12, the heatmap of Figure 12 was converted to a histogram. The thickness was quantified by ImajeJ.

A Normal Culture



B ALI Culture



Supplementary Figure S3. ALI culture creates a convex structure. We observed how the morphology of hIC changed over time. A Cell3iMager Estier was used for imaging, and the same locations were imaged every 12 hours until day 10 of culture. Dead cells on the hIC were removed prior to imaging to allow clear observation of the structure, and the maxima of the villi were marked by ImajeJ. White dots indicate convexity.