SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Paired shedding of neighboring cells. Images overlaying ZO-1 (*red*) and Hoechst 33342 (*blue*) are shown in a representative time course. White dashed rectangle encloses two shedding cells. By 13 min, cell 1 starts shedding, manifested by ZO-1 redistribution and nucleus displacement from the baseline (dotted blue line along the adjacent cell nuclei) and eventually leading to cell extrusion by 26 min. Nine min after this shedding event, the neighboring epithelial cell (2) starts shedding as well. Scale bar = 5 µm. (actually I would like to keep this figure)

Supplemental Figure 2. Cell nucleus condensation after shedding. *A)* Average intensity of nucleus fluorescence (Hoechst 33342) was measured during cell shedding (closed circles), in comparison with non-shedding control cells (open circles). *B*) Compiled data demonstrate that the size of the nucleus of the shedding cell (left panel) is not measurably different comparing before the start of ZO-1 redistribution (b) and during cell shedding (d), but increases directly at the first time point after the cell has been detached from the monolayer (a). Adjacent cells that are not shed (control, right panel) do not change throughout the shedding of the neighboring cell. *$P < 0.01$ comparing 'b' *vs* 'a' mean values (n = 18).

Supplemental Figure 3. Proposed model of tight junction rearrangement during cell shedding of an epithelial cell in the mouse jejunal villus. The model starts at t = 0 min, when ZO-1 redistribution starts. By 15 min the cell nucleus starts to move upwards while ZO-1 has moved downward to regions of the basolateral membrane that surround the nucleus in a funnel-shape. The cells is extruded at 30 min, leaving an expanded space underneath the shed cell location, with a residual ZO-1 patch in the epithelial layer. By 45 min, ZO-1 has returned to a normal distribution at the apex of the epithelial layer, and the gap has been closed at the site of shedding.

Supplemental Figure 4. Distinct staining patterns comparing *en face* views between the nuclear stains Hoechst 33258 (*A*) and Hoechst 33342 (*B*). Hoechst stains are shown *blue-cyan*, ZO-1 *red*. "True gaps" (circles) do not reveal any nucleus staining with either Hoechst dye throughout Z-axis microscopy and show remnants/patches of ZO-1 at the surface of the epithelial layer where they contact neighboring cells. "False gaps" (arrows) show faintly stained deeply located nuclei (8-16 μm depth) with Hoechst 33342, no visible nuclei with Hoechst 33258, and have no accumulated ZO-1 in the cytoplasmic periphery. Scale bar = 5 μm.