Marchiando et al., http://www.jcb.org/cgi/content/full/jcb.200902153/DC1

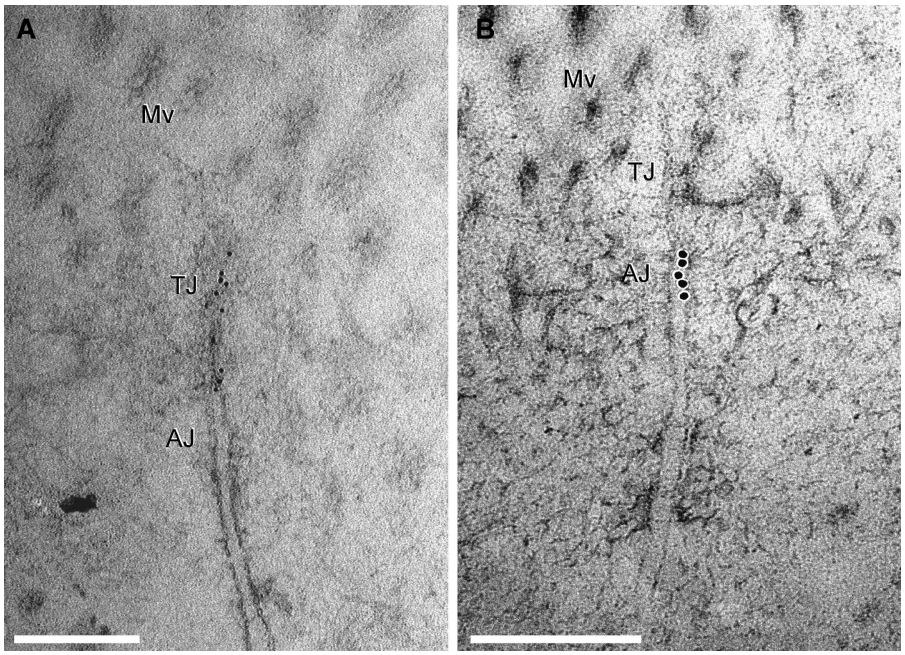


Figure S1. **Controls for occludin and caveolin-1 immunogold double labeling in jejunal enterocytes of wild-type mice.** High pressure–frozen, freeze-substituted, cryoembedded specimens were double immunolabeled with either anti–caveolin-1 or antioccludin omitted followed by secondary 10 nm and 15 nm gold-conjugated antisera to detect antioccludin and anti–caveolin-1, respectively. (A) Occludin is labeled with 10 nm gold, but no 15 nm gold is detected when anti–caveolin-1 antiserum is omitted. (B) Caveolin-1 is labeled with 15 nm gold, but no 10 nm gold is detected when antioccludin antiserum is omitted. AJ, adherens junction; Mv, microvilli; TJ, tight junction. Bars, 300 nm.

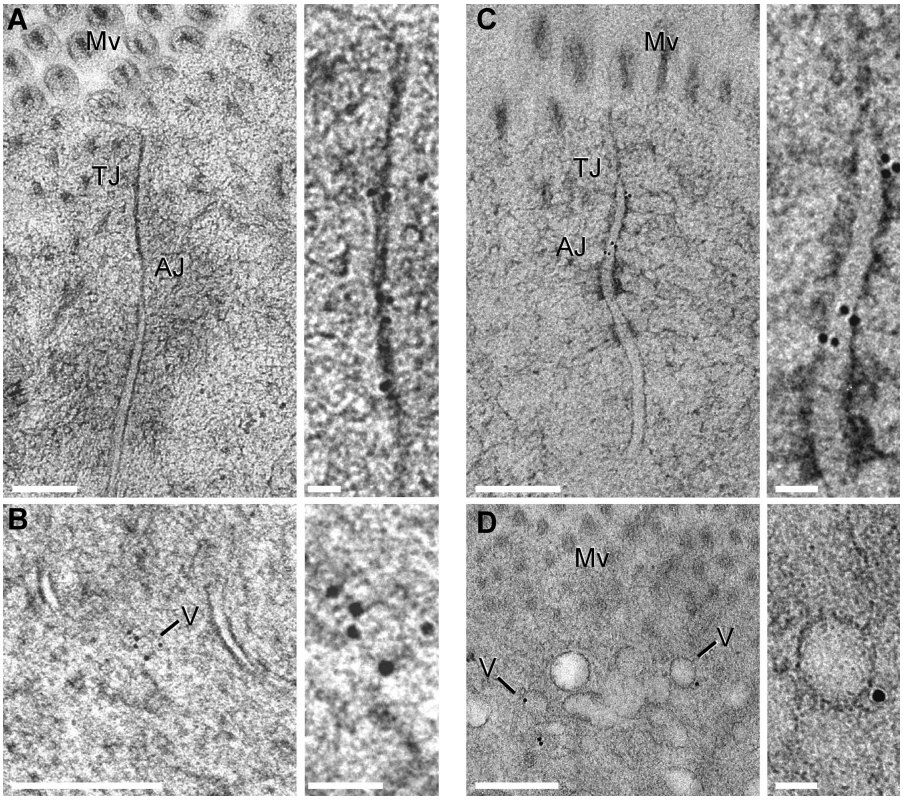
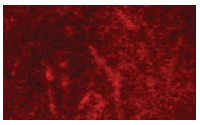
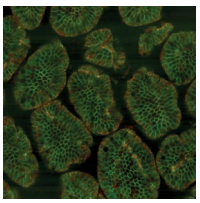


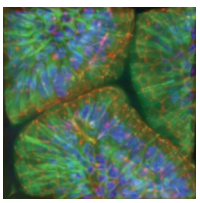
Figure S2. **Immunogold labeling of occludin and caveolin-1 in jejunal enterocytes of 90-min TNF-treated wild-type mice.** High pressure–frozen, freeze-substituted, cryoembedded specimens were immunolabeled. (A and B) Antioccludin, detected with 10 nm gold-conjugated secondary antisera, labels the tight junction (TJ) and small vesicles (best appreciated in the enlarged region [right]). (C) Anti–caveolin-1, detected with 10 nm gold-conjugated secondary antisera, labels the adherens junction (AJ). (D) Anti–caveolin-1, detected with 15 nm gold-conjugated secondary antisera, labels small vesicles (V). Mv, microvilli. Bars: (left) 300 nm; (right) 50 nm.



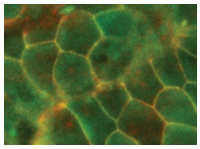
Video 1. **Blood flowing through villus capillaries.** Individual blood cells can be seen as streaks moving through a capillary in a single villus. Confocal reflectance is shown in red. Frames were collected at a rate of 10 frames per second. Single images from this video are shown in Fig. 2 B.



Video 2. **Localization of fluorescent tight junction proteins EGFP-occludin and mRFP1 –ZO-1 in a transgenic mouse.** The z series shows several jejunal villi and the epithelia of these villi. EGFP-occludin is shown in green, and mRFP1–ZO-1 is shown in red. The luminal space appears black. The images were collected every 0.1 µm for 9.2 µm. Single images from this video are shown in Fig. 2 C.



Video 3. **3D reconstruction of three jejunal villi from a transgenic mouse.** This video was created from a single z series. Both villous structure and localization of fluorescent tight junction proteins EGFP-occludin and mRFP1–ZO-1 in the epithelium can be appreciated in these images. EGFP-occludin is shown in green, mRFP1–ZO-1 is shown in red, and nuclei are shown in blue. The luminal space appears black. The images were collected every 0.1 µm. A single image from this video is shown in Fig. 2 D.



Video 4. **Endocytosis of EGFP-occludin in response to TNF in a transgenic mouse.** Jejunal epithelial cells were imaged over time and demonstrate the formation of occludin-containing vesicles and the movement of these vesicles into the cytoplasm. EGFP-occludin is shown in green, and mRFP1–ZO-1 is shown in red. Images were collected from a single z plane every 30 s from 85–180 min after injection of TNF. Single images from this video are shown in Fig. 2 E.