Supporting Information

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SI Materials and Methods

Immunofluorescence. Sandwich cultures were fixed in 4% paraformaldehyde in PBS for 10 to 15 min followed by incubation with methanol for 15 min at −20 °C (for GFP, GFP-Rap1GAP cells were only fixed in 4% paraformaldehyde for 15–20 min), blocked, and permeabilized with 1% BSA and 0.5% Triton X-100 for 1 h, and incubated with primary antibodies overnight at 4 °C. After washing with PBS, cells were incubated with anti-mouse IgG $F(ab')_2$ fragment–Cy3 or Alexa Fluro 488 goat anti-rabbit IgG antibodies for 2 h, followed by extensive washing in PBS. Confocal images were taken using Zeiss 510 Meta confocal microscopy with 63× oil objective and LSM 510 program (Zeiss). All images were projections of *z*-stacks, and analyzed using Image J program (National Institutes of Health, Bethesda, MD). All samples were coded and scored according to morphological criteria (canalicular presence, canalicular length per cell, tight junction staining appearance, and distribution of P-gp) by at least two investigators. At least three imaged areas of confluent cells were randomly selected from each culture dish. In no instance did scoring differ among investigators or samples. The Image J program was used to measure the length of canaliculi within the image field. Canalicular length was summed and divided by the number of cells to obtain the canalicular length per cell. Mean ± SD was calculated from three to five individual experiments.

Western Blot Analysis. Cultures were harvested and lysed in lysis buffer (150 mM NaCl, 1% Triton X-100, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, and protease inhibitor mixture), and sonicated 15 times for 1 s on ice, followed by centrifugation at 16,100 × *g* at 4 °C for 30 min. Fifty micrograms of total protein extracts were subjected to 8% SDS/PAGE gel. Following overnight transfer at 4 °C, PDVF membranes were blocked in 5% BSA for 1 h, and incubated with primary antibodies overnight at 4 °C. After washing in TBS-T, membranes were incubated with HRP-conjugated secondary antibody for 1 h. After washing, proteins were detected using ECL-Plus chemiluminescence detection system (GE Healthcare). Densitometry was measured using the Image J program.

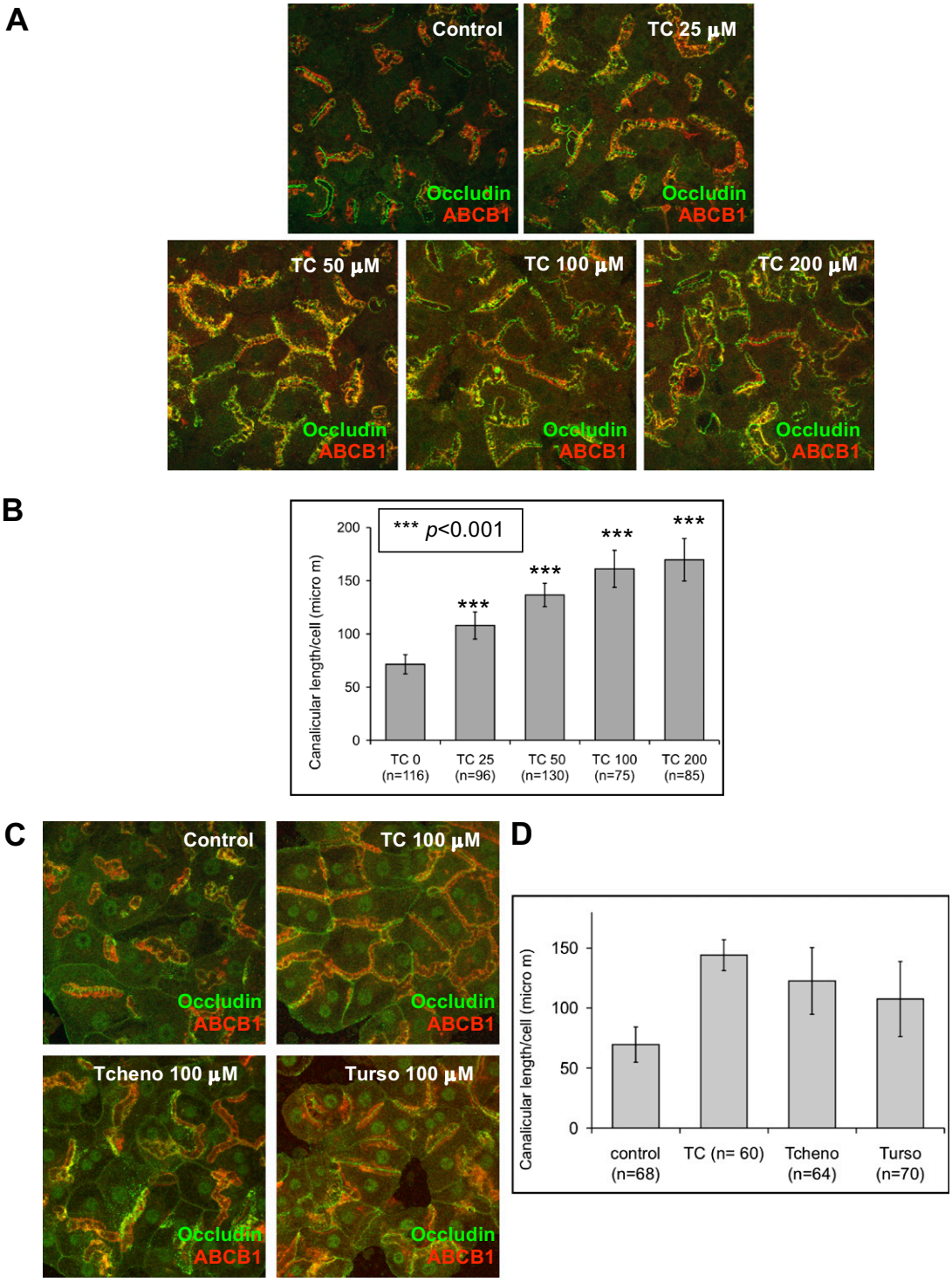

Fig. S1. Taurocholate effect on canalicular network formation is dose dependent. Day 2 cells were treated with different concentrations of taurocholate (0, 25, 50, 100, 200 μM) for 24 h. (*A*) Immunofluorescence of tight junction marker, occludin (green), and apical marker, ABCB1 (red), was used to study canalicular structure. (*B*) Mean canalicular length per cell (±SD) from three individual experiments (*n*, cell number; ***$P < 0.001$). (*C*) Day 2 cells were treated with taurocholate (100 μM), taurochenodeoxycholate (Tcheno, 100 μM) and tauroursodeoxycholate (Turso, 100 μM) for 24 h. Immunofluorescence of tight junction marker, occludin (green), and apical marker, ABCB1 (red). (*D*) Mean canalicular length per cell (±SD, from three repeats) (*n*, cell number).

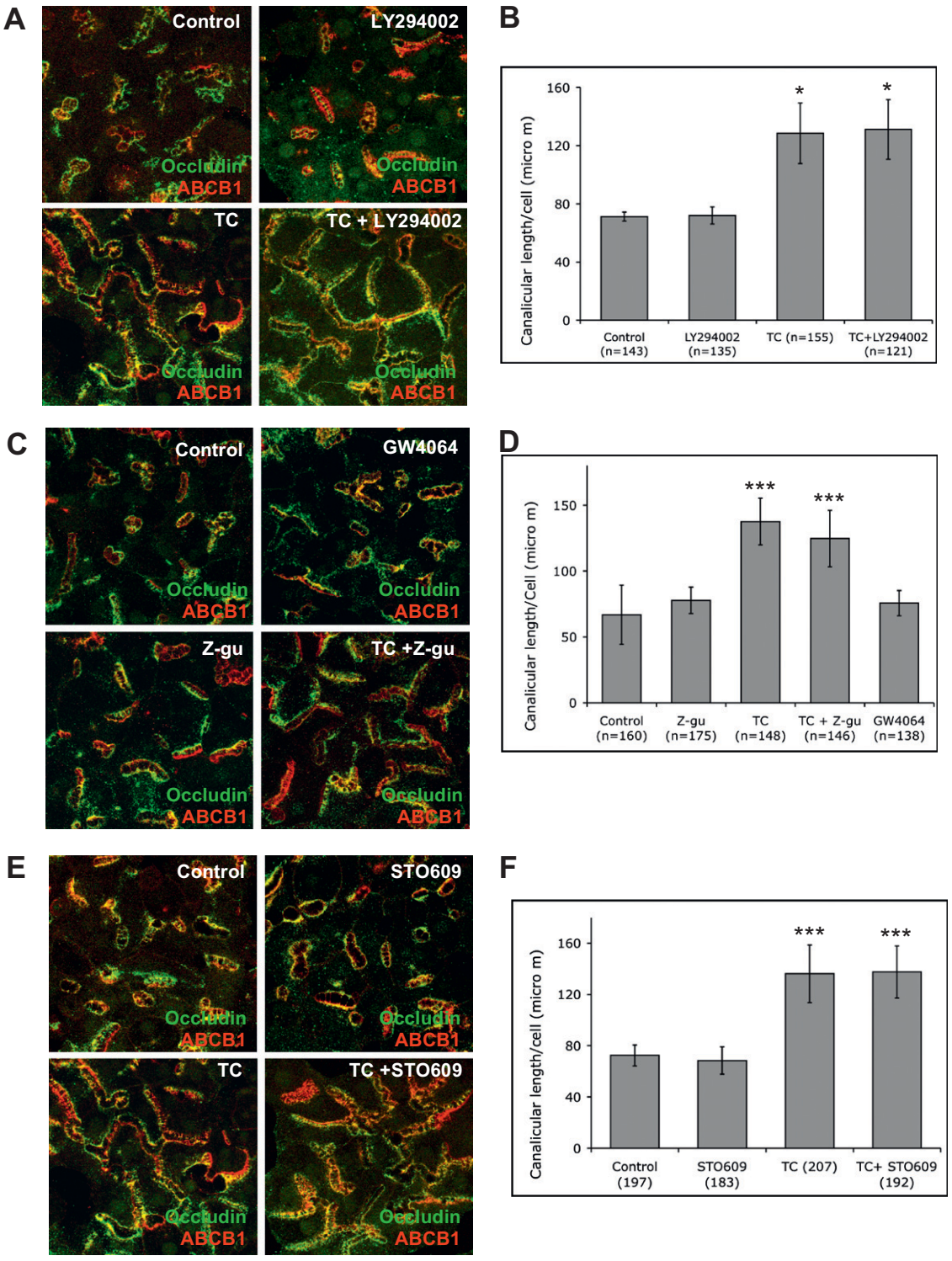

Fig. S2. Taurocholate-accelerated canalicular network formation is PI3K, FXR, and CaMKK independent. Immunofluorescence of tight junction marker, occludin, and apical marker, ABCB1 was used to study canalicular structure after various treatment on day 2 cells. (*A*) Day 2 cells were treated with PI3K inhibitor (LY294002) in the presence or absence of taurocholate (100 μM) for 24 h. (*B*) Mean canalicular length per cell (±SD) from three individual experiments (*n*, cell number; $*P < 0.05$). (*C*) Day 2 cells were treated with either inhibitor (Z-Gu) or activator (GW4064) of FXR in the presence or absence of taurocholate for 24 h, respectively. (*D*) Mean canalicular length per cell (±SD) from three individual experiments (*n*, cell number; $***P < 0.001$). (*E*) Day 2 cells were treated with CaMKK inhibitor (STO 609) in the presence or absence of taurichолate (100 μM) for 24 h. (*F*) Mean canalicular length per cell (±SD) from three individual experiments (*n*, cell number; $***P < 0.001$).

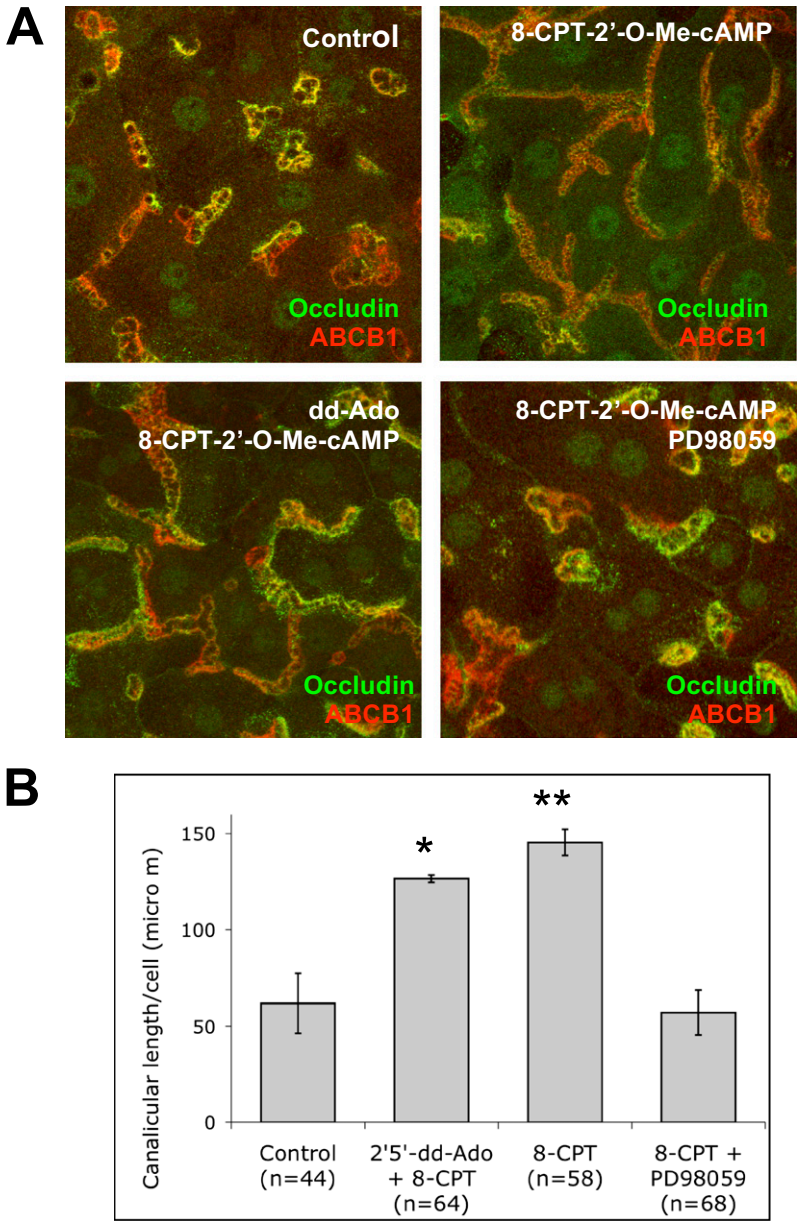

Fig. S3. Effect of Epac activator on canalicular network formation. Day 2 cells were treated with Epac activator 8-CPT-2′-O-Me-cAMP (8-CPT, 3 μM, 24 h) in the presence or absence of either adenylate cyclase inhibitor (2′5′-dd-Ado, 200 μM) or MEK inhibitor (PD98059, 100 μM). (*A*) Immunofluorescence of occludin and ABCB1. (*B*) Mean canalicular length per cell (±SD) (*n*, cell number; $*P < 0.05$, $**P < 0.01$).

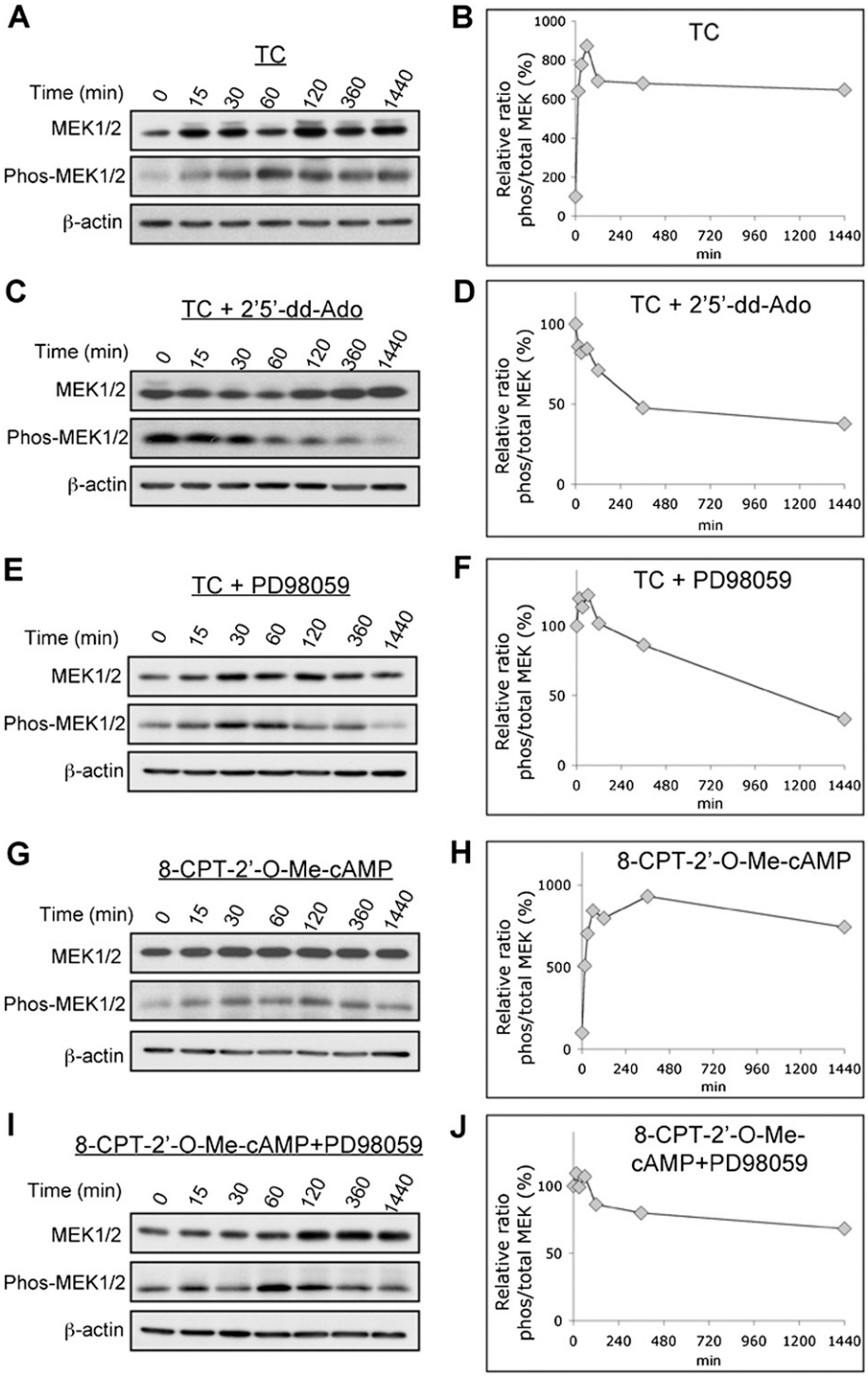

Fig. S4. Protein levels of total and phosphorylated MEK1/2 (Ser-217/221). Day 2 cells were studied after various treatments for 0, 15, 30, 60, 120, 360, and 1,440 min. Total proteins were extracted from the cells and Western blot for MEK and phosphorylated-MEK (Ser-217/221) were performed. Densitometric measurements were from three individual experiments. (*A*) Taurocholate- (100 μM) treated cells. (*B*) Ratio of phos-MEK/total MEK in taurocholate-treated cells. (*C*) Taurocholate and adenylate cyclase inhibitor- (2′5′-dd-Ado, 200 μM) treated cells. (*D*) Ratio of phos-MEK/total MEK in TC and 2′5′-dd-Ado–treated cells. (*E*) Taurocholate and MEK inhibitor- (PD98059, 100 μM) treated cells. (*F*) Ratio of phos-MEK/total MEK in taurocholate- and PD98059-treated cells. (*G*) Epac activator- (8-CPT-2′-O-Me-cAMP, 3 μM) treated cells. (*H*) Ratio of phos-MEK/total MEK in 8-CPT-2′-O-Me-cAMP–treated cells (*I*) Epac activator- (8-CPT-2′-O-Me-cAMP, 3 μM) plus MEK inhibitor- (PD98059, 100 μM) treated cells. (*J*) Ratio of phos-MEK/total MEK in 8-CPT-2′-O-Me-cAMP– and PD98059-treated cells.