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**Graphical abstract**

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T cell protein tyrosine phosphatase protects intestinal barrier function by restricting epithelial tight junction remodeling

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Genome-wide association studies revealed that loss-of-function mutations in protein tyrosine phosphatase non-receptor type 2 (PTPN2) increase the risk of developing chronic immune diseases, such as inflammatory bowel disease (IBD) and celiac disease. These conditions are associated with increased intestinal permeability as an early etiological event. The aim of this study was to examine the consequences of deficient activity of the PTPN2 gene product, T cell protein tyrosine phosphatase (TCPTP), on intestinal barrier function and tight junction organization in vivo and in vitro. Here, we demonstrate that TCPTP protected against intestinal barrier dysfunction induced by the inflammatory cytokine IFN-γ by 2 mechanisms: it maintained localization of zonula occludens 1 and occludin at apical tight junctions and restricted both expression and insertion of the cation pore-forming transmembrane protein, claudin-2, at tight junctions through upregulation of the inhibitory cysteine protease, matriptase. We also confirmed that the loss-of-function PTPN2 rs1893217 SNP was associated with increased intestinal claudin-2 expression in patients with IBD. Moreover, elevated claudin-2 levels and paracellular electrolyte flux in TCPTP-deficient intestinal epithelial cells were normalized by recombinant matriptase. Our findings uncover distinct and critical roles for epithelial TCPTP in preserving intestinal barrier integrity, thereby proposing a mechanism by which PTPN2 mutations contribute to IBD.

Introduction
The epithelial barrier is essential for intestinal homeostasis and is critical in preserving intestinal functions such as maintaining ionic balance, establishing a protective layer between the intestinal microbiota and the mucosa, and absorbing water and nutrients (1, 2). Disruption of apical tight junctions increases paracellular permeability to electrolytes and macromolecules, such as dietary antigens and bacterial products (3). This can induce activation of the mucosal immune system, resulting in inflammation and tissue damage (1, 4, 5). Moreover, loss of barrier integrity contributes to diarrheal and inflammatory symptoms in patients with inflammatory bowel disease (IBD; refs. 1, 6). While the 2 subtypes of IBD, Crohn’s disease (CD) and ulcerative colitis (UC), display several distinct pathological features, they both feature a dysfunctional intestinal barrier early in disease, before the onset of gross inflammation (5, 7). Thus, a compromised epithelial barrier is a hallmark of IBD pathogenesis. While initially presumed to be entirely a consequence of elevated inflammatory cytokine expression, increased intestinal permeability actually precedes inflammation in animal models of colitis and serves as a predictor of disease onset in first-degree relatives and of relapse in patients with IBD (8–13). Cumulatively, this suggests that reduced epithelial barrier function plays an essential early role in IBD pathogenesis. However, while increased permeability by itself is insufficient to cause colitis in animal models, it is required for intestinal inflammation (8, 9, 14). The exact mechanisms involved in the initiation of barrier dysfunction, and how they interact with other IBD risk factors, remain elusive.

SNPs in over 200 gene loci are associated with increased IBD risk (15, 16). One such gene, protein tyrosine phosphatase non-receptor type 2 (PTPN2), is associated with CD, UC, and other chronic immune conditions, including celiac disease, type 1 diabetes, and rheumatoid arthritis, all of which exhibit increased intestinal permeability early in disease (15–19). The disease-associated SNPs give rise to loss-of-function mutations in the PTPN2 product, T cell protein tyrosine phosphatase (TCPTP; refs. 20, 21).

TCPTP belongs to a family of protein tyrosine phosphatases that share a conserved catalytic domain (22). TCPTP is ubiquitously expressed and is essential for normal cellular function in many
cell types, including hematopoietic and epithelial cells (23–25). TCPTP dephosphorylates — thereby deactivating — members of the janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathways activated by interferon-γ (IFN-γ; i.e., JAK1, STAT1, -3) and other cytokines (JAK3, STAT5, -6; ref. 26). Dysregulated STAT signaling can contribute to IBD pathogenesis while STAT3 is also an IBD candidate gene (27, 28). Importantly, IFN-γ increases TCPTP expression as part of a negative feedback loop to restrict IFN-γ signaling (29).

Building on our observations that TCPTP promotes intestinal epithelial barrier properties in vitro (25, 29, 30), we now identify that TCPTP protects the intestinal epithelial barrier in vivo and in vitro through bimodal restriction of the claudin-2 cation pore, and preventing mislocalization of occludin and zonula occludens proteins at the apical tight junction, thus reducing permeability to electrolytes and macromolecules. These data confirm epithelial TCPTP as a key regulator of barrier function and suggest a mechanism by which PTPN2 loss-of-function mutations contribute to chronic inflammatory diseases, such as IBD.

Results
Loss of TCPTP compromises intestinal barrier function. TCPTP loss compromises epithelial barrier function in vitro (25, 29, 30). To determine whether this occurs in vivo, we assessed intestinal barrier function in whole-body Tcptp-deficient mice. To precede the described onset of systemic inflammation in Tcptp-knockout mice, intestinal tissues were isolated from 18- to 21-day-old Tcptp wild-type (WT), heterozygous (HET), and homozygous constitutive knockout (KO) mice and probed for TCPTP expression (31, 32). As expected, KO mice exhibited loss of TCPTP expression in the ileum, cecum, and proximal and distal colon (Supplemental Figure 1A; supplemental material available online with this article; https://doi.org/10.1172/JCI138230DS1). Concomitantly, phosphorylation of the TCPTP substrates JAK3 and STAT1 was increased (Supplemental Figure 1, B–D), while KO mice also displayed body weight loss and splenomegaly (Supplemental Figure 1, E and F). In vivo intestinal permeability to 4 kDa FITC-dextran (FD4) was significantly increased in HET mice and further increased in KO mice (~4-fold) compared with WT cohoused littermates (Figure 1A). Serum rhodamine B-dextran 70 kDa (RD70) flux was not significantly increased in HET or KO mice compared with WT littermates, indicating that “unrestricted” and tight junction-independent permeability was not a factor in the elevated permeability to FD4 (Figure 1B). Histological analysis (H&E staining) revealed an intact epithelium in small and large intestine in KO and HET mice (Figure 1C), although a decrease in colon length and increased crypt depths were seen in the cecum and proximal colon, but not distal colon, of KO mice (Figure 1, D and E). Ex vivo electrophysiological analyses of transepithelial electrical resistance (TER) (which measures resistance to the passive flux of electrolytes, such as sodium) and FD4 flux across isolated small and large intestinal tissues mounted in Ussing chambers revealed regional differences (Figure 2, A–J). Significant decreases in TER occurred in the distal ileum (Figure 2B) and cecum from HET and KO mice (Figure 2C), while KO mice also showed significantly reduced TER in the distal colon (Figure 2E). Mucosal FD4 permeability was significantly increased in the cecum and proximal colon from Tcptp-KO mice (Figure 2, H and I). Cumulatively, these data demonstrate a compromised epithelial barrier with increased tight junction–dependent electrolyte flux and macromolecule permeability in specific gut segments of Tcptp-deficient mice.

TCPTP loss promotes intestinal epithelial tight junction remodeling in vivo. Intestinal epithelial cells (IECs) isolated from Tcptp-deficient mice showed normal expression of the integral transmembrane tight junction protein, occludin, and the junction peripheral membrane scaffolding protein, zonula occludens 1 (ZO-1; Figure 3, A–D). However, the normally clear focal localization of ZO-1 (shown in green) and occludin (shown in red) at apical tight junctions was disrupted in KO mice, which instead showed more diffuse staining in the apical and subapical membranes (Figure 3E).

TNF-α/IFN-γ-induced internalization of tight junction proteins, and increased paracellular permeability, require phosphorylation of the myosin light chain (MLC) protein (33–35). Immunostaining of the colon and Western blotting of IECs isolated from the ileum, cecum, and colon showed increased MLC activation (phosphorylated Ser19) in KO mouse epithelium (Figure 3, F–H). Overall, these data indicate that loss of TCPTP leads to tight junction remodeling and increased MLC phosphorylation, which is consistent with the elevated FD4 permeability observed in vivo and ex vivo (c.f. Figure 2).

Tcptp-deficient mice exhibit elevated mucosal inflammatory cytokine profiles. We next determined if TCPTP loss altered regional expression of mucosal cytokines capable of altering barrier function. By multiplex expression analysis, we identified a gene–titratable increase in IFN-γ in HET mice, and significantly higher levels of IFN-γ, TNF-α, and IL-6 in the colon of KO mice, compared with WT and HET mice (Supplemental Figure 2, A–C). Ileal cytokine levels were below the level of detection. IL-22 was significantly elevated in the distal colon of KO mice versus WT (Supplemental Figure 2D). The Th2 cytokines IL-5 and IL-4 were significantly decreased in KO mouse cecum and distal colon, respectively (Supplemental Figure 2, E and F), whereas IL13 was unchanged (Supplemental Figure 3A). Serum cytokine analysis showed increased IFN-γ and TNF-α levels in KO mice (Supplemental Figure 3, B and C), both of which are elevated in IBD, particularly CD (31, 32, 36, 37). In addition, serum levels of IL-5 were higher in KO mice despite reduced expression in intestinal mucosal tissues (Supplemental Figure 3D). No change was observed in serum levels of IL-4 or IL-6 between genotypes (Supplemental Figure 3, E and F). These data identify increased serum inflammatory cytokines in KO mice but also discrete changes in intestinal protein levels of mucosal Th1- and Th2-associated cytokines.

Tcptp-deficient mice exhibit altered tissue lymphocyte and myeloid populations. To identify the source of inflammatory cytokines, we performed flow cytometric analysis of immune cell populations in the cecum, colon, mesenteric lymph nodes, and spleen (Supplemental Figure 4). We identified tissue-specific increases in IFN-γ CD4+ and CD8+ T cells, TNF-α/CD8+ T cells, and IL-22/CD4+ T cells in KO mice (Supplemental Figure 5). Decreases in IL-17/CD4+ T cells were observed in the cecum, colon, and lymph nodes, while IL-4/CD4+ and IL-10+ Tregs were also reduced in KO mice, although there was no overall decrease in Treg numbers in any tissue (Supplemental Figure 5). Myeloid cell characterization showed no change in overall murine Ly6G+ or Ly6C+ monocytes or
In vivo assessment of FD4 permeability showed no difference between adult \textit{Ptpn2} \( \Delta \) IEC and \textit{Ptpn2} fl/fl control mice following TMX-induced epithelial \textit{Ptpn2} deletion (Figure 4A). However, ex vivo analysis showed significant changes in underlying permeability with reduced TER (Figure 4, B–D) and increased FD4 flux (Figure 4, E–G) in the cecum and proximal colon — but not distal colon — of \textit{Ptpn2} \( \Delta \) IEC mice. We next determined the effect of in vivo cytokine challenge (24 hours) to \textit{Ptpn2} \( \Delta \) IEC mice and \textit{Ptpn2} fl/fl mice on regional barrier function in colonic tissues subsequently isolated and mounted in Ussing chambers. IL-6 induced a significant decrease in TER in the cecum (Figure 4B) and distal colon (Figure 4D) of \textit{Ptpn2} \( \Delta \) IEC mice, while it exacerbated the underlying defect in the proximal colon (Figure 4C). IL-6 also increased the relative abundance of dendritic cells (Supplemental Figure 6, A–C). The proportion of macrophages was increased in KO mouse cecum and colon (Supplemental Figure 6D). In KO mice, all tissues showed a larger proportion of M1-like (MHCII\(^{\text{hi}}\)CD206\(^{\text{lo}}\)) macrophages and decreased abundance of M2-like (MHCII\(^{\text{lo}}\)CD206\(^{\text{hi}}\)) macrophages (Supplemental Figure 6, E and F). Thus, global TCPTP loss provokes increased intestinal Th1 T lymphocytes and proinflammatory macrophage polarization.

\textbf{Intestinal epithelial-specific TCPTP loss in vivo predisposes to increased intestinal permeability.} To distinguish between immune cell and epithelial contributions to the permeability defects in \textit{Tcptp}-deficient mice, we generated tamoxifen-inducible (TMX-inducible) epithelial TCPTP-KO mice (\textit{Ptpn2} \(^{\Delta \text{IEC}}\); see Supplemental Figure 1). In vivo assessment of FD4 permeability showed no difference between adult \textit{Ptpn2} \(^{\Delta \text{IEC}}\) and \textit{Ptpn2} \(^{\Delta \text{IEC}}\) \textit{Ptpn2} WT control mice following TMX-induced epithelial \textit{Ptpn2} deletion (Figure 4A). However, ex vivo analysis showed significant changes in underlying permeability with reduced TER (Figure 4, B–D) and increased FD4 flux (Figure 4, E–G) in the cecum and proximal colon — but not distal colon — of \textit{Ptpn2} \(^{\Delta \text{IEC}}\) mice. We next determined the effect of in vivo cytokine challenge (24 hours) to \textit{Ptpn2} \(^{\Delta \text{IEC}}\) mice and \textit{Ptpn2} \(^{\Delta \text{IEC}}\) mice on regional barrier function in colonic tissues subsequently isolated and mounted in Ussing chambers. IL-6 induced a significant decrease in TER in the cecum (Figure 4B) and distal colon (Figure 4D) of \textit{Ptpn2} \(^{\Delta \text{IEC}}\) mice, while it exacerbated the underlying defect in the proximal colon (Figure 4C). IL-6 also increased
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erated by Western blotting of IECs isolated from cytokine-treated
mice, which showed elevated STAT1, STAT3, and NF-κB (p65)
phosphorylation, respectively, and this was further elevated in
Ptpn2\textsuperscript{ΔIEC} mice (Supplemental Figure 9, A–D). In agreement with
in vitro studies, we observed that loss of epithelial TCPTP in vivo
was sufficient to increase expression of the cation pore-forming
tight junction protein, claudin-2 (Supplemental Figure 9, A–D, and
the constitutive elevation in FD4 permeability in Ptpn2\textsuperscript{2\textsuperscript{ΔIEC}} mouse
cecum, but no difference was found in colonic regions (Figure 4E).
IFN-γ or TNF-α significantly reduced TER in the cecum and proximal
colon, while IFN-γ but not TNF-α increased FD4 permeability
in the cecum and proximal colon (Figure 4, E and F). IFN-γ and
TNF-α in combination significantly reduced TER in all 3 regions
and increased FD4 flux in cecum and proximal colon. Of note,
no underlying increase in FD4 permeability was found in distal
colon, and this was not significantly altered by cytokine treatment
(Figure 4G). Ex vivo tissue viability was confirmed by electrogenic
transport responses to Ca\textsuperscript{2+} and cAMP stimuli that were unaffect-
ed in the different genotypes (Supplemental Figure 8). Confirmation
of in vivo IFN-γ, IL-6, or TNF-α-induced signaling was generated by Western blotting of IECs isolated from cytokine-treated
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**Figure 2.** Tcptp-deficient mice display regional variations in macromolecule and electrolyte intestinal permeability ex vivo. In Ussing chambers, mucosal transepithelial electrical resistance (TER) (A–E) and ex vivo FD4 permeability (F–J) were measured across isolated (A and F) jejunum, (B and G) distal ileum, (C and H) cecum, (D and I) proximal colon, and (E and J) distal colon (n = 5–11). Data are expressed as mean ± SEM. Numbers of mice shown in parentheses. Comparisons between groups were by 1-way ANOVA and Student-Newman-Keuls posttest. *P < 0.05, and **P < 0.01 vs. WT; #P < 0.05 vs. HET.
data confirm that loss of TCPTP expression in IECs alone was sufficient to compromise intestinal epithelial barrier function. A loss-of-function PTPN2 mutant promotes cytokine-induced tight junction remodeling and increased permeability. To identify if a TCPTP loss-of-function mutation can precipitate tight junction reorganization, we generated a serine substitution of the cysteine at position 216 of the 45 kDa TCPTP isoform (TC45), the key site of ref. 30). This was further increased by IL-6 and IFN-γ (alone or in combination with TNF-α) but not TNF-α alone. These data demonstrate: (a) loss of TCPTP in IECs induced region-specific intestinal permeability defects that were not revealed by global assessment of intestinal permeability in vivo; (b) IEC loss of TCPTP increased susceptibility to cytokine-induced permeability defects; and (c) this susceptibility was cytokine and region specific. Overall, these data confirm that loss of TCPTP expression in IECs alone was sufficient to compromise intestinal epithelial barrier function.

A loss-of-function PTPN2 mutant promotes cytokine-induced tight junction remodeling and increased permeability. To identify if a TCPTP loss-of-function mutation can precipitate tight junction reorganization, we generated a serine substitution of the cysteine at position 216 of the 45 kDa TCPTP isoform (TC45), the key site of...
TCPTP enzymatic activity and one with clinical relevance (24, 38). This C216S-TC45 construct with a hemagglutinin (HA) tag, under a doxycycline-inducible tetracycline repressor protein (TetR) promoter (Tet-On), was transfected into HCA-7 IECs. Doxycycline treatment induced expression of the HA-tagged TCPTP (Supplemental Figure 10A and Figure 5A). Reduced cellular TC45 enzymatic activity was confirmed following IFN-γ treatment of C216S IECs (+DOX induction), which significantly increased STAT1 phosphorylation above control vector–transfected IECs (Figure 5, B and C). Induction of C216S-TC45 expression was sufficient to cause functional disruption of tight junctions (reduced TER and increased FD4 permeability) that was further accentuated by IFN-γ (Figure 5, D and E). IFN-γ treatment of C216S-TC45–transfected cells also disrupted tight junction composition and decreased levels of ZO-1 and occludin in IEC membranes (Figure 5, F and G), indicative of the “leak pathway” barrier defect. ZO-1/occludin, and ZO-1/claudin-4, colocalization at IEC junctions was significantly decreased in IFN-γ–treated C216S IECs compared with IFN-γ–treated control cells (Figure 5, G and H, and Supplemental Figure 11). These data indicate that a loss-of-function mutation in TCPTP predisposes to greater tight junction reorganization in response to IFN-γ, and increased permeability, consistent with observations of barrier disruption in TCPTP-deficient mice.

TCPTP suppresses expression of the cation-selective pore, claudin-2, in intestinal epithelium. The “pore pathway” of increased paracellular flux of electrolytes (i.e., Na+) and water into the intestinal lumen can promote diarrhea and is mediated by select members of the claudin superfamily, most prominently the cation-selective protein, claudin-2 (25, 30, 39–41). We report increased epithelial claudin-2 mRNA in \( \text{Tc p t p} \)-HET and -KO mice across intestinal regions (Supplemental Figure 10B). Claudin-2 immunostaining was elevated in the cecum of both HET and KO mice compared with WT (Figure 6A). Localization of claudin-2 was strongest at the crypt base. The ileum, cecum, and proximal and distal

Figure 4. In vivo and ex vivo intestinal permeability measurements in cytokine-treated Ptpn2\( ^{\Delta IEC} \) KO mice. (A) FD4 was administered by oral gavage to Ptpn2\( ^{\Delta IEC} \) and Ptpn2\( ^{fl/fl} \) control mice. Serum was collected after 5 hours and FD4 concentration determined (\( n = 7–10 \)). Mice were injected i.p. with IL-6, IFN-γ, TNF-α, or IFN-γ + TNF-α for 24 hours prior to tissue isolation and mounting in Ussing chambers. TER and FD4 permeability were measured in (B and C) cecum, (D and E) proximal colon, and (F and G) distal colon (\( n = 4 \)). Data are expressed as mean ± SD. Comparisons between genotypes and within treatment groups were by unpaired 2-tailed Student’s t test. *\( P < 0.05; **P < 0.01; ***P < 0.001. \)
Figure 5. A TCPTP loss-of-function mutation increases cytokine-induced tight junction remodeling. (A) Fluorescence micrographs of TCPTP (green) and HA (red) expression in doxycycline-treated (DOX-treated) (15 μg/mL; 48 hours) control and C216S-TC45–transfected HCA-7 IECs (merge image includes DAPI nuclear staining shown in blue). Scale bars: 10 μm. (B) Western blots showing STAT1 tyrosine phosphorylation (Y701) after basolateral addition of IFN-γ (200 U/mL; 6 hours) to DOX-pretreated control or C216S-TC45 HCA-7 IECs. Total STAT1 and β-actin levels shown for all conditions. (C) STAT1 phosphorylation relative to total STAT1 protein was determined by densitometry. (D) Control and C216S HCA-7 cells were cultured on Transwells prior to treatment with vehicle (PBS) or DOX (15 μg/mL; 48 hours) and subsequent treatment with IFN-γ (1000 U/mL, 24 hours). TER was measured and expressed as Ω/cm². (E) FD4 permeability was calculated after 6 hours of IFN-γ treatment and expressed as mg/mL. (F) Representative image showing ZO-1 and occludin localization following IFN-γ treatment of DOX-pretreated control and C216S-TC45 IECs (merge image includes DAPI nuclear staining shown in blue). Scale bars: 10 μm. (G and H) Color conversion (in Adobe Photoshop) and quantification (ImageJ software, NIH) of ZO-1/Occludin colocalization (black arrows) in DOX-pretreated control (top row) and C216S-TC45 IECs (bottom row) exposed to IFN-γ (200 U/mL, 6 hours). Original magnification, 63×. Data shown as mean ± SD. Statistical significance was calculated by 1-way ANOVA and Student-Newman-Keuls posttest. Comparisons between 2 groups were by 2-tailed Student’s t test (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
was increased in the distal colon of KO mice while claudin-3 and -4 were unchanged (Figure 6B and Supplemental Figure 12, B–D). Moreover, an additional cation pore, claudin-15, was increased in the colon of KO mice (Figure 6B and Supplemental Figure 12E).

Colon had increased claudin-2 protein in IECs across all regions in KO versus WT mice, with higher claudin-2 also observed in cecum and distal colon of HET versus WT mice (Figure 6B and Supplemental Figure 12A). Of the barrier-promoting claudins, claudin-1 was increased in the distal colon of KO mice while claudin-3 and -4 were unchanged (Figure 6B and Supplemental Figure 12, B–D). Moreover, an additional cation pore, claudin-15, was increased in the colon of KO mice (Figure 6B and Supplemental Figure 12E).
The physiological relevance of increased claudin-2 expression in Tcptp-HE and -KO mice was demonstrated in colonic tissues from CD patients harboring the loss-of-function IBD-associated PTPN2 rs1893217 SNP (Table 1). Patients homozygous (GG/CC) or heterozygous (GA/CT) for this SNP displayed increased claudin-2 expression in colonic and ileal (Figure 6, C and D, and Supplemental Figure 13A) epithelium compared with patients with WT PTPN2 alleles (AA/TT). Using stable PTPN2-knockdown (PTPN2-KD) Caco-2BBe IECs, we also confirmed increased claudin-2 immunofluorescence staining at cell membranes compared with control cells while claudin-1 was unchanged (Figure 6E).

Table 1. Patient characteristics

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Tcptp limits claudin-2 by restricting STAT1-mediated transcription of claudin-2 and hepatocyte growth factor activator inhibitor-1, a suppressor of the integral membrane serine protease, matriptase. In addition to the transcriptional control of CLDN2, claudin-2 levels at the tight junction are also modulated by the serine protease matriptase (ST14; ref. 44). We found that Tcptp loss modulated this mode of claudin-2 regulation since matriptase mRNA and protein levels were significantly reduced in colonic tissue from Tcptp-KO mice compared with WT mice (Figure 8A and Supplemental Figure 14A). Moreover, IFN-γ treatment of C216+TC45-expressing IECs significantly reduced matriptase expression and concomitantly increased membrane claudin-2 (Figure 8B). Tcptp-KD IECs (Supplemental Figure 14B) were more susceptible to IFN-γ induction of claudin-2 expression, and we demonstrated that IFN-γ selectively reduced matriptase protein and mRNA levels in Tcptp-deficient but not control IECs (Figure 8C and Supplemental Figure 15). To further elucidate the mechanism of Tcptp regulation of matriptase and claudin-2, we performed siRNA knockdown of STAT1 or HAI-1/SPINT1, which acts as a physiological inhibitor and regulator of matriptase activity and expression (45). Knockdown of STAT1 or HAI-1 normalized claudin-2 expression, and reversed matriptase suppression, in Tcptp-KD (shRNA) Caco-2 IECs, following IFN-γ challenge (Figure 8C and Supplemental Figure 15). Thus, Tcptp limits claudin-2 levels by (a) inhibiting STAT1-mediated transcription of (a) claudin-2 and (b) HAI-1, as well as (c) inhibiting STAT1-induced, HAI-1-mediated suppression of matriptase (ST14) transcription.

Recombinant matriptase rescues TER and normalizes claudin-2 expression in Tcptp-deficient IECs. To confirm a functional role for matriptase loss in the TER barrier defect observed in Tcptp-KD IECs, we performed rescue experiments where Tcptp-stable SD HT-29 IECs were treated with recombinant human matriptase (rMAT). Basolateral, but not apical, application of rMAT to Tcptp-KD monolayers significantly increased TER over 24 hours, indicating rescue of baseline TER (Figure 8D). Elevated claudin-2 in Tcptp-KD cells was reversed by basolateral addition of rMAT, indicating that rMAT rescued the decreased TER in Tcptp-deficient IEC by reducing claudin-2 (Figure 8, E and F). Recombinant matriptase had no effect on Tcptp levels in control or KD cells (Supplemental Figure 14C). This demonstrates that Tcptp suppresses claudin-2 levels by restricting cytokine-induced repression of matriptase expression in IECs, thus preserving a key regulatory signaling pathway to control claudin-2. Moreover, the decrease in TER due to loss of Tcptp activity is mediated by elevated claudin-2, and this defect can be rescued by recombinant matriptase.

Discussion

One of the initial and fundamental disturbances in intestinal homeostasis that contributes to IBD pathogenesis is increased intestinal permeability, which occurs primarily through disruption of cellular tight junction composition (6, 11). An altered complement of claudin family members — such as increased claudin-2 in inflammatory states — increases charge-selective paracellular electrolyte permeability via the “pore” pathway. The “leak” pathway, which facilitates increased paracellular permeability to macromolecules and non–charge-selective passage of electrolytes, is increased by removal of proteins such as occludin and ZO-1 from the tight junction. An “unrestricted” mode of permeability, which displays neither charge nor size selectivity, can also occur.
due to IEC apoptosis or increased cell shedding (4, 7, 12, 46, 47). While unrestricted permeability — assessed by RD70 flux — was not increased in Tc pt p-deficient mice, we did observe both forms of tight junction-mediated defect (pore and leak). Intriguingly, different intestinal regions revealed distinct patterns of barrier defect, with the cecum exhibiting a titrated increase in macromolecule permeability and reduced TER based on Ptpn2/Tc pt p genotype. This aligns with described differences in TER in midcolonic versus distal colonic tissues from control mice (48). In addition to the tight junction-regulated paracellular route, transcellular routes of solute passage across the gut epithelium have also been identified, including endocytosis, antigen transcytosis by Peyer patch M cells, and goblet cell–associated antigen passages (GAPs; ref. 49). While small intestinal GAPs could contribute to FD4 permeability in Tc pt p-KO mice, it is notable that they are actually reduced during inflammation (50).

While expression of inflammatory cytokines varied across intestinal regions, IFN-γ was consistently elevated in Tc pt p-HET and -KO mice in all large intestinal regions examined. In addition, increased crypt depth, possibly indicating increased epithelial proliferation, was apparent in the cecum and proximal colon but not the distal colon of KO mice. Factors responsible for these segmental variations may include altered signaling between IECs and a variety of mucosal immune cells, as well as disruption of homeo-

![Figure 7. Claudin-2 mediates altered TER but not FD4 permeability in TCPTP-deficient epithelial cells. Control-shRNA and TCPTP-KD Caco-2BBe IECs cultured on Transwells for 7 days prior to treatment for 48 hours with no siRNA (open black circle) or control/scrambled (filled black circle) or CLDN2 (open red circle) siRNA. (A) TER and (B) FD4 permeability was measured (n = 4). (C) Tight junction freeze fracture morphology of control-shRNA (top) and TCPTP-KD (bottom) Caco-2BBe cells. The overall morphology of both the control and PTPN2-KD cells appeared similar in both the control and TCPTP-KD cells. Comparable fracture planes show continuous strands consistently segregating to the protoplasmic (P-) face (indicated with arrows) and grooves left in the exoplasmic (E-) face. However, more discontinuities in the P-face strands were observed in TCPTP-KD cells. Microvilli indicated by asterisks. Scale bar: 500 nm. Data are expressed as mean ± SD (n = 4). Comparisons between multiple groups were by 1-way ANOVA and Tukey’s posttest. *P < 0.05; **P < 0.01; ***P < 0.001.](https://doi.org/10.1172/JCI138230)
Figure 8. TCPTP promotes matriptase regulation of claudin-2, and recombinant matriptase rescues the claudin-2–mediated barrier defect in TCPTP-deficient IECs. (A) Western blot of matriptase and β-actin in proximal colon (whole tissue) from Tcptp WT and KO mice. Densitometric quantification of Western blot colonic whole tissue matriptase expression normalized to tissue β-actin (*P < 0.05 vs. WT; n = 4). (B) Fluorescence micrograph showing line scan analysis of fluorescence intensity of claudin-2 (red, white arrows) and matriptase (green) expression in IFN-γ–treated (200 U/mL, 48 hours) control-shRNA and C216S-TC45 HCA-7 IECs. Representative of 4 to 6 scans per treatment condition (n = 4). (C) Western blots of control-shRNA and TCPTP-KD Caco-2 IECs transfected with control/scrambled siRNA (siCtr), or siRNA targeting STAT1 or hepatocyte growth factor activator inhibitor-1 (HAI-1), and probed for claudin-2, matriptase, HAI-1, phosphorylated (Y701) and total STAT1, and β-actin. (D) TCPTP-shRNA– (TCPTP-KD) or control-shRNA–transfected HT-29 IEC monolayers grown on Transwells were treated apically or basolaterally with recombinant human matriptase (rMAT; 5 nM), and TER change after 24 hours was measured and compared with unchallenged monolayers (–). (E) Expression of claudin-2 in control-shRNA and TCPTP-KD HT-29 monolayers was determined by Western blotting and (F) quantified by densitometry. Data expressed as mean ± SD. Comparisons between multiple groups were by 1-way ANOVA and Student-Newman-Keuls posttest (n = 4). Comparison between 2 groups was by 2-tailed Student’s t test. *P < 0.05; **P < 0.01.
significant loss ($P < 0.05$) of occludin and ZO-1 membrane colocalization following IFN-γ challenge. Induction of TC45-C216S by itself was sufficient to reduce TER and increase FD4 flux and exacerbate IFN-γ’s effects on both parameters. While IFN-γ disrupted ZO-1 colocalization with occludin and claudin-4, membrane ZO-1 was not disrupted in non-cytokine-treated C216S cells, suggesting that the functional defects in untreated cells cannot be attributed to ZO-1 mislocalization. The C216s site is a highly conserved and critical part of the protein tyrosine phosphatase catalytic region (38). Of clinical relevance, a loss-of-function mutation of the C216s site (C216G) is a monogenic driver of autoimmune enteropathy, thus emphasizing the disease-causing potential of deficient PTPN2 activity (38). Mechanistically, removal of ZO-1 and occludin can occur by endocytic processes in response to MLC phosphorylation, which was also elevated in Tcptp-KO, and to a lesser extent in Tcptp-HET, mice. MLC phosphorylation is a point of convergence for both IFN-γ-Rho kinase-mediated tight junction protein internalization as well as TNF-α-MLC kinase-mediated epithelial permeability, and its increase is consistent with the elevated IFN-γ and TNF-α levels we detected in TCPTP-deficient mouse intestine (33, 56).

The claudin family of tetraspanning membrane proteins is essential for homeostatic electrolyte flux, fluid uptake, and sodium recycling in electrolyte-coupled nutrient absorption (41, 57–59). Increased epithelial expression of the cation pore-forming molecule claudin-2 is a feature of IBD but can also have protective roles against pathogen infection (39, 46). Claudin-2 shows a region-specific distribution pattern in the intestine with higher expression in leaky epithelia of the small intestine and lower levels in tight epithelia of the large intestine (60, 61). Thus, elevated claudin-2 expression in the large intestine is of functionally greater significance than in a leakier epithelium, as it facilitates fluid loss consistent with IBD-associated diarrhea (39, 40, 62). Here, we observed increased claudin-2 expression in large intestine from Tcptp-KO and -HET mice. This is consistent with the elevated levels of IL-6 in all regions and IL-22 in the distal colon of Tcptp-deficient mice, because both of these cytokines are known inducers of claudin-2 expression (Supplemental Figure 2 and refs. 46, 63). We previously identified that IFN-γ induces claudin-2 expression in TCPTP-deficient IECs by STAT1 activation and binding to STAT-binding motifs in the CLDN2 promoter (30). This was supported by data from Ptpn2ΔIEC mice confirming increased baseline claudin-2 expression, and phosphorylation of STAT1 and STAT3 that was accentuated by in vivo IL-6 or IFN-γ administration, while claudin-2 was also increased in CD patients with the PTPN2 rs1893217 SNP. Of note, the cation pore, claudin-15, was also increased in colonic epithelial cells in whole-body Tcptp-KO mice, thus potentially offering an additional route for paracellular Na+ flux.

Our present findings indicate that TCPTP is a positive regulator of the serine metalloproteinase matriptase (ST14). Matriptase regulates epithelial barrier integrity by promoting the removal of claudin-2 from the tight junction, leading to its subsequent proteasomal degradation (44, 64). In IECs expressing the TCPTP loss-of-function mutant, we observed a reversal of the normal homeostatic equilibrium between high matriptase and low claudin-2 seen in control IECs. IFN-γ significantly reduced matriptase expression in C216S-TC45 IECs while increasing membrane claudin-2 levels (Figure 8B). We found that TCPTP restricted the capacity of IFN-γ to reduce matriptase mRNA levels through STAT1-dependent induction of the matriptase inhibitor, HAI-1, and that claudin-2 functionally mediated the reduced TER in TCPTP-deficient IECs. Rescue of TER in PTPN2-KD IECs with recombinant matriptase supports previous findings of matriptase as a negative regulator of claudin-2 but also reveals that matriptase signaling can be targeted to correct elevated claudin-2 arising from reduced TCPTP activity.

In summary, we show that TCPTP protects the intestinal epithelial barrier by restricting cytokine-induced tight junction remodeling and increased permeability to macromolecules. In addition, TCPTP exerts bimodal inhibition of the cation pore-forming molecule claudin-2 to restrict paracellular flux of sodium and water. These findings identify mechanisms by which insufficient TCPTP expression, and/or loss-of-function mutations in the PTPN2 gene, can contribute to elevated intestinal permeability that is a feature of chronic intestinal inflammatory diseases and plays a critical role in their onset.

Methods

Macroscopic assessment of constitutive Tcptp-deficient mice. Tcptp WT and constitutive, whole-body heterozygous (HET) and knockout (KO) BALB/c mouse littersmates (age matched at 17–24 days old) of both sexes were weighed (31, 32). Spleen weight, colon length, and colon wet weight were also measured.

Generation of inducible Ptpn2ΔIEC KO mice. Ptpn2ΔIEC/EUCOMM[Fl]+/Wtsi mice (obtained from Wellcome Trust) have exon 3 of the Ptpn2 gene flanked by loxP sites and a neoR-lacz cassette flanked by FRT sites in intron 2 (Supplemental Figure 7A). To generate mice with inducible deletion of Ptpn2 in IECs, we crossed these mice with Vil-Cre/ERT2 mice [B6.Cg-Tg(Vill-cre/ERT2)2Syr/j] (provided by Sylvie Robine, Institut Curie, Paris, France) that carry a tamoxifen-inducible ERT2 gene under the control of the Villin promoter (65). The resulting double-heterozygous mice were crossed with transgenic mice expressing FLP recombinase in the germline [B6.129S4-Gt(Rosa)26Sart1Frt1(LacZ)-m2/fl;Rain]; the Jackson Laboratory] to remove the neoR-lacz cassette. Mice were backcrossed to a C57BL/6j background for 10 generations and made homozygous for Ptpn2ΔIEC/ΔIEC without or with hemizygous Vil-Cre/ERT2 and maintained in that state. Cre-mediated recombination was induced by i.p. injection in 6– to 10-week-old mice of 1 mg TMX (50 mg/kg body weight; 15648, MilliporeSigma), dissolved in sterile corn oil (C8267, MilliporeSigma), for 5 consecutive days. Tissues were harvested 4 weeks after the final administration of TMX.

In vivo cytokine administration. For in vivo cytokine administration, 6- to 8-week-old Ptpn2-Villin-Cre/ERT2ΔIEC and Ptpn2ΔIEC littermates were injected with TMX on 5 consecutive days (as above). Four weeks later, the mice were injected once with IL-6 (100 ng), IFN-γ (1000 U) or TNF-α (100 ng), or IFN-γ and TNF-α in combination and intestinal tissues harvested 24 hours later for Ussing chamber (Physiological Instruments) measurements.

Mouse barrier function studies. Tcptp wild-type (WT), heterozygous (HET), and constitutive knockout (KO) Balb/c mice (littersmates, age matched within genotypes; males and females, 17–21 days old) were housed under conventional housing conditions. In vivo permeability of FD4 (MilliporeSigma; catalog 46944; hydrodynamic diameter, 28 Å; 80 mg/mL) and RD70 (MilliporeSigma; catalog R9379; 20 mg/mL) was determined following oral gavage (100 μL volume).
After 5 hours, serum was harvested retro-orbitally. Fluorescence of fluorescein and rhodamine in samples loaded into a black plate was measured using excitation wavelengths of 495 nm and 555 nm, and emission wavelengths of 525 nm and 585 nm, respectively, using a SpectraMax iD3 plate reader ( Molecular Devices), aligned with established protocols (46).

For ex vivo permeability studies, mice were sacrificed by cervical dislocation, and full-thickness segments of jejenum, distal ileum, cecum, proximal colon, and distal colon were mounted in Ussing chambers. Tissues were bathed bilaterally with Ringer’s physiological solution (composition in mM: 140 Na+, 5.2 K+, 1.2 Ca2+, 0.8 Mg2+, 120 Cl−, 25 HCO3−, 2.4 H2PO4−, 0.4 HPO4−2, and 10 glucose) with 10 mM mannitol substituted for glucose in apical bathing media for small intestinal tissues to prevent activation of Na+-glucose cotransport. A transepithelial current pulse of 10 μA was administered to assess tissue viability, and TER was calculated using Ohm’s law (66). To test for permeability, FD4 was added to the mucosal medium at a final concentration of 1 mg/mL. Serosal samples were collected at 0 and after 2 hours (t20) and calculated for change in resistance relative to the initial FD4 concentration in mucosal samples (t0). Fluorescence was analyzed with a SpectraMax iD3 microplate reader. Tissue viability at the end of the experiment was confirmed by challenge with the cAMP agonist forskolin (20 μM, bilaterally) and the Ca2+ agonist carbachol (300 μM, serosally). Electric field transport responses were measured and expressed as the change in short-circuit current (ΔIsc), in μA/cm2.

Histology and immunohistochemistry of mouse tissue. Intestinal segments (distal ileum, cecum, proximal and distal colon) from WT, HET, and KO mice (as above) were fixed in 4% paraformaldehyde (PFA) and exposed to a graded ethanol series before embedding in paraffin. Tissue blocks were cut into 5 μm sections, deparaffinized, and rehydrated in a descending ethanol series. Intestinal cross-sections were used for morphometric measurements. Only well oriented sections with bottom-to-top crypt-villus axis visibility were used in the analysis. The straight line tool from FIJI software was calibrated using the scale bar from the image being measured. A straight line was drawn measuring crypt depth, villus length, and crypt width. All data for each parameter per mouse were averaged per mouse with a minimum of 4 measurements for each parameter per mouse. For cell counting, total number of IECs per crypt was counted using the Cell Counter tool in FIJI software. Immunostaining of sections for phosphorylated STAT1 was performed by incubation with streptavidin-coupled HRP (N100, Thermo Fisher Scientific) for 1 hour and subsequent addition of DAB substrate according to the manufacturer’s protocol (Cell Signaling Technology, catalog 8059). Slides were mounted with Permount mounting medium (Thermo Fisher Scientific) and visualized with a Leica DM550B microscope with DFC450 C camera. All confocal immunofluorescence microscopy was conducted at the UCR or UCSD microscopy core facilities.

Immunohistochemistry of human intestinal sections. Human samples were obtained from patients with IBD attending the IBD Center at Cedars-Sinai Medical Center after informed consent was received. Samples with active inflammation were excluded following pathologist review. Diagnoses of IBD were made using standard clinical, endoscopic, radiological, and histological criteria. Genotyping and quality control for the rs1893217 SNP were performed as previously described utilizing the Immunochip (Illumina) platform (67). Formalin-fixed, paraffin-embedded human colon and ileal tissues were sectioned at 5 μm and processed for immunohistochemistry as follows: after deparaffinizing, antigen epitopes were retrieved in 10 mM sodium citrate buffer pH 6.0 (20 minutes at ~96°C). Endogenous peroxidase was quenched with 3% hydrogen peroxide. Nonspecific antigens were blocked with blocking buffer (2% normal donkey serum [NDS], 1% albumin, 0.1% Triton X-100, 0.05% Tween 20, and 0.05% sodium azide in PBS) for 30 minutes at room temperature. The biotinylated primary antibody (Clausd-2; Abcam; catalog ab53032; 1:400 in PBS, 5% NDS) was applied for 1 hour at room temperature. Detection was performed by incubation with streptavidin-coupled HRP (N100, Thermo Fisher Scientific) for 1 hour and subsequent addition of DAB substrate according to the manufacturer’s protocol (Cell Signaling Technology, catalog 8059). Slides were mounted with Permount mounting medium (Thermo Fisher Scientific) and visualized with a Leica DM550B microscope with DFC450 C camera. All confocal immunofluorescence microscopy was conducted at the UCR or UCSD microscopy core facilities.

Tissue cytokine analysis. For cytokine protein detection, Luminex inflammatory cytokine kits (Affymetrix) were processed according to manufacturer’s instructions and quantified on Luminex MagPix (Luminex Corp.). IL13 mRNA expression was determined by reverse transcription PCR.

Tissue RNA isolation and quantitative PCR. Total RNA was extracted from intestinal segments from mice using TRIzol reagent according to the manufacturer’s instructions (Invitrogen, Thermo Fisher Scientific). RNA purity and concentration were assessed by absorbance at 260 and 280 nm. One microgram of total RNA was transcribed into cDNA using qScript cDNA SuperMix (Quanta Biosciences). Two microliters of 5X-diluted cDNA was amplified using gene-specific primers and GoTag Green, 2X mix (Promega; sequences listed in Supplemental Table 1). Gene-specific primers were used with the following conditions: initial denaturation 95°C for 5 minutes, followed by 30 cycles 95°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing), and 72°C for 30 seconds (extension). The final extension was 72°C for 5 minutes. Mouse GAPDH was used as an endogenous control.

Flow cytometry. Lamina propria immune cells were isolated as described (68). For analysis of myeloid immune cells, the cells were washed in PBS; incubated with FCr blocking antibody (130-092-575, Miltenyi Biotech) for 10 minutes; and stained with Pacific Blue anti-mouse CD45 (clone 30-F11, catalog 103125), Brilliant Violet 650 (BV650) anti-CD3 (clone 17A2, catalog 100229), anti-NK1.1-BV650
(clone PK136, catalog 108735), anti-B220–BV650 (clone RA3-6B2, catalog 101257), anti-CDC11c-PECy7 (clone N418, catalog 117338), PerCP-Cy5.5 anti-mouse anti-Ly6C (clone HK1.4, catalog 128011), APC anti-mouse anti-F4/80 (clone BM8, catalog 123115), and anti-mouse CD64–PE (clone X54-5-7,1, catalog 139303), all from BioLegend, and Alexa Fluor 700 anti-mouse MHC-II (clone M5/114.15.2, catalog 56-5321-80, Thermo Fisher Scientific), for 15–30 minutes. Zombie NIR live dead stain (BioLegend) was used for discrimination between live and dead cells. For cytokine staining, the cells were incubated with ionomycin and PMA in the presence of Brefeldin A (eBioscience, Thermo Fisher Scientific) for 3.5 hours prior to surface staining with anti–CD25–Alexa Fluor 700 (clone PC61, catalog 102024), anti–CD3–PerCP-Cy5.5 (clone 17A2, catalog 100217), anti–CD4–BV510 (clone GK1.5, catalog 100449), and anti–CD8–BV570 (clone 53-6,7, catalog 100739), all from BioLegend, for 15 minutes. Cells were then fixed with the FoxP3 staining kit (catalog 00-5522-00, eBioscience, Thermo Fisher Scientific) according to the manufacturer’s instruction; stained with anti–FoxP3–Pacific Blue (clone MF-14, catalog 128011, BioLegend), anti–IFN-γ–PECy7 (clone XMG1.2, catalog 505825), anti–IL-17–APC (clone TC11-18H10.1, catalog 506915), anti–TNF-α–BV650 (clone MP6-XT22, catalog 506333), and anti–IL-22–PE (clone Polycl64, catalog 516404) for 30 minutes; and washed in PermWash buffer (catalog 554723, BD). Samples were acquired on an LSRII cytometer (BD) and analyzed using FlowJo (Tree Star, Inc.).

**Lentiviral vectors.** A vector containing the predominant 45 kDa splice variant of TCPTP (EGFP-C1-TC45) was provided by Tony Tiganis (Monash University, Melbourne, Victoria, Australia). pCMV-HA construct and Lenti-X Tet-On Advanced Vector Set (Clontech) and doxycycline (MilliporeSigma) were obtained from the suppliers listed. All other reagents were of analytical grade and acquired commercially. EGFP-TC45 constructs were used to form the C216S mutant (site-directed mutagenesis kit, Clontech). Resulting constructs were subcloned into pCMV-HA (Clontech) and doxycycline (MilliporeSigma) were obtained from the suppliers listed. The human colonic epithelial cell line, HCA7 (obtained from the late Martin F. Kagnoff, UCSD), transfected with dominant-negative TCPTP (C216S), was cultured in a humidified atmosphere with 5% CO₂ in Dulbecco’s modified Eagle’s/F-12 medium (Mediatech, Inc.) supplemented with 5% Tet-approved new-born calf serum (Clontech) in 75 cm² flasks and grown in selection media supplemented with G418 and puromycin per Clontech protocol. Culture medium was changed twice a week. Cells were separated by trypsinization and 0.5 x 10⁶ cells were seeded onto 12 mm Millicell-HA semipermeable filter supports (pore size 0.45 μm) with a surface area of 0.6 cm² (MilliporeSigma). The cell monolayers were incubated for 48 hours with media (vehicle) or doxycycline (15 μg/mL) followed by coinubcation for 6 or 72 hours with doxycycline (15 μg/mL; apically) and IFN-γ (200 or 500 U/mL; 6 or 24 hours; basolaterally).

**In vitro rescue experiments with recombinant matrkapase.** HT-29 control-shRNA (obtained in-house) and TCPTP-KD cells were seeded on 12-well Transwells (0.5 x 10⁶ cells; ref. 30). On day 5, media were changed, cells were treated with rMAT in serum-free media either apically or basolaterally (5 nM), and TER was measured. The percentage change in TER after 24 hours was calculated from the TER at 0 hours of untreated cells (control and KD, respectively).

**Western blot analysis of whole-tissue lysates, isolated IECs, and cell cultures.** Isolated whole intestinal tissues were everted and incubated in Cell Recovery Solution (354253, Corning) on ice for 2 hours, then vigorously shaken by hand to release IECs. IECs were washed twice with ice-cold PBS, then lysed with radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) supplemented with 1x protease inhibitor (Roche), 2 mM sodium fluoride, 1 mM PMSF, and phosphatase inhibitors (2 mM sodium orthovanadate, Phosphatase Inhibitor Cocktail 2 and 3, MilliporeSigma) for at least 10 minutes on ice. Cells were homogenized on ice using the QI25 Sonicator (QSonica Sonicators), lysates centrifuged at 16,200g at 4°C for 10 minutes, and supernatants collected into new microcentrifuge tubes. Protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Loading samples were prepared by mixing the same amount of total protein from each sample with Laemmli loading buffer (60 mM Tris-HCl pH 6.8, 2% SDS, 5% β-mercaptoethanol, 0.01% bromophenol blue, and 10% glycerol), then boiling the samples at 95°C for 10 minutes.

IEC monolayers were removed from insert membranes, suspended in ice-cold RIPA lysis buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 20 μg/mL NaF, 1 mM EDTA, 1 μg/mL anti-pain, 1 μg/mL leupeptin, 1 μg/mL NaVO₃, 100 μg/mL PMSF), vortexed thoroughly, and subjected to lysis. Lysates were centrifuged at 12,000g for 10 minutes at 4°C to remove insoluble material, and protein content was determined as above. Samples were resuspended in loading buffer (50 mM Tris at pH 6.8, 2% SDS, 100 mM dithiothreitol, 0.2% bromophenol blue, 20% glycerol) and boiled for 5 minutes. Equal protein amounts were separated on 4% to 15% polyacrylamide gradient gels to resolve proteins (Mini-protean, Bio-Rad) and transferred onto polyvinylidene difluoride membranes (DuPont-New England Nuclear). Membranes were blocked with a 1% BSA solution in 0.1% Tween/Tris buffer for 30 minutes, followed by incubation with a 0.1% BSA/Tris buffer solution containing primary antibodies at 1:1000 dilution in wash buffer (0.1% Tween 20, in Tris buffer). After washing, secondary antibody HRP-conjugated goat anti-mouse (catalog 115-036-062) or goat anti-rabbit IgG (111-036-045; both Jackson Immunoresearch), at 1:1000 dilution in wash buffer, was added for 60 minutes followed by washing (x3). The membrane was treated with chemiluminescence solution per manufacturer's directions (ECL, Pierce, Thermo Fisher Scientific) and exposed to radiographic film (LabScientific). Densitometric analysis was performed using ImageJ. Primacy antibodies used for Western blot analysis were mouse monoclonal anti-TCPTP antibody CF-4 (catalog PH031; Calbiochem, Merck), rabbit anti-TCPTP (catalog 589395), anti–phospho-STAT1 (Tyr701; catalog 9167), anti–STAT1 (catalog 9175), anti–phospho-STAT3 (Tyr705; catalog 9145), anti–STAT3 (catalog 9139), anti–phospho-MLC (Ser19, myosin light chain 2; catalog 3671), and anti–MLC (catalog 3672) (Cell Signaling Technology); rabbit anti–CLDN1 (catalog 31-9000), anti–CLDN2 (catalog 32-5600), anti–CLDN3 (catalog 34-1700), anti–CLDN4 (catalog 32-9400), anti–CLDN15 (catalog 32-9800), anti–CLDN18 (catalog 32-9800), and anti–CLDN20 (catalog 32-9800).
and mouse anti-HA (catalog 26183) (Thermo Fisher Scientific); and rabbit anti-ST14/matriptase (catalog ab28266) and HAI-1 (catalog ab228661) (Abcam); and mouse monoclonal anti-β-actin (catalog number A5316; MilliporeSigma).

In vitro TCPTP KD and quantitative PCR. TCPTP-KD studies were performed in HCA-7 cells in accordance with previously published protocols (30). HCA-7 cells were processed using the Direct-zol RNA extraction kit from ZymoResearch. Transcript was purified using Turbo DNase (Invitrogen, Thermo Fisher Scientific) and converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit from Life Technologies, Thermo Fisher Scientific. Quantitative PCR was processed and analyzed using SYBR green and the step-one system. Fold change was assessed using the 2^{ΔΔCt} method. Knockdown efficiency was confirmed by quantitative PCR using predesigned primer sets from Integrated DNA Technologies.

Claudin-2 siRNA KD. Caco-2BBe (from Robert H. Whitehead, Vanderbilt University, Nashville, Tennessee, USA) were grown to confluence in 60 mm dishes and fixed with 2% glutaraldehyde in PBS for 1 hour. After fixation, cells were lifted from dishes using a LifeCell CF-100 “slam freezer.” Freeze fracture was performed using a Balzer freeze fracture apparatus at –110°C, shadowed with platinum at a 45° angle, and stabilized with a carbon backing. The replicas were imaged using a JEOL 2100 transmission electron microscope operating at 200 kV equipped with a Gatan Orius 832 charge-coupled device camera. Strand discontinuities were characterized by comparing the number of gaps in P-face strands with their total length (calculated in 20 strand segments totaling 8–9 μm per condition).

Statistics. All statistical tests were performed using GraphPad Instat or Prism (v6) using, where appropriate, 2-tailed Student’s t test, 1-way ANOVA or 2-way ANOVA, and Student-Newman-Keuls, Tukey’s, or Bonferroni’s posttest. P ≤ 0.05 was considered not significant (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001).

Study approval. For animal studies, the experimental protocols were approved by the IACUC of UCR under protocol A-20120003B and at UCSD under protocol S02190. For human studies, the collection of intestinal biopsies, DNA preparation, and genotyping were approved by the Cedars-Sinai Medical Center Institutional Review Board (number 3358). Written informed consent was obtained from all study participants.

Author contributions
RRM, MK, MRS, and DFM designed the experiments. RRM, MK, TWP, YSP, RA, ASB, VC, MRS, LHFB, SM, EK, AS, and DFM performed the experiments. RRM, MK, RA, ASB, VC, MRS, EK, CRW, and DFM analyzed the data. CRW analyzed pathological specimens. DPBM provided human specimens. MLT provided constitutive Tcpp-KO mice. RRM and DFM wrote the manuscript. DFM conceived the study and obtained funding. All the authors critically reviewed and approved the final version of the manuscript. RRM, MK, and MRS share joint first authorship as RRM was the lead experimenter at the beginning of the study, MK performed critical experiments to complete the initial submission, and MRS performed critical experiments to complete extensive revisions to the study.

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