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Epithelial chemerin–CMKLR1 signaling restricts microbiota-driven colonic neutrophilia and tumorigenesis by up-regulating lactoperoxidase

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Intestinal barrier immunity is essential for controlling gut microbiota without eliciting harmful immune responses, while its defect contributes to the breakdown of intestinal homeostasis and colitis development. Chemerin, which is abundantly expressed in barrier tissues, has been demonstrated to regulate tissue inflammation via CMKLR1, its functional receptor. Several studies have reported the association between increased expression of chemerin–CMKLR1 and disease severity and immunotherapy resistance in inflammatory bowel disease (IBD) patients. However, the pathophysiological role of endogenous chemerin–CMKLR1 signaling in intestinal homeostasis remains elusive. We herein demonstrated that deficiency of chemerin or intestinal epithelial cell (IEC)-specific CMKLR1 conferred high susceptibility to microbiota-driven neutrophilic colon inflammation and subsequent tumorigenesis in mice following epithelial injury. Unexpectedly, we found that lack of chemerin–CMKLR1 signaling specifically reduced expression of lactoperoxidase (LPO), a peroxidase that is predominantly expressed in colonic ECs and utilizes H_2O_2 to oxidize thiocyanates to the antibiotic compound, thereby leading to the outgrowth and mucosal invasion of gram-negative bacteria and dysregulated CXCL1/2-mediated neutrophilia. Importantly, decreased LPO expression was causally linked to aggravated microbiota-driven colitis and associated tumorigenesis, as LPO supplementation could completely rescue such phenotypes in mice deficient in epithelial chemerin–CMKLR1 signaling. Moreover, epithelial chemerin–CMKLR1 signaling is necessary for early host defense against bacterial infection in an LPO-dependent manner. Collectively, our study reveals that the chemerin–CMKLR1/LPO axis represents an unrecognized immune mechanism that potentiates epithelial antimicrobial defense and restricts harmful colonic neutrophilia and suggests that LPO supplementation may be beneficial for microbiota dysbiosis in IBD patients with a defective innate antimicrobial mechanism.

chemerin–CMKLR1 | lactoperoxidase | colitis | epithelial antimicrobial defense | CXCL1/2

Significance

Epithelial antimicrobial defense is an important innate mechanism to maintain intestinal homeostasis and closely involved in the pathogenesis of inflammatory bowel disease (IBD). Chemerin, which is abundantly expressed in barrier tissues, regulates tissue inflammation via CMKLR1, its functional receptor. We herein reveal a role of epithelial chemerin–CMKLR1 signaling in sustaining epithelial antimicrobial defense, thereby conferring protection from microbiota-driven neutrophilic colitis and subsequent tumorigenesis following epithelial injury. Mechanistically, we identify lactoperoxidase (LPO), which is highly expressed in murine colonic epithelium, as the downstream effector of chemerin–CMKLR1 signaling to restrict the outgrowth and invasion of gram-negative bacteria, thereby preventing dysregulated CXCL1/2 production and pathological mucosal neutrophilia. Thus, targeting chemerin–CMKLR1/LPO axis may be beneficial for improving dysbiosis in IBD patients.

Inflammatory bowel disease (IBD) is a complex disorder caused by the breakdown of intestinal homeostasis resulting from dysregulated interactions between intestinal epithelial cells (IECs), gut microbiota, and immune cells (1, 2). Intestinal epithelium, which lies at the center of such interaction, plays an active role in orchestrating the host–microbiota mutualism at steady state and initiating immune responses to invading pathogens and tissue injury (3, 4). IECs respond to microbes, metabolites, and cytokines by expressing various receptors, which is critical for epithelial barrier functions, including antimicrobial peptide synthesis and mucus secretion. Deficiency in such epithelial receptors often leads to excessive microbiota-driven immune responses and dysbiosis, two key events in the initiation of IBD (5–8). Recently, a critical epithelial antimicrobial defense mediated by epithelium-specific NADPH oxidase 1 (NOX1) and dual oxidase 2 (DUOX2) via generating superoxide and hydrogen peroxide (H_2O_2), respectively, has been implicated in intestinal homeostasis and colitis development (9). Moreover, IECs could relay dietary and microbial signals by secreting various cytokines and chemokines to recruit myeloid cells such as neutrophils, which further amplify the immune response against invading pathogens (10, 11).

Neutrophils, as a vital component of the innate immune system, are critical for maintaining intestinal homeostasis by eliminating invading microbes (12). However, dysregulated neutrophil recruitment is causally linked to pathological intestinal inflammation (13). Dense neutrophil infiltration together with extensive mucosal injury and bacterial invasion are often observed in patients with IBD, particularly ulcerative colitis (UC) (14). Inhibition of aberrant neutrophil migration by interrupting the critical neutrophil chemokine receptor CXCL1/CXCR2 could effectively ameliorate colitis and inflammation-driven tumorigenesis (15, 16). Therefore, identification of the critical

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regulatory mechanisms that prevent massive neutrophil recruitment into colonic mucosa would be helpful to develop new therapeutic targets for IBD.

Chemerin is abundantly expressed in barrier tissues and closely associated with tissue inflammation (17). Early studies identified chemerin as a chemoattractant for plasmacytoid dendritic cells and natural killer (NK) cells via its functional receptor CMKLR1 (18, 19). CMKLR1 was also expressed by several tissue structural cells to control their production of cytokines in response to different stimuli, thereby indirectly influencing immune cell activation and recruitment in the microenvironment (20, 21). Several preclinical studies demonstrated inhibitory effects of chemerin–CMKLR1 signaling on tissue neutrophilia (22, 23). A newly published study demonstrated therapeutic effects of agonist anti-CMKLR1 monoclonal antibodies on mouse models of colitis and colon cancer (24), which is in contrast to our previous finding showing that administration of recombinant chemerin exacerbated dextran sulfate sodium (DSS)–induced colitis in chemerin-intact wild-type (WT) mice (25). Such paradoxical results could be due to systemic administration of exogenous chemerin, which may act on different cell types that express CMKLR1 at varying levels in different tissues. Emerging studies reported elevated chemerin levels locally and systemically in UC patients (25, 26) and the association between CMKLR1 expression and resistance to immunotherapies in IBD patients (24). However, the pathophysiological role of endogenous chemerin–CMKLR1 signaling in intestinal homeostasis and colitis development is yet to be thoroughly investigated.

Here, we demonstrated a critical role of epithelial chemerin–CMKLR1 signaling in restricting microbiota-driven neutrophilic colon inflammation and subsequent tumorigenesis as well as enteric bacterial infection. Importantly, we identified lactoperoxidase (LPO), an epithelial peroxidase that utilizes H_2O_2 to oxidize thiocyanates into an antibiotic compound (27), as the downstream effector of chemerin–CMKLR1 signaling to mediate such protection by inhibiting outgrowth and mucosal invasion of gram-negative bacteria and thereby preventing dysregulated CXCL1/2 production.

Results

Chemerin Deficiency Renders Mice Susceptible to DSS-Induced Colitis and Severe Neutrophilic Inflammation. To determine the role of endogenous chemerin in colitis development, *Rarres2*$^{-/-}$ mice and WT littermates were subjected to DSS-induced colitis. *Rarres2*$^{-/-}$ mice displayed significantly greater weight loss, which persisted until day 21 post-DSS, while WT mice almost completely recovered on day 21 (Fig. 1*A*). *Rarres2*$^{-/-}$ mice also had greater colon length shortening and higher scores of disease activity index (DAI) than WT littermates (Fig. 1*B* and *C*). Histological analysis revealed no morphological sign of damage or detectable inflammation in colons from WT and *Rarres2*$^{-/-}$ mice untreated and on day 3, but obvious increases in inflammatory infiltrates, edema, and disrupted epithelial structure in colons of *Rarres2*$^{-/-}$ mice on days 7 and 11 post-DSS (Fig. 1*D*). Furthermore, flow cytometry analysis revealed that numbers of neutrophils, but not other immune cell types, significantly increased in colons of *Rarres2*$^{-/-}$ mice compared with WT littermates post-DSS (Fig. 1*E* and *SI Appendix*, Fig. S1*A*). Accordingly, significantly increased colonic production of proinflammatory cytokines, including TNF-α, IL-6, and IL-1β, as well as the neutrophil chemokines CXCL1 and CXCL2, were detected in *Rarres2*$^{-/-}$ mice post-DSS (Fig. 1*F*). Although we previously reported that exogenous chemerin exacerbated DSS-induced colitis in chemerin-intact WT mice (25), intraperitoneal (i.p.) administration of exogenous chemerin rescued aggravated phenotypes in *Rarres2*$^{-/-}$ mice (*SI Appendix*, Fig. S1 *B*–*G*). Together, these data demonstrate that endogenous chemerin is protective against DSS-induced colitis and neutrophilic colon inflammation.

Chemerin Deficiency Promotes Colitis-Associated Tumorigenesis, which Is Primarily Dependent on CXCR2-Mediated Neutrophilia during Chronic Colitis. IBD is known as a strong predisposing factor for the development of colitis-associated cancer (CAC). We then investigated the role of endogenous chemerin in inflammation-driven intestinal tumorigenesis by using a well-established azoxymethane (AOM) + DSS model. *Rarres2*$^{-/-}$ mice exhibited significantly greater and more persistent weight loss throughout the chronic DSS treatment (Fig. 1*G*). Moreover, *Rarres2*$^{-/-}$ mice were more susceptible to colitis-associated tumorigenesis as evidenced by significantly increased numbers of tumors in different sizes and histological analysis showing colon tumors with higher grade (Fig. 1*H* and *I*). Given that significantly increased colonic infiltration of neutrophils and levels of CXCL1/2 occurred early in *Rarres2*$^{-/-}$ mice on day 3 before colitis onset (Fig. 1*E* and *F*), we attempted to determine whether colonic neutrophilia plays a causative role in aggravated colitis and associated tumorigenesis in *Rarres2*$^{-/-}$ mice. To this end, CXCR2 antagonist SB225002 was i.p. administered into WT and *Rarres2*$^{-/-}$ mice during DSS exposure. SB225002 treatment effectively inhibited colonic infiltration of neutrophils (Fig. 1*J*) and eliminated the differences in clinical symptoms, histology, and inflammatory cytokine levels between WT and *Rarres2*$^{-/-}$ mice post-DSS (Fig. 1*K*–*O*). Importantly, increased tumorigenesis was more obviously abrogated by SB225002 treatment during the chronic colitis phase (days 1 to 54) than that during the tumor phase (days 54 to 80) (Fig. 1*H* and *I*). These data demonstrate that dysregulated CXCR2-mediated colonic neutrophilia accounts for aggravated colitis and subsequent tumorigenesis in *Rarres2*$^{-/-}$ mice.

Specific Deletion of CMKLR1 in IECs Aggravates CXCL1/2-Mediated Neutrophilic Colon Inflammation and Subsequent Tumorigenesis. We next determined which receptor mediates the protective effect of endogenous chemerin on neutrophilic colon inflammation. CMKLR1 was expressed at higher levels in colons of naïve WT mice than CCRL2, the second receptor that is responsible for chemerin enrichment (28), and DSS exposure greatly up-regulated both on day 7 with much higher levels in CMKLR1 (Fig. 2*A*). Notably, *Cmklr1*$^{-/-}$, but not *Ccrl2*$^{-/-}$ mice, recapitulated the severe phenotypes in *Rarres2*$^{-/-}$ mice post-DSS (Fig. 2*B*–*G*), suggesting CMKLR1 as the functional receptor to restrict DSS-induced colitis and colonic neutrophilia. We then attempted to investigate whether lack of the chemerin–CMKLR1 signaling causes dysregulated CXCL1/2 production. We isolated IECs and CD45^{+} lamina propria leukocytes (LPLs) from colons of DSS-exposed WT mice and found that IECs were the primary source of CXCL1/2 (Fig. 2*H*). Moreover, *Rarres2*$^{-/-}$ IECs expressed significantly higher *Cxcl1/2* expression than WT controls (Fig. 2*I*). Notably, obvious CMKLR1 expression was detected in IECs by immunohistochemical staining of WT, but not *Cmklr1*$^{-/-}$, colons (Fig. 2*J*). Flow cytometry analysis further supported CMKLR1 expression in IECs (Fig. 2*K*). These data suggest IECs as the potential target cell of CMKLR1 signaling to control CXCL1/2 production. To seek direct evidence, *Cmklr1*$^{\Delta IEC}$ mice with specific deletion of CMKLR1 in IECs (*SI Appendix*, Fig. S2*A* and *B*) were subjected to DSS-induced colitis. *Cmklr1*$^{\Delta IEC}$ mice phenocopied both *Rarres2*$^{-/-}$ and *Cmklr1*$^{-/-}$ mice (Fig. 2*L*–*O* and *SI Appendix*,

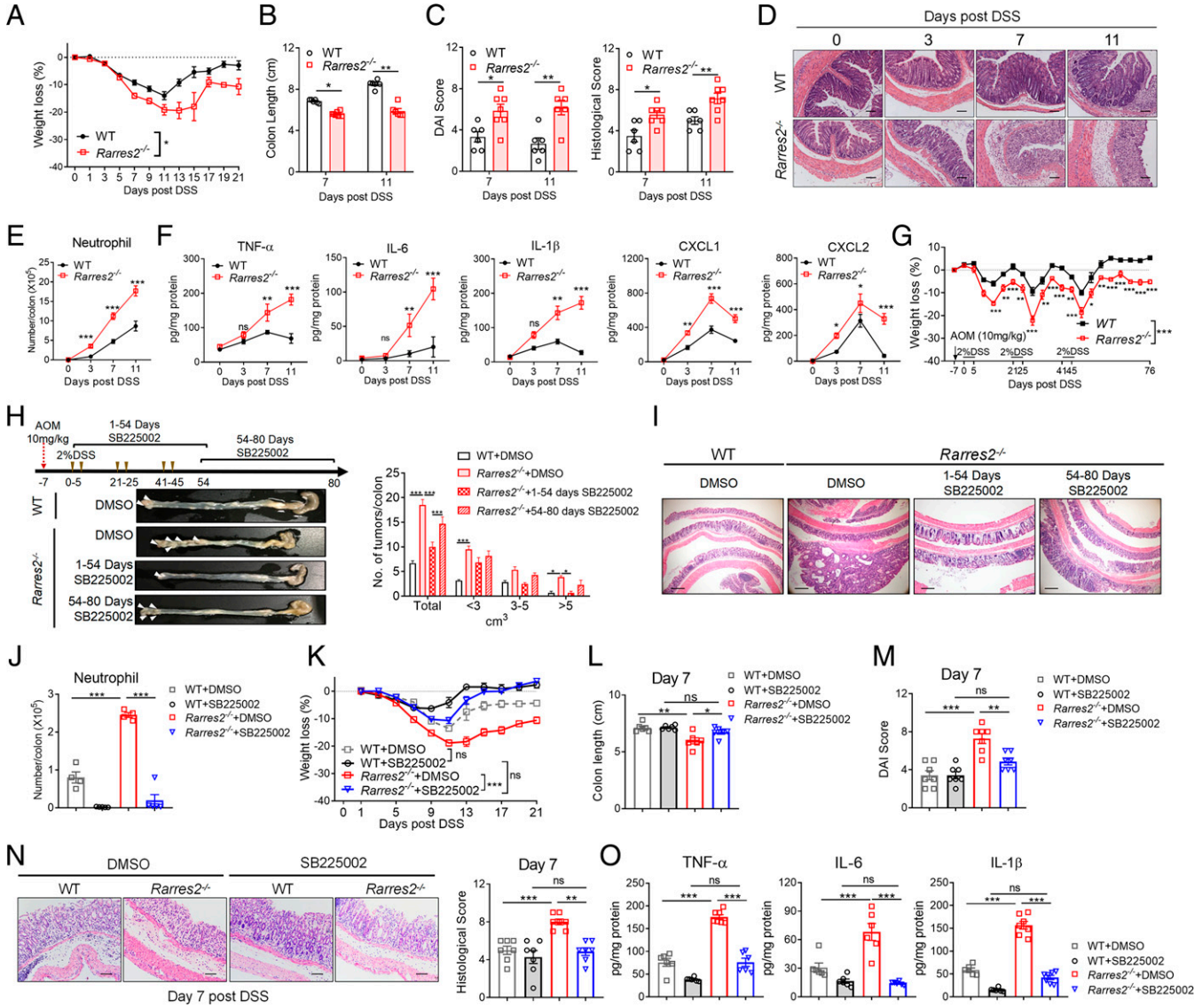

Fig. 1. *Rarres2*$^{-/-}$ mice are highly susceptible to DSS-induced colitis and subsequent tumorigenesis, which is attributed to dysregulated CXCR2-dependent neutrophilia. (*A*–*F*) Weight loss (*A*), colon length (*B*), DAI score (*C*), H&E analysis of colons (*D*), colonic neutrophil numbers (*E*), and colonic levels of proinflammatory cytokines (*F*) of WT and *Rarres2*$^{-/-}$ mice at different time points post-DSS. (*G*) Weight loss during AOM/DSS-induced CAC model. (*H* and *I*) CXCR2 antagonist SB225002 was administered at colitis phase (days 1 to 54) or tumor phase (days 54 to 80). Pictures of colon tumors and tumor numbers in different sizes (*H*) as well as H&E analysis of colons (*I*) of mice in the CAC model. (*J*–*O*) Neutrophil number (*J*), weight loss (*K*), colon length (*L*), DAI score (*M*), H&E analysis of colons (*N*), and levels of inflammatory cytokines in colon tissues (*O*) of WT and *Rarres2*$^{-/-}$ mice on day 7 post-DSS treated with SB225002. (Scale bars: 100 μm [original magnification, 100×].) Dimethylsulfoxide (DMSO) was used as vehicle control for SB225002 treatment. Data are shown as mean ± SEM; $n = 6$ to 7. Significance was determined using one-way ANOVA (*H*) or two-way ANOVA (*A*–*C*, *E*–*G*, and *J*–*O*) followed by Tukey's multiple comparison test. Results are representative of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.

Fig. S2*C*–*I*). SB225002 treatment eliminated the differences in colitis symptoms between *Cmklr1*$^{\Delta IEC}$ mice and *Cmklr1*$^{fl/fl}$ littermates post-DSS (Fig. 2*L*–*O*). *Cmklr1*$^{\Delta IEC}$ mice were also highly susceptible to colitis-associated tumorigenesis, which was effectively reversed by SB225002 treatment during the chronic colitis phase (Fig. 2*P* and *Q*). Together, these results demonstrate that epithelial chemerin–CMKLR1 signaling is necessary for restricting colonic neutrophilia via suppressing CXCL1/2 production post-DSS.

Lack of Chemerin–CMKLR1 Signaling Suppresses LPO Expression in IECs, Leading to Aggravated Colonic Neutrophilia and Tumorigenesis. Inflammatory signaling induced by proinflammatory cytokines or Toll-like receptor (TLR) agonists are potent stimuli to induce CXCL1/2 production by IECs (10). We first excluded the possibility that chemerin directly inhibited CXCL1/2 production, as chemerin pretreatment had no effect on CXCL1/2 levels at baseline or following stimulation with TNF-α or lipopolysaccharide (LPS) in the culture of primary murine colonic ECs (Fig. 3*A*). Similarly, chemerin did not inhibit CXCL1/2 production by intestinal EC lines CT26 or MC38, which expressed CMKLR1 (*SI Appendix*, Fig. S3*A* and *B*). Furthermore, *Cmklr1*$^{-/-}$ IECs showed no intrinsic defect in CXCL1/2 production in response to inflammatory stimuli (Fig. 3*B*). These data raised another possibility that chemerin–CMKLR1 signaling may regulate some upstream factors of CXCL1/2 production. To interrogate the specific target gene of the chemerin–CMKLR1 signaling in IECs, we performed RNA sequencing (RNA-seq) of IECs isolated from colons of *Rarres2*$^{-/-}$ mice and WT littermates on day 2 when there was no detectable inflammation and on day 7 when the colitis was full-blown. There were no differences in genes associated with mucus and tight junction, all of which contribute to

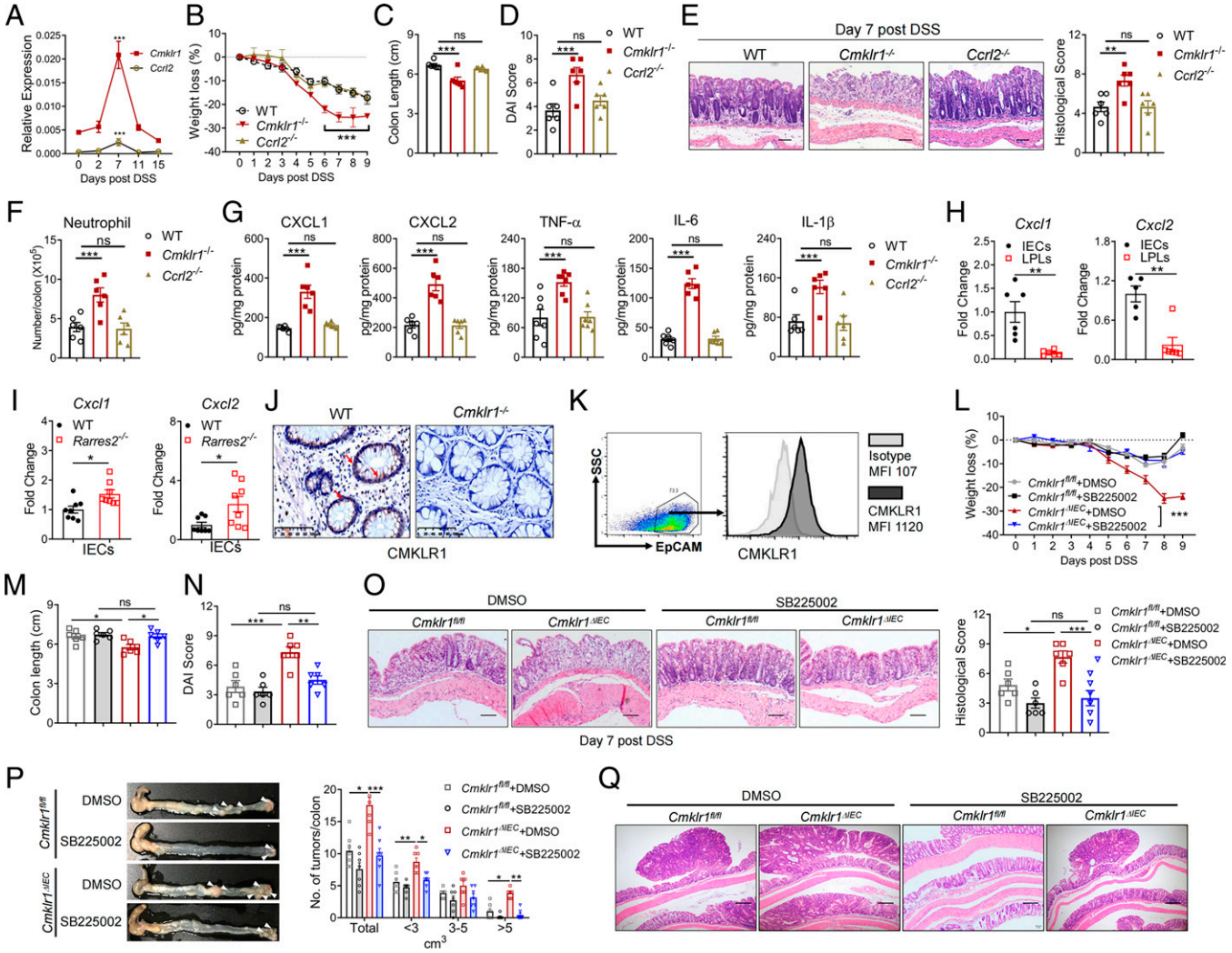

Fig. 2. Specific deletion of epithelial CMKLR1 aggravates CXCL1/2-mediated colonic neutrophilic inflammation and subsequent tumorigenesis. (*A*) qPCR analysis of *Cmklr1* and *Ccrl2* expression in colons of WT mice. (*B*–*F*) Weight loss (*B*), colon length (*C*), DAI score (*D*), H&E analysis of colons and histological score (*E*), neutrophil numbers (*F*), and levels of inflammatory cytokines in colon tissues of different genotypes (*G*). (*H* and *I*) Fold change of *Cxcl1/2* expression in IECs and LPLs isolated from colons of WT mice (*H*) and in IECs of WT and *Rarres2*$^{-/-}$ mice on day 3 post-DSS (*I*). (*J* and *K*) CMKLR1 expression analyzed by IHC analysis in colons of WT and *Cmklr1*$^{-/-}$ mice (*J*) and by flow cytometry in EpCAM^{+} IECs of WT mice (*K*). (*L*–*O*) Weight loss (*L*), colon length (*M*), DAI score (*N*), and H&E analysis of colons and histological score (*O*) of *Cmklr1*$^{\Delta IEC}$ mice on day 7 post-DSS treated with SB225002. (*P* and *Q*) Pictures of colon tumors and tumor numbers in different sizes (*P*) and H&E analysis of colons (*Q*) of mice in the CAC model. Data are shown as mean ± SEM; n = 5 to 7. Mann–Whitney U test (*H* and *I*), one-way ANOVA (*C*–*G*), and two-way ANOVA (*A*, *B*, and *L*–*P*) followed by Tukey's multiple comparison test were used. Results are representative of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.

barrier integrity function, between WT and *Rarres2*$^{-/-}$ mice on day 2 (*SI Appendix*, Fig. S4*A*). There were no differences in the number of Ki67^{+} proliferating cells and apoptotic cells determined by terminal deoxynucleotidyl transferase dUTP nick end labeling staining of colons from WT and *Rarres2*$^{-/-}$ mice on days 0 and 3 post-DSS before colitis onset (*SI Appendix*, Fig. S4*B* and *C*). Significantly decreased Ki67^{+} cells in colons of *Rarres2*$^{-/-}$ mice were observed on day 11 (*SI Appendix*, Fig. S4*B*), which was very likely due to aggravated colonic inflammation, as the previous study has demonstrated that prolonged intestinal inflammation inhibits IEC proliferation (29). We also noted that some antimicrobial peptides such as S100a8/9 and Reg3β/γ increased, albeit at very low levels, in *Rarres2*$^{-/-}$ IECs on day 2, which was further up-regulated to higher levels on day 7 (Fig. 3*C* and *SI Appendix*, Fig. S4*A*). Surprisingly, we found that LPO, which was reported to exert antimicrobial activities as an epithelial peroxidase (27), was dramatically down-regulated in *Rarres2*$^{-/-}$ IECs on day 2 and remained one of the top down-regulated genes on day 7 post-DSS (Fig. 3*C*). Mouse genome study and a previous study demonstrated predominant LPO expression in murine colons, particularly epithelial cells (30). Consistently, we showed high baseline levels of *Lpo* in colonic ECs but very little, if any, *Lpo* in LPLs isolated from WT mice (Fig. 3*D*). This was further confirmed by analyzing recently published single-cell RNA-seq data of murine colon cells (31, 32) (*SI Appendix*, Fig. S4*D* and *E*), although analyzing the published single-cell RNA-seq data of normal human colons showed very low levels of LPO expression (*SI Appendix*, Fig. S4*F*) (33, 34). We further validated blunted *LPO* expressions at gene and protein levels in colonic ECs and total colons from *Rarres2*$^{-/-}$ or *Cmklr1*$^{\Delta IEC}$ mice by qPCR, immunohistochemistry (IHC) staining, and Western blotting (Fig. 3*D*–G). It has been reported that LPO teams up with DUXO2 to generate antibacterial compounds (35). In contrast to dramatically decreased LPO expression, slightly increased gene expression of DUXO1/2 was detected in *Rarres2*$^{-/-}$ IECs on day 2 with no change on day 7 post-DSS (*SI Appendix*, Fig. S4*G* and *H*). These data suggest that chemerin–CMKLR1 signaling specifically inhibits LPO expression in colonic ECs.

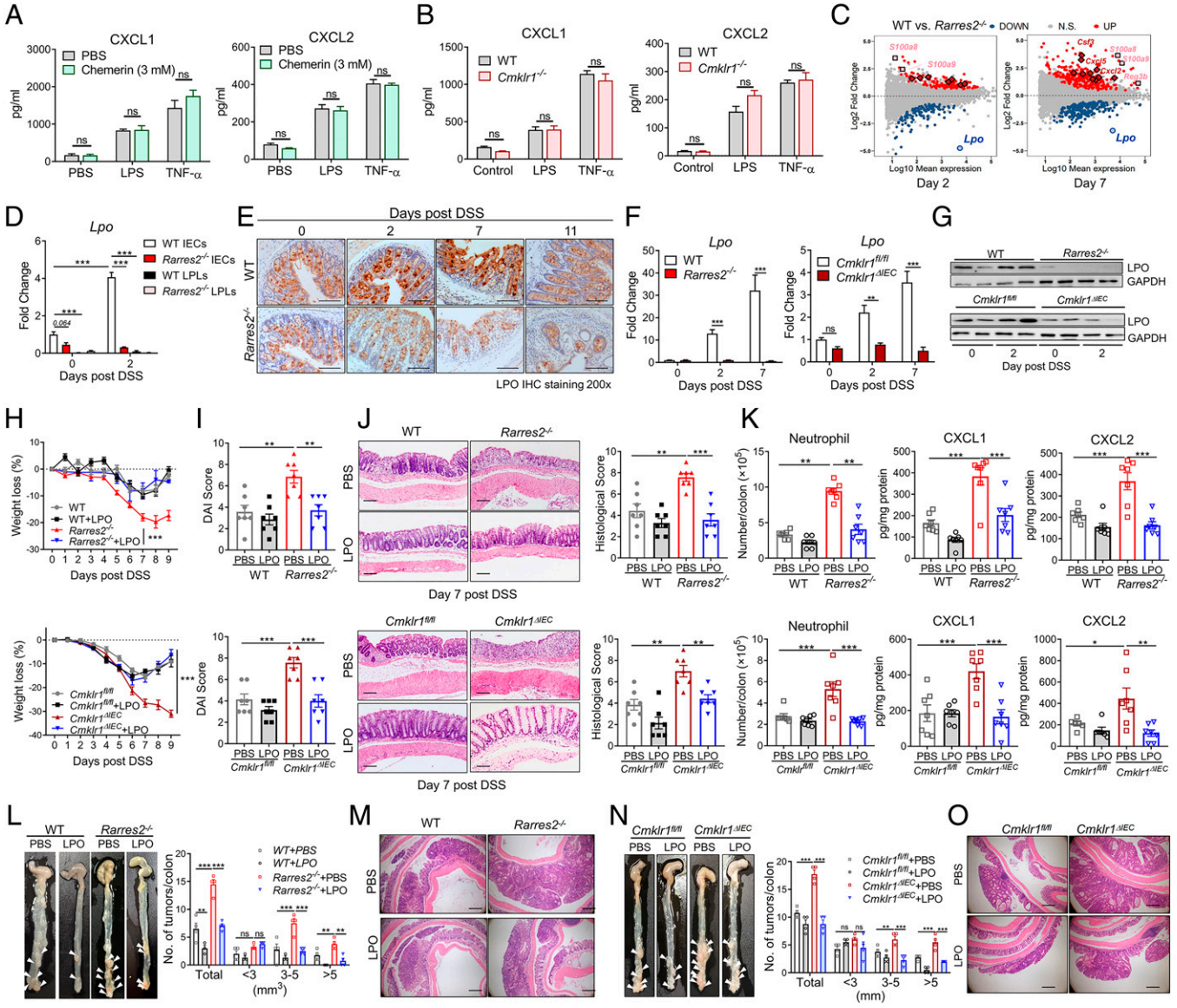

Fig. 3. Lack of chemerin–CMKLR1 signaling suppresses LPO expression in IECs post-DSS, leading to aggravated colonic neutrophilia and associated tumorigenesis. (*A* and *B*) CXCL1/2 levels in cultures of WT colonic ECs that were pretreated with chemerin (3 nM) (*A*) and *Cmklr1*$^{-/-}$ colonic epithelial cells following stimulation with TNF-α (20 ng/mL) or LPS (100 ng/mL) (*B*); $n = 3$ to 4. (*C*) M-versus-A (MA) plot of RNA-seq data of colonic ECs on days 2 and 7 post-DSS. (Diamonds indicate genes encoding proinflammatory factors; pink squares indicate genes of antimicrobial peptides); $n = 3$. (*D*) Fold change of *Lpo* expression. (*E*) IHC analysis of LPO expression in colons. (Scale bars: 100 μm.) (*F* and *G*) Fold change of *Lpo* expression (*F*) and immunoblot analysis of LPO in colon tissues of different genotypes (*G*); $n = 5$. (*H–J*) Weight loss (*H*), DAI score (*I*), H&E analysis of colons and histological score (*J*), neutrophil numbers and CXCL1/2 protein levels (*K*) in the colon tissues of DSS-exposed mice on different genotypes with or without LPO treatment; $n = 6$ to 7. (*L–O*) Pictures of colon tumors and tumor numbers in different sizes (*L* and *N*), and H&E analysis of colons (*M* and *O*) of mice on different genotypes in the CAC model with or without LPO treatment; $n = 4$. Data are shown as mean ± SEM. Two-way ANOVA followed by Tukey's multiple comparison test were used. Results are representative of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns or N.S., not significant.

To investigate whether decreased LPO expression accounts for aggravated colonic neutrophilia and subsequent tumorigenesis in the absence of chemerin–CMKLR1 signaling, *Rarres2*$^{-/-}$ or *Cmklr1*$^{\Delta IEC}$ mice were supplemented with LPO by gavage during DSS exposure. LPO supplementation effectively reversed the severity of DSS-induced colitis in both genotypes and reduced colonic infiltration of neutrophils and expression of CXCL1/2 to similar levels observed in corresponding littermate controls (Fig. 3*H–K*). However, LPO supplementation had no significant effects on WT littermates (Fig. 3*H–K*). Furthermore, LPO supplementation reversed the increased tumorigenesis in both *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice, but only had mild effects on WT littermates (Fig. 3*L–O*). Collectively, these data demonstrate that LPO serves as the downstream effector of epithelial chemerin–CMKLR1 signaling to suppress CXCL1/2-mediated colon neutrophilia and subsequent tumorigenesis.

Both Chemerin–CMKLR1 Signaling and Microbiota-Derived TLR4/Myd88 Signaling Are Indispensable for LPO Expression in Colonic Epithelial Cells. To clarify the mechanism that regulates LPO expression in colonic ECs, we first performed a longitudinal analysis of gene expression profiles of CMKLR1 and LPO in the gut of mice kept in our animal facility. As shown in Fig. 4*A*, *Cmklr1* was expressed in all intestinal segments with the highest levels in the colon and the rectum, while *Lpo* was expressed at very low levels in the small intestine but at very high levels in the colon and the rectum. Notably, i.p. administration of exogenous chemerin almost completely restored *Lpo*

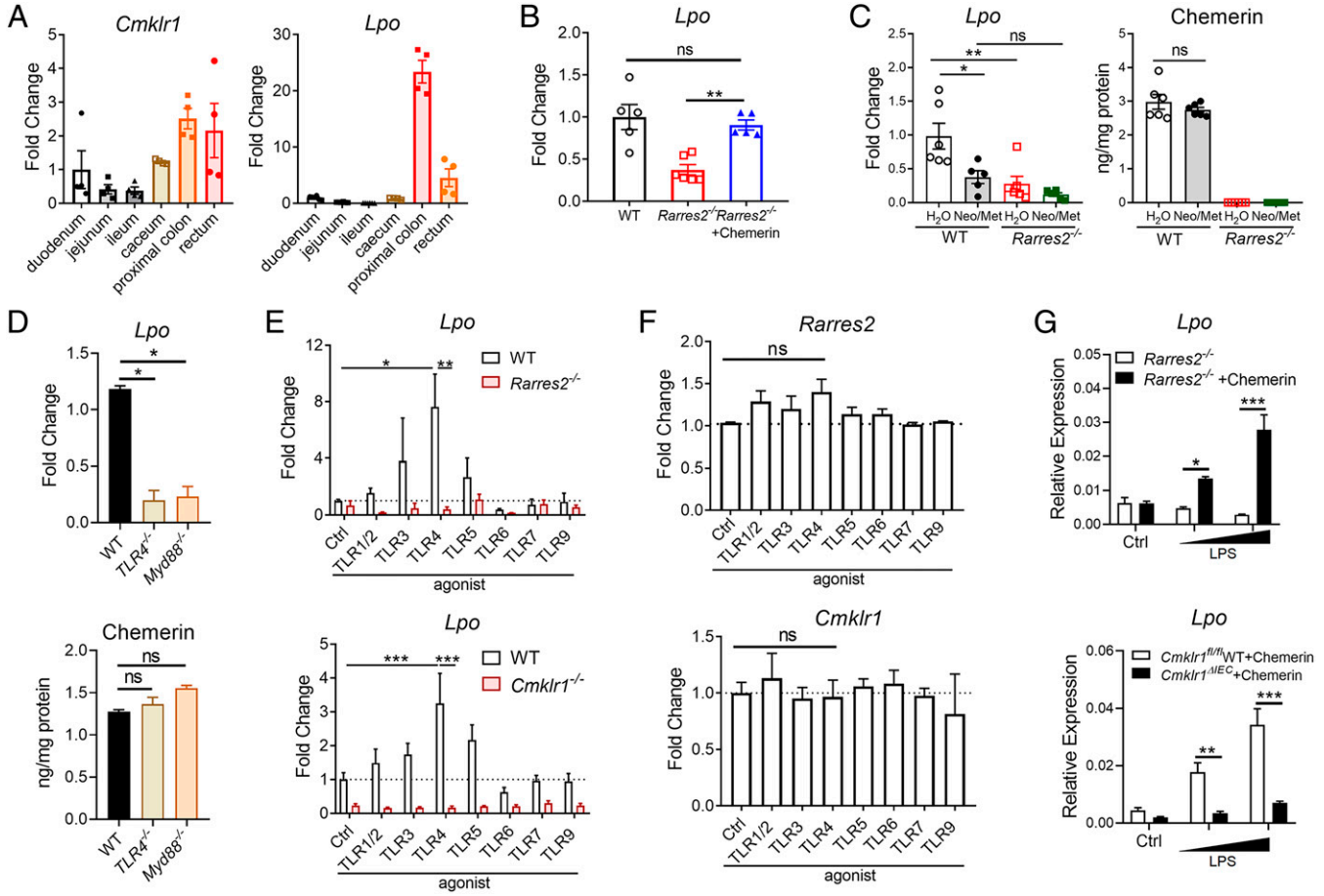

Fig. 4. Both chemerin–CMKLR1 signaling and microbiota-derived TLR4-Myd88 signaling are indispensable for LPO expression. (*A*) *Cmklr1* and *Lpo* expression in different intestinal segments of WT mice; $n = 4$. (*B*) Fold change of *Lpo* expression in colons of WT mice and *Rarres2*$^{-/-}$ mice treated with recombinant mouse chemerin (50 ng/mice) during DSS exposure; $n = 5$. (*C* and *D*) Fold change of *Lpo* expression and chemerin protein levels in colons of Neo/Met-pretreated WT and *Rarres2*$^{-/-}$ mice on day 7 (*C*) as well as WT, *Tlr4*$^{-/-}$, and *Myd88*$^{-/-}$ mice on day 2 post-DSS; $n = 3$ to 6 (*D*). (*E* and *F*) Fold change of *Lpo* expression (*E*) and chemerin protein levels and fold change of *Cmklr1* expression (*F*) in colon explants stimulated with different TLR agonists; $n = 3$. (*G*) *Lpo* expression in colon explants stimulated with chemerin (3 nM) together with LPS at different doses; $n = 3$. Data are shown as mean ± SEM. One-way ANOVA (*B*, *D*, and *F*) and two-way ANOVA (*C*, *E*, and *G*) followed by Tukey's multiple comparison test were used. Results are representative of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.

expression in colons of DSS-exposed *Rarres2*$^{-/-}$ mice (Fig. 4*B*), emphasizing the indispensable role of chemerin–CMKLR1 signaling for LPO expression. Given that the colon contains the most abundant gut microbiota and selectively expresses high levels of LPO, we hypothesized that microbial signaling might also be required for LPO expression. Depletion of gram-negative bacteria and anaerobic bacteria that are abundant in the colon by antibiotic neomycin (Neo) and metronidazole (Met) significantly reduced *Lpo* expression in colons of DSS-exposed WT mice with intact CMKLR1 signaling (Fig. 4*C*). IECs are known to sense microbiota through TLRs and their common signaling adaptor MyD88 to regulate the expression of mucus and some antimicrobial peptides that are critical for epithelial barrier functions (5, 6). Since TLR4 was recently reported to be predominantly expressed in colonic ECs and increased LPS contents found in DSS-exposed colons (10), we then investigated whether TLR4/MyD88 signaling is required for microbiota regulation of LPO expression by exposing *Myd88*$^{-/-}$ and *Tlr4*$^{-/-}$ mice to DSS. The absence of TLR4 or MyD88 significantly decreased *Lpo* expression in colons (Fig. 4*D*) with no difference between two genotypes, suggesting TLR4 signaling may represent the major TLR responsible for *Lpo* expression. In contrast, Neo/Met treatment or the absence of MyD88 or TLR4 had no effect on gene expression of chemerin in colons (Fig. 4*C* and *D*), suggesting that chemerin is unlikely to act as the downstream effector of microbiota-dependent microbial signaling to mediate LPO expression. Furthermore, we found that stimulation with the TLR4 agonist significantly up-regulated *Lpo* expression in WT colon explants, which was completely blunted in those from *Rarres2*$^{-/-}$ or *Cmklr1*$^{-/-}$ mice (Fig. 4*E*). Additionally, mildly increased *Lpo* expressions were detected in WT colon explants stimulated with agonists of TLR1/2, TLR3, and TLR5, which were abrogated by the absence of the chemerin–CMKLR1 axis, whereas no effects were observed with agonists of TLR6, TLR7, and TLR9 (Fig. 4*E*). Similarly, stimulation with TLR agonists did not affect gene expression of chemerin and CMKLR1 in WT colon explants (Fig. 4*F*). Moreover, adding exogenous chemerin together with LPS at increasing concentrations significantly up-regulated *Lpo* expression in *Rarres2*$^{-/-}$, but not *Cmklr1*$^{\Delta IEC}$ colon explants, whereas adding chemerin or LPS alone lacked such an effect in *Rarres2*$^{-/-}$ colon explants (Fig. 4*G*). Collectively, these results suggest that LPO expression in colonic ECs requires both chemerin–CMKLR1 signaling and microbial TLR4/Myd88 signaling.

Decreased LPO Leads to Impaired Epithelial Antimicrobial Defense and the Outgrowth of Gram-Negative Bacteria in Mice with Chemerin–CMKLR1 Signaling Deficiency. LPO is known as a peroxidase that mainly functions to oxidize thiocyanate into an antibiotic compound, particularly against gram-negative

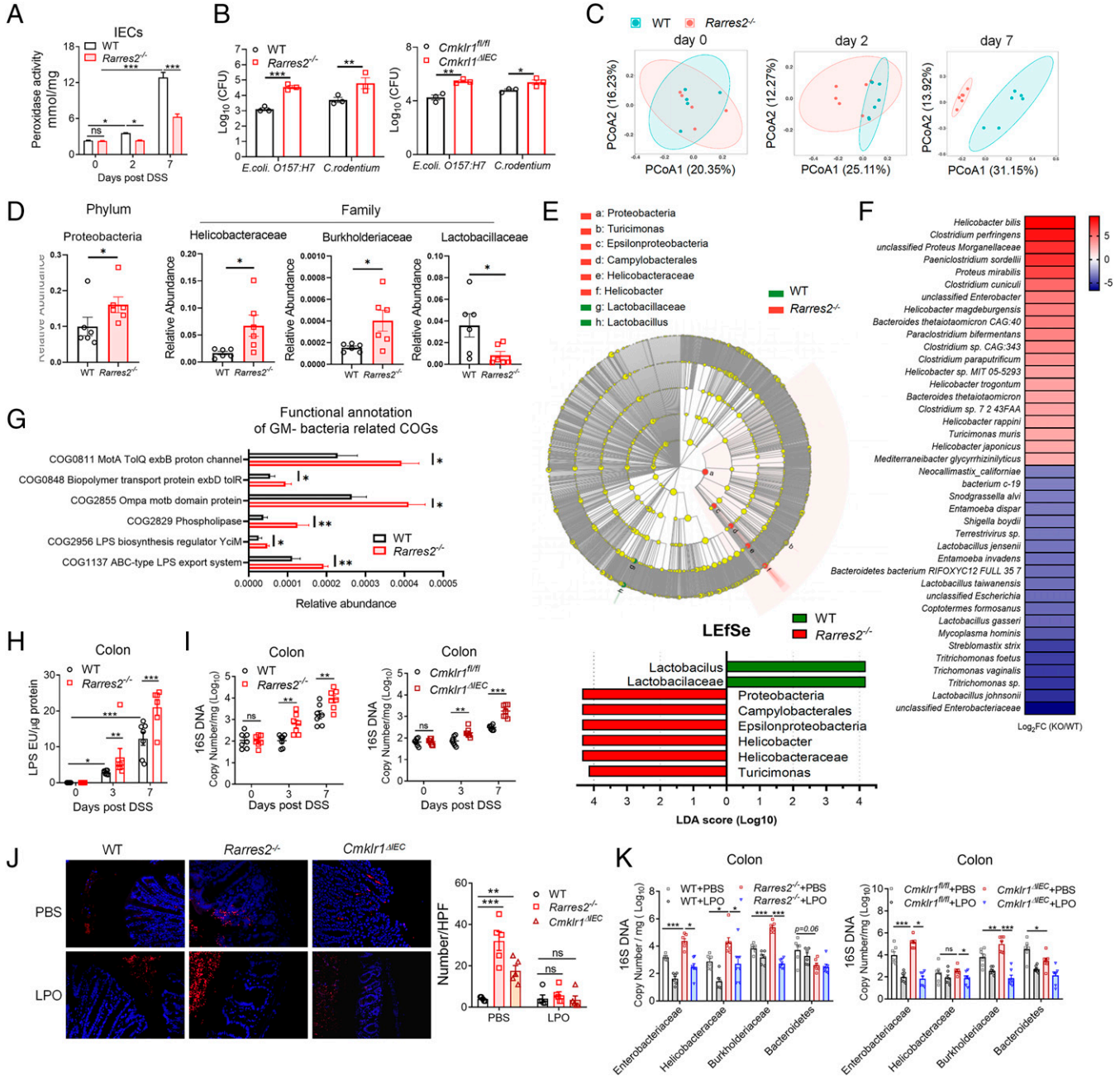

Fig. 5. Decreased LPO leads to impaired epithelial antimicrobial defense and the outgrowth of gram-negative bacteria in the absence of chemerin–CMKLR1 signaling. (*A*) Peroxidase activity of colonic ECs. (*B*) Bacterial survival determined by counting CFUs following incubation with supernatants of colon explants of DSS-exposed mice on different genotypes. (*C*) Fecal DNA was isolated and analyzed by 16S rRNA sequencing. PCoA plots showing the differences between microbiota composition of WT and *Rarres2*$^{-/-}$ mice. (D0, $R = 0.04$, $P = 0.48$; D2, $R = 0.49$, $P = 0.007$; D7, $R = 0.80$, $P = 0.006$) (*D*–*G*) Metagenomic sequencing of feces from WT and *Rarres2*$^{-/-}$ mice on day 7 post-DSS. Relative bacterial abundance at phylum and family levels (*D*), LEfSe cladogram and bar plot of differential bacterial taxa between WT and *Rarres2*$^{-/-}$ mice (*E*), heatmap of the top 20 differentially enriched species ($P < 0.05$) (*F*), and functional annotation of COGs related to gram-negative bacteria in WT and *Rarres2*$^{-/-}$ mice (*G*). (*H*) LPS levels in colons. (*I*) Colon tissue–associated bacteria were quantified by qPCR determination of 16S rRNA gene copy number. (*J* and *K*) FISH using EUB338 probe (red) and DAPI (blue) with cell number per high-power field (*J*), and quantification of colon tissue–associated specific bacteria by qPCR (*K*) in colons of mice on day 7 post-DSS. Data are shown as mean ± SEM; $n = 6$ to 7. Mann–Whitney *U* test (*D* and *F*) and two-way ANOVA followed by Tukey's multiple comparison test (*A*, *B*, and *G*–*K*) were used. Analysis of similarities was used to test the similarity of different groups in PCoA analysis (*C*). Nonparametric factorial Kruskal–Wallis sum-rank test and LDA were used in LEfSe (*E*). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.

bacteria (27). Compared with WT controls, significantly lower peroxidase activities were detected in colonic ECs isolated from DSS-exposed *Rarres2*$^{-/-}$ mice (Fig. 5*A*), which was paralleled to drastically decreased *Lpo* expression. To investigate whether reduced epithelial peroxidase activity results in impaired antibacterial defense, supernatants of colon explants from DSS-exposed mice on different genotypes were collected and incubated with enterohemorrhagic *Escherichia coli* O157:H7 or *Citrobacter rodentium*. Incubation with supernatants from *Rarres2*$^{-/-}$ or *Cmklr1*$^{\Delta IEC}$ colon explants led to significantly increased bacterial survival as determined by counting colony-forming units (CFUs) (Fig. 5*B*). Given that supernatants from colon explants of *Cmklr1*$^{\Delta IEC}$ mice had increased chemerin levels but decreased ability to kill bacteria (*SI Appendix*, Fig. S5*A*), the direct bacteria-killing effect of chemerin described previously (36) was not likely involved in our experimental system. We then determined

whether weakened peroxidase activity of controlling bacteria due to decreased LPO expression alters gut microbiota composition in *Rarres2*$^{-/-}$ mice. Principal coordinates analysis (PCoA) of 16S rRNA gene sequencing data of stool samples revealed no significant difference in the diversity and community structure of the baseline microbiota between WT and *Rarres2*$^{-/-}$ mice, but significant alteration occurred in *Rarres2*$^{-/-}$ mice on day 2, which became more obvious on day 7 post-DSS (Fig. 5*C*). Relative abundance of bacterial taxa annotated based on the SILVA database displayed significant abundance differences between WT and *Rarres2*$^{-/-}$ mice post-DSS. For instance, we observed bloomed Proteobacteria at the phylum level as well as an increase of disease-associated Burkholderiaceae families in the inflamed gut of *Rarres2*$^{-/-}$ mice compared to those of WT littermates (*SI Appendix*, Fig. S5*B*). To further confirm these findings, metagenomic sequencing of stool samples of WT and *Rarres2*$^{-/-}$ mice on day 7 post-DSS was performed. Consistently, we found significant increases in relative abundance of Proteobacteria at the phylum level and that of disease-associated gram-negative bacteria, including Helicobacteraceaea and Burkholderiaceae, but a decrease in that of health-associated Lactobacillaceae at the family level in the feces of *Rarres2*$^{-/-}$ mice compared to those of WT littermates (Fig. 5*D*). Linear discriminant analysis effect size (LEfSe) with a linear discriminant analysis (LDA) score of >4.0 further revealed that, at the genus level, the differentially abundant bacterial taxa in feces of *Rarres2*$^{-/-}$ mice were *Helicobacter* and *Turicimonas* with very high LDA scores, while *Lactobacillus* was markedly lost in *Rarres2*$^{-/-}$ mice (Fig. 5*E*). Moreover, at the species level, many helicobacter and clostridium species were more enriched in feces of *Rarres2*$^{-/-}$ mice, including *Helicobacter bilis*, *Helicobacter magdeburgensis*, *Clostridium perfringens*, and *Clostridium cuniculi*, while *Lactobacillus johnsonii*, and *Lactobacillus gasseri* were more enriched in feces of WT mice post-DSS (Fig. 5*F* and *SI Appendix*, Fig. S5*C*). The Clusters of Orthologous Groups (COG) annotation indicated that many COGs associated with gram-negative bacteria, including LPS export and synthesis, phospholipase synthesis, the TonB system, and the OmpA family, were more enriched in *Rarres2*$^{-/-}$ mice (Fig. 5*G*). The Virulent Factor Database (VFDB) annotation of metagenomic sequencing data also revealed three LPS virulent factors were more enriched in *Rarres2*$^{-/-}$ mice (*SI Appendix*, Fig. S5*D*). In support of these sequencing data, significantly increased LPS contents were detected in homogenized colon tissues from DSS-exposed *Rarres2*$^{-/-}$ mice (Fig. 5*H*). Furthermore, substantially amplified bacterial DNA was detected in colon tissues, but not feces, from *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice post-DSS (Fig. 5*I* and *SI Appendix*, Fig. S5*E*). Analysis of bacterial localization by fluorescence in situ hybridization (FISH) revealed obviously more intestinal bacteria in direct contact with the epithelial layer in the colons of *Rarres2*$^{-/-}$ or *Cmklr1*$^{\Delta IEC}$ mice than corresponding littermate controls (Fig. 5*J*). We also found significantly increased copy numbers of several specific subgroups of gram-negative bacteria that belong to Proteobacteria, including Enterobacteriaceae, Helicobacteraceae, and Burkholderiaceae, but unaltered or decreased Bacteroidaceae, in feces and colon tissues of *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice (*SI Appendix*, Fig. S5*F* and Fig. 5*K*). Importantly, LPO supplementation effectively abrogated alterations in localization and abundance of these gram-negative bacteria in *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice (*SI Appendix*, Fig. S5*F* and Fig. 5*J* and *K*), suggesting that decreased LPO expression results in defective control of some pathobionts. Together, these data suggest that epithelial chemerin–CMKLR1 signaling is critical for barrier antibacterial function and for preventing microbiota dysbiosis via up-regulating LPO expression.

Altered Microbiota Contributes to Colitis Susceptibility in *Rarres2*$^{-/-}$ Mice. We next determined whether altered gut microbiota directly mediates aggravated colitis in *Rarres2*$^{-/-}$ mice. To this end, *Rarres2*$^{-/-}$ mice and WT littermates were cohoused after weaning and throughout DSS treatment. Notably, cohousing equilibrated the severity of DSS-induced colitis, neutrophilic inflammation, and CXCL1/2 levels in *Rarres2*$^{-/-}$ mice and WT littermates (Fig. 6*A*–*F*), despite higher LPO expression in cohoused WT littermates (Fig. 6*G*). We found that cohousing also equilibrated copy numbers of several specific subgroups of gram-negative bacteria, including Enterobacteriaceae, Helicobacteraceae, Burkholderiaceae, and Bacteroidaceae between WT and *Rarres2*$^{-/-}$ mice (Fig. 6*H*). These data suggest that altered microbiota contributes to the severity of colitis downstream of defective LPO induction in *Rarres2*$^{-/-}$ mice.

To further confirm the impact of alterations in microbiota composition on aggravated colitis in the absence of chemerin–CMKLR1 signaling, mice on different genotypes were treated with Met/Neo antibiotics. Depletion of gram-negative bacteria and anaerobic bacteria completely abrogated the differences in the severity of colitis symptoms between *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice and corresponding littermate controls (Fig. 6*I*–*L* and *SI Appendix*, Fig. S6*A*–*F*). Moreover, antibiotic Met/Neo treatment reduced neutrophil infiltration and CXCL1/2 production in different genotypes to similarly low levels post-DSS (Fig. 6*M* and *N* and *SI Appendix*, Fig. S6*G* and *H*), suggesting the dependency of aggravated colonic neutrophil recruitment on gut microbiota. Together, these results suggest that altered microbiota is responsible for colonic overproduction of CXCL1/2 and consequent neutrophilia, leading to excessive tissue damage and severe colitis in the absence of epithelial chemerin–CMKLR1 signaling.

Epithelial Chemerin–CMKLR1 Signaling Contributes to Early Host Defense against Enteric Infection in an LPO-Dependent Manner. Finally, we investigated whether chemerin–CMKLR1 signaling protects the host against bacterial infection. Significantly up-regulated *Lpo* expression was detected in colons of WT mice, but not *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice, on day 5 postinfection with *C. rodentium* or *E. coli* O157:H7 (Fig. 7*A* and *B*). Both *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice showed significantly increased CFU counts in colons, mesenteric lymph nodes (MLNs), and blood, suggesting more local penetration and systemic dissemination of *C. rodentium* (Fig. 7*C* and *D*) or O157:H7 *E. coli* (*SI Appendix*, Fig. S7*A* and *B*). Consistently, increased DNA amplification of *C. rodentium* (*SI Appendix*, Fig. S7*C* and *D*) or O157:H7 *E. coli* (*SI Appendix*, Fig. S7*E* and *F*) was detected in colon tissues and feces of *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice. Increased colonic neutrophil infiltration was detected at a later time point (day 10) postinfection with *C. rodentium* in *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice (Fig. 7*E* and *F*), indicating increased recruited neutrophils as a compensatory mechanism to augment host defense response due to decreased LPO expression. Furthermore, LPO supplementation almost completely reverted the increases in bacteria loads and colonic neutrophilia in *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice, but had minimal effects on WT controls postinfection (Fig. 7*C* and *D* and *SI Appendix*, Fig. S7*A*–*F*). These results demonstrate that epithelial chemerin–CMKLR1 signaling is sufficient for early host defense against enteric bacterial infection by sustaining LPO-mediated epithelial antibacterial defense.

Discussion

In this study, we reveal an unrecognized function of epithelial chemerin–CMKLR1 signaling to potentiate barrier antimicrobial

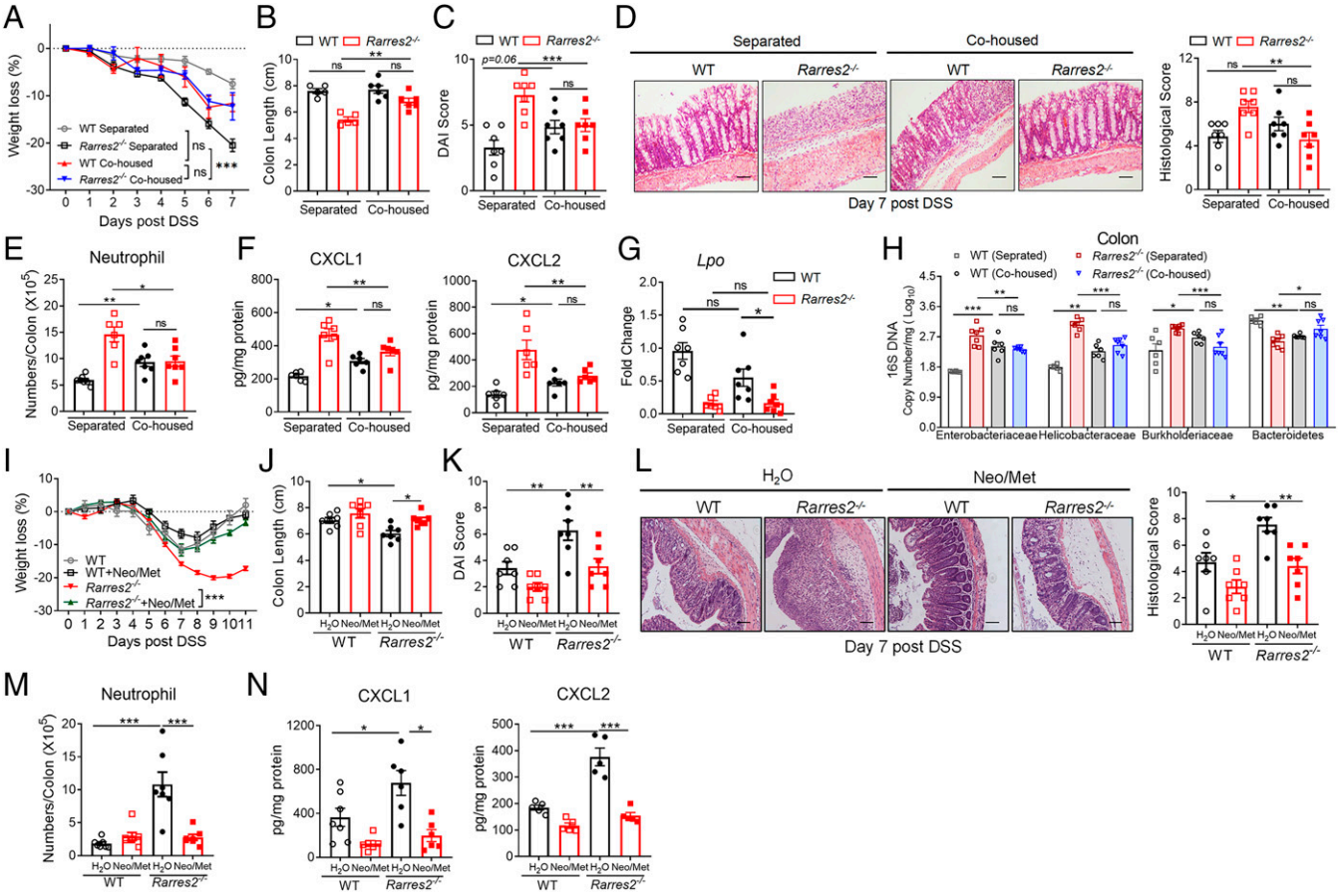

Fig. 6. Altered microbiota contributes to colitis susceptibility in *Rarres2*$^{-/-}$ mice. (*A*–*G*) Weight loss (*A*), colon length (*B*), DAI score (*C*), H&E analysis of colons and histological score (*D*), neutrophil numbers (*E*), CXCL1/2 levels (*F*), fold change of *Lpo* expression in colon tissues (*G*), and quantification of colon tissue–associated specific bacteria (*H*) of WT and *Rarres2*$^{-/-}$ mice, which were cohoused or separated after weaning on day 7 post-DSS. (*I*–*M*) Weight loss (*I*), colon length (*J*), DAI score (*K*), H&E analysis of colons and histological score (*L*), neutrophil numbers (*M*), and CXCL1/2 levels (*N*) in colons of Neo/Met-pretreated WT and *Rarres2*$^{-/-}$ mice on day 7 post-DSS. Data are shown as mean ± SEM; $n = 6$. Two-way ANOVA followed by Tukey's multiple comparison test were used. Results are representative of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.

defense via inducing LPO expression, thereby restricting microbiota-driven neutrophilic colon inflammation and subsequent tumorigenesis (*SI Appendix*, Fig. S7*G*).

A recent study showed that up-regulation of CXCL1/2 is one of the most consistent readouts of epithelial TLR signaling (10), which may lead to augmented colonic neutrophilia against encroaching or translocated bacteria. We found that deficiency of epithelial chemerin–CMKLR1 signaling significantly up-regulated colonic CXCL1/2 production even before colitis onset, leading to excessive colonic infiltration of neutrophils, thereby aggravating DSS-induced colitis and associated tumorigenesis. Although activation of CMKLR1 was previously shown to inhibit inflammatory NF-κB signaling and downstream proinflammatory cytokine expression (37, 38), our data did not support that chemerin–CMKLR1 signaling regulated CXCL1/2 production by IECs in a cell-intrinsic manner. Instead, we identified LPO as the major effector targeted by epithelial chemerin–CMKLR1 signaling to limit the outgrowth of potentially pathogenic bacteria and their mucosal invasion, thereby controlling colonic CXCL1/2 production.

LPO has long been known as an important component of the natural defense system and is widely applied in food preservation (39). Early studies demonstrated that LPO teams up with DUOXs to generate bactericidal hypothiocyanite, which is responsible for bacterial killing in airways (40, 41). We found predominant expression of LPO in murine colonic ECs, which was further enhanced upon epithelial injury. Moreover, LPO expression requires both chemerin–CMKLR1 signaling and gut microbiota–derived TLR4/MyD88 signaling. The expression of various antimicrobial molecules, including DUOX2, the main H_2O_2 provider for LPO, was shown to require microbial TLR4/MyD88 signaling in IECs (42). However, chemerin–CMKLR1 signaling was dispensable for DUOX2 expression. Lack of chemerin–CMKLR1 signaling even increased expression of some antimicrobial peptides in IECs of DSS-exposed mice, which may reflect the compensatory reaction to aggravated microbiota dysbiosis and colitis. These results indicate epithelial chemerin–CMKLR1 signaling as a specific inducer of LPO expression.

Importantly, reduced LPO expression due to lack of epithelial chemerin–CMKLR1 signaling was causally linked to dysregulated CXCL1/2-mediated colonic neutrophilia and subsequent tumorigenesis. The lack of chemerin–CMKLR1 signaling caused more obvious microbiota dysbiosis characterized by increased abundance of gram-negative bacterial populations and their mucosal adherence, which could be reversed by LPO supplementation. Moreover, we proved that microbiota dysbiosis resulting from impaired LPO-mediated epithelial antimicrobial defense is a cause rather than the effect of aggravated colitis in *Rarres2*$^{-/-}$ mice. First, altered microbiota composition was observed between *Rarres2*$^{-/-}$ and WT littermates on day 2 post-DSS when there was no detectable colonic inflammation. Second, cohousing eliminated the differences in colonic production of CXCL1/2 and infiltration of neutrophils between WT and *Rarres2*$^{-/-}$ mice. Third, antibiotic treatment abrogated aggravated colitis in both *Rarres2*$^{-/-}$ and

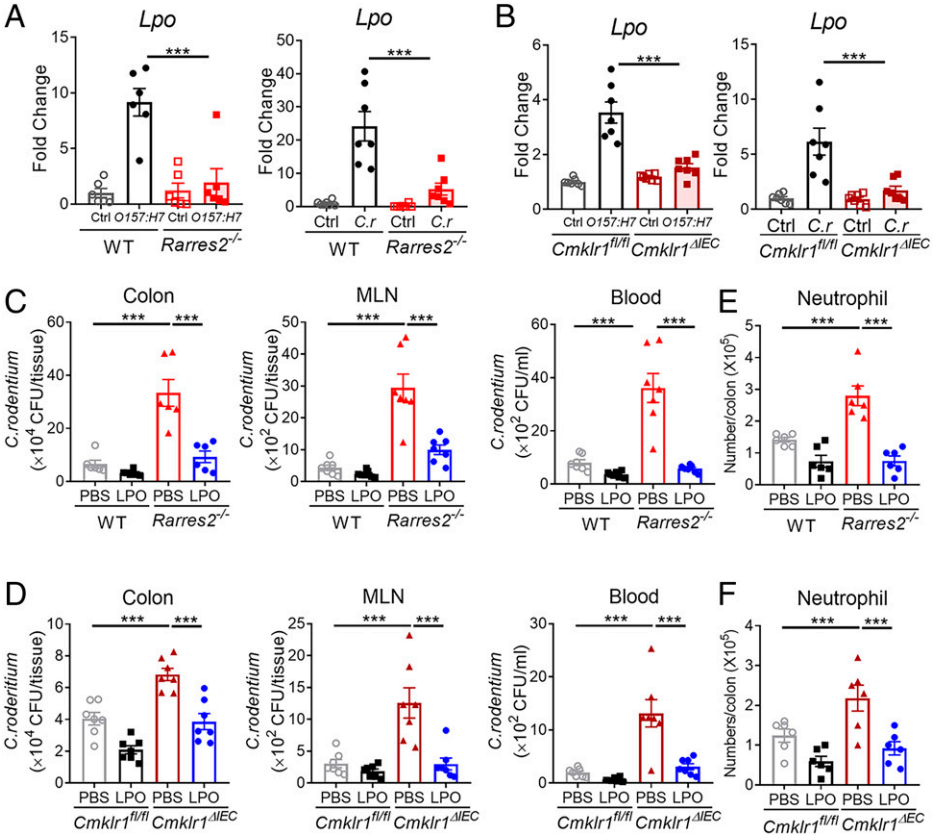

Fig. 7. Epithelial chemerin–CMKLR1 signaling contributes to early host defense against *C. rodentium* infection in an LPO-dependent manner. (*A* and *B*) Fold change of *Lpo* expression in colons of different genotypes on day 5 postinfection with *C. rodentium* or with O157:H7 *E. coli*. (*C* and *D*) Bacterial loads in different sites of different genotypes on day 5 postinfection with *C. rodentium*. (*E* and *F*) Neutrophil numbers in colons of different genotypes on day 10 postinfection with *C. rodentium*. Data are shown as mean ± SEM; $n = 6$ to 7. Two-way ANOVA followed by Tukey's multiple comparison test were used. Results are representative of three independent experiments. ***$P < 0.001$.

Cmklr1$^{\Delta IEC}$ mice. Thus, our study here highlights a critical role of LPO-mediated epithelial antimicrobial defense in controlling microbiota dysbiosis following epithelial injury.

In contrast to LPO expression in human saliva glands and trachea (43), a recent study reported undetectable LPO in colons of healthy subjects and IBD patients (44), suggesting LPO expression in a species- and tissue-specific manner. The differential LPO expression between colons of human and mouse may reflect the differences in intestinal innate host defense mechanisms between different species, which have evolved in response to different environmental challenges. This is the same for differential expression of defensins in Paneth cells of human and mouse (45). Interestingly, we noted that LPO supplementation had only minimal effects on DSS-induced colitis in WT mice, suggesting that up-regulated LPO expression is sufficient enough to control translocated bacteria following epithelial injury so that supplementation of exogenous LPO lacks further effect. In contrast, LPO supplementation markedly ameliorated colitis and associated tumorigenesis in *Rarres2*$^{-/-}$ and *Cmklr1*$^{\Delta IEC}$ mice, both of which have defective LPO expression. Considering that LPO has long been used in food preservation with high safety (39), oral supplementation of LPO may represent a potential therapeutic strategy to improve microbiota dysbiosis and related colitis in IBD patients, particularly for those with defective innate antimicrobial mechanism.

In summary, our study reveals the importance of the chemerin–CMKLR1/LPO axis in epithelial innate defense for preventing microbiota dysbiosis and aberrant colonic inflammation.

Materials and Methods

Animals. *Villin-cre* (#004586) transgenic mice were purchased from The Jackson Laboratory. *Rarres2*$^{-/-}$, *Ccrl2*$^{-/-}$, and *Cmklr1*$^{-/-}$ mice were generated by Cyagen as previously described (20, 46). Cmklr1-floxed (*Cmklr1*$^{fl/fl}$) mice were generated by Shanghai Model Organisms Center, Inc. by inserting the sequence of flox at axon 3 of murine *Cmklr1* gene (MGI#109603). The genotyping primers and the length of PCR production are listed in *SI Appendix*, Table S1. *Cmklr1*$^{fl/fl}$:*Villin-cre* (*Cmklr1*$^{\Delta IEC}$) were generated by crossing *Cmklr1*$^{fl/fl}$ mice with *Villin-cre* transgenic mice. Age and sex-matched littermates were used for all experiments. All mice used were on a C57BL/6J background and housed under specific pathogen-free conditions. All studies were approved by the Animal Care and Use Committee of the Fudan University Shanghai Medical College.

Mouse Models. Mice were given 2% DSS (36 000-50 000 MW; #216011090, MP Biomedicals) in their drinking water for 5 d, followed by plain water to induce colitis. The DAI was scored according to the average of three parameters: stool consistency (0, 2, 4), fecal blood (0, 2, 4), and percentage of weight loss (0 to 4). Recombinant chemerin (50 ng/mice) was i.p. administrated into *Rarres2*$^{-/-}$ mice every 2 d starting from 1 d prior to DSS treatment. To establish a CAC model, mice were i.p. administrated once with 10 mg/kg AOM (Sigma) and then three cycles of 2% DSS for 5 d with a 2-wk interval. For blocking neutrophil recruitment, CXCR2 antagonist SB225002 (3 mg/kg) was i.p. injected daily starting from 1 d prior to DSS treatment and lasting through the colitis model or starting from the third cycle of DSS in the CAC model. For LPO supplementation, LPO (500 ng/mice) was administered by daily gavage starting from 1 d prior to DSS treatment. For enteric infection, mice were fasted for 8 h followed by infecting with 10^9 CFUs of *C. rodentium* strain DBS100 (ATCC51459) or *E. coli* O157:H7 (ATCC700728) by oral gavage. For the cohousing experiment, WT and *Rarres2*$^{-/-}$ mice (1:1 per cage) were cohoused after weaning for 2 mo followed by DSS treatment.

Statistics. Statistical analyses were performed by Graph Prism 7. Results are presented as means ± SEM. Comparisons between two groups were performed using Mann–Whitney *U* test. Multiple-group comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test. Multiple-group comparisons were performed using two-way ANOVA followed by Tukey's correction to compare each group. *P* value of <0.05 was considered statistically significant. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; ns, not significant.

Detailed information for the materials and methods used in this study is provided in *SI Appendix, SI Methods*.

Data Availability. 1) RNA sequencing data of colon epithelial cells can be accessed from Gene Expression Omnibus with accession number of GSE178308 (47); 2) 16srRNA sequencing data of feces from WT and Rarres2-KO mice can be accessed from Sequence Read Archive (SRA) at accession number of PRJNA736316 (48); and 3) metagenomic sequencing data of fecal microbiota from WT and Rarres2-KO mice at 7 d post-DSS can be accessed from SRA at accession number of PRJNA801447 (49).

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The authors declare no competing interest.

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