Supplementary Material

**Impaired COMMD10-Mediated Regulation of Ly6Chi Monocyte-Driven Inflammation Disrupts Gut Barrier Function**

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# Supplementary Figures and Tables

**Figure S1**

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**Figure S1. Inducible depletion of infiltrating Ly6Chi monocytes using the anti-CCR2 MC-21 antibody**

*LysMΔCommd10* mice were i.p. injected with LPS (0.2 mg per mouse of similar weight). Some of the mice were treated with MC-21 12 h prior to LPS stimulation. (**A**) Representative flow cytometry images showing gating strategy of liver Ly6Chi monocytes, KCs and neutrophils out of CD45+ non-parenchymal immune cells**.** Top panels: *LysMΔCommd10* mice treated with LPS alone. Bottom panels: *LysMΔCommd10* mice treated with LPS and MC-21. (**B**) Representative flow cytometry images showing gating strategy of colonic Ly6Chi monocytes, resident lpMFs and neutrophils out of CD45+CD11b+ immune cells. Top panels: *LysMΔCommd10* mice treated with LPS alone. Bottom panels: *LysMΔCommd10* mice treated with LPS and MC-21.

**Figure S2.**

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**Figure S2. LPS-challenged COMMD10-deficient BM neutrophils exhibit declined production of IL-1.**

Neutrophils were isolated from BM of *Commd10fl/fl* (blue) or *LysMΔCommd10* (red) mice and subjected to LPS (100ng/ml) for 3h. ATP was added in the last 30 min of the experiment. (**A**) Immunoblots showing the expression of COMMD10. β-actin was utilized as control (n=3). (**B**) ELISA analysis of IL-1β from cell free supernatants (n=3). Data were analyzed by unpaired, two-tailed *t-test*, comparing *Commd10fl/fl* and *LysMΔCommd10* and are presented as mean ± SEM. Data in panels A and B represent a single experiment.

**Figure S3.**

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**Figure S3. *Commd10* gene expression is near background levels in blood neutrophils and significantly higher in blood Ly6Chi monocytes**

Bar graph showing the raw gene expression of COMMD10 (*Commd10*) in comparison with that of the adipocyte marker adiponectin (*Adipoq*) and the hepatocyte and cholangiocyte marker Cytokeratin18 (*Krt18*), both were used to set background expression levels. Gene-expression data were extracted from the ImmGen Consortium database (GSE37448) (n=3 for Ly6Chi monocytes, n=4 for blood neutrophils). Data were analyzed by unpaired, two-tailed *t-test*, comparing blood Ly6Chi monocytes and blood neutrophils and presented as mean ± SEM with significance: \*\*\* p < 0.001.

**Table S1.**

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**Table S1. List of primers**

A table showing the sequences of all primers used.

**Movie S1. *LysM*Δ*Commd10* but not *Cx3cr1*Δ*Commd10* mice exhibit increased intestinal inflammation during DSS-induced colitis**

*Commd10fl/fl*, *LysM*Δ*Commd10* or *Cx3cr1*Δ*Commd10* mice were treated with DSS (1.5% in drinking water) for 7 days. Colonoscopy movie showing representative captures of the five parameters which indicate colitis severity grade: thickening of the colon wall, changes in the normal vascular pattern, presence of fibrin, mucosal granularity and stool consistency**.**