

Supplementary Materials for
**MAIT cells monitor intestinal dysbiosis and contribute to host protection
during colitis**

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The PDF file includes:

Figs. S1 to S8

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S6

Data S1

MDAR Reproducibility Checklist

El Morr et al, Figure S1

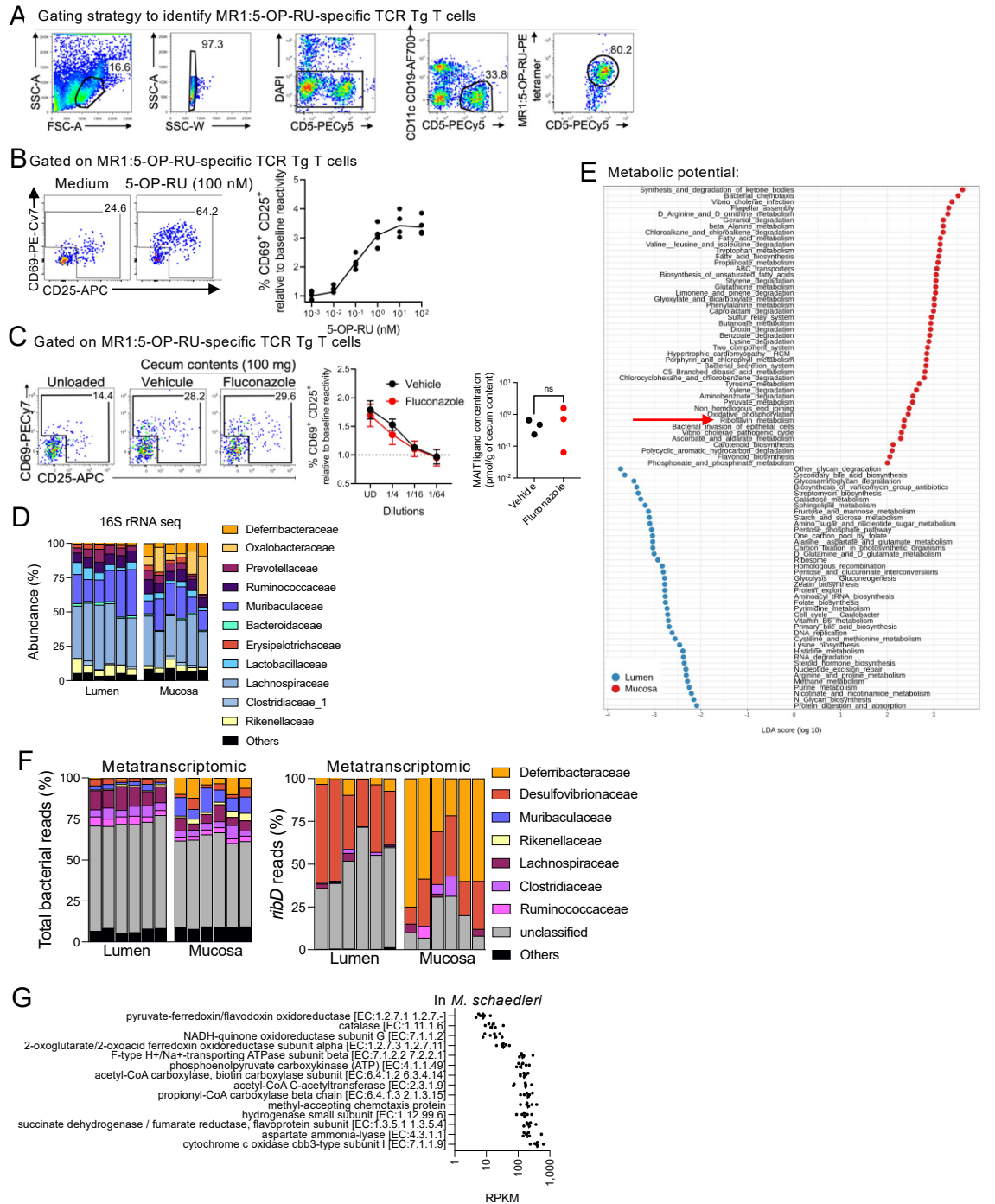


Figure S1: Bacterial production of MAIT antigens in the colonic mucosa. **A.** Gating strategy used to identify 5-OP-RU-specific TCR Tg T cells after co-culture with WT3-MR1 cells pulsed with intestinal contents. **B.** Left: Activation of MR1:5-OP-RU-specific TCR Tg T cells after overnight incubation with WT3-MR1 cells pulsed with 5-OP-RU (100 nM). Right: Relative expression of CD25 and CD69 by 5-OP-RU-specific TCR Tg T cells in the indicated conditions (data pooled from three independent experiments). **C.** Left: Activation of 5-OP-RU-specific TCR Tg T cells after overnight incubation with WT3-MR1 cells unloaded or pulsed with 100 mg of luminal contents from the cecum of control (vehicle) or of mice treated for 7d with fluconazole. Right: Relative expression of CD25 and CD69 by 5-OP-RU-specific TCR Tg T cells in the indicated conditions (means \pm SEMs, $n = 3$ mice per group). The dotted line indicates baseline reporter T cell activation in absence of intestinal contents. MAIT antigen concentrations as estimated using a standard curve with synthetic 5-OP-RU. Data from 1 experiment. **D.** Abundance of the indicated bacterial *Families* in the cecum lumen and mucosa based on 16S rRNA gene sequencing (columns indicate individual mice). **E.** Pathway enrichment analysis using PICRUST-predicted KEGG orthologs between the lumen and mucosal niches in the cecum. Significance in pathway enrichment was tested using LEfSe analysis. **F.** Taxonomy of total (left) and *ribD* (right) bacterial reads at the *Family* level. **G.** Normalized counts (Reads per Kb per million) of the indicated genes expressed by *M. schaedleri* in the mucosa.

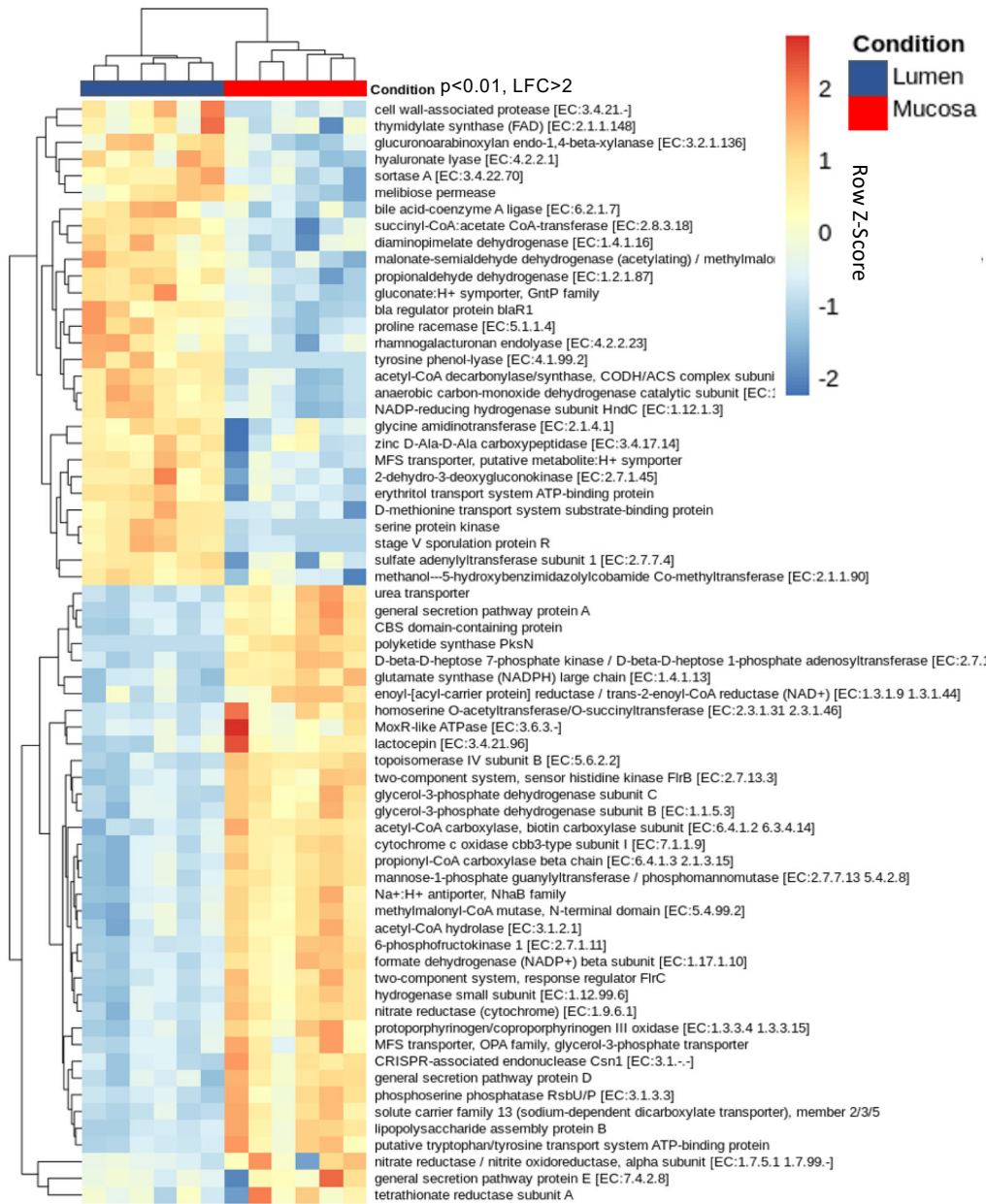
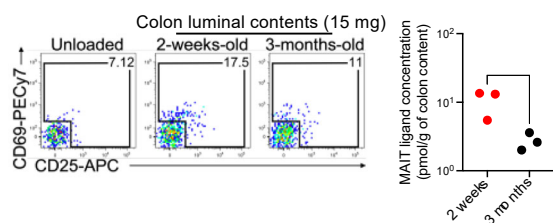


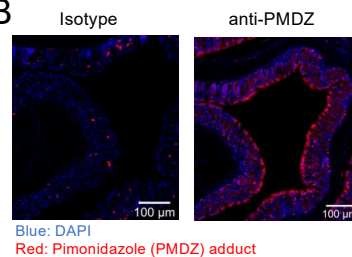
Figure S2: Bacterial gene expression in lumen and mucosa. Heatmap showing the top differentially expressed bacterial genes between the lumen and mucosal niches of the cecum (adjusted $p < 0.01$ by Mann-Whitney U test, $LFC > 2$).

El Morr et al, Figure S3

A Gated on MR1:5-OP-RU-specific TCR Tg T cells



B



C

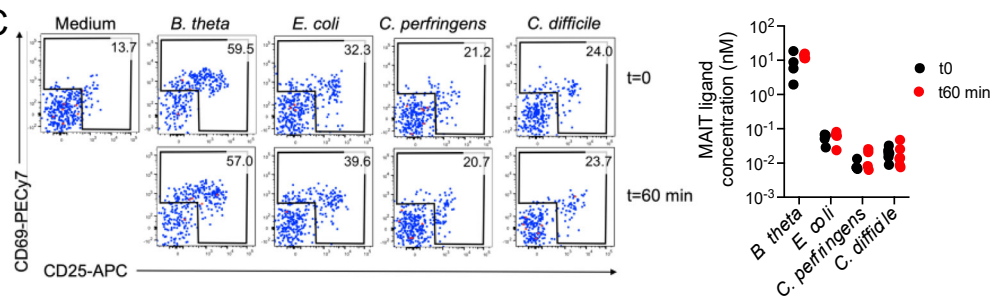


Figure S3: MAIT ligand production upon bacterial exposure to air. **A.** Left: Activation of MR1:5-OP-RU-specific TCR Tg T cells after overnight incubation with WT3-MR1 cells unloaded or pulsed with luminal contents from the colon of mice at the indicated age. Right: Summary of the results. MAIT antigen concentrations were estimated using a standard curve with synthetic 5-OP-RU. Data from 1 experiment. * $p < 0.05$ by unpaired Student's t test. **B.** Representative images of the colon from a naive mouse stained with control or anti-PMDZ antibody (red). DNA staining with DAPI is shown in blue. Representative of 3 independent experiments. **C.** Left: Activation of MR1:5-OP-RU-specific TCR Tg T cells after overnight incubation with WT3-MR1 cells unloaded or pulsed with filtered bacterial culture supernatants. Bacteria were grown anaerobically until late exponential growth phase prior to exposure to air at 37°C for 1h. Right: MAIT antigen concentration in the indicated conditions, estimated using a standard curve of synthetic 5-OP-RU. Data pooled from 2 independent experiments.

El Morr et al, Figure S4

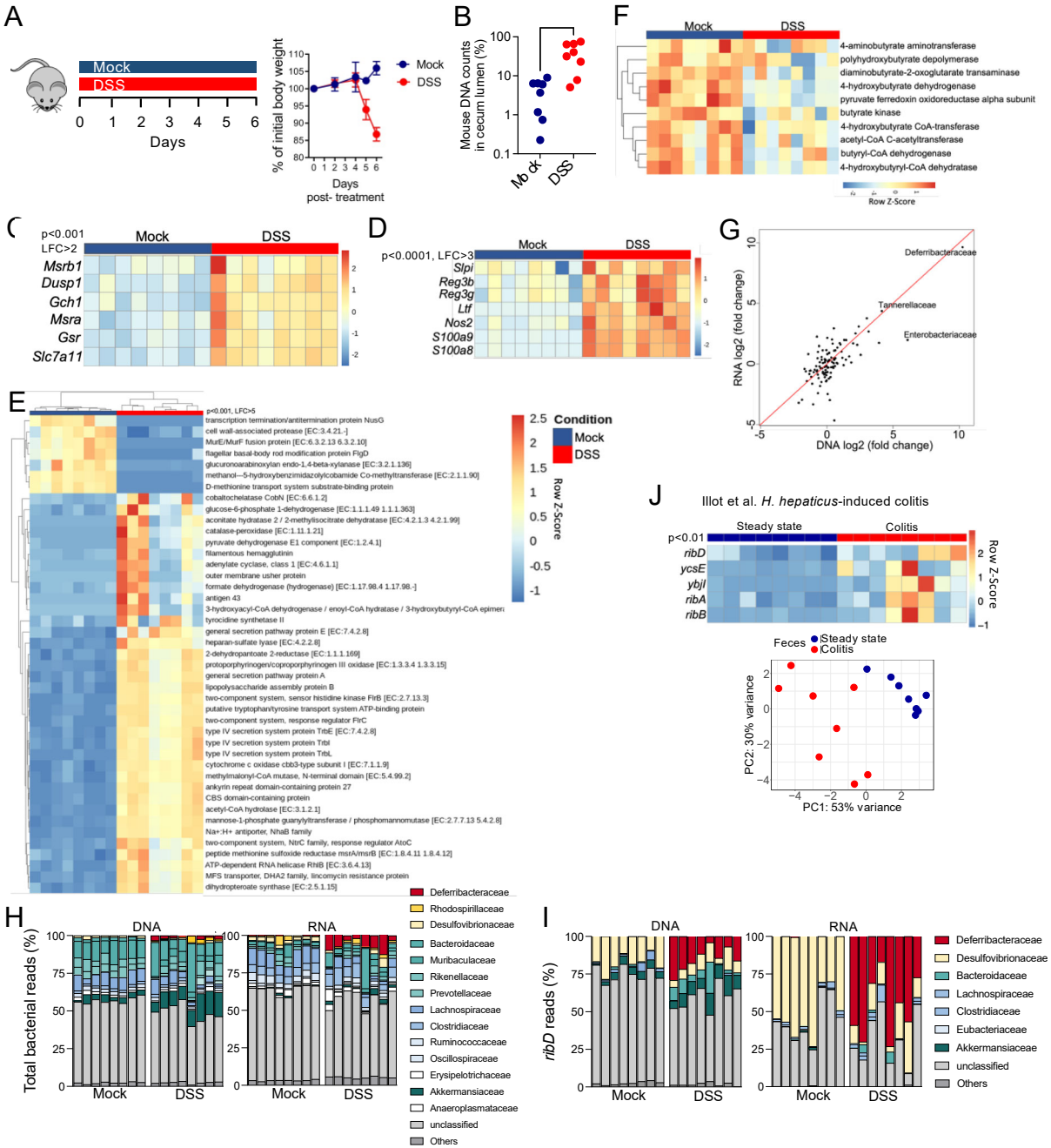


Figure S4: Bacterial gene expression in response to DSS-induced colitis. **A.** Left: Scheme of the acute DSS model of colitis. Right: Mouse weight as percentage of initial body weight (means \pm SEMs, $n=6$ mice per group). Representative of 3 independent experiments. **B.** Percentage of cecal DNA reads aligned to the mouse genome in the indicated mice. $**p < 0.01$ by unpaired Mann-Whitney *U* test. **C.** Heatmap showing mouse genes with oxidative stress resistance function found up-regulated ($p < 0.001$ by Mann-Whitney *U* test; LFC > 2) in the cecum lumen during colitis. **D.** Heatmap showing mouse genes with anti-bacterial functions found over-expressed (adjusted $p < 0.0001$ by Wald test, LFC > 3) in the cecum lumen during colitis. **E.** Heatmap showing the top differentially expressed bacterial genes in the cecum lumen between control (mock) and DSS-treated mice ($p < 0.001$ by Mann-Whitney *U* test, LFC > 5). **F.** Heatmap showing expression of the bacterial genes involved in butyrate metabolism in mock- and DSS-treated mice. **G.** Correlation of fold changes between metagenomic and metatranscriptomic analyses. The red line represents the linear model fit. **H.** Family-level taxonomy of total bacterial DNA (left) or RNA (right) reads from the cecum of the indicated mice. **I.** Family-level taxonomy of *ribD* DNA (left) or RNA (right) reads from the cecum of the indicated mice. **J.** Analysis of metatranscriptomic data from Ilott et al, ISEM J. 2016. Top: Heatmap showing the genes of the riboflavin biosynthetic pathway found up-regulated in the feces upon colitis induction ($p < 0.01$ by Mann-Whitney *U* test). Bottom: Principal component analysis (PCA) of the riboflavin biosynthesis pathway genes expressed in the feces before (steady state, blue) or after *Helicobacter hepaticus*-induced colitis (red) ($n = 8$ mice).

El Morr et al, Figure S5

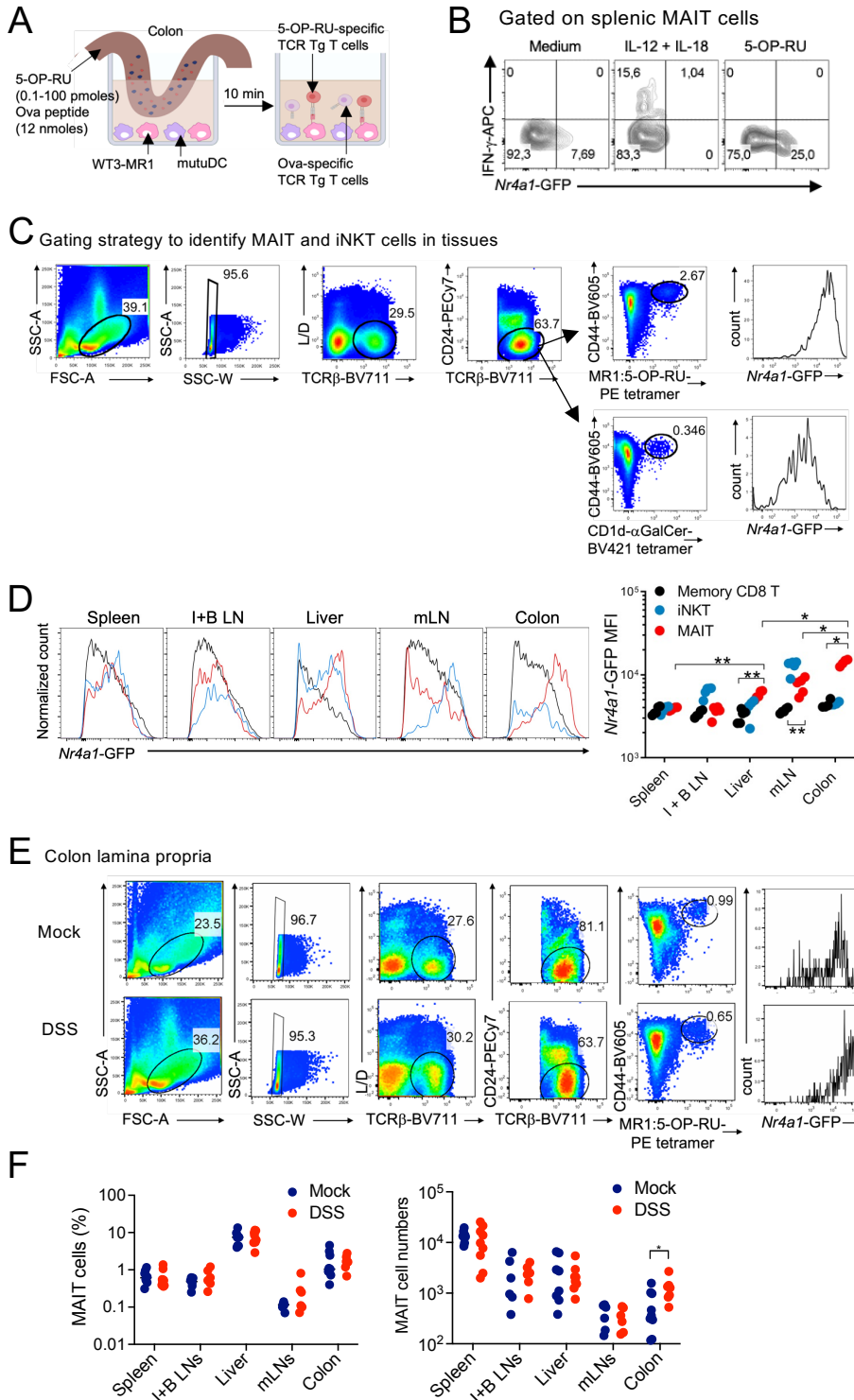


Figure S5: Nur77 expression in MAIT cells at steady-state and upon DSS-induced colitis. **A.** Scheme of the assay used to assess intestinal permeability to ligands. The ligand mixture deposited in the lumen contained a fixed amount of Ova peptide and variable amounts of 5-OP-RU. **B.** Interferon (IFN)- γ and *Nr4a1*-GFP expression in splenic MAIT cells untreated or incubated overnight with interleukin (IL)-12 (10 ng/ml) and -18 (12.5 ng/ml) or with WT3-MR1 cells pulsed with 5-OP-RU (10 nM). MAIT cells were identified using MR1:5-OP-RU tetramer. Data from 1 experiment. **C.** Gating strategy used to identify MAIT and iNKT cells in tissues, here in colon lamina propria. Representative of at least 8 independent experiments. **D.** Left: *Nr4a1*-GFP expression in the indicated T cell subsets and tissues; representative of at least 3 independent experiments. I+B LN: pooled inguinal and brachial lymph nodes. mLN: pooled mesenteric lymph nodes. Right: Mean fluorescence intensity (MFI) in the indicated cell subsets and tissues ($n = 4$ mice; representative of at least 3 independent experiments). **E.** Gating strategy used to identify MAIT cells in the colon lamina propria of Mock- and DSS-treated mice. Data representative of 3 independent experiments. **F.** MAIT cell frequencies (left) and numbers (right) in the indicated tissues of mice treated with DSS for 7d. Data pooled from 3 independent experiments. * $p < 0.05$ and ** $p < 0.01$ by Mann-Whitney U tests.

El Morr et al, Figure S6

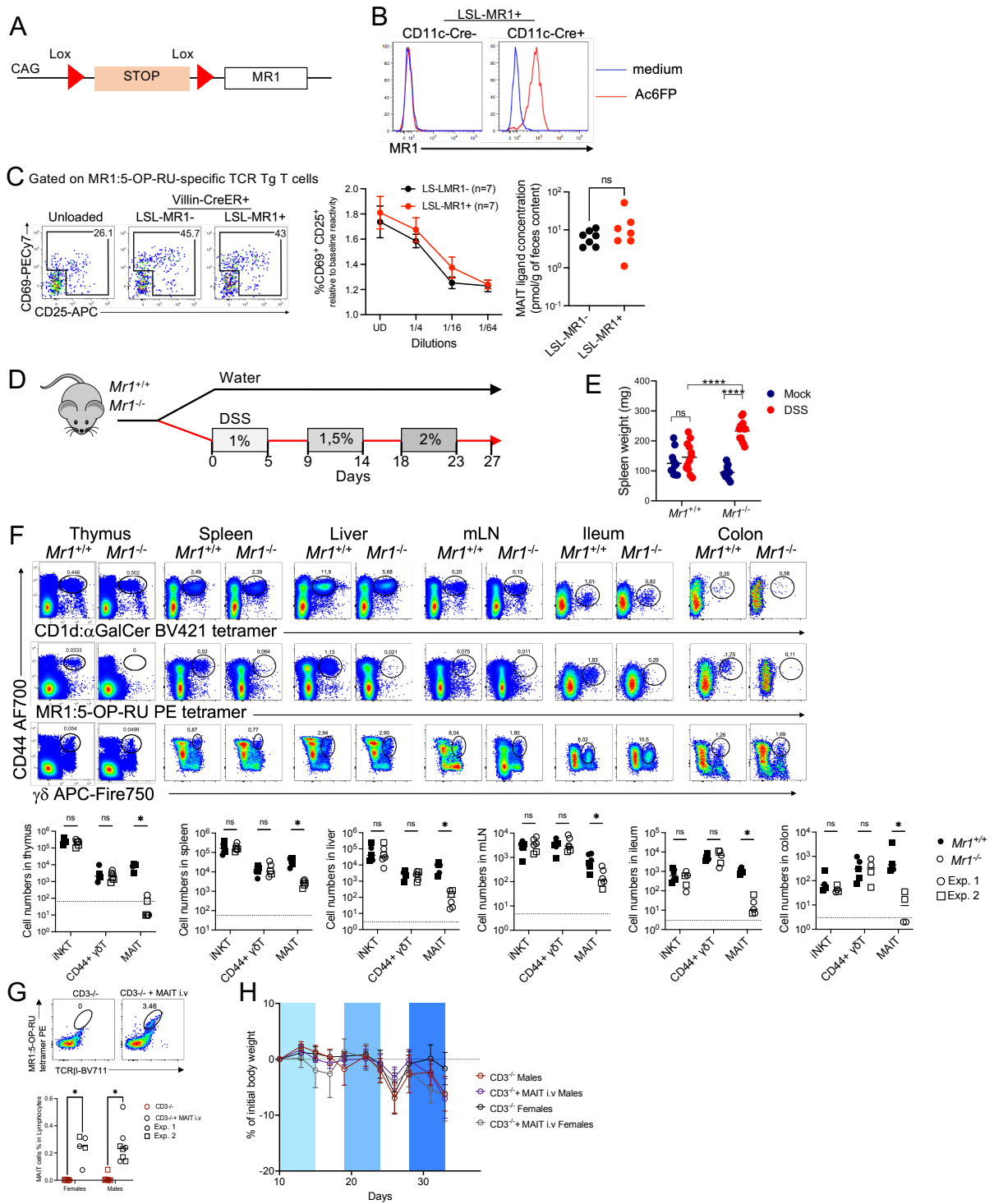


Figure S6: *Mr1*^{-/-} mice are more sensitive to chronic DSS-induced colitis. **A.** Scheme of the lox-stop-lox-MR1 transgenic construct. **B.** Surface MR1 expression on splenic CD11c⁺ MHCII⁺ dendritic cells from LSL-MR1⁺ CD11c⁻ or LSL-MR1⁺ CD11c⁺ mice after overnight incubation with Ac6-FP (10 μM). Representative of 2 independent experiments. **C.** Left: Activation of MR1:5-OP-RU-specific TCR Tg T cells after overnight incubation with WT3-MR1 cells unloaded or pulsed with 100 mg of feces from the indicated mice. Both LSL-MR1⁺ and LSL-MR1⁻ mice were crossed to the VillinCreER mouse and received tamoxifen injections. Right: MAIT antigen concentrations in feces estimated using synthetic 5-OP-RU. n=7 mice per group, data from 1 experiment. **D.** Scheme of the chronic DSS-induced colitis model used to assess disease severity in *Mr1*^{+/+} and *Mr1*^{-/-} mice. **E.** Spleen weight in the indicated mice with chronic colitis. n=12-13 mice per group; data pooled from 3 independent experiments. ns p ≥ 0.05, *p < 0.05, **p < 0.01 and ****p < 0.0001 by Student's *t* tests. **F.** Top: Representative flow cytometry dotplots showing identification of iNKT, MAIT and γδ T cells (defined as TCRβ⁺ γδTCR⁺ CD44⁺) in the indicated tissues of B6-MAIT^{Cast} *Mr1*^{-/-} and *Mr1*^{+/+} mice. Data are representative of two independent experiments. Bottom: iNKT, MAIT and γδ T cell numbers in the indicated tissues. ns p ≥ 0.05, *p < 0.05, **p < 0.01 and ****p < 0.0001 by multiple Mann-Whitney tests. Pooled data from 2 independent experiments. Dotted lines indicate the limit of quantification for each tissue. **G.** Top: Representative flow cytometry showing tetramer⁺ cells in the spleen of CD3^{-/-} mice engrafted with *in vitro*-expanded MAIT cells. Cells were gated on live CD45⁺ CD19⁻ CD11c⁻ cells. Bottom: Summary of the data pooled from 2 independent experiments, represented as percentage among live cells. **H.** Body weight of control and recipient CD3^{-/-} mice. Data pooled from 2 independent experiments.

El Morr et al, Figure S7

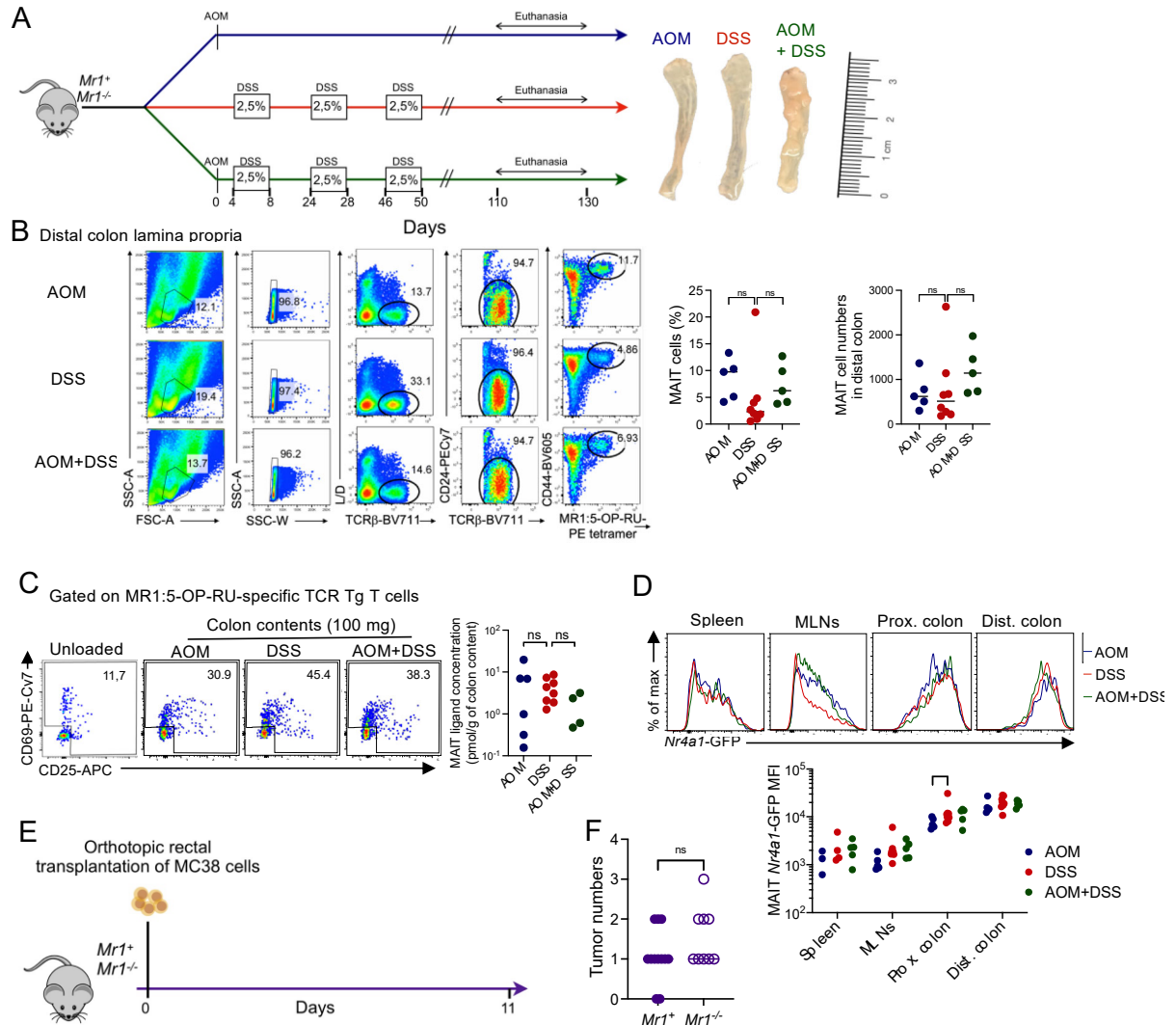
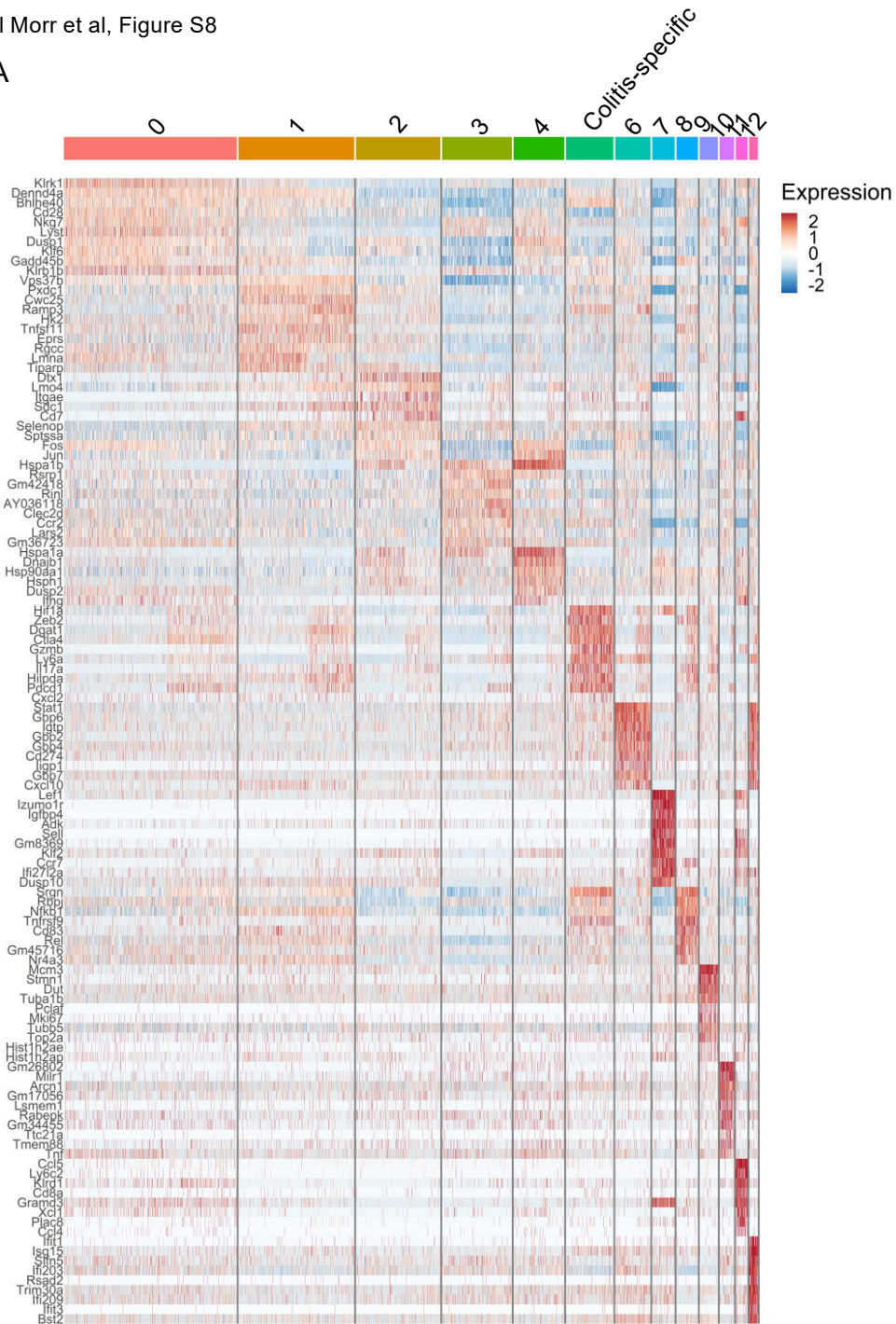


Figure S7: *Mr1*^{-/-} mice develop more rectal tumors in the AOM/DSS colitis-induced colorectal tumor model. **A.** Left: Scheme of the AOM/DSS model of inflammation-induced colorectal cancer. Right: Representative images of the distal colon of WT mice at the end of the indicated protocol. Representative of 3 independent experiments. **B.** Left: Gating strategy used to identify MAIT cells in the distal colon lamina propria of the indicated B6-MAIT^{Cast} mice. Data representative of 2 independent experiments. Right: MAIT cell frequencies and numbers in the distal colon of the indicated B6-MAIT^{Cast} mice. Results are pooled from 2 independent experiments. **C.** Left: Activation of reporter T cells after incubation with WT3-MR1 cells unloaded or pulsed with 100 mg of colon contents from indicated mice. Right: MAIT antigen concentration in the indicated conditions as estimated using synthetic 5-OP-RU. Pooled data from 2 independent experiments. n=4-8 mice per group. **D.** Top: *Nr4a1*-GFP expression in MAIT cells from the indicated tissues. Bottom: Data pooled from 2-3 independent experiments. **E.** Scheme of the orthotopic colorectal tumor model. **F.** Tumor numbers in the rectum of the indicated mice 11d after orthotopic rectal injection of MC38 cells. Pooled data from 2 independent experiments. ns p≥ 0.05 by ANOVA (C) or by Student's *t* test (B, F), *p<0.05 by Mann-Whitney test (D).

A



B

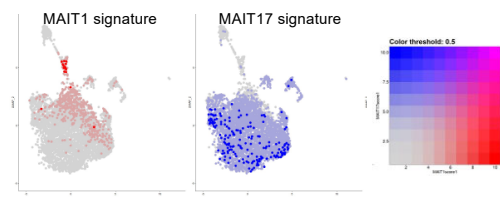


Figure S8: Gene expression in colonic MAIT cells upon colitis. A. Heatmap showing the 10 most differentially expressed genes in cells from the indicated clusters. Colonic MAIT cells isolated from the colon of AOM- or DSS-treated mice were analyzed by single-cell RNAseq. Cells from both conditions were integrated using default Seurat integration parameters and were analyzed jointly using 30 principal components and a clustering resolution of 0.8. **B.** Expression of a MAIT1 (red) and a MAIT17 (blue) gene signature by colonic MAIT cells. The signatures were defined by bulk analysis of thymic MAIT1 and MAIT17 cells in Legoux et al, Nat. Immunol 2019.