Bile acids regulate intestinal antigen presentation and reduce graft-*versus*-host disease without impairing the graft-*versus*-leukemia effect

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Bile acids regulate intestinal antigen presentation and reduce graft-versus-host disease without impairing the graft-versus-leukemia effect

Eileen Haring[*1,2], Franziska M. Uhl[*1,2], Geoffroy Andrieux[3,4], Michele Proietti[5], Alla Bulashevska[5], Barbara Sauer[1], Lukas M. Braun[1,2], Enrique de Vega Gomez[1], Philipp R. Esser[6], Stefan F. Martin[6], Dietmar Pfeifer[1], Marie Follo[1], Annette Schmitt-Graeff[7], Joerg Buescher[8], Justus Duyster[1], Bodo Grimbacher[5,9,10,11], Melanie Boerries[3,4,12], Erika L. Pearce[8], Robert Zeiser[1,4,9] and Petya Apostolova[1]

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Figure S1

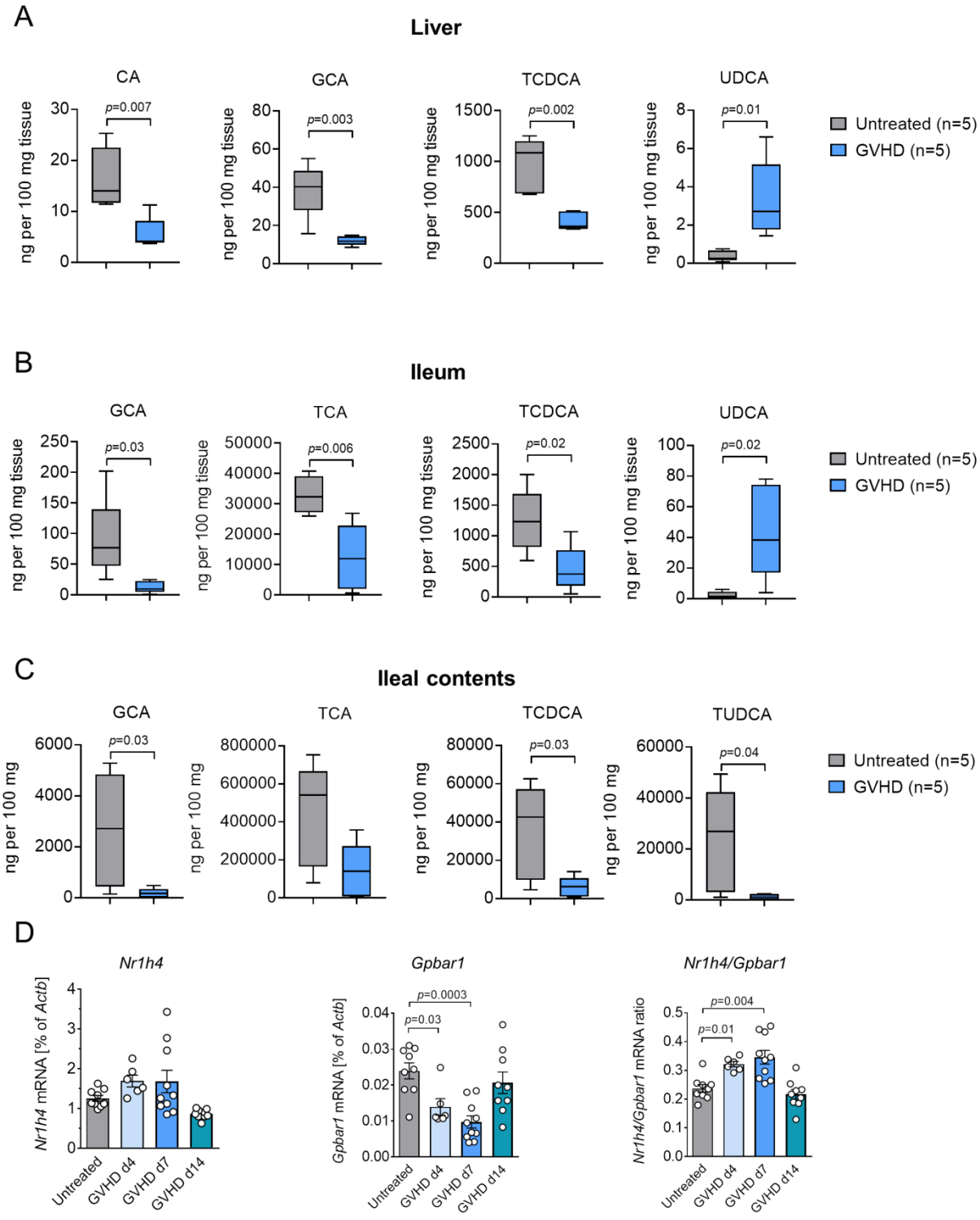

Figure S1. GVHD induction modulates the bile acid composition and bile acid receptor expression in various compartments

(A)–(C) Metabolomic profiling was performed as shown in Figure 1 in untreated mice and mice developing GVHD. Levels of the four most significantly regulated bile acids in the liver (A), ileum (B) and ileal contents (C) are shown. CA: cholic acid, GCA: glycocholic acid,

TCDCA: taurochenodeoxycholic acid, UDCA: ursodeoxycholic acid, TCA: taurocholic acid, TUDCA: tauroursodeoxycholic acid. N numbers represent individual mice. Whiskers were created using the Tukey method. *P*-values were calculated using the two-tailed unpaired Student's t-test.

(D) Quantitative real-time PCR analysis of the mRNA expression of the bile acid receptors *Nr1h4* (encodes for the FXR protein) and *Gpbar1* (encodes for the TGR5 protein) in the colon of untreated mice and mice developing GVHD. Data are pooled from n=10 mice in the untreated, GVHD d7 and GVHD d14 groups and n=5 mice in the GVHD d4 group. The *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons.

Figure S2

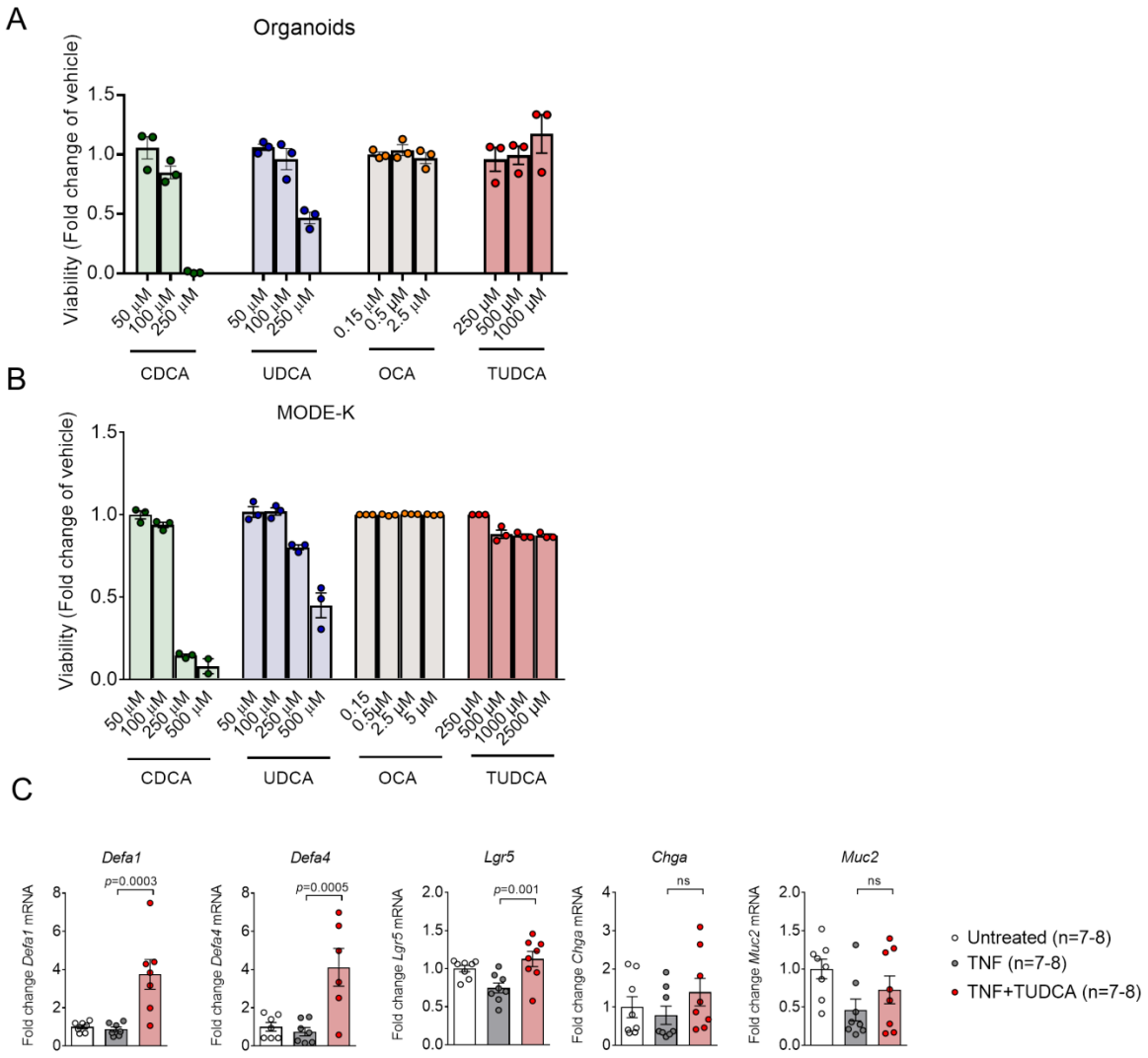

Figure S2. Impact of bile acids on intestinal organoids and MODE-K cells

(A) Viability of small intestinal organoid cells after treatment with increasing concentrations of bile acids determined by flow cytometry. Each dot represents an independent biological replicate. Data were normalized to vehicle treatment.

(B) Viability of MODE-K cells after treatment with increasing concentrations of bile acids determined by flow cytometry. Each dot represents an independent biological replicate. Data were normalized to vehicle treatment.

(C) Intestinal organoids were cultured and treated with TNF ± TUDCA as described in Figure 2A. Quantitative real-time PCR analysis of the mRNA expression of the ISC markers *Lgr5*, the Paneth cell markers *Defa1* and *Defa4*, the enteroendocrine cell marker *Chga* and the goblet cell marker *Muc2* with *Actb* as a reference gene. Data were pooled from 7-8 single experiments with at least 3 organoid wells/condition. The *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons.

Figure S3

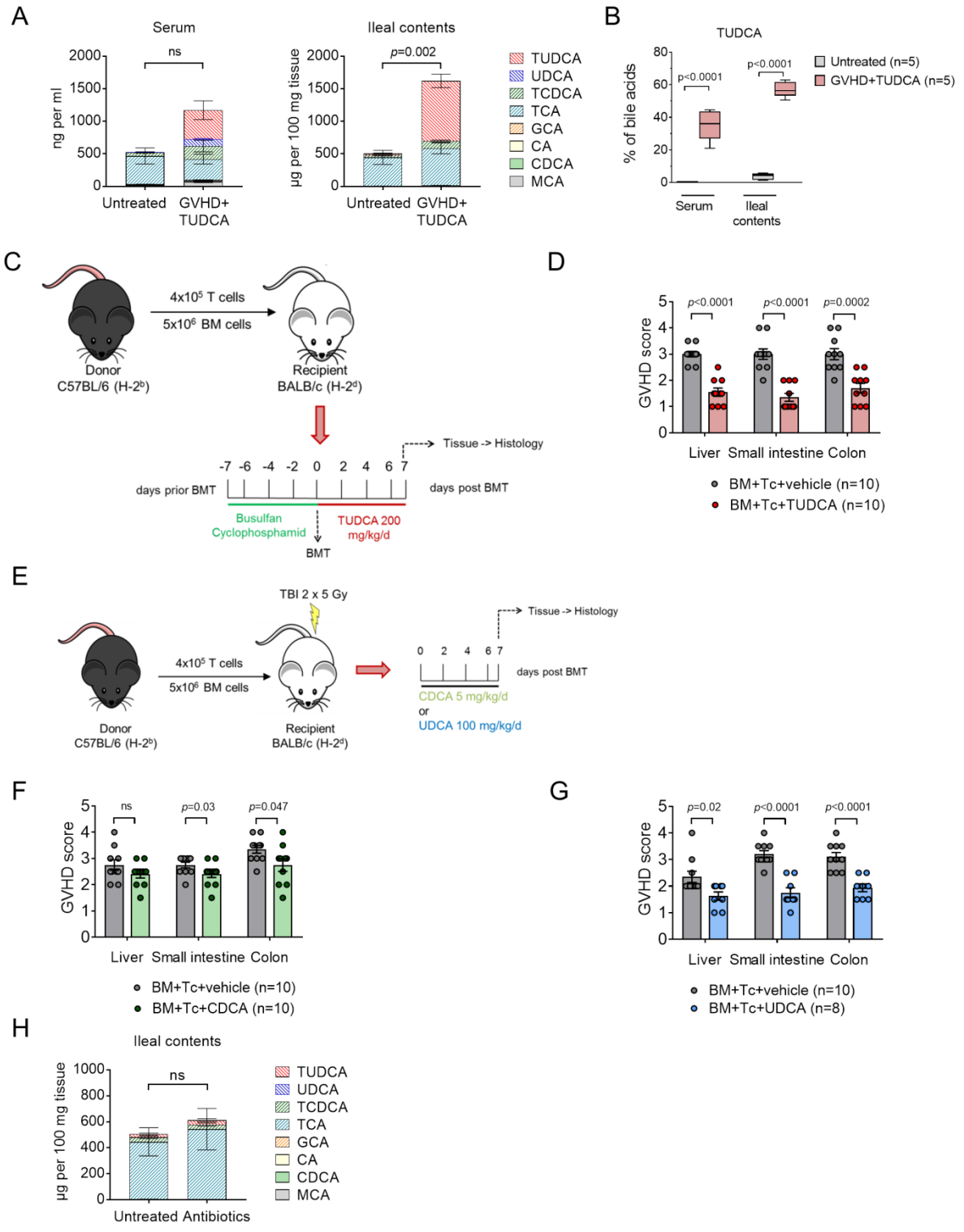

Figure S3. Bile acid treatment in a preclinical murine model of GVHD

(A) Serum and ileal contents were isolated from untreated mice or mice treated with TUDCA on day 14 after BMT (C57BL/6 in BALB/c model, see Figure 3A). Quantification of bile acids in the serum (left) and ileal contents (right) by LC-MS. Data were pooled from n=5 individual

mice/group and are presented as mean±SEM. *P*-values were calculated with regard to the total amount of bile acids using the two-tailed unpaired Student's t-test, ns: not significant.

(B) Quantification of the proportion of TUDCA within the measured bile acid pool. Data were pooled from n=5 individual mice. Whiskers were created using the Tukey method. *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons.

(C) Transplantation model with chemotherapy conditioning treatment. Recipients were treated with 200 mg/kg body weight TUDCA or an equal volume of vehicle from day 0 until day 7 after BMT by a daily intraperitoneal injection.

(D) GVHD histopathology scores of liver, small intestine and colon assessed on day 7 after BMT as shown in (C). N numbers represent individual mice. *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons.

(E) Transplantation model for treatment with UDCA and CDCA. Recipient mice were treated with 100 mg/kg body weight UDCA or 5 mg/kg body weight CDCA or an equal volume of vehicle from day 0 until day 7 after BMT by a daily intraperitoneal injection.

(F), (G) GVHD histopathology scores of liver, small intestine and colon assessed on day 7 after BMT as shown in (E). N numbers represent individual mice. *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons.

(H) Quantification of bile acids in the ileal contents of untreated BALB/c mice and mice exposed to an antibiotic cocktail by LC-MS. Data were pooled from n=5 individual mice/group and are presented as mean±SEM. *P*-values were calculated with regard to the total amount of bile acids using the two-tailed unpaired Student's t-test; ns: not significant.

Figure S4

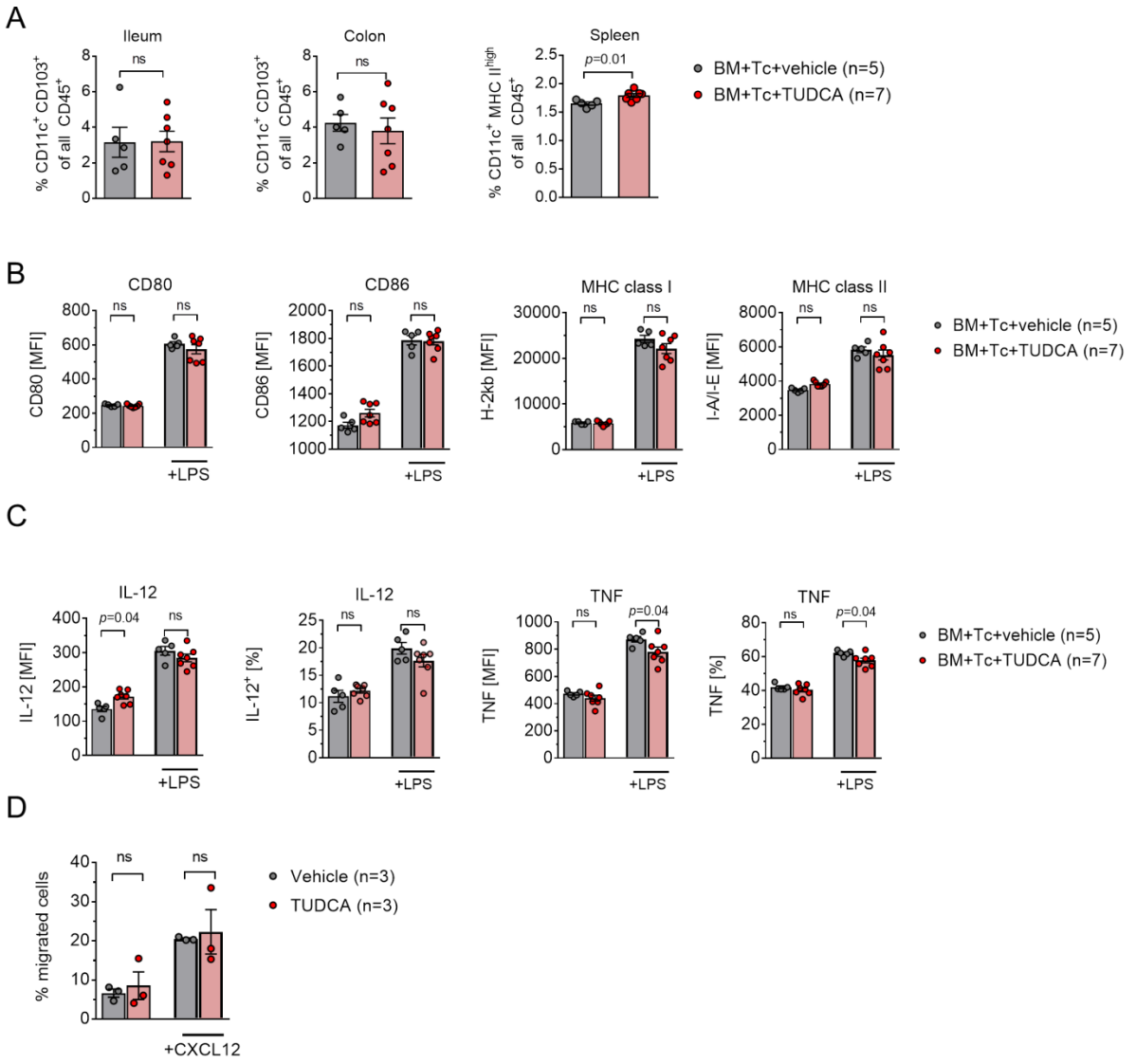

Figure S4. Effects of TUDCA treatment on hematopoietic antigen-presenting cells

(A) – **(C)** Ileum, colon and spleen were isolated from recipient mice, treated with vehicle or TUDCA, on day 14 after BMT (C57BL/6 in BALB/c model).

(A) Quantification of the percentage of professional hematopoietic $CD11c^+$ antigen-presenting cells by flow cytometry. N numbers represent individual mice. *P*-values were calculated using the two-tailed unpaired Student's t-test, ns: not significant.

(B), (C) Splenocytes were isolated from recipient mice and stimulated with 20 ng/ml LPS overnight. Flow cytometry evaluation of costimulatory molecule **(B)** and cytokine **(C)** expression on $CD11c^+$ MHC class II^+ cells by flow cytometry. N numbers represent individual mice. *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons.

(D) Migration ratio of bone marrow-derived dendritic cells treated with 500 µM TUDCA or vehicle for 24 hours along a CXCL12 gradient (60 ng/ml). One representative result from three independent experiments performed in technical triplicates is presented. *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons.

Figure S5

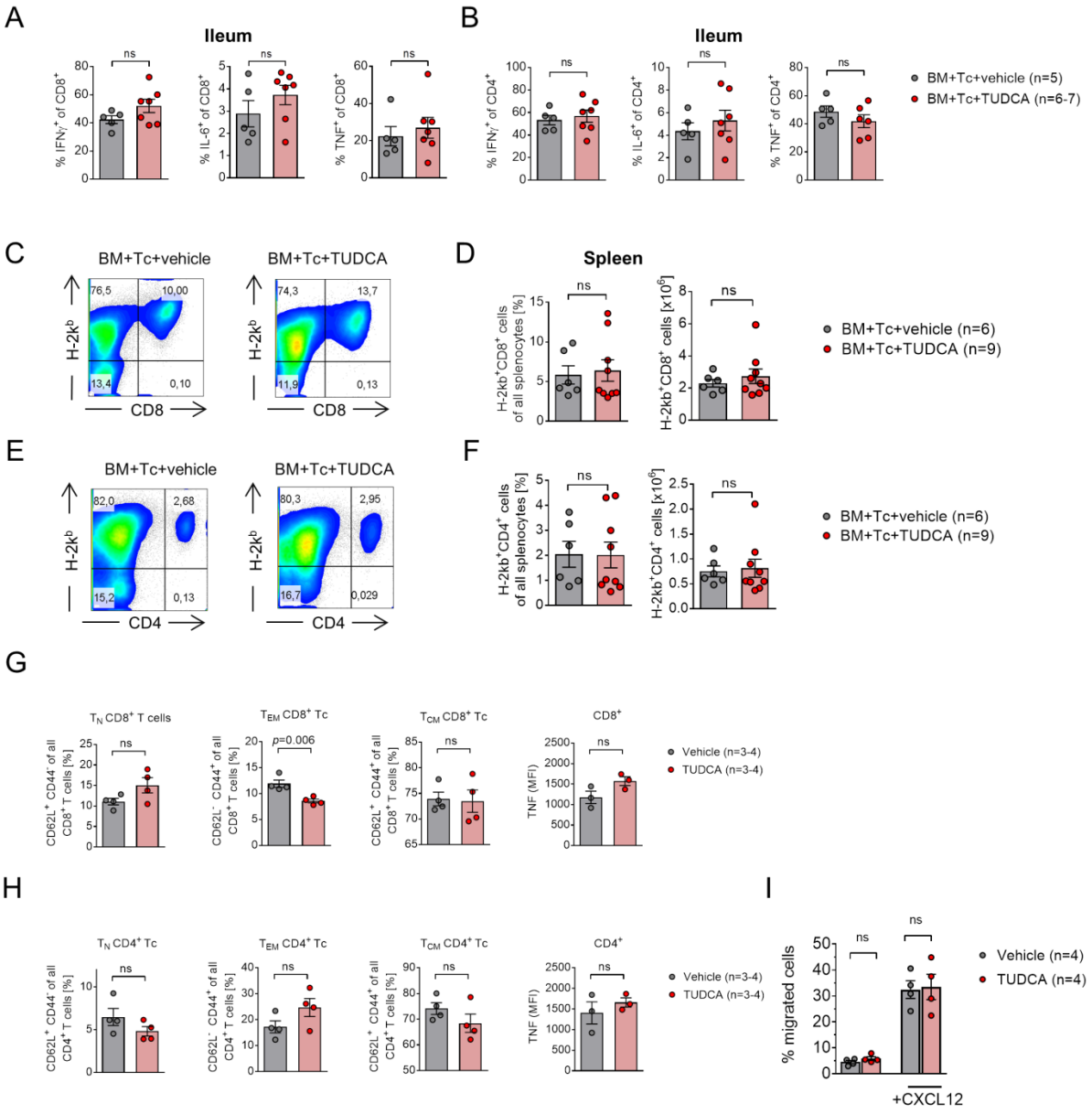

Figure S5. Impact of TUDCA treatment on T cell activation and differentiation

(A) – (F) Ileum and spleen were isolated from recipient mice, treated with vehicle or TUDCA, on day 14 after BMT (C57BL/6 in BALB/c model). N numbers indicate individual mice. P-values were calculated using the two-tailed unpaired Student's t-test; ns: not significant.

(A) – (B) Flow cytometric analysis of cytokine expression of $CD8^+$ (A) and $CD4^+$ (B) cells in the ileum. Representative data from one of two independent experiments.

(C) – (F) Flow cytometric analysis of splenic T cells isolated from recipient spleens on day 14 after BMT (C57BL/6 in BALB/c model) was performed. Data were pooled from two independent experiments.

(C) Representative flow cytometry plots for $CD8^+$ T cells.

(D) Quantification of the relative (left panel) and absolute (right panel) numbers of $CD8^+$ T cells.

(E) Representative flow cytometry plots for $CD4^+$ T cells.

(F) Quantification of the relative (left panel) and absolute (right panel) numbers of $CD4^+$ T cells.

(G) – (I) $CD4^+$ and $CD8^+$ T cells were isolated from the spleen of C57BL/6 mice and activated *in vitro* via incubation with anti-CD3/CD28 beads and treated with 500 µM TUDCA or vehicle for 48h. N numbers represent individual biological replicates performed in 2-3 technical replicates. *P*-values were calculated using the two-tailed unpaired Student's t-test.

(G) Flow cytometric analysis of $CD8^+$ cells.

(H) Flow cytometric analysis of $CD4^+$ cells.

(I) Migration ratio of $CD4^+$ and $CD8^+$ T cells along a CXCL12 gradient (60 ng/ml).

Figure S6

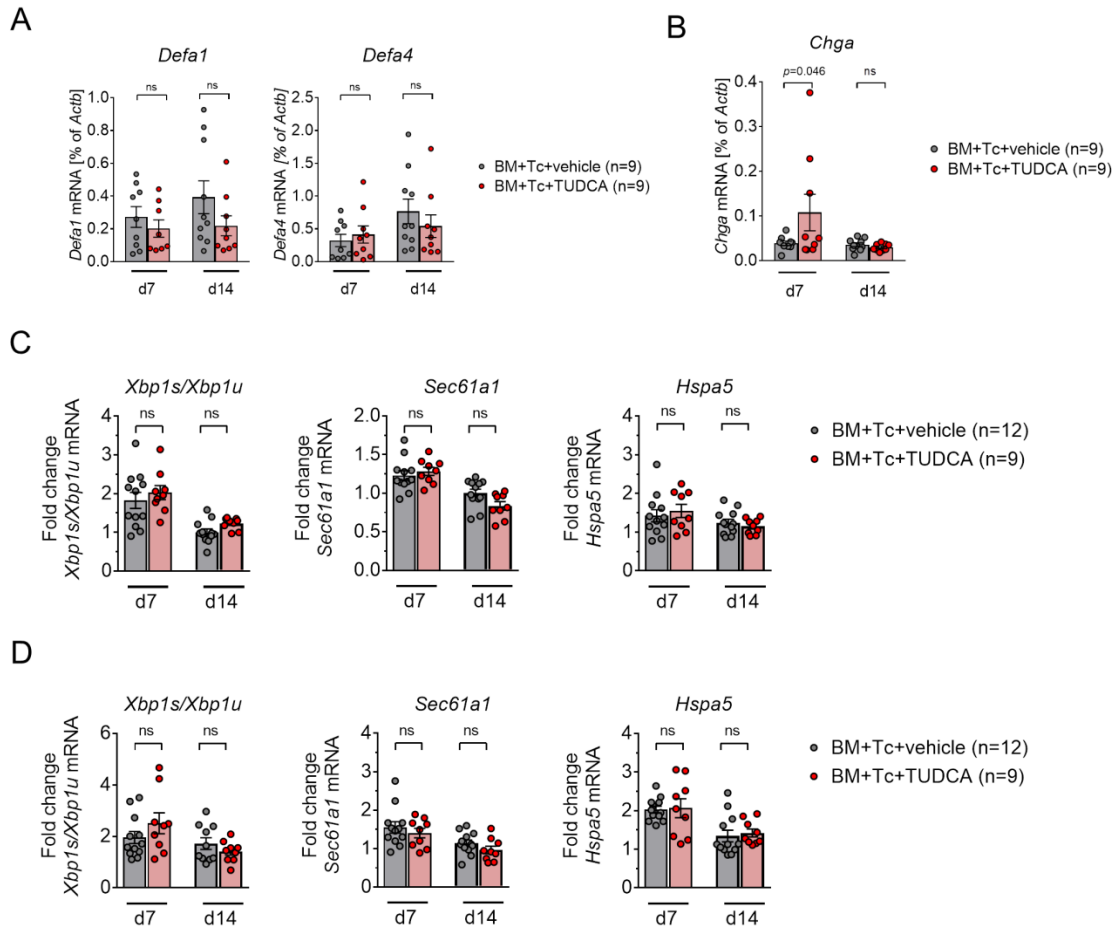

Figure S6. Paneth and enteroendocrine cell markers as well as ER stress levels after treatment with TUDCA

(A) - (B) Small intestinal samples were isolated from recipient mice, treated with vehicle or TUDCA, on day 7 and 14 after BMT (C57BL/6 in BALB/c model). Quantitative real-time PCR analysis of the mRNA expression of the Paneth cell markers *Defa1* and *Defa4* (A) and the enteroendocrine cell marker Chga (B) with *Actb* as a reference gene. Data were pooled from two independent experiments, N numbers represent individual mice. *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons; ns: not significant.

(C) – (D) Small intestinal (C) and colon (D) samples were isolated from recipient mice, treated with vehicle or TUDCA, on day 14 after BMT (C57BL/6 in BALB/c model). Quantitative real-time PCR analysis of the mRNA expression of the ER stress markers *Xbp1s/Xbp1u*, *Sec61a1* and *Hspa5* with *Actb* as a reference gene. Data were pooled from three independent experiments, N numbers represent individual mice. *P*-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons; ns: not significant.

Figure S7

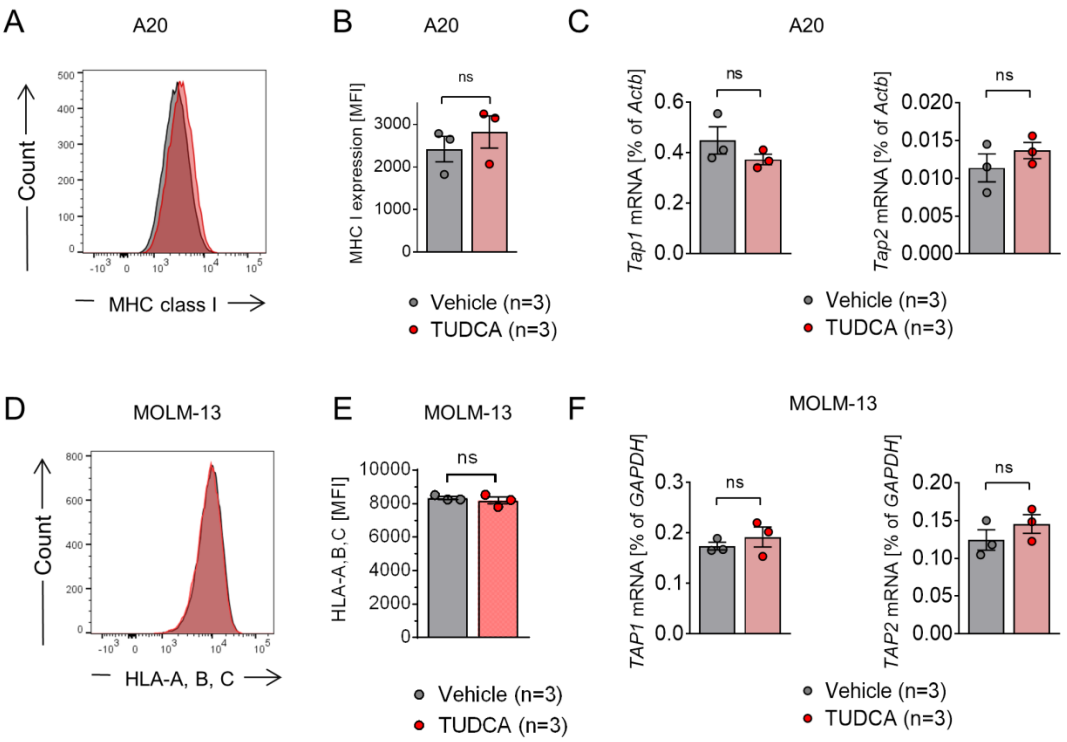

Figure S7 TUDCA does not reduce antigen presentation-related molecules in leukemic cells

(A)-(F) The murine leukemia/lymphoma cell line A20 and the human cell line MOLM-13 were cultured for 72 hours with or without addition of TUDCA. One representative result from three independent experiments performed in technical triplicates is shown for panel (A) and (D). In panels (B), (C), (E) and (F) data were pooled from three independent experiments. *P*-values were calculated using the unpaired two-tailed Student's t-test, ns: not significant.

(A) Representative histograms from a flow cytometric analysis of MHC class I expression on A20 cells.

(B) Quantification of MHC class I expression on A20 cells.

(C) Quantitative PCR analysis of the expression of the antigen presentation-related genes *Tap1* and *Tap2* in A20 cells with *Actb* as reference gene.

(D) Representative histograms from flow cytometric analysis of HLA-A, B, C expression on MOLM-13 cells.

(E) Quantification of HLA-A, B, C expression on MOLM-13 cells.

(F) Quantitative PCR analysis of the expression of the antigen presentation-related genes *TAP1* and *TAP2* in MOLM-13 cells with *GAPDH* as reference gene.

Figure S8

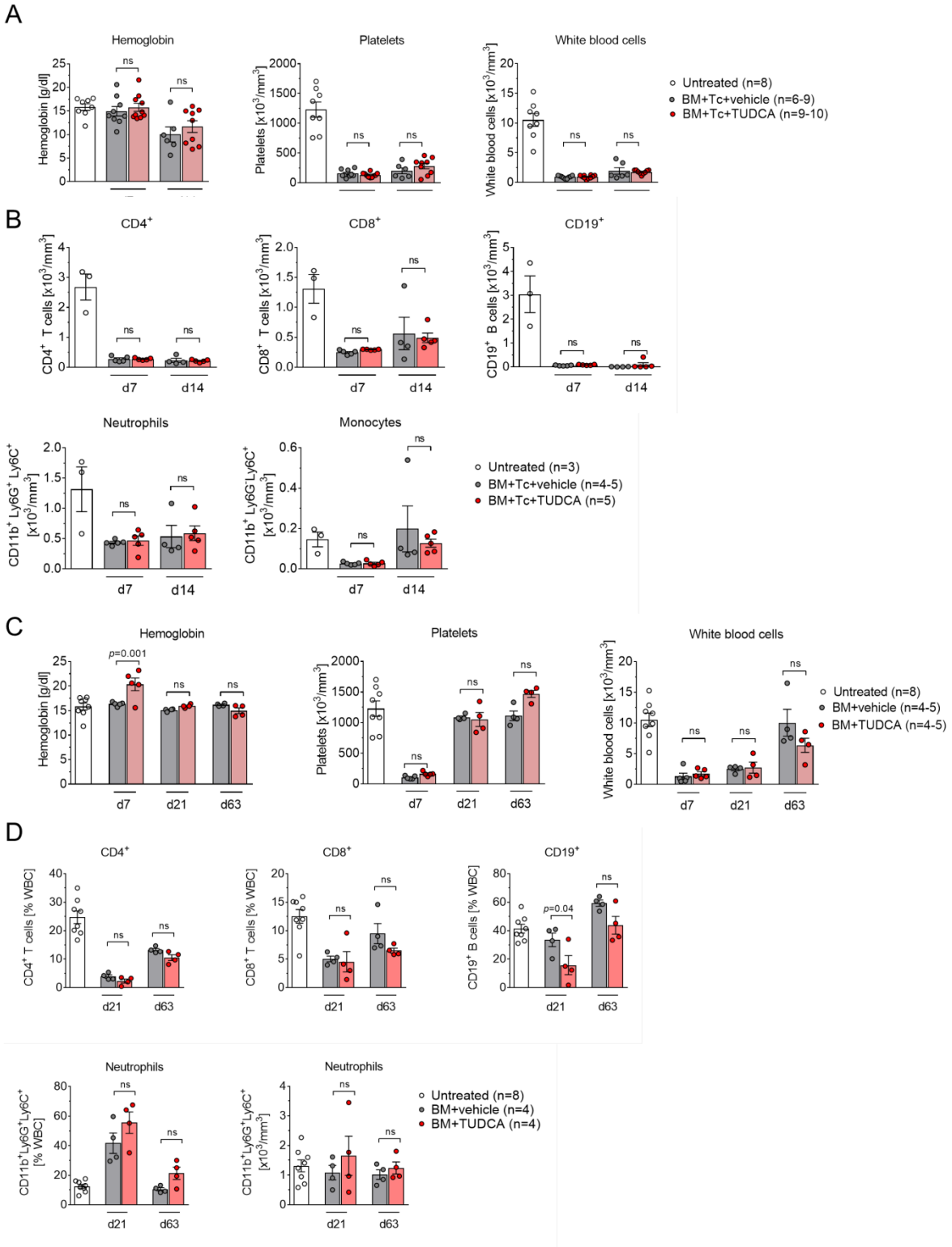

Figure S8. TUDCA does not impair hematopoietic regeneration after allo-HCT

(A) Peripheral blood counts in recipient mice on day 7 and day 14 after BMT (C57BL/6 in BALB/c model) compared to untreated mice. Data were pooled from two independent experiments.

(B) Flow cytometry-based quantification of the absolute numbers of T cells, B cells, granulocytes and monocytes in recipient mice on day 7 and day 14 after BMT (C57BL/6 in BALB/c model).
(C) Peripheral blood counts in recipient mice on day 7, 21 and 63 after BMT (C57BL/6 in BALB/c model, transfer only of BM without T cells).
(D) Flow cytometry-based quantification of the absolute numbers of T cells, B cells, granulocytes and monocytes in recipient mice on day 21 and day 63 after BMT (C57BL/6 in BALB/c model, transfer only of BM without T cells).
(A) – (D) N numbers indicate individual mice. P-values were calculated using the ordinary one-way ANOVA test with correction for multiple comparisons; ns: not significant.

Supplementary Methods

Antibiotic treatment

Drinking water was supplemented with Cefoxitin Sodium Salt (Santa Cruz Biotechnology, USA), Gentamicin sulfate salt (Sigma Aldrich, Germany), Neomycin Sulfate (Merck, Germany) and Metronidazole (Sigma Aldrich, Germany) at a concentration of 1 mg/ml and administered to recipient C57BL/6 mice for 14 days prior to BMT. For the bile acid metabolomic analysis, BALB/c mice received the antibiotic cocktail for 7 days prior to isolation of the ileal contents.

Bioluminescence imaging

Bioluminescence imaging was performed as described previously [1]. Briefly, $CD4^+$ and $CD8^+$ T cells from a luciferase-transgenic C57BL/6 donor were used for GVHD induction. Recipient mice received an intraperitoneal injection of 150 µg/g body weight luciferin in a total volume of 200 µl PBS. After 10 minutes, bioluminescence imaging was performed using an IVIS charge-coupled device for 5 minutes. Data acquisition and analysis were performed using the Living Image Software (Xenogen). Light emission was quantified in photons/second/mouse.

Blood cell counter

Blood samples were drawn via tail vein puncture or terminal heart puncture and cell counts were measured in the fresh samples using the scil Vet Animal Blood Counter (scil animal care company, Germany).

BMT, induction of aGVHD and GvL

Recipient mice were used between 6 and 12 weeks of age for gender-matched transplantations in an MHC major mismatch model of aGVHD. Recipient BALB/c mice received myeloablative conditioning treatment by total body irradiation (TBI; IBL 637C Cis bio international caesium source, France) with 10 Gy in total and recipient C57BL/6 mice received myeloablative conditioning treatment by TBI with 12 Gy in total. In both cases TBI was applied in two equal split doses, at least 4 h apart. After the second TBI, the recipient mice were injected intravenously with 5×10^6 bone marrow (BM) cells which were purified from the femora and tibiae of donor mice. In experiments with chemotherapy conditioning, mice first received Busulfan at a concentration of 20 mg/kg via daily intraperitoneal injection

for four days from d -7 to d -4 prior to BMT. From d -3 to d -2 the animals received 100 mg/kg Cyclophosphamide. On d-1 there was no treatment and on d0 the animals were injected intravenously with 5 x 10^6 bone marrow (BM) cells

For induction of aGVHD, $CD4^+$ and $CD8^+$ T cells were isolated via positive selection with anti-CD4/CD8 MACS Micro Beads or negative selection with Pan T Cell Isolation Kit II and LS Columns (Miltenyi Biotec) from the spleens of donor mice. The number of T cells injected varied in dependence of the utilized transplantation model and is indicated in the respective figure legend. To investigate the GVL effect *in vivo*, BALB/c recipients received myeloablative conditioning treatment by total body irradiation with 10 Gy in two equally split doses and were transplanted intravenously with 1000 leukemia cells (BA/F3-ITD^{GFP+}) and 5x10^6 C57BL/6 BM. Two days post transplantation, 3x10^5 T cells from C57BL/6 mice were administered to induce the GVL effect.

Cell culture

Mouse intestinal organoids were isolated based on the description by Sato and Clevers [2]. After removal of the mucus and villi with a microscopic slide, a 10 cm-long piece from the proximal part of the murine small intestine was repetitively washed in cold PBS. Crypts were isolated from the proximal part of the murine small intestine after a digestion in a 2 mM EDTA buffer for 30 minutes and subsequent shaking steps with ice-cold PBS. Fractions which were free of villi and enriched for crypts were pooled, passed through an Easystrainer 70 µm (Greiner Bio One, Germany) and resuspended in Corning™ Matrigel™ GFR Basement Membrane (Corning, USA). Fifty µl of crypt suspension was pipetted on the bottom of a 24-well flat-bottom TC plate allowing formation of a hemispherical droplet. After incubation for 10 minutes at 37°C, 500 µl Intesticult Organoid Growth Medium (Stem Cell Technology, Canada), supplied with 100 U/ml penicillin and 100 µg/ml streptomycin was applied. Intestinal organoids were cultured at 37 °C and 5% CO_2. Media change was performed every 3-4 days and the organoids were split once a week. Organoid digestion was performed after washing out the Matrigel™ by incubation on a shaking platform in pre-warmed TrypLE Express Enzyme (Gibco-Thermo Fisher Scientific, Germany) for 30 minutes at 37°C.

The MODE-K cell line, an immortalized mouse small intestinal epithelial cell line, was kindly provided by D. Kaiserlian (INSERM, France) and cultured in RPMI, supplemented with 1 mM sodium pyruvate, 1% HEPES, 1% MEM Non-essential Amino Acids, 50 µM 2-mercaptoethanol, 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin [3].

Ba/F3-ITD, a murine pro-B cell line (transfected with a FLT3-ITD mutation), was kindly provided by Prof. J. Duyster (Medical Center – University of Freiburg, Germany) and cultured in RPMI 1640 supplemented with 10 % FCS, 100 U/ml penicillin and 100 µg/ml streptomycin.

Bone marrow-derived dendritic cells (BM-DC) were isolated by flushing out the femora and tibiae of untreated donor mice. 6 x 10^6 bone marrow cells were placed in a 100 mm cell culture dish in RPMI 1640 + L-Glutamine, supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin and 40 ng/ml mGM-CSF. BM-DC were cultured at 37°C and 5% CO_2. On day 3 and 5 of culture, medium was replaced and fresh mGM-CSF was added. BM-DC were used after 7-8 days of culture.

For analysis of TUDCA effects on malignant cell lines, the murine cell lines A20 and RMB-1, and the human cell lines MV-4-11 and MOLM-13 were used. The A20 cell line was purchased from ATCC (#TIB-208, USA) and cultured in RPMI 1640+L-Glutamine, supplemented with 10% FCS, 1% sodium pyruvate, 1% MEM non-essential amino acids, 1% MEM Vitamin, 1% HEPES, 100 U/ml, 100 µg/ml streptomycin and 50 mM 2-mercaptoethanol. The RMB-1 cell line was purchased from DSMZ (#: ACC 391, Germany) and was cultured in RPMI 1640+L-Glutamine, supplemented with 10% FCS and 1% penicillin/streptomycin. The human cell line MV-4-11 was purchased from ATCC (#CRL 9591, USA) and cultured in IMDM supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. MOLM-13, a human acute myeloid leukemia cell line, was purchased from DSMZ (ACC 554) and cultured in RPMI 1640 supplemented with 10 % FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. All cell lines were cultured at 37 °C and 5% CO_2.

Chemicals

Chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), 6-ethylchenodeoxycholic acid (obeticholic acid; OCA) and tauroursodeoxycholic acid (TUDCA) were purchased from Sigma Aldrich. Freshly prepared TUDCA solutions were stored at 4°C, the other bile acids were stored at -20°C according to the manufacturer's instructions. For cell culture experiments, CDCA was used at 50 µM, UDCA at 100 µM, OCA at 0.5 µM and TUDCA at 500 µM concentrations after titration to determine the viability after bile acid exposure.

Mice were treated with 200 mg/kg body weight TUDCA applied as an intraperitoneal injection once daily. Control animals received an equal volume of Aqua ad iniectabilia as vehicle. UDCA was administered at a concentration of 100 mg/kg in Aqua ad iniectabilia with 5% ethanol once daily via intraperitoneal injection. CDCA was administered at a concentration of 5 mg/kg in Aqua ad iniectabilia with 5% ethanol once daily via intraperitoneal injection. Control animals received an equal volume of Aqua ad iniectabilia with 5% ethanol as vehicle.

Busulfan (Sigma Aldrich) was prepared in 100% DMSO at a concentration of 50 mg/ml and cyclophosphamide (Pharmacy at the Medical Center – University of Freiburg) was prepared in NaCl at a concentration of 20 mg/ml.

LPS from *Salmonella enterica* was purchased from Sigma Aldrich and used at a concentration of 1 µg/ml for DC maturation and at a concentration of 20 ng/ml for cell re-stimulation in flow cytometric experiments.

Recombinant murine TNFα was purchased from PeproTech and used at a concentration of 20 ng/ml for organoid treatment. Recombinant murine IFNγ (PeproTech) was used at a final concentration of 2.5 ng/ml in organoid culture experiments. For migration assays, recombinant murine CXCL12/SDF1α (R&D Systems) was employed at a concentration of 60 and 100 ng/ml.

Cytometric Bead Array

Serum was collected from mice developing aGVHD on day 14 after BMT. Serum cytokine concentrations were determined with the flow cytometry-based Cytometric Bead Array Mouse Inflammation Kit (BD Biosciences, Germany) according to the manufacturer's instructions.

Fecal microbial DNA isolation

Upon collection, fecal samples were immediately stored at -80 °C. Microbial DNA was extracted from a 2 ml aliquot using QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturer's instructions with modifications. The temperature of lysis was increased from 70 °C (suggested in the protocol) to 95 °C for the lysis of bacteria that are known to be difficult to lyse as e.g. Gram-positive bacteria. In a later step, 400 µl supernatant (instead of suggested volume 200 µl) were pipetted to 15 µl of proteinase K and 400 µl AL buffer (instead of suggested volume of 200µl) were added followed by thoroughly mixing and incubation at 70 °C for 10 minutes. Afterwards, spin column was loaded twice with 400 µl of the lysate.

Flow Cytometry

Prior to flow cytometry staining, samples were washed with PBS. In general, 200 000 to 500 000 cells were used per staining. For analysis of intracellular cytokines, splenocytes or intestinal cell fractions isolated from mice developing aGVHD were stimulated for 5 h either with Cell Stimulation Cocktail (Invitrogen-Thermo Fisher Scientific, Germany) for the

identification of T cell cytokines or overnight with 20 ng/ml LPS and GolgiPlug (1:1000) for identification of myeloid cell cytokines. For intracellular stainings, fixation and permeabilization was performed using the BD Cytofix/Cytoperm kit (BD Biosciences, Germany) according to the manufacturer's instructions. Dead cells were marked using the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, Germany) or the Red Zombie Fixable Viability Kit (Biolegend, USA) according to the manufacturer's instructions. Cells were then washed with FACS buffer (PBS supplemented with FCS, EDTA and NaN_3) and Fc block was performed using the purified anti-CD16/CD32 antibody (dilution 1:25, eBioscience-Thermo Fisher Scientific). Antibodies were added at titrated concentrations for 30 minutes. After washing, analysis was performed on a BD LSRFortessa Cell Analyzer (BD Biosciences, Germany). All antibodies utilized for flow cytometric analyses can be obtained from Tables S1-3. Data were acquired using the BD FACSDiva Software and analyzed with FlowJo_V10.1 (TreeStar, USA). Naïve T cells (T_N) were defined as $CD62L^+$ $CD44^-$. Effector memory T cells (T_{EM}) were defined as $CD62L^-$ $CD44^+$. Central memory T cells (T_{CM}) were defined as $CD62L^+$ $CD44^+$. Professional antigen-presenting dendritic cells in the intestine were defined as $CD45^+$ $CD11c^+$ $CD103^+$. Professional antigen-presenting cells in the spleen were defined as $CD45^+$ $CD11c^+$ MHC class II^+.

Histology

Liver, small intestine and colon were isolated from the mice at indicated time points, flushed with PBS and fixed in 4% formaldehyde solution overnight. Following standard dehydration and paraffin embedding procedures (Leica TP1020 and Leica EG1150 H Tissue embedding center), 5 µm-thick sections were prepared.

For aGVHD histopathology scoring, the sections were stained with Hematoxylin/Eosin (HE) with a Leica Autostainer (Infinity H&E Staining system). Sections were scored by an experienced pathologist (A. S.-G.) blinded to the treatment groups as previously described [4]. Liver GVHD was scored based on the tissue involved (0, none; 1, few involved tracts; 2, numerous involved tracts; 3, injury of the majority of tracts; 4, severe involvement of most tracts). Intestinal GVHD was scored on the basis of crypt apoptosis (0, rare to none; 1, occasional apoptotic bodies per 10 crypts; 2, few apoptotic bodies per 10 crypts; 3, the majority of crypts contain an apoptotic body; 4, the majority of crypts contain >1 apoptotic body) and inflammation (0, none; 1, mild; 2, moderate; 3, severe, without ulceration; 4, severe, with ulceration).

Apoptosis staining was performed using the In Situ Cell Death Detection Kit, Fluorescein (Roche, Germany) according to the manufacturer's instructions. All nuclei were stained with DAPI (Sigma Aldrich, Germany). Analysis of apoptotic nuclei was performed on an Olympus

ScanR High Content Screening Station. Using the analysis software, 50 000 to 100 000 cells per slide were analyzed. The percentage of apoptotic nuclei (green fluorescence) in all nuclei ($DAPI^+$) was calculated using the ScanR Analysis software.

Anti-GFP staining was performed to quantify ISC using the B6.129P2-*Lgr5*$^{tm1(cre/ERT2)Cle/J}$ mice for *in vivo* experiments (GFP fluorescence in $Lgr5^+$ cells). Antigen retrieval was performed by pretreatment with Target Retrieval Solution citrate buffer pH 6.0 (Dako, Germany). The polyclonal chicken anti-GFP antibody (ab13970, Abcam) was used at a dilution of 1:2000 according to the manufacturer's instructions. The AlexaFluor 488-coupled goat anti-chicken IgY secondary antibody (ab150169, Abcam) was used in a dilution of 1:2000 according to the manufacturer's instructions. Analysis was performed on a Leica TCS SP2 AOBS Confocal microscope.

Intestinal leukocyte isolation

Intestinal leukocytes were isolated as described [5]. In brief, 4 cm intestinal segments were dissected and Peyer's patches removed. The segments were opened longitudinally and rinsed in PBS to remove remaining feces. Epithelial cells were separated from the lamina propria using cell dissociation (CD) buffer (HBSS without $Mg2^+$, $Ca2^+$, 5 mM EDTA, 10 mM HEPES). The remaining tissue was digested with digestion buffer (HBSS with $Mg2^+$, $Ca2^+$, D 0.5 mg/ml, DNAse 0.5 mg/ml) to obtain single cell suspensions which were then further processed for flow cytometric analyses.

Killing Assay

For evaluation of the *in vitro* killing capacity of $CD8^+$ T cells, these were isolated from the spleens of naïve C57BL/6 mice and stimulated with allogeneic (BALB/c) BM-DC for 72 h with or without TUDCA as indicated. For the evaluation of the *ex vivo* killing capacity, T cells were isolated from the spleens of GVHD mice treated with TUDCA or vehicle for 10 days *in vivo*.

In both assays, T cells were then co-cultured with GFP^+ A20 cells at the indicated ratios and incubated for 24 h. The percentage of dead A20 cells was determined by flow cytometry using the Live Dead Staining Red Zombie Fixable Viability Kit.

Metabolomics Analysis

For quantification of bile acids, liver, small intestine, ileal contents and serum were isolated. Organs and the ileal content were weighed and put into ice-cold methanol (MeOH) (50 mg liver, 25 mg small intestine and 10 mg ileal content in each 1.5 ml MeOH). Blood samples

were allowed to clot, centrifuged and 20 µl of serum were mixed with 100 µl MeOH. Organs and the ileal content were mechanically disrupted and serum samples were carefully mixed in MeOH. All samples were incubated on ice for at least 1 hour. Subsequently, samples were centrifuged using a tabletop centrifuge at maximum speed for 5 min. Clear supernatants were transferred to glass vials and ileal content samples were additionally diluted 1:2 in MeOH before storing the samples at -80 °C until further analysis by LC-MS. A standard solution with known concentration of the bile acids of interest was prepared and diluted 1:10, 1:100 and 1:1000 to obtain four separate standard solutions. Targeted quantification of bile acids by LC-MS was carried out using an Agilent 1290 II UHPLC inline with an Agilent 6495 QQQ-MS operating in MRM mode. MRM settings were optimized separately for the selected bile acids using pure standards and transferred for the detection of their respective isomers. LC separation was on a Waters CSH C18 column (100 x 2.1 mm, 1.8 µm particles) using a solvent gradient of 70% buffer A (10 mM ammonium formate in water) to 97% buffer B (2:1 2-propanol:acetonitrile). Flow rate was 300 µl/min. Autosampler temperature was 4°C and injection volume was 3 µl. Data processing was performed using Agilent MassHunter Software. Peak areas from the four standard solutions were used to generate standard curves after logarithmic transformation in Microsoft Excel. Unknown sample concentrations were extrapolated from the standard curves.

Microarray analysis

Microarray analysis of murine small intestine samples was performed as described previously [6] using Clariom S Assays (Thermo Fisher Scientific, Germany) according to the manufacturer's instructions. CEL files were processed with the oligo R package [7] and normalized with Robust Multi-Array Average (RMA) approach. Relatedness between samples was visualized on a principal component analysis (PCA), where two clear outliers were excluded from downstream analysis. Finally we adjusted RNA intensity for batch effect using Combat method from SVA R package [8]. Differential expression analysis was performed using a linear model-based approach (limma R package [9]). The significantly regulated genes between "TUDCA" and "vehicle" groups were selected with the following criteria: adjusted *p* value (benjamini Hochberg procedure) < 0.05. We performed a hypergeometric test on the differentially regulated genes between "TUDCA" and "vehicle" groups (q-value < 0.05) to find the enriched gene-sets. We used the Gene Ontology (GO) [10] and ConsensusPathDB [11] as gene-sets and the whole set of quantified genes in the microarray as background for the statistical analysis. The threshold for significant adjusted *p* value was set to 0.05. Differentially regulated genes that belong to relevant gene-sets were visualized using heatmaps. To compare gene expression across the samples we scaled

each row using Z-score. Samples and genes were clustered using hierarchical clustering with complete agglomeration method on Euclidean distance.

Migration assay

Migration assay of BM-DC was performed using 24-well flat-bottom transwell migration plates (Costar, USA). Five-hundred thousand BM-DC were pipetted into the insert (3 µm) of the transwell plate and allowed to migrate for 5 h at 37 °C and 5% CO_2 with or without a chemotactic CXCL12 gradient (60 ng/ml). The percentage of cells migrated to the bottom of the well in comparison to the total number in the well and the insert was quantified using flow cytometry.

For evaluation of T cell migration, T cells were isolated from spleens of naïve C57BL/6 mice, treated with 500 µM TUDCA or vehicle (aqua ad iniectabilia) as indicated and stimulated with Dynabeads® Mouse T-Activator CD3/CD28 (Life Technology – Thermo Fisher Scientific, Germany) according to the manufacturer's instructions for 48 h in 6-well-plates. The migration assay was performed using 24-well flat-bottom transwell migration plates (Costar, USA). Five-hundred thousand T cells were pipetted into the insert (5 µm) of the transwell plate and allowed to migrate for 2.5 h at 37 °C and 5% CO_2 with or without a chemotactic CXCL12 gradient (100 ng/ml). The percentage of cells migrated to the bottom of the well in comparison to the total number in the well and the insert was quantified using flow cytometry.

RNA isolation and quantitative real-time PCR

RNA was isolated using the miRNeasy Mini Kit from Qiagen according to the manufacturer's instructions. RNA concentration and quality were determined by Nanodrop measurement and RNA was stored at -80°C. For reverse transcription, between 200 and 2000 ng of RNA were utilized and reverse transcription was performed with the High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems, Thermo Fisher Scientific, Germany) following the supplied protocol.

For real-time quantitative PCR (qPCR), SybrGreen assays were performed on a Roche Light Cycler 480 with the Light Cycler 480 Sybr Green I Master kit (Roche, Germany) according to the manufacturer's instructions with 45 cycles of amplification. Primer design was performed using the Beacon Designer software. All primer sequences are listed in Tables S4-S5. Analysis was performed using the ΔΔCt method with normalization to *Actb* for murine samples and *GAPDH* for human samples as a reference gene.

T cell differentiation assay

For evaluation of T cell differentiation, cells were isolated from spleens of naïve C57BL/6 mice, treated with 500 µM TUDCA or vehicle (aqua ad iniectabilia) as indicated and stimulated with Dynabeads® Mouse T-Activator CD3/CD28 (Life Technology – Thermo Fisher Scientific, Germany) according to the manufacturer's instructions for 48 h in 6-well-plates. After incubation, T cells were recovered from the Dynabeads® and further processed for flow cytometric analysis.

Western Blot

Small intestinal samples were lysed using T-PER Tissue Protein Extraction Reagent (Thermo Fisher scientific) supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher scientific). The protein concentration was determined using Pierce™ BCA Protein Assay Kit (Life Technologies, Germany). After running and resolving total proteins using 4-12% sodium dodecyl sulphate-polyacrylamide electrophoresis gels (NuPAGE, Invitrogen, Germany), the samples were transferred onto PVDF membranes (Perkin Elmer, Germany). The membrane was incubated with blocking buffer (5% BSA in 1X tris buffer saline containing 0.1% tween-20 (TBS-T) or 5% milk in TBS-T) followed by incubation with primary antibodies diluted in the respective blocking buffer. Anti rabbit IgG-HRP linked antibody was used as secondary antibody. WesternBright Sirius Chemiluminescent Detection Kit (Advansta, USA) was used as the chemoluminescent substrate. Protein signals from the blot were captured using the ChemoCam Imager 3.2 (Intas Science Imaging Instruments GmbH, Germany) and quantified using LabImage 1D software. All antibodies can be obtained from Table S6.

16s ribosomal RNA gene sequencing

Variable (V) region 3 and 4 amplicons of 16S rRNA gene were sequenced following 16S sequencing library protocol by Illumina. 16S Amplicon PCR Forward Primer 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 16S Amplicon PCR Reverse Primer 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATCC were chosen as specific primers for the regions of interest of the 16 S rRNA gene (V3 and V4). The Amplicon Polymerase chain reaction (PCR) was set up with 2.5 µl of 5 ng/µl concentrated DNA, 5 µl 16S Amplicon PCR Forward Primer, 5 µl 16S Amplicon PCR Reverse Primer and 12.5 µl 2x KAPA HiFi HotStart ReadyMix (final volume: 25 µl). PCR products were purified using magnetic beads (Beckman Coulter, Agencourt AMPure XP-Kit). The Index PCR was set up with 5 µl PCR product, 5 µl Nextera Index

Primer 1,1.5 µl Nextera Index Primer 2 (different combination of primers for every sample), 25 µl 2x KAPA HiFi HotStart ReadyMix and 10 µl nuclease-free water (final volume: 50 µl). After a second PCR clean up, the size of the PCR product was visualized by gel electrophoresis. In addition, loading on a Tape Station using the D1000 High Sensitivity Reagents from Agilent Technologies allowed quantification of the PCR product and preparation of final library by equimolar pooling of 96 samples. Library was denatured with NaOH, diluted, mixed with 5% PhiX control and finally loaded on MiSeq for high-throughput sequencing (2 x 300 cycle V3 kit).

16S rRNA data analysis was performed to describe the diversity of bacterial clades in the sample and to provide a rough estimate of their relative abundance. The preprocessing of raw reads included the removal of low-quality bases, ambiguous bases and adapter sequences, the stitching together of paired reads and the detection of chimeric reads. The stitching was done using the program FLASH (Fast Length Adjustment of Short reads) as a software tool to merge paired-end reads from next-generation sequencing experiments [12] (https://ccb.jhu.edu/software/FLASH/). The removal of low-quality sequences was conducted with the help of FASTQ Quality Filter Tool from the FASTX-Toolkit, http://hannonlab.cshl.edu/fastx_toolkit/. Detection of chimeric reads was done using the tool UCHIME [13], available at http://www.drive5.com/.

An OTU (Operational Taxonomic Units) approach involves clustering the sequences together by similarity into OTUs and selecting a representative sequence for each OTU (e.g. the most abundant sequence). Each OTU is then classified by comparison to a reference database and a taxonomic identity is assigned to each representative sequence. The tools UCLUST and UPARSE were employed at this step [14, 15].

R package phyloseq was used to import, store and analyze phylogenetic sequencing data and estimate diversity [16]. R packages RcolorBrewer, ggplot2, reshape, scales, colorspace, grid and ape were used for visualization.

Statistics

Statistical analysis was performed using the GraphPad Prism Lab Software V7.0 and Microsoft Excel. Comparisons of two groups were performed by two-tailed unpaired Student's t tests. Comparisons of more than two groups were performed by a one-way ANOVA with Dunnett's, Bonferroni- or Sidak's correction for multiple testing as appropriate. When data from independent experiments were to be pooled, we set a control group that was consistent among all experiments and normalized the values of all samples as fold change of the control group in order to compensate for a batch effect. Differences in survival

(Kaplan-Meier survival curves) were evaluated using the Mantel Cox (log-rank) test. The Grubbs' test was utilized to determine outlier values. Data are presented as mean ± SEM if not otherwise indicated. A p value <0.05 was considered to be significant.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. The microarray data are available at GEO (ID GSE124426).

Table S1. Murine FACS antibodies for extracellular antigens

Antibody	Clone	Company
anti-H2kb	AF6-88.5	BD Biosciences
anti-H-2kd	SF1-1.1	Biolegend
anti-CD4	GK1.5 and RM4-5	Biolegend
anti-CD8	53-6.7	eBioscience
anti-CD11b	M1/70	Biolegend
anti-CD11c	N418	Biolegend
anti-CD19	6D5	Biolegend
anti-CD40	3_23	Biolegend
anti-CD44	IM7	Biolegend
anti-CD45	30-F11	Biolegend
anti-CD62L	MEL14	Biolegend
anti-CD80	16-10A1	eBioscience
anti-CD86	GL-1	Biolegend
anti-CD103	2E7	Biolegend
anti-CD107a	1D4B	Biolegend
anti-MHCII (I-A/I-E)	M5/114.15.2	eBioscience
Anti-Ly6C	HK1.4	Biolegend

Table S2. Murine FACS antibodies for intracellular antigens

Antibody	Clone	Company
anti-IL 6	MP5-20F3	eBioscience
anti-IL12	C15.6	Biolegend
anti-Foxp3	FJK-16s	eBioscience
anti-IFN-γ	XMG1.2	eBioscience
anti-TNFα	MP6-XT22	eBioscience

Table S3. Human FACS antibodies for extracellular antigens

Antibody	Clone	Company
anti-HLA-A, B, C	W6/32	Biolegend
anti-HLA-DR	L243	Biolegend

Table S4. Murine primers for SybrGreen qPCR

Gene	Primer/Probe	Sequence
Actb	Forward primer	5’ CTC AGG AGG AGC AAT GAT CTT GAT 3’
	Reverse primer	5’ TAC CAC CAT GTA CCC AGG CA 3’
Chga	Forward primer	5’ TGA ATG TCA GCA CAG ATT 3’
	Reverse primer	5‘ AAG GTC AGA AGT TAT TGC 3‘
Ciita	Forward primer	5’ CTC TCC AGT GTC CTA ATC TAC 3’
	Reverse primer	5’ GAT ACT GAG GCT GCT TGA 3’
Defa1	Forward primer	5’ CTT GTC CTG CTT GGC TTC C 3’
	Reverse primer	5’ TTC TCC TGG CTG CTC CTC 3’
Defa4	Forward primer	5’ GGC TGT GTC TAT CTC CTT 3’
	Reverse primer	5’ TGG TTG TCA TAT CTT TGT CAT 3’
Gpbar1	Forward primer	5’ TGG AAC TCT GTT ATC GCT 3’
	Reverse primer	5’ AAT GCT GCA TTG GCT ACT 3’
Hspa5	Forward primer	5' TCA TCG GAC GCA CTT GGA A3'
	Reverse primer	5' CAA CCA CCT TGA ATG GCA AGA 3'
Lgr5	Forward primer	5’ CGA GTC TGC TGT CCA TTA 3’
	Reverse primer	5’ AAG GCA AGT CAT AGG CTA T 3’
Muc2	Forward primer	5‘ GTG AAG ACC GAG ATT GTG 3‘
	Reverse primer	5’ TGG CAC TTG TTG GAA TAC 3’
Nr1h4	Forward primer	5’ CTG GCT CCT TGT CCT CCT 3’
	Reverse primer	5’ GGG AAA GCA GTT CTA TCT AGT CT 3’
Phlda1	Forward primer	5‘ TCG GAA AGT GGG AAG AGT 3‘
	Reverse primer	5’ GTC GGG TGA AGT CCA AAC 3’
Tap1	Forward primer	5’ TTC TGG TTG TCT TGA TTC T 3’
	Reverse primer	5’ GGA ACT GTC TTA TCC TGA A 3’
Tap2	Forward primer	5‘ GGC TAG GGT CAG GAT AAA GA 3‘
	Reverse primer	5’ ACC ACA AAC CAC CCA CTC 3’
Tapbp	Forward primer	5’ AAG TCA CAG TAA AGA AGT 3’

	Reverse primer	5‘ GGA GAA GAA GAG AAG AAG 3‘
Tapbpl	Forward primer	5’ AAG CAC ATT GGT TCA TCA 3’
	Reverse primer	5’ AGA GGT AGG TTC AGT GTA G 3’
Sec61a1	Forward primer	5' CTA TTT CCA GGG CTT CCG AGT 3'
	Reverse primer	5' AGG TGT TGT ACT GGC CTC GGT 3'
Xbp1 u	Forward primer	5' GAC AGA GAG TCA AAC TAA CGT GG 3'
	Reverse primer	5' GTA CAG CAG GAC AGA AGG T 3'
Xbp1 s	Forward primer	5' AAG AAC ACG CTT GGG AAT GG 3'
	Reverse primer	5' CTT TTT TGC ACC TGC TGC GGA C 3'

Table S5. Human primers for SybrGreen qPCR

Gene	Primer/Probe	Sequence
TAP1	Forward primer	5’ ACT TGC CTT GTT CCG AGA G 3’
	Reverse primer	5‘ AGG CGG TAG GGT GAC TTC 3‘
TAP2	Forward primer	5’ AAT GAA CGA ATA TGT GGA A 3’
	Reverse primer	5’ TGA TAC GAC TTT GGG ATA 3’
GAPDH	Forward primer	5‘ CTCCTCCACCTTTGACGCTG 3‘
	Reverse primer	5’ ACCACCCTGTTGCTGTAGCC 3’

Table S6. Murine Western Blot antibodies

Antibody	Cat. Number	Company
Anti-mouse β Aktin	4970	Cell Signaling
Anti-mouse Tap1	12341	CellSignaling
Anti-rabbit IgG HRP-linked	7074	CellSignaling

Supplementary References

1. Tsukamoto H, Chernogorova P, Ayata K, et al. Deficiency of CD73/ecto-5'-nucleotidase in mice enhances acute graft-versus-host disease. Blood. 2012;119(19):4554-4564.
2. Sato T, Clevers H. Primary mouse small intestinal epithelial cell cultures. Methods in molecular biology. 2013;945(319-328.
3. Vidal K, Grosjean I, evillard JP, Gespach C, Kaiserlian D. Immortalization of mouse intestinal epithelial cells by the SV40-large T gene. Phenotypic and immune characterization of the MODE-K cell line. J Immunol Methods. 1993;166(1):63-73.
4. Kaplan DH, Anderson BE, McNiff JM, Jain D, Shlomchik MJ, Shlomchik WD. Target antigens determine graft-versus-host disease phenotype. Journal of immunology. 2004;173(9):5467-5475.
5. Hulsdunker J, Zeiser R. In Vivo Myeloperoxidase Imaging and Flow Cytometry Analysis of Intestinal Myeloid Cells. Methods Mol Biol. 2016;1422(161-167.
6. Schwab L, Goroncy L, Palaniyandi S, et al. Neutrophil granulocytes recruited upon translocation of intestinal bacteria enhance graft-versus-host disease via tissue damage. Nature medicine. 2014;20(6):648-654.
7. Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. Bioinformatics. 2010;26(19):2363-2367.
8. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics. 2012;28(6):882-883.
9. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
10. Gene Ontology C. Gene Ontology Consortium: going forward. Nucleic Acids Res. 2015;43(Database issue):D1049-1056.
11. Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics. 2009;10(161.
12. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957-2963.
13. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194-2200.
14. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26(19):2460-2461.
15. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature methods. 2013;10(10):996-998.
16. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one. 2013;8(4):e61217.