Supplemental Methods

Urinary Indoxyl Sulfate Analysis Urine samples from mice were diluted 1:10 in water and indoxyl sulfate (Indican) measured by spectrophotometric Indican assay (SIGMA).

Colonization with *E. coli* strains Construction of *E. coli* strains (K12Str^{R}Nal^{R} or K12ΔTnaAStr^{R}Nal$^{R)}$ is previously described [1,2]. Strains were introduced by a single oral gavage (450 µL of overnight culture per mouse, pelleted and resuspended in 200 µL sterile PBS) administered 24 hours after initiation of streptomycin treatment (5g/L) and mice remained on streptomycin for the duration of the experiment, including post-BMT. Colonization was assessed by serial dilution plating of fecal samples on MacConkey Agar containing streptomycin and nalidixic acid (100µg/ml, 50µg/ml). Colonies were checked for indole production by Kovacs reagent following overnight growth in Luria Broth.

Administration of ICA Indole-3-Carboxaldehyde (Sigma) was dissolved in DMSO and delivered by oral gavage at a dose of either 100 or 150 mg/kg/day in a final mixture of DMSO (20%), PEG400 (40%), Citric Acid (2%). ICA treatment was started 24 hours prior to irradiation / myeloablative conditioning and continued throughout the experiment. In some experiments, ICA administration was stopped when the last TCD-BM+T cell (GvHD) mouse receiving vehicle succumbed (day 38-50) and survival of the remaining mice was monitored in the absence of ICA.

Bacterial Translocation Assay Mesenteric lymph nodes were removed with sterile instruments, weighed and immediately homogenized in 0.25 ml sterile PBS. Homogenates were plated (neat, 100µl), in duplicate, on Blood Agar plates to determine CFU/g tissue.

Chemotherapy Busulfan was administered IP at 20 mg/kg/day for 4 days (80 mg/kg total), followed by Cyclophosphamide at 100 mg/kg/day for 2 days (200 mg/kg total).

Histology Colons and livers from BMT mice were assessed on day 21 post-transplant. All tissues were formalin fixed, paraffin embedded and stained with H&E by standard procedures (Winship Research Pathology Core, Emory University). Samples were scored in a blinded fashion. For colon, a 1 cm long piece of tissue harvested form the distal colon (starting at the rectum), cut longitudinally, was scored for crypt loss, apoptotic cells per crypt, and degree of inflammation. Crypt loss score: Degree of crypt loss (0=no crypt loss; 1=mild crypt loss, most crypts still visible; 2=medium severity, fewer crypts visible in large areas; 3=total crypt loss) multiplied by percent of tissue at each score. Inflammation score: Degree of inflammation (0=no evidence of inflammatory infiltrate; 1=very low level of cells infiltrating the tissue; 2=thickening of lamina propria, and clear infiltrating lymphocytes into epithelial tissue; 3=thickening of lamina propria and large boluses of inflammatory infiltrates that correspond with areas a crypt loss) multiplied by the percent of tissue

at each score. Apoptotic nuclei were identified by morphology and enumerated per crypt.

Livers were assessed for the average degree of immune cell aggregates surrounding portal triads (0=no infiltrates, 1=mild, 2=medium, 3=severe). Intestines from lethally irradiated mice were scored for villus atrophy (villus height) on day 3 post-irradiation and for the size (height x width) of surviving, regenerating crypts (regenerating foci) [3] on day 4 post-irradiation.

Transepithelial electrical resistance (TER) Caco-2 cells , treated with ICA (100 µm) and/or TNF α (50 ng/ml, Peprotech) for 48 hrs. Caco-2s were grown on permeable supports (Transwell, Corning ,Sigma-Aldrich group. 0.4µm pore size), and monolayers were monitored for electrical resistance using an epithelial volt-ohmmeter (EVOM2/EndOhm; World Precision Instruments). Caco-2 cells were treated with ICA (100 µm) and/or TNFα (50 ng/ml, Peprotech) for 48 hrs.

Diarrhea Severity Stool samples from irradiated mice were assessed on day 3 post-irradiation and scored for stool consistency (0=normal stool, 1 = semi-soft, 2 = very soft, 3 = runny) and for presence of blood in the stool using Hemoccult Sensa (Beckman Coulter) diagnostic test (0 = no color change, 1 = light blue, 2 = medium blue, 3 = dark blue). Stool consistency and hemoccult scores for each mouse were combined for a total diarrhea severity score. Scale = 0 (normal) to 6 (severe diarrhea).

Cytokine Analysis Intestinal tissues and plasma were collected on d21 post-transplant, snap frozen and stored at -80˚ prior to processing. Tissue protein lysates were obtained by homogenizing tissue in T-PER lysis buffer (Pierce/Thermo Fisher) according to manufacturer's protocol. Protein concentrations were determined by BCA protein assay (Pierce/Thermo Fisher) and adjusted to 1mg/ml with lysis buffer. Luminex Magnetic bead assay was performed by the Human Immune Monitoring Center (HIMC) at Stanford University. Mouse 38 plex kits (eBiosciences/Affymetrix) were used according to manufacturer's recommendations. Each sample was measured in duplicate. Plates were read using a Luminex 200 instrument with a lower bound of 50 beads per sample per cytokine. Custom assay Control beads by Radix Biosolutions were added to all wells.

T Cell Flow Cytometry and Cytokine Analyses: Spleens from recipient mice were harvested on d21 post-transplant. Splenocytes were stained using fluorescent antibodies to distinguish T-cells derived from the original mature T-cells in the graft (CD45.1), CD4, CD8, CD69 and PD1. For intracellular cytokine analyses, splenocytes were cultured in complete RPMI 1640 supplemented with 10% FBS, 100 U/mL of penicillin, 100µg/mL of streptomycin, and 50µM of 2-mercaptoethanol, nonessential amino acids, glutamine,

HEPES, and sodium pyruvate (complete media) for 4 hours at 37 °C in the presence of BD Leukocyte Activation Cocktail. Intracellular analysis of T cells was performed using the BD Bioscience Cytofix/Cytoperm kit and anti-mouse IFNy, IL4, and IL-17 antibodies.

T Cell Proliferation Assay – CFSE The proliferation of donor T cells in recipient spleen was analyzed by CFSE dilution as described previously [4,5]. In brief, donor T cells were stained with CFSE before transfer, then recipient spleens were harvested 3 days later and cells suspensions prepared. Proliferation of donor T cells was determined by flow cytometric analysis of CFSE dilution profiles gated on donor T cell populations using FlowJo software (TreeStar). The replication index for gated CD4 or CD8 T cells was calculated by the software, and represents the fold expansion of the responding cell subset [6].

Mixed lymphocyte reactions: In a mixed lymphocyte reaction, 200,000 responder splenocytes were co-cultured with 200,000 stimulator splenocytes in the presence of IL-2 (1U/mL), in triplicate, in complete media as described above. At 48 hours post-MLR, intra-nuclear T cell analysis was performed using the eBiosciences Fixation/Permeabilization kit and anti-mouse Ki67.

***In vivo* bioluminescent imaging** Bioluminescent imaging was performed according to standard procedures. In brief, anesthetized mice were injected intraperitoneally with luciferin (10 µg/g body weight). Ten minutes later, mice were imaged using an IVIS100 charge-coupled device imaging system (PerkinElmer). Imaging data were analyzed with Living Image Version 3.2 software (PerkinElmer).

Whole-Transcript Expression Analysis (Gene ST Array) RNA was isolated from whole intestinal tissue using TRIzol (Invitrogen) and RNEasy Plus Mini kit (Qiagen). Sample quality profiles were assessed on the Agilent 2100 by RNA 6000 Nano assay. Microarray analysis was performed by the Emory Integrated Genomics Core as follows: RNA samples were reverse transcribed using Ambion WT Expression Kit and cDNA was processed and labelled using Affymetrix GeneChip WT Terminal Labeling and Hybridization kit, all according to manufacturer protocols. Labeled cDNA was hybridized to whole transcriptome Gene ST GeneChip microarrays (Mouse Gene ST 2.0 (Affymetrix)) at 45 degrees C for 16-18 hours. Hybridized microarrays were washed and stained on an Affymetrix GeneChip 450 fluidics station using the appropriate chip dependent fluidics script. Arrays were scanned and intensity data extracted using an Affymetrix 7G scanner and the Command Console software suite. Preliminary data analysis was performed by the Emory Integrated Computing Core using Partek Genome Studio (http://www.partek.com). Affymetrix array data were RMA normalized and grouped according to treatment: TCD-BM+VEH, TCD-BM+ICA, TCD-BM+T+VEH, and TCD-BM+T+ICA. PCA analysis and unsupervised clustering were used to verify sample grouping. 1-way and 2-way ANOVA tests were performed

to determine significant differential expression of genes among groups and identify common genes. Differential expression filtering was performed using an unadjusted p-value < 0.05 and absolute fold-change greater than 1.5. GEO Accession number GSE111512

Hierarchical clustering, PCA and Gene Ontology Hierarchical clustering and Principle Component Analysis (PCA) of genes determined to be significantly up- or down-regulated between various experimental conditions were conducted using ClustVis (https://biit.cs.ut.ee/clustvis/) [7] and were based on Z-score normalization of log2 signal intensity values for each gene across all samples. A Euclidian distance metric was used to assess clustering and generate heatmaps. Gene Ontology was assessed using AmiGO (http://amigo.geneontology.org/amigo) [8].

Type I interferon Stimulated Genes (ISGs) were identified using the Interferome database: (http://www.interferome.org/interferome/home.jspx) [9].

Statistics Significance was assessed using Graph Pad software and the following tests: log rank Mantel-Cox (Survival), Mann-Whitney non-parametric, Kruskal-Wallace ANOVA with Tukey's post-test, or two-way ANOVA with Sidak's post-test. All analyses were two-tailed. A p value < 0.05 was considered significant.

1. Leatham MP, Stevenson SJ, Gauger EJ, et al. Mouse intestine selects nonmotile flhDC mutants of Escherichia coli MG1655 with increased colonizing ability and better utilization of carbon sources. *Infect Immun*. 2005;73(12):8039-8049.
2. Sonowal R, Swimm A, Sahoo A, et al. Indoles from commensal bacteria extend healthspan. *Proc Natl Acad Sci U S A*. 2017;114(36):E7506-E7515.
3. Withers HR, Elkind MM. Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1970;17(3):261-267.
4. Giver CR, Montes RO, Mittelstaedt S, et al. Ex vivo fludarabine exposure inhibits graft-versus-host activity of allogeneic T cells while preserving graft-versus-leukemia effects. *Biol Blood Marrow Transplant*. 2003;9(10):616-632.
5. Li JM, Petersen CT, Li JX, et al. Modulation of Immune Checkpoints and Graft-versus-Leukemia in Allogeneic Transplants by Antagonizing Vasoactive Intestinal Peptide Signaling. *Cancer Res*. 2016;76(23):6802-6815.
6. Roederer M. Interpretation of cellular proliferation data: avoid the panglossian. *Cytometry A*. 2011;79(2):95-101.
7. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic Acids Res*. 2015;43(W1):W566-570.
8. Carbon S, Ireland A, Mungall CJ, et al. AmiGO: online access to ontology and annotation data. *Bioinformatics*. 2009;25(2):288-289.
9. Rusinova I, Forster S, Yu S, et al. Interferome v2.0: an updated database of annotated interferon-regulated genes. *Nucleic Acids Res*. 2013;41(Database issue):D1040-1046.

Supplemental Data

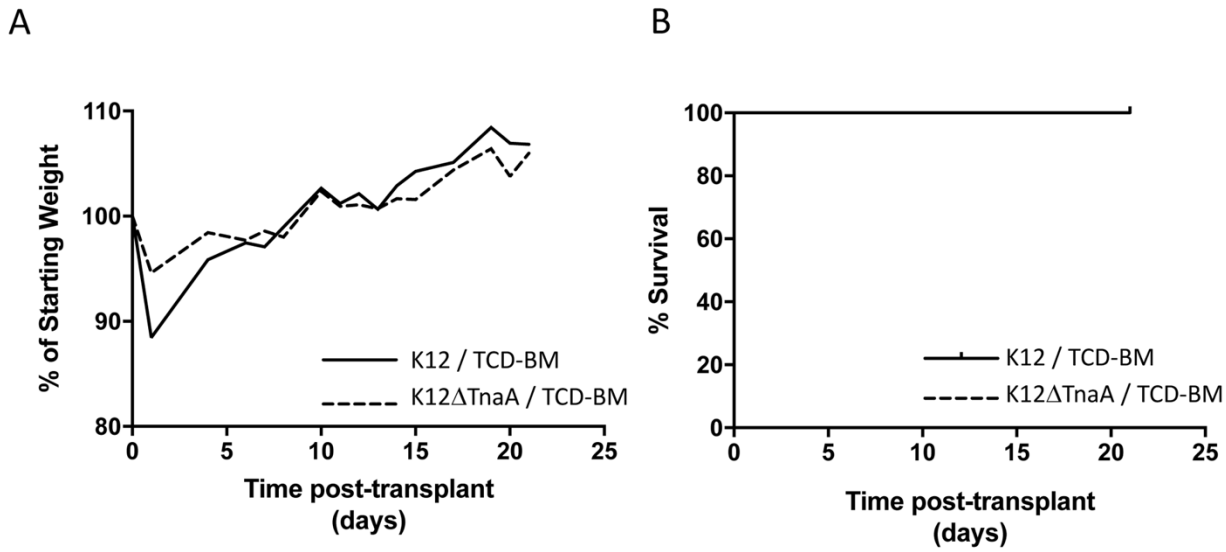

Figure S1.

Microbiota-derived indoles do not affect morbidity or mortality in control mice transplanted with T cell depleted BM only. In **A** and **B**, B10.BR recipient mice were treated with streptomycin and then colonized with streptomycin and nalidixic acid resistant K12 or K12ΔTnaA one week prior to lethal irradiation and allo-BMT with TCD-BM from C57Bl/6 donor mice. **A.** Weight loss (n = 10 per condition) **B**. Kaplan-Meier Survival Curve (n = 10 per condition). All control animals receiving TCD-BM only were sacrificed for analysis on day 21 post-transplant, but had 100% survival up until that point. Statistics: Mantel Cox Log-rank (Survival curve) Results were not significant.

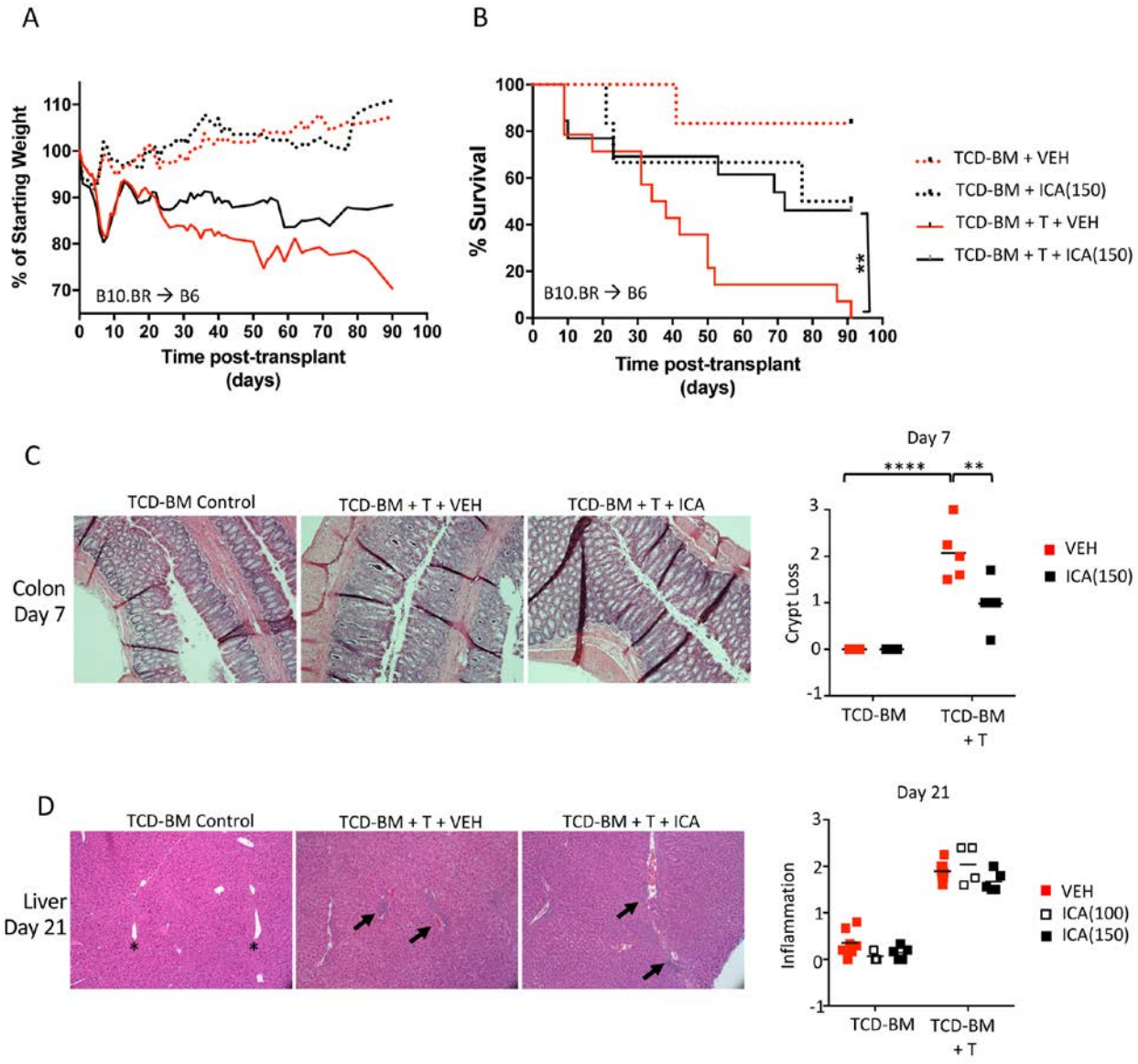

Figure S2.

A and B. ICA reduces GvHD-related morbidity and mortality in the B10.BR → C56Bl/6 transplant model. C57Bl/6 recipients were lethally irradiated and subjected to allo-BMT with T cell depleted BM alone or in combination with purified T cells (TCD-BM + T) from B10.BR donor mice to induce GvHD. Mice received daily oral gavage with 150mg/kg ICA or VEH starting one day prior to irradiation. A. Weight loss. B. Kaplan-Meier survival curve (n = 6 for TCD-BM groups and n=14 for TCD-BM + T groups). **C. ICA mitigates gut damage in early stages of GvHD.**

Left panel: H&E staining of colon sections in animals receiving 150 mg/kg ICA or VEH. (images are 20X magnification) Right panel: Quantitation of crypt loss, n=5 per condition. **D. ICA does not significantly affect hepatic histopathology at day 21 post-transplant.** Left panel: H&E staining of liver sections in animals receiving 100mg/kg ICA or VEH. All images are 200X magnification. Asterisks in liver panels indicate normal portal areas and arrows show immune cell infiltration (darker purple) of periportal areas. Right panel**:** Quantitation of degree of cellular infiltrates in portal areas. Statistics: Mantel Cox Log-rank (Survival curve), 2-way ANOVA. ****p < 0.0001, ***p = 0.0001 to 0.001, **p = 0.001 to 0.01.

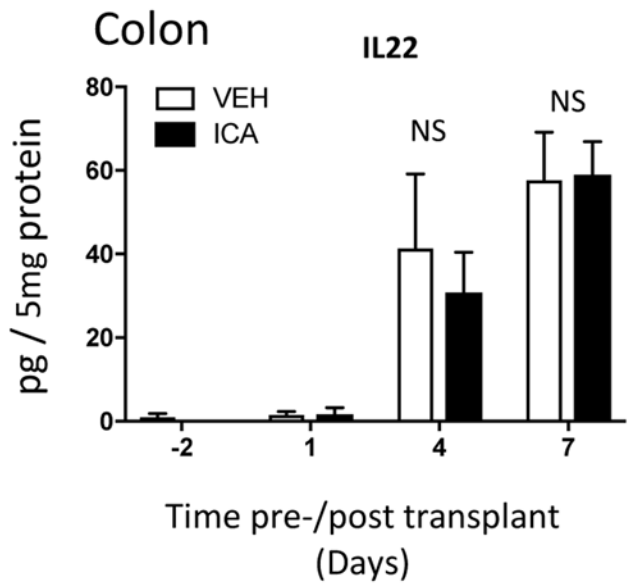

Figure S3.

ICA does not alter levels of IL22 in the gut in the early stages of GvHD.

Lethally irradiated B10.BR recipients were transplanted with TCD-BM +T cells from C57Bl/6 mice to induce GvHD. Mice received daily oral gavage of 150mg/kg ICA or vehicle. Samples were collected day 1, 4 and 7 post-transplant. n=5 per condition. Statistics: Kruskal-Wallis ANOVA. Error bars represent standard deviations. NS = not significant.

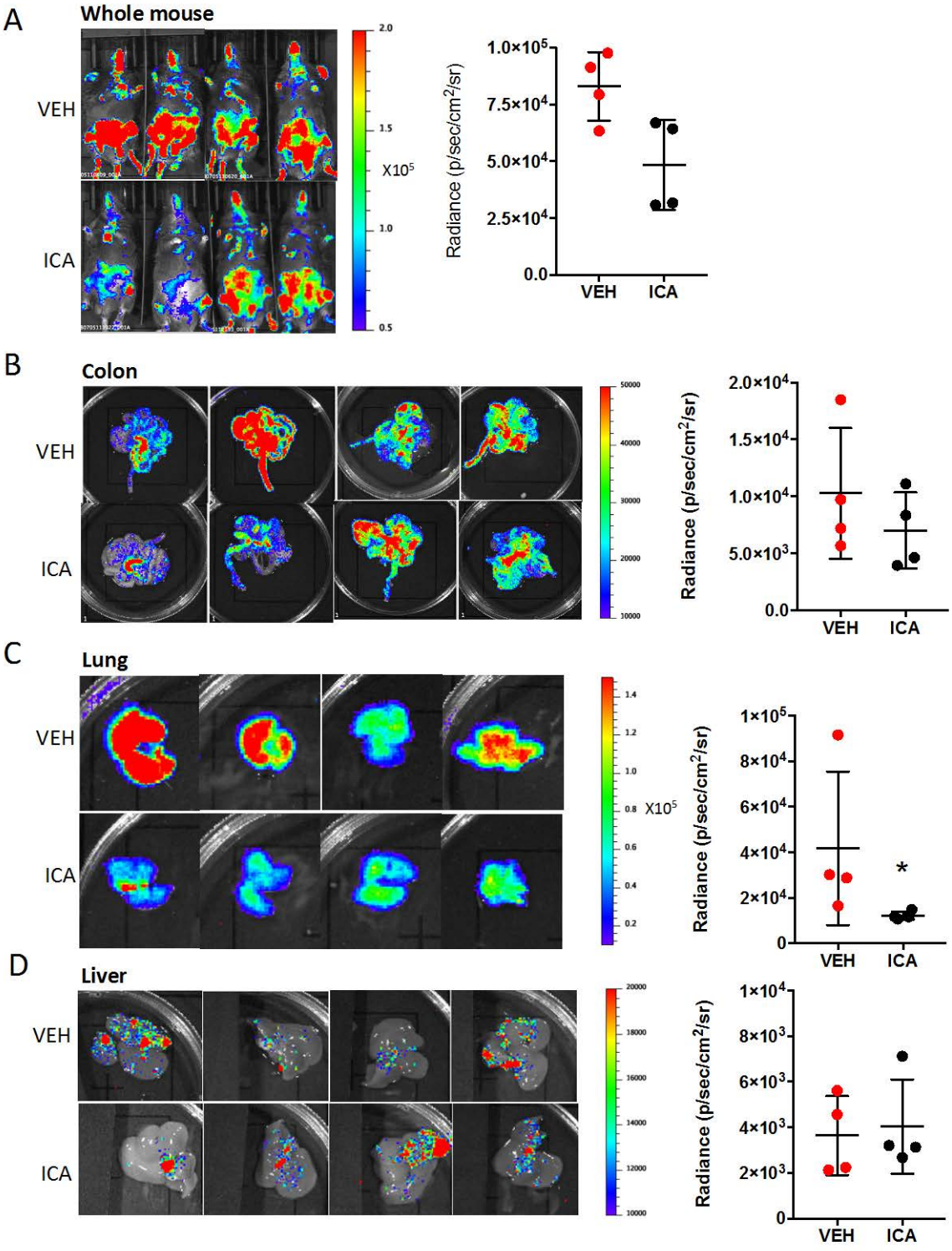
A
Whole mouse
VEH
ICA
2.0
1.5
X10^5
1.0
0.5
Radiance (p/sec/cm²/sr)
1.0×10^5
7.5×10^4
5.0×10^4
2.5×10^4
0.0
VEH
ICA
B
Colon
VEH
ICA
50000
40000
30000
20000
10000
Radiance (p/sec/cm²/sr)
2.0×10^4
1.5×10^4
1.0×10^4
5.0×10^3
0.0
VEH
ICA
C
Lung
VEH
ICA
1.4
1.2
1.0
0.8
X10^5
0.6
0.4
0.2
Radiance (p/sec/cm²/sr)
1×10^5
8×10^4
6×10^4
4×10^4
2×10^4
0
*
VEH
ICA
D
Liver
VEH
ICA
20000
18000
16000
14000
12000
10000
Radiance (p/sec/cm²/sr)
1×10^4
8×10^3
6×10^3
4×10^3
2×10^3
0
VEH
ICA

Figure S4

BLI of recipient mice (C56Bl/6→B10.BR) at day 21 post-transplant. A-C: Lethally irradiated B10.BR recipients were transplanted with TCD-BM + luc^+ T cells from C57Bl/6 mice to induce GvHD. Mice received daily oral gavage of 150mg/kg ICA or vehicle and were imaged on day 21 post-transplant. **A**. BLI (left) and radiance (right) of whole mouse. **B**. BLI (left) and radiance (right) of colon. **C**. BLI (left) and radiance (right) of lung. n = 4 per condition. **D**. BLI (left) and radiance (right) of liver. n = 4 per condition. Statistics: Mann-Whitney rank sum, * p = 0.01 to 0.05.

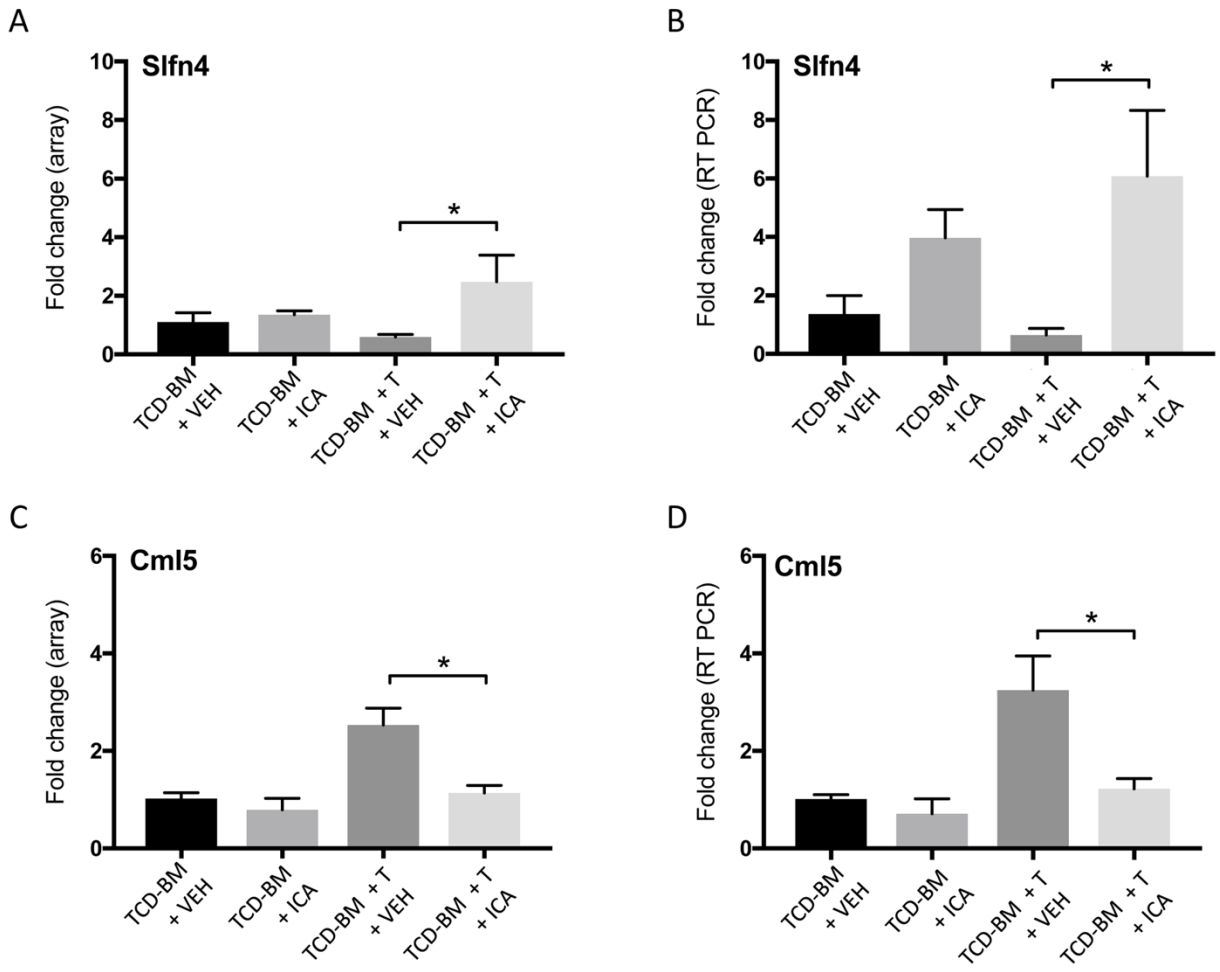

Figure S5

Validation of microarray gene expression changes by qRT-PCR.

A-D Changes in *Slfn4* and *Cml5* gene expression (fold change normalized to BM + VEH controls) in intestinal samples. **A** and **C** microarray, or qRT-PCR, **B** and **D**. **A-D**. TCD-BM n = 4 per condition, TCD-BM + T, n = 5 per condition. Statistics: Kruskal-Wallis ANOVA. * p = 0.01 to 0.05.

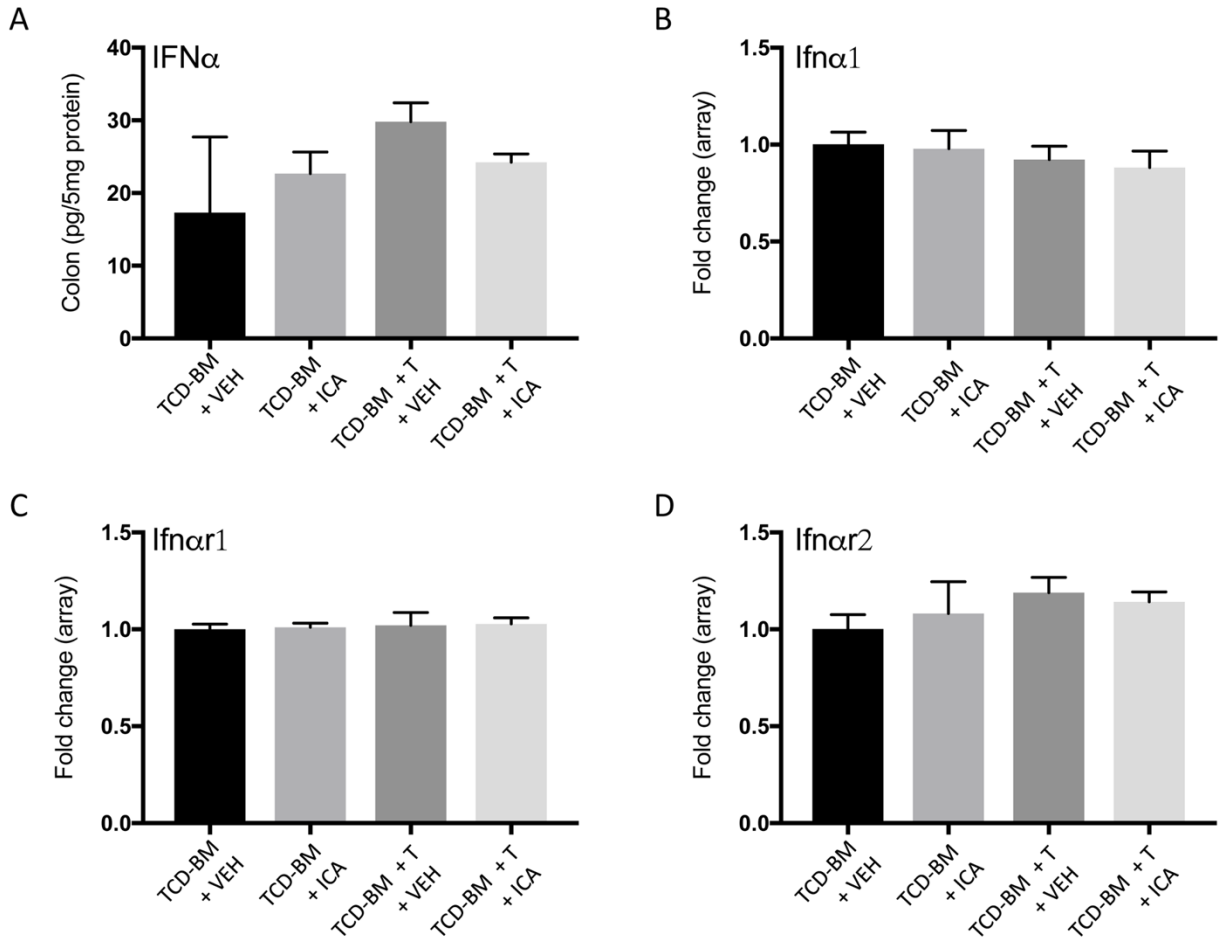

Figure S6

No changes in IFN1 cytokines or receptors were evident with GvHD or ICA

A. IFNα in colon homogenates (d21). **B - D**. Gene expression changes (d21) (fold change compared to TCD-BM + VEH controls) of IFN1 cytokines and receptors in intestinal samples as determined by microarray. **B.** Ifnα1 **C.** Ifnαr1 **D.** Ifnαr2.

A-D. TCD-BM n = 4 per condition, TCD-BM + T n = 5 per condition. Statistics: Kruskal-Wallis ANOVA. No statistical significance between experimental groups.

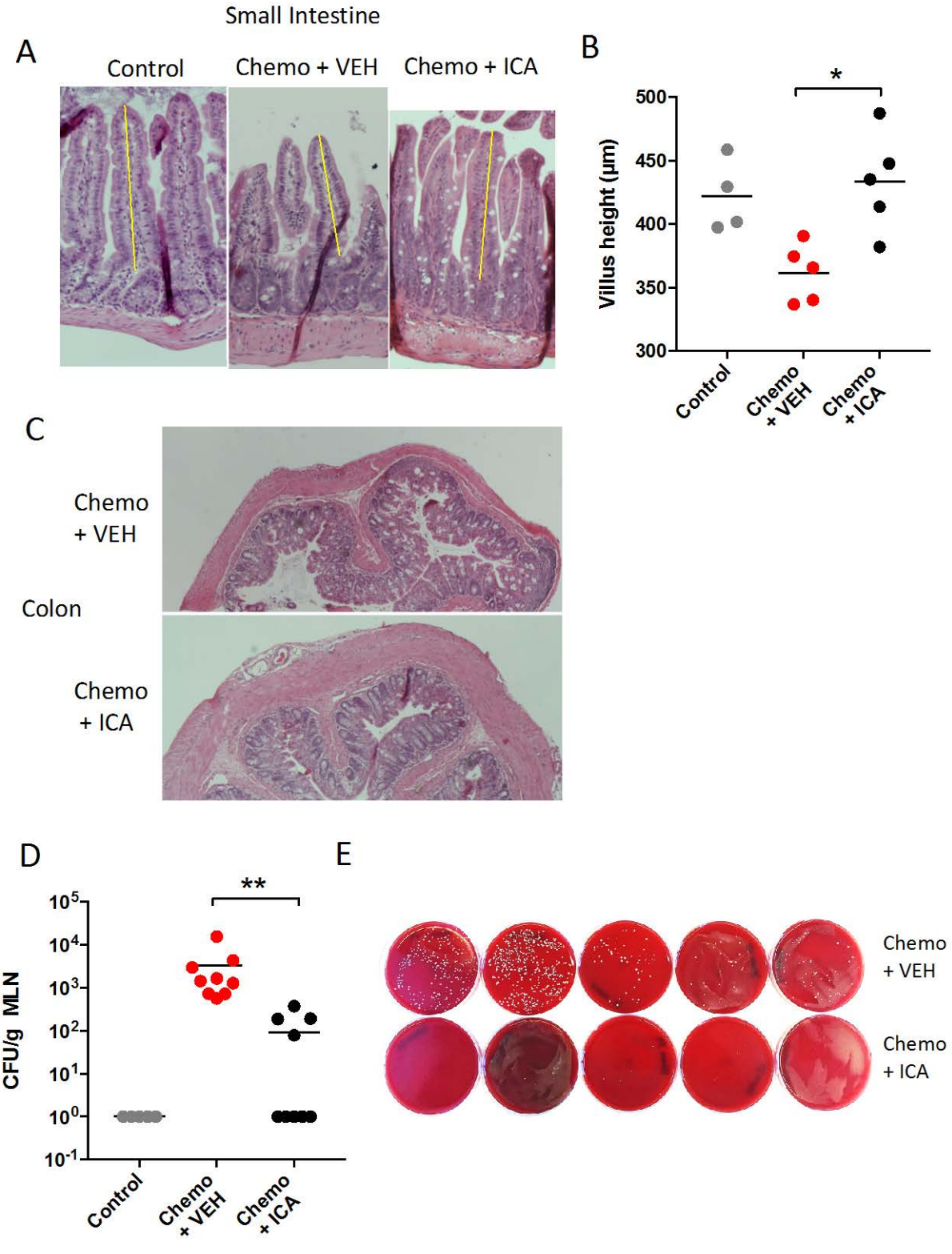
Small Intestine
A
Control
Chemo + VEH
Chemo + ICA
B
Villus height (µm)
500
450
400
350
300
*
Control
Chemo + VEH
Chemo + ICA
C
Chemo + VEH
Colon
Chemo + ICA
D
CFU/g MLN
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
**
Control
Chemo + VEH
Chemo + ICA
E
Chemo + VEH
Chemo + ICA

Figure S7

ICA reduces intestinal damage following Chemotherapy. In **A-D**, Mice were administered a myeloablative dose of Busulfan and Cyclophosphamide (80 mg/kg and 200 mg/kg total) without subsequent BMT. Control animals received PBS. Mice received daily oral gavage with 150mg/kg ICA or vehicle (VEH) starting one day prior to initiation of chemotherapy. Samples were collected 3 days post-chemotherapy. **A**. H&E staining of distal ileum on day 3 post-chemotherapy showing rescue of villus blunting by ICA. Typical villus height measurements are indicated by yellow lines. Images are 200X. **B**. Villus height (distal ileum, day 3 post-irradiation) measured in H&E stained sections (n = 5). **C.** Representative images of H&E stained cross sections of colon showing reduced crypt loss, inflammation and damage following chemotherapy in animals treated with ICA. Images are 10X. **D**. Quantitation of CFU per gram mesenteric lymph node (day 3 post-chemotherapy) (n = 9). **E.** Image of representative result of dilution plating of MLN homogenates to determine CFU/g MLN. Representative data from 2 experiments. Statistics: Kruskal-Wallis ANOVA **p = 0.001 to 0.01, *p = 0.01 to 0.05.