

SUPPLEMENTARY METHODS

Materials and methods

Diarrhoea scores

Diarrhoea score ranged from 0 to 3, 0 being no and 3 being frank diarrhoea¹⁹; rectal bleeding ranged from 0 to 3, 0 no bleeding and 3 frank rectal bleeding. For DSS induced colitis in *Hprt*^{-/-} mice the composite disease activity index (DAI) score was used. This comprised diarrhoea, rectal bleeding and body weight percent loss subscores (0 <1% loss; 1= 1-5%loss; 2= 5-15% loss; 3 >15% loss).

RNA extraction, cDNA synthesis and gene expression

An equal amount of extracted and purified RNA from both proximal and distal colon (PC and DC) using TRIzol® (Invitrogen) and RNAeasy mini kit (QIAGEN), was reverse transcribed to cDNA by using a iScript cDNA synthesis kit (Bio-Rad Laboratories). Relative gene expression to housekeeping genes (*β-actin* or *Gapdh*) was determined by using RT-PCR (Supp. Table 1).

Sample collection and microbiome analysis

Caecal content (CC), caecal mucosa (CM) of WT, *Hprt*^{-/-} and *Winnie* mice were collected at day 14 post-TG administration at doses of 0, 0.5 mg/kg or 2.5 mg/kg (*Winnie* only).

DNA was extracted from CM and CC at day 14, using our previously described method^{20 21}. DNA extracts were used as the template for PCR-based amplification of the bacterial V1-V3 region of the 16S rRNA gene using a barcoded pyrotagging approach. The sequences obtained were processed using QIIME²² and MOTHUR²³.

The samples-by-OTUs matrix was sub-sampled 100 times to a common depth (the lower quartile of the dataset's coverage) and the averaged matrix was kept, and values less than 1.01 were recoded as 0²⁴. Sample specific data sets having fewer sequences than the chosen threshold were analysed in their entirety. The resulting normalized dataset was used, in conjunction with the distance matrix describing the phylogenetic relationships between all OTU representative sequences, to perform double principal coordinate analyses (DPCOA) which ordines the samples based on the phylogenetic relationships and abundances of the species present in their resident community. Possible differences between groups of samples (e.g. day 0 vs. day 14, control vs. treatment) were tested by between class analysis^{25 26}. Then, the relative abundances of chosen phylogenetic groups across the samples were recovered, and observed differences between the sample classes were further analysed using a statistical test (Wilcoxon test) with significance set at P=0.05.

FACS analysis

At culling point immune organs were collected and transferred in ice cold media and they were harvested and teased apart into single cell suspension by pressing with plunger of a syringe into a 40 µM sterile cell strainer (BD Falcon). Cells were pre-incubated with 1:200 of anti-CD16/CD32 (Fc block from eBioscience) for 5-10 mins on ice prior to staining. After washing and removing supernatant containing Fc block, cells were resuspended in cocktail mix which comprised the following antibodies: CD11c, MHCII, CD11b, CD3e, CD4, CD69, CD8 and CD19 (BD-Pharmingen, Australia). 7AAD was used in order to exclude debris and necrotic cells and was read in the PerCP-Cy5-5 channel. Flow cytometry analysis was performed on LSRII (BD Biosciences), and data was analyzed using FlowJo software 8.8.6.

TGN measurement

For *in vitro* experiments with bacteria (*Escherichia coli* PC1101, *Enterococcus faecalis* ATCC 19433 and *Bacteroides thetaiotaomicron* AMC0002), TGN in 3 x washed bacterial pellet was measured as TG riboside (TGr) by converting the intracellular TGN to TGr. This was achieved by acid phosphatase (Sigma) incubation of cellular sonicates for 30 minutes at 37°C. TGr was measured by HPLC-UV using an Altima HP C18 column.

In mouse samples, a modified Dervieux method²⁷ was used for measurement of TGN by LC-MS/MS (Shimadzu LC 20 HPLC system coupled to API 3200 tandem mass spectrometer). After tissue homogenization perchloric acid 35% and DTT 100 mg/mL were added and the samples were centrifuged at 3500g at 4°C for 60 s. The supernatant was divided into two aliquots of equal volume; one of these aliquots was boiled at 100°C for 60 minutes. TGN was quantification was performed by subtracting the quantity of TG in the unboiled sample from that of the boiled sample (in which all TGNs are reduced to TG).

Cell cultures

HeLa, HCT116, HT29, HepG2 and RAW cells were obtained from ATCC and grown in DMEM supplemented with GlutaMAX (Life Technologies), 10% fetal calf serum and 20 µg/ml penicillin/streptomycin. HeLa cells stably expressing LC3-GFP have been described previously. Primary fibroblasts isolated from WT and *Hprt*^{-/-} mice were generated from ear punch samples after dissociation with trypsin and cultured in DMEM supplemented with Glutamax and 20% fetal calf serum with antibiotics and passaged up to four times to ensure that they were axenic.

Bulk autophagy assay/Western Blot.

To measure autophagy induction, cell lines were plated at 70% confluence and treated for indicated time with TG 10, 50 and 100 µM with or without the addition of 10 µg/ml of E64D -

Pepstatin A (Sigma) or mock treated with DMSO 0.1%. Following treatment cells were lysed with TNN (Tris-HCl, NaCl, NP40) buffer. Western blotting to demonstrate LC3 lipidation was performed after equalization of protein amounts and SDS-PAGE electrophoresis on a 4-12% NuPAGE BisTris gel (Invitrogen). Following transfer to PVDF membranes (Invitrogen), detection was performed using rabbit anti-LC3 primary (Sigma), mouse anti-Actin (Sigma) and appropriate fluorescent secondary antibodies (LI-COR Biosciences) and imaged on a LICOR fluorescent imaging system.

Antibacterial autophagy assay

S. Typhimurium infections of HeLa cells and gentamycin protection assays were performed as previously described²⁸. HeLa cells were plated in 12-well plates containing 18 mm glass coverslips at a density of 1×10^5 cells per well in DMEM 24 hours before infections. Cells were treated with TG or mock treated with DMSO for 16 hours prior to infection. *S. enterica* serovar Typhimurium SL1344 carrying a DsRed2 expression plasmid was grown overnight in Luria-Bertani broth containing 100 µg/mL carbenicillin at 37°C with aeration and subcultured at a dilution of 1/33 for a further 3 h in Luria-Bertani carbenicillin broth. This culture was further diluted in DMEM 10% serum without antibiotics to yield a multiplicity of infection (MOI) of 100 and added to HeLa LC3-GFP cells. Infections were allowed to proceed for 20 minutes and the cells were washed once in complete medium containing 100 µg/mL gentamicin sulfate and then incubated in fresh high gentamicin medium for an additional 40 minutes. Cells were washed twice in PBS before methanol fixation/permeabilization, nuclear counterstaining with Hoechst 33342 (Invitrogen) and mounting with ProlongGold (Invitrogen). Slides were viewed for counting under wide-field fluorescence illumination with a 60x lens (Olympus). The fraction of LC3-GFP-positive bacteria per cell was assessed in randomly chosen fields with at least 90 cells counted for each condition. Bacteria were scored as within autophagosomes only when a complete and closely conforming LC3-GFP “capsule” was visible.

Bacterial Replication assay

Gentamicin protection replication assays in HeLa cells were performed by using bioluminescent *Salmonella typhimurium* containing Xen33 (Perkin-Elmer). HeLa cells were infected as above in 96 well plates and after 40 minutes cells were washed twice with PBS and then media containing 20 µg/mL gentamicin was added to wells. Plates were read at 12h.

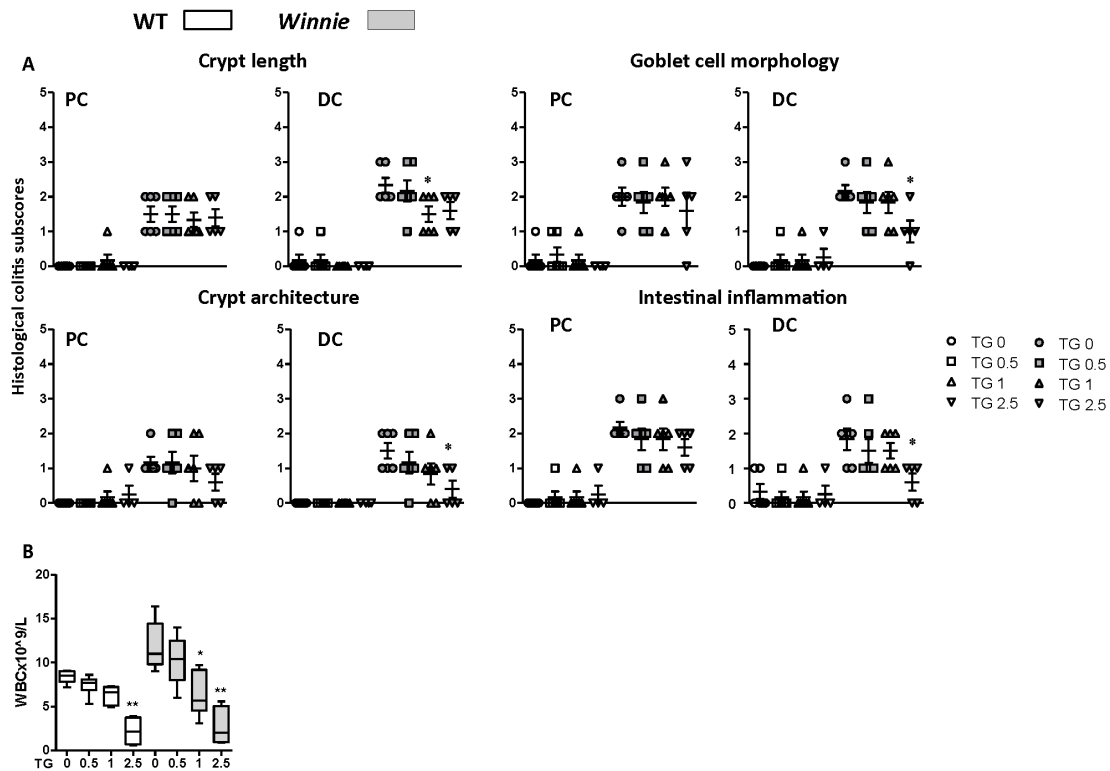
Organoid cultures and treatment

Immediately after sacrifice each intestinal segment was flushed with ice cold PBS. Segments were sliced into 3mm pieces and incubated in ice cold PBS with 8mM EDTA for

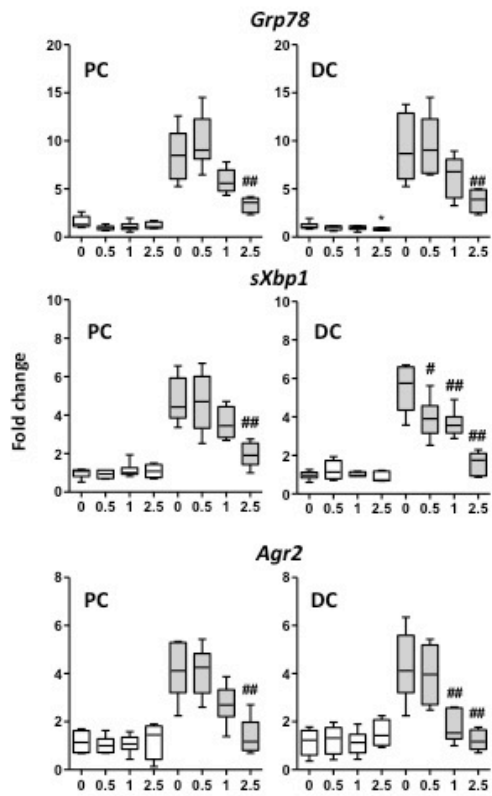
45 minutes on a roller. After vortexing, the supernatant was removed and spun at 500rpm for 5 minutes to capture any dissociated epithelial elements. The fragments were digested in collagenase 2mg/ml (Invitrogen, New York, USA) in DMEM/F12 (with 10% fetal calf serum, 100U/ml penicillin, 0.1ml/ml streptomycin and 2mM L-glutamine, known as washing medium)(Invitrogen), at 37°C until numerous dissociated crypts were identified after gentle pipetting (approximately 5 minutes). The supernatant was removed, added to the previously generated cell pellet, and washed twice in washing solution, spinning for 5 minutes at 200rpm each time. The resulting pellet was resuspended in a small volume of Advanced DMEM/F12 (with 20% fetal calf serum, penicillin, streptomycin and glutamine)(Invitrogen) and mixed with defrosted Matrigel (BD Biosciences, San Jose, USA) in a ratio of approximately 2:1. 15µl per well of the mix was pipetted into a 24 well plate, spread with a pipette tip into a disc, and inverted and incubated at 37°C until solid. 400µl of media was added to each well, made up of a 50:50 mix of Advanced DMEM (with 20% fetal calf serum, penicillin, streptomycin and glutamine) and conditioned media generated by culture of L-WRN cells²⁹, supplemented with 10µM ROCK inhibitor Y-27632 (Tocris Bioscience, Minneapolis, USA), 10µM TGFBR1 inhibitor SB431542 (Tocris Bioscience) and 5µM GSK-3 inhibitor CHIR99021 (Sigma-Aldrich, St. Louis, USA), and changed second daily. L-WRN cells were the kind gift of Prof. T. Stappenbeck of Washington University, St. Louis, USA. Differentiation media used once spheroid culture was established consisted of 5% of conditioned media as above, with 5µM DAPT (Sigma) and 10µM ROCK inhibitor Y-27632 (Tocris Bioscience) without SB431542 or CHIR99021. After 3 days of differentiation DMSO 0.1% and TG 50 µM were added for 16h.

MTS cell viability assay

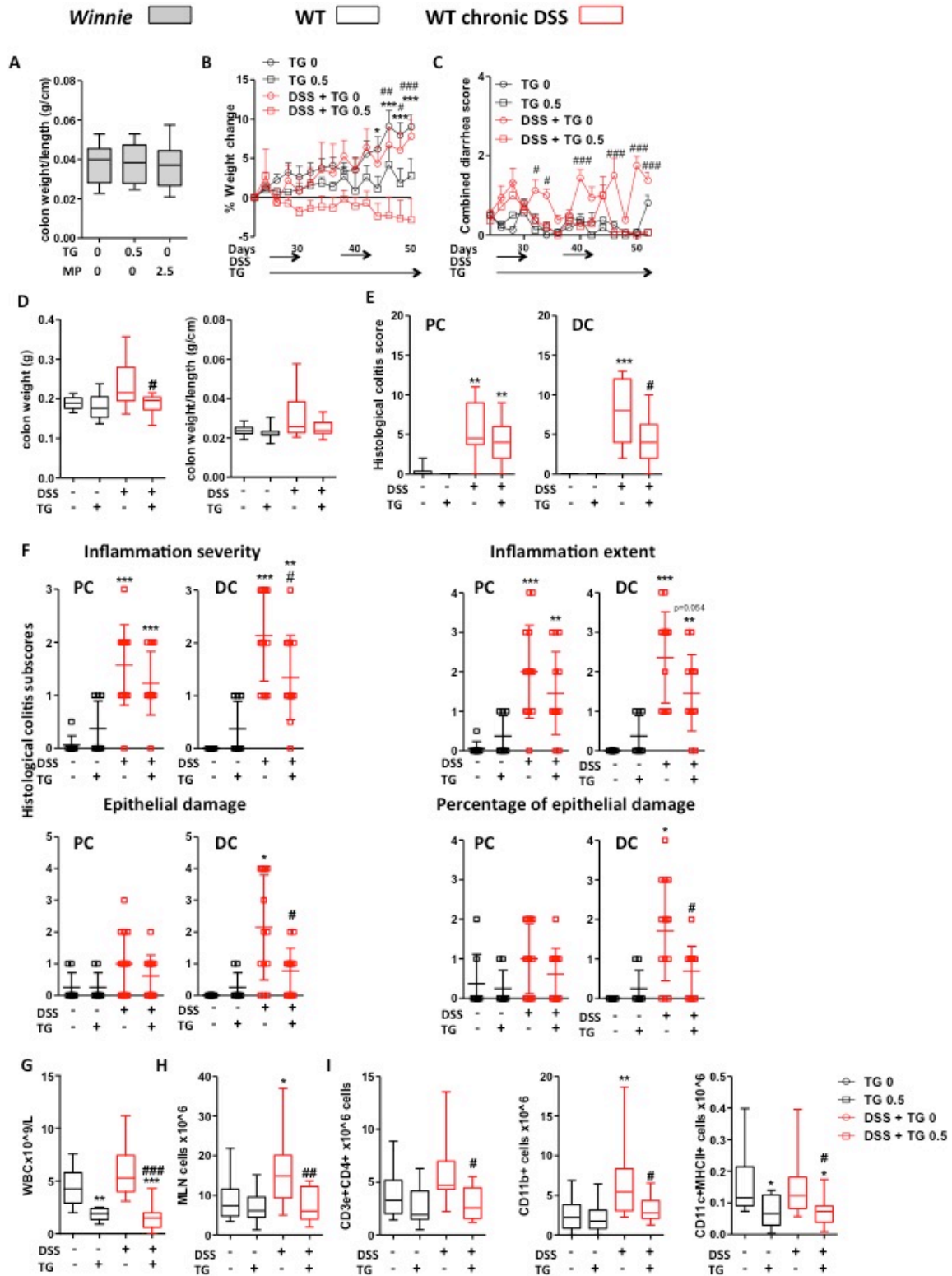
HeLa, RAW, HCT116 and HT29 were plated in 96 well plates and treated with DMSO 0.1% and 1, 10 and 50 µM TG for 16h. 20 µL of a tetrazolium compound (Promega) was added and plates were incubated for 2h, recording the absorbance at 490nm with a 96-well plate reader (Polarstar).



Supplementary figure 1. Acute administration of TG improved *Winnie* colitis. C57Bl/6 (WT) and *Winnie* daily gavaged TG 0, 0.5, 1 or 2.5 mg/kg/day for up to 14 days. A) Histological colitis subscores; B) Peripheral blood leukocytes (WBC). Box and whiskers plots of median, quartiles and range, N=4-6. Symbols: * versus WT TG 0 mg/kg; # versus *Winnie* TG 0 mg/kg. Statistical analysis: Mann-Whitney non-parametric test.

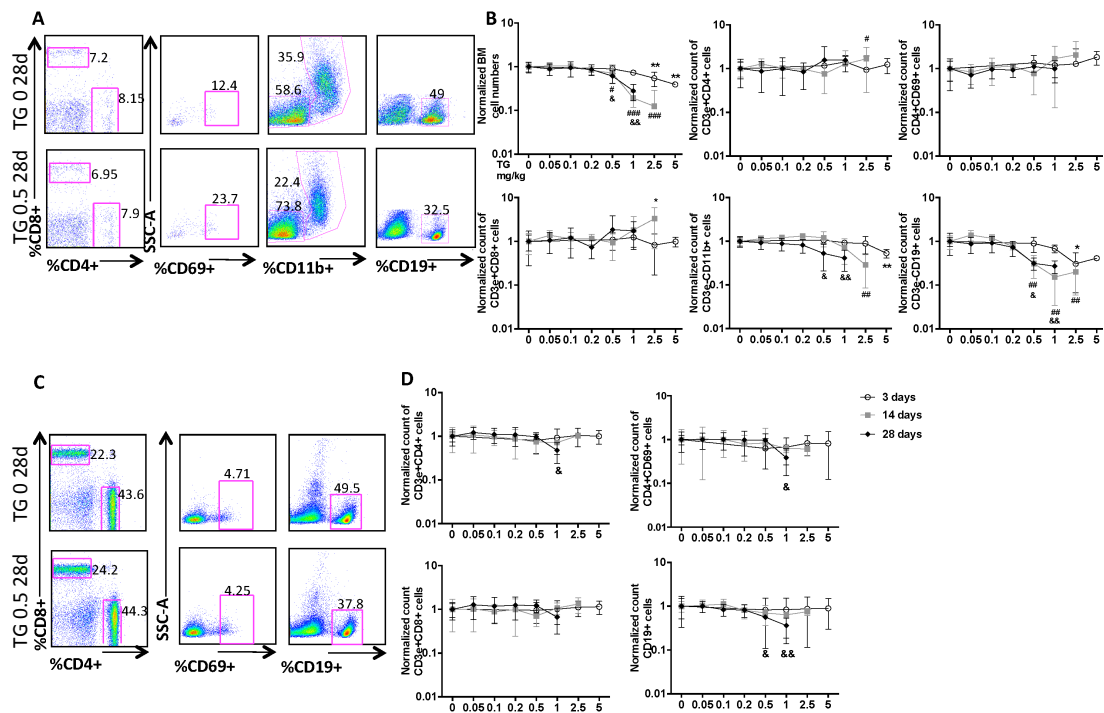


Supplementary figure 2. Acute administration of TG in *Winnie* colitis. WT and *Winnie* proximal (PC) and distal (DC) colon. *Grp78*, *sXbp1* and *Agr2* mRNA fold change normalised to house-keeping gene and to WT control.

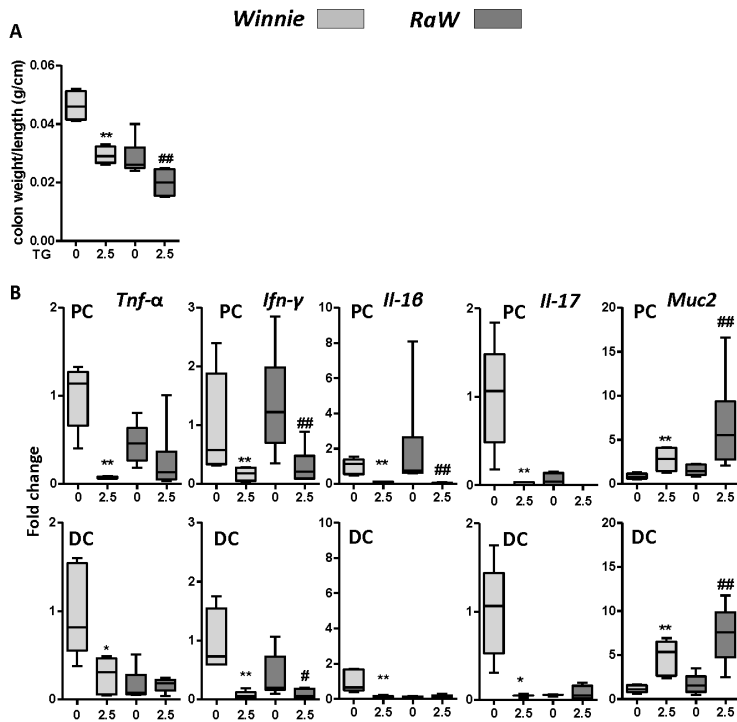


Supplementary figure 3 Administration of low dose of TG over 28 days in *Winnie* or chronic DSS induced colitis. A) *Winnie* colon weight/length ratio; B-I: WT mice chronic DSS colitis. B) % body weight change over the last 2 cycles; C) combined diarrhea score; D) colon weight and colon weight/length ratio; E) histological colitis scores for PC and DC; F) Histological

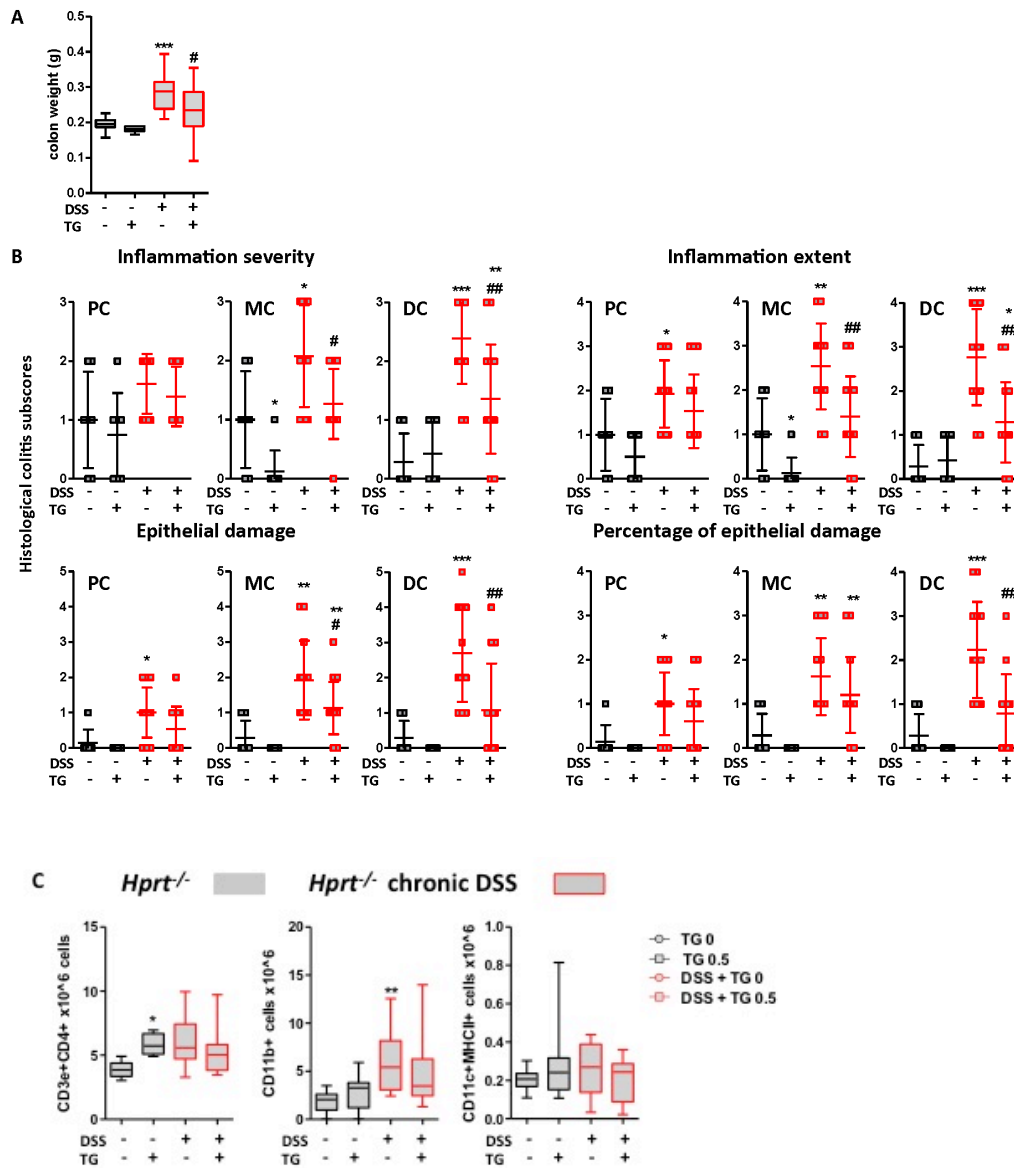
colitis subscores for PC and DC; G) white blood cell numbers; H) mesenteric lymph node numbers; I) mesenteric node CD3e+CD4+ T lymphocyte, myeloid (CD3e-CD11b+) and dendritic cell counts (CD11c+MHCII+) were decreased in MLN of mice treated with DSS and TG. N=2-8, from two to three experiments. Symbols: * versus WT TG 0 mg/kg; # versus WT DSS TG 0 mg/kg. Statistical analysis: Mann-Whitney non-parametric test.



Supplementary figure 4. Administration of TG but not MP affected diverse cell populations in the bone marrow and spleen. A) Representative BM FACS analysis plots for CD3e+CD8+, CD3e+CD4+, CD3e+CD4+CD69+, CD3e-CD11b+ and CD3e-CD19+ cells of mice treated with TG 0 or 0.5 mg/kg for 28 days. B) Effect of TG on bone marrow total cell number normalised to TG 0 mg/kg controls, and respective CD3e+CD8+, CD3e+CD4+, CD3e+CD4+CD69+, CD3e-CD19+, CD3e-CD11b+ cell subpopulations; C) Representative spleen FACS analysis plots for CD3e+CD8+, CD3e+CD4+, CD3e+CD4+CD69+ and CD3e-CD19+ cells of mice treated with TG 0 or 0.5 mg/kg for 28 days. D) Effect of TG on spleen cell numbers normalised to TG 0 mg/kg controls: CD3e+CD4+, CD3e+CD4+CD69+, CD3e+CD8+, CD3e-CD19+. Statistical analysis: Mann-Whitney non-parametric test.

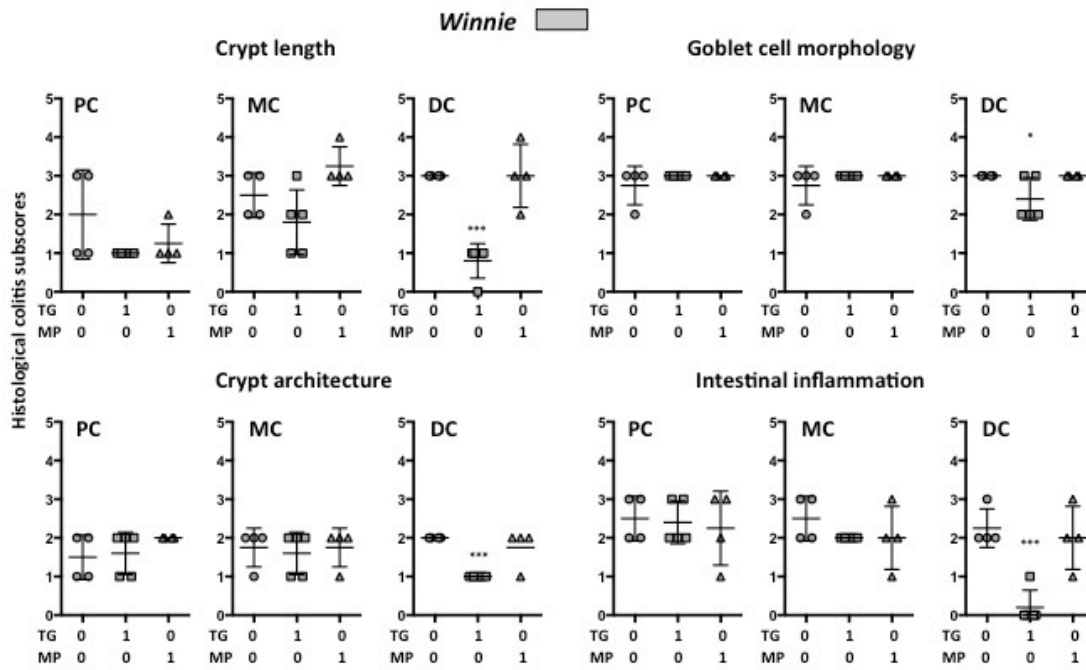


Supplementary figure 5. Acute administration of TG improved colitis in *RaW* mice. *Winnie* and *RaW* mice were daily gavaged TG 0 or 2.5 mg/kg/day for up to 14 days (dark symbols) as were *Winnie* controls (light grey). A) Colon weight/length ratio (g/cm); B) *Tnf-α*, *Ifn-γ*, *Il-18*, *Il-17*, *Muc2* fold change mRNA in PC and DC normalised to house-keeping gene and *Winnie* TG 0 mg/kg. Statistical analysis: Mann-Whitney non-parametric test. Symbols: * v *Winnie* TG 0 mg/kg; # v *RaW* TG 0 mg/kg.

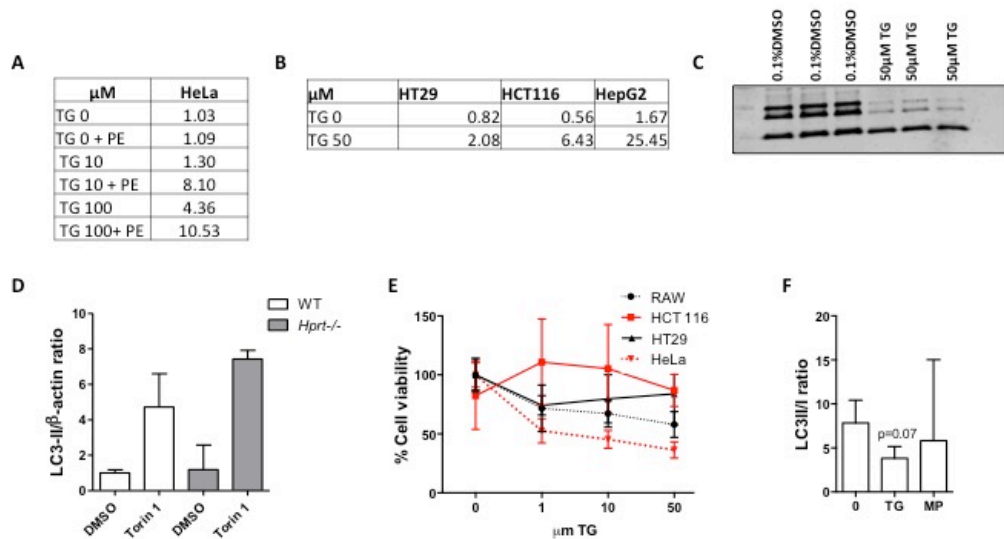


Supplementary figure 6. In *Hprt*^{-/-} mice, administration of low dose of TG over 28 days improved chronic DSS induced colitis without immunosuppression. *Hprt*^{-/-} mice were administered either water or 0.5% DSS in drinking water for 4 cycles and gavaged daily TG 0 or 0.5 mg/kg for the last two cycles (28 days): A) colon weight; B) histological colitis subscores for PC, MC and DC; C) TG did not alter CD3e+CD4+ T lymphocyte, CD3e-CD11b+ myeloid or CD3-CD11c+MHCII+ dendritic cell numbers in MLN of *Hprt*^{-/-} mice. N=2-8, from two to three experiments.

Symbols: * versus *Hprt*^{-/-} TG 0 mg/kg; # versus *Hprt*^{-/-} DSS TG 0 mg/kg. Statistical analysis: Mann-Whitney non-parametric test.



Supplementary figure 7. *Winnie* were daily treated with intra-rectal TG 0, 1 or MP 1 mg/kg/day for 14 days. TG, but not MP, improved *Winnie* colitis. Histological colitis subscores. Scatter dot plots, N=4-5. Symbols: * versus TG 0 mg/kg. Statistical analysis: one-way Anova with multiple comparisons.



Supplementary figure 8. TG *in vitro* administration enhanced autophagy. A and B: quantification data on epithelial cell western blots A) Densitometry derived LC3-II to LC3-I ratios of HeLa cells treated with DMSO 0.1% or TG (10 or 100 μM) +/- PE for 16h; B) LC3-II to LC3-I ratios of HT29, HCT 116 and HepG2 cells line treated with DMSO 0.1% or TG 50 μM for 16h; C) representative western blot for RAW macrophages treated with DMSO 0.1% or TG 50 μM for 16h; D) LC3-II to actin ratios in WT (open symbols) and Hprt- deficient primary mouse fibroblasts induced by the mTor inhibitor, Torin1; E) % Cell viability for RAW, HCT116, HT29 and HeLa treated with TG 1, 10 and 50 μM for 16h; F) Densitometry derived LC3-II to LC3-I ratios of distal colon protein extracts of Winnie mice treated with intrarectal vehicle control, TG or MP 1mg/kg/day for 14 days.

Targets	Forward (F) and reverse (R) primer sequences
Mouse <i>β-actin</i>	F: AGCACTGTGTTGGCATAGAGGTC
	R: CTTCTTGGGTATGGAATCCTGTG
Mouse <i>Gapdh</i>	F: GACATGCCGCCTGGAGAAAC
	R: AGCCCAGGATGCCCTTTAGT
Mouse <i>Tnf-α</i>	F: CATCTTCTCAAAATTCGAGTGACAA
	R: TGGGAGTAGACAAGGTACAACCC
Mouse <i>Il-1b</i>	F: CAACCAACAAGTGATATTCTCCATG
	R: GATCCACACTCTCCAGCTGCA
Mouse <i>Ifn-γ</i>	F: AGCTCTTCCTCATGGCTGTTTC
	R: ATGTTGTTGCTGATGGCCTGA
Mouse <i>Il-17</i>	F: CTCCAGAAGGCCCTCAGACTAC
	R: AGCTTCCCTCCGCATTGACACAG
Mouse <i>Muc2</i>	F: CCATTGAGTTTGGGAACATGC
	R: TTCGGCTCGGTGTTTCAGAG
Mouse <i>Grp78</i>	F: TGCTGCTAGGCCTGCTCCGA
	R: CGACCACCGTGCCACATCC
Mouse <i>Agr2</i>	F: CGGTGAGGGCAGACATCACTGGA
	R: CCGGTGCGCAGTTGGCTCTA

Conditions	Hold cycle 95°C for 10' and then 45x(95°C for 15", 60°C for 60", 72°C for 20")
Reagents	Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen)
Instrument	Rotor-Gene 6000
Expression	Normalized to the housekeeping gene using the Pfaffl equation and expressed relative to the mean of a relevant control group

Supplementary table 1 Forward and reverse primer sequences for mouse *B-actin*, *Gapdh*, *Tnf-α*, *Il-1b*, *Ifn-γ*, *Il-17*, *Muc2*, *Grp78*, *Agr2* and qPCR conditions, reagents, instrument and quantification