SUPPLEMENTARY DATA

Supplementary Fig. 1: LGG is not radioprotective of the bone marrow. (A, B) Mice received fractionated TBI (4Gy X 4 days). LGG was given by gavage on 6 consecutive days; 2 days before radiation and at 1h before each radiation dose. Mice were sacrificed 24 hours after the last dose of irradiation and the number of (A) $CD4^+$ and (B) $CD8^+$ cells in the peripheral blood was assessed. Pre-treatment with LGG had no effect on the number of surviving $CD4^+$ or $CD8^+$ cells. Data are means ± SEM for 3 mice per treatment group. ***P<.0001 compared with unirradiated control. (C) Mice were gavaged on 3 consecutive days with LGG, or LTA, or drinking water followed by a single dose of 4Gy TBI. 24 hours later the femurs were analyzed for the number of surviving $CD34^+$ bone marrow stem cells. Data are means ± SEM for 3 mice per treatment group.

SUPPLEMENT METHODS

Enteroid culture and assessment of proliferation

Small intestinal enteroid cultures from WT C57BL/6 mice were established and maintained per published protocols.[19, 20] A 1-cm^2 segment of ileal tissue was removed and washed in DMEM/F12 containing 10% FBS to inactivate endogenous proteases. The tissue was then minced with fine sterile scissors, incubated with collagenase for 10 min with vigorous pipetting every 5 min, filtered using a 40 µm cell strainer, and then washed once with DMEM/F12 in a 15 ml centrifuge tube. The pellet was resuspended in DMEM/F12 and centrifuged at 200 g for 5 min. The pellet was then resuspended in Matrigel. Matrigel (15 µl) containing the epithelial cells was placed in the center of each well of a 24-well plate and incubated in a tissue culture incubator for 10 min upside down to avoid the cells attaching to the bottom of the plate. After this incubation, 500 µl of 50% L-WRN conditioned media (containing Wnt3a, Noggin, and R-spondin) supplemented with 10 µM Y27632 and 10 µM SB431542 was added. Experiments were performed using purified epithelial spheroid populations.

Cell proliferation was assessed using two methods. First, the 3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay kit (ATCC, Manassas, VA) was used to measure cell growth as we have previously described with the following modifications.[15, 20] Cells were pretreated with lipoteichoic acid (LTA, from Staphylococcus aureus, 10µg/ml) or vehicle in medium for 2 hours, then irradiated with zero, 4, 8, or 12 Gy. LTA or vehicle was removed 2 hours post irradiation, and cells were cultured for 72 hours, and proliferation was assessed by MTT incorporation using a Biotek Synergy 2 microplate reader (Winooski, VT).

In later experiments, the CCK-8 proliferation assay (Dojindo Molecular Technologies, Inc) was used to measure cell proliferation. WT enteroids were plated in 50% CM for 12h, then grown in 5% CM supplemented with 10 µM Y27632 for 24h. Enteroids were then treated with the indicated compounds for 1h in 5% CM, followed by 6Gy irradiation. Culture medium was immediately replaced with 50% CM and enteroids were grown for 48h. Proliferation was measured by CCK-8 proliferation assay (Dojindo Molecular Technologies, Inc). Absorbance was measured using a Biotek Synergy 2 microplate reader (Winooski, VT).

In another set of experiments, ileum enteroids or small intestine mesenchymal stem cells (MSC) were incubated in media alone, or in media containing LTA (10µg/ml) or indomethacin (50µM), or NS398 (5µM). Conditioned media was collected after 24h incubation and PGE_2 concentration was determined by a PGE_2-specific enzyme-linked immunoassay according to the manufacturer's directions.

LTA and Bone Marrow Protection

C57BL/6J mice were given vehicle or LTA by gavage on 2 consecutive days before the start of the fractionated irradiation protocol. Thereafter, mice received 4Gy TAI on 4 consecutive days and vehicle or LTA by gavage at 1h before each radiation dose and were sacrificed 24h after the final radiation dose. Equal volumes (50µl) of peripheral blood from mice in each treatment group were analyzed for $CD4^+$ and $CD8^+$ by flowcytometry. C57BL/6J mice were given vehicle or LTA by gavage on 3 consecutive days. Mice received a single dose of 4Gy TBI at 1h after the final gavage and were sacrificed 24h later. Bone marrow was obtained from femurs and equal numbers of cells (1×10^6) from each treatment group were analyzed for $CD34^+$ cells by flow cytometry.