Supplemental methods

Confocal Laser Microscopy of mice

C57BL/6 male mice were housed in specific pathogen free conditions with a 12:12 hour light, dark cycle. To prevent possible diurnal rhythm effects, mice were all anaesthetised at 9:30 am. A laparotomy was performed to exteriorise a 2cm segment of small intestine as previously described.[26] The exteriorised small intestine was bathed in either an isosmolar (Na^+ 87, K^+ 15, Ca^{2+} 2, Cl^- 79, HCO_3^- 23, Glucose 90 mM; 300mOsm) or hyposmolar (Na^+ 60, K^+ 15, Ca^{2+} 2, Cl^- 52, HCO_3^- 23, Glucose 90 mM; 246mOsm) solution containing FITC-conjugated dextran (4KDa; 20µg/ml; Sigma). Hoechst 33342 (5mg/kg; Invitrogen) as a nuclear dye and Alexa-fluor conjugated dextran 647 (10KDa; 4mg/kg; Invitrogen, Paisley, Scotland) as an intravenous permeability probe were administered intravenously immediately prior to imaging. Multiphoton and confocal images were obtained using a Leica DMIRE2 laser scanning confocal microscope and a x20 air objective. Image stacks were collected every 90 seconds for 2 hour periods. Nuclei stained by Hoechst 33342 were visualised by 748nm multi-photon excitation and 435-485 emission. Luminally administered dextrans were imaged by 488nm argon/krypton laser excitation and 508-569nm emission. Intravenous dextrans were visualised by 633nm neon laser excitation and 653-795nm emission. Images were analysed and movies generated using Leica software and Image J (Imagej.nih.gov.il/, NIH, Bethesda, USA).