Supplementary Information

Materials and Methods

Animal models

Gucy2c$^{-/-}$ mice were generated by neomycin resistance gene insertion as previously described.[1, 2] *Gucy2c*$^{-/-}$ mice used in the experiments were backcrossed to C57BL/6 mice (The Jackson Laboratory) for 14 generations. Sibling *Gucy2c*$^{+/+}$ and *Gucy2c*$^{-/-}$ mice produced from at least three different breeding pairs of the 14th generation of *Gucy2c*$^{+/-}$ mice were used to generate colony mice used in the experiments. *Akt1*$^{+/-}$*Gucy2c*$^{+/+}$ and *Akt1*$^{+/-}$*Gucy2c*$^{-/-}$ mice were generated by crossing *Akt1*$^{-/-}$*Gucy2c*$^{+/-}$ male with *Akt1*$^{+/+}$*Gucy2c*$^{+/-}$ female mice, *Akt1*$^{-/-}$*Gucy2c*$^{+/+}$ male with *Akt1*$^{+/+}$*Gucy2c*$^{+/+}$ female mice or *Akt1*$^{-/-}$*Gucy2c*$^{-/-}$ male with *Akt1*$^{+/+}$*Gucy2c*$^{-/-}$ female mice. *Akt*$^{-/-}$*Gucy2c*$^{+/+}$ and *Akt*$^{-/-}$*Gucy2c*$^{-/-}$ mice were generated from *Akt1*$^{-/-}$ male and *Akt1*$^{+/-}$ female with specific homozygous *Gucy2c* genotype.[3] *Rosa-STOP*^{flox}*-Guca2a* mice were generated by standard transgenic procedures in the Thomas Jefferson University transgenic mouse facility.[4] Expression of the GUCY2C ligand, guanylin (GUCA2A), is regulated by the *Rosa26* promoter followed by a STOP codon flanked by two *loxP* sites upstream of full length *Guca2a* in *Rosa-STOP*^{flox}*-Guca2a* mice. Removal of the STOP codon by Cre recombinase activates constitutive transcription of *Guca2a* driven by the *Rosa26* promoter. The murine villin promoter targets stable and homogeneous expression of transgenes in small and large intestine along the crypt-villus axis, in differentiated enterocytes, as well as in the immature, undifferentiated cells of the crypt. *Villin-Cre*-ER^{T2} mice were obtained from S. Robine (Institut Curie, Paris, France).[5] *Villin-Cre*ER^{T2} mice express Cre recombinase in intestinal epithelial cells after intraperitoneal injection of tamoxifen (20 mg/Kg BW) for 5 consecutive days.[5] *Villin-*

*Cre*ER^{T2} mice were crossed with *Rosa-STOP^{flox}-Guca2a* mice to generate heterozygous *Rosa-STOP^{flox}-Guca2a-vil-Cre-ER^{T2}* mice. *Villin-Cre*ER^{T2} mice were paired with heterozygous *Rosa-STOP^{flox}-Guca2a-vil-Cre-ER^{T2}* mice to produce *Rosa-STOP^{flox}-Guca2a-vil-Cre-ER^{T2}* mice and corresponding littermate controls lacking the *Rosa-STOP^{flox}-Guca2a* transgene. Both *Villin-Cre*ER^{T2} and *Rosa-STOP^{flox}-Guca2a* mice were in the C57BL/6 background. All mice were housed in light-cycled and climate-controlled conventional animal facilities. All experiments were performed in compliance with the Thomas Jefferson University Animal Care and Use Guidelines.

Gucy2c genotype was confirmed by primers (forward: 5'-AGGTCATGACGTCACTGCTGGGCC-3'; reverse: 5'-TGTCCAGTCCTTCCTC CACAG-3'; neomycin: 5'-GGTGGGCTCTATGGCTTC-3').[1, 2] (Li, AJP 2007, Lin, Gastro 2010). *Akt1* genotype was confirmed by PCR with *Akt1* primers (Cp325: 5'-GCTCCATAAGCACACCTTCAG-3'; Cp326: 5'-GTGGATGGGAA TATGTGCGAG-3'; Cp328: 5'-ACAAGCTCTTCTTCCACCTGTC-3').[3] *Rosa-STOP^{flox}-Guca2a* genotype was confirmed by PCR with primers (forward: 5'-CCGCCGTTGTTGTTATTGTAG-3'; reverse: 5'-GTTGTGGTGATAGGTGGCAAG-3'). *Villin-Cre*-ER^{T2} genotype was confirmed by primers (forward: 5'-GAAAATGCTTCTGTCCGTTTG-3'; reverse: 5'-ATTGCTGTCACTTGGTCGTG-3').

DSS colitis

Age matched *Gucy2c$^{+/+}$* and *Gucy2c$^{-/-}$* mice were treated with 3.0% (w/v) DSS (Sigma-Aldrich) in the drinking water for 7 d followed by normal access to water.[6] Preliminary dose-finding experiments exploring the colitic effects of 0-5% DSS permitted selection of DSS doses for these studies that achieved inflammation within 7 d. *Rosa-STOP^{flox}-Guca2a-vil-Cre-ER^{T2}*, and corresponding littermate control *Villin-Cre*ER^{T2}, mice were injected intraperitoneally with tamoxifen (20 mg/Kg BW) for 5 d. Gene expression was analyzed 7 d after the last tamoxifen

treatment by mRNA and protein expression. DSS (3.0%; w/v) was administrated in the drinking water for 7 d followed by normal access to water. For ligand supplementation experiments, 8 wk old C57BL/6 mice were purchased from NCI and gavaged with ST or control peptide for 14 d before receiving 3.5% DSS for 7 d. Body weight and physical activity were monitored daily.

Barrier integrity

Mice were gavaged with 200 µL of FITC-dextran (75 mg/mL; 4kD; Sigma-Aldrich) and blood was collected 90 min later by cardiac puncture and added to EDTA-coated tubes. Hemolysis-free serum was collected by centrifugation and evaluated immediately for fluorescence (485/525 nM) in black opaque plates. FITC-dextran (7.5 mg/mL) was used as 90% for gain adjustment. Background fluorescence was determined by serum from mice without FITC-dextran gavage.[7]

Caco2 human colon cancer cells were infected with wtAKT1 or MyrAKT1 delivered by adenovirus, or stably transfected with shAKT1 and MSCV empty vector delivered by retrovirus, grown into monolayers on 24-well PET transwell permeable inserts (BD Biosciences, 1.0 µm pore size, coated with fibrillar collagen), and treated with ST (1 µM) and IBMX (50 µM) or IBMX alone for 6 d. Complete growth media and reagents were exchanged every other day. After 6 d of treatment, 40 µL of 75 mg/mL FITC-dextran was added into the upper chamber containing 400 µL of complete growth media, while 800 µL of complete growth media was present in the lower chamber. FITC-dextran (7.5 mg/mL) was used as 90% for gain adjustment. After 60 min of incubation, the fluorescence of the media in the bottom chamber was assessed to determine permeability.

Electron microscopy

Intestinal sections 10 cm after the pyloric sphincter were cleaned, collected and fixed with 2.5% glutaraldehyde/2% formaldehyde with 0.1 M sodium cacodylate and stored at 4°C until embedding. Post-fixation with 2% osmium tetroxide and an increasing gradient dehydration step using ethanol and propylene oxide was followed by embedding in LX-112 medium (Ladd Research). Sections (90 nm) were placed on uncoated copper grids and stained with 0.2% lead citrate and 1% uranyl acetate. Images were collected in a blinded fashion with a JEOL-1010 electron microscope employing 80 kV of acceleration.[3]

Microarray analysis

Microarrays, using the Affymetrix Mouse 430 2.0 3'-IVT platform, were run on RNA extracted from colon and small intestine of *Gucy2c*$^{+/+}$ and *Gucy2c*$^{-/-}$ littermates (n=4).[3] The quality of RNA, as well as hybridization to chips, was assessed by hybridization levels of spike-in controls, 3'-5' RNA degradation profiles, and modeling the probe-level data to investigate consistency across arrays.[8] Robust Multichip Analysis (RMA[9]) was used to background-correct and normalize the probe-level data and summarize it at the probeset level. The processed data was evaluated for differential expression of genes using permutations tests and libraries from the Bioconductor project.[10] Global tests of differential expression of groups of genes (genesets) determined from KEGG pathways or from prior biological interest were performed as described.[11] Gene ontology-based scoring was performed using pathway sets curated from the Biocarta and KEGG databases (www.biocarta.com; http://www.genome.jp/kegg/ pathway.html).[12]

Supplemental References

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