Supplemental Methods

Animals

Generation of *Il22bp*$^{-/-}$ mice: The gene encoding *Il22bp* (*Il22ra2*) was ablated in ES cells using the Velocigene method[31]. Briefly, a bacterial artificial chromosome (BAC) containing the *Il22ra2* gene (clone 305f24 from Incyte Genomics mouse "BAC ES release 2" library) was isolated and bacterial homologous recombination (BHR) was performed to replace 9.4 kb of the *Il22ra2* gene with a cassette containing a TM-lacZ reporter gene and a floxed neomycin resistance selectable marker gene. The resulting mutant allele (designated VG437) encodes a fusion protein containing 7 amino acids of the mature form of IL22BP, fused to a transmembrane segment, fused to *E. coli* beta-galactosidase under transcriptional control of the *Il22ra2* gene. BHR resulted in a large targeting vector (BACvec) in which the reporter/selection cassette was flanked with homology arms of 155 and 45 kb. The BACvec was electroporated into VGF1[31] ES cells and G418 resistant colonies were screened for targeting using a LONA assay with two qPCR probe/primer sets within the deleted region: 437TU (primers= 437TUF: GGGACCTTCAGCTTCCTGC and 437TUR: CTAAGCAAGTGGCTGCCAGC and probe= 437TUP: TGCACAAGGGCTCCTCAGTATGTCAAATG) and 437TD (primers= 437TDF: CCAGCCCATGTTTGACAGAAG and 437TDR: TAGCGCCTCAGACCAGTTCAT and probe= 437TDP: TGCACACATCTCTCCTTGCTTCTTGGG) and appropriate reference probes as described[31]. Correctly targeted ES cells were microinjected into C57/BL6 blastocysts to produce chimeras that were subsequently bred to generate knockout mice. *Il22bp*$^{-/+}$ mice were backcrossed for 12 generations on C57/BL6 background. For all experiments age- and sex-matched KO mice and co-housed in house breed C57/BL6 wild type

animals between 8 and 14 weeks of age were used. *Casp1*$^{-/-}$ were generated in our laboratory[32]. *Apc*$^{min/+}$, *Il18*$^{-/-}$, *Il1r*$^{-/-}$, *Myd88*$^{-/-}$, *Trif*$^{-/-}$, *Tlr4*$^{-/-}$, *Tlr5*$^{-/-}$ were obtained from 'The Jackson Laboratory'. *Nlrp3*$^{-/-}$, *Nlrp6*$^{-/-}$, *Asc*$^{-/-}$[24], and *Il22*$^{-/-}$[9] are described elsewhere. For antibiotic treatment, mice were given either a combination of ciprofloxacin (0.2□g□l−1) and metronidazole (1□g□l−1) or a combination of vancomycin (1 g/l), ampicilin (1 g/l), kanamycin (1 g/l), and metronidazole (1 g/l) for 4□weeks in the drinking water[24].
Mice were cared for in accordance with institutional animal care and use committee-approved protocols at the Yale University School of Medicine animal facility.

Tumor induction

Mice were injected intraperitoneally with AOM (Sigma) at a dose of 7.5mg/kg body weight. After 5 days, mice were fed 2.5% DSS (MP biomedicals, M.W. =36,000-50,000 Da) in the drinking water for 5 days, followed by 16 days of regular water. This cycle was repeated twice times[33]. Mice were sacrificed on day 80 of the experiment.

DSS colitis

For acute DSS colitis induction mice were administered 2.5% DSS in the drinking water for 7 days, followed by 3 days of regular water. For chronic DSS colitis induction mice were fed 2.5% DSS for 5 days followed by 16 days of regular water. This cycle was repeated three times. According to the animal protocol mice were sacrificed, if they lost more then 20% of their initial body mass.

Endoscopic Procedures

Colonoscopy was performed in a blinded fashion for colitis and tumor monitoring using the Coloview system (Karl Storz, Germany) as previously described[6]. Briefly: Colitis scoring was based on granularity of mucosal surface, stool consistence, vascular pattern, translucency of the colon and fibrin visible (0-3 points for each). Tumor sizes were graded from 1 to 5. Tumors observed during endoscopy were counted to obtain the total number of tumors per animal. The total tumor score per mouse was calculated as sum of all tumor sizes. The colon was wounded using the endoscopy and a biopsy forceps as described previously[34, 8]. A rebiopsy was taken two days after the wounding either close to the initial biopsy or in a distance of about 0.5 cm.

Histopathology procedures

Colons were evaluated and were assigned scores by investigators blinded to experimental manipulation. Each section was evaluated by a semi quantitative criterion-based method (score 0–5) essentially as described before[35]. For immunohistochemistry Paraffin-embedded sections were stained with anti-BrdU (Sigma) or anti-Ki67 (Thermo (Lab Vision)). DAKO EnVision™ System was used for detection. All sections were counterstained with hematoxylin. TUNEL staining was performed using ApoAlert® DNA Fragmentation Assay Kit according to the manufacturer's instruction. BrdU was injected four hours before the animals were sacrificed.

Isolation of EC and hematopoietic cells from the intestine

Epithelial cells and hematopoietic cells were isolated from the freshly obtained colon. After removal of the Payer's patches and the adventitial fat, the colon was cut longitudinally and washed with PBS. For disruption of the epithelial cells the colon was

incubated in HBSS/EDTA in a 37°C. The supernatant was collected and further separated in CD45 positive (IEL) and negative cells using MACS. The remaining colon was digested using Collagenase/DNase incubation in 37°C. CD45 positive cells (LPL) were purified using MACS. In a second step CD45 positive IEL and LPL cells were further purified using a MoFlo. The purity of CD45 positive cells was > 95%, EC were >98% CD45 negative.

Colon explant culture

One centimeter of the distal colon was removed, washed with PBS, scaled, and cultured for three days in X-Vivo medium containing Penicillin, Streptomycin and Tetracycline. IL-6 was measured using CBA.

RNA Analysis

Total RNA was extracted from colon tissue, tumors, colon biopsies or cells using Trizol® Reagent, followed by RNA clean up using the RNeasy Kit (Quiagen) or the Dynabeads mRNA Kit (Applied Biosystems). The High capacity cDNA synthesis Kit (Applied Biosystems) was used for synthesis of cDNA. Real-time PCR analysis using TaqMan® Fast Universal PCR Mater Mix and TaqMan® Gene Expression Assays (Applied Biosystems) was performed on 7500 Fast Real-time PCR system machine (Applied Biosystems).

Human subjects

Informed consent was obtained from healthy participants per protocol approved by the institutional review board at Yale University.

References

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