Interleukin-22 protects intestinal stem cells against genotoxic stress

Konrad Gronke1,2,3,4,5,6,16, Pedro P. Hernández5,6,7,16, Jakob Zimmermann5, Christoph S. N. Klose1,5,8, Michael Kofode-Branzki1,2,3,4,5, Fabian Guendel1,2,3,4,5, Mario Wittkowski1,2,3,4, Caroline Tzianabos1,2,3,4, Lukas Amann5,15, Fabian Schumacher9,10, Hansruedi Glatt11,12, Antigoni Triantafyllopoulou13,14 & Andreas Diefenbach1,2,3,4,5

Environmental genotoxic factors pose a challenge to the genomic integrity of epithelial cells at barrier surfaces that separate host organisms from the environment. They can induce mutations that, if they occur in epithelial stem cells, contribute to malignant transformation and cancer development1–3. Genome integrity in epithelial stem cells is maintained by an evolutionarily conserved cellular response pathway, the DNA damage response (DDR). The DDR culminates in either transient cell-cycle arrest and DNA repair or elimination of damaged cells by apoptosis4–6. Here we show that the cytokine interleukin-22 (IL-22), produced by group 3 innate lymphoid cells (ILC3) and γδ T cells, is an important regulator of the DDR machinery in intestinal epithelial stem cells. Using a new mouse model that enables sporadic inactivation of the IL-22 receptor in colon epithelial stem cells, we demonstrate that IL-22 is required for effective initiation of the DDR following DNA damage. Stem cells deprived of IL-22 signals and exposed to carcinogens escaped DDR-controlled apoptosis, contained more mutations and were more likely to give rise to colon cancer. We identified metabolites of glucosinolates, a group of phytochemicals contained in cruciferous vegetables, to be a widespread source of genotoxic stress in intestinal epithelial cells. These metabolites are ligands of the aryl hydrocarbon receptor (AhR)6, and AhR-mediated signalling in ILC3 and γδ T cells controlled their production of IL-22. Mice fed with diets depleted of glucosinolates produced only very low levels of IL-22 and, consequently, the DDR in epithelial cells of mice on a glucosinolate-free diet was impaired. This work identifies a homeostatic network protecting stem cells against challenge to their genome integrity by AhR-mediated ‘sensing’ of genotoxic compounds from the diet. AhR signalling, in turn, ensures on-demand production of IL-22 by innate lymphocytes directly regulating components of the DDR in epithelial stem cells.

To model colitis-associated colon cancer (CAC) we challenged mice with the pro-carcinogenic azoxymethane (AOM), and then treated them with dextran sodium sulfate (DSS) resulting in intestinal inflammation fuelling tumour growth. AOM is an alkylating agent that generates mutagenic adducts of O6-methylguanine (O6meG) in DNA that lead to O6meG:T mismatches and, eventually, to mutations7. These mutations often affect the glyco-kinase (GSK)-3β phosphorylation site in exon 3 of the β-catenin gene, similar to the mutations8–10 found in most human colon cancers. Mice genetically and constitutively lacking IL-22 developed larger numbers of tumours than wild-type mice11 (Extended Data Fig. 1a, b), mainly in the distal part of the colon (Extended Data Fig. 1c). IL-22 is a cytokine that acts selectively on non-haematopoietic cells, including epithelial stem cells12. Although these results suggest that IL-22 may restrain tumour development, Il22−/− mice also present dysbiotic changes in microbial communities13 and increased inflammation14 after DSS application (Extended Data Fig. 1d–f). Therefore, the increased number of tumours in Il22−/− mice could be a result of the enhanced inflammation.

Il22−/− mice also developed more tumours when treated with AOM injections only (Fig. 1a). To distinguish between direct effects of IL-22 signalling on tumour transformation in colon stem cells and confounding differences in inflammation, microbiota and/or other stem cell-extrinsic factors inherent in comparisons between mutant and wild-type mice, we designed a mouse model that enables inducible sporadic inactivation of the IL-22 receptor in colon intestinal epithelial stem cells (Fig. 1b, Extended Data Fig. 1g). We used FACS to sort Lgr5creERT2+; Il22ra1fl/fl mice to enriched populations of Lgr5+ crypts, as IL22RA1 subunit (Il22ra1) in intestinal epithelial stem cells (Fig. 1b, Extended Data Fig. 1g), the primary target of carcinogenesis15,16. To achieve this, we first generated Il22ra1fl/fl mice (Extended Data Fig. 2a–g) and crossed these to Lgr5creERT2+/− mice, enabling tamoxifen-inducible Cre activity in colonic stem cells in the same mouse (Extended Data Fig. 2j). Notably, Confetti+ tumours from Il22ra1fl/fl/Lgr5creERT2+/−, R26R-Confetti mice (Extended Data Fig. 2j). Notably, Confetti+ tumours from Il22ra1fl/fl/Lgr5creERT2+/−, R26R-Confetti mice did not express the α subunit of the IL-22 receptor (Il22Rα1), whereas Confetti+ tumours in the same mouse were Il22Rα1-positive (Extended Data Fig. 3a–e). This approach allowed us to interrogate IL-22-dependent programs in stem cells within the same mouse by creating a mosaic of IL22Rα1+ and IL22Rα1− crypts (Fig. 1b).

Considering the labelling efficiency (Fig. 1c, Extended Data Fig. 2h, i), we expected 15–30% of tumours to exhibit Confetti labelling. This was consistent with our observations in mice having one functional allele of Il22ra1 (Fig. 1d). By contrast, mice with sporadic inactivation of Il22ra1 had a markedly increased fraction of Confetti+ tumours, demonstrating that absence of IL-22 signalling in colon epithelial cells predisposes them for tumour development (Fig. 1d–f). In the colon of C57BL/6 mice obtained from a commercial vendor, approximately half of the IL-22 producers were CD4+ T cells, a third were ILC3 cells, approximately 6% were FOXP3+ CD4+ T cells and approximately 6% were γδ T cells (Extended Data Fig. 4a). ILC3 cells were the dominant

1Laboratory of In innate Immunity, Department of Microbiology, Infectious Diseases and Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany. 2Berlin Institute of Health (BIH), Berlin, Germany. 3Mucosal and Developmental Immunology, Deutsches Rheuma-Forschungszentrum, Berlin, Germany. 4Department of Medical Microbiology, University of Duisburg-Essen, Essen, Germany. 5Max Planck Institute for Immunobiology and Epigenetics, Freiburg, Germany. 6Macrophages and Development of Immunity, Institute Pasteur, Paris, France. 7ILL Roberts Institute for Research in Inflammatory Bowel Disease, Joan and Sanford I. Weill Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA. 8Department of Tumour Epigenetics, German Cancer Research Center, Heidelberg, Germany. 9Department of Molecular Biology, University of Duisburg-Essen, Essen, Germany. 10German Institute for Human Nutrition Potsdam-Rehbruecke (DIfE), Potsdam, Germany. 11Department of Medical Microbiology, University of Mainz, Mainz, Germany. 12Department of Medical Microbiology, University of Freiburg, Freiburg, Germany. 13National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA. 14Department of Pathology, Institute of Neurology, University College London, London, UK. 15Present address: Institute of Neuropathology, Medical Faculty, University of Freiburg, Freiburg, Germany. 16These authors contributed equally: Konrad Gronke, Pedro P. Hernández. *e-mail: andreas.diefenbach@charite.de
source of IL-22 in the small intestine (Extended Data Fig. 4b). Mice from a non-barrier, specific pathogen-free (SPF) facility showed a larger fraction (79–88%) of CD4+ T cells among IL-22 producers (Extended Data Fig. 4c). Collectively, the data demonstrate that IL-22 signalling in colon epithelial cells is a substantial barrier to tumour development. Cancer originates in stem cells, but IL-22 is continuously produced at the steady state, but the IL-22-controlled transcriptional networks in colon stem cells remain unknown. Using RNA sequencing (RNA-seq) analysis, we found that during the steady state, 350 genes exhibited greater than twofold change in expression between LGR5+ colon stem cells of IL22+/+ and IL22−/− mice (Extended Data Fig. 5a, b). Gene set enrichment analysis (GSEA) revealed that stem cells from IL22+/− mice were depleted of transcripts associated with 'DNA repair' (Fig. 2a) and 'DNA double-strand break processing' terms (Extended Data Fig. 5c). Considering these biological pathways, we performed RNA-seq of sorted colonic LGR5+ stem cells from IL22+/+ and IL22−/− mice 24 h after inducing DNA double-strand breaks by irradiation, when the cellular response to DNA damage was maximal (Extended Data Fig. 5d). GSEA of the expressed genes showed marked enrichment of gene signatures such as ‘hallmark apoptosis’ and ‘DNA damage response effector genes’ in LGR5+ stem cells from IL22+/+ but not from IL22−/− mice (Fig. 2b, Extended Data Fig. 5e). IL22BP is an antagonist of IL-22 that blunts excessive and potentially pathologic IL-22 production. Production of IL22BP was reduced following DNA damage, resulting in higher concentrations of bioactive IL-22 (Extended Data Fig. 5f). These results indicate that stem cells deprived of IL-22 signals lack transcriptional signatures of the DDR and DNA repair pathways. To identify the IL-22-dependent processes within the DDR, we performed a systematic analysis of critical checkpoints. Expression of MRN-complex genes (Mre11a, Rad50 and Nbn), which sense DNA

**Fig. 1 | IL-22 protects stem cells against malignant transformation.**

- **a.** Colon tumours in mice injected with AOM only (IL22+/+, n = 9; IL22−/−, n = 10; mean ± s.e.m.; P = 0.0059). b–f. Indicated strains were treated for 2 weeks with tamoxifen diet and subjected to AOM or DSS. b. Sporadic deletion of IL22RA1 after Cre activation. c. Labelling efficiency of crypts (IL22ra1fl/+; n = 27; IL22ra1fl/fl, n = 29 visual fields; mean ± s.e.m.; P = 0.07256). d. Fraction of Confetti-positive tumours (IL22ra1fl/fl, n = 7; IL22ra1fl/fl, n = 9; mean ± s.e.m.; P = 0.0001). e. Quantification of all tumours (IL22ra1fl/fl, n = 10; IL22ra1fl/fl, n = 19; mean ± s.e.m.; P = 0.0003). Scale bar, 1 cm. f. Representative immunohistology of colon carcinomas. Scale bars, 200 μm. Data are representative of two (a, d–f) or three (c) biologically independent experiments.

**Fig. 2 | IL-22 regulates key components of the DDR.**

- **a, b.** IL22+/+:Lgr5-GFP+/+ and IL22−/−:Lgr5-GFP+/+ mice (n = 3) were left untreated (a) or irradiated with 8 Gy (b). LGR5(GFP)+ colon epithelial stem cells were purified. Gene expression was analysed by RNA-seq. GSEA on all expressed transcripts at steady state (a) or 24 h after irradiation (b). Nominal P value (Kolmogorov–Smirnov test) was calculated for the terms Kauffmann DNA repair genes (P = 0.0099), hallmark apoptosis (P = 0.05) and DDR effector genes (P = 0.004). c. Atm gene expression in untreated colon epithelial stem cells determined by quantitative reverse transcription PCR (qRT-PCR), relative to Hprt expression (IL22+/+, n = 8; IL22−/−, n = 5 (P = 0.005); IL22ra1fl/fl; Vil1-cre, n = 6 (P = 0.0009); mean ± s.e.m.). d. ATM expression in untreated colon epithelial (EpCam+) cells or after IL-22 injection. AF555, AlexaFluor555; MFI, mean fluorescence intensity. IL22+/+, n = 6; IL22−/−, n = 3 (P = 0.04); IL22+/+ + IL-22, n = 3 (P = 0.0001); mean ± s.e.m. e, f. γH2AX formation in colon epithelial (EpCam+) cells 4 h and 8 h after mice were exposed to 8 Gy irradiation. γH2AX+ nuclei (IL22+/+ 8 h, n = 6; Atmfl/fl; Rorc(γt)-cre 8 h, n = 7; other, n = 3; mean ± s.e.m.). f. Representative immunohistology 8 h after irradiation. Scale bars, 50 μm. Data in c–f are representative of two biologically independent experiments.
damage, was independent of IL-22 (Extended Data Fig. 5g). The MRN complex recruits and activates a group of kinases (that is, ATM, ATR and DNA-PK) that initiate the DDR cascade and are expressed in almost all living cells for an immediate response to genotoxic stress.18 We found a reduction of Atm expression in colon stem cells from Il22−/− mice in the absence of genotoxic stress (Fig. 2c,d). Atm is a STAT3-target gene and IL-22 induces STAT3 signalling in epithelial cells.20 Chromatin immunoprecipitation (ChIP) of STAT3 using primers for three STAT3-binding sites in Il22ra1 or STAT3 in all intestinal epithelial cells (Fig. 3a,b) or AOM treatment (Extended Data Fig. 7c). Increased expression of these genes after DNA damage required IL-22 signalling in epithelial cells, as documented in mice with deletion of Il22r1 or STAT3 in all intestinal epithelial cells (Fig. 3a, b, Extended Data Fig. 7d). Exogenous application of IL-22 rescued IL22R1-dependent Puma levels in colon stem cells (Fig. 3c).

We hypothesized that colon stem cells experiencing DNA damage in the absence of IL-22 signals do not undergo PUMA-mediated apoptosis or p21-regulated cell cycle arrest. Genetic inactivation of Il22r1 in epithelial cells or antibody-mediated neutralization of IL-22 resulted in a lower fraction of apoptotic (that is, cleaved (c)Casparase-3+) LGR5+ stem cells in colon crypts following irradiation (Fig. 3d, e, Extended Data Figs. 7c–g, 8a–d). Using sporic inactivation of Il22r1 in colon epithelial stem cells, we found a profound reduction of apoptosis in Il22r1−/− (Deficient (Confetti+)) crypts compared to Il22r1−/−-proficient (Confetti--) crypts within the same mouse (Fig. 3f, g). Inhibition of apoptosis with Z-V AD-FMK (zV AD), a pan-caspase inhibitor, in 1 Gy Il22−/− mice led to substantially increased numbers of tumours comparable to those observed in Il22−/− mice (Fig. 3h). These data indicate that the reduced rate of apoptosis observed after DNA damage in IL-22-deprived colon stem cells may explain the increase in tumour count.

Cells that continue to cycle in the presence of DNA breaks are prone to mitotic defects that lead to the formation of micronuclei, a sign of genomic instability. Colonic epithelial cells of irradiated Il22−/− mice showed increased numbers of micronuclei in comparison to wild-type
controls (Fig. 3i). Conversely, treatment of an intestinal epithelial cell line with IL-22 reduced the numbers of micronuclei after irradiation (Extended Data Fig. 8e). IL-22 has also been shown to promote proliferation of damaged stem cells and sustain proliferation of already-transformed cells. We found an increase in proliferation after DNA damage in II22+/− mice which was absent in II22−/− mice (Extended Data Fig. 8f). The DDR is a powerful cellular program that prevents accumulation of mutations during genotoxic stress. We isolated colon epithelial cells from mice eight weeks after AOM administration and quantified the frequency of exon 3 mutations in the Ctnnb1 gene, which are strongly associated with various forms of human cancer (Extended Data Table 1). Colon epithelial cells from II22−/− mice accumulated a much higher frequency of Ctnnb1 mutations than cells from II22+/− mice (Fig. 3j). Thus, IL-22, a cytokine produced by lymphocytes, acts as an important, epithelial cell-extrinsic regulator of the DDR in colon stem cells, preventing the accumulation of potentially dangerous mutations.

Mutations accumulate in colon stem cells, partly due to environmental genotoxic compounds. Cruciferous vegetables of the Brassicaceae family contain high levels of a group of phytochemicals referred to as glucosinolates; some metabolites of glucosinolates are potent genotoxins. For example, the treatment with the glucosinolate metabolite 1-methoxy-3-indolylmethyl alcohol (1-MIM-OH) leads to the formation of DNA adducts and activates the DDR (Extended Data Fig. 9a). We applied a single dose of 1-MIM-OH by gavage and monitored the expression of Puma and p21 (Fig. 4a, Extended Data Fig. 9b), which were induced in colon epithelial stem cells in an IL-22-dependent manner. Indole 3-carbinol (I3C), a glucosinolate metabolite that does not damage DNA, did not trigger the DDR (Fig. 4b). Regardless of their DNA-damaging qualities, a single application of 1-MIM-OH or I3C significantly increased the production of IL-22 by ILC3 and γδ T cells, but not by CD4+ T cells (Fig. 4c, d and Extended Data Fig. 9c, d). IL-10 production by FOXP3+ T cells was not affected (Extended Data Fig. 9e). Glucosinolate-induced IL-22 expression increased expression of Reg3γ, an antimicrobial gene regulated by IL-22 (Extended Data Fig. 9f, g).

The increase of IL-22 production in response to 1-MIM-OH or I3C required AhR expression in ILC3 and T cells (Extended Data Fig. 9h). Mice genetically lacking AhR in ILC3 and T cells exhibited a phenotype similar to that of IL-22-deficient mice, with reduced expression of Atm (Fig. 4e). Following genotoxic stress, these mice also showed reduced apoptosis of crypt-resident stem cells (Extended Data Fig. 9i). The increase of IL-22 production in response to 1-MIM-OH or I3C was almost fully restored in mice with a functional AhR (Fig. 4f).

We investigated whether glucosinolate-deficient diets would reduce the resistance of mice to consecutive genotoxic stress. We fed adult mice with either an experimental defined diet (EDD) lacking phytochemicals (including glucosinolates; EDD−) or with a defined diet supplemented with I3C (EDD+) (Extended Data Fig. 9b). Normal mouse chow, which contains bioactive concentrations of AhR ligands, served as a control diet. Colon ILC3 and γδ T cells from mice on EDD− diet...
did not produce IL-22, but they produced high levels of IL-22 when the mice were fed EDD+ or the control diet (Fig. 4g, Extended Data Fig. 10a, b). Epithelial cells from mice on the EDD diet showed reduced expression of Atm (Fig. 4h) and, following DNA damage, profound reductions in H2AX (Fig. 4i, Extended Data Fig. 10c), p21 and Puma expression (Fig. 4j) and in apoptosis in colon crypts (Fig. 4k, Extended Data Fig. 10d). Further, addition of i3c to the diet restored all of these functions. Therefore, AhR-mediated regulation of IL-22 provides a substantial barrier against DNA-damaging agents in the diet.

These data reveal a novel homeostatic control circuit by which IL-22-producing and tissue-resident innate lymphocytes regulate the response of epithelial stem cells to genotoxic dietary compounds at surfaces bordering the environment, thereby effectively limiting the number of mutations (Extended Data Fig. 10e). AhR expressed by ILC3 and γδ T cells serves as a ‘sensor’ of genotoxic phytochemicals for on-demand production of IL-22, which regulates the DDR machinery in epithelial stem cells. The AhR may turn out to be an important node for cancer development, as AhR signalling within epithelial cells was recently shown to reduce development of colon cancer36. Finally, we expect such communication between innate lymphocytes and their tissue client cells to be operative at other border surfaces. Such communication may be exploited as an adjunctive therapy to avoid genotoxic sequelae associated with various forms of clinical therapy such as chemotherapy, radiation therapy and bone marrow transplantation.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-0899-7.

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METHODS

Data reporting. For power calculations, the D value (difference in means)/(standard deviation) was calculated in preliminary experiments. The exact sample size was determined under consideration of the D value, available resources (genotypes) and ethical aspects (implementing the three Rs). If an insufficient number of animals was available for a reliable significance prediction, biologically independent repetition experiments were performed and data pooled for analysis. We did not use randomization to assign animals to experimental groups. Investigators were not blinded to group allocations during the experiment and data analysis. Because treatment and experimental analysis could not be separated, blinding of the investigators was not possible. Histological scoring was performed by two researchers according to pre-set criteria. For quantification pre-defined algorithms/analysis sequence was performed by image analysis software and specific criteria were documented.

Mice. Conventional (specific pathogen-free) C57BL/6N mice were purchased from Janvier Laboratories. Lgr5-EGFP-ΔloxP (referred to here as Lgr5-ΔloxPtERT2) or Lgr5-ΔloxPtm1a mice37, R26R-Confi†® and Rorc(+/−)/Il22ra1−/− mice33 were purchased from Jackson Laboratories and crossed in our animal facility. Lgr5−/− mice32 were a gift from J.-C. Renaudel and L. Dumontier (Ludwig Institute for Cancer Research, Brussels, Belgium). Mice with a floxed Stat3 allele33 were kindly provided by V. Seyl (University of Veterinary Medicine, Vienna) with permission of V. Poli (Universita di Torino, Torino, Italy). Vil1−/− mice34 were provided by M. Stemmler (Max-Planck-Institute of Immunobiology & Epigenetics, Freiburg, Germany) with permission of P. S. Robine (Institute Curie, Paris, France). Mice with an intestinal epithelial cell (IEC)-specific deletion of Stat3 (Stat3(−/−)) were generated by breeding Stat3tm1a mice with Vil1−/− mice. I22ra1−/− mice were generated by crossing Lgr5−/− mice with Rorc(+/−)/Il22ra1−/− mice33. Ahrα−/− mice33 and AhRβ−/− mice were provided by C. Esser (Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany). Ahrβ−/−Rorc(+/−)/Il22ra1−/− mice were obtained by crossing Ahrβ−/− mice with Rorc(+/−)/Il22ra1−/− mice33. To identify LGR5+ stem cells, we back-crossed I22ra1−/− and I22−/− mice to Lgr5-EGFP-ΔloxP tm1a (referred to as I22−/−ILgr5EGFP+ mice or I22−/−ILgr5EGFP− mice). Mice were 8 to 10 weeks of age, unless indicated otherwise. Cohoused littermates were used as controls whenever possible. Animals were housed in accordance with the guidelines by the Federation for Laboratory Animal Science Associations and the national animal welfare body Gesellschaft für Versuchstierkunde. Experiments were in compliance with the German animal protection law (TierSchG) and were approved by the local animal care committee (G15-1-034, Landesuntersuchungsamt Rheinland-Pfalz, Koblenz).

Generation of I22ra1−/− mice. I22ra1−/− mice were generated using EUCOMM embryonic stem (ES) cells subclone JM8.N4 (I22ra1tm1a(EUCOMM)Wtsi)37. In brief, a floxed I22ra1 allele was created by inserting an FRT-flanked IRES-LacZ-Neo resistance cassette into intron 1 of the I22ra1 gene. Loxp sites were inserted to flank exon 2 (Extended Data Fig. 2a–g). After germline transmission, the FRT cassette was removed by crossing to FlPe deleter mice33. For inactivation of the I22ra1 gene, I22ra1−/− mice were back-crossed to Lgr5−/− mice to Vil1−/− mice. Ahrα−/− mice33 and AhRβ−/− mice were provided by C. Esser (Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany). Ahrβ−/−Rorc(+/−)/Il22ra1−/− mice were obtained by crossing Ahrβ−/− mice with Rorc(+/−)/Il22ra1−/− mice33. To identify LGR5+ stem cells, we back-crossed I22ra1−/− and I22−/− mice to Lgr5-EGFP-ΔloxP tm1a (referred to as I22−/−ILgr5EGFP+ mice or I22−/−ILgr5EGFP− mice). Mice were 8 to 10 weeks of age, unless indicated otherwise. Cohoused littermates were used as controls whenever possible. Animals were housed in accordance with the guidelines by the Federation for Laboratory Animal Science Associations and the national animal welfare body Gesellschaft für Versuchstierkunde. Experiments were in compliance with the German animal protection law (TierSchG) and were approved by the local animal care committee (G15-1-034, Landesuntersuchungsamt Rheinland-Pfalz, Koblenz).

RNA-seq analysis of LGR5+ and LGR5− colon stem cells. The colon, including the anorectal section, was opened longitudinally and cleaned of faeces and fat tissue. Epithelial cells were harvested by incuba-
inhibitors without EDTA (Thermo Fisher)) for 1 h on ice and additionally sonicated for 3 cycles using Bioruptor Plus (Diagenode). SDS–PAGE was performed with gels containing 5% or 10% polyacrylamide and blotted for 16 h at 40 mA on a PVDF membrane (Carl Roth). Detection antibodies p21 (FS; Santa Cruz), PUMA, p-p53 (all Abcam), actin (AC15; Sigma-Aldrich), p-SMC (D7S87), mouse ATM (DD2E2), SMC (86E6) and ±H2AX (D1A4) (T33) (all Cell Signaling Technology) were diluted in 5% BSA and incubated overnight at 4 °C. Secondary antibodies were coupled to HRP (anti-mouse–HRP anti-rabbit–HRP; Jackson Immuno Research) and visualized using enhanced chemiluminescence (PerkinElmer) and Hyperfilm (GE Healthcare). All antibodies used are listed in Supplementary Table 2.

**STAT3 CHIP.** Mice were injected with 1 μg recombinant IL-22 (Peprotech) or PBS intraperitoneally 1 h before epithelial cell isolation. Epithelial cells were isolated as described above. Cells were resuspended in PBS and fixed with 0.75% paraformaldehyde. Fixation was stopped with glycine and cells were washed and lysed in 50 mM Tris, 2.5 mM EDTA, 0.1% NP-40 and 10% glycerol. Nuclei were lysed in 50 mM Tris, 5 mM EDTA and 0.25% SDS and subsequently sonicated in a Bioruptor Plus (Diagenode). Lysates were cleared by centrifugation and stored at −80 °C. Protein A–coated magnetic beads (Diagenode) were blocked with yeast tRNA (Life Technologies) and BSA (New England Biolabs). Nuclear lysates were split as 10% input and 90% immunoprecipitation solutions. The latter were incubated with STAT3 antibody (C-20; Santa Cruz Biotechnology) overnight, with rotation at 4 °C. Protein A–coated magnetic beads were added and incubated for another 30 min with rotation at 4 °C. Beads were purified and washed repeatedly. Bound DNA was eluted in 10 mM TrIS, 1 mM EDTA and 2% SDS at 37 °C for 15 min. De-cross-linking was performed overnight at 65 °C. DNA was removed by 1 h incubation with RNAase A (PeproTech) at 37 °C, followed by 1 h incubation with proteinase K (PeproTech) at 42 °C. DNA was purified using PCR Purification Kit (Qiagen) according to the manufacturer’s instructions. All antibodies used are listed in Supplementary Table 2.

**Micronucleus assay.** Mycoplasma-free IEC6 cells48 (a gift from M. Horner, RWTH Aachen) were cultured for 48 h in DMEM (Life Technologies) with 10% FCS, 80 μM 2-mercaptoethanol, 10 mM HEPES, 1 mM sodium pyruvate, non-essential amino acids (Gibco), 100 μM penicillin, 0.4 mg/ml gentamicin, 100 mg/ml streptomycin, and 8 mg/ml glucose and 0.1 μM insulin (Sigma-Aldrich), with or without 100 ng/ml IL-22 (Peprotech). After culturing cells in the presence of IL-22 for three days, cells were irradiated with 2 Gy. They were then cultured for another 48 h in the presence of cytochalasin B (4.5 μg/ml; Sigma-Aldrich) to stop cytokinesis. Nuclei of cells that had undergone division, and the occurrence of micronuclei within these cells, were counted under a fluorescence microscope. Between 500 and 1,000 nuclei per replicate were analysed. For analysis of primary epithelium ex vivo, mice were irradiated with 2 Gy and epithelial cells were isolated by EDTA-based dissociation as described above. For micronucleus assessment cells were settled on glass slides and fixed for 2 min in ice-cold methanol. DNA was stained with DAPI (Sigma-Aldrich) and images were acquired on an Axiovert 200M microscope (Zeiss).

**Ctnnb1 mutations.** Mice were co-housed and injected with 10 mg/kg body weight (Tvo) RNA (Sigma-Aldrich) intraperitoneally on day 1, day 8 and day 15. Mice were sacrificed on day 75, when no macroscopic tumour development could be observed. Epithelial cells from the colon were isolated as described above and genomic DNA was extracted using QIAamp Fast DNA Tissue Kit (Qiagen) according to the manufacturer’s instructions. CastPCR (Thermo Fisher) was used to detect single base-pair mutations in the Ctnnb1 gene. Reported AOM-induced mutations were found from the literature42 and compared to the OMIM criteria. Reported AOM-induced mutations were found from the literature42 and compared to the OMIM criteria.

**Data availability**

RNA-seq data are available at ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) under accession number E-MTAB-6723. All datasets generated and/or analysed during the current study are presented in this published article, the accompanying Source Data or Supplementary Information files, or are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | Increased inflammation in Il22−/− mice during AOM–DSS carcinogenesis. a–f, Mice were given one dose of AOM intraperitoneally, followed by one week of 2% DSS in drinking water. a, Tumour development in Il22+/+ and Il22−/− mice. Scale bar, 1 cm. Tumour number (b) (Il22+/+, n = 9; Il22−/−, n = 8; mean ± s.e.m.; P = 0.022) and regional distribution of tumours (c) (Il22+/+, n = 7; Il22−/−, n = 10; mean ± s.e.m.; rectum, P = 0.0007; total, P = 0.0078). d, Body weight as percentage of initial weight (Il22+/+, n = 7; Il22−/−, n = 10; mean ± s.e.m.). e, Expression of the indicated inflammatory and tumour-promoting genes (n = 4–6; mean ± s.e.m.) in colonic mucosa was assessed by qRT–PCR two days after DSS treatment was stopped. f, Expression of the indicated genes was analysed by qRT–PCR within tumours (n = 3–9, mean ± s.e.m.). g, Schematic representation of the CAC treatment in mice with sporadic inactivation of the Il22ra1 gene (Il22ra1fl/fl;Lgr5creERT2+/--;R26R-Confetti and Il22ra1fl/fl;Lgr5creERT2+/--;R26R-Confetti). Data are representative of two biologically independent experiments.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Generation and validation of Il22ra1flox mice.
a, Schematic representation of the Il22ra1tm1a(EUCOMM)Wtsi allele in targeted ES cells. The position of PCR primers and Southern blot probes is indicated. b, Verification of construct integration into JM8.N4 ES cells by genomic PCR. DNA from untargeted ES cells (Bruce4) served as a control. c, Southern blot after BamHI digestion, confirming the correct integration of the construct, which introduced a novel BamHI recognition site into the genomic DNA. d, Germline transmission was assessed by genomic PCR in the F1 generation. e, LacZ reporter activity was assessed in F1 mice. f, Gene expression of the IL22RA1 binding partners in colonic epithelial cells of the indicated mouse strains was assessed by qRT–PCR (Il22+/+, n = 5; Il22−/−, n = 4; Il22ra1+/+, Vili1-cre, n = 4; Ahr+/+; Rorc(γt)-cre, n = 3; mean ± s.e.m.). g, Representative immunofluorescence of IL22RA1 (red) in colonic epithelial cells. Scale bars, 50 µm. h, i, Il22ra1+/+, Lgr5ERT2Δ; R26R-Confetti or Il22ra1−/−; Lgr5ERT2Δ; R26R-Confetti mice were fed with a tamoxifen-containing diet for two weeks. h, Labelling efficiency of colon crypts at different time points after stopping the tamoxifen treatment (day 1: Il22ra1+/+, n = 8; Il22ra1−/−, n = 10; day 7: Il22ra1+/+, n = 7; Il22ra1−/−, n = 8; day 14: Il22ra1+/+, n = 7; Il22ra1−/−, n = 7 visual fields; mean ± s.e.m.). i, Fraction of Confetti+ crypts (mean) with one, two or three colours. j, The indicated mouse strains were fed with tamoxifen-containing diets and subjected to AOM–DSS treatment as indicated. Body weight is shown as percentage of initial weight (Il22ra1−/−, n = 6; Il22ra1+/+, n = 10; mean ± s.e.m.). Data in f–j are representative of two biologically independent experiments.
Extended Data Fig. 3 | IL22RA1 expression in tumours from Il22ra1^{fl/fl}; Lgr5^{creERT2/2+}; R26R-Confetti mice. a–d, Representative IL22RA1 immunofluorescence (violet) by Confetti-negative (a, c) and Confetti-positive (b, d) colon tumours from Il22ra1^{fl/+}; Lgr5^{creERT2/2+}; R26R-Confetti (a, b) and Il22ra1^{fl/fl}; Lgr5^{creERT2/2+}; R26R-Confetti (c, d) mice. e, MFI of IL22RA1 fluorescence across tumour area of the indicated mouse strains with and without prior Cre activation (n = 7, except Il22ra1^{fl/fl} Confetti-negative, n = 8; mean ± s.e.m.).
Extended Data Fig. 4 | Identification of IL-22-producing lymphocytes. 
a–c, Mice of the indicated genotypes or strains were injected with AOM or PBS (control). Twenty-four hours later, IL-22+ cells among the indicated lymphocyte subsets and absolute numbers of IL-22+ cells were determined. a, Colon of C57BL/6 mice (n = 6; mean ± s.e.m.; P = 0.0154). b, Small intestine of C57BL/6 mice (n = 6; mean ± s.e.m.). c, Colon of C57BL/6 mice (n = 2; mean ± s.e.m.). Data are representative of two biologically independent experiments.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Initiation of the DDR is impaired in Il22−/− mice. a–c, Il22+/+;Lgr5GFP and Il22−/−;Lgr5GFP mice (n = 3 each) were irradiated with 8 Gy (DNA damage) or left untreated (steady-state). LGR5(GFP)+ colon epithelial stem cells were highly purified and gene expression was analysed by RNA-seq. a, Sorting strategy for colonic LGR5+ stem cells. Stem cells were highly purified (>98%) for RNA-seq analysis. b, Modified Venn diagram. All expressed genes with fold change greater than 2 are represented. The bottom half of the diagram represents genes differentially expressed between Il22+/+ and Il22−/− mice at the steady state. 'Core expression' contains all genes with detectable transcripts that were not differentially expressed. The top half represents genes differentially expressed 24 h after irradiation in comparison to untreated mice. Of these, 596 genes differed in both genotypes, whereas 638 genes were only regulated in stem cells of Il22+/+ mice and 392 genes were regulated in stem cells of Il22−/− mice. c, GSEA was performed on all expressed transcripts represented in LGR5+ colon stem cells from Il22+/+ (n = 3) and Il22−/− (n = 3) mice at the steady state. Nominal P value (Kolmogorov–Smirnov) was calculated for 'Double strand break processing' (P = 0.01). d, Mice were irradiated with 8 Gy. Expression of DDR effector genes p21 and Puma in colonic epithelial cells was determined by qRT–PCR at the indicated time points after irradiation (n = 3; mean ± s.e.m.). e, List of genes included in the GSEA gene set 'DDR effector genes'4 (Fig. 2b). f, Mice were irradiated with 8 Gy. Gene expression of Il22bp (also known as Il22ra2) in lamina propria or colon epithelial cells was determined by qRT–PCR at the indicated time points (n = 3; mean ± s.e.m.). g, h, Expression of the indicated genes in colon epithelial cells was determined by qRT–PCR at steady state: MRN complex genes (g, Il22+/+, n = 7; Il22−/−, n = 6; mean ± s.e.m.); DDR transducer genes (h, Il22+/+, n = 10, except Atmin, n = 7 and Parp1, n = 5; Il22−/−, n = 7, except Atmin, n = 4 and Parp1, n = 5; mean ± s.e.m.).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | IL-22 regulates ATM expression and formation of γH2AX. **a,** Immunoblot analysis of ATM from colon epithelial cells. Densitometric quantification of signal intensity normalized to loading controls (n = 3; mean ± s.e.m.; P = 0.004). **b,** Schematic representation of STAT3-binding sites in the Atm gene locus. **c,** STAT3 ChIP was performed to determine specific pulldown at the transcription factor-binding sites (start, mid, end) in the promoter regions of the Atm gene and at a control site within the Atm gene (cold). Genomic DNA from colon epithelial cells of Il22+/+ and Il22−/− mice before and after in vivo stimulation with recombinant IL-22. Results represent specific pulldown relative to a DNA input sample (start, n = 14; mid, n = 7; end, n = 12; cold, n = 8; mean ± s.e.m.). **d, e,** Mice of the indicated genotypes were irradiated with 8 Gy. **d,** Immunofluorescence analysis for γH2AX (red) and EpCam (blue) in the entire colon was performed 8 h after irradiation. Scale bars, 500 µm. **e,** Quantification (control, 4 h and 24 h, n = 2; 8 h, n = 3; mean ± s.e.m.) of γ-H2AX+ nuclei per colon before and at the indicated time points after irradiation. **f,** Immunoblot analysis of H2AX protein and densitometric quantification of signal intensity (normalized to loading controls) in primary colon epithelial cells of Il22+/+ and Il22−/− mice (mean ± s.e.m.: n = 2). **g, h,** Mice were irradiated with 8 Gy or injected intraperitoneally with AOM. Immunofluorescence analysis of colon sections was performed 8 h after damage. Quantification (g; n = 3, mean ± s.e.m.) of γ-H2AX+EpCam+ cells and representative immunofluorescence images (h) after γ-H2AX (red) and EpCam (blue) staining. Scale bars, 25 µm.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Impairment of p53 activation and DDR effector genes in Il22−/− mice. a–g. Mice were irradiated with 8 Gy or injected with AOM. a, b, Immunoblot analysis of the indicated proteins from primary colon epithelial cells 24 h after irradiation. Densitometric quantification of signal intensity (mean ± s.e.m.; n = 2). Phosphorylated (p)-p53 and actin, p-SMC and SMC, PUMA and actin, and p21 immunoblots were derived from one gel each. Band densities were normalized to loading (p-p53, PUMA) or sample-processing (p-SMC, SMC, p21) controls. c, d, Fold induction of p21 and Puma gene expression in colon epithelial cells 24 h after irradiation or AOM treatment was determined by qRT–PCR (AOM, n = 10; 8 Gy, n = 5; Stat3fl/fl, n = 4; Stat3fl/fl;Vil1-cre, n = 3; mean ± s.e.m.). e, Apoptosis (red, cCaspase-3) in LGR5+ (blue) colon stem cells (arrows). Representative immunofluorescence images 8 h after irradiation. f, g. Quantification of cCaspase-3+ EpCam+ colonic epithelial cells 8 h after treatment with AOM or 8 Gy irradiation (f; n = 3; mean ± s.e.m.) and representative immunofluorescence images (g; cCaspase-3, red; EpCam, blue). Scale bars, 25 μm. Data are representative of two (d–g) or three (a–c) biologically independent experiments.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Impairment of DDR-triggered apoptosis in Il22<sup>−/−</sup> mice. a–c, Mice of the indicated genotypes were irradiated with 8 Gy and analysed 8 h later. a, Immunofluorescence for cCaspase-3 (red) and EpCam (blue). Scale bars, 50 µm. Quantification of cCaspase-3<sup>+</sup> cells by histology (b; Il22<sup>+/+</sup>, n = 3; Il22<sup>−/−</sup>, n = 6; Il22ra1<sup>fl/fl</sup>;Vil1-cre, n = 3; Ahr<sup>fl/fl</sup>;Rorc<sup>(γt)-cre</sup>, n = 4, mean ± s.e.m.) or by flow cytometry (c; n = 3; mean ± s.e.m.; P = 0.003). d, IL-22 neutralizing antibody or control IgG was injected intraperitoneally 36 h and 12 h before, and 12 h after AOM application. Representative immunofluorescence images of the colon 24 h after AOM treatment, stained for LGR5 (blue) and cCaspase-3 (red; arrows indicate apoptotic stem cells). Right, quantification of cCaspase-3<sup>+</sup> crypts (n = 3; mean ± s.e.m.; P = 0.0039). e, IL-22-responsive IEC6 cells were cultured with or without recombinant IL-22. Cells were irradiated with 2 Gy or left untreated and cultured in the presence of cytochalasin B. DNA was visualized with DAPI 48 h after irradiation and number of cells containing one or more micronuclei was counted (n = 4, mean ± s.e.m.). Representative images of untreated (ctrl) or irradiated (2 Gy) IEC6 cells. Arrow indicates a micronucleus. f, Mice of the indicated genotypes were irradiated with 8 Gy. Quantification of BrdU<sup>+</sup> cells in colonic crypts (n = 3; mean ± s.e.m.). Data are representative of two biologically independent experiments.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Dietary AhR ligands promote IL-22 production. 

a, Wild-type mice were given 1-MIM-OH or carrier control (ctrl) by gavage. Quantification of DNA adducts at the indicated time points (control 4 h, n = 2; control 8 h, n = 4; control 24 h, n = 2; 1-MIM-OH 4 h, n = 4; 1-MIM-OH 8 h, n = 8; 1-MIM-OH 24 h, n = 6; mean ± s.e.m.). 

b, Schematic representation of the different treatment regimens. 

c–g, Mice of the indicated genotypes were exposed to 1-MIM-OH (c, f) or I3C (d, e, g) by gavage and analysed 8 h later. 

c, d, Representative flow cytometry analysis of IL-22 production by the indicated lymphocyte populations after gavage with 1-MIM-OH (c) or I3C (d). CD4+ T cells were defined as CD45+ lineage marker (Lin)+CD4+ cells, ILC3 as CD45+Lin−RORγt+ cells, and γδ T cells as CD45+Lin−γδ TCR+ cells. Numbers indicate percentage of cells in gate. 

e, Representative flow cytometry analysis and quantification of IL-22 and IL-10 production by Lin−CD4+FOXP3+ Treg cells 8 h after gavage with I3C (control, n = 4; I3C, n = 4; mean ± s.e.m.). 

f, g, Expression of the IL-22 response gene Reg3g in primary colon epithelial cells was determined by qRT–PCR 8 h after gavage with 1-MIM-OH (f; Il22+/− control, n = 5; Il22+/− 1-MIM-OH, n = 4; Il22−/− control, n = 3; Il22−/− 1-MIM-OH, n = 6; mean ± s.e.m.) or I3C (g; n = 5; mean ± s.e.m.). 

h, Percentage of IL-22+ cells among the indicated lymphocyte subsets 8 h after 1-MIM-OH treatment of Ahr−/− mice (control, n = 3; 1-MIM-OH, n = 4; mean ± s.e.m.). 

i, Numbers of IL-22+ ILC3 cells at different time points after 1-MIM-OH gavage (control, n = 2; 1-MIM-OH, n = 4; 1-MIM-OH 24 h, n = 6; mean ± s.e.m.). 

Data are representative of two biologically independent experiments.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Diet-derived AhR-ligands control IL-22 production. a–d, Mice were fed for 6 weeks with either normal, grain-based chow (CD), EDD− or EDD+. a, Representative flow cytometry analysis of IL-22 production by the indicated lymphocyte populations. Numbers indicate percentage of cells in gate. b, Expression of the IL-22 responsive gene Reg3g in primary colon epithelial cells was determined by qRT–PCR (n = 5; mean ± s.e.m.). c, d, Representative colon immunofluorescence images 8 h after irradiation stained for γH2AX (red, c) or cCaspase-3 (red, d) and EpCam (blue). Scale bars, 50 μm. Data are representative of three biologically independent experiments. e, Model of IL-22-mediated protection of epithelial stem cells against genotoxic stress. Epithelial stem cells at the intestinal barrier are continuously exposed to a variety of insults. Among them are threats to genomic integrity represented by genotoxins contained in diets or produced by bacteria. Genome integrity in stem cells requires a functioning DDR pathway to limit damage to the genetic code in order to prevent malignant transformation. Our data implicate a homeostatic regulatory loop that assesses the degree of ‘genotoxic danger’ by AhR-mediated sensing of nutrient-derived genotoxins that serve as AhR ligands. AhR signalling in ILC3 and γδ T cells controls IL-22 production. IL-22 signalling in intestinal epithelial stem cells in turn controls components of the DDR. In particular ATM, a PI3-kinase-like molecule and an important upstream transducer of the DDR, was upregulated by IL-22 signalling in stem cells. Following genotoxic stress, ATM controls downstream events of the DDR such as p53 activation and expression of the DDR effector molecules p21 and PUMA, which control DNA repair and apoptosis, respectively. Disturbances of this pathway in mice genetically lacking IL-22, the IL-22 receptor or the AhR led to an impaired DDR to various forms of genotoxic stress. Consequently, intestinal epithelial stem cells from mice with impairment of IL-22 signalling or production accumulated cancer-promoting mutations and showed accelerated development of cancer.
Reporting Summary

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LAS X (Leica); FACS Diva (BD Biosciences)

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FlowJo V10 software (TreeStar); Graph Pad Prism V7 (GraphPad Software); Fiji 2.0.0 (ImageJ); Cufflinks 2.2.1; R Studio 3.3.2, GSEA v2.2.3, GIMP 2.8

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RNA-Seq data is available at the ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) with the accession number E-MTAB-6723.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
Three to twenty mice were used per experiment. The precise numbers are stated in the Figure legends and are visible as separate dots in the graph. In preliminary experiments the D value (Difference in means) / (Standard Deviation) was calculated. The exact sample size was determined under consideration of the D value, available resources (genotypes) and ethical aspects (implementing the three Rs). If an insufficient number of animals was available for a reliable significance prediction, biologically independent repetition experiments were performed and data pooled for analysis.

**Data exclusions**
Data points that fulfilled Peirce’s criterion for outliers were considered carefully. If the investigators concluded that they were presented with an uncorrectable measurement error, it was not considered for further analysis. Exclusion of outliers, as they happen in biomedical research with living animals, was pre-established in the laboratory.

**Replication**
Experiments were repeated that the presented data is based on at least two to three biologically independent experiments with similar results (for most important conclusions up to ten times e. g. apoptosis induction). If group size was small (due to limited availability of specialized reagents and mouse strains), data from replicate experiments were pooled for graphical representation. All replicates are biological replicates obtained from biologically independent experiments.

**Randomization**
We did not use randomization to assign animals to experimental groups. As whenever possible littermate controls were used, age did not constitute a variable (and was matched for non-littermates) and sex ratios were distributed evenly among experimental groups. Furthermore, we did not observe sex-specific differences in our findings.

**Blinding**
Investigators were not blinded to group allocations during the experiment and data analysis. Since treatment and experimental analysis could not be separated, blinding of the investigators was not possible. Histological scoring was performed by two researchers according to pre-set criteria. For quantification pre-defined algorithms/analysis sequence was performed by image analysis software and specific criteria were documented.

Reporting for specific materials, systems and methods

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Unique biological materials |
| ☑   | Antibodies |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq |
| ☑   | Flow cytometry |
| ☑   | MRI-based neuroimaging |

#### Unique biological materials

Policy information about availability of materials

**Obtaining unique materials**

1-MIM-OH, Detailed description for synthesis has been provided in PMID: 20846518. All other Materials are available at commercial vendors.

**Antibodies**

| Antibodies used | Flow Cytometry |
|-----------------|----------------|
| Target Clone Vendor Cat number Most recent Lot# |
| CD3e 145-2C11 eBioscience 13-0031-82 E02347-1633 |
| CD5 53-7.3 eBioscience 13-0051-82 4319149 |
Validation

All antibodies were validated by the commercial manufacturers (according to the vendor’s websites).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

IEC6 were kindly provided by M. Hornef (Aachen, Germany)

Authentication

The cells were not authenticated, but were tested to be specifically responsive to IL-22.

Mycoplasma contamination

All cell lines in the laboratory are randomly tested for Mycoplasma contamination by PCR. IEC6 were Mycoplasma negative.

Commonly misidentified lines (See CLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

C57BL/6N, Lgr5EGFP-IRES-CreERT2/+, Rosa26R-Confetti, Ahr fl/fl;Rorc(γt)-CreTg, Il22-/-, Stat3fl/fl;Vil1-Cre, Il22ra1fl/fl;Vil1-Cre, Ahr-/-: All mice had been crossed onto a C57BL/6N background for at least 10 generations.
Mice were 8 to 10 weeks of age. Male and female mice were used in a ratio 1:1. Glucosinolate-chow experiments were performed with only female C57BL/6N.

Wild animals
No wild animals were used in this study.

Field-collected samples
No field-collected samples were used in this study.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
This information is provided in detail in the M&M section.

Instrument
BD FACS CANTO II for Analysis, BD FACS ARIA III for Cell Sorting

Software
FACS DIVA (BD Biosciences) and FlowJo V10 (TreeStar)

Cell population abundance
Purity of sorted populations was assessed by post-sort analysis on the BD FACS ARIA III. Purity of over 98% was routinely achieved.

Gating strategy
An example for the Lgr5 sorting strategy is provided in Fig S4a. For lymphocyte analysis cells were gated the following way: SSC-A/FSC-A (lymphocytes)--SSC-A/SSC-W (Singlets)--CD45+(lymphocyte marker)--seperated into Lin(CD3,CD5,CD19,Gr-1,CD64,F4/80)pos CD4+ (CD4+ Tcells), Lin neg RORgt+ (ILC3), Lin pos gdTCR+ (gd Tcells), Lin pos CD4+ FoxP3+ (FoxP3+ Tcells)

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.