Supplementary Methods

Sample collection and assessment

The first 10cm of the small intestine was excised and preserved in formalin fixed paraffin embedded blocks for histological evaluation. In our experience (Bond et al 2018), most murine serrated lesion are concentrated in this region. Intestinal epithelial cells were scraped using a scalpel blade from the mucosa of the next ten centimeters were snap-frozen for molecular analyses, with the remainder of the intestine preserved for histological assessment. If macroscopic murine serrated lesions were identified in the tissue section usually reserved for DNA methylation, care was taken to collect tissue >1cm from the margin of the lesion. Four micron sections were cut and stained with hematoxylin and eosin using standard methods. Histological features were assessed by a specialist gastrointestinal pathologist (CL), who previously established the histological criteria for assessing murine serrated neoplasia[23]. From the frozen samples, DNA/RNA was extracted using the Qiagen AllPrep Mini Kit (Qiagen, CA, USA) as per the manufacturer's protocol. DNA quality was assessed using QuBit BR dsDNA assay kit (Thermofisher, MA, USA). We extracted RNA from formalin fixed, paraffin embedded lesions and hyperplasia using the TruXTRAC RNA Plus Magnetic bead kit as per the manufacturers instructions (Covaris, USA). RNA was subsequently concentrated and purified using ethanol precipitation.

Immunohistochemistry

Immunostaining for β-Catenin was performed as per Kane et al (2020). Sections were dewaxed and rehydrated, before undergoing heat-induced antigen retrieval in a declocking chamber for 8 minutes at 121º in Dako high antigen retrieval solution (Agilent, USA). Sections were incubated in 5% hydrogen peroxide for peroxidase blocking. Sections were subsequently stained overnight with an anti-β-Catenin antibody (1:500, Abcam, UK). Slides were incubated with the MACH1 universal polymer HRP detection reagent (Biocare Medical, USA) for an hour, prior to incubation with DAB for 8 minutes. Sections were counterstained with hematoxylin. For analysis, slides were digitized using a Turbo XT slide scanning instrument (Aperio, USA), and analyzed using the QuPath software.

Organoid Culture

10 month old BrafCA/Villin-CreERT2 that had not been induced by Tamoxifen in vivo were sacrificed and a 0-25cm portion of the small intestine was harvested, washed with PBS and fragmented for organoid culturing where briefly, intestinal pieces were incubated in chelating buffer (2mM EDTA) before being shaken to release crypts. Isolated crypts were passed through a 70uM filter, mechanically dissociated and suspended in 25ul 100% matrigel droplets in 48 well plates. These were overlaid with organoid media consisting of Advanced DMEM/F12, 1X Antibiotic/Antimycotic, 50ug/ml Gentamicin, 10mM Hepes, 1X GlutaMAX, 1X B27, 1X N2 (all Life Technologies). Niche factors included 20% Rspo conditioned media, 50ng/ml EGF (Peprotech) and 100ng/ml Noggin (Peprotech). For the first 2 days post isolation and passaging, 10uM Y27632 (In Vitro), 3uM GSK-3i XVI (Calbiochem #361559) and 3uM Thiazovivin/iPSC (Calbiochem #420220) were also included in the organoid media. Cultures were passaged twice weekly where organoids were collected in cold PBS, incubated in TryplE before organoid pieces were split at a 1:3 ratio and plated in matrigel. Organoid media was changed every 2-3 days.

Approximately 1-2 weeks after organoid culture was established, DNA methylation was inhibited by treating organoids with 250nmol 5-Azacytidine for 48 hours, with media and drug replacement at 24 hours. After which, oncogenic Braf was induced using by exposing organoids to 1uM 4-OHT (Sigma #H7904) for 24 hrs. Converted organoids were selected and maintained in organoid media without EGF growth factor.

Organoids were fixed in 10% formalin, washed in PBS, pelleted in 2% agarose and dehydrated in 70% ethanol for paraffin embedding and sectioning. H&E stains were applied as per standard protocol.

Reduced representation bisulfite sequencing and analysis

We assessed genome scale DNA methylation using the NuGen Ovation RRBS Methyl-Seq system. In brief, DNA is digested by the methylation insensitive MspI restriction enzyme. This enzyme digests DNA at CCGG motifs, resulting in fragments of ~250-300bp that correspond to regions with high CpG content. Sequencing these fragments allows for the assessment of hundreds of thousands to millions of CpG sites without sequencing the entire genome.

RRBS Sequencing preprocessing and data analysis

We performed single-end 100bp sequencing on libraries using the NovaSeq 6000 S1 flowcell with a target of 30 million reads per sample. Output BCL files were converted to fastq format, and demultiplexed using bcl2fastq2. Adapters were removed and reads trimmed for poor quality using TrimGalore and the Nugen Diversity adaptor trimming script (trimRRBSdiversityAdaptCustomers.py). Reads were aligned to the mm10 using Bismark (v0.20.0)[64]. Positions with poor coverage (<10X in any sample) were filtered and methylation fractions extracted using the methyKit R package (1.8.1)[65]. Epigenetic age was modelled by elastic net regression using the glmNet R package using an alpha of 0.5. The cv.glmnet function was used for ten-fold cross validation and the identification of the minimum lambda. For human samples the model was trained using data from Luebeck et al (2019)[47] and validated using data from Barrow et al (2018)[48]. Murine epigenetic age was also assessed using two external epigenetic age models and a model developed in-house. For analysis with the Stubbs et al epigenetic clock, clock sites were extracted and missing clock sites imputed using the K-Nearest neighbor method. Data was quantile normalized with data from Stubbs et al prior to epigenetic age estimation. For analysis with the epigenetic clock proposed by Meer et al clock sites were extracted and sites not present in any individual sample were excluded from all samples prior to epigenetic age estimation. For construction of our in house epigenetic age model, epigenetic age was modelled by elastic net regression using the glmNet R package using an alpha of 0.5. The cv.glmnet function was used for ten-fold cross validation and the identification of the minimum lambda. The model was trained on wild type animals aged 24 days to 20 months. The coefficients generated from these analyses are available as supplementary materials. CpG sites were annotated using the AnnotatePeaks.pl script contained within the HOMER software suite.

RNA-Sequencing and data analysis

Transcript expression was assessed in a subset of animals (Table 1) by RNA-Sequencing. Total RNA sequencing libraries were prepared using the TruSeq Stranded Total RNA with

RiboZero Gold kit (Illumina, CA, USA) with 500ng of input total RNA. Input RNA was quality assessed using the TapeStation platform (Agilent, CA, USA), and RNA fragmentation and PCR cycling optimized as per the manufacturers protocol. Libraries were sequenced on an Illumina NextSeq 550 platform to an average target depth of 50,000,000 75bp paired end reads per sample. For data processing, including quality control, adaptor trimming, alignment (to mm10), and transcript quantification we used nf-core rnaseq pipeline (v1.4.2). As the library is stranded the –reverseStranded flag was used. In brief, this pipeline trims adaptors with TrimGalore and aligns reads with STAR, marks duplicates and calculates mean fragment sizing using Piccard, and quantifies gene expression using Subread. Various quality control metrics are produced using RseQC, FastQC, Qualimap, Preseq, DupRadar, EdgeR and MultiQC. These reports were assessed to maintain pre and post alignment quality control. Counts generated using featureCounts (Subread) were normalized to fragment length and library size (FPKM) using countToFPKM. Differential expression analysis was performed using DeSEQ2.

Nanostring Gene Expression Assay

To assess transcript expression of FFPE derived RNA from mSLs and hyperplastic tissue we used the nCounter system (Nanostring, USA). The nCounter system is a probe-based technology that is optimized for analysis of degraded samples, including those from FFPE samples. 5uL of RNA was hybridized to probes mapping to 750 cancer-associated genes (PanCancer Pathway Panel, Nanostring, USA) for 24 hours at 55ºC. Samples were then processed on the Nanostring Prep Station as per the manufacturers instructions, and quantified using the Nanostring Digitial Analyzer. For analysis, samples were processed using the standard, Nanostring-endorsed pipeline on the ROSALIND platform.

Statistical analysis

Statistical analyses were performed in R and JMP (v13) and are reported throughout the manuscript. For ANCOVA analysis between wild type and *Braf* mutant animals the model was as follows: Chronological Age ~ Epigenetic Age * *Braf* Status. Differential DNA methylation and Transcript expression was assessed using MethylKit and DEseq2, respectively. For enrichment analysis we used the PANTHER Enrichment tool, with gene lists from the Gene Ontology database (release date: 08/10/2019) and PATHER pathways database (release date: 03/12/2019). Where appropriate, P values were corrected for multiple testing using the false discovery rate method. $P<0.05$ was the threshold for statistical significance.