SUPPLEMENTARY MATERIALS AND METHODS

Virus production and organoid transduction

pLenti6-miRNA control and pLenti6-miRNA KMT9α constructs were used to produce recombinant lentiviruses as previously described (1). Organoid transduction with lentivirus was performed as previously described with some modifications (2). Briefly, patient-derived CRC samples were harvested with ice-cold PBS and dissociated using trypsin for 5min at 37°C. After trypsin inactivation and centrifugation, single cell suspensions were incubated with lentivirus and 8 μg/mL polybrene. Cells were spinoculated at 600g for 1h at 37°C and then incubated for 3h at 37°C. Transduced cells were collected and plated in Matrigel domes (1:1 growth factor-reduced Matrigel/Advanced DMEM-F12 medium). After Matrigel polymerization, domes were covered with the appropriate medium supplemented with 10μM Y27632 (Sigma).

Organoid size assessment

Pictures of organoids were taken using the EVOS™ FL cell imaging system. To determine organoid size, the diameter was measured in two different axes with Adobe Photoshop (RRID:SCR_014199) and the organoid volume was determined by the formula: $V = 4/3 \times \pi \times r^3$. The volume of Kmt9α WT organoids was normalized to 100% and the volume of Kmt9α KO organoids was calculated accordingly.

Colonic epithelial cells isolation

The colons of Kmt9α$^{IEC\text{-}WT}$ and Kmt9α$^{IEC\text{-}KO}$ mice were harvested after 3 months of feeding with Tamoxifen food. Colons were opened lengthwise, washed twice with cold PBS and incubated with chelation buffer (distilled water with 5.6mM Na_2HPO_4, 8mM KH_2PO_4, 96.2mM NaCl, 1.6mM KCl, 43.4mM sucrose, 54.9mM D-sorbitol, 0.5mM DTT and 2mM EDTA). After incubating for 15 min at 4°C with gentle agitation, samples were thoroughly vortexed to dissociate the colonic epithelial cells, and the supernatant was collected and kept on ice. The remaining tissue fragments were incubated for another 15 min with fresh chelation buffer and

the entire procedure was repeated twice. The supernatants were pooled and colonic epithelial cells were pelleted by centrifugation at 1000g for 10 min at 4°C. Protein extraction was performed with RIPA buffer and RNA was isolated using RNeasy Mini columns (Qiagen).

Core histone isolation

Organoids were dissociated with TrypLE for 20 min at 37°C, washed with PBS and resuspended in Triton Extraction Buffer (TEB; PBS, Triton X100, 2 mM PMSF, 0.02% NaN_3). After 10 min of incubation on ice, cell lysates were centrifuged and supernatants were discarded. After washing a second time with TEB, cell pellets were resuspended in 0.2 N HCl and the histones were extracted overnight at 4°C. Core histone concentration was determined by Bradford assay.

Western blot analysis

The following antibodies were used for Western blot analysis: anti-KMT9α (#27630, lot 27022018, Schüle Lab) ; anti-KMT9β (#28358, lot 18122018, Schüle Lab, Fig. 1C), anti-KMT9β (TRMT112, #A14310, lot 0066060201, AB clonal, RRID:AB_2761174, Supplementary Fig. S1E); anti-Casp3 (Caspase3, #9662S, Cell Signaling, RRID:AB_331439); anti H4 (#ab10158, lot GR3267840-1, Abcam, RRID:AB_296888); anti H4K12me1 (#27429, lot 27062017, Schüle Lab); anti-α-Tubulin (alpha tubulin, #T6074, lot 03714804V, Sigma, RRID:AB_477582); anti-β-actin (#A1978, lot 012M4821, Sigma, RRID:AB_476692).

Cell proliferation assay

Cell proliferation was determined using Click-iT EdU Alexa Fluor 647 Flow Cytometry Assay Kit according to the manufacturer's instructions (Invitrogen). AOM/DSS tumour organoids were treated with 1μM Tam or EtOH (vehicle) and 7 days later, organoids were incubated with 10μM EdU for 6h. The organoids were then dissociated into a single cell suspension with TrypLE, washed twice with 1% BSA in PBS and processed for EdU staining according to the

manufacturer's protocol. EdU fluorescence was determined by flow cytometry using a BD LSR-Fortessa Cell Analyzer and data were analysed with FlowJo software.

Flow cytometry

For apoptosis assays and cell cycle phase distribution analyses, AOM/DSS tumour organoids were treated with 1μM Tam or EtOH (vehicle) as a control. Five days later, the organoids were dissociated into single cell suspensions using TrypLE for 20 min at 37°C. After dissociation, single cell suspensions were washed twice with PBS (300g, 5 min). For apoptosis analysis, cells were stained with AnnexinV-APC in binding buffer together with 2μg/mL DAPI for 30 min at room temperature according to manufacturer's protocol (Biolegend, Apoptosis Detection Kit). The percentage of early apoptotic cells (Annexin V positive and DAPI negative cells) were analysed with FlowJo software. Cell cycle phase distribution was determined according to manufacturer's protocol (BD Pharmingen, APC BrdU Flow Kit). Flow cytometry was performed using BD LSR-Fortessa Cell Analyzer and data were analysed with FlowJo software (RRID:SCR_008520).

Quantitative RT-PCR analysis

Quantitative RT-PCR was performed using the Abgene SYBR Green PCR kit (Invitrogen) according to the supplier's protocol. *Hprt* and *HPRT* were used for normalization. Primers used for the qRT-PCR are described in Supplementary Table S1.

Single-cell mRNA sequencing (scRNA-seq)

Filtered raw-count matrices were further analysed using the Seurat (v3) R package (RRID:SCR_016341) (3) for data integration and unbiased clustering of single cell transcriptomes. Low-quality single cell libraries with less than 500 detected genes and more than 10% mitochondrial counts were excluded (Supplementary Fig. S3A-D). In addition, two low-quality clusters with low numbers of detected genes and high percentages of mitochondrial genes that lacked expression of biologically relevant cell type markers were removed after

initial clustering. Cell cycle phases (G1/S/G2M) were assigned to each cell using the *CellCycleScoring* function and meta-signature scores for apoptosis were assigned to each cell using the *AddModuleScore* function with gene lists derived from the Molecular Signature Database (https://www.gsea-msigdb.org/gsea/index.jsp) and Supplementary Table S3.

Both Tam and EtOH (vehicle) datasets were normalized independently using the SCTransform method (4). Technical (percent.mt, nFeature_RNA) and biological confounders (S.Score, G2M.Score) were regressed out using the *var.to.regress* argument in the *SCTransform* function. Both datasets were harmonized using canonical correlation analysis (CCA Seurat v3) with default parameters. Principal component analysis (PCA) was used for dimensionality reduction and the K-nearest neighbor (KNN) graph was constructed using the first 30 principal components (PCs). Cells were clustered using the Louvain algorithm and embedded in two-dimensional space (30 PCs) using Uniform Manifold Approximation and Projection (UMAP). Differentially expressed genes between clusters were identified using Wilcoxon Rank Sum test and clusters were assigned to four major cell types according to expressed marker genes and their similarity to intestinal cell types (Supplementary Fig. S3E-K). We merged transcriptional clusters if their average gene expression profiles were highly correlated and if they were characterized by similar cell type-specific marker genes. Pseudotime analysis was computed by Monocle3 [https://cole-trapnell-lab.github.io/monocle3/, (RRID:SCR_018685)], a plugin for genesorteR as previously described (5-7) and trajectories were visualized using Cerebro-app (8). Plots were generated with Seurat, genesorteR or custom R script using ggplot2.

Hematoxylin and eosin and immunohistochemical staining

Colon sections were harvested, fixed in 10% formalin for 24h, washed twice in PBS and then embedded in paraffin. Paraffin blocks were sliced into 5μm thick sections and slides were stained using a standard procedure for hematoxylin and eosin staining. For immunohistochemical staining, slides were deparaffinized with xylene, rehydrated in a descending alcohol series, and finally washed with PBS and H_2O. Antigen retrieval was performed by pressure cooking in 20mM Citrate Buffer (pH 6.0) and the samples were

incubated with 3% H_2O_2 for 30 min to reduce endogenous peroxidase activity. After blocking for 1h in PBS containing 3% milk and 0.1% Tween®20, samples were incubated overnight at 4°C with primary antibodies: 7.5μg/ml anti-KMT9α (#27630, lot 27022018, Schüle Lab), 1μg/ml anti-KI67 (#M7249, Dako, RRID:AB_2250503) or IgG control (#Kch-504-250, Diagenode, RRID:AB_2722554). Slides were washed and primary antibodies were detected using Vectastain ABC KIT, Rabbit IgG (#PK-4001, Vector Laboratories, RRID:AB_2336810) according to the manufacturer's instructions. Peroxidase substrate (Vector NovaRED, #SK-4800, Vector Laboratories, RRID:AB_2336845) was added and the slides were washed with H_2O, counterstained with hematoxylin and then mounted with Fluoromount-G Mounting Medium (Southern Biotech).

TUNEL assay

Apoptotic activity in AOM/DSS tumours of Kmt9α$^{IEC\text{-}WT}$ and Kmt9α$^{IEC\text{-}KO}$ mice was determined in 5μm thick sections of paraffin-embedded tissue using an *In Situ* Cell Death Detection Kit (#11684795910, Sigma Aldrich). For manual analysis, Fiji software (RRID:SCR_002285) was used.

References

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