Supplementary Note 2: Supplementary Methods

Mouse jejunum samples

Mice were housed in ventilated polycarbonate cages, and given *ad libitum* sterile food (Harlan 2918X) and water. Adult mice were housed by sex in groups of 2-5 littermates. The vivarium was maintained under controlled temperature (21°C±1°C) and humidity (50-60%), with a 12-h diurnal cycle (lights on: 0700-1900).

mRNA analyses

Sections of small intestine (~30 mg) were homogenized with a hand-held homogenizer on ice (VWR; human jejunum samples) or a ceramic bead-based homogenizer (Precellys; for mouse jejunum samples) in 1 mL RLT-buffer (Qiagen) supplemented with 10 µl β-mercaptoethanol. Total RNA was subsequently extracted using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Purified RNA samples were treated with RNase-free DNase I (Qiagen) at room temperature for 30 minutes and then repurified with the RNeasy Mini Kit. RNA yield was quantified using UV spectrophotometry (NanoDrop ND-1000, Thermo Fisher Scientific), and RNA integrity was verified on an Agilent Bioanalyzer 2100 system (Agilent Technologies).

Total RNA from human epithelial colorectal adenocarcinoma (Caco-2, ATCC) cells was extracted using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. Extracted RNA samples were treated with DNase I (Qiagen), repurified using the RNeasy Mini Kit (Qiagen), and quantified and analyzed as described above.

Total RNA (750 ng) was converted to cDNA using High-Capacity RNA-to-cDNA Kit (Life Technologies). For each sample, a negative control (without reverse transcriptase treatment) was prepared to confirm the absence of DNA contamination. Steady-state mRNA levels were assayed with TaqMan Gene Expression Master Mix (Life Technologies) using Applied Biosystems ViiA 7 Real-time PCR system. qPCR cycling conditions were: 50°C for 2 min, 95°C for 10 min and 40 cycles of (95°C for 15 sec, 60°C for 1 min). Samples were ran in triplicate for each gene and a reference sample was

used for inter-plate normalization. TaqMan gene expression probes (Life Technologies) for mouse and human are detailed in Supplementary Table 3. mRNA of villin 1 and sucrase-isomaltase were used as endogenous controls. ΔΔCt was used to calculate the relative steady-state mRNA levels of each sample.

Detailed statistical analyses of the qPCR data were performed using Statistica (Statsoft). The data were analyzed using repeated-measure (RM) ANOVA with the appropriate between-subjects and within-subject factors. Significant effects and interactions were further analyzed by Tukey's honest significant difference (HSD) post hoc comparisons or by unpaired two-tailed Student's t-test to 95% confidence intervals. mRNA levels are expressed as mean ± standard error of the mean (s.e.m.).

Intestinal epithelial cell isolation and DNA extraction

To isolate epithelial cells (enterocytes) from the villi of the jejunum we optimized a method based on a chelating approach[23]. Jejunum samples were washed four times in 1 mL citrate buffer (27 mM Na-citrate, 5.6 mM Na_2HPO_4, 96 mM NaCl, 8 mM KH_2PO_4, 1.5 mM KCl, pH 7.4) containing 10 µl of DNase inactivation reagent (Life Technologies) with gentle agitation. Enterocytes were released by exposing jejunum samples to 1 mL Ca^{2+}-chelating buffer (1.5 mM EDTA, 0.5 mM DTT, 10 mM NaH_2PO_4, 154 mM NaCl) at 37°C for 15 min with agitation (700 rpm). The remaining jejunum was separated from the released enterocytes and the previous step was repeated two additional times using fresh Ca^{2+}-chelating buffer. For each individual, the released enterocytes were collected by centrifugation at 1000 rpm for 5 min at 4°C, and the pellet was used for DNA isolation. DNA was extracted separately for enterocytes and the jejunum lacking enterocytes for each individual using standard phenol-chloroform DNA extraction methods in combination with Phase-Lock tubes (5 Prime). DNA quantity was measured by UV spectrophotometry (NanoDrop ND-1000, Thermo Fisher Scientific), and DNA quality was verified on a 1% agarose gel.

For DNA isolation from white blood cells, blood samples were first mixed with 2.5% Triton X-100 and cells were pelleted by centrifugation at 400 x g for 15 min at 4°C. The pelleted white blood cells were washed in a buffer (10 mM Tris-HCl pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, 2 mM EDTA),

centrifuged again and then DNA was extracted using standard phenol-chloroform methods. Sperm DNA isolation initially involved mixing sperm cells with 0.5% Triton X-100 and 10% SDS and centrifugation at 400 x g for 10 min at 4°C. Sperm cell pellets were washed once gently in PBS, pelleted again, and DNA was isolated with a standard phenol-chloroform approach. DNA quantity and quality was measured as described above.

mTAG-click method for DNA unmethylome enrichment

Enrichment of the unmodified DNA fraction was performed using the mTAG-click method, as described previously[24]. Briefly, genomic DNA was sonicated using a Covaris S220 instrument (200 cycles/burst, 10% duty factor, 3 min/sample) in 10 mM Tris-HCl (pH 8.5) to yield fragments with a peak size of 200 bp. Sheared DNA fragments were blunt-ended using the Fast DNA End Repair Kit (Thermo Scientific). DNA was then purified using the ZR-96 DNA Clean & Concentrator-5 kit (Zymo Research). For each sample, mTAG-click labeling was performed by mixing 300 ng of sheared and end repaired DNA with 25 µM Ado-6-azide cofactor and 90nM eM.SssI in Labelling buffer (10 mM Tris-HCl pH 7.6, 50 mM NaCl, 0.1 mg/ml BSA) in a 25 µl reaction. Reactions were incubated at 37°C for 30 min, followed by heating at 80°C for 10 min. Labelled unmodified cytosines were then biotinylated with 200 µM dibenzylcyclooctyne-S-S-PEG3-biotin (Click Chemistry Tools) in a 2 h incubation at 37˚C. Samples were again purified using the ZR-96 DNA Clean & Concentrator-5 kit (Zymo Research).

Biotinylated DNA fragments were captured with 1.25 mg/ml Dynabeads MyOne Streptavidin T1 (Invitrogen) in 5X BW buffer (25 mM Tris-HCl (pH 7.5), 2.5 mM EDTA, and 5 M NaCl). Samples were mixed with beads at 900 rpm for 2 h at room temperature. The beads were washed three times with 3X BW buffer and twice with 1X BW buffer. A qPCR reaction comparing an aliquot of each sample bound and unbound to beads confirmed the desired 25% labelling efficiency[24]. DNA was then released from the beads and biotin tags by a 1 h incubation in 50 mM DTT and 60 mM Tris pH 7.8 with mixing at 900 rpm.

The recovered DNA was ligated to adaptors (A-25 5'-AGTTACATCTTGTAGTCAGTCTCCA and A-19 5'-TGGAGACTGACTACAAGAT) in a reaction containing 0.5 mM ATP, 10 mM $MgCl_2$, 5% PEG, 3.3 µM adaptors and 10 a.u. T4 Ligase (Thermo Scientific). Samples were ligated overnight at 22˚C followed by an enzyme inactivation step of 65˚C 15 min and gradual cooling to 25 ˚C -1˚C/10 sec. Immediately afterwards, samples were treated with 32 mM β-mercaptoethanol for 10 min at room temperature and PCR-amplified. PCR amplification was done in two rounds. The first PCR was performed by adding buffer (63 mM Tris-HCl pH 8.8, 17 mM $(NH_4)_2SO_4$, 0.084% (v/v) Tween 20), 587 µM dNTPs, 1.5 µM adaptor-specific A-25 primer and 6.3U Taq polymerase (Thermo Scientific) for a 100 µl reaction. PCR cycling conditions were: 50°C for 1 min, 72°C for 2 min, 94°C for 5 min, 65°C for 1 min, 72°C for 5 min, 15 cycles of (94°C for 1 min, 65°C for 1 min, 72°C for 1 min), and 72°C for 2 min. The second round of PCR amplification contained 5 µl of the PCR product from the first round in Buffer (75 mM Tris-HCl (pH 8.8 at 25°C), 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20), 2 mM $MgCl_2$, 0.4 mM dNTPs with 0.02 mM dUTP, 1.5 µM A-25 primer and 6.3 U Taq polymerase (Thermo Scientific) in a 50 µl reaction. PCR cycling conditions: 95°C for 1 min, 10 cycles of (93°C for 30 sec, 65°C for 1 sec, 68°C for 90 sec + 5 sec/cycle), and 72°C for 5 min. PCR products were verified on a 2% agarose gel and purified with the QIAquick 96 PCR Purification kit (Qiagen).

Tiling microarrays and analysis

For the microarray hybridization, 7 µg of purified uracil-containing amplicons were fragmented to 50–100 bp using GeneChip Double-stranded DNA Terminal Labeling kit (Affymetrix). Individual samples were hybridized to a separate GeneChip Human Tiling 2.0R Array (B array, covering chromosomes 2, 9 and 19) or GeneChip Mouse Tiling 2.0R Array (A array, chromosome 1, 9 and 19). The arrays were washed, stained and scanned using an Affymetrix GeneChip Scanner as described in the Affymetrix ChIP Assay protocol.

For the human samples, the tiling array data of isolated enterocytes and enterocyte-deficient jejunum from 56 individuals were analyzed, along with 10 input control samples (no mTAG-click enrichment). Replicates were included for 22 samples (7 sample pairs of enterocytes and 15 pairs of

enterocyte-deficient jejunum). For the mouse samples, enterocytes from mice at postnatal day 6 (n = 14 mice) and 60 (n =15 mice) were examined. Tiling arrays intensities were extracted using bioconductor R package “AffyTiling". The mean intensities across input tracks were subtracted, and the intensities for the relevant chromosomes (human Hs-NCBIv36:chr2, mouse Mm-NCBIv33:chr1) were quantile normalized and log(2) transformed. Probe sequences were re-aligned to the genomes for each species (human GRCh37/hg19, mouse GRCm38/mm10) using mrfast-2.6.1.0 with no mismatches allowed. Reference genomic sequences were downloaded from the UCSC, with repetitive sequences masked (“random" and “chrUn" chromosomes were ignored). Only intensities for uniquely-mapping probe sequences were considered. For each sample, normalized intensities were then smoothed using a running pseudomedian filter[48] with span of 200 bp.

The mean correlation of each sample to other samples of the same tissue was calculated, and samples with correlations less than -2σ were excluded from further analysis (n = 4 human, n = 1 mouse). One additional human sample was excluded due to abnormally high *LCT* mRNA levels ($> \mu + 4\sigma$).

Probe filtering for the human samples involved a bootstrapping procedure that was used to identify the probes that were significantly more consistent between pairs of replicates than random samples of the same tissue. After filtering, 20 pairs of replicate samples remained (6 for enterocytes, 14 for enterocyte-deficient jejunum). First, the Pearson correlation was computed between each pair of replicates for every probe. Then the significance of this correlation was assessed against an empirical null distribution, which was calculated by measuring the correlation of 20 random pairs of samples, for each of 1000 iterations. Random pairs were chosen in a stratified fashion, such that they were always from the same class (both enterocyte or both enterocyte-deficient jejunum), while maintaining the same ratio of enterocyte and enterocyte-deficient jejunum samples as in the original replicates. Probes were kept for further analysis only if the average correlation between replicates was greater than or equal to that of 99% of random samplings, resulting in n = 57,583 probes. As expected, replicates clustered tightly together after this filtering step, but samples also clustered distinctly according to the tissue type. Remaining replicates were then averaged together, resulting in

a single data point per sample (to avoid imbalance in the statistical analysis). Finally, probes were filtered to the 5,482 probes that were significantly different between enterocytes and rest of jejunum, based on a paired Mann-Whitney test at each probe, Benjamini-Hochberg multiple testing correction, and a false discovery rate (FDR) threshold of $q < 0.01$.

In the analysis of the human samples, the significance of correlation between DNA modification and *LCT* steady-state mRNA was calculated using Kendall's nonparametric correlation test at each probe, followed by Benjamini-Hochberg multiple testing correction. For the mouse samples, age-associated probes were identified by performing a Mann-Whitney test at each probe and then applying the Benjamini-Hochberg correction for multiple testing with a threshold of $q < 0.01$ (n = 2,680,563 sites). The Mann-Whitney and Kendall non-parametric tests were used to assess significance because the array intensity values were not normally distributed. Due to the limited sample size and correlation between nearby probes, the Benjamini-Hochberg method was used to correct for multiple testing. Chromosomes 9 and 19 were subsequently analyzed using the same method.

Bisulfite padlock probes for fine-mapping of DNA modification in humans and mice

The bisulfite padlock probe technique[26] was used to examine DNA modifications at single nucleotide resolution in the *LCT* and *MCM6* loci of humans and mice. Padlock probes for target DNA regions (excluding repetitive elements and SNP sites) were designed for bisulfite converted DNA using the ppDesigner software[26]. CpG sites within probes were modified with a mixed nucleotide (CR, R = even mixture of A and G) to capture both modified and unmodified cytosines. Probe sequences are described in Supplementary Table 4.

Padlock probes were synthesized using a programmable microfluidic microarray platform (LC Sciences). Each probe consisted of 20-25 bp probe segments (used to interrogate a ~180 bp bisulfite-converted region of interest) on either end of a 30 bp common linker region (used for PCR amplification of captured regions). To prepare the probes, 1 nM of the mixed template oligonucleotides were PCR-amplified in the presence of 400 nM of each eMIP_CA1_F primer and eMIP_CA1_R primer

(Supplementary Table 4), and 1X KAPA SYBR fast Universal qPCR Master Mix. The PCR conditions were: 95°C for 30 sec, 5 cycles of (95°C for 5 sec, 50°C for 1 min, 60°C for 30 sec), 12 cycles of (95°C for 5 sec, 60°C for 30 sec, 72°C for 30 sec), and 72°C for 10 min. PCR amplicons were purified with QIAquick PCR purification columns (Qiagen). The purified amplicons were then re-amplified in 15 PCR reactions each containing 0.02 nM of amplicon, 400 nM each of eMIP_CA1_F primer and eMIP_CA1_R primer, and 1X KAPA SYBR fast Universal qPCR Master Mix. The PCR conditions were: 95°C for 30 sec, 15 cycles of (95°C for 5 sec, 60°C for 30 sec, 72°C for 30 sec), and 72°C for 10 min. These PCR reactions were pooled and amplicons were purified with QIAquick PCR purification columns (Qiagen). Probes (4 µg) were then digested with wild type AlwI (10 U/µl, NEB) and Nb.BrsDI (10 U/µl, NEB). Both digestions were performed in cutSmart buffer (NEB) at 37°C for 1 h, 55°C for 20 min, 60°C for 20 min, 65°C for 1 h, followed by an enzyme inactivation step of 80°C for 20 min. The probes were purified with a QIAquick PCR purification column (Qiagen). Further purification was completed by running the probes on a 6% denaturing Urea-PAGE gel and electro-eluted using D-Tube Dialyzer 6-8KDa tubes (Novagen).

Genomic DNA was bisulfite-converted and purified using the EZ DNA Methylation-Lightning Kit (Zymo Research). In tandem, whole genome amplified DNA (WGA1 kit, Sigma), a genome devoid of DNA modifications, was also bisulfite-converted and used to verify the high efficiency of the bisulfite conversion reactions (i.e. ~98% conversion in human samples). For each sample, 200 ng of bisulfite-converted DNA was hybridized to 0.12 ng of probes in 1X Ampligase Buffer (EpiCentre), using the following reaction conditions: 98°C for 3 min, 85°C for 30 min, gradual cooling to 60°C (-1°C/min), 60°C for 60 min, gradual cooling to 56°C (-1°C/min), 56°C for 300 min, gradual cooling to 45°C , (-1°C/min), and 45°C for a minimum of 120 min. Target region extension and circularization was completed in a reaction containing 1X Ampligase Buffer (Epicentre), 2.5 U PfuTurbo C_x (Agilent Technologies), 0.4 mM dNTPs, 5 U Ampligase (Epicentre), 200 µM NAD (NEB) that was incubated at 56°C for 1 hr, followed by enzyme inactivation at 72°C for 20 min. Each sample was then digested with exonucleases, using 8U exonuclease I (USB), 40 U exonuclease III (USB), 6 U RecJf, 2.5 U Lambda exonuclease, and 0.33X exonuclease III buffer (USB) in an incubation of 37°C for 30 min,

80°C for 20 min, 95°C for 5 min, and kept at 4°C. The samples were then PCR-amplified by mixing an 8 µl aliquot of DNA template with 1X PfuTurbo C_x Buffer, 0.25 mM dNTPs, 2.5 U PfuTurbo C_x DNA polymerase (Agilent Technologies), 0.45 µM AmpF6.4Sol Primer, 0.45 µM Reverse barcode primer (primers detailed Supplementary Table 4) in a 50 µl reaction. PCR conditions were: 98°C for 3 min, 35 cycles of (98°C for 30 sec, 50°C for 30 sec, 72°C for 1 min) and 72°C for 10 min. The PCR amplicons were visualized on a 2% agarose gel. Library pools were generated, containing ≤ 68 samples per pool. For each library pool, the desired ~330 bp band was excised from the agarose gel and purified using the QIAquick Gel Extraction kit (Qiagen). The purified libraries were then submitted for next-generation sequencing on an Illumina HiSeq 2500 machine in Rapid Run mode at the Donnelly Sequencing Centre in Toronto, Canada. Technical replicates contained in different sample pools confirmed a high reproducibility between the sequencing runs ($r > 0.99$).

Analysis of bisulfite padlock probes data

Using a custom pipeline[49] based on the Bismark tool[50], DNA modification density was interrogated at every cytosine covered by padlock probes. Reads were first trimmed and residual adapter sequences removed using Trimmomatic-0.32. To remove residual phiX DNA spiked-in as a sequencing control, reads were aligned to the phiX reference genome (NC_001422.1) using Bismark (560 bp from the beginning of the phiX genome was appended to the end to accommodate the circular nature of the genome for read alignment). Reads that did not align to phiX were aligned to the target reference genome (GRCh37/hg19 or GRCm38/mm10). Percent modification was estimated as the fraction of spanning reads that retained the reference “C”, and were not converted to “T” from the bisulfite treatment. Modification estimates were only considered if 30 or more reads spanned the cytosine.

For human intestine (enterocytes and enterocyte-deficient jejunum), sperm, and blood samples, DNA modification density was interrogated at 891 CpGs (14,830 cytosines) across 301 samples (plus 46 replicates). CpG sites were filtered for effect size and sparsity by excluding sites with an interquartile range < 0.05 (376 CpGs) or with coverage in fewer than 30 intestinal samples (233 CpGs). Exceptionally sparse samples with more than $\mu + 2\sigma$ missing data (18 samples) and outliers

(14 samples with a mean pairwise Pearson correlation with other samples of the same tissue < μ - 2σ) were excluded. Remaining replicates (n = 35) were merged by taking the mean across replicates at each CpG site, resulting in 58 blood, 18 sperm, 100 enterocyte, and 104 enterocyte-deficient jejunum samples (280 samples overall). To avoid genomic variants confounding the bisulfite sequencing results, we extracted the positions of all common polymorphisms called as part of the 1000 Genomes Project[51] (phase 3, v5a release). Polymorphisms were filtered to those with allele frequencies ≥ 5% either across all populations or within just the European (EUR) population. Cytosines within 1 bp of these polymorphisms were excluded from further analysis (17 CpGs). The correlation was measured between the DNA modification density at each remaining CpG (n = 282) and relative steady-state *LCT* mRNA using Kendall's nonparametric correlation test. Bonferroni multiple testing correction was then performed and CpGs were considered significant at a familywise error rate threshold $\alpha < 0.01$. CpGs exhibiting cell-type specific expression were identified by performing a nonparametric Mann-Whitney test at each CpG between the sets of enterocyte and enterocyte-deficient jejunum samples, followed by Bonferroni multiple testing correction and a significance threshold of $\alpha < 0.01$. The Kendall and Mann-Whitney tests were selected because neither the DNA modification density nor the steady-state *LCT* mRNA was normally distributed.

For mouse samples, DNA modification density was interrogated at 859 CpGs (11,611 cytosines) in 29 enterocyte samples. CpG sites were filtered for effect size and sparsity by excluding sites with an interquartile range < 0.05 (236 CpGs) or with coverage in fewer than 15 enterocyte samples (498 CpGs). The significance of association between DNA modification and age was measured separately at each CpG (n = 298) by comparing the modification levels of mice at postnatal day 6 (n = 16 mice) and day 60 (n = 13 mice) with a Mann-Whitney nonparametric test. This test was selected because the DNA modification densities were not normally distributed. Multiple testing correction was performed using the Benjamini-Hochberg method with a FDR threshold $q < 0.05$.

RNAi technique

The relationship between lncRNA *LOC100507600* and *LCT* mRNA levels was examined using RNA interference (RNAi) in Caco-2 cells (ATCC). One day prior to transfection, Caco-2 cells (passage number 3) were passaged into 6-well plates containing Eagle's minimum essential medium (EMEM, ATCC) supplemented with 20% (v/v) fetal bovine serum (FBS, ATCC) at 2.2 x 10^4 cells/cm^2 confluency. Immediately prior to transfection, the media was replaced with EMEM (without 20% FBS). Cells were transfected with either an equimolar pool of 4 siRNAs (30 pmol) directed to *LOC100507600* (Qiagen FlexiTube GeneSolution; SI05737893, SI05737900, SI0737907 and SI0737914) or a scrambled-control siRNA (Qiagen AllStars Neg. siRNA AF488, SI03650318) using RNAiMAX (Life technologies) according to the manufacturer's instructions. FBS (final 20% v/v) was added to the transfected-cell containing media 6 h after transfection. Prior to harvesting the cells at 48 h post-transfection, transfection efficiency was confirmed for siRNAs conjugated to AlexaFluor-488 using an Olympus FV1200 confocal microscope with a FITC filter (excitation 490 nm, emission 525 nm) and FluoView 1200 software (Olympus). The media was removed and cells were then harvested in 1 mL of TRIzol (Life Technologies). RNA was extracted and used for qPCR (as described above) by researchers blind to experimental conditions. mRNA levels were analyzed to evaluate the consequence of *LOC100507600* knockdown on *LCT* expression. Sample sizes (n = 8 scrambled control-treated, 11 RNAi-treated cell cultures) were comparable to other studies involving RNAi (Gong, C. & Maquat, L.E. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3' UTRs via Alu elements. *Nature* **470**, 284-8 (2011)).

CRISPR-Cas9n genome-editing of the Caco-2 human cell line

Genetic modifications in the Caco-2 cell line (ATCC), a model of human intestinal epithelial cells, were completed using the CRISPR-Cas9n system, as previously described[54,55]. We generated deletions in *MCM6* intron 13, *LCT* intron 2 or *LCT* intron 1 in the human cells. sgRNAs for the CRISPR-Cas9n system were selected using the MIT CRISPR Design tool[55], avoiding exons, splicing regions and repetitive elements, as described above.

Deletions in the human cells were generated using the pSpCas9n(BB)-2A-GFP plasmid[54,55] (gift of Feng Zhang, Addgene 48140) and pU6-sgRNA-BB plasmid, a derivative of the pX335 plasmid (gift of Feng Zhang, Addgene 42235). To engineer a pU6-sgRNA-BB plasmid encoding sgRNA backbone, the *cas9* gene was eliminated by XbaI and PsiI double digestion of pX335 vector. The plasmids expressing targeting sgRNA were prepared by ligating oligoduplexes (Supplementary Table 3) in to pU6-sgRNA-BB vector over BbsI sites, as described (Cong, L. *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819-23 (2013)). The PCR fragments encoding U6 promoter and targeting sgRNAs were generated with the GG-701 and GG-702 primer pair (Supplementary Table 3).

Caco-2 cells were plated and grown in 100 mm dishes containing culture medium composed of EMEM (ATCC) supplemented with 20% FBS (ATCC) and 2X Antibiotic-Antimycotic (Life Technologies). Cells at 70% confluency were passaged at a ratio of 1:4 using 1X trypsin-EDTA (Life Technologies). Cells at passage number 3 were used in the CRISPR-Cas9n experiment. At 22 h before transfection, Caco-2 cells were passaged, filtered to obtain single cells, and seeded into 24-well cell culture plates (1.5 X 10^5 cells/well) containing culture medium without antibiotic/antimycotic. For each transfection, 2 µg of the pSpCas9n(BB)-2A-GFP plasmid was pre-mixed with the appropriate sgRNA plasmids (1.2 µg each, *LCT* intron 1 and 2) or PCR fragments (80 ng each, *MCM6* intron 13) for each mutation. Two transfection replicates were prepared for the three separate mutations or Cas9n-GFP control, and these were randomly assigned to cell wells. Immediately before transfection, culture medium was replaced with 500 µl EMEM and the cells were transfected with Lipofectamine LTX according to the manufacturer's instructions. At 6 h after transfection, 500 µl EMEM with 20% FBS was added to each cell well. Transfection efficacy (≥ 70%) was confirmed 72 h, as discussed above. At 72 h post-transfection, the cells were prepared for fluorescence-activated cell sorting (FACS), which involved cell trypsinization, resuspension of cells in 2 mL of culture medium, filtering for single cells and a 30 min incubation in 0.5 ug/mL 7-AAV (Sigma) to detect dead cells. Seeding of 7-AAV negative and GFP-positive single cells into 96-well cell culture plates (1 cell per well) was performed by the University of Toronto's Faculty of Medicine Flow Cytometry Facility, Canada. Cells

were grown for ~4 weeks (each well containing a genetically homogenous population), followed by genotyping analysis and expansion into 24-well cell culture plates. Cell lines carrying homozygous deletions were confirmed by Sanger sequencing (ACGT Corporation in Toronto, Canada) and were used in further experiments.

At 70% confluency, cells were transferred into 6-well plates and then 100 mm dishes. For analysis of *LCT* mRNA levels, cells were seeded into 6-well plates (1.5×10^5 cells/well). Caco-2 cells containing a deletion in *MCM6* intron 13, *LCT* intron 1 or *LCT* intron 2, as well as controls (transfected with Cas9n) were collected at day 6 (undifferentiated state) or day 15 (differentiated, enterocyte-like state). On collection day, cells were rinsed three times with PBS pH 7.2 (Life Technologies), and then scraped and mixed with 1 mL TRIzol. After a 5 min incubation in TRIzol, the homogenates were snap frozen and stored at -80°C until further analysis. RNA was extracted and investigated in a qPCR assay (described above) by researchers blind to experimental conditions. *LCT* steady-state mRNA levels were measured in the CRISPR-Cas9n-modified Caco-2 cells. Cell culture sample sizes were comparable to other studies of CRISPR-Cas9-modified cell lines (Liao, J. *et al.* Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. *Nat Genet* **47**, 469-78 (2015)).

Genotyping

Human jejunum samples were genotyped for the genetic variants C/T-13910, G/A-22018 and the deletion in *LCT* intron 1. For each site, genomic DNA (50 ng) was amplified by PCR using the KAPA2G Fast kit (Kapa Biosystems) and 0.6 μM of each forward and reverse primer (Primers detailed in Supplementary Table 3) in a 25 μl reaction. PCR cycling conditions were: 95°C for 3min, 30 cycles of (98°C for 20 sec, 58.5°C (C/T-13910) or 61.5°C (G/A -22018, *LCT* intron 1 deletion) for 15 sec, 72°C for 15 sec) and 72°C for 1 min. The PCR amplicon for the C/T-13910 SNP was Sanger sequenced (ACGT Corporation in Toronto, Canada), and genotypes were identified by chromatogram analysis with the Sequencher program (GeneCodes). For the G/A -22018 SNP, PCR amplicons were digested with BstUI (NEB), which cleaved the G allele (225 and 393 bp fragments), but not the A allele (618 bp fragment). The presence of a deletion in *LCT* intron 1 was identified by PCR using a

primer set that overlapped the deleted site (244 bp band for non-deletion carriers) and a primer set that flanked the deletion site (944 bp band for deletion carriers) (for more details see ref[5]). Linkage disequilibrium between these genetic variants was examined using the Haploview 4.2 program.

For the CRISPR-Cas9 modified mice, genotyping was conducted with genomic DNA isolated from mice tail-clippings using a standard high salt method. Genomic DNA (100 ng) was PCR-amplified with 1X Herculase II buffer (Agilent Technologies), 1 mM dNTPs, 0.3 µM of each primer (Supplementary Table 3) and 1 U Herculase II Fusion DNA polymerase (Agilent Technologies) in a 50 µl reaction. PCR conditions were: 95°C for 7 min, 10 cycles of (95°C for 20 sec, 61°C (*Mcm6*, *Lct* intron 1) or 57°C (*Lct* intron 2) for 20 sec, 68°C for 5 min), 20 cycles of (95°C for 20 sec, 61°C (*Mcm6*, *Lct* intron 1) or 57°C (*Lct* intron 2) for 20 sec, 68°C for 5 min + 20 sec/cycle), and 68°C for 8 min. Mice carrying a CRISPR-Cas9 induced deletion in *Mcm6* intron 13, *Lct* intron 1 or *Lct* intron 2 were genotyped by surveying PCR amplicon size (detailed in Supplementary Table 3) on a 1.5% agarose gel.

For the CRISPR-Cas9n modifications in Caco-2 cells, genomic DNA was isolated for each cell colony with QuickExtract solution (15 µl/cell pellet, Epicentre) and incubated at 65°C for 6 min, and 98°C for 2 min. Screening for deletions in *MCM6* intron 13 and *LCT* intron 2 involved generating PCR amplicons by combining 200 ng of genomic DNA with 1X Herculase II buffer (Agilent Technologies), 1 mM dNTPs, 0.5 µM of each primer (Supplementary Table 3), 1 U of Herculase II fusion DNA polymerase (Agilent Technologies) in a 50 µl reaction. PCR conditions were: 95°C for 2 min, 10 cycles of (95°C for 20 sec, 54.4°C (*MCM6*) or 58.8°C (*LCT* intron 2) for 20 sec, 68°C for 5 min), 20 cycles of (95°C for 20 sec, 54.4°C (*MCM6*) or 58.8°C (*LCT* intron 2) for 20 sec, 68°C for 5 min + 20 sec/cycle), 68°C for 8 min. Deletion in *LCT* intron 1 involved PCR containing 200 ng of genomic DNA mixed with 1X ThermoPol buffer (NEB), 2 mM $MgCl_2$, 0.2 µM each primer (Supplementary Table 3), 0.2 mM dNTPs, 5 U Taq (NEB) in a 100 µl reaction, and PCR cycling conditions were: 95°C for 10min, 40 cycles of (95°C for 30 sec, 58°C for 35 sec, 68°C for 75 sec), 68°C for 4min. Deletions were determined by examining PCR amplicon size (described in Supplementary Table 3) on a 1.5% agarose gel.

Off-target analysis for CRISPR-Cas9-induced deletions

The MIT CRISPR Design tool[54,55] scores the likelihood of off-target activity for each sgRNA guide, providing a ranked list of putative off-target sites. We examined the most likely candidate regions for sgRNA off-target activity in both the mice with CRISPR-Cas9-induced deletions (2 sgRNAs/deletion) and Caco-2 human cells with CRISPR-Cas9n-mediated deletions (4 sgRNA guides/deletion). A total of 46 potential off-target sites were tested in the genetically-modified mice and 172 sites were tested in the Caco-2 human cell lines (Supplementary Table 3). Candidate off-target regions were amplified via PCR in a 25 µl reaction containing a final concentration of 1X KAPA2G Fast HotStart ReadyMix (KAPA Biosystems), 0.5 µM forward and reverse primers (Supplementary Table 3) and 100 ng of input DNA. The following cycling conditions were implemented for all reactions: 95°C for 3 min, 30 cycles of (95°C for 15 sec, 56°C for 15 sec, 72°C for 6 sec), 72°C for 90 sec. PCR products were run on a 1.5% agarose gel to verify fragment size. An enzymatic purification was then performed by adding 30 U of exonuclease I (NEB) and 3 U of FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific), and incubating for 15 min at 37°C, followed by a 5 min inactivation at 75°C. Purified amplicons underwent Sanger sequencing (ACGT Corporation in Toronto, Canada) using the forward primer (Supplementary Table 3). Sequences were analyzed for off-target activity by alignment with the mouse (GRCm38/mm10) or human (GRCh37/hg19) reference genome. To display the regions investigated we used the CIRCOS program version 0.67[56].