**Supplementary Materials and Methods**

RRBS (Step 1a)

Two separate bisulfite sequencing experiments were performed on esophageal tissues; the first focused on comparing adenocarcinomas to normal squamous mucosa and the second looked at Barrett’s cases (non-dysplastic, low grade and high grade dysplasia). Each discovery sequencing experiment included rigorously selected age- and gender-matched cases and cancer free controls. Normal buffy coat samples were used in each of the sequencing experiments to control for inflammation related background methylation. Tissue specimen types used in the discovery experiments are listed in methods section of the manuscript.

Fresh frozen (FF) tissues, formalin fixed paraffin embedded (FFPE) tissues, and buffy coat samples were provided by IRB-approved patient registries. Tumor and Barrett’s tissue sections were reviewed by an expert GI pathologist to confirm diagnosis and estimate abnormal cellularity. Sections were then macro-dissected. Genomic DNA was purified using the QiaAmp Mini kit (Qiagen, Valencia CA) and subsequently re-purified with the AMPure XP kit (Beckman Coulter, Brea CA). DNA (300 ng) was fragmented by digestion with 10 Units of MspI, a methylation-specific restriction enzyme which recognizes CpG-containing motifs, to enrich sample CpG content and eliminate redundant areas of the genome. Digested fragments were end-repaired and A-tailed with 5 Units of Klenow fragment (3’-5’ exo-), and ligated overnight to methylated TruSeq adapters (Illumina, San Diego CA) containing barcode sequences (to link each fragment to its sample ID.) Size selection of 160-340bp fragments (40-220 bp inserts) was performed using AMPure XP SPRI beads/buffer (Beckman Coulter, Brea CA). Buffer cutoffs were 0.7X - 1.1X sample volumes of beads/buffer. Final elution volume was 22 uL (EB buffer – Qiagen); qPCR was used to gauge ligation efficiency and fragment quality on a small sample aliquot. Samples then underwent bisulfite conversion (twice) using a modified EpiTect protocol (Qiagen). qPCR and conventional PCR (PfuTurbo Cx hotstart – Agilent, Santa Clara CA) followed by Bioanalyzer 2100 (Agilent) assessment on converted sample aliquots determined the optimal PCR cycle number prior to final library amplification. The following conditions were used for final PCR: 1.) each 50uL reaction contained 5uL of 10X buffer, 1.25uL of 10 mM each deoxyribonucleotide triphosphate (dNTP), 5uL primer cocktail (~5uM), 15uL template (sample), 1uL PfuTurbo Cx hotstart and 22.75 water; temperatures and times were 95C-5min; 98C-30sec; 16 cycles of 98C-10sec, 65C-30sec, 72C-30sec, 72C-5min and 4C hold, respectively. Samples were combined (equimolar) into 4-plex libraries based on the randomization scheme and tested with the bioanalyzer for final size verification, and with qPCR using phiX standards and adaptor-specific primers.

Samples were loaded onto flow cells according to a randomized lane assignment with additional lanes reserved for internal assay controls. Sequencing was performed by the Next Generation Sequencing Core at the Mayo Clinic Medical Genome Facility on the Illumina HiSeq 2000 platform. Reads were unidirectional for 101 cycles. Each flow cell lane generated 100-120 million reads, sufficient for a median coverage of 30-50 fold sequencing depth (read number per CpG) for aligned sequences. Standard Illumina pipeline software called bases and sequenced read generation in the fastq format. SAAP-RRBS, a Mayo developed analysis and annotation pipeline for reduced representation bisulfite sequencing, was used for sequence alignment and methylation extraction.

Candidate CpGs were filtered by a priori read-depth and variance criteria, significance of differential %-methylation between cases and controls, and discrimination of cases from controls based on area under the receiver operating characteristics curve (AUC) and target to background ratio. The primary comparison of interest was the methylation difference between cancer and Barrett’s cases, normal esophagus controls and leukocyte controls at each mapped CpG. CpG islands are biochemically defined by an observed to expected CpG ratio >0.6. However, for this model, tiled units of CpG analysis “differentially methylated regions (DMRs)” were created based on distance between CpG site locations for each chromosome. Islands with only single CpGs were excluded. Individual CpG sites were considered for differential analysis only if the total depth of coverage per disease group was ≥200 reads (an average of 10 reads/subject) and the variance of %-methylation was >0 (non-informative CpGs were excluded). Read-depth criteria were based on the desired statistical power to detect a 10% difference in the %-methylation between any two groups in which the sample size of each group was 18 individuals. Statistical significance was determined by logistic regression of the methylation percentage per DMR, based on read counts. To account for varying read depths across individual subjects, an over-dispersed logistic regression model was used, where dispersion parameter was estimated using the Pearson Chi-square statistic of the residuals from fitted model. DMRs, ranked according to their significance level, were further considered if % methylation in benign esophagus and leukocyte controls, combined, was ≤1% but ≥10% in Barrett’s cases.

Step 3

*Inclusion criteria:*

1. Subjects with known BE.
2. Patient between the ages 18 – 85.
3. Patients with a BE segment ≥ 1cm in maximal extent endoscopically.
4. Histology showing evidence of intestinal metaplasia with or without presence of dysplasia.
5. Undergoing clinically indicated endoscopy.
6. Subjects without known evidence of BE
7. Undergoing clinically indicated diagnostic endoscopy.

*Exclusion criteria:*

a. Subjects with known BE.

1. Patients with prior history of ablation (photodynamic therapy, radiofrequency ablation, cryotherapy, argon plasma coagulation). *Patients with history of endoscopic mucosal resection alone will not be excluded.*
2. Patients with history of esophageal or gastric resection.

b. Subjects with or without known evidence of BE (on history or review of medical records).

1. Pregnant or lactating females.
2. Patients who are unable to consent.
3. Patients with current history of uninvestigated dysphagia.
4. History of eosinophilic esophagitis, achalasia.
5. Patients on oral anticoagulation including Coumadin, Warfarin.
6. Patients on antiplatelet agents including Clopidogrel, unless discontinued for five days prior to the sponge procedure.
7. Patients on oral thrombin inhibitors including Dabigatran and oral factor X a inhibitors such as rivaroxaban, apixaban and edoxaban, unless discontinued for five days prior to the sponge procedure.
8. Patients with history of known varices or cirrhosis.
9. Patients with history of esophageal or gastric resection.
10. Patients with congenital or acquired bleeding diatheses.
11. Patients with a history of esophageal squamous dysplasia.

Patients were randomized to either the more compressible 25 mm/10 ppi sponge, or the less compressible 25 mm/20 ppi sponge. The capsule was swallowed with a few sips of water and patients were given the option of pharyngeal anesthesia with lidocaine spray before or after the procedure. The sponge was removed using the attached cord after 10 minute interval. Following retrieval, the sponge was placed in a vial containing 20mL of cell preservation buffer (PreservCyt, Cytyc Corporation, Marlborough MA) and vortexed. The suspension was removed and the process repeated with an additional 20 ml buffer. The 40mL suspension was centrifuged at 500 x g for 5 minutes to pellet the cells. DNA was extracted using the Gentra Puregene Buccal cell kit, as above. Following bisulfite conversion (Zymo Research), the samples were assayed by QuARTs (quantitative allele-specific, real-time, target and signal amplification)(ref). Briefly, QuARTS is a highly sensitive and specific multiplexed amplification technique which combines a polymerase-based target amplification with an invasive cleavage-based signal cascade. The fluorescence signal is detected in a fashion similar to real-time PCR. Oligos, including primers, invasive oligos, and detection probes, were designed for 27 markers, the 22 original candidates and five additional markers from the tissue validation phase. Standards were constructed from cloned plasmid-derived marker sequences where absolute copy numbers were determined by Poisson modeling after serial dilution. Samples were preamplified using corresponding primer sets, diluted, and amplified by QuARTs using the LightCycler 480. QuARTS reactions were performed using 400–600 nmol/L of each of the primers and detection probes, 100 nmol/L invasive oligo, 600–700 nmol/L of each FAM (Hologic), Yakima Yellow (Hologic), and Quasar 670 (BioSearch Technologies) FRET cassettes, 6.675 ng/μl cleavase 2.0 (Hologic), 1 U hot-start GoTaq polymerase (Promega), 10 mmol/L MOPS, 7.5 mmol/L MgCl2, and 250 μmol/L of each dNTP. QuARTS cycling conditions consisted of 95 °C for 3 min, 10 cycles at 95 °C for 20 s, 67 °C for 30 s, and 70 °C for 30 s, 37 cycles at 95 °C for 20 s, 53 °C for 1 min, and 70 °C for 30 s, and 40 °C hold for 30 s.