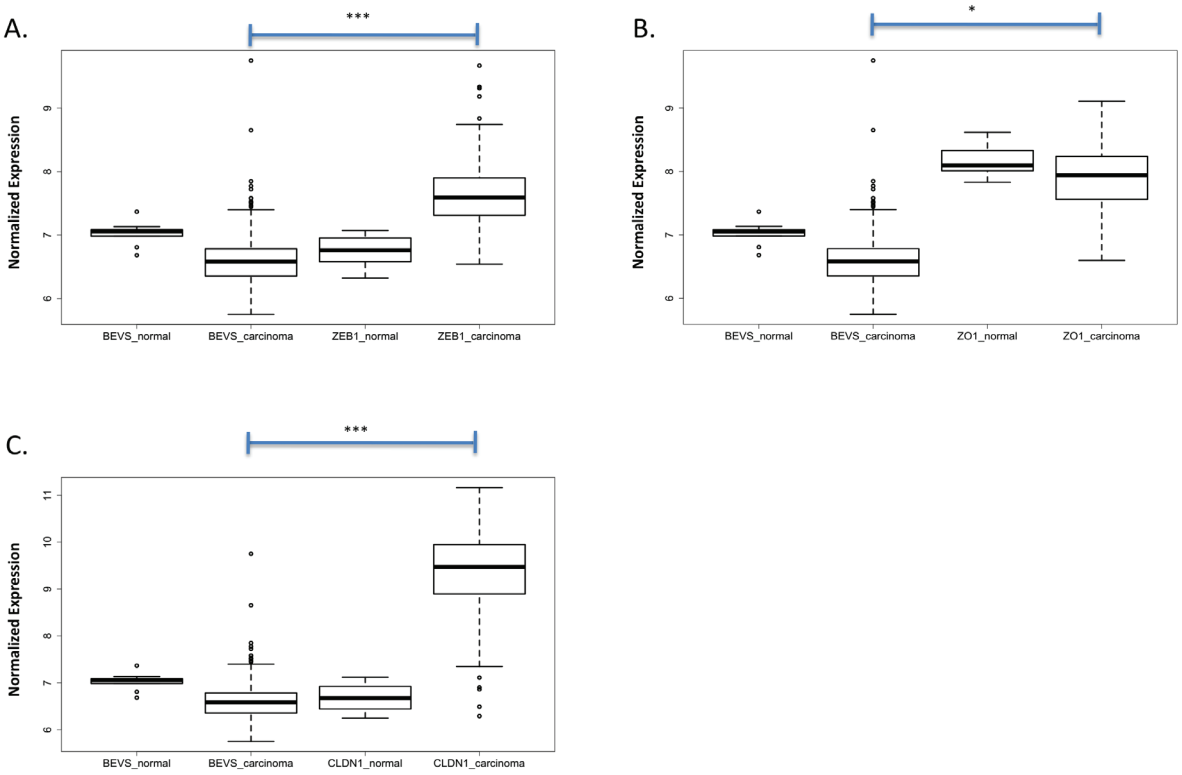
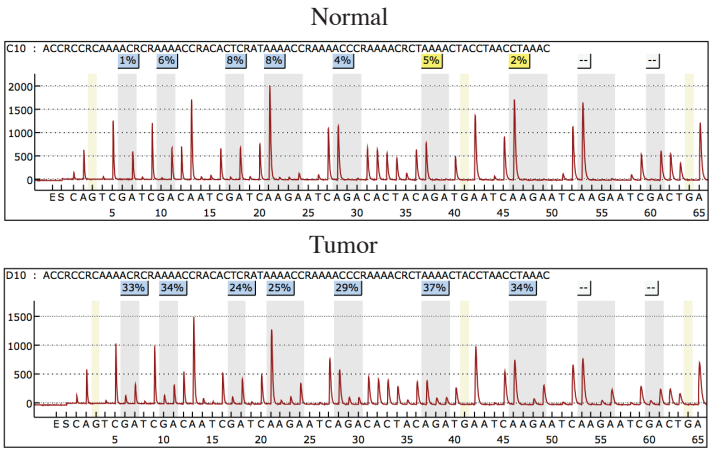
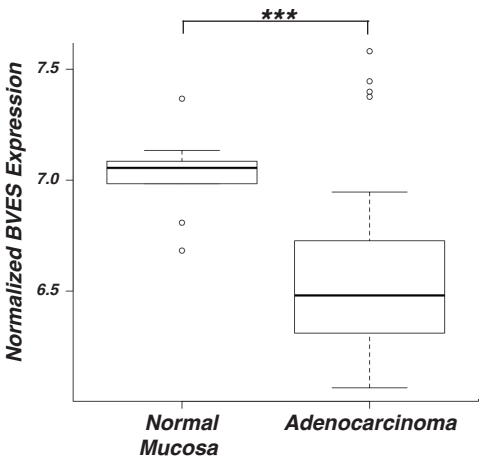


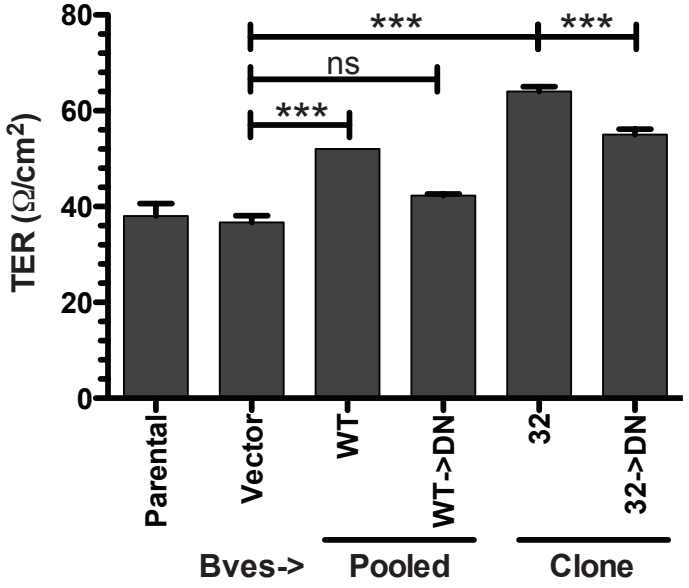
Supplemental Figure S1: Inverse relationship between BVES, ZEB1 and Claudin-1 and parallel down-regulation of ZO-1 in adenocarcinoma. Vanderbilt and Moffitt combined colon cancer expression array sub-set analysis comparing the relationship between BVES expression in carcinoma to ZEB1 (A) and Claudin-1 (C) and ZO-1 (B). *** P<0.001; * P<0.05.



Supplemental Figure S2 BVES CpP island pyrosequencing. Representative sequence tracings from bisulfate modified DNA from either adjacent normal mucosa or CRC.

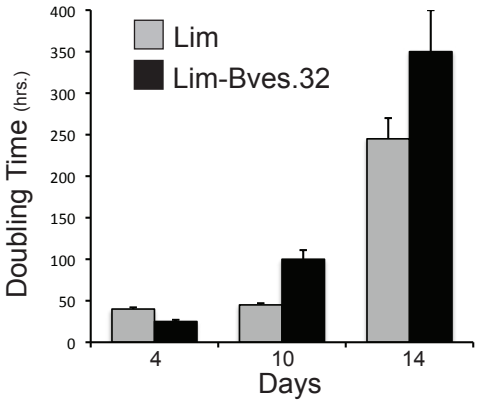


Supplemental Figure S3 BVES expression in CRC samples showing BVES hypermethylation. Sub-set BVES expression analysis of CRC samples with BVES hypermethylation (N=18). *** *P*=0.001.

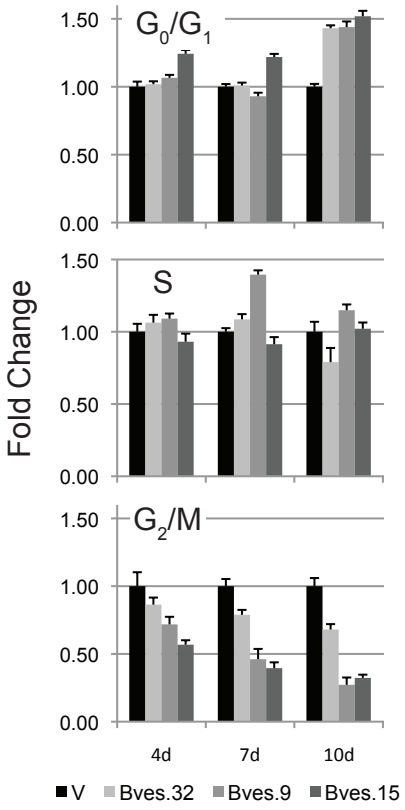


Supplemental Figure S4 BVES increases TER in Lim2405 cells. Pooled BVES transfected, Pooled BVES + DN BVES transfected Lim2405 lines and BVES stable clones 32 and BVES stable clone 32 transfected with DN-BVES were grown to confluence and the transepithelial resistance was measured. Experiments performed in triplicate. (*** *P*=0.001, Student's t test.)

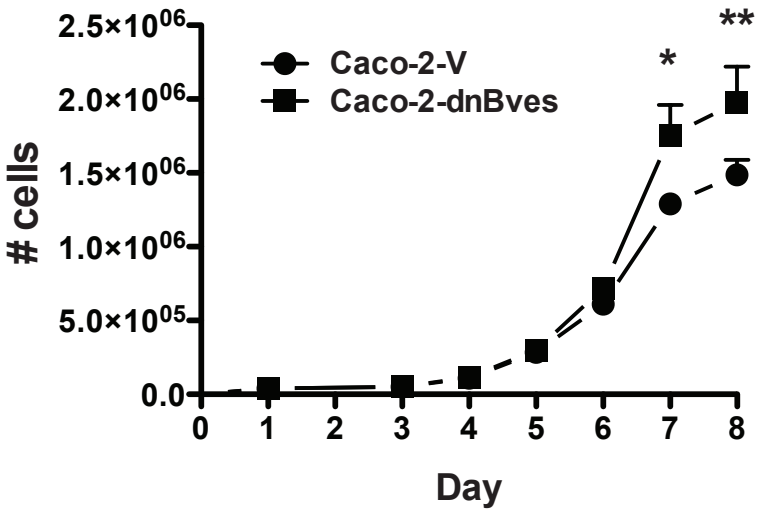
A)



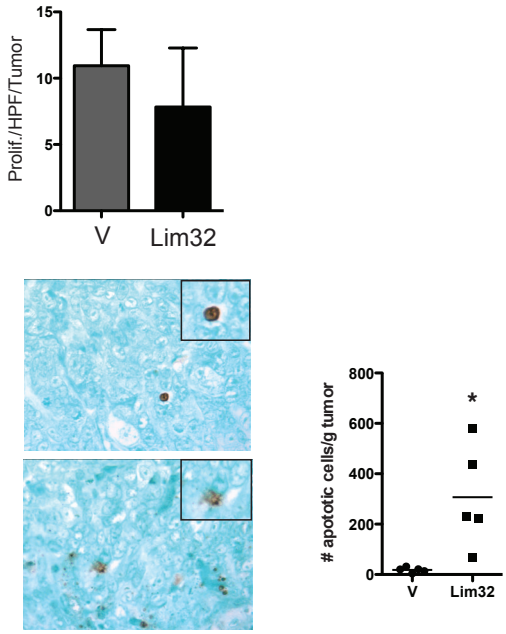
B)



Supplemental Figure S5 BVES induces G1 cell cycle arrest in LIM2405 Cells. A) LIM2405-V or LIM2405-BVES.32 were plated at 5000 cell/cm2 density in replicates. Cell number was determined after 4, 10, and 14 days of culturing and doubling times were calculated. B) Cell cycle analysis in LIM2405-Vector (V) or each of three LIM2405 cell lines overexpressing WT BVES (BVES.32, BVES.9, BVES.15) (a.) Cells were plated in replicate and harvested at 4, 7, 10, and 13 days and analysis performed. Representative flow cytometry tracings from LIM2405-Vector and BVES.32 line at 10 days (b.) Bars represent mean ± S.D. C) Lysates were obtained from LIM2405-V and BVES over-expressing clones and western analysis performed using p21 specific antibody. β−actin was used as a loading control. This suggests that BVES may play a role in regulating cell-cycle progression in CRC cells and restoration of BVES allows for contact mediated growth arrest.

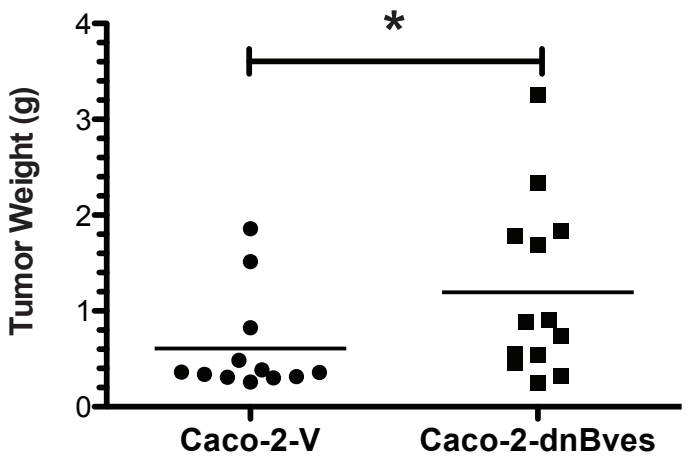


Supplemental Figure S6 DN-BVES augments growth of Caco-2 cells. Caco-2 cells stably transfected with a dominant negative BVES construct (Caco-2-dnBves) or vector control (Caco-2-V). Cell counts were performed in triplicate at the indicated days post-seeding. (* $P<0.05$, ** $P<0.01$, Student's t test.)



Supplemental Figure S7 BVES enhanced intra-tumoral apoptosis. Tumors proliferation and apoptosis rates were determined by anti-Ki67 IHC and in situ TUNEL staining performed according to the manufacturers protocol (ApoTag, Calbiochem). Upper: Ki67 positive cells per HPF per tumor were counted using a 400x magnification at least 20 fields/tumor were counted. Lower: TUNEL (-) serial sections were used as negative controls. Representative high power field (400x) images are shown. The total number of TUNEL positive cells were counted and adjusted for tumor mass and summarized in the chart shown at right (*P*=0.01, Student's t test).

A)



Supplemental Figure S8 BVES modulates tumor growth. A) Pooled BVES-Vector CACO2bbe cells were implanted into the dorsal flank of aythmic mice. Chart shows weights of dissected tumors. (*P<0.05, Student's t test)

SUPPLEMENTARY TABLE 1	*VMC (N=55 Colorectal adenocarcinomas)*	*MCC (N=195 Colorectal adenocarcinomas)*	*Combined (N=250 Colorectal adenocarcinomas)*	*MCC-NORMAL (N=10 Normal adjacent colon specimens)*	*VMC-Adenoma (N=6 adenoma specimens)*
Mean Age (s.d.)	62.3 (14.1)	65.3 (12.9)	n/a	61.9 (16.3)	61.7 (15.2)
Sex (%male)	30 (54.5%)	106 (54.4%)	n/a	7 (70%)	2 (33.3%)
Normal adjacent colon specimens	n/a	n/a	n/a	10	n/a
Stage I	4 (7.3%)	29 (14.9%)	33 (13.2%)	2	n/a
Stage II	15 (27.3%)	61 (31.3%)	76 (30.4%)	5	n/a
Stage III	19 (34.5%)	63 (32.3%)	82 (32.8%)	3	n/a
Stage IV	17 (30.9%)	42 (21.5%)	59 (23.6%)	0	n/a
Caucasian (%)	50 (90.9%)	165 (84.6%)	215 (86%)	9 (90%)	5 (83.3%)
Black (%)	4 (7.3%)	11 (5.6%)	15 (6%)	0 (0%)	0 (0%)
Other (%)	1 (1.8%)	19 (9.8%)	20 (8%)	1 (10%)	1 (16.7%)

Supplemental Table 1 Human colorectal, normal adjacent specimen and adenoma microarray dataset demographics. Colorectal cancer patients from Vanderbilt Medical Center (VMC, n=55 adenocarcinomas and n = 6 adenomas)), Moffitt Cancer Center (MCC, n=195) and 10 MCC normal adjacent colon specimen patients used for microarray analyses are displayed. All patients were diagnosed with colorectal adenocarcinoma and staged according to American Joint Commission on Cancer (AJCC) guidelines (stages I-IV) and the 10 normal adjacent and 6 adenoma specimens were evaluated by a pathologist and determined to contain no adenocarcinoma contribution (only normal colonic mucosa or adenoma). The normal specimens were normal adjacent colon mucosa specimens from patients whose colons were resected for colon cancer. The VMC 55 cohort includes 14 patients from the University of Alabama-Birmingham Medical Center. Other in the VMC medical record implies ‘not otherwise specified’ and implies ‘Hispanic, not otherwise specified’ in the MCC medical record.