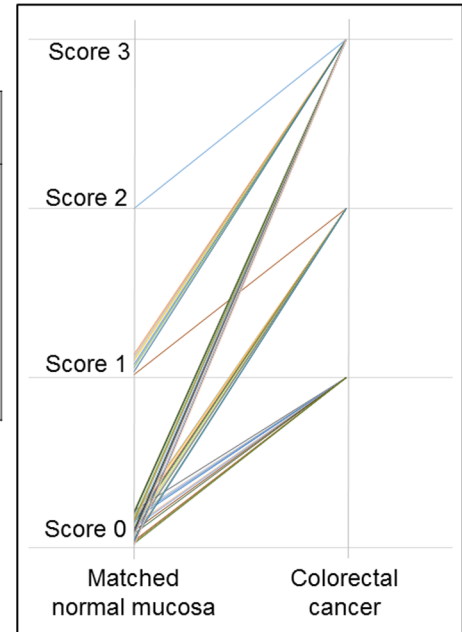


SUPPLEMENTARY FIGURES AND TABLES

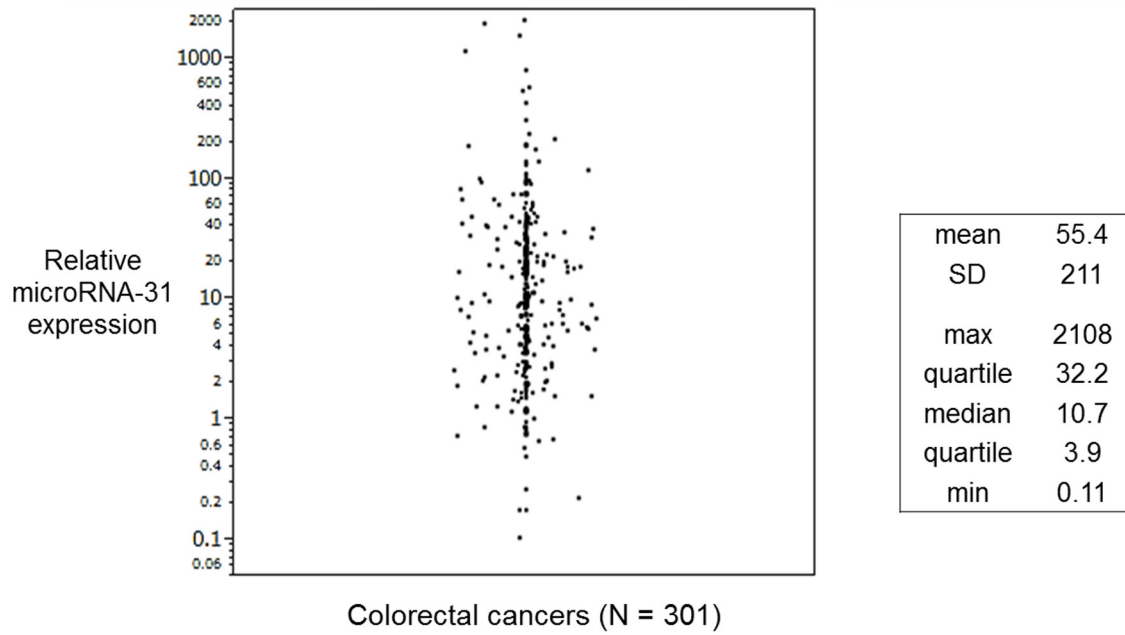
EZH2 expression in colorectal cancer tissue and matched normal mucosa

	Score 0 (Negative)	Score 1 (Weak)	Score 2 (Moderate)	Score 3 (Strong)	Total	<i>P</i>
Colorectal cancer	3	10	14	23	50	< 0.0001
Matched normal mucosa	42	7	1	0	50	



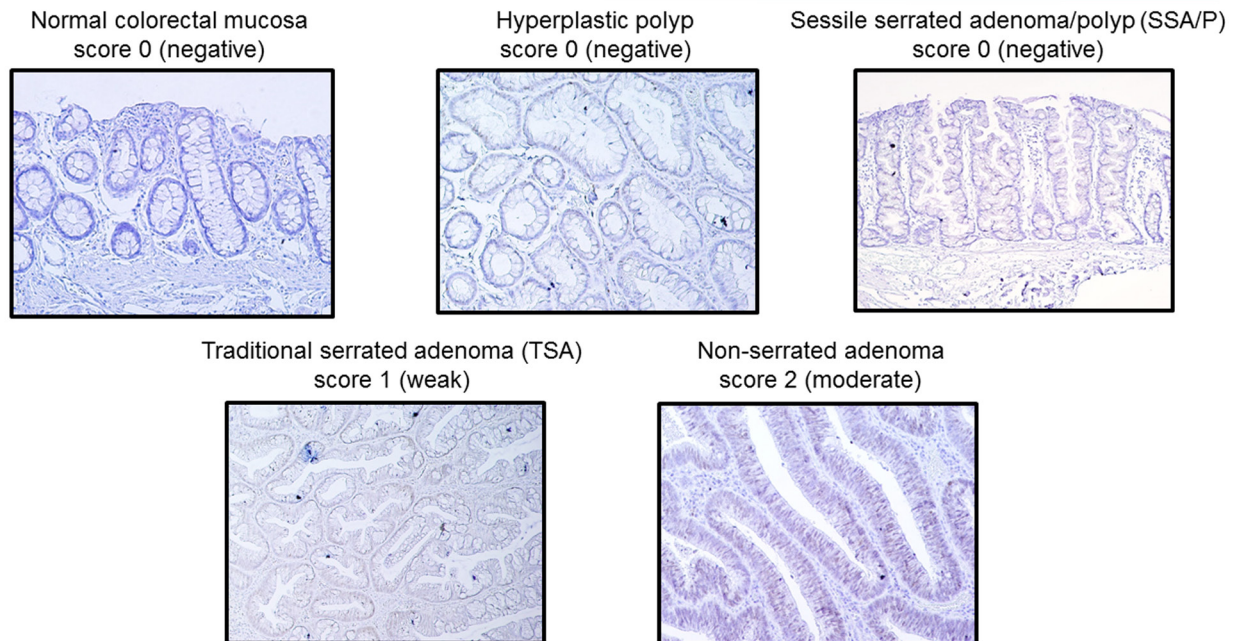
**Supplementary Figure 1: EZH2 expression in 50 colorectal cancer tissues and matched normal mucosa tissues.** EZH2 expression in colorectal cancer tissues was significantly higher than that in normal mucosa ( $P < 0.0001$ ).

### The distributions of microRNA-31 expression in 301 colorectal cancers



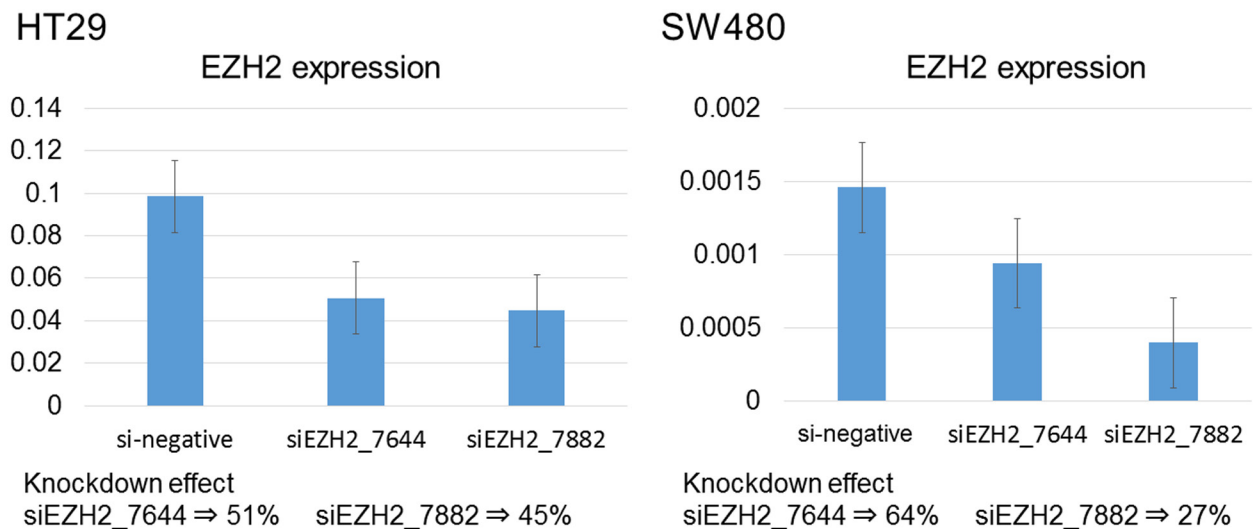
**Supplementary Figure 2: The distributions of microRNA-31 expression in 301 colorectal cancers.** Mean 55.4; median 10.7; standard deviation (SD) 211; range 0.11–2108; interquartile range 3.9–32.2.

## EZH2 expressions in normal colorectal mucosa and premalignant lesions

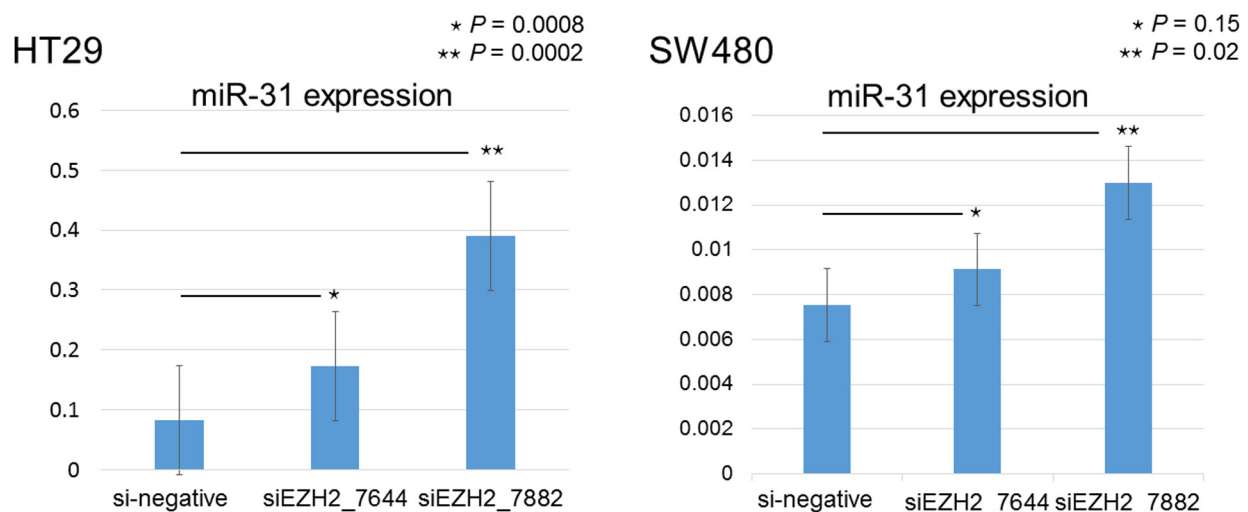


**Supplementary Figure 3: EZH2 expressions in normal colorectal mucosa and premalignant lesions.** Negative EZH2 expression (score 0) was frequently detected in SSA/Ps (76%), compared with HPs (36%), TSAs (25%), and non-serrated adenomas (36%). Moderate (score 2) or strong (score 3) EZH2 expression was not detected in any SSA/P. HP, hyperplastic polyp; SSA/P, sessile serrated adenoma/polyp; TSA, traditional serrated adenoma.

A The siRNA-mediated knockdown of EZH2 caused a significant reduction in EZH2 expression in HT29 and SW480 transfected with EZH2 siRNAs

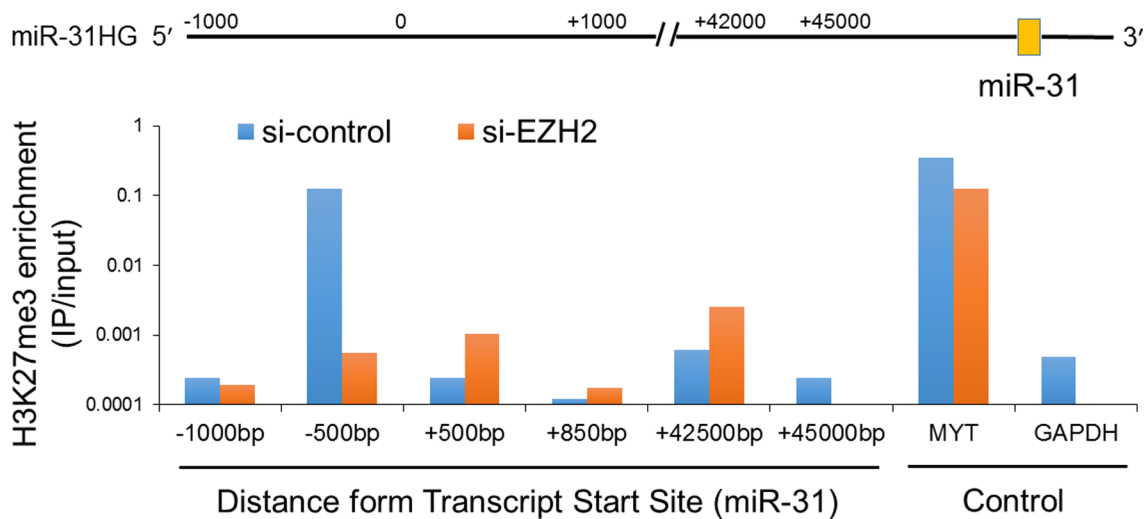


B There was a considerable increase in microRNA-31 (miR-31) expression in HT29 and SW480 cells transfected with EZH2 siRNAs



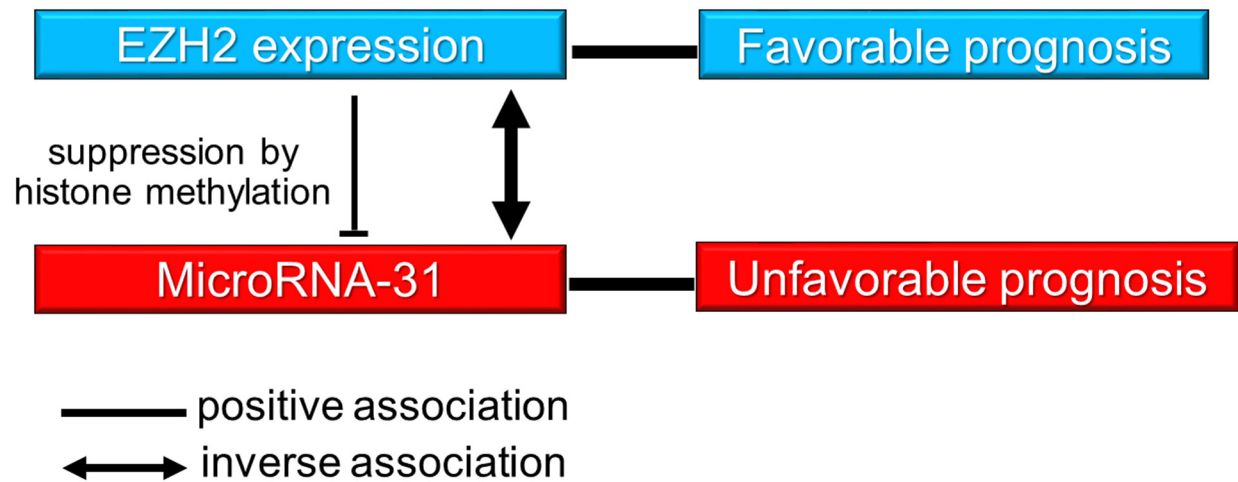
**Supplementary Figure 4: EZH2 knockdown caused microRNA-31 overexpression on quantitative RT-PCR.** A. The siRNA-mediated knockdown of EZH2 caused a significant reduction in EZH2 expression in HT29 and SW480 cells transfected with EZH2 siRNAs (siEZH2\_7644 and siEZH2\_7882). B. There was a considerable increase in miR-31 expression in HT29 and SW480 cells transfected with EZH2 siRNAs (siEZH2\_7644 and siEZH2\_7882). The  $P$ -value was analyzed using paired T-test.

### Histone H3 lysine 27 trimethylation (H3K27me3) levels around the promoter region of microRNA-31 (miR-31) by ChIP assay



**Supplementary Figure 5: H3K27me3 levels around the promoter region of microRNA-31 by performing ChIP assay.** H3K27me3 was steadily enriched at the promoter region of microRNA-31 in RKO cells. The H3K27me3 levels were decreased after knockdown of EZH2. H3K27me3, histone H3 lysine 27 trimethylation; ChIP, chromatin immunoprecipitation.

## The association of EZH2 expression with microRNA-31 and colorectal cancer-specific survival



**Supplementary Figure 6: The association of EZH2 expression with microRNA-31 and colorectal cancer-specific survival.** Similar to previous studies, our current data showed that high EZH2 expression was significantly associated with favorable survival. These results are reasonable because we recently reported that high microRNA-31 expression, which is inversely correlated with EZH2 expression, was an unfavorable prognostic factor in patients with colorectal cancer.

Supplementary Table 1: Association of EZH2 expression with patient-mortality in colorectal cancers

EZH2 status	Total N	Cancer-specific survival		
		Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage- stratified HR (95% CI)
Low expression group (score 0–2)	178	1 (referent)	1 (referent)	1 (referent)
High expression group (score 3)	123	0.48 (0.29-0.77)	0.51 (0.29-0.77)	0.46 (0.27-0.76)
<i>P</i>		0.0018	0.0022	0.0022

The multivariate, stage-stratified Cox model included the EZH2 expression variable stratified by gender, age at diagnosis, tumor size, year of diagnosis, tumor location, tumor differentiation, CIMP status, MSI status, mutations of *BRAF*, *KRAS*, and *PIK3CA*, and microRNA-31 expression.

CI, confidence interval; CIMP, CpG island methylator phenotype; HR, hazard ratio; MSI, microsatellite instability.

Supplementary Table 2: Sequences of chromatin immunoprecipitation (ChIP) primers

Name	Forward primer	Reverse primer
-1,000 bp	CCGATGACCTAGCCAGAAGT	CCCCACCCTTCAACTCGTAG
-500 bp	TATCCTCAACCCTCCGTGTC	CATACACCTGAAGGGGCAGT
+500 bp	CAATTTTGGCCCAGGAGATA	TTTCCGGGGACCTCTAGTTT
+850bp	TTTGATTCCGCCCTCTTTGG	AACGATGGCCCATGCATTC
+42,500 bp	TGGCCTATTTGCTGTTCTAATGAC	GCAAGCCAACCCCAACA
+45,000 bp	AATGGGCCCTGCATTCTCT	AAAACCCACACCCTCACCAC
MYT	AGGCACCTTCTGTTGGCCGA	AGGCAGCTGCCTCCCGTACA
GAPDH	CGGCTACTAGCGGTTTTACG	AAGAAGATGCGGCTGACTGT