

1 **Supplementary Figure Legends**

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3 **Figure S1** Comparison of subjects' age distributions (A) and gender distributions (B)
4 among HC, Early and Mid-Ad ESCC groups. HC, healthy controls; ESCC, esophageal
5 squamous cell carcinoma; Mid-Ad, middle to advanced.

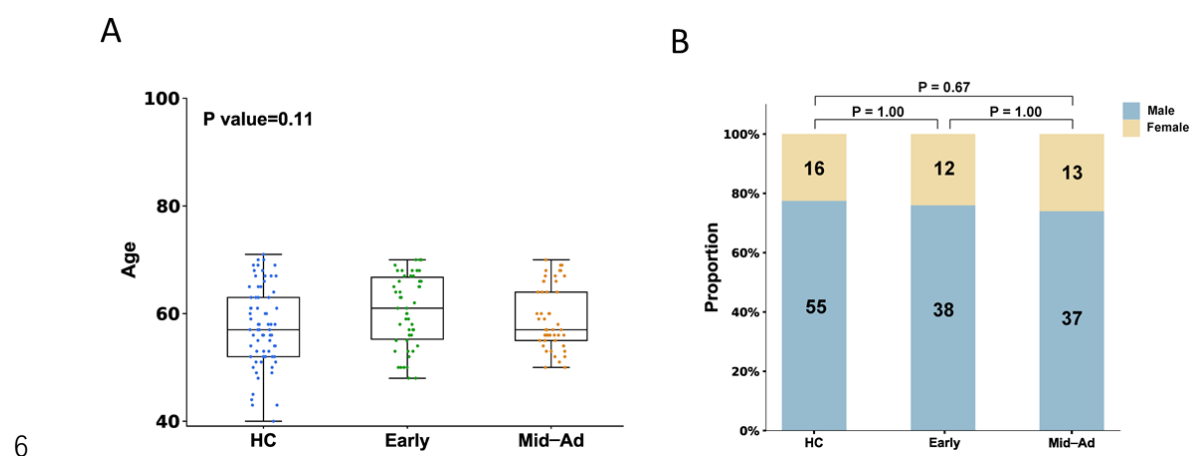


Figure S1

Figure S2 Comparison of the total number of 5hmC peaks between ESCC (green, n=100) and HC (blue, n=50) cohorts. Each dot depicts an individual cfDNA sample. *P* value shows statistical significance by Mann-Whitney U test. HC, healthy controls; ESCC, esophageal squamous cell carcinoma.

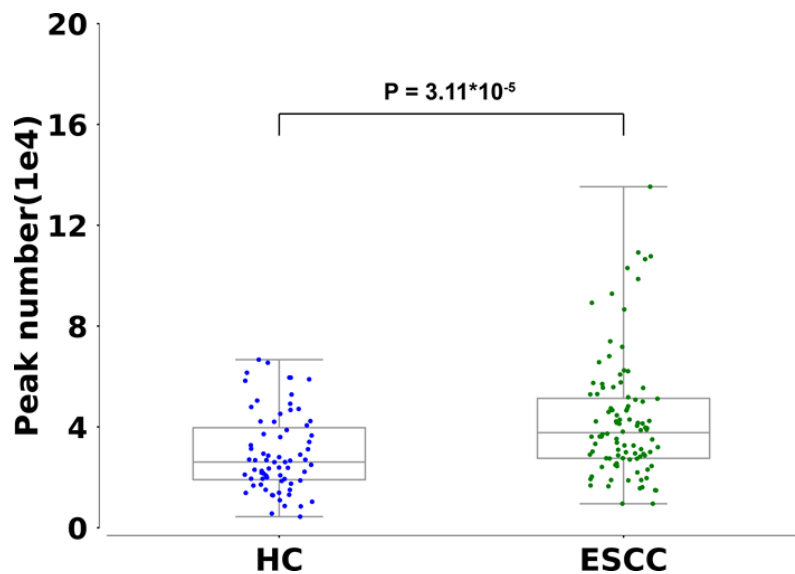
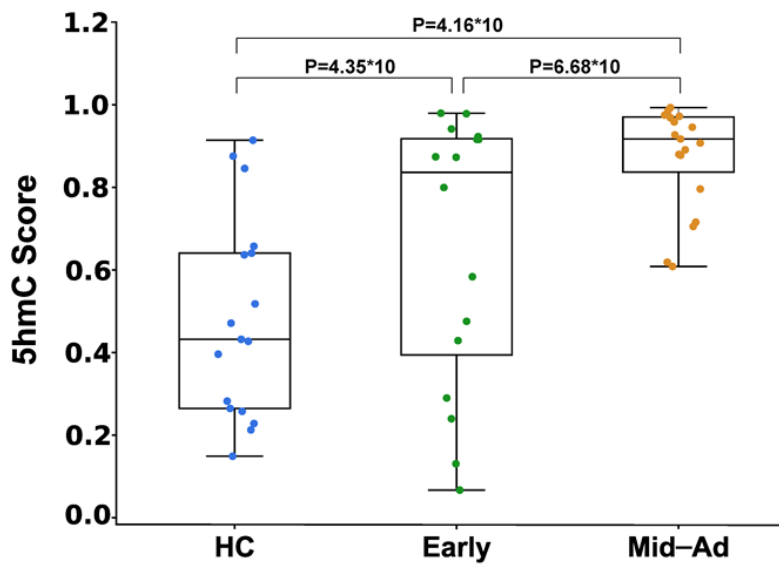


Figure S2

18 **Figure S3** Comparison of predictive scores of 5hmC classifier among HC, Early ESCC
 19 and Mid-Ad ESCC groups in the internal test set. HC, healthy controls; ESCC,
 20 esophageal squamous cell carcinoma; Mid-Ad, middle to advanced.



23 **Figure S3**

Figure S4 The diagnostic powers of individual genomic features and 5hmC classifier and combined classifier in the test set. (A) The table of diagnostic sensitivity and specificity of individual genomic features in ESCC vs HC of the test set. (B) The table of the diagnostic accuracy of 5hmC classifier and combined classifier in different clinical stages of ESCC. Sen, sensitivity; Spe, specificity; HC, healthy controls; ESCC, esophageal squamous cell carcinoma; NF, nucleosome footprint.

A	Motif-test			NF-test		
	Classes	Actual_ESCC	Actual_HC	Classes	Actual_ESCC	Actual_HC
	Predicted_ESCC	25	3	Predicted_ESCC	31	5
	Predicted_HC	9	14	Predicted_HC	3	12
	Sen/Spe	73.5%	82.4%	Sen/Spe	91.2%	70.6%
	Fragment-test			5hmC-test		
	Classes	Actual_ESCC	Actual_HC	Classes	Actual_ESCC	Actual_HC
	Predicted_ESCC	27	3	Predicted_ESCC	26	3
	Predicted_HC	7	14	Predicted_HC	8	14
	Sen/Spe	79.4%	82.4%	Sen/Spe	76.5%	82.4%

B	Stage	5hmC classifier accuracy	Stage	Combined classifier accuracy
	P0 (n=6)	33.3% (2/6)	P0 (n=5)	80.0% (4/5)
	I (n=10)	70.0% (7/10)	I (n=10)	80.0% (8/10)
	II (n=9)	88.9% (8/9)	II (n=9)	88.9% (8/9)
	III (n=8)	87.5% (7/8)	III (n=8)	75.0% (6/8)
	IV (n=2)	100% (2/2)	IV (n=2)	100% (2/2)
	Total (n=35)	74.3% (26/35)	Total (n=34)	82.4% (28/34)

35 **Supplementary Table legends**

36 **Table S1** Clinicopathological characteristics of esophageal cancer patients and healthy
37 controls. Twenty one healthy controls downloaded from a previously published article
38 listed in Table S1B.

39 **Table S2** The top 10-significantly enrichment 5hmC motifs in the Southern ESCC
40 group, Northern ESCC group and CRC group. CRC, colorectal cancer; ESCC,
41 esophageal squamous cell carcinoma.

42 **Table S3** List of annotated 5hmC marker genes used in model construction.

43 **Table S4** List of 5' end motif (A), NF (B) and fragmentation (C) biomarkers used in
44 model construction. NF, nucleosome footprint.

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