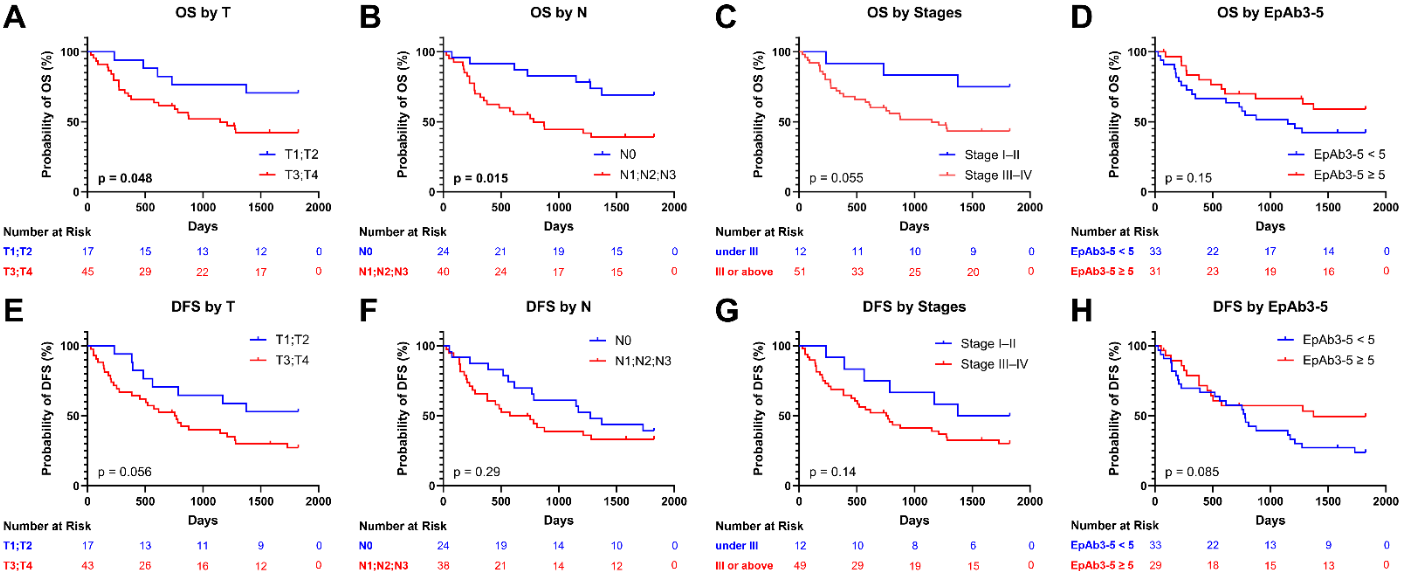

Figure S1 Kaplan-Meier analysis of overall survival (OS) and disease-free survival (DFS) in HNSCC patients.

(A–C) OS stratified by T stage (T1–T2 vs. T3–T4), nodal status (N0 vs. N1–3), and overall clinical stage (Stage I–II vs. Stage III–IV).

(D) OS stratified by epithelial cell adhesion molecule (EpCAM) expression (cutoff < 5 vs. ≥ 5, assessed using EpAb3-5).

(E–G) DFS stratified by T stage, nodal status, and overall clinical stage.

(H) DFS stratified by EpCAM expression (cutoff < 5 vs. ≥ 5, assessed using EpAb3-5).

Survival curves were generated using the Kaplan-Meier method and compared with the log-rank test. All analyses were truncated at five years (1,825 days), with events beyond this point censored. Statistical significance was determined using the log-rank (Mantel-Cox) test, with significant p-values shown in bold.

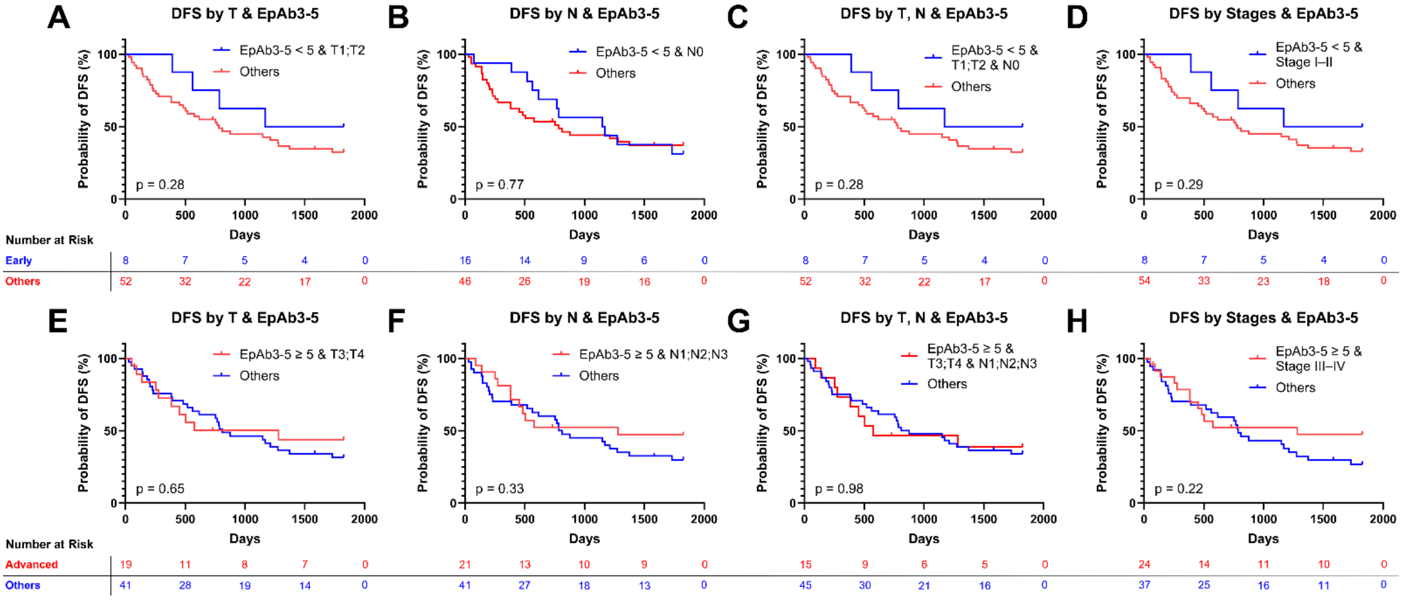

Figure S2 Disease-free survival (DFS) analysis using combined EpAb3-5–assessed epithelial cell adhesion molecule (EpCAM) expression and tumor staging conditions in HNSCC patients.

(A–D) DFS curves comparing patients with low EpCAM expression (< 5, assessed using EpAb3-5) in early-stage categories [T1–T2 (A), N0 (B), combined T1–T2 and N0 (C), and Stage I–II (D)] against all other patients.

(E–H) DFS curves comparing patients with high EpCAM expression (≥ 5, assessed using EpAb3-5) in advanced-stage categories [T3–T4 (E), N1–3 (F), combined T3–T4 and N1–3 (G), and Stage III–IV (H)] against all other patients.

Kaplan-Meier survival curves were generated and compared using the log-rank test. All analyses were truncated at five years (1,825 days), with events beyond this time censored. Statistical significance was determined using the log-rank (Mantel-Cox) test, with significant p-values shown in bold.

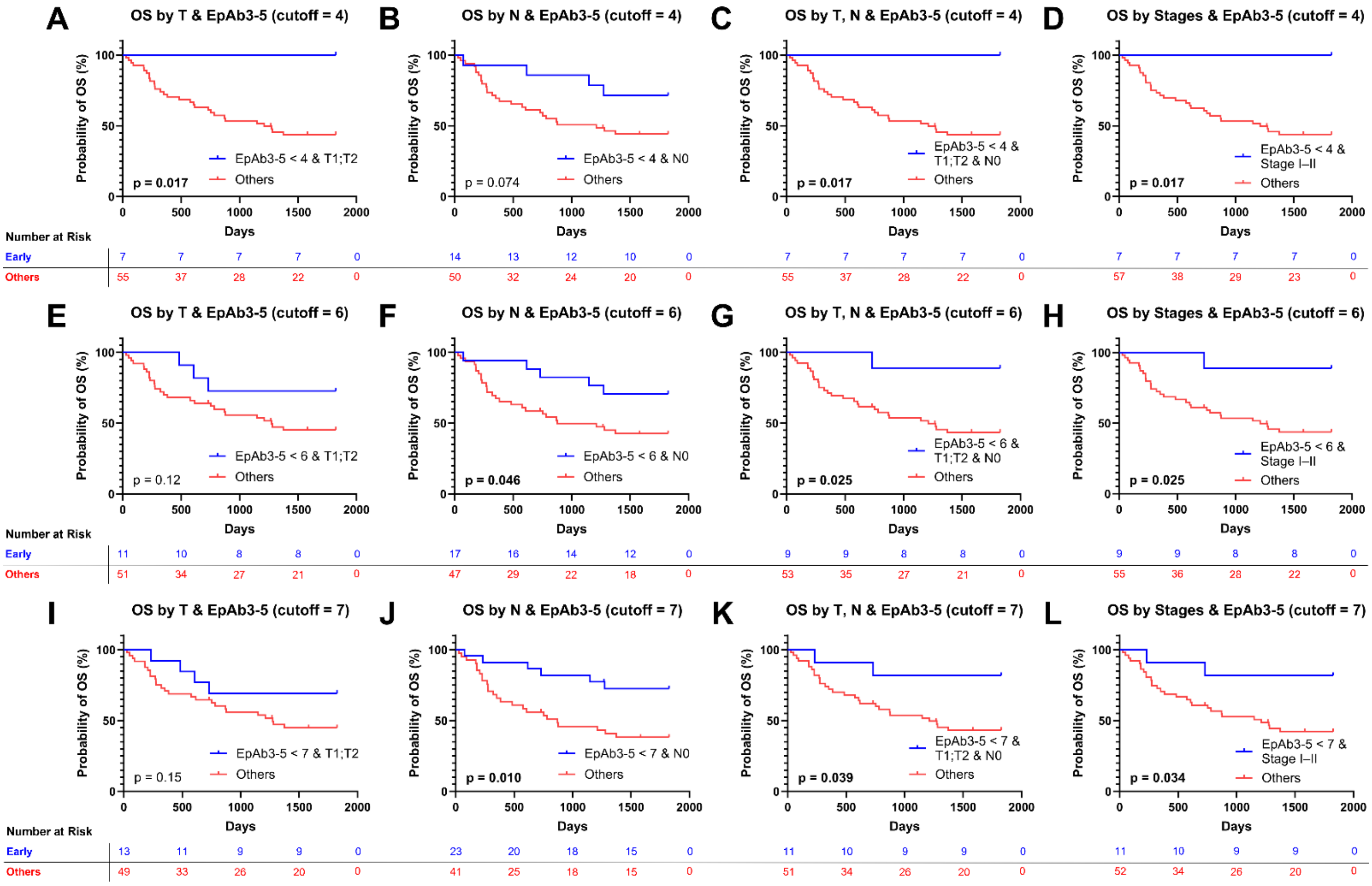

Figure S3 Sensitivity analysis of overall survival (OS) using alternative EpAb3-5 cutoffs in early-stage HNSCC.

(A–D) cutoff = 4; (E–H) cutoff = 6; (I–L) cutoff = 7.

Within each cutoff set, panels correspond respectively to T1–T2, N0, T1–T2 & N0, and Stage I–II. Survival curves were compared using the log-rank (Mantel–Cox) test and truncated at five years (1,825 days), with events beyond this time censored.

Table S1 Summary of variables available in the study dataset.

Variable	Description	Type / Coding
Patient number	Unique identifier assigned to each patient	Integer
Primary tumor site	Anatomical location of the primary tumor	Categorical
TNM classification	Tumor stage according to the TNM system	T, N, M categories
Clinical stage	Overall stage grouping based on TNM classification	Stage I–IV
p16 status	p16 IHC result used as an HPV surrogate marker	Positive / Negative
CTC count (EpAb3-5)	CTC count detected using the EpAb3-5 anti-EpCAM antibody	Integer
CTC count (MACS)	CTC count detected using the MACS EpCAM-based platform	Integer
OS days	Time from diagnosis to death or last follow-up	Continuous
OS status	OS event indicator	1 = death, 0 = censored
DFS days	Time from diagnosis to recurrence, death, or last follow-up	Continuous
DFS status	DFS event indicator	1 = recurrence or death, 0 = censored

IHC = **I**mmuno**h**isto**c**hemistry, HPV = **H**uman **P**apilloma**v**irus, CTC = **C**irculating **T**umor **C**ell, EpCAM = Epithelial Cell Adhesion Molecule, MACS = **M**agnetic-**A**ctivated **C**ell **S**orting, OS = **O**verall **S**urvival; DFS = **D**isease-**f**ree **S**urvival.

Table S2 Cox regression analysis of grouping indicators and their association with five-year overall survival after exclusion of the p16-positive patient (n = 61).

Grouping Indicator	HR	CI (Lower)	CI (Upper)	p-value
T1;T2 vs. T3;T4	1.509053	0.328140	6.939857	0.597104
N0 vs. N1;N2;N3	2.672531	0.905079	7.891489	0.075169
Tumor Stage I;II vs. III;IV	1.118321	0.129577	9.651700	0.919000
EpAb3-5–assessed EpCAM expression < vs. ≥ 5	0.517090	0.233560	1.144807	0.103851

HR = **H**azard **R**atio, CI = **C**onfidence **I**nterval, EpCAM = Epithelial Cell Adhesion Molecule.

Table S3 Cox regression analysis of grouping indicators and their association with five-year disease-free survival after exclusion of the p16-positive patient (n = 59).

Grouping Indicator	HR	CI (Lower)	CI (Upper)	p-value
T1;T2 vs. T3;T4	1.926671	0.426713	8.699207	0.393848
N0 vs. N1;N2;N3	1.424164	0.618175	3.281016	0.406321
Tumor Stage I;II vs. III;IV	0.871640	0.139007	5.465577	0.883395
EpAb3-5–assessed EpCAM expression < vs. ≥ 5	0.583795	0.283911	1.200434	0.143390

HR = **H**azard **R**atio, CI = **C**onfidence **I**nterval, EpCAM = Epithelial Cell Adhesion Molecule.