Supplemental Table S1: Clinicopathologic characteristics of the TCGA-HNSC cohort.

Parameter	all patients	HPV-negative	HPV-positive	P-value
Total	331	286	45	
Age				**.004**
<=61	176 (53.2%)	143 (81.2%)	33 (18.8%)	
>61	155 (46.8%)	143 (92.3%)	12 (7.7%)	
Gender				**.04**
Female	85 (25.7%)	79 (92.9%)	6 (7.1%)	
Male	246 (74.3%)	207 (84.1%)	39 (15.9%)	
Smoking				.87
Non-Smoker	110 (34.5%)	94 (85.5%)	16 (14.5%)	
Smoker	209 (65.5%)	180 (86.1%)	29 (13.9%)	
NA	12	12	0	
AJCC Stage				.51
Stage I	15 (4.9%)	15 (100%)	0 (0%)	
Stage II	48 (15.8%)	41 (85.4%)	7 (14.6%)	
Stage III	48 (15.8%)	41 (85.4%)	7 (14.6%)	
Stage IV	193 (63.5%)	169 (87.6%)	24 (12.4%)	
NA	27	20	7	
WHO Grade				**.002**
G1	46 (13.9%)	45 (97.8%)	1 (2.2%)	
G2	204 (61.6%)	179 (87.7%)	25 (12.3%)	
G3	81 (24.5%)	62 (76.5%)	19 (23.5%)	
WHO Subtype				**.005**
basaloid	24 (7.3%)	15 (62.5%)	9 (37.5%)	
conventional	305 (92.1%)	269 (88.2%)	36 (11.8%)	
papillary	1 (0.3%)	1 (100%)	0 (0%)	
verrucous	1 (0.3%)	1 (100%)	0 (0%)	
Brandwein-Gensler score				.17
low	12 (3.9%)	9 (75%)	3 (25%)	
intermediate	194 (63.2%)	173 (89.2%)	21 (10.8%)	
high	101 (32.9%)	85 (84.2%)	16 (15.8%)	
NA	24	19	5	
pN				**.03**
N0	120 (42%)	112 (93.3%)	8 (6.7%)	
N1/2/3	166 (58%)	140 (84.3%)	26 (15.7%)	
NA	45	34	11	
pT				**.007**
T1/T2	115 (37.1%)	93 (80.9%)	22 (19.1%)	
T3/T4	195 (62.9%)	179 (91.8%)	16 (8.2%)	
NA	21	14	7	
cpM				.35
M0	320 (99.1%)	278 (86.9%)	42 (13.1%)	
M1	3 (0.9%)	2 (66.7%)	1 (33.3%)	
NA	8	6	2	
Localization				**< .001**
Hypopharynx	6 (1.8%)	4 (66.7%)	2 (33.3%)	
Larynx	87 (26.3%)	84 (96.6%)	3 (3.4%)	
Oral cavity and lips	202 (61%)	186 (92.1%)	16 (7.9%)	
Oropharynx	36 (10.9%)	12 (33.3%)	24 (66.7%)	
L1				.08
absent	323 (97.6%)	281 (87%)	42 (13%)	
present	8 (2.4%)	5 (62.5%)	3 (37.5%)	
Pn1				.11
absent	264 (79.8%)	224 (84.8%)	40 (15.2%)	
present	67 (20.2%)	62 (92.5%)	5 (7.5%)	
Margin status				.2
negative/close	263 (85.9%)	234 (89%)	29 (11%)	
positive	43 (14.1%)	35 (81.4%)	8 (18.6%)	
NA	25	17	8	

Supplemental Table S2: Association of tumor budding (TB) with the clinicopathologic tumor characteristics in the HPV-negative subcohort of TCGA-HNSC.

Parameter	TB absent	TB weak	TB moderate	TB strong	P-value
Age					.66
<=61	15 (10.5%)	47 (32.9%)	45 (31.5%)	36 (25.2%)	
>61	20 (14%)	52 (36.4%)	39 (27.3%)	32 (22.4%)	
Gender					.36
Female	12 (15.2%)	22 (27.8%)	27 (34.2%)	18 (22.8%)	
Male	23 (11.1%)	77 (37.2%)	57 (27.5%)	50 (24.2%)	
Smoking					.52
Non-Smoker	10 (10.6%)	35 (37.2%)	31 (33%)	18 (19.1%)	
Smoker	23 (12.8%)	59 (32.8%)	51 (28.3%)	47 (26.1%)	
NA	2	5	2	3	
AJCC Stage					.39
Stage I	2 (13.3%)	3 (20%)	5 (33.3%)	5 (33.3%)	
Stage II	3 (7.3%)	18 (43.9%)	15 (36.6%)	5 (12.2%)	
Stage III	4 (9.8%)	18 (43.9%)	9 (22%)	10 (24.4%)	
Stage IV	21 (12.4%)	56 (33.1%)	46 (27.2%)	46 (27.2%)	
NA	5	4	9	2	
WHO Grade					.13
G1	10 (22.2%)	15 (33.3%)	12 (26.7%)	8 (17.8%)	
G2	17 (9.5%)	67 (37.4%)	56 (31.3%)	39 (21.8%)	
G3	8 (12.9%)	17 (27.4%)	16 (25.8%)	21 (33.9%)	
WHO Subtype					.41
basaloid	3 (20%)	6 (40%)	4 (26.7%)	2 (13.3%)	
conventional	31 (11.5%)	92 (34.2%)	80 (29.7%)	66 (24.5%)	
papillary	0 (0%)	1 (100%)	0 (0%)	0 (0%)	
verrucous	1 (100%)	0 (0%)	0 (0%)	0 (0%)	
Brandwein-Gensler score					**< .001**
low	5 (55.6%)	4 (44.4%)	0 (0%)	0 (0%)	
intermediate	22 (12.7%)	63 (36.4%)	55 (31.8%)	33 (19.1%)	
high	5 (5.9%)	24 (28.2%)	23 (27.1%)	33 (38.8%)	
NA	3	8	6	2	
pN					**.009**
N0	17 (15.2%)	48 (42.9%)	29 (25.9%)	18 (16.1%)	
N1/2/3	13 (9.3%)	40 (28.6%)	44 (31.4%)	43 (30.7%)	
NA	5	11	11	7	
pT					.19
T1/T2	11 (11.8%)	26 (28%)	34 (36.6%)	22 (23.7%)	
T3/T4	20 (11.2%)	69 (38.5%)	45 (25.1%)	45 (25.1%)	
NA	4	4	5	1	
cpM					.38
M0	34 (12.2%)	96 (34.5%)	81 (29.1%)	67 (24.1%)	
M1	1 (50%)	1 (50%)	0 (0%)	0 (0%)	
NA	0	2	3	1	
Localization					.36
Hypopharynx	0 (0%)	0 (0%)	1 (25%)	3 (75%)	
Larynx	15 (17.9%)	26 (31%)	27 (32.1%)	16 (19%)	
Oral cavity and lips	19 (10.2%)	68 (36.6%)	53 (28.5%)	46 (24.7%)	
Oropharynx	1 (8.3%)	5 (41.7%)	3 (25%)	3 (25%)	
L1					.8
absent	35 (12.5%)	96 (34.2%)	83 (29.5%)	67 (23.8%)	
present	0 (0%)	3 (60%)	1 (20%)	1 (20%)	
Pn1					.37
absent	31 (13.8%)	78 (34.8%)	65 (29%)	50 (22.3%)	
present	4 (6.5%)	21 (33.9%)	19 (30.6%)	18 (29%)	
Margin status					.8
negative/close	27 (11.5%)	78 (33.3%)	72 (30.8%)	57 (24.4%)	
positive	4 (11.4%)	13 (37.1%)	8 (22.9%)	10 (28.6%)	
NA	4	8	4	1	

Supplemental Table S3: Association of tumor budding (TB) with the clinicopathologic tumor characteristics in the HPV-positive subcohort of TCGA-HNSC.

Parameter	TB absent	TB weak	TB moderate	TB strong	P-value
Age					.3
<=61	10 (30.3%)	16 (48.5%)	2 (6.1%)	5 (15.2%)	
>61	6 (50%)	3 (25%)	2 (16.7%)	1 (8.3%)	
Gender					.25
Female	1 (16.7%)	2 (33.3%)	1 (16.7%)	2 (33.3%)	
Male	15 (38.5%)	17 (43.6%)	3 (7.7%)	4 (10.3%)	
Smoking					.66
Non-Smoker	5 (31.2%)	8 (50%)	2 (12.5%)	1 (6.2%)	
Smoker	11 (37.9%)	11 (37.9%)	2 (6.9%)	5 (17.2%)	
AJCC Stage					.09
Stage I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Stage II	5 (71.4%)	1 (14.3%)	0 (0%)	1 (14.3%)	
Stage III	4 (57.1%)	2 (28.6%)	1 (14.3%)	0 (0%)	
Stage IV	4 (16.7%)	12 (50%)	3 (12.5%)	5 (20.8%)	
NA	3	4	0	0	
WHO Grade					.76
G1	0 (0%)	1 (100%)	0 (0%)	0 (0%)	
G2	7 (28%)	11 (44%)	3 (12%)	4 (16%)	
G3	9 (47.4%)	7 (36.8%)	1 (5.3%)	2 (10.5%)	
WHO Subtype					.41
basaloid	5 (55.6%)	4 (44.4%)	0 (0%)	0 (0%)	
conventional	11 (30.6%)	15 (41.7%)	4 (11.1%)	6 (16.7%)	
papillary	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
verrucous	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Brandwein-Gensler score					.19
low	3 (100%)	0 (0%)	0 (0%)	0 (0%)	
intermediate	9 (42.9%)	9 (42.9%)	1 (4.8%)	2 (9.5%)	
high	3 (18.8%)	7 (43.8%)	2 (12.5%)	4 (25%)	
NA	1	3	1	0	
pN					.08
N0	6 (75%)	2 (25%)	0 (0%)	0 (0%)	
N1/2/3	6 (23.1%)	11 (42.3%)	4 (15.4%)	5 (19.2%)	
NA	4	6	0	1	
pT					.6
T1/T2	9 (40.9%)	7 (31.8%)	3 (13.6%)	3 (13.6%)	
T3/T4	4 (25%)	8 (50%)	1 (6.2%)	3 (18.8%)	
NA	3	4	0	0	
cpM					.21
M0	15 (35.7%)	19 (45.2%)	3 (7.1%)	5 (11.9%)	
M1	0 (0%)	0 (0%)	0 (0%)	1 (100%)	
NA	1	0	1	0	
Localization					**.02**
Hypopharynx	0 (0%)	0 (0%)	0 (0%)	2 (100%)	
Larynx	2 (66.7%)	1 (33.3%)	0 (0%)	0 (0%)	
Oral cavity and lips	3 (18.8%)	7 (43.8%)	4 (25%)	2 (12.5%)	
Oropharynx	11 (45.8%)	11 (45.8%)	0 (0%)	2 (8.3%)	
L1					.36
absent	16 (38.1%)	16 (38.1%)	4 (9.5%)	6 (14.3%)	
present	0 (0%)	3 (100%)	0 (0%)	0 (0%)	
Pn1					.58
absent	15 (37.5%)	17 (42.5%)	3 (7.5%)	5 (12.5%)	
present	1 (20%)	2 (40%)	1 (20%)	1 (20%)	
Margin status					.38
negative/close	11 (37.9%)	9 (31%)	3 (10.3%)	6 (20.7%)	
positive	2 (25%)	5 (62.5%)	1 (12.5%)	0 (0%)	
NA	3	5	0	0	

Supplemental Table S4: Multivariate survival analysis of cellular dissociation grading (CDG) including age, sex, HPV status, localization, AJCC stage, and tumor margin status.

Endpoint	Variable	Hazard ratio	Confidence interval	P
PFI	**CDG (high vs. low)**	**1.55**	**1.05 to 2.28**	**.03**
	Age (>61 vs. ≤61)	1.03	0.7 to 1.52	.87
	Sex (female vs. male)	1.15	0.74 to 1.8	.53
	HPV status (pos. vs. neg.)	1.36	0.69 to 2.66	.37
	Localization (oropharynx vs. other)	0.52	0.21 to 1.27	.15
	AJCC stage (IV vs. I-III)	**1.9**	**1.22 to 2.93**	**.004**
	tumor margin status (pos. vs. neg./close)	**2.48**	**1.57 to 3.92**	**< .001**
OS	**CDG (high vs. low)**	**1.74**	**1.21 to 2.49**	**.003**
	Age (>61 vs. ≤61)	**1.46**	**1.02 to 2.08**	**.04**
	Sex (female vs. male)	**1.46**	**1 to 2.13**	**.05**
	HPV status (pos. vs. neg.)	1.67	0.91 to 3.07	.1
	Localization (oropharynx vs. other)	0.82	0.39 to 1.75	.61
	AJCC stage (IV vs. I-III)	**1.88**	**1.27 to 2.78**	**.002**
	tumor margin status (pos. vs. neg./close)	**1.99**	**1.27 to 3.13**	**.003**

Supplemental Table S5: Multivariate survival analysis of WHO grading including age, sex, HPV status, localization, AJCC stage, and tumor margin status.

Endpoint	Variable	Hazard ratio	Confidence interval	P
PFI	**WHO grade (G2/G3 vs. G1)**	1.18	0.64 to 2.19	.6
	Age (>61 vs. ≤61)	0.99	0.67 to 1.46	0.97
	Sex (female vs. male)	1.17	0.74 to 1.83	.5
	HPV status (pos. vs. neg.)	1.23	0.62 to 2.41	.55
	Localization (oropharynx vs. other)	0.48	0.19 to 1.2	.12
	AJCC stage (IV vs. I-III)	**1.88**	**1.21 to 2.93**	**.005**
	tumor margin status (pos. vs. neg./close)	**2.37**	**1.5 to 3.75**	**< .001**
OS	**WHO grade (G2/G3 vs. G1)**	1.43	0.81 to 2.52	.22
	Age (>61 vs. ≤61)	1.36	0.96 to 1.94	.08
	Sex (female vs. male)	1.45	0.99 to 2.11	.055
	HPV status (pos. vs. neg.)	1.43	0.78 to 2.62	.25
	Localization (oropharynx vs. other)	0.75	0.35 to 1.61	.47
	AJCC stage (IV vs. I-III)	**1.85**	**1.25 to 2.75**	**.002**
	tumor margin status (pos. vs. neg./close)	**1.83**	**1.17 to 2.88**	**.009**

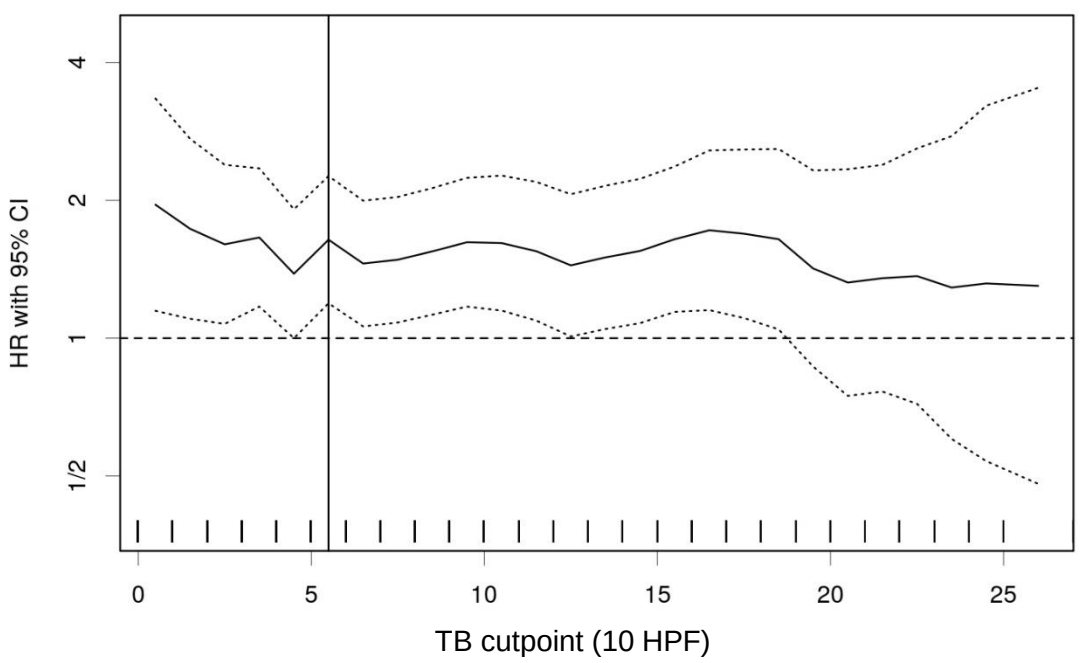
Supplemental Table S6: Multivariate survival analysis of Brandwein-Gensler (BG) score including age, sex, HPV status, localization, AJCC stage, and tumor margin status.

Endpoint	Variable	Hazard ratio	Confidence interval	P
PFI	**BG score (high vs. low/intermediate)**	1.49	1 to 2.23	.051
	Age (>61 vs. ≤61)	1	0.67 to 1.49	0.99
	Sex (female vs. male)	1.07	0.67 to 1.7	.79
	HPV status (pos. vs. neg.)	1.08	0.51 to 2.28	.84
	Localization (oropharynx vs. other)	0.58	0.23 to 1.48	.26
	AJCC stage (IV vs. I-III)	**1.63**	**1.05 to 2.55**	**.03**
	tumor margin status (pos. vs. neg./close)	**2.2**	**1.37 to 3.53**	**.001**
OS	**BG score (high vs. low/intermediate)**	**1.48**	**1.03 to 2.13**	**.03**
	Age (>61 vs. ≤61)	1.41	0.98 to 2.03	.06
	Sex (female vs. male)	1.31	0.88 to 1.94	.18
	HPV status (pos. vs. neg.)	1.6	0.84 to 3.04	.15
	Localization (oropharynx vs. other)	0.74	0.35 to 1.6	.45
	AJCC stage (IV vs. I-III)	**1.69**	**1.14 to 2.51**	**.009**
	tumor margin status (pos. vs. neg./close)	**1.65**	**1.04 to 2.62**	**.03**

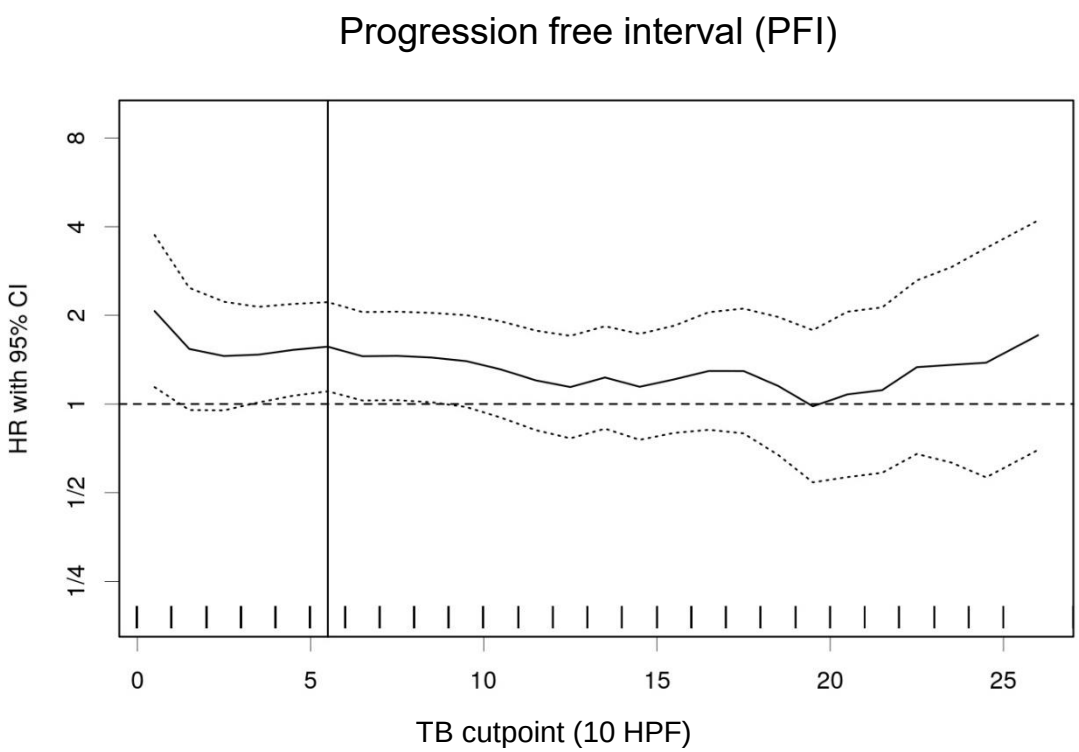
Supplemental Table S7: Multivariate survival analysis of cellular dissociation grading (CDG), WHO grading, Brandwein-Gensler (BG) score as well as age, sex, HPV status, localization, AJCC stage, and tumor margin status.

Endpoint	Variable	Hazard ratio	Confidence interval	P
PFI	**CDG (high vs. low)**	1.42	0.94 to 2.14	.09
	BG score (high vs. low/intermediate)	1.38	0.91 to 2.09	.12
	WHO grade (G2/G3 vs. G1)	1.14	0.61 to 2.13	.68
	Age (>61 vs. ≤61)	1.04	0.7 to 1.55	.85
	Sex (female vs. male)	1.06	0.66 to 1.69	.81
	HPV status (pos. vs. neg.)	1.12	0.53 to 2.37	.76
	Localization (oropharynx vs. other)	0.65	0.26 to 1.65	.36
	AJCC stage (IV vs. I-III)	**1.62**	**1.03 to 2.54**	**.04**
	tumor margin status (pos. vs. neg./close)	**2.29**	**1.42 to 3.69**	**< .001**
OS	**CDG (high vs. low)**	**1.53**	**1.06 to 2.21**	**.02**
	BG score (high vs. low/intermediate)	1.37	0.95 to 1.99	.09
	WHO grade (G2/G3 vs. G1)	1.35	0.76 to 2.41	.3
	Age (>61 vs. ≤61)	**1.53**	**1.06 to 2.21**	**.02**
	Sex (female vs. male)	1.3	0.88 to 1.92	.2
	HPV status (pos. vs. neg.)	1.67	0.88 to 3.16	.11
	Localization (oropharynx vs. other)	0.85	0.4 to 1.83	.68
	AJCC stage (IV vs. I-III)	**1.63**	**1.09 to 2.43**	**.02**
	tumor margin status (pos. vs. neg./close)	**1.75**	**1.1 to 2.78**	**.02**

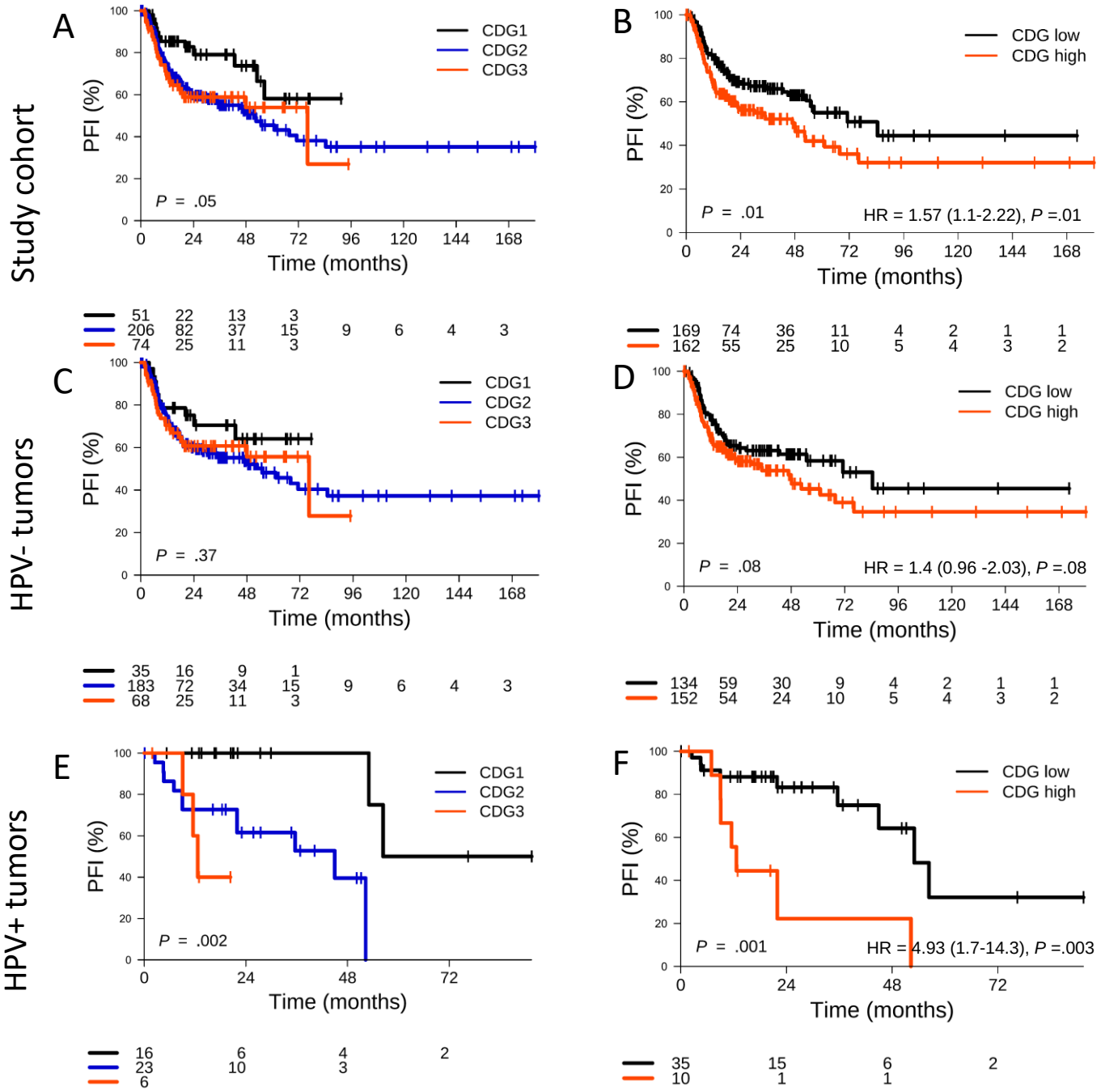
A



B

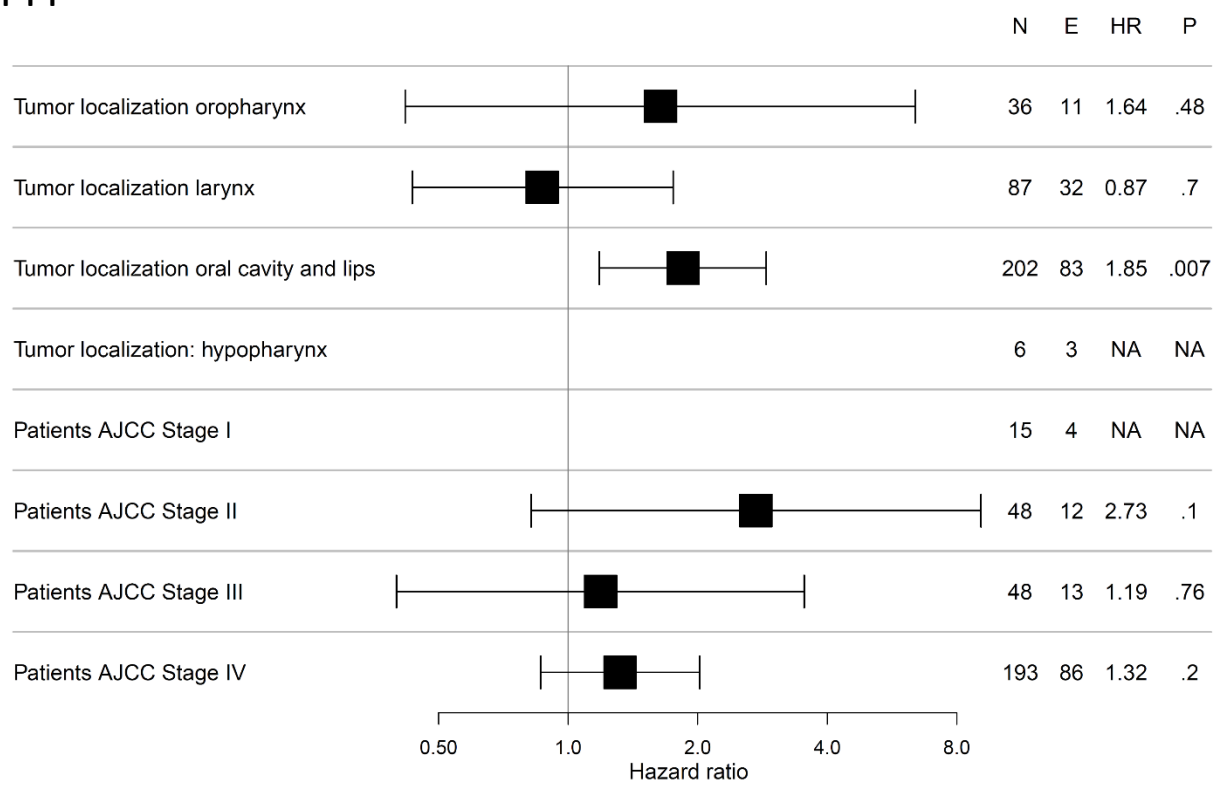


Supplemental Figure S1: Analysis of the prognostic significance of different TB cutpoints (1, …, 27 budding foci). For both, OS **(A)** and PFI **(B)** the most significant difference in survival was achieved by separating tumors with ≥ 6 budding foci from tumors with < 6 budding foci (10 HPF approach).

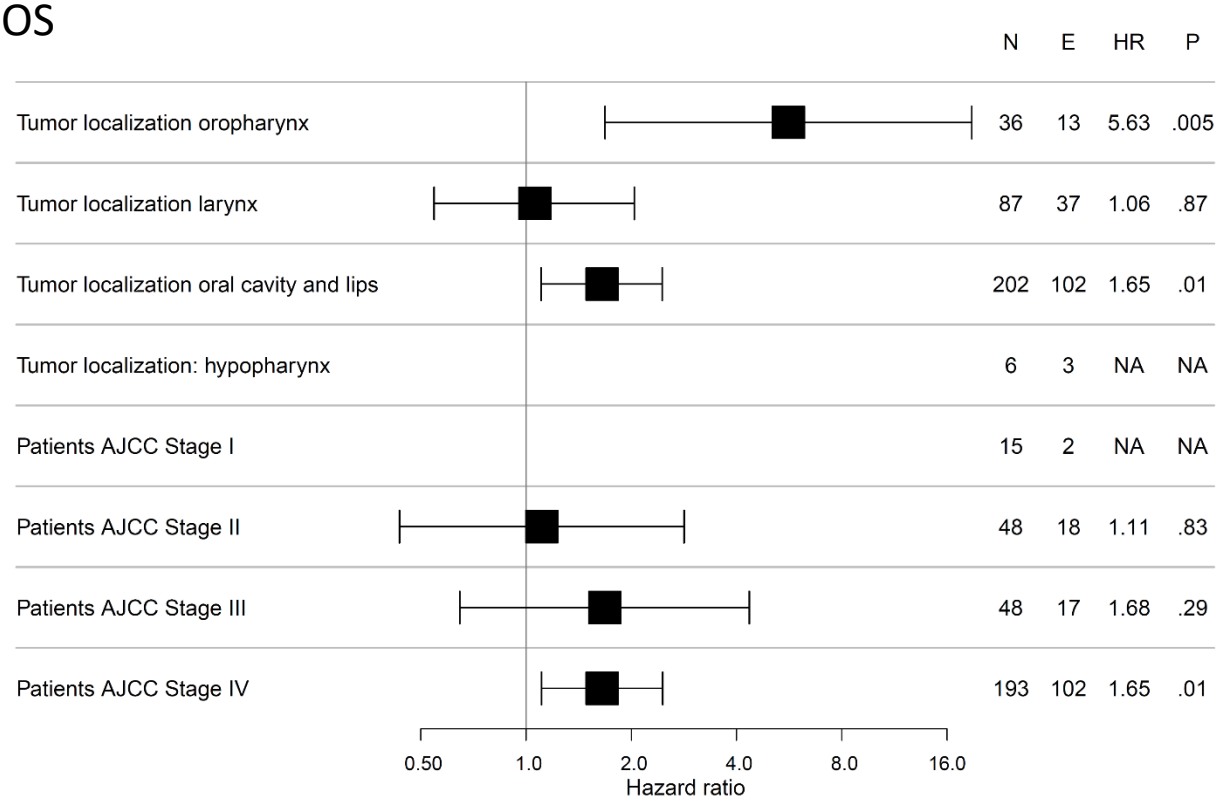


Supplemental Figure S2: Association of progression-free interval (PFI) with cellular dissociation grading (CDG). Performance of the 3-tiered CDG system and of the 2-tiered CDG system in the study cohort **(A/B)**, in the subcohort of HPV-negative tumors **(C/D)** and in the subcohort of HPV-positive tumors **(E/F)**.

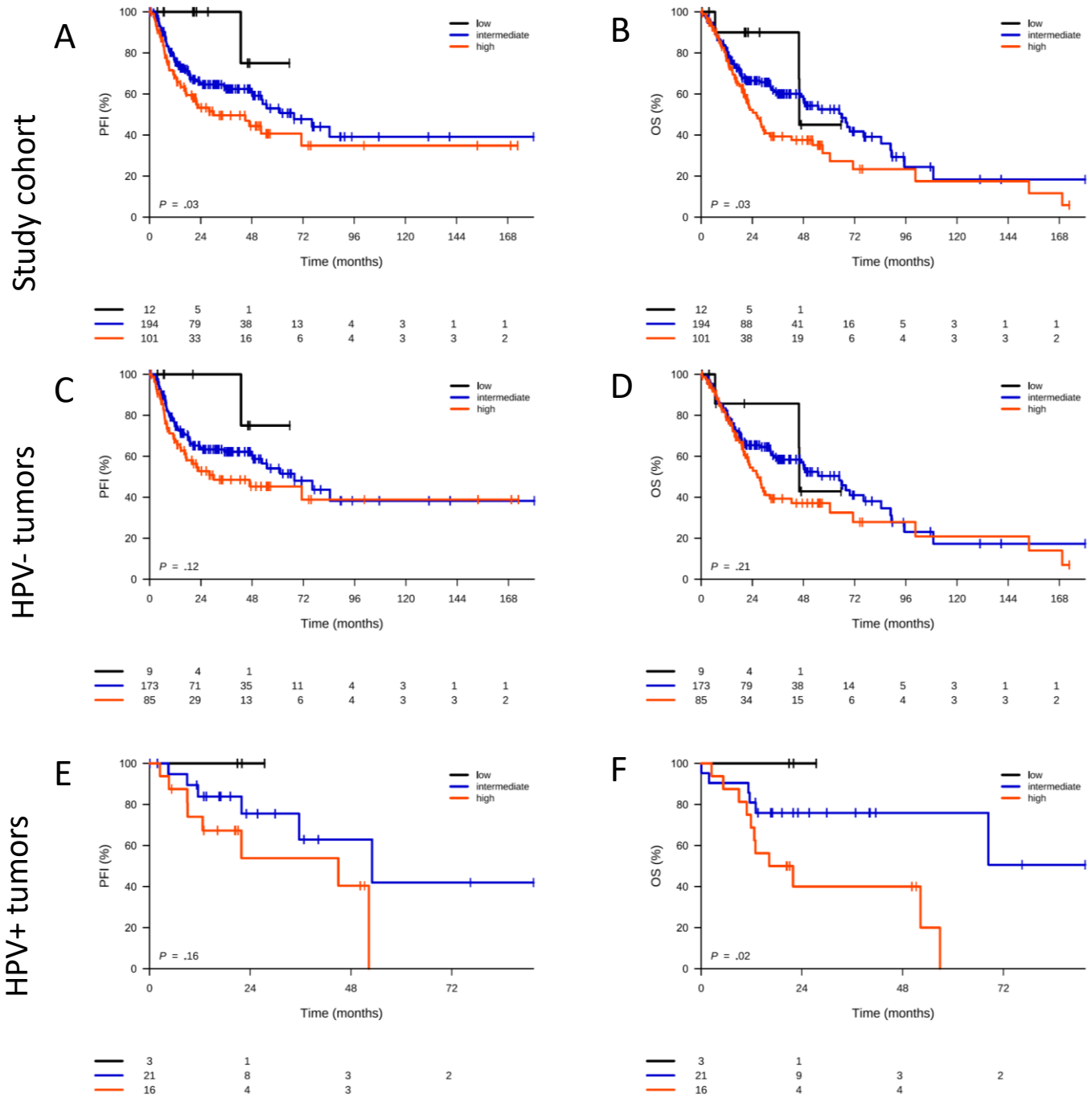
A: PFI



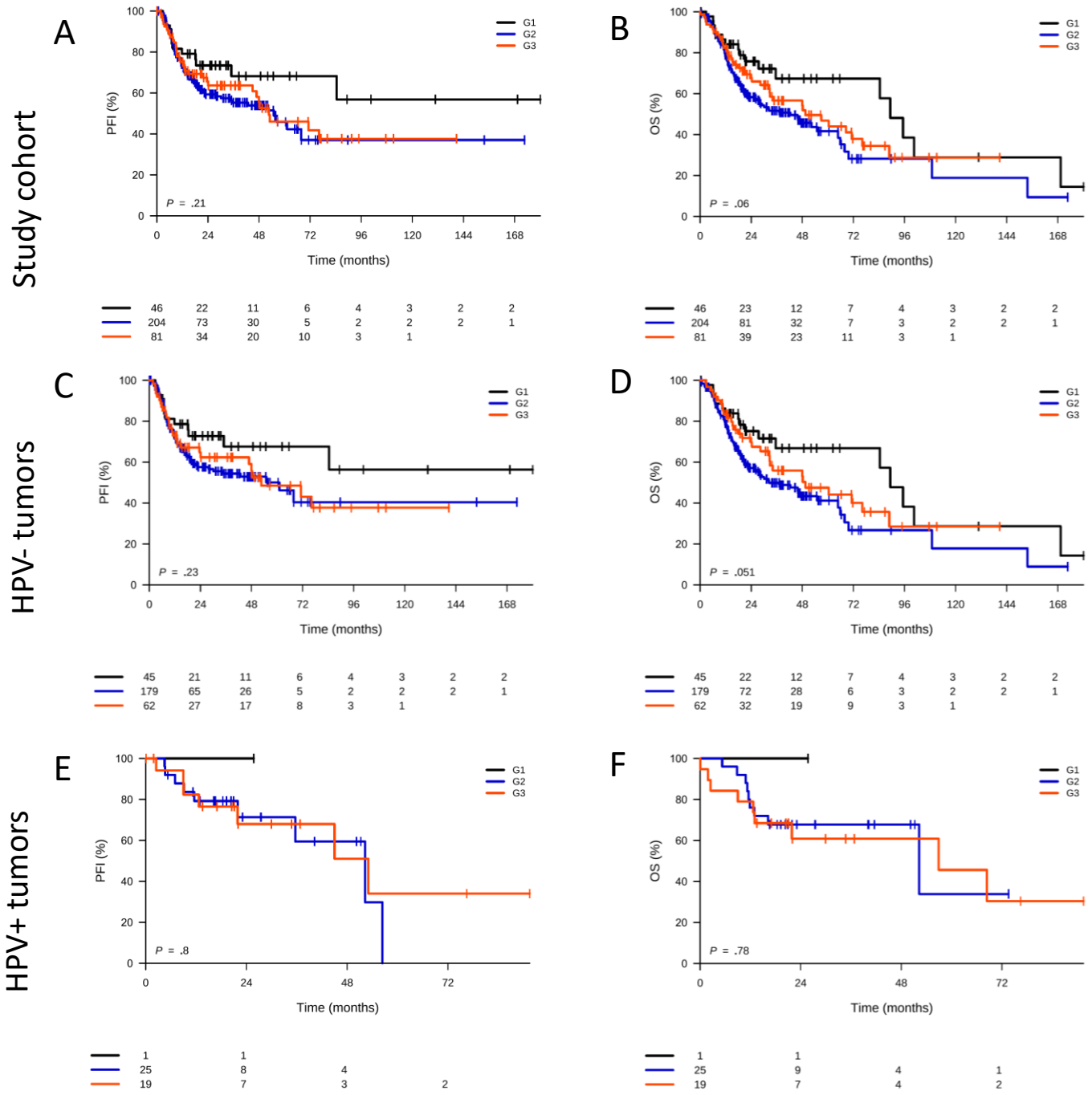
B: OS



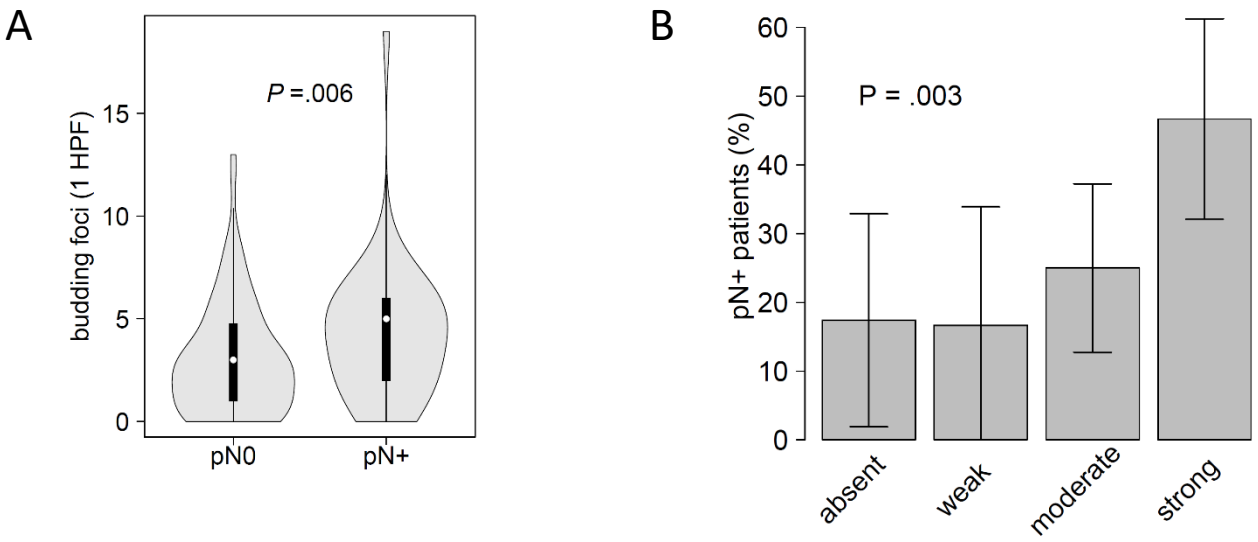
Supplemental Figure S3: Extended subgroup analysis of the prognosticity of cellular dissociation grading (CDG). Comparison of CDG high with CGD low tumors with respect to PFI **(A)** and OS **(B)**. N = number of patients, E = number of events, HR = hazard ratio, CI = 95% confidence interval, NA = not applicable because of too few events.



Supplemental Figure S4: Prognostic impact of the Brandwein-Gensler (BG) score. Analysis of PFI and OS in the study cohort **(A/B)**, in the subgroup of HPV-negative tumors **(C/D),** and in the subgroup of HPV-positive tumors **(E/F)**.



Supplemental Figure S5: Prognostic impact of WHO grading. Analysis of PFI and OS in the study cohort **(A/B)**, in the subgroup of HPV-negative tumors **(C/D),** and in the subgroup of HPV-positive tumors **(E/F)**.



Supplemental Figure S6: Analysis of tumor budding (TB) in 1 high-power field (HPF) of clinically nodal-negative (cN0) patients. A Higher numbers of budding foci (1 HPF) in pathologically nodal-positive (pN+) compared to pN0 patients. **B** Increasing percentages of pN+ patients with increasing TB (1 HPF).