

Neutrophils in HNSCC can be associated with both a worse or favorable prognosis

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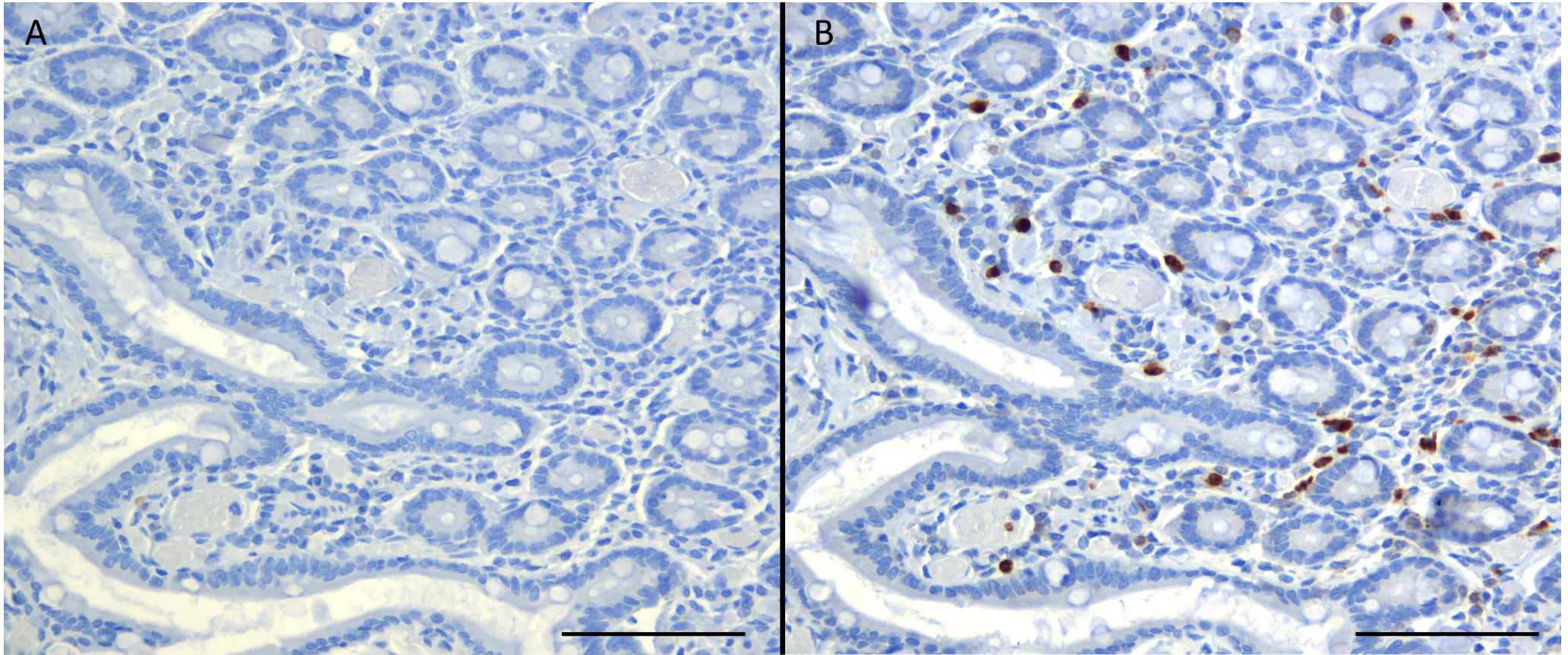
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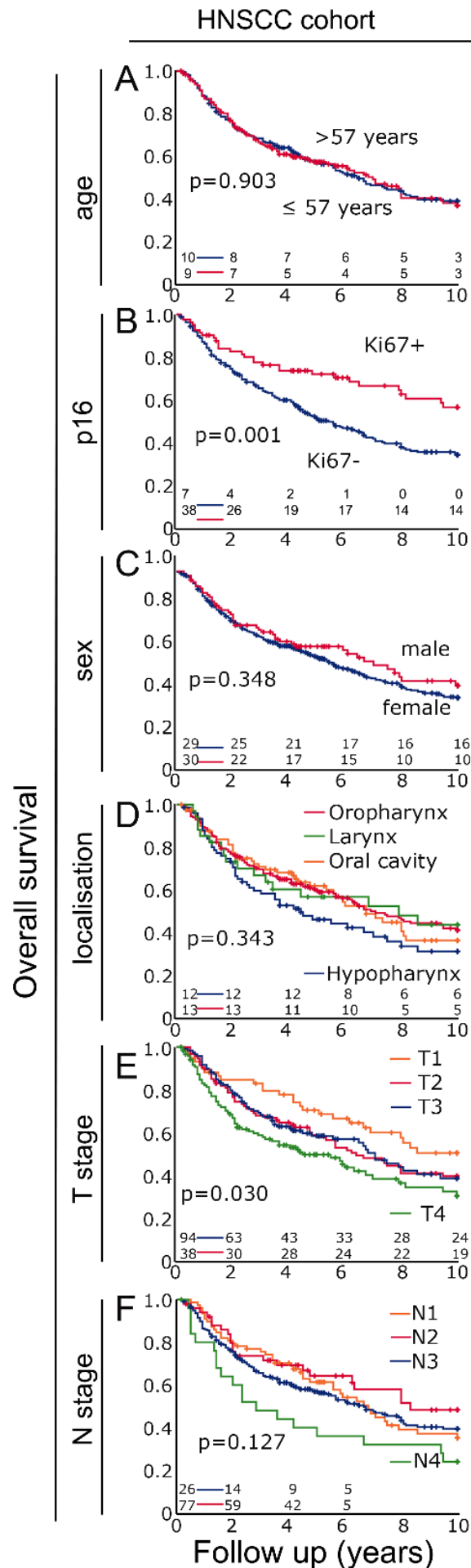
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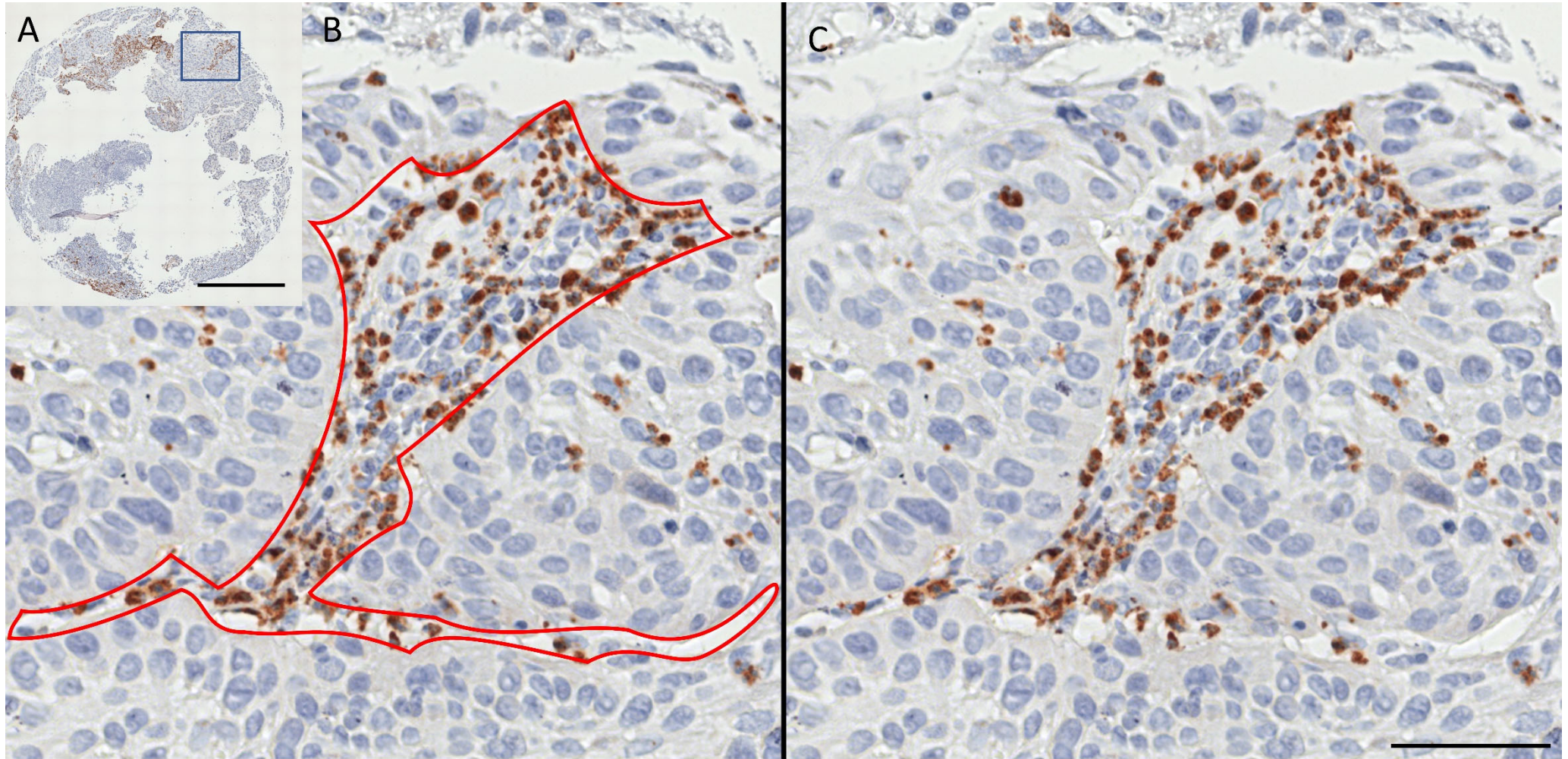
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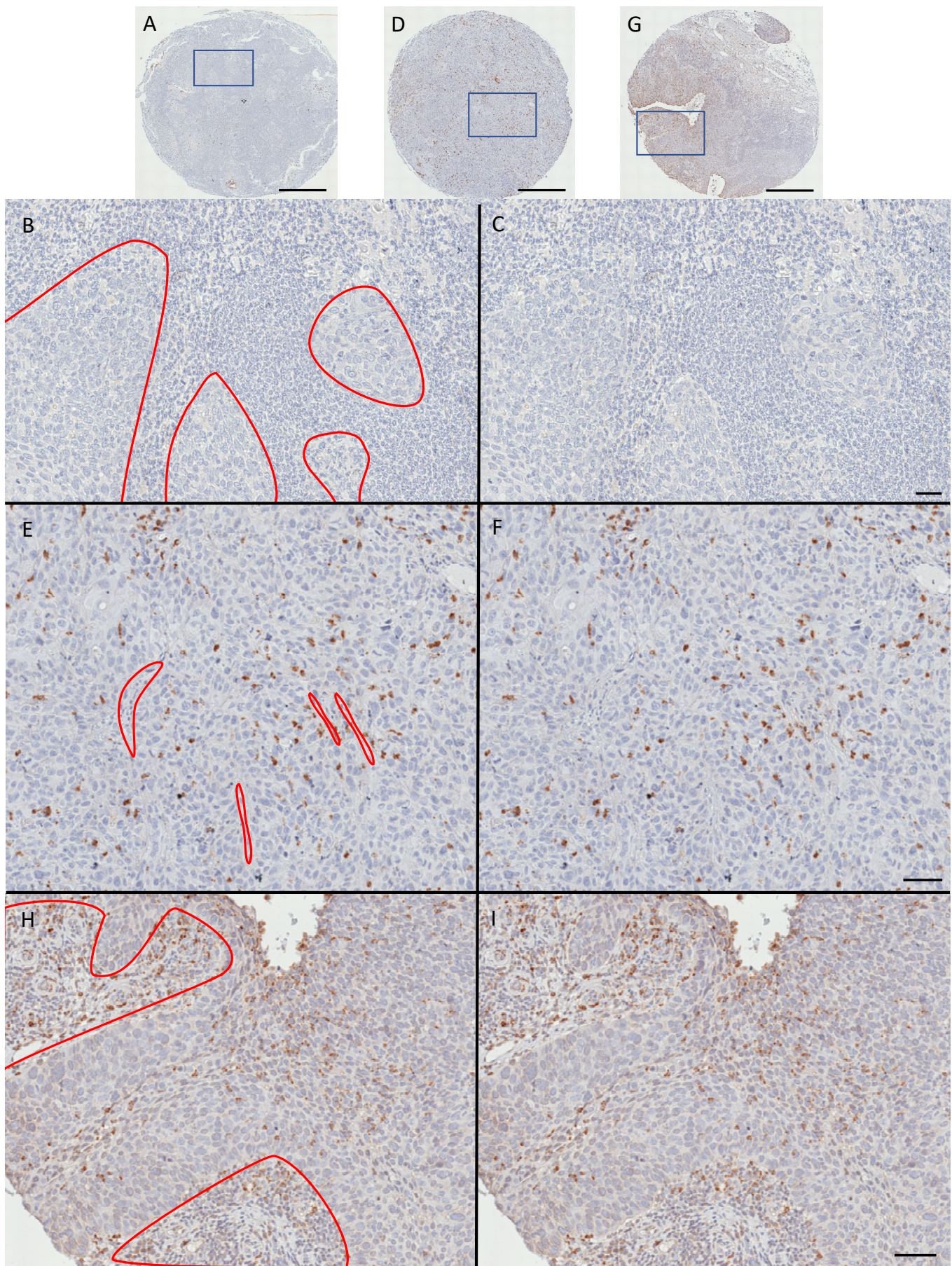
Supplementary Figure S1: (A) Isotype controls were performed with biotinylated goat anti-mouse IgM secondary antibodies, omitting the primary antibodies. Otherwise, the staining procedure was identical to the primary antibody staining. (B) The staining procedure was performed in accordance with the protocol used in the study. Control studies have been performed on tissue from the jejunum. The scale bar in (B) indicates 100 μm .



Supplementary figure S2 Kaplan-Meier plots of overall survival in the HNSCC cohort according to neutrophil densities depending on (A) age, (B) p16-status, (C) sex, (D) tumor localization, (E) T-stage and (F) N-stage.

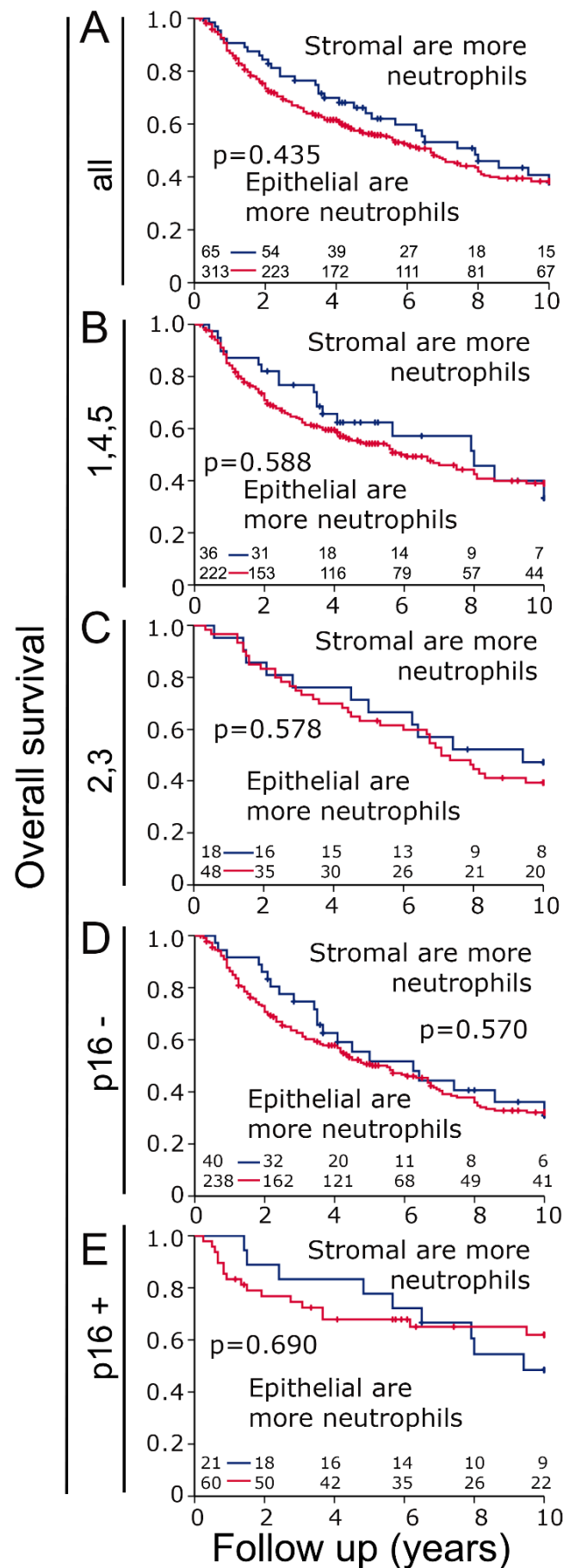


Supplementary Figure S3: HNSCC tissue stained for CD66b+ neutrophils. (A) The whole spot of the tissue microarray with the blue square marking the area of the enlarged section, (B) a section with separately labeled epithelial and stromal compartments. (red), (C) the section without labeled compartments. A tissue with neutrophils predominantly in the stromal compartment and to a much lesser extent in the epithelial compartment. The scale bar in (A) indicates 500 μm and in (C) 50 μm .



Supplementary Figure S4: HNSCC tissue stained for CD66b+ neutrophils. (A, D, G) The whole spots of the tissue microarray with the blue square marking the area of the enlarged sections, scale bars indicates 500 μm . (B, E, H) sections with separately labeled epithelial and stromal compartments (red). (C, F, I) the section without labeled compartments. (A, B, C) A tissue with neutrophils predominantly in the epithelial compartment. (D, E, F) A tissue with neutrophils predominantly in the epithelial compartment. (G, H, I) A tissue with neutrophils in the stromal and epithelial compartments. The scale bars indicate 50 μm .

HNSCC cohort



Supplementary figure S5 Kaplan-Meier plots of overall survival in the HNSCC cohort according to neutrophil densities depending on the stromal to epithelial ratio in (A) all patients, (B) the 1,4,5 cohort, (C) the 2,3 cohort, (D) p16 negative patients and (E) p16 positive patients.

Supplementary table S1: Mean neutrophils in the stromal and epithelial compartments of different locations and different subgroups. Number of patients in brackets.

	variable	all locations		oral cavity		oropharynx		hypopharynx		larynx	
		stromal	epithelial	stromal	epithelial	stromal	epithelial	stromal	epithelial	stromal	epithelial
age	all	813.7 (387)	1478.8 (397)	394.5 (80)	1648.3 (83)	823.6 (198)	1295.4 (203)	1161.4 (73)	1794.1 (77)	559.3 (31)	1252.4 (34)
	≤ 57	512.2 (205)	1291.9 (214)	310.1 (46)	1436.3 (48)	593.9 (101)	1234.4 (103)	540 (40)	1265.3 (43)	356.4 (18)	1344.7 (20)
	>57	1059.8 (176)	1647.5 (182)	482.9 (34)	1922.7 (35)	1004.9 (96)	1321.7 (99)	1606.9 (33)	2148.3 (34)	771.1 (13)	1130 (14)
sex	male	861.2 (323)	1466.6 (330)	390.8 (63)	1739.3 (65)	894.6 (164)	1358.8 (169)	1193 (62)	1534.8 (64)	577.9 (29)	1291.4 (32)
	female	506.4 (64)	1543.9 (67)	420.3 (17)	1298.9 (18)	211.9 (34)	897.5 (34)	1001.5 (11)	2819.4 (13)	4.9 (2)	238.2 (2)
T stage	T1	1232.3 (58)	1873.3 (59)	603.8 (9)	3302.1 (10)	414.8 (39)	1214 (39)	2940.1 (9)	1732.3 (9)	0 (1)	0 (1)
	T2	412.8 (94)	1477.8 (96)	198.1 (23)	673.8 (23)	393.2 (52)	1009.3 (53)	648 (14)	2960.7 (14)	218 (5)	467.3 (6)
	T3	877.9 (115)	1333.7 (119)	445.5 (18)	1355.3 (18)	1059.3 (60)	1455.6 (62)	697.1 (24)	1162.1 (25)	733.5 (13)	1094 (14)
	T4	745.8 (115)	1390.4 (123)	384 (30)	1306.9 (32)	1047.6 (47)	1382.9 (49)	426.4 (26)	1425.9 (29)	484.3 (12)	1649.9 (13)
N stage	N0	811.8 (75)	1637.2 (79)	464.6 (16)	2800.6 (17)	994.5 (38)	788.2 (40)	722.6 (17)	1573.9 (17)	162 (4)	265.8 (5)
	N1	371 (51)	909.7 (52)	153.2 (13)	682.4 (13)	413.6 (30)	909.9 (30)	512.4 (6)	794 (6)	222.3 (2)	1567.1 (3)
	N2	904.9 (230)	1542.9 (240)	417.7 (50)	1209.3 (52)	859.6 (111)	1481.2 (114)	1396 (45)	2002.1 (49)	624.8 (24)	1300.8 (25)
Grade	Grade 1	1185.2 (21)	961.6 (22)	76.3 (8)	331.6 (8)	1690.5 (10)	1204.2 (11)	23 (2)	14.6 (2)	0 (1)	0 (1)
	Grade 2	903.6 (222)	1533.8 (231)	451.3 (48)	1820.1 (51)	781.2 (113)	1132.7 (113)	1539.9 (39)	2044.2 (43)	646.1 (22)	1420.5 (24)
	Grade 3	546.8 (131)	1426.6 (136)	317.3 (24)	1484.8 (24)	669.6 (71)	1456.3 (75)	399 (28)	1505.1 (28)	187.5 (8)	568 (9)
	Grade 4	1003.9 (8)	1903 (8)	- (0)	- (0)	1384.7 (4)	2765.1 (4)	123.6 (4)	780.4 (4)	- (0)	- (0)