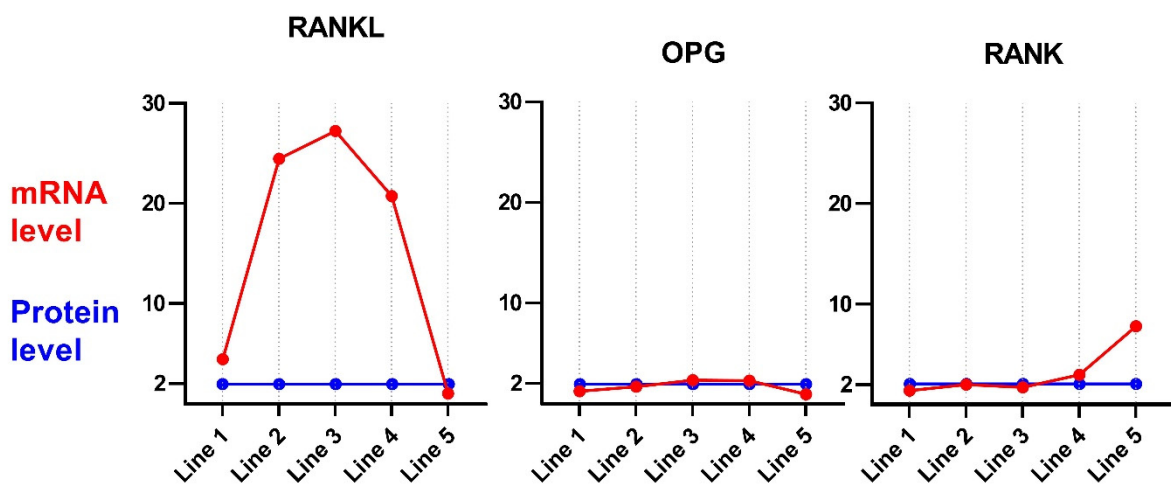
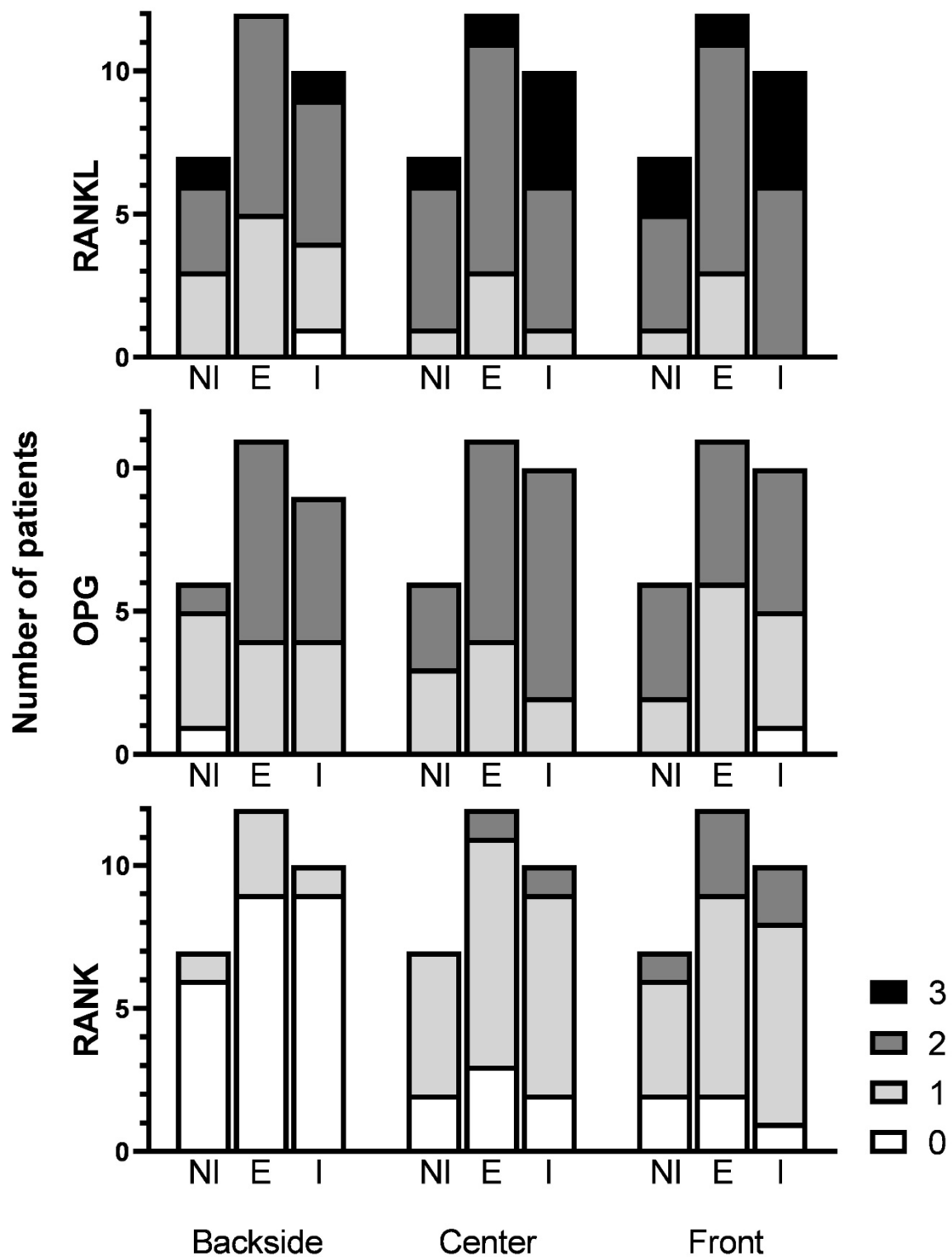


**Figure S1.** Results of 2<sup>nd</sup> quantitative PCR, \*indicates HPV positive organoid line. Red: indicates organoid line of wildtype tongue epithelium used as control; Blue: indicates organoid line with bone invasion; Grey: indicates 4 organoid lines without bone invasion.



**Figure S2.** Protein versus mRNA expression, Immunohistochemical protein expression of RANKL, OPG and RANK was compared with mRNA expression assessed with qPCR.



**Figure S3.** Differences in expression between patient groups per tumor side. Stacked bar charts of RANKL/OPG/RANK staining intensity score. Legend at the right displays color per staining intensity. X-axis displays three tumor sides with subdivision per patient group; NI: No Invasion, E: Erosion, I: Invasion. Y-axis displays number of patients. Groups were analyzed using Kruskal-Wallis H test, differences between groups were not statistically significant. Important note: RANKL intensity scored 0-3, OPG and RANK intensity scored 0-2.

**Table S1.** Primers for qPCR

primer name		primer sequence	length of product
Human RANKL(TNFSF11)	Forward 1	CAACATATCGTTGGATCACAGCA	161
Human RANKL(TNFSF11)	Reverse 1	GACAGACTCACTTTATGGGAACC	
Human RANKL(TNFSF11)	Forward 2	CCCATAAAGTGAGTCTGTCC	256
Human RANKL(TNFSF11)	Reverse 2	CAATACTGGTGCTTCCTCC	
Human OPG(TNFRSF11B)	Forward 1	CACAAATTGCAGTGTCTTTGGTC	216
Human OPG(TNFRSF11B)	Reverse 1	TCTGCGTTTACTTTGGTGCCA	
Human OPG(TNFRSF11B)	Forward 2	GAAGGGCGCTACCTTGAGAT	102
Human OPG(TNFRSF11B)	Reverse 2	GCAAACGTGATTTCGCTCTGG	
Human RANK(TNFRSF11A)	Forward 1	TCCTCCACGGACAAATGCAG	92
Human RANK(TNFRSF11A)	Reverse 1	CAAACCGCATCGGATTCTCT	
Human RANK(TNFRSF11A)	Forward 2	CACCAAATGAACCCCATGTTTAC	182
Human RANK(TNFRSF11A)	Reverse 2	GGACTCCTTATCTCCACTTAGGC	

**Table S2:** Differences in expression in tumor compared to expression in normal mucosa

Friedman test			Multiple comparison with Bonferroni correction		
	Test statistic	p-value test statistic	Tumor front versus Normal mucosa	Tumor center versus Normal mucosa	Tumor backside versus Normal mucosa
<b>RANKL</b>					
No invasion	$\chi^2(3) = 12.60$	0.006	<b>0.013</b>	<b>0.042</b>	0.668
Erosion	$\chi^2(3) = 17.855$	<0.001	<b>0.004</b>	<b>0.022</b>	0.054
Invasion	$\chi^2(3) = 17.468$	0.001	<b>0.003</b>	<b>0.016</b>	1.000
<b>OPG</b>					
No invasion	$\chi^2(3) = 6.480$	0.09	*	*	*
Erosion	$\chi^2(3) = 3.766$	0.288	*	*	*
Invasions	$\chi^2(3) = 10.705$	0.013	0.877	0.061	0.152
<b>RANK</b>					
No invasion	$\chi^2(3) = 7.235$	0.065	*	*	*
Erosion	$\chi^2(3) = 19.857$	<0.001	<b>0.015</b>	0.116	1.000
Invasion	$\chi^2(3) = 7.174$	0.067	*	*	*

**Table S2:** Statistical testing of RANKL/OPG/RANK expression comparing tumor front, tumor center and tumor backside with expression in normal mucosa. As the expression score is ordinal data and expression is compared within a patient, the non-parametric Friedman's test was used. If the Friedman's test was statistically significant, multiple comparison with Bonferroni correction was executed. A p-value of  $\leq 0.05$  was interpreted as statistical significant and is displayed in bold.

**Table S3:** Differences in expression within tumor

Friedman test			Multiple comparison with Bonferroni correction		
	Test statistic	p-value test statistic	Tumor front versus Tumor backside	Tumor center versus Tumor backside	Tumor front versus Tumor center
<b>RANKL</b>					
No invasion	$\chi^2(2) = 2.333$	0.311	*	*	*
Erosion	$\chi^2(2) = 2.571$	0.276	*	*	*
Invasion	$\chi^2(2) = 10.571$	0.005	<b>0.042</b>	0.076	1.000
<b>OPG</b>					
No invasion	$\chi^2(2) = 2.947$	0.229	*	*	*
Erosion	$\chi^2(2) = 4.000$	0.135	*	*	*
Invasions	$\chi^2(2) = 2.273$	0.321	*	*	*
<b>RANK</b>					
No invasion	$\chi^2(2) = 7.538$	0.023	0.247	0.425	1.000
Erosion	$\chi^2(2) = 13.923$	0.001	<b>0.024</b>	0.157	1.000
Invasion	$\chi^2(2) = 14.000$	0.001	<b>0.016</b>	0.076	1.000

**Table S3:** Statistical testing of RANKL/OPG/RANK expression comparing tumor front, tumor center and tumor backside. As the expression score is ordinal data and expression is compared within a patient, the non-parametric Friedman's test was used. If the Friedman's test was statistically significant, multiple comparison with Bonferroni correction was executed. A p-value of  $\leq 0.05$  was interpreted as statistical significant and is displayed in bold.

**Table S4:** Differences in expression between patient groups per tumor side

	Mean Rank			Kruskal Wallis Test	
	No invasion	Erosion	Invasion	Test statistic	p-value
<b>RANKL</b>	N=7	N=12	N=10		
Tumor-front	15.50	11.79	18.50	4.587	0.101
Tumor-center	14.57	12.63	18.15	3.102	0.212
Tumor-Backside	15.64	14.58	15.05	0.085	0.958
<b>OPG</b>	N=6	N=11	N=10		
Tumor-front	16.17	13.41	13.35	0.743	0.690
Tumor-center	11.75	13.59	15.80	1.536	0.464
Tumor-backside	8.50	15.45	14.44	4.397	0.111
<b>RANK</b>	N=7	N=12	N=10		
Tumor-front	12.93	15.58	15.75	0.733	0.693
Tumor-center	13.64	15.04	15.90	0.440	0.802
Tumor-backside	14.57	16.13	13.95	0.885	0.642

**Table S4:** Statistical testing of RANKL/OPG/RANK expression comparing patient groups; no invasion, erosion and invasion per tumor side (tumor front, tumor center and tumor backside). As the expression score is ordinal data and expression is compared from different patients, the non-parametric Kruskal-Wallis H test was used. As none of these tests were statistically significant, post-hoc testing was not executed. A p-value of  $\leq 0.05$  was interpreted as statistical significant and is displayed in bold.