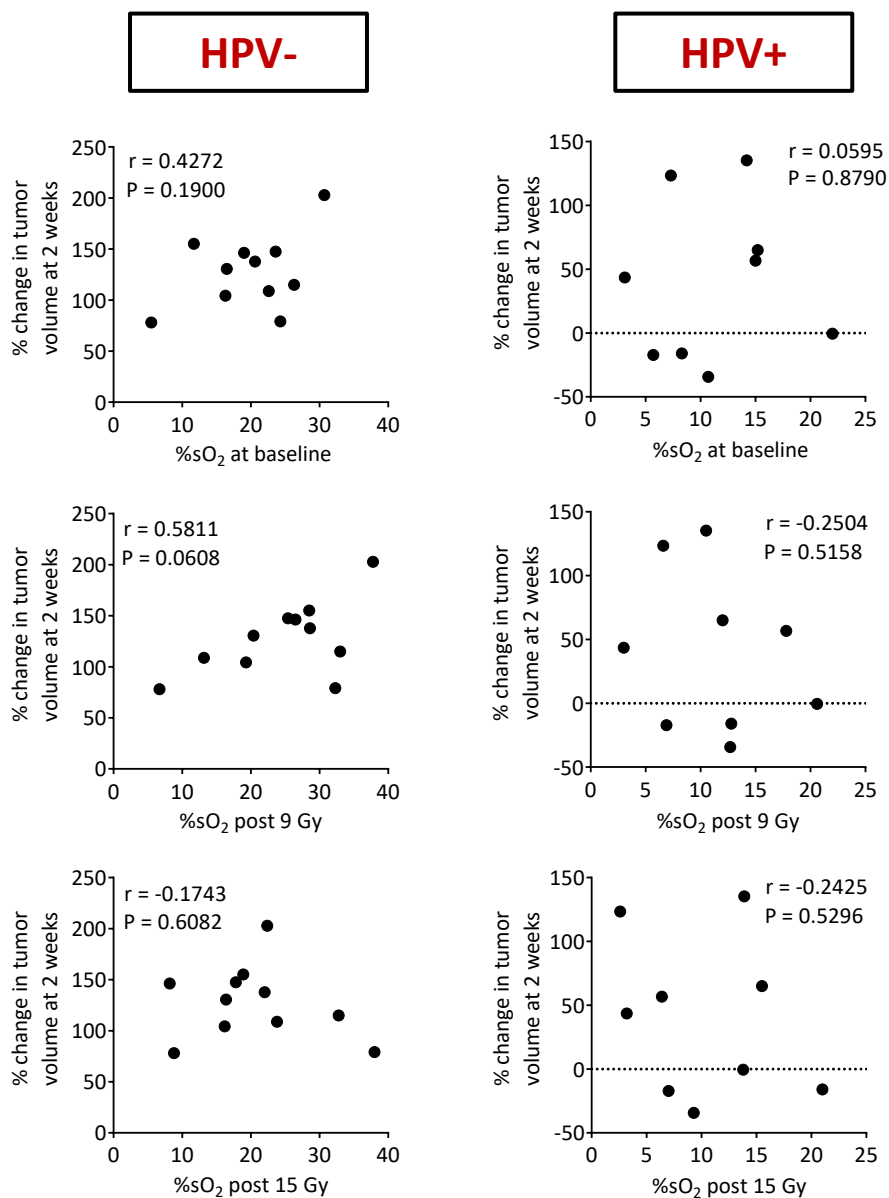


**Supplementary Information**

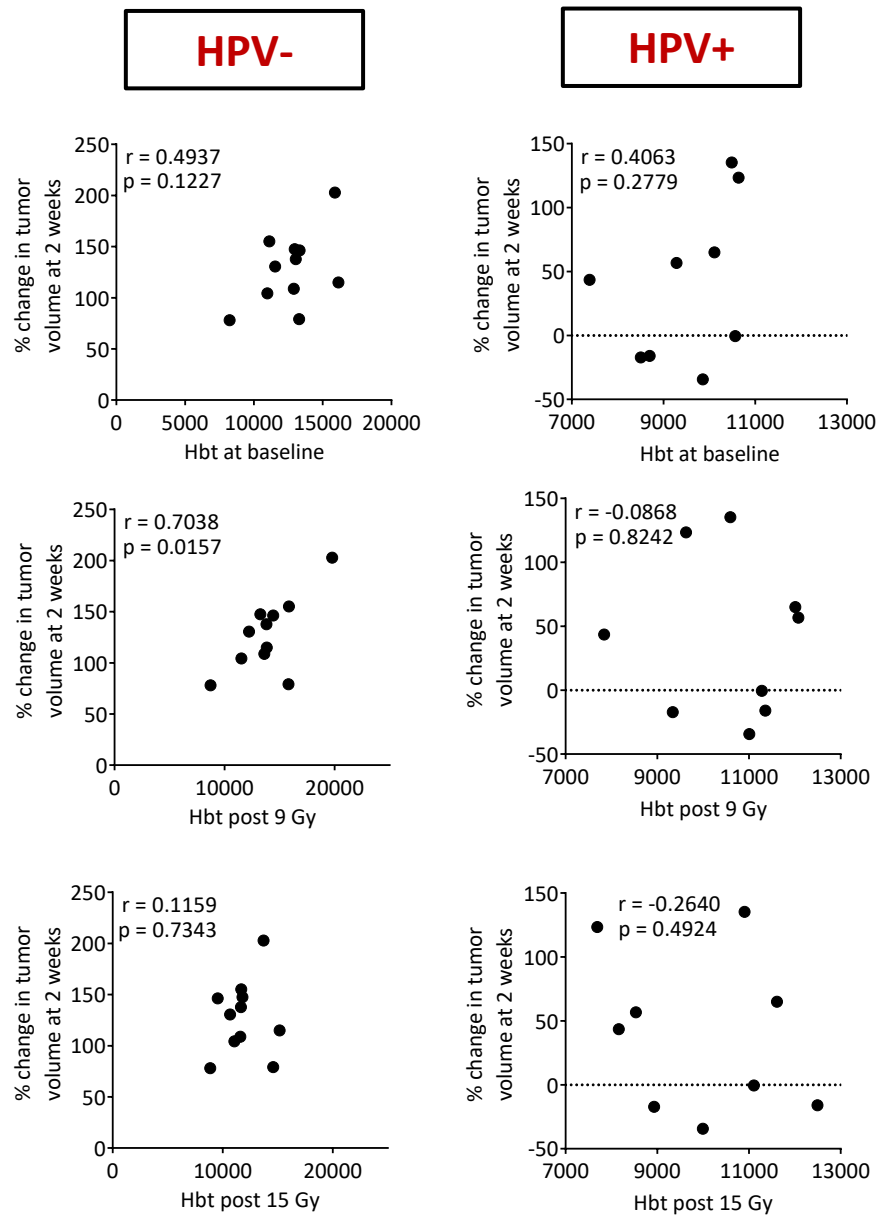
**PHOTOACOUSTIC IMAGING AS AN EARLY BIOMARKER OF RADIOTHERAPEUTIC EFFICACY IN HEAD AND NECK CANCER**

Laurie. J. Rich<sup>1,2</sup>, Austin Miller<sup>3</sup>, Anurag K. Singh<sup>4</sup>, Mukund Seshadri<sup>1,2,5\*</sup>

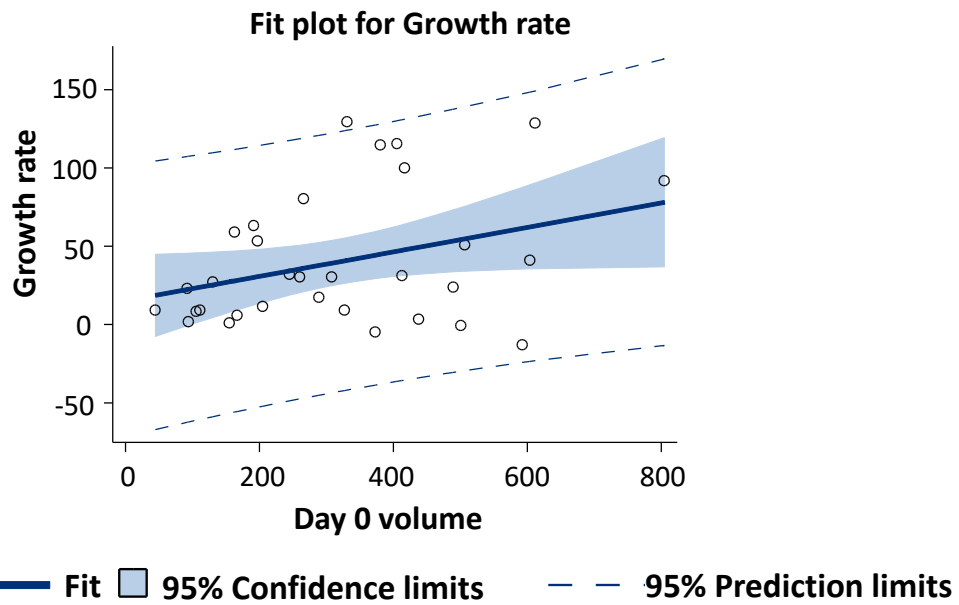
## Supplementary Results



**Figure S1.** Correlation between absolute measurements of oxygen saturation of HPV- and HPV+ xenografts at individual time points before, during and immediately post fRT and change in tumor volume at two weeks.

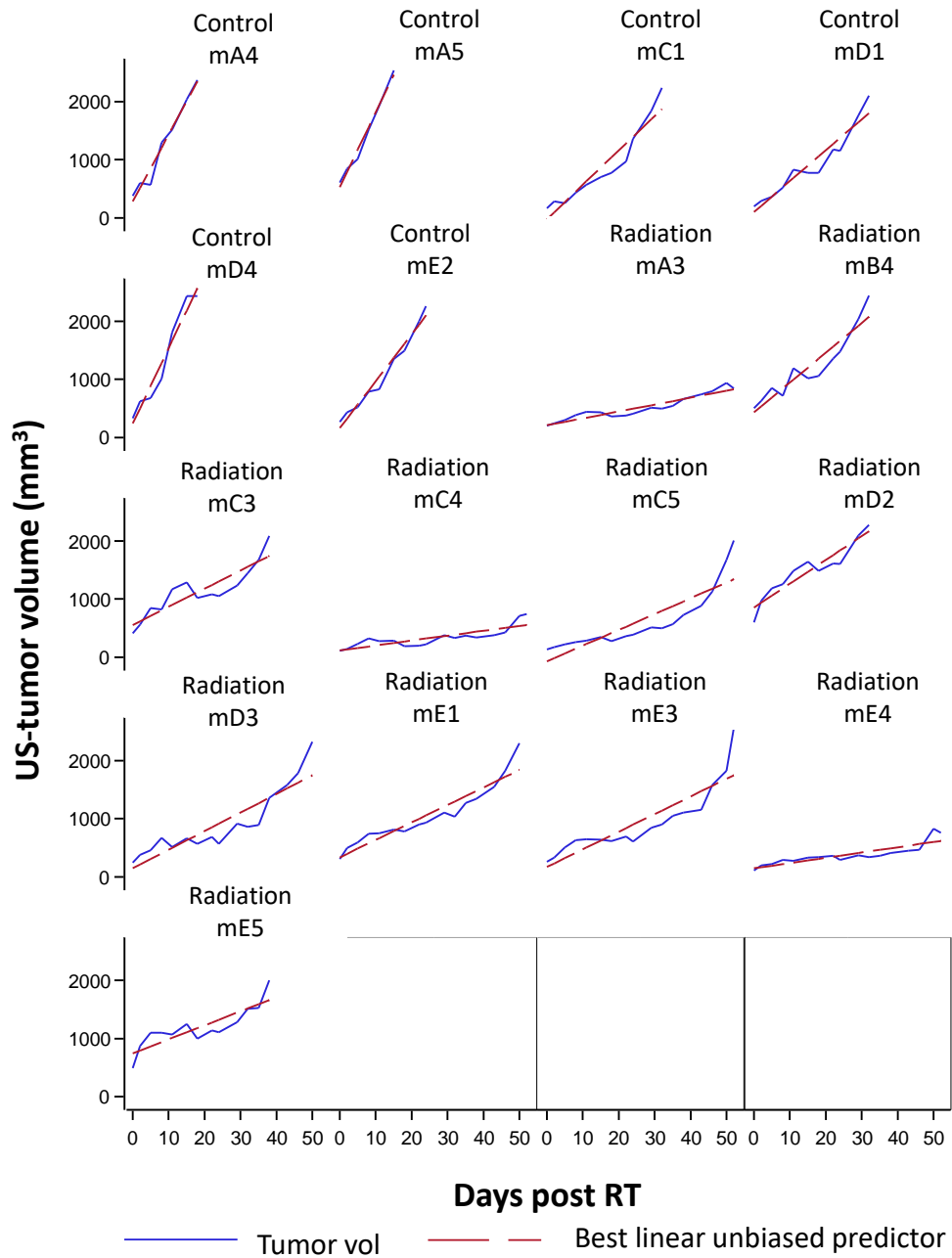


**Figure S2.** Correlation between absolute measurements of hemoglobin concentration of HPV- and HPV+ xenografts at individual time points before, during and immediately post fRT and change in tumor volume at two weeks.

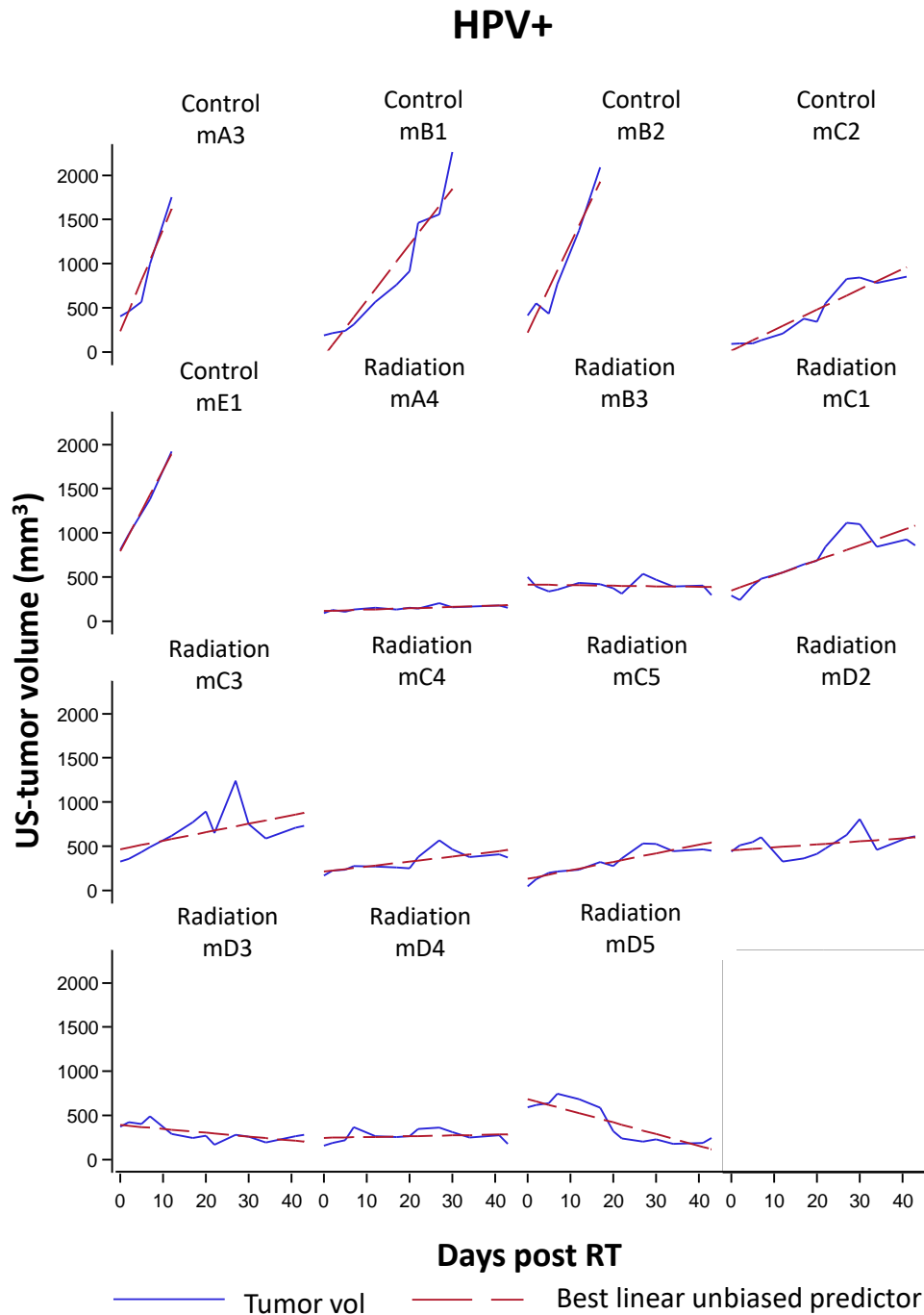


**Figure S3.** Plot shows correlation between pre-treatment tumor volumes (day 0) and growth rate for all tumors in the study. No evidence of association between baseline tumor volume was observed with growth rates or HPV and radiation treatment.

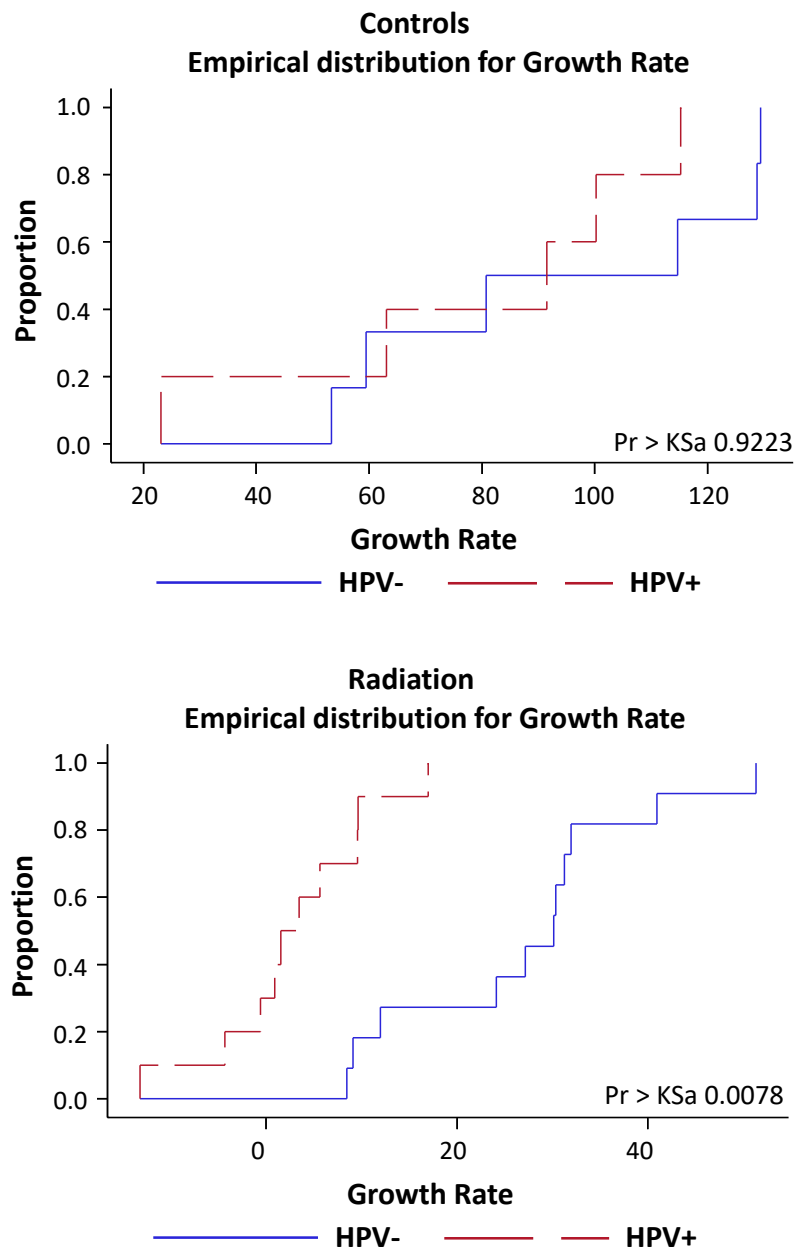
# HPV-



**Figure S4.** US-based measurements of individual growth rates of HPV- xenografts of all 17 animals (control n = 6; fRT n = 11) as a function of time post RT. Tumor growth rate was quantified as the slope from Ordinary Least Squares regression of tumor volume as a function of time.



**Figure S5.** US-based measurements of individual growth rates of HPV+ xenografts of all 15 animals (control n = 5; fRT n = 10) as a function of time post RT. Tumor growth rate was quantified as the slope from Ordinary Least Squares regression of tumor volume as a function of time.



**Figure S6. Long-term treatment outcome following fRT in HPV+ and HPV- PDX models of HNSCC.** The effects of radiation on tumor growth rate in HPV- and HPV+ xenografts were estimated using an ANOVA model with main effects for radiation, tumor type and the interaction. In control animals (top), distribution of growth rates showed faster growth rates in the HPV- PDX model compared to the HPV+ PDX. A differential response to fRT (bottom) was seen between HPV- and HPV+ tumors with a significantly greater growth rate inhibition in the HPV+ PDX model compared to the HPV- PDX model.

**Association between PAI Marker and Tumor Growth Rates**  
**Slope Estimate (95% Confidence Interval)**

PAI Marker	Any PAI effect?	Treatment	Slope Estimate (95% Confidence Interval)	
			HPV(-)	HPV(+)
SO2 Tot (day 0)	p=0.22	Control	1.29 (-3.49 to 6.06)	-3.17 (-5.89 to -0.44)
		RT	-0.04 (-2.00 to 1.92)	-0.53 (-3.09 to 2.03)
SO2 Tot (day 2)	p=0.13	Control	-3.21 (-5.63 to -0.80)	-0.70 (-5.22 to 3.81)
		RT	-0.40 (-1.87 to 1.07)	-0.12 (-1.81 to 1.58)
SO2 Tot (day 5)	p=0.06	Control	-3.89 (-6.46 to -1.32)	-1.89 (-20.33 to 16.55)
		RT	-0.36 (-1.81 to 1.08)	-0.55 (-2.89 to 1.79)
SO2 Total (day 0-2)	p< 0.01	Control	-3.00 (-4.68 to -1.32)	5.51 (2.76 to 8.27)
		RT	-0.78 (-2.37 to 0.81)	0.27 (-1.58 to 2.12)
SO2 Total (day 0-5)	p< 0.01	Control	-2.56 (-4.32 to -0.79)	3.78 (1.27 to 6.28)
		RT	-0.54 (-2.16 to 1.09)	-0.04 (-2.08 to 2.00)
SO2 Total (day 2-5)	p= 1.00	Control	0.01 (-3.09 to 3.10)	0.79 (-5.19 to 6.77)
		RT	0.06 (-1.73 to 1.85)	-0.16 (-2.19 to 1.86)
SO2 Tot (day 0-12)	p< 0.01	Control	-3.03 (-4.55 to -1.52)	4.09 (1.35 to 6.83)
		RT	0.15 (-1.23 to 1.53)	0.41 (-2.15 to 2.98)
HBT (day 0)	p= 0.39	Control	3.51 (-11.35 to 18.37)	-11.11 (-22.61 to 0.38)
		RT	0.20 (-6.21 to 6.62)	1.39 (-11.40 to 14.17)
HBT (day 2)	p= 0.67	Control	-7.48 (-20.06 to 5.10)	-5.29 (-17.49 to 6.91)
		RT	-0.78 (-6.08 to 4.53)	0.18 (-7.49 to 7.84)
HBT (day 5)	p= 0.02	Control	-19.27 (-32.71 to -5.84)	-21.75 (-44.06 to 0.57)
		RT	-2.12 (-8.44 to 4.19)	-2.61 (-10.80 to 5.58)
HBT (day 12)	p= 0.63	Control	-3.87 (-10.84 to 3.10)	-5.35 (-23.80 to 13.11)
		RT	1.53 (-10.90 to 13.95)	-6.85 (-22.09 to 8.39)
HBT (day 0-2)	p= 0.10	Control	-14.62 (-28.37 to -0.86)	16.25 (-1.18 to 33.69)
		RT	-1.96 (-8.94 to 5.03)	-0.50 (-9.54 to 8.54)
HBT (day 0-5)	p= 0.05	Control	-9.43 (-18.35 to -0.51)	16.88 (0.56 to 33.20)
		RT	-4.46 (-13.42 to 4.51)	-2.71 (-10.35 to 4.93)
HBT (day 2-5)	p= 0.94	Control	-4.64 (-18.05 to 8.78)	1.19 (-14.74 to 17.11)
		RT	-0.44 (-7.37 to 6.50)	-1.71 (-9.50 to 6.07)
HBT (day 0-12)	p= 0.06	Control	-7.94 (-16.00 to 0.13)	14.97 (1.44 to 28.49)
		RT	0.21 (-5.36 to 5.78)	-4.70 (-14.56 to 5.16)

**Table S1.** Summary of statistical modeling of the relationship between PAI parameters and tumor growth rate in PDX models of HNSCC. Positive slope estimates indicate faster tumor growth among mice with higher PAI marker values. The 95% confidence interval indicates the plausible range for the true, population-level average that is supported by the data. Confidence intervals excluding 0 can be interpreted as statistically significant at the p<0.05 level.



## Supplementary Methods

Statistical significance of the PAI effect was obtained using a partial *F*-test. The Full Model included main effects for PAI, HPV and radiation, and all 2<sup>nd</sup> and 3<sup>rd</sup> order interactions. The Reduced Model included main effects for HPV and radiation and the interaction. All models were fit using ordinary least squares. Statistical modeling was performed using SAS/STAT software, Version 9.4 (SAS Institute Inc. Cary, NC, USA). The association between the PAI read-outs and mouse-level tumor growth rate was determined using Ordinary Least Squares slope estimate, interpreted as the change in growth rate expected from a unit increase in the PAI read-out. For HBT, a unit increase indicates a 1,000 point increase. The PAI Effect p-value assesses the null hypothesis of no PAI association with the growth rates using a partial *F*-Test from a model specified with main effects for Treatment, HPV status and up to 3<sup>rd</sup> order interactions.