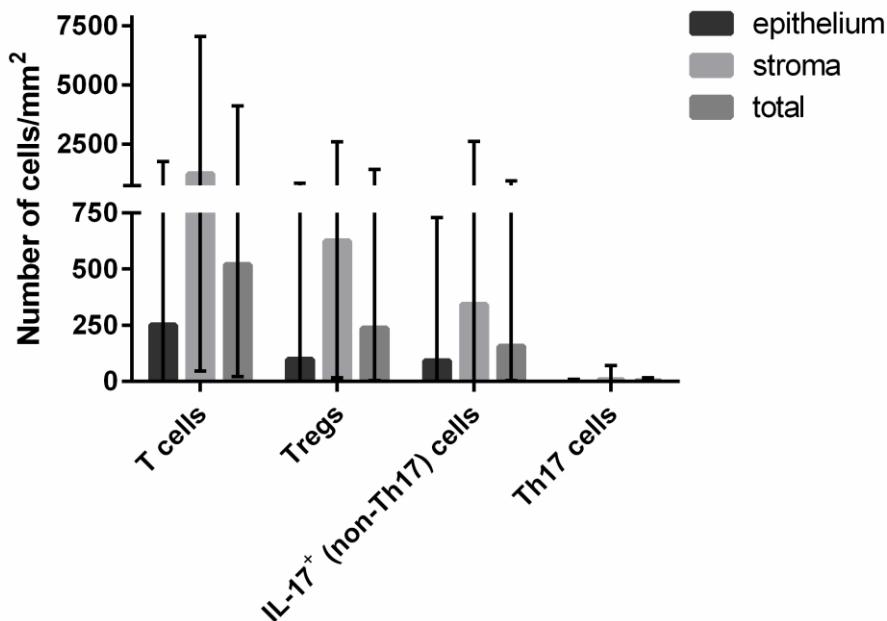
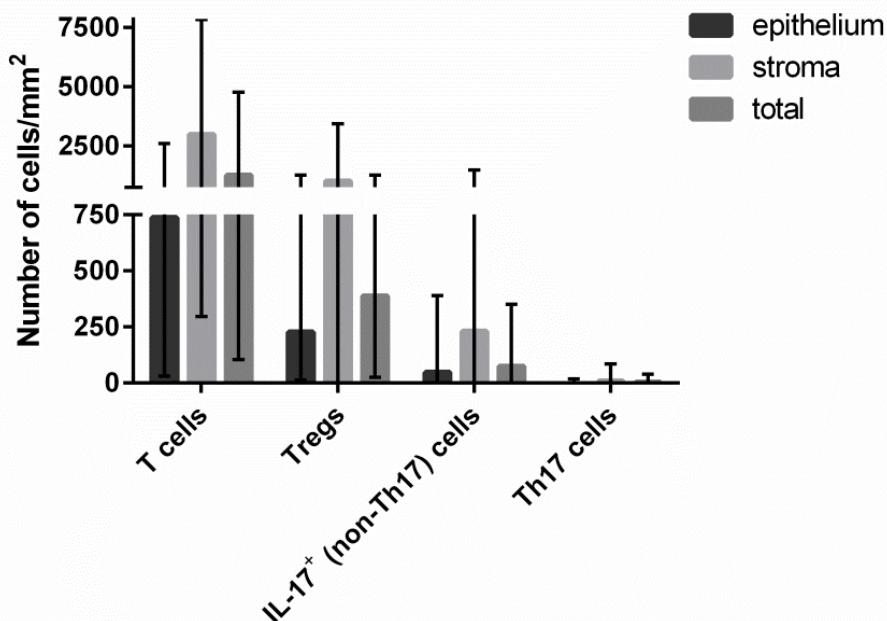


Supplementary Figure 1. Quantification of tumor infiltrating cells

a

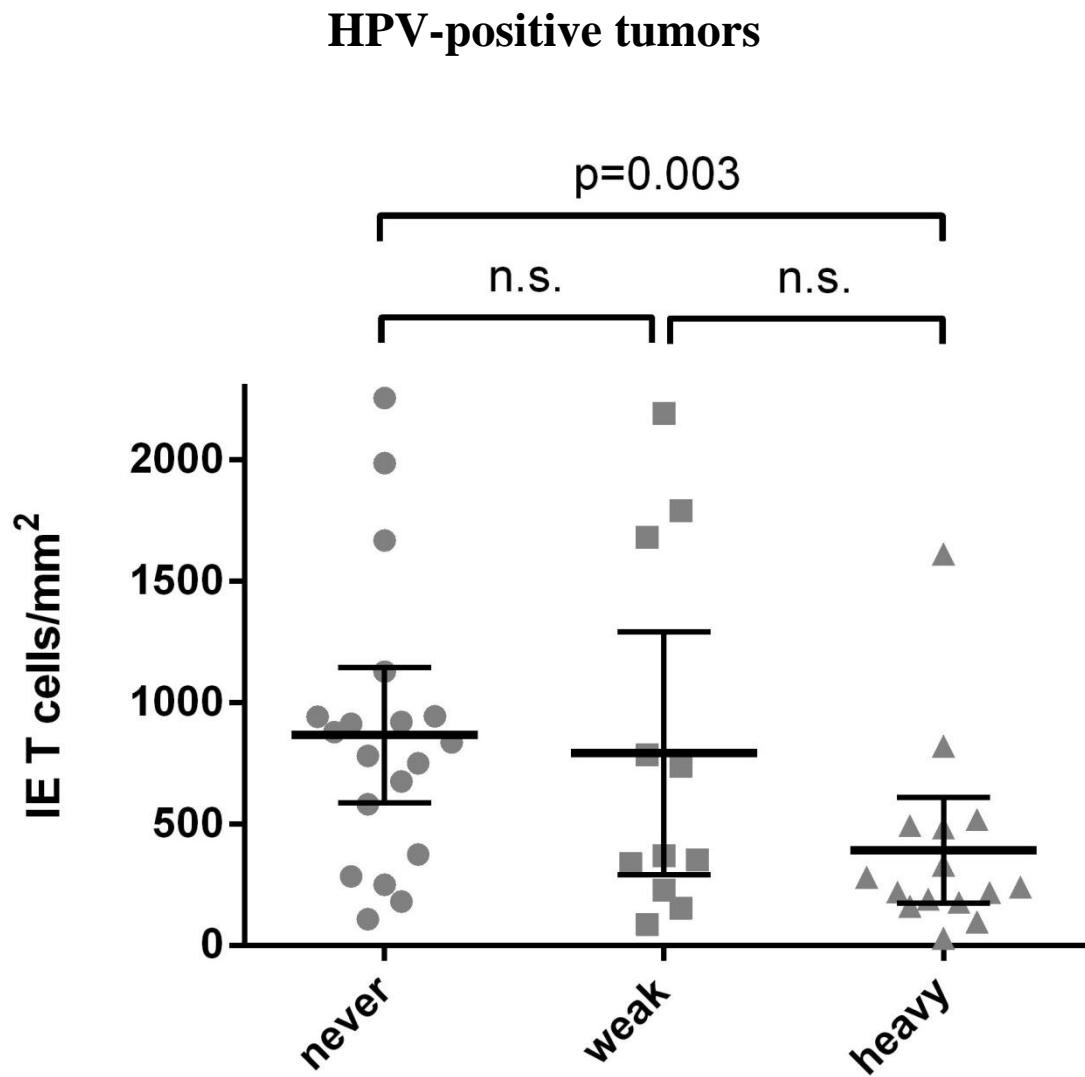


b



The mean and range of the number of CD3⁺ T cells, FoxP3⁺CD3⁺ Tregs, CD3⁺IL-17⁺ cells and CD3⁺IL-17⁺ Th17 cells infiltrating in the tumor epithelium, tumor stroma and combined total area per mm² is shown in HPV-negative tumors (**a**, n=99) and HPV-positive tumors (**b**, n=63).

Supplementary Figure 2. Decreased intraepithelial T-cell frequency in HPV-positive OPSCC of heavy compared to never smokers



The number of total CD3⁺ T cells/mm² tumor epithelium in HPV-positive tumors is shown for never, weak (1-24 packyears) and heavy smokers (>24 packyears). The bars indicate the mean and 95% confidence interval; n.s. = not significant.

Supplementary Table 1. Number of T cells, Tregs and IL-17⁺ cells present in squamous oropharyngeal carcinoma

| | Location | Total T cells | | Tregs | | IL-17 ⁺ (non-Th17) cells | | Th17 cells | | Total T cells/Tregs | |
|---------------------|------------|--------------------|--------|------------------|--------|--|--------|-----------------|--------|---------------------|--------|
| | | Mean (range) | Median | Mean (range) | Median | Mean (range) | Median | Mean (range) | Median | Mean (range) | Median |
| HPV-negative tumors | Epithelium | 251 (0-1767) | 151 | 98 (0-863) | 49 | 92 (0-730) | 41 | 1 (0-9) | 0 | 4 (1-37) | 2 |
| | Stroma | 1237 (47-7042) | 971 | 624 (16-2596) | 520 | 343 (0-2613) | 237 | 6 (0-70) | 0 | 2 (1-8) | 2 |
| | Total | 519 (21-4113) | 362 | 237 (4-1438) | 193 | 156 (3-956) | 99 | 2 (0-17) | 0 | 2 (1-8) | 2 |
| HPV-positive tumors | Epithelium | 738 (30-2596) | 545 | 226 (12-1272) | 150 | 47 (0-389) | 14 | 1 (0-17) | 0 | 6 (1-84) | 3 |
| | Stroma | 2985 (295-7831) | 2633 | 1017 (0-3437) | 972 | 229 (0-1479) | 121 | 7 (0-85) | 0 | 4 (1-35) | 3 |
| | Total | 1251 (104-4775) | 1005 | 387 (24-1272) | 335 | 73 (0-351) | 39 | 2 (0-38) | 0 | 5 (1-63) | 3 |

The mean, minimum, maximum and median number of total CD3⁺ cells, FoxP3⁺CD3⁺ Tregs, CD3⁺IL-17⁺ cells and CD3⁺IL-17⁺ Th17 cells observed infiltrating in the tumor epithelium, tumor stroma and combined total area per mm² is indicated for HPV-negative and HPV-positive tumors.

Supplementary Table 2. Effect of 6 different types of T cell infiltrations on disease-free survival regarding HPV status

| | High intraepithelial T cell frequency | | High total T cell frequency | | High total Treg frequency | | High total nonTreg T cell frequency | | High intraepithelial nonTreg T cell frequency | | High total IL-17 ⁺ cell frequency | |
|-----------------------------------|---------------------------------------|-----------------|-----------------------------|-----------------|---------------------------|-----------------|-------------------------------------|-----------------|---|-----------------|--|-----------------|
| Univariate Hazard Ratio | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value |
| HPV negative (p16-) | 1.46 (0.73-2.93) | 0.282 | 1.11 (0.58-2.12) | 0.751 | 1.65 (0.81-0.36) | 0.165 | 1.62 (0.81-3.26) | 0.174 | 1.15 (0.89-1.50) | 0.292 | 0.96 (0.51-1.82) | 0.912 |
| HPV positive (p16+/PCR+) | 0.22 (0.08-0.64) | 0.006 | 0.28 (0.10-0.82) | 0.021 | 0.21 (0.07-0.64) | 0.006 | 0.27 (0.09-0.78) | 0.015 | 0.35 (0.12-1.03) | 0.056 | 0.50 (0.17-1.51) | 0.220 |
| Multivariate Hazard Ratio* | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value | <i>HR</i> (95%CI) | <i>p</i> -value |
| HPV negative (p16-) | 1.52 (0.74-3.18) | 0.256 | 1.13 (0.57-2.21) | 0.721 | 1.77 (0.83-3.74) | 0.134 | 1.66 (0.81-3.41) | 0.168 | 1.16 (0.84-1.88) | 0.258 | 1.09 (0.54-2.18) | 0.806 |
| HPV positive (p16+/PCR+) | 0.25 (0.07-0.95) | 0.043 | 0.34 (0.10-1.13) | 0.080 | 0.28 (0.08-1.02) | 0.054 | 0.40 (0.12-1.32) | 0.135 | 1.18 (0.24-5.97) | 0.835 | 0.53 (0.17-1.69) | 0.284 |

*Adjusted for comorbidity, prior tumor and smoking status using Cox proportional hazards regression analysis. This analysis was performed 6 times, entering a different type of T cell infiltration each time, but adjusting for the same variables (comorbidity, prior tumor and smoking status).

HR= hazard ratio, 95%CI = 95% confidence interval. ‘High’= upper 3 qrt vs lowest qrt.