

## Figure legends of Supplemental Figures

### **Supplemental Figure S1: Overview showing the prognostic relevance of perineural invasion for HNSCC and its different primary sites**

(A) Kaplan-Meier plots for 5-years overall survival (OS, left), disease-specific survival (DSS, middle) and progression-free intervals (PFI, right) for patients of TCGA-HNSC with an annotated PNI-status. Number of patients at risk at the indicated time points are given below. (B) Forest plot for OS, DSS and PFI based on univariate Cox regression models for subgroups of oral (OSCC), laryngeal (LASCC) and oropharyngeal (OPSCC) tumors of TCGA-HNSC. <sup>1</sup> reference group.

### **Supplemental Figure S2: Flow chart visualizing experimental design of our study**

### **Supplemental Figure S3: Identification of PNI-related DEGs for TCGA-HNSC**

Volcano plots illustrates DEGs, which were calculated by limma-voom (A) or edgeR (B), and candidate genes with the significance level of adj. p val. < 0.05 and log<sub>2</sub>FC < -1, > 1 are indicated in green (up in PNI+) and red (down in PNI+). (C) Venn diagram demonstrates 60 overlapping DEGs for the limma-voom and edgeR analysis. (D) Heatmap shows an unsupervised hierarchical clustering based on gene expression data for the 60 DEGs and demonstrates a cluster of co-expressed muscle-related genes (n =16).

**Supplemental Figure S4: Analysis of the PNI-related 44-gene signature with independent HNSCC cohorts, best performing classification models** Heatmaps show an unsupervised hierarchical clustering based on gene expression data of the PNI-related 44-gene signature and confirm a classification into cluster A (red) and sub-clusters B1 (blue) and B2 (green) in independent HNSCC cohorts: GSE117973 (A), GSE65868 (B), and GSE41613 (C). (D-F) ROC curves for calculation of a suitable machine learning classification model (random forest, neural network, logistic regression) illustrated by AUCs.

**Supplemental Figure S5: Transmission of our machine learning model and our 44-gene signature to other tumor entities demonstrating the relevance of the model**

(A) Forest plot for overall survival (OS) based on univariate Cox regression models for indicated TCGA cohorts (CESC, LUAD; PAAD, COAD). <sup>1</sup> indicates the reference group. (B) Heatmap shows an unsupervised hierarchical clustering of the 44-gene signature based on gene expression data confirming a classification into cluster A (red) and sub-clusters B1 (blue) and B2 (green) in TCGA-CESC.

**Supplemental Figure S6 + S7: Differences In copy number alterations between ML A and ML B2:**  
Zoom-in to illustrate the deletions (blue) and amplifications (red) in the comparison between ML A and ML B2, exemplified here by the regions containing hot spots with the lowest p-values (chromosome 1 (6A), 7 (6B), 13 (7A), 16 (7B)).

**Supplemental Figure S8: Analysis of immune and stromal cells as part of the comprehensive analysis of multi-omics data to show the potential impact distinct immune cell subsets**

Spider plot demonstrating statistical significance by showing calculated p-values between ML A and ML B2 for different immune and stromal cells (B cells, T cells, CD4 T cells, Fibroblasts, Endothelium).