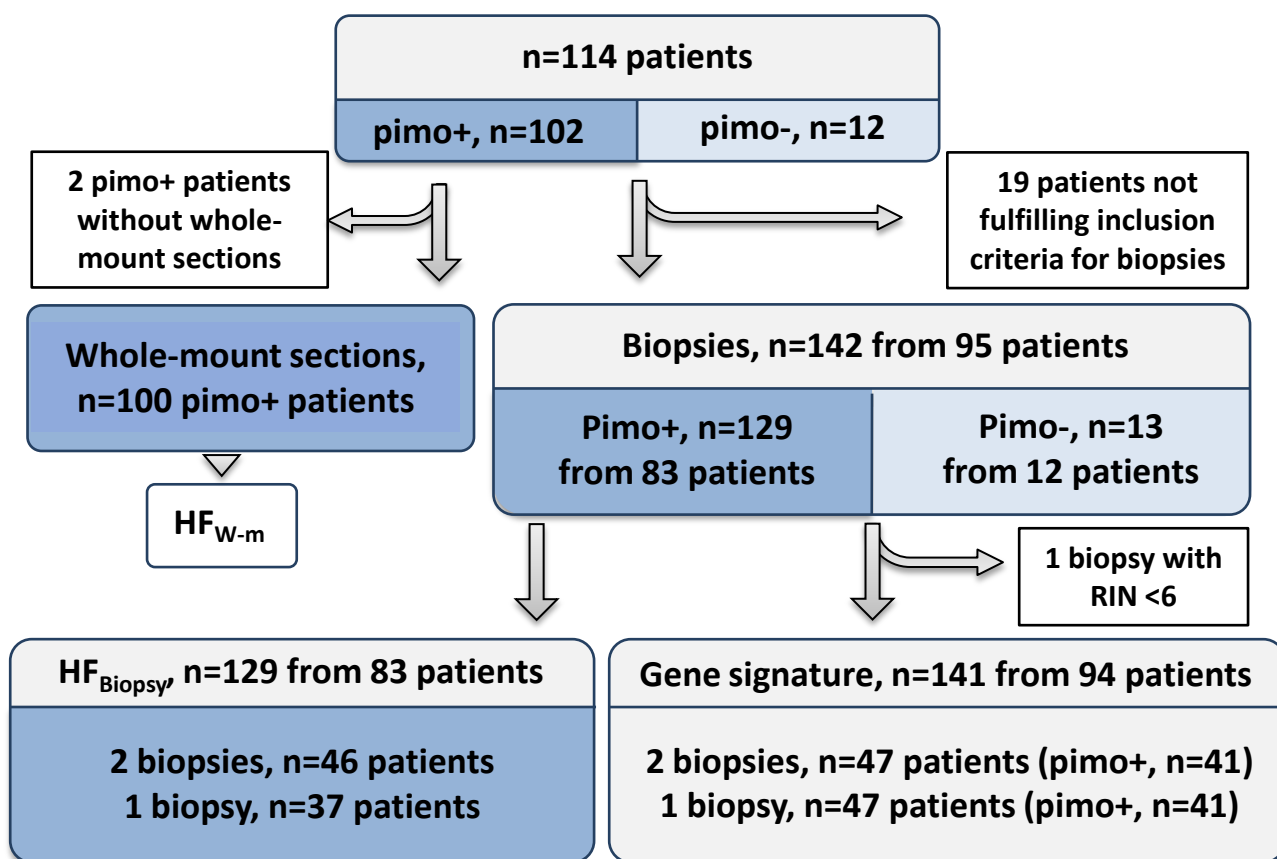
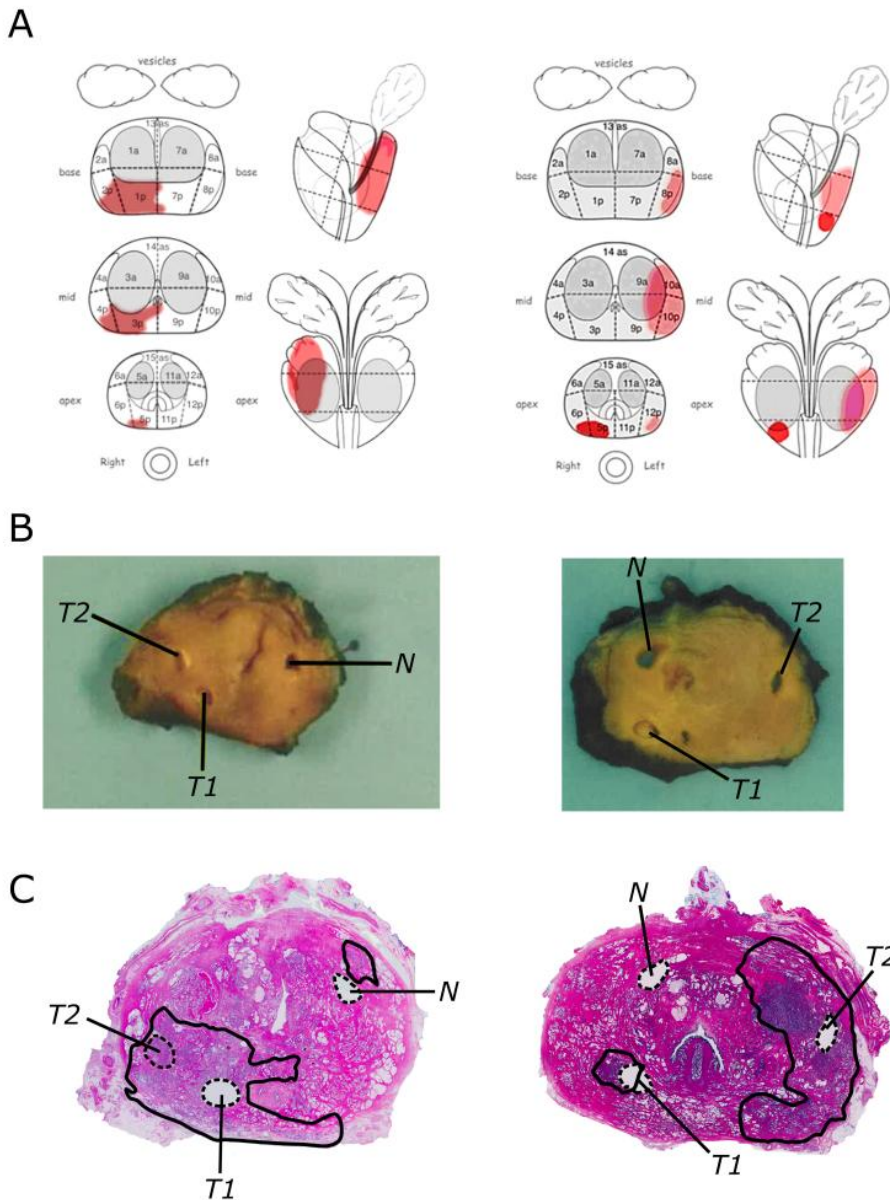


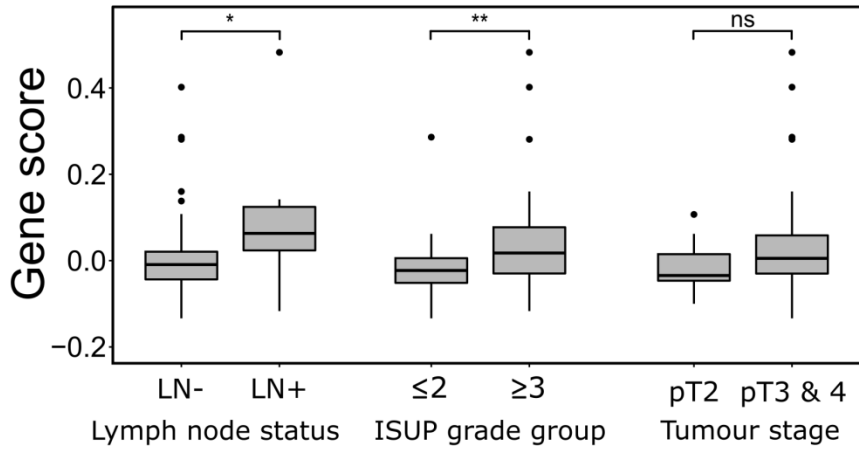


Supplementary Figure S1. Overview of patients and biopsies included in the study. Out of totally 114 patients, 102 patients received pimonidazole (pimo+). Whole-mount sections from 100 of these patients were available and used to assess HF_{W-m} . Totally 142 biopsies from 95 out of the 114 patients (83 pimo+, 12 pimo-) were used to assess HF_{W-m} (n=129 biopsies) and gene signature (n=141). Analysis of intratumor heterogeneity was performed in 52 patients with 2 biopsies from the index lesion (HF_{Biopsy} , n=46; gene signature, n=47; n=41 patients in common for both assays).

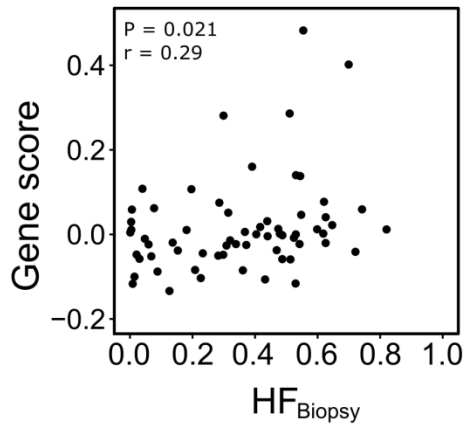


Supplementary Figure S2. Localization of tumour biopsies. The procedure is illustrated for a patient with two biopsies from the index lesion (left) and a patient with one biopsy from the index lesion and one biopsy from another lesion (right). **(A)** MRI reports of the prostate, indicating the site of one (left patient) and two (right patient) intra-prostatic tumour lesions. **(B)** Macro images of the prostatectomy specimens, indicating the site of the punch biopsies. **(C)** HE-stained whole-mount sections of the prostatectomy specimens with the tumour lesions delineated (solid line) and the locations of punch biopsies indicated (dotted line). T1, the biopsy collected first; T2, the second biopsy; N, biopsy collected outside tumour.

A

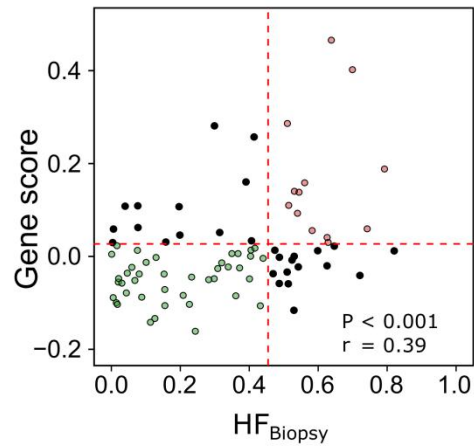


B

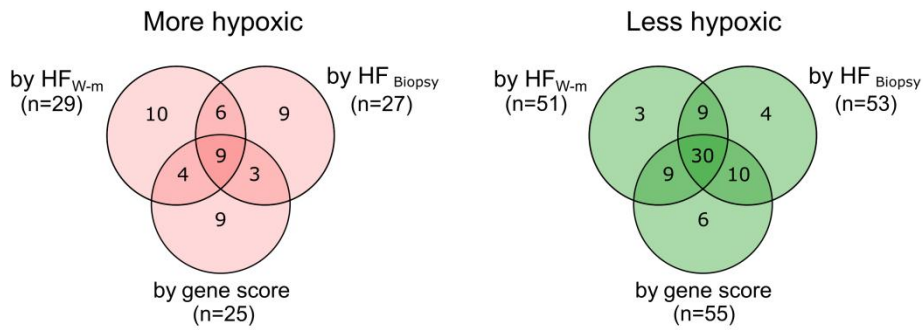


Supplementary Figure S3. Gene score analyses based on biopsies not included in previous work. (A) Box plots of gene score for patients with positive and negative lymph node (LN) status (n=74), ISUP grade group of ≤ 2 and ≥ 3 (Gleason grade $\leq 3+4$ and $\geq 4+3$) (n=75) and pathological tumour stage T2 and T3 or T4 (n=75). The boxes extend from the first to the third quartile with the median value indicated. Significant difference between groups by Mann-Whitney U-test is indicated; **, $P=0.0028$, adj $P=0.0084$; *, $P=0.013$, adj $P=0.0195$; ns, not statistically significant $P=0.076$, adj $P=0.076$. (B) Association between HF_{Biopsy} and gene score for 76 patients with both parameters available. Pearson correlation coefficient (r) and P-value are indicated.

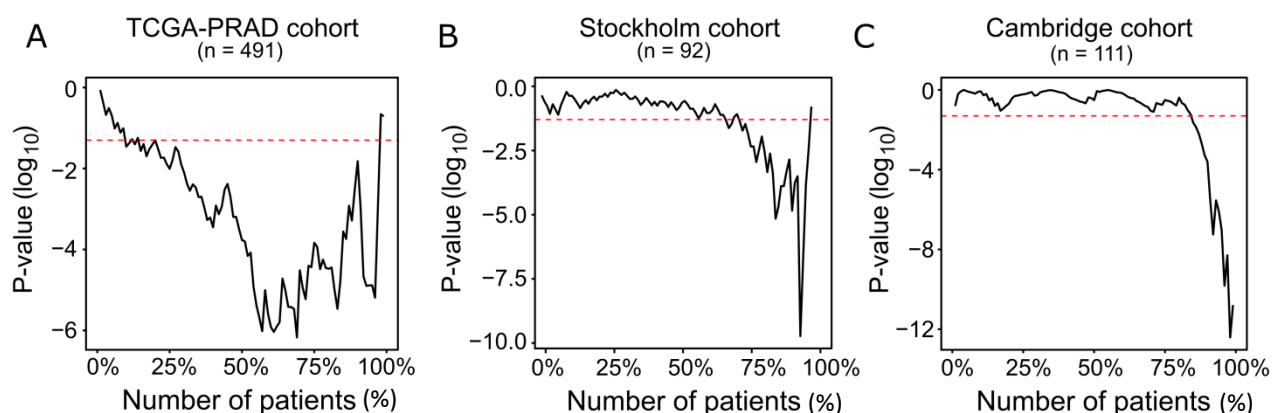
A



B



Supplementary Figure S4. Relationship between pimonidazole- and gene-defined hypoxia in biopsies. (A) Association between HF_{Biopsy} and gene score for 82 patients with both parameters available. The red dotted lines indicate the separation between more and less hypoxic tumours based on the 67% percentile of the distribution in Figure 2B and D. Pearson correlation coefficient (r) and P-value are indicated. (B) Venn-diagram showing the overlap in classification of 80 patients with HF_{W-m}, HF_{Biopsy} and gene score available. The 67% percentiles indicated in (A) were used as classification cutoffs for HF_{Biopsy} and gene score, while the 67% percentile of 0.27 was used for HF_{W-m}.



Supplementary Figure S5. Screening of gene score cutoff for patient classification in external data sets. P-value in log rank test *versus* number (%) of patients in the group with a low gene score, based on the TCGA-PRAD cohort (**A**), Stockholm cohort (GSE70769 (**B**), and Cambridge cohort (GSE70768 (**C**). Red line indicates a P-value of 0.05. Number of patients in each cohort is indicated.