

**tRNA-derived RNA fragments are novel biomarkers for diagnosis, prognosis, and
tumor subtypes in prostate cancer**

Supplementary Materials

Table S1 5'-tRFs are differentially expressed between PRAD tumor and adjacent normal tissues*

tRFs	Log(FC)	P value
Upregulated		
5'-M-tRNA-Glu-TTC-1-2_L30	3.676	8.74E-31
5'-M-tRNA-Glu-TTC-2-2_L30	3.166	2.18E-29
5'-M-tRNA-Gly-GCC-5-1_L30	2.888	6.07E-90
5'-M-tRNA-Gly-GCC-5-1_L29	2.787	3.95E-26
5'-M-tRNA-Val-CAC-5-1_L30	2.581	1.37E-28
5'-M-tRNA-Gly-GCC-5-1_L28	2.56	1.86E-28
5'-M-tRNA-Glu-CTC-2-1_L29	2.551	1.99E-26
5'-M-tRNA-Gly-GCC-1-5_L30	2.432	3.30E-25
5'-M-tRNA-Glu-CTC-2-1_L30	2.405	1.07E-29
5'-M-tRNA-His-GTG-1-9_L30	2.32	4.50E-29
5'-M-tRNA-Gln-CTG-5-1_L30	2.306	3.26E-24
5'-M-tRNA-Leu-CAG-2-2_L30	2.051	3.64E-31
5'-M-tRNA-Cys-GCA-4-1_L29	2.01	2.74E-25
5'-M-tRNA-Gln-TTG-4-1_L30	1.978	6.40E-30
5'-M-tRNA-Tyr-GTA-chr21-2_L30	1.932	8.47E-24
5'-M-tRNA-Gln-CTG-5-1_L29	1.918	2.24E-29
5'-M-tRNA-Ala-TGC-4-1_L24	1.865	1.23E-31
5'-tRNA-Gly-TCC-1-1_L30	1.857	2.82E-29
5'-M-tRNA-Gly-CCC-2-2_L29	1.828	1.37E-24
5'-M-tRNA-Gly-GCC-5-1_L27	1.809	3.03E-26
5'-M-tRNA-Gln-TTG-3-3_L30	1.709	1.10E-28
5'-M-tRNA-Lys-TTT-5-1_L30	1.69	5.22E-26
5'-M-tRNA-Arg-TCG-1-1_L19	1.663	1.42E-11
5'-M-tRNA-Leu-TAG-1-1_L26	1.631	6.42E-32
5'-M-tRNA-Ser-TGA-4-1_L24	1.627	1.33E-28
5'-M-tRNA-Tyr-GTA-3-1_L25	1.612	4.58E-31
5'-M-tRNA-Pro-TGG-3-5_L30	1.576	3.61E-29
5'-M-tRNA-Ser-TGA-4-1_L25	1.558	9.97E-32
5'-M-tRNA-Glu-CTC-2-1_L28	1.555	5.34E-26
5'-M-tRNA-Leu-CAG-2-2_L25	1.511	3.38E-32
5'-M-tRNA-Val-TAC-1-2_L30	1.478	8.31E-28
5'-M-tRNA-Leu-TAG-1-1_L30	1.473	6.35E-27
5'-M-tRNA-Val-CAC-5-1_L23	1.469	8.03E-28
5'-M-tRNA-Leu-TAG-1-1_L21	1.462	1.45E-12
5'-tRNA-Val-CAC-8-1_L30	1.426	5.45E-29
5'-M-tRNA-Gly-GCC-1-5_L29	1.422	4.97E-18
5'-M-tRNA-Tyr-GTA-7-1_L25	1.416	4.03E-28
5'-M-tRNA-Leu-TAG-1-1_L25	1.412	3.20E-32
5'-M-tRNA-Ser-GCT-5-1_L24	1.398	9.71E-32
5'-tRNA-Arg-TCT-1-1_L19	1.349	1.08E-09
5'-M-tRNA-Gly-TCC-4-1_L23	1.305	5.11E-27

5'-tRNA-Leu-TAA-1-1_L25	1.269	4.82E-32
5'-M-tRNA-iMet-CAT-2-1_L30	1.264	2.54E-30
5'-M-tRNA-Leu-CAG-2-2_L26	1.246	1.18E-30
5'-tRNA-Pro-TGG-2-1_L30	1.234	3.29E-29
5'-M-tRNA-Glu-TTC-2-2_L28	1.221	2.54E-25
5'-tRNA-Glu-TTC-9-1_L30	1.209	1.54E-29
5'-M-tRNA-Lys-CTT-3-1_L30	1.194	1.59E-25
5'-M-tRNA-Thr-CGT-4-1_L24	1.187	3.03E-27
5'-tRNA-Arg-CCG-2-1_L30	1.185	8.86E-26
5'-M-tRNA-Arg-ACG-2-4_L19	1.184	6.47E-12
5'-M-tRNA-Lys-CTT-4-1_L30	1.181	3.57E-28
5'-tRNA-Val-AAC-2-1_L20	1.174	3.19E-11
5'-M-tRNA-Arg-TCT-5-1_L19	1.148	1.14E-11
5'-M-tRNA-Gly-TCC-2-6_L30	1.136	4.30E-25
5'-M-tRNA-Leu-TAG-1-1_L24	1.069	1.86E-31
5'-M-tRNA-Ser-CGA-2-1_L25	1.052	5.89E-31
5'-tRNA-Val-TAC-2-1_L30	1.036	4.94E-23
5'-tRNA-Val-AAC-2-1_L23	1.031	7.78E-27
5'-M-tRNA-Gln-CTG-5-1_L23	1.029	6.64E-29
5'-M-tRNA-Leu-TAG-1-1_L22	1.018	2.59E-24
5'-M-tRNA-Ala-TGC-4-1_L22	1.013	1.17E-23
5'-M-tRNA-Tyr-GTA-7-1_L23	1.006	4.27E-20
Dowregulated		
5'-M-tRNA-Leu-TAG-3-1_L15	-1.005	4.38E-12
5'-M-tRNA-Glu-TTC-1-2_L16	-1.129	4.85E-27
5'-M-tRNA-Val-TAC-3-1_L15	-1.271	2.59E-18
5'-M-tRNA-Ser-TGA-4-1_L18	-1.296	2.58E-19
5'-M-tRNA-Gly-CCC-2-2_L15	-1.409	6.11E-26
5'-M-tRNA-Ser-TGA-4-1_L15	-1.431	1.63E-13
5'-M-tRNA-Val-CAC-7-1_L15	-1.518	1.38E-17

*Note: The top 13 upregulated 5'-tRFs (highlighted by bold font) were used to build tRF classifiers for diagnosis of PRAD.

Table S2 Univariate Cox regression analysis confirms 5'-tRF association with PRAD prognosis*

tRF	Z-score	HR	Low95%CI	High95%CI	Wald test	Log-rank test
5'-tRNA-Arg-TCG-3-1_L18	-2.79	0.49	0.30	0.81	0.005	0.001
5'-M-tRNA-Val-AAC-6-1_L20	-2.21	0.60	0.39	0.95	0.027	0.003
5'-M-tRNA-Glu-TTC-1-2_L16	-2.56	0.48	0.28	0.84	0.01	0.004
5'-tRNA-Glu-TTC-9-1_L30	2.16	1.47	1.04	2.07	0.031	0.006
5'-M-tRNA-Ser-TGA-4-1_L15	-2.36	0.75	0.59	0.95	0.018	0.007
5'-M-tRNA-Leu-TAG-1-1_L17	-2.84	0.36	0.18	0.73	0.005	0.010
5'-M-tRNA-Arg-CCT-4-1_L18	-2.74	0.60	0.42	0.87	0.006	0.014
5'-M-tRNA-Ala-TGC-4-1_L23	2.15	1.29	1.02	1.63	0.032	0.016
5'-M-tRNA-Lys-TTT-5-1_L22	2.49	1.73	1.12	2.65	0.013	0.024
5'-tRNA-Arg-TCG-3-1_L19	-1.98	0.83	0.68	1.00	0.048	0.025
5'-M-tRNA-Ala-TGC-4-1_L16	-2.60	0.49	0.29	0.84	0.009	0.028
5'-M-tRNA-Arg-TCG-1-1_L18	-2.24	0.74	0.57	0.96	0.025	0.032
5'-M-tRNA-Ala-TGC-4-1_L24	2.09	1.34	1.02	1.77	0.037	0.034
5'-M-tRNA-Gly-GCC-1-5_L22	2.00	1.43	1.01	2.04	0.046	0.037
5'-M-tRNA-Gly-TCC-4-1_L18	-2.41	0.63	0.43	0.92	0.016	0.042
5'-M-tRNA-Lys-TTT-5-1_L23	2.64	1.79	1.16	2.76	0.008	0.05

*Note: These identified 5'-tRFs with marginal significance were further subjected to variable selection using LASSO (the one highlighted by bold font).

Table S3 Multivariate Cox regression analysis reveals eight 5'-tRFs showing significant association with PRAD prognosis*

tRFs	coef	HR	Z-score	P value	Low95%CI	High95%CI
5'-tRNA-Arg-TCG-3-1_L18	-0.4808	0.6183	-1.4631	0.1434	0.3247	1.1774
5'-M-tRNA-Glu-TTC-1-2_L16	-0.8764	0.4163	-3.0229	0.0025	0.2358	0.7348
5'-tRNA-Glu-TTC-9-1_L30	0.3224	1.3805	1.6809	0.0928	0.9479	2.0106
5'-M-tRNA-Leu-TAG-1-1_L17	-0.5624	0.5698	-1.5320	0.1255	0.2775	1.1701
5'-M-tRNA-Arg-CCT-4-1_L18	-0.3822	0.6824	-1.5064	0.1320	0.4150	1.1220
5'-M-tRNA-Ala-TGC-4-1_L23	0.2152	1.2402	1.6799	0.0930	0.9648	1.5942
5'-M-tRNA-Lys-TTT-5-1_L22	0.7238	2.0623	2.8348	0.0046	1.2503	3.4018
5'-M-tRNA-Ala-TGC-4-1_L16	-0.4262	0.6530	-1.4640	0.1432	0.3691	1.1553

*Note: Coefficients estimated from the multivariate Cox regression model were used to calculate tRF score.

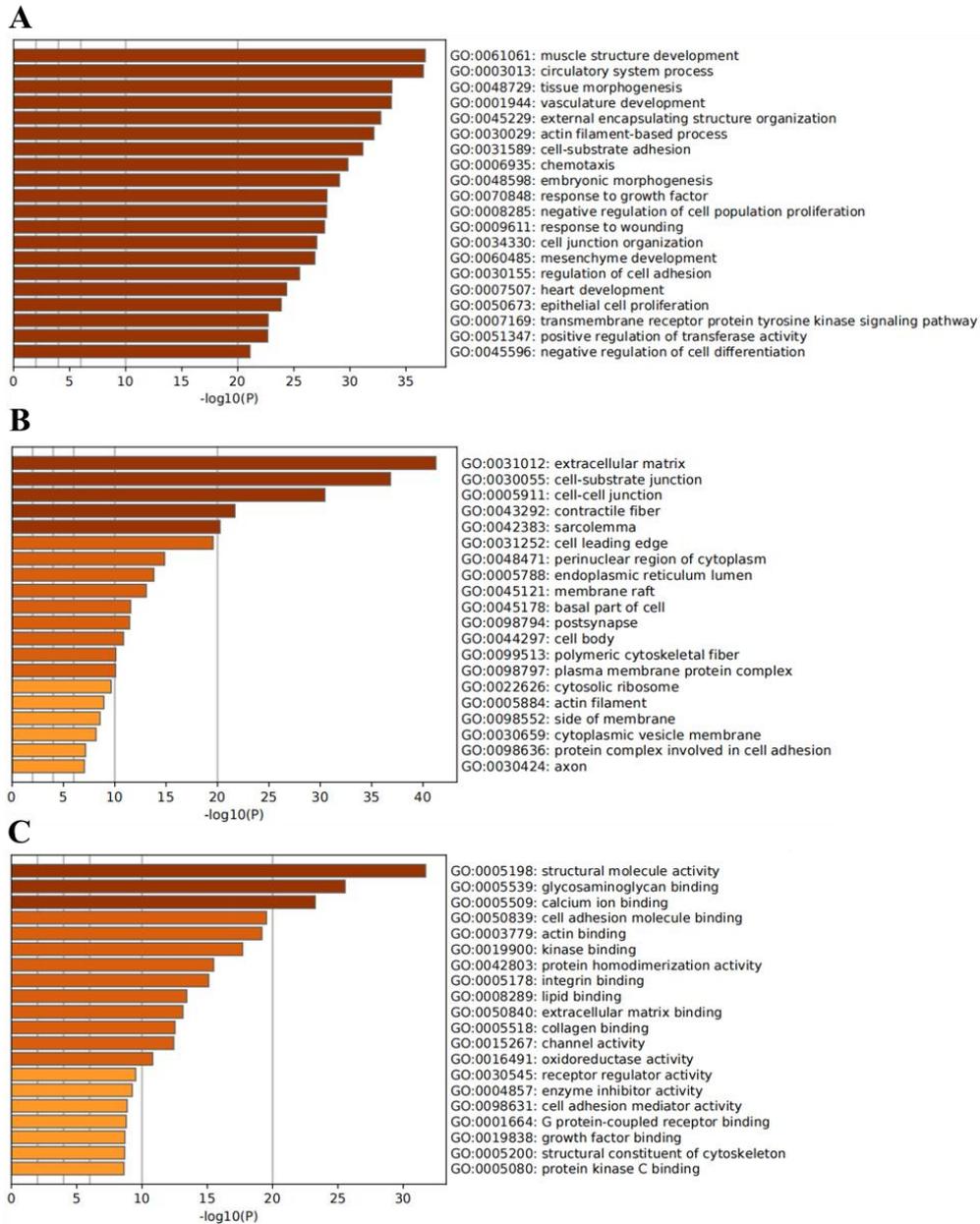


Figure S1 GO terms enriched for mRNA genes that were co-expressed with significantly dysregulated 5'-tRFs. Related to Figure 1. (A) Biological process. (B) Cellular component. (C) Molecular function.

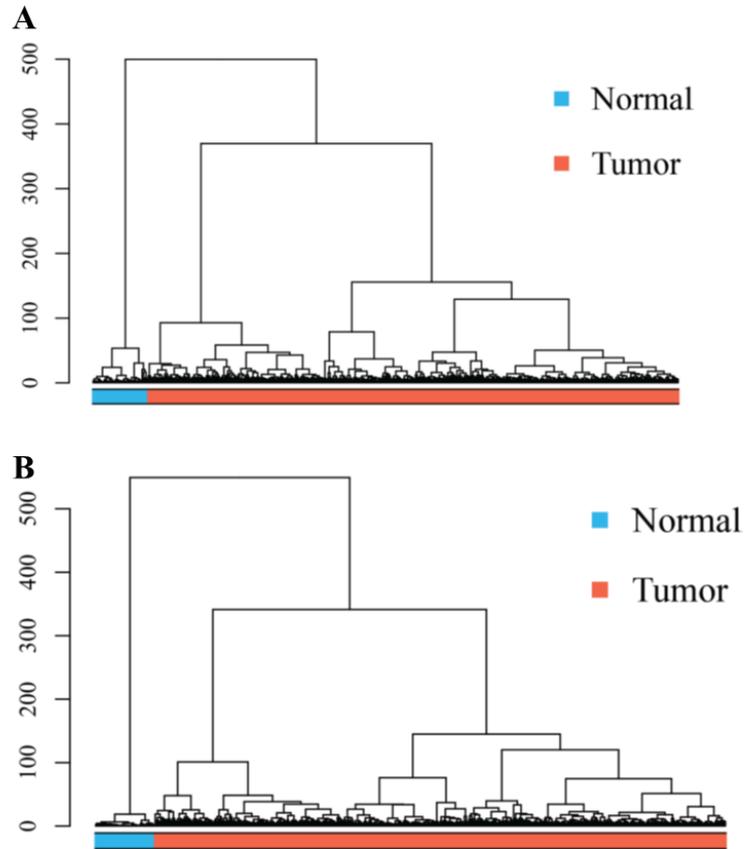


Figure S2 Hierarchical cluster analyses of PRAD specimens using 5'-tRF expression profiles. Related to Figure 2. **(A)** 70 5'-tRFs differentially expressed between PRAD tumor and adjacent normal tissues. **(B)** 63 5'-tRFs upregulated in PRAD tumors.

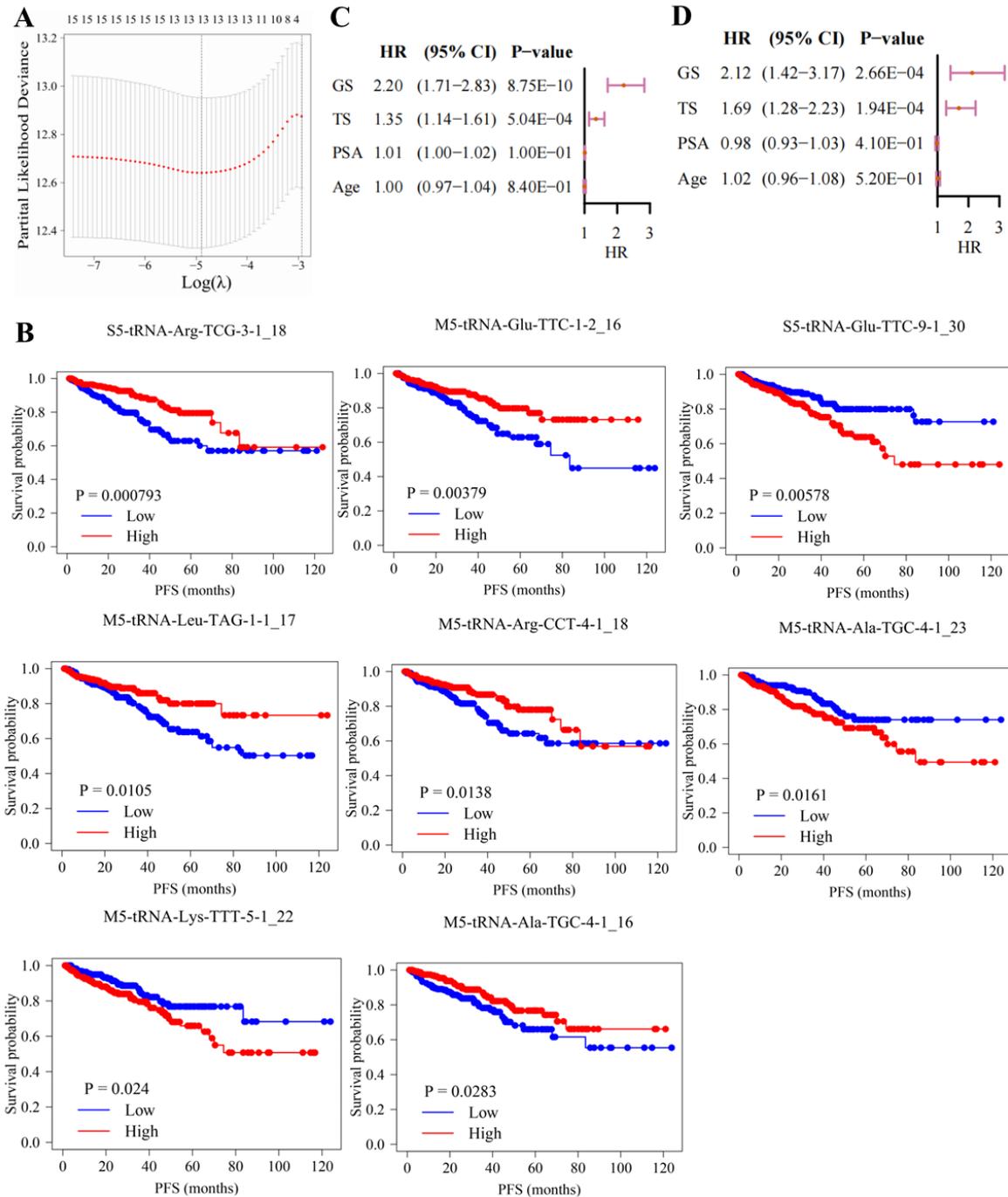


Figure S3 5'-tRFs are associated with the prognosis of PRAD. Related to Figure 3. **(A)** Partial likelihood deviance versus lambda relationship in LASSO. According to the partial likelihood deviance, a subset of 13 5'-tRF that minimizes prediction error of PFS were identified. **(B)** Kaplan-Meier curves of PFS of PRAD patients with low vs high 5'-tRF expression. Eight 5'-tRFs included in the tRF score were analyzed separately, including S5-tRNA-Arg-TCG-3-1_18, M5-tRNA-Glu-TTC-1-2_16, M5-tRNA-Leu-TAG-1-1_17, M5-tRNA-Arg-CCT-4-1_18, M5-tRNA-Ala-TGC-4-1_16, S5-tRNA-Glu-TTC-9-1_30, M5-tRNA-Ala-TGC-4-1_23, and M5-tRNA-Lys-TTT-5-1_22. **(C, D)** HRs and their 95% CI of GS, TS, PSA and age estimations from the multivariate Cox regression model for PFS **(C)** and DFS **(D)**.

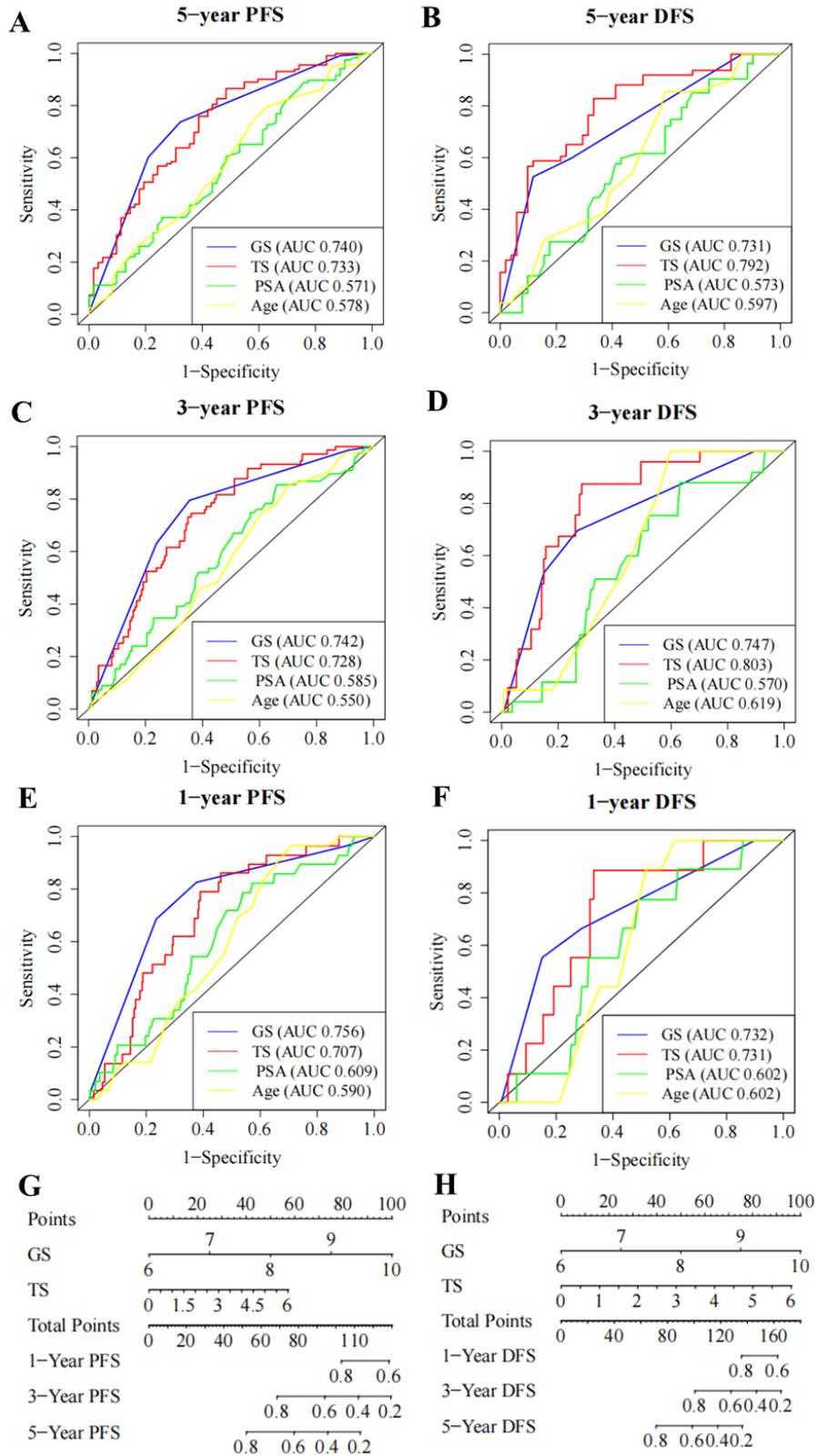


Figure S4 Prognostic performance of Gleason score, tRF score, PSA and age. Related to Figure 4. (A, C, E) ROC curves to evaluate the prognostic performance of 5-year (A), 3-year (C) and 1-year (E) PFS using TS, GS, PSA, or age. (B, D, F) ROC curves to evaluate the prognostic performance of 5-year (B), 3-year (D) and 1-year (F) DFS using TS, GS, PSA, or age. (G, H) Nomograms to estimate the risk of PFS (G) and DFS (H) in PRAD patients.

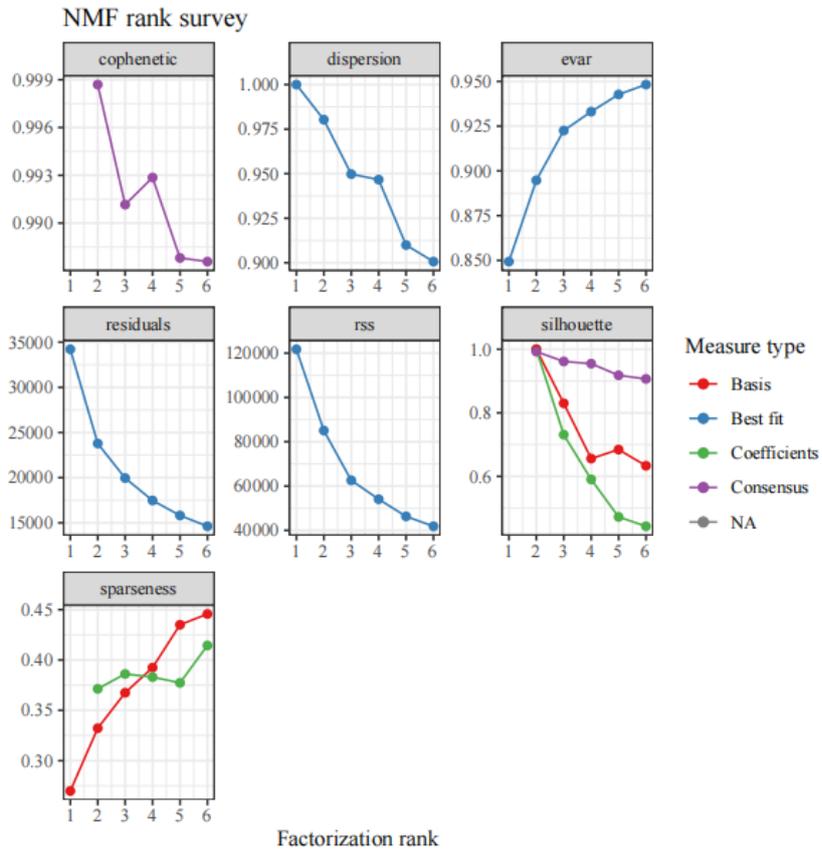


Figure S5 Determining an optimal number of tRF subtypes PRAD in HNSC. Related to Figure 5. The cophenetic correlation coefficient is a measure of the dispersion of the consensus matrix based on the average of connectivity matrices. It measures the stability of the 5'-tRF clusters obtained from NMF analysis of 5'-tRF expression data in PRAD.

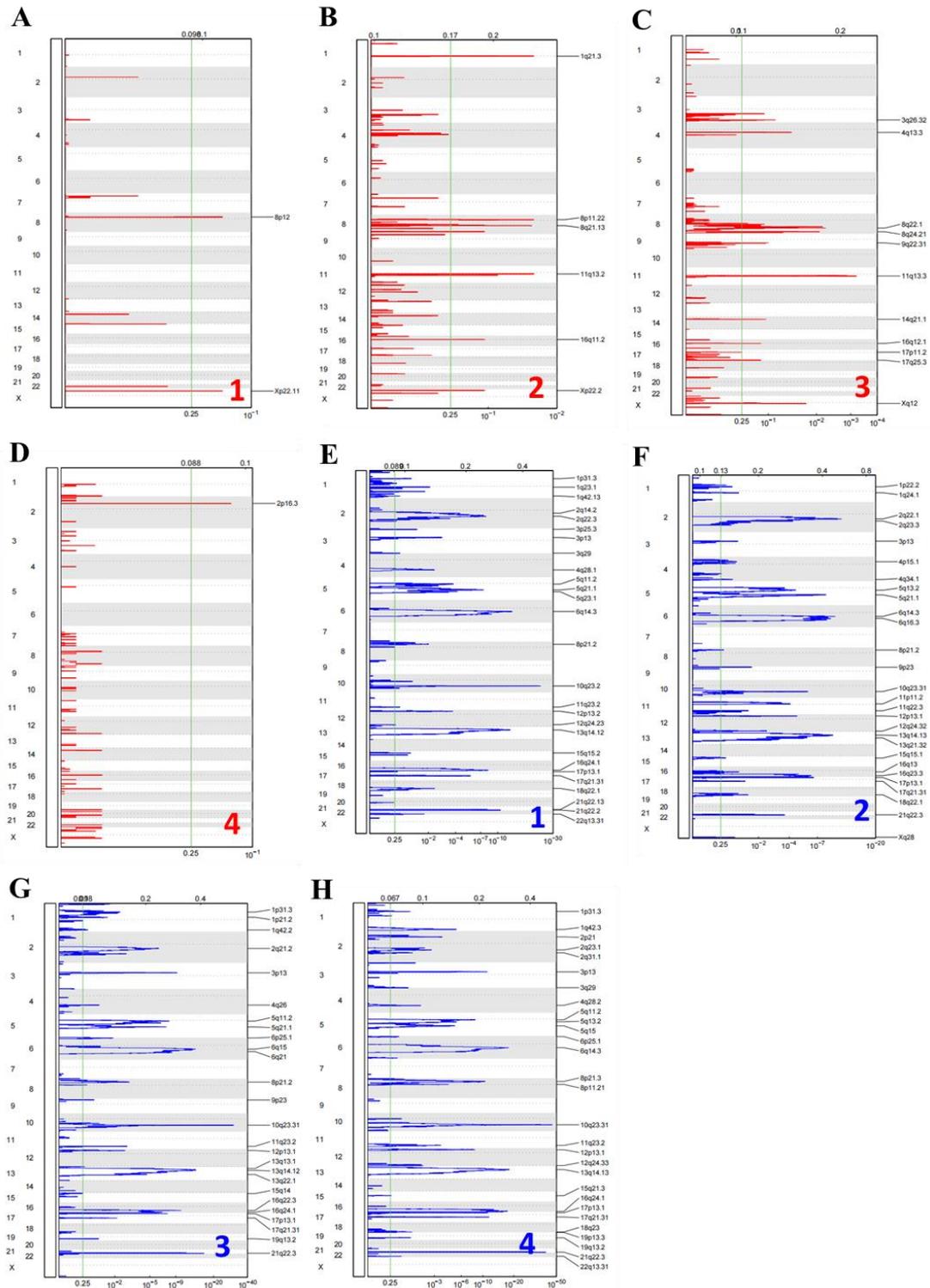


Figure S6 Copy number gain and loss in 5'-tRFs tumor subtypes. Related to Figure 6. (A-D) Somatic gain of copy number in four 5'-tRFs tumor subtypes. (E-H) Somatic loss of copy number in four 5'-tRFs tumor subtypes. The numbers 1, 2, 3 and 4 at the lower right corner of each panel indicate 5'-tRFs tumor subtypes, tRF-1, tRF-2, tRF-3, and tRF-4, respectively.