

Figure S1. mRNA expression of FOXO3 in different subgroups. (A) mRNA expression of FOXO3 in HCC in TNM I/II and TNM III/IV groups from TCGA dataset. (B) mRNA expression of FOXO3 in HCC with or without vascular invasion from TCGA dataset. (C) mRNA expression of FOXO3 in HCC in TNM I/II and TNM III/IV groups from the ICGC dataset. (D) mRNA expression of FOXO3 in HCC with or without vascular invasion from the ICGC dataset. FOXO3, Forkhead box O3; HCC, hepatocellular carcinoma; VIHCC, vascular invasion hepatocellular carcinoma; TNM, tumor, node, metastasis; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium.

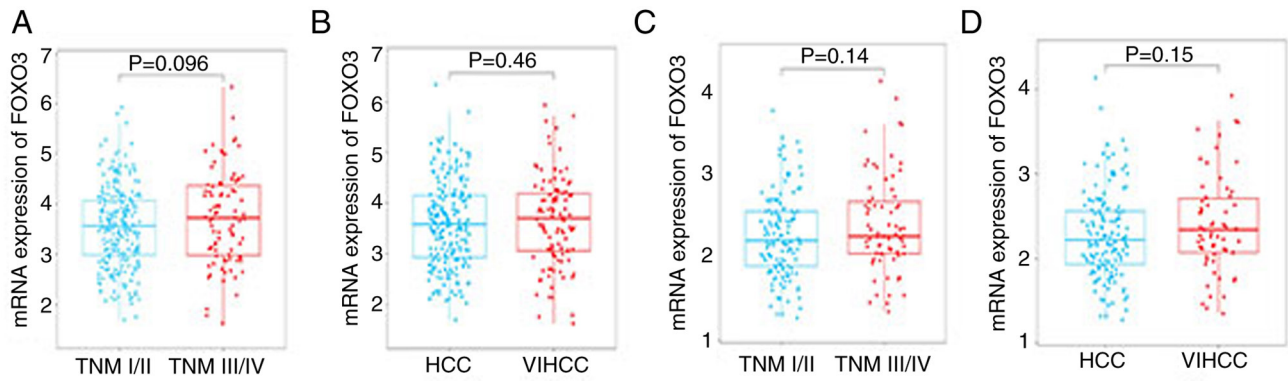


Figure S2. Evaluation of the 5-gene signature model. (A) Calibration curves at 1, 3 and 5 years of the model in The Cancer Genome Atlas dataset. (B) Calibration curves at 1, 3 and 4 years of the model in the International Cancer Genome Consortium dataset.

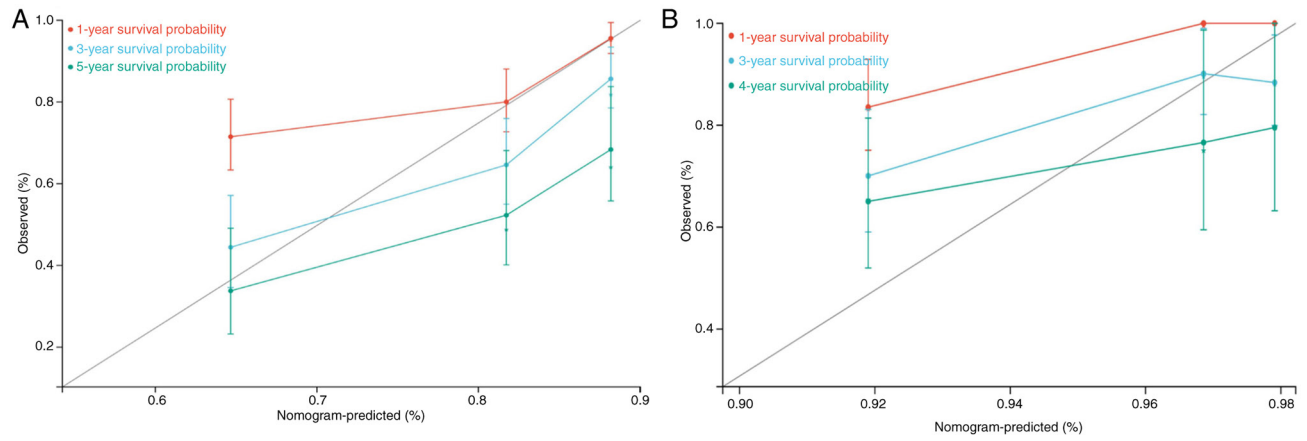


Figure S3. Analysis of the carcinogenic pathways and tumor microenvironment between high- and low-RS groups in the ICGC dataset. Gene set enrichment analysis of the high-RS group vs. low-RS group for (A) the G2/M checkpoint, (B) E2F targets and (C) PI3K/AKT/MTOR signaling. (D) Distribution of 22 immune cell types in the tumor microenvironment of each hepatocellular carcinoma sample. (E) Comparison of 22 immune cell types infiltrated in the tumor microenvironment in the high- and low-RS groups. -, not significant; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001. RS, risk score; FDR, false discovery rate; NES, normalized enrichment score.

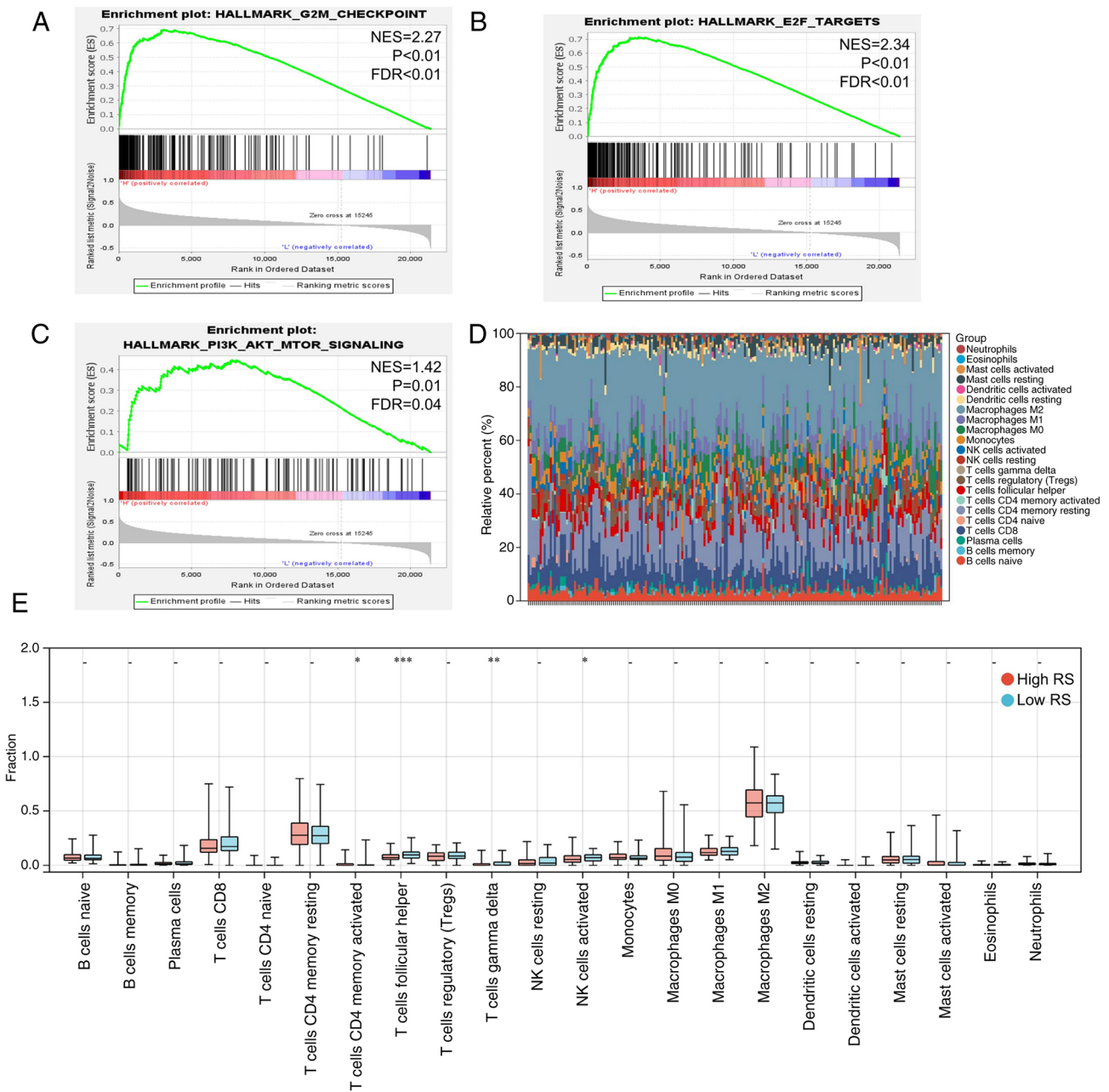


Figure S4. Validation of five HCC biomarkers in the ICGC dataset. (A) mRNA expression of DDX55, RAB10, RAB7A, TAF1B and TAF3 in HCC and normal tissues from the ICGC dataset. Overall survival curves of patients with HCC with high or low expression of (B) DDX55, (C) RAB10, (D) RAB7A, (E) TAF1B and (F) TAF3 from the ICGC dataset. The best cut-off value for each gene was obtained using X-tile software. (G) The knockdown efficiency of RAB10, RAB7A and TAF3 in Huh7 cells was assessed using western blot analysis. RAB10 si#1, RAB7A si#1 and TAF3 si#2 were selected for subsequent experiments. \*\*\*\* $P < 0.0001$ . DDX55, DEAD-box helicase 55; RAB10, member RAS oncogene family; TAF1B, TATA-box binding protein associated factor, RNA polymerase I subunit B; TAF3, TATA-box binding protein associated factor 3; HCC, hepatocellular carcinoma; ICGC, International Cancer Genome Consortium; NC, negative control.

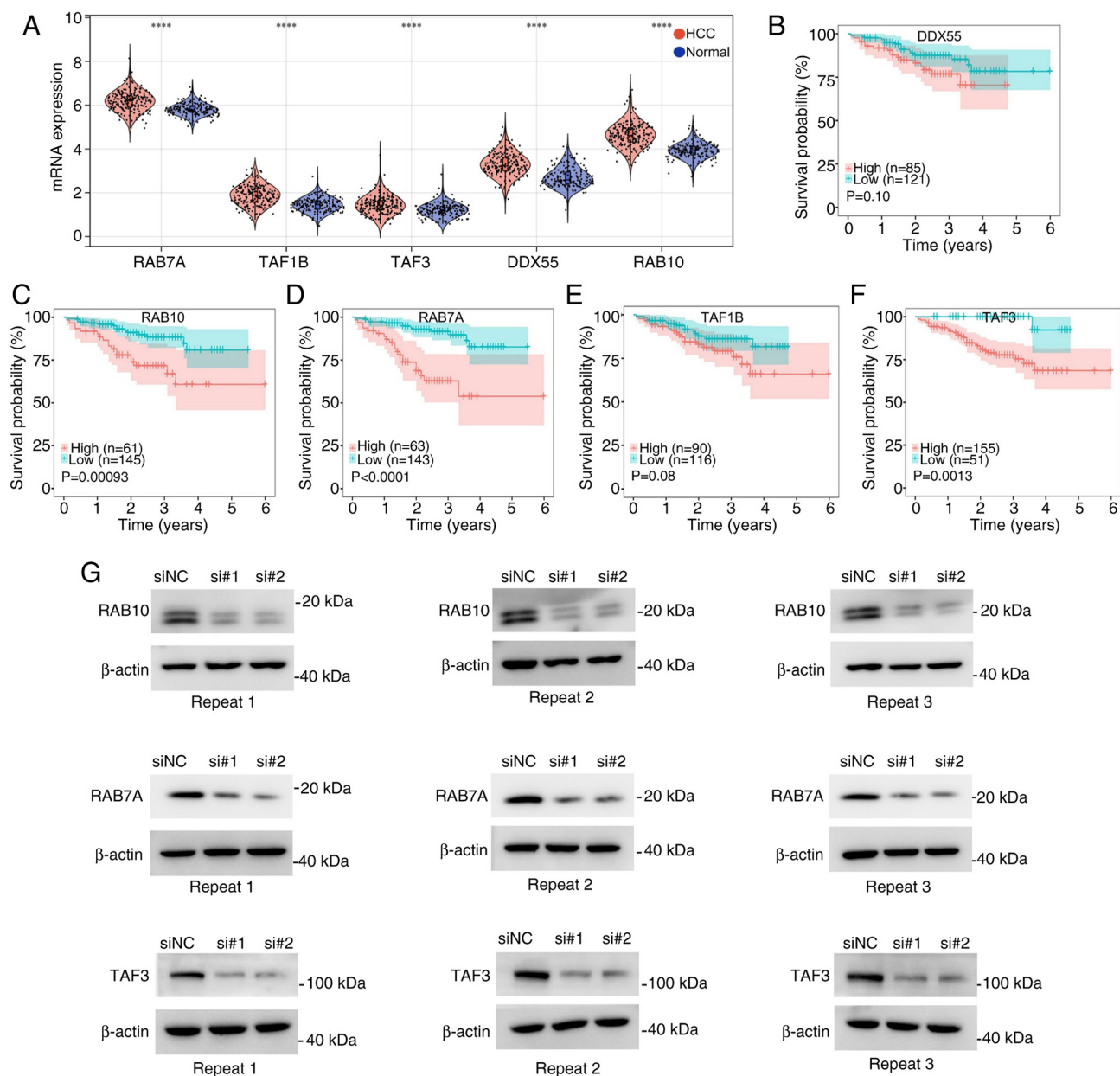


Figure S5. The association between FOXO3 and the five signature genes, RS and TNM in (A) TCGA and (B) ICGC datasets. (C) The knockdown efficiency of FOXO3 in Huh7 cells using siRNA. (D) Western blotting showed that FOXO3 knockdown decreased the expression of DDX55, RAB10, RAB7A, TAF1B and TAF3 in Huh7 cells. FOXO3, Forkhead box O3; DDX55, DEAD-box helicase 55; RAB10, RAB10, member RAS oncogene family; RAB7A, RAB7A, member RAS oncogene family; TAF1B, TATA-box binding protein associated factor, RNA polymerase I subunit B; TAF3, TATA-box binding protein associated factor 3; AUC, area under the curve; RS, risk score; TNM, tumor, node, metastasis.

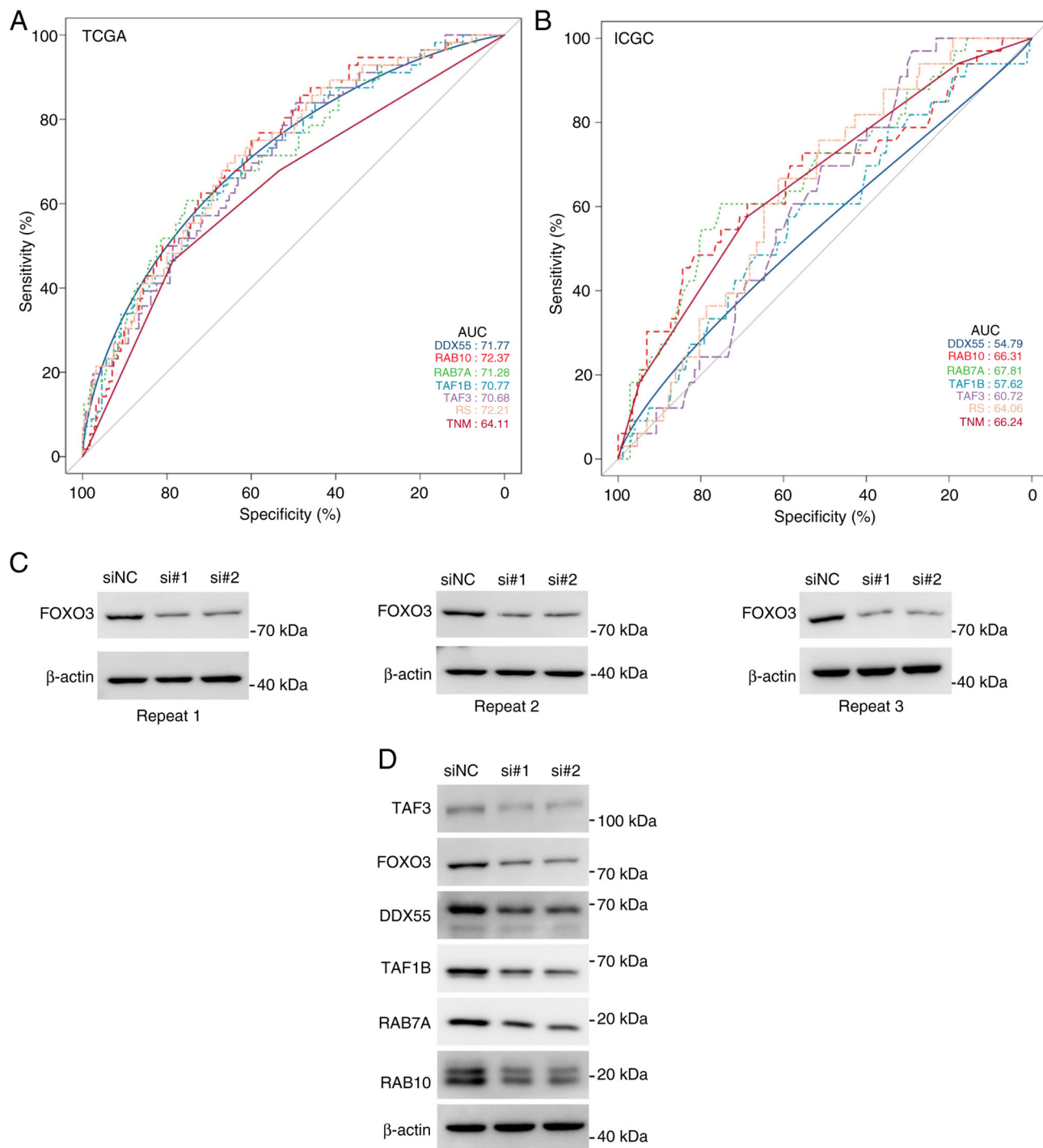


Figure S6. The correlation between FOXO3 and DDX55, RAB10, RAB7A, TAF1B and TAF3 genes. The spearman correlation analysis of FOXO3 and the five signature genes in (A) The Cancer Genome Atlas and (B) International Cancer Genome Consortium datasets. FOXO3, Forkhead box O3; DDX55, DEAD-box helicase 55; RAB10, RAB10, member RAS oncogene family; TAF1B, TATA-box binding protein associated factor, RNA polymerase I subunit B; TAF3, TATA-box binding protein associated factor 3.

