Clin. Lab. 2025;71:1-14

ORIGINAL ARTICLE

Screening of Metabolism-Related Biomarkers and Construction of Prognostic Models in Patients with Neuroblastoma

Feng-Zhi Gu [1, *], Liang-Yu Mi [2, *], Bin Liu [3], Xian-Wu Yang [1], Ying-Quan Zhuo [1], Hua-Jian Gu [1]

[*] *Feng-Zhi Gu and Liang-Yu Mi contributed equally to this work*
[1] *Department of Pediatric Surgery, The Affiliated Hospital of Guizhou Medical University, Guiyang City, Guizhou Province, China*
[2] *Department of Rheumatology and Immunology, Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Taiyuan City, Shanxi Province, China*
[3] *Department of Endocrinology, Huai'an Hospital of Traditional Chinese Medicine, Huai'an City, Jiangsu Province, China*

SUMMARY

Background: **Energy metabolism (EM) genes play crucial roles in tumor development and progression. While neuroblastoma (NBL) cells exhibit high proliferation rates requiring efficient energy metabolism, the underlying mechanisms remain incompletely understood.**
Methods: **Transcriptomic analysis of the TARGET-NBL dataset was performed to stratify samples based on EM-related gene expression. Differential expression analysis and weighted gene co-expression network analysis (WGCNA) were integrated to identify critical gene modules. Prognostic biomarkers were determined through univariate and multivariate Cox regression analyses. Functional enrichment analysis and drug prediction were conducted for the identified biomarkers. Expression levels of candidate genes (GRIA2, FBXO32, GNG12, and PHLDA2) were validated using qRT-PCR. The biological function of GNG12 was investigated through gain- and loss-of-function studies in neuroblastoma cell lines.**
Results: **The analysis identified 1,675 differentially expressed genes and two critical modules (MEblack and MEturquoise) through WGCNA. Four prognostic biomarkers (GRIA2, FBXO32, GNG12, and PHLDA2) were established and integrated into a nomogram with clinical parameters. Functional analysis revealed their involvement in extracellular matrix organization, DNA replication, and nucleocytoplasmic transport. Drug prediction identified potential therapeutic compounds targeting GRIA2 and FBXO32. Experimental validation demonstrated elevated expression of all four biomarkers in neuroblastoma cell lines compared to normal controls. Notably, GNG12 knockdown significantly suppressed while its overexpression enhanced proliferation and migration of SH-SY5Y cells.**
Conclusions: **This study identified and validated four EM-related prognostic biomarkers in neuroblastoma, with GNG12 functionally implicated in tumor cell proliferation and migration. These findings provide potential therapeutic targets and prognostic indicators for neuroblastoma management.**
(Clin. Lab. 2025;71:1-14. DOI: 10.7754/Clin.Lab.2025.250323)

Correspondence:
Hua-Jian Gu
Department of Pediatric Surgery
The Affiliated Hospital of Guizhou Medical University
No. 28 Guiyi Street
Guiyang City
Guizhou Province, 550004
China
Email: Guhuajian_gululu@hotmail.com

Manuscript accepted April 3, 2025

Supplementary Data

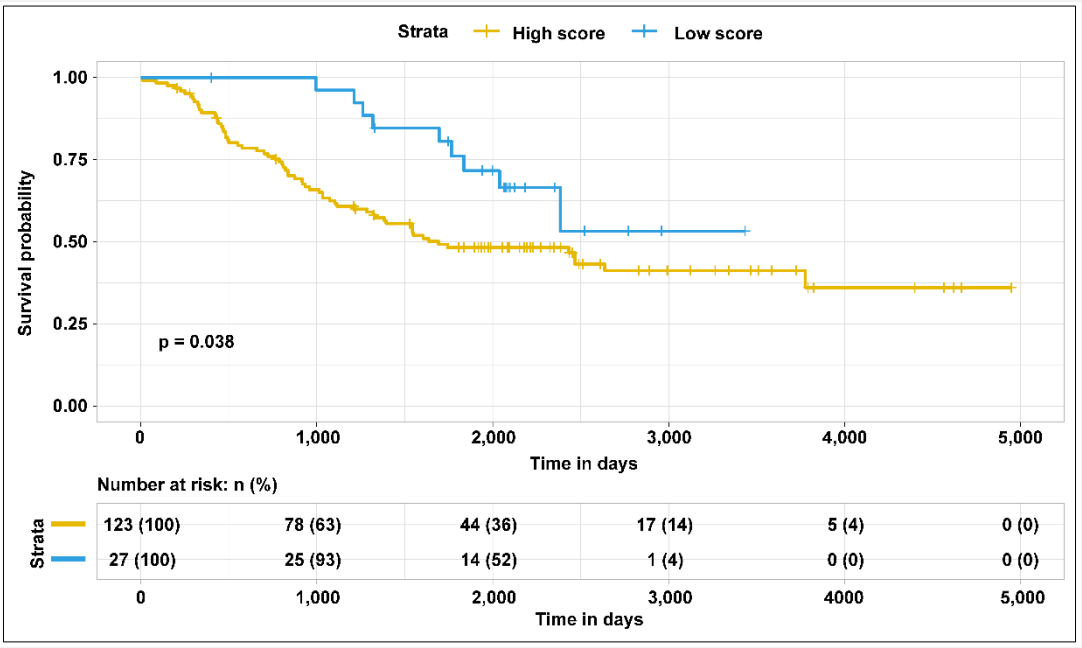

Figure S1. Significant difference in survival between score subgroups ($p < 0.05$), with higher survival in the low-score group.

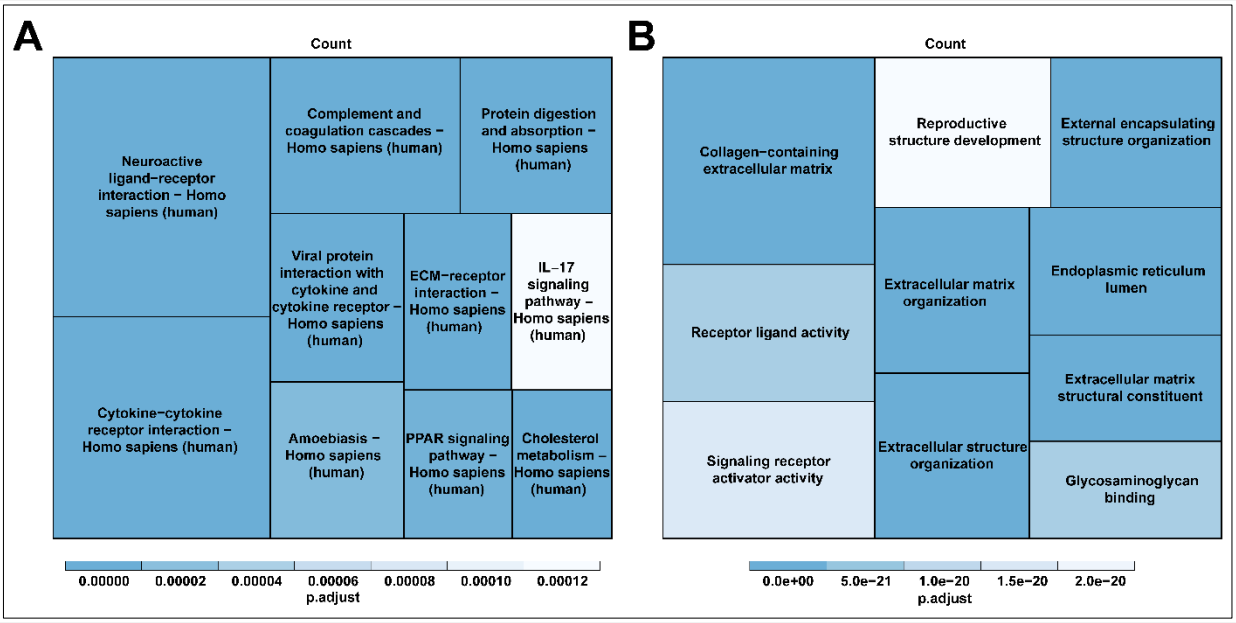

Figure S2. Enrichment analysis of DEGs.

A and B - Functional enrichment analysis, including KEGG pathways and GO terms.

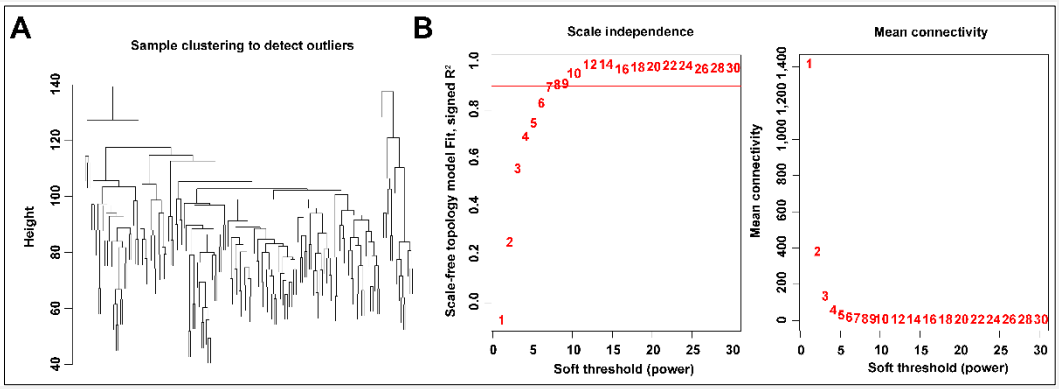

Figure S3. Acquisition of critical module.

A - Clustering results of samples indicating no outlier samples. B - The soft-thresholding power (β) was set to 12, ensuring the gene network exhibited scale-free topology.

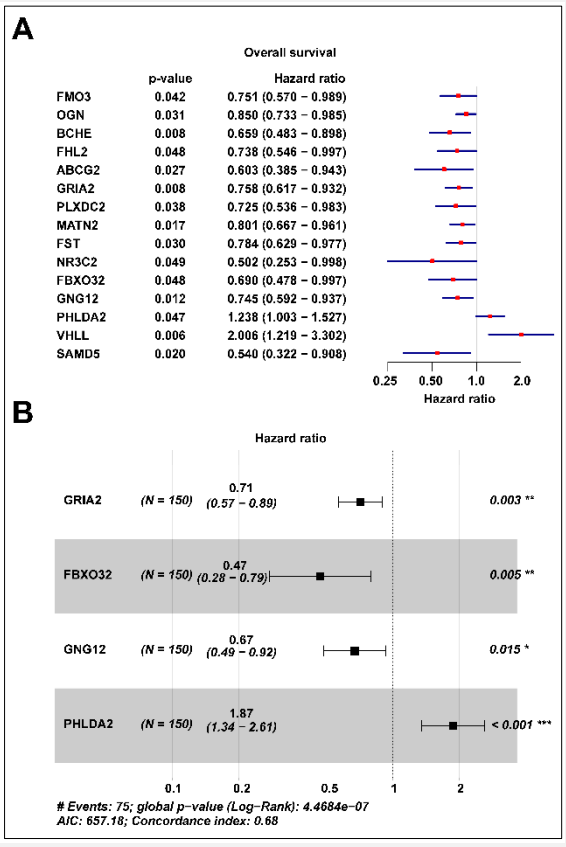

Figure S4. Identification of four biomarkers.

A - Identification of 15 candidate genes, including FMO3 and OGN. B - Multivariate Cox analysis revealed 4 biomarkers: GRIA2, FBXO32, GNG12, and PHLDA2.

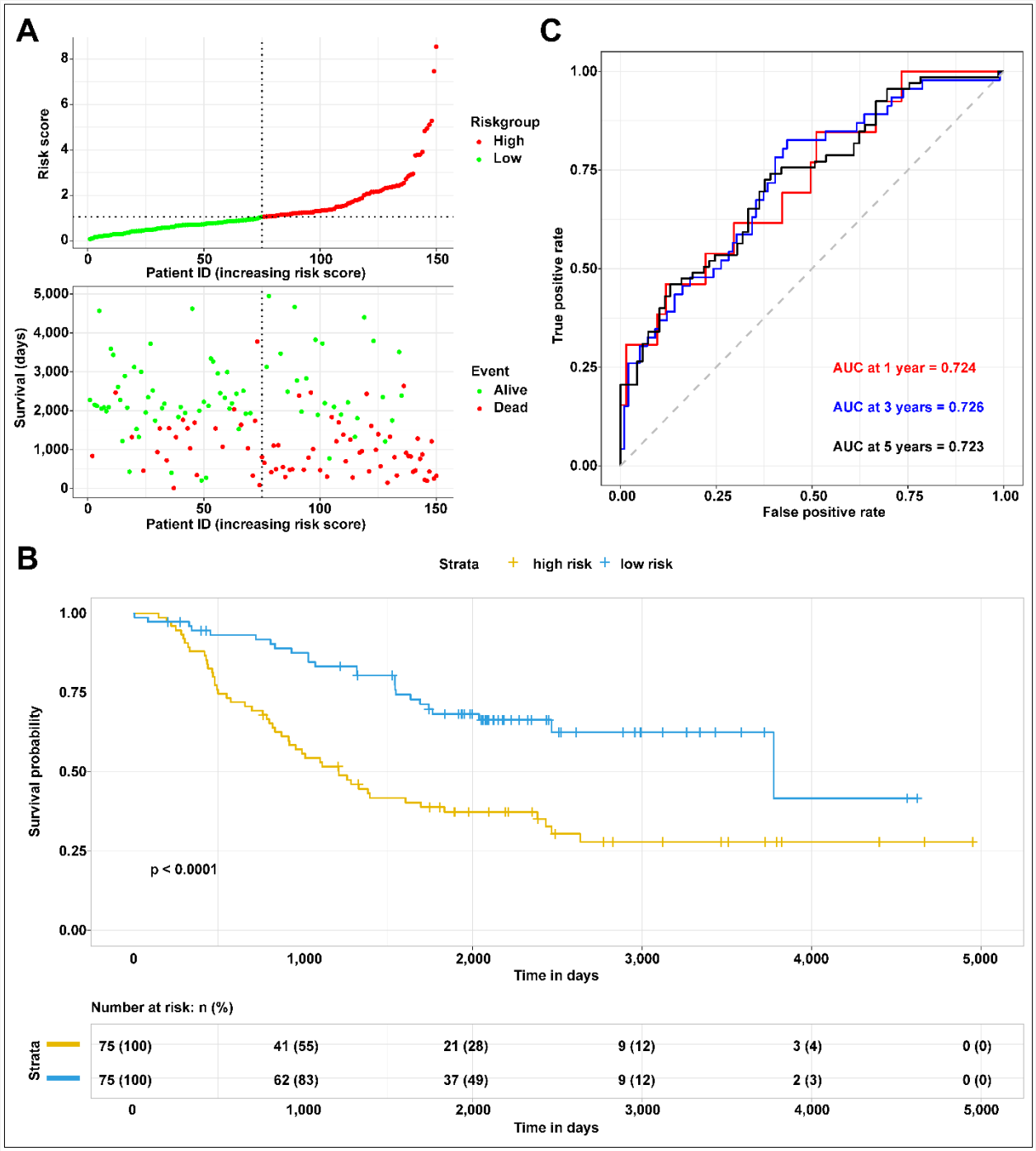

Figure S5. TARGET-NBL dataset analysis.

A - NBL samples in the TARGET-NBL dataset were classified into high- and low-risk groups. Higher risk scores corresponded to increased mortality. B - Significant difference in survival between high- and low-risk subgroups ($p < 0.0001$), with higher survival rates in the low-risk group. C - AUC values for 1-, 3-, and 5-year survival rates all above 0.7, indicating the risk model's strong predictive ability for NBL patient survival.

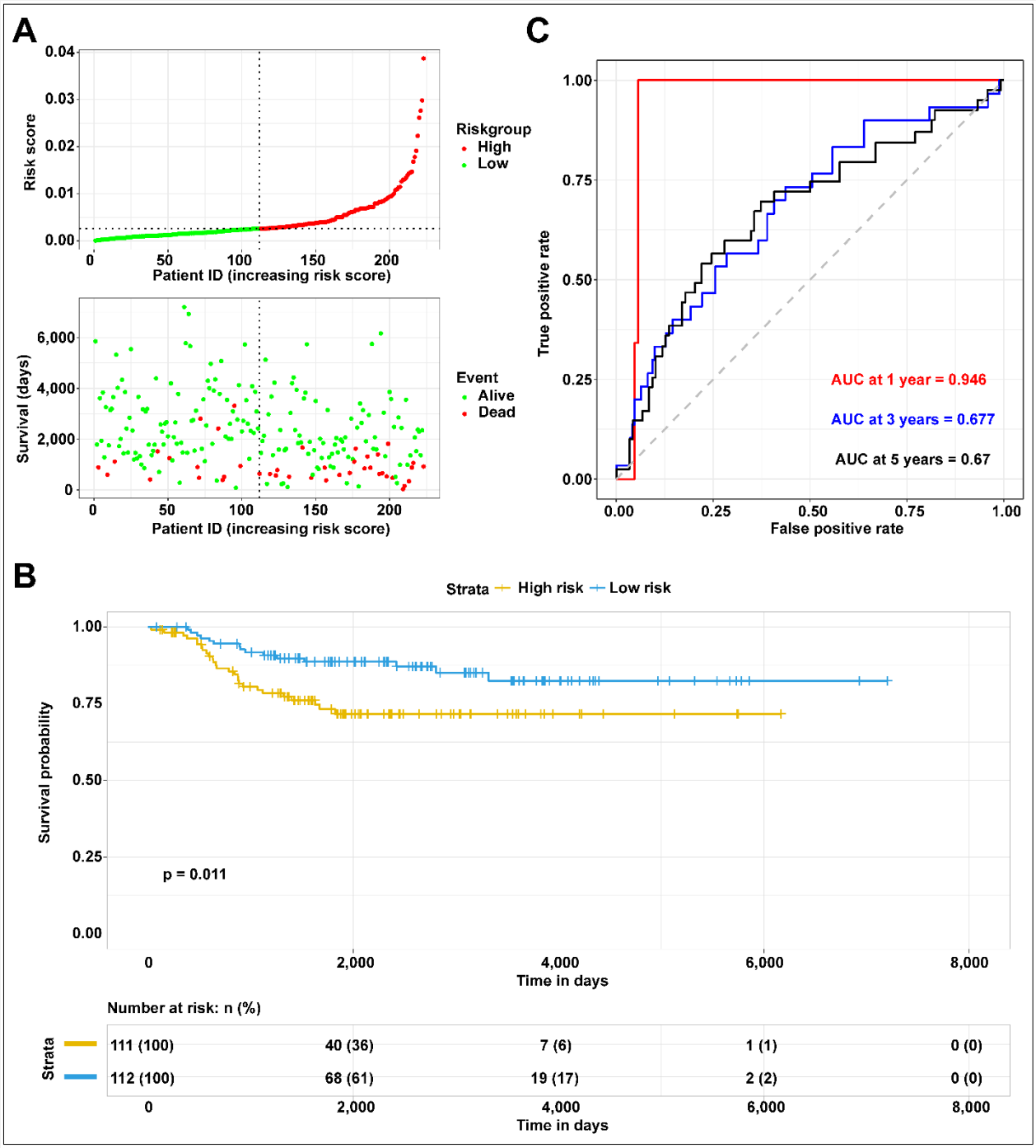

Figure S6. E-MTAB-8248 dataset analysis.

A - C - Validation of the risk model in the E-MTAB-8248 dataset showed consistent results with the TARGET-NBL dataset.

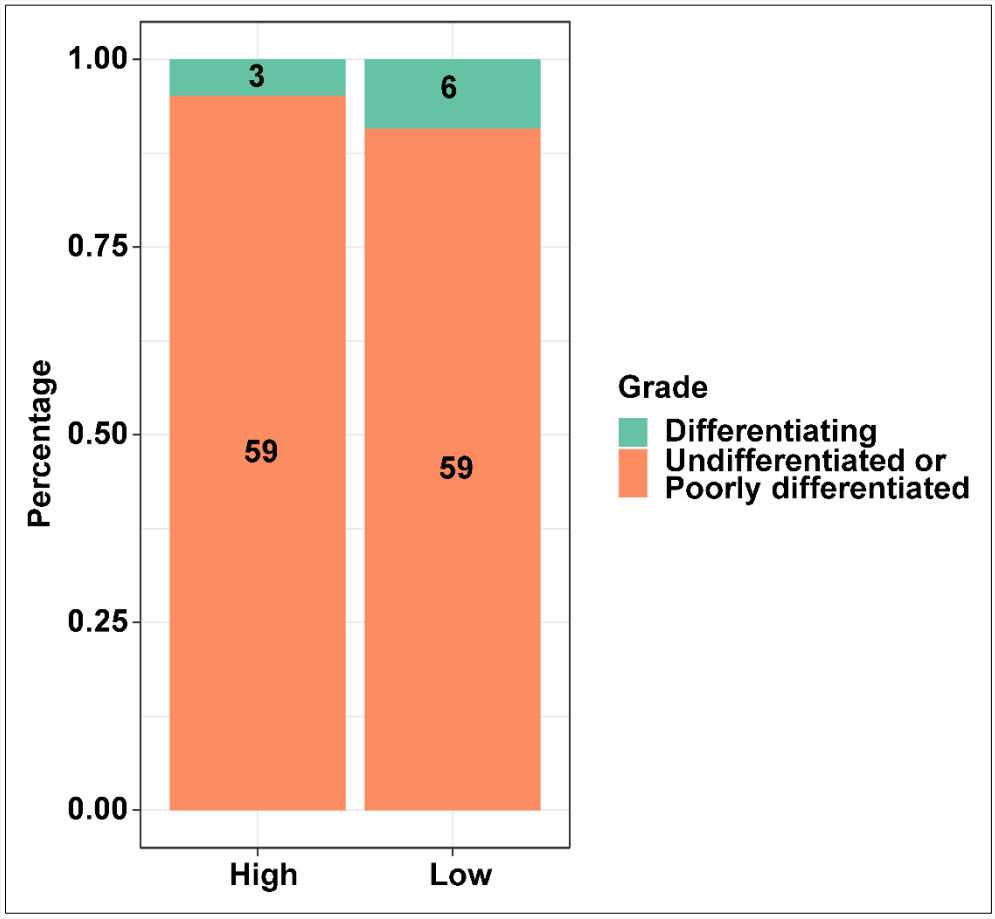

Figure S7. The distribution of grade (differentiated, undifferentiated or poorly differentiated) between two risk subgroups.

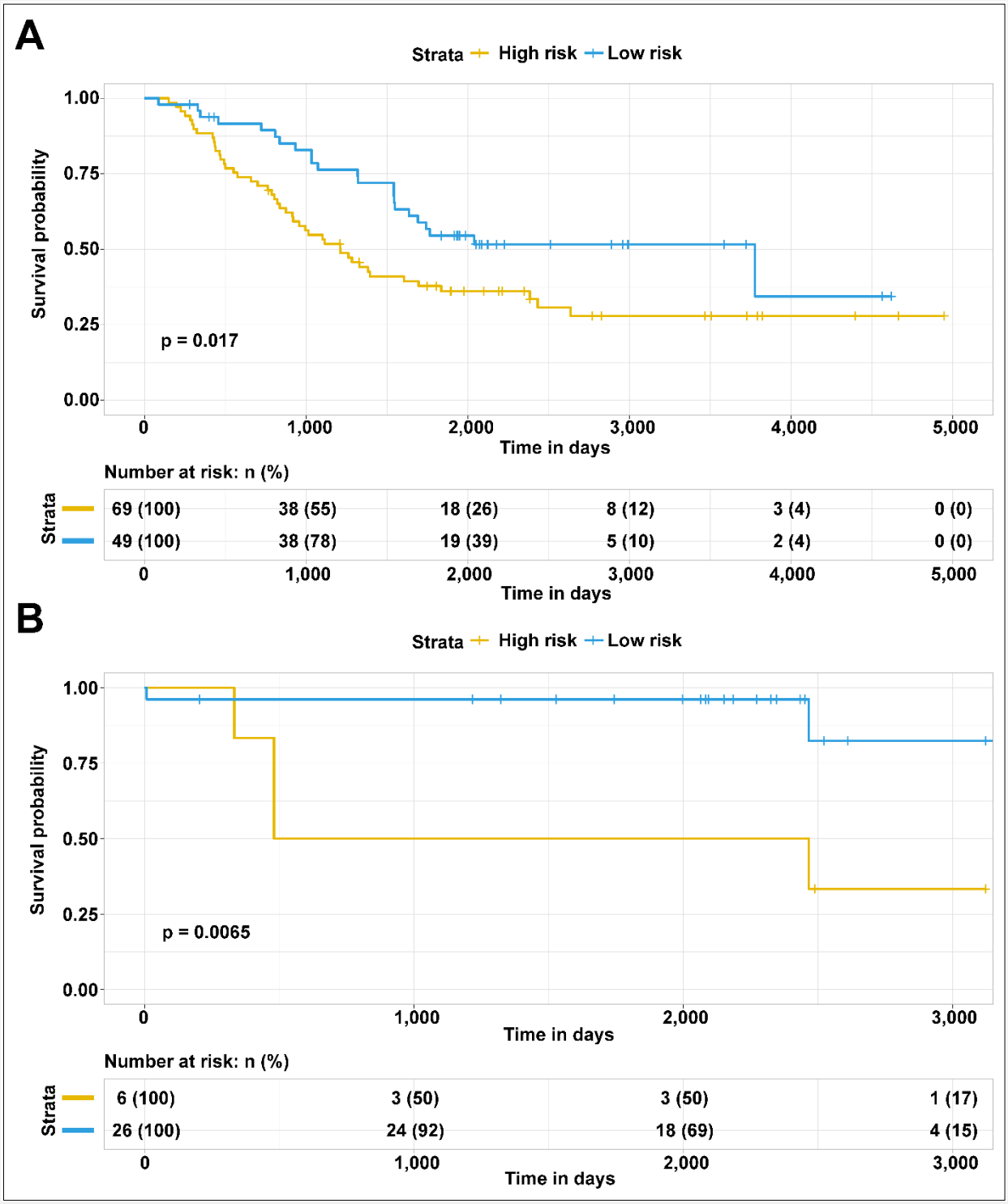

Figure S8. The survival difference analysis between two risk subgroups for age.

A and B - Significant survival differences between risk subgroups for age (≥ 1.5 and < 1.5).

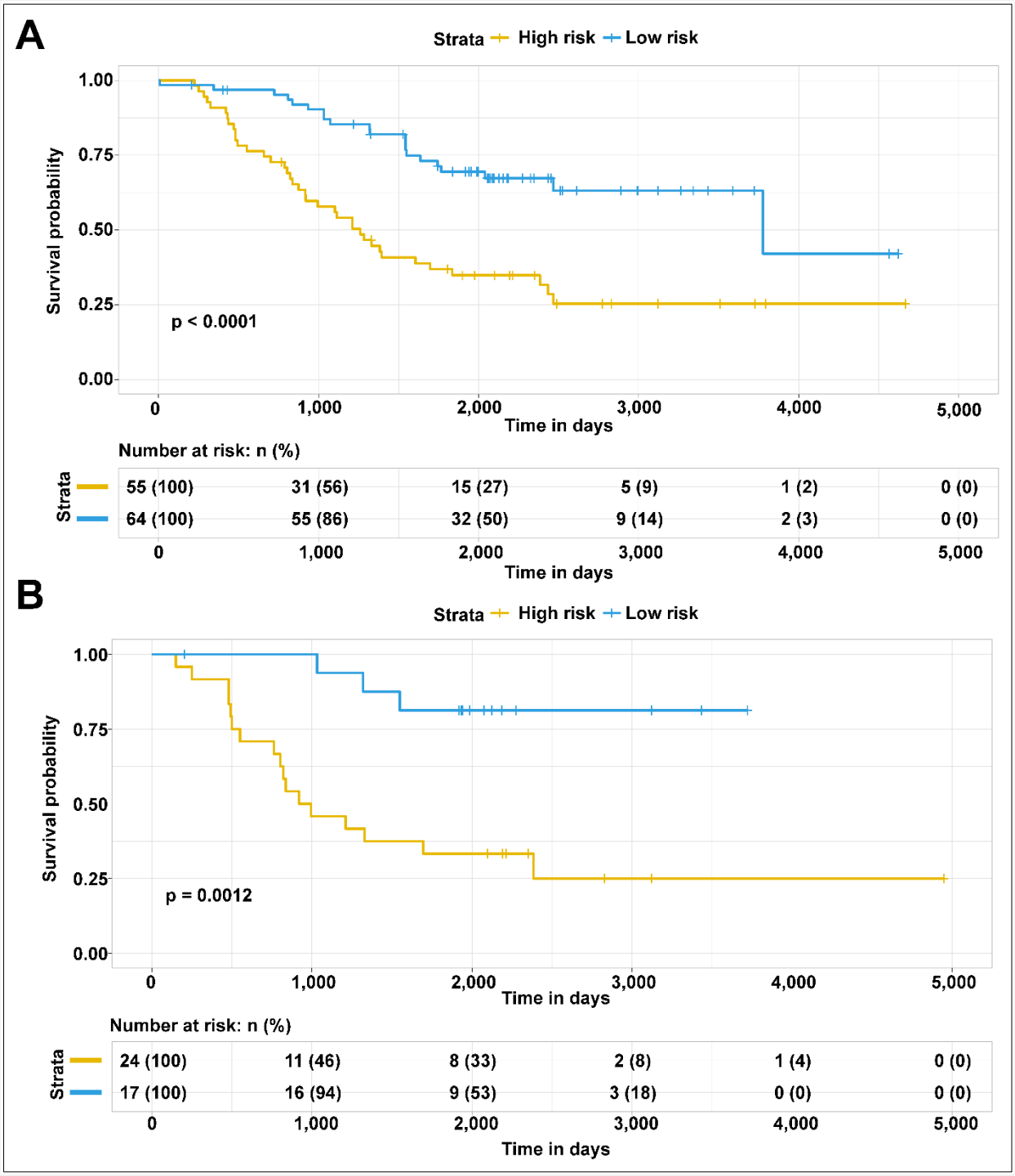

Figure S9. The survival difference analysis between two risk subgroups for MYCN and MKI.

A - Significant survival differences between risk subgroups for MYCN (not amplified). B - Significant survival differences between risk subgroups for MKI (intermediate).

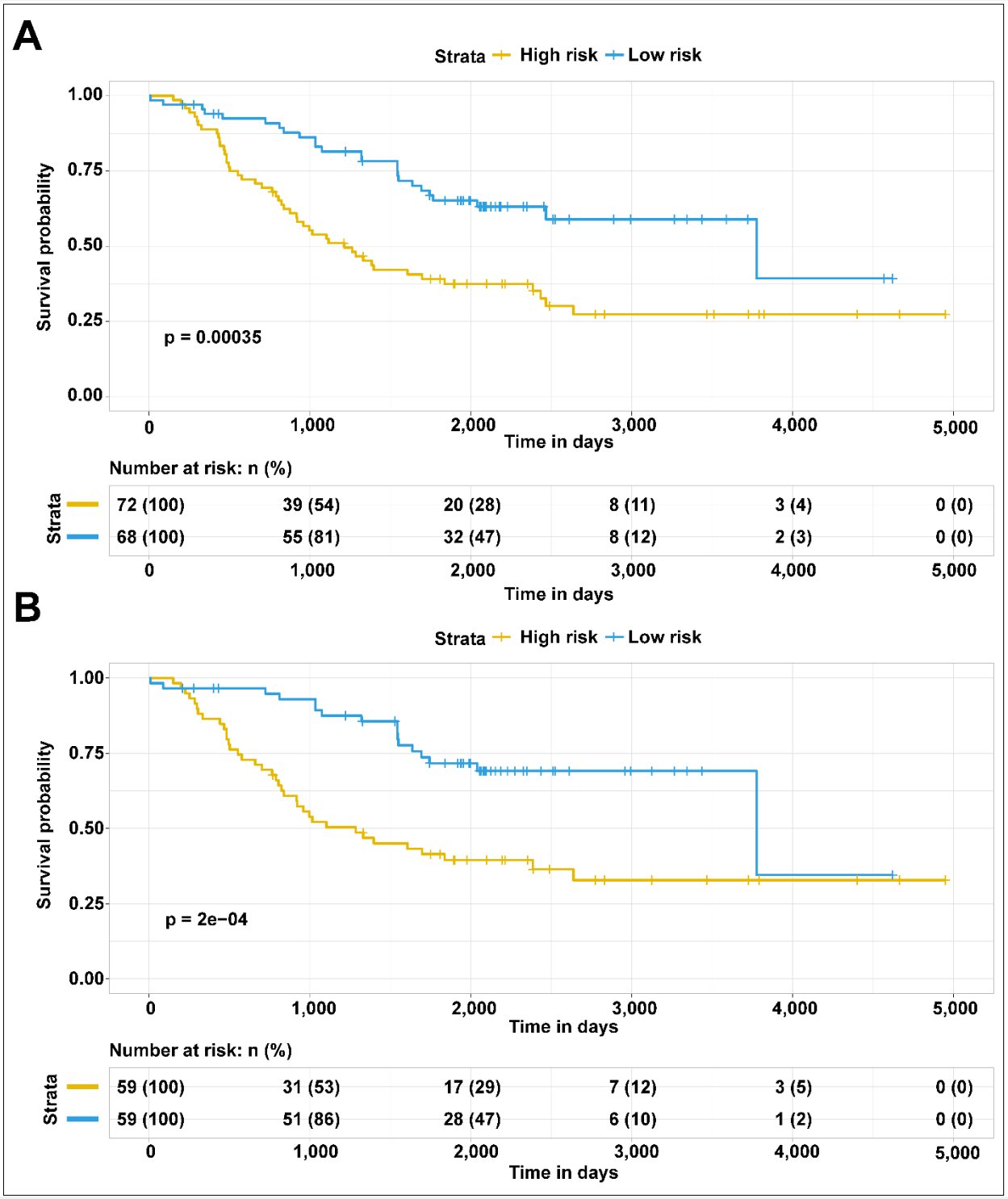

Figure S10. The survival difference analysis between two risk subgroups for INSS and grade.

A - Significant survival differences between risk subgroups for INSS (stage 4). B - Significant survival differences between risk subgroups for grade (undifferentiated or poorly differentiated).

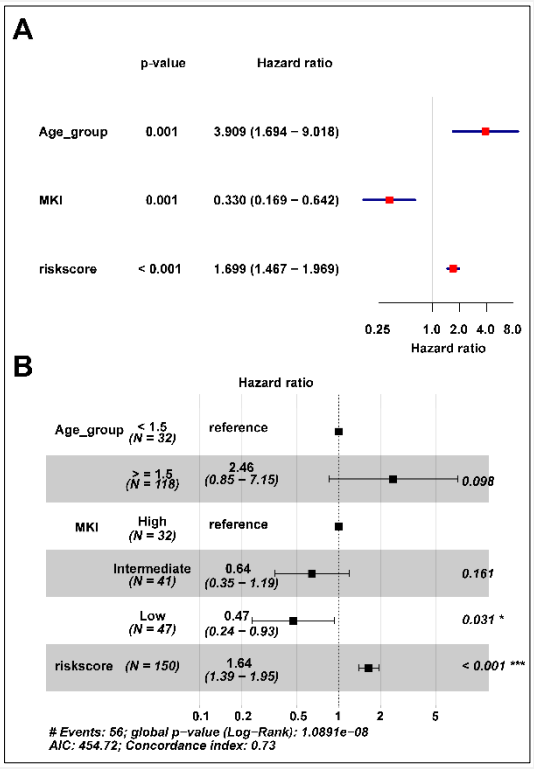

Figure S11. Cox analysis.

A - Univariate Cox analysis identified risk score, age, and MKI as significant factors (p < 0.05). B - Independent prognostic model constructed using age, risk score, and MKI.

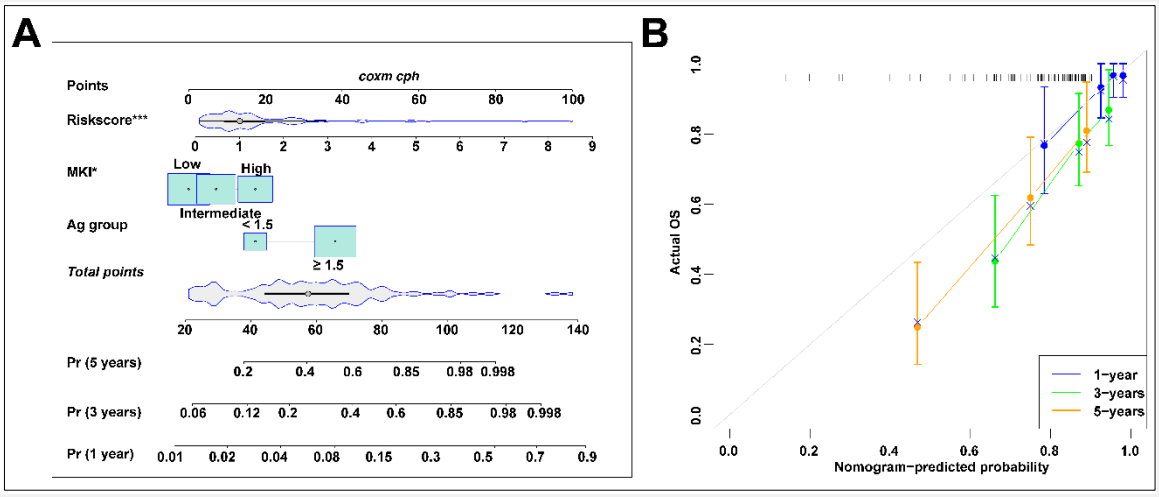

Figure S12. Nomogram.

A - Nomogram created for 1-, 3-, and 5-year survival prediction in NBL patients based on age, risk score, and MKI. B - Calibration curve showing the nomogram's favorable prediction accuracy.

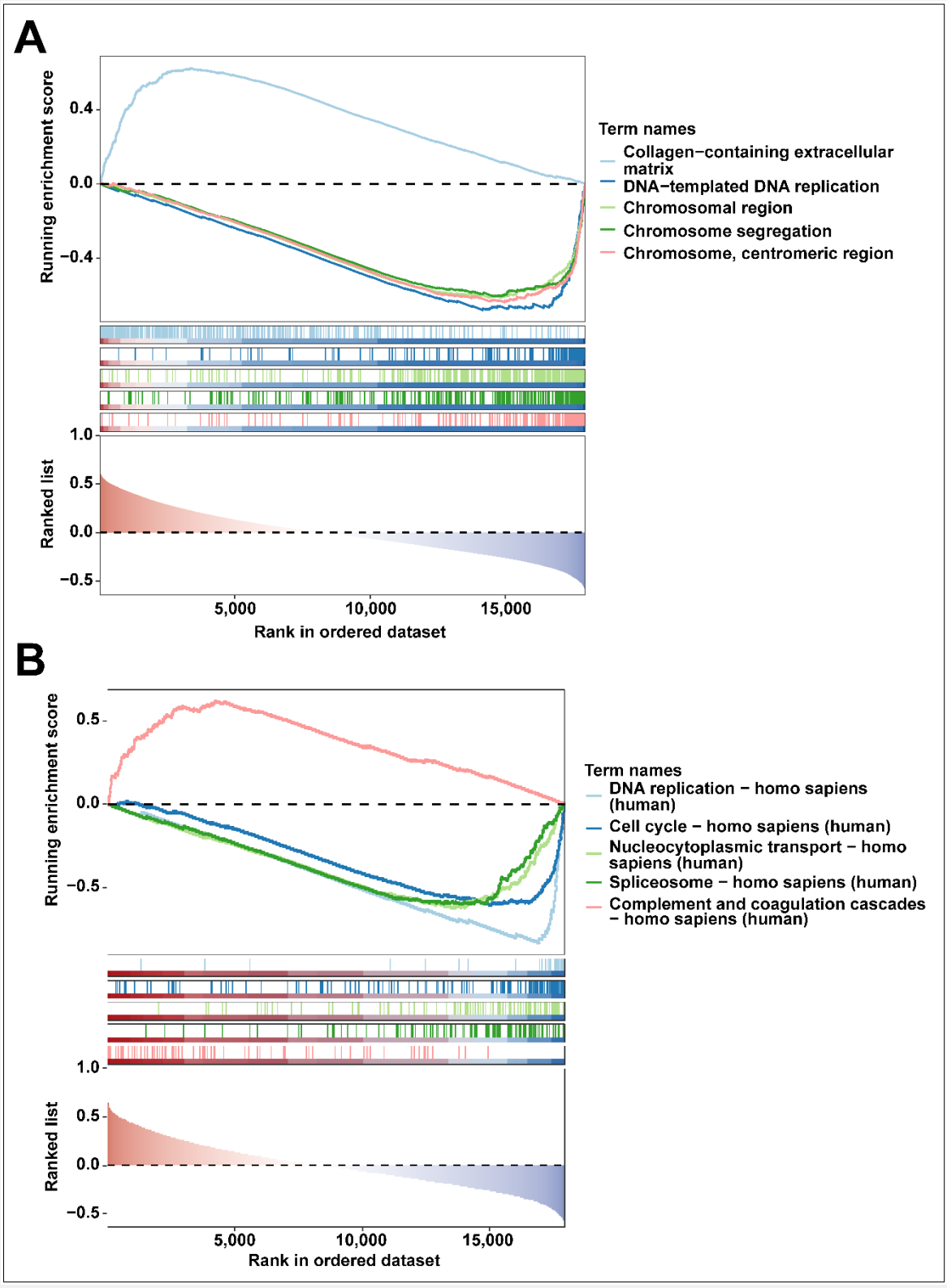

Figure S13. The GSEA of FBXO32.

A - GO enrichment analysis of FBXO32-associated genes in neuroblastoma. B - KEGG pathway enrichment analysis of FBXO32-associated genes in neuroblastoma.

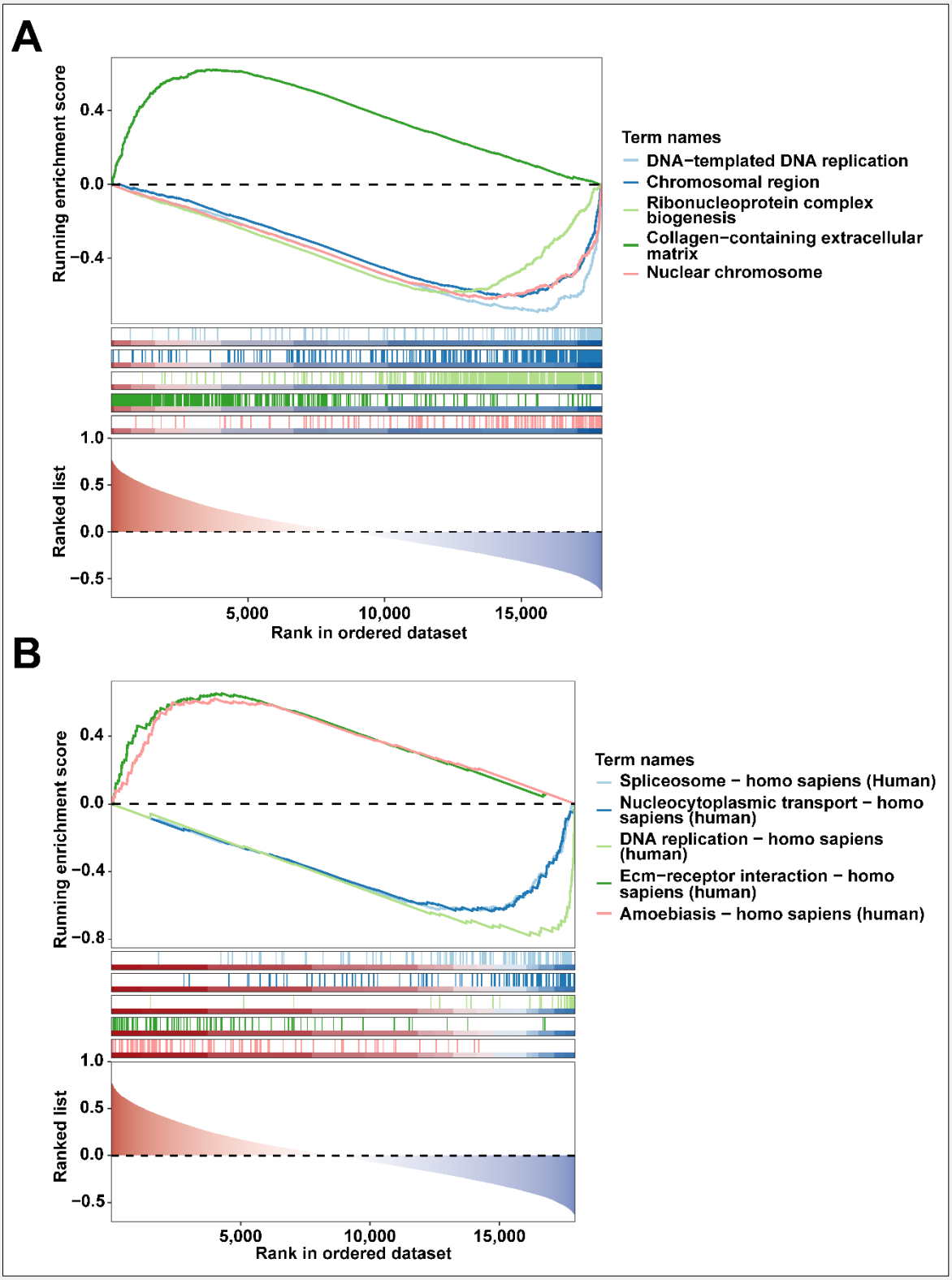

Figure S14. The GSEA of GNG12.

A - GO enrichment analysis of GNG12-associated genes in neuroblastoma. B - KEGG pathway enrichment analysis of GNG12-associated genes in neuroblastoma.

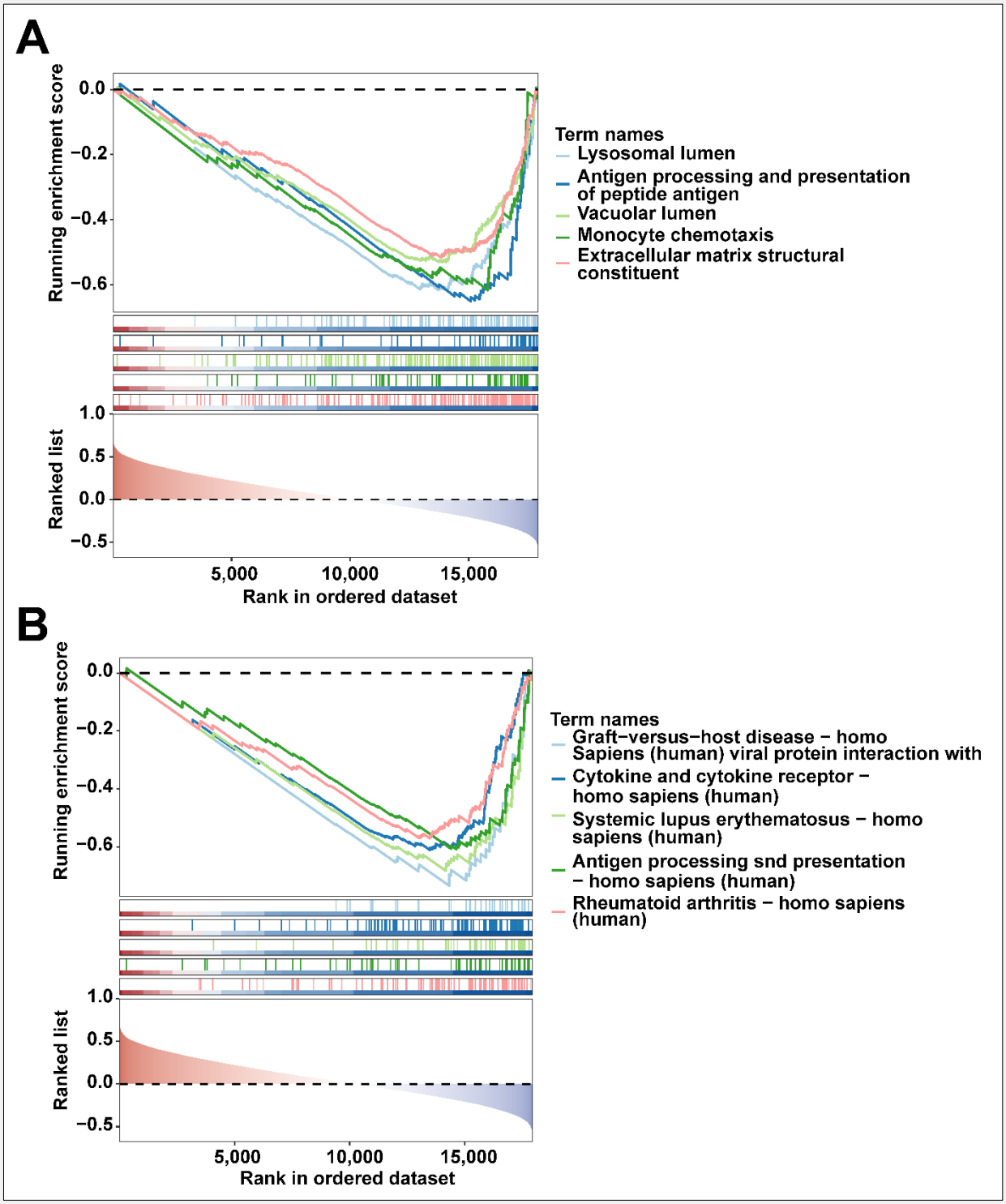

Figure S15. The GSEA of GRIA2.

A - GO enrichment analysis of GRIA2-associated genes in neuroblastoma. B - KEGG pathway enrichment analysis of GRIA2-associated genes in neuroblastoma.

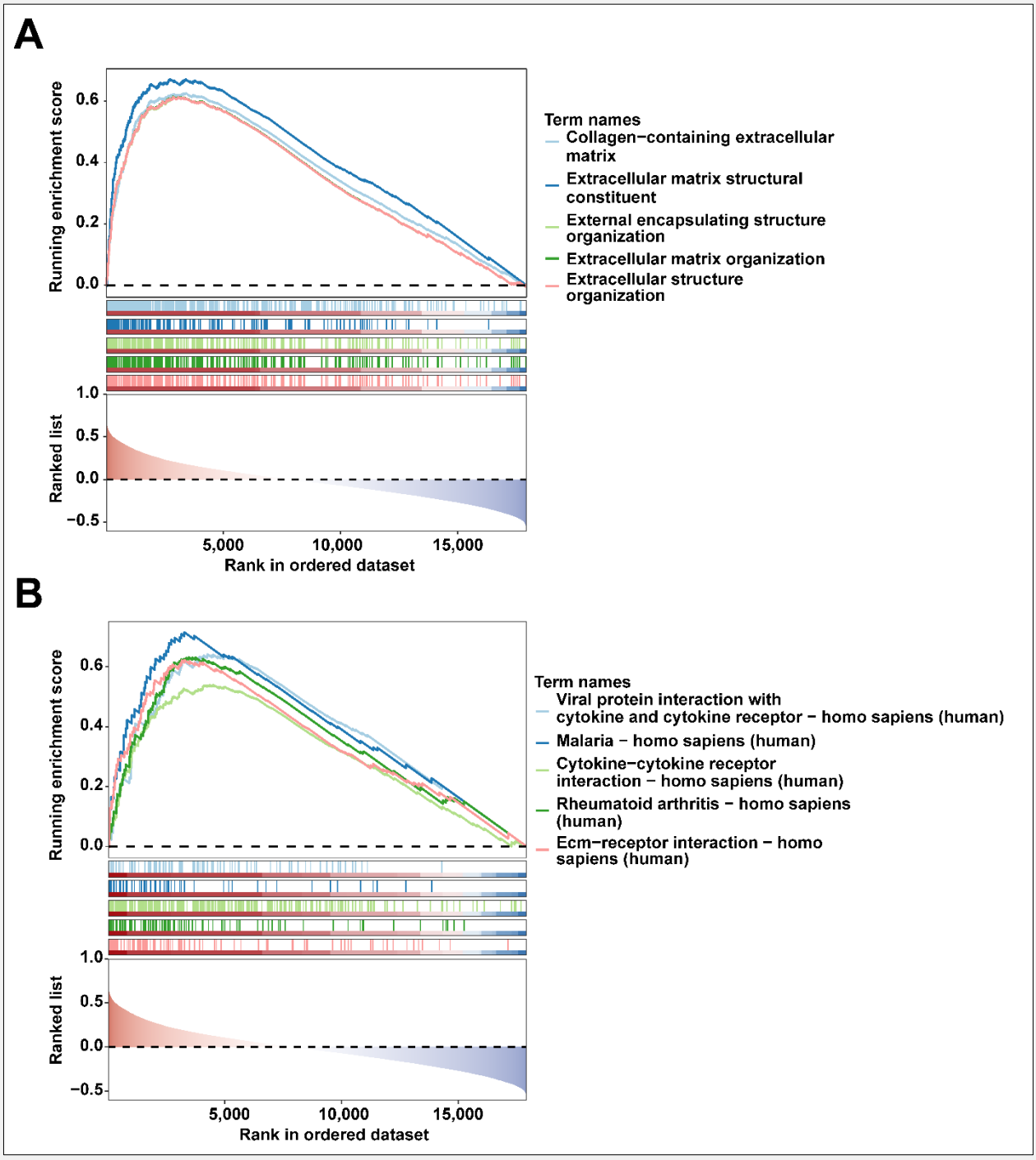

Figure S16. The GSEA of PHLDA2.

A - GO enrichment analysis of PHLDA2-associated genes in neuroblastoma. B - KEGG pathway enrichment analysis of PHLDA2-associated genes in neuroblastoma.