# Low FTO expression serves as a potential biomarker for diagnosis and prognosis of patients with liver cancer

**Hongyun Shi**
The First Hospital of Jilin University
**Chang Fu**
The First Hospital of Jilin University
**Panpan Quan**
The First Hospital of Jilin University
**Liyue Zhang**
The First Hospital of Jilin University
**Yunpeng Liu**
The First Hospital of Jilin University
**Yan Jiao** ( jiaoyan@jlu.edu.cn )
The First Hospital of Jilin University

**Article**

**Keywords:** Liver cancer, N6-methyladenosine, FTO, biomarker, prognosis, diagnosis

**Posted Date:** April 19th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1408109/v2

# Abstract

**Background:** As an important part of m$^6$A modification, *FTO* plays a carcinogenic effect in cancer. In this study, we aim to explore whether *FTO* could be a new target for diagnosis and prognostic of patients with liver cancer.

**Material and Methods:** We obtained and analysis data from TCGA database by using R software (version 3.5.1). The box plot was used to analyze the pattern of *FTO* expression. Receiver operating characteristic (ROC) curve was performed to evaluate diagnostic value of *FTO* expression in liver cancer. Fisher's exact and chi-square tests were used to analyze relationship between *FTO* expression and clinical pathological characteristics. Kaplan-Meier curve and Cox regression analysis were used to explore the prognostic value of *FTO* in liver cancer.

**Results:** We observed that *FTO* was down-regulated in liver cancer and was associated with patients' age. ROC curve showed that *FTO* expression had good clinical diagnostic value. Our results also revealed that *FTO* expression was associated with age, histologic grade, M classification, vital status, and overall survival (OS) of patients. Low *FTO* expression decreased OS, and was an independent predictor of poor prognosis in patients with liver cancer.

**Conclusion:** *FTO* could serves as a potential biomarker for the diagnosis and prognosis of liver cancer


# Introduction

Liver cancer is a malignant tumor associated with high recurrence and metastasis rate[1–3]. Despite tremendous progress had been made in the development of early diagnosis and comprehensive therapy, its incidence and mortality have been steadily increasing[4,5]. The main reason is local recurrence and distant organ metastasis after surgical treatment, leading to poor prognosis and shortened survival time of patients[6]. With the research on cancer treatment strategies and prognosis prediction, molecular targets had become a novel clinical strategy[7]. Therefore, it is very meaningful and important to explore new molecular markers related to liver cancer.

N$^6$-methyladenosine (m$^6$A) is a dominant RNA methylation modification. This methylation modification has been proven to be reversible, and it was involved in three types of main proteins, including transmethylase (m$^6$A"writer"),demethylase(m$^6$A "eraser") and methylation reading protein (m$^6$A "reader")[8,9]. The m$^6$A "writer" was responsible for the methylation of target RNA transcripts[10]. The main function of m$^6$A "reader" was to identify m$^6$A modifications to guide RNA translation, splicing, export, degradation and microRNA processing

[8] .The m$^6$A "eraser" was responsible for removing m$^6$A from the target transcript[8,10,11].Studies have shown that the dysregulation of m$^6$A-related molecules affects the tumorigenesis, indicating that m$^6$A was closely relevant to tumor occurrence and tumor progression[12].

*FTO* (fat mass and obesity associated protein), the first identified m$^6$A demethylase, was able to control mRNA splicing by inhibiting SRSF2 binding at the splice site[13–15]. In addition, *FTO* also blocked YTHDF2-mediated mRNA degradation by reducing m$^6$A levels of cyclin A2 and cyclin-dependent kinase 2 (CDK2), leading to promote adipocyte cycle progression and fat formation[16,17]. Recently, studies have shown that *FTO* played a carcinogenic effect in cancer[18–20]. It was reported that *FTO* induced drug resistance in tumor cells after drug treatment[21]. For example, knocking out *FTO* can significantly enhance response of acute myelocytic leukemia (AML) cells to all-transretinoic acid (ATRA) treatment, thereby promoting differentiation[22]. Similarly, in cutaneous squamous cell carcinoma (CSCC), *FTO* increased the resistance of tumor cells to radiotherapy and chemotherapy by removing m$^6$A from beta-catenin[23]. At present, the expression level of *FTO* has been studied as a potential biomarker for various cancers, such as gastric cancer[24], intrahepatic cholangiocarcinoma[25]and endometrial cancer[26]. However, the potential role of *FTO* expression in the clinical diagnosis and prognostic evaluation of liver cancer patients has not been determined.

In the current study, we compared the expression of *FTO* mRNA between liver cancer patients and healthy individuals, and analyzed the diagnostic and prognostic significance of *FTO* expression. We also studied the correlation between *FTO* expression

and clinic-pathological parameters. The results revealed that *FTO* expression is an independent risk factor for poor survival, indicating that *FTO* may be a useful biomarker for the prognosis of liver cancer.

## Materials And Methods

# Database selection and data collection

We obtained RNA-Seq of *FTO* and clinical information of liver cancer patients from The Cancer Genome Atlas (TCGA) dataset. RNA-Seq was transformed to RSEM by estimating as log2(x + 1) normalized counts and used for subsequent analysis by selecting R software (version 3.5.1)[27].

# Statistical analysis

The package ggplot2 in R software is used for visual analysis. The box plot showed the expression of *FTO* in the LIHC dataset. Chi-square test was used to evaluate the correlation between *FTO* expression and clinical features of liver cancer patients. The ROC curve was used to evaluate the diagnostic significance of *FTO* expression in liver cancer. The Kaplan-Meier curve was performed to assess the effect of *FTO* expression on patient's survival (OS and RFS). The univariate and multivariate Cox analysis was used to analyze the prognostic value of *FTO* expression. $P < 0.05$ is considered statistically significant.

## Results

### The expression pattern of FTO in liver cancer patients

Through using R software, clinical data of 373 patients were collected from the TCGA database, including the patient's age, gender, histological type, histologic grade, clinical stage, and TNM classification, as well as radiation therapy, residual tumor, vital status, sample type overall survival and relapse-free survival (Table 1). Subsequently, in order to analyze the expression pattern of *FTO*, we analyzed the expression of *FTO* in 52 healthy individuals and 373 patients. As shown in Fig. 1, *FTO* expression was significantly lower in tumor tissues than that in normal tissues ($P = 0.026$). In addition, differences in *FTO* expression was found according to age ($P = 0.039$).

Table 1
Demographic and clinical characteristics of TCGA-LIHC cohort

| characteristics | Numbers of cases(%) |
|---|---|
| Age | |
| < 55 | 117(31.45) |
| >=55 | 255(68.55) |
| NA | 1(0) |
| Gender | |
| Female | 121(32.44) |
| Male | 252(67.56) |
| Histological type | |
| Fibrolamellar Carcinoma | 3(0.8) |
| Hepatocellular Carcinoma | 363(97.32) |
| Hepatocholangiocarcinoma (Mixed) | 7(1.88) |
| Histologic grade | |
| NA | 5(1.34) |
| G1 | 55(14.75) |
| G2 | 178(47.72) |
| G3 | 123(32.98) |
| G4 | 12(3.22) |
| Stage | |
| NA | 24(6.43) |
| I | 172(46.11) |
| II | 87(23.32) |
| III | 85(22.79) |
| IV | 5(1.34) |
| T classification | |
| NA | 2(0.54) |
| T1 | 182(48.79) |
| T2 | 95(25.47) |
| T3 | 80(21.45) |
| T4 | 13(3.49) |
| TX | 1(0.27) |
| N classification | |
| NA | 1(0.27) |

Abbreviation: NA, not available.

| characteristics | Numbers of cases(%) |
|---|---|
| N0 | 253(67.83) |
| N1 | 4(1.07) |
| NX | 115(30.83) |
| M classification | |
| M0 | 267(71.58) |
| M1 | 4(1.07) |
| MX | 102(27.35) |
| Radiation therapy | |
| NA | 25(6.7) |
| No | 340(91.15) |
| Yes | 8(2.14) |
| Residual tumor | |
| NA | 7(1.88) |
| R0 | 326(87.4) |
| R1 | 17(4.56) |
| R2 | 1(0.27) |
| RX | 22(5.9) |
| Vital status | |
| Deceased | 130(34.85) |
| Living | 243(65.15) |
| Sample type | |
| Primary Tumor | 371(99.46) |
| Recurrent Tumor | 2(0.54) |
| Overall survival | |
| No | 237(64.58) |
| Yes | 130(35.42) |
| Relapse-free survival | |
| No | 179(55.94) |
| Yes | 141(44.06) |
| HK3 | |
| high | 282(75.6) |
| low | 91(24.4) |
| Abbreviation: NA, not available. | |

**Diagnostic significance of FTO expression in liver cancer**

To evaluate the diagnostic significance of *FTO* expression, we established ROC curve using the expression value of liver cancer patients and healthy individuals from TCGA. We found that *FTO* expression had diagnostic value overall (AUC = 0.597; Fig. 2). Subsequently, we analyzed the diagnostic value of *FTO* expression in different stages of liver cancer, including stage I (AUC = 0.610), stage II (AUC = 0.628), stage III (AUC = 0.550) and stage IV (AUC = 0.672).

**Correlation between** FTO **expression and clinical characteristics of liver cancer.**

To further analyze the correlation between *FTO* expression and clinical characteristics, we divided patients into two groups (high expression and low expression of *FTO* expression) according to the ROC curve threshold (Fig. 2A). As shown in Table 2, the chi-square test indicated low *FTO* expression was significantly associated with age (P = 0.0079), histologic grade (P = 0.0088), M classification(P = 0.0023), vital status (P = 0.0227) and overall survival of patients (P = 0.0216).

Table 2
Correlation between the expressions of *FTO*and the clinic pathologic characteristics in liver cancer

| Parameter | variables | N | *FTO* | | | | X2 | P-value | Fish |
|---|---|---|---|---|---|---|---|---|---|
| | | | high | prop | low | prop | | | |
| Age | < 55 | 117 | 78 | (27.66) | 39 | (43.33) | 7.0642 | 0.0079 | 0.0063 |
| | >=55 | 255 | 204 | (72.34) | 51 | (56.67) | | | |
| Gender | Female | 121 | 91 | (32.27) | 30 | (32.97) | 0 | 1 | 0.8982 |
| | Male | 252 | 191 | (67.73) | 61 | (67.03) | | | |
| Histological_type | Fibrolamellar Carcinoma | 3 | 3 | (1.06) | 0 | (0) | 1.387 | 0.4998 | 1 |
| | Hepatocellular Carcinoma | 363 | 273 | (96.81) | 90 | (98.9) | | | |
| | Hepatocholangiocarcinoma (Mixed) | 7 | 6 | (2.13) | 1 | (1.1) | | | |
| Histologic_grade | G1 | 55 | 47 | (16.91) | 8 | (8.89) | 11.4123 | 0.0097 | 0.0088 |
| | G2 | 178 | 142 | (51.08) | 36 | (40) | | | |
| | G3 | 123 | 81 | (29.14) | 42 | (46.67) | | | |
| | G4 | 12 | 8 | (2.88) | 4 | (4.44) | | | |
| Stage | I | 172 | 136 | (51.91) | 36 | (41.38) | 5.5806 | 0.1339 | 0.1195 |
| | II | 87 | 63 | (24.05) | 24 | (27.59) | | | |
| | III | 85 | 61 | (23.28) | 24 | (27.59) | | | |
| | IV | 5 | 2 | (0.76) | 3 | (3.45) | | | |
| T_classification | T1 | 182 | 145 | (51.79) | 37 | (40.66) | 4.4394 | 0.3498 | 0.324 |
| | T2 | 95 | 69 | (24.64) | 26 | (28.57) | | | |
| | T3 | 80 | 55 | (19.64) | 25 | (27.47) | | | |
| | T4 | 13 | 10 | (3.57) | 3 | (3.3) | | | |
| | TX | 1 | 1 | (0.36) | 0 | (0) | | | |
| N_classification | N0 | 253 | 185 | (65.84) | 68 | (74.73) | 2.5685 | 0.2769 | 0.207 |
| | N1 | 4 | 3 | (1.07) | 1 | (1.1) | | | |
| | NX | 115 | 93 | (33.1) | 22 | (24.18) | | | |
| M_classification | M0 | 267 | 194 | (68.79) | 73 | (80.22) | 12.0013 | 0.0025 | 0.0023 |
| | M1 | 4 | 1 | (0.35) | 3 | (3.3) | | | |
| | MX | 102 | 87 | (30.85) | 15 | (16.48) | | | |
| Radiation_therapy | No | 340 | 256 | (97.34) | 84 | (98.82) | 0.1429 | 0.7054 | 0.6851 |
| | Yes | 8 | 7 | (2.66) | 1 | (1.18) | | | |
| Residual_tumor | R0 | 326 | 242 | (88) | 84 | (92.31) | 5.1227 | 0.163 | 0.2034 |
| | R1 | 17 | 14 | (5.09) | 3 | (3.3) | | | |
| | R2 | 1 | 0 | (0) | 1 | (1.1) | | | |
| | RX | 22 | 19 | (6.91) | 3 | (3.3) | | | |

| Parameter | variables | N | *FTO* | | | | X2 | P-value | Fish |
|---|---|---|---|---|---|---|---|---|---|
| | | | high | prop | low | prop | | | |
| Vital_status | Deceased | 130 | 89 | (31.56) | 41 | (45.05) | 4.9396 | 0.0262 | 0.0227 |
| | Living | 243 | 193 | (68.44) | 50 | (54.95) | | | |
| Sample_type | Primary Tumor | 371 | 280 | (99.29) | 91 | (100) | 0 | 1 | 1 |
| | Recurrent Tumor | 2 | 2 | (0.71) | 0 | (0) | | | |
| Overall survival | No | 237 | 189 | (67.99) | 48 | (53.93) | 5.2222 | 0.0223 | 0.0216 |
| | Yes | 130 | 89 | (32.01) | 41 | (46.07) | | | |
| Relapse-free survival | No | 179 | 140 | (56.22) | 39 | (54.93) | 0.0034 | 0.9534 | 0.8925 |
| | Yes | 141 | 109 | (43.78) | 32 | (45.07) | | | |

**Effect of** FTO **expression on patient survival in liver cancer**

We performed survival analysis to evaluate the effect of *FTO* expression on the survival of liver cancer patients. As shown in Fig. 3, Kaplan-Meier curves showed that the patients of low *FTO* expression significantly had the poor overall survival (P = 0.011). Subgroups analysis found that low *FTO* expression significantly affects patient OS in stage G1/G2, stage Ⅲ/Ⅳ (P = 0.0098), T1 (P = 0.033), N0 (P = 0.016) and M0 (P = 0.018). We also observed that female patients with low *FTO* expression had shorter OS (P = 0.014), and elder patient (P = 0.0025). However, the low expression of *FTO* had no significant effect on patients' RFS (Fig. 4).

**Low** FTO **expression is an independent risk factor for patient's prognosis.**

To assess the prognostic significance of *FTO* expression in liver cancer, univariate Cox analysis and multivariate Cox analysis were performed. We selected potential variables that make sense in univariate analysis to conduct multivariable Cox analysis (Tables 3 and 4). We found that low *FTO* expression is an independent risk factor for poor OS (hazard ratio [HR] = 0.62, 95% confidence interval [CI]:0.43–0.91, P = 0.014). In addition, in our results, *FTO* expression cannot be used as an independent predictor of RFS.

Table 3
Summary of univariate and multivariate Cox regression analyses of overall survival duration

| Parameters | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Hazard Ratio | 95%CI (lower ~ upper) | *P* value | Hazard Ratio | 95%CI (lower-upper) | *P* value |
| Age | 1 | 0.69–1.45 | 0.997 | | | |
| Gender | 0.8 | 0.56–1.14 | 0.22 | | | |
| Histologicgrade | 1.04 | 0.84–1.3 | 0.698 | | | |
| stage | 1.38 | 1.15–1.66 | 0.001 | 0.84 | 0.67–1.05 | 0.123 |
| Tclassification | 1.66 | 1.39–1.99 | 0 | 1.85 | 1.47–2.34 | 0 |
| Nclassification | 0.73 | 0.51–1.05 | 0.086 | | | |
| Mclassification | 0.72 | 0.49–1.04 | 0.077 | | | |
| Radiationtherapy | 0.51 | 0.26–1.03 | 0.06 | | | |
| Residualtumor | 1.42 | 1.13–1.8 | 0.003 | 1.45 | 1.13–1.86 | 0.004 |
| FTO | 0.62 | 0.43–0.9 | 0.011 | 0.62 | 0.43–0.91 | 0.014 |

Table 4
Summary of univariate and multivariate Cox regression analyses of relapse-free survival duration

| Parameters | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Hazard Ratio | 95%CI (lower ~ upper) | *P* value | Hazard Ratio | 95%CI (lower-upper) | *P* value |
| Age | 0.9 | 0.63–1.28 | 0.55 | | | |
| Gender | 0.99 | 0.7–1.41 | 0.966 | | | |
| Histologicgrade | 0.98 | 0.8–1.21 | 0.883 | | | |
| stage | 1.66 | 1.38–1.99 | 0 | 1.13 | 0.88–1.46 | 0.333 |
| Tclassification | 1.78 | 1.49–2.12 | 0 | 1.63 | 1.26–2.13 | 0 |
| Nclassification | 0.97 | 0.67–1.4 | 0.874 | | | |
| Mclassification | 1.17 | 0.79–1.74 | 0.432 | | | |
| Radiationtherapy | 0.74 | 0.26–2.16 | 0.584 | | | |
| Residualtumor | 1.28 | 1.01–1.61 | 0.042 | 1.33 | 1.05–1.69 | 0.017 |
| FTO | 0.9 | 0.61–1.34 | 0.619 | | | |

# Discussion

Based on the TCGA-LIHC data set analysis revealed that *FTO* was down-regulated in liver cancer and was associated with patients' age. The ROC curve showed that *FTO* expression was able to be clinical diagnosis marker in the liver cancer. In the analysis of clinical characteristics, *FTO* expression was related to the patient's age, histological grade, M classification, vital status and OS. Through the survival curve, we found that liver cancer patients with low *FTO* expression had a worse OS. Univariate and multivariate Cox regression analysis confirmed that *FTO* is an independent predictor of poor prognosis in patients with liver cancer.

As the first m$^6$A “eraser”, indeed, *FTO* played an oncogenic role in a number of acute myeloid leukemias by enhancing leukemic oncogene-mediated cell transformation: acts by mediating m6A demethylation of target transcripts such as MYC, CEBPA, ASB2 and RARA, leading to promote their expression[18,28]. Many studies suggested that *FTO* was up-regulated in several tumors, including breast cancer[29], gastric cancer[30]and glioblastoma[31]. However, in this study, our results show that *FTO* played a tumor suppressor effect in liver cancer, which suggested that *FTO* may play an important role as acontext-related regulator in network signal of tumorigenesis. In addition, we also observed that *FTO* expression gradually decreased with increasing of histological grade, which meant that it might have a potential negative impact on the progression of liver cancer.

Considering the relationship between immune regulation and anti-tumor effects, it is clear that tumor cells are up-regulated with many new antigens, which may be recognized by the immune system[32]. In the process of tumorigenesis, tumor cells promote their proliferation and metastasis by escaping from immune host surveillance and attack. It had been reported that *FTO* promoted the occurrence of gastric cancer by promoting tumor cell proliferation, migration and lymph node metastasis[24]. Zhang et al. found that *FTO* induced the growth and invasion of endometrial cancer cells by regulating the PI3K/AKT and MAPK signaling pathways, which indicated that *FTO* played an important role in evading immune surveillance in tumors[33].These findings indicated that *FTO* played an important role in tumor proliferation, invasion and metastasis, although no previous studies had directly detected the specific function of *FTO* in tumor progression. In the present study, we found that the low *FTO* expression significantly shortened the OS of liver cancer patients, and found that low *FTO* expression significantly affects patient OS inG1/G2, I/II, T1, N0 and M0. This indicated that *FTO* expression had predictive prognostic value for specific types of patients, which was beneficial to the selection and supervision of prognostic treatment for patients. Interestingly, *FTO* expression did not have significant correlation with patients' RFS. We guess this may be related to the limited number of samples or patient's lifestyle. However, the specific reason needs to be further clarified.

Gene expression and genetic characteristics in tumors are related to clinical characteristics, pathological characteristics and patient prognosis[34]. In liver cancer, several biomarkers related to tumor proliferation signals are associated with poor prognosis of patients, such as *TGF-β*, *KI-67* and *PTEN*[35–37]. Previous studies showed that a reproducible gene-expression signature correlated with survival is present in liver tissue adjacent to the tumor in HCC patients, and can predict the risk of tumor recurrence after postoperative in HCC patients[38].Additionally, a large number of studies based on tumor tissue (gene expression profiles and mutation models) are being conducted to discover potential biomarkers for predicting HCC patients[39]. These studies indicate the importance and feasibility of studying biomarkers of tumor tissue. Therefore, it is necessary to increase efforts to verify the genetic biomarkers related to the prognosis of liver cancer in clinical test.

To our comprehension, this study is the first time to identify the correlation between *FTO* expression and clinical application value in liver cancer patients. We revealed that *FTO* may be used as a biomarker for clinical diagnosis and poor prognosis of liver cancer. However, due to the limited number of samples, it is difficult to establish a more valuable predictive model between *FTO* expression and clinicopathological data of liver cancer patients. In future studies, we need to expand the sample size in order to generate an appropriate prediction model for the prognosis of liver cancer patients.

# Declarations

Competing interests: All authors have completed the ICMJE uniform disclosure form.The authors have no conflicts of interest to declare.

Ethics approval and consent to participate: Ethics committee approval was not necessary because all clinical data used in this study were obtained from a public database and are available for research.

Consent for publication: Not applicable

Availability of data and materials: The results shown here are partly based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.

Funding: This project was supported by the foundation of Jilin Provincial Finance Department of China (Grant Number: JLSWSRCZX2021-071 and JLSCZD2019-021) and The First Hospital of Jilin University (Grant Number: JDYY11202008).

Authors' contributions: Hongyun Shi and Chang Fu wrote the manuscript. Panpan Quan contributed to data collection and analysis. Yan Jiao and Yunpeng Liu had a significant role in the study design and manuscript review. Liyue Zhang revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements: Not applicable

## References

1 Marengo, A., Rosso, C. & Bugianesi, E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Annu Rev Med* **67**, 103-117, doi:10.1146/annurev-med-090514-013832 (2016).

2 Aufhauser, D. D. *et al.* Incidence of Occult Intrahepatic Metastasis in Hepatocellular Carcinoma Treated With Transplantation Corresponds to Early Recurrence Rates After Partial Hepatectomy. *Ann. Surg.* **267**, 922-928, doi:10.1097/sla.0000000000002135 (2018).

3 Su, Y. H., Kim, A. K. & Jain, S. Liquid biopsies for hepatocellular carcinoma. *Transl Res* **201**, 84-97, doi:10.1016/j.trsl.2018.07.001 (2018).

4 Cheng, A. L., Shen, Y. C. & Zhu, A. X. Targeting Fibroblast Growth Factor Receptor Signaling in Hepatocellular Carcinoma. *Oncology-Basel* **81**, 372-380, doi:10.1159/000335472 (2011).

5 Joliat, G. R., Hahnloser, D., Demartines, N. & Schafer, M. Future development of gastrointestinal cancer incidence and mortality rates in Switzerland: a tumour registry- and population-based projection up to 2030. *Swiss Med Wkly* **145**, doi:ARTN w14188

10.4414/smw.2015.14188 (2015).

6 Zhang, X. F., Li, J., Shen, F. & Lau, W. Y. Significance of presence of microvascular invasion in specimens obtained after surgical treatment of hepatocellular carcinoma. *J Gastroen Hepatol* **33**, 347-354, doi:10.1111/jgh.13843 (2018).

7 Lachenmayer, A., Alsinet, C., Chang, C. Y. & Llovet, J. M. Molecular approaches to treatment of hepatocellular carcinoma. *Digest Liver Dis* **42**, S264-S272, doi:Doi 10.1016/S1590-8658(10)60515-4 (2010).

8 He, L. E. *et al.* Functions of N6-methyladenosine and its role in cancer. *Mol Cancer* **18**, doi:ARTN 176

10.1186/s12943-019-1109-9 (2019).

9 Chen, B., Li, Y., Song, R. F., Xue, C. & Xu, F. Functions of RNA N6-methyladenosine modification in cancer progression. *Molecular Biology Reports* **46**, 2567-2575, doi:10.1007/s11033-019-04655-4 (2019).

10 Yue, Y. N., Liu, J. Z. & He, C. RNA N-6-methyladenosine methylation in post-transcriptional gene expression regulation. *Genes Dev.* **29**, 1343-1355, doi:10.1101/gad.262766.115 (2015).

11 Alarcon, C. R., Lee, H., Goodarzi, H., Halberg, N. & Tavazoie, S. F. N-6-methyladenosine marks primary microRNAs for processing. *Nature* **519**, 482-+, doi:10.1038/nature14281 (2015).

12 Yang, J., Chen, J., Fei, X., Wang, X. & Wang, K. N6-methyladenine RNA modification and cancer. *Oncology letters* **20**, 1504-1512, doi:10.3892/ol.2020.11739 (2020).

13 Zhao, X. *et al.* FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. *Cell Res* **24**, 1403-1419, doi:10.1038/cr.2014.151 (2014).

14 Ben-Haim, M. S., Moshitch-Moshkovitz, S. & Rechavi, G. FTO: linking m(6)A demethylation to adipogenesis. *Cell Res* **25**, 3-4, doi:10.1038/cr.2014.162 (2015).

15 Mathiyalagan, P. *et al.* FTO-Dependent N-6-Methyladenosine Regulates Cardiac Function During Remodeling and Repair. *Circulation* **139**, 518-532, doi:10.1161/Circulationaha.118.033794 (2019).

16 Wu, R. F. *et al.* FTO regulates adipogenesis by controlling cell cycle progression via m(6)A-YTHDF2 dependent mechanism. *Bba-Mol Cell Biol L* **1863**, 1323-1330, doi:10.1016/j.bbalip.2018.08.008 (2018).

17 Wu, R. F. *et al.* Epigallocatechin gallate targets FTO and inhibits adipogenesis in an mRNA m(6)A-YTHDF2-dependent manner. *Int J Obesity* **42**, 1378-1388, doi:10.1038/s41366-018-0082-5 (2018).

18 Li, Z. J. *et al.* FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N-6-Methyladenosine RNA Demethylase. *Cancer Cell* **31**, 127-141, doi:10.1016/j.ccell.2016.11.017 (2017).

19 Chen, J. L. & Du, B. Novel positioning from obesity to cancer: FTO, an m(6)A RNA demethylase, regulates tumour progression. *J Cancer Res Clin* **145**, 19-29, doi:10.1007/s00432-018-2796-0 (2019).

20 Huang, Y. *et al.* Small-Molecule Targeting of Oncogenic FTO Demethylase in Acute Myeloid Leukemia. *Cancer Cell* **35**, 677-+, doi:10.1016/j.ccell.2019.03.006 (2019).

21 Yan, F. *et al.* A dynamic N-6-methyladenosine methylome regulates intrinsic and acquired resistance to tyrosine kinase inhibitors. *Cell Res* **28**, 1062-1076, doi:10.1038/s41422-018-0097-4 (2018).

22 Cui, Q. *et al.* m(6)A RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Glioblastoma Stem Cells. *Cell Reports* **18**, 2622-2634, doi:10.1016/j.celrep.2017.02.059 (2017).

23 Zhou, S. *et al.* FTO regulates the chemo-radiotherapy resistance of cervical squamous cell carcinoma (CSCC) by targeting -catenin through mRNA demethylation. *Mol Carcinogen* **57**, 590-597, doi:10.1002/mc.22782 (2018).

24 Xu, D. *et al.* FTO expression is associated with the occurrence of gastric cancer and prognosis. *Oncology Reports* **38**, 2285-2292, doi:10.3892/or.2017.5904 (2017).

25 Rong, Z. X. *et al.* Downregulation of Fat Mass and Obesity Associated (FTO) Promotes the Progression of Intrahepatic Cholangiocarcinoma. *Front. Oncol.* **9**, doi:ARTN 369

10.3389/fonc.2019.00369 (2019).

26 Delahanty, R. J. *et al.* Association of Obesity-related Genetic Variants With Endometrial Cancer Risk: A Report From the Shanghai Endometrial Cancer Genetics Study. *Am J Epidemiol* **174**, 1115-1126, doi:10.1093/aje/kwr233 (2011).

27 Shim, S. R. & Kim, S. J. Intervention meta-analysis: application and practice using R software. *Epidemiology and health* **41**, e2019008, doi:10.4178/epih.e2019008 (2019).

28 Su, R. *et al.* R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m(6)A/MYC/CEBPA Signaling. *Cell* **172**, 90-+, doi:10.1016/j.cell.2017.11.031 (2018).

29 Tan, A. H., Dang, Y. W., Chen, G. & Mo, Z. N. Overexpression of the fat mass and obesity associated gene (FTO) in breast cancer and its clinical implications. *Int J Clin Exp Patho* **8**, 13405-13410 (2015).

30 Li, Y. *et al.* Expression of Demethylase Genes, FTO and ALKBH1, Is Associated with Prognosis of Gastric Cancer. *Dig. Dis. Sci.* **64**, 1503-1513, doi:10.1007/s10620-018-5452-2 (2019).

31 Deng, X. L., Su, R., Stanford, S. & Chen, J. J. Critical Enzymatic Functions of FTO in Obesity and Cancer. *Front Endocrinol* **9**, doi:UNSP 396

10.3389/fendo.2018.00396 (2018).

32 Tang, H. D., Qiao, J. & Fu, Y. X. Immunotherapy and tumor microenvironment. *Cancer Lett* **370**, 85-90, doi:10.1016/j.canlet.2015.10.009 (2016).

33 Zhang, Z. B. *et al.* Estrogen induces endometrial cancer cell proliferation and invasion by regulating the fat mass and obesity-associated gene via PI3K/AKT and MAPK signaling pathways. *Cancer Lett* **319**, 89-97, doi:10.1016/j.canlet.2011.12.033 (2012).

34 Jiao, Y., Li, Y. Q., Lu, Z. Y. & Liu, Y. H. High Trophinin-Associated Protein Expression Is an Independent Predictor of Poor Survival in Liver Cancer. *Dig. Dis. Sci.* **64**, 137-143, doi:10.1007/s10620-018-5315-x (2019).

35 Zucman-Rossi, J., Villanueva, A., Nault, J. C. & Llovet, J. M. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology* **149**, 1226-+, doi:10.1053/j.gastro.2015.05.061 (2015).

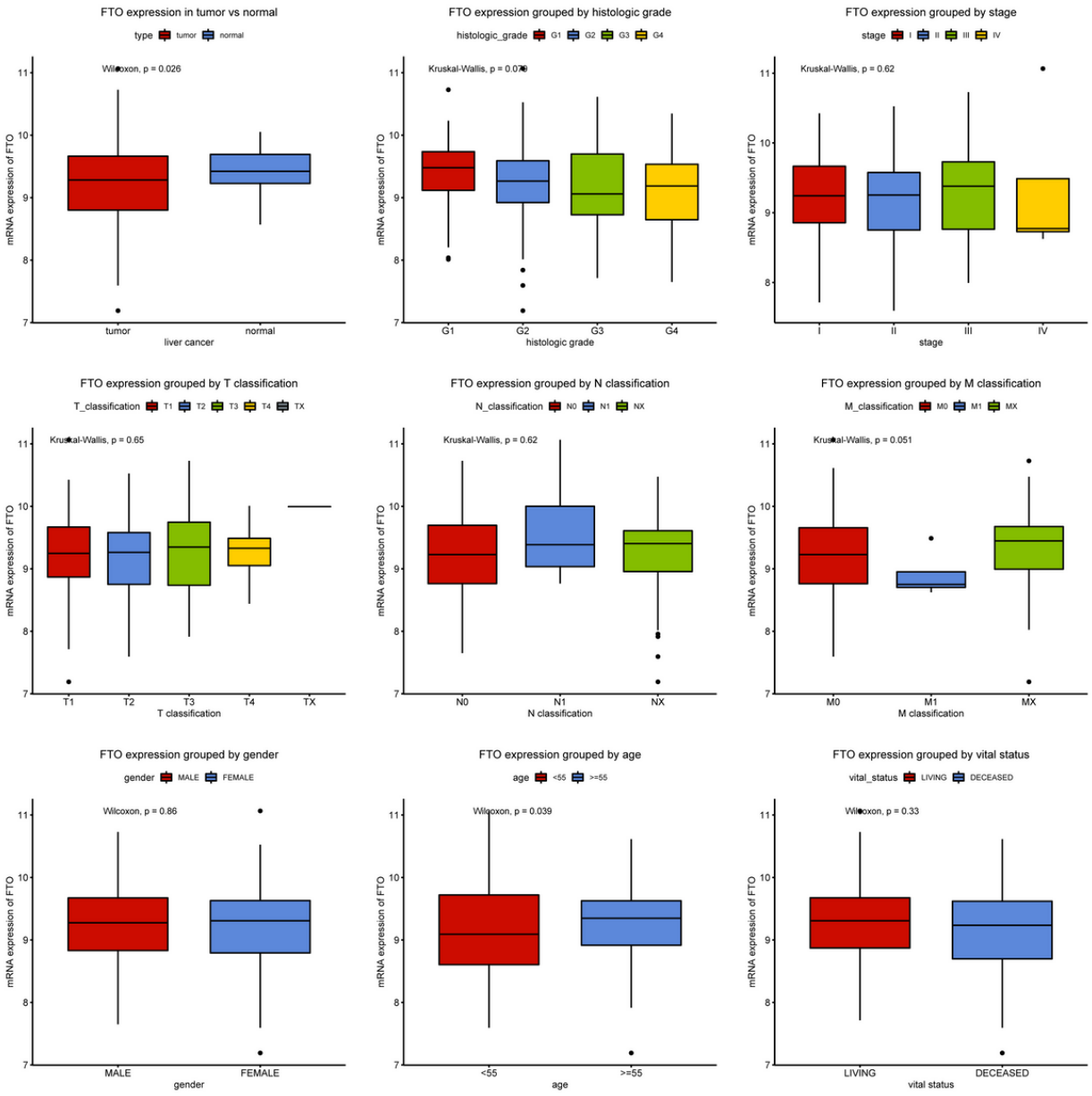
36 Denkert, C. *et al.* Strategies for developing Ki67 as a useful biomarker in breast cancer. *Breast* **24**, S67-S72, doi:10.1016/j.breast.2015.07.017 (2015).

37 Li, S. T. *et al.* Loss of PTEN expression in breast cancer: association with clinicopathological characteristics and prognosis. *Oncotarget* **8**, 32043-32054, doi:10.18632/oncotarget.16761 (2017).

38 Hoshida, Y. *et al.* Gene Expression in Fixed Tissues and Outcome in Hepatocellular Carcinoma. *New Engl J Med* **359**, 1995-2004, doi:10.1056/NEJMoa0804525 (2008).

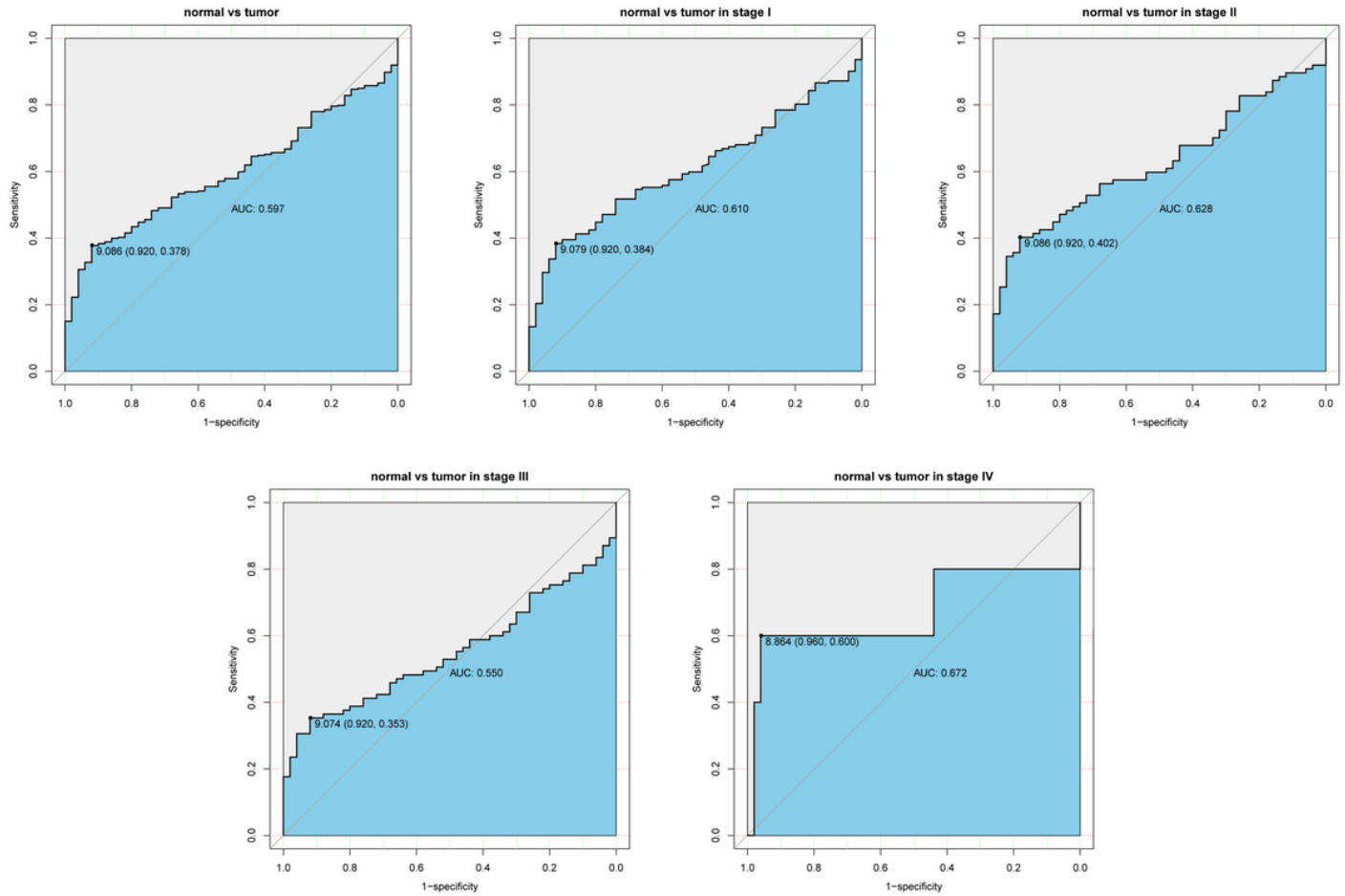
39 King, L. Y. *et al.* A genomic and clinical prognostic index for hepatitis C-related early-stage cirrhosis that predicts clinical deterioration. *Gut* **64**, 1296-1302, doi:10.1136/gutjnl-2014-307862 (2015).

# Figures



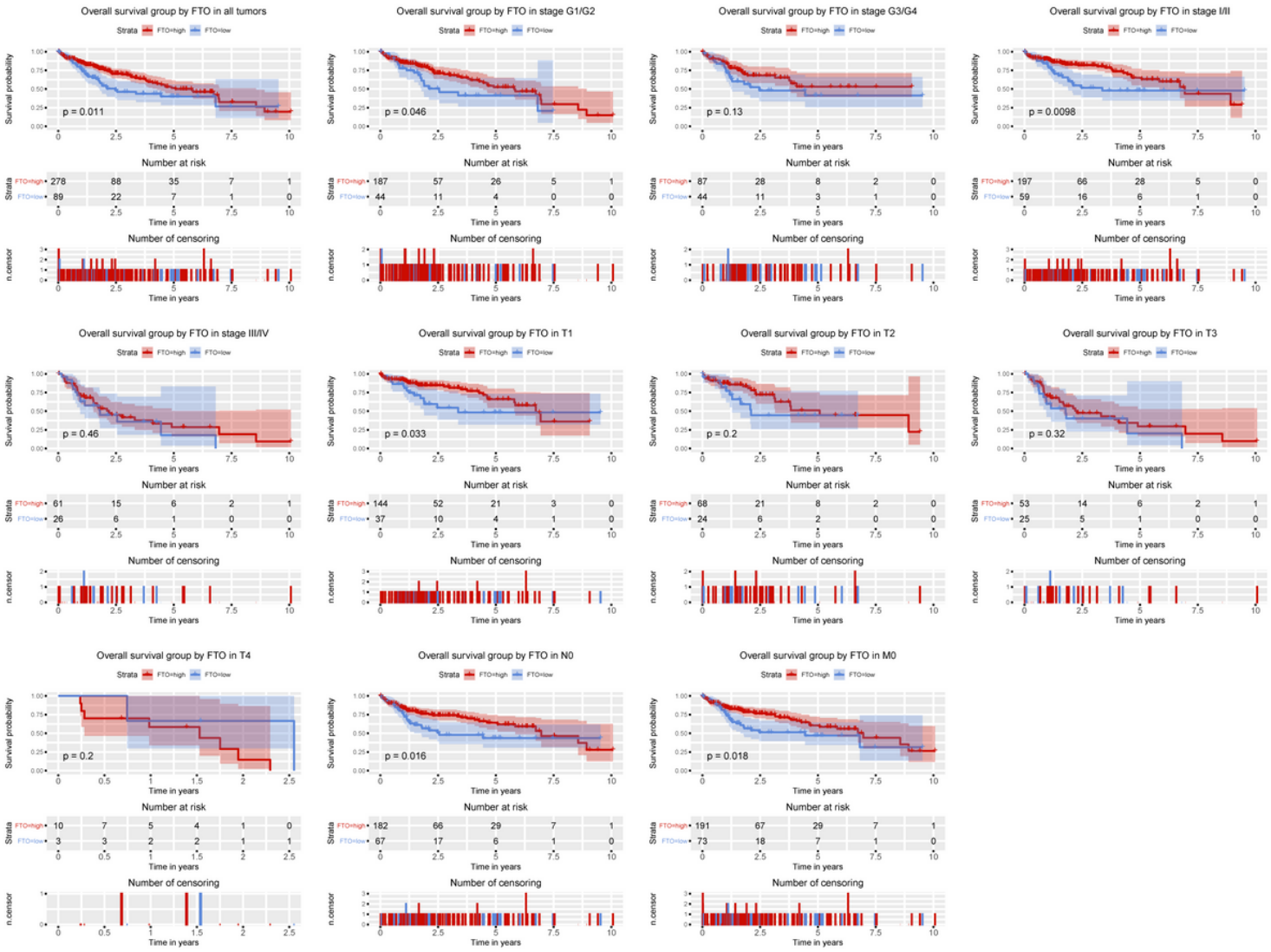

## Figure 1

**Expression of *FTO* in liver cancer.** Expression of *FTO* between tumor and normal tissue was compared. The expression of *FTO* was compared according to different age, gender, histologic grade, histological type, T/N/M classification, as well as radiation therapy, residual tumor, sample type, stage, and vital status.



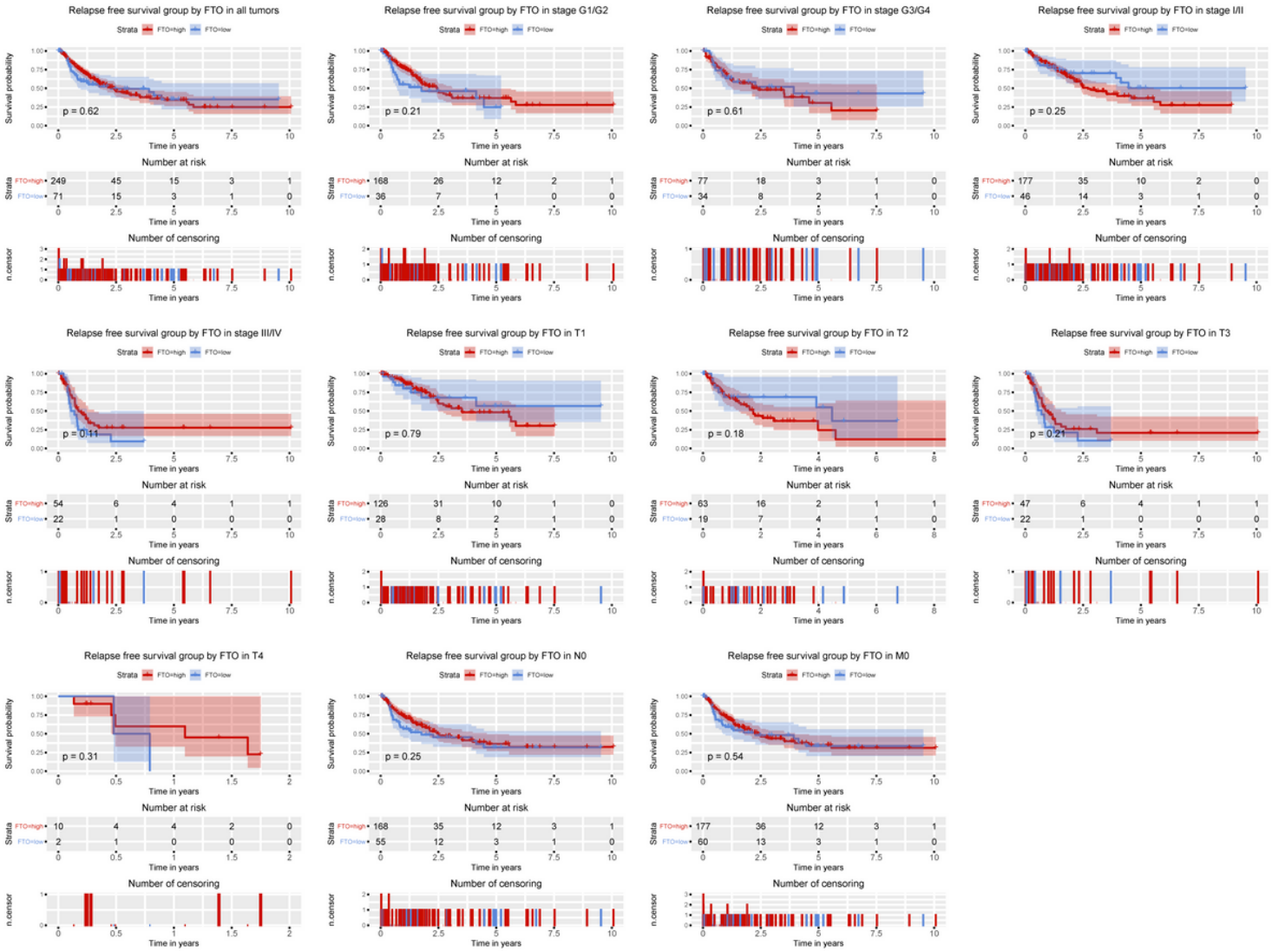

## Figure 2

**Diagnosis value of *FTO* expression in liver cancer.** The ROC curves of *FTO* expression in cancerous vs. normal liver tissues was generated, and cancerous vs. normal liver tissues was analyzed in different stages of liver cancer.




## Figure 3

**The effect of *FTO* expression on OS in liver cancer.** Kaplan-Meier curves of *FTO* expression in all patients and subgroup.




## Figure 4

**The effect of *FTO* expression on RFS in liver cancer.** (a)Kaplan-Meier curves of *FTO* expression in all patients and subgroup.