

## Original Article

# Multi-omics characterization of the unsaturated fatty acid biosynthesis pathway in colon cancer

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**Abstract:** The biosynthesis of unsaturated fatty acids is involved in the initiation and progression of colon adenocarcinoma (COAD). In this study, we aimed to investigate the multi-omics characteristics of unsaturated fatty acid biosynthesis-related genes and explore their prognostic value in colon cancer by analyzing the data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. An unsaturated fatty acid biosynthesis pathway related-genes enrichment score (BUFAS) was constructed utilizing the single sample gene set enrichment analysis (ssGSEA). We discovered that a high BUFAS was associated with longer overall survival (OS) in both the training and the validation sets. Multivariable analysis including the clinical characteristics further verified the independent prognostic value of the BUFAS in both the TCGA-COAD and the GSE39582 datasets. In addition, GSEA analysis revealed that BUFAS was positively associated with several signaling pathways, including MTORC1, peroxisome, and pathways related to fatty acid metabolism, while was negatively associated with other signaling pathways, such as hedgehog, NOTCH, and Wnt/beta-catenin pathway. Furthermore, in the COAD cell lines of the Genomics of Drug Sensitivity in Cancer (GDSC) database, we found that BUFAS was positively correlated with the drug sensitivities of cisplatin, gemcitabine, camptothecin, lapatinib, and afatinib, while was negatively correlated with that of ponatinib. Moreover, in the COAD single-cell transcriptomic dataset (GSE146771), the BUFAS varied among different cell types and was enriched in mast cells and fibroblasts. Taken together, the BUFAS we constructed could be used as an independent prognostic signature in predicting the OS and drug resistance of colon cancer. Unsaturated fatty acid biosynthesis pathway might serve as potential therapeutic targets for cancer treatment.

**Keywords:** Unsaturated fatty acids biosynthesis, colon cancer, prognostic signature, overall survival, multi-omics characterization

## Introduction

Colon cancer is one of the leading causes of cancer-related deaths worldwide, accounting for almost 10% of cancer mortality in 2020 [1]. In the newly diagnosed colon cancer patients, one in five has already developed metastasis, and more than a quarter of them will experience regional or distant metastases later even with the initial diagnosis of localized disease [2], attributing to the dismal clinical outcomes [3]. Thus, stratifying patients at the time of diagnosis is critical in selecting the most appro-

priate treatment options and cancer management. However, except the generally accepted clinical features such as age and tumor stage, biomarkers for predicting the prognosis of colon cancer are yet limited. Therefore, identifying novel and reliable signatures for improving the overall outcome of colon cancer are urgently needed.

Lipid metabolism is known to be involved in tumorigenesis. As the product of lipid metabolism, triacylglycerol is served as a substrate in energy metabolism to ensure adequate energy

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supply for tumor cell growth [4]. The biosynthesis of other lipids, such as cholesterol, phospholipids, and sphingolipids, provides indispensable materials for cytomembrane generation during cancer cell proliferation [5]. Hence, the abnormal lipid metabolism is a distinct feature of malignant cells [6], which is supported by the findings of altered lipid component in tumors, including breast [7], lung [8], renal [9], and liver cancers [10]. Consistently, the activated expression of genes encoding lipid synthesis-related enzymes has been found in colon cancer cells [11]. Furthermore, *in vitro* studies have demonstrated the potential of using lipid synthesis inhibitors in cancer therapy [12], suggesting the pivotal role of lipid metabolism in colon cancer.

Consistent with the importance of lipid metabolism in tumor initiation and progression, fatty acids, as one of the most important productions of lipid synthesis, have been suggested to associate with the cellular survival, malignancy, metastasis, and immune phenotype of different tumors, including glioma [13], cervical cancer [14], and myeloid leukemia [15]. In addition, extra intake of unsaturated fatty acids has been demonstrated to have significant benefits in cardiovascular diseases [16], inflammatory diseases [17], and diet-related tumors including colon cancer [18], whereas an excessive dietary intake of saturated fatty acids is an important risk factor for colon cancer [19]. Furthermore, unsaturated fatty acid supplementation helps sensitize the multi-drug resistant colon cancer cells to chemo treatment [20], although the functional mechanism of unsaturated fatty acids in colon cancer development remains elusive. Collectively, unsaturated fatty acids may have potential protective functions in colon cancer development, which provides the rationale for further investigations on the role of unsaturated fatty acid biosynthesis-related genes in tumorigenesis, which may provide new insights into colon cancer management.

Using public databases, including The Cancer Genome Atlas (TCGA) project and the Gene Expression Omnibus (GEO) database, we depicted the multi-omics characteristics of unsaturated fatty acid biosynthesis pathways in colon cancer, and further constructed a prognostic signature, which might reveal the poten-

tial molecular mechanisms underlying the function of unsaturated fatty acids in the development of colon cancer.

## Methods and materials

### *Data collection*

Three cohorts were included in this study. HTSeq-FPKM gene expression matrix of The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) was obtained via the R package “TCGAbiolinks”, together with the survival and clinical information [21]. Mutation annotation format, copy number variation (CNV) information, and methylation sequencing results by Illumina HumanMethylation450 BeadChip (450K) were retrieved from UCSC XENA database (<https://xenabrowser.net/>). Survival and clinical of COAD samples in the GSE39582 cohort were acquired via the R package “GEOquery” [22]. Raw expression microarray “CEL” data files of the GSE39582 cohort were directly downloaded from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>), which was further normalized by the R package “affy” [23]. The transcriptomic data of single-cell sequencing (Smart-seq2) in colon cancer patients (GSE-146771) were downloaded from the GEO database [24]. The unsaturated fatty acid biosynthesis-related gene set (hsa01040) was downloaded from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database via the R package “KEGGREST”, and 27 genes involved in the biosynthesis of unsaturated fatty acid were obtained from the gene set. The 50 gene sets of cancer hallmark pathways were obtained from the Molecular Signature Database v.7.4 (MSigDB). In the TCGA-COAD and GSE39582 cohorts, patients were excluded if their survival status was unknown, or survival time was 0. Patients with both clinical information and expression profiling data were included for further analysis.

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The expression and methylation levels of the 27 genes related to the biosynthesis of unsaturated fatty acids were extracted from the expression matrix and methylation matrix, respectively. Mann-Whitney test was applied to evaluate the difference in expression and

methylation levels of these 27 genes between normal and tumor tissues. The CNV amplification and deletion frequency of unsaturated fatty acid biosynthesis-related genes were extracted and calculated from the sequencing results, while the somatic single-nucleotide variation of these genes was calculated via the R package “maftools” based on sequencing results [25].

### *Development of a prognostic score via single sample gene set enrichment analysis (ssGSEA)*

The unsaturated fatty acid biosynthesis pathway gene set enrichment score (BUFAS) of every sample was calculated via the R package “GSVA” through “ssGSEA” method in the TCGA-COAD cohort [26, 27]. Samples were further divided into high- and low-BUFAS groups according to the median of BUFAS. Kaplan-Meier analysis was utilized to calculate the overall survival (OS), and the log-rank test was applied to compare the difference in survival curves between the high- and low-BUFAS groups through the R package “survival”. BUFAS as an independent prognostic factor was verified by univariable and multivariable Cox regression analyses, which was applied by the *coxph* function in the R package “survival”. Furthermore, model validation was conducted by applying the same methods to the GSE39582 cohort. The consensus molecular subtypes (CMSs) of colon cancer patients in both the TCGA-COAD and GSE39582 cohorts were assessed through the R package “CMS-caller” [28]. The BUFAS of different clinical characteristics was compared to evaluate the association between the BUFAS and the clinical information of the TCGA-COAD cohort, as well as the estimated CMS subtypes.

### *Pathway correlation analysis*

The enrichment scores for many hallmarks of cancer were calculated by the ssGSEA analysis based on the gene expression matrix in the TCGA-COAD cohort. The correlation between the BUFAS and 50 hallmarks was quantified by the Spearman’s correlation analysis according to their ssGSEA scores. The *corAndPvalue* function in the R package “WGCNA” was exploited to evaluate the correlation between each gene in the unsaturated fatty acid biosynthesis pathway gene set and 50 hallmarks of cancer based on single gene expression and

the ssGSEA scores of these hallmarks [29]. GSEA analysis was conducted utilizing the java GSEA Desktop Application (<http://software.broadinstitute.org/gsea/index.jsp>) to associate the signaling pathway enrichment in *c2.cp.kegg.v7.5.symbols.gmt* in the BUFAS-high and BUFAS-low groups. The normalized enrichment score (NES) was examined to identify significant pathways with a *P*-value <0.05, which was adjusted by the false discovery rate (FDR) method.

### *Drug sensitivity analysis*

The pRRophetic algorithm was originally exploited to predict drug response based on gene expression microarray data and the half maximal inhibitory concentration (IC<sub>50</sub>) information from the Genomics of Drug Sensitivity in Cancer (GDSC) database [30, 31]. The R package “pRRophetic” was applied to estimate the drug sensitivity of 138 drugs based on the mRNA expression data of those samples in the TCGA-COAD cohort, which was shown as IC<sub>50</sub> prediction. The correlation between the estimated drug sensitivity and the BUFAS was estimated for every drug through the Spearman’s correlation analysis, and a *P*<0.05 was considered statistically significant. Furthermore, the *corAndPvalue* function in the R package “WGCNA” was utilized for conducting the Pearson’s correlation analysis between the drug susceptibility and gene expression [29]. Genes that were potentially related to the drug response were selected by the Pearson’s correlation coefficient (*r*) with a *P*-value <0.05.

### *Single-cell transcriptomic analysis*

Each single cell in the GSE146771 dataset was assigned with a BUFAS score by the ssGSEA method via the R package “GSVA”, together with the annotation by the Tumor Immune Single-cell Hub (TISCH) database (<http://tisch.comp-genomics.org>). Uniform manifold approximation and projection (UMAP) plots of the BUFAS for single cell in the GSE146771 dataset were displayed under two resolutions: “Cell type malignancy” and “Cell type major lineage”. The differences in the BUFAS among cell types were calculated by the Kruskal-Wallis test, and *P*-value <0.05 was considered statistically significant. Finally, the BUFAS in each cell type was compared with the others for calculation of the log<sub>2</sub> Fold Change (log<sub>2</sub>FC), which

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was used to predict the cell subpopulations on which unsaturated fatty acid biosynthesis pathway mainly targeted.

### *Statistical analysis*

Statistical analyses, as well as analyses that used various R packages, were all conducted in R (version 4.1.2). Visualization of the results was achieved by R and GraphPad Prism (version 9). The median value (range) and the exact number (percentage) were presented for continuous variables and categorical variables, respectively. The distribution of continuous variables was compared among different groups by the Wilcoxon test. The proportions of categorical variables were examined by the Chi-Square test or the Fisher's exact test. Statistical significance was considered as  $P$ -value  $<0.05$ .

### **Results**

#### *Multi-omics characterization of unsaturated fatty acid biosynthesis-related genes in colon cancer*

A total of 27 gene candidates involved in the unsaturated fatty acid biosynthesis were derived from the KEGG hsa01040 gene set ([Supplementary Table 1](#)). The expression and methylation levels of the unsaturated fatty acid biosynthesis-related genes were analyzed in 453 cancer and 41 normal samples from the TCGA-COAD dataset (**Figure 1A** and **1B**). Since the methylation information was only available in 23 genes, the correlation between the gene expression and the methylation levels was only analyzed in these genes. Six genes showed negative regulation of the methylated modification in gene expression, including *ACOT4* and *ELOVL4* (hypermethylated and down-regulated in tumor tissues), and *ACOT7*, *SCD*, *ELOVL3*, and *HSD17B12* (hypomethylated and up-regulated in tumor tissues), while an inverted correlation was found in another six genes, including *FADS1*, *FADS2*, and *BAAT* (hypermethylated and up-regulated in tumors), and *ACOT1*, *ACOX1*, and *ACOX3* (hypomethylated and down-regulated in tumors), suggesting additional gene expression regulatory mechanisms other than methylation were involved. Furthermore, the CNV and mutational characteristics of these genes were also included in the multi-omics analyses. Copy number

amplification was observed in *ELOVL1* and *ACOX1*, while *ACAA1*, *ACOT7*, *HSP17B14*, *ELOVL2*, *FADS2*, *SCD*, and *ELOVL3* exhibited significant copy number deletion (**Figure 1C**). Various gene mutations were detected in 77 (17.0%) tumor samples, and three genes, *ELOVL2* (14%), *ACOX1* (12%), and *FADS2* (12%) exhibited the high mutational frequency (**Figure 1D**).

#### *Prognostic value of unsaturated fatty acid biosynthesis score in colon cancer*

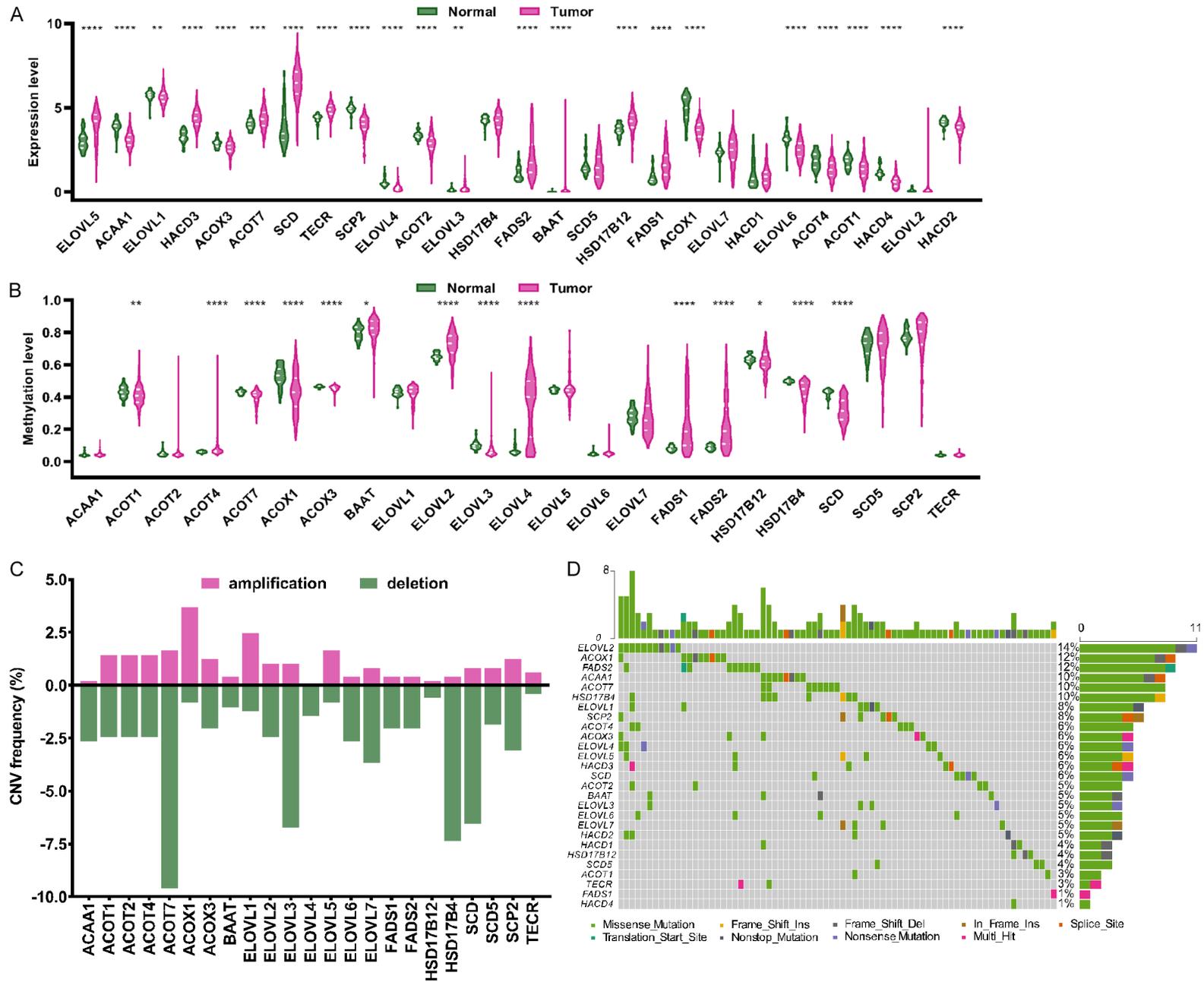
Based on the ssGSEA algorithm, an unsaturated fatty acid biosynthesis-related gene enrichment score (BUFAS) was calculated and assigned to each tumor sample in the TCGA-COAD dataset. To investigate the potential prognostic value of BUFAS, samples were divided into the high- and low-BUFAS groups by the median value for Kaplan-Meier analysis. In the TCGA-COAD cohort, a high BUFAS was associated with longer overall survival (OS, log-rank  $P=0.003$ ; HR, 0.54; 95% CI, 0.35-0.81, **Figure 2A**). To verify the independent prognostic value of the BUFAS, univariable and multivariable analyses were performed on the clinical features, including age, sex, TNM stage, location of the primary tumor, microsatellite status, and consensus molecular subtype (CMS). Patients' age and clinical stage exhibited statistical significance ( $P<0.05$ , **Figure 2B**) in the univariable models and were integrated into the following multivariable model, in which BUFAS remained to be significantly associated with OS (HR, 0.59, 95% CI, 0.38-0.90;  $P=0.01$ , **Figure 2C**), suggesting BUFAS as an independent prognostic marker in colon cancer.

Importantly, similar results were obtained in the validation set (GSE39582). A high BUFAS was associated with longer OS (HR, 0.72, 95% CI, 0.54-0.96;  $P=0.03$ , **Figure 2D**), and, moreover, this prognostic effect remained significant in the multivariable model (HR, 0.73, 95% CI, 0.55-0.98;  $P=0.03$ , **Figure 2E, 2F**), confirming BUFAS as an independent prognostic factor for predicting the OS of colon cancer.

#### *Association between BUFAS and clinical features in COAD*

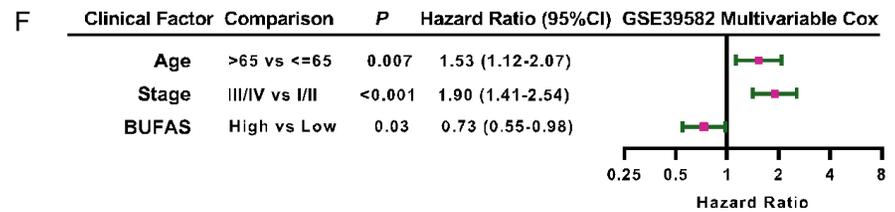
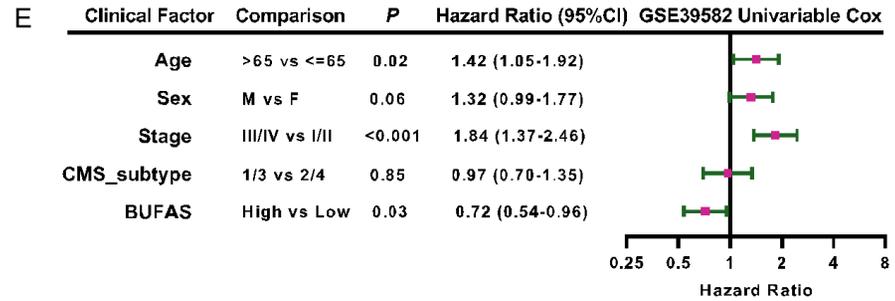
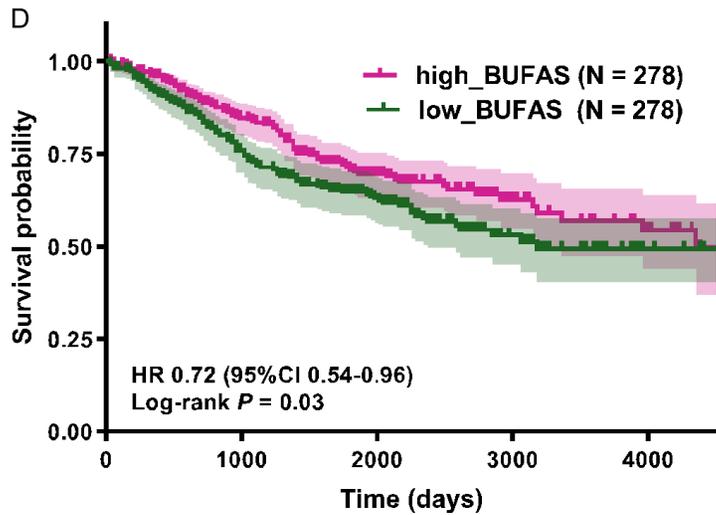
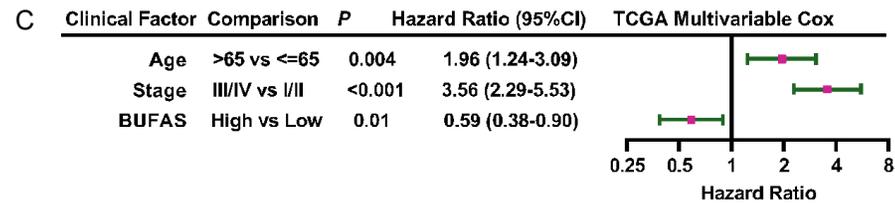
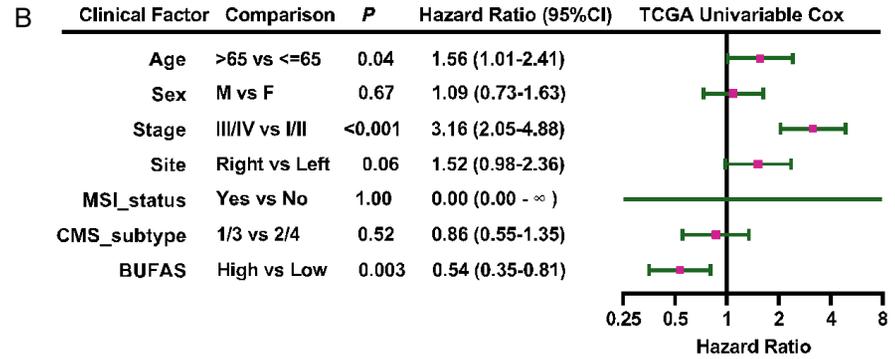
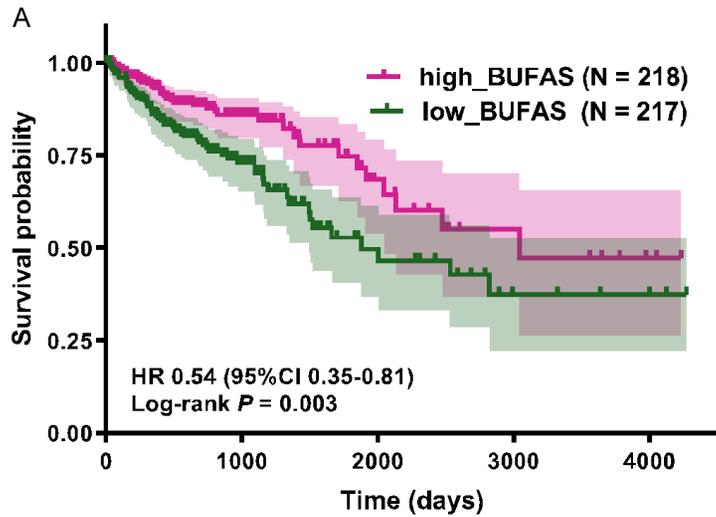
The BUFAS was compared with different clinical features, including age, sex, tumor site, TNM stage, CMS, microsatellite status, *KRAS* muta-

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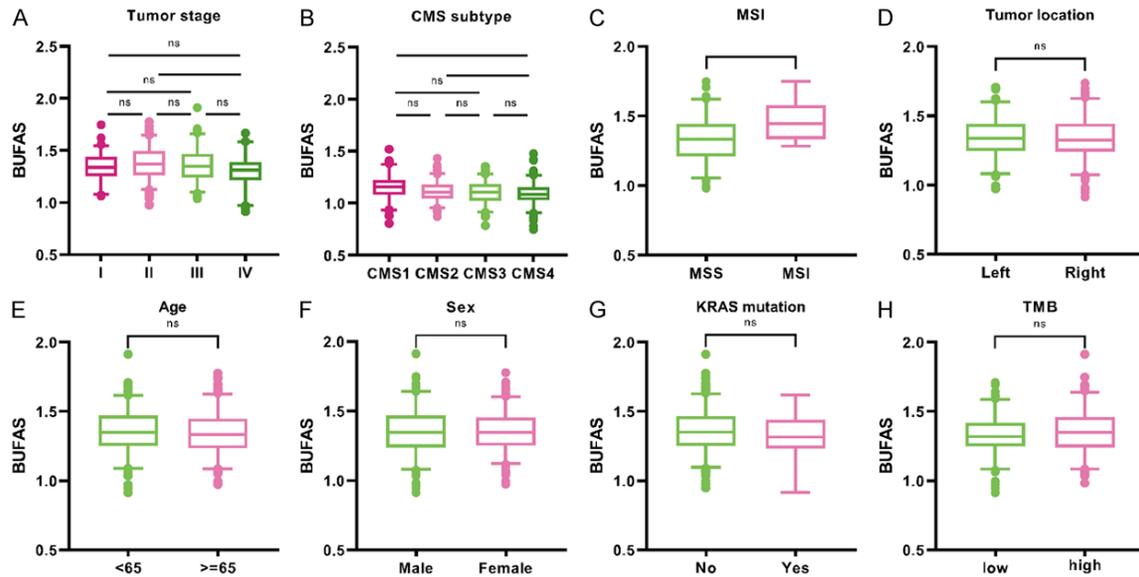
# Unsaturated fatty acid biosynthesis in colon cancer

**Figure 1.** Characterization of unsaturated fatty acid biosynthesis-related genes in the TCGA-COAD cohort. (A, B) Boxplots depicting the expression (A) and methylation status (B) of the genes involved in unsaturated fatty acid biosynthesis. (C, D) Histogram of the copy number variation (CNV) features (C) and oncoprint showing the mutation characteristics (D) of the unsaturated fatty acid biosynthesis-related genes in tumor tissues. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; and \*\*\*\* $P < 0.0001$  by Mann-Whitney test.



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**Figure 2.** Prognostic value of BUFAS in COAD. (A) The overall survival (OS) of colon cancer patients compared between the high- and low-BUFAS groups, with the Kaplan-Meier survival curves showing the longer OS in patients with high-BUFAS (3042 vs. 1881 days). (B, C) The univariable (B) and multivariable (C) analyses of the BUFAS and the clinical characteristics in the TCGA-COAD dataset. (D) The OS of colon cancer patients compared between the high- and low-BUFAS groups, with the Kaplan-Meier survival curves showing the longer OS in patients with high-BUFAS (4350 vs. 3180 days). (E, F) The univariable (E) and multivariable (F) analyses of the BUFAS and clinical characteristics in the GSE39582 dataset. HR, hazard ratio; 95% CI, 95% confidence interval.



**Figure 3.** The association between BUFAS and clinical characteristics. (A-H) Boxplot showing the distribution of BUFAS in in different groups of TNM stages (A), consensus molecular subtypes (CMS, B), microsatellite status (C), tumor sites (D), age (E), sex (F), K-Ras mutations (G), and tumor mutational burden (TMB, H). ns (non-significant)  $P > 0.05$ ,  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ ; and  $****P < 0.0001$  by Wilcoxon test.

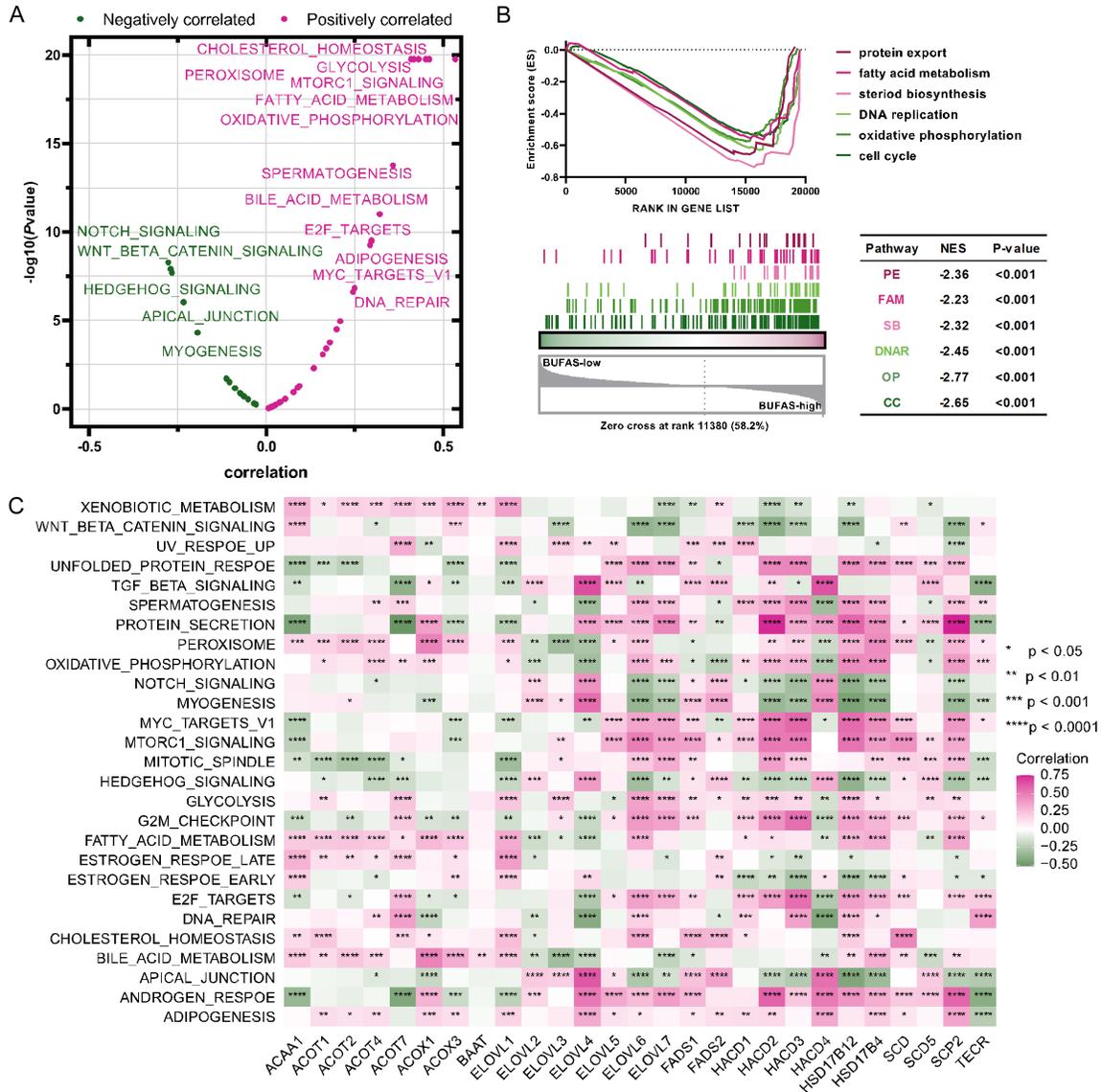
tion status, and tumor mutational burden among patients (TMB, **Figure 3**). A relatively lower BUFAS was observed in the patients with stage IV (**Figure 3A**), CMS4 subtype (**Figure 3B**), and microsatellite stability (**Figure 3C**), while the BUFAS was not associated with tumor sites (**Figure 3D**), age (**Figure 3E**), sex (**Figure 3F**), KRAS mutations (**Figure 3G**), and TMB (**Figure 3H**).

### Functional mechanisms of unsaturated fatty acid biosynthesis

Considering that other cancer-related pathways may be involved in the unsaturated fatty acid biosynthesis, the correlation between the BUFAS and other pathways was determined on the samples in the TCGA-COAD cohort by assigning a group of cancer hallmark ssGSEA scores. We found that the BUFAS was positively associated with MTORC1 signaling, fatty acid metabolism, and peroxisome, while BUFAS was negatively correlated with such signaling

pathways as hedgehog, NOTCH, and Wnt/beta-catenin (**Figure 4A** and [Supplementary Table 2](#)). Besides, all the genes constructing the BUFAS were analyzed. The samples with upregulated expression of *HACD4* and *ELOVL4* showed higher enrichment scores in the pathways of KRAS signaling upregulation, angiogenesis, and TGF-beta signaling, but showed lower enrichment scores in the pathways of DNA repair and MYC targets (**Figure 4B**). These results suggested that *HACD4* and *ELOVL4* might be the core genes in unsaturated fatty acid biosynthesis. In addition, GSEA analysis further revealed the prominent enrichment of signatures in the high-BUFAS group, which were related to oxidative phosphorylation, steroid biosynthesis, fatty acid metabolism, nucleotide excision repair, DNA replication, and mismatch repair (**Figure 4C**). Together, these data suggested the relationship between unsaturated fatty acid synthesis and other metabolism pathways, as well as the pathways related to authentic DNA replication.

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**Figure 4.** Potential cancer hallmarks associated with unsaturated fatty acids biosynthesis. (A) Volcano plot showing the cancer hallmark pathways that were most correlated with BUFAS score. (B) GSEA enrichment of KEGG pathways in the BUFAS-high group. (C) The association of cancer hallmark pathways with the genes involved in unsaturated fatty acid biosynthesis. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; and \*\*\*\* $P < 0.0001$  by spearman correlation.

To explore the potential association between immune response and the BUFAS, the fraction of 22 types of tumor-infiltrating immune cells, which reflected the immune microenvironment of tumor samples, was estimated by utilizing the R package “CIBERSORT”. A lower proportion of Treg cells and higher infiltration levels of activated CD4<sup>+</sup> memory T cells and macrophage M1 were observed in the high-BUFAS groups. The proportions of the other 19 immune cell types were not significantly differed between the high- and the low-BUFAS groups

(Supplementary Figure 1). Furthermore, among the 14 immune checkpoints, only the expression of *TNFRSF9* was upregulated in the high-BUFAS group (Supplementary Figure 2). These findings suggested a weak association between unsaturated fatty acid biosynthesis and immunoregulation.

### Drug sensitivity

To help in drug selection for cancer treatment, the association between the BUFAS and the

drug sensitivity was evaluated using GDSC database. A total of 69 drugs were identified to have significantly different  $IC_{50}$  levels between the low- and high-BUFAS groups. We further analyzed the potential association between the BUFAS score and the  $IC_{50}$  of agents with analogues that are currently used for colon cancer treatment. Significant results were obtained as we found that the BUFAS was negatively associated with the  $IC_{50}$  of several chemotherapeutics, including cisplatin (analogues of oxaliplatin) (**Figure 5A**), gemcitabine (analogue of capecitabine) (**Figure 5B**), and camptothecin (analogue of irinotecan) (**Figure 5C**). In addition, increased sensitivity to Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) such as lapatinib (**Figure 5D**) and afatinib (BIBW2992) (**Figure 5E**) was associated with increased BUFAS, suggesting the beneficial effect of these drugs in patients with higher BUFAS. In contrast, the  $IC_{50}$  of ponatinib (AP.24534) (**Figure 5F**), a tumor angiogenesis inhibitor, exhibited a positive correlation with BUFAS, suggesting a better response to ponatinib in patients with lower BUFAS who had poor overall survival.

Moreover, we investigated the association between  $IC_{50}$  values and the expression of all the unsaturated fatty acid biosynthesis-related genes and identified a significant association between drug sensitivity and the expression of 8 genes: *ACOT7*, *HACD2*, *ELOVL4*, *HACD4*, *ACOX1*, *SCP2*, *HSD17B12*, and *HACD3* ( $P < 0.05$  and  $|\text{correlation}| > 0.5$ ) (**Figure 5G**). These genes might be the potential core genes of unsaturated fatty acid biosynthesis involved in drug response.

### *Dysregulated unsaturated fatty acid biosynthesis*

Since the dysregulation of unsaturated fatty acid biosynthesis plays important roles during oncogenesis, we explored the possible origin of abnormal unsaturated fatty acid biosynthesis by utilizing the single-cell sequencing dataset (GSE146771). Classification of the cell types was conducted based on the annotation information from the TISCH database, and the cells were classified as malignant cells, immune cells, and stromal cells (**Figure 6A**), or presented as 13 detailed subsets (**Figure 6B**). The expression of the unsaturated fatty acid bio-

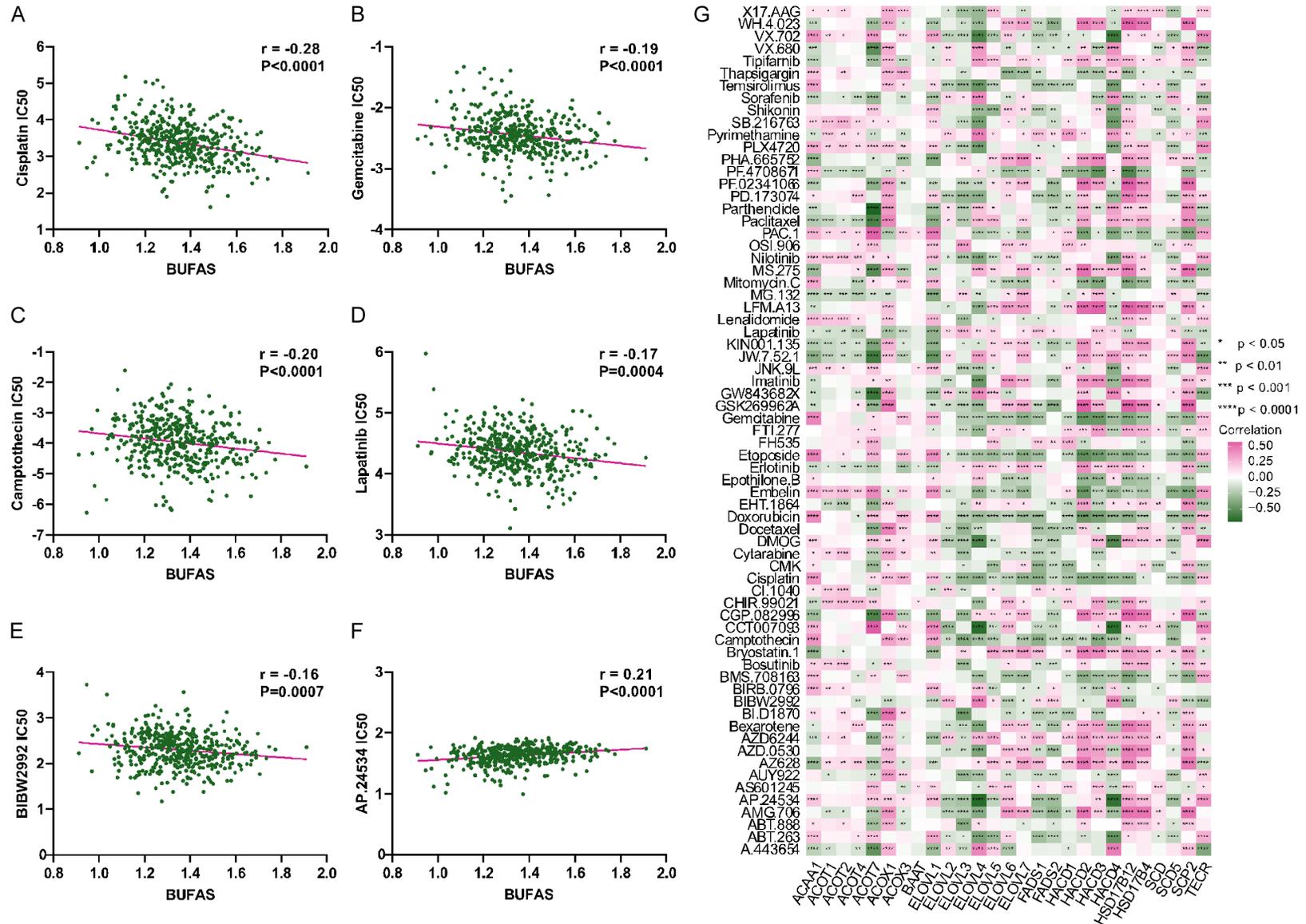
synthesis-related genes was analyzed in these cells, and the BUFAS was assigned to each cell type by ssGSEA (**Figure 6C**). The BUFAS was different among different cell subsets ( $P < 0.001$ ), as the B cells and plasma cells exhibited lower BUFAS distribution, while the BUFAS score was higher in mast cells and fibroblasts (**Figure 6D**). A  $\log_2FC$  value of BUFAS distribution was calculated in each cell subset for enrichment analysis of the score, and a significant enrichment of BUFAS was observed in mast cells and fibroblasts, suggesting that the unsaturated fatty acid biosynthesis alteration might originate from or accumulate on specific cell types (**Figure 6E**).

### Discussion

As early as in the 1920s, the German physiologist Otto Warburg and his colleagues first reported metabolic alterations in tumors [32]. Over the past decade, the idea of cancer cell metabolic reprogramming has become one of the focuses in cancer research [33] and has been considered as one of the hallmarks of cancer by facilitating tumorigenesis [34-36]. In recent years, growing evidence has revealed that the cancer metabolism is complex with heterogeneity and plasticity to facilitate the rapid proliferation of cancer cells [37-39]. In support this, fatty acid metabolism is involved in many aspects of cancer cell biology. Fatty acid metabolism influences the synthesis of lipid building blocks of membrane and the signaling molecules. Fatty acids are also the substrate for ATP and NADH synthesis. Additionally, the acceleration of polysaturated fatty acid (PUFA) can induce ferroptosis in different cancer types [40-42]. All studies suggest that fatty acid synthesis and metabolism have multifaceted roles in cancer [43, 44].

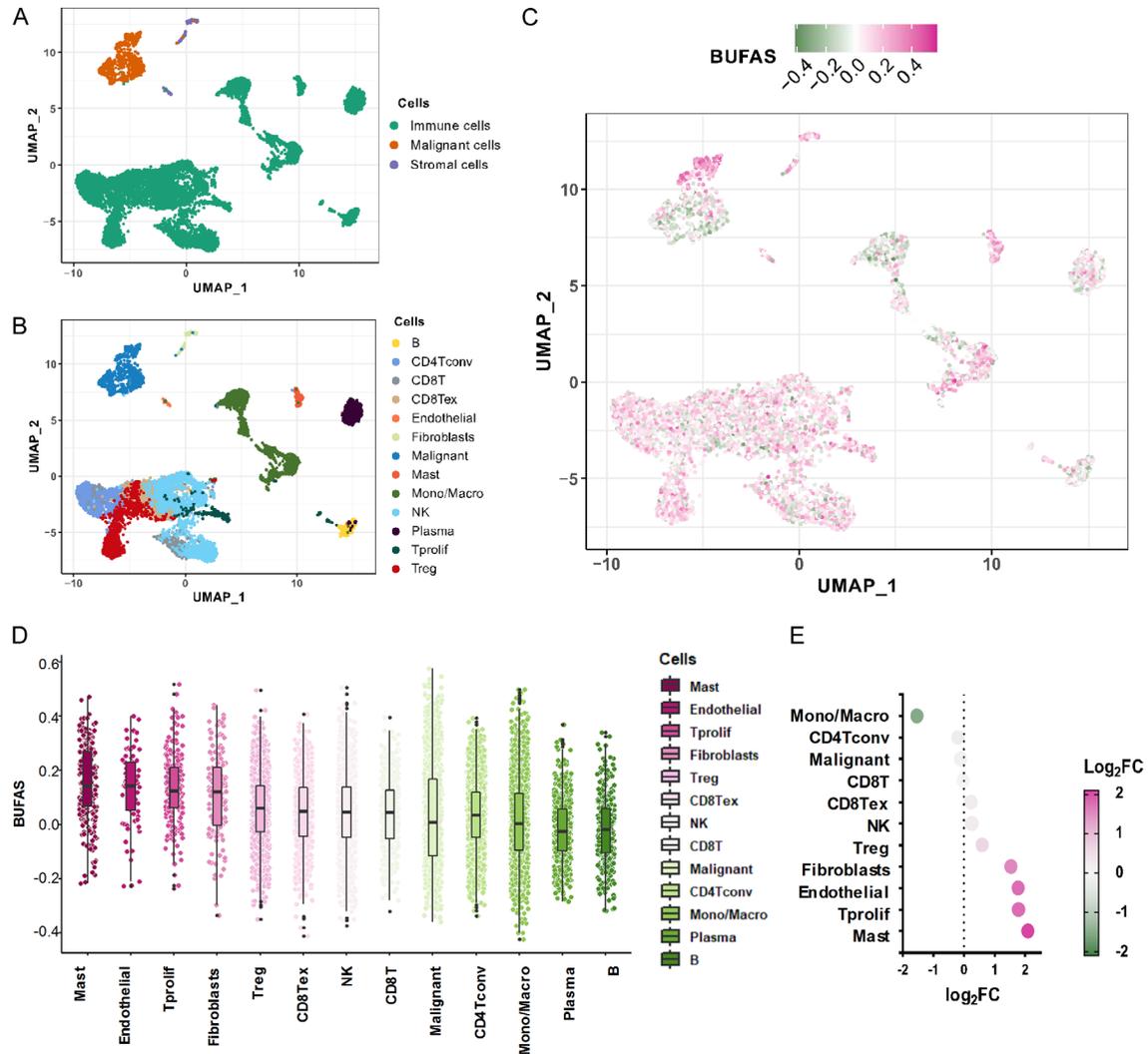
Cancer cells can acquire fatty acids through intracellular and extracellular sources under different circumstances. For example, fatty acid de novo synthesis uses non-lipid substrates to produce palmitate, which can be further converted into unsaturated fatty acids through desaturases and elongases, such as fatty acid desaturase 1 (FADS1) and very long-chain fatty acid protein 5 (ELOVL5). Based on the annotation information in KEGG database [45], a total of 27 genes were identified to be involved in the biosynthesis of unsaturated

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**Figure 5.** Association between BUFAS and drug sensitivity. (A-F) The correlation between BUFAS of cell samples and the estimated IC<sub>50</sub> value of medications, including cisplatin (A), gemcitabine (B), camptothecin (C), lapatinib (D), BIBW2992 (E), and AP.24534 (F). (G) The association of IC<sub>50</sub> value estimated in 69 medications with the genes involved in unsaturated fatty acid production. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; and \*\*\*\* $P < 0.0001$  by spearman correlation.

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**Figure 6.** Biosynthesis of unsaturated fatty acids in single cell cohorts. (A, B) The tumor microenvironment (TMB) in the GSE146771 datasets identified by the single cell sequencing markers and the calculation of uniform manifold approximation and projection (UMAP). The cells are displayed in the malignant-immune-stromal sequencing (A) or more detailed subsets of TMB (B). (C) The BUFAS distribution of each cell sample in the GSE146771 dataset. (D) The Kuskal-Wallis test revealed a varied distribution of BUFAS among 13 cell subgroups. (E) Log<sub>2</sub> fold change (log<sub>2</sub>FC) of BUFAS in cell subsets.

fatty acids, such as in the production of long-chain omega-3 fatty acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA). These genes, including acyl-CoA thioesterase (ACOT) [46], acyl-CoA oxidase (ACOX) [47], and elongation of very-long-chain-fatty acids (ELOVL) [48] gene families, regulate both lipid metabolism and unsaturated fatty acid biosynthesis; however, little is known about the integrated roles of these unsaturated fatty acid biosynthesis-related genes in the initiation and progression of colon cancer. To tackle these questions and further understand the multifaceted roles of

unsaturated fatty acids in colon cancer, in this study, we developed a prognostic model, BUFAS, based on the 27 unsaturated fatty acid biosynthesis-related genes retrieved from the KEGG hsa01040 gene set by ssGSEA algorithm.

To date, this is the first study on developing a prognostic factor based on unsaturated fatty acid biosynthesis-related genes enrichment score in colon cancer. Although there were existing prognostic signatures based on different signaling pathways or biological molecules

in colon cancer [49], most studies focused on immune-related biological functions. For example, a tumor microenvironment risk score (TMRS) was established by LASSO Cox regression in the GEO cohort, which was a robust tool not only for prognostic prediction but also for the tailored therapy in colon cancer [50]. Xu et al. identified 11 key immune-related genes and constructed a prognostic model by random forest algorithm. They further validated their model in an independent validation set and investigated the molecular mechanism of their model in a single-cell sequencing dataset, which proved that their model was accurate and reliable [51].

However, so far, very few prognostic models in colon cancer are based on the fatty acid synthesis or metabolism-related genes. Ding et al. extracted the differentially expressed fatty acid metabolism-related genes between colon cancer and normal tissues. They further developed a prognostic model based on these fatty acid-related genes using LASSO Cox regression analysis [52]. Nevertheless, given the multifaceted roles and different categories of fatty acids, our study particularly focused on the function and prognostic value of unsaturated fatty acid biosynthesis-related genes in colon cancer. Previous studies have revealed the important roles of PUFA in different cancer types. For example, it has been reported that the biosynthesis of arachidonic acid (AA) and adrenic acid (AdA) by ELOVL5 and FADS1 may be a critical checkpoint in the ferroptosis pathway in gastric cancer [40]. This study supports the notion that the PUFA biosynthesis pathway plays an essential role in ferroptosis which may, in turn, predict the efficacy and prognosis after ferroptosis-mediated cancer therapy. In colon cancer, the intake of long-chain omega-3 fatty acid, a type of PUFA, could improve the DFS in patients with high COX2 expression [53]. Therefore, in our study, we constructed our prognostic factor based on the unsaturated fatty acid biosynthesis pathway-related gene enrichment score in the TCGA cohort and validated it in the GSE39582 cohort, which was proved to be an independent prognostic signature for the OS of colon cancer.

Pathway correlation analysis revealed that the unsaturated fatty acid biosynthesis was strongly correlated with some important hallmarks of

cancer, such as the peroxisome pathway and the mTOR complex 1 pathway. Specifically, in peroxisome pathway, Peroxisome Proliferator-Activated Receptor  $\gamma$  (PPAR $\gamma$ ) is a nuclear receptor and is involved in regulating lipid metabolism-related gene expression. Several PUFAs are the natural ligands of this receptor [54, 55]. Importantly, colon cancer patients with PPAR $\gamma$  expressing in the tumor tissues have better overall survival, which is consistent with our prognostic factor and correlation analysis results [56]. Furthermore, PUFA, such as those in fish oil, can activate PPAR $\gamma$ , and their consumption is associated with the prevention of colon cancer [57]. It has been reported that Eicosapentaenoic acid (EPA), one type of PUFA, can inhibit the growth of human colon cancer HT-29 cells, and this process may be associated with the downregulation of PI3K/Akt/mTOR signaling pathway [58]. Several genes in our gene set, such as ELOVL5 and FADS1, were also reported to be associated with ferroptosis in cancer, and PUFA is reported to induce ferroptosis in various cancer cells [59-61]. According to these studies, we further investigated whether our BUFAS was associated with the ssGSEA score of the ferroptosis signaling pathway by using Spearman correlation analysis. We found that our BUFAS was positively associated with the ferroptosis pathway in colon cancer ( $Rho=0.35$ ,  $P<0.001$ , [Supplementary Figure 3](#)).

The immune microenvironment is critically involved in the tumor formation, development, and therapeutic efficacy in various cancer types including colon cancer [62, 63]. Therefore, the association of immune cell infiltration in tumor microenvironment with the BUFAS was evaluated in this study ([Supplementary Figure 1](#)). There was a significantly higher infiltration proportion of activated CD4<sup>+</sup> memory T cells and macrophage M1 in the high-BUFAS group, while the fraction of Treg cells was lower in the high-BUFAS group. Interestingly, a low intraepithelial CD3<sup>+</sup>/FoxP3<sup>+</sup> cell ratio is associated with poor survival in patients with colon cancer, which is consistent with our results [64]. Finally, our analyses into the previous single-cell sequencing dataset indicated that the biosynthesis of the unsaturated fatty acid pathway might intervene in the behaviors and activities of mast cells and macrophages in colon cancer, which is consistent with the findings of

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increased M1 macrophages infiltration in the high-BUFAS group.

There are several limitations in our study. First, we developed our BUFAS prognostic factor via ssGSEA to estimate the overall enrichment score of a certain gene set, which made it difficult to identify key genes of prognostic value. Second, the analyses were mainly conducted under *in silico* circumstances, which needs further experimental validation and clinical validation. Finally, since the signature was constructed in retrospective cohorts, further validation on its performance in prospective studies should be conducted.

## Conclusions

In this study, a novel unsaturated fatty acid biosynthesis-related signature was constructed as an independent prognostic signature in COAD. This signature was correlated with the sensitivity to chemotherapeutic drugs and was identified to originate from mast cells and macrophages. These results may provide rationale for the stratification and therapeutic treatment selection in the management of colon cancer.

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## Disclosure of conflict of interest

None.

## Abbreviations

TCGA, The Cancer Genome Atlas; GEO, Gene Expression 21 Omnibus database; BUFAS, unsaturated fatty acid biosynthesis pathway gene set enrichment score (BUFAS); ssGSEA, single sample gene set enrichment analysis; OS, overall survival; IC50, half maximal inhibitory concentration; CMS, consensus molecular subtypes; NES, normalized enrichment score; FDR, false discovery rate; GDSC, Genomics of Drug Sensitivity in Cancer database; TISCH, Tumor Immune Single-cell Hub database;

UMAP, Uniform manifold approximation and projection; CNV, copy number variations; TMB, tumor mutational burden; EGFR, Epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; VEGFR, vascular endothelial growth factor receptor; PUFA, polysaturated fatty acids; PPAR $\gamma$ , Peroxisome Proliferator Activated Receptor  $\gamma$ .

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## References

- [1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209-249.
- [2] Biller LH and Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. *JAMA* 2021; 325: 669-685.
- [3] Sagaert X, Vanstapel A and Verbeek S. Tumor heterogeneity in colorectal cancer: what do we know so far? *Pathobiology* 2018; 85: 72-84.
- [4] Santos CR and Schulze A. Lipid metabolism in cancer. *FEBS J* 2012; 279: 2610-2623.
- [5] Matsushita Y, Nakagawa H and Koike K. Lipid metabolism in oncology: why it matters, how to research, and how to treat. *Cancers (Basel)* 2021; 13: 474.
- [6] Vriens K, Christen S, Parik S, Broekaert D, Yoshinaga K, Talebi A, Dehairs J, Escalona-Noguerro C, Schmieder R, Cornfield T, Charlton C, Romero-Pérez L, Rossi M, Rinaldi G, Orth MF, Boon R, Kerstens A, Kwan SY, Faubert B, Méndez-Lucas A, Kopitz CC, Chen T, Fernandez-Garcia J, Duarte JAG, Schmitz AA, Steigemann P, Najimi M, Hägebarth A, Van Ginderachter JA, Sokal E, Gotoh N, Wong KK, Verfaillie C, Derua R, Munck S, Yuneva M, Beretta L, DeBerardinis RJ, Swinnen JV, Hodson L, Cassiman D, Verslype C, Christian S, Grünewald S, Grünewald TGP and Fendt SM. Evidence for an alternative fatty acid desaturation pathway increasing cancer plasticity. *Nature* 2019; 566: 403-406.
- [7] Cifková E, Holčápek M, Lída M, Vrána D, Gatěk J and Melichar B. Determination of lipidomic differences between human breast cancer and

## Unsaturated fatty acid biosynthesis in colon cancer

- surrounding normal tissues using HILIC-HPLC/ESI-MS and multivariate data analysis. *Anal Bioanal Chem* 2015; 407: 991-1002.
- [8] Eggers LF, Müller J, Marella C, Scholz V, Watz H, Kugler C, Rabe KF, Goldmann T and Schwudke D. Lipidomes of lung cancer and tumour-free lung tissues reveal distinct molecular signatures for cancer differentiation, age, inflammation, and pulmonary emphysema. *Sci Rep* 2017; 7: 11087.
- [9] Melana JP, Mignolli F, Stoyanoff T, Aguirre MV, Balboa MA, Balsinde J and Rodriguez JP. The hypoxic microenvironment induces stearoyl-CoA desaturase-1 overexpression and lipidomic profile changes in clear cell renal cell carcinoma. *Cancers (Basel)* 2021; 13: 2962.
- [10] Nakagawa H, Hayata Y, Kawamura S, Yamada T, Fujiwara N and Koike K. Lipid metabolic reprogramming in hepatocellular carcinoma. *Cancers (Basel)* 2018; 10: 447.
- [11] Zaytseva YY, Harris JW, Mitov MI, Kim JT, Butterfield DA, Lee EY, Weiss HL, Gao T and Evers BM. Increased expression of fatty acid synthase provides a survival advantage to colorectal cancer cells via upregulation of cellular respiration. *Oncotarget* 2015; 6: 18891-18904.
- [12] Peck B, Schug ZT, Zhang Q, Dankworth B, Jones DT, Smethurst E, Patel R, Mason S, Jiang M, Saunders R, Howell M, Mitter R, Spencer-Dene B, Stamp G, McGarry L, James D, Shanks E, Aboagye EO, Critchlow SE, Leung HY, Harris AL, Wakelam MJO, Gottlieb E and Schulze A. Inhibition of fatty acid desaturation is detrimental to cancer cell survival in metabolically compromised environments. *Cancer Metab* 2016; 4: 6.
- [13] Qi Y, Chen D, Lu Q, Yao Y and Ji C. Bioinformatic profiling identifies a fatty acid metabolism-related gene risk signature for malignancy, prognosis, and immune phenotype of glioma. *Dis Markers* 2019; 2019: 3917040.
- [14] Zhang C, Liao Y, Liu P, Du Q, Liang Y, Ooi S, Qin S, He S, Yao S and Wang W. FABP5 promotes lymph node metastasis in cervical cancer by reprogramming fatty acid metabolism. *Theranostics* 2020; 10: 6561-6580.
- [15] Tabe Y, Konopleva M and Andreeff M. Fatty acid metabolism, bone marrow adipocytes, and AML. *Front Oncol* 2020; 10: 155.
- [16] Chang CL and Deckelbaum RJ. Omega-3 fatty acids: mechanisms underlying 'protective effects' in atherosclerosis. *Curr Opin Lipidol* 2013; 24: 345-350.
- [17] Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr* 2002; 21: 495-505.
- [18] Maskrey BH, Megson IL, Rossi AG and Whitfield PD. Emerging importance of omega-3 fatty acids in the innate immune response: molecular mechanisms and lipidomic strategies for their analysis. *Mol Nutr Food Res* 2013; 57: 1390-1400.
- [19] Bairey O, Shaklai M and Inbal A. Haemarthrosis in patients with mild coagulation factor deficiency. *Blood Coagul Fibrinolysis* 1991; 2: 669-671.
- [20] Gelsomino G, Corsetto PA, Campia I, Montorfano G, Kopecka J, Castella B, Gazzano E, Ghigo D, Rizzo AM and Riganti C. Omega 3 fatty acids chemosensitize multidrug resistant colon cancer cells by down-regulating cholesterol synthesis and altering detergent resistant membranes composition. *Mol Cancer* 2013; 12: 137.
- [21] Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, Sabedot TS, Malta TM, Pagnotta SM, Castiglioni I, Ceccarelli M, Bontempi G and Noushmehr H. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res* 2016; 44: e71.
- [22] Davis S and Meltzer PS. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. *Bioinformatics* 2007; 23: 1846-1847.
- [23] Gautier L, Cope L, Bolstad BM and Irizarry RA. Affy-analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 2004; 20: 307-315.
- [24] Zhang L, Li Z, Skrzypczynska KM, Fang Q, Zhang W, O'Brien SA, He Y, Wang L, Zhang Q, Kim A, Gao R, Orf J, Wang T, Sawant D, Kang J, Bhatt D, Lu D, Li CM, Rapaport AS, Perez K, Ye Y, Wang S, Hu X, Ren X, Ouyang W, Shen Z, Egen JG, Zhang Z and Yu X. Single-cell analyses inform mechanisms of myeloid-targeted therapies in colon cancer. *Cell* 2020; 181: 442-459, e429.
- [25] Mayakonda A, Lin DC, Assenov Y, Plass C and Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res* 2018; 28: 1747-1756.
- [26] Hänzelmann S, Castelo R and Guinney J. GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics* 2013; 14: 7.
- [27] Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, Fröhling S, Chan EM, Sos ML, Michel K, Mermel C, Silver SJ, Weir BA, Reiling JH, Sheng Q, Gupta PB, Wadlow RC, Le H, Horsch S, Wittner BS, Ramaswamy S, Livingston DM, Sabatini DM, Meyerson M, Thomas RK, Lander ES, Mesirov JP, Root DE, Gilliland DG, Jacks T and Hahn WC. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* 2009; 462: 108-112.

## Unsaturated fatty acid biosynthesis in colon cancer

- [28] Eide PW, Bruun J, Lothe RA and Sveen A. CMS-caller: an R package for consensus molecular subtyping of colorectal cancer pre-clinical models. *Sci Rep* 2017; 7: 16618.
- [29] Langfelder P and Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 2008; 9: 559.
- [30] Geeleher P, Cox NJ and Huang RS. Clinical drug response can be predicted using baseline gene expression levels and in vitro drug sensitivity in cell lines. *Genome Biol* 2014; 15: R47.
- [31] Geeleher P, Cox N and Huang RS. pRRophetic: an R package for prediction of clinical chemotherapeutic response from tumor gene expression levels. *PLoS One* 2014; 9: e107468.
- [32] Warburg O, Wind F and Negelein E. The metabolism of tumors in the body. *J Gen Physiol* 1927; 8: 519-530.
- [33] Faubert B, Solmonson A and DeBerardinis RJ. Metabolic reprogramming and cancer progression. *Science* 2020; 368: eaaw5473.
- [34] Pavlova Natalya N and Thompson Craig B. The emerging hallmarks of cancer metabolism. *Cell Metab* 2016; 23: 27-47.
- [35] Zhu J and Thompson CB. Metabolic regulation of cell growth and proliferation. *Nat Rev Mol Cell Biol* 2019; 20: 436-450.
- [36] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646-674.
- [37] Fendt SM, Frezza C and Erez A. Targeting metabolic plasticity and flexibility dynamics for cancer therapy. *Cancer Discov* 2020; 10: 1797-1807.
- [38] Martinez-Reyes I and Chandel NS. Cancer metabolism: looking forward. *Nat Rev Cancer* 2021; 21: 669-680.
- [39] Wu H, Han Y, Rodriguez Sillke Y, Deng H, Siddiqui S, Treese C, Schmidt F, Friedrich M, Keye J, Wan J, Qin Y, Kuhl AA, Qin Z, Siegmund B and Glauben R. Lipid droplet-dependent fatty acid metabolism controls the immune suppressive phenotype of tumor-associated macrophages. *EMBO Mol Med* 2019; 11: e10698.
- [40] Lee JY, Nam M, Son HY, Hyun K, Jang SY, Kim JW, Kim MW, Jung Y, Jang E, Yoon SJ, Kim J, Kim J, Seo J, Min JK, Oh KJ, Han BS, Kim WK, Bae KH, Song J, Kim J, Huh YM, Hwang GS, Lee EW and Lee SC. Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer. *Proc Natl Acad Sci U S A* 2020; 117: 32433-32442.
- [41] Blomme A, Ford CA, Mui E, Patel R, Ntala C, Jamieson LE, Planque M, McGregor GH, Peixoto P, Hervouet E, Nixon C, Salji M, Gaughan L, Markert E, Repiscak P, Sumpton D, Blanco GR, Lilla S, Kamphorst JJ, Graham D, Faulds K, MacKay GM, Fendt SM, Zanivan S and Leung HY. 2,4-dienoyl-CoA reductase regulates lipid homeostasis in treatment-resistant prostate cancer. *Nat Commun* 2020; 11: 2508.
- [42] Perez MA, Magtanong L, Dixon SJ and Watts JL. Dietary lipids induce ferroptosis in *Caenorhabditis elegans* and human cancer cells. *Dev Cell* 2020; 54: 447-454, e444.
- [43] Hoy AJ, Nagarajan SR and Butler LM. Tumour fatty acid metabolism in the context of therapy resistance and obesity. *Nat Rev Cancer* 2021; 21: 753-766.
- [44] Rohrig F and Schulze A. The multifaceted roles of fatty acid synthesis in cancer. *Nat Rev Cancer* 2016; 16: 732-749.
- [45] Kanehisa M, Sato Y, Kawashima M, Furumichi M and Tanabe M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* 2016; 44: D457-462.
- [46] Xie X, Chen C, Feng S, Zuo S, Zhao X and Li H. Acyl-CoA thioesterase 7 is transcriptionally activated by kruppel-like factor 13 and promotes the progression of hepatocellular carcinoma. *J Hepatocell Carcinoma* 2021; 8: 1623-1641.
- [47] Perfiliev A, Dahlman I, Gillberg L, Rosqvist F, Iggman D, Volkov P, Nilsson E, Riserus U and Ling C. Impact of polyunsaturated and saturated fat overfeeding on the DNA-methylation pattern in human adipose tissue: a randomized controlled trial. *Am J Clin Nutr* 2017; 105: 991-1000.
- [48] Ofman R, Dijkstra IM, van Roermund CW, Burger N, Turkenburg M, van Cruchten A, van Engen CE, Wanders RJ and Kemp S. The role of ELOVL1 in very long-chain fatty acid homeostasis and X-linked adrenoleukodystrophy. *EMBO Mol Med* 2010; 2: 90-97.
- [49] Ahluwalia P, Kolhe R and Gahlay GK. The clinical relevance of gene expression based prognostic signatures in colorectal cancer. *Biochim Biophys Acta Rev Cancer* 2021; 1875: 188513.
- [50] Zhou R, Zeng D, Zhang J, Sun H, Wu J, Li N, Liang L, Shi M, Bin J, Liao Y, Huang N and Liao W. A robust panel based on tumour microenvironment genes for prognostic prediction and tailoring therapies in stage I-III colon cancer. *EBioMedicine* 2019; 42: 420-430.
- [51] Xu J, Dai S, Jiang K, Xiao Q, Yuan Y and Ding K. Combining single-cell sequencing to identify key immune genes and construct the prognostic evaluation model for colon cancer patients. *Clin Transl Med* 2021; 11: e465.
- [52] Ding C, Shan Z, Li M, Chen H, Li X and Jin Z. Characterization of the fatty acid metabolism in colorectal cancer to guide clinical therapy. *Mol Ther Oncolytics* 2021; 20: 532-544.
- [53] Blarigan EV, Fuchs CS, Niedzwiecki D, Ye X, Zhang S, Song M, Saltz L, Mayer RJ, Mowat RB, Whittom R, Hantel A, Benson AB, Atienza DM, Messino MJ, Kindler HL, Venook AP, Ogino S, Willett WC, Giovannucci EL and Meyerhardt JA.

## Unsaturated fatty acid biosynthesis in colon cancer

- Long-chain omega-3 fatty acid and fish intake after colon cancer diagnosis and disease-free, recurrence-free, and overall survival in CALGB 89803 (Alliance). 2017; 35: 585.
- [54] Koeffler HP. Peroxisome proliferator-activated receptor  $\gamma$  and cancers. *Clin Cancer Res* 2003; 9: 1.
- [55] Forman BM, Chen J and Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci U S A* 1997; 94: 4312-4317.
- [56] Ogino S, Shima K, Baba Y, Nosho K, Irahara N, Kure S, Chen L, Toyoda S, Kirkner GJ, Wang YL, Giovannucci EL and Fuchs CS. Colorectal cancer expression of peroxisome proliferator-activated receptor gamma (PPARG, PPARgamma) is associated with good prognosis. *Gastroenterology* 2009; 136: 1242-1250.
- [57] Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM and Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U S A* 1997; 94: 4318-4323.
- [58] Tang FY, Cho HJ, Pai MH and Chen YH. Concomitant supplementation of lycopene and eicosapentaenoic acid inhibits the proliferation of human colon cancer cells. *J Nutr Biochem* 2009; 20: 426-434.
- [59] Dierge E, Debock E, Guilbaud C, Corbet C, Mignolet E, Mignard L, Bastien E, Dessy C, Larondelle Y and Feron O. Peroxidation of n-3 and n-6 polyunsaturated fatty acids in the acidic tumor environment leads to ferroptosis-mediated anticancer effects. *Cell Metab* 2021; 33: 1701-1715, e1705.
- [60] Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE, Noel K, Jiang X, Linkermann A, Murphy ME, Overholtzer M, Oyagi A, Pagnussat GC, Park J, Ran Q, Rosenfeld CS, Salnikow K, Tang D, Torti FM, Torti SV, Toyokuni S, Woerpel KA and Zhang DD. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 2017; 171: 273-285.
- [61] Zou Y, Henry WS, Ricq EL, Graham ET, Phadnis VV, Maretich P, Paradkar S, Boehnke N, Deik AA, Reinhardt F, Eaton JK, Ferguson B, Wang W, Fairman J, Keys HR, Dančík V, Clish CB, Clemons PA, Hammond PT, Boyer LA, Weinberg RA and Schreiber SL. Plasticity of ether lipids promotes ferroptosis susceptibility and evasion. *Nature* 2020; 585: 603-608.
- [62] Schmitt M and Greten FR. The inflammatory pathogenesis of colorectal cancer. *Nat Rev Immunol* 2021; 21: 653-667.
- [63] Ganesh K, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH and Diaz LA Jr. Immunotherapy in colorectal cancer: rationale, challenges and potential. *Nat Rev Gastroenterol Hepatol* 2019; 16: 361-375.
- [64] Sinicrope FA, Rego RL, Ansell SM, Knutson KL, Foster NR and Sargent DJ. Intraepithelial effector (CD3+)/regulatory (FoxP3+) T-cell ratio predicts a clinical outcome of human colon carcinoma. *Gastroenterology* 2009; 137: 1270-1279.

## Supplemental Materials

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**Supplementary Table 1.** The 27 unsaturated fatty acid biosynthesis-related genes used for the development of BUFAS

Gene	Description	Entrez Gene Summary
<i>ACOT2</i>	acyl-CoA thioesterase 2	<i>ACOT2</i> encodes a member of the acyl-CoA thioesterase protein family, and is one of four acyl-CoA hydrolase genes located in a cluster on chromosome 14.
<i>ACOT7</i>	acyl-CoA thioesterase 7	<i>ACOT7</i> encodes a member of the acyl coenzyme family. The encoded protein hydrolyzes the CoA thioester of palmitoyl-CoA and other long-chain fatty acids.
<i>ACOT4</i>	acyl-CoA thioesterase 4	Enables acyl-CoA hydrolase activity and succinyl-CoA hydrolase activity. Involved in carboxylic acid metabolic process; saturated monocarboxylic acid metabolic process; and succinyl-CoA metabolic process.
<i>HACD2</i>	3-hydroxyacyl-CoA dehydratase 2	The protein encoded by <i>HACD2</i> can catalyze the third step (dehydration) in the conversion of long chain fatty acids to very long chain fatty acids.
<i>ACAA1</i>	acetyl-CoA acyltransferase 1	<i>ACAA1</i> encodes an enzyme operative in the beta-oxidation system of the peroxisomes. Deficiency of this enzyme leads to pseudo-Zellweger syndrome.
<i>HSD17B4</i>	hydroxysteroid 17-beta dehydrogenase 4	The protein encoded by <i>HSD17B4</i> is a bifunctional enzyme that is involved in the peroxisomal beta-oxidation pathway for fatty acids. It also acts as a catalyst for the formation of 3-ketoacyl-CoA intermediates from both straight-chain and 2-methyl- branched-chain fatty acids.
<i>FADS1</i>	fatty acid desaturase 1	The protein encoded by <i>FADS1</i> is a member of the fatty acid desaturase (FADS) gene family. Desaturase enzymes regulate unsaturation of fatty acids through the introduction of double bonds between defined carbons of the fatty acyl chain.
<i>HACD4</i>	3-hydroxyacyl-CoA dehydratase 4	Enables 3-hydroxyacyl-CoA dehydratase activity and enzyme binding activity. Involved in fatty acid elongation and very long-chain fatty acid biosynthetic process.
<i>ACOX1</i>	acyl-CoA oxidase 1	The protein encoded by <i>ACOX1</i> is the first enzyme of the fatty acid beta-oxidation pathway, which catalyzes the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs. It donates electrons directly to molecular oxygen, thereby producing hydrogen peroxide.
<i>HSD17B12</i>	hydroxysteroid 17-beta dehydrogenase 12	<i>HSD17B12</i> encodes a very important 17beta-hydroxysteroid dehydrogenase (17beta-HSD) that converts estrone into estradiol in ovarian tissue. This enzyme is also involved in fatty acid elongation.
<i>HACD3</i>	3-hydroxyacyl-CoA dehydratase 3	Enables 3-hydroxyacyl-CoA dehydratase activity and enzyme binding activity. Involved in fatty acid biosynthetic process; positive regulation by virus of viral protein levels in host cell; and positive regulation of viral genome replication.
<i>ELOVL2</i>	ELOVL fatty acid elongase 2	Enables fatty acid elongase activity. Involved in fatty acid elongation, polyunsaturated fatty acid and very long-chain fatty acid biosynthetic process.
<i>BAAT</i>	bile acid-CoA: amino acid N-acyltransferase	The protein encoded by <i>BAAT</i> is a liver enzyme that catalyzes the transfer of C24 bile acids from the acyl-CoA thioester to either glycine or taurine, the second step in the formation of bile acid-amino acid conjugates. The bile acid conjugates then act as a detergent in the gastrointestinal tract, which enhances lipid and fat-soluble vitamin absorption.
<i>ELOVL5</i>	ELOVL fatty acid elongase 5	<i>ELOVL5</i> belongs to the <i>ELO</i> family. It is highly expressed in the adrenal gland and testis, and encodes a multi-pass membrane protein that is localized in the endoplasmic reticulum. This protein is involved in the elongation of long-chain polyunsaturated fatty acids.

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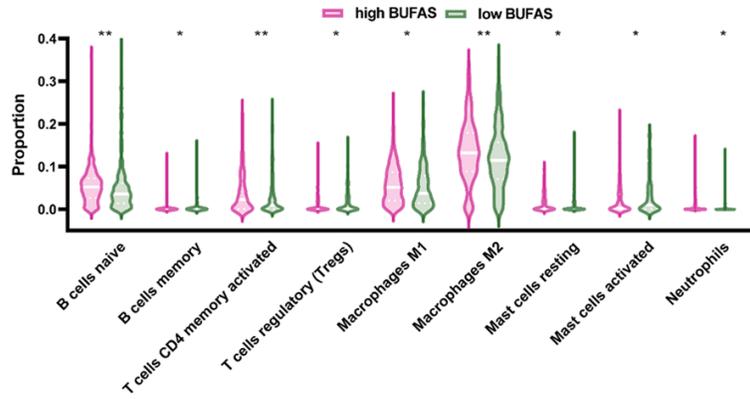
<i>SCD</i>	stearoyl-CoA desaturase	<i>SCD</i> encodes an enzyme involved in fatty acid biosynthesis, primarily the synthesis of oleic acid. The protein belongs to the fatty acid desaturase family and is an integral membrane protein located in the endoplasmic reticulum.
<i>SCP2</i>	sterol carrier protein 2	<i>SCP2</i> encodes two proteins : sterol carrier protein X (SCPx) and sterol carrier protein 2 (SCP2), as a result of transcription initiation from 2 independently regulated promoters. The SCPx protein is a peroxisome-associated thiolase that is involved in the oxidation of branched chain fatty acids, while the SCP2 protein is thought to be an intracellular lipid transfer protein.
<i>ACOT1</i>	acyl-CoA thioesterase 1	Enables acyl-CoA hydrolase activity. Involved in acyl-CoA metabolic process; long-chain fatty acid metabolic process; and very long-chain fatty acid metabolic process.
<i>ELOVL1</i>	ELOVL fatty acid elongase 1	Enables fatty acid elongase activity. Involved in fatty acid biosynthetic process and sphingolipid biosynthetic process.
<i>ELOVL4</i>	ELOVL fatty acid elongase 4	<i>ELOVL4</i> encodes a membrane-bound protein which is a member of the ELO family, proteins which participate in the biosynthesis of fatty acids.
<i>ELOVL6</i>	ELOVL fatty acid elongase 6	<i>ELOVL6</i> encodes a fatty acid elongases, which uses malonyl-CoA as a 2-carbon donor in the first and rate-limiting step of fatty acid elongation.
<i>SCD5</i>	stearoyl-CoA desaturase 5	Stearoyl-CoA desaturase ( <i>SCD</i> ; EC 1.14.99.5) is an integral membrane protein of the endoplasmic reticulum that catalyzes the formation of monounsaturated fatty acids from saturated fatty acids. <i>SCD</i> may be a key regulator of energy metabolism with a role in obesity and dyslipidemia.
<i>ELOVL7</i>	ELOVL fatty acid elongase 7	Enables fatty acid elongase activity. Involved in fatty acid elongation, polyunsaturated fatty acid; fatty acid elongation, saturated fatty acid; and very long-chain fatty acid biosynthetic process.
<i>ACOX3</i>	acyl-CoA oxidase 3, pristanoyl	Acyl-Coenzyme A oxidase 3 also known as pristanoyl-CoA oxidase ( <i>ACOX3</i> ) is involved in the desaturation of 2-methyl branched fatty acids in peroxisomes.
<i>ELOVL3</i>	ELOVL fatty acid elongase 3	<i>ELOVL3</i> encodes a protein that belongs to the GNS1/SUR4 family. Members of this family play a role in elongation of long chain fatty acids to provide precursors for synthesis of sphingolipids and ceramides.
<i>HACD1</i>	3-hydroxyacyl-CoA dehydratase	The protein encoded by <i>HACD1</i> contains a characteristic catalytic motif of the protein tyrosine phosphatases (PTPs) family. The PTP motif of this protein has the highly conserved arginine residue replaced by a proline residue; thus it may represent a distinct class of PTPs.
<i>FADS2</i>	fatty acid desaturase 2	The protein encoded by <i>FADS2</i> is a member of the fatty acid desaturase ( <i>FADS</i> ) gene family. Desaturase enzymes regulate unsaturation of fatty acids through the introduction of double bonds between defined carbons of the fatty acyl chain.
<i>TECR</i>	trans-2,3-enoyl-CoA reductase	<i>TECR</i> encodes a multi-pass membrane protein that resides in the endoplasmic reticulum, and belongs to the steroid 5-alpha reductase family. The elongation of microsomal long and very long chain fatty acid consists of 4 sequential reactions. This protein catalyzes the final step, reducing trans-2,3-enoyl-CoA to saturated acyl-CoA.

## Unsaturated fatty acid biosynthesis in colon cancer

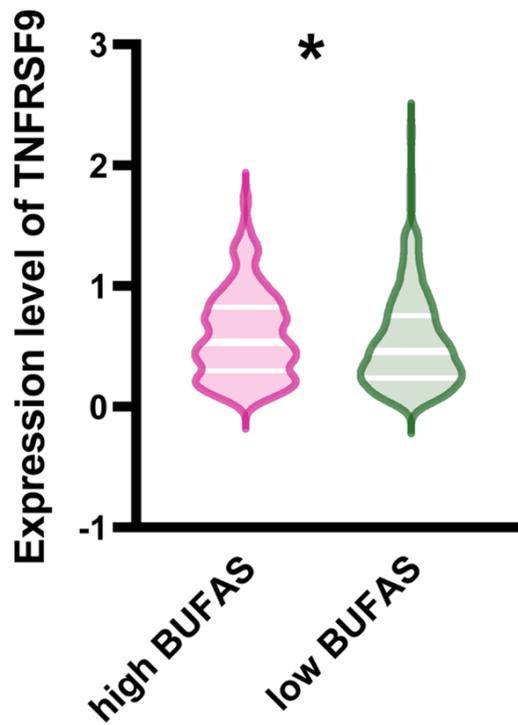
**Supplementary Table 2.** Cancer hallmark pathways correlated to BUFAS

Cancer hallmarks	cor	p value	correlation	-LOG10 (P value)
HALLMARK_HEDGEHOG_SIGNALING	-0.27647	5.23E-09	negative	8.281141
HALLMARK_NOTCH_SIGNALING	-0.27001	1.21E-08	negative	7.918701
HALLMARK_WNT_BETA_CATENIN_SIGNALING	-0.26602	2.00E-08	negative	7.699414
HALLMARK_APICAL_JUNCTION	-0.23333	9.28E-07	negative	6.032497
HALLMARK_MYOGENESIS	-0.19374	4.91E-05	negative	4.309354
HALLMARK_TGF_BETA_SIGNALING	-0.11231	0.019162	negative	1.717565
HALLMARK_ESTROGEN_RESPONSE_EARLY	-0.11076	0.020899	negative	1.679868
HALLMARK_MITOTIC_SPINDELE	-0.10342	0.031085	negative	1.507444
HALLMARK_KRAS_SIGNALING_DN	-0.08805	0.066574	negative	1.176695
HALLMARK_P53_PATHWAY	-0.0745	0.120744	negative	0.918136
HALLMARK_UV_RESPONSE_DN	-0.07129	0.137625	negative	0.861304
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	-0.06295	0.189985	negative	0.72128
HALLMARK_COAGULATION	-0.06169	0.198984	negative	0.701183
HALLMARK_APICAL_SURFACE	-0.05114	0.287142	negative	0.541903
HALLMARK_TNFA_SIGNALING_VIA_NFKB	-0.03385	0.481216	negative	0.31766
HALLMARK_ANGIOGENESIS	-0.02844	0.554013	negative	0.25648
HALLMARK_PANCREAS_BETA_CELLS	0.006758	0.888176	positive	0.051501
HALLMARK_IL6_JAK_STAT3_SIGNALING	0.013085	0.785436	positive	0.104889
HALLMARK_KRAS_SIGNALING_UP	0.01532	0.749917	positive	0.124987
HALLMARK_IL2_STAT5_SIGNALING	0.016989	0.723729	positive	0.140424
HALLMARK_INTERFERON_ALPHA_RESPONSE	0.020697	0.66673	positive	0.17605
HALLMARK_HYPOXIA	0.024337	0.612575	positive	0.212841
HALLMARK_ALLOGRAFT_REJECTION	0.025787	0.591558	positive	0.228003
HALLMARK_INTERFERON_GAMMA_RESPONSE	0.037744	0.432174	positive	0.364341
HALLMARK_PI3K_AKT_MTOR_SIGNALING	0.041463	0.388171	positive	0.410977
HALLMARK_INFLAMMATORY_RESPONSE	0.041487	0.387896	positive	0.411285
HALLMARK_COMPLEMENT	0.053466	0.265714	positive	0.575586
HALLMARK_APOPTOSIS	0.053841	0.262374	positive	0.58108
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	0.076976	0.108865	positive	0.963112
HALLMARK_HEME_METABOLISM	0.089306	0.062764	positive	1.202292
HALLMARK_MYC_TARGETS_V2	0.093967	0.050195	positive	1.29934
HALLMARK_ESTROGEN_RESPONSE_LATE	0.134446	0.005002	positive	2.300828
HALLMARK_PROTEIN_SECRETION	0.159905	0.000828	positive	3.081943
HALLMARK_UV_RESPONSE_UP	0.16974	0.000384	positive	3.416106
HALLMARK_XENOBIOTIC_METABOLISM	0.179361	0.000173	positive	3.760853
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.198653	3.12E-05	positive	4.50617
HALLMARK_ANDROGEN_RESPONSE	0.209391	1.11E-05	positive	4.952792
HALLMARK_G2M_CHECKPOINT	0.245032	2.49E-07	positive	6.603092
HALLMARK_DNA_REPAIR	0.249421	1.50E-07	positive	6.824605
HALLMARK_MYC_TARGETS_V1	0.293111	5.53E-10	positive	9.257123
HALLMARK_ADIPOGENESIS	0.296762	3.31E-10	positive	9.479776
HALLMARK_E2F_TARGETS	0.297565	2.96E-10	positive	9.529161
HALLMARK_BILE_ACID_METABOLISM	0.320582	9.75E-12	positive	11.01104
HALLMARK_SPERMATOGENESIS	0.358116	1.70E-14	positive	13.76922
HALLMARK_GLYCOLYSIS	0.408606	1.70E-20	positive	19.76922
HALLMARK_PEROXISOME	0.417655	1.70E-20	positive	19.76922
HALLMARK_OXIDATIVE_PHOSPHORYLATION	0.430355	1.70E-20	positive	19.76922
HALLMARK_FATTY_ACID_METABOLISM	0.450569	1.70E-20	positive	19.76922
HALLMARK_CHOLESTEROL_HOMEOSTASIS	0.460148	1.70E-20	positive	19.76922
HALLMARK_MTORC1_SIGNALING	0.533759	1.70E-20	positive	19.76922

## Unsaturated fatty acid biosynthesis in colon cancer

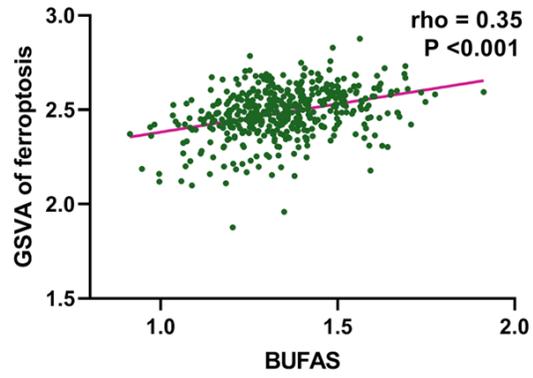


**Supplementary Figure 1.** Comparison of the immune infiltration cells between the high- and low-BUFAS groups in the TCGA-COAD cohort. Violin plot showing the proportion of immune infiltration cells between the high- and low-BUFAS groups in the TCGA-COAD cohort. \* $P < 0.05$  and \*\* $P < 0.01$  by Wilcoxon test.



**Supplementary Figure 2.** Comparison of the TNFRSF9 expression between the high- and low-BUFAS groups in the TCGA-COAD cohort. Violin plot showing the *TNFRSF9* expression between the high- and low-BUFAS groups in the TCGA-COAD cohort. \* $P < 0.05$ .

## Unsaturated fatty acid biosynthesis in colon cancer



**Supplementary Figure 3.** Correlation between the BUFAS and ferroptosis. The correlation between the BUFAS and ferroptosis by the Spearman correlation analysis.