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RESEARCH

# GUCA2A dysregulation as a promising biomarker for accurate diagnosis and prognosis of colorectal cancer

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## Abstract

Colorectal cancer is a leading cause of global mortality and presents a significant barrier to improving life expectancy. The primary objective of this study was to discern a unique differentially expressed gene (DEG) that exhibits a strong association with colorectal cancer. By achieving this goal, the research aims to contribute valuable insights to the field of translational medicine. We performed analysis of colorectal cancer microarray and the TCGA colon adenoma carcinoma (COAD) datasets to identify DEGs associated with COAD and common DEGs were selected. Furthermore, a pan-cancer analysis encompassing 33 different cancer types was performed to identify differential genes significantly expressed only in COAD. Then, comprehensively in-silico analysis including gene set enrichment analysis, constructing Protein–Protein interaction, co-expression, and competing endogenous RNA (ceRNA) networks, investigating the correlation between tumor-immune signatures in distinct tumor microenvironment and also the potential interactions between the identified gene and various drugs was executed. Further, the candidate gene was experimentally validated in tumoral colorectal tissues and colorectal adenomatous polyps by qRael-Time PCR. *GUCA2A* emerged as a significant DEG specific to colorectal cancer (|log2FC|> 1 and adjusted q-value < 0.05). Importantly, *GUCA2A* exhibited excellent diagnostic performance for COAD, with a 99.6% and 78% area under the curve (AUC) based on TCGA-COAD and colon cancer patients. In addition, *GUCA2A* expression in adenomatous polyps equal to or larger than 5 mm was significantly lower compared to smaller than 5 mm. Moreover, low expression of *GUCA2A* significantly impacted overall patient survival. Significant correlations were observed between tumor-immune signatures and *GUCA2A* expression. The ceRNA constructed included *GUCA2A*, 8 shared miRNAs, and 61 circRNAs. This study identifies *GUCA2A* as a promising prognostic and diagnostic biomarker for colorectal cancer. Further investigations are warranted to explore the potential of *GUCA2A* as a therapeutic biomarker.

**Keywords** Colorectal cancer · GUCA2A · Prognostic biomarker · Diagnostic biomarker · CeRNA network · Integrated bioinformatics analysis

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**Abbreviations**

| | |
|---|---|
| Act-B cell | Activated B cell |
| AUC | Area under the ROC curve |
| BMI | Body mass index |
| BP | Biological process |
| CC | Cellular component |
| Ce-RNA | Competing endogenous RNA |
| COAD | Colon adenocarcinoma |
| CRC | Colorectal cancer |
| DEG | Differentially expressed genes |
| FC | Fold Change |
| GO | Gene ontology |
| GTEx | Genotype-tissue expression |
| GUCA2A | Guanylate cyclase activator 2A |
| HPA | Human protein atlas |
| IHC | Immunohistochemistry |
| KEGG | Kyoto encyclopedia of genes and genomes |
| MF | Molecular function |
| NGS | Next-generation sequencing |
| NK | Natural killer cells |
| OS | Overall survival |
| PPI | Protein–protein interaction |
| RMA | Robust multi-array average |
| RT-qPCR | Reverse transcription polymerase chain reaction |
| TCGA | The cancer genome atlas |
| Tcm_CD4 | Central memory CD4 + T cell |
| Tem-CD4 | Effector memory CD4 + T cell |
| Th2 | Type 2 T helper cell |
| Th17 | Type 17 T helper cell |
| TIME | Tumor immune microenvironment |

## Introduction

Cancer is a leading cause of mortality worldwide and poses a substantial challenge to increasing life expectancy [1]. Among the various types of cancer, colorectal cancer (CRC) ranks as one of the most prevalent globally. Recent epidemiological statistics indicate that CRC constitutes 10.2% of all malignant tumors, making it the third most common cancer, with the number of deaths accounting for 9.2%, ranking second [2, 3]. The incidence of CRC continues to rise, necessitating a deeper understanding of its molecular basis.

The development and progression of colon cancer involve complex interactions among multiple genes and molecular alterations in somatic cell genomes [4]. In recent decades, extensive data mining analyses have been conducted on various human cancers, including mRNA, microRNA, long noncoding RNA, and DNA methylation studies [5–8]. The identification of diagnostic and prognostic biomarkers in cancer has become increasingly important, aided by advancements in sequencing technologies and bioinformatics tools [9–11]. Consequently, the identification of additional potential biomarkers related to colon cancer progression expands the options for diagnosis and treatment.

Several studies have utilized quantitative reverse transcription polymerase chain reaction (RT-qPCR) and gene microarray profiling to identify genes associated with recurrence risk and prognosis in colon cancer patients [12, 13]. Gene microarray profiling, a high-throughput method for assessing mRNA expression in tissues, has emerged as a promising tool in medical oncology [14]. It allows for the analysis of differential gene expression between tumor tissues and normal control tissues, providing insights into the molecular pathogenesis of various cancer types and facilitating the identification of potential target genes and signaling pathways for precision therapy [15]. Previous studies utilizing microarray technology have examined gene expression profiles in CRC and identified differentially expressed genes (DEGs) [16, 17]. Numerous gene expression datasets for CRC are available in the Gene Expression Omnibus (GEO) database, which have been utilized to identify DEGs in CRC [18–21]. However, individual studies have reported inconsistent results due to variations in sample collection, platform types, and analysis methods Additionally, large-scale studies evaluating the prognostic value of DEGs in CRC are lacking [22]. In recent years, RNA-seq analysis based on next-generation sequencing (NGS) has become the gold standard for whole transcriptome gene expression analysis. This approach enables the generation of mechanistic hypotheses regarding molecular events in cells and tissues [23]. Databases such as The Cancer Genome Atlas (TCGA) provide RNA-Seq-based transcriptome data for various cancer types, including primary cancer and matched normal samples, offering valuable resources for comprehensive analyses.

Here, we aimed to identify a gene differentially expressed just in colon adenocarcinoma. We employed GEO datasets and validated our findings using the TCGA COAD dataset to determine DEGs in CRC compared to noncancerous tissues. Subsequently, pan-cancer analysis was performed to identify colon adenocarcinoma-specific DEGs. Our investigation led to the identification of *GUCA2A* as a specific diagnostic and prognostic marker for COAD. In addition, we investigated *GUCA2A* gene expression in CRC tissues and adenomatous polyps. *GUCA2A* gene exerts a significant influence on the activity of infiltrating lymphocytes, holding promising clinical implications for the diagnosis, treatment, and prognosis prediction of colon adenocarcinoma.

## Materials and methods

To gain insights into the expression, regulation, interactions, and potential therapeutic implications of a unique differentially expressed gene in COAD, a series of analysis were employed (Fig. 1): **(1)** Identification of Differentially

Expressed Genes (DEGs): Four microarray datasets were analyzed, and DEGs were identified. Intersection analysis with the GEPIA2 database led to the identification of 88 common DEGs; **(2)** Differential Expression Analysis of *GUCA2A*: Using the GEPIA2, OncoDB, and cBioPortal databases, we analyzed the expression levels of *GUCA2A* in COAD. This analysis provided valuable information on the transcriptional activity and differential expression of *GUCA2A* in COAD samples; **(3)** Analysis of Methylation and Genetic Alterations: The methylation status and genetic alterations of *GUCA2A* were explored using available data from the GEPIA2, OncoDB, and cBioPortal databases. This analysis provided insights into potential epigenetic and genomic regulatory mechanisms associated with *GUCA2A* in COAD; **(4)** Co-expression Analysis and Protein–Protein Interaction (PPI) Networks: Co-expression genes and protein–protein interaction networks associated with *GUCA2A* were investigated using the LinkedOmics and STRING databases, respectively. These analyses allowed us to explore potential functional associations and interactions between *GUCA2A* and other genes in COAD; **(5)** Pathway Enrichment Analysis: The Enrichr database was utilized to perform pathway enrichment analysis on *GUCA2A* and its co-expression genes. This analysis provided a comprehensive understanding of the biological processes and pathways in which *GUCA2A* may be involved; **(6)** Correlation Analysis of Tumor-Immune Signatures: The TISIDB database was used to explore the correlation between tumor-immune signatures in different tumor microenvironments and *GUCA2A* expression. This analysis provided insights into the potential interactions between *GUCA2A* and the tumor immune response in COAD; **(7)** Identification of miRNAs and ceRNAs: Using the miRDB, miRWalk, TargetScan, and circBank databases, we identified miRNAs targeting *GUCA2A* and their corresponding competing endogenous RNAs (ceRNAs). This analysis revealed the regulatory network involving *GUCA2A*, miRNAs, and circRNAs; **(8)** Examination of *GUCA2A*-Interacted Drugs: The DGIdb database was explored to identify drugs that interact with *GUCA2A*, potentially offering therapeutic implications for COAD; and **(9)** *GUCA2A* gene expression was experimentally investigated in tumor/adjacent normal tissue and colorectal adenomatous polyps/normal adjacent tissues.

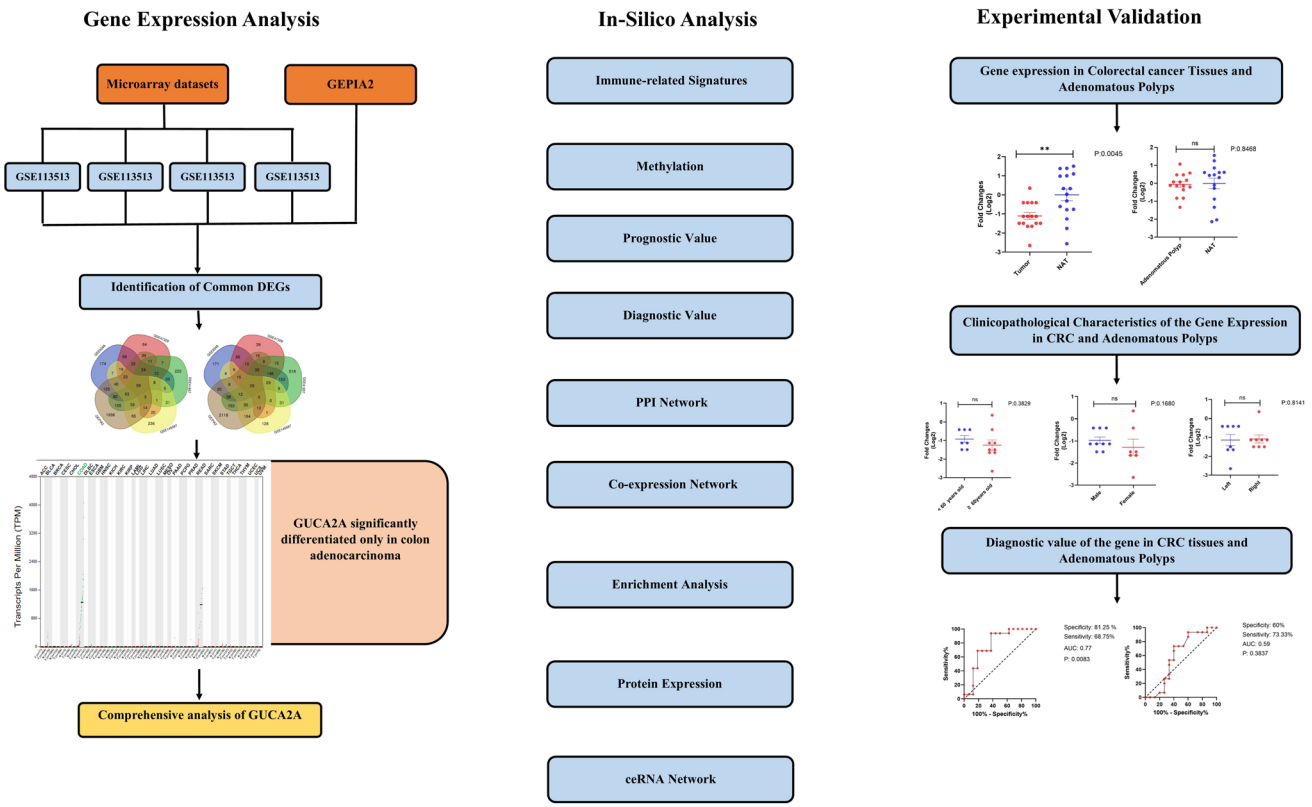



**Fig. 1** Study Workflow for Comprehensive Analysis of *GUCA2A* in colorectal and cancer. After pan cancer analyzing of 88 common DEGs between 4 microarray datasets and GEPIA2 database, *GUCA2A* was found as a gene which is significantly differentiated only in COAD. Then, via several bioinformatics databases and experimentally validation we comprehensively and systematically studied the roles of *GUCA2A* in colorectal cancer and polyps

## Bioinformatics analysis

### Microarray datasets and identification of DEGs

To acquire the gene expression datasets of CRC, the microarray data were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/https://www.ncbi.nlm.nih.gov/gds/) [24]. The datasets were selected based on the following inclusion criteria: "Colorectal cancer", and "Expression profiling by array", and "Homo sapiens", and "tissues". Next, profiles which examined a particular CRC stage or used drugs were excluded. After comprehensive analysis, GSE9348 [platforms: 570, [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array, and colorectal tumoral tissue: colorectal healthy tissue = 70;12], GSE113513 [platforms: GPL15207] Affymetrix Human Gene Expression Array, and colorectal tumoral tissue: noncancerous surrounding tissue = 14:14], GSE41657 [platforms: GPL6480, Agilent-014850 Whole Human Genome Microarray 4 × 44K G4112F, and colorectal tumoral tissue: colorectal normal mucosa = 25:12], and GSE146587 [platforms: GPL17077, Agilent-039494 SurePrint G3 Human GE v2 8 × 60K Microarray 039381,and colorectal tumoral tissue: noncancerous tissue = 6:6] were selected for further analysis (Table 1). CEL files from Affymetrix microarrays were pre-processed using the Affy package (version 1.74.0; http://bioconductor.org/packages/release/bioc/html/affy.html) in R software (version 4.4.2; http://www.r-project.org/). The Robust Multi-array Average (RMA) method [25] was used for the pre-processing, which included background correcting, normalizing and calculating expression. The latest annotation files were downloaded for re-annotation. The Limma package (version 3.52.2) [26] in R software was subsequently used to screen DEGs between CRC and matched normal tissues in the microarray. A |log2 (FC)| value of $\geq 1$ and a q-value of $< 0.05$ were considered as the cut-off criteria for the identification of DEGs.

### GEPIA2 database and identification of DEGs

Next, the TCGA COAD dataset were investigated using GEPIA2 (http://gepia2.cancer-pku.cn/http://gepia2.cancerpku.cn) to determine all COAD-associated DEGs among high throughput RNA-Seq data. GEPIA2 is a web-based tool for assessing the transcriptional profiles of human cancers and normal tissues utilizing the TCGA database and the Genotype-Tissue Expression (GTEx) projects [27]. Genes with |log2FC|> 1 and adjusted q-value < 0.05 were considered significant. All the CRC-associated DEGs in these datasets were selected for further study.

### Identification of a gene significantly differentiated only in colon adenocarcinoma

Subsequently, a Venn diagram (https://bioinformatics.psb.ugent.be/webtools/Venn/) was created to identify the common DEGs between the four datasets and the GEPIA2 COAD-TCGA data. To identify a gene significantly differentiated only in colon adenocarcinoma, all the identified common DEGs were investigated in a pan cancer model among 33 cancer types through utilizing the GEPIA2 database.

### OncoDB

OncoDB (https://oncodb.org/) is an online database resource to explore abnormal patterns in gene expression as well as viral infection that are correlated to clinical features in cancer. All the analysis results are presented in OncoDB with a flexible interface to search for data related to RNA expression, DNA methylation, viral infection, and clinical features of the cancer patients [28]. Expression and methylation analyzing of the identified gene was performed based on clinical profiles include gender, pathological stages, and Body mass index (BMI). Some viruses such as cytomegalovirus and herpes virus can cause changes in the expression of genes in colon adenocarcinoma, so onco-analyzing of the gene was performed using OncoDB database.

**Table 1** Characteristic of the studied microarray dataset

| No | GSE no | GPL/platform | No. of sample CRC NCT (n) | Sample type | Update (year) | Race | Total |
|---|---|---|---|---|---|---|---|
| 1 | GSE113513 | Affymetrix Human Gene Expression Array | 14 | 14 | Colorectal tissue | 2018 | Unknown [a] 28 |
| 2 | GSE41657 | Agilent-014850 Whole Human Genome Microarray 4 × 44K G4112F | 25 | 12 | Colorectal tissue | 2019 | Unknown 37 |
| 3 | GSE146587 | Agilent-039494 SurePrint G3 Human GE v2 8 × 60K Microarray 039381 | 6 | 6 | Colorectal tissue | 2021 | Unknown 12 |
| 4 | GSE9348 | Affymetrix Human Genome U133 Plus 2.0 Array | 70 | 12 | Colorectal tissue | 2019 | Unknown 82 |

[a] The data were collected in the USA/South San Francisco, but the race of subjects is unknown

CRC, Colorectal Cancer; NCT, Non-cancerous tissue

### UALCAN

To investigate the prognostic values of *GUCA2A* in COAD patients, data from the TCGA COAD datasets of TCGA were used to perform the survival analyses utilizing UALCAN database (http://ualcan.path.uab.edu/) [29].

### Investigating diagnostic value of *GUCA2A*

To evaluate the diagnostic feature of *GUCA2A*, gene expression profile of *GUCA2A* in COAD and normal tissue was obtained from OncoDB database. Furthermore, Then, we calculate sensitivity, specificity, and Area under the ROC Curve (AUC) and evaluated the diagnostic value of *GUCA2A* for COAD tissues and normal counterparts using https://analysistools.cancer.gov/biomarkerTools.

### Co-expression analysis and PPI network

In this study top 50 positively and negatively correlated genes with *GUCA2A* in colon cancer was retrieved using LinkedOmics [30]. Protein–protein interaction (PPI) network for GUCA2A was constructed using STRING (https://string-db.org), and results were visualized in Cytoscape software (version 3.9.1; https://cytoscape.org). Also, top genes that have similar expression pattern with *GUCA2A* obtained from GEPIA2 database [27]. Then, intersection analysis between proteins interacted with *GUCA2A* and the top 100 similar expression genes was performed.

### Gene set enrichment analysis

To evaluate potential *GUCA2A* gene functional annotation and pathway enrichment, Gene Oncology (GO) including biological processes (BP), molecular functions (MF), and cellular component (CC), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were analyzed by using Enrichr (https://maayanlab.cloud/Enrichr/) database [31]. Enrichment analyzes were performed by *GUCA2A* and its interacting genes.

### miRNA, ceRNA, and ceRNA network

The miRNAs targeting *GUCA2A* were predicted based on four different databases, including miRDB [32], miRWalk[33], and TargetScan [34], and miRTarBase [35]. Next, common miRNAs were identified through intersection analysis. The circRNAs that regulate *GUCA2A* were identified using the circBank databases [36]. CircRNAs with a total score of over 1000 were chosen as a cutoff and subjected to further analysis. A competitive endogenous RNA (ceRNA) network was created using cytoscape [37]. ceRNA included *GUCA2A*, miRNAs targeting *GUCA2A*, and circRNAs sponging identified miRNAs.

### Genetic alteration of *GUCA2A*

The cBioPortal (https://www.cbioportal.org/) was searched for genetic alteration information of *GUCA2A* [38]. All Colorectal Adenocarcinoma studies were included. Somatic mutation frequency and genomic information of *GUCA2A* mutation were explored. Also, the mutations sites were obtained from "mutations" modules.

### Human Protein Atlas

The Human Protein Atlas (HPA) database (http://www.proteinatlas.org) was utilized to gather immunohistochemistry data for the *GUCA2A* protein in COAD and normal tissues [39]. The evaluation of protein expression in the HPA is based on both the fraction of stained cells and the intensity of the staining. In the HPA database, protein expression ranks are categorized by staining intensity levels (strong, moderate, weak, negative) and the fraction of stained cells (greater than 75%, between 25 and 75%, and less than 25%). This results in classifications of high expression (strong with > 25% stained cells), medium expression (strong with 25% stained cells), low expression (moderate with 25% stained cells), and not detected (weak or negative with < 25% stained cells).

### TISIDB

TISIDB (http://cis.hku.hk/TISIDB/) is a web portal for tumor and immune system interaction, which integrates multiple heterogeneous data types [40]. We explored associations between *GUCA2A* expression and immune-related signatures including immune cells, immunoinhibitors, immunstimulators and HLA molecules across human cancers.

### DGIdb

The Drug-Gene Interaction Database (https://www.dgidb.org/) is a web resource that provides information on drug-gene interactions from publications, databases, and other web-based sources [41]. We identified possible therapeutic medicines utilizing DGIBD.

## Experimental validation

### Human sample collection

In this study, polyp and colorectal cancer samples were obtained from patients referred to Taleghani Hospital. Based

on the inclusion and exclusion criteria, 15 biopsy samples of colorectal adenomatous polyps and 15 normal adjacent tissues (NATs) samples, as well as 16 colon cancer tissues with normal adjacent tissues were obtained. Patients undergoing chemotherapy and taking special drugs (anti-inflammatory) were excluded from this study. The clinical information of the patients was collected using a questionnaire. Tissues were stored in nitrogen and at -80°C for future evaluation. The ethical committee of the Institute of Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, approved the study (IR. SBMU.RIGLD.REC.1399.036), and written informed consent was obtained from all participants before entering the study.

#### RNA extraction and quality control

Total RNA was extracted from all samples Favor PrepTM total RNA extraction kit (FAVORGEN, Taiwan) according to the kit instructions. RNA concentration and purity ratios (OD260/280, OD260/230) were evaluated by NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The integrity of RNA was determined by electrophoresis on a denaturing 1.5% agarose gel.

#### cDNA synthesis

For cDNA synthesis, total RNA was reversed transcribed using AddScript cDNA synthesis kit (Add bio,Korea) according to the protocol kit is following:. the tubes were placed on ice where 4 µL of 5 × primer script buffer, 1 µL RT enzyme, 2 µL oligo dt primer or 2 µL random hexamer, 2 µL dNTP, 1 µg RNA template, and up to 20 µL RNA free distilled water (dH2O) were added. The cDNA synthesis was performed as follows: 25 °C for 10 min, 50 °C for 60 min, 80 °C for 5 min for inactivation of the reverse transcriptase enzyme and 12 °C for ∞ for hold temperature. Next, cDNA products were stored at − 20 °C. Note that in all reactions, the same concentrations of RNA samples were used (RNA adjustment).

#### Primer design

*GUCA2A* and Glyceraldehyde 3 phosphate dehydrogenase (*GAPDH*) primers were designed by Gene Runner software (version 6.5). Also, the specificity of the primers has been examined in the http://blast.ncbi.nlm.nih database. Primers for *GUCA2A* and *GAPDH* (housekeeping gene) were as follow: Forward *GUCA2A*: 5′- ATGAATGCCTTCCTG CTCTC -3′, Reverse *GUCA2A*: 5′-TTCCATCCTGCACGG TGAC-3′, ForwardGAPDH:5′-CTCAAGATCATCAGC AATGCCT-3′, Reverse *GAPDH*: 5′-ACAGTCTTCTGG GTGGCAGT 3′.

#### Real time-PCR

The qPCR was performed by Rotor-Gene Q real-time PCR cycler (Qiagen, Germany) and RealQ plus 2 × Master Mix Green (Ampliqon, Denmark). The Real-time PCR conditions were: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 61 °C for 35 s, and 72 °C for 20 s. Fold change of gene expression was evaluated by 2-ΔΔct method.

#### Statistical analysis

All results were analyzed by Graph pad Prism software version 8 (Graph Pad Software, California, USA). The data were non-normally distributed and the non-parametric test was used. Specifically, student t-test and one-way ANOVA test were performed. *p-value* < 0.05 was considered statistically significant.

## Results

### Identification of genes differentially expressed among microarray datasets

To acquire the gene expression CRC datasets, the microarray data were downloaded from the GEO database. Based on the inclusion/exclusion criteria, CRC and normal or adjacent mucosa tissue gene expression profile of GSE41328, GSE41657, GSE9348 and GSE146587 were selected. By using q-value < 0.05 and [logFC] > 1 as cut-off criterion, 861, 1932, 1568, and 1107 differentially expressed genes (DEGs) were extracted from the expression profile datasets of GSE41328, GSE41657, GSE9348 and GSE146587, respectively (Supplementary Table 1).

### *GUCA2A* was founded as a DEG which is significantly differentiated only in colon adenocarcinoma

To determine a DEG which is significantly differentiated only in colon adenocarcinoma, we first extracted TCGA-COAD associated DEGs from GEPIA2 database. With the q-value < 0.01 and [logFC] > 1 as cut-off criterion, 5356 DEGs were detected (Supplementary Table 1). After integrated bioinformatics analysis, a total of 88 common DEGs were identified among the five profile datasets (Supplementary Table 1), including 59 up-regulated genes and 29 down-regulated genes in the colorectal cancer tissues compared to normal colon tissues (Fig. 2A and B, respectively).

Second, all 88 common were analyzed in a pan cancer model using the GEPIA2 database. Finally, *GUCA2A* was founded as a DEG which is significantly differentiated only in colon adenocarcinoma (Fig. 3A). *GUCA2A* was not only expressed at low levels in tumor tissues compared to

adjacent normal tissues, but also than the normal tissues from normal samples (Fig. 3B). The box plots for *GUCA2A* expression in COAD patients compared to adjacent normal colon tissue and normal colon tissue in normal patients are shown in Fig. 3C and D.

Oncovirus analyzing of *GUCA2A* was performed using OncoDB database. Cytomegalovirus and Herpes virus do not significantly change the expression of *GUCA2A* in colon adenocarcinoma (Supplementary File 2 Fig. 1). In the clinical parameter analysis of *GUCA2A* by expression level, *GUCA2A* expression in was significantly different between races ($p$ value = 0.003) (Supplementary File 2 Fig. 2). There were no significant differences in *GUCA2A* expression between gender, BMI, and pathological stages (Supplementary File 2 Fig. 2).

## Investigating the diagnostic and prognostic value of *GUCA2A*

To investigate the diagnostic values of *GUCA2A* in colon adenocarcinoma, expression level of *GUCA2A* in COAD and normal patients in OncoDB database was used. The Area under the ROC Curve (AUC) was used to calculate sensitivity and specificity. With 98% sensitivity, 95% Specificity and 99.6% AUC, *GUCA2A* can be used as a diagnostic factor for COAD (Fig. 4A). To investigate the prognostic values of *GUCA2A* in colon adenocarcinoma, data from colon adenocarcinoma TCGA datasets were used to perform the survival analyses utilizing UALCAN database. Low expression of *GUCA2A* was significantly affected on patient's overall survival (Fig. 4B).

## Methylation of *GUCA2A*

Further, we examined the Methylation levels within the *GUCA2A* gene using OncoDB database. The results showed significant differential methylated levels of the gene between normal and COAD tissues in ''cg13452215'' probe which was hypo methylated (Fig. 5). In addition, in the clinical parameter analysis of *GUCA2A* by methylation level, significant differences were seen between BMI ($p$ value = 0.029) and pathological stages ($p$ value = 0.0093). There were no significant differences in *GUCA2A* methylation regarding Race and gender (Supplementary File 2 Fig. 3).

## Association between *GUCA2A* with immune-related signatures

It is well established that the immune system plays an important role in tumor development, progression, and therapeutic response, and immune modulators in the tumor immune microenvironment (TIME) may have a significant influence on the infiltrating lymphocytes' activity [42, 43]. Also, immune cells may serve as an independent predictor of survival and response to chemotherapy [44, 45]. Therefore, it is necessary to investigate the relationship between *GUCA2A* with TIME. The correlation between *GUCA2A* expression and immune signatures in colon adenocarcinoma performed via TISIDB (Fig. 6A). There was significant correlation between *GUCA2A* expression and CD4 + T cell (Act-CD4), central memory CD4 + T cell (Tcm_CD4), Effector memory CD4 + T cell (Tem-CD4), Type 17 T helper cell (Th17), Type 2 T helper cell (Th2), Activated B cell (Act-B cell), Natural killer cell (NK), Natural killer T cell (NKT), Eosinophil, Mast cell, Monocyte, and Neutrophil (Fig. 6B).

Additionally, the correlation analysis was performed between *GUCA2A* expressions and immune stimulators in colon adenocarcinoma (Fig. 7A). A significant correlation was observed between *GUCA2A* expression and CD27, CD40LG, CD48, CXCR4, ENTPD1, HHLA2, ICOSLG, IL6, KLKR1, MICB, NT5E, PVR, TMEM173, TMIGD2,

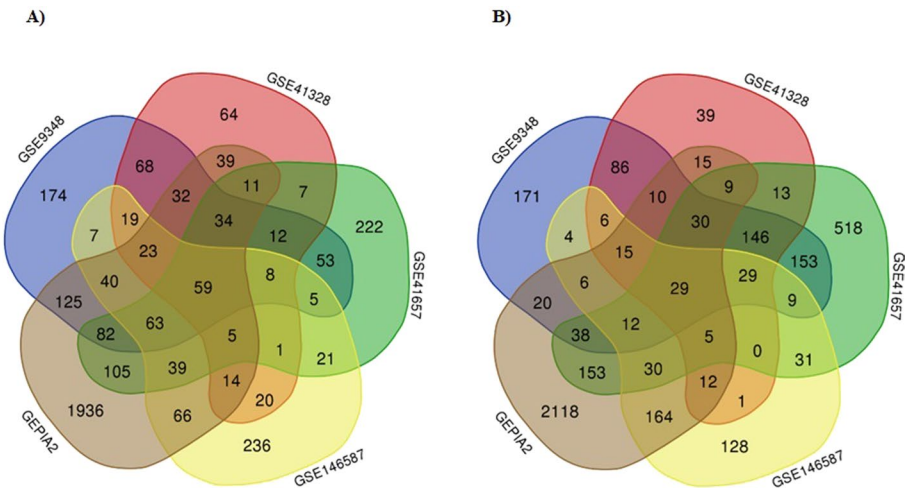



**Fig. 2** A total of 88 common DEGs were identified among the five profile datasets. **A** Common up-regulated DEGs between datasets. **B** Common down-regulated DEGs between datasets

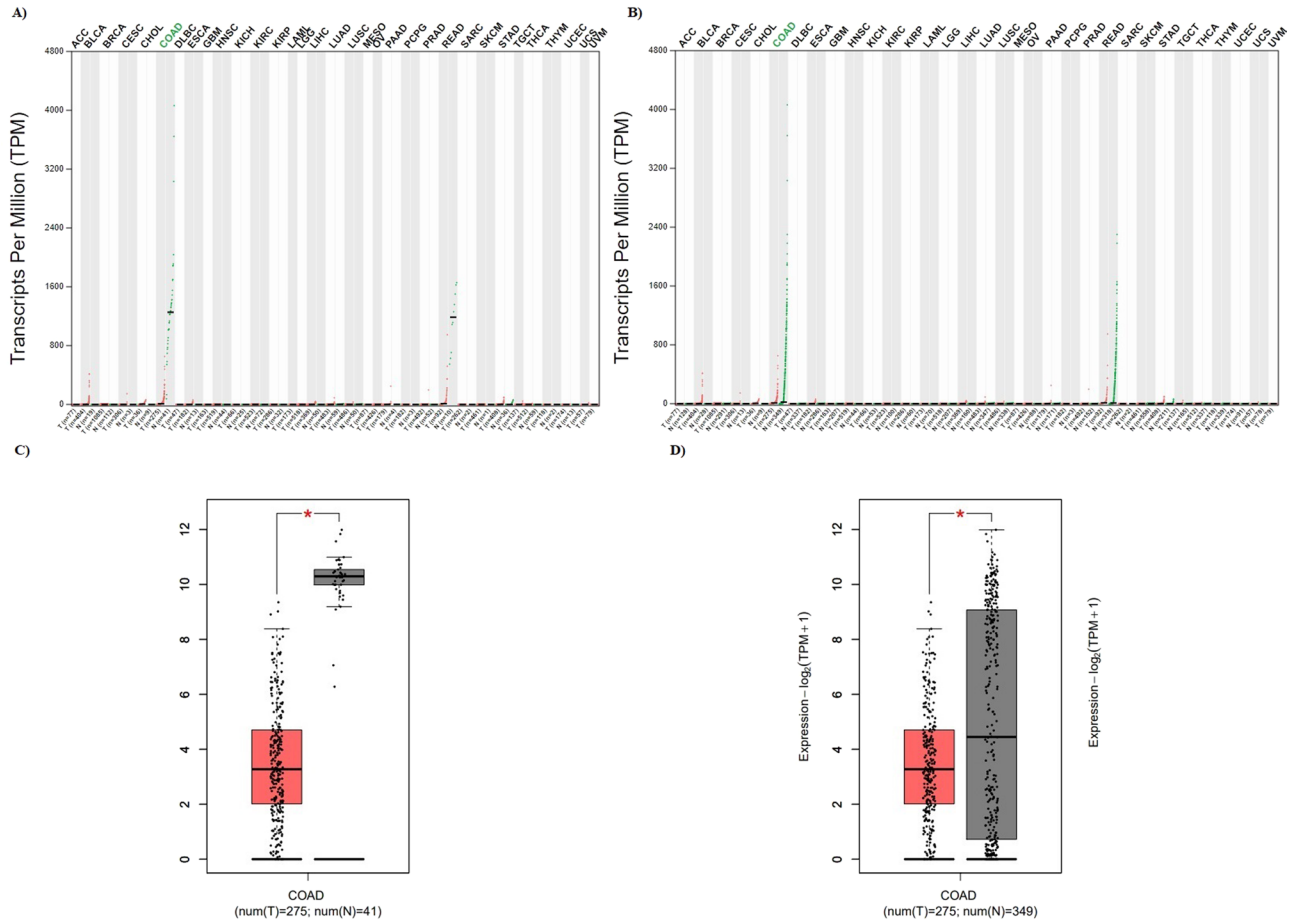
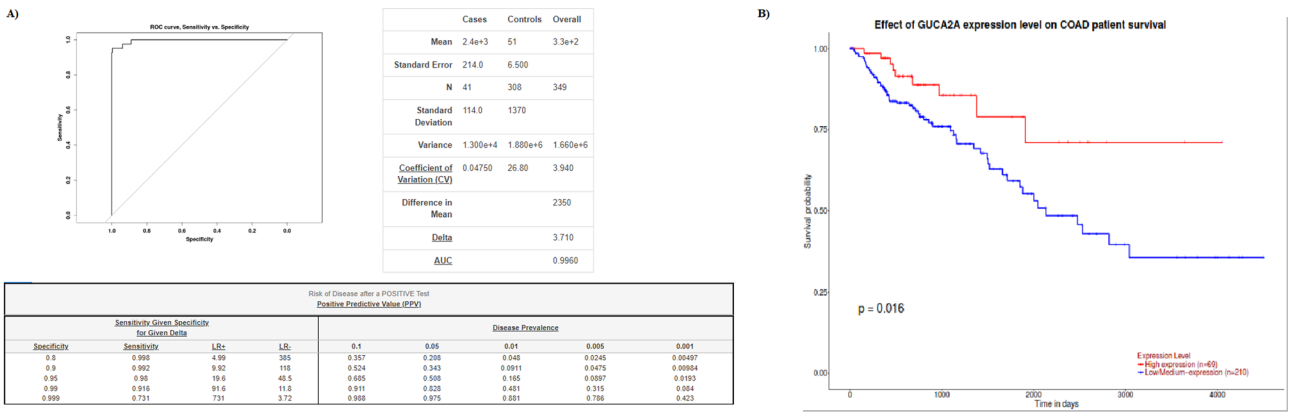



**Fig. 3** Pan cancer model for *GUCA2A* expression. **A** *GUCA2A* was founded as a DEG which is significantly differentiated only in colon adenocarcinoma among 33 cancers in tumor tissues compared to adjacent normal tissues and normal tissues from normal samples. **B** *GUCA2A* was founded as a DEG which is significantly differentiated only in colon adenocarcinoma among 33 cancers in tumor tissues compared to normal tissues from normal samples. Box plots show that *GUCA2A* significantly downregulated in COAD samples compared to adjacent normal tissues **C** and normal tissues in normal patients **D**




| | Cases | Controls | Overall |
|---|---|---|---|
| Mean | 2.4e+3 | 51 | 3.3e+2 |
| Standard Error | 214.0 | 6.500 | |
| N | 41 | 308 | 349 |
| Standard Deviation | 114.0 | 1370 | |
| Variance | 1.300e+4 | 1.880e+6 | 1.660e+6 |
| Coefficient of Variation (CV) | 0.04750 | 26.80 | 3.940 |
| Difference in Mean | | | 2350 |
| Delta | | | 3.710 |
| AUC | | | 0.9960 |

Risk of Disease after a POSITIVE Test
Positive Predictive Value (PPV)

| Sensitivity Given Specificity for Given Delta | | | | Disease Prevalence | | | | |
|---|---|---|---|---|---|---|---|---|
| Specificity | Sensitivity | LR+ | LR- | 0.1 | 0.05 | 0.01 | 0.005 | 0.001 |
| 0.8 | 0.998 | 4.99 | 385 | 0.357 | 0.208 | 0.048 | 0.0245 | 0.00497 |
| 0.9 | 0.992 | 9.92 | 118 | 0.524 | 0.343 | 0.0911 | 0.0475 | 0.00964 |
| 0.95 | 0.98 | 19.6 | 48.5 | 0.685 | 0.508 | 0.165 | 0.0897 | 0.0193 |
| 0.99 | 0.916 | 91.6 | 11.8 | 0.911 | 0.828 | 0.481 | 0.315 | 0.084 |
| 0.999 | 0.731 | 731 | 3.72 | 0.988 | 0.975 | 0.881 | 0.786 | 0.423 |

**Fig. 4** Diagnostic and prognostic value of *GUCA2A*. **A** ROC curve of *GUCA2A* for colon adenocarcinoma. With 0.95 sensitivity, 0.98 specificity, and 0.996 AUC, *GUCA2A* can be identified as a diagnostic biomarker for colon adenocarcinoma. **B** Survival analysis of *GUCA2A*. Low expression of *GUCA2A* was significantly affected on patient's overall survival

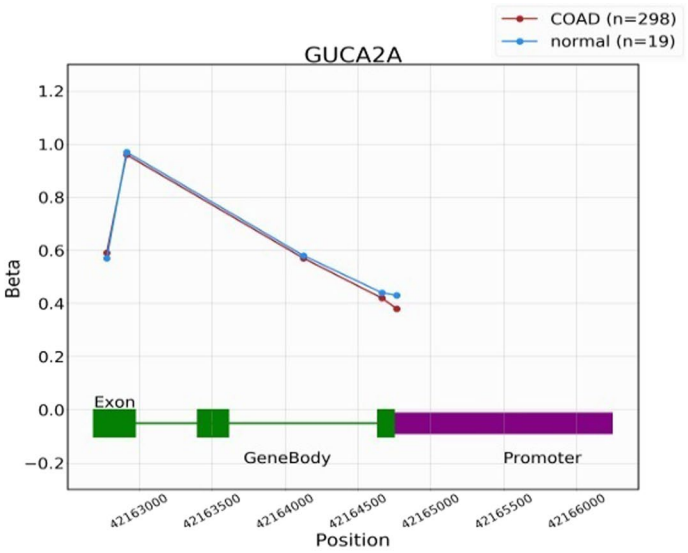



**Fig. 5** *GUCA2A* methylation levels. Significant hypo methylation in ''cg13452215'' probe was observed in COAD tissues compared to normal

**Significant probe highlighted (p<0.05)**

| Gene | Probe | Chr | Position | Average of Cancer Sample | Average of Normal Sample | p-value |
|---|---|---|---|---|---|---|
| GUCA2A | cg04701869 | chr1 | 42162778 | 0.59 | 0.57 | 2.6e-01 |
| GUCA2A | cg23022656 | chr1 | 42162914 | 0.96 | 0.97 | 3.8e-01 |
| GUCA2A | cg06003187 | chr1 | 42164127 | 0.57 | 0.58 | 7.2e-01 |
| GUCA2A | cg05398883 | chr1 | 42164664 | 0.42 | 0.44 | 2.9e-01 |
| GUCA2A | cg13452215 | chr1 | 42164765 | 0.38 | 0.43 | 1.8e-02 |

TNFRSF17, TNFRSF25, TNFRSF4, TNFSF13B, TNFSF4, TNFSF9, and ULBP1 (Fig. 7B).

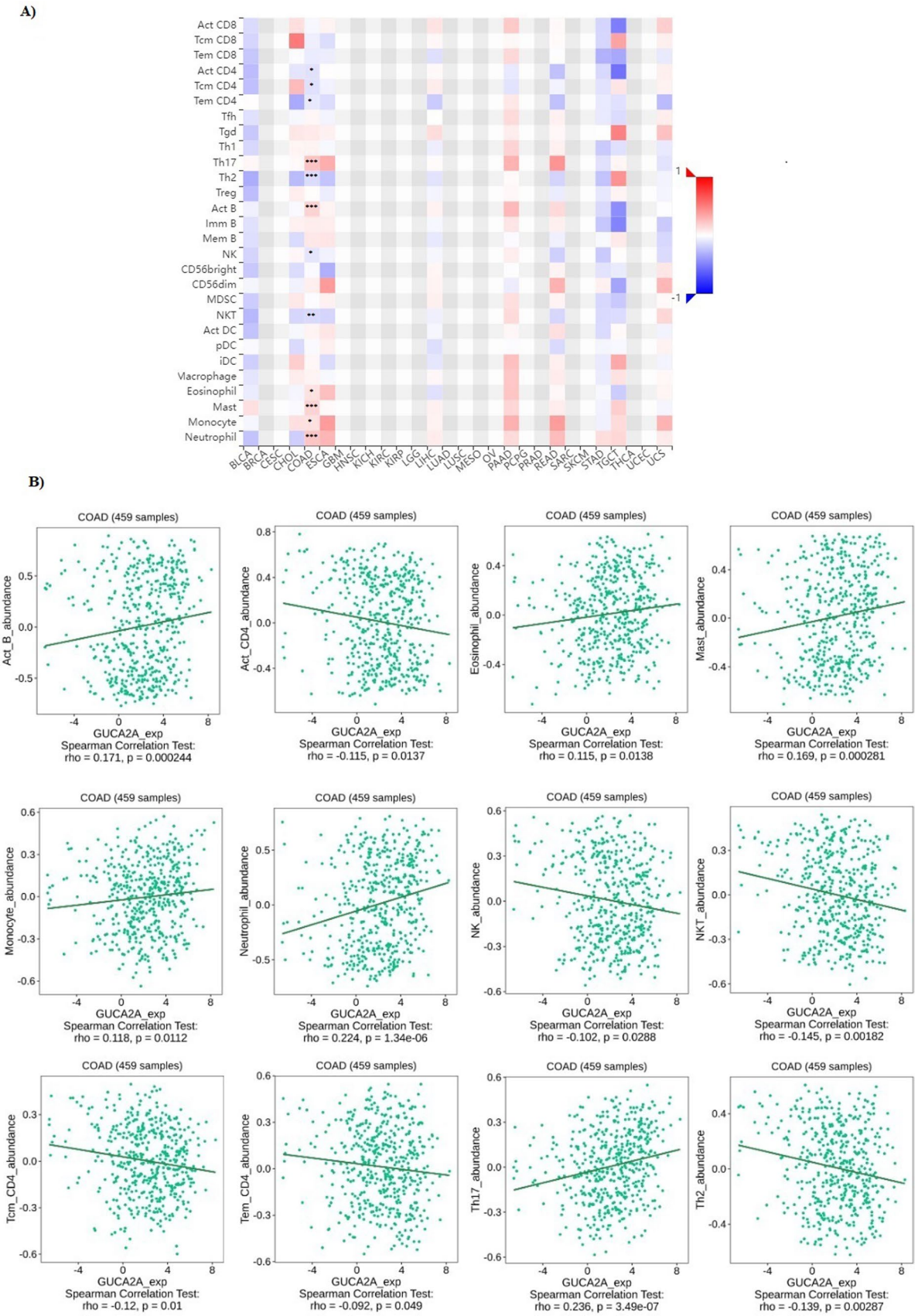
Also, correlation *GUCA2A* expression with immunoinhibitors in colon adenocarcinoma performed (Fig. 8A). There was significant correlation between *GUCA2A* expression and ADORA2A, CD160, CD244, CD274, IDO1, IL10RB, TGFBR1, and VTCN1 (Fig. 8B).

Furthermore, correlation between *GUCA2A* expression with MHC molecules in colon adenocarcinoma showed significant correlation between *GUCA2A* expression and HLA-A, HLA-DMA, HLA-DQA2, HLA-G, and TAP2 (Fig. 9).

In addition, the role of *GUCA2A* expression on immune and molecular subtypes among human cancers was determined using TISIDB website. Immune subtypes were classified into six types, including C1 (wound healing), C2 (IFN-gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet) and C6 (TGF-b dominant). The results showed that *GUCA2A* expression was related to different immune subtypes in COAD (Fig. 10A). Furthermore, *GUCA2A* expression differed in different immune subtypes of COAD (Fig. 10B). Based on the above results, we concluded that *GUCA2A* expression significantly differs in various immune subtypes as well as molecular subtypes of COAD.

## *GUCA2A* co-expression analysis and PPI network

*GUCA2A* co-expressed genes were retrieved using LinkedOmics [30], which provided the data from TCGA-COAD cohort. The heatmap of the top 50 positively and negatively *GUCA2A* correlated genes are demonstrated in Fig. 11A and B, respectively. Besides, the volcano plot of the genes positively and negatively correlated with *GUCA2A* is shown in Fig. 11C. While GUCA2B, CA4, and TMIGD1 were found to be the highest positively correlated genes, CLUAP1, C5orf46, and KIR2DL3 were found to be the top 3 highest negatively correlated genes with *GUCA2A*. *GUCA2A* interacting genes were screened for protein–protein interactions (PPI) network construction using STRING database. A PPI network containing 11 genes with 5.82 average node degree and average local clustering coefficient of 0.824 was constructed (Fig. 11D). We also performed an intersection analysis between these 11 genes and the top 100 genes that have similar expression pattern with *GUCA2A* obtained from GEPIA2, which identified TMIGD1, SLC26A3, PDZD3 (NHERF4), and GUCA2B as common genes.


A)
Act CD8
Tcm CD8
Tem CD8
Act CD4
Tcm CD4
Tem CD4
Tfh
Tgd
Th1
Th17
Th2
Treg
Act B
Imm B
Mem B
NK
CD56bright
CD56dim
MDSC
NKT
Act DC
pDC
iDC
Macrophage
Eosinophil
Mast
Monocyte
Neutrophil
BLCA BRCA CESC CHOL COAD ESCA GBM HNSC KICH KIRC KIRP LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA UCEC UCS
1
-1
B)
COAD (459 samples)
Act_B_abundance
GUCA2A_exp
Spearman Correlation Test:
rho = 0.171, p = 0.000244
Act_CD4_abundance
rho = -0.115, p = 0.0137
Eosinophil_abundance
rho = 0.115, p = 0.0138
Mast_abundance
rho = 0.169, p = 0.000281
Monocyte_abundance
rho = 0.118, p = 0.0112
Neutrophil_abundance
rho = 0.224, p = 1.34e-06
NK_abundance
rho = -0.102, p = 0.0288
NKT_abundance
rho = -0.145, p = 0.00182
Tcm_CD4_abundance
rho = -0.12, p = 0.01
Tem_CD4_abundance
rho = -0.092, p = 0.049
Th17_abundance
rho = 0.236, p = 3.49e-07
Th2_abundance
rho = -0.139, p = 0.00287

◂**Fig. 6** The relationship between *GUCA2A* expression and immune cells infiltration in colon adenocarcinoma. (A) Analysis between *GUCA2A* expression and immune infiltration levels. (B) Significant Correlations between *GUCA2A* expression and immune infiltration levels. * $p$ value < 0.05, ** $p$ value < 0.01, *** $p$ value < 0.001

## GO and KEGG pathway enrichment analysis

GO and KEGG pathway enrichment analysis were performed with *GUCA2A* and its interacting genes. For GO enrichment analysis, *GUCA2A* and its interacting genes were significantly enriched in 31 Biological processes (BPs) and 17 Molecular functions (MFs). Although sixteen cellular components were enriched, none of them were significant. The most significant biological process and molecular function were receptor guanylyl cyclase signaling pathway (GO:0007168; $q$-value = 8.52E-4, RF = 0.2) (Fig. 12A) and guanylate cyclase activator activity (GO:0030250; $q$-value = 7.69E-5, RF = 0.4) (Fig. 12B). Also, the most significant KEGG pathway was pancreatic secretion ($q$-value = 0.01, RF = 0.02).

## Integrated analysis of miRNA, mRNA, CircRNA ceRNA network

Upstream regulation of *GUCA2A* was analyzed by screening miRNAs targeted *GUCA2A*. Of 1003 miRNAs predicted targeting *GUCA2A*, 16 was found from miRDB, 966 from miRWalk, and 103 from Target Scan databases (Supplementary Table 1). No miRNA was detected by miRTarBase. As a result, *hsa-miR-4747-5p, hsa-miR-4722-5p, hsa-miR-5196-5p, hsa-miR-4731-5p, hsa-miR-1207-5p, hsa-miR-4763-3p, hsa-miR-6851-3p,* and *hsa-miR-558* were screened as the most vital miRNA regulators by overlapping predictions of miRDB, miRWalk, and Target Scan databases (Fig. 13A). Additionally, 61 circRNAs were identified regarding 8 potential miRNAs regulating *GUCA2A* (Supplementary Table 1). The ceRNA constructed included *GUCA2A*, 8 shared miRNAs, and 61 circRNAs (Fig. 13B).

## SNPs/Mutations of *GUCA2A*

Five mutation sites between amino acids 0 and 115, including 5 missense mutations were identified utilizing CbioPortal database (Table 2). Proteins changed by mutation of *GUCA2A* expression in COAD were P75S, E96K, A98T, A108V, and G114R. By analyzing whether an amino acid substitution affects protein function, P75S, A98T, A108V, and G114R were found as a deleterious mutation [46–48]. Also, A98T, A108V, and G114R were found as a probably damaging mutation.

## Lack of GUCA2A protein expression level in colorectal cancer tissues

We focused on the levels of GUCA2A protein and its effects on COAD utilizing HPA database. Based on the IHC data, GUCA2A Protein is expressed in normal colon cells with moderate cytoplasmic and membranous staining intensity in endocrine cells, low moderate staining intensity in enterocytes (less than 25%), and low moderate staining intensity in goblet cells (also less than 25%). In contrast, GUCA2A was not detected in tumor cells in any of the aforementioned cell types, indicating a lack of expression in the tumor microenvironment (Fig. 14).

### Gene drug interaction

According to the results obtained from DGIdb, Lactose anhydrous[50], Atropin[51], and Volanesorsen sodium [52] were identified as a drug that has interactions with *GUCA2A* (Table 3).

### *GUCA2A* expression levels in colorectal cancer tissues have shown significant down-regulation compared to NATs

The clinicopathological characteristics of colorectal cancer patients and those with colorectal adenomatous polyps are presented in Table 4. We investigated the expression levels of the *GUCA2A* gene in cancerous tissues and colorectal adenomatous polyps in comparison to normal adjacent tissues (NATs). Our results indicate that *GUCA2A* expression is significantly down-regulated in colorectal cancer tissues compared to NATs ($p$ value = 0.0045, Fig. 15a). In contrast, the expression level of *GUCA2A* in colorectal adenomatous polyps did not show a significant difference compared to NATs ($p$ value = 0.8468, Fig. 15b).

### The expression of *GUCA2A* in adenomatous polyps equal to or larger than 5 mm has shown a significant decrease compared to polyps smaller than 5 mm

The expression of the *GUCA2A* gene was analyzed in relation to various demographic and tumor characteristics of colorectal cancer patients, specifically age (younger than 60 years and 60 years or older), sex (male and female), and tumor location (left side and right side). None of these characteristics showed statistically significant differences (Fig. 16a–c). Similarly, the *GUCA2A* gene expression in colorectal adenomatous polyps was evaluated based on age (($< 65$ and $\geq 65$), sex (male and female), polyp location (left side and right side), and type of polyps (tubular and tubulovillus/villous). No statistically significant differences were found in these analyses (Fig. 17a–d). Importantly, the expression of the *GUCA2A* gene in

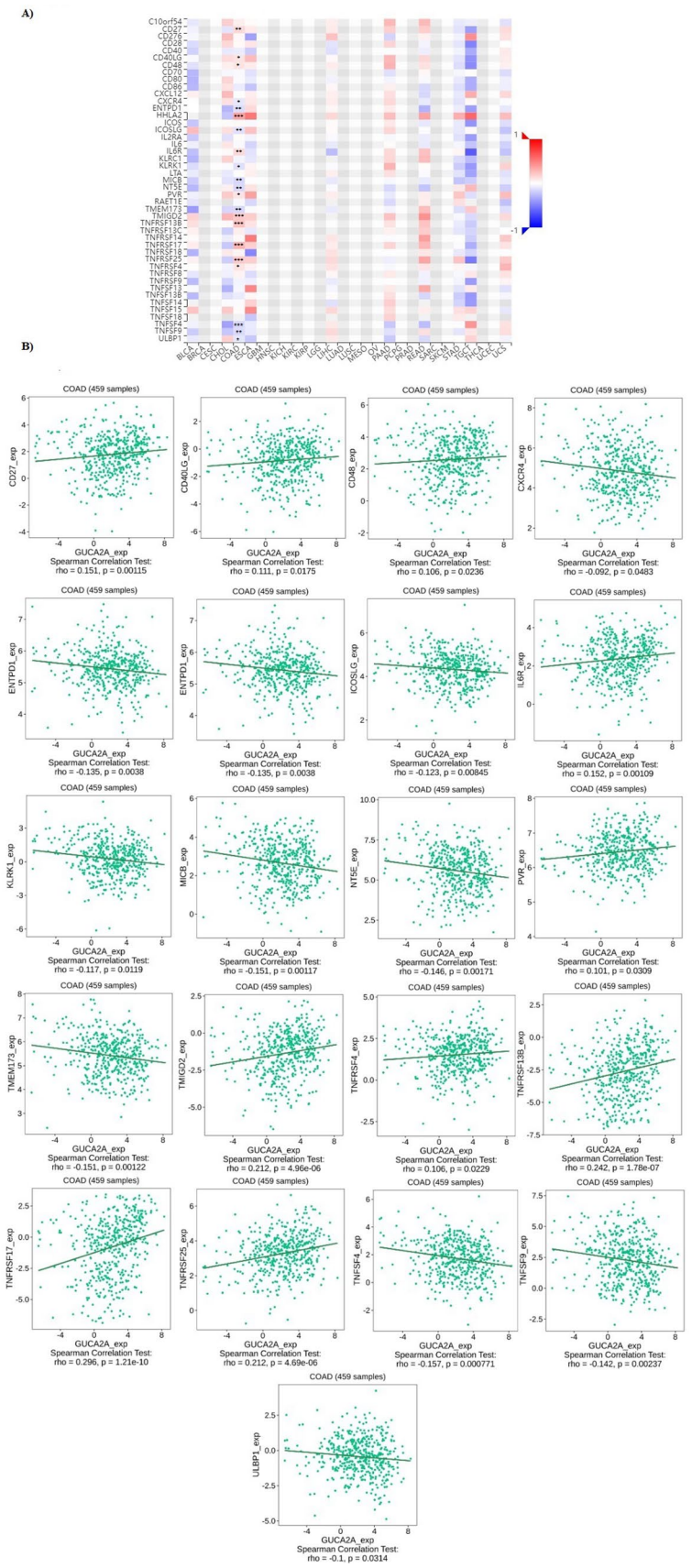



**Fig. 7** The relationship between *GUCA2A* expression and Immune stimulators in colon adenocarcinoma. **A** Analysis between *GUCA2A* expression and immune stimulators levels. **B** Significant Correlations between *GUCA2A* expression and immune stimulators levels. * $p$ value < 0.05, ** $p$ value < 0.01, *** $p$ value < 0.001

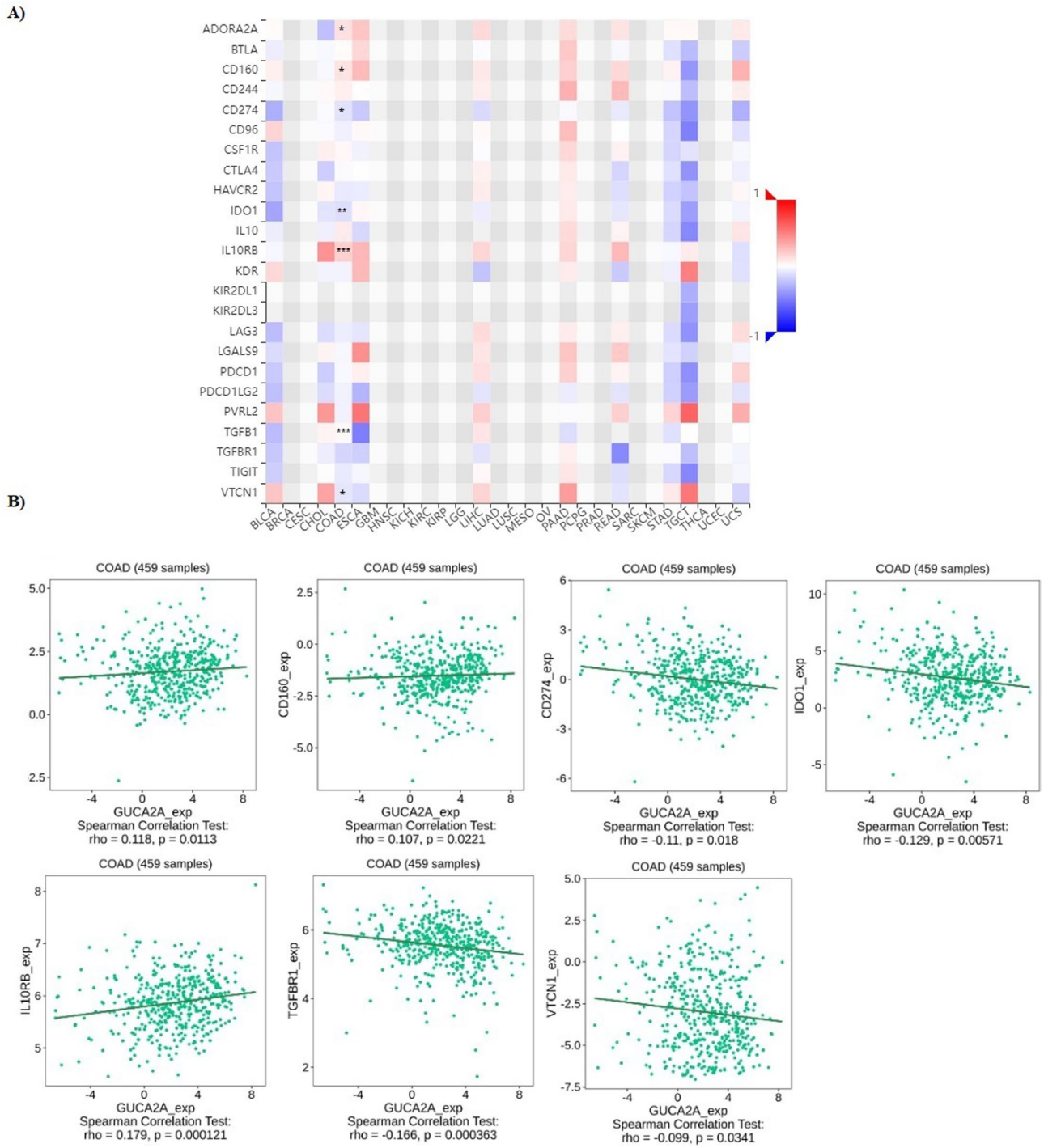



**Fig. 8** The relationship between *GUCA2A* expression and immune-related signatures in COAD. (A) Analysis between *GUCA2A* expression and immune infiltration levels. **B** Analysis between *GUCA2A* expression and immuno-inhibitors levels. **P*-value < 0.05, ***p* value < 0.01, ****p* value < 0.001

adenomatous polyps was also assessed based on polyp size ((< 5 mm and ≥ 5 mm). Our findings indicate that *GUCA2A* expression was significantly decreased in polyps measuring 5 mm or larger compared to those smaller than 5 mm (Fig. 17e).

## *GUCA2A* expression can be used as a biomarker for CRC diagnosis

To investigate and analyze the diagnostic value of *GUCA2A* expression in colorectal cancer tissues and colorectal

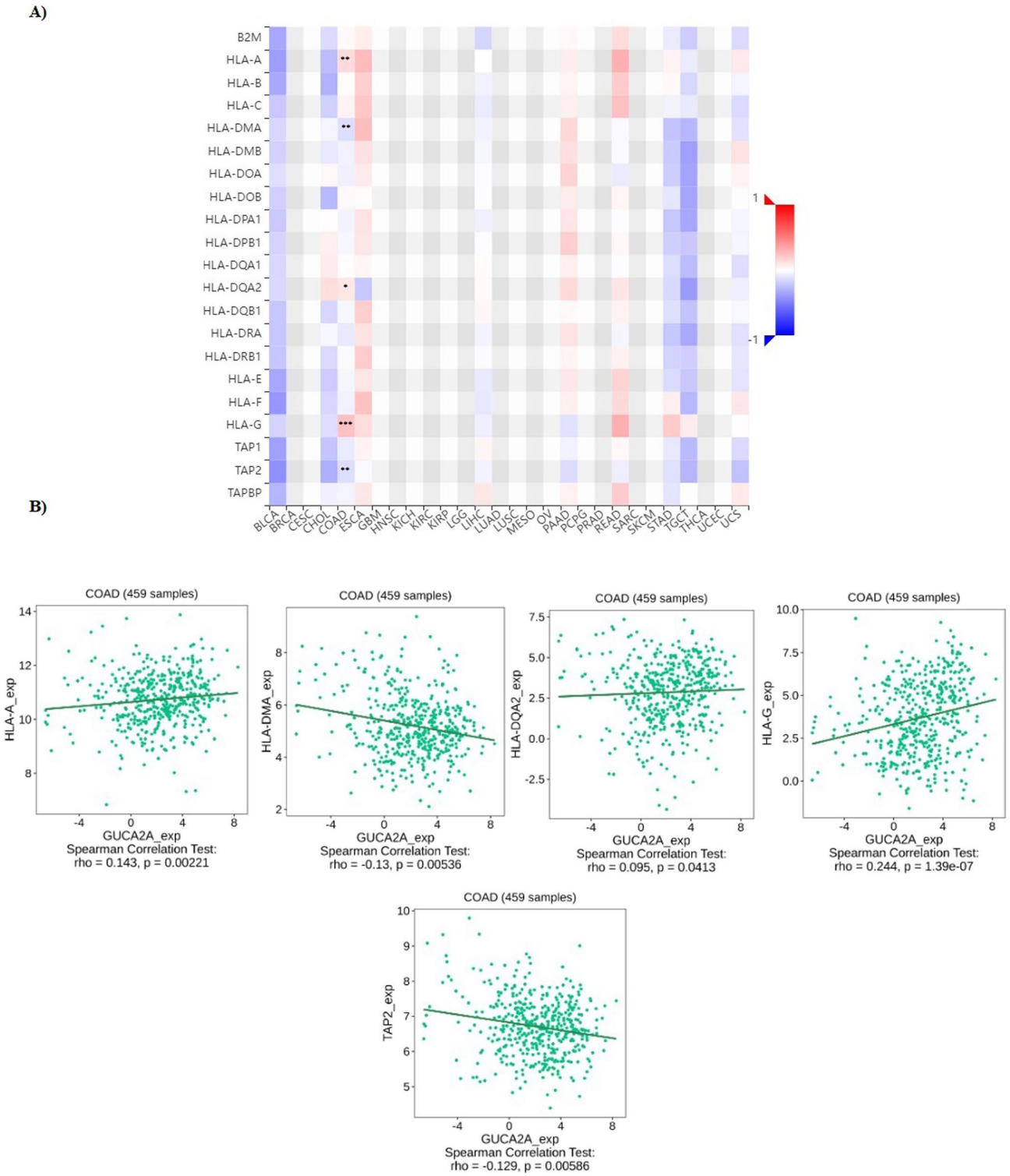



**Fig. 9** The relationship between *GUCA2A* expression and MHC molecules in COAD. (A) Analysis between *GUCA2A* expression and MHC molecule levels. **B** Significant Correlations between *GUCA2A* expression and MHC molecules levels. **P*-value < 0.05, ***p* value < 0.01, ****p* value < 0.001

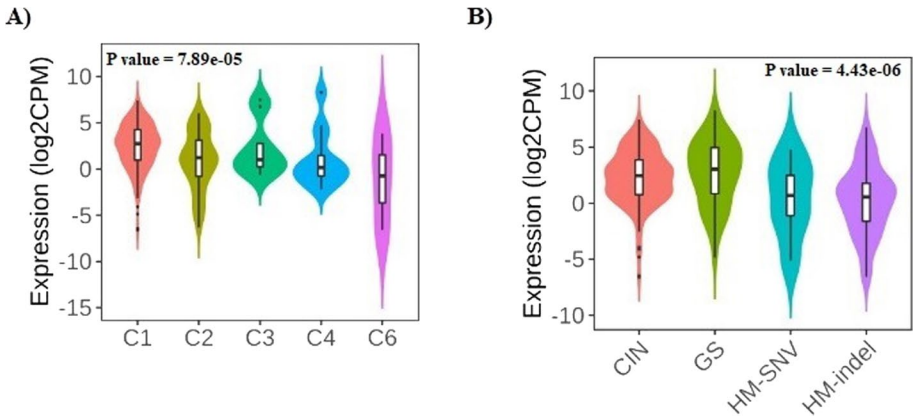



**Fig. 10** *GUCA2A* expression and immune and molecular subtypes in colon adenocarcinoma. (A) The relationship between *GUCA2A* expression and COAD immune subtypes. **B** The relationship between *GUCA2A* expression and COAD molecular subtypes

adenomatous polyps, we performed a Receiver Operating Characteristic (ROC) curve analysis for each group (Fig. 18a and b). Our results revealed that AUC for colorectal cancer tissues was 77%, which is statistically significant ($p$ value: 0.0083). The analysis indicated a sensitivity of 68.75% and a specificity of 81.25%. In contrast, the ROC curve analysis for colorectal adenomatous polyps showed an AUC of 59%, with a sensitivity of 73.33% and a specificity of 60%. However, this finding was not statistically significant ($p$ value = 0.3837).

## Discussion

Colorectal cancer (CRC) stands as a significant global health burden, ranking third among the leading causes of cancer-related mortality for both males and females. In 2020 alone, an estimated 515,637 deaths occurred among males, while females accounted for 419,536 deaths [53]. The overall survival (OS) rates for patients diagnosed with metastatic colorectal cancer indicate a challenging scenario, with approximately 70–75% surviving beyond one year, 30–35% surviving beyond three years, and less than 20% surviving beyond five years post-diagnosis [54]. Early diagnosis of colorectal cancer is vital because it opens up a wider array of treatment options and greatly influences patient survival rates [55]. The primary treatment approach for unresectable metastatic colorectal adenocarcinoma (COAD) involves systemic therapy, encompassing cytotoxic chemotherapy, biological therapies targeting cell growth factors, immunotherapy, and their combinations. Notably, recent clinical trials have demonstrated improved OS by tailoring treatments to the molecular and pathological features of individual tumors, underscoring the importance of analyzing genomic, transcriptomic, and proteomic profiles to identify effective treatments for specific somatic types [56–58].

Here, we employed an integrative analysis approach to identify COAD-specific differentially expressed genes by analyzing common DEGs from the GEO and GEPIA2 datasets within a pan-cancer model. Our investigation highlighted *GUCA2A* as a significantly differentiated gene specific to COAD. Through an examination of TCGA-COAD data, we observed downregulation of *GUCA2A* mRNA levels in COAD tissues compared to adjacent normal tissues and normal colon samples. Notably, our predictive analysis revealed a strong association between lower *GUCA2A* expression levels and shorter OS in COAD patients, indicating the potential prognostic value of *GUCA2A* in COAD. Additionally, the ROC curve analysis demonstrated the robust diagnostic performance of *GUCA2A* expression in differentiating COAD tissue from normal tissue, with 98% sensitivity, 95% specificity, and an impressive 99.6% AUC using TCGA-COAD data and 81.25% sensitivity, 68.75% specificity, and 0.77% AUC in CRC tissues, further supporting its potential as a robust diagnostic marker for COAD. These findings align with the study by Zhang et al.[54], which reported a significantly shorter OS in COAD patients with lower *GUCA2A* expression levels compared to those with higher expression levels. Furthermore, Liu et al. [59] employed Cox regression analysis to identify genes associated with colorectal cancer prognosis and constructed a 3-gene signature, including *CLCA1-CLCA4-GUCA2A*, which exhibited predictive power for prognosis in colorectal cancer. In this study, *GUCA2A* was significantly downregulated in adenoma polyps larger or equal to 5mm compared to smaller than 5mm which indicates its potential to predict adenocarcinoma in these polyps. In addition, later research identified areas of adenocarcinoma within adenomatous polyps, known as "adenomas," indicating that these polyps could potentially evolve into cancers [60]. A longitudinal study on patients who refused surgical removal of colonic polyps larger than 1 cm reported that there was a 24% chance of developing invasive adenocarcinoma at the original polyp site and a 35% chance of carcinoma occurring at any location within the colon over a period of 20 years [61].

To gain insights into the genomic alterations associated with *GUCA2A* in COAD, we conducted further investigations. Our analysis revealed the occurrence of five *GUCA2A*

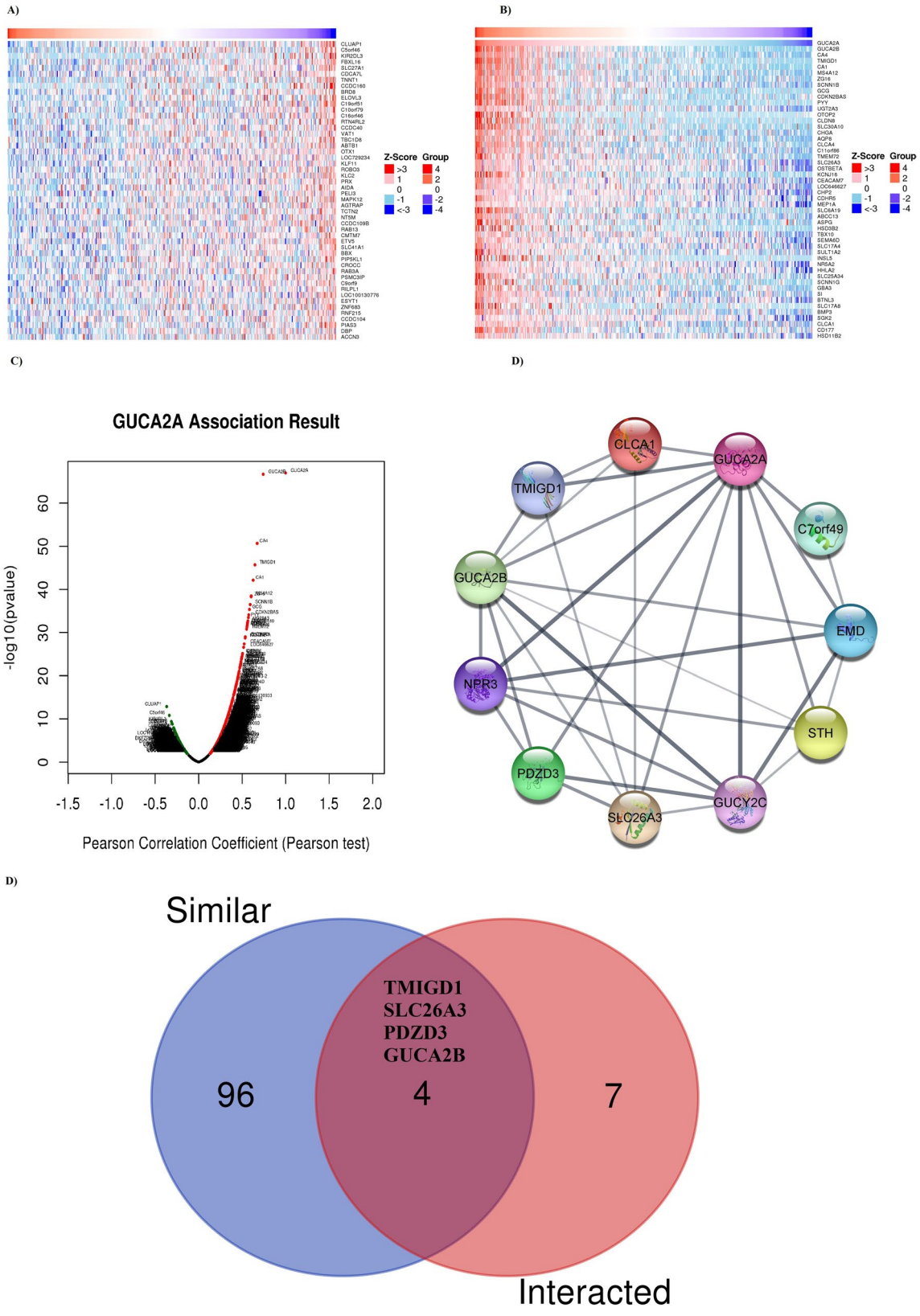



**Fig. 11** Co-expressed genes with *GUCA2A* and interaction network of proteins. Top 50 positively **A** and negatively **B** similar genes with *GUCA2A* in COAD. **C** Volcano plot demonstrating the positively and negatively correlated genes with *GUCA2A*. (**D**) Protein–protein interaction network of *GUCA2A* **E** Intersection analysis of *GUCA2A* similar and interacted genes

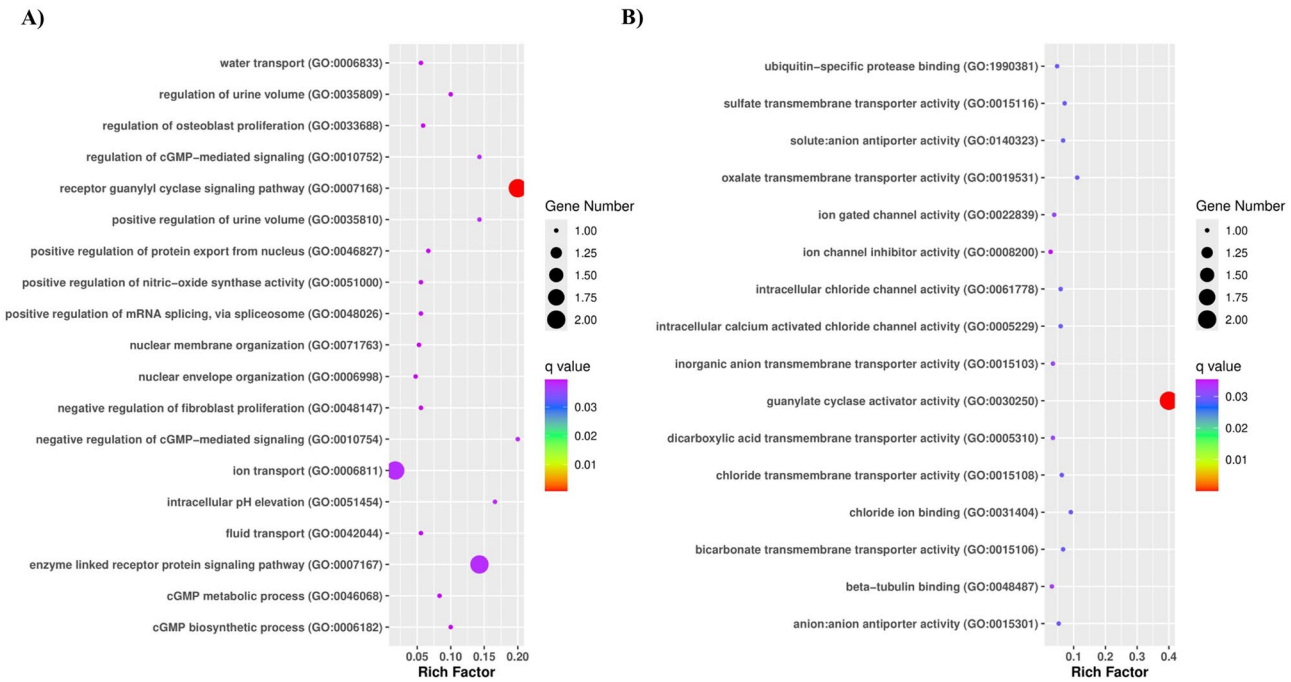



**Fig. 12** GO enrichment analysis. **A** Biological process and **B** Molecular function (MF) of interacting genes

missense mutations in COAD, with P75S, A98T, A108V, and G114R classified as deleterious mutations, while A98T, A108V, and G114R were classified as probably damaging mutations. Additionally, the analysis of *GUCA2A* methylation data revealed differential methylation patterns in COAD compared to healthy individuals, with one probe exhibiting significant differential methylation. In line with the expression profile, we observed a significant downregulation of *GUCA2A* promoter methylation in COAD, indicating the potential involvement of mutations and methylation in *GUCA2A* in altering the expression of *GUCA2A* protein and contributing to CRC pathogenesis.

The co-expression analysis conducted in our study revealed a strong positive correlation between *GUCA2A* and *GUCA2B*. These two genes encode peptide hormones that function as endogenous ligands for the guanylate cyclase-C (*GUCY2C*) receptor [62]. Specifically, *GUCA2A* and *GUCA2B* encode guanylin (GU) and uroguanylin (UG), respectively, while *GUCY2C* encodes guanylyl cyclase C (GC-C). The activation of GC-C occurs through GN and UG, which share structural and functional similarities. The GN and UG peptides play a crucial role in the transduction signaling that regulates homeostasis, as well as the transport and secretion of fluids and electrolytes in the gastrointestinal tract during the process of digestion [52, 63].

Previous studies have highlighted the significance of *GUCY2C* signaling in the mediation of mucosal wounding and inflammation by controlling the production of resistin-like molecule β [64]. Downregulation of *GUCA2A, GUCA2B,* and *GUCY2C* has been observed in inflammatory bowel disease, suggesting their potential involvement in the pathogenesis of this condition [65]. Furthermore, recent research has demonstrated that *GUCY2C* can exert inhibitory effects on tumor progression in the intestine, and the loss of *GUCY2C* signaling cascade increases susceptibility to colorectal cancer (CRC) [66, 67]. The disruption of intestinal homeostasis and the development of CRC are often associated with the loss of *GUCA2A* and *GUCA2B* [68–70]. Bashir et al. presented evidence suggesting that the loss of *GUCA2A* could lead to the silencing of *GUCY2C*, thereby contributing to the development of microsatellite instability tumors [71]. These findings collectively indicate the potential involvement of *GUCA2A, GUCA2B*, and *GUCY2C* in crucial biological processes, including gastrointestinal fluid regulation, inflammation mediation, and CRC development. Further investigation into the precise mechanisms underlying the interplay between these genes and their roles in intestinal homeostasis and tumorigenesis is warranted. Understanding these processes at a molecular level could potentially lead to the development of novel therapeutic strategies and interventions targeting *GUCY2C* signaling for the management and prevention of CRC.

Moreover, we performed an intersectional analysis to identify genes and proteins that share a similar expression pattern with *GUCA2A*. Through this analysis, we constructed a *GUCA2A* protein–protein interaction (PPI) network consisting of 11 proteins. Among the identified common genes, *TMIGD1, SLC26A3, NHERF4,* and *GUCA2B*

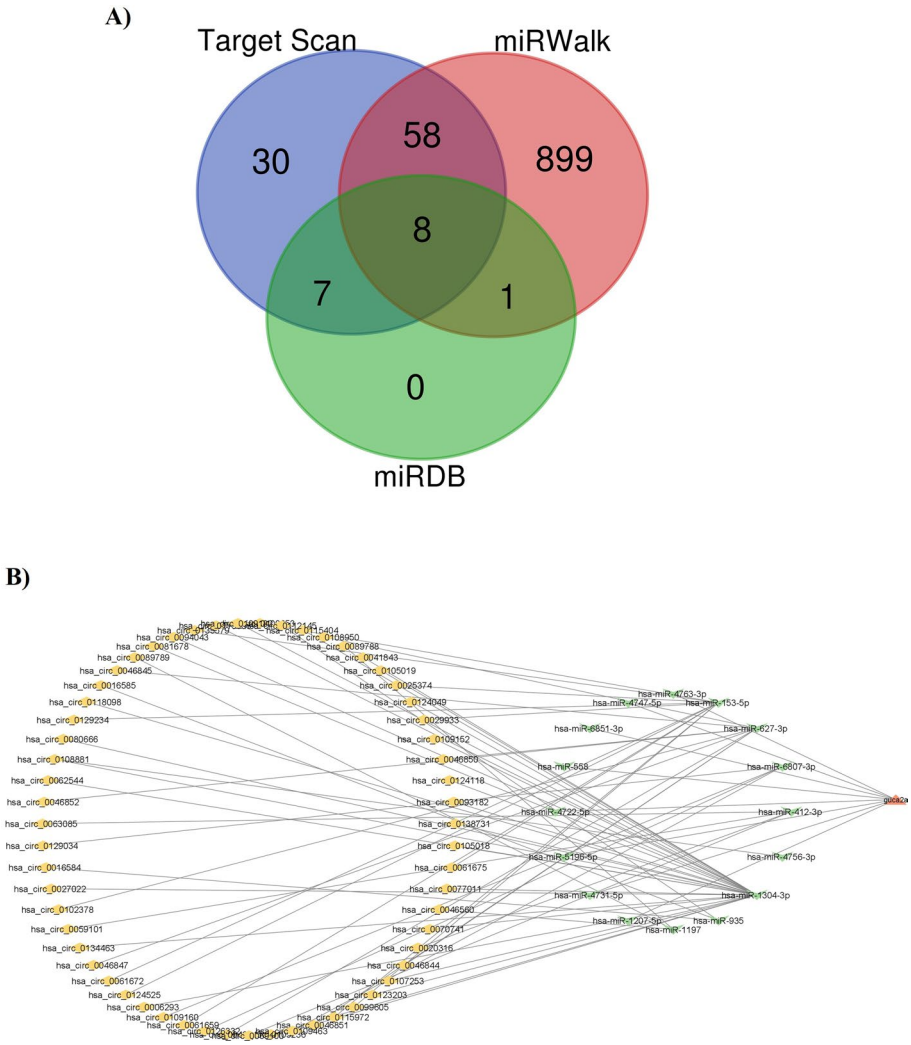



**Fig. 13** Integrated analysis of miRNA, mRNA, CircRNA, and ceRNA network. **A** miRNAs that target *GUCA2A* were screened using the data of mirDIP, miRWalk and Target Scan. Eight miRNAs were detected as the most vital miRNA regulators. **B** The ceRNA network includes *GUCA2A*, 8 miRNAs targeting *GUCA2A*, and 61 circRNAs sponging identified miRNAs

stood out for their potential significance in colorectal cancer (CRC). *TMIGD1* has been implicated as a tumor suppressor gene that plays a crucial role in the intestinal epithelium. De La Cena et al. reported that the loss of *TMIGD1* leads to adverse effects on the brush border membrane, junctional polarity, and maturation of the intestinal epithelium [72]. Their study demonstrated that *TMIGD1* acts as a tumor suppressor by inhibiting tumor cell proliferation and migration, and by arresting the cell cycle at the G2/M phase. Moreover, *TMIGD1* was found to induce the expression of key cell cycle inhibitor proteins, *p21CIP1* and *p27KIP1*, which are responsible for regulating cell cycle progression. Importantly, *TMIGD1* expression was progressively downregulated in sporadic human CRC, and its downregulation correlated with poor overall survival. These findings suggest that *TMIGD1* could serve as a potential therapeutic target and a novel tumor suppressor gene, shedding light on the pathogenesis of CRC [72]. Similarly, another study by Mu et al. identified *TMIGD1* as one of the highly downregulated genes in CRC, indicating its potential role in promoting CRC progression and invasion [73]. *SLC26A3* is a transporter protein involved in the exchange of chloride and bicarbonate ions in intestinal cells, predominantly expressed in the apical domain of various intestinal segments. Studies have consistently reported a significant decrease in *SLC26A3* expression levels in patients with CRC, suggesting its potential involvement in CRC progression. However, some studies propose that *SLC26A3* is primarily expressed in differentiated colon cells rather than proliferating cells, potentially serving as a marker for differentiation [74, 75]. *NHERF4* is a regulatory protein that interacts with *GUCY2C* and negatively modulates its activation induced by heat-stable enterotoxin [76]. Additionally, *NHERF4* stimulates the activity of SLC9A3 in the presence of high calcium ions [77]. NHERF1, a closely related member of the *NHERF* family, has been identified as a key regulator of CRC progression through its interaction with the VEGFR2 pathway. High expression of *NHERF1* has been associated with CRC progression, metastasis, and significantly worse overall survival, recurrence-free survival, and disease-specific survival. Knockdown of *NHERF1* has

**Table 2** Detailed analysis of mutations of *GUCA2A* gene in TCGA-COAD[a]

| Protein Change | Mutation Type | Start Pos.[b] | End Pos[b] | Ref | Variant Type | Functional Impact | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Mutation Assessor[c] | SIFT[d] | PolyPhen-2[e] | Sex | Ref |
| A98T | Missense | 42,628,633 | 42,628,633 | C | SNP | Medium | deleterious | Probably damaging | Female | Giannakis et al. [46] |
| G114R | Missense | 42,628,585 | 42,628,585 | C | SNP | Medium | deleterious | Probably damaging | Male | Giannakis et al.[46] |
| P75S | Missense | 42,629,134 | 42,629,134 | G | SNP | Medium | deleterious | Possibly damaging | n/a | Seshagiri et al. [47] |
| A108V | Missense | 42,628,602 | 42,628,602 | G | SNP | Medium | deleterious | Probably damaging | n/a | Seshagiri et al. [47] |
| E96K | Missense | 42,628,639 | 42,628,639 | C | SNP | neutral | neutral | neutral | Male | Weinstein et al. [49] |

a:Colon Adenocarcinoma b: position; c: Predicts the functional impact of amino-acid substitutions in proteins, such as mutations discovered in cancer or missense polymorphisms, d: predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids, e: (Polymorphism Phenotyping v2) is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations

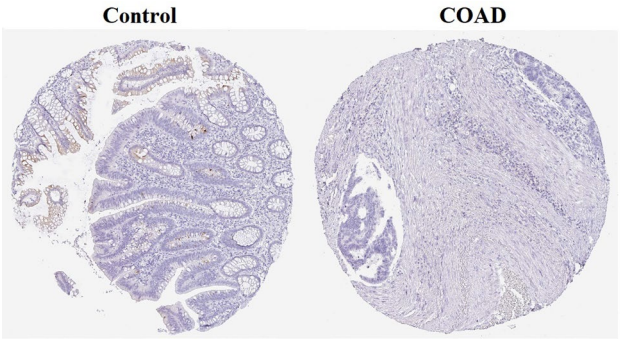



**Fig. 14** The immunohistochemical staining results from HPA. There was medium GUCA2A straining in endocrine cells and low straining in enterocytes and goblet cells in normal tissues. However, no GUCA2A straining was detected in colon cancer tissues

**Table 3** Candidate drugs target GUCA2A

| Drug | Interaction type | Sources | Interaction Score | Ref |
|---|---|---|---|---|
| Lactose anhydrous | n/a | NCI | 5.89 | Steinbrecher et al. [50] |
| Atropine | n/a | NCI | 3.75 | Furuya et al.[51] |
| Volanesorsen sodium | n/a | NCI | 3.43 | Kita et al. [52] |

been shown to increase apoptosis and reduce the expression of XIAP/survivin, underscoring the critical role of *NHERF1* in CRC cell survival [78, 79]. The identification of *TMIGD1*, *SLC26A3*, and *NHERF4* in our *GUCA2A* PPI network suggests their potential involvement in CRC pathogenesis.

Recent studies have emphasized the crucial role of different classes of non-coding RNAs, including mRNAs, miRNAs, and lncRNAs, in various biological processes and their associations with human diseases [80, 81]. Computational models have been developed to predict potential associations between miRNAs/lncRNAs and human diseases, providing valuable tools for disease-association prediction [82–85]. In 2011, the concept of competitive endogenous RNA (ceRNA) was introduced, which involves non-coding RNAs, such as lncRNAs or circRNAs, acting as competitive binding partners for miRNAs, thereby reducing the repression of target mRNAs by miRNAs [86]. In our study, we focused on investigating the involvement of miRNAs and circRNAs in the regulatory network of *GUCA2A*, aiming to identify more effective biomarkers and gain insights into the pathogenesis of colorectal adenocarcinoma (COAD) at different molecular levels. We constructed a comprehensive ceRNA network comprising 8 miRNAs that target *GUCA2A* and 183 circRNAs acting as miRNA sponges. Among the identified

**Table 4** Clinicopathological characteristics of colorectal adenomatous polyp and colorectal cancer patients

| Feature | Colorectal adenomatous polyps | | | Colorectal cancer |
|---|---|---|---|---|
| | n (%) | *P*-value | n (%) | *P*-value |
| Tissue Adenomatous polyp /Tumor NAT | 15 15 | 0.8468 | 16 16 | 0.0045 |
| Age < 65 / < 60 ≥ 65 ≥ 60 | 6 (40%) 9 (60%) | 0.2274 | (43.7%) 9 (56.2%) | 0.3829 |
| Sex Male Female | 9 (60%) 6 (40%) | 0.6105 | 9 (56.2%) 7 (43.7%) | 0.1680 |
| Location Left side Right side | 9 (60%) 6 (40%) | 0.7381 | 8 (50%) 8 (50%) | 0.8141 |
| Size 5 mm ≥ 5 mm | 10 (66.6%) 5 (33.3%) | 0.0416 | – | |
| Type of polyp Tubular Tubulovillus /Villous | 9 (60%) 6 (40%) | 0.7657 | – | |

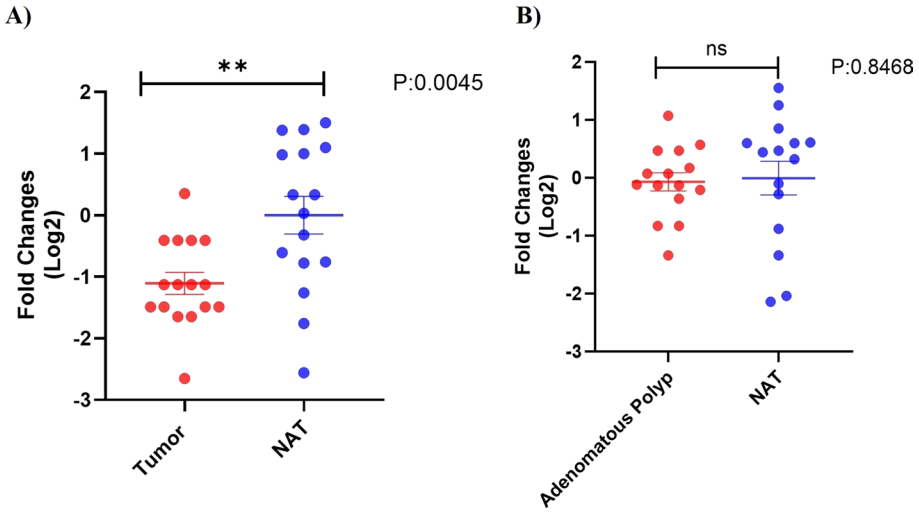



**Fig. 15** The scatter diagram shows the expression levels of *GUCA2A* gene in colorectal cancer tissues and colorectal adenomatous polyps compared to their normal adjacent tissues (NATs). (A) The average level of *GUCA2A* expression in colorectal cancer tissues showed a significant decrease compared to NATs. **B** *GUCA2A* expression levels in colorectal adenomatous polyps were not significantly different from NATs. **: $p$ value < 0.01, ns: non- significant

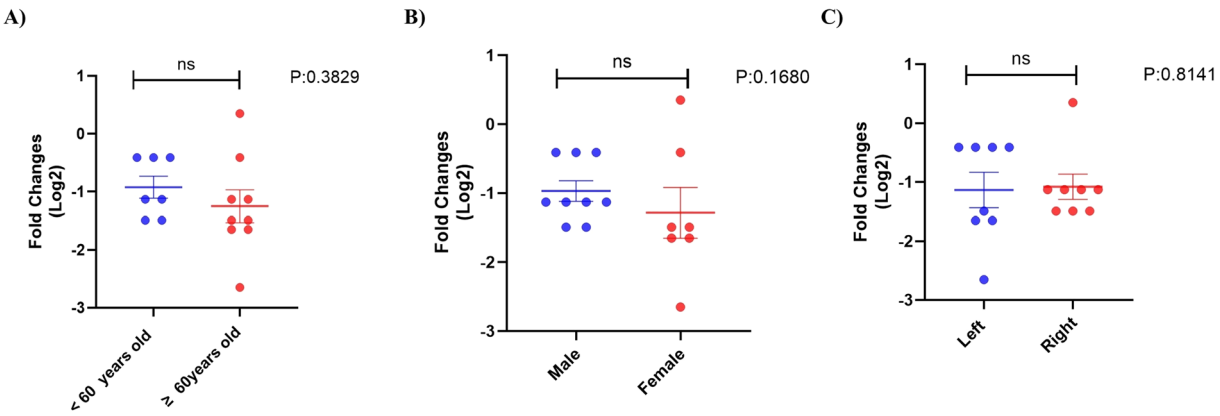



**Fig. 16** Comparison of *GUCA2A* expression levels in colorectal cancer tissues based on clinicopathological characteristics. Expression levels *GUCA2A* were compared based on (A) age, (B) sex, and (C) tumor location colorectal cancer patients. It was shown that there is no significant difference in the expression of *GUCA2A* based on the parameters of age, sex and tumor location. ns: non- significant

miRNAs, *hsa-miR-1207-5p* has been reported to promote the proliferation of breast cancer cells by directly regulating *STAT6* [87]. Moreover, studies have demonstrated a significant decrease in circulating *miR-1207-5p* levels, which is associated with poor prognosis and serves as a highly diagnostic marker in colorectal cancer (CRC) [88]. Additionally, Ng et al. found a correlation between high tumor levels of *miR-187-3p* and poor prognosis in colorectal cancer [89].

We further aimed to explore the relationship between *GUCA2A* expression and the immune properties of the tumor microenvironment in colorectal adenocarcinoma. Our

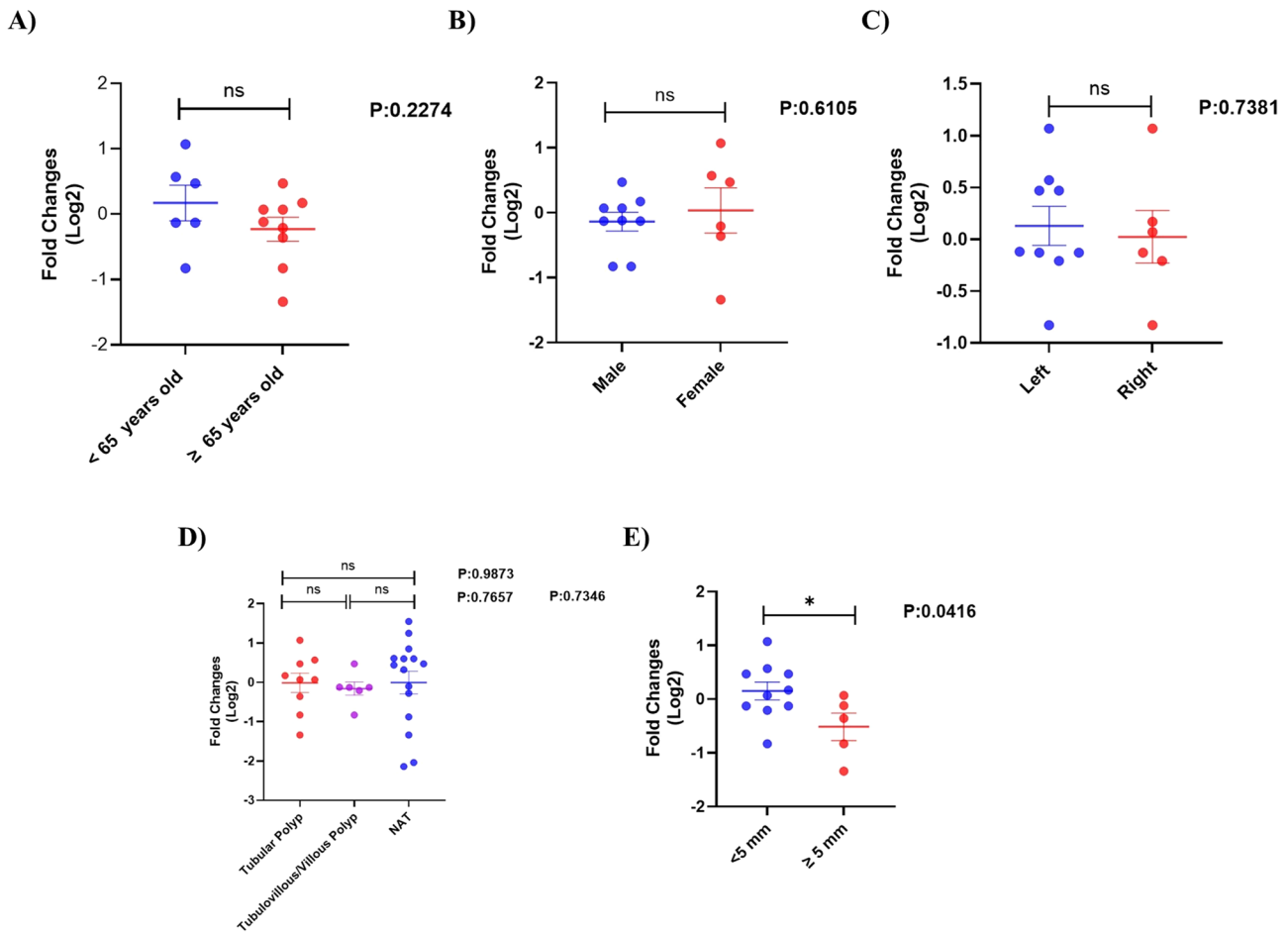



**Fig. 17** Comparison of *GUCA2A* expression levels in colorectal adenomatous polyps based on clinicopathological characteristics. Expression levels *GUCA2A* were compared based on (**A**) age, (**B**) sex (**C**) polyp location, (**D**) type of polyps and (E) polyp size in patients with colorectal adenomatous polyps. It was found that there is no significant difference in the expression of the *GUCA2A* gene based on the parameters of age, sex, location of the polyp and the type of polyp but the level of expression of *GUCA2A* in polyps equal to 5 mm and larger than 5 mm was significantly lower compared to smaller than 5 mm. *: $p$ value < 0.05, ns: non- significant

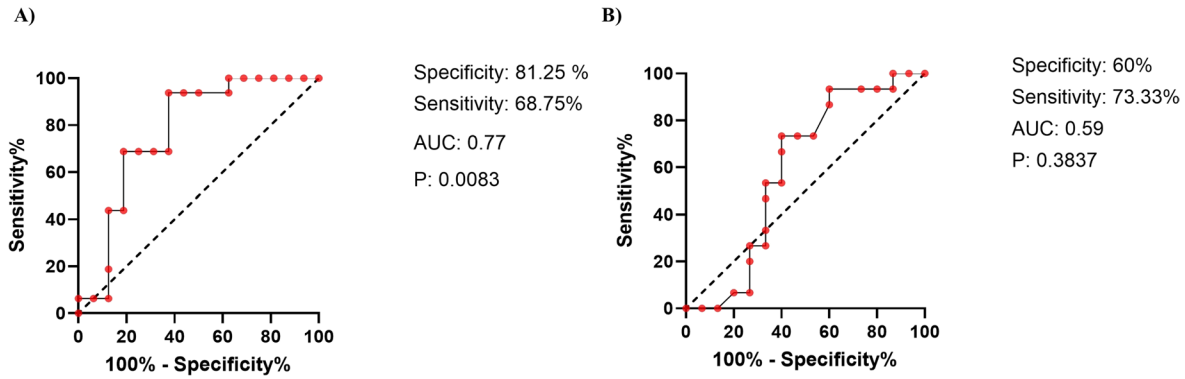



**Fig. 18** Investigating the biomarker property and diagnostic value of *GUCA2A* expression in colorectal cancer tissues, and colorectal adenomatous polyps using ROC curve analysis and based on the area under the curve (AUC). **A** AUC for *GUCA2A* expression levels in colon cancer tissues was 77% which is statistically significant. (B) *GUCA2A* expression levels in colon adenomatous polyps with AUC of 59% were not statistically significant

findings revealed significant correlations between *GUCA2A* expression and various immune cell populations within the tumor microenvironment. We observed significant correlations between *GUCA2A* expression and the abundance of several immune cell subsets, including CD4 + T cells, central memory CD4 + T cells, effector memory CD4 + T cells, type 17 T helper cells, type 2 T helper cells, activated B cells, natural killer (NK) cells, natural killer T cells, eosinophils, mast cells, monocytes, and neutrophils. These correlations highlight the potential involvement of *GUCA2A* in modulating the immune response in COAD. Previous studies have indicated the significance of T helper 17 cells in the progression of colorectal cancer [90]. The presence of these cells within the tumor microenvironment has been associated with disease progression. Furthermore, infiltration of B cells, NK cells, and macrophages has been linked to a favorable prognosis in colorectal cancer [91–94]. The significant correlations observed between T and B cells, as well as activated NK cells, and *GUCA2A* expression suggest that these immune cell populations may contribute to the impact of *GUCA2A* on the survival of COAD patients. Our findings suggest that *GUCA2A* may interact with immune cells and influence the immune landscape within the COAD tumor microenvironment. Further investigations are warranted to elucidate the underlying mechanisms by which *GUCA2A* influences immune cell populations and its potential implications for the prognosis and treatment of COAD. Understanding the intricate interplay between *GUCA2A* expression and immune cell populations will provide valuable insights into the immune-mediated mechanisms driving COAD progression. This knowledge could potentially contribute to the development of immunotherapeutic strategies targeting *GUCA2A* and its associated immune pathways in the treatment of COAD patients.

We also conducted a gene-drug analysis to identify drugs that interact with *GUCA2A*, and we identified three drugs: lactose anhydrous, atropine, and volanesorsen sodium. The associations between these drugs and *GUCA2A* provide insights into potential therapeutic strategies and shed light on the underlying molecular mechanisms in colorectal cancer. Lactose intolerance has been found to have a significant relationship with sporadic CRC, suggesting that lactose intolerance may act as a risk factor for CRC development [95]. Interestingly, lactose consumption has been shown to lower the risk of CRC by activating the guanylate signaling pathway. The interaction between lactose anhydrous and *GUCA2A* highlights the potential role of this drug in modulating *GUCA2A*-mediated signaling pathways and its implications in CRC prevention. Another chemotherapy drug commonly used in colorectal cancer treatment is irinotecan. It has been observed that irinotecan can induce diarrhea as a side effect. Atropine, an anticholinergic agent, is used to prevent the development of irinotecan-induced diarrhea [96]. The interaction between atropine and *GUCA2A* suggests a potential mechanism through which atropine may modulate *GUCA2A*-associated pathways to alleviate diarrhea in patients receiving irinotecan-based chemotherapy. Further, Volanesorsen sodium is a drug used in the treatment of familial chylomicronemia syndrome or hypertriglyceridemia [97]. This drug targets apolipoprotein C3 (apoC3) to increase the clearance of chylomicrons and other triglyceride-rich lipoproteins, leading to a significant reduction in triglyceride (TG) levels by 70–80%. Elevated TG levels have been associated with an increased risk of pancreatitis, and there is also a significant relationship between hypertriglyceridemia and CRC [98]. The interaction between Volanesorsen sodium and *GUCA2A* suggests a potential link between *GUCA2A* and TG metabolism pathways, highlighting the importance of *GUCA2A* in modulating lipid-related pathways that may impact CRC risk. Additionally, our *GUCA2A* KEGG pathway analysis identified pancreatic secretion as a significant pathway associated with *GUCA2A*. The downregulation of *GUCA2A* expression may contribute to an elevation in TG levels, potentially increasing the risk of CRC. These findings provide valuable insights into the molecular pathways influenced by *GUCA2A* and its potential role in CRC development. The identification of drugs that interact with *GUCA2A* and the exploration of associated pathways provide a foundation for further research and potential therapeutic interventions. Understanding the mechanisms underlying the interactions between *GUCA2A* and these drugs may lead to the development of novel treatment approaches targeting *GUCA2A*-mediated pathways in CRC. Further investigations are warranted to validate these findings and explore the clinical implications of targeting *GUCA2A* and its associated pathways in the prevention and treatment of CRC. The identification of *GUCA2A* as a potential therapeutic target opens up new possibilities for precision medicine strategies in CRC management.

## Limitations

While this study contributes to the understanding of *GUCA2A* in COAD, it is important to acknowledge the limitations. Firstly, the microarray data obtained from the GEO database and the TCGA-RNASeq data were acquired from the GEPIA2 database. Utilizing data from different laboratories with diverse platforms may introduce systematic biases and variations in the results. Although efforts were made to minimize these biases through data processing and normalization, it is important to consider these potential limitations when interpreting the findings. Secondly, no anti-*GUCA2A* therapeutic monoclonal antibodies have been evaluated in clinical trials to date. Therefore, there is a lack of specific data available to assess the potential

benefits of anti-*GUCA2A* targeting drugs in terms of the survival of COAD patients or inhibition of tumor growth. Future investigations should focus on exploring the feasibility and efficacy of anti-*GUCA2A* therapies, including the development and evaluation of novel anti-tumor immunotherapy drugs that specifically target *GUCA2A*. This would provide valuable insights into the clinical applicability of *GUCA2A* as a therapeutic target in COAD. In the future, a prospective study examining *GUCA2A* expression and its impact on immune infiltration in COAD patients is needed. By integrating comprehensive analyses of *GUCA2A* expression and immune cell infiltration, a deeper understanding of the interplay between *GUCA2A* and the tumor microenvironment can be obtained. Furthermore, testing newly developed anti-tumor immunotherapy drugs that target *GUCA2A* would provide valuable data on their efficacy, safety, and potential synergistic effects with existing treatment modalities. Addressing these limitations through further experimental studies, clinical trials, and prospective investigations will enhance our understanding of *GUCA2A*'s functional role, clinical significance, and therapeutic potential in COAD management.

## Conclusions

In conclusion, this study sheds light on the potential the significance of *GUCA2A* as a valuable prognostic and diagnostic biomarker in colon adenocarcinoma (COAD). As a COAD-specific gene, *GUCA2A* exhibited significant downregulation and hypomethylation, along with the five missense mutations in COAD patients. The significant downregulation of GUCA2A in cancerous colon tissues compared to normal colon tissues, as well as in adenomatous polyps $\geq 5$ mm compared to those $< 5$ mm, indicates its potential as a predictive biomarker for the development of colorectal polyps into cancerous tissues. Moreover, significant correlations were revealed between *GUCA2A* and immune-related signatures in this gastrointestinal cancer type. As co-expressed genes with similar expression patterns, *GUCA2A TMIGD1, SLC26A3, PDZD3 (NHERF4),* and *GUCA2B* could be considered as significant gene signature in COAD. These findings further emphasize the importance of investigating the collective role of these genes in COAD pathogenesis. Additionally, the analysis of drug-gene interactions indicated a potential beneficial effect of lactose anhydrase and volanesorsen in CRC risk reduction through the regulation of *GUCA2A* expression. These findings provide insights into potential therapeutic avenues for COAD management. However, to fully elucidate the underlying mechanisms and functional roles of *GUCA2A* in COAD, further in vivo and in vitro studies are warranted. These investigations will contribute to a deeper understanding of *GUCA2A*'s involvement in COAD progression and facilitate the development of targeted therapeutic strategies.

## Decelerations

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**Data availability** The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material. The experimental data is available upon request from the corresponding authors.

## Declarations

**Competing interest** The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

The authors declare no competing interests.

**Ethics approval and consent to participate** The ethical committee of the Institute of Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, approved the study (IR. SBMU. RIGLD.REC.1399.036), and written informed consent was obtained from all participants before entering the study.

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