# Identification of potential glioma drug resistance target proteins based on ultra-performance liquid chromatography-mass spectrometry differential proteomics

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In this study, to screen for candidate markers of Temozolomide (TMZ) resistance in glioblastoma, we artificially established TMZ drug-resistant glioblastoma (GBM) cell lines, U251-TMZ and U87-TMZ. In the U251-TMZ and U87-TMZ cell lines, we screened and analyzed differentially expressed proteins using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) differential proteomics. Compared with the U251 and U87 control cell lines, 95 differential proteins were screened in the U251-TMZ and U87-TMZ cell lines, of which 28 proteins were upregulated and 67 proteins were down-regulated. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of the co-upregulated proteins showed that most of the differentially expressed proteins were located in the cytoplasm and were significantly upregulated in the biological processes related to vesicular transport in the intimal system and inflammatory response mediated by myeloid leukocytes. Seven candidates were identified as potential GBM markers of TMZ resistance. Combined with existing research findings, our study supports that UAP1L1 and BCKDK are promising potential markers of TMZ resistance in GBM. This is important for further understanding the molecular mechanisms that drive the development and enhancement of TMZ resistance.

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4 **proteomics**

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## Abstract

In this study, to screen for candidate markers of Temozolomide (TMZ) resistance in glioblastoma, we artificially established TMZ drug-resistant glioblastoma (GBM) cell lines, U251-TMZ and U87-TMZ. In the U251-TMZ and U87-TMZ cell lines, we screened and analyzed differentially expressed proteins using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) differential proteomics. Compared with the U251 and U87 control cell lines, 95 differential proteins were screened in the U251-TMZ and U87-TMZ cell lines, of which 28 proteins were upregulated and 67 proteins were down-regulated. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of the co-upregulated proteins showed that most of the differentially expressed proteins were located in the cytoplasm and were significantly upregulated in the biological processes related to vesicular transport in the intimal system and inflammatory response mediated by myeloid leukocytes. Seven candidates were identified as potential GBM markers of TMZ resistance. Combined with existing research findings, our study supports that UAP1L1 and BCKDK are promising potential markers of TMZ resistance in GBM. This is important for further understanding the molecular mechanisms that drive the development and enhancement of TMZ resistance.

## Introduction

Glioblastoma (GBM) is one of the most common and recurrent primary malignant brain tumors. GBM accounts for approximately 46% of all diagnosed brain tumors. According to statistics, 2.7% of cancer-related deaths are caused by GBM . The average survival time of GBM patients is only 12–15 months . Extensive studies have been performed to understand and treat GBM, but the results remain unsatisfactory. The short survival time is related to the anatomical location of the tumor, heterogeneity of GBM cells, and rapid growth of GBM(Shergalis et al. 2018). The incidence of GBM increases with age, and the incidence in men is slightly higher than that in women(Yang et al. 2019a). It is listed as the third most common cause of cancer-related death in patients aged 15–34 years. Clinically, standard GBM treatment includes the maximum safety of surgical resection of tumors, radiotherapy, and adjuvant chemotherapy(Delgado-Lopez & Corrales-Garcia 2016).

Since Temozolomide (TMZ) was approved by the FDA in 2005, it has been widely used as a standard chemotherapy regimen for GBM diagnosis (Hanif et al. 2017; Xu et al. 2020). TMZ can cross the blood-brain barrier and can be administered orally. It is activated by conversion to the metabolite 5-(3-methyltriazen1-yl) imidazole-4-carboxamide (MTIC) at physiological pH values (Stupp et al. 2014). However, TMZ is also a key factor in drug resistance and recurrence. Due to the high heterogeneity and mutagenicity of GBM and extensive exposure to TMZ, > 50% of GBM patients treated with TMZ do not respond to treatment(Lee 2016). However, TMZ remains the key to the treatment of high-level gliomas. TMZ resistance is a significant issue that must be overcome for the successful treatment of GBM.

At present, proteomics technology can reflect the tumor phenotype more accurately, and its advantage is better than genome and transcriptome analyses(Khalil 2007). The level of gene

54  expression is not always related to the level of protein expression, and the process of post-
55  translational repair cannot be detected(Niclou et al. 2010). Because of the limited predictive
56  markers of TMZ resistance(Arora & Somasundaram 2019), anti-TMZ resistance has become
57  more complex for GBM treatment(Wick & Platten 2014). Therefore, it is urgent to identify
58  reliable biomarkers that can be used to determine TMZ resistance.
59  In this study, two classic GBM cell lines, U251 and U87, were used to identify reliable
60  biomarkers for TMZ resistance. Firstly, TMZ-resistant U251 and U87 GBM cell lines were
61  constructed, and the protein expression profiles of U251-TMZ and U87-TMZ were compared
62  using the non-quantitative labeling proteomic technique ultra-performance liquid
63  chromatography-mass spectrometry (UPLC-MS). Twenty-seven upregulated differential proteins
64  were screened, among which seven were identified according to the expression difference
65  multiple and the credibility of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment
66  analysis. Bioinformatics analysis of differentially expressed proteins showed that the upregulated
67  differentially expressed proteins were mainly related to energy metabolism, ribosome synthesis,
68  and processing, and were enriched in endocytosis, carbon metabolism, and antibiotic synthesis.
69  Our study summarizes the protein expression profiles in U251 and U87TMZ drug-resistant cell
70  lines, which provides a basis for a better understanding of TMZ resistance in disease.
71

## Materials & Methods

72

**Materials**

73

74  U251 and U87 cell lines were purchased from Tianjin Genink Biotechnology Co.,Ltd; DMEM and fetal
75  bovine serum were purchased from Gibco; Cell Counting Kit-8 was purchased from Dojindo
76  Laboratories.

**Screening of the U251 U87-TMZ cell line**

77

78  Glioma cells in the logarithmic phase were added with 200 $\mu$M TMZ solution (diluted with DMEM
79  complete medium). After 24 h, the medium was replaced, and 200 $\mu$M TMZ solution was added after 2 d
80  of culture. When glioma cells proliferate and there is no significant change in cell morphology, glioma
81  cells were treated with a higher concentration of TMZ according to the procedure described above.
82  Repeat the above steps as the TMZ concentration gradually increases, such as 300, 400, 500 $\mu$M. to
83  obtain TMZ resistant cell lines for 60 days (Lee 2016). Viability curve of U87-TMZ and U251-TMZ for
84  TMZ resistance compared with their parental cell lines were demonstrated in Supplementary Fig. 1.
85

**Liquid-phase MS**

86

87  The type of liquid chromatography was EASY-nLC1200 (Thermo Fisher) and the tandem mass
88  spectrometer was Q Exactive HF (Thermo Fisher). The peptides were digested with phase A (0.1%
89  formic acid) loaded onto a self-made Trap column (100um × 2 cm C18 packing specification is 3umline
90  120A). Different gradients of phase B (80% acetonitrile, 0.1% formic acid, and 19.9% water) were used
91  to elute the trap column. The eluted peptides were separated using an analytical column (150um × 15 cm
92  C18 packing specification, 1.9um120A) to form a charged spray, which was detected using MS. The B
93  phase gradient was 0 min, 7%; 8 min, 14%; 60 min,28%; 72 min, 42%; 73 min, 95%; 78 min, 95%; and
94  79 min, 30%, and the flow rate was 600 nL/min. Mass spectrum parameters: The first-order detector type
95  was an orbital trap (Orbitrap) with a resolution of 120000; Orbitrap scan range, 300–1400m/z; max

96   injection time, 80ms; and AGC Target, 3E6. The second stage adopted a data-dependent scanning mode
97   and the top 20 precursor ions were selected for secondary acquisition in each scan. The secondary
98   detector was the same Orbitrap;  resolution, 15000 at 120m/z; maximum injection time, 19 ms; AGC
99   Target, 2E4; scan range, 200–2000m/z; included charge state, 2–6; collision energy duration, 15 s; mass
100  tolerance, ±7 ppm; isolation window, 1.6 m/z; activation type, HCD; and normalized collision energy,
101  27%.
102  **Data processing and statistical analysis**
103  Microsoft Excel 2016 was used for preliminary data collation. We performed all statistical analyses using
104  GraphPad Prism 8. We used unpaired t test for comparison of cell viability between cell lines. The results
105  were expressed as means ± standard deviations, and $P < 0.05$ was considered significant. We considered p
106  $< 0.05$ to indicate statistical significance. *** $p < 0.001$.**** $p < 0.0001$.
107  MaxQuant software (version 1.6.1.0) was used to search the database of the original data collected using
108  MS. Restriction endonuclease digestion method: trypsin/P, and a maximum of two missing sites were
109  allowed. Variable modification: oxidation (M) and acetyl (protein N-term). Fixed modification:
110  Carbamidomethyl (C), a maximum of five variable modifications, was allowed for each peptide. The
111  first-level quality deviation was 20ppm. The second-level quality deviation was 20ppm. The database was
112  Uniprot's SWISS database (containing 20431 annotated proteins, released2019.07). The minimum peptide
113  length was seven amino acids, and the maximum peptide mass was 5000 Da. The false discovery rate
114  (FDR) of spectrum and protein matching was set to 1% and the protein quantitative value was obtained
115  using the iBAQ algorithm.
116  After the median normalization of the original quantitative value, log2 transformation was performed to
117  ensure that it met the normal distribution criteria, and the quantitative value of each sample met the
118  normal distribution criteria. Proteins that could be statistically analyzed were selected (quantification of at
119  least three samples in one group) and the minimum value for vacancies worthy of interpolation was
120  selected. The difference in protein was analyzed using the R software t-test and function, and the p value
121  was corrected using the Benjamini-Hochberg algorithm. Proteins with adjusted $P < 0.05$, and fold change
122  $> 2$ were considered differentially expressed proteins. The upregulated differential proteins were analyzed
123  using the Cluster Profiler Package for GO and KEGG pathway enrichment analyses. Protein-protein
124  interaction (PPI) analysis (STRING database, version 11.0) was also performed.
125

# Results

**Results 1. Screening of TMZ-resistant cell lines**

128  U251 and U87 glioma cell lines in the logarithmic growth phase were added to the 200 $\mu M$ TMZ solution
129  in the beginning and cultured for 2 d followed by the addition of TMZ solution. The above procedures
130  were repeated until the proliferation and morphology of glioma cells did not change significantly at this
131  TMZ concentration. Replace higher concentration of TMZ sequentially and repeat the abovementioned
132  steps to construct TMZ-resistant U251-TMZ and U87-TMZ cell lines. In addition to observing the
133  morphological changes in cells to confirm the state of drug resistance, we also determined the OD value
134  of cell-counting kit-8 (CCK8). As shown in Fig.1A and B, there was no significant difference in
135  morphology and quantity between the control and + TMZ groups of the U251-TMZ and U87-TMZ cell
136  lines. The OD values of CCK8 in the U251-TMZ and U87-TMZ cell lines were significantly higher than
137  those of their respective control groups. Therefore, we successfully constructed and screened TMZ-
138  resistant glioma U251 and U87 cell lines.
139

**Results 2. Data quality control**

To better analyze the differential protein expression between TMZ drug-resistant cell lines U251 and
U87, the quantitative data were normalized to the median. A comparison of the results before and after
normalization is shown in Fig.2A. Normalized data were analyzed using Pearson's correlation coefficient
(Fig.2B). The correlation coefficients between the samples were higher than those between the groups,
and there were significant differences in protein expression between the two groups. Additionally, the
quantitative data were also analyzed by cluster analysis, and the results showed that each group of
biological repeats was clustered together, indicating high similarity and good data repeatability (Fig.2C).
Analysis of the coefficient of variation of the normalized data (Fig.2D) showed that the coefficient of
variation was low, and the quantitative stability of the protein was high.

**Results 3. Protein quantitative analysis**

In this study, some of the 4160 proteins detected were statistically available for analysis, including 3308
in the U251 group and 3621 in the U87 group. We screened the differentially expressed proteins in the
four groups, particularly the upregulated proteins, and focused on screening and analyzing the
differentially upregulated proteins in the U251-TMZ and U87-TMZ cell lines.

**Results 3.1 Cross-comparison of differential proteins between the U251-TMZ and U87-TMZ cell
lines**

A total of 1403 differential proteins were detected in the U251 group, of which 467 were upregulated and
936 were down-regulated in the U251-TMZ cell line. A total of 676 differentially expressed proteins were
detected in the U87 group, of which U87-TMZ upregulated 386 proteins and down-regulated 290 (see
Supplementary Data). Because of the heterogeneity of GBM, we only screened 28 proteins upregulated
from the upregulated proteins of the U251-TMZ and U87-TMZ cell lines (three repetitive samples), and
screened 67 down-regulated proteins from their respective down-regulated proteins. A total of 95
differential proteins were screened, as shown in the differential protein clustering thermogram (Fig. 3A).
Owing to the lack of statistical significance of the down-regulated proteins, we did not perform follow-up
bioinformatics analysis of the down-regulated proteins, but focused on the 28 proteins that were
upregulated. For each quantifiable protein, the fold change was logarithmically calculated from a base of
2, and the P value was logarithmically calculated from a base of 10 to create a volcano plot ( Fig. 3B).

**Results 4. Functional annotation enrichment analysis of protein**

**4.1 GO and KEGG pathway analysis of the U251-TMZ and U87-TMZ cell lines**

A total of 28 proteins upregulated in the U251-TMZ and U87-TMZ cell lines were analyzed using GO
and KEGG pathways, respectively. The possible pathways from biological process (BP), cellular
component (CC), and molecular function (MF) were screened. As shown in Fig.4A, these differentially
expressed proteins were significantly upregulated in biological processes related to myeloid leukocyte
activation, myeloid leukocyte-mediated immunity, leukocyte degranulation, and inflammation. Based on
GO cell composition analysis, most of them were located in the cytoplasm and may be involved in vesicle
transport in the intimal system. In addition, these proteins exhibited GO molecular functions of "catalytic
activity" and "binding," such as aminopeptidase activity and coenzyme binding. These results suggest that
the development of TMZ drug resistance may be related to intracellular immune regulation and energy
metabolism(Fu et al. 2021; St-Coeur et al. 2015).

183    In addition, the enrichment of the 28 protein KEGG pathways upregulated in the U251-TMZ and U87-

184    TMZ cell lines mainly included carbon metabolism, antibiotic biosynthesis, secondary metabolite

185    biosynthesis, glyoxylic acid and dicarboxylic acid metabolism, and endocytosis (Fig.4B).

186    **Results 4.2 Differential protein interaction network**

187    In the interaction network of proteins upregulated in the U251-TMZ and U87-TMZ cell lines (Fig.4C),

188    the changes in these proteins are related to immune regulation and vesicular transport, as well as to energy

189    metabolism, such as the mitochondrial stress response.

190    **Results 5 Potential GBM markers of TMZ resistance**

191    In the MS analysis of differentially expressed proteins in the U251/U251-TMZ and U87/U87-TMZ cell

192    lines, 28 proteins were upregulated. Through manual screening, seven proteins with the highest

193    differential expression in the two groups were identified: UGT1A7, BCKDK, UAP1L1, RAB3GAP1,

194    VWA8, HLA-B, and VPS26B (Table.1). At present, there is no research on the relationship between these

195    seven proteins and TMZ resistance. Among these proteins, only UAP1L1 was related to GBM studies,

196    and the expression of UAP1L1 in glioma tissues was significantly upregulated. Increased expression of

197    UAP1L1 is associated with higher tumor grade and poor prognosis. UAP1L1 may promote the

198    proliferation of glioma cells by regulating glycosylation of key proteins(Yang et al. 2021b).

199    Another noteworthy protein, BCKDK, has recently been found to regulate the catabolism of branched

200    chain amino acids (BCAA) and enhance MEK/ERK signal transduction, which is a key pathway driving

201    the growth and proliferation of cancer cells(Xue et al. 2017). Among the remaining five candidate

202    proteins, it has been reported that UGT1A7 may affect the progression of pancreatic cancer(Yilmaz et al.

203    2015). Rab3GAP1 was first isolated from the synaptic soluble section of the rat brain as an Rab3A-

204    binding protein, and it exhibits GAP activity against Rab3(Handley & Aligianis 2012). Rab3GAP

205    deficiency leads to neurological disorders(Muller et al. 2011). VWA8 is expressed in organs with high

206    energy requirements, including those with a high density of mitochondria(Luo et al. 2017). Upregulation

207    of VWA8 expression was negatively correlated with brain metastasis associated with breast cancer(Yuan

208    et al. 2018). HLA is necessary for T-cell activation and is associated with multiple sclerosis (Hollenbach

209    et al. 2016). VPS26 is one of the components of the cargo recognition core of the reverse transcriptase

210    complex, which is significantly decreased in the hippocampus of patients with Alzheimer's disease.

211    Changes in reverse transcriptase composition are related to an increased risk of Alzheimer's disease(Small

212    et al. 2005; Vagnozzi & Pratico 2019).

213    **Results 6 Verification of potential markers of TMZ resistance**

214    To verify the results of MS analysis of the U251/U251-TMZ and U87/U87-TMZ cell lines, we performed

215    western blotting on seven selected markers: UGT1A7, BCKDK, UAP1L1, RAB3GAP1, VWA8, HLA-B,

216    and VPS26B. The results showed that in the four cell lines, the above seven proteins were successfully

217    upregulated in the U251-TMZ and U87-TMZ cell lines compared to the control groups (Fig. 5). Tet-On

218    systems for doxycycline (Dox)-inducible gene expression were applied in our two resistant cell lines. The

219    addition of Dox reduced the expression of these potential target genes. The cell growth was inhibited after

220    the addition of Dox at Day 4 to reduce the target gene and was restored after removal of Dox at Day

221    10 (Figure 6, Supplementary Fig. 2). Furthermore, the results of RT-qPCR suggested higher expression of

222    BCKDK and UAP1L1 in TMZ resistant glioma tissues than in TMZ sensitive glioma tissues

223    (Supplementary Fig. 3). The aforementioned results verified that upregulation of these target gene

224    possibly mediated the TMZ resistance.

225

## Discussion

226
227 Neoplasm remains the main killer worldwide (Li et al. 2023; Li et al. 2018; Ma et al. 2016; Sun et al.
228 2021; Wu et al. 2018; Wu et al. 2017; Yang et al. 2019b; Zhang et al. 2023). TMZ is the preferred
229 alkylating agent for the treatment of GBM, and it induces cell death through DNA damage. Most patients
230 develop TMZ resistance during treatment. Activation of the DNA repair pathway is the main mechanism
231 underlying this phenomenon. With the deepening of research, newly proposed mechanisms of drug
232 resistance, such as the regulation of microRNAs, membrane transporters, gap junction activity, and
233 autophagy, still cannot solve this issue. There is a lack of effective treatments to maintain the effects of
234 TMZ. In the present study, we screened and analyzed the differentially expressed proteins using UPLC-
235 MS differential proteomics in two TMZ-resistant GBM cell lines, namely U251-TMZ and U87-TMZ.
236 Compared with the U251 and U87 cell lines, 95 differential proteins were screened in the U251-TMZ and
237 U87-TMZ cell lines, of which 28 proteins were upregulated and 67 proteins were down-regulated. The
238 GO analysis demonstrated that the development of TMZ drug resistance may be related to intracellular
239 immune regulation and energy metabolism of leukocytes. The KEGG analysis showed that the enriched
240 pathways were related to energy metabolism. These preliminary data indicated the key role of energy
241 metabolism in the development of TMZ resistance in GBM cell lines(Fu et al. 2021; Manni & Min 2022;
242 Mao et al. 2022). Through manual screening, we further identified seven proteins as potential GBM
243 markers of TMZ resistance. Following this investigation and analysis of existing studies, we propose for
244 the first time that UAP1L1 and BCKDK are potential markers of TMZ resistance in GBM.
245           UAP1L1 is a collateral homologue of UAP1 that participates in protein glycosylation and
246 promotes the proliferation of some tumors. UAP1L1 is significantly upregulated in hepatocellular
247 carcinoma tissues, and a high level of UAP1L1 expression is associated with poor prognosis (Lai et al.
248 2019). Recent studies have found that the expression of UAP1L1 is significantly upregulated in glioma
249 tissues. Increased expression of UAP1L1 is associated with higher tumor grade and poor prognosis.
250 UAP1L1 may promote the proliferation of glioma cells by regulating glycosylation of key proteins(Yang
251 et al. 2021b). However, the specific mechanism by which UAP1L1 regulates glioma cell proliferation,
252 apoptosis, and TMZ drug resistance is still poorly understood. BCKDK is a key regulator of branched-
253 chain ketoacid dehydrogenase complex activity, and inactivates branched-chain ketoacid dehydrogenase
254 through phosphorylation. Recent studies have shown that BCKDK regulates the catabolism of branched
255 chain amino acids (BCAA) and enhances MEK/ERK signal transduction, which is the key pathway that
256 drives the growth and proliferation of cancer cells(Xue et al. 2017). In patients with colorectal cancer,
257 high BCKDK expression is associated with poor prognosis(Wang et al. 2021). In addition, BCKDK can
258 alter the metabolism of non-small cell lung cancer(Wang et al. 2021).
259           Owing to time constraints, we found only these two possible markers. GBM is one of the most
260 difficult cancers to overcome in solid tumors. Photothermal therapy (PTT), photodynamic therapy (PDT),
261 and sonodynamic therapy (SDT) are used in the field of oncology treatment as minimally invasive
262 methods, as well as immunotherapies that are considered the most promising treatments. These treatments
263 are very dependent on the use of active drugs, which are often designed for delivery into tumors. In
264 addition, novel peptide-based molecular probes and multifunctional nanomaterials are used as different
265 biomarkers and applied to tumor imaging during treatment. Therefore, nanomaterials based on self-
266 assembled peptides have great potential to effectively improve the therapeutic effect on tumors by
267 maximizing immunotherapy and non-invasive treatments(Guo et al. 2022; Liu et al. 2022; Ma et al. 2020;
268 Ren et al. 2020; Yang et al. 2021a). In a follow-up study, it will be necessary to conduct an in-depth study

269 of BCKDK and UAP1L1 based on the mechanism of TMZ resistance in GBM. This will be of great
270 significance for the diagnosis, development, and application of TMZ resistance in GBM in the future.
271

## Conclusions

273 In this study, we established TMZ drug-resistant cell lines, U251-TMZ and U87-TMZ, in GBM. Through
274 the screening and analysis of differential proteins using UPLC-MS differential proteomics technology, 95
275 differential proteins were screened using the U251-TMZ and U87-TMZ cell lines, of which 28 proteins
276 were upregulated and 67 proteins were down-regulated. The co-upregulated proteins were studied using
277 GO and KEGG analyses. Most of the co-upregulated differentially expressed proteins were located in the
278 cytoplasm and were significantly upregulated in biological processes related to endocytosis and myeloid
279 leukocyte-mediated immune response, and may be involved in vesicle transport in the intimal system.
280 These findings suggest that TMZ resistance may be related to immune regulation of leukocytes and
281 intimal system transport. Subsequently, we screened and identified seven potential candidates for TMZ
282 resistance. Combined with existing research, we propose for the first time that UAP1L1 and BCKDK are
283 promising potential markers of TMZ resistance in GBM.

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401

# Figure 1

Fig. 1. Screening of TMZ-resistant glioma cell lines U251 and U87.

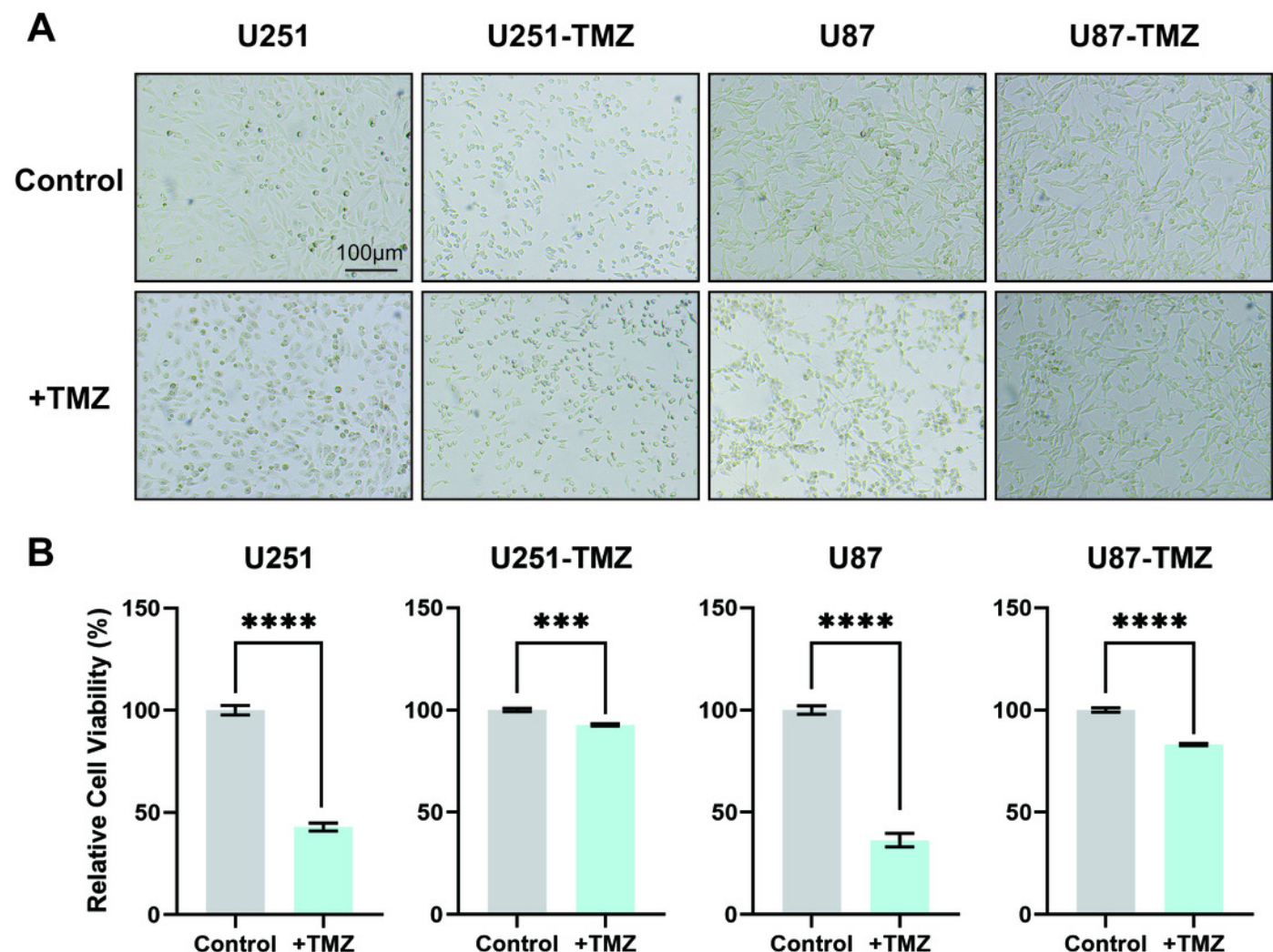
The culture status of U251-TMZ, U87-TMZ cells and control cell lines. (A) .The relative cell viability of U251-TMZ, U87-TMZ cells and control cell lines, as measured by CCK-8 assay (B).

# Figure 2

Fig. 2. Quantitative protein data quality control.

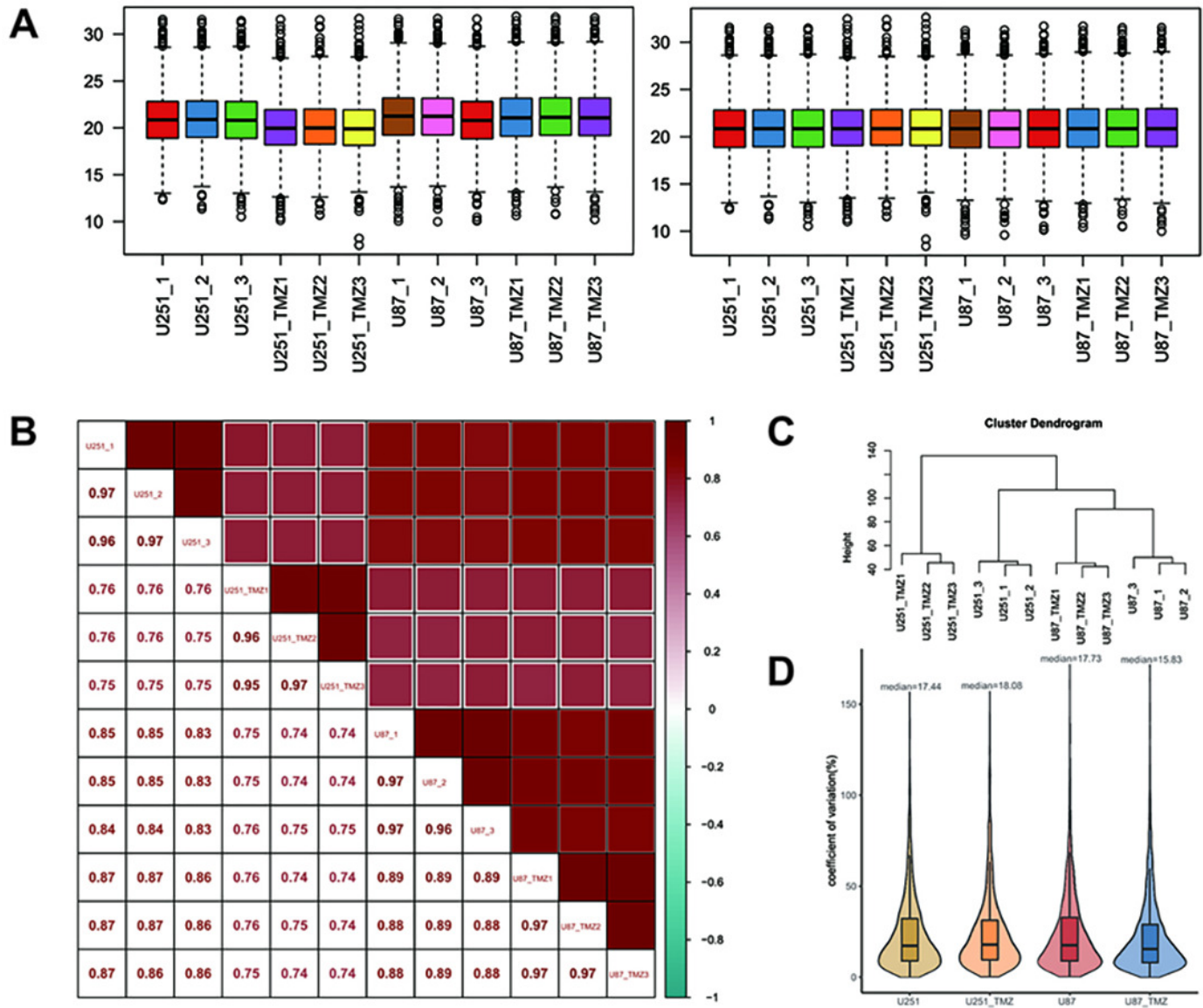
Before and after the median normalization (A left and right). The correlation between samples (B). The correlation between U251-1 and U251-2 is 0.97, the correlation between U251-1 and U251-3 is 0.96, the correlation between U251-2 and U251-3 is 0.97, the higher the correlation is, the closer the sample is. Cluster analysis of U251, U87, U251-TMZ and U87-TMZ samples (C). The coefficients of variation of U251, U87, U251-TMZ and U87-TMZ proteins were analyzed (D).

# Figure 3

Fig. 3. Differential protein cross-comparison.

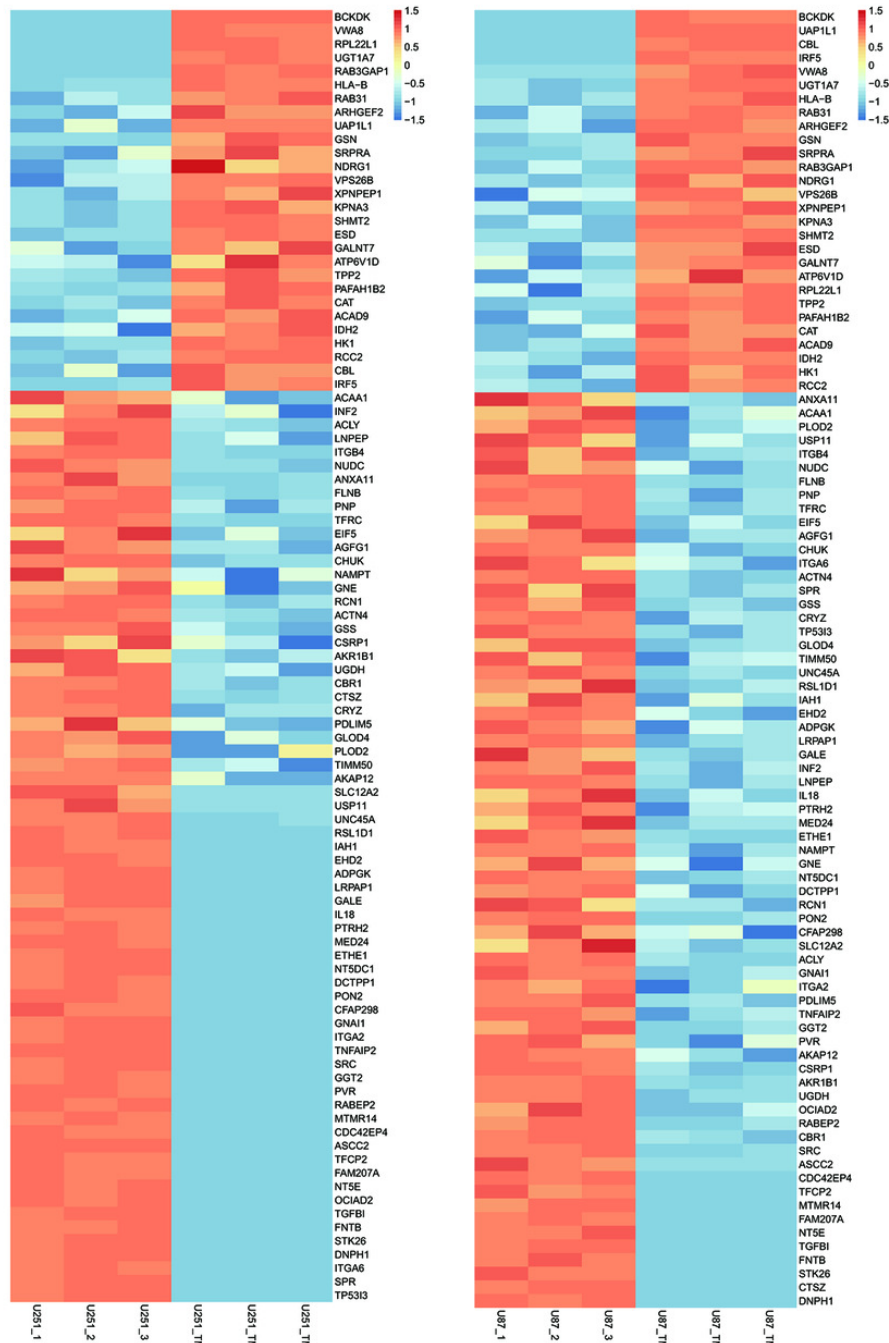
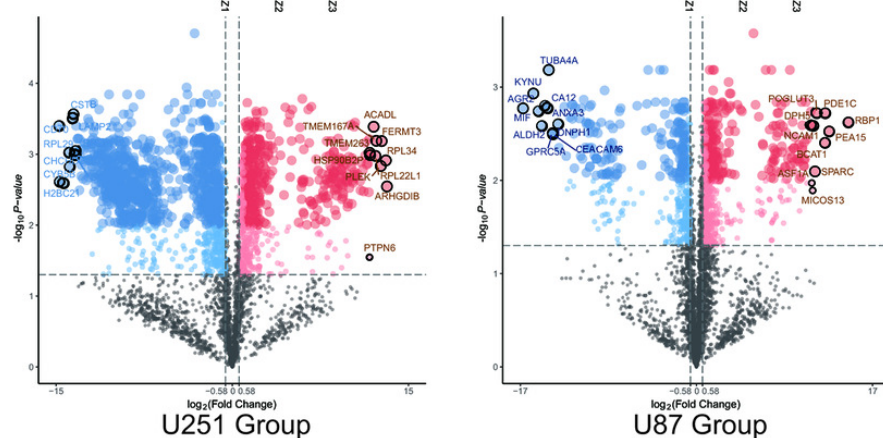
The differential proteins of U251-TMZ and U251 (left), U87-TMZ and U87 (right) were clustered respectively. U251-TMZ and U87-TMZ up-regulated 28 proteins and down-regulated 67 proteins (A). U251-TMZ and U251, U87-TMZ and U87 can be statistically analyzed protein volcano diagram (B).

# Figure 4

Fig. 4. Functional analysis of proteins up-regulated by U251-TMZ and U87-TMZ.

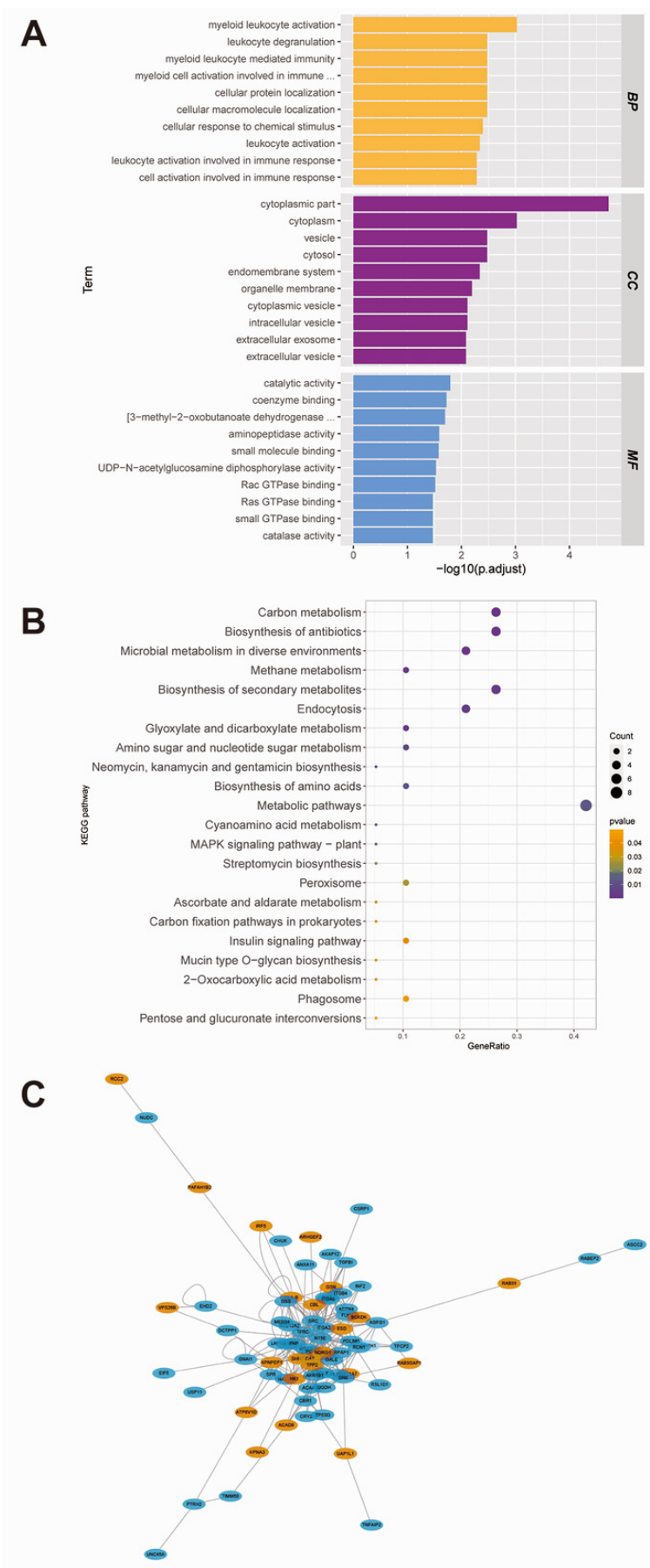
GO analysis of proteins up-regulated by U251-TMZ and U87-TMZ, including biological processes (Biological Process BP), cellular components (CC), and molecular function MF (A). KEGG signal pathway enrichment analysis of proteins up-regulated by U251-TMZ and U87-TMZ (B). Differential protein interaction network (C).

# Figure 5

Fig. 5. Validation protein level expression of these upregulated genes by western blots.

(A) Protein expression of UGT1A7, BCKDK, UAP1L1, RAB3GAP1, VWA8, HLA-B and VPS26B in U251 and U251-TMZ cells. (B) Protein expression of UGT1A7, BCKDK, UAP1L1, RAB3GAP1, VWA8, HLA-B and VPS26B in U87 and U87-TMZ cells. (C) Quantification of three replicated data of each gene normalized to GAPDH from (A). (D) Quantification of three replicated data of each gene normalized to GAPDH from (B).

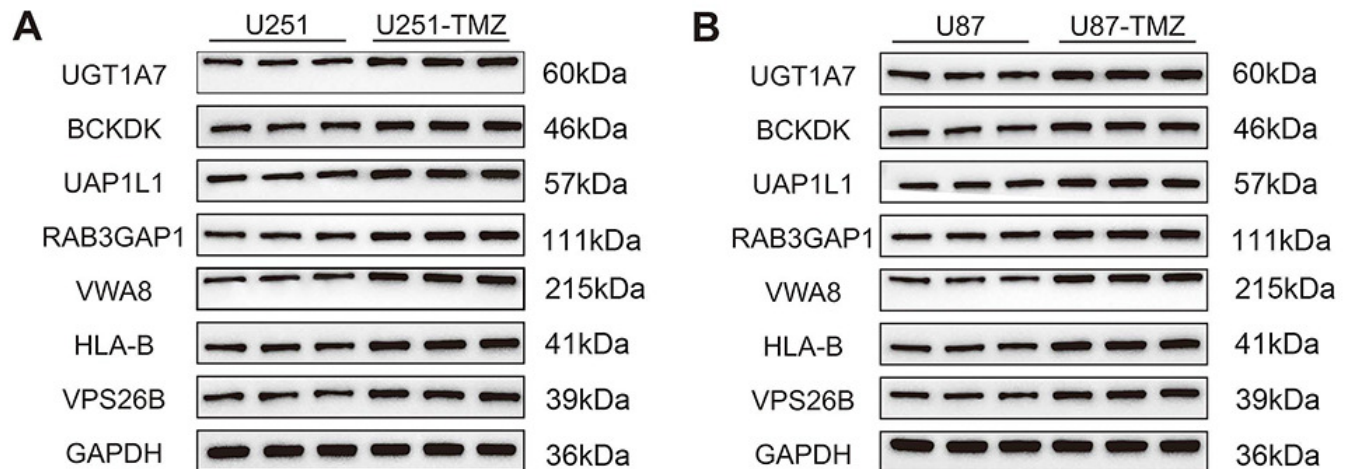



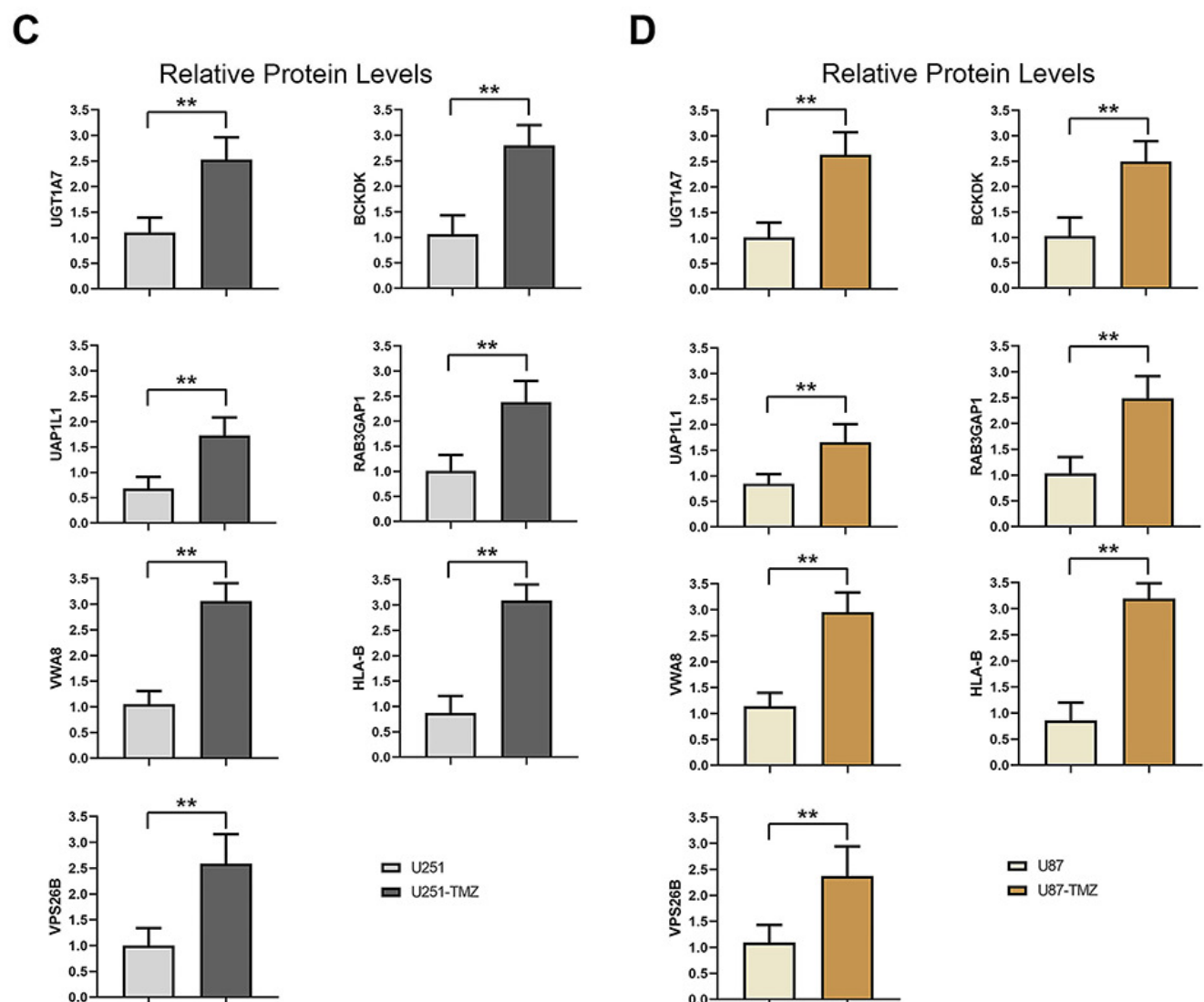
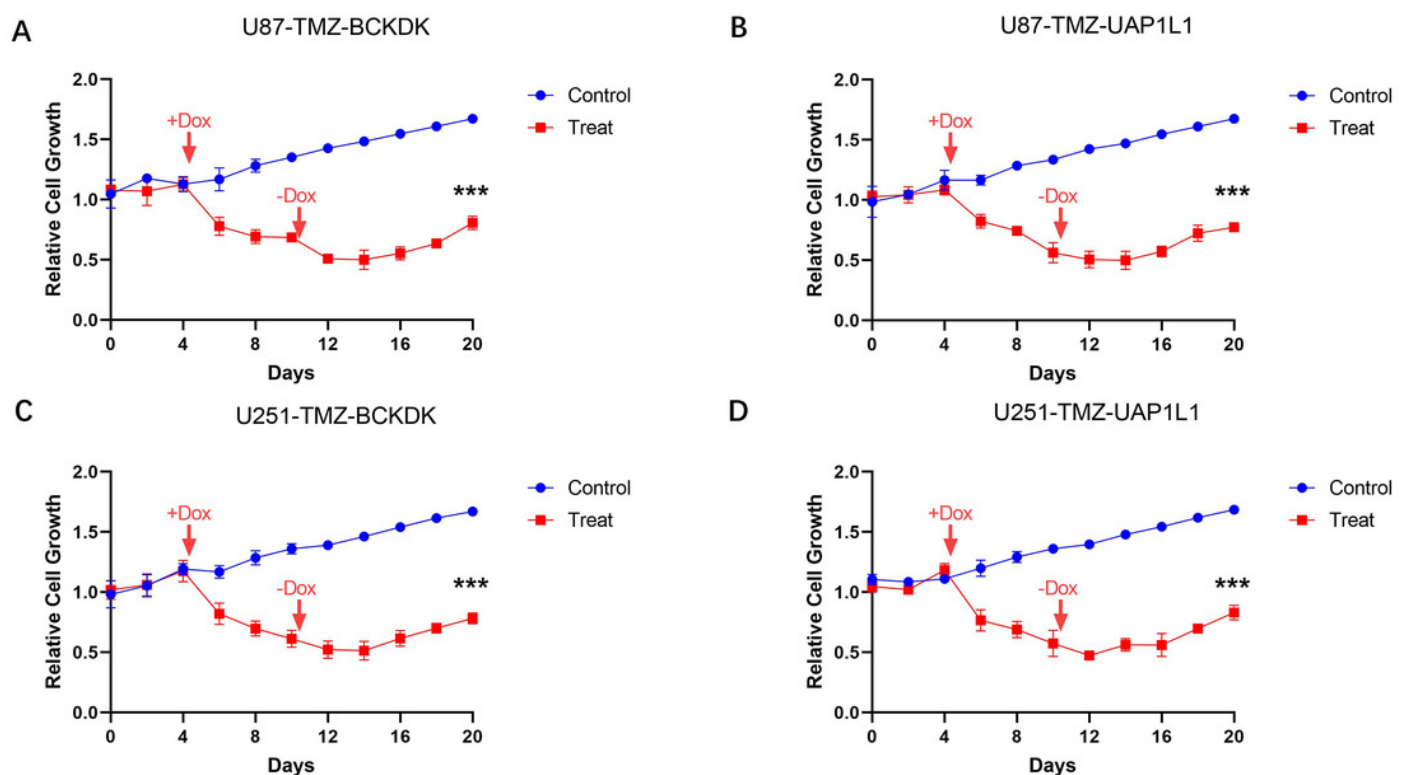
Fig. 5. Western Blotting showing the levels of upregulated proteins in U251/U251-TMZ and U87/U87-TMZ

# Figure 6

Fig. 6. Verification of potential markers of TMZ resistance through knockdown of upregulated genes.

The doxycycline were added at Day 4 to reduce the expression of these four potential target gene, BCKDK (A), UAP1L (B) in U87-TMZ cells and BCKDK (C), UAP1L (D) in U251-TMZ cells and removed at Day 10.

Potential markers for TMZ resistance in GZM

1                  Table 1. Potential markers for TMZ resistance in GZM

| Gene Symbol | Gene ID | Description |
| --- | --- | --- |
| UGT1A7 | 54577 | UDP glucuronosyltransferase family 1 member A7 |
| BCKDK | 10295 | branched chain keto acld dehydrogenase kinase |
| UAP1L1 | 91373 | UDP-N-acelylglucosamine pyrophosphorylase 1 like 1 |
| RAB3GAP1 | 22930 | RAB3 GTPase aclivating protein catalyic subunit 1 |
| WWA8 | 23078 | von Willebrand factor A domain containing 8 |
| HL A-B | 3106 | major histocompatblity complex, class I, B |
| VPS26B | 112936 | VPS26, retromer complex component B |