1   *Manuscript for (Article)*

2

Integrative Omics Uncovers Low Tumorous Magnesium Content as A Driver Factor of Colorectal Cancer

5

6   Rou Zhang[1,#], Meng Hu[1,#], Yu Liu[1,#], Wanmeng Li[1], Zhiqiang Xu[1], Siyu He[1], Ying Lu[1], Yanqiu

7   Gong[1], Xiuxuan Wang[1], Shan Hai[1], Shuangqing Li[1], Shiqian Qi[1], Yuan Li[4], Yang Shu[2], Dan

8   Du[3], Huiyuan Zhang[1], Heng Xu[1], Zongguang Zhou[2,4], Peng Lei[1,*], Hai-Ning Chen[2,*], Lunzhi

9   Dai[1,*]

10

11   *[1] National Clinical Research Center for Geriatrics and General Practice Ward/International*

12   *Medical Center Ward, General Practice Medical Center, State Key Laboratory of Biotherapy,*

13   *West China Hospital, Sichuan University, Chengdu 610041, China*

14   *[2] Colorectal Cancer Center, Department of General Surgery, West China Hospital, Sichuan*

15   *University, Chengdu 610041, China*

16   *[3] Advanced Mass Spectrometry Center, Research Core Facility, Frontiers Science Center for*

17   *Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu 610041,*

18   *China*

19   *[4] Institute of Digestive Surgery, West China Hospital, Sichuan University, Chengdu 610041, China*

20

21   # Equal contribution.

22   * Corresponding authors.

23   E-mail: lunzhi.dai@scu.edu.cn (Dai L), hnchen@scu.edu.cn (Chen HN), peng.lei@scu.edu.cn

24   (Lei P).

25

26   **Running title**: *Zhang R et al / The Role of Magnesium in Colorectal Cancer*

27

28   Total word counts (from "Introduction" to "Materials and methods"): 8109

29   References: 83

30   Article title: 84 characters

31   Running title: 47 characters

32   Keywords: 5

33   Abstract: 200

34   Total figures: 7

35   Total tables: 0

36   Total supplementary figures: 8

37   Total supplementary tables: 8

38   Total supplementary files: 0

39

Abstract

Magnesium (Mg) deficiency is associated with increased risk and malignancy of colorectal cancer (CRC), yet the underlying mechanisms remain elusive. Here we used genomic, proteomic, and phosphoproteomic data to elucidate the impact of Mg deficiency on CRC. Genomic analysis identified 160 genes with higher mutation frequencies in Low-Mg tumors, including key driver genes such as *KMT2C* and *ERBB3*. Unexpectedly, initiation driver genes of CRC, such as *TP53* and *APC*, displayed higher mutation frequencies in High-Mg tumors. Additionally, proteomics and phosphoproteomics indicated that low tumorous Mg content may activate epithelial-mesenchymal transition (EMT) by modulating inflammation or remodeling the phosphoproteome of cancer cells. Notably, we observed a negative correlation between the phosphorylation of DBN1 at S142 (DBN1^{S142p}) and Mg content. A mutation in S142 to D (DBN1^{S142D}) mimicking DBN1^{S142p} upregulated MMP2 and enhanced cell migration, while treatment with MgCl$_2$ reduced DBN1^{S142p}, thereby reversing this phenotype. Mechanistically, Mg^{2+} attenuated the DBN1-ACTN4 interaction by decreasing DBN1^{S142p}, which, in turn, enhanced the binding of ACTN4 to F-actin and promoted F-actin polymerization, ultimately reducing MMP2 expression. These findings shed new light on the crucial role of Mg deficiency in CRC progression and suggest that Mg supplementation may offer a promising preventive and therapeutic strategy for CRC.

KEYWORDS: Magnesium deficiency; colorectal cancer; genome instability; tumor metastasis; phosphorylation; DBN1

Introduction

61 Metal ions are crucial in both physiological and pathological processes within living organisms[1,

62 2]. Magnesium (Mg), a predominant intracellular divalent cation, is crucial for maintaining cellular

63 homeostasis and participates in nearly all cellular processes[3, 4]. The intracellular Mg content is

64 up to 10−30 mM, however, the concentration of free Mg^{2+} in cells is only 0.5−1.2 mM[5]. Mg^{2+}

65 can bind to ATP, ribosomes or nucleotides as a cofactor and serves as an activator of numerous

66 enzymes involved in glycolysis, phosphorylation events, DNA repair, DNA stabilization, and

67 protein synthesis[6−8]. Many proteins, such as MRS2, TRPM6/7, MAGT1, SCL41A1, and

68 CNNMs, are well-established Mg^{2+} transporters[9]. Dysregulation of these transporters may lead

69 to Mg^{2+} imbalance and diseases.

70

71 Mg is crucial in controlling cancer initiation and progression[5, 10]. Disruptions in Mg

72 homeostasis contribute to cancer progression by promoting proliferation, angiogenesis, and

73 invasion of cancer cells into surrounding tissues[11−13]. Additionally, changes in Mg levels can

74 impair immune function, compromising the body's ability to detect and eliminate cancerous

75 cells[14, 15]. Furthermore, Mg's dysregulation affects chemoresistance, rendering cancer cells less

76 susceptible to chemotherapy[16, 17]. Colorectal cancer (CRC) ranks as the third most common

77 cause of cancer-related deaths globally. A meta-analysis of 29 studies demonstrated that increased

78 Mg intake is linked to a reduced risk of CRC[18−21]. Previous investigations showed that Mg's

79 anti-inflammatory properties may help reduce inflammation in the colon[22, 23], which is a risk

80 factor for CRC[24]. Moreover, Mg functions as an antioxidant, protecting colon cells against

81 oxidative stress and preventing DNA damage[25−27]. In CRC treatment, Mg can enhance the

82 effectiveness of chemotherapy by improving drug uptake by cancer cells and protecting healthy

83 cells from damage[28].

84 Despite some progress, many questions about the role of Mg in CRC remain unanswered.

85 First, there is a lack of systematic investigation on how Mg affects tumor progression at the

86 molecular level. Second, it is unclear whether Mg has a differential effect on left- and right-sided

87 CRC at the molecular level[29]. Third, tumor metastasis is a leading cause of mortality in CRC

88 patients. Mg deficiency not only reduces intracellular ATP[30] but also affects kinase and

89 phosphatase activities[31],[32]. Dysregulated protein phosphorylation is common in CRC and

90 linked to unfavorable outcomes[33−36]. However, the direct link between Mg deficiency and

91 tumor metastasis through affecting protein phosphorylation still lacks direct evidence.

92   With the rapid development of omics technologies, the functional interpretation of metal ions

93   in aging using omics data has been achieved[37]. In this study, we utilized an integrative genomic,

94   proteomic, and phosphoproteomic approach to uncover the roles of Mg in CRC and found that low

95   Mg content in tumors affected both genomic stability and metastasis. Genomics revealed that low

96   Mg content in tumors increased the gene mutations associated with tumor progression rather than

97   initiation. Proteomics and phosphoproteomics analyses indicated that low Mg content in tumors

98   could activate epithelial-mesenchymal transition (EMT) by activating the complement pathway

99   and inducing inflammation or by directly remodeling protein phosphorylation in cancer cells.

100   Furthermore, we demonstrated that Mg^{2+} weakened the interaction between DBN1 and ACTN4

101   by reducing the phosphorylation of DBN1 at S142 ($DBN1^{S142p}$), which enhanced the interaction

102   of ACTN4 to F-actin and promoted F-actin polymerization, ultimately leading to downregulated

103   MMP2 and reduced cancer cell migration.

Results

Low Mg content in tumors predicts unfavorable prognosis of CRC patients

106   To uncover the clinical significance of low Mg content in tumors and its impact on tumor

107   progression, omics data of 230 paired tissue samples collected from 115 treatment-naive CRC

108   patients were used (**Figure 1**A; Table S1). Proteomics and phosphoproteomics employed a TMT-

109   based quantitative approach (Figure S1A). Correlation analysis of quality control (QC) samples,

110   internal standard (IS) samples in proteomics and phosphoproteomics, and replicate samples in

111   proteomics demonstrated the stability of the instrument as well as the reliability and reproducibility

112   of mass spectrometry (MS) data (Figure S1B−H). A total of 9652 proteins and 12,988 phosphosites

113   were identified. Of them, 5322 proteins (with > 1 unique peptide) and 2162 phosphosites detected

114   in > 50% of samples were utilized for further data analysis (Figure S1E, F, and S1I−L).

115   Additionally, an inductively coupled plasma-mass spectrometry (ICP-MS) analysis of Mg content

116   was conducted on 115 paired samples, and correlation analysis of QC samples indicated the

117   reliability of the measurements (Figure S1M). Whole-exome sequencing (WES) data were

118   obtained from a previous work[38], in which 16,234 mutated genes with less than 5000 amino

119   acids in 76 paired samples were reported.

120   Our findings showed that the intra-tumoral Mg content ranged from 162−920 μg per gram of

121   extracted protein (Figure 1B). The Wilcoxon rank-sum test identified higher levels of Mg content

122   in tumors than in distant normal tissues (DNTs) (Figure 1C), and the levels of Mg between the

123   left- and right-sided tumors were significantly different (Figure 1D and E). Our Kaplan−Meier

124   survival analysis of 115 CRC patients revealed that lower levels of Mg in tumors were associated

125   with poor overall survival (OS) (Figure 1F). Multivariable Cox regression models demonstrated

126   that Mg content remains significantly associated with CRC survival even after adjusting for

127   prognostic factors, such as renal function and liver function (Figure S2A−J), indicating that Mg

128   content serves as an independent predictor of survival. Moreover, we conducted an independent

129   analysis to investigate the relationship between Mg levels and prognosis in left-sided and right-

130   sided CRC cases. Interestingly, we observed a notable connection between Mg levels and the

131   prognosis of CRC patients specifically in the right-sided cases, while a less significant association

132   was found in the left-sided cases (Figure 1G and H). Additionally, we also did not observe any

133   significant variation in Mg content between tumors in female and male patients (Figure 1I).

134   Therefore, the following correlation analysis between Mg and omics data did not take into account

135   the influence of gender.

136   **Low Mg content in tumors is linked to genome instability**

137   Mg is important for DNA stabilization, DNA replication and DNA repair[39, 40]. To investigate

138   the impact of low Mg content on CRC at the genomic level, we analyzed Mg-associated genomic

139   data. Of the 76 colon cancer cases analyzed by WES, 20 showed hypermutation in tumors (> 10

140   Mut/Mb) (Table S2)[38]. To exclude hypermutations caused by mutated mismatch repair (MMR)

141   genes and major replicase genes, including *MSH2*, *MSH6*, *MLH1*, *PSM2*, *POLD,* and *POLE*, we

142   removed 14 hypermutated samples with mutations in the above genes[38, 41]. Statistics showed

143   that *TP53* had the highest frequency of mutations (48%), followed by *APC* (40%) and *KRAS* (39%),

144   in the remaining 62 colon cancer cases (**Figure 2**A). The total percentage of single nucleotide

145   variants (SNVs) differed between the High-Mg (39 cases) and Low-Mg (23 cases) groups, with

146   the most frequent thymine to cytosine (T > C) transition in the Low-Mg group (Figure 2B).

147   Additionally, the classes of mutations were different between the two groups, and more types of

148   mutations were observed in the High-Mg group (Figure 2C). Association analysis of mutated genes

149   in the two groups using the somatic interactions algorithm showed that mutations in the High-Mg

150   group were mutually exclusive, while mutations in the Low-Mg group mainly co-occurred (Figure

151   2D and E), suggesting that low Mg content may cause simultaneous mutations in many genes,

152   possibly due to increased genome instability and the dysregulation of DNA replication and repair

153   processes[39, 40, 42−44]. In addition, somatic copy number alteration (SCNA) analysis revealed

6

154   distinct patterns in the cytobands at the locations of the variant sites. In the High-Mg group,
155   amplified regions were observed at 5p15.33 and 8p23.1, while in the Low-Mg group, amplified
156   regions were found at 4p16.1, 15q11.1, and 15q11.2. Conversely, deleted regions were identified
157   at 1p36.21, 1p36.33, and 9p11.2 in the High-Mg group and at 17q12 and 17q21.31 in the Low-Mg
158   group (Figure 2F). Among the identified 7,173 mutated genes with less than 5000 amino acids in
159   62 paired samples, the mutation frequencies of 162 genes were associated with Mg content, of
160   which 160 genes exhibited a higher frequency of mutations in the Low-Mg group (Figure 2G and
161   H; Table S2), including 6 known CRC driver genes such as *KMT2C*, *BCL9*, *ERBB3*, *EP300*, *FAT3,*
162   and *CARD11* (Table S2)[45]. To our surprise, the mutation frequencies of *TP53* and *APC* were
163   notably higher in the High-Mg group (Figure 2I).

164   Next, after excluding 4 tumors with unknown locations, we analyzed the impact of low Mg
165   content on the genomic landscape of left-sided and right-sided colon cancer using the remaining
166   58 cases. Initially, we investigated the frequency of gene mutations in colon cancer and observed
167   that the mutation frequencies of *TP53* and *APC* were higher on the left side, whereas *KRAS*
168   exhibited a higher mutation frequency on the right side (Figure 2J), aligning with previous
169   findings[29]. Subsequently, we explored the influence of Mg levels on gene mutations in left-sided
170   and right-sided colon cancer separately. The results revealed that the majority of genes displayed
171   higher mutation frequencies in the Low-Mg group on both sides. Notably, *TP53* demonstrated a
172   higher mutation frequency in the High-Mg group for both left- and right-sided colon cancer,
173   whereas *APC* exhibited a higher mutation frequency exclusively in the High-Mg group of left-
174   sided tumors (72% cases in High-Mg group *vs* 20% cases in Low-Mg group) but not right-sided
175   tumors (5 cases in High-Mg group *vs* 4 cases in Low-Mg group) (Figure 2K). Collectively, these
176   results indicate that low Mg content in tumors probably increases the mutations of genes associated
177   with CRC progression rather than initiation.

178   **Low Mg content in tumors is associated with EMT activation**
179   To further explore the potential roles of Mg in CRC, we conducted a screening of the Mg-
180   associated proteome in tumors according to a previously described strategy[37]. The correlation
181   analysis of the levels of 5322 proteins and Mg content in the tumors revealed 1347 proteins that
182   were positively correlated with Mg content and 1252 proteins that were negatively correlated with
183   Mg content (**Figure 3**A; Table S3). Pathway enrichment analysis revealed that the 1252 proteins
184   negatively correlated with Mg primarily belonged to cell adhesion-related pathways. Conversely,

185 the 1347 proteins positively correlated with Mg were found to be associated with mRNA
186 processing and translation pathways (Figure S3A−D). Further GSEA using 5322 proteins revealed
187 that pathways negatively correlated with Mg content mainly included immune- and metastasis-
188 related pathways (Figure 3B−D), such as the coagulation cascade, complement system, IL6-JAK-
189 STAT3 signaling, TNFα signaling *via* NF-κB, EMT and angiogenesis (Figure 3B and D). On the
190 other hand, pathways positively correlated with Mg content were predominantly related to cell
191 proliferation and the cell cycle (Figure 3C).

192   Additionally, we conducted separate screenings of Mg-associated proteins in left-sided and
193 right-sided CRC (Figure 3E and F), and performed pathway enrichment analysis on these proteins
194 (Figure 3G). The results indicated that proteins negatively correlated with Mg levels in either left-
195 sided or right-sided CRC, or in both sides, were primarily associated with cell adhesion-associated
196 pathways, such as regulation of actin cytoskeleton, actin cytoskeleton organization, focal adhesion,
197 actin filament-based process, and extracellular matrix organization (Figure 3G). It is noteworthy
198 that the complement and coagulation cascades were enriched exclusively in right-sided colon
199 cancer, suggesting a more prominent influence of Mg on the inflammatory response in the right
200 side (Figure 3G). As Mg have demonstrated variations between left-sided and right-sided CRC,
201 we next asked whether Mg has impacts on different subtypes of CRC patients. To achieve this
202 goal, we classified these patients into three subtypes utilizing the top 25% most variable proteins
203 (Figure S4A−C). While the OS did not exhibit a significant difference among the three subtypes,
204 further analysis indicated that subtype II displayed a comparatively lower OS rate and notably
205 lower Mg content in comparison to subtypes I and III (Figure S4D and E). Proteins upregulated in
206 subtype II (subtype II vs non-subtype II) were predominantly enriched in migration-related
207 pathways (Figure S4F). Consistent with prior findings, Mg content closely correlates with the cell
208 adhesion of CRC tumors.

209   Considering the findings from GSEA (Figure 3B and C), it can be inferred that low Mg content
210 in tumors potentially affects tumor metastasis through the regulation of cell adhesion-related
211 pathways. Consistent with our assumption, many proteins linked to cell-cell adhesion, such as tight
212 junction proteins CLDN3, TJP2, and CGN, adherens junction proteins CDH1, CTNNB1, and
213 CTNND1, and desmosome proteins DSC2, DSG2, and JUP, showed a significant positive
214 correlation with Mg content. These proteins were crucial to maintain of the epithelial phenotype
215 (**Figure 4**A; Table S4). In contrast, many cell-matrix adhesion proteins that were critical for

216 maintaining the mesenchymal phenotype exhibited a significant negative correlation with Mg

217 content, such as VCL, ITGA1, ITGB3, FN1, and FGA (Figure 4A). Further immunoblots

218 confirmed increased N-cadherin, vimentin, vinculin, MMP2, and reduced E-cadherin, cingulin in

219 Low-Mg tumors (Figure 4B), indicating the possible role of Mg in the EMT process.

220   To validate the role of Mg in the regulation of tumor metastasis, transwell and wound healing

221 assays were conducted using HCT116 and DLD-1 cell lines. ICP-MS analysis confirmed the

222 successful uptake of Mg^{2+} into colon cancer cell lines (Figure 4C). Immunoblot analysis revealed

223 that treatment with magnesium chloride ($MgCl_2$) increased E-cadherin, and reduced the vimentin,

224 MMP2, and N-cadherin expression in HCT116 and DLD-1 cells (Figure 4D). In line with the

225 changes observed in EMT markers, both the transwell and wound healing assays demonstrated

226 that treatment with $MgCl_2$ significantly decreased the migratory ability of colon cancer cells

227 (Figure 4E−G). Taken together, our findings suggest that low Mg content in tumors may activate

228 EMT by disturbing the homeostasis of cell adhesion molecules.

229 **Potential mechanisms of EMT-related cell adhesion molecule alteration induced by low Mg**

230 **content**

231 Numerous studies have provided evidence indicating that inflammation is a key factor contributing

232 to the loss of cell adhesion molecules[46−48]. Our proteomics analysis of Mg-associated proteins

233 indicated that the expression of 44 complement and coagulation components, such as C1R, C1Q,

234 C2, C3, and C5, was negatively correlated with Mg content and significantly increased in Low-

235 Mg tumors and DNTs (Figure S3C)[49], suggesting the activation of the complement pathway in

236 these tumors and paired DNTs[50], which is known to induce inflammation[51] and affect the

237 expression of cell adhesion molecules (Figure S3D)[52−54].

238   Additionally, previous studies have shown that changes in protein phosphorylation are also

239 important for the regulation of cell adhesion molecules[55, 56]. Mg^{2+} is an important regulator of

240 protein phosphorylation. When Mg is deficient, it not only reduces intracellular ATP[30], but also

241 impacts kinase and phosphatase activities[31]`[32], leading to alterations in phosphorylation

242 signaling in cancer cells. Therefore, in addition to its impact on maintaining the homeostasis of

243 cell adhesion molecules by regulating inflammation, it is conceivable that low Mg content may

244 also modulate the levels of cell adhesion molecules through the modulation of phosphorylation in

245 cancer cells. To investigate the impact of Mg on tumors through phosphorylation mechanisms, we

246 analyzed the Mg-associated phosphoproteome of colon cancer, leading to the identification of 332

247 and 465 phosphosites that were positively and negatively correlated with the Mg content in tumors,

248 respectively (**Figure 5**A; Table S5). Enrichment analysis showed that proteins, such as ACTN4,

249 DBN1, and VIM, corresponding to the 465 negatively correlated phosphosites were implicated in

250 actin cytoskeleton organization, signaling by Rho GTPases, regulation of actin filament-based

251 process, focal adhesion and cell-matrix adhesion (Figure 5B), while proteins, such as CDK11B,

252 CDK12, and THRAP3, related to the 332 positively correlated phosphosites exhibited significant

253 enrichment in tight junction and signaling by Rho GTPases pathways (Figure 5C), indicating that

254 the Mg-associated phosphoproteome in colon cancer is closely linked to EMT-associated pathways

255 (Figure 5D).

256 Furthermore, we separately screened Mg-associated phosphosites in left-sided and right-sided

257 colon cancer. The results revealed that 18 phosphosites were significantly correlated with Mg

258 content in both left-sided and right-sided colon cancer, while 45 phosphosites were only associated

259 with Mg content in the left-sided colon cancer, and 734 phosphosites were specifically related to

260 Mg content in the right-sided colon cancer (Figure 5E and F). Pathway enrichment analysis of the

261 corresponding phosphorylated proteins unique to the left-sided and right-sided colon cancer

262 demonstrated that the Mg-associated phosphorylated proteins on both sides were associated with

263 cell adhesion pathways, including cell-cell adhesion, tight junction, cell junction organization, and

264 actin cytoskeleton organization pathways (Figure 5G). Furthermore, through an integrative

265 analysis of both the proteome and phosphorylome data, we identified 128 phosphosites positively

266 correlated and 118 phosphosites negatively correlated with Mg content. These correlations were

267 observed independently of protein levels (Figure S5A−C). Furthermore, enrichment analysis of

268 phosphoproteins exhibiting significant correlations with Mg content highlighted their association

269 with cell adhesion pathways, including actin cytoskeleton organization and cytoskeleton

270 organization pathways (Figure S5D and E).

271 To validate the direct impacts of Mg on cancer cells via phosphorylation, we analyzed the

272 phosphoproteome of colon cancer cells treated with and without $MgCl_2$. As a result, of the 15,235

273 identified phosphosites, 564 sites were increased in the $MgCl_2$-treated group, and 516 sites were

274 reduced (**Figure 6**A and Table S6). Notably, cell adhesion pathways such as actin filament-based

275 processes, regulation of actin cytoskeleton organization and signaling by Rho GTPases were

276 enriched using proteins with differentially changed phosphosites (Figure 6B and C), consistent

277 with the above phosphoproteomic data in CRC tissues (Figure 5B and C). Overlapping analysis

278 indicated that the expression of 28 phosphosites were significantly changed with Mg content in

279 both HCT116 and CRC tissues (Figure 6D and E). Among them, 4 phosphosites, including

280 DBN1^{S142p}, ARHGAP1^{S51p}, TNS1^{S1177p}, and FLNA^{S1459}, were involved in cell adhesion (Figure

281 6E). Additionally, a high degree of phosphorylation at these four sites was found to be a predictor

282 of unfavorable prognosis among CRC patients (Figure 6E and F, Figure S6A). Taken together, our

283 results indicate that low Mg content in tumors can reshape the protein phosphorylation network of

284 proteins associated with EMT and may ultimately affect tumor cell migration.

285 **Mg-regulated DBN1^{S142p} reduces the interaction between DBN1 and ACTN4 and contributes**

286 **to EMT**

287 DBN1 is a protein that binds to F-actin, which is essential for maintaining the levels of cell

288 adhesion molecules[57, 58]. However, the functions of DBN1^{S142p} in CRC and the mechanism by

289 which Mg-regulated DBN1^{S142p} contributes to the EMT process are completely unknown. The

290 confident identification of DBN1^{S142p} was verified through the MS/MS spectrum (Figure 6G).

291 Statistical analysis revealed a significant decrease in DBN1^{S142p} in the MgCl$_2$-treated group (Figure

292 6H). Moreover, a remarkable negative correlation was observed between DBN1^{S142p} and Mg

293 content in CRC, irrespective of its sidedness (Figure 6I). To explore the role of DBN1^{S142p} in the

294 regulation of EMT, we transfected wild-type DBN1 or DBN1 mutants in *DBN1*-knockdown

295 HCT116 cells that mimic the phosphorylation (S142D) or de-phosphorylation (S142A) states

296 (Figure 6J). Given that DBN1 is an F-actin-binding protein and that the assembly of F-actin can

297 promote MMP2 expression[57, 58], we assumed that the Mg-regulated decrease in DBN1^{S142p}

298 might serve as a signal triggering F-actin disassembly and promoting MMP2 degradation.

299 Immunoblotting results showed that DBN1^{S142D} remarkably upregulated MMP2 compared with

300 DBN1^{S142A} (Figure 6K). Consistently, both transwell and wound healing assays demonstrated that

301 the DBN1^{S142A} mutation significantly inhibited cell migration (Figure 6L and M). Further studies

302 showed that MgCl$_2$ treatment, which was able to reduce the level of DBN1^{S142p} (Figure 6H),

303 obviously enhanced the formation of F-actin (**Figure 7**A, Figure S6B). Consistent with these

304 findings, DBN1^{S142A}, which mimics the de-phosphorylation state, increased the formation of F-

305 actin compared with DBN1^{S142D} (Figure 7B). In addition, we further explored the function of

306 ARHGAP1^{S51A}. While the ARHGAP1^{S51A} mutation also notably inhibited cell migration, it did

307 not impact F-actin formation in contrast to ARHGAP1^{S51D}. This suggests that ARHGAP1^{S51p}

308 inhibits cell migration through mechanisms other than F-actin formation (Figure S6C−E).

309   To elucidate the impact of Mg-regulated $DBN1^{S142p}$ on F-actin polymerization, we employed

310   immunoprecipitation coupled MS analysis. This approach allowed us to identify proteins binding

311   to DBN1, the interactions of which were influenced by $DBN1^{S142p}$ and Mg^{2+} (Figure 7C). As a

312   result, we identified 376 proteins that bind to DBN1, with 30 and 46 proteins being enriched in the

313   $DBN1^{S142D}$ and $DBN1^{S142A}$ groups, respectively (Figure S7A and B; Table S7). Notably, some of

314   these proteins are involved in actin assembly and disassembly, suggesting that the level of

315   $DBN1^{S142p}$ may affect its binding to cytoskeleton-related proteins. Upon comparing the differential

316   binding proteins between the $DBN1^{WT}$ and $DBN1^{WT}+Mg^{2+}$ groups, we noted that 27 proteins

317   exhibited increased interactions with DBN1, while interactions with 56 proteins were diminished

318   in response to $MgCl_2$ treatment (Figure S7C). Of the 83 differential proteins, 73 proteins

319   demonstrated no notable changes between the $DBN1^{S142A}$ and $DBN1^{S142A}+Mg^{2+}$ groups (Figure

320   S7C), indicating that Mg^{2+} might modulate the interactions of these 73 proteins with DBN1 by

321   altering $DBN1^{S142p}$. Among these 73 proteins, 53 whose interactions were weakened by Mg^{2+} were

322   primarily associated with the actin cytoskeleton (Figure S7D), indicating that Mg^{2+} could affect

323   the interaction between DBN1 and these cytoskeletal proteins, which may further impact the

324   depolymerization of F-actin. Additional comparisons were performed to identify the proteins that

325   bind to DBN1 in both the phosphorylated and dephosphorylated states at the S142 residue. (Figure

326   S7E and F). The results indicated that Mg suppressed $DBN1^{S142p}$ to inhibit the interaction of three

327   proteins, namely, ACTN4, CDC42, and PARK7 (Figure 7D and E, Figure S7E), while it enhanced

328   the interaction of six proteins, namely, ALDH1B1, DCAF7, PLOD1, PGM3, NDUFV2, and PISD

329   (Figure 7D, Figure S7 F and G). Among these proteins, ACTN4 and CDC42 are well known for

330   their close association with the cell cytoskeleton[59].

331   Previous studies have indicated that ACTN4 participates in the formation of F-actin and that

332   F-actin stability is compromised when ACTN4 is detached from F-actin[59]. Thus, we propose

333   that the interaction of DBN1 to ACTN4 results in the detachment of ACTN4 from F-actin. The

334   de-phosphorylation of DBN1 at S142 could weaken the interaction between DBN1 and ACTN4,

335   thereby promoting the interaction of ACTN4 to F-actin and enhancing F-actin stability. To

336   investigate this assumption, we overexpressed Flag-tagged vector, $DBN1^{WT}$, $DBN1^{S142A}$, and

337   $DBN1^{S142D}$ in *DBN1*-knockdown HCT116 cells and performed immunoprecipitation under

338   identical experimental conditions. Immunoblotting results confirmed ACTN4's ability to interact

339   with DBN1 (Figure 7E). Moreover, the interaction between the mutant DBN1^{S142A} and ACTN4

340   was weakened, indicating that DBN1^{S142p} may affect the DBN1-ACTN4 interaction (Figure 7F).

341   To confirm that Mg^{2+} reduces the interaction between DBN1 and ACTN4 by decreasing the

342   level of DBN1^{S142p}, we constructed stable cell lines overexpressing Flag-tagged vector, DBN1^{WT},

343   and DBN1^{S142A}. These cell lines were treated with and without $MgCl_2$, followed by Flag-targeted

344   immunoprecipitation assays. The immunoblotting results showed that $MgCl_2$ treatment weakened

345   the interaction between DBN1^{WT} and ACTN4, while DBN1^{S142A}-ACTN4 remained consistent

346   after $MgCl_2$ treatment (Figure 7G). These findings imply that Mg^{2+} may decrease the interaction

347   between DBN1 and ACTN4 by reducing DBN1^{S142p}. Then, we generated stable HCT116 cell lines

348   with *ACTN4* knockdown and examined the effect of ACTN4 on F-actin polymerization (Figure

349   S7H). The results showed that *ACTN4* knockdown inhibited F-actin formation (Figure 7H). In

350   contrast, knockdown of *DBN1* resulted in a significant increase in colocalization between ACTN4

351   and F-actin (Figure 7I), suggesting that the interaction between DBN1 and ACTN4 inhibits the

352   formation of F-actin.

353   Collectively, these findings suggest that Mg^{2+} diminishes the interaction between DBN1 and

354   ACTN4 by decreasing the level of DBN1^{S142p}. This, in turn, enhances the binding of ACTN4 to F-

355   actin and stabilizes F-actin, ultimately resulting in reduced MMP2 expression and decreased

356   migratory ability of colon cancer cells (Figure 7J).

Discussion

358   In summary, for the first time, we presented an atlas of the Mg-associated genome, proteome and

359   phosphoproteome in CRC and demonstrated that low Mg content in tumors promoted genome

360   instability and tumor metastasis. This work has yielded some novel findings. First, our study

361   revealed that a large number of genes in Low-Mg tumors exhibited higher mutation frequencies

362   and co-occurred, including several driver genes associated with tumor progression. Conversely,

363   High-Mg tumors displayed high mutation frequencies in well-known initiation driver genes of

364   CRC, such as *TP53* and *APC*, indicating that the lack of Mg content may primarily impact tumor

365   progression rather than initiation at the genomic level. Second, our study showed that Low-Mg

366   tumors activated EMT by disrupting the homeostasis of adhesion molecules in cancer cells. This

367   disruption was attributed not only to complement pathway activation and elevated inflammation

368   in Low-Mg tumors but also to changes in the phosphorylation signaling of proteins associated with

369   cell adhesion. Third, we discovered a novel phosphosite, DBN1^{S142p}, that is regulated by Mg^{2+}.

13

370   Our results showed that Mg^{2+} reduced the interaction between DBN1 and ACTN4 by decreasing

371      $DBN1^{S142p}$. This reduction in $DBN1^{S142p}$ resulted in increased binding of ACTN4 to F-actin,

372   thereby stabilizing F-actin and ultimately leading to reduced MMP2 expression and inhibition of

373   colon cancer cell migration. Overall, this work uncovers the roles of Mg in CRC and demonstrates

374   that low Mg content in tumors could be a potential driver factor of CRC.

375   Cancer cells require a large amount of Mg to support their glycolytic metabolism, ATP

376   production and protein synthesis, which are essential for sustaining rapid proliferation[60].

377   Insufficient Mg uptake not only slows cancer cell proliferation but also leads to genome

378   instability[61], as we demonstrated in this work. Mg deficiency is known to induce low-grade

379   systemic inflammation and cause increased production of ROS and proinflammatory cytokines

380   such as IL-6, TNF-α, and IL-1β[8, 62], leading to genome instability. Moreover, Mg also plays a

381   crucial role in regulating DNA replication and repair processes[39, 40, 42−44], which may

382   partially explain why a significant number of CRC driver genes, such as *KMT2C* and *ERBB3*, are

383   mutated in tumors with low Mg content. Tumor initiation and progression are determined by the

384   inactivation of the tumor suppressors *APC* and *TP53* and the activation of the oncogene *KRAS*[63].

385   Surprisingly, the mutation frequencies of these genes were much higher in tumors with high Mg

386   content (High-Mg/Low-Mg frequencies: *APC*, 51%/22%, $P = 0.032$; *TP53*, 62%/26%, $P = 0.0091$;

387   *KRAS*, 44%/30%, $P = 0.42$). However, the mechanism by which high levels of Mg induce

388   mutations in the *APC* and *TP53* genes remains to be investigated. In addition, the screening of Mg-

389   related gene mutations in left- or right-sided colon cancer, or on both sides, requires validation in

390   larger CRC cohorts.

391   EMT, characterized by loss of cell-cell junctions, disruption of cell-matrix attachments and

392   cytoskeleton remodeling, can increase the mobility of cells. Many signaling pathways, such as

393   TGFβ, Wnt and Hippo, are associated with the regulation of EMT[64]. Mg deficiency is well

394   known to cause inflammation by disturbing the coordination of the innate immune system and the

395   adaptive immune response[14], leading to rapid loss of cell adhesion molecules[46−48].

396   Alternatively, phospho-signals, serving as the first wave of response to intracellular and

397   extracellular changes, extensively exist during the EMT process[65, 66]. For example,

398   phosphorylation of SNAIL[67], GSK3β[68], and EGFR[69] contributes to the regulation of EMT.

399   Our findings, for the first time, systematically highlight the significance of Mg's direct regulation

400   of phosphorylation in cancer cells, which is critical to maintain the homeostasis of cell adhesion

14

401 molecules and is closely linked to the EMT process. Specifically, our experiments revealed that

402 Mg^{2+} played a crucial role in reducing the interaction between DBN1 and ACTN4 by lowering the

403 level of DBN1^{S142p}. This, in turn, had a significant impact on F-actin stability and the homeostasis

404 of cell adhesion molecules. However, the exact mechanism by which Mg affects DBN1^{S142p} is not

405 yet fully understood, although it may impact the activity of phosphatases, thereby influencing

406 DBN1^{S142p}. Additionally, the precise binding mode between DBN1 and ACNT4 remains elusive,

407 and further biochemical experiments are necessary to determine how DBN1^{S142p} affects the

408 interaction between DBN1 and ACNT4.

409 Adults typically consume a daily Mg intake of approximately 330−350 mg. Previous studies

410 have showed that cirrhotic patients with hepatocellular carcinoma (HCC) exhibit reduced serum

411 Mg levels compared to those without HCC, independent of confounding factors such as dietary

412 Mg intake and medications affecting Mg levels[70]. Moreover, mouse experiments have validated

413 that the growth of primary tumors sequesters Mg from the extracellular environment, leading to

414 hypomagnesemia[60]. Additionally, both our work and other studies have described the higher

415 preference of Mg absorption in tumors than normal tissues, which may raise the possibility of

416 reducing serum Mg levels[60, 70]. However, this speculation requires further evidence.

417 Previous clinical studies have demonstrated the potential benefits of Mg in the prevention and

418 treatment of CRC. A population-based prospective study suggests that a high Mg intake may

419 reduce the risk of CRC in women[21]. Moreover, Mg supplementation can enhance the effect of

420 drug treatment and minimize serious side effects in CRC patients. For example, a combination of

421 25(OH)D3 and Mg is essential in reducing the risk of mortality in CRC patients[71]. Peripheral

422 neuropathy is a common side effect caused by chemotherapy in CRC patients, but a high dietary

423 Mg intake can reduce its prevalence and severity[28]. Notably, in late-stage CRC patients treated

424 with cetuximab[72] or bevacizumab[73], EGFR inhibition can result in Mg wasting due to

425 decreased renal reabsorption, and circulating Mg^{2+} reduction may act as a predictive factor of

426 treatment efficacy and outcome[74, 75]. The extracellular matrix (ECM) is a complex network of

427 proteins and other molecules that surround cells and play important roles in cell signaling,

428 migration, and proliferation[76]. Abnormalities in ECM composition and organization are often

429 associated with cancer progression and metastasis. ECM proteins themselves and ECM protein-

430 interacting proteins such as integrins are known targets of cancer treatment[77, 78]. Interestingly,

431 by referring to known drug targets with FDA-approved drugs or candidate drugs in clinical trials,

15

432 we identified 24 clinically actionable cell-matrix proteins, such as COL3A1, FGA, and ITGA5,

433 whose expression was negatively correlated with Mg content in tumors (Spearman's rank

434 correlation test, $P < 0.05$, Figure S8; Table S8), suggesting that Mg might serve as an adjuvant

435 drug for precise CRC treatment by affecting the levels of these potential drug targets. Our research

436 revealing the functions of Mg is expected to promote the application of Mg reagents in the

437 prevention and treatment of CRC.

Materials and methods

Sample collection and preparation

440 A total of 115 paired tumors and corresponding DNTs wereobtained from treatment-naive CRC

441 patients at West China Hospital of Sichuan University. The samples were rapidly snap-frozen in

442 liquid nitrogen and then stored at -80 °C for long-term preservation. This study was carried out

443 with the approval of the Research Ethics Committee (Approval No. 2020 (374)). Informed consent

444 and approvals were obtained from each patient and reviewed accordingly. Detailed clinical

445 information, such as age, gender, tumor region, OS status, OS (months) and TNM stage, is

446 systematically recorded in Table S1. Patient follow-up was conducted over a median period of

447 67.25 months. OS was the duration from surgery to either the patient's demise or the last follow-

448 up visit. We used genomic, proteomic, and phosphoproteomic data of these CRC patients to

449 elucidate the impact of Mg deficiency on CRC. Notably, the genome, proteome, and

450 phosphoproteome data for each patient were generated using a same sample.

Proteomic and phosphoproteomic analyses

452 Protein extraction and digestion procedures were as follows: Tissues were homogenized and lysed

453 using gentleMACS Dissociators (Catalog No. 130-093-235, Miltenyi Biotec, Nordrhein-

454 Westfalen, Germany) with RIPA buffer (Catalog No. P0013C, Beyotime, Shanghai, China). Cell

455 samples, on the other hand, were directly lysed in RIPA buffer. Subsequently, the prepared lysates

456 were sonicated for 5 min at 227.5 W, with a 3-second on and 10-second off cycle. After

457 centrifugation at 20,000 g for 20 min at 4 °C, the supernatant was transferred to a new tube, and

458 the Bradford protein assay was taken to measure protein concentration. For each sample, 100 μg

459 of protein lysates were first reduced using 10 mM tris (2-carboxyethyl) phosphine (TCEP) at 56 °C

460 for 60 min. Subsequently, alkylated with 20 mM iodoacetamide for 30 min in the dark at 25 °C.

461 The protein samples were subsequently precipitated using CH_3OH, $CHCl_3$, and H_2O (CH_3OH:

462 $CHCl_3$: H_2O = 4:1:3, v/v). Finally, the proteins were digested with trypsin at a 1:50 (trypsin/protein,

463 w/w) for 12 hours.

464 Tandem Mass Tag (TMT)-10 labeling of peptides was conducted as follows: The TMT-10

465 plex Isobaric Label Reagent (Catalog No. 90110, Thermo Fisher Scientific, Waltham, MA) was

466 dissolved in anhydrous acetonitrile (ACN) after reaching room temperature. 10 μg and 40 μg

467 digested peptides of each sample for proteome and phosphoproteome analysis were labeled with

468 TMT reagents following the manufacturer's instructions, respectively. Subsequently, the TMT-

469 labeled peptides were combined and dried.

470 TMT-labeled peptide fractionation was performed as follows: Reversed-phase high-

471 performance liquid chromatography (RP-HPLC) was used to fractionate peptides with a basic

472 mobile phase in proteomics. The separation was carried out with a flow rate of 1 mL/min using a

473 mixture of buffer A (98% H_2O, 2% ACN, pH = 10) and buffer B (90% ACN, 10% H_2O, pH = 10).

474 The LC gradient run spanned 120 min and followed this pattern: 3%−35% buffer B in 95 min,

475 35%−60% buffer B in 10 min, 60%−100% buffer B in 15 min. The eluates were collected in 120

476 fractions, which were subsequently merged into 15 fractions for each batch of CRC samples. These

477 fractions were then dried using a vacuum centrifuge. Following desalting with C18 ZipTips, the

478 TMT-labeled peptides were subjected to LC-MS/MS analysis. For phosphoproteomics, the TMT-

479 labeled peptides from tissue samples were initially fractionated into 15 fractions using a C18 solid-

480 phase extraction (SPE) columns (100 mg/1 mL). Later, these fractions were further combined into

481 5 fractions and dried. In the case of cell phosphoproteomics, each sample was divided into 9

482 fractions using a C18 SPE columns, which were then merged into 3 fractions prior to drying.

483 The enrichment of phosphorylated peptides was performed as follows: Phosphopeptides were

484 enriched using PureCube Fe-NTA Agarose Beads (Catalog No. 31403-Fe, Cube Biotech,

485 Monheim, Germany) according to the manufacturer's instructions. The peptides, dissolved in 300

486 μL of loading buffer (85% ACN, 0.1% TFA), were then incubated with the prepared beads for

487 about 60 min on a 3D shaker at room temperature. Subsequently, the agarose beads were washed

488 4 times with washing buffer (80% ACN, 0.1% TFA) and then eluted with 150 μL of elution buffer

489 (composed of 40% ACN and 15% ammonium hydroxide). To neutralize the eluate, 8 μL of 20%

490 TFA was added. The elution buffer were subsequently dried by vacuum, and subjected to LC-

491 MS/MS analysis after desalting using C18 ZipTips.

492   For proteomics, LC-MS/MS analysis was conducted using a Nano EASY-nLC 1200 liquid

493   chromatography system LC instrument coupled with an Orbitrap Exploris 480 mass spectrometer

494   (Catalog No. BRE725539, Thermo Fisher Scientific). After desalting with a Ziptip columns, the

495   samples were dried and reconstituted in buffer A (98% H_2O, 2% ACN, 0.1% formic acid (FA)).

496   They were then loaded onto an in-house pulled and packed analytical column (75 μm × 30 cm)

497   packed with C18 particles. Samples were analyzed using a 65-minute gradient of 4% to 100%

498   buffer B (0.1% FA in 80% ACN) at a flow rate of 300 nL/min in positive ion mode. The MS1 full

499   scans (m/z 350−1800) were acquired with a resolution of 60,000. The automatic gain control (AGC)

500   value was set to 300%, and the maximum injection time (MIT) was 50 ms. For MS/MS scans, the

501   top 20 most abundant parent ions were selected under an isolation window of 0.7 m/z, and

502   fragmentation was performed using a normalized collision energy (NCE) of 36%. The normalized

503   AGC value for MS/MS was set to 75%, and the MIT was 80 ms. Precursor ions with charge states

504   of z = 1, 8, or unassigned charge states were excluded from further fragmentation.

505   For phosphoproteomics, LC-MS/MS analysis was conducted using a Nano EASY-nLC 1200

506   liquid chromatography system LC instrument coupled with a Q Exactive HF-X high-resolution

507   mass spectrometer (Catalog No. BR64966, Thermo Fisher Scientific). After desalting with Ziptip

508   columns, the samples were dried and reconstituted in buffer A, consisting of 2% ACN and 0.1%

509   FA. They were then separated by a homemade trap column (2.5 cm × 75 μm) packed with Spursil

510   C18 particles and an analytic column (25 cm × 75 μm) packed with Reprosil-Pur C18-AQ particles.

511   Samples were analyzed using a 65-min gradient of 6% to 100% buffer B (0.1% FA in 80% ACN)

512   at a flow rate of 330 nL/min in positive ion mode. The MS1 full scans (m/z 350−1600) were

513   acquired with a resolution of 60,000. The AGC value was set to 3e6, and the MIT was 20 ms. For

514   MS/MS scans, the top 20 most abundant parent ions were selected under an isolation window of

515   0.6 m/z, and fragmentation was performed using stepped NCE of 25% and 31%. Precursor ions

516   with charge states of z = 1, 8, or unassigned charge states were excluded from further fragmentation.

517   For label-free cell phosphoproteomics, samples were analyzed using a 90-min gradient of 12% to

518   100% buffer B (0.1% FA in 80% ACN) at a flow rate of 330 nL/min in positive ion mode. The

519   MS1 full scans (m/z 350−1800) were acquired with a resolution of 60,000. The AGC value was

520   set to 3e6, and the MIT was 20 ms. For MS/MS scans, the top 20 most abundant parent ions were

521   selected under an isolation window of 1.6 m/z, and fragmentation was performed using stepped

522 NCE of 25% and 27%. Precursor ions with charge states of $z = 1$, 8, or unassigned charge states
523 were excluded from further fragmentation.

MS database searching

525 All mass spectrometry raw data files of proteomics and phosphoproteomics were analyzed by
526 using MaxQuant (version 1.6), aligned against the Swiss-Prot human protein sequence database
527 comprising 20,413 entries (updated 04/2019). For MS2 reporter ion quantification, the reporter
528 mass tolerance was set at 0.02 Da. Peptide mass tolerance was set at 10 ppm, and only peptides
529 and proteins with a false discovery rate (FDR) lower than 1% were kept for further data processing.
530 Up to 2 trypsin-missing cleavage sites were allowed. Cysteine carbamidomethylation was
531 specified as a fixed modification, while oxidation of methionine and protein N-terminal acetylation
532 were considered variable modifications. For phosphoproteomics analysis, phosphorylation
533 (+79.9663 Da) of serine, threonine, and tyrosine residues were also added to the above variable
534 modifications.

Data cleaning of proteome data

536 R (version 4.2.1) was used to process proteome data to minimize systematic errors based on the
537 "peptides.txt" from MaxQuant output. Several preprocessing steps were performed to refine the
538 data. First, potential contaminants and reverse proteins were excluded. Subsequently, only proteins
539 with $\geqslant$ 2 unique peptides were selected for further analysis. To ensure comparability across
540 samples within the same batch, the total protein abundance of each sample was adjusted to an
541 equal level. To reduce the impact of noise, the protein intensity values of the tumor or DNT
542 samples were divided by the protein intensity of an IS sample, yielding protein sample-to-standard
543 (S/S) values. This step helped normalize the data and mitigate potential confounding factors. All
544 data from the 27 batches were combined into a matrix, with samples represented as columns and
545 proteins as rows. Additionally, the normalized values underwent an additional log2-transformation
546 to facilitate subsequent analyses. The values in the matrix were transformed to column z-scores
547 and row z-scores to normalize the data distribution across samples and proteins, respectively. Any
548 values that were originally zero were considered missing and replaced with "NA". To ensure data
549 quality, proteins with > 50% missing values were excluded from the dataset, resulting in a refined
550 and reliable set of proteins for further analysis.

Data cleaning of phosphoproteome data.

19

552 R (version 4.2.1) was used to process phosphoproteome data to minimize systematic errors based

553 on the "Phospho (STY) Sites.txt". Several preprocessing steps were performed to refine the data.

554 First, potential contaminants and reverse peptides were excluded. In addition, phosphopeptides

555 with localization probability > 0.75 were kept. To ensure comparability across samples within the

556 same batch, the total intensities of phosphopeptides in each sample was adjusted to an equal level.

557 To reduce the impact of noise, the phosphopeptide intensities of the tumors or DNT samples were

558 divided by the phosphopeptide intensity of an IS sample, resulting in sample-to-standard (S/S)

559 values. This step helped normalize the data and mitigate potential confounding factors. All data

560 from the 20 batches were combined into a matrix, with samples represented as columns and

561 phosphopeptides as rows. Additionally, the normalized values underwent an additional log2-

562 transformation to facilitate subsequent analyses. The values in the matrix were transformed to

563 column z-scores and row z-scores to normalize the data distribution across samples and

564 phosphopeptides, respectively. Any values that were originally zero were considered missing and

565 replaced with "NA". To ensure data quality, phosphopeptides with $> 50\%$ missing values were

566 excluded from the dataset.

567 **Measurement of magnesium content in tissues**

568 Tissues samples were homogenized and lysed using gentleMACS Dissociators (Catalog No. 130-

569 093-235, Miltenyi Biotec) with RIPA buffer. Subsequently, the tissue lysates were sonicated for 5

570 min at 227.5 W, with a 3-second on and 10-second off cycle. Bradford protein assay was taken to

571 measure protein concentration, and 350 μL of each sample were taken and used for the

572 measurement of Mg content. Mg content in each tissue was quantified by normalizing to protein

573 concentration. The lyophilized samples were then treated with a suitable amount of 65% nitric acid

574 (HNO_3) overnight at room temperature. This process was continued by heating the samples in a

575 heating block at 90 °C for about 20 min. Subsequently, an equivalent volume of 30% H_2O_2 was

576 added to each sample. The reaction was stopped after an additional 30 min, followed with a further

577 heating step at 70 °C for 15 min. The mean reduced volume was established, and subsequently,

578 the samples were diluted with 1% HNO_3. Measurements were conducted using an Agilent 7700

579 series ICP-MS instrument under standard multi-element operating conditions, employing a helium

580 reaction gas cell. Calibration of the instrument was performed using certified multi-element ICP-

581 MS standard calibration solutions with concentrations spanning 0, 5, 10, 50, 100, and 500 ppb for

582 various elements. Moreover, a certified IS solution containing 200 ppb yttrium was employed for

583 an internal control.

Whole-exome sequencing and data processing

585 In our previous study, we have utilized a total of 76 paired tumors and corresponding DNTs from

586 CRC patients for WES analysis[35]. The detailed methods have been illustrated in the previous

587 publication. Briefly, genomic DNA was quantified by a Qubit® DNA Assay Kit in Qubit® 2.0

588 Fluorometer (Catalog No. Q32866, Thermo Fisher Scientific). A total amount of 0.6 µg genomic

589 DNA per sample was used as input material for the DNA sample preparation. Subsequently, WES

590 libraries were prepared and captured using an Agilent SureSelect Human All Exon kit (Catalog

591 No. 5191-5735, Agilent Technologies, Santa Clara, CA). The DNA library, featuring 150 bp

592 paired-end reads, underwent sequencing using an Illumina NovaSeq 6000 System. The initial

593 fluorescence image files acquired from the HiSeq platform were conversed to raw data through

594 base calling and subsequently transformed into FASTQ format. This format includes both

595 sequence information and corresponding sequencing quality details.

Somatic copy number alteration analysis

597 The exome sequencing data were initially aligned to the human genome hg19. Following the

598 alignment, copy number variations (CNVs) were detected from the BAM files derived from WES.

599 These CNVs were then utilized for SCNA analysis. To integrate the results obtained from

600 individual patients and identify recurrently amplified or deleted focal genomic regions in our

601 samples, we employed the GISTIC 2.0 software (https://cloud.genepattern.org)[79]. Additionally,

602 the R package "maftools" was used to display Q values less than 0.1. Moreover, to minimize false

603 positives, the related parameters were set as follows: refgene file = Human_Hg19.mat, focal length

604 cutoff = 0.50, gene gistic = yes, confidence level = 0.99. All other parameters were maintained at

605 their default settings.

Differential abundance analysis

607 The cleaned proteome and phosphoproteome data underwent a differential abundance analysis

608 between tumors and DNTs through the Wilcoxon rank-sum test. Variables that contain > 50%

609 missing values are excluded. Significance was considered when $P < 0.05$, and fold change (FC)

610 was calculated as the median $\log_2$(FC). Differential proteins and phosphosites in cell lines were

611 detected through the Student's *t*-test. Significance was considered when $P < 0.05$, and FC was

612 calculated as the median $\log_2$(FC). Pathway enrichment analysis was performed based on the

21

613   DAVID Bioinformatics Resources (https://david.ncifcrf.gov/)[80, 81] and Metascape database

614   (http://metascape.org)[82].

615   **Correlation analysis**

616   Pearson's correlation analysis was performed between IS samples or QC samples to assess the

617   quality of the proteome and phosphoproteome data. Mg content and the levels of proteins or

618   phosphosites were calculated by the Spearman's rank correlation analysis.

619   **Univariate survival analysis**

620   The optimal cutoff point for the selected samples was calculated by the R package "survminer".

621   To assess differences between the categorical variables, the log-rank test was applied, and two-

622   tailed tests and P values < 0.05 were used for significance evaluation. In the R package

623   "survminer," survival curves were created through the Kaplan−Meier method for specific variables

624   of interest. The estimation of hazard ratios (HRs) and their 95% confidence intervals (CIs) was

625   performed using the "coxph" function from the R package "Survival."

626   **Western blotting analysis**

627   Proteins were extracted from tissues and cells with RIPA buffer and then quantified by the

628   Bradford assay. SDS-PAGE (10%) was used to separate the protein samples, which were then

629   transferred to PVDF membranes (Catalog No. ISEQ00010, Darmstadt, Germany). Following that,

630   PVDF membranes were blocked using 5% milk in PBST and then incubated with an E-cadherin

631   antibody (1:1000; Catalog No. 20874-1-AP, Proteintech, Wuhan, China), N-cadherin antibody

632   (1:1000; Catalog No. 22018-1-AP, Proteintech), Vimentin antibody (1:1000; Catalog No. 10366-

633   1-AP, Proteintech), Vinculin antibody (1:1000; Catalog No. 66305-1-Ig, Proteintech), MMP2

634   antibody (1:1000; Catalog No. 10373-2-AP, Proteintech), Cingulin antibody (1:1000; Catalog No.

635   21369-1-AP, Proteintech), DBN1 antibody (1:1000; Catalog No. 10260-1-AP, Proteintech),

636   ACTN4 antibody (1:1000; Catalog No. 19096-1-AP, Proteintech) or GAPDH antibody (1:5000;

637   Catalog No. 60004-1-Ig, Proteintech) overnight at 4 °C. Following overnight incubation,

638   underwent three washes with PBST, followed by incubation with the appropriate secondary

639   antibody at room temperature for 1 hour. Finally, the membrane was subjected to

640   chemiluminescent detection using the Immobilon Western HRP Substrate (Catalog No.

641   WBKLS0500, Sigma).

642   **Cell culture and generation of stable cell lines**

643 The HCT116 and DLD-1 human CRC cell lines were obtained from the Cell Bank/Stem Cell Bank,

644 Chinese Academy of Sciences. Additionally, HCT116 and DLD-1 cells were cultured in DMEM

645 (Catalog No. C11995-065, Gibco, Grand Island, NY) and RPMI 1640 (Catalog No. 10270-106,

646 Gibco) with 10% FBS (Catalog No. FCS500, ExCell, Shanghai, China), 100 units of penicillin,

647 and 100 μg/mL streptomycin (Catalog No. 15140-122, Gibco), respectively.

648 To construct DBN1-GFP-tagged and DBN1-Flag-tagged cells, the cDNA of *DBN1* was

649 inserted into the pCDH-LGFP and pCDH-3 × Flag vectors, respectively. The PLKO.1 vector was

650 used for *DBN1* and *ACTN4* knockdown. To generate cells overexpressing *DBN1* or cells with

651 silenced *DBN1* and *ACTN4*, a co-transfection approach was employed using HEK293T cells. The

652 pSPAX2, pMD2. G, pCDH-LGFP-DBN1, pCDH-3 × Flag-DBN1, shDBN1, shACTN4, and their

653 respective control plasmids were co-transfected into HEK293T cells. 48 hours later, the medium

654 containing the virus was collected and filtered. To enhance transfection efficiency, 10 mg/mL

655 Polybrene (Catalog No. S2667, Sigma) was added. Subsequently, cells underwent infection and

656 selection with 1 μg/mL puromycin (Catalog No. ST551, Beyotime) for 48 hours. The primer

657 sequences for *DBN1* and *ACTN4* are listed as follows:

658 shDBN1 # 1: 5'-CCGGCTGTGGAAATGAAGCGGATTACTCGAGTAATCCGCTTCAT

659 TTCCACAGTTTTTG-3'

660 shACTN4 # 1: 5'-CCGGCATCGCTTCCTTCAAGGTCTTCTCGAGAAGACCTTGAAG

661 GAAGCGATGTTTTTG-3'

662 shACTN4 # 2: 5'-CCGGCCTGTCACCAACCTGAACAATCTCGAGATTGTTCAGGTT

663 GGTGACAGGTTTTTG-3'

664 The primer sequences for *DBN1*-overexpressing were as follows:

665 DBN1-PCDH-GFP: 5'-GATTCTAGAGCTAGCGAATTCATGGCCGGCGTCAGCTTCA

666 GC-3'

667 DBN1-PCDH-Flag: 5'-GATGACAAGTCTAGAGAATTCATGGCCGGCGTCAGCTTCA

668 GC-3'

669 The primer sequences for *DBN1* mutants were as follows:

670 DBN1^{S142A}: 5'-CGCGACTCTCCGCCCCTGTGCTGCA-3'

671 DBN1^{S142D}: 5'-CGCGACTCTCCGACCCTGTGCTGCA-3'

672 **Cell migration assay**

673

23

674 In the cell migration assay, 8.0 mm, 24-well plate chamber inserts were used (Catalog No. 354578,

675 Corning Life Sciences, Corning, NY). In the upper chamber of the inserts, a total of 3×10^5 cells

676 suspended in 200 μL of serum-free medium were added, and 800 μL of 10% FBS medium was

677 added to the bottom chamber. 24 hours later, the cells were fixed with 4% PFA for 15 min and

678 then stained with 0.5% crystal violet for another 15 min. Cells on the upper surface of the inserts

679 were retained, while cells on the underside were gently removed using a cotton swab. The captured

680 images were analyzed using ImageJ software to quantify the number of cells.

681 **Wound healing assay**

682 HCT116 and DLD-1 cells were plated in a 6-well plate, and a 10 μL pipette tip was used to generate

683 a straight-line wound. The wells were washed with PBS and replenished with serum-free medium.

684 Subsequently, the wounds were photographed at 0 hours and 48 hours after the injury. ImageJ

685 software was utilized to measure the width of the gap.

686 **Immunofluorescence**

687 Immunofluorescence assays were performed in 96-well plates (Catalog No. 6055300, PerkinElmer,

688 Waltham, MA), and 1×10^4 cells were plated and incubated for 24 hours. Subsequently, the cells

689 were fixed with 4% PFA for 15 min, three washes with PBS, and then permeabilized and blocked

690 with 0.5% Triton X-100 (Catalog No. T8200, Solarbio, Beijing, China) for 5 min. Next, the cells

691 were incubated with Actin-Tracker Red-594 (Catalog No. C2205S, Beyotime) for about 30 min,

692 followed by 4',6-diamidino-2-phenylindole (Catalog No. C0060, Solarbio) for about 5 min.

693 Images were photographed by Opera Phenix Plus (Catalog No. HH14001000, PerkinElmer), and

694 then quantified by using Harmony software.

695 **Coimmunoprecipitation**

696 Cells were lysed using RIPA buffer on ice for 30 min, then centrifuged at 20,000 g for 10 min. To

697 pull down DBN1-Flag, anti-Flag magnetic beads (Catalog No. HYK0207, MedChemExpress,

698 Monmouth Junction, NJ) were utilized. The elution of interacting proteins was carried out using 1

699 × SDS loading buffer (Catalog No. P0015A, Beyotime) and subjected to heating at 95 °C for 5

700 min. The eluted products were subsequently analyzed either through Western blotting or LC-

701 MS/MS.

702 **Ethical statement**

703 Written informed consents and approvals for all tissue specimens were obtained from the Research

704 Ethics Committee (Permission number: 2020 (374)).

24

Data availability

The MS proteomics data of CRC have been deposited to the ProteomeXchange Consortium via the iProX partner repository with the dataset identifier PXD039360. The mass spectrometry phosphoproteomics data of CRC and cells have been deposited in the ProteomeXchange Consortium and are available using the iProX accession number PXD042746. The raw GSE sequence data reported in this paper have been deposited in the Genome Sequence Archive[83] in the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA003386), which are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.

Code availability

The source code is freely available at the National Genomics Data Center (NGDC) BioCode (https://ngdc.cncb.ac.cn/biocode/tools/BT007400).

Competing financial interests

The authors have declared no competing interests.

CRediT authorship contribution statement

Rou Zhang: Investigation, Validation, Visualization, Writing– original draft. **Meng Hu:** Software, Formal analysis, Visualization, Writing – original draft. **Yu Liu:** Investigation, Validation, Visualization. **Wanmeng Li:** Visualization. **Zhiqiang Xu:** Investigation. **Siyu He:** Visualization. **Ying Lu:** Visualization. **Yanqiu Gong:** Validation. **Xiuxuan Wang:** Validation. **Shan Hai**: Resources, Funding acquisition. **Shuangqing Li**: Resources, Funding acquisition. **Shiqian Qi**: Supervision. **Yuan Li:** Resources. **Yang Shu:** Data curation, Software. **Dan Du**: Resources. **Huiyuan Zhang**: Resources. **Heng Xu:** Resources, Funding acquisition. **Zongguang Zhou:** Resources, Funding acquisition. **Peng Lei:** Resources, Project administration, Conceptualization. **Hai-Ning Chen:** Resources, Writing – review & editing, Supervision. **Lunzhi Dai:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. All authors have read and approved the final manuscript.

Acknowledgments

25

735 of West China Hospital (No. Z2021JC005) and the 135 project of West China Hospital (No.

736 ZYYC23013, ZYYC23025, ZYGD20006, and 2016105).

737 **ORCID**

738 ORCID 0009-0001-5056-8345 (Rou Zhang)

739 ORCID 0000-0001-5113-2888 (Meng Hu)

740 ORCID 0000-0001-5359-1906 (Yu Liu)

741 ORCID 0009-0009-1357-3629 (Wanmeng Li)

742 ORCID 0000-0002-8133-1228 (Zhiqiang Xu)

743 ORCID 0009-0007-4350-4232 (Siyu He)

744 ORCID 0000-0002-6424-5424 (Ying Lu)

745 ORCID 0000-0001-7890-5158 (Yanqiu Gong)

746 ORCID 0000-0002-4925-7048 (Xiuxuan Wang)

747 ORCID 0009-0007-7718-4249 (Shan Hai)

748 ORCID 0000-0001-8510-7568 (Shuangqing Li)

749 ORCID 0000-0002-7589-8877 (Shiqian Qi)

750 ORCID 0000-0003-0274-855X (Yuan Li)

751 ORCID 0000-0002-8304-1531 (Yang Shu)

752 ORCID 0000-0001-9745-8197 (Dan Du)

753 ORCID 0000-0002-1532-2462 (Huiyuan Zhang)

754 ORCID 0000-0002-7748-2621 (Heng Xu)

755 ORCID 0000-0002-7616-1199 (Zongguang Zhou)

756 ORCID 0000-0001-5652-1962 (Peng Lei)

757 ORCID 0000-0003-0104-8498 (Hai-Ning Chen)

758 ORCID 0000-0002-3003-8910 (Lunzhi Dai)

759

760 **References**

761 [1] Fang X, Ardehali H, Min J, Wang F. The molecular and metabolic landscape of iron and

762 ferroptosis in cardiovascular disease. Nat Rev Cardiol 2023;20:7−23.

763 [2] Jomova K, Makova M, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, et al. Essential

764 metals in health and disease. Chem Biol Interact 2022;367:110173.

765   [3] Daw CC, Ramachandran K, Enslow BT, Maity S, Bursic B, Novello MJ, et al. Lactate Elicits

766   ER-Mitochondrial Mg(2+) Dynamics to Integrate Cellular Metabolism. Cell 2020;183:474−89

767   e17.

768   [4] Sissi C, Palumbo M. Effects of magnesium and related divalent metal ions in topoisomerase

769   structure and function. Nucleic Acids Res 2009;37:702−11.

770   [5] de Baaij JH, Hoenderop JG, Bindels RJ. Magnesium in man: implications for health and disease.

771   Physiol Rev 2015;95:1−46.

772   [6] Ban C, Junop M, Yang W. Transformation of MutL by ATP binding and hydrolysis: a switch

773   in DNA mismatch repair. Cell 1999;97:85−97.

774   [7] Rubin H. Central role for magnesium in coordinate control of metabolism and growth in animal

775   cells. Proc Natl Acad Sci U S A 1975;72:3551−5.

776   [8] Barbagallo M, Veronese N, Dominguez LJ. Magnesium in Aging, Health and Diseases.

777   Nutrients 2021;13:463.

778   [9] Auwercx J, Rybarczyk P, Kischel P, Dhennin-Duthille I, Chatelain D, Sevestre H, et al. Mg(2+)

779   Transporters in Digestive Cancers. Nutrients 2021;13:210.

780   [10] Gupta AA, Shekatkar M, Raj AT, Kheur S. Potential Role of Magnesium in Cancer Initiation

781   and Progression. Pathol Oncol Res 2020;26:2001−2.

782   [11] Kumar G, Chatterjee PK, Madankumar S, Mehdi SF, Xue X, Metz CN. Magnesium

783   deficiency with high calcium-to-magnesium ratio promotes a metastatic phenotype in the CT26

784   colon cancer cell line. Magnes Res 2020;33:68−85.

785   [12] Nasulewicz A, Wietrzyk J, Wolf FI, Dzimira S, Madej J, Maier JA, et al. Magnesium

786   deficiency inhibits primary tumor growth but favors metastasis in mice. Biochim Biophys Acta

787   2004;1739:26−32.

788   [13] Li T, Yu Y, Shi H, Cao Y, Liu X, Hao Z, et al. Magnesium in combinatorial with valproic

789   acid suppressed the proliferation and migration of human bladder cancer cells. Front Oncol

790   2020;10:589112.

791   [14] Mazur A, Maier JA, Rock E, Gueux E, Nowacki W, Rayssiguier Y. Magnesium and the

792   inflammatory response: potential physiopathological implications. Arch Biochem Biophys

793   2007;458:48−56.

794   [15] Sun L, Li X, Xu M, Yang F, Wang W, Niu X. In vitro immunomodulation of magnesium on

795   monocytic cell toward anti-inflammatory macrophages. Regen Biomater 2020;7:391−401.

27

796 [16] Willox JC, McAllister EJ, Sangster G, Kaye SB. Effects of magnesium supplementation in

797 testicular cancer patients receiving cis-platin: a randomised trial. Br J Cancer 1986;54:19−23.

798 [17] Castiglioni S, Cazzaniga A, Trapani V, Cappadone C, Farruggia G, Merolle L, et al.

799 Magnesium homeostasis in colon carcinoma LoVo cells sensitive or resistant to doxorubicin. Sci

800 Rep 2015;5:16538.

801 [18] Meng Y, Sun J, Yu J, Wang C, Su J. Dietary intakes of calcium, Iron, magnesium, and

802 potassium elements and the risk of colorectal cancer: a meta-analysis. Biol Trace Elem Res

803 2019;189:325−35.

804 [19] Chen GC, Pang Z, Liu QF. Magnesium intake and risk of colorectal cancer: a meta-analysis

805 of prospective studies. European Journal of Clinical Nutrition 2012;66:1182−6.

806 [20] van den Brandt PA, Smits KM, Goldbohm RA, Weijenberg MP. Magnesium intake and

807 colorectal cancer risk in the Netherlands Cohort Study. British Journal of Cancer 2007;96:510−3.

808 [21] Larsson SC, Bergkvist L, Wolk A. Magnesium intake in relation to risk of colorectal cancer

809 in women. Jama 2005;293:86−9.

810 [22] Shahi A, Aslani S, Ataollahi M, Mahmoudi M. The role of magnesium in different

811 inflammatory diseases. Inflammopharmacology 2019;27:649−61.

812 [23] Gilca-Blanariu GE, Trifan A, Ciocoiu M, Popa IV, Burlacu A, Balan GG, et al. Magnesium-

813 A Potential Key Player in Inflammatory Bowel Diseases? Nutrients 2022;14.

814 [24] Schmitt M, Greten FR. The inflammatory pathogenesis of colorectal cancer. Nat Rev

815 Immunol 2021;21:653−67.

816 [25] Scanlan BJ, Tuft B, Elfrey JE, Smith A, Zhao A, Morimoto M, et al. Intestinal inflammation

817 caused by magnesium deficiency alters basal and oxidative stress-induced intestinal function. Mol

818 Cell Biochem 2007;306:59−69.

819 [26] Liu M, Dudley SC, Jr. Magnesium, Oxidative Stress, Inflammation, and Cardiovascular

820 Disease. Antioxidants (Basel) 2020;9.

821 [27] Petrović J, Stanić D, Dmitrašinović G, Plećaš-Solarović B, Ignjatović S, Batinić B, et al.

822 Magnesium Supplementation Diminishes Peripheral Blood Lymphocyte DNA Oxidative Damage

823 in Athletes and Sedentary Young Man. Oxid Med Cell Longev 2016;2016:2019643.

824 [28] Wesselink E, Winkels RM, van Baar H, Geijsen AJMR, van Zutphen M, van Halteren HK, et

825 al. Dietary Intake of Magnesium or Calcium and Chemotherapy-Induced Peripheral Neuropathy

826 in Colorectal Cancer Patients. Nutrients 2018;10:398.

[29] Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet 2019;394:1467−80.

[30] Hardy S, Kostantin E, Wang SJ, Hristova T, Galicia-Vázquez G, Baranov PV, et al. Magnesium-sensitive upstream ORF controls PRL phosphatase expression to mediate energy metabolism. Proc Natl Acad Sci U S A 2019;116:2925−34.

[31] Zou Z-G, Rios FJ, Montezano AC, Touyz RM. TRPM7, Magnesium, and Signaling. International Journal of Molecular Sciences 2019;20:1877.

[32] Yu L, Xu L, Xu M, Wan B, Yu L, Huang Q. Role of Mg2+ ions in protein kinase phosphorylation: insights from molecular dynamics simulations of ATP-kinase complexes. Molecular Simulation 2011;37:1143−50.

[33] Vasaikar S, Huang C, Wang X, Petyuk VA, Savage SR, Wen B, et al. Proteogenomic Analysis of Human Colon Cancer Reveals New Therapeutic Opportunities. Cell 2019;177:1035−49.e19.

[34] Li C, Sun YD, Yu GY, Cui JR, Lou Z, Zhang H, et al. Integrated Omics of Metastatic Colorectal Cancer. Cancer Cell 2020;38:734−47 e9.

[35] Zhang R, Hu M, Chen HN, Wang X, Xia Z, Liu Y, et al. Phenotypic Heterogeneity Analysis of APC-Mutant Colon Cancer by Proteomics and Phosphoproteomics Identifies RAI14 as a Key Prognostic Determinant in East Asians and Westerners. Mol Cell Proteomics 2023;22:100532.

[36] Gao L, Lu Y, Chen HN, Li Z, Hu M, Zhang R, et al. Deciphering the Clinical Significance and Kinase Functions of GSK3α in Colon Cancer by Proteomics and Phosphoproteomics. Mol Cell Proteomics 2023;22:100545.

[37] Morel JD, Sauzéat L, Goeminne LJE, Jha P, Williams E, Houtkooper RH, et al. The mouse metallomic landscape of aging and metabolism. Nat Commun 2022;13:607.

[38] Campbell BB, Light N, Fabrizio D, Zatzman M, Fuligni F, de Borja R, et al. Comprehensive analysis of hypermutation in human cancer. Cell 2017;171:1042−56 e10.

[39] Kiefer JR, Mao C, Braman JC, Beese LS. Visualizing DNA replication in a catalytically active Bacillus DNA polymerase crystal. Nature 1998;391:304−7.

[40] Doublié S, Tabor S, Long AM, Richardson CC, Ellenberger T. Crystal structure of a bacteriophage T7 DNA replication complex at 2.2 Å resolution. Nature 1998;391:251−8.

[41] Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487:330−7.

857   [42] Blaszczyk U, Duda-Chodak A. Magnesium: its role in nutrition and carcinogenesis. Rocz

858   Panstw Zakl Hig 2013;64:165−71.

859   [43] Hartwig A. Role of magnesium in genomic stability. Mutat Res 2001;475:113−21.

860   [44] Wolf FI, Maier JA, Nasulewicz A, Feillet-Coudray C, Simonacci M, Mazur A, et al.

861   Magnesium and neoplasia: from carcinogenesis to tumor growth and progression or treatment.

862   Arch Biochem Biophys 2007;458:24−32.

863   [45] Martínez-Jiménez F, Muiños F, Sentís I, Deu-Pons J, Reyes-Salazar I, Arnedo-Pac C, et al. A

864   compendium of mutational cancer driver genes. Nat Rev Cancer 2020;20:555−72.

865   [46] Huang HW, Chang CC, Wang CS, Lin KH. Association between inflammation and function

866   of cell adhesion molecules influence on gastrointestinal cancer development. Cells 2021;10.

867   [47] Reglero-Real N, Colom B, Bodkin JV, Nourshargh S. Endothelial Cell Junctional Adhesion

868   Molecules: Role and Regulation of Expression in Inflammation. Arterioscler Thromb Vasc Biol

869   2016;36:2048−57.

870   [48] Weber C, Fraemohs L, Dejana E. The role of junctional adhesion molecules in vascular

871   inflammation. Nat Rev Immunol 2007;7:467−77.

872   [49] Lötscher J, Martí ILAA, Kirchhammer N, Cribioli E, Giordano Attianese GMP, Trefny MP,

873   et al. Magnesium sensing via LFA-1 regulates CD8(+) T cell effector function. Cell

874   2022;185:585−602.e29.

875   [50] Roumenina LT, Daugan MV, Petitprez F, Sautès-Fridman C, Fridman WH. Context-

876   dependent roles of complement in cancer. Nat Rev Cancer 2019;19:698−715.

877   [51] Reis ES, Mastellos DC, Hajishengallis G, Lambris JD. New insights into the immune

878   functions of complement. Nat Rev Immunol 2019;19:503−16.

879   [52] Nitta H, Murakami Y, Wada Y, Eto M, Baba H, Imamura T. Cancer cells release

880   anaphylatoxin C5a from C5 by serine protease to enhance invasiveness. Oncol Rep

881   2014;32:1715−9.

882   [53] Cho MS, Rupaimoole R, Choi HJ, Noh K, Chen J, Hu Q, et al. Complement Component 3 Is

883   Regulated by TWIST1 and Mediates Epithelial-Mesenchymal Transition. J Immunol

884   2016;196:1412−8.

885   [54] Abdelbaset-Ismail A, Borkowska-Rzeszotek S, Kubis E, Bujko K, Brzeźniakiewicz-Janus K,

886   Bolkun L, et al. Activation of the complement cascade enhances motility of leukemic cells by

887   downregulating expression of HO-1. Leukemia 2017;31:446−58.

[55] Larsen M, Tremblay ML, Yamada KM. Phosphatases in cell-matrix adhesion and migration. Nat Rev Mol Cell Biol 2003;4:700−11.

[56] Mitra SK, Hanson DA, Schlaepfer DD. Focal adhesion kinase: in command and control of cell motility. Nat Rev Mol Cell Biol 2005;6:56−68.

[57] Butkevich E, Bodensiek K, Fakhri N, von Roden K, Schaap IAT, Majoul I, et al. Drebrin-like protein DBN-1 is a sarcomere component that stabilizes actin filaments during muscle contraction. Nat Commun 2015;6:7523.

[58] Howard EW, Crider BJ, Updike DL, Bullen EC, Parks EE, Haaksma CJ, et al. MMP-2 expression by fibroblasts is suppressed by the myofibroblast phenotype. Exp Cell Res 2012;318:1542−53.

[59] Huang Z, Zhou JK, Wang K, Chen H, Qin S, Liu J, et al. PDLIM1 Inhibits Tumor Metastasis Through Activating Hippo Signaling in Hepatocellular Carcinoma. Hepatology 2020;71:1643−59.

[60] Wolf FI, Cittadini AR, Maier JA. Magnesium and tumors: ally or foe? Cancer Treat Rev 2009;35:378−82.

[61] Trapani V, Wolf FI. Dysregulation of Mg(2+) homeostasis contributes to acquisition of cancer hallmarks. Cell Calcium 2019;83:102078.

[62] Wang P, Yu X, Guan P-P, Guo J-W, Wang Y, Zhang Y, et al. Magnesium ion influx reduces neuroinflammation in Aβ precursor protein/Presenilin 1 transgenic mice by suppressing the expression of interleukin-1β. Cellular & Molecular Immunology 2017;14:451−64.

[63] Paterson C, Clevers H, Bozic I. Mathematical model of colorectal cancer initiation. Proc Natl Acad Sci U S A 2020;117:20681−8.

[64] Huang Z, Zhang Z, Zhou C, Liu L, Huang C. Epithelial-mesenchymal transition: The history, regulatory mechanism, and cancer therapeutic opportunities. MedComm (2020) 2022;3:e144.

[65] Serrano-Gomez SJ, Maziveyi M, Alahari SK. Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. Molecular Cancer 2016;15:18.

[66] Zhong Q, Xiao X, Qiu Y, Xu Z, Chen C, Chong B, et al. Protein posttranslational modifications in health and diseases: Functions, regulatory mechanisms, and therapeutic implications. MedComm (2020) 2023;4:e261.

[67] Zheng H, Shen M, Zha YL, Li W, Wei Y, Blanco MA, et al. PKD1 phosphorylation-dependent degradation of SNAIL by SCF-FBXO11 regulates epithelial-mesenchymal transition and metastasis. Cancer Cell 2014;26:358−73.

919 [68] Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, et al. Dual regulation of Snail by GSK-
920 3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol
921 2004;6:931−40.

922 [69] Wang ZC, Gao Q, Shi JY, Guo WJ, Yang LX, Liu XY, et al. Protein tyrosine phosphatase
923 receptor S acts as a metastatic suppressor in hepatocellular carcinoma by control of epithermal
924 growth factor receptor-induced epithelial-mesenchymal transition. Hepatology 2015;62:1201−14.

925 [70] Parisse S, Ferri F, Persichetti M, Mischitelli M, Abbatecola A, Di Martino M, et al. Low
926 serum magnesium concentration is associated with the presence of viable hepatocellular carcinoma
927 tissue in cirrhotic patients. Sci Rep 2021;11:15184.

928 [71] Wesselink E, Kok DE, Bours MJL, de Wilt JHW, van Baar H, van Zutphen M, et al. Vitamin
929 D, magnesium, calcium, and their interaction in relation to colorectal cancer recurrence and all-
930 cause mortality. The American Journal of Clinical Nutrition 2020;111:1007−17.

931 [72] Vincenzi B, Santini D, Galluzzo S, Russo A, Fulfaro F, Silletta M, et al. Early magnesium
932 reduction in advanced colorectal cancer patients treated with cetuximab plus irinotecan as
933 predictive factor of efficacy and outcome. Clin Cancer Res 2008;14:4219−24.

934 [73] Schulz C, Heinemann V, Heinrich K, Haas M, Holch JW, Fraccaroli A, et al. Predictive and
935 prognostic value of magnesium serum level in FOLFIRI plus cetuximab or bevacizumab treated
936 patients with stage IV colorectal cancer: results from the FIRE-3 (AIO KRK-0306) study.
937 Anticancer Drugs 2020;31:856−65.

938 [74] Streb J, Püsküllüoğlu M, Glanowska I, Ochenduszko S, Konopka K, Łupkowski R, et al.
939 Assessment of frequency and severity of hypomagnesemia in patients with metastatic colorectal
940 cancer treated with cetuximab, with a review of the literature. Oncol Lett 2015;10:3749−55.

941 [75] Tejpar S, Piessevaux H, Claes K, Piront P, Hoenderop JG, Verslype C, et al. Magnesium
942 wasting associated with epidermal-growth-factor receptor-targeting antibodies in colorectal cancer:
943 a prospective study. Lancet Oncol 2007;8:387−94.

944 [76] Multhaupt HA, Leitinger B, Gullberg D, Couchman JR. Extracellular matrix component
945 signaling in cancer. Adv Drug Deliv Rev 2016;97:28−40.

946 [77] Huang J, Zhang L, Wan D, Zhou L, Zheng S, Lin S, et al. Extracellular matrix and its
947 therapeutic potential for cancer treatment. Signal Transduct Target Ther 2021;6:153.

948 [78] Li M, Wang Y, Li M, Wu X, Setrerrahmane S, Xu H. Integrins as attractive targets for cancer
949 therapeutics. Acta Pharm Sin B 2021;11:2726−37.

950 [79] Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhim R, Getz G. GISTIC2.0

951 facilitates sensitive and confident localization of the targets of focal somatic copy-number

952 alteration in human cancers. Genome Biol 2011;12:R41.

953 [80] Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, et al. DAVID: a web server for

954 functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic

955 Acids Res 2022.

956 [81] Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene

957 lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44−57.

958 [82] Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape

959 provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun

960 2019;10:1523.

961 [83] Chen T, Chen X, Zhang S, Zhu J, Tang B, Wang A, et al. The Genome Sequence Archive

962 Family: Toward Explosive Data Growth and Diverse Data Types. Genomics Proteomics

963 Bioinformatics 2021;19:578−83.

964

965 **Figure legends**

966 **Figure 1 Low Mg content in tumors predicts unfavorable prognosis of CRC**

967 **A.** The Study's concept and workflow. **B.** Intra-tumorous Mg content of CRCs. **C.** Statistics of Mg

968 content in the tumors and paired DNTs of 115 CRCs. *, $P < 0.05$. Wilcoxon rank-sum test. **D.**

969 Comparisons of Mg content between left- and right-sided tumors or between left- and right-sided

970 DNTs. ***, $P < 0.001$; **, $P < 0.01$. Wilcoxon rank-sum test. **E.** Comparisons of Mg content

971 between tumors and paired DNTs located on the left or right side. *, $P < 0.05$; ns, means no

972 significance. Wilcoxon rank-sum test. **F.** Survival analysis of 115 CRC patients with different Mg

973 contents in tumors. Log-rank test. **G.** Survival analysis of 53 left-sided CRC patients with different

974 Mg contents in tumors. Log-rank test. **H.** Survival analysis of 49 right-sided CRC patients with

975 different Mg contents in tumors. Log-rank test. **I.** Comparisons of Mg content between females

976 and males in tumors or DNTs. ns, means no significance. Wilcoxon rank-sum test.

977

978 **Figure 2 Low Mg content in tumors is linked to genome instability**

979 **A.** Genetic profile of the top 20 mutated genes in High-Mg and Low-Mg tumors. Somatic

980   mutations in the 20 genes were observed in 49 of 62 tumors. The top bar plot illustrates the overall

981   count of somatic mutations in each patient, while the right bar plot depicts the distribution and

982   composition of mutation types in each gene. **B.** Percentages of SNVs in High-Mg and Low-Mg

983   groups. **C.** Percentages of the classes of mutations in High-Mg and Low-Mg groups. **D.** and **E.**

984   The interactions among the top 20 mutated genes in the High-Mg (D) and Low-Mg (E) groups. *,

985    $P < 0.01$; ▲, $P < 0.05$. Fisher's exact test. **F.** Focal peaks exhibiting significant somatic copy-

986   number amplification (red) and deletion (blue) (GISTIC2 Q-values < 0.1) are displayed in both

987   High-Mg and Low-Mg groups. The top 5 amplified and deleted cytobands are labeled. **G.** Statistics

988   of genes with significant variations in mutation frequencies between the High-Mg and Low-Mg

989   groups. $P < 0.05$. Fisher's exact test. **H.** Density plot displaying the mutation frequencies of genes

990   in the High-Mg and Low-Mg groups. **I.** Genetic profile of the CRC driver genes with significant

991   mutation frequencies between the High-Mg and Low-Mg groups. $P < 0.05$. Fisher's exact test. **J.**

992   Comparisons of gene mutation frequencies between left- and right-sided tumors. Genes with

993   mutations in more than 7 out of 62 patients are displayed. **K.** Comparisons of gene mutation

994   frequencies between High-Mg and Low-Mg tumors on the left (lower) or right (upper) side. Genes

995   with mutations in more than 5 out of 62 patients are displayed.

996

997   **Figure 3 Low Mg content in tumors is associated with EMT activation**

998   **A.** Spearman's rank correlation analysis of protein levels and Mg content. GSEA pre-ranked

999   hallmark analysis of the 5322 proteins. $P < 0.05$. Spearman's rank correlation test. **B.** and **C.** Blue

1000   and red represent the pathways negatively (B) or positively (C) associated with Mg content,

1001   respectively. NES represents normalized enrichment score. **D.** Hallmark pathways related to

1002   immunity and metastasis. ES means enrichment score. **E.** and **F.** Venn diagrams displaying the

1003   overlapping proteins negatively (E) or positively (F) associated with Mg content between the left-

1004   and right-sided tumors. Spearman's rank correlation analysis, $P < 0.05$. **G.** Heatmap showing the

1005   levels of Mg-related proteins in the left- and right-sided tumors. The correlation patterns of the

1006   proteins with Mg content in distinct modules are shown. Z-score of protein levels were mapped

1007   along Mg content in the left- and right-sided tumors. The top 5 pathways enriched by Metascape

1008   database using Mg-related proteins are shown. The gender, age, TNM stage, OS status and Mg

1009   content are annotated above the heatmap.

1010

Figure 4 Functional validation of Mg in EMT activation in tumor cells

A. The diagram illustrates the schematic of the EMT and cell adhesion pathway. Spearman's coefficients between Mg content and the levels of core components involved in cell-cell adhesion and cell-matrix adhesion are shown. The correlations were analyzed using all 104 tumor samples (A), 54 left-sided tumor samples (L), and 48 right-sided tumor samples (R). **B.** Immunoblots showing the expression of *N*-cadherin, vimentin, vinculin, MMP2, *E*-cadherin and cingulin in High-Mg and Low-Mg tumors. **C.** The measurements of Mg content in HCT116 and DLD-1 cells with or without $MgCl_2$ treatment by ICP-MS. ***, $P < 0.001$; **, $P < 0.01$. Student's *t* test. **D.** Western blotting analysis of vimentin, E-cadherin, N-cadherin and MMP2 in colon cancer cell lines with (10 mM) or without $MgCl_2$ treatment. **E.** Representative images (left) and quantification results (right) of the migration assays using colon cancer cells treated with increasing concentrations of $MgCl_2$. Scale bars, 500 μm. ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Student's *t* test. **F.** and **G.** Representative images (left) and quantification results (right) of the wound healing assays using HCT116 (F) and DLD-1 (G) cells treated with (10 mM) or without $MgCl_2$. Scale bars, 200 μm. ****, $P < 0.0001$; **, $P < 0.01$. Student's *t* test.

Figure 5 Impacts of Mg on the phosphoproteome of CRCs

A. Spearman's rank correlation analysis of the levels of phosphosites and Mg content. $P < 0.05$. Spearman's rank correlation test. **B.** and **C.** Pathway enrichment analysis of the proteins containing Mg negatively associated phosphosites (B) and positively associated phosphosites (C) by Metascape. **D.** Mg-correlated pathways related to cell adhesion are shown with Spearman's coefficients obtained from (A). **E.** Venn diagrams displaying the overlapping phosphosites significantly correlated with Mg content between the left- and right-sided tumors. Spearman's rank correlation analysis, $P < 0.05$. **F.** Heatmap displaying the levels of 18 common Mg-related phosphosites in left- and right-sided tumors. **G.** Heatmap showing the levels of phosphosites specifically correlated with Mg content in tumors on the left and right sides. The top 5 enriched pathways by Metascape using the proteins with Mg significantly correlated phosphosites are shown. Annotations above the heatmap include information such as gender, age, TNM stage, OS status, and Mg content. The heatmap illustrates the relative expression of phosphosites, utilizing the z-score for representation.

35

Figure 6 Mg-regulated DBN1^{S142p} reduction inhibits cell migration in CRC cells

A. The statistics of the number of phosphosites occurring in the +Mg^{2+} group or in the control group (left). The phosphosites mainly identified in the Mg^{2+} and control groups were directly considered upregulated (484) and downregulated (150), respectively. Additionally, Volcano plot showing 80 upregulated and 366 downregulated phosphosites in the +Mg^{2+} group and control group (right), respectively, using phosphosites identified in at least two replicates of each group. $P < 0.05$. Cutoff, ratio(+Mg^{2+}/Control) > 1.2 or < 0.83. Student's t test. Blue and red dots represent significantly increased phosphosites in the control and +Mg^{2+} groups, respectively. **B.** and **C.** Pathway enrichment analysis of the corresponding proteins of downregulated (516) (B) and upregulated (564) (C) phosphosites in the +Mg^{2+} group using Metascape. **D.** Venn diagram showing the number of overlapping phosphosites quantified in HCT116 and CRC. **E.** Venn diagram showing the number of overlapping phosphosites with significant changes in Mg^{2+}-treated HCT116 cells and significant correlations with Mg content in CRC. **F.** Survival analysis of 79 CRC patients with different DBN1^{S142p} levels in tumors. $P < 0.05$. Log-rank test. **G.** MS/MS spectrum of the identified phosphopeptide containing DBN1^{S142p}. **H.** The statistics of the levels of DBN1^{S142p} in the control and +Mg^{2+} groups. *, $P < 0.05$. Student's t test. **I.** Spearman's rank correlation analysis of DBN1^{S142p} and Mg content in all (72), left (25)- and right (46)-sided tumors. **J.** Mutation information of the DBN1^{WT}, DBN1^{S142A} and DBN1^{S142D} genes and sequencing results. **K.** Immunoblotting assays determined the effects of DBN1 mutations on MMP2 expression. *, $P < 0.05$. Student's t test. **L.** and **M.** Transwell migration (L) and wound healing assays (M) were performed in cells transfected with vector, DBN1^{WT}, DBN1^{S142A} and DBN1^{S142D}. Scale bars, 500 μm (L) and 200 μm (M). **, $P < 0.01$; *, $P < 0.05$. Student's t test.

Figure 7 Mg-regulated DBN1^{S142p} reduces the interaction between DBN1 and ACTN4 and contributes to EMT

A. Effects of MgCl$_2$ treatment on the formation of F-actin determined by immunofluorescence assays in HCT116 cells. Scale bars, 500 μm. *, $P < 0.05$. Student's t test. **B.** Effects of DBN1^{WT}, DBN1^{S142A} and DBN1^{S142D} on the formation of F-actin determined by immunofluorescence assays. Scale bars, 500 μm. *, $P < 0.05$. Student's t test. **C.** Schematic representation of the identification of DBN1-binding proteins affected by DBN1^{S142p} and Mg^{2+} through affinity purification followed by MS analysis. **D.** Heatmap showing 9 DBN1-binding proteins affected by Mg^{2+} through

36

regulating DBN1^{S142p}. Z-score of protein levels were used. **E.** The relative intensity of the DBN1-binding protein ACTN4 determined by MS in immunoprecipitation assays under different experimental conditions. ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, means no significance. Student's t test. **F.** Immunoblotting assays determined the effects of DBN1^{S142p} on the interactions of ACTN4 and CDC42 with DBN1. **G.** Immunoblotting assays determined the effects of Mg on the interaction between ACTN4 and DBN1 affected by DBN1^{S142p}. **H.** Effects of ACTN4 on the formation of F-actin determined by immunofluorescence assays. Scale bars, 500 μm. **, $P < 0.01$; *, $P < 0.05$. Student's t test. **I.** Co-localization of ACTN4 and F-actin was determined by immunofluorescence assays. Scale bars, 500 μm. ***, $P < 0.001$; **, $P < 0.01$. Student's t test. **J.** Potential mechanism of Mg-regulated cell migration *via* modulation of DBN1^{S142p}.

Supplementary material

Supplementary Figure Legends

Figure S1 Quality control analysis of proteome and phosphoproteome data and Mg content detection

A. Workflow of proteome and phosphoproteome experiments. **B.** Pearson's correlation analysis of 15 QC samples of the proteome to evaluate machine stability. A scatterplot matrix was generated for pairwise calculation of Pearson's correlation coefficients among the samples, with density plots on the diagonal and correlation values displayed in the upper triangle. **C.** Pearson's correlation analysis of 27 internal standards to evaluate the robustness of TMT-based quantification in the proteomic study. Top right: pairwise calculation of Pearson's correlation coefficients among the 27 ISs. Bottom left: elliptic chart shows pairwise comparison of the 27 ISs. **D.** Pearson's correlation analysis of replicate samples. **E.** Distribution of unique peptides among 27 TMT-labeled batches. **F.** Protein counts of each batch (light blue), counts of common proteins (blue) and cumulative protein counts among the 27 batches. **G.** Pearson's correlation analysis of 6 QC samples of the phosphoproteome to evaluate machine stability. A scatterplot matrix was generated for pairwise calculation of Pearson's correlation coefficients among the samples, with density plots on the diagonal and correlation values displayed in the upper triangle. **H.** Pearson's correlation analysis of 20 ISs to evaluate the robustness of TMT-based quantification in the phosphoproteomic study. Top-right half: pairwise calculation of Pearson's correlation coefficients among the 20 ISs.

37

1104   Bottom-left half: elliptic chart shows pairwise comparison of the 20 ISs. **I.** Identified (blue) and

1105   quantified (orange) phosphoprotein counts of each batch and cumulative phosphoprotein counts

1106   among 20 batches. **J.** Number of identified and quantified phosphosites (blue) and

1107   phosphoproteins (red). **K.** Localization and distribution of phosphosites. **L.** Statistics of the

1108   number of phosphosites in a protein. **M.** Coefficient of variation of Mg content for the QC samples.

1109   **Figure S2 Association analysis between Mg content and TNM stage (or overall survival)**

1110   **A.−C.** Forest plots of univariable cox models for clinical information with CRC patients in all

1111   samples (A), or left-sided (B) or right-sided (C) colon samples. Data are presented as hazard ratio

1112   (HR) ± 95% confidence interval. **D.−F.** Cox multivariable regression models designed to test for

1113   prognostic factors. Low Mg content was an independent predictor of outcome in all CRC patients

1114   (D) and right-sided CRC patients (E) but not left-sided CRC patients (F). Data are presented as

1115   hazard ratio (HR) ± 95% confidence interval. **G.−I.** Mg content among four TNM stages in tumors

1116   and DNTs of all samples (G) or left-sided (H) or right-sided (I) colon samples. **J.** Violin plots

1117   illustrating the levels of clinical information, encompassing variables such as age, ALT, AST, etc.,

1118   for both the High-Mg and Low-Mg groups.

1119   **Figure S3 Analysis of Mg-associated proteome and phosphoproteome**

1120   **A.** GOBP term enrichment analysis of proteins negatively associated with Mg content using

1121   DAVID database. **B.** GOBP term enrichment analysis of proteins positively associated with Mg

1122   content using DAVID database. **C.** The expression of complement-associated proteins in the

1123   tumors and paired DNTs. **D.** The expression of cell adhesion-associated proteins in the tumors and

1124   paired DNTs. GOBP, Gene ontology biological process.

1125   **Figure S4 Proteomic classification of CRC tumors**

1126   **A.** Consensus matrix of unsupervised clustering based on the top 25 most variable proteins (k = 2,

1127   k =3 and k = 4). **B.** The consensus CDF of unsupervised clustering based on the top 25 most

1128   variable proteins. **C.** Delta area (change in CDF area) plots for 2-10 clusters. **D.** Kaplan–Meier

1129   curves of OS for each proteomic subtype. **E.** Boxplot showing the difference of Mg content among

1130   proteomic subtypes. **F.** GOBP enrichment analysis of proteins upregulated in subtype II (subtype

1131   II *vs* subtype I and subtype III) using DAVID.

1132   **Figure S5 Integrative analysis of proteome and phosphoproteome data**

1133   **A.** Comparison between Mg-protein correlations and their Mg-phosphosite correlations. $P < 0.05$.

1134   Spearman's rank correlation test. **B.** and **C.** Top 20 phosphosites positively (B) or negatively (C)

associated with Mg content independent of protein level. **D.** GOBP enrichment analysis of phosphoproteins with negatively Mg-associated phosphosites. **E.** GOBP enrichment analysis of phosphoproteins with positively Mg-associated phosphosites.

Figure S6 Mg-regulated phosphosites related to cell adhesion

A. Survival analysis of 79 CRC patients with different ARHGAP1^{S51p}, FLNA^{S1459p} and TNS1^{S1177p} levels in tumors. Log-rank test. **B.** Effects of MgCl$_2$ treatment on the formation of F-actin determined by immunofluorescence assays in DLD-1 cells. Scale bars, 500 μm. *, $P < 0.05$, Student's t test. **C.** Immunoblotting assays determined the expression of ARHGAP1 mutations. *, $P < 0.05$, Student's t test. **D.** Transwell assays were performed in cells transfected with empty vector, ARHGAP1^{WT}, ARHGAP1^{S51A} and ARHGAP1^{S51D}, respectively. Scale bars, 500 μm. **, $P < 0.01$; *, $P < 0.05$. Student's t test. **E.** Effects of empty vector, ARHGAP1^{WT}, ARHGAP1^{S51A} and ARHGAP1^{S51D} on F-actin formation determined by immunofluorescence assays. Scale bars, 500 μm. *, $P < 0.05$. Student's t test.

Figure S7 The DBN1-binding proteins affected by DBN1^{S142p} and Mg^{2+}

A. and **B.** Venn diagram showing the number of proteins strongly and specifically bound to DBN1^{S142D} (A) and DBN1^{S142A} (B), respectively. Fold changes of proteins between groups > 1.2, $P < 0.05$. Student's t test. Gene annotation enrichment analysis of the proteins obtained in protein-protein interaction network analysis. **C.** Schematic of screening Mg-regulated DBN1 binding proteins affected by DBN1^{S142p}. **D.** GO biological process enrichment analysis of Mg-regulated DBN1 binding proteins. **E.** and **F.** Venn diagram showing the number of interacting proteins affected by both DBN1^{S142p} and Mg^{2+}. **G.** The levels of the DBN1-binding protein CDC42 under different treatment and immunoprecipitation conditions. ****, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$; ns means no significance. Student's t test. **H.** Immunoblots showing the expression of ACTN4.

Figure S8 Mg-associated cell-matrix adhesion proteins as drug targets

Cell-matrix adhesion-associated proteins with clinical drugs were negatively associated with Mg content. Spearman's rank correlation analysis, $P < 0.05$ indicating a significant correlation. The gender, TNM stage, survival event, age and Mg content are annotated above the heatmap. The heatmap depicts the intensity of proteins using the z-score. The corresponding subcellular location (middle) and clinical drugs (right) are listed.

1166 **Supplementary Table Legends**

1167 **Table S1. Clinical information of CRC patients and Mg content in tumors and DNTs**.

1168 **Table S2. Genomic data of CRC**.

1169 **Table S3. Proteomics data of CRC**.

1170 **Table S4. Spearman's rank correlation analysis of the levels of the cell adhesion-related**

1171 **proteins and Mg content**.

1172 **Table S5. Phosphoproteomics data of CRC**.

1173 **Table S6. Phosphoproteome in Mg-treated HCT116 cells**.
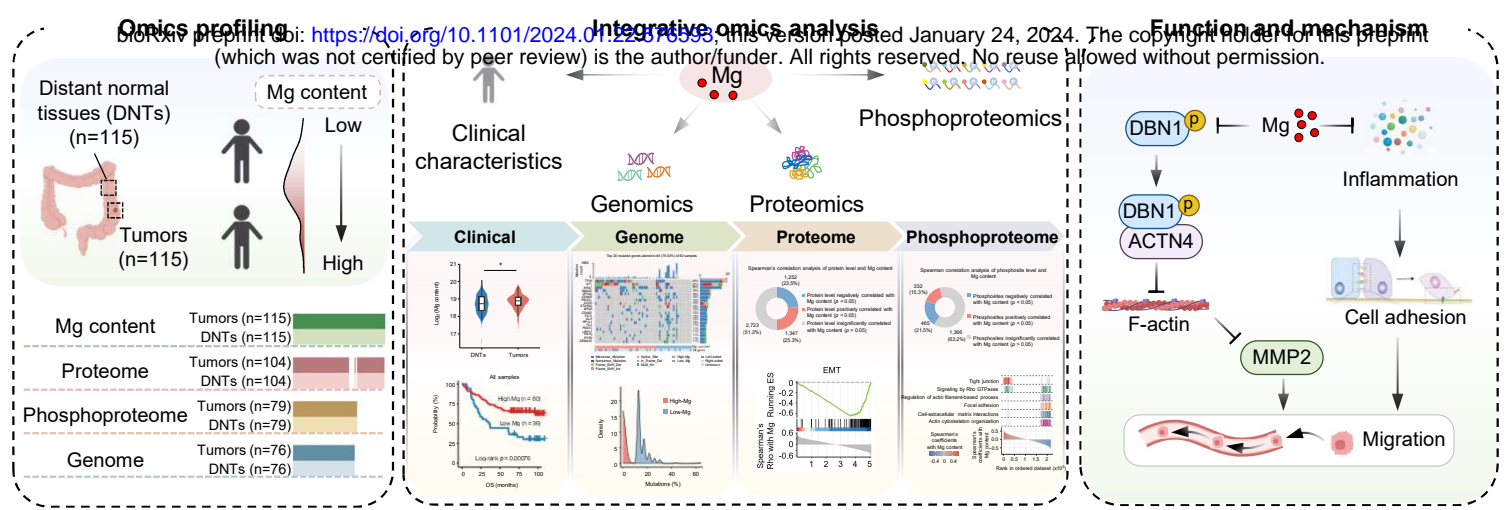
1174 **Table S7. Proteins strongly and specifically bound to DBN1 and affected by DBN1^{S142p} and**

1175 **Mg^{2+}**.

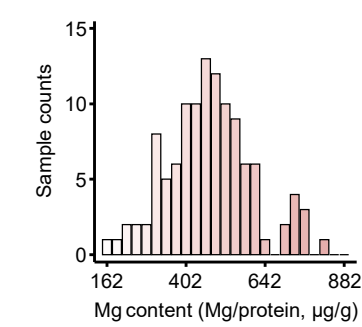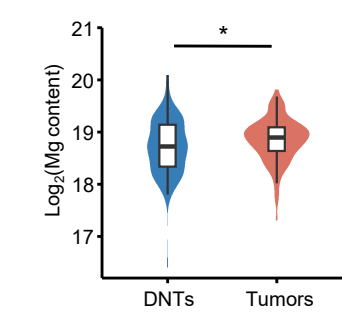1176 **Table S8. The subcellular location and drugs of targets.**

1177

Figure 1

Figure 2

Figure 3

A

Spearman correlation analysis of protein level and Mg content

1252 (23.5%)

2723 (51.2%)

1347 (25.3%)

- Protein level negatively correlated with Mg content (*p* < 0.05)
- Protein level positively correlated with Mg content (*p* < 0.05)
- Protein level insignificantly correlated with Mg content (*p* > 0.05)

B

Epithelial mesenchymal transition
Coagulation
Myogenesis
Complement
Apical junction
TNFα signaling via NF-κB
Angiogenesis
Heme metabolism
IL6-JAK-STAT3 signaling
Hypoxia

NES −2 −2.5 −3

−Log$_{10}$(*p* value) 0 2.5 5 7.5 10

C

MYC targets v1
E2F targets
Oxidative phosphorylation
MYC targets v2
G2M checkpoint
DNA repair
Fatty acid metabolism
Unfolded protein response
mTORC1 signaling

NES 1.5 2 2.5 3

−Log$_{10}$(*p* value) 0 2.5 5 7.5 10

D

EMT Apical junction TNFα signaling *via* NF-κB

Running ES Spearman's Rho

Coagulation Complement IL6-JAK-STAT3 signaling

Rank in ordered dataset (×10^3)

E

Proteins negatively correlated with Mg content (*p* < 0.05)

Left-sided (671)
209
462 — Overlap
719
Right-sided (1181)

F

Proteins positively correlated with Mg content (*p* < 0.05)

Left-sided (731)
257
475 — Overlap
754
Right-sided (1229)

G

Left-sided (n = 53) Right-sided (n = 48)

Gender
Age
TNM stage
OS status
Mg content

Left-sided Specific

Overlap

Right-sided specific

Ubiquitin mediated proteolysis
Nucleoside diphosphate phosphorylation
Actin cytoskeleton organization
Membrane Trafficking
Regulation of protein modification by small protein conjugation or removal

Asparagine N-linked glycosylation
Translation
Processing of Capped Intron-Containing Pre-mRNA
Protein processing in endoplasmic reticulum
Intra-Golgi vesicle-mediated transport

Actin filament-based process
Focal adhesion
Regulation of actin cytoskeleton
Hemostasis
Smooth Muscle Contraction

Translation
Mitochondrial translation
Ribonucleoprotein complex biogenesis
mRNA processing
Ribonucleoprotein complex assembly

Complement and coagulation cascades
Extracellular matrix organization
Hemostasis
NABA CORE MATRISOME
Vesicle-mediated transport

Metabolism of RNA
Ribonucleoprotein complex biogenesis
Translation
Mitochondrion organization
Metabolism of amino acids and derivatives

Mg content

Relative abundance (Z-score) −1 0 1

Gender: Female, Male

TNM stage: I, II, III, IV

OS status: 0, 1

Age: 20 60 100

Mg content (×10^5): 2 6 10

Figure 4

Figure 5

Figure 6

Figure 7

Figure S1

Figure S2

Figure S3

Figure S4

A

Consensus matrix (k=2)   Consensus matrix (k=3)   Consensus matrix (k=4)

B

Consensus CDF

C

Delta area

D

Log-rank *p* = 0.51

E

F

Figure S5

A

Phosphosites positively associated with Mg independent of protein level
Phosphosites negatively associated with Mg independent of protein level

n= 128

n= 118

Spearman's Rho of Mg and phosphosites

Spearman's Rho of Mg and phosphoproteins

B

Top 20 phosphosites positively associated with Mg independent of protein level

Spearman's R

-Log$_{10}$(p value) 1 3 5

-Log$_{10}$(p value) 0 0.4

○ Phosphosite   □ Phosphoprotein

C

Top 20 phosphosites negatively associated with Mg independent of protein level

Spearman's Rh

-Log$_{10}$(p value) 1 3 5

-Log$_{10}$(p value) -0.4 0 0.4

○ Phosphosite   □ Phosphoprotein

D

Phosphoproteins negatively associated with Mg only in phosphorylation level

Protein transport
mRNA splicing, via spliceosome
RNA splicing
Regulation of small GTPase mediated signal transduction
mRNA transport
Regulation of RNA splicing
Nucleocytoplasmic transport
Cellular response to ionizing radiation
Fatty acid biosynthetic process
Cellular response to hydrogen peroxide

-Log$_{10}$(p value)   Counts

Fold enrichment 4 8 12 16

Counts 3 5 7

-Log$_{10}$(p value) 2 3

E

Phosphoproteins positively associated with Mg only in phosphorylation level

Negative regulation of transcription, DNA-templated
Actin cytoskeleton organization
Endocytosis
Cytoskeleton organization
Response to virus
Protein import into nucleus
Activation of innate immune response
Positive regulation of type I interferon production
Retinoic acid receptor signaling pathway
Lipophagy

-Log$_{10}$(p value)   Counts

Fold enrichment 50 100 150 200

Counts 2 4 6 8

-Log$_{10}$(p value) 2.4 3.2

Figure S6

Figure S7

A Proteins favored binding to DBN1^{S142D} over DBN1^{S142A} (DBN1^{S142D} vs DBN1^{S142A}, $p < 0.05$)

Proteins strongly and specifically bound to DBN1^{S142D}

69 / 30 / 346

Proteins specifically bound to DBN1^{WT} (DBN1^{WT} vs Vector, $p < 0.05$)

- MCODE 1
- MCODE 2
- MCODE 3

Top3 GO-terms significantly enriched in each MCODE

MCODE	GO Description
1	Parkinson disease
	Amyotrophic lateral sclerosis
	Pathways of neurodegeneration
2	Actin filament organization
	Actin cytoskeleton organization
	Supramolecular fiber organization

B Proteins favored binding to DBN1^{S142A} over DBN1^{S142D} (DBN1^{S142D} vs DBN1^{S142A}, $p < 0.05$)

Proteins strongly and specifically bound to DBN1^{S142A}

66 / 46 / 330

Proteins specifically bound to DBN1^{WT} (DBN1^{WT} vs Vector, $p < 0.05$)

- MCODE 1

Top3 GO-terms significantly enriched in each MCODE

MCODE	GO Description
1	Actin filament depolymerization
	Actin filament-based process
	Actin filament organization

C

Vector vs DBN1^{WT} — 376 proteins specifically bound to DBN1^{WT}

Vector+Mg^{2+} vs DBN1^{WT}+Mg^{2+} — 407 proteins specifically bound to DBN1^{WT} with addition of Mg^{2+}

459 DBN1-binding proteins

DBN1^{WT} vs DBN1^{WT}+Mg^{2+}

Upregulated in DBN1^{WT}+Mg^{2+} → Enhanced interaction by Mg^{2+} (27 proteins)

Downregulated in DBN1^{WT}+Mg^{2+} → Weakened interaction by Mg^{2+} (56 proteins)

DBN1^{S142A} vs DBN1^{S142A}+Mg^{2+}

No significant change → Enhanced interaction dependent on the reduction of DBN1^{S142p} (20 proteins)

No significant change → Weakened interaction dependent on the reduction of DBN1^{S142p} (53 proteins)

D Weakened interaction dependent on DBN1^{S142p} (53 proteins)

- Actin cytoskeleton organization
- Actin filament organization
- Negative regulation of protein kinase activity
- Arp2/3 complex−mediated actin nucleation
- Vesicle transport along actin filament

Protein counts — $-Log_{10}(p\ value)$ 3, 4, 5, 6

Enhanced interaction dependent on DBN1^{S142p} (20 proteins)

- Aerobic respiration
- Mitochondrial ATP synthesis coupled proton transport

Protein counts — $-Log_{10}(p\ value)$ 3, 4, 5, 6

E Proteins strongly and specifically bound to DBN1^{S142D} (30 proteins)

27 / 3 / 50

Weakened interaction dependent on the reduction of DBN1^{S142p} (53 proteins)

F Proteins strongly and specifically bound to DBN1^{S142A} (46 proteins)

40 / 6 / 14

Enhanced interaction dependent on the reduction of DBN1^{S142p} (20 proteins)

G

CDC42 intensity ($\times 10^7$)

a: Vector
b: DBN1^{WT}
c: DBN1^{S142A}
d: DBN1^{S142D}
e: Vector+Mg^{2+}
f: DBN1^{WT}+Mg^{2+}
g: DBN1^{S142A}+Mg^{2+}

ns, **, *, ****

H

Scramble, shACTN4#1, shACTN4#2

ACTN4
GAPDH
HCT116

Figure S8