

Loss of IP3R3 Expression Is Associated with Histological Dedifferentiation in Colorectal Adenocarcinoma

Diego Chianese

University of Ferrara

Bianca Vezzani

University of Parma

Veronica Angela Maria Vitto

University of Ferrara

Mariasole Perrone

University of Ferrara

Alberto Campagnaro

Azienda Unità Sanitaria Locale di Ferrara

Roberta Gafà

University Hospital of Ferrara

Giovanni Lanza

University Hospital of Ferrara

Luigi Abelli

University of Ferrara

Gabriele Anania

Azienda Unità Sanitaria Locale di Ferrara

Massimo Bonora

University of Ferrara

Carlotta Giorgi

University of Ferrara

Paolo Pinton

paolo.pinton@unife.it

University of Ferrara

Research Article

Keywords: Colorectal cancer, Inositol 1,4,5-trisphosphate receptor type 3 (IP3R3), Calcium signaling, Apoptosis, Tumor dedifferentiation, Biomarkers

Posted Date: August 30th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10481167/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations:

No competing interests reported.

Tables are available in the Supplementary Files section.

Loss of IP3R3 Expression Is Associated with Histological Dedifferentiation in Colorectal Adenocarcinoma

Diego Chianese ¹, Bianca Vezzani ^{1,2}, Veronica Angela Maria Vitto ¹, Mariasole Perrone ¹, Alberto Campagnaro³, Roberta Gafà ^{4,5}, Giovanni Lanza ^{4,5}, Luigi Abelli ⁶, Gabriele Anania ^{3,7}, Massimo Bonora ¹, Carlotta Giorgi ¹ and Paolo Pinton ^{1,8,*}

Affiliations:

¹ Department of Medical Sciences, Section of Experimental Medicine, Laboratory for Technologies of Advanced Therapies, University of Ferrara, 44121 Ferrara, Italy.

² Department of Medicine and Surgery, University of Parma, 43125 Parma, Italy.

³ Department of Surgery, AUSL of Ferrara, 44121, Ferrara, Italy.

⁴ Department of Translational Medicine, University of Ferrara, 44121, Ferrara, Italy.

⁵ Anatomic Pathology Unit, University Hospital of Ferrara, 44124, Cona, FE, Italy.

⁶ Department of Life Sciences and Biotechnology, University of Ferrara, via L. Borsari, 46, Ferrara 44121, Italy.

⁷ Department of Medical Science, University of Ferrara, Ferrara, Italy.

⁸ Maria Cecilia Hospital, GVM Care & Research, Cotignola, RA, 48033, Italy.

* **Corresponding Author:** Paolo Pinton, University of Ferrara, Via Fossato di Mortara 70, Ferrara, 44121, Italy. E-mail: paolo.pinton@unife.it

24 **ABSTRACT**

25 **Background:** Colorectal adenocarcinoma remains one of the leading causes of cancer-related
26 mortality worldwide despite substantial advances in diagnosis and treatment. Resistance to apoptosis
27 is a hallmark of tumor progression and therapeutic failure. Inositol 1,4,5-trisphosphate receptor type
28 3 (IP3R3), a key mediator of endoplasmic reticulum-to-mitochondria Ca^{2+} transfer and intrinsic
29 apoptosis, has been implicated in tumor biology, but its clinicopathological significance in colorectal
30 adenocarcinoma remains poorly defined. This study investigated IP3R3 expression in human
31 colorectal adenocarcinoma and evaluated its association with clinicopathological features.

32 **Methods:** A prospective cohort of 179 patients with colorectal adenocarcinoma was enrolled. IP3R3
33 protein expression was assessed by Western blotting and immunohistochemistry in paired tumor and
34 matched adjacent normal colonic tissues. The findings were independently validated using publicly
35 available Clinical Proteomic Tumor Analysis Consortium (CPTAC) datasets.

36 **Results:** IP3R3 expression was significantly reduced in colorectal adenocarcinoma compared with
37 matched normal colonic mucosa. Loss of IP3R3 expression was independent of TNM stage and
38 primary tumor location but was strongly associated with histological dedifferentiation, progressively
39 declining from well- and moderately differentiated tumors to poorly differentiated lesions, in which
40 IP3R3 expression was frequently undetectable. Immunohistochemical analysis confirmed
41 progressive loss of IP3R3 during disruption of glandular architecture, while independent CPTAC
42 proteomic datasets validated the observed expression pattern.

43 **Conclusions:** Loss of IP3R3 expression is closely associated with histological dedifferentiation in
44 colorectal adenocarcinoma, independently of TNM stage and primary tumor location. These findings
45 identify IP3R3 as a candidate biomarker of tumor differentiation and suggest that reduced IP3R3
46 expression reflects impaired apoptotic competence and the biological state of colorectal tumors.

Further studies are warranted to determine whether restoration of IP3R3-dependent ER-to-mitochondria Ca^{2+} signaling may improve therapeutic responsiveness in colorectal cancer.

KEYWORDS

Colorectal cancer; Inositol 1,4,5-trisphosphate receptor type 3 (IP3R3); Calcium signaling; Apoptosis; Tumor dedifferentiation; Biomarkers.

BACKGROUND

Colorectal cancer is the third most commonly diagnosed malignancy and the second leading cause of cancer-related death worldwide ¹. More than 90% of CRCs are adenocarcinomas ². Although early detection, surgical resection, and advances in systemic therapies have significantly improved patient outcomes, CRC remains a major cause of cancer mortality ^{1,3}. A substantial proportion of patients develop recurrent or metastatic disease requiring systemic therapy, whose efficacy is frequently limited by intrinsic or acquired resistance. Consequently, identifying biomarkers that improve patient stratification, predict therapeutic response, and uncover novel therapeutic vulnerabilities remains a major clinical challenge.

Current prognostic assessment relies primarily on TNM staging ⁴⁻⁸; histological grade ⁹, and an expanding panel of molecular biomarkers. While these parameters have substantially improved clinical decision-making, they remain insufficient to fully capture the biological heterogeneity of CRC and accurately predict treatment response. Among the mechanisms driving disease progression and therapeutic resistance, escape from apoptosis is increasingly recognized as a hallmark of aggressive colorectal tumors ¹⁰⁻¹².

Intracellular Ca^{2+} signaling is a fundamental regulator of apoptosis ^{13, 14}. Sustained mitochondrial Ca^{2+} accumulation represents a critical trigger of the intrinsic apoptotic pathway and

70 depends on efficient Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria at
71 specialized contact sites known as mitochondria-associated membranes (MAMs), where the two
72 organelles are closely apposed^{15–17}. This unique structural organization generates local Ca^{2+}
73 microdomains that enable rapid mitochondrial Ca^{2+} uptake and activation of apoptotic signaling¹⁸.

74 Inositol 1,4,5-trisphosphate receptors (IP3Rs) are the principal ER Ca^{2+} -release channels
75 responsible for initiating this signaling cascade^{19–22}. Among the three IP3R isoforms, IP3R3 is
76 preferentially enriched at MAMs²³, where it promotes ER-to-mitochondria Ca^{2+} transfer and
77 activation of mitochondrial apoptosis^{18, 23–28}. Beyond its physiological role in apoptosis,
78 accumulating evidence indicates that dysregulation of IP3R3-dependent Ca^{2+} signaling contributes to
79 tumor progression and therapeutic resistance in multiple malignancies^{23–26, 29–33}.

80 Despite the recognized role of IP3R3 in regulating apoptotic competence, its expression
81 pattern and clinicopathological significance in human colorectal adenocarcinoma remain poorly
82 defined. We hypothesized that progressive loss of IP3R3 expression accompanies CRC
83 dedifferentiation and reflects impaired pro-apoptotic Ca^{2+} signaling.

84 To test this hypothesis, we analyzed IP3R3 protein expression in paired colorectal
85 adenocarcinoma and matched adjacent normal colonic tissues from a prospective patient cohort using
86 complementary biochemical and histopathological approaches. We further examined the relationship
87 between IP3R3 expression and clinicopathological features to define its clinical significance and
88 evaluate its potential as a biomarker of tumor differentiation and impaired apoptotic competence in
89 CRC.

90 **MATERIALS AND METHODS**

91 **Study Design and Patient Enrollment**

92 This prospective observational study enrolled patients between 2020 and 2025 at the Department of
93 General Surgery, University Hospital of Ferrara (Ferrara, Italy). Eligible participants were adult

94 patients undergoing elective surgery for primary or recurrent colorectal adenocarcinoma, regardless
95 of disease stage. Exclusion criteria included: (i) neoadjuvant therapy; (ii) previous chemotherapy or
96 radiotherapy; (iii) immunosuppression or severe chronic inflammatory or autoimmune diseases; (iv)
97 previous malignant neoplasms at other sites; and (v) concomitant second primary malignancies.

98 The study was conducted in accordance with the Declaration of Helsinki and approved by the
99 local Ethics Committee (Comitato Etico Area Vasta Emilia Centro, CE-AVEC; protocol no.
100 1103/2020/Oss/AUOFe). Written informed consent was obtained from all participants before surgery.

101 For each patient, paired specimens consisting of colorectal adenocarcinoma tissue (T) and
102 macroscopically normal colonic mucosa (H), collected at a distance of at least 8 cm from the tumor
103 margin, were obtained during surgical resection. Tissue specimens were transported in physiological
104 saline at 4°C to the Laboratory for Technologies of Advanced Therapies (University Hospital of
105 Ferrara) and processed within 24 hours of collection.

106 **Clinicopathological Data Collection**

107 Clinicopathological variables collected for each patient included age, sex, tumor recurrence status,
108 primary tumor location, pathological TNM stage ⁸, and histological grade.

109 Histological grading was classified according to the WHO classification as low grade
110 (well/moderately differentiated) and high grade (poorly differentiated and undifferentiated).

111 For subgroup analyses, according to pathological TNM stage, patients were stratified into low-
112 stage (LOW) and intermediate/high-stage (INT/HIG), as detailed below.

113 Moreover, primary tumors were also categorized as proximal (cecum, ascending colon,
114 hepatic flexure, transverse colon and splenic flexure) or distal (descending colon, sigmoid colon, and
115 rectum) colon cancers.

116 **CPTAC Proteomic Dataset Analysis**

117 Independent validation was performed using publicly available datasets from the Clinical Proteomic
118 Tumor Analysis Consortium (CPTAC; <https://pdc.cancer.gov>).

119 For the analyses shown in Supplementary Figures S1A and S1B, dataset PDC000116 was
120 interrogated. Protein expression was reported as fold change relative to matched normal tissue. The
121 original dataset comprised 197 samples annotated for multiple clinicopathological variables,
122 including tumor stage. Three samples annotated as stage II without subclassification were excluded.

123 For Supplementary Figure S1C, dataset PDC000111 was analyzed. Protein abundance was
124 reported as normalized precursor area. Samples originating from the rectum or with unspecified
125 anatomical origin were excluded. Tumors arising from the cecum and ascending colon were classified
126 as proximal, whereas tumors from the descending and sigmoid colon were classified as distal.

127 Because all CPTAC datasets are publicly available and de-identified, no additional ethical
128 approval was required.

129 **Western Blot Analysis**

130 Approximately 3 mm³ of tissue from each biopsy was homogenized in lysis buffer containing 150
131 mM NaCl, 20 mM Tris-HCl (pH 7.5), 1% Triton X-100, 0.5% SDS, 1 mM EDTA, 1 mM PMSF,
132 protease inhibitor cocktail, and phosphatase inhibitors. Protein concentration was determined using
133 the RC DC Protein Assay (Bio-Rad).

134 Equal amounts of protein (20 µg) were separated by SDS-PAGE, transferred to nitrocellulose
135 membranes, and blocked with 5% nonfat milk in TBS-T. Membranes were incubated overnight at
136 4°C with antibodies against IP3R3 (BD Biosciences, #610312) and GAPDH (Cell Signaling
137 Technology, #2118), followed by horseradish peroxidase-conjugated secondary antibodies.
138 Immunoreactive bands were visualized using enhanced chemiluminescence and acquired with an
139 ImageQuant LAS4000 imaging system.

140 Each biopsy was analyzed in three independent technical replicates. Band intensities were
141 quantified using ImageJ software. IP3R3 expression was normalized to GAPDH, and the mean
142 normalized value for each biopsy was used for subsequent analyses.

143 Samples in which IP3R3 was undetectable in both tumor tissue and matched normal mucosa
144 could not be reliably classified according to differential expression and were therefore excluded from
145 quantitative analyses.

146 **Immunohistochemistry**

147 Formalin-fixed, paraffin-embedded tumor specimens from 10 patients representing different
148 histological grades (Table 2) were analyzed by immunohistochemistry. Four-micrometer tissue
149 sections were subjected to heat-induced antigen retrieval, endogenous peroxidase blocking, and
150 incubation with a mouse monoclonal anti-IP3R3 antibody (BD Biosciences, 610312).
151 Immunoreactivity was detected using the VECTASTAIN Elite ABC system followed by nickel-
152 enhanced diaminobenzidine development.

153 Sections were examined using a Nikon Eclipse 80i bright-field microscope equipped with a
154 digital camera. Representative images were acquired using the software package Lucia G 4.81
155 (Laboratory Imaging Ltd., Praha, Czech Rep.) under identical acquisition settings.

156 **Statistical Analysis**

157 Normality was assessed using the Shapiro–Wilk test. Because continuous variables were not normally
158 distributed, data are presented as median with minimum–maximum values unless otherwise
159 indicated.

160 Differences between paired samples were analyzed using the Wilcoxon matched-pairs signed-
161 rank test, whereas comparisons between independent groups were performed using the Mann–
162 Whitney U test. Multiple-group comparisons were analyzed using the Kruskal–Wallis test followed
163 by Dunn's multiple-comparison correction.

164 All statistical analyses were performed using GraphPad Prism. Two-sided *P* values <0.05
165 were considered statistically significant (*= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$).

166 **RESULTS**

167 **Clinical Characteristics of the Study Cohort**

168 A total of 179 patients with colorectal adenocarcinoma were prospectively enrolled, including 92 men
169 and 87 women. The clinicopathological characteristics of the study cohort are summarized in Table
170 1. The median follow-up was 13.3 ± 8.5 months (range, 0.1–30.1 months), during which 28 patients
171 (15.6%) died.

172 To investigate the clinicopathological significance of IP3R3 expression, patients were
173 stratified according to TNM stage, histological grade, and primary tumor location. Based on TNM
174 classification, tumors were categorized into a low-stage group (stages I–IIA; $n = 94$) and an
175 intermediate/high-risk group (stages IIB–IVC; $n = 85$).

176 Histopathological evaluation identified 133 well/moderately differentiated (low grade) and 46
177 poorly differentiated (high grade) adenocarcinomas.

178 Finally, tumors were classified according to anatomical location as proximal ($n = 123$) or
179 distal ($n = 56$) CRCs.

180 **Loss of IP3R3 Expression Characterizes Human Colorectal Adenocarcinoma**

181 To determine whether IP3R3 expression is altered in colorectal adenocarcinoma, we quantified IP3R3
182 protein levels by Western blot analysis in paired tumor (T) and matched adjacent normal colonic
183 mucosa (H). Based on the relative abundance of IP3R3 in each paired specimen, samples were
184 classified as showing reduced expression in tumors ($H > T$), unchanged expression ($H = T$), or
185 increased expression in tumors ($H < T$) (Figure 1A). Differences within $\pm 10\%$ between paired
186 samples were considered unchanged.

187 Reduced IP3R3 expression in tumor tissue was observed in 46.93% of patients, whereas only
188 25.14% exhibited higher IP3R3 levels in tumors than in the corresponding normal mucosa (Figure
189 1B). In 26.26% of paired samples, IP3R3 was undetectable in both tumor and matched normal tissue.
190 Because these samples could not be reliably classified as exhibiting differential expression, they were
191 analyzed separately and excluded from subsequent quantitative analyses.

192 Quantitative analysis of the remaining paired samples demonstrated a significant reduction in
193 IP3R3 expression in colorectal adenocarcinoma compared with matched normal colonic mucosa
194 (Figure 1C).

195 To validate these findings in an independent patient cohort, we interrogated the CRC
196 proteomic dataset generated by the Clinical Proteomic Tumor Analysis Consortium (CPTAC) and
197 analyzed ITPR3 protein expression reported by Vasaikar *et al.*³⁴. Consistent with our observations,
198 ITPR3 protein abundance was significantly lower in colorectal adenocarcinoma than in matched
199 normal tissue. Here we observed that the medianlog2FC is -0.08, significantly differing from 0,
200 independently confirming our observation that IP3R3 is downregulated in human CRC (Figure S1A).

201 Collectively, these findings demonstrate that loss of IP3R3 expression is a common feature of
202 human colorectal adenocarcinoma and establish the robustness of this observation through
203 independent proteomic validation.

204 **IP3R3 Downregulation Occurs Independently of TNM Stage**

205 To determine whether IP3R3 downregulation was associated with disease stage, patients were
206 stratified into low-stage (stages I–IIA) and intermediate/high-stage (stages IIB–IVC) groups
207 according to the TNM classification, as described in the Methods.

208 Consistent with the overall cohort analysis, IP3R3 expression remained significantly lower in
209 tumor tissue than in the corresponding matched normal mucosa in both TNM groups (Figure 2A, B).
210 Comparison of tumor specimens between low- and intermediate/high-stage patients revealed no
211 significant difference in IP3R3 expression, although a modest trend toward lower levels was observed

212 in advanced-stage disease (Figure 2C). A similar trend was observed in the corresponding normal
213 mucosa (Figure 2D) but did not reach statistical significance.

214 As above, to independently validate these findings, we analyzed the CRC proteomic dataset
215 generated by CPTAC ³⁴. Samples were stratified using the same TNM-based classification, and
216 tumor-to-normal ITPR3 protein expression was compared between the two groups. Consistent with
217 our cohort, no significant association was observed between ITPR3 expression and TNM stage
218 (Figure S1B).

219 These findings indicate that IP3R3 downregulation is an early and persistent feature of
220 colorectal adenocarcinoma that occurs independently of disease stage.

221 **IP3R3 Expression Is Independent of Tumor Sidedness**

222 Because primary tumor location represents an important determinant of CRC biology and therapeutic
223 response, we next investigated whether IP3R3 expression differed between proximal and distal colon
224 cancers.

225 As observed in the overall cohort, IP3R3 expression was significantly lower in tumor tissue
226 than in matched normal mucosa regardless of tumor location (Figure 3A, B). Although proximal
227 tumors tended to express lower IP3R3 levels than distal tumors, this difference did not reach statistical
228 significance (Figure 3C). Likewise, no significant differences were detected in the corresponding
229 healthy mucosa (Figure 3D).

230 Independent analysis of the TCGA/CPTAC CRC cohort, in which tumor samples are
231 annotated according to the anatomical site of resection, confirmed that ITPR3 expression did not
232 differ significantly between proximal and distal CRCs ^{35,36}(Figure S1C).

233 Together, these data demonstrate that loss of IP3R3 expression is independent of the
234 anatomical location of the primary tumor, suggesting that IP3R3 reflects biological properties shared
235 across colorectal adenocarcinomas rather than tumor sidedness.

236 **Loss of IP3R3 Expression Correlates with Tumor Dedifferentiation**

237 Having established that IP3R3 downregulation is independent of both TNM stage and tumor
238 sidedness, we next investigated whether IP3R3 expression was associated with histological
239 differentiation, one of the principal indicators of tumor aggressiveness.

240 Patients were stratified into well/moderately differentiated (Low Grade) and poorly
241 differentiated (High Grade) tumors as described in the Methods. Consistent with the overall cohort
242 analysis, IP3R3 expression remained significantly lower in tumor tissue than in the corresponding
243 matched normal mucosa in both groups (Figure 4A, B). Notably, poorly differentiated tumors
244 exhibited a further significant reduction in IP3R3 expression compared with well/moderately
245 differentiated lesions (Figure 4C). In contrast, only a nonsignificant trend toward lower IP3R3
246 expression was observed in the corresponding normal mucosa from patients with G3 tumors (Figure
247 4D).

248 These findings identify progressive loss of IP3R3 expression as a feature of tumor
249 dedifferentiation rather than disease stage or anatomical location, indicating that IP3R3 more closely
250 reflects the biological state of colorectal tumors than their anatomical extent.

251 **Immunohistochemistry Reveals Progressive Loss of IP3R3 During Tumor** 252 **Dedifferentiation**

253 To validate the biochemical findings at the tissue level, IP3R3 expression was evaluated by
254 immunohistochemistry in representative colorectal adenocarcinoma specimens.

255 Moderately differentiated tumors retained detectable IP3R3 immunoreactivity despite partial
256 alteration of glandular architecture. Residual glandular epithelial cells displayed numerous IP3R3-
257 positive cytoplasmic vesicles, particularly in areas of epithelial erosion. In addition, abundant IP3R3-
258 immunoreactive material was detected within intraluminal dirty necrosis, where granular cellular
259 debris indicated release of IP3R3-positive epithelial components (Figure 5A).

260 In contrast, poorly differentiated adenocarcinomas showed extensive loss of glandular
261 organization accompanied by a marked reduction in IP3R3 immunoreactivity. Only occasional
262 weakly positive cells were detected, whereas IP3R3-positive material within dirty necrosis was
263 markedly reduced or absent (Figure 5B).

264 These qualitative observations were consistently reproduced throughout the
265 immunohistochemical cohort (Table 2) and closely paralleled the biochemical analyses,
266 demonstrating that progressive loss of IP3R3 accompanies the morphological dedifferentiation of
267 colorectal adenocarcinoma.

268 **DISCUSSION**

269 Numerous experimental studies have established IP3R3 as a key regulator of ER-to-mitochondria
270 Ca^{2+} transfer and apoptosis^{14, 16–18, 27}. Consistent with this role, several molecular mechanisms have
271 been shown to regulate IP3R3 abundance, stability, and activity, thereby influencing susceptibility to
272 apoptosis^{24–31}. Collectively, these observations support the concept that impairment of IP3R3
273 function may confer a selective advantage during tumorigenesis.

274 However, studies investigating IP3R3 expression in human cancers have yielded heterogeneous
275 results, suggesting that the biological contribution of IP3R3 is highly context-dependent^{32, 37–43}.

276 The present study demonstrates that progressive loss of IP3R3 expression is a characteristic feature
277 of human colorectal adenocarcinoma and is closely associated with tumor dedifferentiation. By
278 analyzing paired tumor and matched normal tissues from a prospective patient cohort and
279 independently validating our findings in the CPTAC proteomic dataset, we show that IP3R3
280 expression shows an overall reduction in CRC. Notably, IP3R3 downregulation was associated with
281 histological grade, whereas no significant relationship was observed with TNM stage or primary
282 tumor location. Collectively, these findings suggest that IP3R3 reflects the biological state of
283 colorectal tumors rather than their anatomical extent.

284 The most significant observation emerging from the present study is the strong association
285 between IP3R3 loss and histological dedifferentiation. Unlike TNM stage, which primarily reflects
286 the anatomical extent of disease, histological grade captures fundamental changes in tumor cell
287 identity and differentiation. The selective association between IP3R3 expression and tumor grade
288 therefore suggests that IP3R3 is linked to intrinsic tumor biology rather than disease burden. This
289 interpretation is reinforced by the absence of significant associations with either TNM stage or tumor
290 sidedness and by the immunohistochemical analyses, which demonstrated progressive disappearance
291 of IP3R3 immunoreactivity together with loss of glandular architecture.

292 This association is biologically plausible. Previous studies have shown that IP3 receptor
293 expression is closely linked to the acquisition and maintenance of differentiated cellular phenotypes,
294 whereas reduced IP3R signaling has been associated with stemness and cellular plasticity ⁴⁴⁻⁴⁷.
295 Although our study does not establish a causal relationship, the observed reduction of IP3R3 in poorly
296 differentiated tumors raises the possibility that impaired ER-to-mitochondria Ca^{2+} signaling either
297 accompanies or contributes to the progressive loss of differentiation characteristic of aggressive CRC.

298 To date, recurrent genetic alterations of ITPR3 have not emerged as a common feature of
299 colorectal adenocarcinoma, indicating that changes in receptor abundance are more likely to arise
300 from transcriptional, post-transcriptional, or post-translational mechanisms than from direct genomic
301 alterations. In this regard, the tumor suppressor BAP1 stabilizes IP3R3 by antagonizing FBXL2-
302 mediated ubiquitination and proteasomal degradation, thereby preserving ER-to-mitochondria Ca^{2+}
303 transfer and apoptotic signaling ²⁶. Our observation that IP3R3 protein levels progressively decline
304 in poorly differentiated tumors is fully consistent with the concept that regulation of receptor
305 abundance contributes to CRC biology. Because receptor abundance directly influences ER-to-
306 mitochondria Ca^{2+} transfer capacity, reduced IP3R3 expression is expected to attenuate mitochondrial
307 apoptotic signaling and thereby confer a selective survival advantage.

308 These observations alone cannot fully explain the biological complexity of IP3R3 in CRC.
309 Previous studies have demonstrated that AKT-, STAT3- and PTEN-dependent phosphorylation
310 profoundly modulates IP3R3 function without necessarily altering protein expression ^{24, 25, 29, 48}.

311 Moreover, the ability of IP3R3 to sustain ER-to-mitochondria Ca^{2+} transfer and trigger
312 mitochondrial Ca^{2+} -dependent apoptosis critically depends on the amount of Ca^{2+} stored within the
313 endoplasmic reticulum. Accordingly, several oncogenes and tumor suppressors regulate apoptotic
314 sensitivity by modulating ER Ca^{2+} homeostasis. Among these, anti-apoptotic members of the BCL-2
315 family reduce ER Ca^{2+} content ⁴⁹, thereby limiting IP3R3-mediated Ca^{2+} transfer to mitochondria,
316 whereas the tumor suppressor p53 has been shown to enhance ER Ca^{2+} loading and promote
317 mitochondrial Ca^{2+} signaling ³⁰. Together, these observations indicate that IP3R3 activity is
318 determined not only by its expression level but also by the receptor's functional state and the ER Ca^{2+}
319 content, both of which are frequently altered during colorectal tumorigenesis.

320 Importantly, our own data supports the existence of heterogeneous IP3R3 regulation, since
321 approximately one quarter of tumors displayed higher IP3R3 expression than the matched normal
322 mucosa. The present findings complement previous histological observations by providing a
323 quantitative assessment of total IP3R3 abundance in paired tumor and matched normal tissues.
324 Several methodological differences distinguish the present study from earlier investigations. First, we
325 quantified total IP3R3 protein abundance in paired tissues by immunoblotting, whereas previous
326 studies primarily relied on immunohistochemical assessment of regional protein expression. Second,
327 matched normal samples were obtained from macroscopically normal colonic mucosa collected at
328 least 8 cm from the visible tumor margin, thereby minimizing the potential influence of local tumor-
329 associated tissue alterations. These differences provide a framework to interpret previous apparently
330 discordant observations ⁴³. Specifically, *Shibao et al.* reported focal enrichment of IP3R3
331 immunoreactivity at the invasive tumor front, where increased staining was associated with poor
332 clinical outcome. Although these findings appear to differ from our overall quantitative analysis, the
333 two studies employed complementary experimental approaches. While immunoblotting provides a

334 quantitative estimate of total IP3R3 abundance in paired tissues, immunohistochemistry preserves
335 tissue architecture and may reveal localized expression within specific tumor compartments.

336 Our findings are consistent with this model and suggest that downregulation of IP3R3
337 represents one mechanism by which CRC cells may evade apoptotic stimuli. At the same time, tumors
338 retaining or even overexpressing IP3R3 may achieve a similar biological outcome through
339 mechanisms that impair receptor activity rather than its expression, underscoring the importance of
340 considering both IP3R3 abundance and functional status when evaluating its contribution to CRC
341 progression.

342 Our study has several limitations. First, the relatively short follow-up precluded a robust
343 evaluation of the relationship between IP3R3 expression and long-term clinical outcome. Second, the
344 observational nature of the study does not establish whether loss of IP3R3 directly contributes to
345 chemoresistance or tumor progression. Finally, functional studies will be required to determine
346 whether restoration of IP3R3 expression or activity enhances sensitivity to chemotherapy and limits
347 disease progression in experimental models of CRC.

348 Despite these limitations, the present work provides a comprehensive protein-level
349 characterization of IP3R3 expression in a large cohort of paired human colorectal adenocarcinomas
350 and matched normal tissues, supported by independent validation in publicly available proteomic
351 datasets. Our findings identify altered IP3R3 abundance as one layer of regulation underlying IP3R3
352 biology in CRC. However, the marked heterogeneity observed both within our cohort and across
353 previous studies indicates that receptor abundance alone is unlikely to fully explain the biological
354 contribution of IP3R3 to CRC, emphasizing the need to consider both expression and functional
355 regulation when evaluating its role in tumor progression.

356 **CONCLUSIONS**

357 We demonstrate that IP3R3 expression is progressively lost during colorectal tumor dedifferentiation
358 and is independent of TNM stage and tumor location. These findings identify IP3R3 as a candidate
359 biomarker of tumor differentiation and provide a rationale for future studies investigating the
360 contribution of IP3R3-dependent ER-to-mitochondria Ca^{2+} signaling to colorectal cancer progression
361 and therapeutic response.

362 **LIST OF ABBREVIATIONS**

- 363 ABC: avidin-biotin complex
- 364 AOM: azoxymethane
- 365 BAP1: BRCA1-associated protein 1
- 366 BCL-2: B-cell lymphoma 2
- 367 BRAF: v-Raf murine sarcoma viral oncogene homolog B
- 368 Ca^{2+} : calcium ion
- 369 CE-AVEC: Comitato Etico Area Vasta Emilia Centro
- 370 CPTAC: Clinical Proteomic Tumor Analysis Consortium
- 371 CRC: colorectal cancer
- 372 DN: dirty necrosis
- 373 DSS: dextran sodium sulfate
- 374 EDTA: ethylenediaminetetraacetic acid
- 375 ER: endoplasmic reticulum
- 376 FBXL2: F-box and leucine-rich repeat protein 2
- 377 GAPDH: glyceraldehyde-3-phosphate dehydrogenase
- 378 H: healthy (matched normal colonic mucosa)
- 379 HRP: horseradish peroxidase

380 IP₃: inositol 1,4,5-trisphosphate
381 IP3R: inositol 1,4,5-trisphosphate receptor
382 IP3R3: inositol 1,4,5-trisphosphate receptor type 3
383 KRAS: Kirsten rat sarcoma viral oncogene homolog
384 MAMs: mitochondria-associated membranes
385 MSIH: microsatellite instability-high
386 PBS: phosphate-buffered saline
387 PMSF: phenylmethanesulphonyl fluoride
388 SDS: sodium dodecyl sulfate
389 SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis
390 STAT3: signal transducer and activator of transcription 3
391 TCGA: The Cancer Genome Atlas
392 TBS-T: Tris-buffered saline with Tween-20
393 TNM: tumor-node-metastasis staging system
394 WB: western blot

395 **DECLARATIONS**

396 **Ethics approval and consent to participate**

397 The study was conducted in accordance with the Declaration of Helsinki and approved by the local
398 Ethics Committee (Comitato Etico Area Vasta Emilia Centro, CE-AVEC; protocol no.
399 1103/2020/Oss/AUOFe).

400 **Consent for publication**

401 Not applicable.

402 **Availability of data and materials**

403 The datasets used and/or analyzed during the current study are available from the corresponding
404 author on reasonable request.

405 **Competing interest**

406 The authors declare that they have no competing interests.

407 **Funding**

408 The Signal Transduction Laboratory is supported by the Italian Association for Cancer Research (IG-
409 23670 to P.P. and IG-19803 to C.G.); by the European Research Council FARE 2020 (INFLA-
410 THROMB to C.G); by the Unione Europea–NextGenerationEU, Mission 4 Component 1 – Italian
411 Ministry of Education, University and Research, PRIN2017E5L5P3 and PRIN2020RRJP5L003,
412 Piano Nazionale di Ripresa e Resilienza, Missione 4 Componente 2 – Investimento 1.3 –
413 NextGenerationEU – PE 00000019 – CUP E83C22004670001, Investimento 1.4 –
414 NextGenerationEU – CN 00000041 – CUP C93C22002780006 to P.P. the Italian Ministry of Health
415 (PNRR-MCNT1-2023-12378355 to C.G); A-ROSE ODV and local funds from the University of
416 Ferrara to P.P., C.G. and M.B. P.P. is grateful to C. degli Scrovegni for her continuous support.

417 **Authors’ Contributions**

418 Conceptualization, Paolo Pinton (P.P.), Carlotta Giorgi (C.G.); methodology, Diego Chianese (D.C.),
419 Massimo Bonora (M.B.), C.G. and P.P.; software, D.C. and M.B.; formal analysis, D.C., Bianca
420 Vezzani (B.V.) and M.B.; investigation, D.C., M.B., Luigi Abelli (L.A.), B.V., Mariasole Perrone
421 (M.P.) and Veronica Angela Maria Vitto (V.VAM.), Alberto Campagnaro (A.C.); resources, P.P.,
422 Gabriele Anania (G.A.), Giovanni Lanza (G.L.) and Roberta Gafà (R.G.); data curation, D.C. and
423 G.A.; writing-original draft preparation, D.C., B.V; writing-review and editing, M.B., L.A. and P.P.;
424 supervision, M.B., C.G. and P.P.; project administration, C.G. and P.P.; funding acquisition P.P. All
425 authors have read and agreed to the published version of the manuscript.

426

427 **REFERENCES**

- 428 1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global
429 Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36
430 Cancers in 185 Countries. *CA Cancer J Clin* 71:209–249
- 431 2. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi DJ, John A, others (2022) Colorectal
432 cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors,
433 preventive and treatment strategies. *Cancers (Basel)* 14:1732
- 434 3. Safiri S, Sepanlou SG, Ikuta KS, et al (2019) The global, regional, and national burden of
435 colorectal cancer and its attributable risk factors in 195 countries and territories, 1990–2017: a
436 systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol*
437 *Hepatol* 4:913–933
- 438 4. Tominaga T, Sakabe T, Koyama Y, Hamano K, Yasutomi M, Takahashi T, others (1996)
439 Prognostic factors for patients with colon or rectal carcinoma treated with resection only: five-
440 year follow-up report. *Cancer* 78:403–408
- 441 5. Greene FL, Stewart AK, Norton HJ (2002) A new TNM staging strategy for node-positive
442 (stage III) colon cancer. *Ann Surg* 236:416–421
- 443 6. Yasuda K, Adachi Y, Shiraishi N, Yamaguchi K, Hirabayashi Y, Kitano S (2001) Pattern of
444 lymph node micrometastasis and prognosis of patients with colorectal cancer. *Ann Surg Oncol*
445 8:300–304
- 446 7. Newland RC, Dent OF, Lyttle MNB, Chapuis PH, Bokey EL (1994) Pathologic determinants
447 of survival associated with colorectal cancer with lymph node metastases: a multivariate
448 analysis of 579 patients. *Cancer* 73:2076–2082
- 449 8. Mahul B. Amin, Frederick L. Greene, Stephen B. Edge, Carolyn C. Compton, Jeffrey E.
450 Gershenwald, Robert K. Brookland, Laura Meyer, Donna M. Gress, David R. Byrd, David P.
451 Winchester (2017) *AJCC Cancer Staging Manual*, 8th ed. Springer Cham

- 452 9. Wiggers T, Arends JW, Volovics A (1988) Regression analysis of prognostic factors in
453 colorectal cancer after curative resections. *Dis Colon Rectum* 31:33–41
- 454 10. Prabhudesai SG, Rekhraj S, Roberts G, Darzi AW, Ziprin P (2007) Apoptosis and chemo-
455 resistance in colorectal cancer. *J Surg Oncol* 96:77–88
- 456 11. Longley D, Johnston P (2005) Molecular mechanisms of drug resistance. *J Pathol* 205:275–
457 292
- 458 12. Fesik SW (2005) Promoting apoptosis as a strategy for cancer drug discovery. *Nat Rev Cancer*
459 5:876–885
- 460 13. Rizzuto R, Pinton P, Ferrari D, Chami M, Szabadkai G, Magalhães PJ, Virgilio F Di, Pozzan
461 T (2003) Calcium and apoptosis: facts and hypotheses. *Oncogene* 22:8619–8627
- 462 14. Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R (2008) Calcium and apoptosis: ER-
463 mitochondria Ca^{2+} transfer in the control of apoptosis. *Oncogene* 27:6407–6418
- 464 15. Perrone M, Caroccia N, Genovese I, et al (2020) The role of mitochondria-associated
465 membranes in cellular homeostasis and diseases. *Int Rev Cell Mol Biol* 350:119–196
- 466 16. Marchi S, Bittremieux M, Missiroli S, et al (2017) Endoplasmic Reticulum-Mitochondria
467 Communication Through Ca^{2+} Signaling: The Importance of Mitochondria-Associated
468 Membranes (MAMs). pp 49–67
- 469 17. Missiroli S, Danese A, Iannitti T, Patergnani S, Perrone M, Previati M, Giorgi C, Pinton P
470 (2017) Endoplasmic reticulum-mitochondria Ca^{2+} crosstalk in the control of the tumor cell
471 fate. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1864:858–864
- 472 18. Giorgi C, Marchi S, Pinton P (2018) The machineries, regulation and cellular functions of
473 mitochondrial calcium. *Nat Rev Mol Cell Biol* 19:713–730
- 474 19. Parys JB, Bultynck G, Vervliet T (2021) IP3 Receptor Biology and Endoplasmic Reticulum
475 Calcium Dynamics in Cancer. pp 215–237
- 476 20. Berridge MJ (2009) Inositol trisphosphate and calcium signalling mechanisms. *Biochimica et*
477 *Biophysica Acta (BBA) - Molecular Cell Research* 1793:933–940

- 478 21. Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis
479 and remodelling. *Nat Rev Mol Cell Biol* 4:517–529
- 480 22. Foskett JK, White C, Cheung K-H, Mak D-OD (2007) Inositol Trisphosphate Receptor Ca^{2+}
481 Release Channels. *Physiol Rev* 87:593–658
- 482 23. Giorgi C, Ito K, Lin H-K, et al (2010) PML Regulates Apoptosis at Endoplasmic Reticulum
483 by Modulating Calcium Release. *Science* (1979) 330:1247–1251
- 484 24. Marchi S, Marinello M, Bononi A, Bonora M, Giorgi C, Rimessi A, Pinton P (2012) Selective
485 modulation of subtype III IP3R by Akt regulates ER Ca^{2+} release and apoptosis. *Cell Death*
486 *Dis* 3:e304–e304
- 487 25. Kuchay S, Giorgi C, Simoneschi D, et al (2017) PTEN counteracts FBXL2 to promote IP3R3-
488 and Ca^{2+} -mediated apoptosis limiting tumour growth. *Nature* 546:554–558
- 489 26. Bononi A, Giorgi C, Patergnani S, et al (2017) BAP1 regulates IP3R3-mediated Ca^{2+} flux to
490 mitochondria suppressing cell transformation. *Nature* 546:549–553
- 491 27. Mendes CCP, Gomes DA, Thompson M, Souto NC, Goes TS, Goes AM, Rodrigues MA,
492 Gomez M V., Nathanson MH, Leite MF (2005) The Type III Inositol 1,4,5-Trisphosphate
493 Receptor Preferentially Transmits Apoptotic Ca^{2+} Signals into Mitochondria. *Journal of*
494 *Biological Chemistry* 280:40892–40900
- 495 28. Hedgepeth SC, Garcia MI, Wagner LE, et al (2015) The BRCA1 Tumor Suppressor Binds to
496 Inositol 1,4,5-Trisphosphate Receptors to Stimulate Apoptotic Calcium Release. *Journal of*
497 *Biological Chemistry* 290:7304–7313
- 498 29. Bononi A, Bonora M, Marchi S, Missiroli S, Poletti F, Giorgi C, Pandolfi PP, Pinton P (2013)
499 Identification of PTEN at the ER and MAMs and its regulation of Ca^{2+} signaling and apoptosis
500 in a protein phosphatase-dependent manner. *Cell Death Differ* 20:1631–1643
- 501 30. Giorgi C, Bonora M, Sorrentino G, et al (2015) p53 at the endoplasmic reticulum regulates
502 apoptosis in a Ca^{2+} -dependent manner. *Proceedings of the National Academy of Sciences*
503 112:1779–1784

- 504 31. Szado T, Vanderheyden V, Parys JB, et al (2008) Phosphorylation of inositol 1,4,5-
505 trisphosphate receptors by protein kinase B/Akt inhibits Ca^{2+} release and apoptosis.
506 Proceedings of the National Academy of Sciences 105:2427–2432
- 507 32. Rosa N, Sneyers F, Parys JB, Bultynck G (2020) Type 3 IP3 receptors: The chameleon in
508 cancer. *Int Rev Cell Mol Biol* 351:101–148
- 509 33. Xue Y, Morris JL, Yang K, et al (2021) SMARCA4/2 loss inhibits chemotherapy-induced
510 apoptosis by restricting IP3R3-mediated Ca^{2+} flux to mitochondria. *Nat Commun* 12:5404
- 511 34. Vasaikar S, Huang C, Wang X, Petyuk VA, Savage SR, Wen B, others (2019) Proteogenomic
512 analysis of human colon cancer reveals new therapeutic opportunities. *Cell* 177:1035-1049.e19
- 513 35. Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, others (2014) Proteogenomic characterization
514 of human colon and rectal cancer. *Nature* 513:382–387
- 515 36. Slebos RJC, Wang X, Wang X, Zhang B, Tabb DL, Liebler DC (2015) Proteomic analysis of
516 colon and rectal carcinoma using standard and customized databases. *Sci Data* 2:150022
- 517 37. Szatkowski C, Parys JB, Ouadid-Ahidouch H, Matifat F (2010) Inositol 1,4,5-trisphosphate-
518 induced Ca^{2+} signalling is involved in estradiol-induced breast cancer epithelial cell growth.
519 *Mol Cancer* 9:156
- 520 38. Guerra MT, Florentino RM, Franca A, Lima Filho AC, dos Santos ML, Fonseca RC, others
521 (2019) Expression of the type 3 InsP3 receptor is a final common event in the development of
522 hepatocellular carcinoma. *Gut* 68:1676–1687
- 523 39. Kang SS, Han K-S, Ku BM, Lee YK, Hong J, Shin HY, others (2010) Caffeine-mediated
524 inhibition of calcium release channel inositol 1,4,5-trisphosphate receptor subtype 3 blocks
525 glioblastoma invasion and extends survival. *Cancer Res* 70:1173–1183
- 526 40. Yang Y-C, Chang T-Y, Chen T-C, Lin W-S, Chang S-C, Lee Y-J (2017) ITPR3 gene haplotype
527 is associated with cervical squamous cell carcinoma risk in Taiwanese women. *Oncotarget*
528 8:10085–10090

- 529 41. Rusinek D, Swierniak M, Chmielik E, Kowal M, Kowalska M, Cyplinska R, others (2015)
530 BRAFV600E-associated gene expression profile: early changes in the transcriptome, based on
531 a transgenic mouse model of papillary thyroid carcinoma. *PLoS One* 10:e0143688
- 532 42. Sakakura C, Hagiwara A, Fukuda K, Shimomura K, Takagi T, Kin S, others (2003) Possible
533 involvement of inositol 1,4,5-trisphosphate receptor type 3 (IP3R3) in the peritoneal
534 dissemination of gastric cancers. *Anticancer Res* 23:3691–3697
- 535 43. Shibao K, Fiedler MJ, Nagata J, Minagawa N, Hirata K, Nakayama Y, others (2010) The type
536 III inositol 1,4,5-trisphosphate receptor is associated with aggressiveness of colorectal
537 carcinoma. *Cell Calcium* 48:315–323
- 538 44. Rönkkö J, Rodriguez Y, Rasila T, Torregrosa-Muñumer R, Pennonen J, Kvist J, others (2023)
539 Human IP3 receptor triple knockout stem cells remain pluripotent despite altered
540 mitochondrial metabolism. *Cell Calcium* 114:102782
- 541 45. Stovall KE, Tran TDN, Suantawee T, Yao S, Gimble JM, Adisakwattana S, others (2020)
542 Adenosine triphosphate enhances osteoblast differentiation of rat dental pulp stem cells via the
543 PLC-IP3 pathway and intracellular Ca²⁺ signaling. *J Cell Physiol* 235:1723–1732
- 544 46. Wu L, Zhang G, Guo C, Pan Y (2020) Intracellular Ca²⁺ signaling mediates IGF-1-induced
545 osteogenic differentiation in bone marrow mesenchymal stem cells. *Biochem Biophys Res*
546 *Commun* 527:200–206
- 547 47. Torre EC, Bicer M, Cottrell GS, Widera D, Tamagnini F (2021) Time-dependent reduction of
548 calcium oscillations in adipose-derived stem cells differentiating towards adipogenic and
549 osteogenic lineage. *Biomolecules* 11:
- 550 48. Avalor L, Camporeale A, Morciano G, Caroccia N, Ghetti E, Orecchia V, Viavattene D, Giorgi
551 C, Pinton P, Poli V (2019) STAT3 localizes to the ER, acting as a gatekeeper for ER-
552 mitochondrion Ca²⁺ fluxes and apoptotic responses. *Cell Death Differ* 26:932–942

553 49. Pinton P, Ferrari D, Rapizzi E, Di Virgilio F, Pozzan T, Rizzuto R (2001) The Ca^{2+}
554 concentration of the endoplasmic reticulum is a key determinant of ceramide-induced
555 apoptosis: significance for the molecular mechanism of Bcl-2 action. EMBO J 20:2690–701
556
557

558 **FIGURE LEGENDS**

559 **Figure 1. Differential IP3R3 expression between H and T tissues.**

560 **A)** Representative WB showing the differential IP3R3 expression levels in the H and the isogenic T
561 colon mucosa. GAPDH was used as housekeeping and loading control. Full-length blots are presented
562 in Supplementary Figure 2. **B)** Slices chart reporting the observed IP3R3 expression patterns
563 distribution, with the respective percentages. **C)** Box plot (min-max) reporting the distribution of the
564 data obtained by WB quantification comparing healthy mucosa with the respective tumor tissue.
565 Lines highlight the means. The p-value was calculated with the paired two-tailed Wilcoxon test.
566 ****=p≤0,01. H= healthy, T= tumor.**

567
568 **Figure 2. IP3R3 expression according to TNM stratification.**

569 **A)** Box plot comparing IP3R3 values (min-max), and sample variation, between healthy mucosa and
570 isogenic tumor tissue in the Low-Stage group. **B)** Box plot comparing IP3R3 values (min-max), and
571 sample variation, between healthy mucosa and isogenic tumor tissue in the Int/High-Stage group. **C)**
572 Box plot comparing IP3R3 values (min-max), and sample variation, of tumor tissue belonging from
573 the Low versus the Int/High-Stage group. **D)** Box plot comparing IP3R3 values (min-max), and
574 sample variation, of healthy colic mucosa belonging from the Low versus the Int/High-Stage group.
575 The p-value was calculated with the paired one-tailed Wilcoxon test (A and B) and with the unpaired
576 two-tailed Mann-Whitney test (C and D). ***=p≤0,05; ns=not significant. H= healthy, T= tumor.**

577
578 **Figure 3. IP3R3 expression according to tumor localization.**

579 **A)** Box plot comparing IP3R3 values (min-max), and sample variation, between healthy mucosa and
580 isogenic tumor tissue in the distal colon carcinoma patients' group. **B)** Box plot comparing IP3R3
581 values (min-max), and sample variation, between healthy mucosa and isogenic tumor tissue in the
582 proximal colon carcinoma patients' group. **C)** Box plot comparing IP3R3 values (min-max), and

sample variation, of tumor tissue belonging from the proximal versus the distal colon carcinoma patients' group. **D)** Box plot comparing IP3R3 values (min-max), and sample variation, of healthy colic mucosa belonging from the proximal versus the distal colon carcinoma patients' group. The p-value was calculated with the paired one-tailed Wilcoxon test (A and B) and with the unpaired two-tailed Mann-Whitney test (C and D). $\ast=p\leq0,05$; ns=not significant. H= healthy, T= tumor.

588

Figure 4. IP3R3 expression according to tumor grading.

A) Box plot comparing IP3R3 values (min-max), and sample variation, between healthy mucosa and isogenic tumor tissue in the Low Grade carcinoma patients' group. **B)** Box plot comparing IP3R3 values (min-max), and sample variation, between healthy mucosa and isogenic tumor tissue in the High Grade carcinoma patients' group. **C)** Box plot comparing IP3R3 values (min-max), and sample variation, of tumor tissue belonging from the Low versus the High Grade colon carcinoma patients' group. **D)** Box plot comparing IP3R3 values (min-max), and sample variation, of healthy colic mucosa belonging from the Low versus the High Grade colon carcinoma patients' group. **E)** Box plot comparing IP3R3 expression levels between healthy mucosa and tumor tissue from both the grading groups (Low and High grade). The p-value was calculated with the paired one-tailed Wilcoxon test (A and B), with the unpaired two-tailed Mann-Whitney test (C and D), and with the Kruskal-Wallis test with Dunn's correction. $\ast=p\leq0,05$; $\ast\ast\ast=p<0.001$; ns=not significant. H= healthy, T= tumor.

601

Figure 5. Immunohistochemical detection of IP3R3 in colon adenocarcinoma.

Representative IP3R3 immunostaining (ABC-peroxidase with nickel enhancement) of paraffin sections from colon carcinomas with different histopathological grading: a moderately differentiated case (#4, Low Grade) and a poorly differentiated case (#9, High Grade) shown in A and B, respectively. Both cases are from the cohort described in Table 2. IR (dark spots) is sharp in some epithelial cells and in the intra-luminal dirty necrosis (DN) in the G2 case, whereas apparently absent in the G3 one. Scale: bar: 50 μ m.

609 **Supplementary Figure 1. Analysis of IP3R3 expression in the CPTAC database.**

610 **A)** Box plot representing the fold change of IP3R3 expression in colon adenocarcinoma according to
611 the CPTAC database (original dataset: PDC000116). **B)** Box plot representing the fold change of
612 IP3R3 expression in colon adenocarcinoma stratified in the Low versus the Int/High stage groups
613 (original dataset: PDC000116). **C)** Box plot representing the relative expression of IP3R3
614 (Normalized Precursor Area) of tumor tissue belonging from the proximal versus the distal colon
615 carcinoma patients' group (original dataset: PDC000111).

616 The p-value was calculated with the one sample Wilcoxon test (**A**) or with the unpaired two-tailed
617 Mann-Whitney test (C and D), and with the Kruskal-Wallis test with Dunn's correction. $\ast = p \leq 0,05$;
618 $\ast\ast\ast = p < 0.001$; ns=not significant. H= healthy, T= tumor.

620 **Supplementary Figure 2. Full-length Western blots corresponding to Figure 1**

621 **A)** Uncropped Western Blot showing IP3R3 and GAPDH on the same membrane. Each lane is
622 labeled with the corresponding sample, including the molecular weight marker, and the approximate
623 molecular weights (kDa) are indicated on the right. The green box delineates the GAPDH band/lanes
624 corresponding to those presented in Figure 1. **B)** Uncropped IP3R3 blot from the same experiment
625 acquired with a longer exposure to enhance signal detection. The green box highlights the IP3R3
626 band/lanes corresponding to those presented in Figure 1.

628 **TABLES LEGENDS**

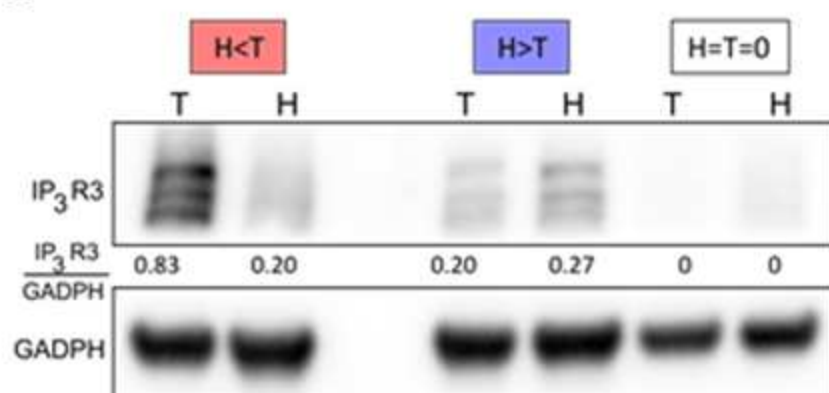
629 **Table 1. Description of the colon tumor cohort.** SD: standard deviation; MSIH: microsatellite
630 instability; *KRAS*: Kirsten rat sarcoma; *BRAF*: v-Raf murine sarcoma viral oncogene homolog.

631

632 **Table 2. Clinicopathological features of the 10 patients included in the immunohistochemistry**
633 **analysis.** Each case was selected among formalin-fixed, paraffin-embedded colon carcinoma biopsies
634 and stratified according to histological grade. These specimens correspond to the cases illustrated in
635 Figure 5.

Figure 1

A



B

- 25.14% H < T
- 26.26% H = T = 0
- 1.68% H = T
- 46.93% H > T



C

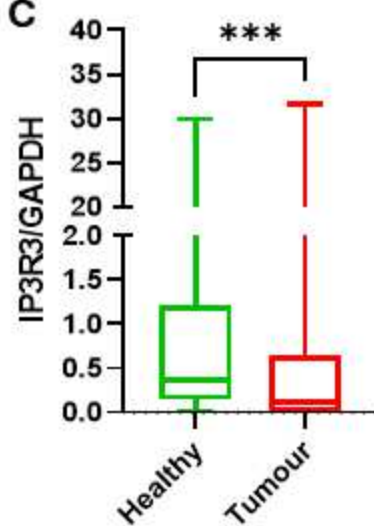


Figure 2

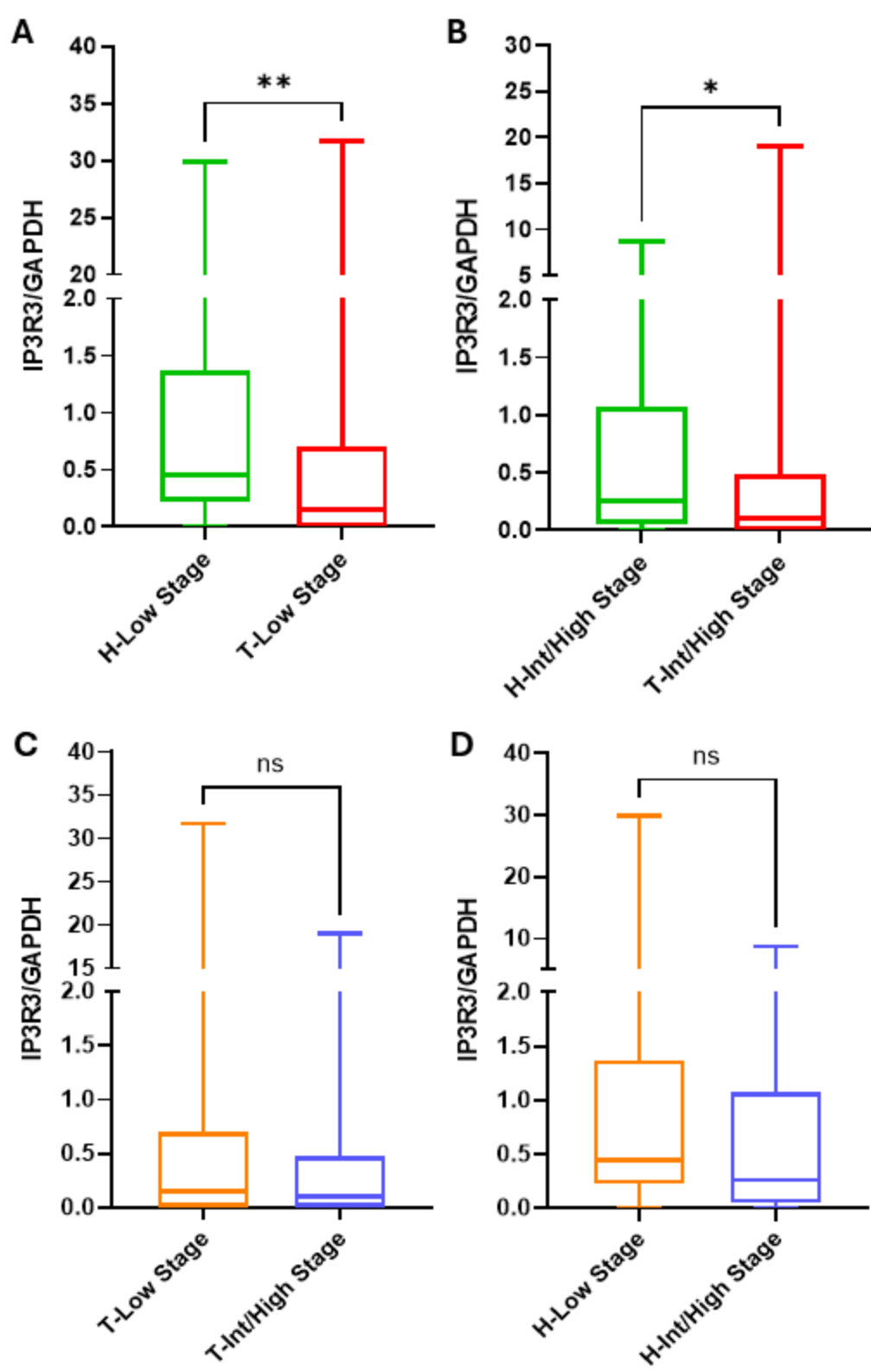


Figure 3

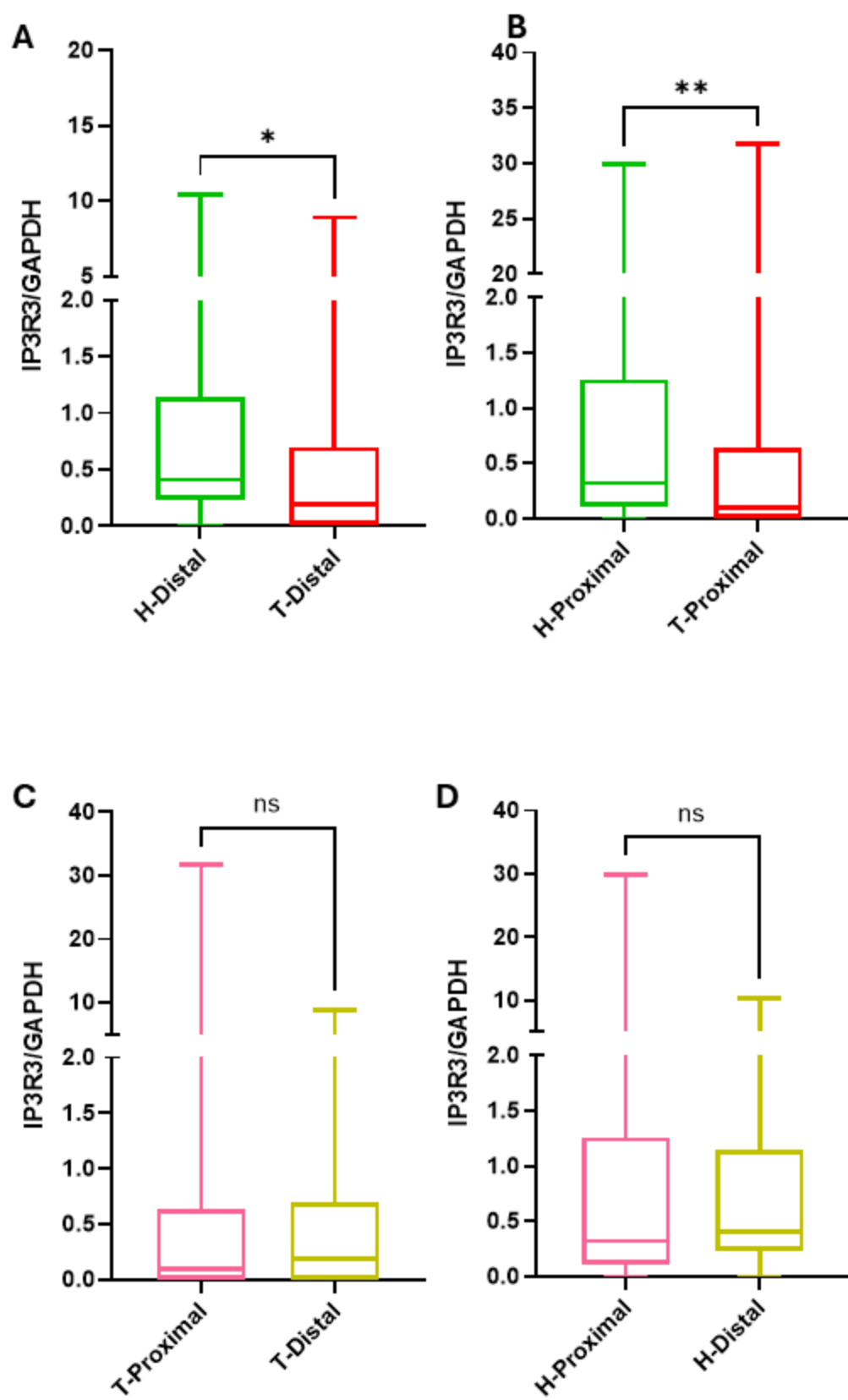


Figure 4

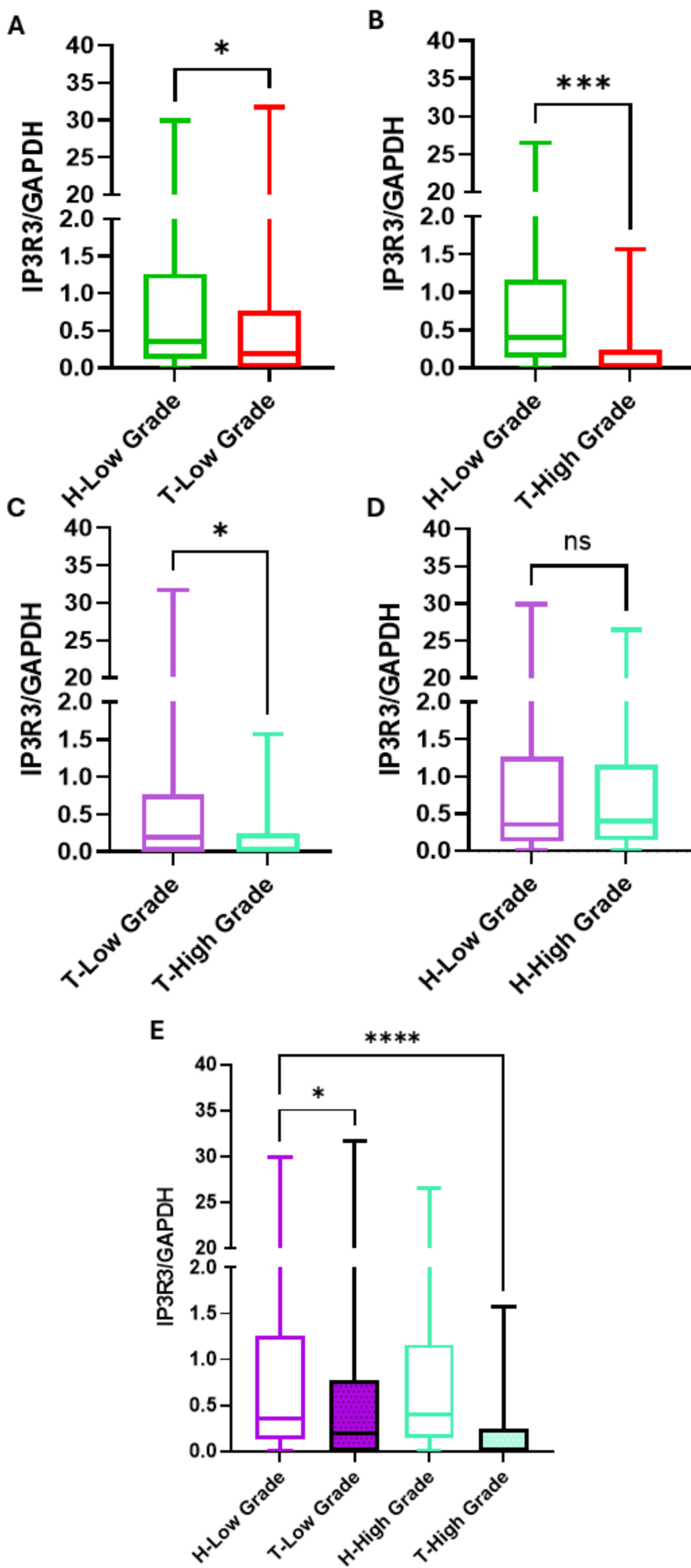
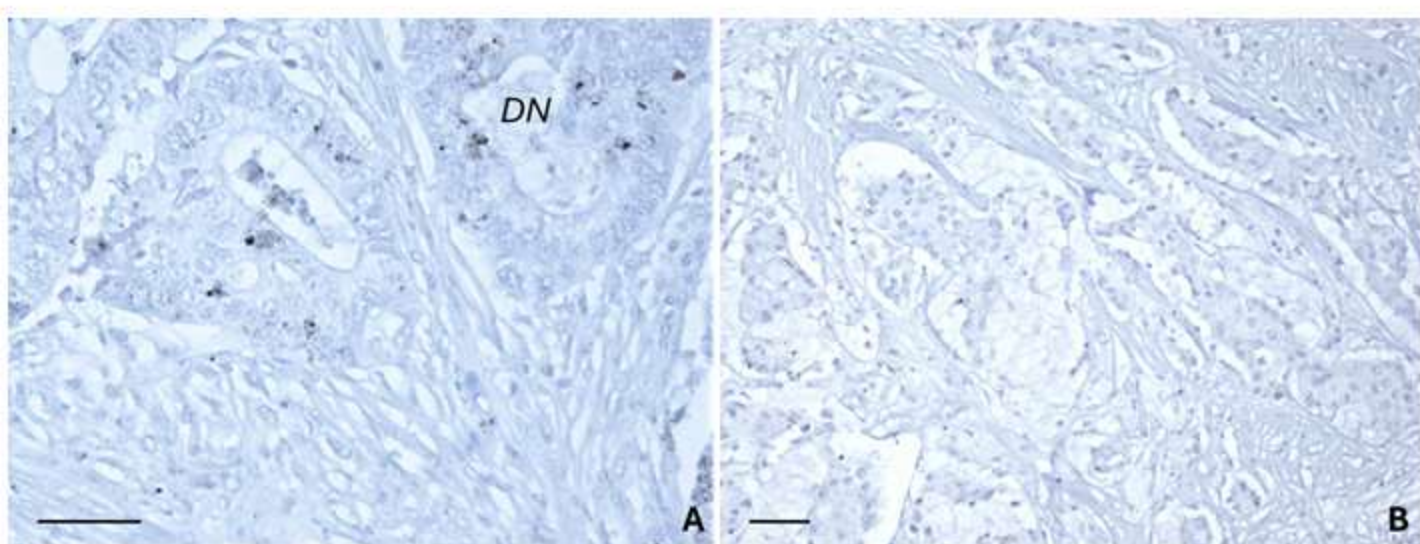


Figure 5



Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.tif](#)
- [Table2.tif](#)
- [FigureS1.tif](#)
- [FigureS2.tif](#)