

**Table S1.** GEO profiles selected gene data sets from human patients used in the study.

| <b>Samples brief description</b>  | <b>Gene data set</b> | <b>GEO series accession number</b> | <b>Original reference / Citation(s)</b> |
|---|----------------------|------------------------------------|---|
| Resected frozen primary tumors from patients with Dukes' stage B colon cancer that recurred in 5 years.   | GDS1263              | GSE2630                            | [50]                                    |
| Serrated and conventional adenocarcinoma.   | GDS2201              | GSE4045                            | [12]                                    |
| Normal-appearing colonic mucosa of early onset colorectal cancer (CRC) patients without a prior family history of CRC.  | GDS2609              | GSE4107                            | [34]                                    |
| Paired colorectal adenomas and normal mucosas.  | GDS2947              | GSE8671                            | [29]                                    |
| Colorectal adenocarcinomas resected from patients treated with celecoxib for 7 days.  | GDS3384              | GSE11237                           | [75]                                    |
| Normal and tumor tissue specimens from Norwegian patients with resectable adenocarcinoma of the rectum, before and after preoperative radio-chemotherapy (RCT).   | GDS3756              | GSE15781                           | [37]                                    |
| Paired colorectal cancer (CRC) tumors and adjacent non-cancerous tissues.   | GDS4382              | GSE32323                           | [30]                                    |
| Patient-derived colorectal cancer (CRC) xenografts with a PIK3CA mutation with enhanced sensitivity to Src inhibitor saracatinib.                                 | GDS4383              | GSE36006                           | [53]                                    |
| Stage III CRCs exhibiting microsatellite-stable (MSS) and mutant p53 phenotypic features.   | GDS4384              | GSE27157                           | [67]                                    |
| CD133-positive and CD133-negative colorectal cancer cells and CAFs isolated from the same stage II patient specimen.  | GDS4385              | GSE34053                           | [41]                                    |
| Primary or metastatic lesions from patients with unresectable colorectal cancer (CRC) prior to FOLFOX6 therapy (oxaliplatin, 5-fluorouracil, leucovorin regimen). | GDS4393;<br>GDS4396  | GSE28702                           | [48]                                    |
| Tumor cells from sporadic stage UICC II colon cancer patients who were treated by elective standard oncological resection but developed relapse during follow-up. | GDS4513              | GSE18088                           | [51]                                    |
| Colorectal adenocarcinomas with microsatellite instability.   | GDS4515              | GSE24514                           | [33]                                    |
| LCM-isolated colorectal cancer (CRC) tumors.  | GDS4516              | GSE21510                           | [47]                                    |
| Homogenized colorectal cancer (CRC) tumors.   | GDS4718              |                                    |   |
| Primary colorectal cancer (CRC) tumors from patients diagnosed at 69 to 87 years.   | GDS5232              | GSE25071                           | [31,32]                                 |

**Table S2.** Selected human gene data sets with respective summary of the samples and relative expression of Tribbles genes represented as fold change to a specific condition.

| Gene data set | Samples description               |  | Relative gene expression - Fold change to Control ( <i>p value</i> ) <sup>test</sup><br>[array probe] |   |   |
|---------------|-----------------------------------|--|---|---|---|
|               | Control / Alternative (n)         | Experimental / Disease (n)             | <i>TRIB1</i>  | <i>TRIB2</i>                                | <i>TRIB3</i>                                |
| GDS2201       | Serrated colorectal carcinoma (8) | Conventional colorectal carcinoma (29) | 0.92x (0.81) <sup>M</sup><br>[202241_at]  | 1.31x (0.086) <sup>M</sup><br>[202478_at]   | 0.83x (0.55) <sup>M</sup><br>[218145_at]    |
|               |                                   |  |   | 1.34x (0.076) <sup>t</sup><br>[202479_s_at] |   |
| GDS1263       | No relapse (10)                   | Tumor relapse (6)                      | 0.48x (0.56) <sup>M</sup><br>[12557]  | 0.82x (0.58) <sup>M</sup><br>[3667]         | 0.53x (0.20) <sup>t</sup><br>[16468]        |
| GDS4513       | No relapse (40)                   | Relapse (13)                           | 1.04x (0.10) <sup>t</sup><br>[202241_at]  | 0.99x (0.98) <sup>t</sup><br>[202478_at]    | 0.99x (0.98) <sup>t</sup><br>[218145_at]    |
|               |                                   |  | 0.46x (0.46) <sup>t</sup><br>[235641_at]  | 0.99x (0.92) <sup>t</sup><br>[202479_s_at]  | 0.99x (0.58) <sup>t</sup><br>[1555788_a_at] |
| GDS4383       | Saracatinib sensitive (2)         | Saracatinib resistant (2)              | 0.95x<br>[8148304]  | 1.04x<br>[8040365]                          | 1.11x<br>[8060344]                          |
| GDS4384       | Wild-type (5)                     | p53 mutant (5)                         | 0.57x (0.021*) <sup>t</sup><br>[202241_at]  | 1.02x (0.42) <sup>M</sup><br>[202478_at]    | 1.09x (0.82) <sup>t</sup><br>[218145_at]    |
|               |                                   |  | 1.03x (0.22) <sup>M</sup><br>[235641_at]  | 1.05x (0.55) <sup>M</sup><br>[202479_s_at]  | 1.04x (0.95) <sup>t</sup><br>[1555788_a_at] |

**Table S3.** List of the GEO profiles selected gene data sets from cell lines used in the study and respective main results.

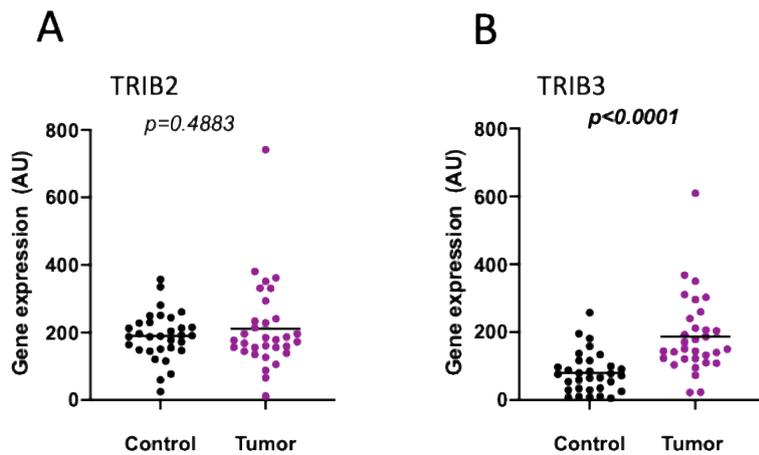
| Samples description   | Main observed results  | Gene data set (Series)              | Original reference / Citation(s) |
|---|--|-------------------------------------|----------------------------------|
| HCT116 colon cancer cells, either fully (p53 <sup>+/+</sup> , n=2), partially (p53 <sup>+/-</sup> , n=2) or not (p53 <sup>-/-</sup> , n=2) expressing p53.  | <i>TRIB1</i> levels revealed no differences between genotypes; <i>TRIB2</i> expression was upregulated in <i>TP53</i> <sup>-/-</sup> when compared to <i>TP53</i> <sup>+/+</sup> .   | GDS170 (GSE90)                      | [62]                             |
| SW480 (n=3), a primary tumor colon cancer cell line, and SW620 (n=3), an isogenic metastatic colon cancer cell line (total RNA; polysomal mRNA).  | From total RNA, <i>TRIB1</i> showed no differences in expression, <i>TRIB2</i> was preferentially expressed in SW480 primary tumor-derived cells, compared to SW620 metastatic cells, and <i>TRIB3</i> showed a reduction in metastatic cells; From polysomal mRNA, <i>TRIB1</i> and <i>TRIB2</i> were downregulated, while <i>TRIB3</i> was overexpressed, in SW620 metastatic cells. | GDS756 (GSE1323); GDS1780 (GSE2509) | [46]                             |
| RKO colorectal carcinoma cells exposed to 4-hydroxy-2-nonenal (HNE), a lipid peroxidation product with anti-cancer potential [81], at 3 different doses (5, 20 and 60 μM) for 6 or 24 hours, compared to control (n=3).   | Dose-dependent downregulation of <i>TRIB1</i> transcript levels, after 6h of HNE treatment compared to control cells; <i>TRIB3</i> expression levels were upregulated in cells treated with 60 μM HNE at both 6h and 24h timepoints.   | GDS1413 (GSE2397)                   | [79]                             |
| HT29 colon adenocarcinoma cells, treated with the cytotoxic natural product apratoxin A (a cyanobacterial metabolite), at 2 different concentrations (2 and 10 nM, which corresponded to the IC50 and IC90 for a 48h treatment) for 3, 6, and 12h, compared to the vehicle control (ethanol-treated cells) (n=2). | <i>TRIB2</i> expression levels were downregulated, only after the 12h treatment, in a dose-independent manner.   | GDS1902 (GSE2742)                   | [80]                             |
| HT29 cells incubated with exogenous hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> ) (100 μM, 12h), to induce oxidative stress in cells, or exposed to UV light (50 J per m <sup>2</sup> ) for 12h, or subjected to a heat shock (42°C) for 1h (n=2).   | <i>TRIB1</i> levels remained unchanged; <i>TRIB3</i> expression was partially downregulated after UV light exposure, and mildly upregulated after H <sub>2</sub> O <sub>2</sub> .  |                                     |                                  |
| HCT116 colon cancer cells, either parental ( <i>PTEN</i> <sup>+/+</sup> , n=2), or in which tumor suppressor <i>PTEN</i> was deleted by gene targeting ( <i>PTEN</i> <sup>-/-</sup> , n=3).   | <i>TRIB1</i> and <i>TRIB3</i> remained unchanged compared to control cells; <i>TRIB2</i> gene expression was upregulated in response to <i>PTEN</i> deletion.  | GDS2446 (GSE6263)                   | [61]                             |
| HT29 colon cancer cells, either sensitive (n=3) or resistant (n=3) to methotrexate (MTX).   | <i>TRIB1</i> was less expressed, and <i>TRIB3</i> was more expressed, in MTX-resistant cells.  | GDS3330 (GSE11440)                  | [54–56]                          |

**Table S3.** List of the GEO profiles selected gene data sets from cell lines used in the study and respective main results (*cont.*).

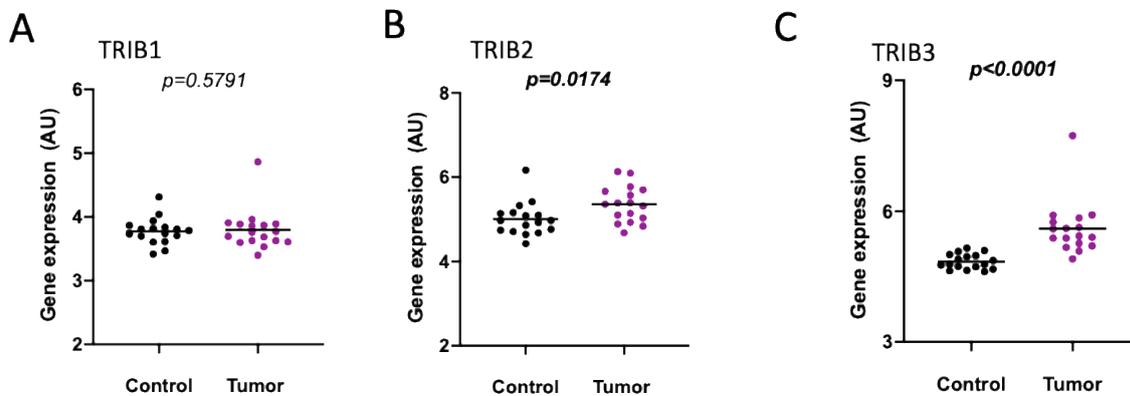
| Samples description   | Main observed results   | Gene data set (Series) | Original reference / Citation(s) |
|---|---|------------------------|----------------------------------|
| Early and late passage HCT116 colon cancer cells depleted for X-linked inhibitor of apoptosis (XIAP), an endogenous caspase inhibitor [109], by knock-down (KD) (n=4).  | <i>TRIB1</i> , <i>TRIB2</i> and <i>TRIB3</i> transcript levels remained unchanged in response to XIAP KD, independently of the cell passage number.   | GDS3482 (GSE11618)     | [65]                             |
| Cell lines from cancer tissue of colon origin from the NCI-60 panel: COLO205, HCC2998, HCT116, HCT15, HT29, KM12 and SW620 colon cancer cell lines (n=3).   | <i>TRIB2</i> was preferentially expressed in COLO205 cells, followed by HCT116, KM12, and SW620; <i>TRIB3</i> was more expressed in HCT15, HT29, and SW620 cells.   | GDS4296 (GSE32474)     | [42–45]                          |
| Ls174T colon cancer cells following doxycycline-induction of a dominant-negative (DN) Tcf4 transgene, or a shRNA against $\beta$ -catenin (knock-down, KD), to block the Wnt signal transduction pathway (n=3).   | <i>TRIB3</i> gene expression was reduced in KD-cells and increased in DN-cells; <i>TRIB2</i> levels did not show expression differences between experimental conditions.  | GDS4386 (GSE18560)     | [63]                             |
| Colonospheres, with cancer stem cell enrichment, from CRC cell line HT29 (n=2).   | <i>TRIB1</i> , <i>TRIB2</i> , and <i>TRIB3</i> transcript levels remained unchanged.  | GDS4511 (GSE14773)     | [60]                             |
| SW480 CRC cells stably overexpressing Snail, an epithelial-mesenchymal transition (EMT) activator highly expressed in CRC colonospheres, which displayed cellular dedifferentiation (n=2).  | No changes in Tribbles family members' gene expression were detected.   | GDS4596 (GSE14773)     |                                  |
| SW480 cancer cells treated with 1 $\mu$ M of the selective Tankyrase inhibitor (TNKSi) NVP-TNKS656, 1 $\mu$ M of the MEK inhibitor (MEKi) selumetinib (AZD3244), or both (duration of 4 and 16 hours) (n=2).  | <i>TRIB1</i> expression was downregulated after both 4- and 16-hour treatment with MEKi; <i>TRIB2</i> and <i>TRIB3</i> expression was preferentially upregulated after 16h of MEKi treatment; Tribbles gene expression was not affected by TNKS inhibition; The relative increase of <i>TRIB2</i> after MEKi was potentiated by TNKSi after 16h of treatment. | GDS5029 (GSE55624)     | [77]                             |
| HCT116 colon cancer cells treated with the potent and selective ATP-dependent CDK inhibitor R547, a diaminopyrimidine compound, at 3 different doses (0.1, 0.2 and 0.6 $\mu$ M, corresponding to the calculated IC50, IC90 and 3x IC90, respectively), and harvested at 5 different time points (1, 2, 4, 6 and 24h) (n=2-4). | <i>TRIB3</i> transcript levels were decreased at the 24h time-point in a dose-dependent manner.   | GDS5268 (GSE15395)     | [78]                             |

**Table S3.** List of the GEO profiles selected gene data sets from cell lines used in the study and respective main results (*cont.*).

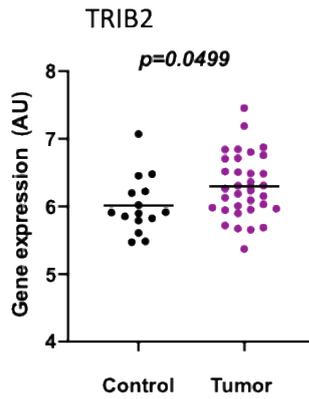
| Samples description  | Main observed results  | Gene data set (Series) | Original reference / Citation(s) |
|--|--|------------------------|----------------------------------|
| SW620 colon cancer cells treated with supercritical Rosemary ( <i>Rosmarinus officinalis</i> L.) extract RE-2 at different doses (30, 60 and 100 µg/mL) compared to non-treated cells (n=2).   | <i>TRIB1</i> and <i>TRIB3</i> were upregulated in increasing doses, compared to control vehicle; <i>TRIB2</i> expression was gradually inhibited upon RE treatment, showing a maximal reduction at the 60 µg/mL concentration.   | GDS5416 (GSE56496)     | [76]                             |
| HCT116 cells either overexpressing wild-type (WT) HLA-F-adjacent transcript 10 (FAT10), a ubiquitin-like modifier protein involved in proteosomal protein degradation [70], or FAT10 mutants with mutations at mitotic arrest-deficient 2 (MAD2)-binding region 1 (M1), region 2 (M2), or at both regions (M12) (n=2). | <i>TRIB1</i> and <i>TRIB2</i> levels remained unchanged between genotypes; <i>TRIB3</i> was downregulated in WT FAT10, compared to parental cells, and this effect was partially or fully lost, in M1 or M2 region mutation and double (M12) region mutations, respectively. | GDS5439 (GSE4167)      | [64]                             |



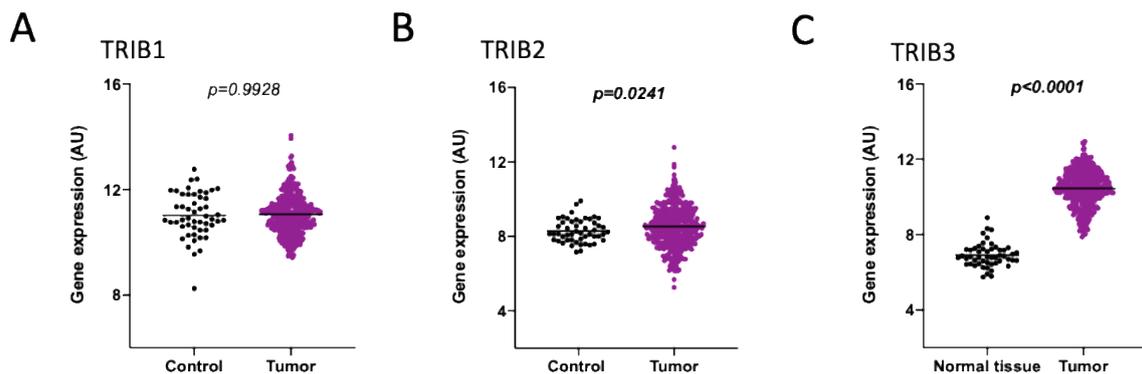
**Figure S1. *TRIB2* and *TRIB3* gene expression in colorectal adenomas compared to normal colon from the same individual.** A-B: Colorectal adenomas (Tumor, n=32) compared to paired normal mucosae (Control, n=32) (GDS2947). *TRIB2* (A – 202479\_s\_at) and *TRIB3* (B – 1555788\_a\_at) gene expression is represented as arbitrary units (AU). Wilcoxon paired test was performed, and the *p* value is represented for each graph. The horizontal bars represent the mean of the values in each group.



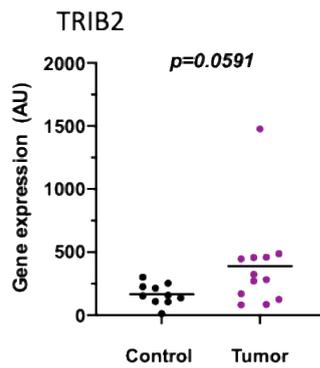
**Figure S2. *TRIB1*, *TRIB2* and *TRIB3* gene expression in colorectal cancer compared to control.** A-C: Colorectal cancer (CRC) tumors (Tumor, n=17) compared to adjacent non-cancerous tissues (Control, n=17) (GDS4382). *TRIB1* (A – 235641\_at), *TRIB2* (B – 202479\_s\_at) and *TRIB3* (C – 1555788\_a\_at) gene expression is represented as arbitrary units (AU). Wilcoxon paired test was performed, and the *p* value is represented for each graph. The horizontal bars represent the mean of the values in each group.



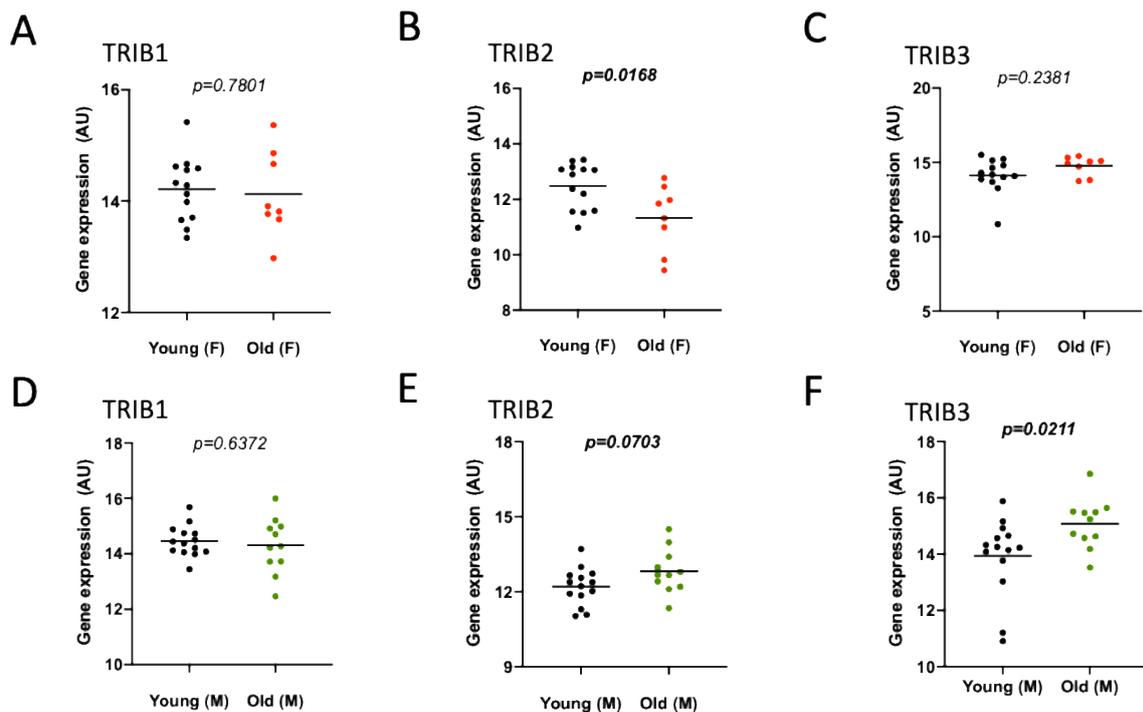
**Figure S3. *TRIB2* gene expression in colorectal cancer compared to control.** Normal colonic mucosa (Control, n=15) compared to colorectal adenocarcinomas with microsatellite instability (Tumor, n=34) (GDS4515). *TRIB2* (202479\_s\_at) gene expression is represented as arbitrary units (AU). Unpaired two-tailed t test was performed, and the *p* value is represented.



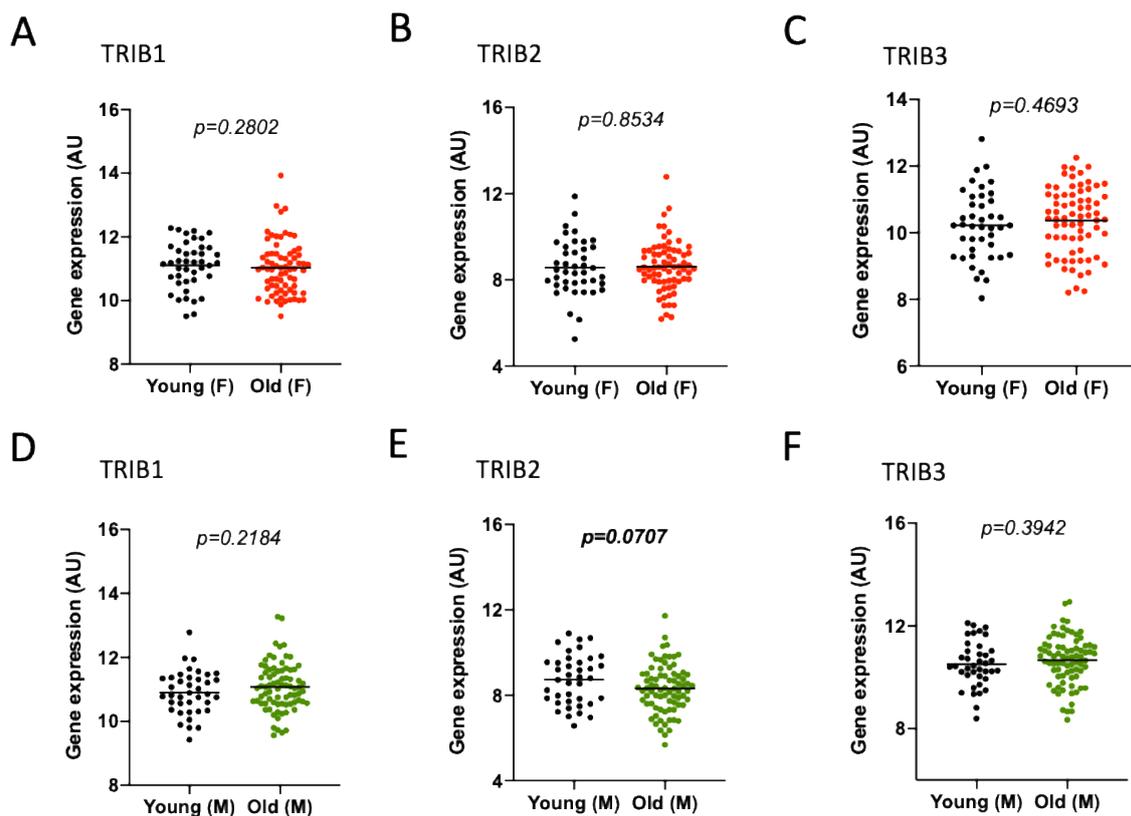
**Figure S4. *TRIB1*, *TRIB2* and *TRIB3* gene expression in colorectal cancer compared to control.** (A) *TRIB1*, (B) *TRIB2* and (C) *TRIB3* expression in colorectal cancer (CRC) tumors (Tumor, n=380) compared to adjacent non-cancerous tissues (Control, n=51) [TCGA colon and rectum adenocarcinoma (COADREAD) cohort]. Gene expression is represented as arbitrary units (AU). The Mann-Whitney test (A, C) and t test with Welch's correction (B) were performed, and the *p* value is represented for each graph. The horizontal bars represent the mean of the values in each group.



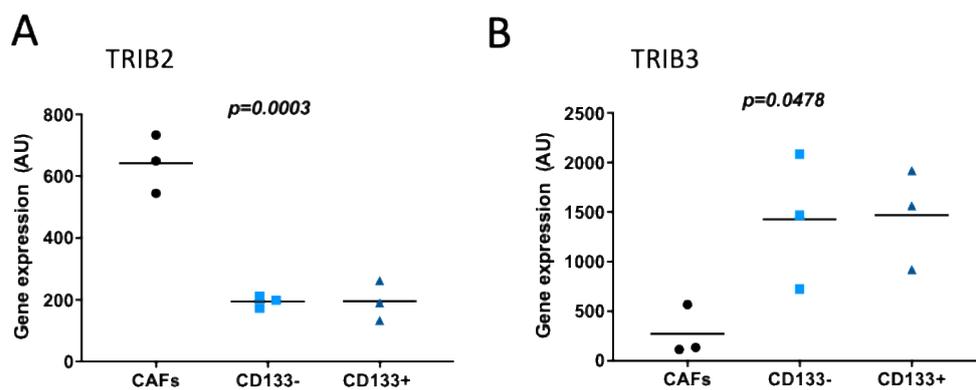
**Figure S5. *TRIB2* gene expression in normal-appearing colonic mucosa from colorectal cancer patients compared to normal colon tissue from healthy individuals.** Normal-appearing colonic mucosa of early onset colorectal cancer (CRC) patients without a prior family history of CRC (Tumor, n=10) compared to healthy control mucosa (Control, n=12) (GDS2609). *TRIB2* (202479\_s\_at) gene expression is represented as arbitrary units (AU). Unpaired Mann-Whitney U test was performed, and the *p* value is represented. The horizontal bars represent the mean of the values in each group.



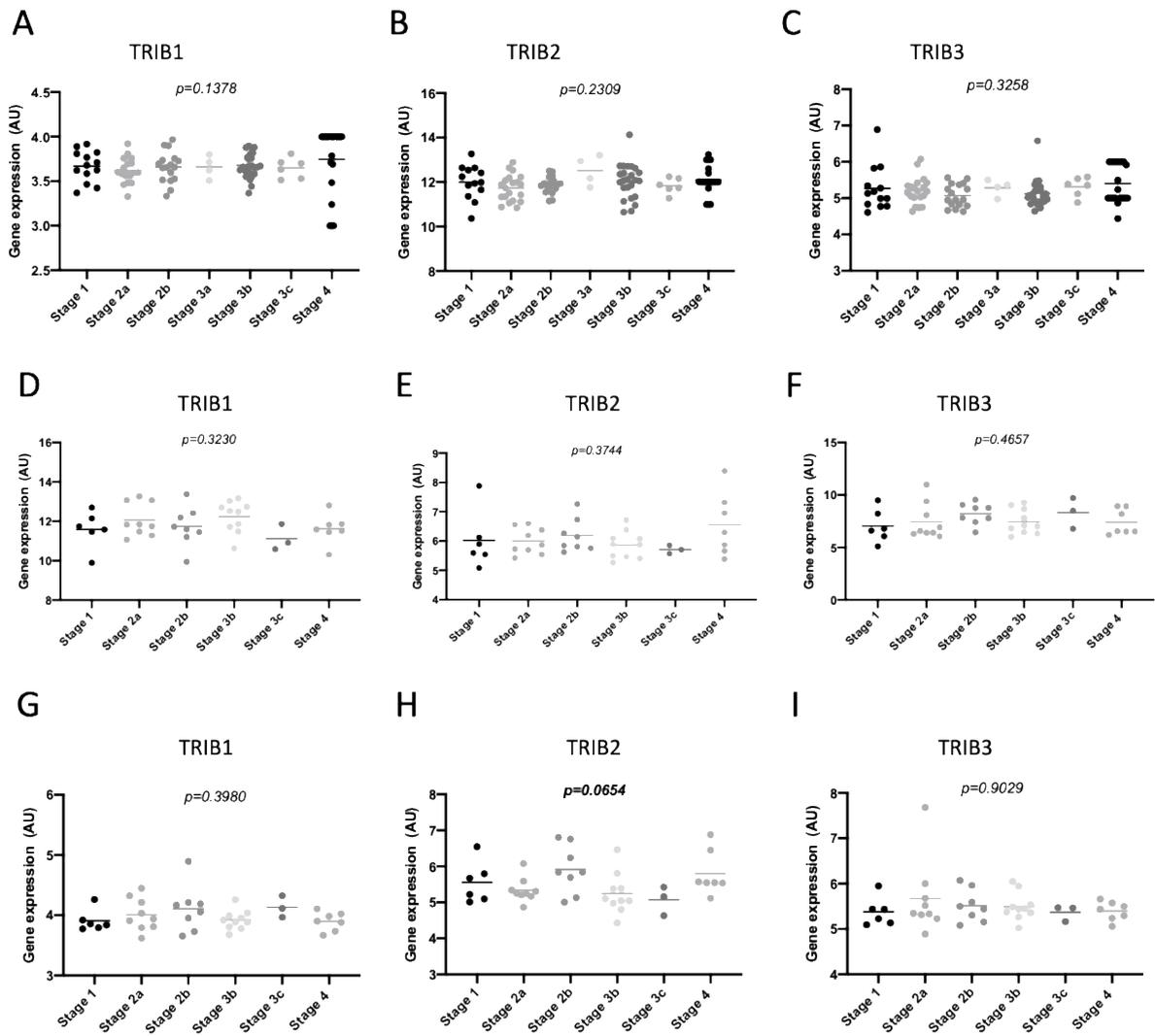
**Figure S6. *TRIB1*, *TRIB2* and *TRIB3* gene expression in primary colorectal cancer (CRC) tumors at different times of diagnosis.** A-C: Female patients diagnosed with CRC at an early age (28 to 53 years of age) (Young (F), n=13) compared with patients diagnosed at a later age (69 to 87 years) (Old (F), n=8) (GDS5232). D-F: Male patients diagnosed with CRC at an early age (28 to 53 years of age) (Young (M), n=14) compared with patients diagnosed at a later age (69 to 87 years) (Old (M), n=11) (GDS5232). Independent datasets were analyzed, and samples plotted individually. *TRIB1* (A, D – 150749), *TRIB2* (B, E – 188922) and *TRIB3* (C, F – 113737) gene expression is represented as arbitrary units (AU). Unpaired two-tailed t test (A, B, D, E), or Mann-Whitney U test (C, F) was performed, and the *p* value is represented for each graph. The horizontal bars represent the mean of the values in each group.



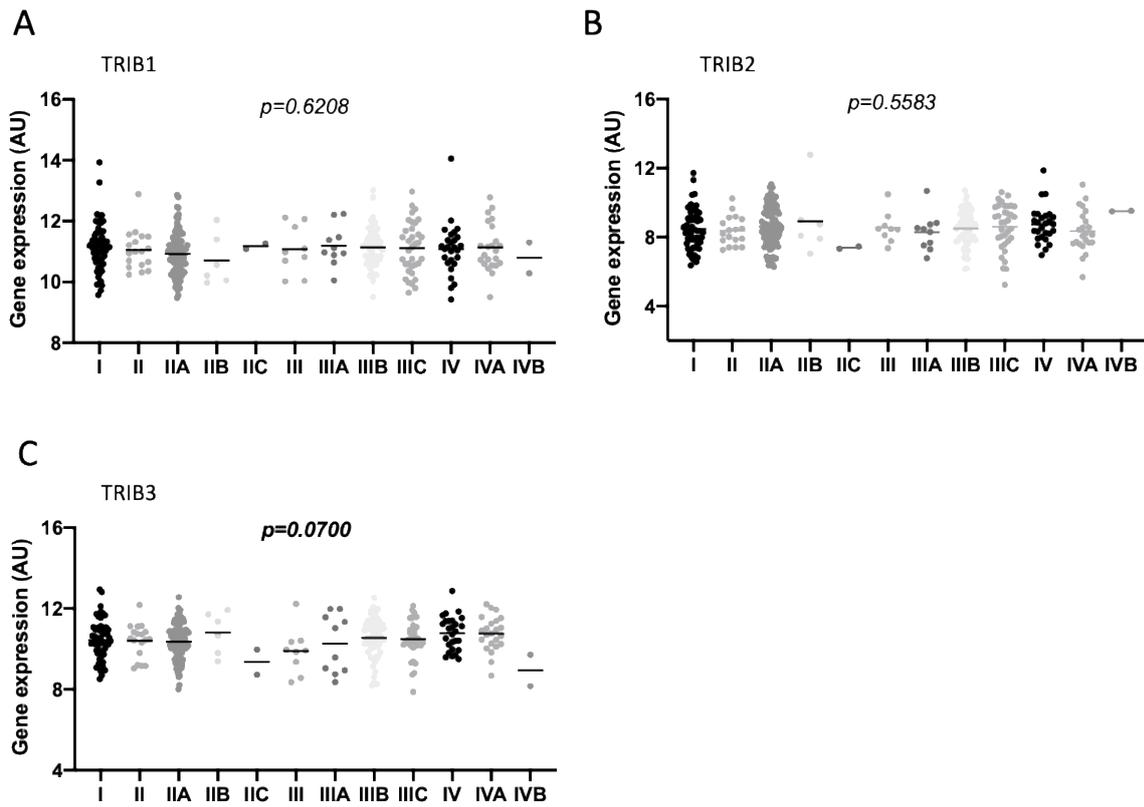
**Figure S7. *TRIB1*, *TRIB2* and *TRIB3* gene expression in primary colorectal cancer (CRC) tumors at different times of diagnose.** (A) *TRIB1*, (B) *TRIB2* and (C) *TRIB3* female patients diagnosed with CRC at an early age (28 to 53 years of age) (Young (F), n=42) compared with patients diagnosed at a later age (69 to 87 years) (Old (F), n=71) [TCGA colon and rectum adenocarcinoma (COADREAD) cohort]. (D) *TRIB1*, (E) *TRIB2* and (F) *TRIB3* male patients diagnosed with CRC at an early age (28 to 53 years of age) (Young (M), n=39) compared with patients diagnosed at a later age (69 to 87 years) (Old (M), n=78) [TCGA colon and rectum adenocarcinoma (COADREAD) cohort]. Gene expression is represented as arbitrary units (AU). The unpaired two-tailed t test was performed, except for *TRIB1* expression in female patients (A) for which the Mann-Whitney test was applied, and the *p* value is represented for each group. The horizontal bars represent the mean of the values in each group.



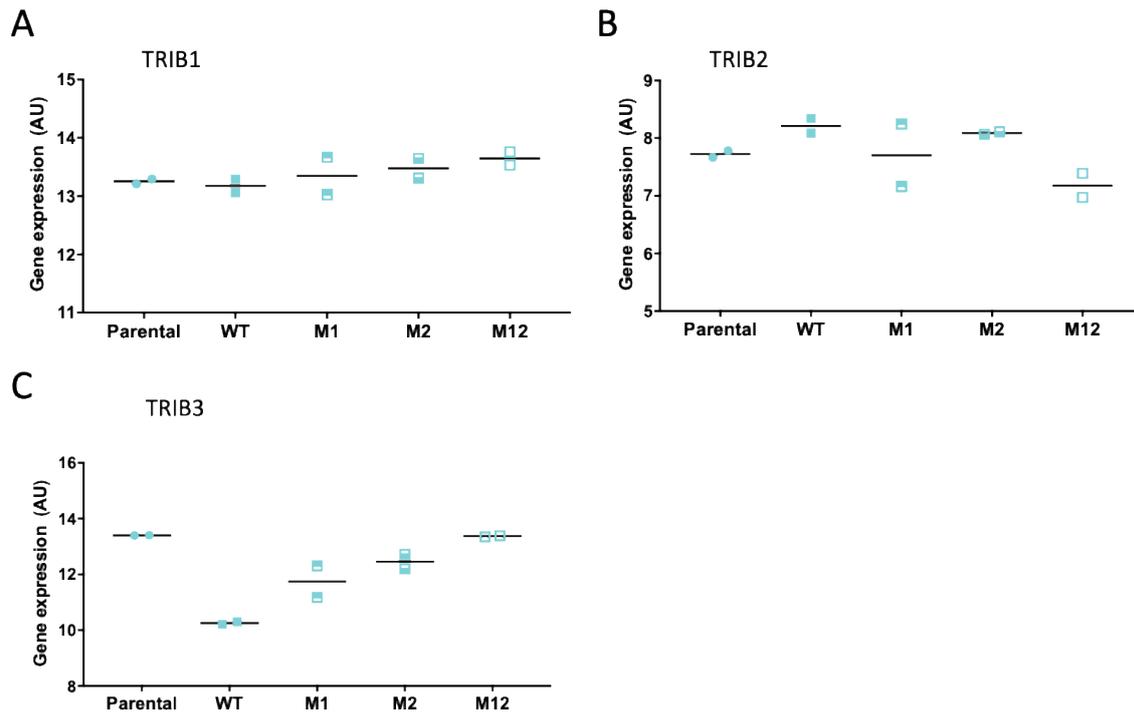
**Figure S8. *TRIB2* and *TRIB3* gene expression in colorectal cancer (CRC) cells compared to carcinoma associated fibroblasts.** A-B: Carcinoma associated fibroblasts (CAFs), CD133-negative and CD133-positive colorectal cancer (CRC) cells isolated from stage II patient samples (GDS4385). *TRIB2* (A – 202479\_s\_at) and *TRIB3* (B – 1555788\_a\_at) gene expression is represented as arbitrary units (AU). Ordinary one-way ANOVA was performed, and the *p* value is represented for each graph. The horizontal bars represent the mean of the values in each group.



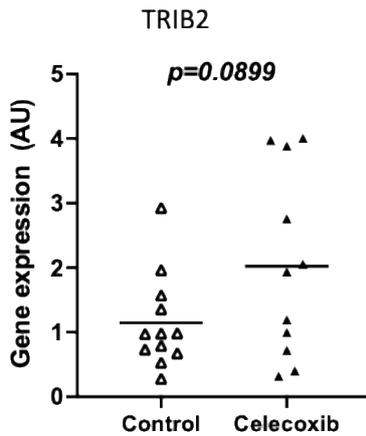
**Figure S9. *TRIB1*, *TRIB2* and *TRIB3* gene expression in CRC tumors with different staging.** Independent datasets GDS4516 (A – C) and GDS4718 (D – I) were analyzed, and samples plotted individually. Laser microdissection (LCM)-isolated (A-C) (n=4-24 each group) or homogenized (D-I) (n=3-10 each group) CRC tumors representing various stages and metastases are shown. *TRIB1* (A, G – 235641\_at; 202241\_at; D – 202241\_at), *TRIB2* (B, H – 202479\_s\_at; E – 202478\_at) and *TRIB3* (C, I – 1555788\_a\_at; F – 218145\_at) gene expression is represented as arbitrary units (AU). Ordinary one-way ANOVA (D, E, H) or Kruskal-Wallis test (A-C, F, G, I) was performed, and the *p* value is represented for each graph. The horizontal bars represent the mean of the values in each group.



**Figure S10. *TRIB1*, *TRIB2* and *TRIB3* gene expression in CRC tumors with different staging.** (A) *TRIB1*, (B) *TRIB2* and (C) *TRIB3* expression in CRC samples classified with different stages (n=2-110 in each group) [TCGA colon and rectum adenocarcinoma (COADREAD) cohort]. *TRIB1*, *TRIB2* and *TRIB3* gene expression are represented as arbitrary units (AU). The Kruskal-Wallis test was performed (A-C), and the *p* value is represented for each graph. The horizontal bars represent the mean of the values in each group.



**Figure S11.** *TRIB1*, *TRIB2* and *TRIB3* gene expression in HCT116 colon cancer cell line overexpressing wild-type (WT) or mutant HLA-F-adjacent transcript 10 (FAT10). A single dataset was analyzed (GDS5439), and samples plotted individually. *TRIB1* (A – ILMN\_1803811), *TRIB2* (B – ILMN\_1714700) and *TRIB3* (C – ILMN\_1787815) gene expression from parental HCT116 colon cancer cells, or either overexpressing a WT or different mutant forms of HLA-F-adjacent transcript 10 (FAT10) (single M1, single M2, double M12) (n=2 each), is represented as arbitrary units (AU). The horizontal bars represent the mean of the values in each group.



**Figure S12. *TRIB2* gene expression in colorectal adenocarcinoma from patients treated with celecoxib.** A single dataset was analysed (GDS3384), and samples plotted individually. *TRIB2* (717\_at) gene expression from patients pre-treated with cyclooxygenase-2 (COX-2) inhibitor celecoxib (400mg twice a day) for 7 days prior to colorectal adenocarcinoma resection (Celecoxib, n = 11), compared to non-treated patients (Control, n = 12), is represented as arbitrary units (AU). Unpaired two-tailed t test with Welch's correction was performed, and the *p* value is represented. There was no additional assay probe for *TRIB1*. There was no assay probe for *TRIB3*. The horizontal bars represent the mean of the values in each group.