

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

Samples brief description	Gene data set	GEO series accession number	Original reference / Citation(s)
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; 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>) ^{test} [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) ^M [202241_at]	1.31x (0.086) ^M [202478_at]	0.83x (0.55) ^M [218145_at]
				1.34x (0.076) ^t [202479_s_at]	
GDS1263	No relapse (10)	Tumor relapse (6)	0.48x (0.56) ^M [12557]	0.82x (0.58) ^M [3667]	0.53x (0.20) ^t [16468]
GDS4513	No relapse (40)	Relapse (13)	1.04x (0.10) ^t [202241_at]	0.99x (0.98) ^t [202478_at]	0.99x (0.98) ^t [218145_at]
			0.46x (0.46) ^t [235641_at]	0.99x (0.92) ^t [202479_s_at]	0.99x (0.58) ^t [1555788_a_at]
GDS4383	Saracatinib sensitive (2)	Saracatinib resistant (2)	0.95x [8148304]	1.04x [8040365]	1.11x [8060344]
GDS4384	Wild-type (5)	p53 mutant (5)	0.57x (0.021*) ^t [202241_at]	1.02x (0.42) ^M [202478_at]	1.09x (0.82) ^t [218145_at]
			1.03x (0.22) ^M [235641_at]	1.05x (0.55) ^M [202479_s_at]	1.04x (0.95) ^t [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 ^{+/+} , n=2), partially (p53 ^{-/+} , n=2) or not (p53 ^{-/-} , n=2) expressing p53.	<i>TRIB1</i> levels revealed no differences between genotypes; <i>TRIB2</i> expression was upregulated in <i>TP53</i> ^{-/-} when compared to <i>TP53</i> ^{+/+} .	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 60μM) 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 60μM 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 ₂ O ₂) (100 μM, 12h), to induce oxidative stress in cells, or exposed to UV light (50 J per m ²) 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 ₂ O ₂ .		
HCT116 colon cancer cells, either parental (PTEN ^{+/+} , n=2), or in which tumor suppressor <i>PTEN</i> was deleted by gene targeting (PTEN ^{-/-} , 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 β -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 μ M of the selective Tankyrase inhibitor (TNKSi) NVP-TNKS656, 1 μ 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 μ M, corresponding to the calculated IC ₅₀ , IC ₉₀ and 3x IC ₉₀ , 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]

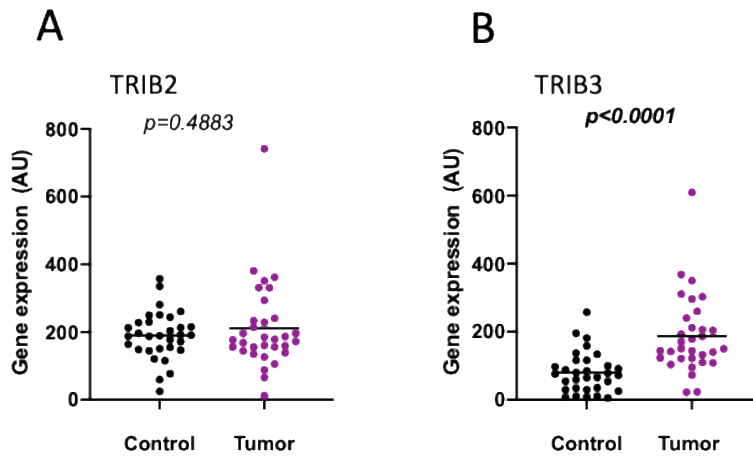


Figure S1. *TRIB2* and *TRIB3* gene expression in colorectal adenomas compared to normal colon from the same individual. A-B: Colorectal adenomas (Tumor, n=32) compared to paired normal mucosas (Control, n=32) (GDS2947). *TRIB2* (A – 202479_s_at) and *TRIB3* (B – 1555788_a_at) gene expression is represented 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.

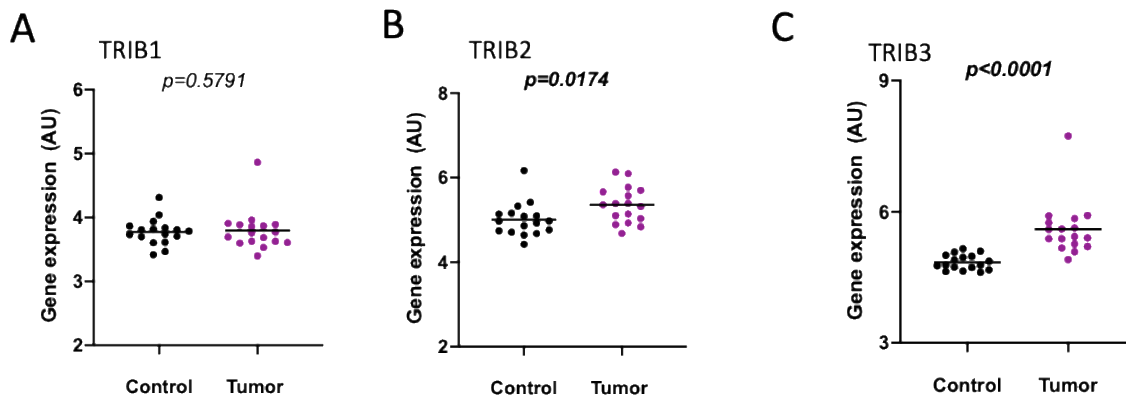


Figure S2. *TRIB1*, *TRIB2* and *TRIB3* gene expression in colorectal cancer compared to control. A-C: Colorectal cancer (CRC) tumors (Tumor, n=17) compared to adjacent non-cancerous tissues (Control, n=17) (GDS4382). *TRIB1* (A – 235641_at), *TRIB2* (B – 202479_s_at) and *TRIB3* (C – 1555788_a_at) gene 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.

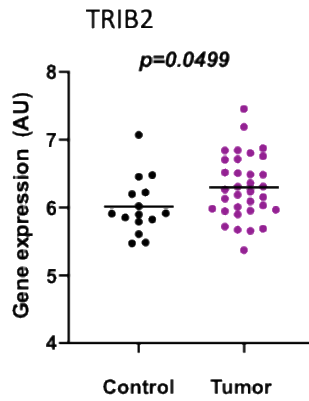


Figure S3. *TRIB2* gene expression in colorectal cancer compared to control. Normal colonic mucosa (Control, n=15) compared to colorectal adenocarcinomas with microsatellite instability (Tumor, n=34) (GDS4515). *TRIB2* (202479_s_at) gene expression is represented as arbitrary units (AU). Unpaired two-tailed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

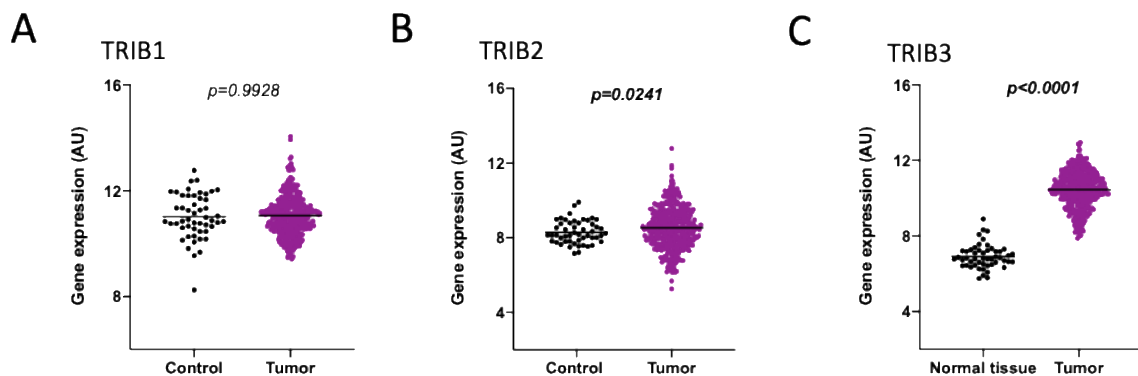


Figure S4. *TRIB1*, *TRIB2* and *TRIB3* gene expression in colorectal cancer compared to control. (A) *TRIB1*, (B) *TRIB2* and (C) *TRIB3* expression in colorectal cancer (CRC) tumors (Tumor, n=380) compared to adjacent non-cancerous tissues (Control, n=51) [TCGA colon and rectum adenocarcinoma (COADREAD) 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.

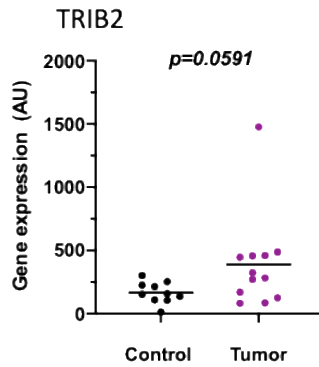


Figure S5. *TRIB2* gene expression in normal-appearing colonic mucosa from colorectal cancer patients compared to normal colon tissue from healthy individuals. Normal-appearing colonic mucosa of early onset colorectal cancer (CRC) patients without a prior family history of CRC (Tumor, n=10) compared 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.

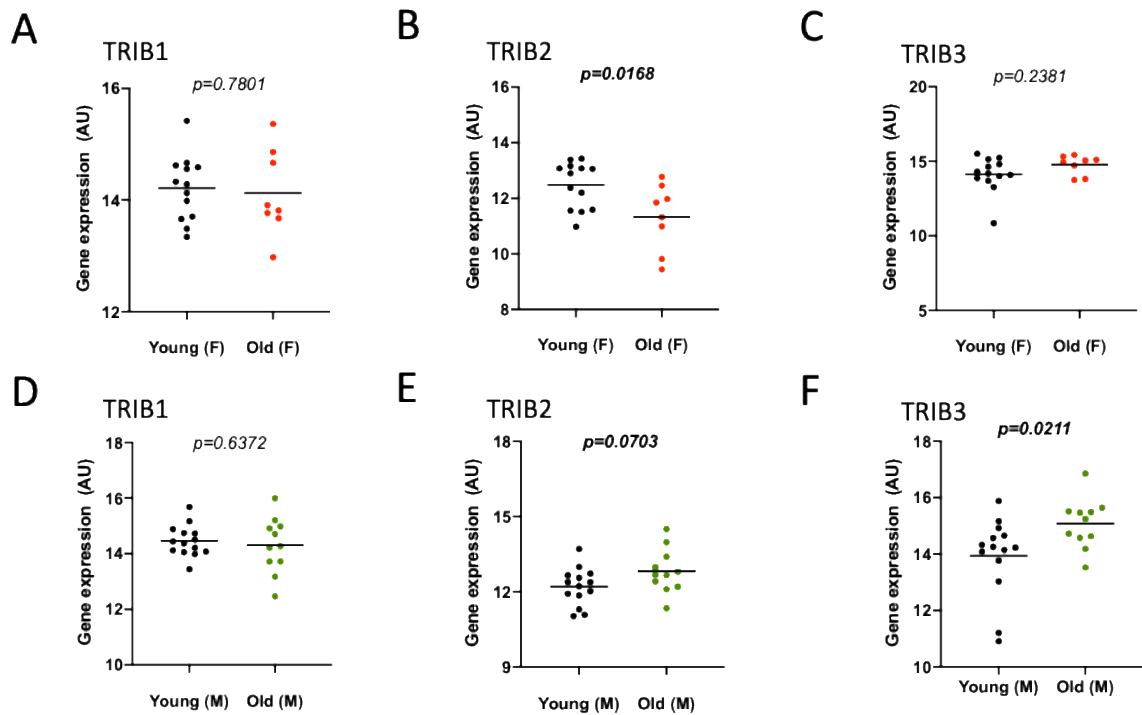


Figure S6. *TRIB1*, *TRIB2* and *TRIB3* gene expression in primary colorectal cancer (CRC) tumors at different times of diagnosis. A-C: Female patients diagnosed with CRC at an early age (28 to 53 years of age) (Young (F), n=13) compared with patients diagnosed at a later age (69 to 87 years) (Old (F), 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.

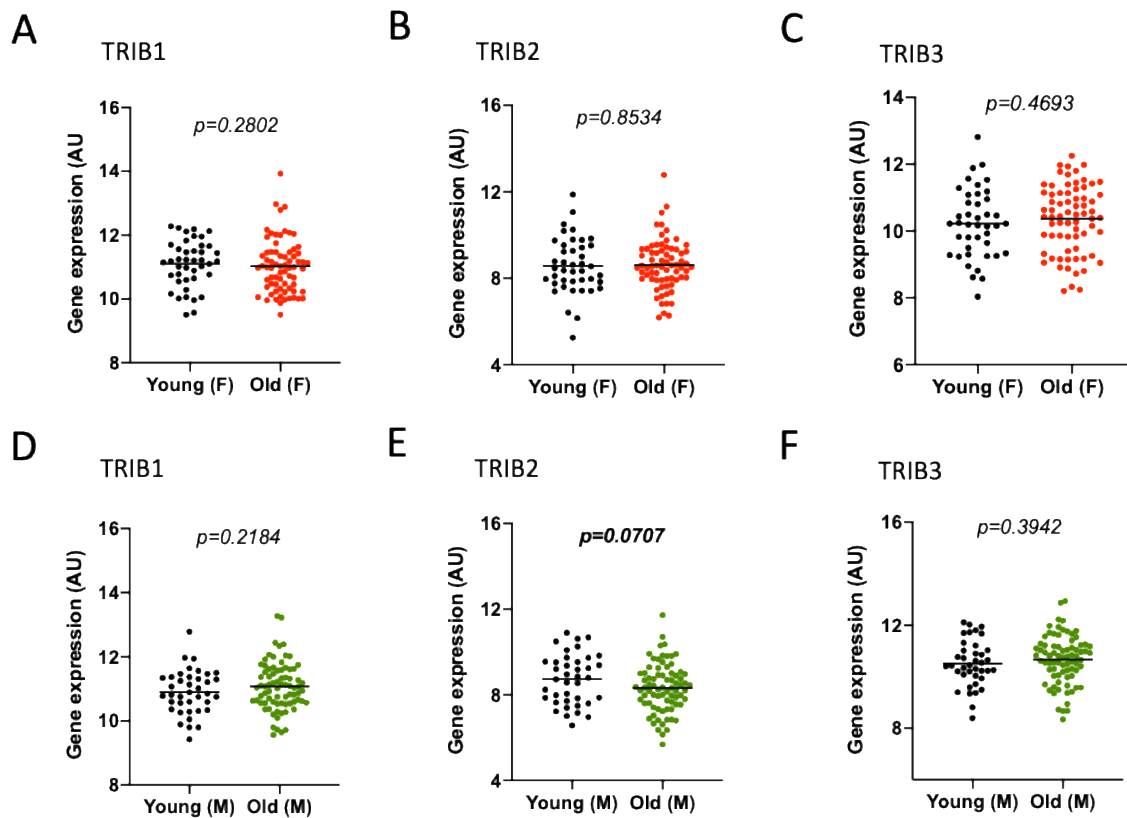


Figure S7. *TRIB1*, *TRIB2* and *TRIB3* gene expression in primary colorectal cancer (CRC) tumors at different times of diagnose. (A) *TRIB1*, (B) *TRIB2* and (C) *TRIB3* female patients diagnosed with CRC at an early age (28 to 53 years of age) (Young (F), n=42) compared with patients diagnosed at a 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 graph. The horizontal bars represent the mean of the values in each group.

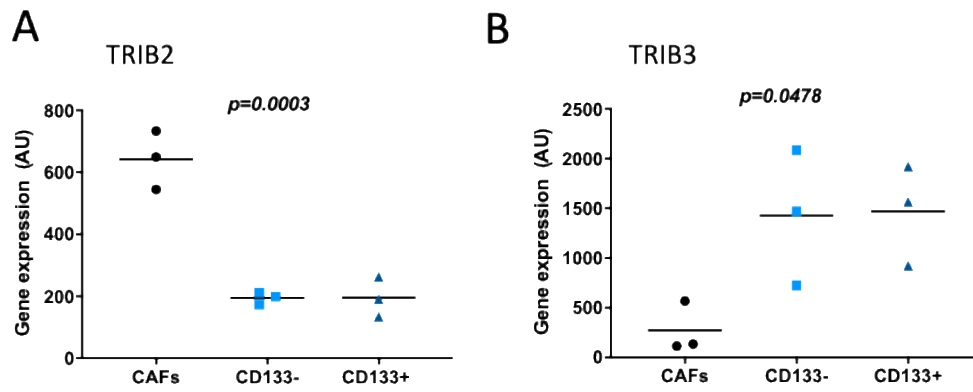


Figure S8. *TRIB2* and *TRIB3* gene expression in colorectal cancer (CRC) cells compared to carcinoma associated fibroblasts. A-B: Carcinoma associated fibroblasts (CAF_s), CD133-negative and CD133-positive colorectal cancer (CRC) cells isolated from stage II patient samples (GDS4385). *TRIB2* (A – 202479_s_at) 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.

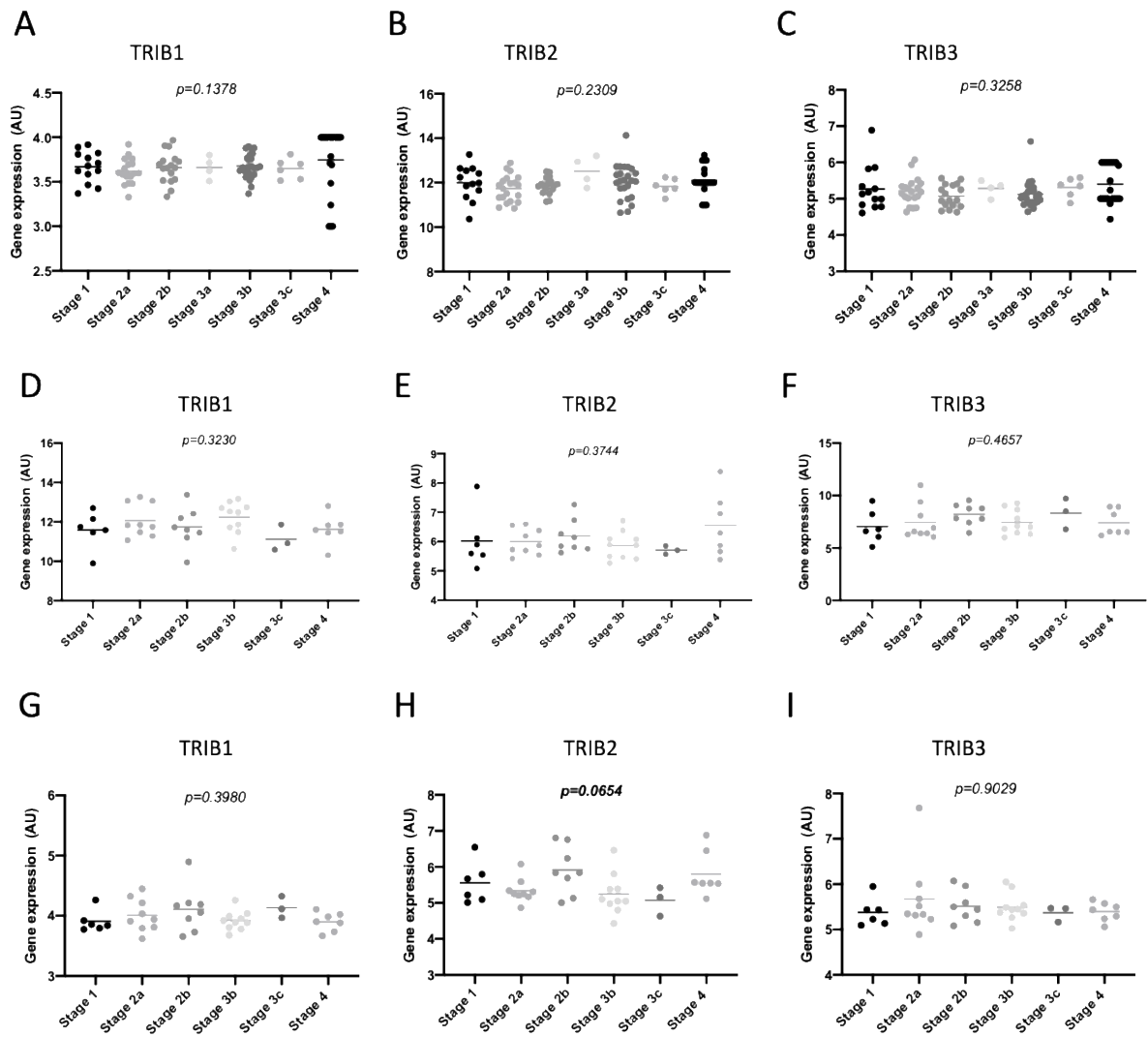


Figure S9. *TRIB1*, *TRIB2* and *TRIB3* gene expression in CRC tumors with different staging. Independent datasets GDS4516 (A – C) and GDS4718 (D – I) were analyzed, and samples plotted individually. Laser microdissection (LCM)-isolated (A-C) (n=4-24 each group) or homogenized (D-I) (n=3-10 each group) 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.

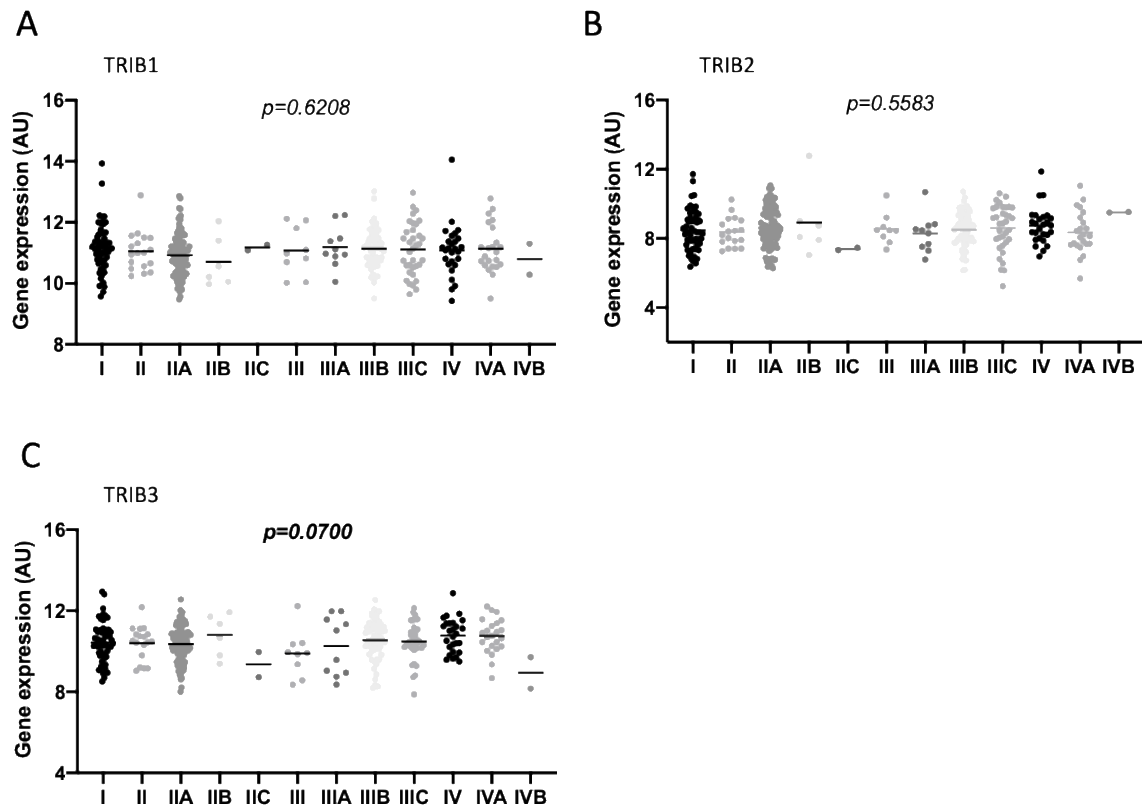


Figure S10. *TRIB1*, *TRIB2* and *TRIB3* gene expression in CRC tumors with different staging. (A) *TRIB1*, (B) *TRIB2* and (C) *TRIB3* expression in CRC samples classified with different stages (n=2-110 in each group) [TCGA colon and rectum adenocarcinoma (COADREAD) cohort]. *TRIB1*, *TRIB2* and *TRIB3* 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.

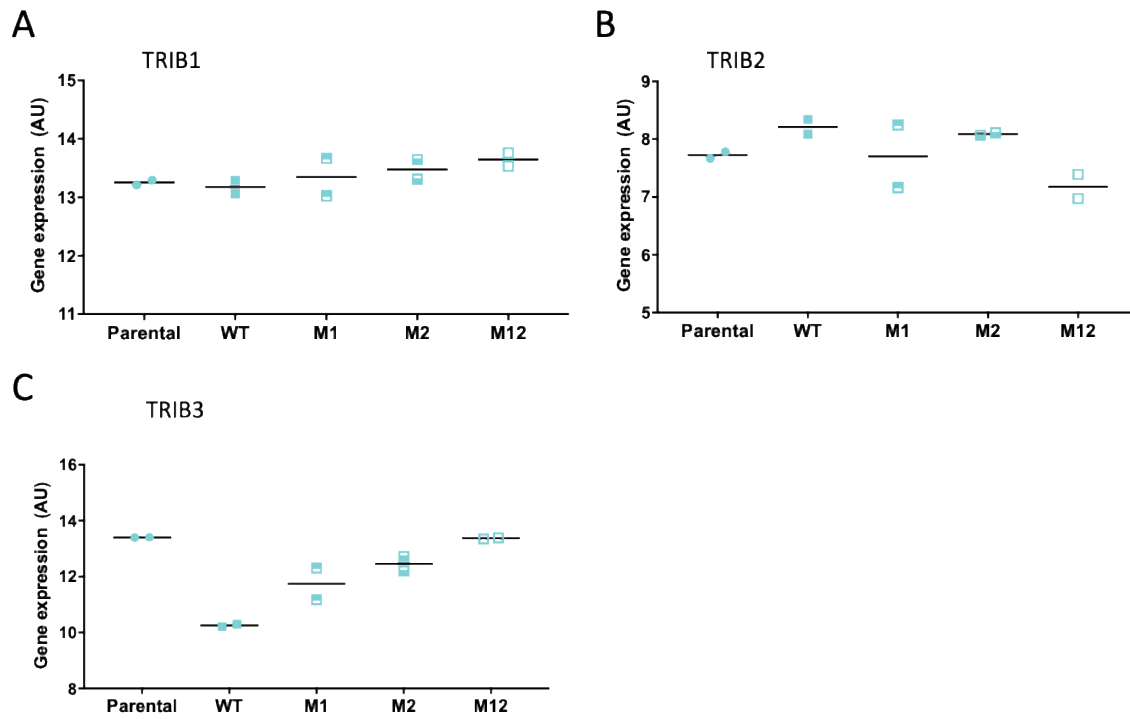


Figure S11. *TRIB1*, *TRIB2* and *TRIB3* gene expression in HCT116 colon cancer cell line overexpressing wild-type (WT) or mutant HLA-F-adjacent transcript 10 (FAT10). A single dataset was analyzed (GDS5439), and samples plotted individually. *TRIB1* (**A** – ILMN_1803811), *TRIB2* (**B** – ILMN_1714700) 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.

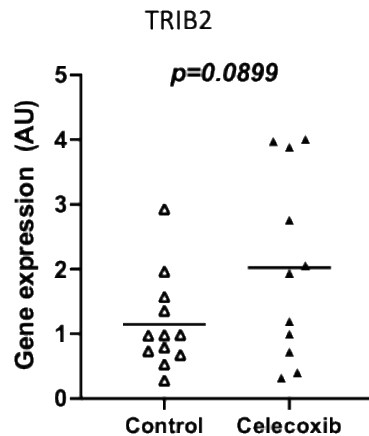


Figure S12. *TRIB2* gene expression in colorectal adenocarcinoma from patients treated with celecoxib. A single dataset was analysed (GDS3384), and samples plotted individually. *TRIB2* (717_at) gene expression from patients pre-treated with cyclooxygenase-2 (COX-2) inhibitor celecoxib (400mg twice a 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.