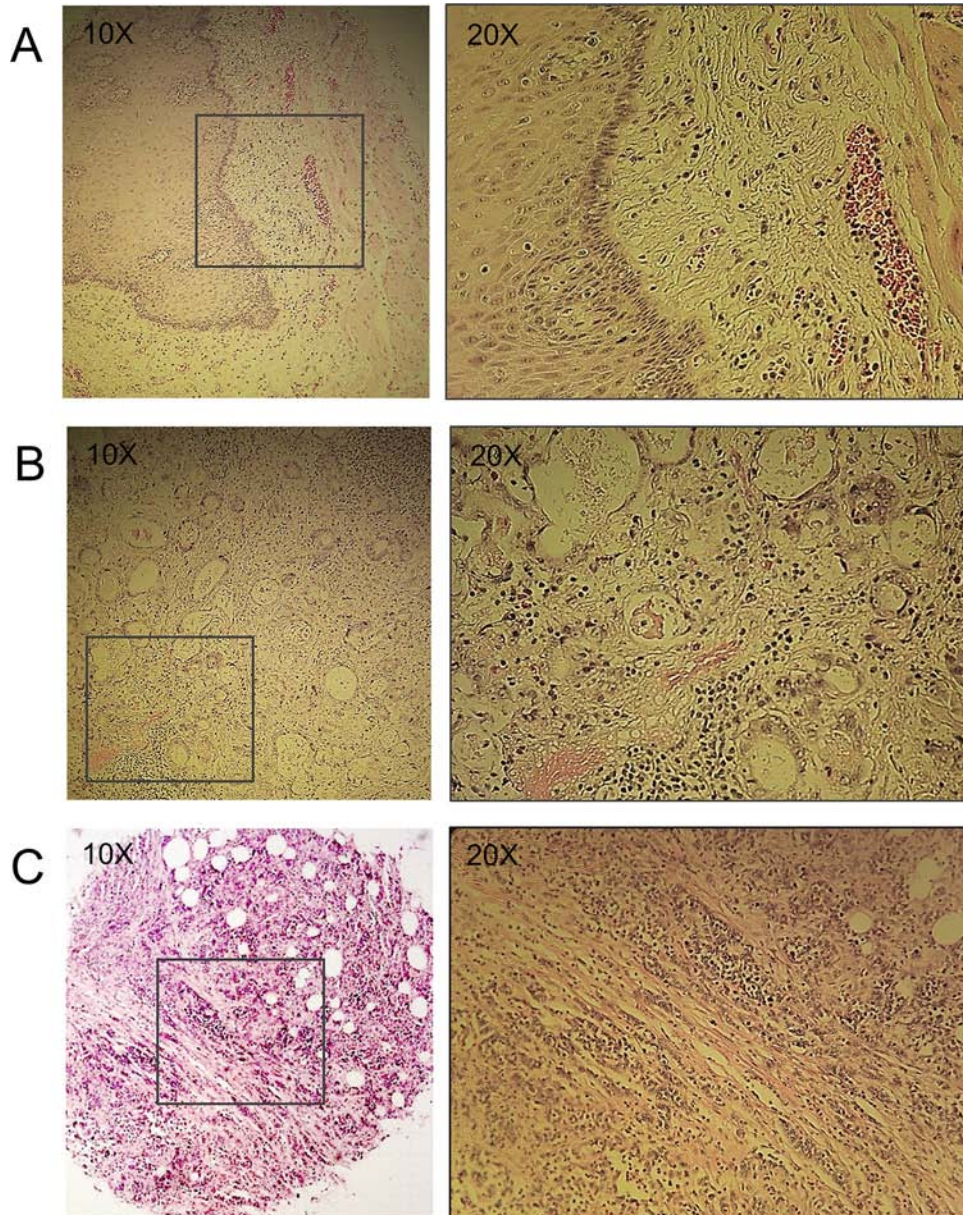# A subset of myofibroblastic cancer-associated fibroblasts regulate collagen fiber elongation, which is prognostic in multiple cancers

Supplementary Materials

Supplementary Figure S1: Low magnification H&E stained images of sections presented in Figure 2 (**A–C**). Normal squamous epithelium and sub-epithelial stroma (A) Sub-mucosal tissue (B) from esophageal tissue samples. (C) Esophageal adenocarcinoma (EAC) tissue.
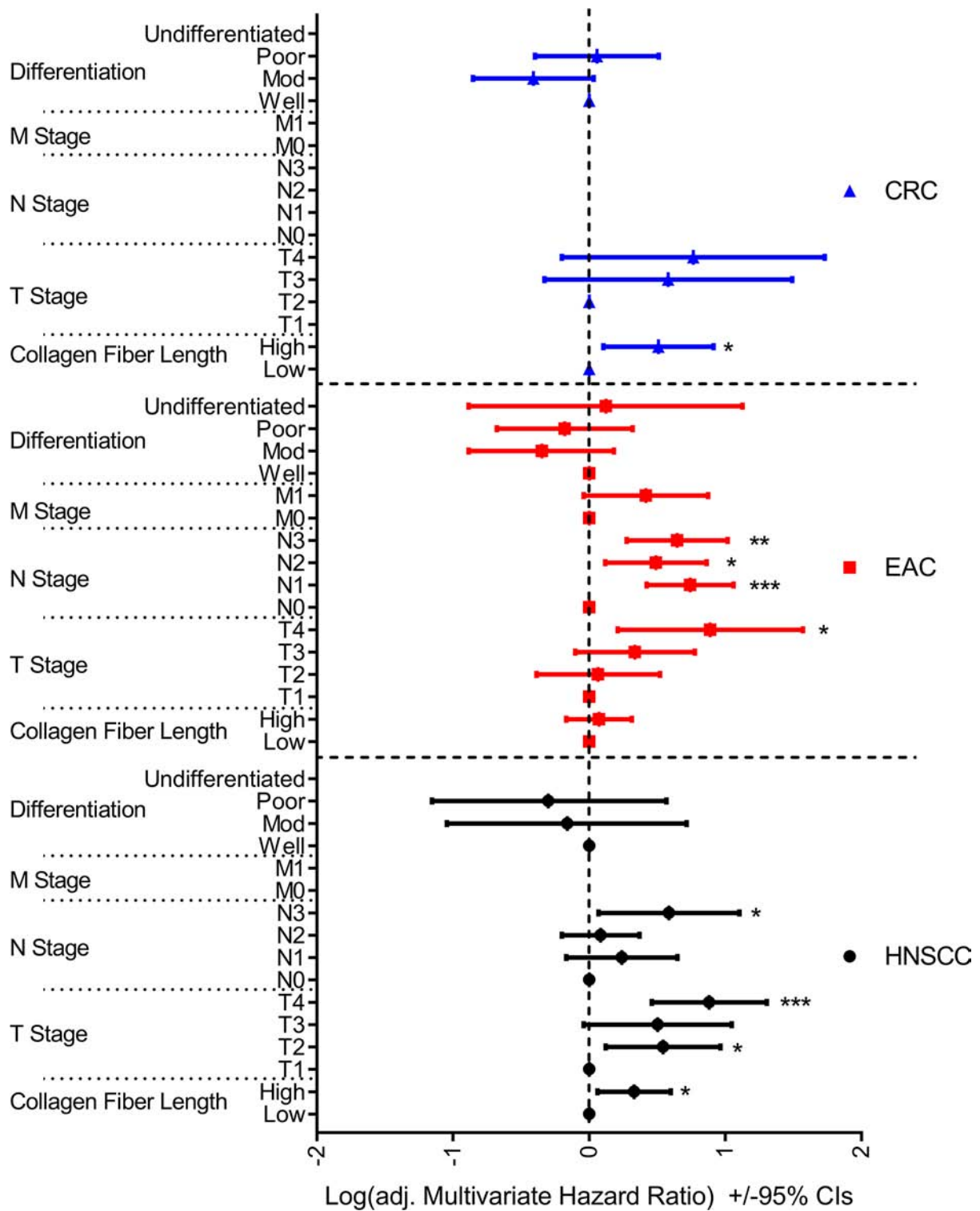
Supplementary Figure S2: Multivariate Cox Regression analysis of survival in the HNSCC, EAC and CRC cohorts adjusted for Collagen fiber length, T/N/M staging and differentiation.
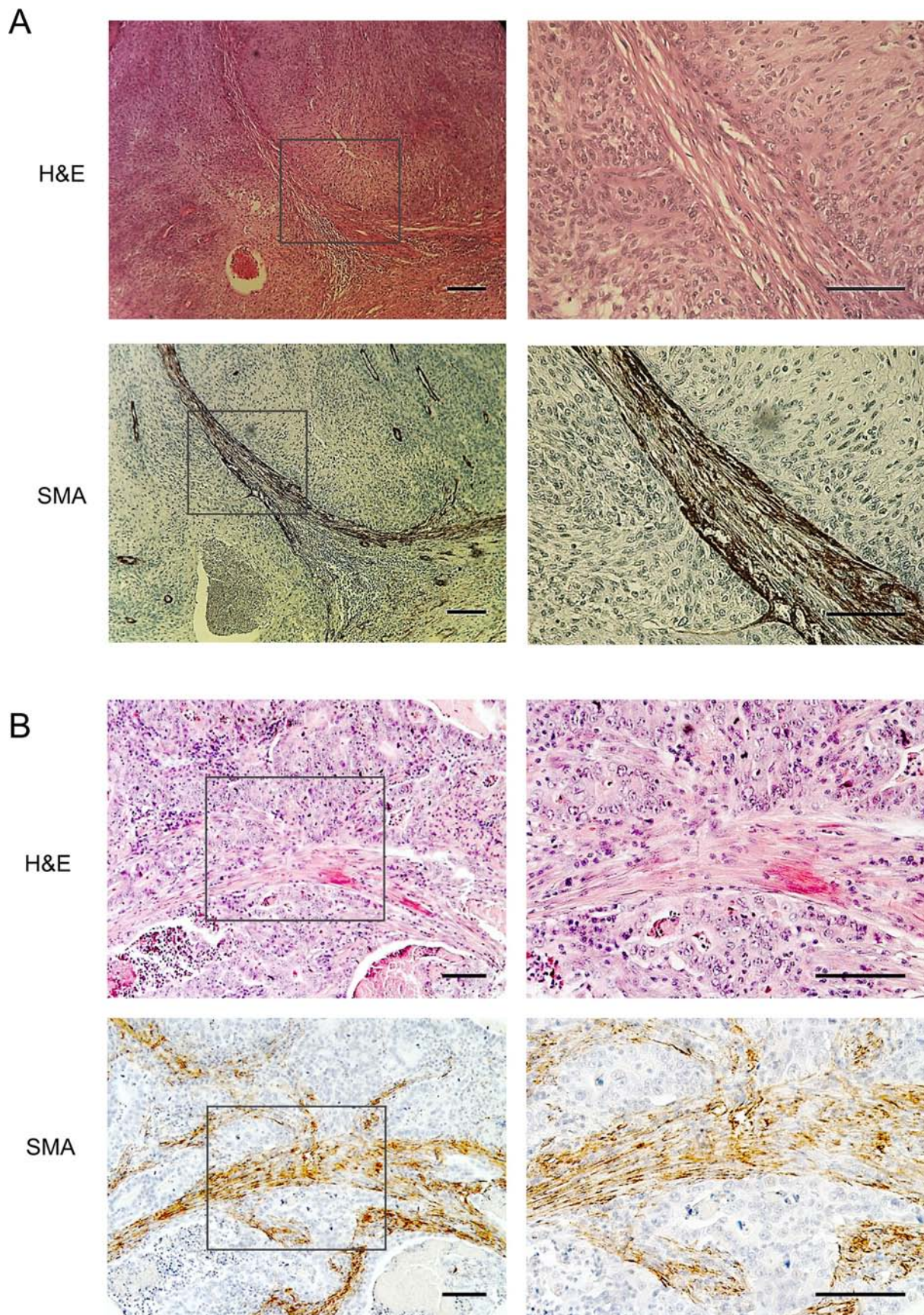
Supplementary Figure S3: Low magnification H & E and SMA stained images of sections presented in Figure 6A: (**A**) HNSCC tissue and (**B**) EAC tissue. Scale Bars represent 100 μm.
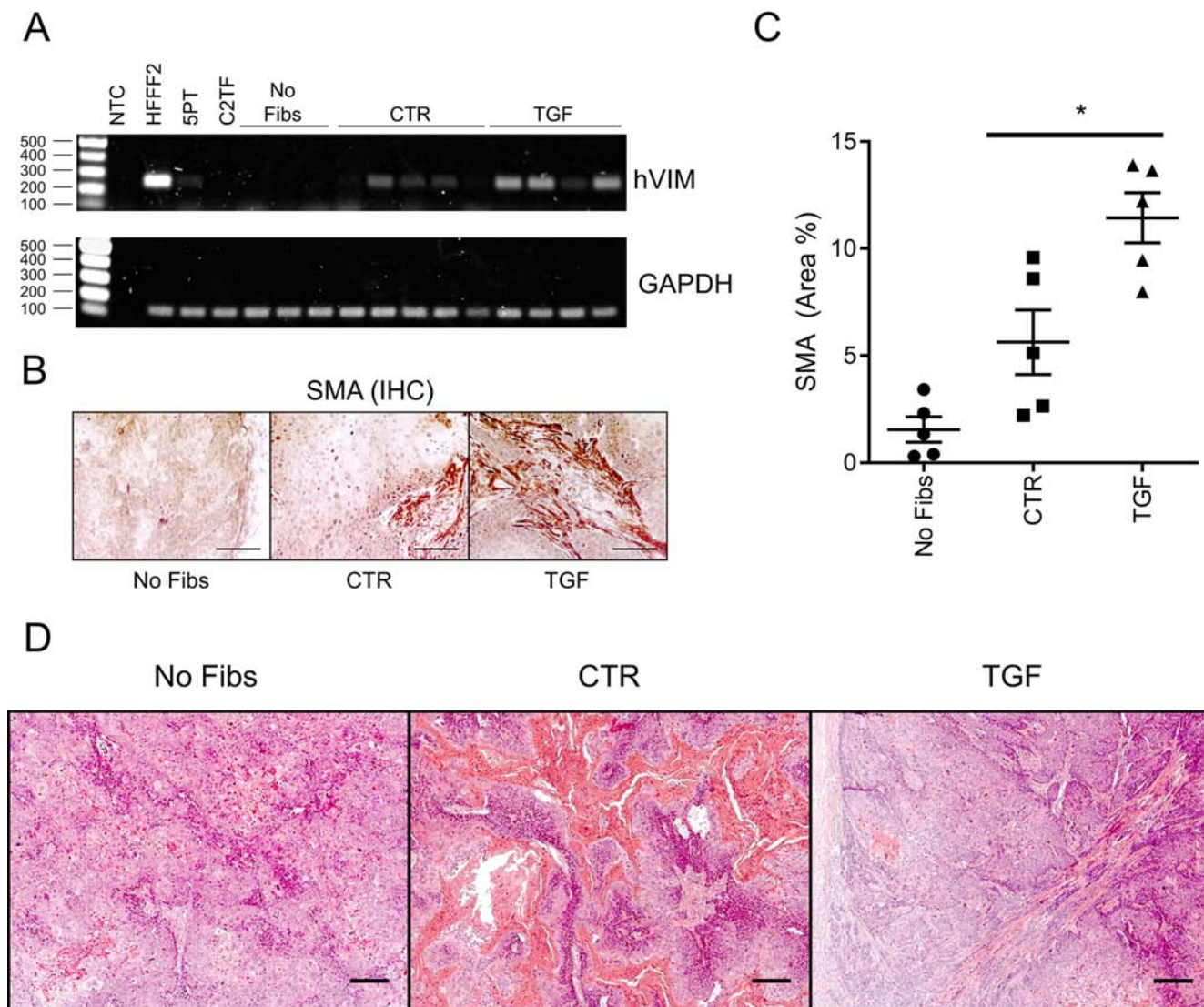
Supplementary Figure S4: Analysis of human fibroblast persistence in 5PT xenograft model. (**A**) Agarose gel electrophoresis showing end-point PCR (40 cycles) of human vimentin (hVIM) mRNA expression, GAPDH was used as a housekeeping gene. Sample descriptions: 5PT – Cancer cell line; C2TF – murine fibroblasts; No fibs – Xenografts injected with 5PT cells alone; CTR/TGF – Xenografts injected with 5PT cells plus control or TGF-β treated HFFF2s respectively. (**B**) SMA Immunohistochemistry staining of 5PT xenograft tumors. (**C**) Histogram showing SMA positive area in each tumor type. SMA positive area was quantified using the "Trainable Weka segmentation" plugin in Fiji. Data is presented as the mean positive area (%) per tumor from 5 20x fields of view. Statistically significant differences were assessed by 2-tailed, unpaired, homoscedastic T-test (*$p < 0.05$). (**D**) Low magnification H&E stained images of sections presented in Figure 6F. Scale bars represent 100 μm.
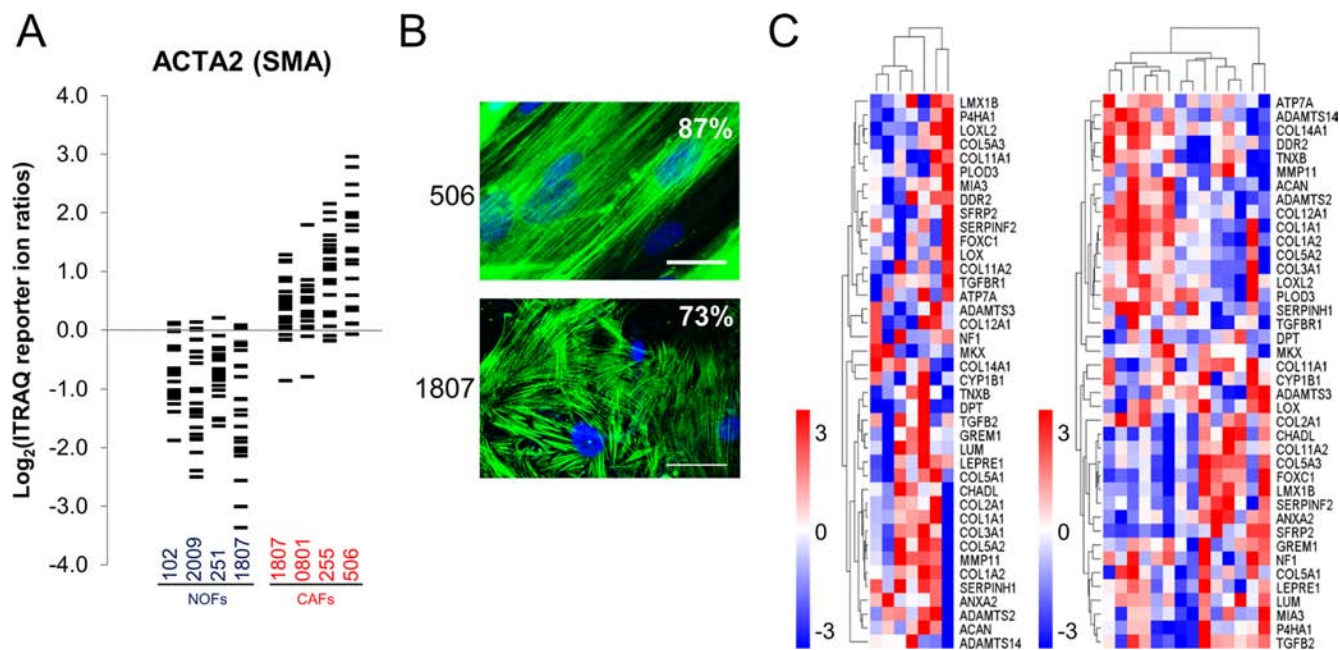
Supplementary Figure S5: Heterogenic expression of CFOGs by myofibroblastic CAFs cultured *ex-vivo*. (**A**) iTRAQ reporter ion ratios derived from 11 surrogate peptides observed in 23 peptide spectral matches (PSMs) for ACTA2 (SMA), demonstrating its overall higher relative expression in each CAF compared to NOFs (*T*-test $p = 0.03$). (**B**) Immunocytochemistry staining for SMA (Green) and DAPI (Blue) in CAF 506 and 1807, the percentage of SMA positive cells (from 6 fields of view) is shown in the top right corner of each image. Scale bar represents 50 μm. (**C**) Unsupervised hierarchical clustering of CAFs isolated from HNSCC (Left) or CRC (Right), using a Euclidean distance measure of pairwise average-linkage based on mRNA expression of CFOGs. Expression levels are row normalized for visualization.

Supplementary Table S1: Baseline clinical and histopatholological features of patients from each cohort.

		HNSCC	EAC	CRC
Number		213	146	64
Median age at diagnosis (range)		57 (28−90)	67 (36−85)	73 (39−89)
Median length of follow-up (months)		36	27	54
Cancer related deaths		59	67	25
Gender	Male	153	124	48
	Female	60	22	16
T Stage	Tis	1		
	T0	0	9	
	T1	51	31	
	T2	88	36	4
	T3	21	67	41
	T4	58	3	19
	Tx	3		
N stage	N0	49	74	64
	N1	26	32	
	N2	128	23	
	N3	8	17	
M stage	M0	211	141	64
	M1	1	5	
TNM	Missing	1	0	0
Grade of differentiation	Well Differentiated	2	13	23
	Moderately Differentiated	71	43	28
	Poorly Differentiated	139	89	13
	Undifferentiated		1	
	Missing	1	0	0
Treatment of Primary Tumour	Surgery only	78	60	46
	Adjuvant Chemo/Radio/ Chemoradio Therapy	95		10
	Neoadjuvant Chemo/Radio/ Chemoradio Therapy	40	86	2
	Adjuvant & Neoadjuvant Chemo/ Radio/Chemoradio Therapy			6

Supplementary Table S2: Cross-tabs showing SMA status and corresponding collagen fiber length.

			Collagen Fiber Length	
			Low	High
HNSCC	SMA	Low	47	41
		Mod	21	62
		High	9	21
		Totals	77	124
EAC	SMA	Low	6	2
		Mod	34	39
		High	19	29
		Totals	59	70
CRC	SMA	Low	11	6
		Mod	15	10
		High	8	5
		Totals	34	21

Supplementary Table S3: Clinicopathological data on tumours from which the CAFs were extracted

	116/CAF1807	118/CAF0801	119/CAF255	121/CAF506
Age	58	61	64	65
Sex	Male	Male	Male	Male
Neoadjuvant chemotherapy	No	Yes	Yes	No
Response to neoadjuvant	N/A	No response	Yes – minimal residual tumour	N/A
Tumour grade	Moderately differentiated adenocarcinoma	Moderately differentiated intestinal-like adenocarcinoma	Poorly differentiated adenocarcinoma	Poorly differentiated adenocarcinoma
Regional metastasis	Yes	Yes	No	Yes
Distant metastasis	No	No	No	No
Pathology stage	PT3N3	PT3N1	PT3N0	PT3N2