Supplemental Figure Legends

Supplemental Figure 1. Validation of anti-TβRII. (A) HSCs transduced with retroviruses that encode either GFP or TβRII-HA were harvested for WB for TβRII expression levels. WB for β-actin was used as a protein loading control. (B) HSCs expressing TβRII-HA were transduced with lentiviruses encoding either NT shRNA or each of different TβRII shRNAs were harvested for WB using anti-TβRII. WB for β-actin was used as a protein loading control. The anti-TβRII antibody specifically recognizes TβRII in HSCs.

Supplemental Figure 2. Thrombin completely removes the GST tag of GST fusion proteins (A) Thrombin was used to remove the GST tag of GST fused IQGAP1 a.a 746-1657. De-tagged IQGAP1 a.a.746-1657 after thrombin treatment and untreated GST-IQGAP1 a.a.746-1657 were subjected to WB using anti-GST. Thrombin completely removed the GST tag of IQGAP1. (B) De-tagged IQGAP1 a.a.746-1657 and GST-IQGAP1 a.a.746-1657 were subjected to WB using anti-IQGAP1. Thrombin treatment resulted in a shorter fragment as detected by anti-IQGAP1.

Supplemental Figure 3. IQGAP1 suppresses TGF-β1/Smad dependent activation of HSCs into myofibroblasts. HSCs transfected with control, or IQGAP1 siRNA, or in combination with a Smad siRNA that targets against both Smad2 and Smad3, were serum starved and stimulated with TGF-β1. Cells were harvested for WB for HSC activation markers (left) or α-SMA IF based analysis (right). IQGAP1 siRNA potentiated TGF-β1 activation of HSCs and this effect was abolished by Smad siRNA. * and #, $P<0.05$ by ANOVA. n=4 randomly picked microscopic fields, each containing 100-200 cells.

Supplemental Figure 4. IQGAP1 knockdown inhibits TGF-β1 downregulation of TβRII. (A) IQGAP1 knockdown by shRNA was assessed by IQGAP1 IF and confocal microscopy. Representative images showing that IQGAP1 shRNA markedly reduced IQGAP1 protein in virtually all HSCs examined. Cell nuclei were counterstained by TOTO-3 (blue). Bars, 20 μM. (B) HSCs that were pretreated with cycloheximide for 1 hr and stimulated with TGF-β1 for indicated times, were collect for WB (left) and densitometric analysis (right). IQGAP1 knockdown inhibited TGF-β1 downregulation of total cellular TβRII in the presence of cycloheximide. Chlo, chloroquine (lysosomal inhibitor). Data represent more than three independent experiments with similar results.

Supplemental Figure 5. IQGAP1 interacts with TβRII in epithelial cells. HepG2 cells that were transduced with TβRII-HA lentiviruses were subjected to IP using anti-HA and IQGAP1 co-precipitated was detected by WB. IQGAP1 interacts with TβRII in HepG2 cells.

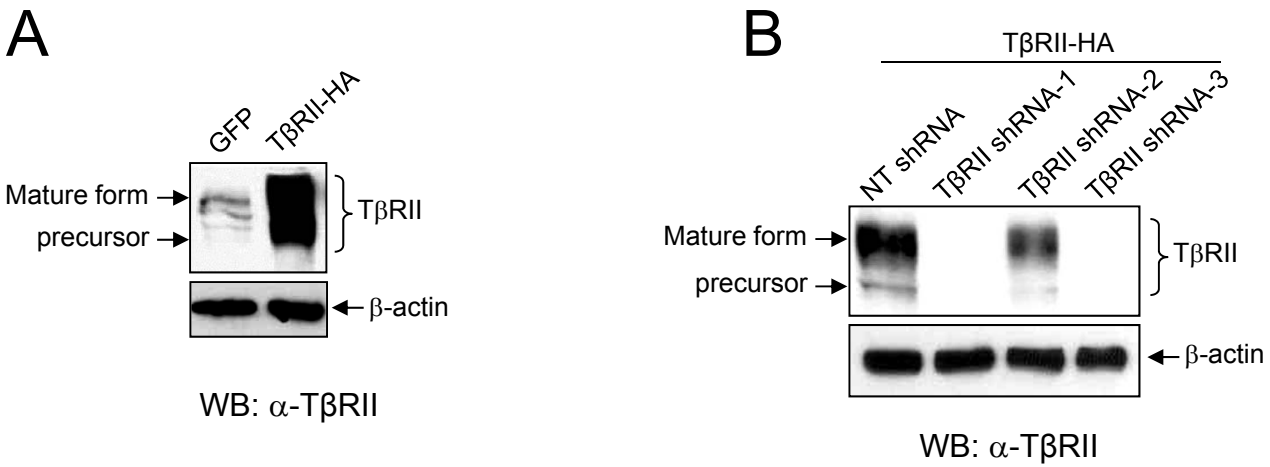
Supplemental Figure 6. Endothelial cell densities in the liver metastases of IQGAP1+/+ and IQGAP1-/- mice are comparable. Frozen sections of liver metastases of mice were subjected to PECAM-1/CD31 IF (green) and confocal microscopy. Representative IF images and H & E images are shown. Blood vessels are indicated by arrows. Cell nuclei were counterstained by TOTO-3 (blue). Bar, 50 μm.

Supplemental Figure 7. IQGAP1 knockdown HSCs promote the proliferation and migration of LLCs. Left, conditioned media collected from activated HSCs that were transduced with NT shRNA (control) or IQGAP1 shRNA were used as a growth stimulant for LLCs in non-radioactive cell proliferation assays. Conditioned

medium of IQGAP1 knockdown HSCs stimulated the proliferation of LLCs as compared to control HSCs. **, P<0.01 by ANOVA. Right, conditioned media collected as described in Left were used as a chemoattractant for LLCs in Boyden chamber assays. Conditioned medium of IQGAP1 knockdown HSCs stimulated the migration of LLCs as comparted to control HSCs. *, P<0.05; **, P<0.01 by ANOVA. CM, conditioned medium. n=3 repeats with similar results.

Supplemental Figure 8. TGF-β1 but not PDGF-BB induces downregulation of IQGAP1 in HSCs. HSCs that were treated with TGF-β1 (5 ng/ml) or PDGF-BB (20 ng/ml) for 24 hrs were subjected to WB for IQGAP1 (top). Densitometric data are shown on the bottom. TGF-β1 but not PDGF-BB induced downregulation of IQGAP1 in HSCs. Data are representative of multiple independent experiments with similar results.

Suppl. Figure 1



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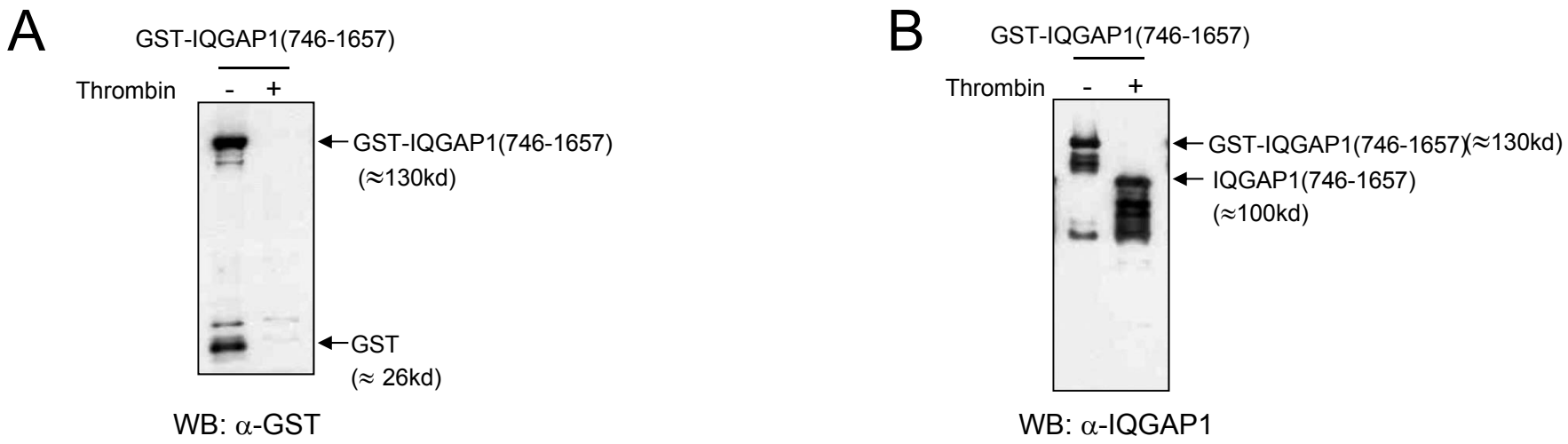

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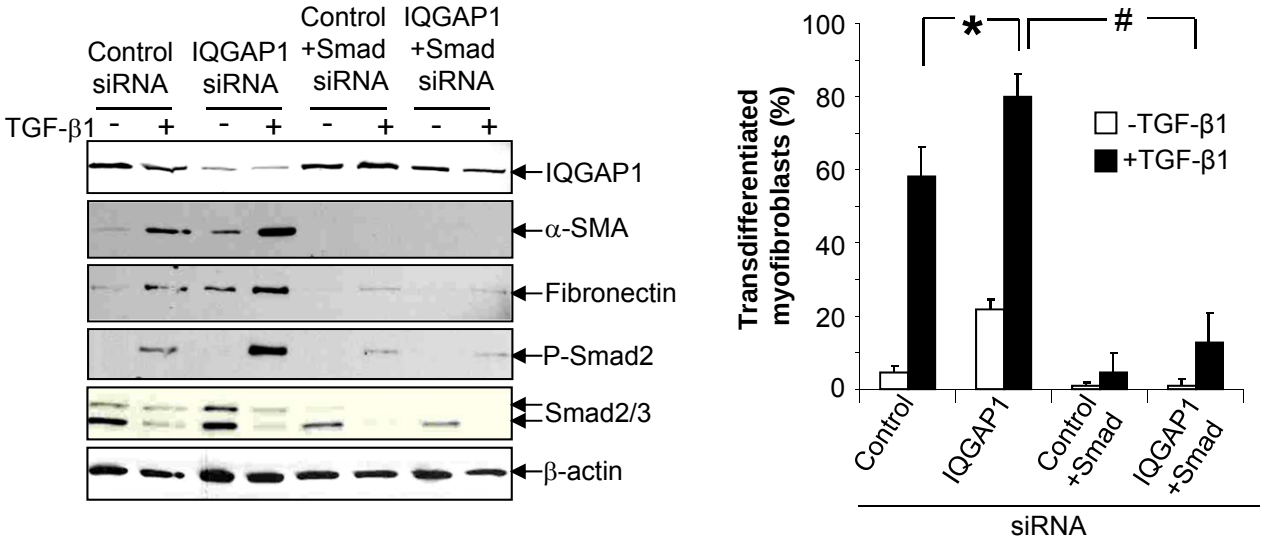
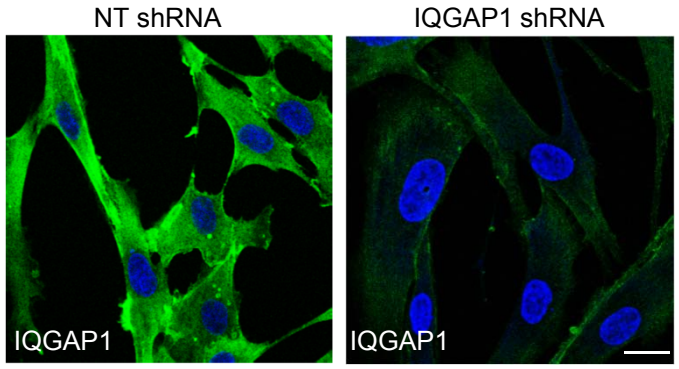


Suppl. Figure 4

A



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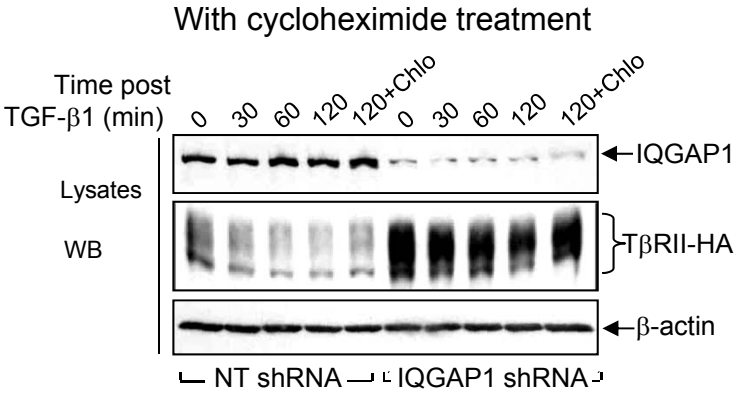

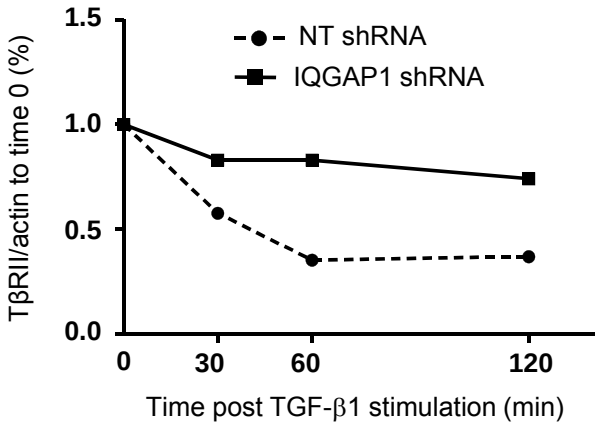

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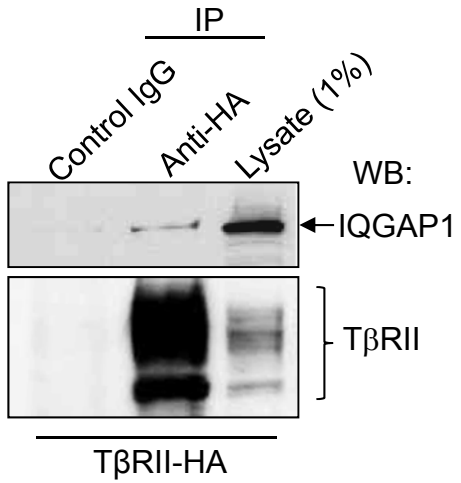

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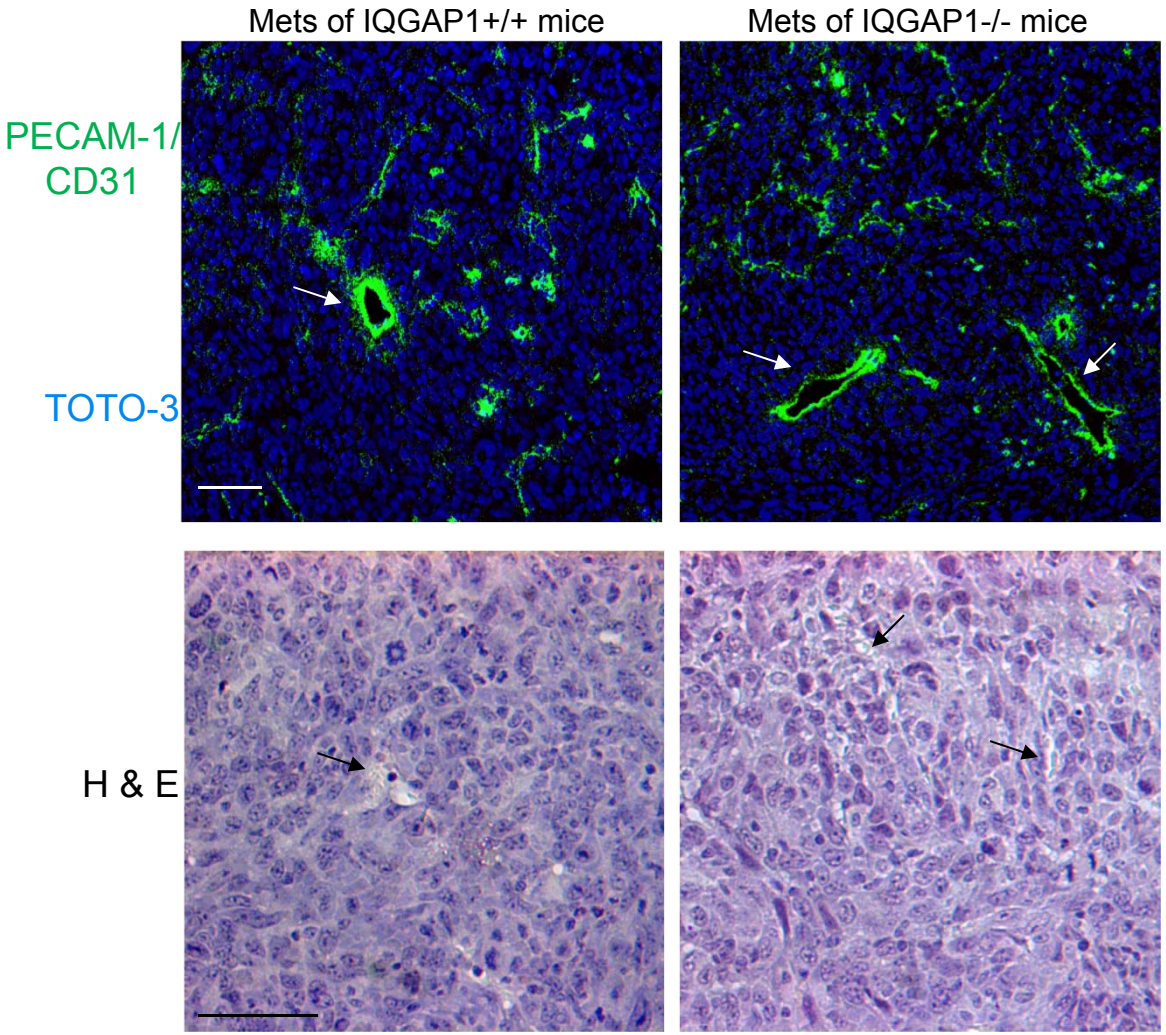

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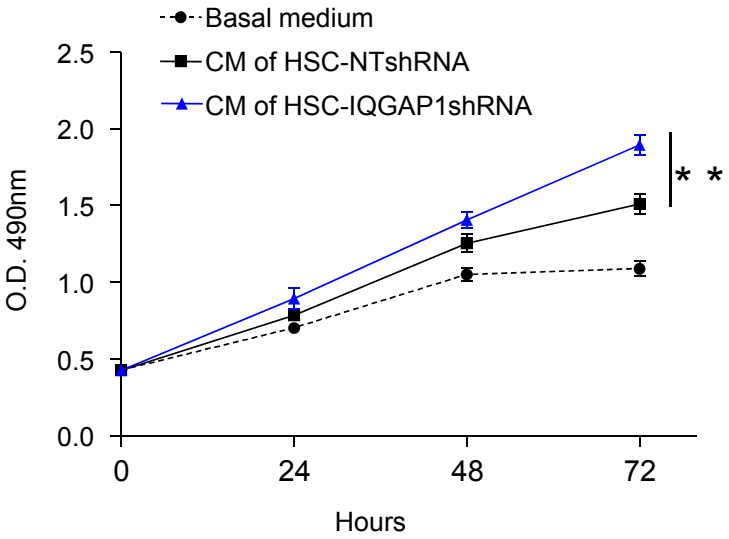
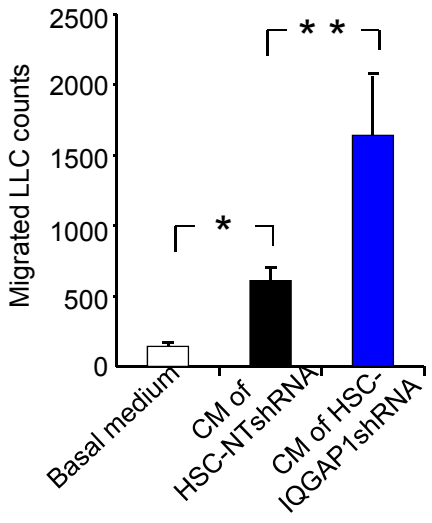
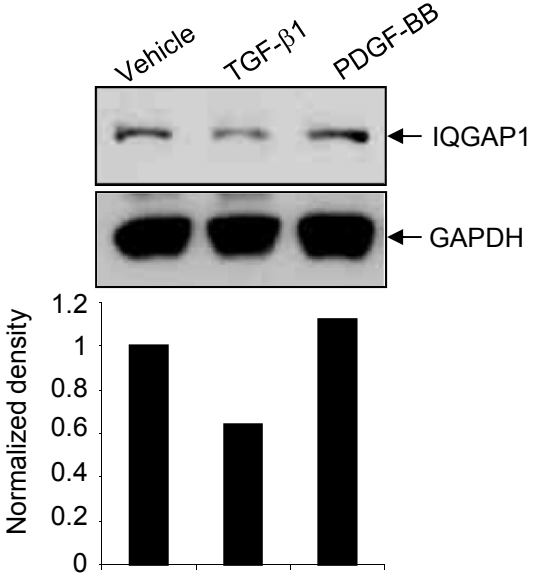




Suppl. Figure 8



Supplemental Table 1

Patient ID	Age	Gender	Diagnosis	Mean of IQGAP1 IF intensity in MFs of Mets (AU)	S.D.	Mean of IQGAP1 IF intensity in MFs of liver (AU)	S.D.	Ratio	
1	52	M	rectum carcinoma	37.05	16.68	53.1	15.32	0.7	#
2	58	F	ascending colon adenocarcinoma	15.99	6.47	71.06	18.16	0.22	
3	74	M	ascending colon adenocarcinoma	15.34	6.78	55.99	10.66	0.27	
4	69	M	ascending colon adenocarcinoma	59.76	13.10	98.74	35.13	0.61	
5	63	F	sigmoid adenocarcinoma	13.42	13.82	67.11	4.02	0.2	
6	59	M	sigmoid adenocarcinoma	34.5	24.48	56.87	8.06	0.61	
7	61	M	rectum adenocarcinoma	18.38	8.24	44.96	2.18	0.41	
8	69	F	cecum adenocarcinoma	24.75	4.16	74.07	20.49	0.35	
9	54	M	sigmoid adenocarcinoma	21.07	5.75	67.86	7.57	0.31	
10	32	F	rectum adenocarcinoma	54.08	11.86	94.46	31.80	0.57	
11	81	F	sigmoid adenocarcinoma	38.72	10.42	94.55	29.72	0.41	
12	75	M	descending colon adenocarcinoma	16.44	5.43	43.47	16.58	0.38	
13	67	F	sigmoid adenocarcinoma	23.8	6.80	92.82	17.54	0.26	
14	69	M	cecum adenocarcinoma	23.48	8.08	60.6	8.48	0.39	
15	58	F	rectum adenocarcinoma	23.55	13.72	36.85	10.10	0.64	
16	72	F	sigmoid adenocarcinoma	19.13	6.31	45.86	14.78	0.42	
17	52	M	sigmoid adenocarcinoma	24.26	6.52	60.5	11.79	0.4	
18	79	F	transverse colon adenocarcinoma	16.5	5.26	29.96	13.40	0.55	
19	54	M	transverse colon adenocarcinoma	24.68	4.82	63.65	8.45	0.39	
20	66	M	rectum adenocarcinoma	25.23	7.91	35.49	7.95	0.71	#
21	39	M	sigmoid adenocarcinoma	9.51	5.90	23.05	6.24	0.41	
22	38	M	rectal adenocarcinoma	19.19	0.14	40.8	5.00	0.47	
23	74	M	right colon adenocarcinoma	22.88	5.40	31.18	7.94	0.73	#
24	90	F	rectal cancer	44.34	12.23	67.92	13.59	0.65	
25	63	F	rectal carcinoma	26.6	5.70	28.02	7.35	0.95	#
26	32	F	rectal carcinoma	29.52	12.79	66.45	21.40	0.44	
27	83	M	sigmoid adenocarcinoma	33.74	10.84	48.12	12.56	0.7	#
28	45	F	cecum carcinoma	38.84	20.24	61	17.06	0.64	
29	84	M	rectosigmoid cancer	17.73	5.66	59.05	10.85	0.3	

MFs: myofibroblasts; AU: arbitrary unit

#, no change