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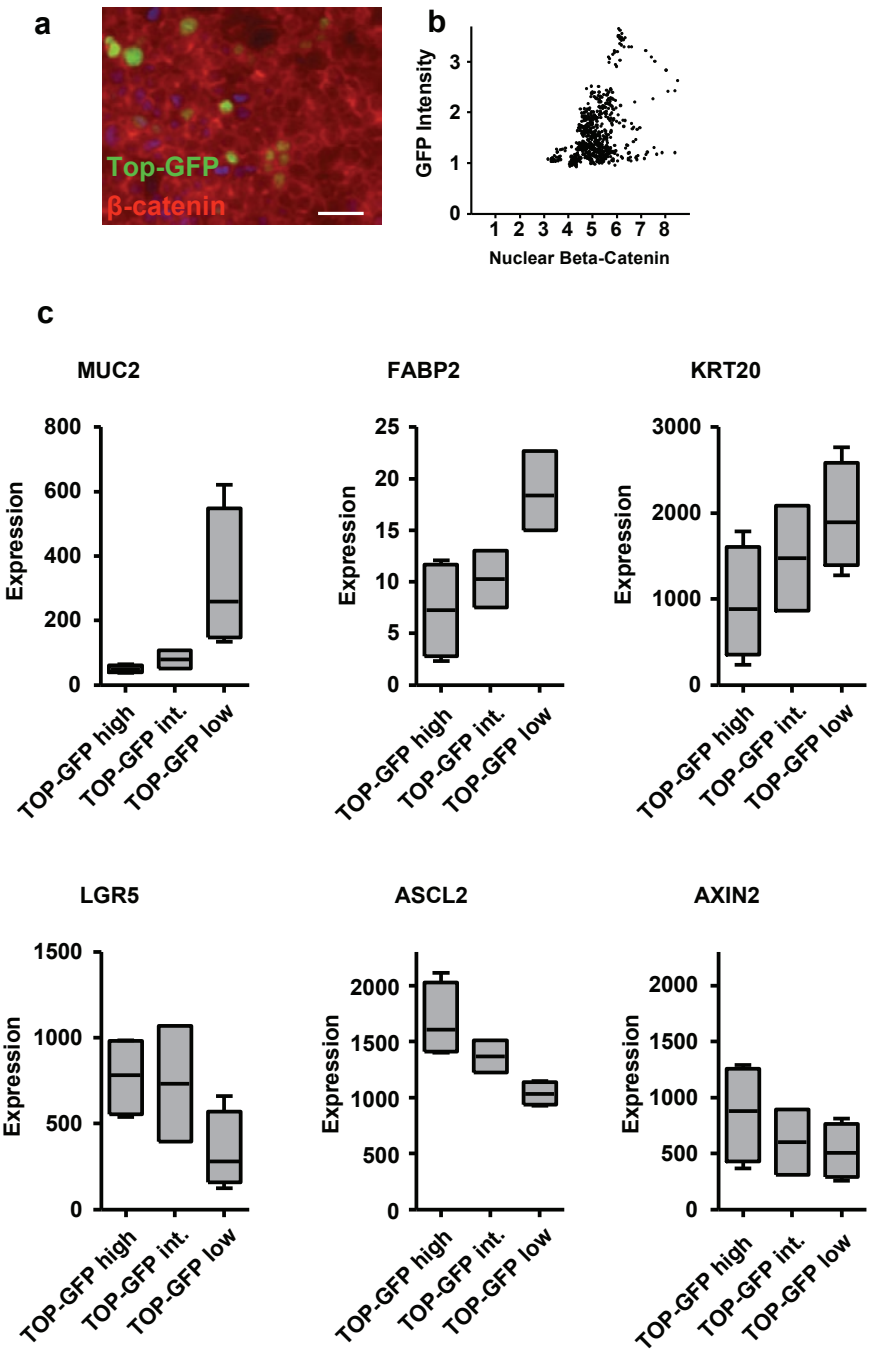

Figure S1 TOP-GFP expression and relation with nuclear β-catenin, wnt targets and differentiation markers. (**a**) Co-immunostaining of TOP-GFP spheroid culture with GFP and β-catenin antibody demonstrates a clear correlation between TOP-GFP levels and nuclear localization of β-catenin (scale bar, 50μm), quantification shown in (**b**). (**c**) Microarray analysis of a specific gene-set in high, intermediate and low TOP-GFP cell fractions indicates gradual increase in differentiation marker expression and a decrease in Wnt target gene expression from TOP-GFP^{high} to TOP-GFP^{low} populations (Each box plot represents a minimal of two data points from separate single-cell cloned TOP-GFP CSC cultures). Genes were picked based on significant differences observed in Fig 1d.

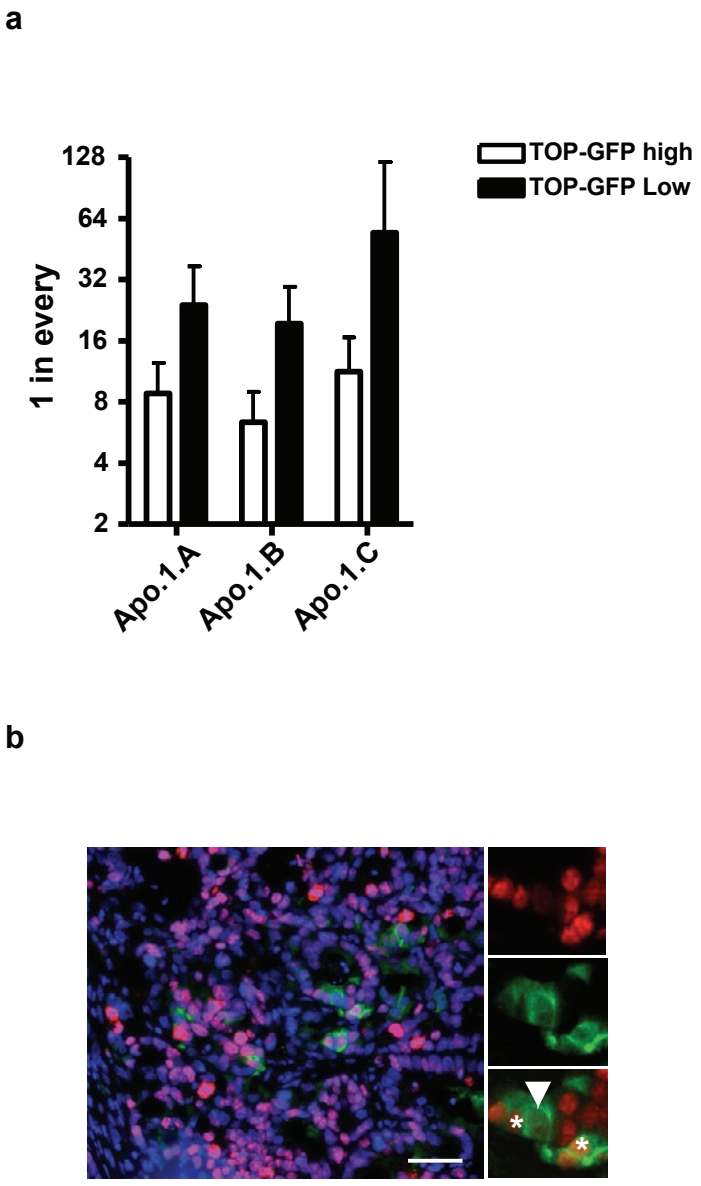

Figure S2 Limiting dilution on various clones, Ki-67 staining. (**a**) Limiting dilution assay and clonogenic potential of 3 independent lines derived from the same patient as Apo.1 (Apo.1.A, Apo.1.B and Apo.1.C). Errors bars represent 95% CI. Representative examples are shown. See Methods for details on limiting dilution assays. (**b**) Ki-67 co-staining with GFP in TOP-GFP xenografts. Ki-67 positivity encompasses both GFP positive and negative cells. Scale bar, 100μm.

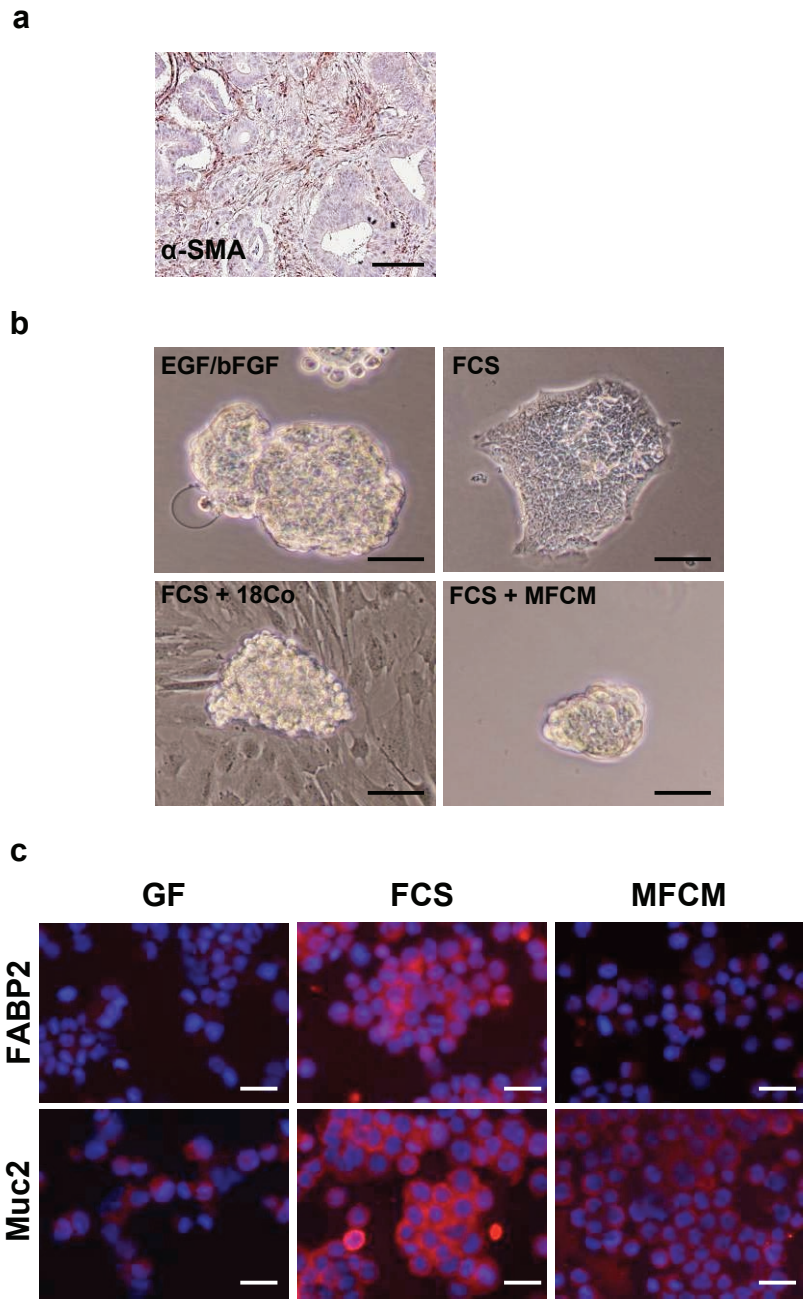

Figure S3 Myofibroblasts prevent differentiation of colon CSCs. (**a**) Immunohistochemistry for α-SMA shows myofibroblasts in the stroma of primary human colorectal malignancies. (Scale bar, 50μm) (**b**) Phase contrast pictures to show morphological differentiation of CSC. Upper left represents a spheroid culture growing in medium containing EGF and bFGF, upper right is after differentiation in 2% FCS. Lower left is differentiation with 2% FCS, but plated on myofibroblasts (18Co) and lower right is differentiation with FCS in the presence of MFCM. (Scale bar, 20μm) (**c**) Immunofluorescence for FABP2 and Muc2 on cytospins of spheroid cells (EGF/bFGF) or cells induced to differentiate with 2% FCS in the absence (middle) or presence of MFCM (right). (Scale bar, 20μm)

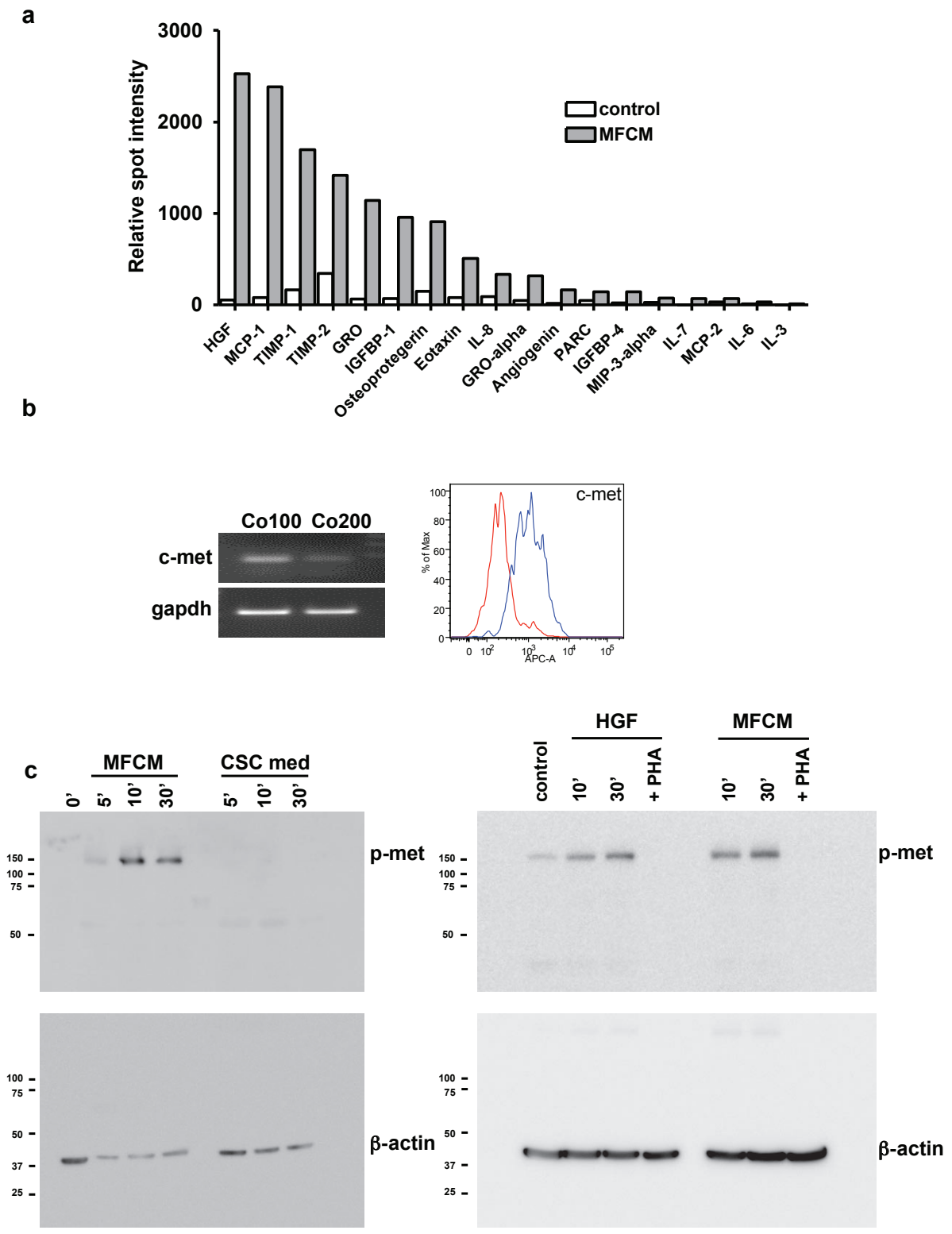

Figure S4 Myofibroblasts produce HGF and human colon CSCs express c-Met. (**a**) A graph depicting the detected secreted factors in MFCM (see Methods for details). (**b**) PCR showing expression of c-Met in spheroid cultured colon CSCs. Right panel shows FACS analysis for c-Met. (red; background and blue; c-Met) (**c**) Full blot of phospho-c-Met and β-actin of Co200 stimulated with 18Co conditioned medium (MFCM) or CSC control medium for the time indicated.

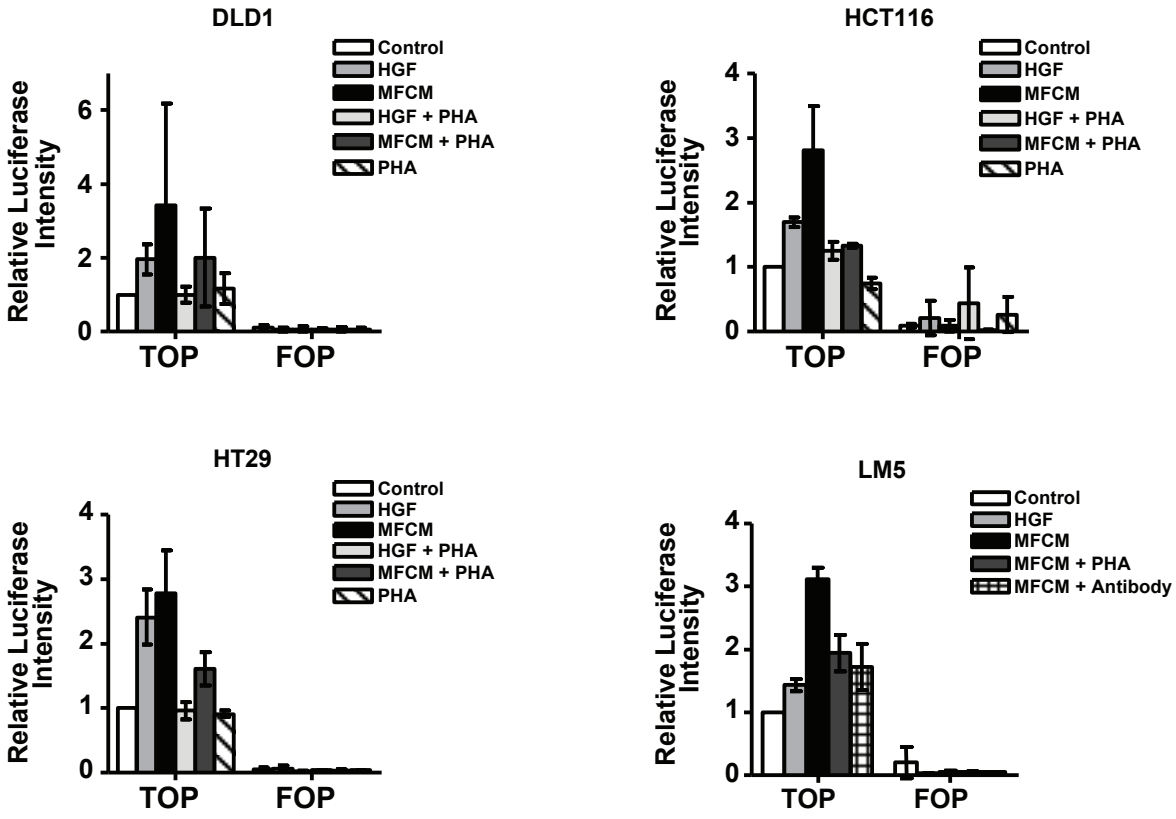

Figure S5 TOP/FOP assay on various lines and with various conditions. Depicted are the results of TOP/FOP assays on different human colon cancer lines including several established colorectal cancer lines (DLD1, HCT116, HT29). LM5 is a liver metastasis derived primary CSC line. The stimulatory effect of MFCM is HGF dependent. Error bars represent SEM (n=3), data from at least 2 replicates is shown.

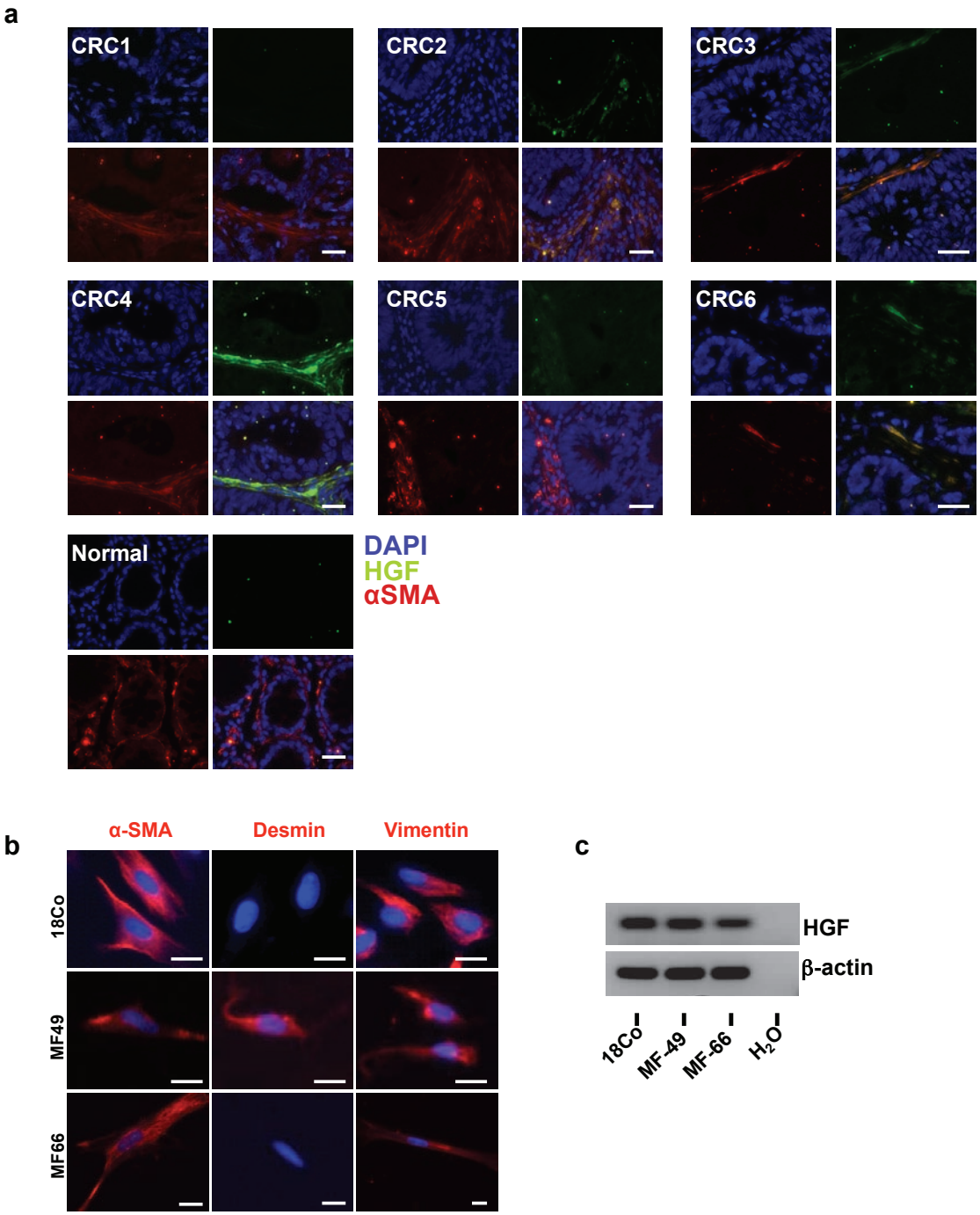

Figure S6 Human colon carcinoma associated myofibroblasts express HGF. Six different human primary colorectal cancer specimens and one normal human colon specimen was stained for both HGF and α-SMA. In 4 out of 6 samples we detected HGF in α-SMA-positive cells. Scale Bars, 50μm. (**b**) Both an established colon myofibroblast line (18Co) as well as two primary lines (MF49, MF66) isolated from colorectal cancer patients express markers associated with myofibroblasts (Scale bars, 20μm) and reveal HGF production by (**c**) PCR.

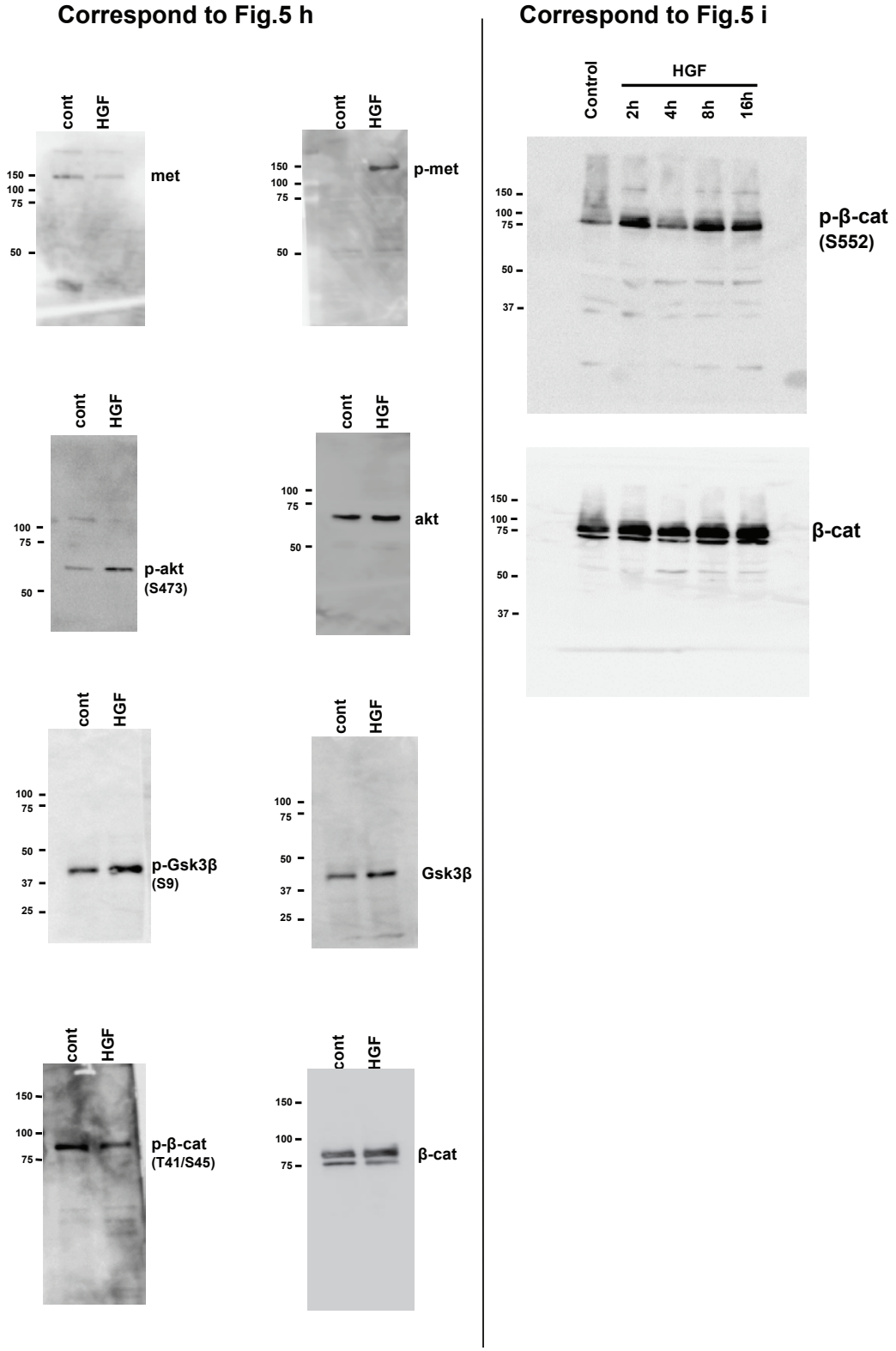

Figure S7 Full blots. Represented are the full Western blots of Fig. 5h and i.

Table S1 Genes showing most differential expression between TOP-GFP^{high} and TOP-GFP^{low} cell populations. A list of the most differentially regulated genes in the TOP-GFP^{high} versus TOP-GFP^{low} fractions from two different single cell cultures is summarized in Table S1, indicated values represent Log_2 fold-changes.

Table S2 List of primers used in this study.

Table S1

Gene	Probeset	G7	A4
FABP1	*205892_s_at*	-4.9	-1.7
AKR1B10	*206561_s_at*	-4.6	-2.5
MUC2	*204673_at*	-3.6	-1.8
ST6GALNAC1	*227725_at*	-3.2	-1.5
GCNT3	*219508_at*	-2.7	-1.3
SPINK4	*207214_at*	-2.4	-1.5
HEPACAM2	*242601_at*	-1.9	-1.7
GMPR	*204187_at*	1.8	1.5
CAB39L	*225915_at*	2.3	1.4
TSPAN5	*209890_at*	2.5	1.5
NEURL1B	*225355_at*	2.7	1.3
TUBB2B	*214023_x_at*	2.8	1.6
LGR5	*213880_at*	2.9	1.3
SERPINI1	*205352_at*	3.0	1.9
LEF1	*221558_s_at*	3.0	1.6
LRP4	*212850_s_at*	3.1	1.4
CXCR4	*217028_at*	3.3	2.7
SP5	*235845_at*	3.7	1.9
DEFA5	*207529_at*	5.1	3.9
APCDD1	*225016_at*	5.2	4.3

Table S2

Primers	*Sequences*	
	Fwd	*Rev*
Lgr5	CTGCCTGCAATCTACAAGGT	CCCTTGGGAATGTATGTCAGA
Survivin	GCCCAGTGTTTCTTCTGCTT	CCGGACGAATGCTTTTTATG
Axin2	CTCCTTATCGTGTGGGCAGT	CTTCATCCTCTCGGATCTGC
Muc2	CGAAACCACGGCCACAACGT	GACCACGGCCCCGTTAAGCA
Krt20	TGTCCTGCAAATTGATAATGCT	AGACGTATTCCTCTCTCACTCTCATA
Fabp2	TGGAAGGTAGACCGGAGT	AGGTCCCCCTGAGTTCAGTT
c-Met	CTGCCTGCAATCTACAAGGT	ATGGTCAGCCTTGTCCCTC
Hgf	CCTATTTCTCGTTGTGAAGGT	TGTTTCGTTTTGGCACAAGA
β-actin	ATGGAAGAAGAGATCGCCGC	TCGTAGATGGGCACCGTGTG