

Supplementary Material

Table S1. Clinical-pathological characteristics of tumor samples from which TASCs used in experiments were derived

Features		Frequency n (%)
Tumor location	Left-sided	5 (62.5)
	Right-sided	3 (37.5)
Grade	G1	3 (37.5)
	G2	5 (62.5)
	G3	0 (0)
	G4	0 (0)
pT stage	T1	1 (12.5)
	T2	1 (12.5)
	T3	6 (75)
	T4	0 (0)
pN stage	N0	3 (37.5)
	N1	4 (50)
	N2	1 (12.5)

Table S2. List of antibodies used for flow cytometry and immunofluorescence.

Name	Clone	Brand	Dilution used
CD90	5E10	BD	1:200
CD73	AD2	BD	1:50
CD105	43A3	Biolegend	1:200
CD29	TS2/16	Biolegend	1:200
CD146	SHM-57	Biolegend	1:200
CD34	581	BD	1:25
CD45	HI30	Biolegend	1:200
CD31	WM59	Biolegend	1:200
CD31	Mec 13.3	BD	1:100
aSMA	E184	Abcam	1:100
aSMA	1A4	SIGMA	1:100
FAP	1E5	Abcam	1:100
FSP-1	EPR2761(2)	Novus Biologicals	1:100
EpCAM	EBA-1	Biolegend, BD	1:50
EpCAM	9C4	Biolegend	1:200
EpCAM	VU1D9	Cell Signalling	1:200
TGFβ	9016	R&D Systems	1:50
PDPN	NC-08	Biolegend	1:200
E-Cadherin	67A4	Biolegend	1:100
N-Cadherin	8C11	Biolegend	1:100
Vimentin	D21H3	Cell Signalling	1:100
αvβ6	53a.2	Abcam	1:100

Ki67	EPR3610	Abcam	1:200
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Table S3. Proteins significantly upregulated in TASCs versus MASCs.

Protein description	UniProt	Gene	NSAF ¹	log2 T/M ratio ²	-log10 adj p-value
Transgelin	Q01995	TAGLN	2.8507	1.369591776	2.024216788
Isoform 2 of Tropomyosin beta chain	P07951-2	TPM2	2.1303	1.146955709	1.930278652
Actin, aortic smooth muscle	P62736	ACTA2	1.0451	0.73306496	1.667646232
Cysteine and glycine-rich protein 1	P21291	CSRP1	0.62694	1.028708326	1.629555599
Isoform 1 of Vinculin	P18206-2	VCL	0.59756	0.731613704	2.072906862
Isoform 2 of Tropomyosin alpha-4 chain	P67936-2	TPM4	0.50352	1.218728987	3.660414337
Calponin-1	P51911	CNN1	0.45455	0.693143643	2.042665302
Four and a half LIM domains protein 2	Q14192	FHL2	0.45161	0.61098913	2.11323767
Myosin regulatory light polypeptide 9	P24844	MYL9	0.29651	0.859685477	2.164914646
Superoxide dismutase [Mn], mitochondrial	P04179	SOD2	0.24324	1.430159513	1.375014938
Myosin-11	P35749	MYH11	0.15314	0.776470331	1.364268629
Aldehyde dehydrogenase X, mitochondrial	P30837	ALDH1B1	0.14313	0.665703034	1.894322199
Isoform 8 of Tropomyosin alpha-1 chain	P09493-8	TPM1	0.12676	0.659642581	1.300274496
Isoform Del-1790 of Myosin light chain kinase	Q15746-6	MYLK	0.11657	0.710862859	1.868401708
Isoform PDE5A2 of cGMP-specific 3',5'-cyclic phosphodiesterase	O76074	PDE5A	0.084034	0.655432099	1.409133562
Tubulin beta-2A chain	Q13885	TUBB2A	0.083146	0.622955915	1.885446624
Isoform 2 of PDZ and LIM domain protein 3	Q53GG5-2	PDLIM3	0.079114	1.664154334	2.97566031
Intercellular adhesion molecule 1	P05362	ICAM1	0.073308	1.928758837	2.40873185
von Willebrand factor A domain-containing protein 5A	O00534	VWA5A	0.062341	0.608349475	2.166147035
Synemin	O15061	SYNM	0.061981	0.634940911	1.419658575
Cell surface glycoprotein MUC18	P43121	MCAM	0.055728	1.088975189	3.069731623
Isoform 2 of CD82 antigen	P27701	CD82	0.053719	0.905728254	3.03133314
Isoform 2 of Sarcolemmal membrane-associated protein	Q14BN4-2	SLMAP	0.039241	1.408815068	1.870052561
Peptidyl-prolyl cis-trans isomerase F, mitochondrial	P30405	PIPF	0.038647	0.598108968	1.346828397
Profilin-2	P35080	PFN2	0.035714	0.609263793	2.21497501
Isoform 2 of Sorbin and SH3 domain-containing protein 2	O94875-2	SORBS2	0.034535	0.721487744	1.333348543
TBC1 domain family member 2A	Q9BYX2	TBC1D2	0.032328	0.676395709	2.311535917
Isoform 4 of Protein MRVI1	Q9Y6F6-4	MRVI1	0.032115	0.873990944	1.768513891
Interferon-induced GTP-binding protein Mx1	P20591	MX1	0.030211	1.0648971	1.657913505
Annexin A3	P12429	ANXA3	0.027864	1.445390134	1.433163419
Interferon-induced protein with tetratricopeptide repeats 3	O14879	IFIT3	0.026531	1.102235479	2.296449251
Na(+)/H(+) exchange regulatory cofactor NHE-RF1	O14745	SLC9A3R1	0.02514	0.613075965	1.37828742
Integrin alpha-3	P26006	ITGA3	0.024738	1.157701255	2.045437517
GDP-mannose 4,6 dehydratase	O60547	GMDS	0.024194	0.642798867	2.121599529
Pyroglutamyl-peptidase 1	Q9NXJ5	PGPEP1	0.023923	0.670435865	1.539127543
Isoform 2 of Redox-regulatory protein FAM213A	Q9BRX8	FAM213A	0.022936	0.97053097	2.749372394
5'-nucleotidase domain-containing protein 3	Q86UY8	NT5DC3	0.021898	1.023362305	1.526041891
Isoform 2 of EGF-like repeat and discoidin I-like domain-containing protein 3	O43854-2	EDIL3	0.019149	0.89064351	1.892695166
C-X-C motif chemokine 14	O95715	CXCL14	0.018018	0.634647798	1.388565252
Junctional protein associated with coronary artery disease	Q9P266	KIAA1462	0.014717	0.815090113	2.01049799
Isoform 2 of Cordon-bleu protein-like 1	Q53SF7	COBLL1	0.01458	1.164800305	1.844518957
Beta-2-glycoprotein 1	P02749	APOH	0.014493	0.6092497	1.77113088
Squalene synthase	P37268	FDFT1	0.014388	0.58771711	1.544439462
Isoform 3 of Endoplasmic reticulum aminopeptidase 2	Q6P179-3	ERAP2	0.014208	0.805458491	1.351017808
Eukaryotic translation initiation factor 1A, Y-chromosomal	O14602	EIF1AY	0.013889	2.116864833	1.704081173
Isoform 2 of Mitogen-activated protein kinase kinase kinase MLT	Q9NYL2-2	ZAK	0.013187	1.284414688	1.462184202
Uncharacterized protein C15orf52	Q6ZUT6	C15orf52	0.013109	0.59257026	1.329147307
Fructosamine-3-kinase	Q9H479	FN3K	0.012945	0.599023216	1.754148201
Isoform 2 of Receptor expression-enhancing protein 2	Q9BRK0-2	REEP2	0.011811	0.786168972	1.604696573
Desmoglein-2	Q14126	DSG2	0.010733	1.113110737	1.413378891
snRNA-activating protein complex subunit 5	O75971	SNAPC5	0.010204	0.660994283	2.095003198
NADP-dependent malic enzyme, mitochondrial	Q16798	ME3	0.0099338	0.650368603	1.508888857

Isoform 2 of Dual specificity protein phosphatase 22	Q9NRW4-2	DUSP22	0.0097561	0.588674525	1.915816667
Ras-related protein Rab-27B	O00194	RAB27B	0.0091743	0.73147857	1.318090928
Synaptopodin-2	Q9UMS6	SYNPO2	0.0091491	1.152318809	2.324063334
Isoform 2 of Neuronal growth regulator 1	Q7Z3B1-2	NEGR1	0.0088496	0.623248463	1.764740807
GMP reductase 1	P36959	GMPR	0.0086957	0.666657304	1.367637382
Ephrin-B1	P98172	EFNB1	0.0086705	0.681586149	2.407998395
Neural cell adhesion molecule 2	O15394	NCAM2	0.0083632	1.075789734	2.521357996
Complement C1q tumor necrosis factor-related protein 5	Q9BXJ0	C1QTNF5	0.0082305	0.63021796	1.901545219
N-acetylgalactosaminyltransferase 7	Q86SF2	GALNT7	0.0076103	0.765409681	1.382277499
Isoform I of Septin-6	Q14141-4	SEPT6	0.0070258	1.174872486	2.666389117
Collectin-12	Q5KU26	COLEC12	0.0067385	0.839409378	2.703170057
Isoform 3 of Alpha-actinin-1	P12814-3	ACTN1	0.0067265	0.653256818	1.551684606
Ninjurin-1	Q92982	NINJ1	0.0065789	0.661403942	2.378112927
Actin-binding LIM protein 1	O14639	ABLIM1	0.0064267	0.927887461	1.874323618
Sodium-dependent phosphate transporter 2	Q08357	SLC20A2	0.006135	1.181213583	1.390201729
Pro-cathepsin H	P09668	CTSH	0.0059701	0.960753372	2.141276676
Isoform 2 of Growth arrest-specific protein 6	Q14393-2	GAS6	0.0058997	1.118955025	2.436266589
Isoform 2 of Serine protease 23	O95084-2	PRSS23	0.005698	1.065393153	3.139927518
Maestro heat-like repeat-containing protein family member 1	Q8NDA8	MROH1	0.0054845	0.605337925	1.785431841
Isoform 2 of Solute carrier family 2, facilitated glucose transporter member 6 GN=SLC2A6	Q9UGQ3	SLC2A6	0.0044944	0.725661842	1.563477258
Isoform 2 of DENN domain-containing protein 3	A2RUS2	DENND3	0.004363	1.50656138	2.896299523
Junctophilin-2	Q9BR39	JPH2	0.0043103	1.917760552	2.367822915
Isoform 2 of Multiple PDZ domain protein	O75970-2	MPDZ	0.0039196	0.653794964	1.713013374
Phosphoinositide-3-kinase-interacting protein 1	Q96FE7	PIK3IP1	0.0038023	1.004159992	2.725592963
Isoform 2 of Disheveled-associated activator of morphogenesis 2	Q86T65	DAAM2	0.0037488	0.625118135	1.526883815
Protein FAM84A	Q96KN4	FAM84A	0.0034247	1.713092913	3.016708531
Kelch-like protein 21	Q9UJP4	KLHL21	0.0033501	1.058100137	2.145910136
Isoform 2 of Xenotropic and polytropic retrovirus receptor 1	Q9UBH6	XPR1	0.0031696	0.690162944	2.434796638
Isoform 3 of Bcl-2-like protein 12	Q9HB09-3	BCL2L12	0.003003	0.628582242	1.521943617
Isoform LCRMP-1 of Dihydropyrimidinase-related protein 1	Q14194-2	CRMP1	0.0029155	0.668038969	1.959832867
Beta-1,4-galactosyltransferase 4	O60513	B4GALT4	0.002907	0.805925093	1.660352963
Zinc finger BED domain-containing protein 1	O96006	ZBED1	0.0028818	0.835505836	1.430145763
Isoform 5 of Disabled homolog 2-interacting protein	Q5VWQ8	DAB2IP	0.0026502	0.613816689	2.113292909
Isoform 2 of Solute carrier family 2, facilitated glucose transporter member 4	P14672	SLC2A4	0.0024096	1.29691691	3.180367016
Phosphatidylcholine:ceramide cholinephosphotransferase 1	Q86VZ5	SGMS1	0.0023866	0.771686724	1.828680742
Isoform 5 of CCR4-NOT transcription complex subunit 4	O95628-5	CNOT4	0.0023095	0.651292955	2.118262217
Integral membrane protein GPR155	Q7Z3F1	GPR155	0.0022989	0.640027315	2.147542393
Interferon-induced protein 44	Q8TCB0	IFI44	0.0022523	0.749256786	1.885455005
Disintegrin and metalloproteinase domain-containing protein 19	Q9H013	ADAM19	0.0020942	0.594223888	1.549447658
Ribosomal protein S6 kinase beta-2	Q9UBS0	RPS6KB2	0.0020747	0.64001956	1.501764907
Isoform 4 of Rho guanine nucleotide exchange factor 10-like protein	Q9HCE6-4	ARHGEF10L	0.0020367	0.901245663	2.134385685
Amphotericin-induced protein 2	Q86SJ2	AMIGO2	0.0019157	0.585036598	2.038956885
SH3KBP1-binding protein 1	Q8TBC3	SHKBP1	0.0014144	0.646003345	1.615812668
Isoform 2 of Telomere-associated protein RIF1	Q5UIP0-2	RIF1	0.0012265	2.074826105	4.306720488
Isoform 2 of Neural cell adhesion molecule 1	P13591-1	NCAM1	0.0011792	0.725111612	1.371141475
DNA (cytosine-5)-methyltransferase 3A	Q9Y6K1	DNMT3A	0.0010965	0.64407483	1.914928422
Isoform 2 of Nesprin-2	Q8WXH0	SYNE2	0.0010135	0.960456358	1.342666208
Sterol regulatory element-binding protein 2	Q12772	SREBF2	0.00087642	0.676395721	1.419569465
Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta	O00750	PIK3C2B	0.000612	0.650472734	2.377016406
Isoform 2 of Dedicator of cytokinesis protein 4	Q8N1I0	DOCK4	0.00051626	0.833251423	1.319558447
Isoform 2 of Myosin-14	Q7Z406-6	MYH14	0.00049116	0.635395693	1.820246093
Isoform 1 of Striated muscle preferentially expressed protein kinase	Q15772	SPEG	0.00031027	0.828858327	1.748434026

¹ Normalized Spectral Abundance Factor (NSAF)² T/M ratio=TASCs/MASCs ratio

Table S4. Previously reported TASC markers

Protein description	UniProt	Gene	NSAF ¹	log2 T/M ratio	-log10 p-value	adj	K-M p value (median) ²	K-M p value (best) ³
Protein S100-A4 FSP1	P26447	FSP1 S100A4	0.24752	0.01300	0.0057		0.33	0.099
Insulin-like growth factor-binding protein 7	Q16270	IGFBP7	0.19858	0.18416	0.2418		0.45	0.12
Isoform 2 of Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	O00469-2	PLOD2	0.11478	0.20681	0.0684		0.77	0.12
Prolyl endopeptidase FAP	Q12884	FAP	0.048684	0.16887	0.2649		0.41	0.043
Isoform 4 of Caldesmon	Q05682-4	CALD1	0.033457	-0.05515	0.3190		0.49	0.006
Periostin	Q15063	POSTN	0.01555	0.33831	0.0894		0.092	0.017
Isoform 4 of Podoplanin	Q86YL7-4	PDPN	0.012712	-0.32283	0.1487		0.71	0.25
Lysyl oxidase homolog 2	Q9Y4K0	LOXL2	0.011628	0.11567	0.6778		0.21	0.14

¹ Normalized spectral abundance factor (NSAF). ² T/M ratio = TASCs/MASCs ratio. ³ *p* value of log-rank test in TCGA COAD cohort (*n* = 483) dichotomized according to median value. ⁴ *p* value of log-rank test in TCGA COAD cohort (*n* = 483) dichotomized according to best cut-off.

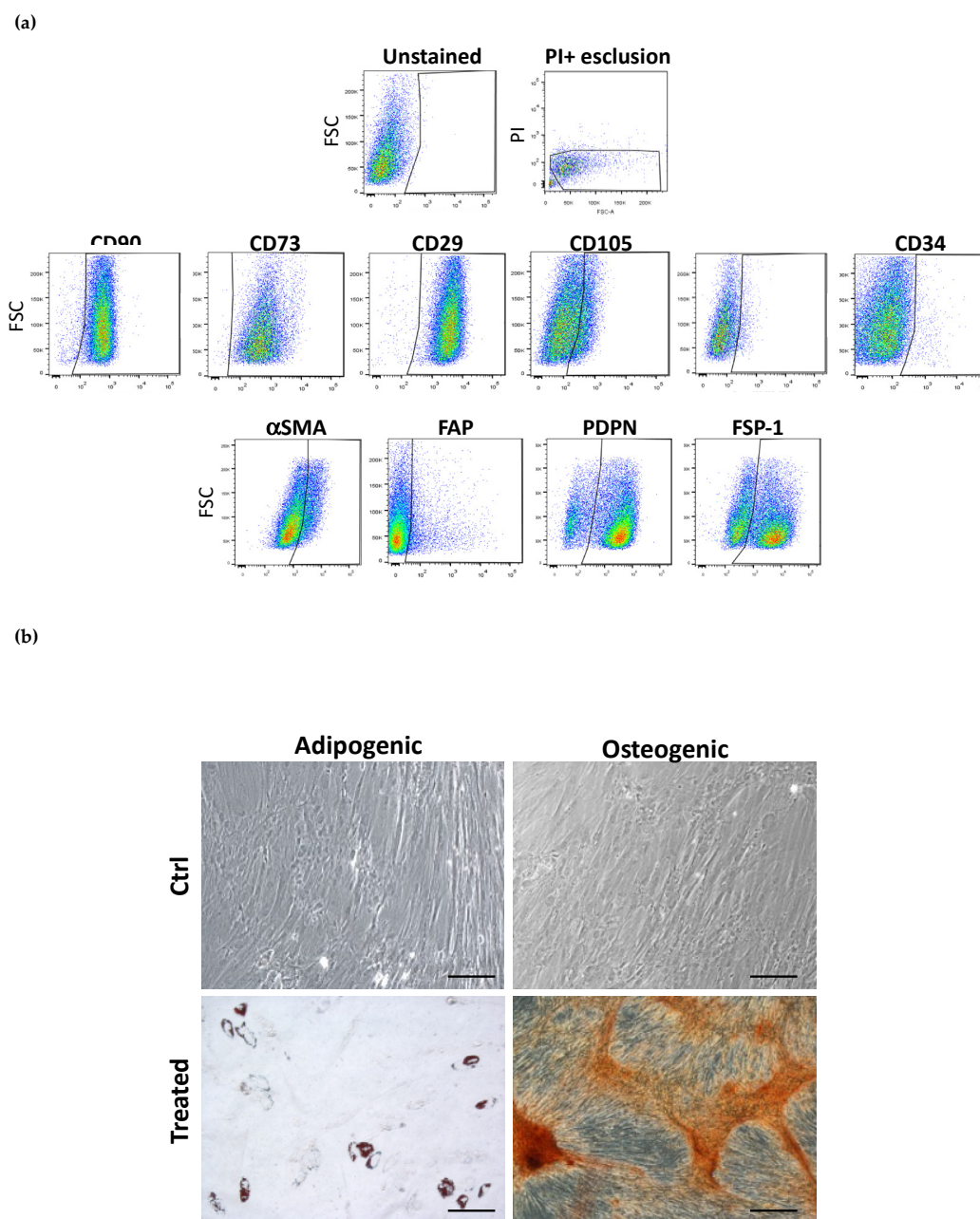


Figure S1. Characterization of colon cancer-derived TASCs. (a) Representative phenotypical analysis of TASCs by flow cytometry. Gates are defined according to the corresponding unstained sample. Dead cells are excluded based on PI incorporation. (b) Representative pictures of TASC differentiation into adipogenic and osteogenic lineages, as evaluated by Oil Red O and Alizarin Red staining, respectively. Magnification 20x, scale bar 100µm. Data refer to 1 representative experiment out of 4 independently performed with 4 different TASC samples, with similar results.

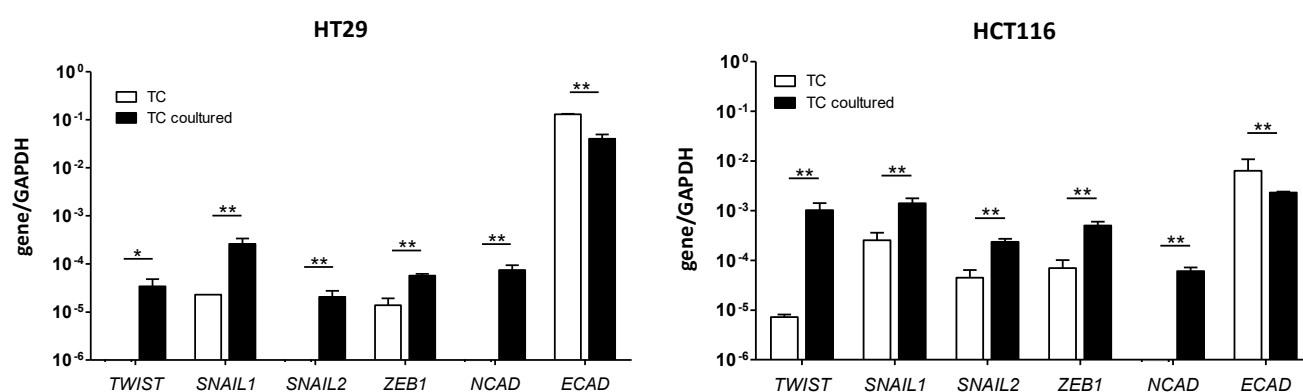


Figure S2. TASCs induce upregulation of EMT-related genes in colon cancer cell lines. Quantitative RT-PCR analysis of EMT-related genes in colon cancer cells from the indicated cell lines cultured alone (TC) or cocultured with TASCs (TC cocultured). Mean \pm SD from triplicates of cultures are shown. * $p \leq 0.05$; ** $p \leq 0.01$.

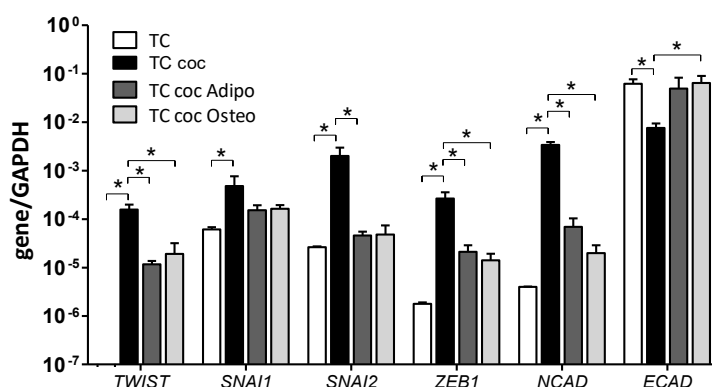


Figure S3. TASCs capacity to induce EMT in tumor cells depends on their differentiation state. Quantitative RT-PCR analysis of the expression of EMT-related genes in LS180 cells cultured alone (TC), with TASCs (TC coc), or with TASCs pre-differentiated into adipocytes (TC coc Adipo) or osteocytes (TC coc Osteo). Cumulative data (mean \pm SD) from four cultures performed with two different TASC samples are shown. Statistical significance of results was evaluated by Mann-Whitney test (* $p \leq 0.05$ ** $p \leq 0.01$).

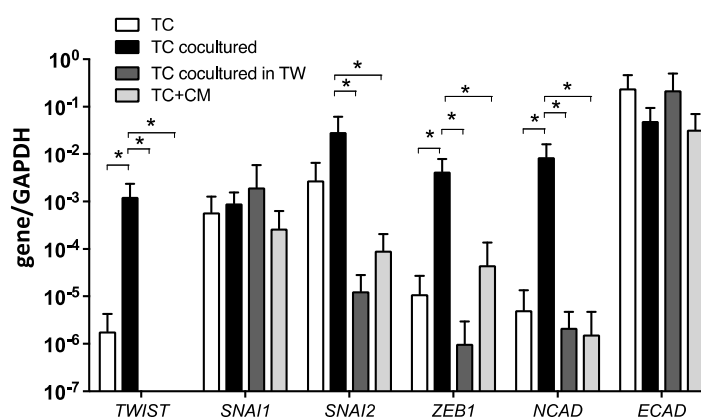


Figure S4. Direct cell-to-cell contact between tumor cells and TASCs is required for EMT induction. Quantitative RT-PCR analysis of the expression of EMT-related genes in LS180 cells cultured alone (TC), with TASCs (TC cocultured), with TASCs in transwell plates (TC cocultured in TW), or alone in the presence of TASC-conditioned medium (CM). Data (mean \pm SD) refer to three independent experiments, performed with TASCs isolated from three different CRC samples. Statistical significance was evaluated by Mann-Whitney test (* $p < 0.05$).

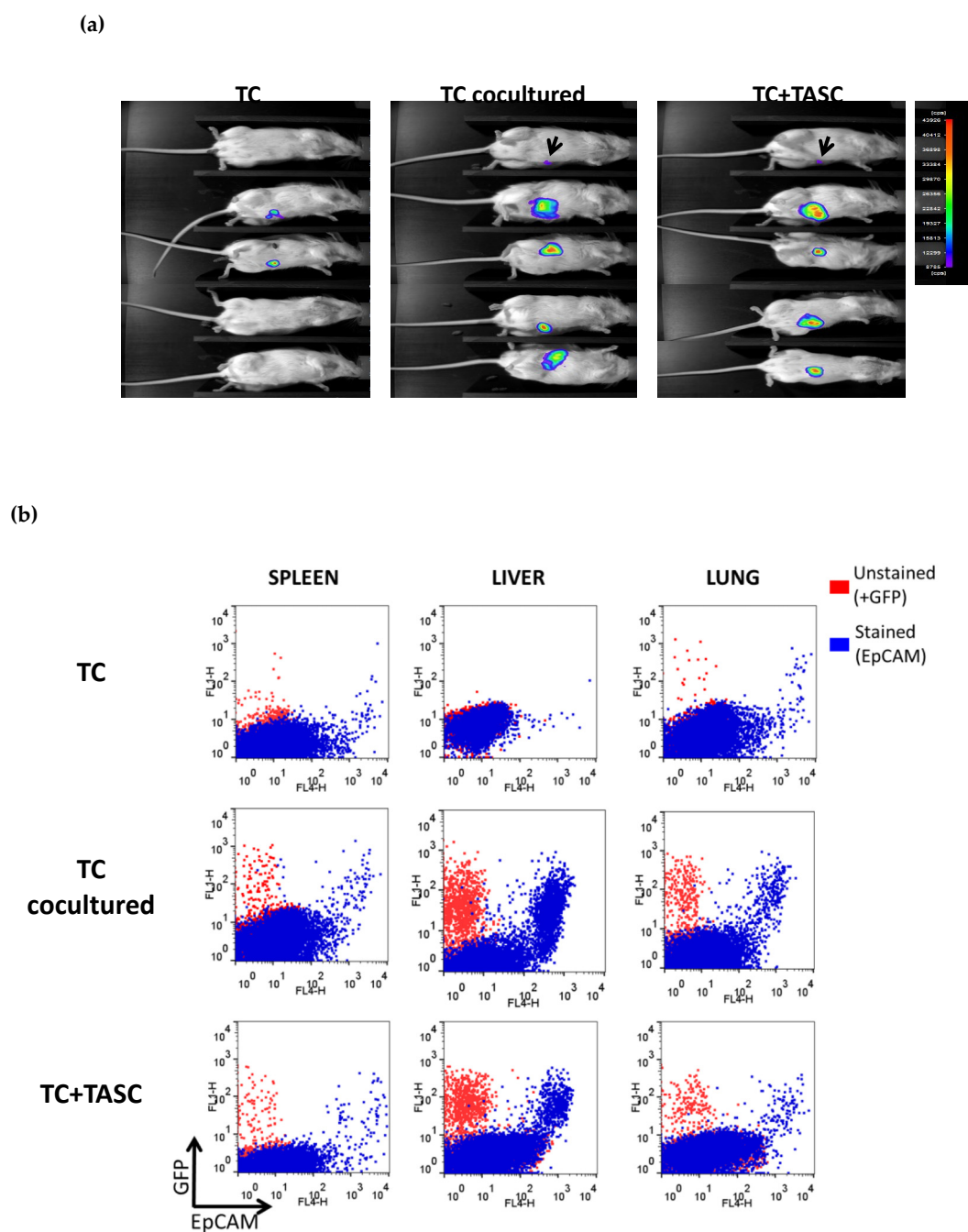


Figure S5. Evaluation of tumor growth and metastasis formation upon development of orthotopic tumor xenografts. (a) Representative images of tumor detection by measurement of luciferase activity in NSG mice upon orthotopic i.c. injection of GFP/Luc-LS180 cells alone (TC), cocultured with TASCs and sorted prior to injection (TC cocultured), or cultured alone and admixed with TASCs prior to injection (TC+TASC). Images are displayed as pseudo-color images of peak bioluminescence, with variations in color representing light intensity at a given location. The color bars indicate relative signal intensity. (b) Representative analysis by flow cytometry of cell suspensions from spleens, lungs and livers of tumor bearing mice injected as detailed above. Metastatic tumor cells are detected as GFP+ in unstained samples (red) or as GFP+ EpCAM+ cells upon staining with EpCAM-specific antibodies (blue).