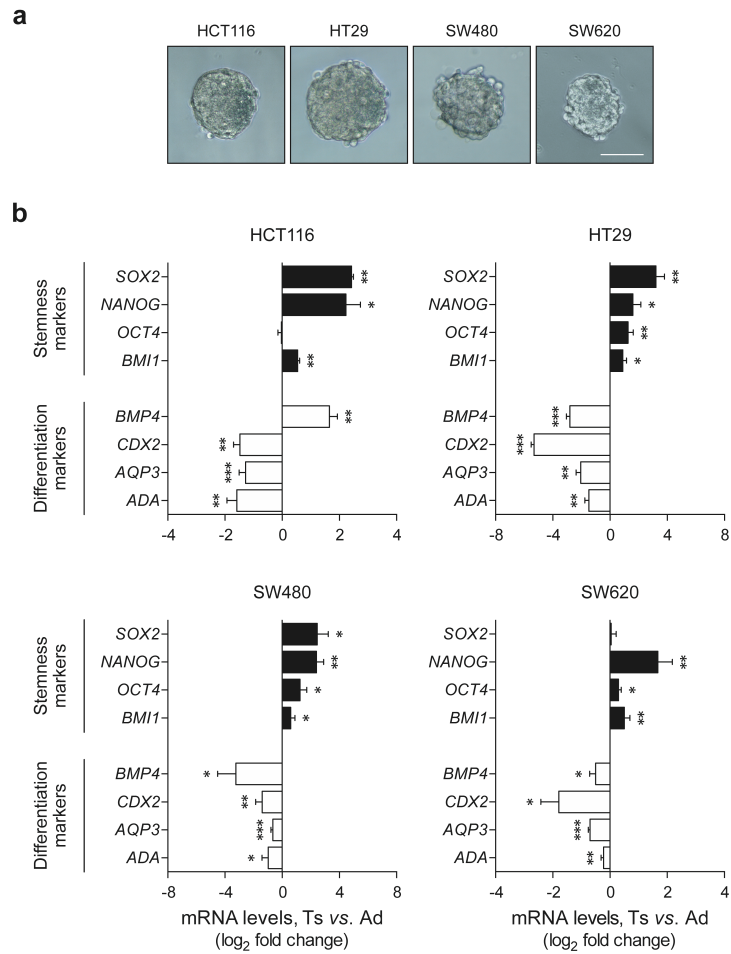
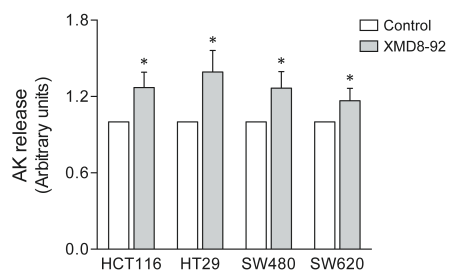


Supplementary Figure S1



Tumorsphere-forming populations are enriched in stem-like colon cancer cells. HCT116, HT29, SW480 and SW620 cells were cultured under sphere-forming or adherent conditions. **(a)** Representative images of 7-day tumorspheres at 100x magnification. Scale bar, 100 μ m. **(b)** The mRNA levels of stemness- and differentiation-associated markers was determined by qRT-PCR. Results are expressed as mean $\pm$ SEM log₂-transformed fold change to respective parental adherent cultures, from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Ad, Adherent; Ts, Tumorsphere.

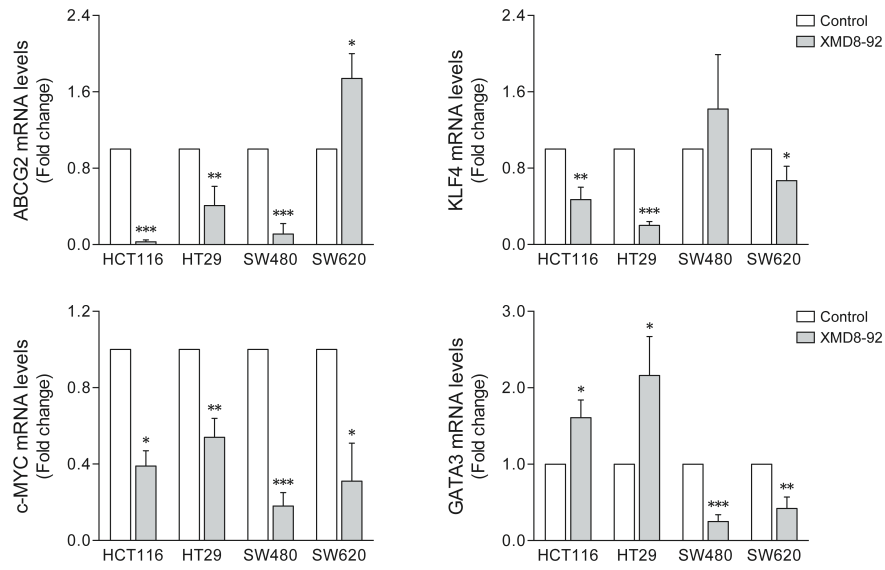
Supplementary Figure S2



ERK5 pharmacological inhibition has minimal cytotoxic effects in tumorsphere cultures.

HCT116, HT29, SW480 and SW620 cells were cultured under sphere-forming conditions in the presence of 4 μ M XMD8-92 or vehicle control. General cell death was evaluated according to adenylate kinase (AK) release using the Toxilight assay. Results are expressed as mean $\pm$ SEM from four independent experiments. * $p < 0.05$ from vehicle control treatment.

Supplementary Figure S3



ERK5 pharmacological inhibition modulates the expression of cancer stem cell-associated genes. Quantitative RT-PCR analysis of a selection of differentially expressed genes between vehicle- and XMD8-92-treated HCT116 tumorspheres, as determined according to the Human Cancer Stem Cells RT² Profiler PCR Array. Results are expressed as mean $\pm$ SEM from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ from vehicle control treatment.

Supplementary Table S1. Primer sequences for quantitative RT-PCR

Gene (human)	Forward primer (5'→3')	Reverse primer (5'→3')
<i>ABCG2</i>	ACGAACGGATTAACAGGGTCA	CTCCAGACACACCACGGAT
<i>ACTB</i>	CTGGAACGGTGAAGGTGACA	AAGGGACTTCCTGTAACAACGCA
<i>ADA</i>	GCCTTCGACAAGCCCAAAGTA	CTCTGCTGTGTTAGCTGGGAG
<i>AQP3</i>	CCGTGACCTTTGCCATGTG	CGAAGTGCCAGATTGCATCATAA
<i>BMI1</i>	TCATCCTTCTGCTGATGCTG	CCGATCCAATCTGTTCTGGT
<i>BMP4</i>	GATCCACAGCACTGGTCTTG	GGGATGCTGCTGAGGTTAAA
<i>CDX2</i>	ACTACAGTCGCTACATCACCA	GAAGACACCGGACTCAAGGG
<i>GATA3</i>	TTAACATCGACGGTCAAGGC	GGTAGGGATCCATGAAGCAG
<i>IL8</i>	CTTGGCAGCCTTCCTGATTT	TTCTTTAGCACTCCTTGGCAAAA
<i>KLF4</i>	CCAATTACCCATCCTTCCTG	CGATCGTCTTCCCCTCTTTG
<i>MYC</i>	ACTCTGAGGAGGAACAAGAA	TGGAGACGTGGCACCTCTT
<i>NANOG</i>	TCTGGACACTGGCTGAATCCT	CGCTGATTAGGCTCCAACCAT
<i>OCT4</i>	TCGAGAACCGAGTGAGAGG	GAACCACACTCGGACCACA
<i>SOX2</i>	GCTAGTCTCCAAGCGACGAA	GCAAGAAGCCTCTCCTTGAA

Supplementary Table S2. Analysis of cancer stem cell-related genes using the RT² Profiler PCR Array

Gene	XMD8-92 vs. Control (log ₂ fold change)	Gene	XMD8-92 vs. Control (log ₂ fold change)
<i>ABCG2</i>	-3,39	<i>ITGB1</i>	0,67
<i>ALCAM</i>	0,04	<i>JAG1</i>	-0,36
<i>ATM</i>	0,30	<i>JAK2</i>	0,93
<i>ATXN1</i>	1,72	<i>KITLG</i>	-1,38
<i>AXL</i>	-0,06	<i>KLF17</i>	2,66
<i>BMI1</i>	0,14	<i>KLF4</i>	-1,18
<i>BMP7</i>	-0,11	<i>LATS1</i>	-0,32
<i>CD44</i>	-0,70	<i>LIN28A</i>	1,22
<i>CHEK1</i>	-0,10	<i>MAML1</i>	0,35
<i>DKK1</i>	0,01	<i>MERTK</i>	-0,53
<i>DNMT1</i>	-0,39	<i>MYC</i>	-1,31
<i>EGF</i>	0,98	<i>NANOG</i>	0,46
<i>EPCAM</i>	-0,37	<i>NFKB1</i>	-1,21
<i>ETFA</i>	0,38	<i>NOTCH2</i>	0,19
<i>FGFR2</i>	-0,08	<i>PLAUR</i>	-1,19
<i>FLOT2</i>	-0,13	<i>PROM1</i>	-1,85
<i>FOXP1</i>	0,11	<i>SAV1</i>	-0,13
<i>FZD7</i>	0,15	<i>SIRT1</i>	0,44
<i>GATA3</i>	1,52	<i>SMO</i>	0,71
<i>GSK3B</i>	0,21	<i>STAT3</i>	0,33
<i>HDAC1</i>	-0,12	<i>TGFBR1</i>	-0,19
<i>ID1</i>	-0,10	<i>TWIST1</i>	-0,34
<i>IKBKB</i>	-0,10	<i>WEE1</i>	-0,42
<i>CXCL8</i>	-1,03	<i>WWC1</i>	-0,66
<i>ITGA2</i>	0,54	<i>YAP1</i>	-0,23
<i>ITGA6</i>	0,52	<i>ZEB1</i>	1,71