OLFM4 (Olfactomedin 4) associates with expression of differentiation markers but not with properties of cancer stemness, EMT nor metastatic spread in colorectal cancer

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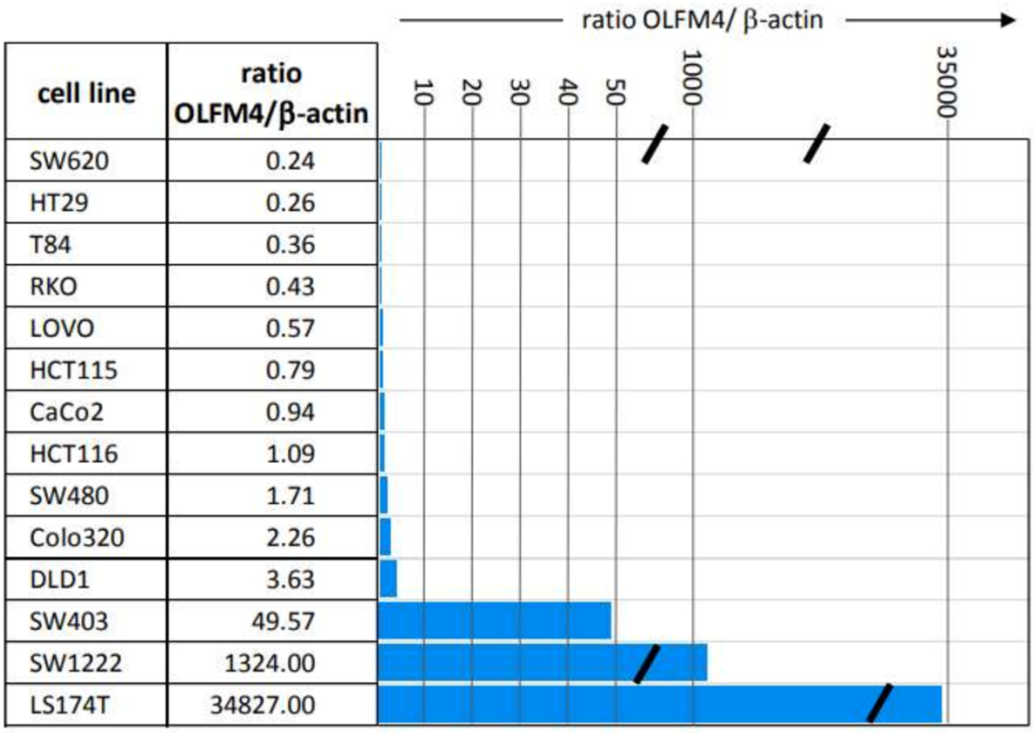
Supplementary Figures S1–S4



cell line	ratio OLFM4/β-actin
SW620	0.24
HT29	0.26
T84	0.36
RKO	0.43
LOVO	0.57
HCT115	0.79
CaCo2	0.94
HCT116	1.09
SW480	1.71
Colo320	2.26
DLD1	3.63
SW403	49.57
SW1222	1324.00
LS174T	34827.00

Figure S1. *OLFM4* expression profiles of cultures human colorectal cell lines
Most human cultivated colorectal cancer cell lines displayed low expression levels of *OLFM4*. For the 14 long term cultivated colorectal cancer cell lines CaCo2, Colo320, DLD1, HCT115, HCT116, HT 29, LOVO, LS 174T, RKO, SW1222, SW403, SW620, SW480, T84 the mRNA levels of OLFM4 and β-actin as the reference gene were determined employing RT-qPCR. Only SW1222 and LS174T harbored reasonable relative amounts of OLFM4 mRNA (ratio: Q = $OLFM4/\beta\text{-}actin$).

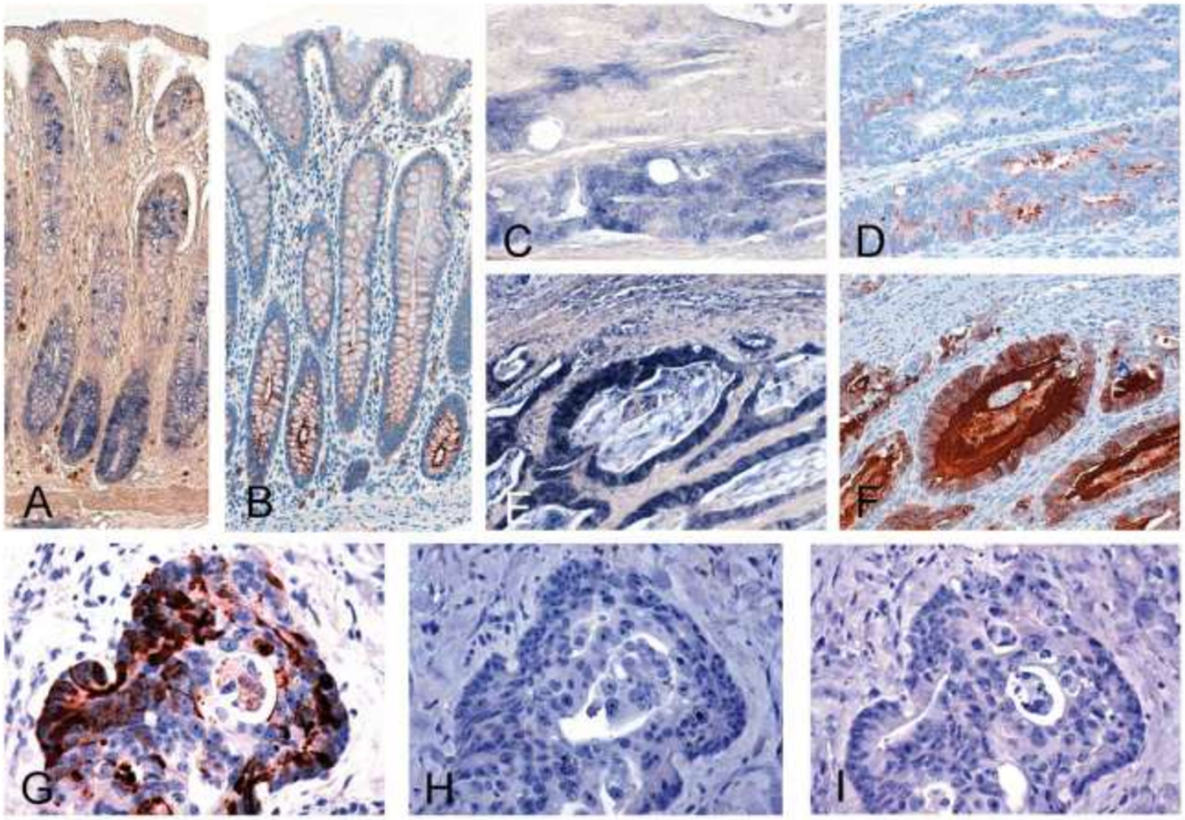


Figure S2. Validation of OLFM4 specific immunohistochemistry
Validation of OLFM4 specific immunohistochemistry by RNA *in situ* hybridization. Serial sections of (A, B) normal mucosa or (C–I) tissue from CRCs were stained using either (A, C, E) *in situ* hybridization or (B, D, F, G–I) immunohistochemistry. OLFM4 was highly expressed in comparable areas at the base of normal crypts (A, B). In CRC, expression patterns of OLFM4 detected by the two methods were overlapped quite well (C, D and E, F). Technical artefacts could also be excluded as OLFM4 detection was only seen in the presence of the OLFM4 specific antibody (G) but not when the primary antibody was omitted (H) or an isotype was used as a control (I). Total magnifications: A–F ×100, G–I ×200.

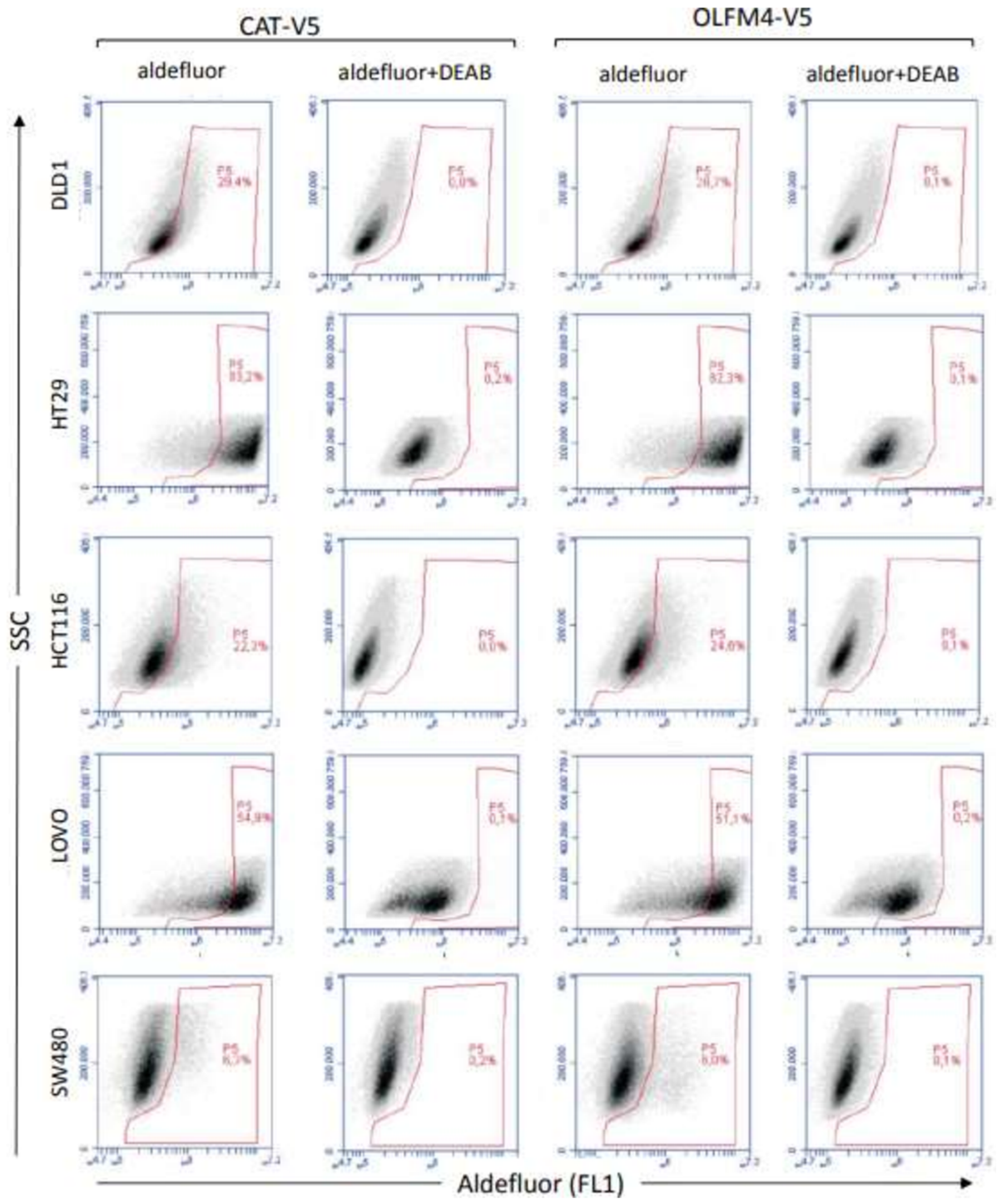


Figure S3. Effect of forced OLFM4 expression on aldefluor activity
Forced OLFM4-V5 expression did not alter the amounts of aldefluor positive cells. Aldefluor is a metabolite that is converted into a green fluorescent dye by the action of ALDH. As ALDH1A1 is an indicator for cancer stem cells the consistency in the amount of aldefluor positive cells in the cell cultures expressing either OLFM4-V5 or CAT-V5 indicated that OLFM4 did not influence the amount of CSC. At the same time the functionality of the aldefluor test set could be seen as specific inhibition of ALDH activity with DEAB (die-ethyl-amino-benz-aldehyd) worked perfectly in all cases (aldefluor+DEAB).

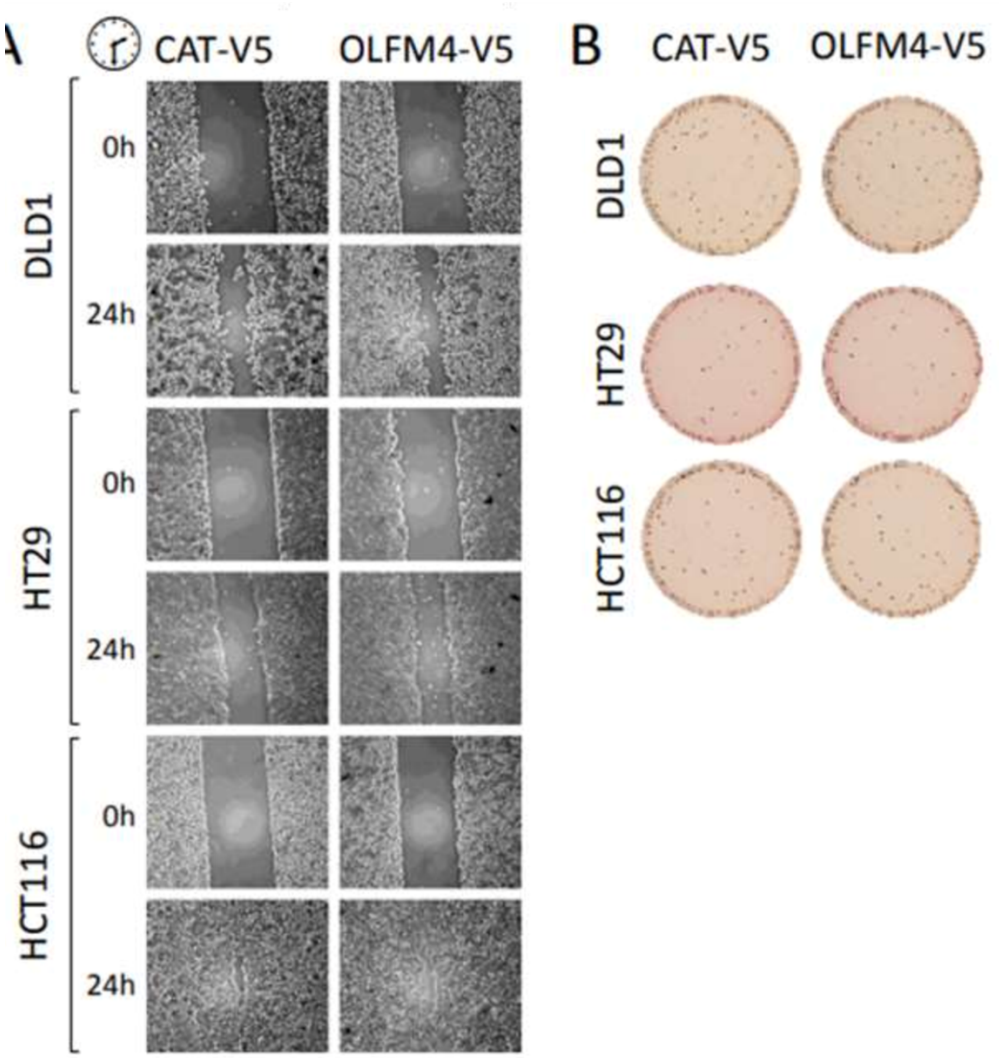


Figure S4. Effect of forced OLFM4 expression on migration and colony formation
Forced expression of OLFM4-V5 did not affect migration nor colony formation in colorectal tumor cells. (A) The capacity for migration of colorectal tumor cells (DLD1, HT29, HCT116) overexpressing either OLFM4-V5 or CAT-V5 was tested employing a wound healing assay together with ibidi-chambers. No difference was seen 24 h after starting the experiment by removing the ibidi-chamber. (B) The same was true for colony formation. No difference in the number nor size of colonies was seen when growing colorectal tumor cells (DLD1, HT29, HCT116) overexpressing either OLFM4-V5 or CAT-V5 in methyl-cellulose for 12–16 days.