Supporting Information

Vermeulen *et al.* 10.1073/pnas.0805706105

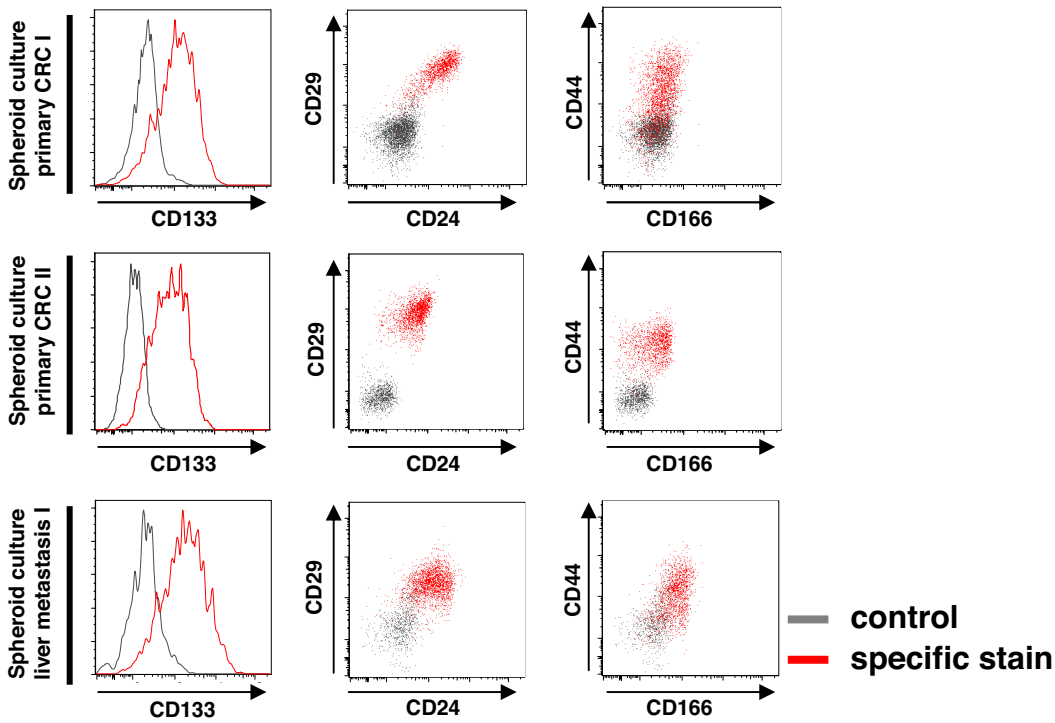

Fig. S1. Colon spheroid cultures show heterogeneous marker expression. Colon CSC cultures show heterogeneous expression of a variety of colon CSC markers, including CD133, CD44/CD166, and CD24/CD29 (isotype in gray, specific stain in red). Representative results shown for two cultures derived from a primary colon carcinoma and one culture derived from liver metastasis.

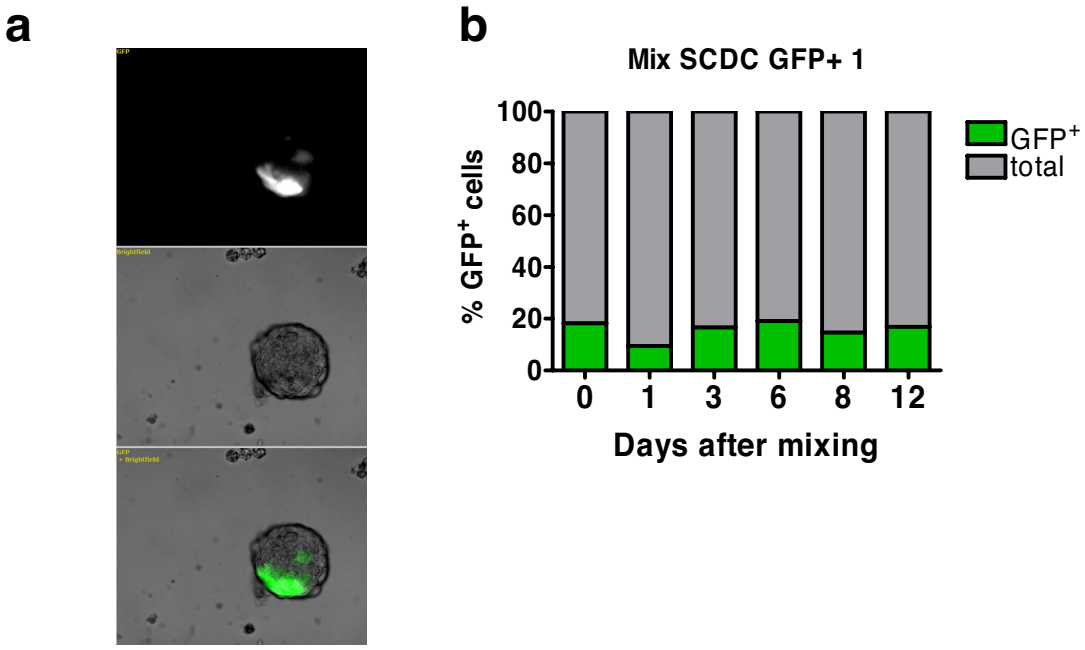

Fig. S2. Exclusion of horizontal GFP transmission. (*a*) GFP^+ and GFP^- spheres were dissociated and mixed to exclude that GFP can spread due to viral production in these cultures (horizontal transmission). After coculturing for 8 days, mixed spheres consisting of both GFP^+ and GFP^- cells were observed, suggesting that infection within a sphere does not occur. (*b*) Moreover, the fraction of GFP^+ cells within these cultures was stable over time as judged by flow cytometry. This confirms that no other transfer of GFP than to offspring of GFP^+ cells is occurring (vertical transfer).

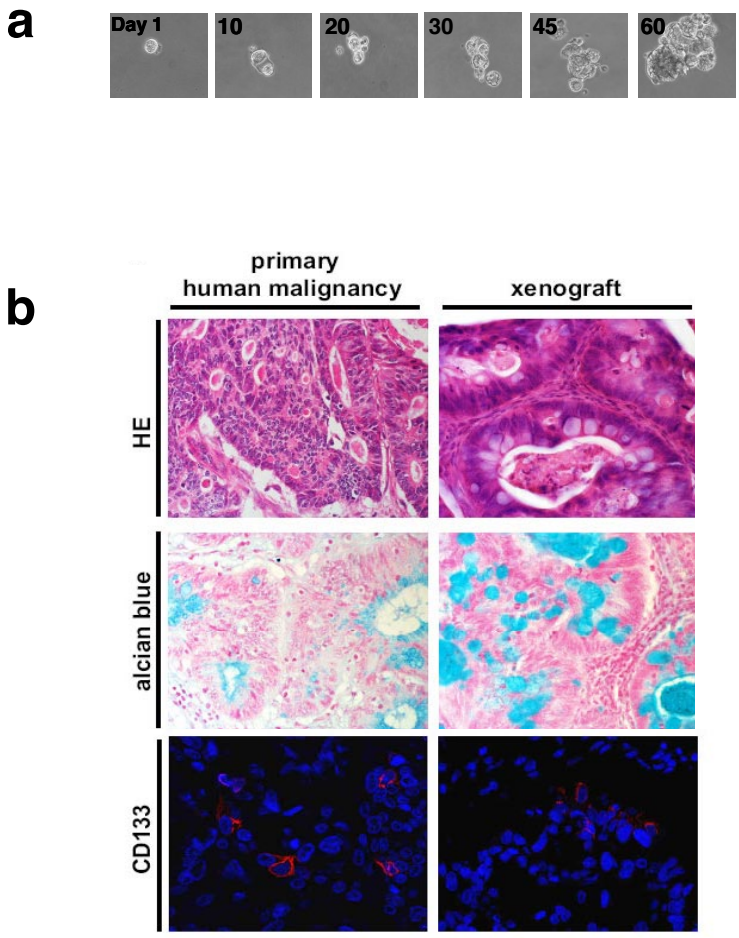

Fig. S3. Direct ex-patient single CD133$^+$ cell plating and SCDC-derived xenograft. (*a*) The CD133 enriched fraction of a dissociated colorectal carcinoma (T3N1M1) was single-cell plated and 116 of 384 wells contained a single cell. (*b*) Injection in immunodeficient mice gave an adenocarcinoma (right) that resembles the original human malignancy (left) as confirmed by HE and Alcian Blue staining. Also heterogeneous CD133$^+$ expression was detected both in the primary tumor and the single cell-derived xenograft.

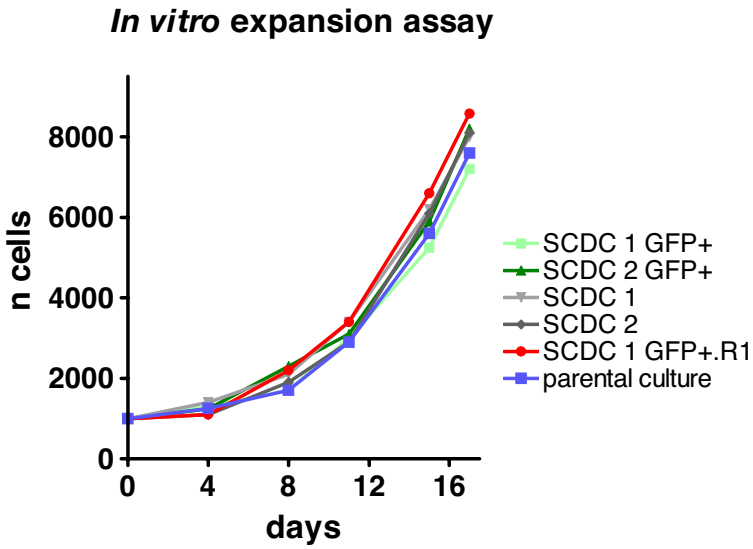

Fig. S4. *In vitro* expansion rate of SCDCs. *In vitro* expansion rate of single cell-derived clones, as measured by cell counting. (GFP^+ SCDC 1 and 2 are derived as shown in Fig. 2*C*, SCDC 1 and 3 are derived as depicted in Fig. 2*A*). SCDC 1 GFP^+.R1 represents a reisolated culture from an SCDC-derived xenograft.

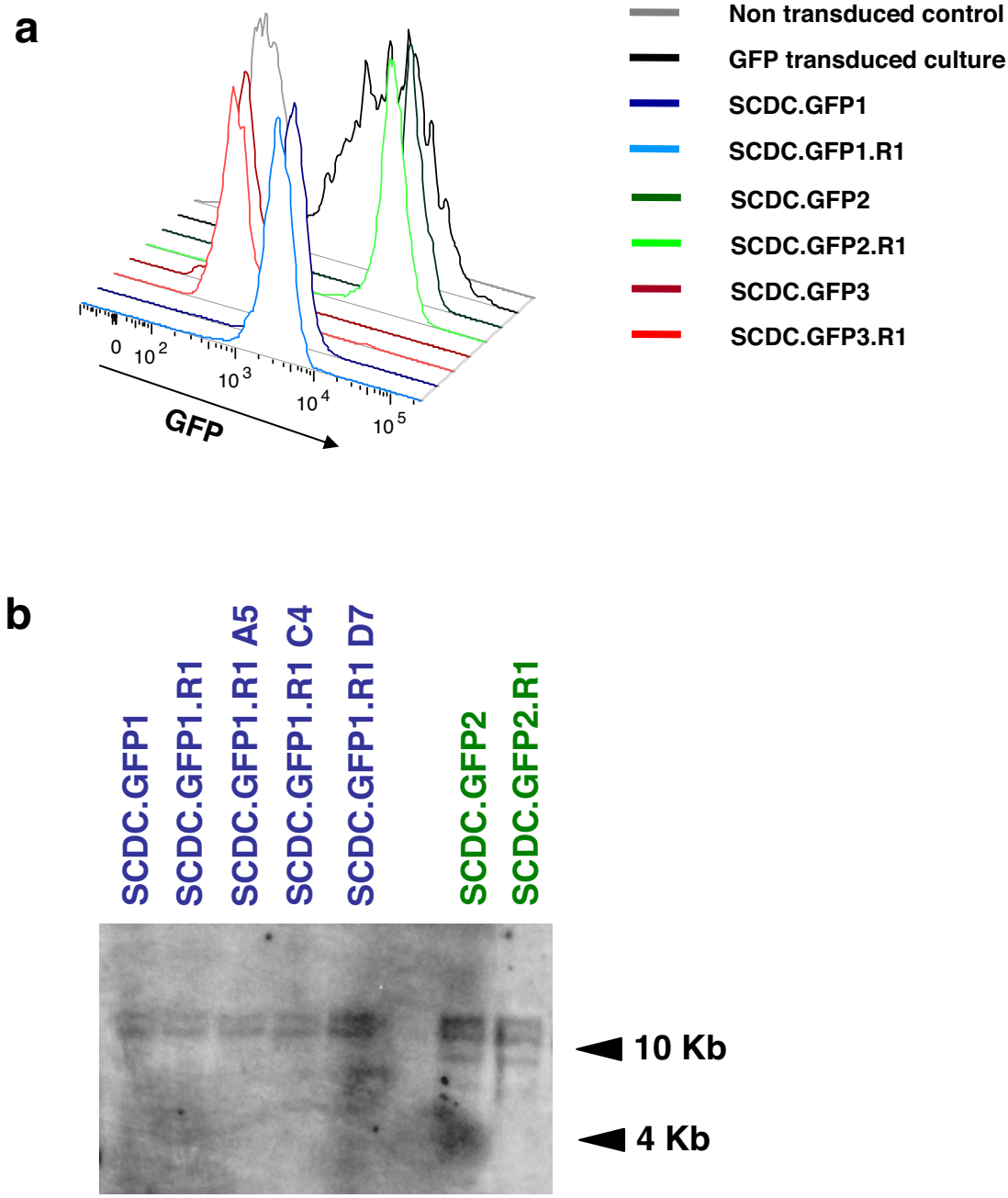

Fig. S5. Evaluation of single-cell nature of SCDCs. (*a*) Lentiviral transduced spheroid culture with constitutively active GFP is shown in black. From this culture, several SCDCs were randomly generated as depicted in Fig. 2C. As is evident from this FACS analysis, every GFP$^+$ SCDC consists only of GFP$^+$ cells. Moreover, GFP expression is restricted to a small-intensity range for every SCDC. This illustrates the single-cell nature of the SCDCs. Importantly, upon deriving spheroid cultures from the SCDC-derived xenografts (SCDC.GFP 1/2/3.R1), GFP expression levels are strictly preserved. (*b*) To confirm the single cell origin of our SCDCs, we performed an EGFP integration sites analysis. The Southern blot shows the insertion profile of two different GFP$^+$ SCDCs. Both the original SCDCs (SCDC.GFP1 and SCDC.GFP2) and the cultures we obtained after a mouse passage of the SCDCs (SCDC.GFP1.R1 and SCDC.GFP2.R1) are shown. It is clear from this analysis that different profiles are obtained from the different cultures that are preserved after a mouse passage. In addition, we single-cell cloned SCDC.GFP1.R1 again by FACS single-cell deposition to exclude that multiple present clones make up the EGFP integration profile. Those re-cloned cultures are shown as SCDC.GFP1.R1 A5, SCDC.GFP1.R1 C4, and SCDC.GFP1.R1 D7. Also the re-cloned SCDCs show conservation of the integration sites. We performed Southern blots according to standard procedures using a DIG PCR kit, the probe spanning 886–1174 bp of EGFP. DNA was cut with XbaII.

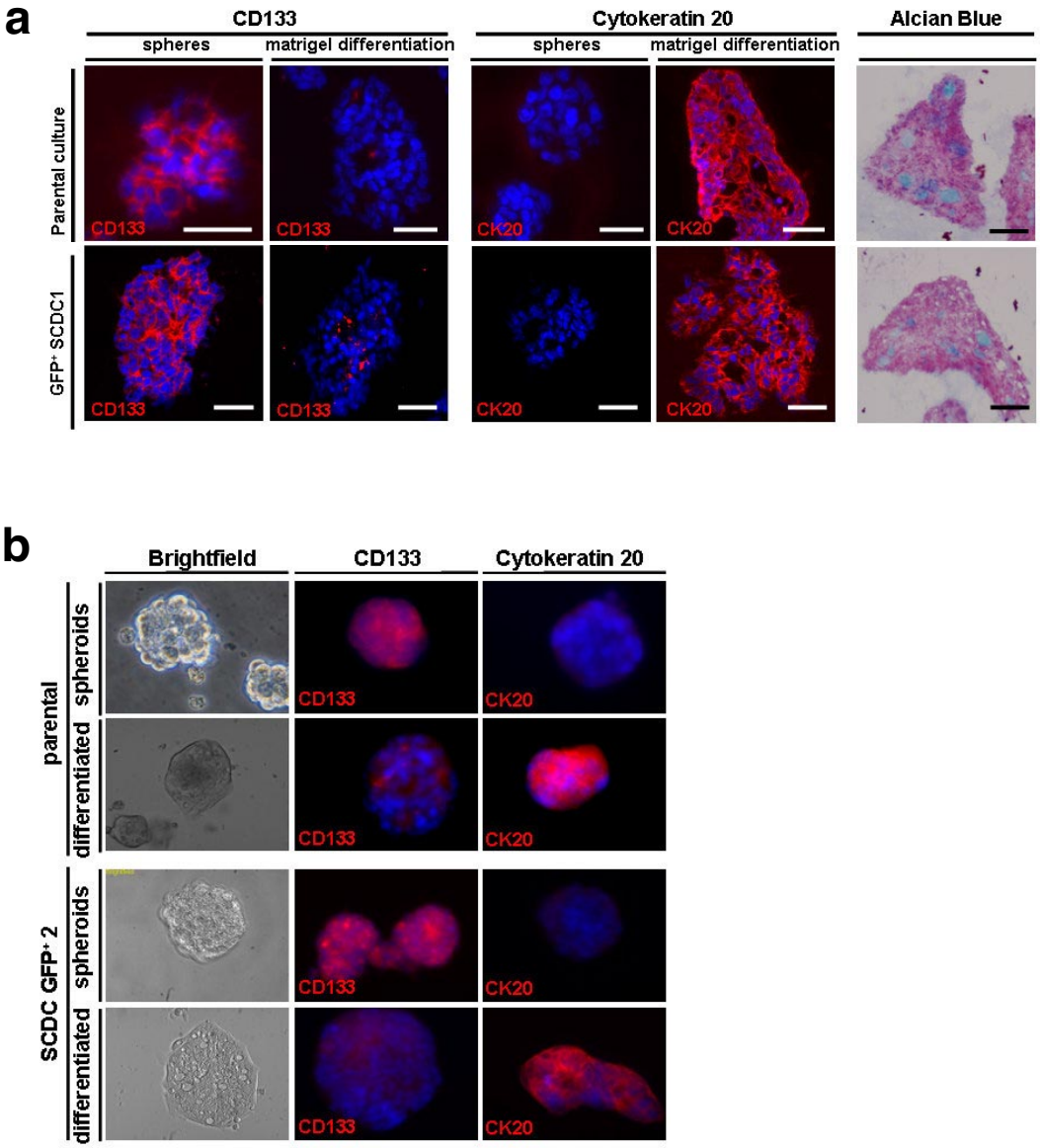

Fig. S6. *In vitro* differentiation. Colon spheroid cultures were subjected to differentiation on an adherent plate with serum containing medium (*a*) or in matrigel overlaid with serum containing medium (*b*). Both the parental culture and a representative SCDC showed loss of CD133 expression and up-regulation of CK20.

a

inhibitor	manufacturer	target	concentration
LiCl	Sigma	GSK-3	30 mM
BIO	EMD Bioscience	GSK-3	5 μM
PKBi V	Calbiochem, Amsterdam, The Netherlands	AKT	10 μM
Rapamycin	Cell Signaling Technology	mTOR	20 nM
UO126	Calbiochem, Amsterdam, The Netherlands	MEK	10 μM
PD98059	Alexis, Breda, The Netherlands	MEK	5 μM
LY294002	Calbiochem, Amsterdam, The Netherlands	PI3K	10 μM

b

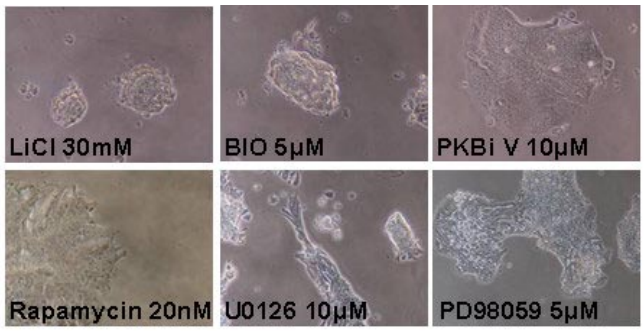

Fig. S7. Effect of various inhibitors on differentiation pattern in vitro. (*a*) Table summarizing inhibitors are used in the screen. (*b*) Representative images of spheroid culture were subjected to differentiation in the presence of the indicated inhibitor.

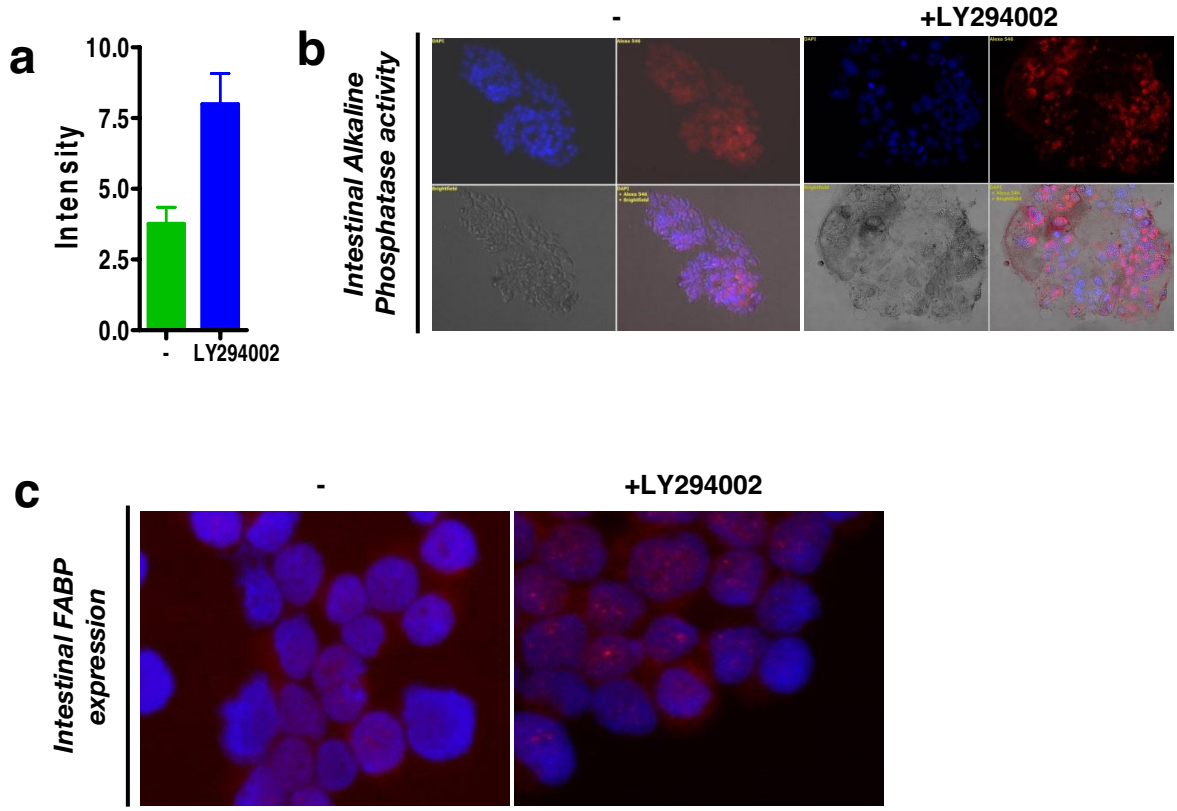

Fig. S8. Enterocyte marker expression. (*a*) Quantification of IAP activity as measured by fluorescence intensity. (*b*) Detection of IAP activity by fluorescence measurement with and without Ly294002. (*c*) Cytospins of differentiated spheroid culture in the presence or absence of Ly294002 stained for I-FABP.