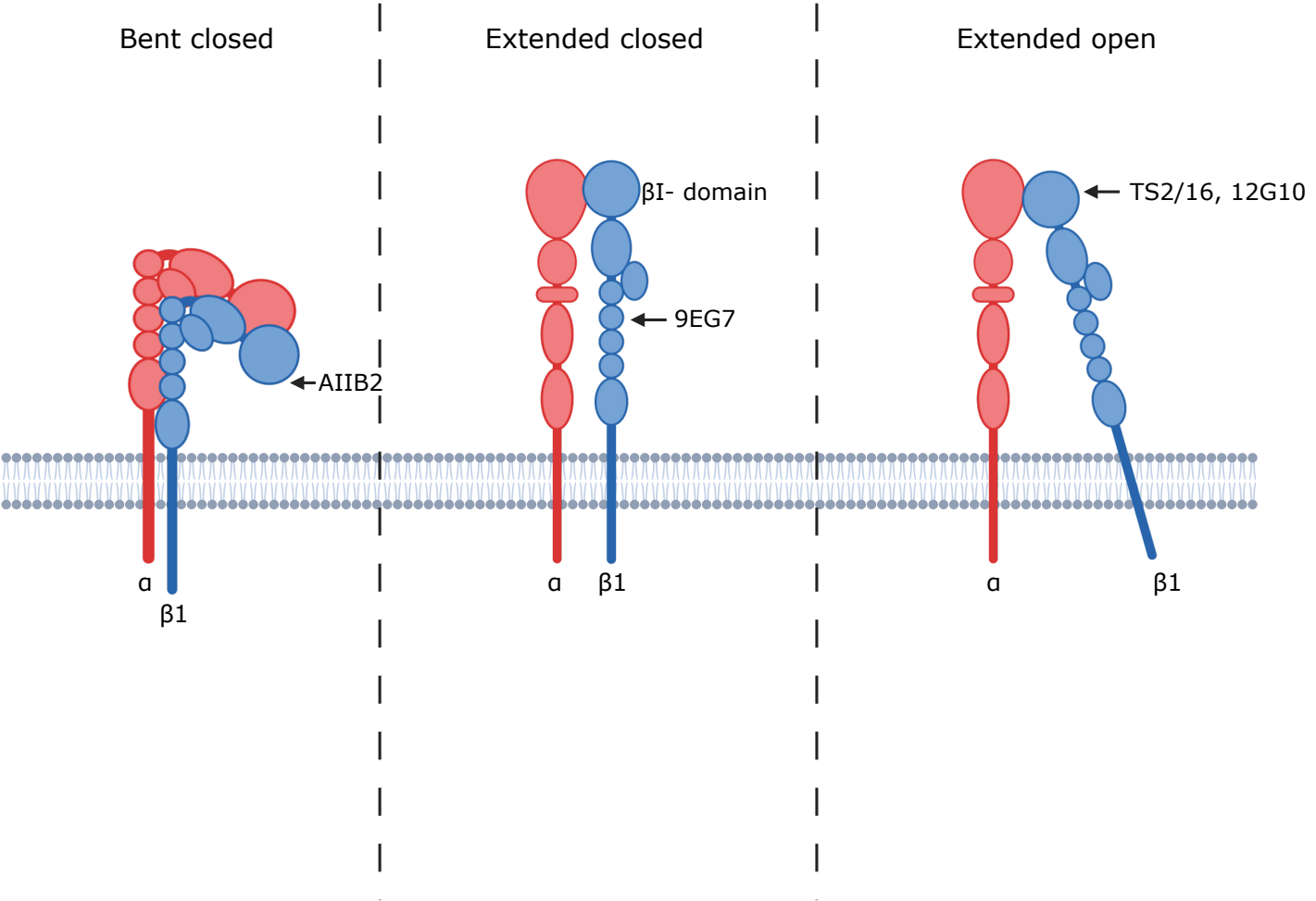


A single-chain derivative of an integrin-activating antibody potentiates organoid growth in Matrigel and collagen hydrogels

In the format provided by the
authors and unedited

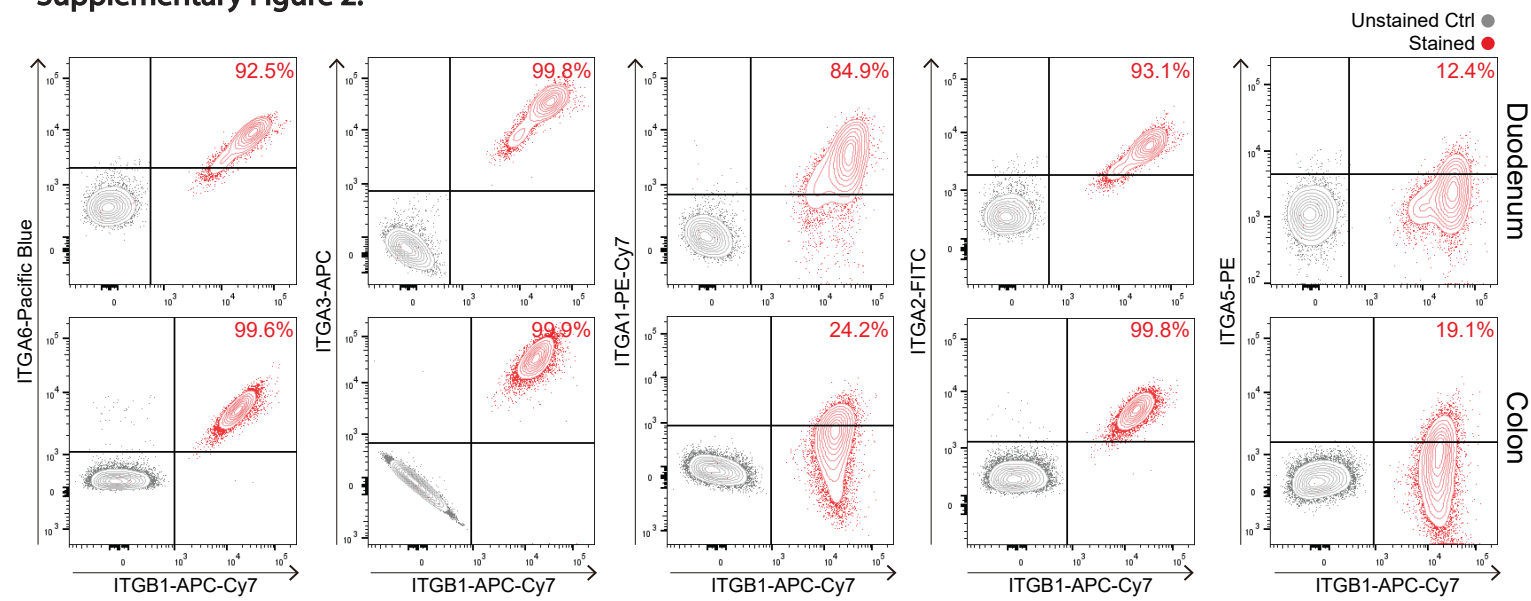
Supplementary Figure 1.



Supplementary Figure 1; Schematic representation of Integrin conformational states relating to ligand affinity, and locations of antibody epitopes.

Ab AIIIB2 stabilizes the non-adhesive bent closed conformation. The 9EG7 Ab stabilizes the adhesive extended closed conformation. The adhesive extended open conformation is stabilized by Ab 12G10. Ab TS2/16 stabilizes the extended-open conformation upon ligand binding.

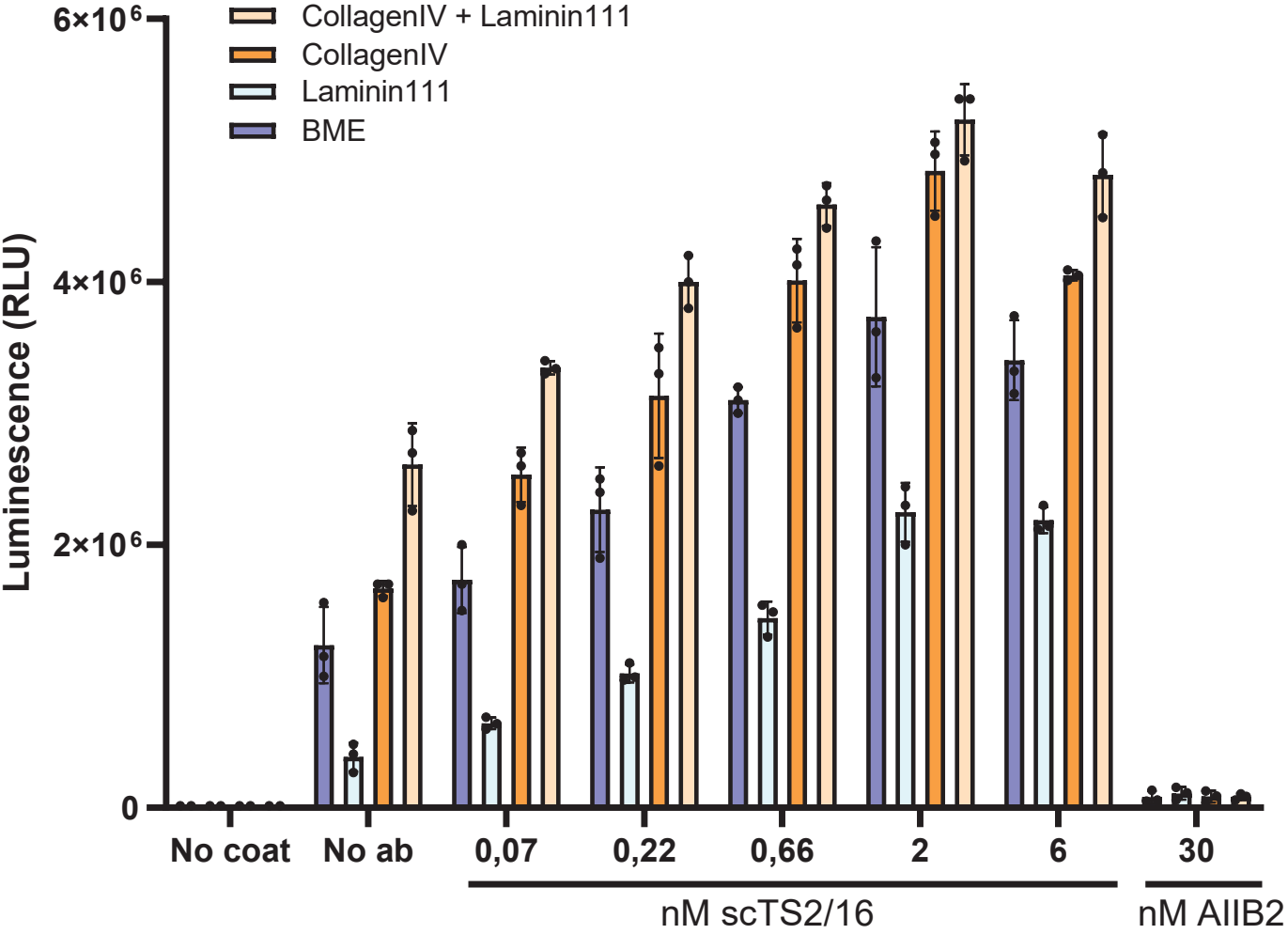
Supplementary Figure 2.



Supplementary Figure 2; FACS analysis of intestinal organoid cells reveals expression of ITGB1, ITGA2, ITGA3, and ITGA6, in all cells.

Colon (P26N) and duodenum (Duodenum) organoids were enzymatically processed to single cells, and incubated with Integrin component-specific antibodies, each directly conjugated to a different fluorophore (as indicated). Stained cells were FSC/SSC gated to remove debris and to focus the integrin analysis on live cells. Analysis of antibody staining (red dots) was performed on 10^4 cells/analysis and compared to the same unstained cells (grey dots). All cells appear to express laminin-specific ITGA6/ITGB1 and ITGA3/ITGB1 integrins, as well as the collagen receptors ITGA2ITGB1. Duodenum cells universally express the $\alpha1\beta1$ collagen receptor. Expression of the RGD-specific ITGA5/ITGB1 was negligible. BD LSRFortessa™ was used for the measurements. These flow-cytometric results are consistent with the extensive single cell mRNA data presented in Extended Data Fig. 1.

Supplementary Figure 3.

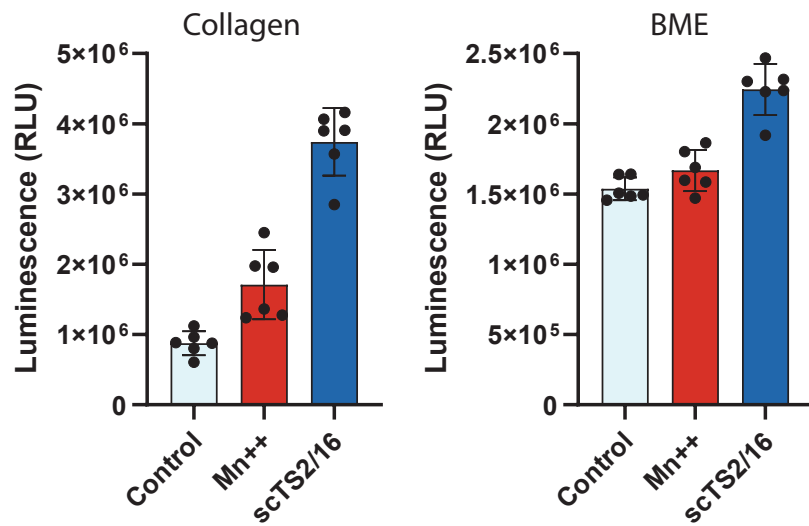


Supplementary Figure 3; Small-intestinal adhesion towards the BME components LN-111 and collagen type-IV (N=2).

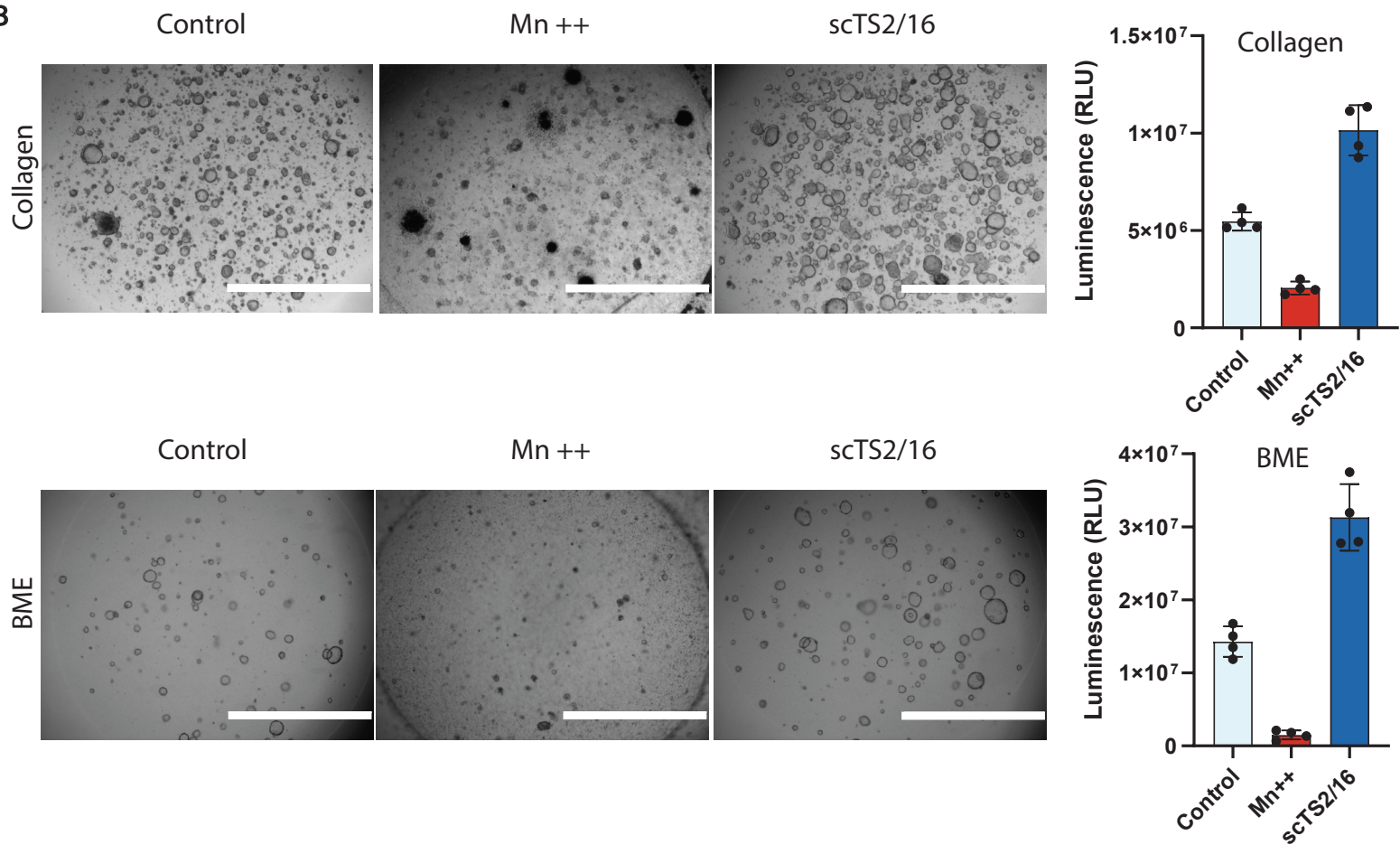
Duodenum-72 organoids were enzymatically processed to single cells and resuspended in DMEM-F12 medium. Cells were plated to a final concentration of 50.000 cells/200 μ l/well in the absence or nM range presence of scTS2/16, as indicated. Wells were coated with BME (100ng/ml), LN-111 (70 ng/ml), collagen IV (30 ng/ml), or a mix of LN-111 and collagen typeIV (70 + 30 ng/ml, resp.). The coat-specific binding of cells was tested by including a no-coating control. To test the relative involvement of integrin β 1. For each of the four coating conditions (from left to right; BME – Laminin111 – collagenIV – collagenIV + laminin111) the inhibitory AIB2 ab (30 nM) was included. Upon incubation for 1,5 hrs (37°C), wells were extensively washed with DMEMF12 medium and remaining cells Quantified by Cell Titer-GLO, an ATP-driven luminescence assay. Mean +/-STDEV are indicated (n=3 biological replicates) (Relative Light Units - RLU).

Supplementary Figure 4.

A



B



Supplementary Figure 4; Mn⁺⁺ and scTS2/16 effects on adhesion and growth of N39 ileum organoid cells. (N=2)

A) Adherence of cells on different coats (Collagen and BME) in presence of 1 mM Mn⁺⁺, 1 nM scTS2/16, or control condition. Mean +/-STDEV of 6 biological replicates are indicated.

B) Growth of organoids in collagen and BME 3D domes for 7 days in the presence of 1 mM Mn⁺⁺, 1 nM scTS2/16, or control condition. Growth is analyzed using brightfield microscopy (left panel, Scale bar is 200 µm) and the viability assay using Cell Titer-Glo (right panel). Mean +/- STDEV of 4 biological replicates are indicated.

P-value adhesion Mn⁺⁺ vs ctr = 1.4×10^{-3} for col , and 4.2×10^{-2} for BME.

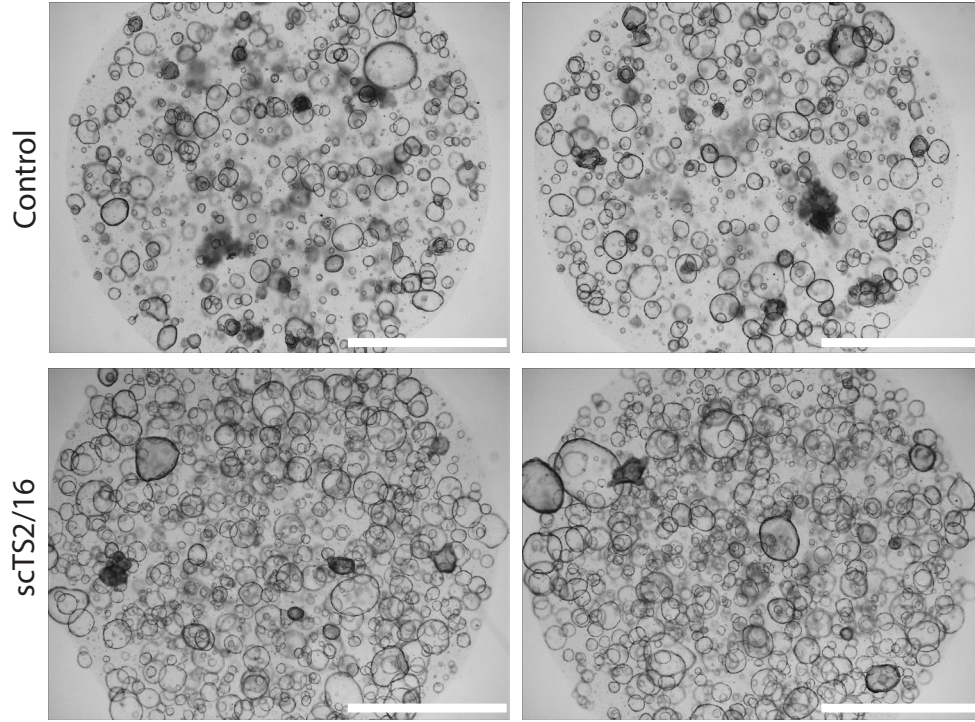
P-value adhesion scFv vs ctr = 4.0×10^{-8} for Col and 2.8×10^{-6} for BME.

P-values growth Mn⁺⁺ vs ctr = irrelevant for Col and BME.

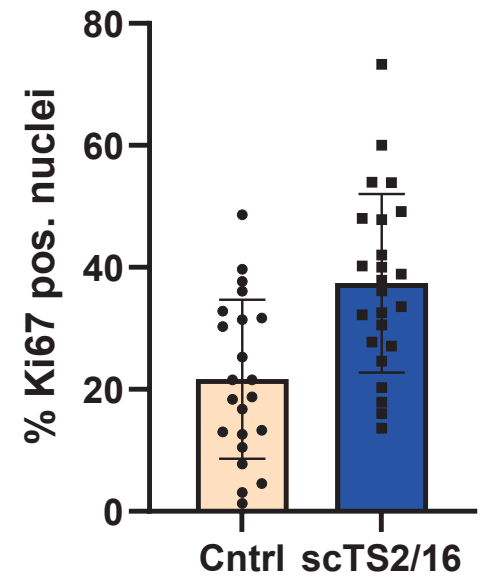
P-values growth scFv vs ctr = 2.5×10^{-4} for Col, and 2.5×10^{-4} for BME.

Supplementary Figure 5.

A



B

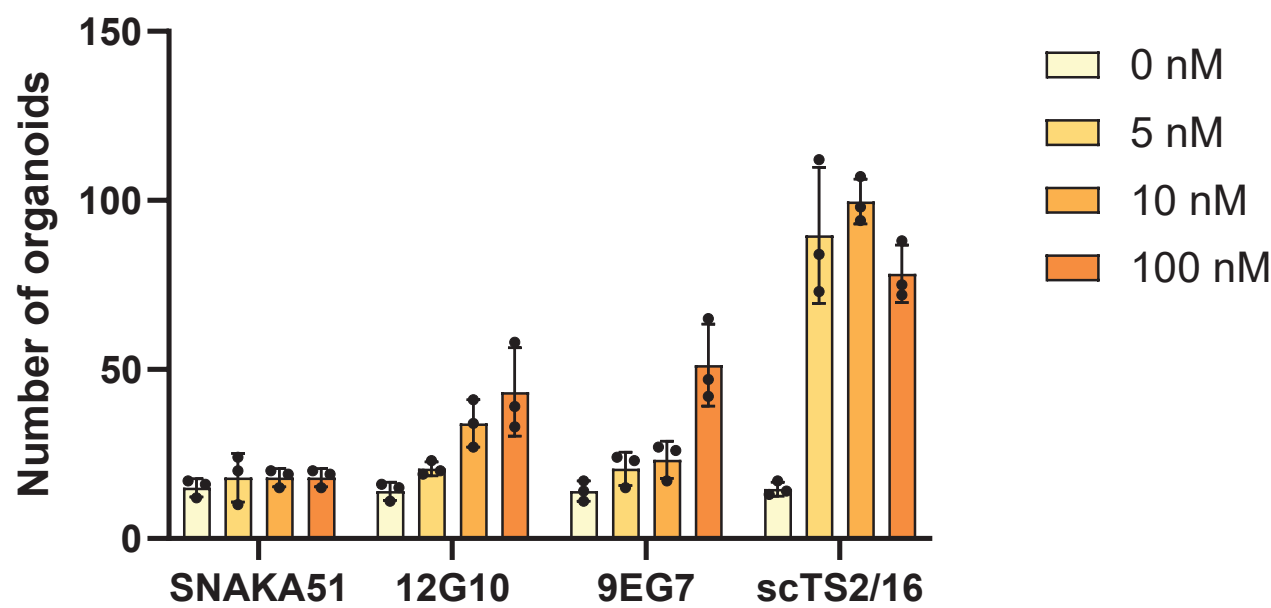


Supplementary Figure 5; Quantification of Ki67-pos nuclei in routine BME-cultured colon organoids. (N=1)

A) P26N colonic organoids were grown in control medium or 5 nM scTS2/16-supplemented medium (n=6, shown are images of two replicates). Scale bar is 200 μm .

B) expression of the nuclear proliferation marker Ki67 protein, in parallel cultures, was visualized using immunofluorescence. Dots represent the percentage of positive nuclei per individual organoid. A total of 3000 nuclei from both control and scTS2/16 cultures, were blindly analysed. Mean $\pm$ STDEV indicated (biological replicates n=19 and 22 for control and scTS2/16, respectively). P-value one-tailed T.test is 4.1×10^{-4} .

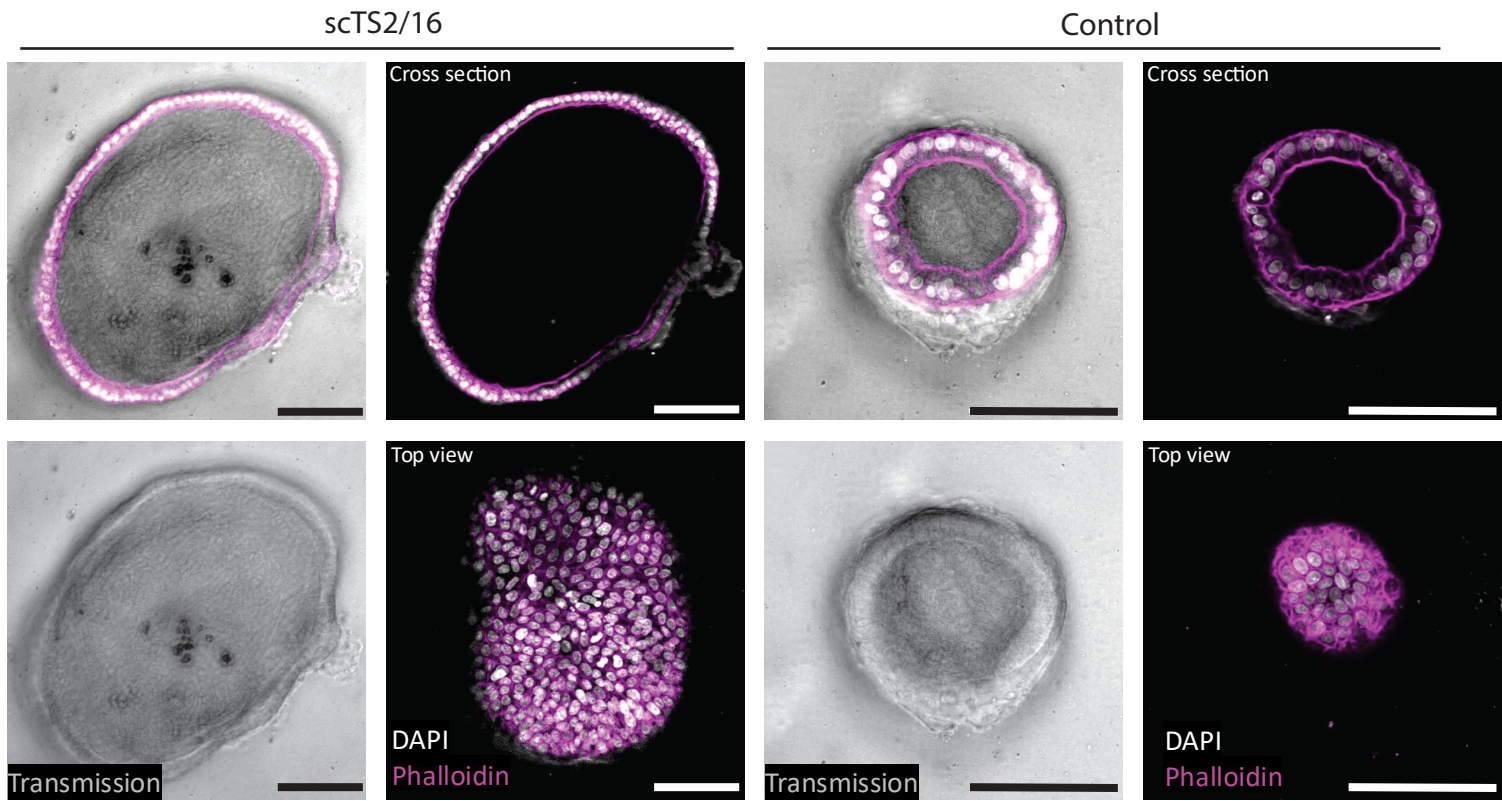
Supplementary Figure 6.



Supplementary Figure 6; Clonal outgrowth of P26N intestinal organoids in the presence of selected integrin-binding antibodies.

Equal numbers of TrypLE-isolated single cells were, in triplo, embedded in BME and cultured in the presence of the indicated integrin antibodies. Test concentrations are indicated. Antibodies 12G10 (Fab) and scTS2/16 (VH-VL) have overlapping epitopes on the ectodomain of $\beta 1$ integrin. Antibody 9EG7 (Fab) binds a different region in the same ectodomain. These antibodies were introduced in Supplementary Fig. 1. The stimulating Fab SNAKA51 binds the integrin $\alpha 5$ subunit, for which the gene is not expressed in these organoids. Emergence of clones was quantified by counting their number at day 10. Average number of clones $\pm$ STDEV indicated (n=3 biological replicates).

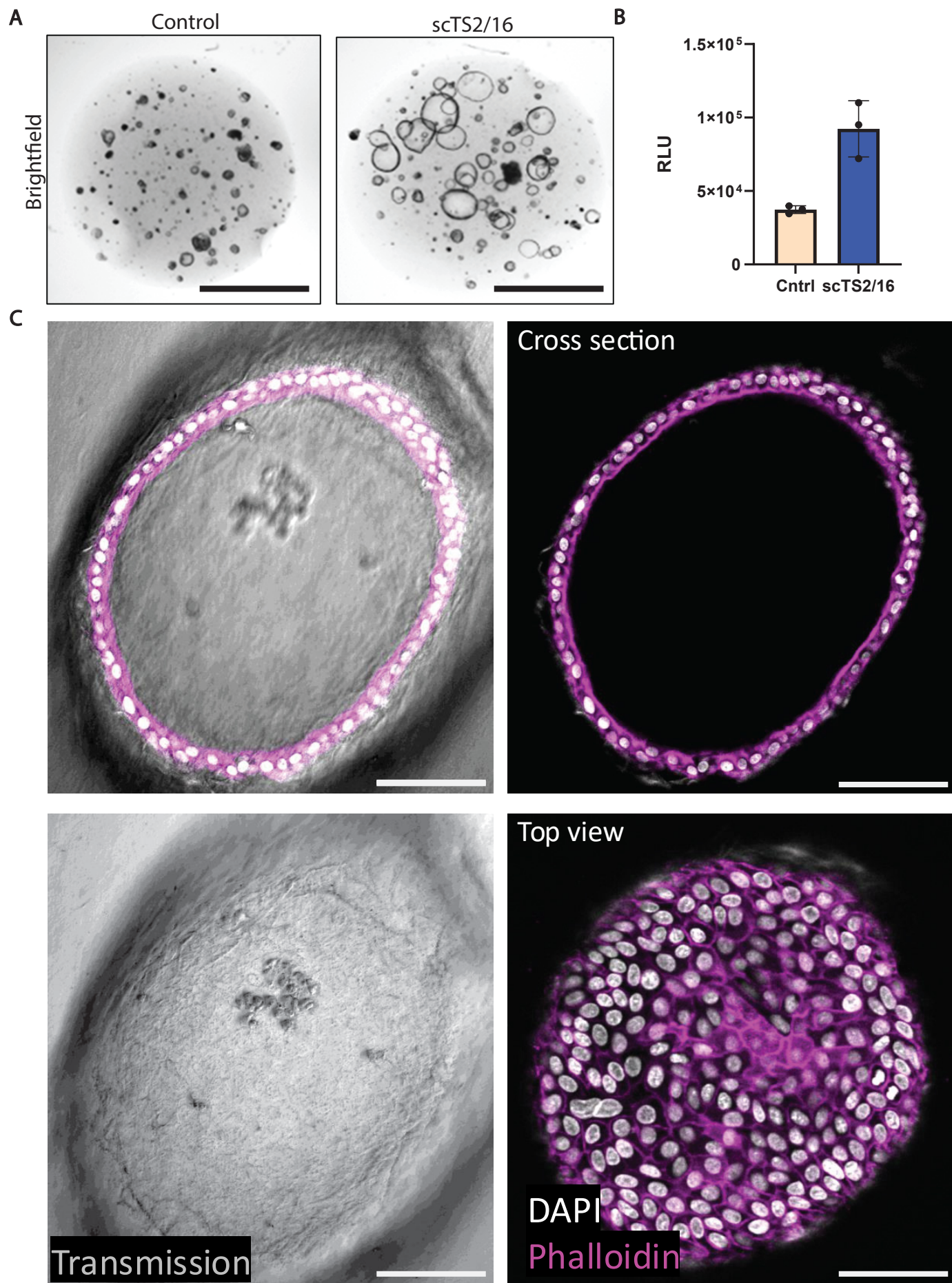
Supplementary Figure 7.



Supplementary Figure 7; Confocal imaging of jejunal organoids in 3D BME.

Transmission and fluorescent confocal imaging (top-view and cross-section) in the presence and absence of 5 nM activating scTS2/16 (scale bar = 100 μm). Organoids were in-gel formaldehyde-fixed, permeabilized, and stained for accumulation of F-actin (Phalloidin) occurring near apical membranes. Nuclei were visualized using the fluorescent DNA dye DAPI.

Supplementary Figure 8.



Supplementary Figure 8; Growth of PureCol®gel-suspended P26N colon organoids (N=2).

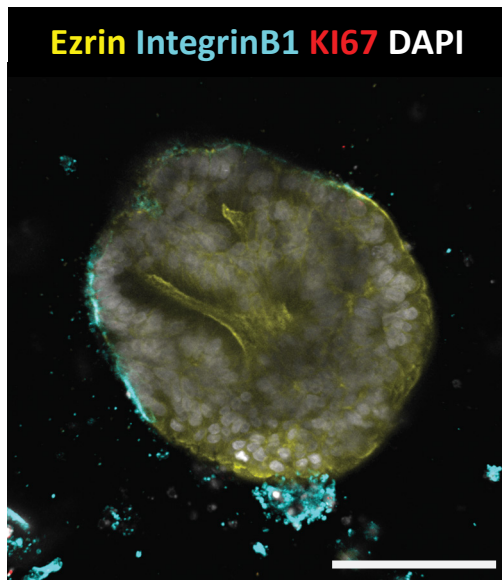
A) Colon-derived organoids, mechanically fragmented, were suspended as triplicates in 20 µl drops in round-bottom 96-well plates. Culture media +/- 5 nM scTS2/16 as indicated. Images made on day 7 (scale bar = 1 mm).

B) Cellular growth was quantified at day 7 by an ATP-driven Luminescence assay. (RLU = Relative Light Units), Mean +/- STDEV indicated. T.test (one tail) calculated p-value for scFv effect = 3.9×10^{-3} .

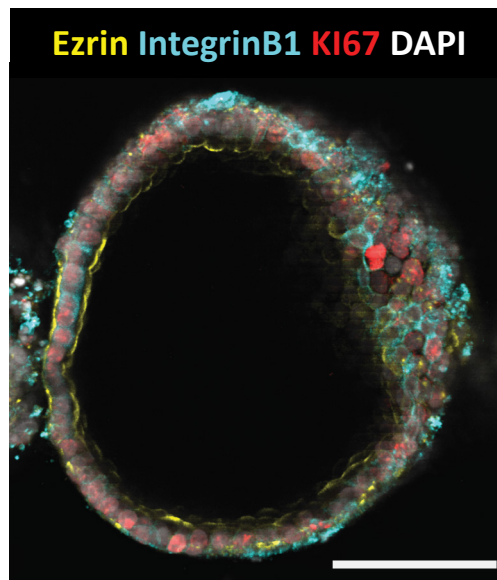
C) Transmission and fluorescent confocal imaging (top-view and cross-section) in the presence of 5 nM activating scTS2/16 (scale bar 100 µm). Organoids were in-gel formaldehyde-fixed, permeabilized, and stained for accumulation of F-actin (Phalloidin) occurring near apical membranes. Nuclei were visualized using the fluorescent DNA dye DAPI.

Supplementary Figure 9.

Collagen



Collagen + scTS2/16



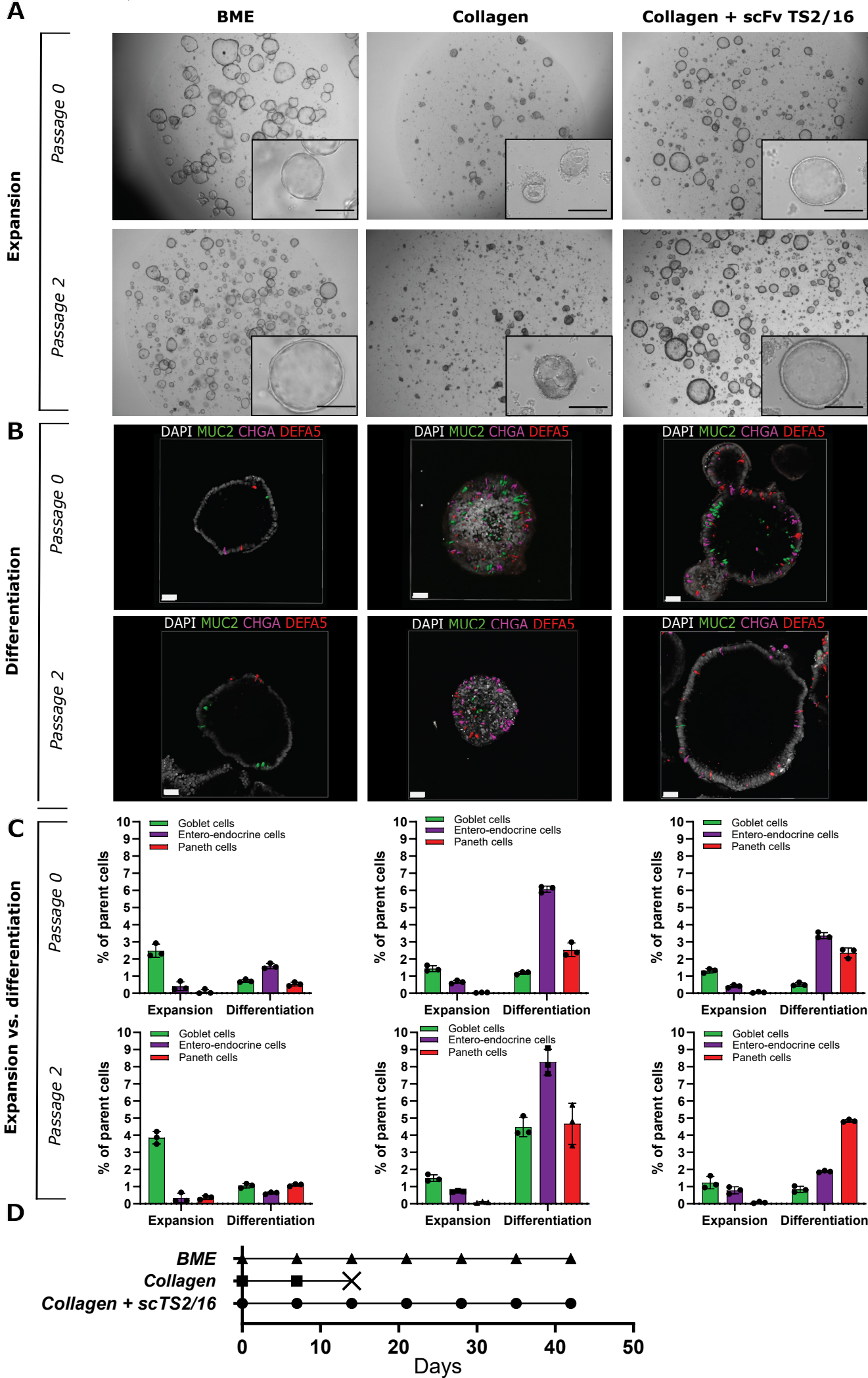
BME



Supplementary Figure 9; Confocal immunofluorescent analysis of intestinal organoids cultured in collagen, collagen + scTS2/16 and BME hydrogels.

N39 ileum organoids were stained for apical marker Ezrin, basal marker Integrin β 1, proliferative marker Ki67 and nuclear marker DAPI. The scale bar is 100 μ m.

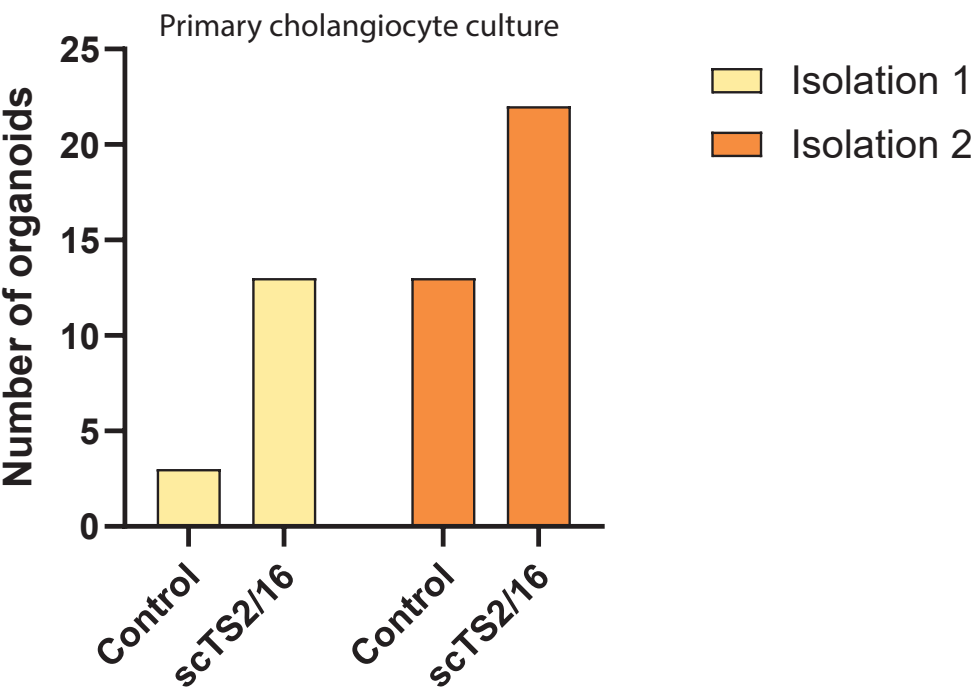
Supplementary Figure 10.



Supplementary Figure 10; N39 triple-reporter ileum organoids during expansion and differentiation.

- A)** Brightfield images of ileum (N39) intestinal organoids, cultured in BME, collagen, collagen + scTS2/16 at P0 and P2 in expansion medium. Only BME and Collagen +scTS2/16 cultures are rich in cystic organoids (see higher magnifications in inserts). Scale bar is 200 μ m.
- B)** Confocal images of differentiated ileum (N39) intestinal organoids cultured in BME, collagen, collagen + scTS2/16 at P0 and P2. Images present levels of endogenously expressed fluorescent tags for marker proteins identifying Goblet cells (MUC2), Enteroendocrine cells (CHGA) and Paneth cells (DEFA5). DAPI marks the nuclei of the cells. Scale bar is 50 μ m.
- C)** Quantification (mean $\pm$ STDEV) of fluorescent signals as illustrated in panel B.
- D)** Testing long-term passaging of ileum organoids in BME, collagen, and collagen + scTS2/16. Each passage is indicated by either a ▲, ■, ● and individual passage ratio's are 1:3.

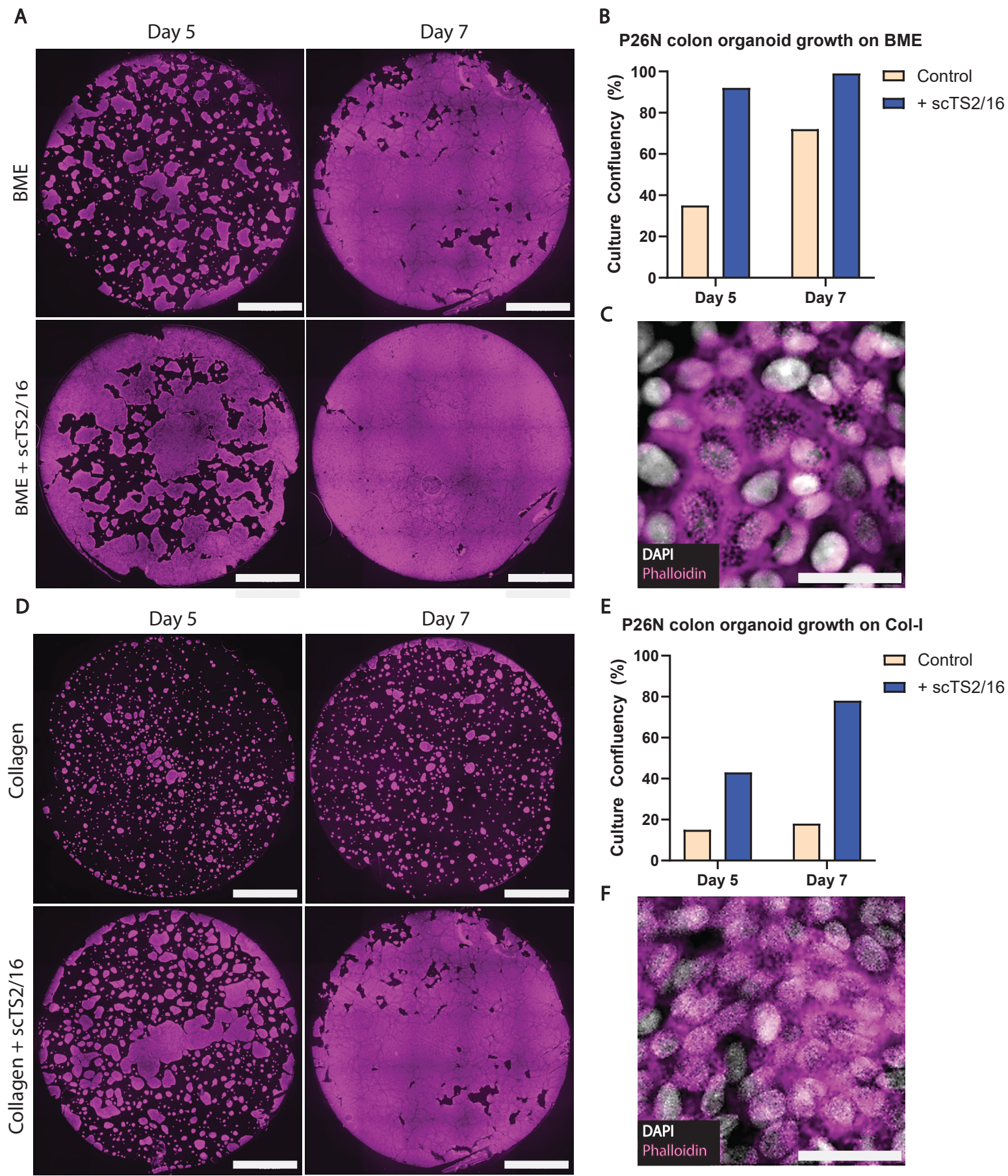
Supplementary Figure 11.



Supplementary Figure 11.

Emerging number of cholangiocyte organoids (N=2) from primary normal liver samples in PureCol EZ hydrogel in the presence and absence of scTS2/16.

Supplementary Figure 12.



Supplementary Figure 12; Growth of P26N colon organoids on BME- and Collagen-type I coated Transwell inserts.

A) F-actin Phalloidin staining of two-dimensional growth on BME at day 5 and day 7, for both control (no treatment) and treatment with 5 nM scTS2/16. Scale bar = 0.25 cm.

B) Graphic representation of growth, indicated as percentage confluency of F-actin stained tissue, for the BME comparison.

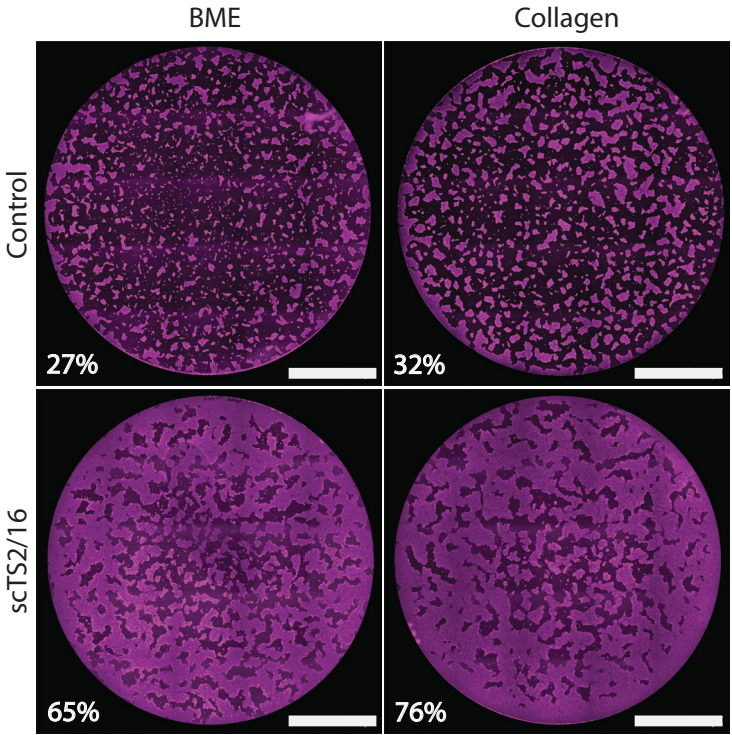
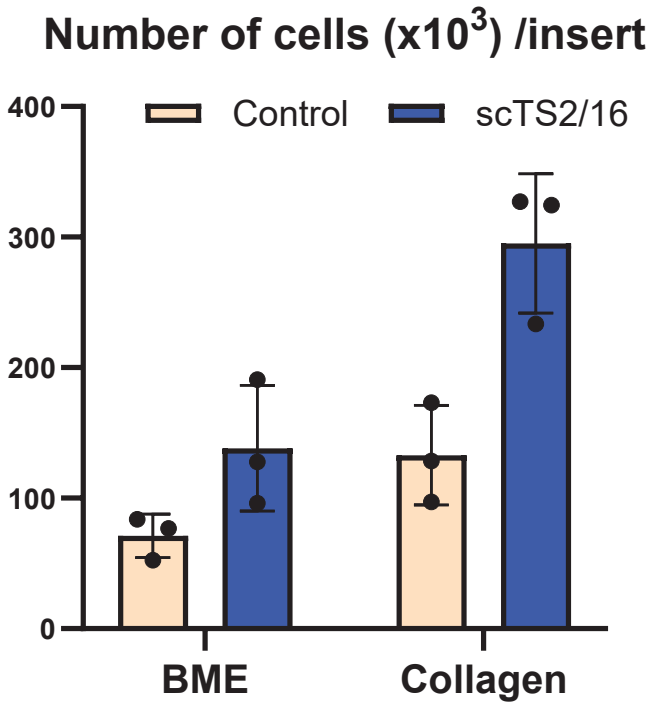
C) Higher magnification image of F-actin-stained BME-grown organoids treated with scTS2/16. Scale bar = 100 μm .

D) F-actin Phalloidin staining of two-dimensional growth on collagen at day 5 and day 7, for both control (no treatment) and treatment with 5 nM scTS2/16. Scale bar = 0.25 cm.

E) Graphic representation of growth, indicated as percentage confluency of F-actin stained tissue, for the collagen comparison.

F) Higher magnification image of F-actin-stained collagen-coated organoids treated with scTS2/16. Scale bar = 100 μm .

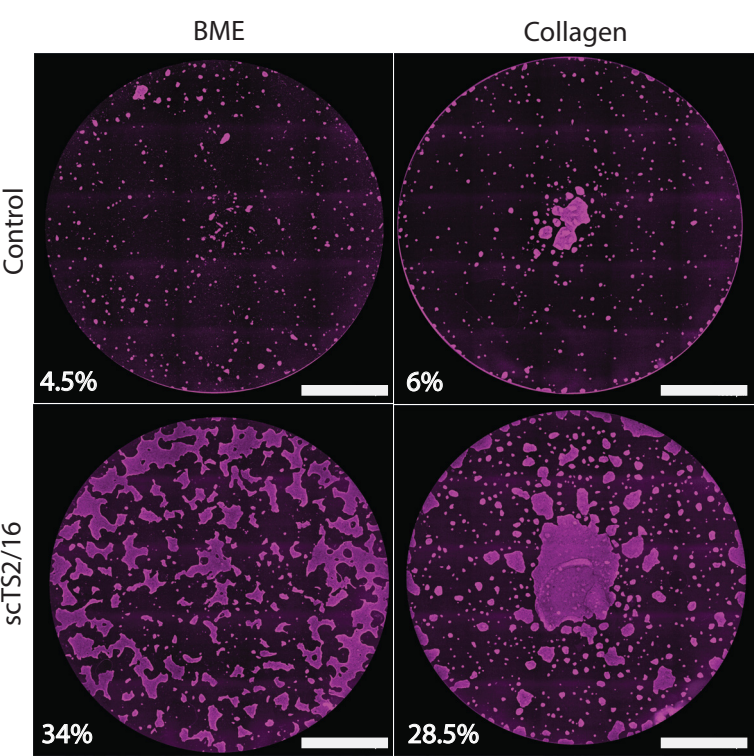
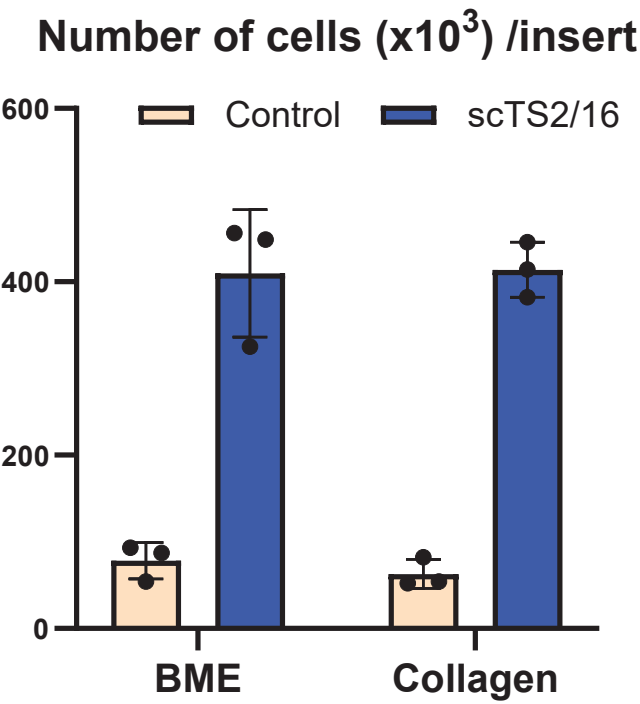
Supplementary Figure 13.



Supplementary Figure 13; Growth of duodenum-72 organoids on BME- and Collagen-type I coated transwell inserts.

The presented graph illustrates cell counts from three biological replicates in both control and scTS2/16-supplemented cultures. Means and standard deviations are provided. P-values for the one-tailed T-test assessing the effect of scFv are 4.5×10^{-2} for BME and 5.8×10^{-3} for collagen, respectively. The right panel displays F-actin-stained tissue samples, with one replicate per condition (scale bar = 0.25 cm) . Organoid growth is further quantified by the percentage of surface area exhibiting F-actin staining.

Supplementary Figure 14.



Supplementary Figure 14; Growth of J2 jejunal organoids on BME- and Collagen-I coated transwell inserts.

Graph shows cell count per insert ($n = 3$). Mean $\pm$ STDEV are shown. P-values for scTS2/16 effect: 9.4×10^{-4} (BME) and 3.4×10^{-5} (collagen). Right panel: F-actin staining and surface area percentage of tissue per insert (scale bar = 0.25 cm) .