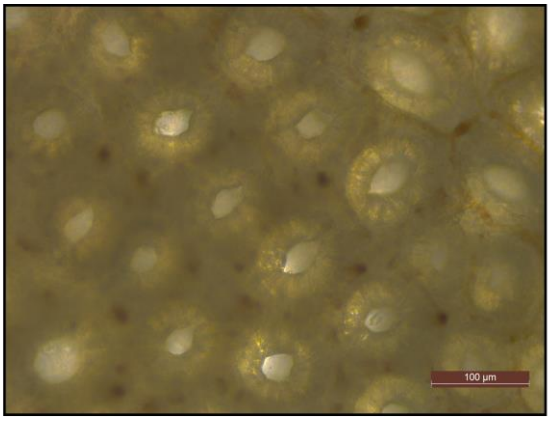
Supplementary materials:

Supplemental Table 1. IHC antibody, dilution and incubation time.

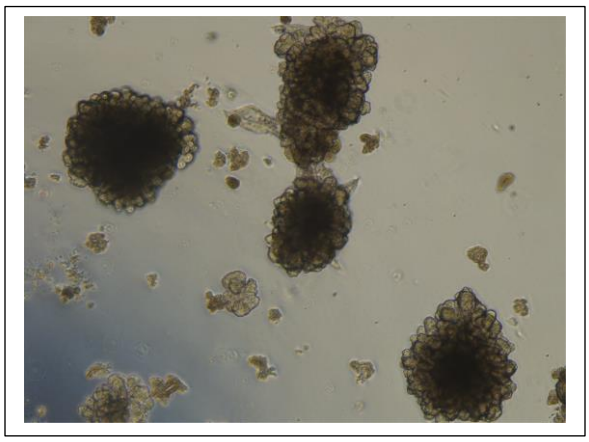
Primary Ab	Source	Pretreatment	Dilution	Incubation	Secondary Ab
Keratin (alt)	Dako	Tris-EDTA pH 9.0, 20 min steam	1:100	90 min	goat anti-mouse (Multi-Link)
T-cell (CD3)	Dako	Tris-EDTA pH 9.0, 20 min steam	1:100	90 min	goat anti-rabbit Multi-Link)
Vimentin	Dako	Citra pH 6.0, 5 min	1:500	30 min	goat anti-mouse (Multi-Link)
Chromogranin A	Santa Cruz	Tris-EDTA pH 9.0, 20 min steam	1:100	60 min	Vector, Gt. ABC kit
CD117, c-kit	Dako	Tris-EDTA pH 9.0, 20 min steam	1:500	120 min	goat anti-rabbit (Multi-Link)
Actin (sm)	BioGenix	None	1:200	30 min	goat anti-mouse (Multi-Link)
Lysozyme	Dako	Tris-EDTA pH 9.0, 20 min steam	1:100	90 min	goat anti-rabbit Multi-Link)

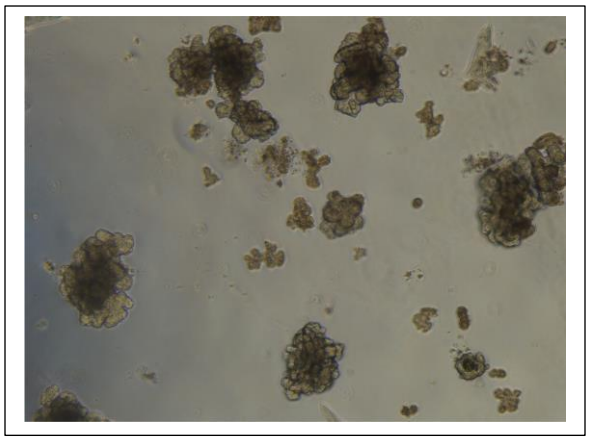
Crypt cell release from tissue



Supplemental Figure 1: Appearance of holes after EDTA incubation indicates release of crypts from intestinal tissue. Representative image of colon tissue after EDTA incubation, showing apparent holes and dense cellular spheroids by phase contrast microscope (20X magnification).

Recovery cell freezing media **90% FBS+10% DMSO media**





Supplemental Figure 2: Comparison of medium for cryopreservation. Representative images of fully differentiated canine enteroids by phase contrast microscope (5X magnification). There was no discernable difference in quality or quantity of organoids recovered after freezing with either commercial cell freezing media (Invitrogen) or 90% FBS with 10% DMSO.

Supplemental methods:

Reagent and ISC media composition:

Composition of 5X CCS (500mL complete chelating solution):

500 mL MilliQ H_2O supplemented with 2.49 g Na_2HPO_4-$2H_2O$, 2.7 g KH_2PO_4, 14 g NaCl, 0.3 g KCl, 37.5 g Sucrose, and 25 g D-Sorbitol.

Composition of 1xCCS (50mL Complete Chelating solution):

40 mL sterile MilliQ H_2O supplemented with 10 mL incomplete chelation solution (5X) and 26ul 1M DTT.

Enteroid Medium:

Composition of CMGF- (500ml complete media without growth factor):

500 mL Advanced DMEM/F12 supplemented with 5 mL Glutamax (100X), 5 mL HEPES (1M) and 1 mL Primocin

Composition of CMGF+ (complete media with growth factors):

40 mL CMGF- supplemented with 800 ul B27 (50X), 400 ul N2 (100X), 80 ul n-acetylcysteine (500mM), 40 ul EGF (1000X: final conc. 50 ng/mL), 40 ul Noggin (1000X: final conc. 100 ng/mL), 40 ul R-spondin-1 (1000X: final conc. 500 ng/mL), 80 ul Wnt 3a (500X: final conc. 100 ng/mL), 40 ul Gastrin (1000X: final conc. 10 nM), 400 ul Nicotinamide (final conc. 10 mM), 4 ul A83-01 (TGFβ type I receptor inhibitor; 10,000X: final conc. 5 mM), 8 ul SB202190 (P38 inhibitor; 5000X: final conc. 50uM) and 8% FBS.