normoxia; H, hypoxia; D1, Day 1 of culture; D2, Day 2; D3, Day 3, D5, Day 5. Values represent mean ± SEM. Two-way ANOVA with Tukey-Kramer pair-wise comparison test.

Figure 6. Schematic summary of *ex vivo* organoid culture experiments. *a*) In the presence of R-spondin 1 and Noggin, addition of HB-EGF leads to increased crypt-villous organoid growth, with promotion of crypt fission and TA zone/villous domain expansion. *b*) In the presence of R-spondin 1 alone, R-spondin 1 plus Noggin, or R-spondin 1 plus Noggin plus HB-EGF in the presence of MEK 1/2 inhibition, crypt-villous organoid growth is limited to small spherical organoids. *c*) In the presence of Noggin alone, HB-EGF alone, or R-spondin 1 plus Noggin plus HB-EGF in the presence of either EGFR or PI3K inhibition, crypt-villous organoid growth is completely abolished. Thus, EGFR and PI3K activation are crucial, and MEK 1/2 activation is important, in HB-EGF-mediated crypt-villous organoid growth. ISCs, green; progenitor cells in TA zone, yellow; paneth cells, brown; enterocytes, blue; goblet cells, red. N, Noggin; RS, R-spondin 1.

SUPPLEMENTARY VIDEOS AND FIGURES

Supplementary Figure 1. Low magnification microscopic images of anti-LGR5 and anti-prominin-1 double immunostaining of ISCs (white arrows) in the intervillous regions of uninjured breast-fed rat pups. ISCs were stained with: *a*) anti-LGR5 (FITC, green); *b*) anti-prominin-1 (Cy3, red); *c*) DAPI nuclear staining (blue); and *d*) merged image. The white dashed lines indicate the base of the mucosal epithelium.

Supplementary Figure 2. Human intestine resected for NEC has loss of ISC staining. Shown are representative photomicrographs of LGR 5 immunostaining of human intestine resected for: *a*) ileal atresia (gestational age 28 weeks, 10 days of life) and *b*) NEC (gestational age 28 weeks, 5 days of life). ISC are indicated by white arrows.

Supplementary Figure 3. HB-EGF protects purified ISCs from hypoxia. (A) Isolation of intervillous epithelia. Villi were separated from intervillous regions as described in Methods. (B) MACS isolation of ISCs. Prominin-1 antibody-conjugated magnetic bead purification was used to purify ISCs. Shown are representative photomicrographs of cells stained for: *a, e*) DAPI nuclear staining; *b, f*) LGR5; *c, g*) prominin-1; *d, h*) merged images. *a-d* represent MACS flow-through fractions; *e-h* represent MACS eluate fractions. (C) Flow cytometric analysis of MACS eluates. MACS-isolated cells were labeled with anti-prominin-1 or anti-LGR5 and analyzed by flow cytometry. Left panel: unstained cells (grey) and cells labeled with prominin-1 antibody (red), with secondary antibody conjugated with Cy3. Right panel, unstained cells (grey) and cells labeled with anti-LRG5 (green), with secondary antibody conjugated with FITC. ~80% of the MACS eluate fractions consist of cells that stain positively for prominin-1 and LGR5. (D) HB-EGF protects prominin-1^{+} ISCs from hypoxia. ISC viability is represented as the % of viable ISCs exposed to normoxia in the absence of HB-EGF, which was arbitrarily designated as 100% viability. NA, medium containing R-spondin 1 and Noggin with no addition of HB-EGF; HB-EGF, medium containing R-spondin 1 and Noggin with HB-EGF (100 µg/ml). Values represent mean ± SEM. One-way ANOVA with Tukey-Kramer pair-wise comparison test.

Supplementary Figure 4. Schematic illustration of HB-EGF signaling in ISCs. The Wnt agonist R-spondin 1 promotes the canonical β-catenin pathway that leads to the expression of c-Myc, Cyclin-D1, growth factors, EGFR and LGR5. HB-EGF and other growth factors, via activation of

EGFR and other TRKs, may provide autocrine signaling that activates the important PI3K/AKT pathway that is complementary to β-catenin signaling. The BMP inhibitor Noggin suppresses PTEN and augments PI3K signaling, thus activating the β-catenin pathway. HB-EGF promotes ISC viability and proliferation through EGFR/PI3K and EGFR/MEK1/2 signalings. Abbreviations: *BMP*, bone morphogenic protein; *BMPR1A*, BMP receptor 1A; *CREB*, cAMP resoponse element-binding protein; *EGFR*, epidermal growth factor receptor; *ETS*, E-twenty-six transcription factor; *FGF*, fibroblast growth factor; *GSK3β*, glycogen synthesis kinase 3β; *LRP*, low-density lipoprotein-receptor related protein; *PI3K*, phosphatidylinositol 3-kinase; *PTEN*, phosphotase and tensin homolog; *RTK*, receptor tyrosine kinase; *TCF*, T-cell factor; *VEGF*, vascular endothelial growth factor; *Wnt*, wingless.

Supplementary Video 1. 3D movie showing confocal reconstruction of intervillous ISCs. ISCs were stained with anti-LGR5 (FITC, green) and anti-prominin-1 (Cy3, red). DAPI, nuclear staining (blue).

Supplementary Video 2. Time lapse recordings of (A) a proliferative crypt forming an organoid (days 0-1) and (B) a degraded crypt cultured from days 1-3.