Supplemental Information for:

Protein-Engineered Scaffolds for *In Vitro* 3D Culture of Primary Adult Intestinal Organoids

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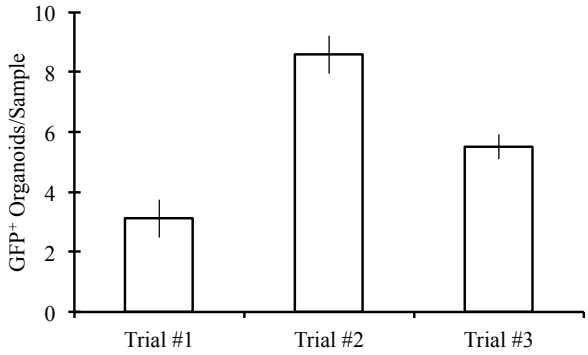

Figure S1. Average organoid counts across multiple independent harvests for primary adult murine intestinal organoids cultured under air-liquid interface configuration for 3 days. Average counts ranged from 3 to 9 organoids per 25 μL collagen gel. Data is shown as mean ± SEM.

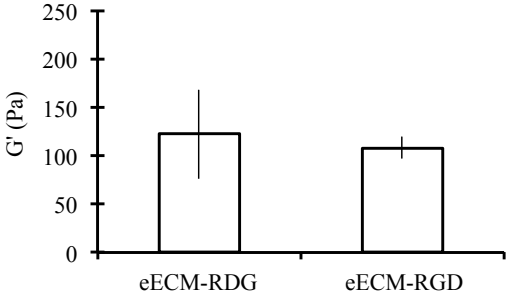

Figure S2. Amino acid sequence within the bioactive domain of eECM did not significantly impact mechanical stiffness, as indicated by equivalent storage moduli, G', measured by oscillatory rheology for RDG- and RGD-containing eECM matrices. Data is shown as mean ± SEM.

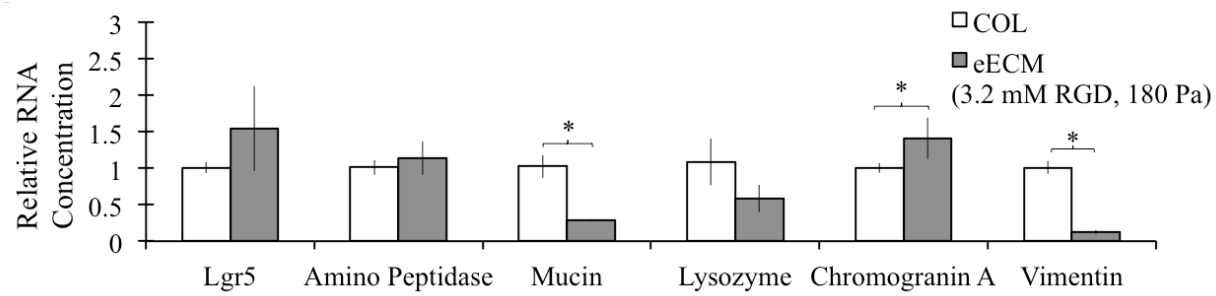

Figure S3. Culture within eECM matrices (3.2 mM RGD, 180 Pa) resulted in enhanced expression of the enteroendocrine marker chromogranin A. Expression of goblet cell marker mucin-2 as well as the myofibroblast marker vimentin were reduced within eECM matrices relative to collagen. No statistical differences were observed in the stem cell marker Lgr5, the intestinal brush border cell marker amino peptidase, and the Paneth cell marker lysozyme. Data is shown as mean ± SEM. *indicates statistical significance (Student's t-test, $p<0.05$).

RNA was isolated from cultures using TRIzol reagent (Life Technologies), and cDNA was synthesized using the High Capacity Reverse Transcription kit (Applied Biosystems), both according to manufacturers' protocols. Quantitative real-time PCR was performed using SYBR Green (Applied Biosystems) on a Step One Plus (Applied Biosystems) instrument, and results were normalized to 18S as endogenous control.