SUPPLEMENTARY INFORMATION

METHODS

Procurement and histopathology of intestinal tissues

Full thickness freshly resected intestinal specimens from subjects with CD and controls, comprising of ulcerative colitis (UC), diverticular disease and apparently healthy tissue (constipation, healthy margin of resections from colorectal cancer patients; termed NL for normal) were procured as previously described[1, 2, 3, 4]. CD specimen were classified based on gross anatomy into strictured (CD_S) and non-strictured (CD_{NS}). CD_{NS} represented purely inflammatory disease without the presence of internal penetrating disease. The CD_S resections included strictures with and without the co-presence of internal penetrating disease (Montreal B2 and B3). We did not find any functional differences within the CD_S group when analyzing both phenotypes separately and hence strictures with and without the presence of internal penetrating disease were combined. Cell isolation was only performed from the area of the stricture. The presence of mesenteric fat was recorded and CF, as opposed to mesenteric fat, was defined by the presence of broad-based fat tissue on the anti-mesenteric serosal aspect of the resected intestine on gross examination[5] *or* in case the anti-mesenteric serosal side was not available on the resection specimen, CF was defined by its aspect of obliteration or loss of the bowel-mesenteric angle[6]. Fat was classified into CF or mesenteric fat. CF can occur in the absence of stricture formation in CD and we were not able to examine this group separately as our approach is bound to the use of resection specimen and in our set CF was exclusively linked with CDs. All but two CD stricture tissues were derived from small bowel resection whereas the controls were derived from the colon. We paid special attention to possible differences between colon and small bowel isolates and all

isolated cells behaved the same and were hence reported in a combined fashion. Our procurement system was validated by histopathologic evaluation performed by a trained IBD pathologist (IOG). Representative histopathologic sections to include the interface of fat and MP were obtained, formalin fixed and embedded in paraffin as previously described[1].

5 µm sections were prepared and slides were stained with hematoxylin and eosin (H&E), Masson trichrome (MT) for connective tissue, movat pentachrome (MOVAT) for various constituents of connective tissue and sirius red for the collagen networks by the standard methods used in the Anatomic Pathology Department of the Cleveland Clinic.

The thickness and area of the matrix scaffold was measured with a DP2-SAL digital camera (Olympus, Tokyo, Japan). Thickness comprised of the three thickest areas of the scaffold perpendicular to the MP. Area was the area of the ECM scaffold within the entire cross section. The thickness and area were analyzed in a blinded fashion and were confirmed by an independent second observer, as reported previously [7].

Isolation and culture of primary human intestinal cells

Primary human intestinal muscularis propria muscle cells (HIMC) were obtained from surgically resected intestinal specimen and isolated based on a previously described method used for esophageal muscularis propria muscle cells [8]. Briefly, after removal of mucosa/submucosa and serosa by sharp mechanical dissection, muscle strips of the MP were procured and their purity (lack of contamination by other tissue types) confirmed by H&E staining (Supplementary Figure 3). Subsequently, strips were incubated in Hank's Balanced Salt Solution (HBSS) with 2500 U potassium penicillin, 2500 µg streptomycin sulfate, 625 µg Amphotericin B (PSF, Lonza, Basel,

Switzerland) for three hours. Strips were then minced into small pieces (2-3 mm^2) using a scalpel and incubated overnight in Dulbecco's modified Eagle's medium (DMEM, Cleveland Clinic media core, Cleveland, OH) supplemented with 10% fetal bovine serum (FBS, Sigma), 2.5% penicillin, streptomycin and fungizone solution (Lonza), 0.1mg/ml DNAse (Worthington, Lakewood, NJ, USA) and 0.1mg/ml collagenase type II (Worthington). After 12 hours digestion with gentle stirring at 37ºC, the digested MP pieces were filtered through a 100 µm cell strainer (Thermo Fisher Scientific, Waltham, MA, USA) and subsequently centrifuged. Cell pellets were resuspended in culture medium (DMEM, 10% FBS, 1000 U potassium penicillin, 1000 µg streptomycin sulfate, 250 µg Amphotericin B, 4mM L-Glutamine and 25mM HEPES (all Lonza)), plated in 25 cm^2 tissue culture flasks and long-term cultures were established, fed twice a week and sub-cultured at confluence[1]. Purity of HIMC cultures was ensured by characterization in immunofluorescence (IF) using specific antibodies as described below. HIMC expressed vimentin, α-smooth muscle actin (SMA), desmin and Sm22, but were negative for vascular endothelial (VE)-cadherin and epithelial (E)-cadherin (Supplementary Figure 4). HIMC were used between passage 3 and 8 to avoid de-differentiation and senescence on tissue culture plastic [9, 10]

Mesenteric pre-adipocytes were isolated as described previously by a protocol kindly provided by Dr. Pothoulakis[11]. In brief, pieces (5 g wet weight) of mesenteric fat or CF were removed from the area right adjacent to the MP by sharp dissection and washed extensively with HBSS to remove debris and subsequently minced into an enzyme solution consisting of HBSS with 4% fatty acid free bovine serum albumin (BSA, Sigma), 2500 U potassium penicillin, 2500 µg streptomycin sulfate, 625 µg Amphotericin B (Lonza) and 0.1 mg/ml collagenase type II (Worthington). Minced fat was then vortexed for 30 seconds in a 50 ml tube (Falcon), and incubated for 50 minutes with

30 seconds vortexing every 10 minutes. Digested samples were filtered through a 100 µm cell strainer (Thermo) and centrifuged to separate adipocytes (floating on surface) from stromal cells (pellet). The cell pellet containing the stromal fraction was resuspended in stromal culture medium consisting of minimum essential medium (MEM) (Thermo), containing 10% FBS, 2.16 g/L NaHCO3 (Thermo), 25mM HEPES (Lonza), 1000 U potassium penicillin, 1000 µg streptomycin sulfate, 250 µg Amphotericin B (Lonza). Pre-adipocytes exhibited fibroblast morphology, were plated in 25 cm^2 tissue culture flasks and long-term cultures were established, fed twice a week and sub-cultured at confluence. pre-adipocytes cultures were characterized first by flow cytometry using specific antibodies as described below. Isolated cells were >99% positive for CD73, CD90, and CD105, but were negative for CD34, CD45, CD11b, CD14, CD19, CD79α or HLA-DR, characterizing them as adipose mesenchymal stem cells[12](Supplementary Figure 12). In addition, IF analysis revealed positivity for Preadipocyte factor 1 (Pref-1) and vimentin, low expression of CCAAT/enhancer-binding protein (CEBP)α, and cells were negative for fatty acid-binding protein 4 (FABP4), α-SMA, desmin, E-cadherin and VE-cadherin. (Supplementary Figure 12A&B) [13, 14]. This established the isolated cell type as pre-adipocytes. Pre-adipocytes were used between passage 3 and 5.

To generate mesenteric adipocytes, in select experiments pre-adipocytes were grown to confluence and their culture medium was changed to adipocyte differentiation medium [DMEM supplemented with 15 mM HEPES (Lonza), 15 mM NaHCO3 (Thermo), 2 mM glutamine (Thermo), 10 mg/L transferrin (Sigma), 33 µM biotin (Sigma), 0.5 µm human insulin (Sigma), 17 µm pantothenate (Sigma), 540 µM 3-Isobutyl-1-methylxanthine (IBMX; Sigma), 0.1 µM dexamethasone (Sigma), 1 mg/ml fetuin (Sigma), 1 µm ciglitazone (Sigma) and 2 nM T3 (3,3′,5-Triiodo-L-thyronine,

Sigma)] [15], which was refreshed twice a week. Intracellular lipid droplets were observed within 14 to 21 days after addition of adipocyte differentiation medium, as shown by Oil Red O lipid staining (performed according to the manufacturer's protocol (Newcomer Supply, Middleton, WI, USA; Supplementary Figure 12C).

Cell culture and induction of matrix production

40.000 HIMC were plated in 24-well cluster plates, incubated with serum-free medium for 24h and stimulated with cytokines or serum-free medium for 72h. After determining optimal stimulatory conditions cytokines were used at the following concentrations: IL-1β, 100U/ml (Peprotech, Rocky Hill, NJ, USA); TNF-α (Peprotech) 100 U/ml; b-FGF (Peprotech), 10ng/ml; TGF-β1, 5 ng/ml (Peprotech); ligands specific for TLR2/6 (synthetic diacylated lipoprotein FSL-1, Peprotech, 1ug/ml), TLR4 (LPS, Peprotech, 1ug/ml) and TLR5 (flagellin from S. Typhimurium, FLA-ST, Peprotech, 10ng/ml) and NOD1 (γ-D-Glu-m dipeptide, DAP, Peprotech, 20ug/ml). Supernatants were harvested and stored at -80ºC for later use in proteomics and migration experiments. For some experiments the supernatants were size fractionated as previously reported[16] using 300, 100, and 10 kDa filters (Nanosep membrane, Pall Corporation, NY, USA) following manufacturers protocols. The obtained filtrates were used for trans-well migration assay. In select cases, immunoprecipitation (IP) of FN in HIMC conditioned medium was performed using IP assay kits (Abcam) according to manufacturer's protocol. HIMC conditioned medium was mixed with G-Sepharose beads that were anti-FN antibody coated (Abcam) and incubated overnight. For the negative control group, the conditioned medium was treated similarly, but was incubated with isotype antibody. The FN-depleted HIMC conditioned medium was collected and

used in transwell-migration assays. The protein G-Sepharose beads with bound FN were washed and boiled for 5 min in lysis buffer. The lysate was used for immunoblot of FN.

Matrisome analysis of human intestinal muscularis propria muscle cell conditioned medium

For liquid chromatography mass spectrometry (LC-MS), HIMC conditioned medium was filtered using a 3K Amicon Ultra 0.5mL centrifugal filter (MilliporeSigma, Burlington, MA, USA), dried and reconstituted in 6M urea buffer, reduced with dithiothreitol (DTT), alkylated with iodoacetamide, incubated with Trypsin/Lys-C Mix (25:1 protein:protease ratio (w/w); Promega, Madison, WI, USA), for 3–4 hours at 37°C and diluted 6-fold with 50 mM Tris-HCl (pH 8). Digestion was continued overnight at 37°C, terminated by adding trifluoroacetic acid (TFA) to a final concentration of 0.5-1%, desalted using a PepClean C18 spin column (Thermo) and resuspended in 1% acetic acid for LC-MS analysis (ThermoScientific Fusion Lumos system) using a Dionex 15cm x 75µm id Acclaim Pepmap C18, 2µm, 100Å reversed- phase capillary chromatography column. 5µL volumes of the extract were injected and the peptides eluted from the column by an acetonitrile/0.1% formic acid gradient at a flow rate of 0.3µL/min were introduced into the source of the LC-MS on-line[17]. The digest was analyzed by using all CID/HCD spectra collected in the experiment to search the human UniProtKB database with the search programs Sequest bundled in Proteome Discoverer 2.2. The protein FDR rate was set to 1%. Label free quantitation intensities were determined using PD2.2.

Wound healing assay of pre-adipocytes on human intestinal muscularis propria derived extracellular matrix

Two dimensional (2D) ECM scaffolds were generated as previously described[18]. Briefly, 400.000 HIMC were plated per well into a 6-well plate, serum deprived overnight, incubated for 14 days with media changes every three days, then washed with HBSS and sequentially exposed to 0.5% Triton-X in PBS at 37°C for 10 minutes followed by 0.025N ammonium hydroxide for 2 minutes at room temperature to lyse and remove the cells while leaving the ECM intact. The residual ECM adherent to the petri-dish surface was then washed 4 times with Ca^{2+}-and Mg^{2+} containing PBS. ECM plates were stored in PBS at 4°C for up to one week (Supplementary Figure 1). The 2D HIMC generated ECM was seeded with pre-adipocytes (25,000 cells per well) into pre-placed IBIDI inserts for overnight incubation (Ibidi GmbH, Martinsried, Germany). After removal of the inserts a cell-free gap of a defined size remained. Fresh serum-free medium was added and gap closure observed at 0, 24 and 48 h, which was quantified using 100x magnification under a Leica microscope (Leica, Wetzlar, Germany) and Image J (NIH).

Immunostaining

Immunostaining was performed as previously described [1, 19]. Briefly, formalin fixed paraffin-embedded (FFPE) intestinal sections of histologically normal control and IBD-involved tissue were cut at 3 µm thickness, deparaffinized then hydrated, blocked for endogenous peroxidase using 3% H_2O_2/H_2O, and subsequently subjected to microwave epitope enhancement using a Dako Target retrieval solution (Dako) at pH 10.00. Non-specific binding was blocked with 3% FBS for 1 hour. The following antibodies were used: Mouse anti-human α-smooth muscle actin at a dilution of 1:25 (Sigma, St. Louis, MO, USA); rabbit anti-human fibronectin (FN) at 1:100 (Abcam, Cambridge, UK); rabbit anti-human collagen I (Col I) at 1:100 (Rockland, Gilbertsville, PA, USA); rabbit anti-human collagen III (Col III) at 1:100 (Rockland, Gilbertsville, PA, USA). Slides were

rinsed three times with PBS and then AlexaFluor 488 or 594 antibody (1:500; Thermo Fisher Scientific, Waltham, MA, USA) was applied for 1 hour at 37ºC. For nuclear counterstaining Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA) was used. Slides were analyzed using an Olympus microscope, ImagePro (Media Cybernetics, Rockville, MD, USA) and Image J software (Bethesda, MD, USA). Tile scan of the tissue slides after IHC was acquired using 20x objective on Leica DM6 B upright microscope equipped with a Leica DFC7000T camera and LAS-X software (Leica Microsystems, GmbH, Wetzlar, Germany).

Immunofluorescence

20,000 cells/well were seeded onto glass 4-well or 8-well chamber slides (Nunc, Naperville, IL, USA). Cells were treated as described in detail in each respective experiment, then slides were rinsed in PBS and fixed with either ice-cold acetone or 4% paraformaldehyde at room temperature for 10 minutes. Before application of the primary antibody, fixed cells were blocked with 3% FBS (Lonza) in PBS. All primary and secondary antibodies were diluted in 1% FBS (Lonza). In the cell monolayers fixed with paraformaldehyde, 0.1% Triton X (Sigma) was used in conjunction with the primary and secondary antibodies. The following antibodies were used: Mouse anti-human α-smooth muscle actin at a dilution of 1:25 (Sigma); rabbit anti-human FN at 1:100 (Abcam); rabbit anti-human Col I at 1:100 (Rockland); rabbit anti-human Col III at 1:100 (Rockland); rabbit anti-human Decorin at 1:100 (Abcam); mouse anti-human Pref-1 at 1:100 (Abcam); rabbit anti-human CEBPα at 1:100 (Abcam) and rabbit anti-human FABP at 1:100 (Abcam). The respective antibody isotypes (Sigma and Santa Cruz) were used as controls for the primary antibody. After 2 h incubation with the primary antibody at room temperature, slides were rinsed three times with PBS and the AlexaFluor 488 or AlexaFluor 594 antibody (Molecular Probes, Eugene, OR) was added

at a dilution 1:500 for 1 hour at 37ºC. For nuclear counterstaining Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA) was used. After staining, the Leica DM2000 microscope (Leica, Wetzlar, Germany) and QImaging® Retiga-2000R digital camera (Teledyne QImaging, British Columbia, Canada) were used to capture images.

Transfection of cell lines

HIMC were transfected with FN1 small interfering RNA (siRNA) and their respective scrambled siRNA (Thermo) using the Amaxa system (Amaxa Biosystems, Köln, Germany). HIMC (0.5 x 10^6) were suspended in 100 µl Nucleofector solution for mesenchymal cells (Lonza), mixed with different concentrations of FN1 or scrambled siRNA, transferred to a cuvette, and nucleofected according to the manufacturer's instructions. Cells then were transferred into 6-well plates containing pre-warmed culture medium. Some cells were left in Nucleofector solution without applying electroporation to serve as negative control. Optimal doses and time points for FN1 knockdown were predetermined by immunoblot using antibodies as described above. HIMC with and without FN1 knockdown were used to generate supernatants for migration experiments as described under cell culture and induction of matrix production.

Migration Assay

Migration assays were performed in the modified 48-well Boyden chamber (Neuro Probe, Gaithersburg, MD, USA) as described previously [2]. A polycarbonate filter (12 µm pore size, polyvinylpyrrolidone-free; Gerbu Biotechnik, Germany) divided the chamber into an upper and a lower compartment. Each test substance was placed into the wells of the lower compartment in triplicates. Culture media alone (DMEM high glucose medium with 1% BSA; Sigma) was used as

a negative control. 20.000 pre-adipocytes or adipocytes per well in DMEM with 1% BSA were seeded into the wells of the upper compartment of the Boyden chamber and incubated at 37°C in 5 % CO_2 atmosphere for 6 hours. The filter was removed from the chamber, and the non-migrated cells on the upper side of the filter were scraped off with a rubber policeman. Migrated cells on the lower side of the filter were fixed and stained with Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA) and automatically counted at a 100-fold magnification and quantified using ImagePro (Media Cybernetics). The total sample size for each individual experiment consisted of at least 3 replicate migration assays for each patient`s pre-adipocytes and adipocyte. Each experiment was repeated at least 3 times.

Quantitative analysis of cell migration

Time-lapse microscopy was performed at 10x magnification with images captured at 15-min intervals for 24 h using a protocol modified from our previous study[20]. Time-lapse recordings were acquired with Leica DMI6000 inverted microscope and LAS X software (Leica, Wetzlar, Germany) equipped with a Hamamatsu Image EM-CCD camera, and a Hamamatsu Flash4 camera, PECON Large Chamber Incubator, heating unit 2000, CO_2 controller, and TempControl 37-2 (PreCon, Germany). Images were transformed into videos by LAS X software, and quantified by ImagePro Plus software (Media Cybernetics). Cell migration distance, displacement, and velocity were calculated for each cell over the 24 h time period. Directionality indices (ratio between the distance between start and end time points in a straight line and the total length of the migratory path) were calculated [21]. Cell coordinates obtained using ImagePro were imported in MATLAB to generate graphs of XY projections of cell migration tracks [22]. More than 50 cells for each condition were analyzed in three independent experiments.

Immunoblotting

Protein extraction was performed using a lysis buffer containing 50 mM TRIS pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1% Na-deoxycholate and 1% protease and phosphatase inhibitor cocktail (all Sigma). The concentration of proteins in each lysate was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) according to manufacturer's recommendations. Immunoblotting was performed as previously described[1]. Equivalent amounts of proteins (20 μg) were fractionated on a 6 or 8% Tris-glycine gel and electrotransferred to a PVDF membrane (Millipore, Billerica, MA). Nonspecific binding was blocked by incubation with 5% milk or 5% BSA in 0.1% Tween 20/Tris-buffered saline (Fisher Scientific, Hanover Park, IL) for 30 min., followed by overnight incubation at 4°C with the primary antibody(s). The following antibodies were used: FN at 1:1000 (BD). Membranes were washed 6 times with 0.1% Tween 20/Tris-buffered saline, incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Sigma), washed again, and incubated with the chemiluminescent substrate (Super Signal; Pierce, Rockford, IL) for 5 minutes, after which they were exposed to film (Kodak).

Enzyme-linked immunosorbent assay

The FN amount in HIMC conditioned media was measured using a commercially available enzyme-linked immunosorbent assay (ELISA; Abcam) according to the manufacture’s protocol.

Flow cytometry analysis

Cell surface expression of pre-adipocytes integrins known to bind FN[23], including α3, α5, αv, αIIb, α8 and β1, β3, β5, β6, and β8 were determined by flow cytometry as previously described[24].

Briefly, single pre-adipocyte suspensions were obtained using a gentle detachment solution (BD Biosciences). Pre-adipocytes were then incubated with human FC block antibody (BD Biosciences) for 10 minutes on ice, followed by incubation with integrin antibody for 30 minutes on ice. Subsequently, cells were washed twice, fixed in 1% paraformaldehyde and integrin expression on the pre-adipocytes surface was analyzed by flow cytometry via a FACS LSR II (BD Biosciences) and FlowJo software (TreeStar, Ashland, OR). Similarly, human mesenchymal stem cell analysis kit (BD Biosciences) including antibodies of CD73, CD90, CD105, CD34, CD45, CD11b, CD14, CD19, CD79α and HLA-DR was used to characterize pre-adipocytes and cells migrated from mesenteric fat.

Additionally used reagents and blocking antibodies

The following blocking antibodies, antagonists and regents were used to inhibit the migration of pre-adipocytes including Boyden chamber migration assay, wound healing assay, *ex vivo* model of human mesenteric fat outgrowth and migration of pre-adipocytes on decellularized human intestine *ex vivo:* anti-integrin β1 (Millipore Sigma), anti-α5 (Abcam), αv antagonists (MedChemExpress, Monmouth Junction, NJ, USA), anti-α3 (Millipore Sigma), GRGDSP (Sigma), GRADSP (Sigma), focal adhesion kinase (FAK) inhibitor (Sigma).

Ethical Statement

Tissues were obtained from histologically normal large-bowel specimens from patients admitted for bowel resection because of malignant and nonmalignant conditions, including colon cancer (healthy margin), benign polyps (non-affected tissue), and diverticulosis. Involved and non-involved CD and UC colonic tissues were also obtained. All diagnoses were confirmed by clinical,

radiologic, endoscopic and histologic criteria. The Institutional Review Board of the Cleveland Clinic approved the human studies.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) for independent groups. Repeated measures for the same experiment were analyzed by using Student's paired t test. Values were expressed as mean ± SEM, and statistical significance was set at $p<0.05$. Analyses were performed using SAS (version 9.3; The SAS Institute Inc., Cary, NC). Relative protein abundance (proportion) was calculated from the LFQ values. Systems levels analyses of proteomics data were conducted using open-access statistical programming language R (version 4.0.1), and specific packages including *ggplot2* to create barplots (part of *tidyverse*), *pheatmap* to create heatmap, *vegan* for diversity (Shannon index) analyses, *ggord* and base R for PCA, *venn* and *RVenn* for creating venn diagrams, and *ggcorrplot* to create corellograms. R Package *PMCMRplus* was used to conduct pairwise multiple comparisons of mean for different datasets (Dunn's non-parametric all-pairs comparison test for Kruskal-type ranked data) with bonferroni correction of multiple P-values. For multiple comparisons, adjusted P-value < .05 was considered statistically significant.

RESULTS

Preadipocytes migrate out of mesenteric fat onto muscle cell-derived ECM

Flow cytometry analysis of the spindle-shaped cells migrating out of fat tissue in response to HIMC-generated ECM showed these cells were > 99% positive for CD90, CD73 and CD105, and negative for CD34, CD11b, CD19, CD45 and HLA-DR (Figure 3D), identifying them as adipose mesenchymal stem cells (aMSC)[12]. This was confirmed by ICC cultures revealing that the cells were > 95% positive for preadipocyte factor 1 (Pref-1) and vimentin, few cells positive for CCAAT/enhancer-binding protein (CEBP)α, and negative for fatty acid-binding protein 4 (FABP4), α-SMA, desmin, E-cadherin and VE-cadherin (Figure 3E). After identifying migrated cells as pre-adipocytes, we allowed them to differentiate by culturing them on HIMC-derived ECM to verify whether they could become mature adipocytes. This indeed was the case, as >85% of the cells developed oil red O-positive lipid droplets after a 14-day differentiation period, while HIMC failed to display any lipid droplets (Figure 3F).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Fibronectin, collagen I and collagen III are present in the 2D HIMC derived ECM scaffolds after decellularization.

Representative immunofluorescence staining of fibronectin, collagen I and collagen III in human intestinal smooth muscle cell (HIMC)-derived 2D matrix before (top) and after (bottom) decellularization. Scale bar: 100um. This technique was used for all experiments involving 2D ECM. Slide is representative for n=4.

Supplementary Figure 2. Characterization of 3D decellularized human intestine.

(**A**) Gross change of human intestinal specimen before and after decellularization. (**B**) Hematoxylin&Eosin (H&E), Masson Trichrome (MT) and DAPI staining of decellularized human intestine. (**C**) RNA concentration of β-actin and GAPDH in human intestine specimen before and after decellularization. N=5. Statistically significant data are indicated by **** $p<0.0001$. (**D**) Immunofluorescence staining of fibronectin, collagen I and collagen III in native human intestinal tissue and decellularized human intestine. Scale bar: 100um. Slide is representative for n=4. Abbreviations: Normal: NL; Ulcerative colitis: UC; Crohn's disease: CD; CD_{NS}: non-strictured; CD_S: strictured; Hematoxylin&Eosin: H&E; Masson Trichrome: MT

Supplementary Figure 3. Dissected muscularis propria muscle strips prior to digestion for isolation of human intestinal muscularis prioria smooth muscle cells.

Representative Hematoxylin&Eosin (H&E) staining of muscularis propria muscle strips after removal of mucosa/submucosa and serosa of human intestinal specimen by sharp mechanical

dissection. Scale bar: 50um. These strips were used for digestion, isolation and culture or primary human intestinal muscularis propria smooth muscle cells.

Supplementary Figure 4. Characterization of human intestinal muscularis propria smooth muscle cells.

Representative immunofluorescence staining of α-smooth muscle actin (α -SMA), vimentin, desmin, SM22, Endothelial(E)-cadherin, vascular endothelial(VE)-cadherin in HIMC. Scale bar: 100um. N=4-5.

Supplementary Figure 5. Diversity in extracellular matrix protein abundance in conditioned medium of HIMC treated with and without TGF-β1.

Shannon diversity index of proteins was calculated using the *vegan* package in R. There was no significant difference in diversity of proteins in the four groups (NL, UC, CD_{NS} and CDs) between untreated and TGF-β1 treated HIMC. Abbreviations: Normal: NL; Ulcerative colitis: UC; Crohn's disease: CD; CD_{NS}: non-strictured; CD_S: strictured; N=5 per group

Supplementary Figure 6. Abundance of extracellular matrix proteins in conditioned medium of HIMC treated with and without TGF-β1.

Data represent statistically significant proteins expressed in the four sample groups (NL, UC, CD_{NS} and CDs). Relative abundance of proteins was compared in conditioned medium of HIMC treated with and without TGF-β1 using Dunn's test for pairwise multiple comparisons between mean rank sums test (with post-hoc correction for multiple comparisons, $P < 0.05$, *PMCMR* package). Box plots show the distribution of relative abundance of extracellular matrix proteins, median levels

and the first and third quartile (whiskers show 1.5x the interquartile range), and dots indicate outliers. Abbreviations: Normal: NL; Ulcerative colitis: UC; Crohn's disease: CD; CD_{NS}: non-strictured; CD_S: strictured; N=5 per group

Supplementary Figure 7. HIMC conditioned medium is responsible for adipocyte migration.

(**A**) Conditioned medium of HIMC exposed to TGF-β1 increases migration of adipocytes in the Boyden chamber. N=5-7 per group (**B**) Filtration of HIMC conditioned medium through a 30, 100 and 300 KDa filter significantly reduces migration of adipocytes in the Boyden chamber. Abbreviations: Normal: NL; Ulcerative colitis: UC; Crohn's disease: CD; CD_{NS}: non-strictured; TGF: Transforming growth factor; N=4-5 per group. Statistically significant data are indicated by *P<0.05, ***P<0.001,****P<0.0001.

Supplementary Figure 8. Fibronectin concentration in conditioned medium of HIMC treated with and without TGF-β1 and data on immunoprecipitation and siRNA knockdown for fibronectin.

(**A**) Fibronectin concentration in conditioned medium of HIMC was significantly increased after exposure to TGF-β1 in all groups. N=5 per group (**B**) Fibronectin concentration was dramatically reduced in conditioned medium after filtration through a 30, 100 and 300 KDa filter. Abbreviations: Normal: NL; Ulcerative colitis: UC; Crohn's disease: CD; CD_{NS}: non-strictured; CD_S: strictured; Transforming growth factor: TGF; N=5; Statistically significant data are indicated by *P<0.05, **P<0.01, ****P<0.0001. **(C)** Immunoblot of fibronectin in lysate from protein G-Sepharose beads after immunoprecipitation, verifying the successful immunoprecipitation of fibronectin in conditioned medium. N=3. **(D)** Representative immunofluorescence staining of fibronectin in

HIMC after FN siRNA knock down compared to scrambled siRNA control. Slide is representative of n=3. scale bar, 100um.

Supplementary Figure 9. Decorin does not increase pre-adipocyte migration and does not suppress fibronectin induced pre-adipocyte migration

Normal pre-adipocytes were exposed to fibronectin, decorin or its combination in the Boyden chamber. Decorin did not affect migration, irrespective of the presence of fibronectin. Abbreviations: Normal: NL; Decorin: DCN; Fibronectin: FN; N=6 per group from two individual pre-adipocyte lines; Statistically significant data are indicated by ***P<0.001.

Supplementary Figure 10. Cell outgrowth from mesenteric fat tissue with and without decorin

Differential, time-dependent progressive outgrowth of cells from mesenteric fat tissue pieces plated on plastic alone (below) or fibronectin coating (above) in the presence or absence of decorin. The % of fat explants with cell outgrowth are depicted and no significant difference was noted between the presence and absence of decorin. N=4.

Supplementary Figure 11. Conditioned medium of HIMC induced adipocyte migration is reduced by GRGD and a focal adhesion kinase (FAK) inhibitor.

Inhibition of HIMC-conditioned medium-induced adipocyte migration in the Boyden chamber by GRGD and a focal adhesion kinase (FAK) inhibitor. Abbreviations: Normal: NL; Ulcerative colitis: UC; Crohn's disease: CD; CD_{NS}: non-strictured; Focal adhesion kinase: FAK; n=4-5 per group; Statistically significant data are indicated by *P<0.05, **P<0.01, ****P<0.0001.

Supplementary Figure 12. Characterization of human mesenteric preadipocytes after mesenteric or creeping fat digestion.

(**A**) Representative flowcytometry images indicate >95% positivity for markers of adipose mesenchymal stem cells (positive for CD73, CD90, and CD105, but be negative for CD34, CD45, CD11b or CD14, CD19 or CD79α, and HLA-DR). Images are representative for n=6. (**B**) Representative immunofluorescence staining of preadipocyte factor 1 (Pref-1), CCAAT/enhancer-binding protein (CEBPα), fatty acid-binding protein 4 (FABP4). Scale bar: 100μm. Images are representative of n=3. (**C**) Isolated pre-adipocytes differentiated into adipocytes after 14 days of adding differentiation medium, as shown by oil red-O staining. Scale bar: 100μm. Images are representative of n=4.

Supplementary Figure 13. Schematic representation of the mechanism of creeping fat formation in Crohn's disease caused by differentiation and numerical expansion of pre-adipocytes migrated out of mesenteric fat in response to ECM products secreted by muscularis propria muscle cells.

In response to soluble mediators, primarily TGF-β, produced by local transmural inflammatory infiltrates, intestinal muscularis propria cells secreted ECM products like fibronectin that, through the preferential ligation of integrin α5β1, induce pre-adipocytes present in the mesenteric fat to migrate out, differentiate into mature adipocytes, and accumulate to ultimately form creeping fat.

VIDEO LEGENDS

Video 1. Representative videos for cell outgrowth from normal mesenteric fat tissue plated (**A**) on plastic or (**B**) on human intestinal smooth muscle cell derived extracellular matrix. Scalebar: 250µm. Duration: 24h

Video 2. Representative videos for tracking of pre-adipocytes seeded on HIMC-derived ECM in (**A**) the absence (control) and (**B**) presence of anti-integrin β1 blocking antibody Scalebar: 500µm. Duration: 24h

Video 3. Representative videos for tracking of fluorescently labelled pre-adipocytes seeded on the decellularized human muscularis propria in (**A**) the absence (control) and (**B**) presence of integrin α5 blocking antibody. Scalebar: 100µm. Duration: 24h

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