SUPPLEMENT

SUPPLEMENTARY RESULTS

Development and validation of decellularized intestinal tissue models

A robust model is essential to perform a relevant functional analysis of intestinal extracellular matrix (ECM), and therefore we developed, tested, and compared three different protocols for human full thickness gut tissue decellularization[1, 2, 3]. Each protocol differed based on the main detergent: sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC) or peracetic acid (PAA) (Figure S1). Prespecified criteria for selection of the optimal protocol included removal of all cellular components as indicated by multiple methods, retention of key ECM molecules, and preservation of structural integrity and ability to re-adhere human intestinal myofibroblasts (HIMF). All three protocols resulted in a translucent whitish tissue that retained the structural integrity of the mucosa, submucosa and muscularis propria with no apparent difference among the protocols (Figure S2). This was confirmed by immunofluorescence (IF) staining of the major ECM components collagen I (COLI), collagen III (COLIII) and fibronectin (FN) irrespective of the decellularization methods (Figure S3). Compared to native tissue, decellularized tissues showed a marked drop in DNA and RNA content, the strongest reduction being observed with the SDC protocol for DNA and the SDC and PAA protocols for RNA (Figure S4A&B). Gene expression levels of housekeeping genes HSP90AB1, UBC, B2M, GUSB, ACTB, GAPDH, HPRT1, TFRC, PPIA, RPLP0 and RPL13A all dramatically dropped in all three protocols compared to native tissues, with the SDC protocol exhibiting undetectable levels of these genes (Figure S4C). Compared to native tissue only the SDC protocol showed complete absence of F-actin by IF

staining (Figure S4D). The cellular proteins E-cadherin, cytokeratin-19, vimentin, β-tubulin, phosphatase and tensin homolog and glyceraldehyde 3-phosphate dehydrogenase showed that all proteins were undetectable in all protocols except for α-smooth muscle actin (α-SMA), which was residual in the SDS and PAA but not the SDC protocol (Figure S4E). Finally, HIMF adhered to SDC- decellularized ECM, indicating that the SDC protocol generated not only a structurally but also a functional intact ECM (Figure S4F).

Matrisome analysis of intestinal tissues using the three tested decellularization protocols

To further understand, how the decellularization protocols differentially affect matrix protein composition and in addition to the extensive validation experiments mentioned above, we performed a pilot proteomic analysis of the three decellularization protocols using one tissue each from ulcerative colitis (UC) and non-strictured (CDns) and subjected them to liquid chromatography mass spectrometry (LC-MS) as previously described[4]. Proteins that passed quality control were interrogated. Principal component analysis (PCA) of the top 500 identified proteins revealed that the native, non-decellularized tissued largely clustered with the PAA and the SDS protocol, whereas the SDC protocol showed the strongest differences in both UC and CD (Figure S3B). To ensure that the ECM remains intact in the decellularization protocols we assessed the protein amount the top 11 distinctive ECM proteins and compared their fold change relative to the native, non-digested tissue. Overall, the relative contribution of ECM molecules to the total pool of proteins increased after decellularization, which is expected given the removal of cellular proteins in the decellularization process (Figure S3C). Of note, the strongest relative increases of the top expressed ECM molecules were observed in the SDC protocol, which together with the PCA data suggests, that in this protocol cellular proteins are removed most effectively, but ECM

composition is retained (Figure S3C). The SDC protocol was therefore selected as the optimal model based on the above results and used for matrisome analysis.

Effect of MFGE8 on human intestinal myofibroblasts cytokine secretion

To evaluate whether the lack of responsiveness of HIMF to MFGE8 was specific for ECM molecules or if this also applied to cytokines, we measured cytokine concentrations in the supernatants of HIMF exposed to MFGE8 or vehicle for 48h using a flow cytometry cytokine assay. We found spontaneous expression of interleukin (IL)-6, IL-8 and monocyte chemotactic protein (MCP)-1 in HIMF. Upon exposure to MFGE8 all tested HIMF lines, (NL, UC, CDns and CDs) reduced expression of IL-6. In contrast IL-8 and MCP-1 either increased or decreased upon exposure to MFGE8 within each experimental group. IL-1β, IL-10 and TNF were not detectable (Figure S9).

Next generation sequencing of HIMF points to integrin signaling of MFGE8

The lack of anti-fibrotic response of CDs HIMF to MFGE8 led us to investigate potential reasons for this observation. We therefore assessed differentially expressed genes in HIMF exposed or not to MFGE8 using next generation RNA sequencing[5] and differential statistical RNA-seq analysis using leading DGE tools such as *edgeR* and *Limma* packages[5, 6, 7].

Upon exposure to MFGE8 107 genes were up- and 44 genes were down-regulated in NL HIMF (2-fold change, $p<0.01$), as displayed in volcano plot (Figure 5A) and Venn diagram (Figure 5B). The top 30 up or downregulated genes were shown in Figure 5C. Pathway enrichment (gProfiler) analysis showed that 93 pathways domains were upregulated and 12 were downregulated upon exposure of NL HIMF to MFGE8 ($p<0.01$) (Table S2). This global gene expression analysis indicates the MFGE8 was a leading regulator of ECM organization and ECM-cell interactions.

We next assessed the response of CDs HIMF to MFGE8, which revealed 6 genes (SNORD43, NBL1, HAPLN4, ZMAT1, CLMAT3, LOC100506606) were up-regulated while 4 genes (SPRY4-AS1, OSTCP1, ZDHHC11B, TAF7L) were downregulated in CDs HIMF (2-fold change, $p<0.01$), as displayed in the volcano plot (Figure 5A) and Venn diagram (Figure 5B). The top 30 up or downregulated genes were shown in Figure 5C. Pathway enrichment analysis indicates enrichment of TGF-β signaling pathways and positive regulation of pathway-restricted SMAD protein phosphorylation (Table S3).

To elucidate pathways potentially involved in the observed differential response of NL and CDs we next investigated the differentially MFGE8-regulated genes in these two groups. Assessing the uniquely upregulated genes in HIMF NL in response to MFGE8 that were not regulated in HIMF CDs as the input, GO genesSet enrichment analysis revealed interferon a/b signaling, cytokine-mediated signaling, and cell surface receptor signaling as the major pathways (Table S4). When focusing on ECM-cell interactions, the pathway analysis indicated induction of ECM organization (HSA-1474244), ECM-integrin interaction (HSA-216083), cell adhesion (HAS-04514), proliferation (GO:0042127) and chemotaxis (GO:0050921) (Figure 5D). Based on these observations, we evaluated the proliferation and migration of NL HIMF in response to MFGE8. Using three different doses of MFGE8, no change in either proliferation or migration of NL HIMF was noted in comparison to untreated NL HIMF (Figure S10). These results suggests that MFGE8 exerts anti-fibrotic effects in NL HIMF, through reduction in ECM production and potentially integrin pathways, but these effects are blunted or missing in CDs HIMF.

SUPPLEMENTARY MATERIALS AND METHODS

Decellularization of colonic tissue sections

After a thorough literature search, we selected three decellularization protocols based on the type of detergent used, the number of publications per protocol and applicability to intestinal resection tissues, which are based on sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC) and peracetic acid (PAA) respectively. The protocols were reviewed and adapted as found applicable to our experimental set-up[1, 2, 3]. For the sake of this publication, we named the protocols after the main detergent in each procedure. Details for each protocol are depicted in Figure S1. Briefly, freshly resected human intestinal tissues (patient demographics can be found in Table S1) were harvested, rinsed in Hank's balanced salt solution (HBSS) and mucus and blood clots were removed. The mesenteric or creeping fat was separated from the intestine by sharp dissection. Each tissue was subsequently cut into three equal segments (approximately 2-5g each) and assigned to each of the three protocols. All three protocols shared similar processes on the first day pertaining to tissue harvest, cleaning and use of anti-microbials. Then, three different protocols were followed for the decellularization of the tissue. Details can be found in Figure S1.

For the protocol with SDS detergent (Sigma, St. Louis, MO, USA) [8], tissues were stored in Belzer UW Cold Storage Solution (Bridge to Life Ltd, Columbia, SC) with 2.5% 2500U penicillin, 2500µg streptomycin sulfate, 625µg amphotericin B (PSF, Loza Basel, Switzerland) overnight at 4 °C. On the second day, they were washed in sterile phosphate-buffered saline (PBS) and then placed in 1% SDS rotating for 6 hours, followed by 10% Triton X-100 (Sigma, St. Louis, MO, USA), for 1 hour at room temperature (RT).

For the protocol with SDC (Sigma, St. Louis, MO, USA)[2] tissues were immersed in double-distilled water (ddH_2O) with 2.5% PSF rotating overnight at RT on the first day and for 3 hours at the beginning of the second day, followed by 4°C for 5 hours after changing the solution. Next,

tissues were incubated in 4% SDC overnight at 4℃, the SDC was changed and applied for another 7 hours at room temperature.

For the protocol with PAA (Pfaltzandbauer, Waterbury, CT) [3], tissues were stored in Belzer UW Cold Storage Solution with 2.5% PSF overnight at 4 ℃ on the first day. On the second day, tissues were rotated in 0.1% PAA for 2 hours at room temperature. Then two cycles of 15 minutes' sterile PBS and ddH2O washes were applied.

DNA and RNA extraction from colon tissue

Up to 25 mg freshly frozen native and decellularized colon tissue were minced prior to DNA extraction using DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA). For RNA extraction, RNeasy Mini Kit (Qiagen, Germantown, MD, USA) was used following the dissociation of the tissue by TissueLyser LT (Qiagen, Germantown, MD, USA) with stainless steel beads (Qiagen, Germantown, MD, USA). DNA and RNA concentrations were determined using the ND-1000 UV/Vis Spectrophotometer (NanoDrop, Thermo Scientific).

Matrisome analysis of human intestinal decellularized tissue blocks

Matrisome analysis of primary human intestinal smooth muscle cells has been previously described by us[9] and the same protocols were used for the decellularized colon tissue cubes. In total, 30mg of decellularized tissues were digested in 8M urea Tris-HCl buffer for homogenization (Sigma, St. Louis, MO, USA). The process of LC-MS was previously described[4]. The samples were filtered using a 3K Amicon Ultra 0.5 mL centrifugal filter (Millipore, UFC500396), dried in a speedvac and reconstituted in 50 µL of 6M urea buffer. The protein sample was reduced with DTT and alkylated with iodoacetamide. The sample was digested by adding Trypsin/Lys-C Mix (Promega # V5071) to the sample at a 25:1 protein:protease ratio (w/w). The sample was mixed

and incubated for 3–4 hours at 37°C. The sample was then diluted 6-fold with 50mM Tris-HCl (pH 8) to reduce urea concentration to 1M or below. Digestion was continued overnight at 37°C. The digestion was terminated by adding trifluoroacetic acid (TFA) to a final concentration of 0.5-1%. The sample was desalted using a PepClean C18 spin column (Thermo Fisher Scientific, Waltham, MA, USA) and resuspended in 1% acetic acid to make up a final volume of ~30 µL for LC-MS analysis. The LC-MS system was a Thermo Scientific Fusion Lumos tribrid mass spectrometer system. The high-performance liquid chromatography column was a Dionex 15cm x75 µm id Acclaim Pepmap C18, 2µm, 100 Å reversed- phase capillary chromatography column. Five µL 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 0.3 µL/min were introduced into the source of the mass spectrometer on-line. The microelectrospray ion source is operated at 2.5 kV. Proteomics analysis was supported by the Cleveland Clinic proteomics core[9].

The digest of the decellularized tissue cubes was analyzed using the data dependent multitask capability of the instrument acquiring full scan mass spectra to determine peptide molecular weights and product ion spectra to determine amino acid sequence in successive instrument scans. CID/HCD spectra collected in the experiment were analyzed using Sequest search program (Proteome Discoverer 2.2), and compared with the human UniProtKB database to determine protein identities (FDR set to 1%). Label free quantitation (LFQ) intensities were determined using PD2.2, and used to calculate relative protein abundance (proportion). 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, ggord and base R for PCA, venn and RVenn for creating venn diagrams. R Package dunn.test was used to conduct pairwise multiple comparisons of mean for different

datasets (Dunn's nonparametric 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.

Dextran sodium-sulfate induced colitis

Acute and chronic dextran sodium sulfate (DSS) colitis was induced as previously described by us [5, 10]. After a dose-finding for each mouse strain used, 3.5% DSS (35–50 000 kDa; MP Biomedicals, OH, USA) in drinking water of wildtype (WT) mice (6-8 weeks of age) for 10 days was chosen as optimal for the BALB/cJ mouse strain (Jackson laboratory, Bar Harbor, ME, USA). Milk fat globule-epidermal growth factor 8 (MFGE8) (R&D, Minneapolis, MN, USA) was administered as enema at a dose of 3,600 ng in 120 µl (R&D, Minneapolis, MN, USA) via Argyle^{TM} Polyurethane Umbilical Vessel Catheter (Covidien LLC, MA, USA) every four days. PBS was used as control. We performed acute DSS colitis with increasing doses of 400ng, 1,200ng and 3,600ng per mouse. Chronic experimental fibrosis was induced by 3.5% DSS in drinking water for 10-12 days followed by a recovery period of 10-14 days with normal tap water, and this was defined as one cycle of DSS[5, 11]. The DSS cycle was repeated twice. Control mice received normal drinking water throughout. In the preventive experiment, enemas of 3,600ng recombinant mouse MFGE8 (mrMFGE8) or PBS every four days was applied from the onset of the first DSS cycle. In the therapeutic experiment, the enema was started at the end of the second DSS cycle and administered all throughout the second recovery period. Clinical disease activity was determined every other day by measuring body weight loss, stool consistency and presence of occult or overt blood in the stools as previously described [5, 12, 13]. Animals were euthanized by CO_2 asphyxiation followed by cervical dislocation at the end of the experiment.

For the MFGE8 knockout (KO) experiments mice were kindly provided by Dr. Kamran Atabai (UCSF). Those mice were previously described[14]. After DSS dose finding experiments, chronic experimental fibrosis was induced by 3% DSS in drinking water for 6 days followed by a recovery period of 10-14 days with normal tap water, and this was defined as one cycle of DSS[5, 11]. The DSS cycle was repeated twice. Control mice received normal drinking water throughout. In the therapeutic experiment, enemas of 3,600ng rmMFGE8 or PBS every four days was applied at the end of the second DSS cycle and administered all throughout the second recovery period. KO mice were age-matched and were co-housed with their WT littermates and per genotype to minimize influence from differences in flora composition[15].

Trinitrobenzene sulfonic acid induced fibrosis

Chronic trinitrobenzene sulfonic acid (TNBS) induced colitis was induced as previously described [10, 16]. After a dose finding exercise, 1% TNBS (administered weekly intrarectally to wildtype (WT) mice (6-8 weeks of age) for 4 weeks was chosen as optimal for the BALB/cByJ mouse strain (Jackson laboratory, Bar Harbor, ME, USA). Milk fat globule-epidermal growth factor 8 (MFGE8) (R&D systems, MN, USA at a dose of 3,600 ng) and/or FAK-inhibitor (Y15, Sigma, St. Louis, MO; 10 mg/kg) was administered as enema in 120 µl via Argyle^{TM} Polyurethane Umbilical Vessel Catheter (Covidien LLC, MA, USA). Control mice received 45% ethanol/PBS only intrarectally weekly. Enemas of 3,600ng recombinant mouse MFGE8 (mrMFGE8) and/or FAK inhibitor were applied every four days from the onset of the first TNBS cycle. Clinical disease activity was determined every other day by measuring body weight loss, stool consistency and presence of occult or overt blood in the stools as previously described [5, 12, 13]. Animals were euthanized by CO_2 asphyxiation followed by cervical dislocation at the end of the experiment.

Endpoints for DSS and TNBS induced colitis experiments

The endpoint and data collection of DSS and TNBS-induced colitis experiment has been previously described. Body weights, stool consistency and occult blood or the presence of gross blood per rectum were recorded every other day[5, 12]. Two investigators blinded to the protocol independently assessed the clinical score as previously described[12]. Briefly, weight loss of 1–5%, 5–10%, 10–20%, and >20% was scored as 1, 2, 3, and 4, respectively. For stool consistency, 0 was scored for well-formed pellets, 2 for pasty and semiformed stools, which did not stick to the anus, and 4 for liquid stools that remained adhesive to the anus. Bleeding was scored 0 for no blood in hemoccult, 2 for positive hemoccult, and 4 for gross bleeding from the rectum. Weight, stool consistency, and bleeding sub-scores were added and divided by 3, resulting in a total clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis). At the end of the experiment animals were euthanized by CO_2 asphyxiation followed by cervical dislocation. The entire colon was removed, cleaned, weighted and measured from the ileocaecal junction to the anus. Tissue was procured from the descending colon. Histology was performed on paraffin embedded, 3 μm-thick transverse sections stained with hematoxylin and eosin, masson trichrome or Sirius red. Slides were scored by an experienced pathologist (IOG or SH) blinded to the experimental groups using one score for inflammation and one for fibrosis as previously described [5]. Briefly, inflammation scoring was performed using hematoxylin & eosin (H&E) slides based on inflammation infiltration (0-3), extent of inflammation (0-3), crypt damage (0-4) and percentage of involved area (0-4). Fibrosis scoring was evaluated using masson trichrome (MT) slides on the whole tissue rings, ranging from 0 to 3. Images were acquired using an Olympus microscope and ImagePro software. Sirius red images were quantified using ImageJ software (Bethesda, MD).

Starting with a non-diseased control slide, threshold was set using the submucosa of a no DSS or no TNBS wildtype animal and remained the same for all following slides and integrated density was measured. The thickness of the intestinal wall layers was calculated as the mean value of four different points per mouse on well oriented cross sections using ImageJ (Bethesda, MD).

Next generation RNA sequencing analysis

RNA was extracted from normal control (NL) (n=4) and Crohn's disease strictured (CDs) (n=3) HIMFs stimulated by recombinant human MFGE8 (rhMFGE8) and PBS. Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Germany). RNA quality was assessed using the Agilent bioanalyzer and samples with RNA quality (RIN) score of >9.0 were used for RNA-sequencing. RNA concentrations were determined using Qubit® 3.0 Fluorometer (Invitrogen, Life Technologies). RNA-sequencing libraries were generated using Illumina TruSEQ kits following the manufacturer's protocol and libraries were sequenced using an Illumina NovaSeq 6000 following Illumina reagents and protocols. Paired-ended 100 base pair reads were trimmed with Trim Galore! (v.0.4.4) and checked for quality with FastQC (v0.11.7) (http://www.bioinformatics.babraham.ac.uk/projects) before alignment to the human genome (GRCh38.p13). Reads were aligned using Rsubread package. Overall, an average of 98.0% of reads aligned uniquely. Gene counts were determined by the number of uniquely aligned, unambiguous reads (Subread: featureCounts, v1.5.2) and annotated (GRCH38, Ensembl 99 release). On average, 70% of reads in each sample were successfully assigned. Raw counts were loaded into R (v3.6.2 and 4.1.2), and subsequent analyses were performed using the packages described below. Counts were filtered to exclude transcripts that were expressed at low levels (counts per million reads mapped [CPM] < 1) before performing differential expression (DE) analysis using R packages *DESeq2*, *edgeR* and *Limma*. Differentially expressed genes (DEGs)

were identify using different statistical RNAseq analysis workflows based on Reads Per Kilobase of transcript, per Million mapped reads (RPKM) and Fragments Per Kilobase of transcript, per Million mapped reads (FPKM) values. DEGs with Benjamini-Hochberg adjusted p-value < 0.05 was considered statistically significant. Volcano plots were created using R package *EnhancedVolcano*. Functional analysis of significant DEGs were performed using R package *clusterProfiler* (v3.14.3) to identify and plot enriched Gene Ontology terms and KEGG pathways. Enrichment plots were created using EnrichmentMap app in Cytoscape (ver 3.8.2, https://cytoscape.org/). Other plots were made using in-house R scripts (available upon request). RNA sequencing data is in the process of being deposited in Gene Expression Omnibus (GEO).

Focal adhesion kinase pathway inhibition

In select experiments, HIMFs were seeded in 6-well or 24-well plates overnight, serum deprived and treated with 10μM focal adhesion kinase (FAK) phosphorylation small molecular inhibitor (Sigma, St. Louis, MO, USA) or vehicle for 1 hour. Subsequently, cells were stimulated with rhMFGE8 (R&D, Minneapolis, MN, USA) or vehicle.

RNA interference

HIMFs were transfected with ITGAV (for integrin αv) and ITGB5 (for integrin β5) small interfering RNA (siRNA) (Horizon Discovery, Cambridge, United Kingdom) and their respective scrambled siRNA using lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The siRNAs are pooled nucleotides with different sequences for each target in the same solution. HIMFs were seeded in 6-well or 24-well plates and cultured overnight. Cell culture medium was removed and replaced with opti-MEM (Thermo Fisher Scientific, Waltham, MA, USA) as well as

lipofectamine 2000 and siRNA mixture per manufacturers recommendations. Subsequently, the media was replaced with HIMF culture media with no serum prior to the experiments.

Migration assay

Migration assays were performed in the modified 48-well Boyden chamber (Neuro Probe, Gaithersburg, MD, USA) as described previously by us[17]. A polycarbonate filter (12 μm pore size, polyvinylpyrrolidone-free; Gerbu Biotechnik, Germany) divided the chamber into an upper and a lower compartment. HIMFs were pretreated with a series of concentrations of MFGE8 (0ng/ml, 100ng/ml, 250ng/ml and 500ng/ml for overnight before the migration assay. 25ug/ml fibronectin (FN) were set up in the lower chambers. Twenty thousand HIMF per well in DMEM with 1% bovine serum albumin (BSA) were seeded into the wells of the upper compartment of the Boyden chamber and incubated at 37°C in 5 % CO2 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. Each experiment was repeated at least 3 times.

Cell Proliferation Assay

A total of 15,000 HIMF/well were seeded onto 24-well plates (Corning), and proliferation assays performed as previously described[18]. Briefly, cells were incubated with rhMFGE8 (100ng/ml, 250ng/ml or 500ng/ml) or PBS for 48 hours and their proliferative potential was evaluated by

measuring DNA synthesis by thymidine incorporation assay. Briefly, cells were incubated with 3H-thymidine (1 µCi/ml; Amersham, Arlington Heights, IL) for 6 hours and washed twice with 5% (vol/vol) trichloroacetic acid before fixation. DNA was precipitated using 0.5 N NaOH, and supernatants were quantified in a γ-counter using basic fibroblast growth factor (bFGF) (10 ng/ml) as positive control.

Quantitative reverse transcriptase polymerase chain reaction procedure

Total RNA was isolated as described previously (RNAEasy Miniprep kit, Qiagen, Germantown, MD, USA)[19], and reverse transcription and quantitative PCR performed according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA) and as described previously by us[5]. The products for all primer pairs were verified by sequencing and relative differences were calculated using the comparative threshold cycle method (ddCt) by normalizing to CT values of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (reference gene). Quantitative RT-PCR was performed on cDNA (synthesized with iScript cDNA Synthesis Kit, Biorad, Hercules, CA, USA) with iQ Sybr Green Supermix (Biorad, Hercules, CA, USA) and gene specific primers. GAPDH was used as the reference gene and the Pfaffl method was used to calculate fold changes in treated versus untreated samples[20]. The primer pairs used for gene expression analysis are summarized in Table S5.

Immunofluorescence

The method for immunofluorescence (IF) was adapted from a previously reported method[4]. For the staining of colon tissue, the formalin fixed paraffin embedded (FFPE) slides were deparaffinized using the following protocol: Clear Rite (Thermo Fisher Scientific, Waltham, MA,

USA) for 3 min, Clear Rite for 3 min, Flex 100 (Thermo Fisher Scientific, Waltham, MA, USA) for 1 min, Flex 100 for 2 min, Flex 95 (Thermo Fisher Scientific, Waltham, MA, USA) for 1 min and Flex 95 for 2 min. Then the slides were washed with ddH_2O for 1min before being incubated with target retrieval solution pH 6 (Dako Denmark, Glostrup, Denmark) at 95°C water bath for 30 min. After that slides were cooled down to room temperature before blocking. For the staining of HIMFs, cells were seeded onto 3-well chamber slides (IBIDI GMBH, Martinsried, Germany). After treatment with MFGE8 or PBS for 48 hours, slides were rinsed in PBS and fixed with 4% paraformaldehyde at room temperature for 10 minutes and 0.5% Triton-X for 5 min. Both tissue and HIMF slides were blocked with 3% FBS in PBS before the application of the primary antibody. All primary and secondary antibodies were diluted in 3% FBS. The primary antibodies were collagen I (COLI) antibody (Rockland, Limerick, PA, USA), collagen III (COLIII) antibody (Rockland, Limerick, PA, USA), FN antibody (BD Biosciences, San Jose, CA, USA), MFGE8 antibody (Abcam, Cambridge, MA, USA) and integrin αvβ5 antibody (Abcam, Cambridge, MA, USA) at a dilution of 1:100. After overnight incubation with the primary antibody at 4°C, slides were rinsed three times with PBS and the AlexaFluor 488 secondary antibody (Molecular Probes, Eugene, OR, USA) was added at a dilution 1:500 for 1 hour at 37ºC. F-actin was stained by fluorescein phalloidin (Thermo Fisher Scientific, Waltham, MA, USA), which did not require the conjugation of fluorescent secondary antibody. For nuclear counterstaining Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA) was used. After staining, the Leica DM5500B microscope (Leica, Wetzlar, Germany) or Olympus IX71 microscope (Olympus Scientific Solutions Technologies Inc, Waltham, MA, USA) were used to capture images. The secondary antibody controls for the entire publication can be found in Figure S13.

Immunoblotting

Protein extraction was performed using a RIPA 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 (Sigma, St. Louis, MO, USA) as previously described by us[4, 5]. The concentration of proteins in each lysate was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA) according to manufacturer's recommendations. Immunoblotting was performed as previously described[5]. Equivalent amounts of proteins (10 µg) were separated using SDS-PAGE on a 10% Tris-glycine gel and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). Nonspecific binding was blocked by incubation with 5% milk or BSA in 0.1% Tween 20/Tris-buffered saline (Thermo Fisher Scientific, Waltham, MA, USA) for 30 min., followed by overnight incubation at 4°C with the primary antibody(s). The following antibodies were used: GAPDH (Trevigen, Gaithersburg, MD, USA) at 1:2000, α-smooth muscle actin (α-SMA) (Sigma, St. Louis, MO, USA) at 1:1000, FN (BD Biosciences, San Jose, CA, USA) at 1:1000, phospho-FAK (p-FAK) (Cell Signaling, Danvers, Massachusetts, USA) at 1:500, FAK (Cell Signaling, Danvers, Massachusetts, USA) at 1:500, integrin αv (Abcam, Cambridge, MA, USA) at 1:1000, integrin β5 (Abcam, Cambridge, MA, USA) at 1:1000, E-cadherin (Abcam, Cambridge, MA, USA) at 1:1000, cytokeratin-19 (Novus, Centennial, CO) at 1:1000, vimentin (Abcam, Cambridge, MA, USA) at 1:1000, β-tubulin (Cell Signaling, Danvers, Massachusetts, USA) at 1:1000 and phosphatase and tensin homolog (PTEN) (Cell Signaling, Danvers, Massachusetts, USA) at 1:1000. Membranes were washed 6 times with 0.1% Tween 20/Tris-buffered saline, incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Sigma, St. Louis, MO, USA), washed again, and incubated with the

chemiluminescent substrate (Super Signal; Pierce, Rockford, IL, USA) for 5 minutes, after which they were exposed to film (Kodak).

Immunohistochemistry

Immunohistochemistry (IHC) staining was performed using the Discovery ULTRA automated stainer from Roche Diagnostics (Indianapolis, IN, USA). In brief, antigen retrieval was performed using a tris/borate/ethylenediaminetetraacetic acid (EDTA) buffer (Discovery CC1, 06414575001; Roche, South San Francisco, CA, USA), pH 8.0 to 8.5. MFGE8 antibody (Abcam, Cambridge, MA, USA) at 1:300, COLI antibody (Abcam, Cambridge, MA, USA) at 1:75, αSMA antibody (Sigma, St. Louis, MO, USA) at 1:100 or FN antibody (BD Biosciences, San Jose, CA, USA) at 1:100 for 0.5 to 1 hour incubation at room temperature were used. The antibodies were visualized using the OmniMap anti-rabbit or anti-mouse HRP (Roche, South San Francisco, CA, USA) in conjunction with the ChromoMap DAB detection kit (Roche, South San Francisco, CA, USA). Lastly, the slides were counterstained with hematoxylin and bluing. Antibodies were diluted with Van Gogh Yellow Diluent (BioCare Medical, Pacheco, CA, USA). The Olympus CX31 microscope (Olympus Scientific Solutions Technologies Inc, Waltham, MA, USA) was used to capture the images. For the quantification of MFGE8 expression within human intestinal tissues, the images of the slides were blindly reviewed by two independent researchers. Scores from 0-4 (0: no expression; 4: highest expression) were assigned according to the expression of MFGE8 in the epithelium and submucosa. In case of discrepancy the two researchers discussed the results jointly and if discrepancies persisted they were resolved by I.O.G. We additionally automatically quantified the expression of MFGE8, Col I, FN and αSMA on IHC using QuPath for determination of the percent area positive in three regions of interest in the submucosa for each tissue section.

HT29 stimulation experiments

HT29 cells ($1x10^5$ cells/well) were seeded in 12-well plate overnight. Then HT29 cells were stimulated with 10ng/ml IL-1β, 10ng/ml TNF, 10ng/ml TGF-β1 (all Peprotech), or 500ng/ml LPS (Sigma) in serum-free DMEM medium for 24 hours. Supernatant were collected, centrifuged and stored at -80°C until use.

Cytokine cytometric bead array

We analyzed cell culture supernatants with the BD Cytometric Bead Array Human Soluble Protein Master Buffer Kit (BD Biosciences, San Jose, CA, USA). This kit is able to detect IL-1β, IL-6, IL-8, IL-10, IL-12, TNF, MCP-1, TGF-β1, IFNγ and RANTES and was performed following manufacturer's instructions.

Enzyme-linked immunosorbent assay

The FN concentration in HIMF supernatant and the MFGE8 concentration in HT-29 supernatants was measured using a commercially available enzyme-linked immunosorbent assay (ELISA; Abcam or R&D Systems, respectively) according to the manufacture's protocol.

Extracellular matrix deposition assay for human intestinal myofibroblasts

Deposition of ECM by intestinal myofibroblasts was assayed using modification of a method described previously [21, 22, 23]. Briefly, cells were plated into 96-well dark walled imaging plates (Greiner) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (described above), and allowed to grow and produce ECM for 5 days. Cells were removed using 0.25 M ammonium hydroxide in 50 mM Tris pH 7.4, and the

deposited ECM was fixed by exposure to 100% methanol at −20°C. Fixed ECM was stained with Alexa Fluor488-conjugated anti-fibronectin (EBioscience, clone FN-3, 1:500 dilution), Alexa Fluor594-conjugated (Invitrogen, 1:1000 dilution) and anti-COLI/III antibodies (EMD, Millipore Corp. 1:100 dilution). Flourescence intensities were obtained by scanning the plates with ECM using Cytation5 scanner. Fluorescence intensities of at least three replicate wells were used to determine the mean ECM levels in an experiment.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Protocol schematic for decellularization of intestinal resection tissue using sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC) or peracetic acid (PAA) based methods. Abbreviations: Hank's balanced salt solution (HBSS); Penicillin, Streptomycin, Fungizone (PSF); Room temperature (RT); Phosphate buffered saline (PBS)

Figure S2. Decellularization of colon tissue from inflammatory bowel disease patients and controls: Hematoxylin & Eosin (H&E) as well as Masson Trichrome (MT) staining. Colon tissue processed by sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC) or peracetic acid (PAA) based decellularization protocols compared to native tissue. Depicted are the gross appearance as well as H&E and MT staining separately for each tissue layer. All three protocols preserved the tissue architecture compared to native tissue. Scalebar = 50µm. Slides representative for n=7.

Figure S3. Decellularization of colon tissue from inflammatory bowel disease patients and controls: Extracellular matrix (ECM) protein expression. (A) Immunofluorescent staining of colon tissues processed by sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC) or peracetic acid (PAA) based decellularization protocols compared to native tissue. Immunofluorescent staining for collagen I (COLI), III and fibronectin (FN). Major ECM molecules remain in the decellularized tissue segments. Scalebar = 100µm. Slides representative for n=8. (B) Principal component analysis (PCA) of the three decellularization protocols considering the top 500 proteins identified in an ulcerative colitis (UC) and Crohn's disease (CD) sample. Each dot in the PCA plot

represents a sample, with different colors representing the different decellularization protocols. The results reveal the strongest separation between native tissue and the decellularized samples for the SDC based protocol. (C) Fold change of the top 11 matrisome proteins in decellularized tissue compared to native tissue in the three different decellularization protocols, relative to the original untreated sample. Overall the relative contribution of ECM molecules to the total pool of proteins increased after decellularization. The strongest relative increases for the top expressed ECM molecules were noted in the SDC protocol. PPA, peracetic acid; SDC, sodium deoxycholate; SDS, sodium dodecyl sulfate.

Figure S4. Decellularization of colon tissue from inflammatory bowel disease patients and controls: DNA and RNA amount, housekeeping gene expression, F-actin staining and re-seeding. (A-D) DNA content (A), RNA content (B), housekeeping gene expression (C), and F-actin staining with phalloidin (D) in colon tissues processed by sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC) or peracetic acid (PAA) based decellularization protocols compared to native tissue. (E) Immunoblot of housekeeping and typical cellular proteins in colon tissues with or without SDC, sodium dodecyl sulfate (SDS) or PAA based decellularization treatment (n=3). (F) Immunoflourescence for vimentin after re-seeding of SDC based decellularized tissue section with primary human intestinal myofibroblasts (HIMF). HIMF populated and aligned with the decellularized extracellular matrix (ECM) (representative for n=3). ****, $p<0.0001$.

Figure S5. Milk fat globule-epidermal growth factor 8 (MFGE8) expression and secretion in intestinal epithelial cells. (A) Immunoblot analysis of MFGE8 in normal (NL) and CD stricture (CDs) primary human intestinal epithelial washes. MFGE8 was increased in CDs epithelial cells

compared to NL (n=12 technical replicates of a total of n=4 patient samples). (B) HT29 intestinal epithelial cells were exposed to interleukin (IL)-1β, tumor necrosis factor (TNF), transforming growth factor (TGF)-β1, lipopolysaccharide (LPS) or left untreated (Unt) for 24h and supernatants collected for MFGE8 detection via ELISA. IL-1β increased and TNF, TGF-β1, LPS decreased MFGE8 secretion compared to untreated. N=3 per group. *, $p<0.05$; ****, $p<0.001$.

Figure S6. Milk fat globule-epidermal growth factor 8 (MFGE8) expression in intestinal resection tissues from normal control (NL) and Crohn's disease stricture (CDs) tissues. Immunoflourescence (IF) for MFGE8 focusing on the mucosa and submucosa separately confirms epithelial cells as the major source of MFGE8 and increased expression in CDs compared to NL in mucosa and submucosa. MFGE8 was found near α-SMA positive cells (myofibroblasts and vascular smooth muscle cells) as marked by white arrows. Slides are representative of n=8.

Figure S7. Milk fat globule-epidermal growth factor 8 (MFGE8) in acute dextran sodium sulfate (DSS)-induced colitis. (A – D) Acute DSS colitis was induced in Balb/C mice by 3.5% DSS administration. 3,600ng of recombinant mouse milk fat globule-epidermal growth factor 8 (rmMFGE8) or vehicle control was applied as enema every four days starting from the first day of DSS administration. The severity of DSS induced colitis was evaluated by measuring (A) body weight loss and (B) calculating the clinical score consisting of blood in stool, weight loss and stool consistency. MFGE8 reduced the clinical score, but not weight loss in DSS treated animals. (C) Colon length was less reduced in MFGE8 treated and DSS exposed mice compared to DSS alone. (D) Inflammation score was determined by an inflammatory bowel disease (IBD) pathologist in a blinded fashion using Hematoxylin & eosin (H&E) sections. There was no difference in DSS

treated animal irrespective of exposure to MFGE8 or not. Data are presented as mean ± SEM (n=5-9 per group from two independent experiments). *, p<0.05, **, p<0.01, ****, p<0.0001.

Figure S8. Milk fat globule-epidermal growth factor 8 (MFGE8) exerts anti-fibrotic properties in chronic trinitrobenzene sulfonic acid (TNBS)-induced colitis. (A to F) Chronic TNBS colitis was induced in Balb/C mice by weekly intrarectal administration of 1% TNBS for a total of 4 weeks. 3,600ng of recombinant mouse milk fat globule-epidermal growth factor 8 (MFGE8) and/or 10 mg/kg Y15 (focal adhesion kinase inhibitor (FAKi)) were applied as enema every four days starting from the first day of TNBS administration. The severity of TNBS induced colitis was evaluated by measuring (A) body weight loss and (B) calculating the clinical score consisting of blood in stool, weight loss and stool consistency. No significant difference was noted between the groups. (C) Colon length was less reduced in MFGE8 and/or Y15 treated TNBS exposed mice compared to TNBS alone. (D) Representative images from mouse colon sections stained with Hematoxylin & eosin (H&E), Masson's trichrome (MT), sirius red (SR), or collagen I (COLI). Slides are representative of n=8 per group. Arrows point towards the area of fibrosis. (E) Inflammation score was determined by an IBD pathologist in a blinded fashion using H&E sections. There was no difference in TNBS treated animal irrespective of exposure to MFGE8 and/or Y15 or vehicle only. Fibrosis score as determined by an IBD pathologist in a blinded fashion using MT sections or SR positive area was analyzed. MFGE8 or MFGE8&Y15 reduced the fibrosis score. SR surface area in TNBS exposed animals was reduced in MFGE8 and Y15 but not in MFGE8&Y15 treated animals compared to TNBS only. The combination of MFGE8&Y15 did not lead to less fibrosis compared to MFGE8 alone. (F) Neither MFGE8, nor Y15 reduced the thickness of the submucosa, muscularis mucosa and muscularis propria in TNBS exposed animals.

Data are presented as mean ± SEM (n=8 per group from two independent experiments). *, p<0.05, **, p<0.01, ***, p<0.001, ****, p<0.0001.

Figure S9. Milk fat globule-epidermal growth factor 8 (MFGE8) reduces IL-6, but not IL-8 or MCP-1 in supernatants normal control primary human intestinal myofibroblasts (NL HIMF). NL HIMF were exposed to MFGE8 or vehicle for 48 and supernatants collected for a cytokine bead array assay (n=3 per group). (A) Absolute concentrations IL-6, IL-8 and MCP-1 are depicted. (B) Relative concentrations to untreated are depicted for IL-6, IL-8 and MCP-1.

Figure S10. Milk fat globule-epidermal growth factor 8 (MFGE8) does not influence migration or proliferation in normal control primary human intestinal myofibroblasts (NL HIMF). (A) NL HIMF were exposed to different concentrations of MFGE8 as the chemoattractant in the lower well of the Boyden chamber. MFGE8 did not influence the migration of NL HIMF (n=4). (B) NL HIMF were exposed to different concentrations of MFGE8 in the 3H-thymidine proliferation assay. MFGE8 did not influence proliferation of NL HMF. Basic fibroblast growth factor (bFGF) was used as a positive control (n=3). *, p<0.05, **, P<0.01

Figure S11. Knockdown efficiency of small interfering RNA (siRNA) for integrin αv and β5 in normal control primary human intestinal myofibroblasts (NL HIMF). NL HIMF were transfected with siRNA using lipofectamine 2000 and optimal conditions were determined. Immunoblot analysis indicates robust knockdown of the proteins of interest. N=3. **, p<0.01, ***, p<0.001.

Figure S12. Milk fat globule-epidermal growth factor 8 (MFGE8) does not reduce cytokine concentrations in supernatants of Crohn's disease (CD) lamina propria mononuclear cells (LPMC). CD LMPC were freshly isolated and exposed to MFGE8 in three different concentrations or left untreated for 24 hours and supernatants collected for a cytokine bead array assay (n=4 replicates per group). (A) Absolute concentrations of the measured cytokines are depicted. There was no difference in cytokine secretion by LPMC regardless of the MFGE8 concentration used.

Figure S13. Isotype controls

To keep the main figures manageable, we added the secondary antibody isotype controls for each of the immunofluorescence images to this figure. The isotype controls are labelled per corresponding figure panel.

SUPPLEMENTARY TABLES

Table S1. Patient demographics

	Combined (n=26)	Controls (n=5)	Crohn's disease (n=11)	Ulcerative colitis (n=10)
Gender				
Female, n (%)	11 (42)	5 (100)	1 (9)	5 (50)
Age at time of surgery, mean ± SD	43 ± 17.5	46 ± 11	44 ± 20	41 ± 17
Surgical indication, n (%)				
Chronic diverticulitis	1 (4)	1 (20)	0 (0)	0 (0)
Refractory constipation	4 (16)	4 (80)	0 (0)	0 (0)
Crohn's disease complications	11 (42)	0 (0)	11 (100)	0 (0)
Refractory ulcerative colitis	10 (38)	0 (0)	0 (0)	10 (100)
Disease location, n (%)				
Colon	15 (58)	5 (100)	0 (0)	10 (100)
Ileum	6 (23)	0 (0)	6 (55)	0 (0)
Ileocolonic	5 (19)	0 (0)	5 (45)	0 (0)
Location of resection, n (%)				
Ileocecal	11 (42)	0 (0)	11 (100)	0 (0)
Hemicolectomy	1 (4)	1 (20)	0 (0)	0 (0)
Total colectomy	14 (54)	4 (80)	0 (0)	10 (100)
Type of biologic or small molecule at any time prior to resection, n (%), non-exclusive				
Anti TNF	16 (62)	0 (0)	7 (64)	9 (90)
Anti integrins	7 (27)	0 (0)	4 (36)	3 (30)
Anti IL12/23	3 (12)	0 (0)	3 (27)	0 (0)
Small molecules	3 (12)	0 (0)	1 (9)	2 (20)
Type of biologic or small molecule at time of reseciton, n (%)				
Anti TNF	2 (8)	0 (0)	2 (18)	0 (0)
Anti integrins	2 (8)	0 (0)	2 (18)	0 (0)
Anti IL12/23	1 (4)	0 (0)	1 (9)	0 (0)
Small molecules	0 (0)	0 (0)	0 (0)	0 (0)

Table S2. Pathways enriched in primary human intestinal myofibroblasts from normal tissue exposed to MFGE8, compared to untreated control

Description	Category	FDR value	Term name	Term Size	Itersection Size
Mixed, incl. interferon alpha/beta signaling, and negative regulators of ddx58/ifih1 signaling	STRING Clusters	2.98E-52	CL:19382	67	35
Mixed, incl. interferon alpha/beta signaling, and negative regulation of type i interferon production	STRING Clusters	7.40E-52	CL:19379	83	36
Interferon alpha/beta signaling, and Negative regulators of DDX58/IFIH1 signaling	STRING Clusters	5.67E-50	CL:19385	61	33
Interferon alpha/beta signaling, and ISG15-protein conjugation	STRING Clusters	6.25E-48	CL:19389	42	30
Interferon alpha/beta signaling, and ISG15-protein conjugation	STRING Clusters	2.45E-45	CL:19391	36	28
Antiviral defense	UniProt Keywords	3.31E-31	KW-0051	127	27
Response to virus	GO Biological Process	7.49E-31	GO:0009615	293	34
Defense response to virus	GO Biological Process	7.49E-31	GO:0051607	210	31
Interferon alpha/beta signaling	Reactome Pathways	8.73E-28	HSA-909733	69	22
Interferon Signaling	Reactome Pathways	8.73E-28	HSA-913531	196	28
Response to type i interferon	GO Biological Process	3.82E-27	GO:0034340	72	22
Interferon alpha/beta signaling	STRING Clusters	8.52E-27	CL:19394	21	17
Microphthalmia with limb anomalies, and Interferon alpha/beta signaling	STRING Clusters	3.55E-26	CL:19397	16	16
Type i interferon signaling pathway	GO Biological Process	4.35E-26	GO:0060337	67	21
Response to other organism	GO Biological Process	1.08E-23	GO:0051707	1256	46
Defense response to other organism	GO Biological Process	7.37E-21	GO:0098542	900	38
Innate immune response	GO Biological Process	4.76E-20	GO:0045087	703	34

Immunity	UniProt Keywords	1.02E-18	KW-0391	522	29
Defense response	GO Biological Process	2.04E-18	GO:0006952	129	41
Innate immunity	UniProt Keywords	5.80E-18	KW-0399	324	24
Interspecies interaction between organisms	GO Biological Process	1.32E-17	GO:0044419	189	47
Response to external stimulus	GO Biological Process	1.58E-17	GO:0009605	231	51
Mixed, incl. 2-5-oligoadenylate synthetase activity, and nephropathia epidemica	STRING Clusters	1.78E-17	CL:19399	11	11
Cytokine Signaling in Immune system	Reactome Pathways	1.84E-17	HSA-1280215	681	31
Mixed, incl. isg15-protein conjugation, and interferon-induced protein 44 family	STRING Clusters	5.61E-17	CL:19437	13	11
Immune response	GO Biological Process	2.97E-16	GO:0006955	158	42
Negative regulation of viral genome replication	GO Biological Process	1.98E-15	GO:0045071	61	14
Immune system process	GO Biological Process	1.12E-14	GO:0002376	248	49
Cytokine-mediated signaling pathway	GO Biological Process	2.12E-14	GO:0019221	678	28
Negative regulation of viral process	GO Biological Process	5.30E-14	GO:0048525	105	15
Response to cytokine	GO Biological Process	1.38E-13	GO:0034097	110	33
Cellular response to cytokine stimulus	GO Biological Process	7.19E-13	GO:0071345	101	31
Antiviral mechanism by IFN-stimulated genes	Reactome Pathways	1.34E-12	HSA-1169410	81	13
Immune response to tuberculosis	WikiPathways	3.27E-11	WP4197	23	9
Type II interferon signaling (IFNG)	WikiPathways	3.27E-11	WP619	37	10
SARS-CoV-2 innate immunity evasion and cell-specific immune response	WikiPathways	5.93E-11	WP5039	66	11
Mixed, incl. interferon-induced protein 44 family, and receptor-transporting protein 4	STRING Clusters	1.29E-10	CL:19438	7	7
Influenza A	KEGG Pathways	2.10E-10	hsa05164	165	14

Immune System	Reactome Pathways	2.28E-10	HSA-168256	195	38
Response to interferon-gamma	GO Biological Process	1.07E-09	GO:0034341	182	14
Interferon gamma signaling	Reactome Pathways	1.63E-09	HSA-877300	88	11
Type I interferon induction and signaling during SARS-CoV-2 infection	WikiPathways	4.23E-09	WP4868	31	8
Response to organic substance	GO Biological Process	6.10E-09	GO:0010033	301	45
Hepatitis C	KEGG Pathways	1.49E-08	hsa05160	156	12
Cellular response to organic substance	GO Biological Process	1.64E-08	GO:0071310	236	39
Response to stress	GO Biological Process	5.36E-08	GO:0006950	348	47
Cellular response to interferon-gamma	GO Biological Process	5.36E-08	GO:0071346	161	12
Interferon-gamma-mediated signaling pathway	GO Biological Process	1.38E-07	GO:0060333	70	9
ISG15 antiviral mechanism	Reactome Pathways	1.50E-07	HSA-1169408	73	9
Host-pathogen interaction of human coronaviruses - interferon induction	WikiPathways	2.06E-07	WP4880	33	7
Microphthalmia with limb anomalies	DISEASES	2.39E-07	DOID:0060861	8	6
Mixed, incl. 4fe-4s single cluster domain, and interferon-induced protein with tetratricopeptide repeats 3	STRING Clusters	3.50E-07	CL:19402	5	5
Response to interferon-beta	GO Biological Process	4.04E-07	GO:0035456	31	7
Cellular response to chemical stimulus	GO Biological Process	4.58E-07	GO:0070887	291	41
Measles	KEGG Pathways	6.84E-07	hsa05162	138	10
OAS antiviral response	Reactome Pathways	1.96E-06	HSA-8983711	9	5
Response to chemical	GO Biological Process	2.12E-06	GO:0042221	433	50
Cell surface receptor signaling pathway	GO Biological Process	2.16E-06	GO:0007166	232	35
NOD-like receptor signaling pathway	KEGG Pathways	4.12E-06	hsa04621	174	10

Regulation of type i interferon production	GO Biological Process	1.83E-05	GO:0032479	130	9
Response to stimulus	GO Biological Process	2.32E-05	GO:0050896	6	70
Double-stranded rna binding	GO Molecular Function	4.28E-05	GO:0003725	75	8
Epstein-Barr virus infection	KEGG Pathways	8.46E-05	hsa05169	193	9
RNA-binding	UniProt Keywords	1.00E-04	KW-0694	670	16
Regulation of response to biotic stimulus	GO Biological Process	0.00011	GO:0002831	406	13
Response to interferon-alpha	GO Biological Process	0.00011	GO:0035455	23	5
Novel intracellular components of RIG-I-like receptor (RLR) pathway	WikiPathways	0.00014	WP3865	59	6
Regulation of ribonuclease activity	GO Biological Process	0.00016	GO:0060700	9	4
RIG-I-like receptor signaling pathway	KEGG Pathways	0.00017	hsa04622	70	6
2-5-oligoadenylate synthetase 1, domain 2, C-terminus	Pfam	0.00018	PF10421	4	4
Pathways of nucleic acid metabolism and innate immune sensing	WikiPathways	0.00032	WP4705	16	4
Cytosolic DNA-sensing pathway	WikiPathways	0.00033	WP4655	73	6
2-5OAS/ClassI-CCAase, nucleotidyltransferase domain	InterPro Domains	0.00034	IPR006116	4	4
2-5-oligoadenylate synthetase, C-terminal conserved site	InterPro Domains	0.00034	IPR006117	4	4
2-5-oligoadenylate synthetase 1, domain 2/C-terminal	InterPro Domains	0.00034	IPR018952	4	4
2-5-oligoadenylate synthase	InterPro Domains	0.00034	IPR026774	4	4
2-5-oligoadenylate synthetase, N-terminal conserved site	InterPro Domains	0.00034	IPR043518	4	4
Response to bacterium	GO Biological Process	0.00049	GO:0009617	634	15
Positive regulation of interferon-beta production	GO Biological Process	0.00056	GO:0032728	34	5
Regulation of defense response	GO Biological Process	0.00097	GO:0031347	674	15
Toll-like receptor signaling pathway	KEGG Pathways	0.001	hsa04620	101	6
Regulation of immune system process	GO Biological Process	0.0012	GO:0002682	151	23

	KEGG	0.001			
Cytosolic DNA-sensing pathway	Pathways	2	hsa04623	62	5
DDX58/IFIH1-mediated induction of	Reactome	0.001	HSA-		
interferon-alpha/beta	Pathways	2	168928	76	6
	GO Biological	0.001	GO:005077		
Regulation of immune response	Process	6	6	896	17
Regulation of response to cytokine	GO Biological	0.001	GO:006075		
stimulus	Process	6	9	175	8
Negative regulation of type i	GO Biological	0.001	GO:003248		
interferon production	Process	7	0	45	5
Positive regulation of type i	GO Biological	0.001	GO:003248		
interferon production	Process	7	1	80	6
Regulation of defense response to	GO Biological	0.001	GO:005068		
virus	Process	7	8	81	6
	UniProt	0.001		502	
Cytoplasm	Keywords	9	KW-0963	8	47
		0.001			
Toll-like receptor signaling pathway	WikiPathways	9	WP75	103	6
SARS coronavirus and innate		0.002			
immunity	WikiPathways	3	WP4912	31	4
	GO Biological	0.002	GO:003545		
Cellular response to interferon-beta	Process	5	8	22	4
	STRING	0.002			
CXCR3 chemokine receptor binding	Clusters	8	CL:18318	5	3
	STRING	0.002			
ISG15-protein conjugation	Clusters	8	CL:19452	5	3
RIG-I-like receptor, C-terminal	InterPro	0.003			
regulatory domain	Domains	2	IPR021673	3	3
P-loop containing nucleoside	InterPro	0.003			
triphosphate hydrolase	Domains	2	IPR027417	861	17
RIG-I-like receptor, C-terminal	InterPro	0.003			
domain superfamily	Domains	2	IPR038557	3	3
	InterPro	0.003			
RIG-I receptor, C-terminal	Domains	2	IPR041204	3	3
Non-genomic actions of 1,25		0.003			
dihydroxyvitamin D3	WikiPathways	2	WP4341	71	5
	COMPARTM	0.003	GOCC:009		
Interferon regulatory factor complex	ENTS	8	7071	13	4
Positive regulation of interferon-	GO Biological	0.004	GO:003272		
alpha production	Process	3	7	26	4
	GO Molecular	0.004	GO:000173		
2-5-oligoadenylate synthetase activity	Function	6	0	3	3
	GO Molecular	0.004	GO:000372		
RNA helicase activity	Function	6	4	74	6
		0.004			
C-terminal domain of RIG-I	Pfam	9	PF11648	3	3

Single-stranded rna binding	GO Molecular Function	0.005 8	GO:000372 7	82	6
CXCR3 chemokine receptor binding	GO Molecular Function	0.005 8	GO:004824 8	5	3
Regulation of toll-like receptor signaling pathway	WikiPathways	0.006 9	WP1449	139	6
Cytoplasmic pattern recognition receptor signaling pathway in response to virus	GO Biological Process	0.007 1	GO:003952 8	9	3
Chemokine receptors bind chemokines	STRING Clusters	0.007 2	CL:18276	28	4
Viral protein interaction with cytokine and cytokine receptor	KEGG Pathways	0.007 4	hsa04061	96	5
Nucleotidyltransferase superfamily	InterPro Domains	0.008	IPR043519	23	4
Cellular response to virus	GO Biological Process	0.008 7	GO:009858 6	32	4
Regulation of innate immune response	GO Biological Process	0.008 8	GO:004508 8	301	9
Negative regulators of DDX58/IFIH1 signaling	Reactome Pathways	0.009 2	HSA-936440	33	4
Regulation of cytokine production	GO Biological Process	0.009 6	GO:000181 7	742	14
Polymerase, nucleotidyl transferase domain	InterPro Domains	0.010 3	IPR002934	7	3
Guanylate-binding protein, C-terminal	InterPro Domains	0.010 3	IPR037684	7	3
Human papillomavirus infection	KEGG Pathways	0.013 1	hsa05165	325	8
Helicase	UniProt Keywords	0.014 2	KW-0347	140	6
Positive regulation of calcium ion transport	GO Biological Process	0.014 8	GO:005192 8	125	6
Interferon regulatory factor DNA-binding domain	InterPro Domains	0.015 9	IPR001346	9	3
Guanylate-binding protein/Atlastin, C-terminal	InterPro Domains	0.015 9	IPR003191	9	3
Interferon regulatory factor, conserved site	InterPro Domains	0.015 9	IPR019817	9	3
Cellular response to interferon-alpha	GO Biological Process	0.016 1	GO:003545 7	13	3
Interferon regulatory factor	SMART Domains	0.016 7	SM00348	8	3
Guanylate-binding protein, C-terminal domain superfamily	InterPro Domains	0.016 7	IPR036543	10	3

TRAF3-dependent IRF activation pathway	Reactome Pathways	0.018 3	HSA-918233	14	3
Guanylate-binding protein, N-terminal	InterPro Domains	0.02	IPR015894	11	3
GB1/RHD3-type guanine nucleotide-binding (G) domain	InterPro Domains	0.02	IPR030386	11	3
Regulation of response to external stimulus	GO Biological Process	0.020 1	GO:003210 1	101 3	16
Nucleotide binding	GO Molecular Function	0.020 5	GO:000016 6	211 9	26
Binding	GO Molecular Function	0.020 5	GO:000548 8	125 16	85
Purine nucleotide binding	GO Molecular Function	0.020 5	GO:001707 6	187 8	24
Purine ribonucleoside triphosphate binding	GO Molecular Function	0.020 5	GO:003563 9	179 9	23
Chemokine receptor binding	GO Molecular Function	0.020 5	GO:004237 9	70	5
Carbohydrate derivative binding	GO Molecular Function	0.023 4	GO:009736 7	222 6	26
Purine ribonucleotide binding	GO Molecular Function	0.026 4	GO:003255 5	186 4	23
Anion binding	GO Molecular Function	0.026 4	GO:004316 8	280 5	30
Protein adp-ribosylase activity	GO Molecular Function	0.026 4	GO:199040 4	15	3
CXC chemokine	InterPro Domains	0.027 3	IPR001089	13	3
CXC chemokine, conserved site	InterPro Domains	0.027 3	IPR018048	13	3
CXC Chemokine domain	InterPro Domains	0.029 8	IPR033899	14	3
Chemokine activity	GO Molecular Function	0.031	GO:000800 9	48	4
Cellular response to exogenous dsrna	GO Biological Process	0.031 3	GO:007136 0	17	3
Swimming behavior	GO Biological Process	0.033 1	GO:003626 9	2	2
Chemokine interleukin-8-like domain	InterPro Domains	0.034	IPR001811	43	4
Chemokine interleukin-8-like superfamily	InterPro Domains	0.034	IPR036048	43	4
Herpes simplex virus 1 infection	KEGG Pathways	0.034 5	hsa05168	479	9
DEAD/DEAH box helicase	Pfam	0.035	PF00270	76	5

Interferon regulatory factor transcription factor	Pfam	0.035	PF00605	9	3
Nucleotidyltransferase domain	Pfam	0.035	PF01909	11	3
Guanylate-binding protein, N-terminal domain	Pfam	0.035	PF02263	11	3
Guanylate-binding protein, C-terminal domain	Pfam	0.035	PF02841	9	3
	UniProt	0.035		176	
Nucleotide-binding	Keywords	1	KW-0547	0	21
	GO Molecular	0.035	GO:001711		
Nucleoside-triphosphatase activity	Function	3	1	760	13
Regulation of pattern recognition	GO Biological	0.037	GO:006220		
receptor signaling pathway	Process	5	7	95	5
Positive regulation of cytokine	GO Biological	0.038	GO:000181		
production	Process	4	9	461	10
Poly(ADP-ribose) polymerase,	InterPro	0.043			
catalytic domain	Domains	2	IPR012317	17	3
Mitochondrial immune response to		0.043			
SARS-CoV-2	WikiPathways	3	WP5038	32	3
	InterPro	0.044			
Interferon-induced protein 44 family	Domains	7	IPR024644	2	2
	UniProt	0.046			
Cytokine	Keywords	4	KW-0202	186	6
	UniProt	0.046			
Nucleotidyltransferase	Keywords	4	KW-0548	69	4

Table S3. Pathways enriched in primary human intestinal myofibroblasts from Crohn's disease stricture tissue exposed to MFGE8, compared to untreated control

Description	Category	FDR.value	Term.name	Term_Size	Itersection_Size
TGF-beta signaling pathway	KEGG Pathways	3.56E-11	hsa04350	91	7
BMP signaling pathway, and activin receptor signaling pathway	STRING Clusters	8.46E-10	CL:21290	99	7
Mixed, incl. chondrogenesis, and dan domain	STRING Clusters	1.44E-08	CL:21347	24	5
TGF-beta propeptide	Pfam	1.80E-08	PF00688	23	5
TGF-beta, propeptide	InterPro Domains	2.37E-08	IPR001111	21	5
Cystine-knot cytokine	InterPro Domains	2.37E-08	IPR029034	75	6
Transforming growth factor-beta-related	InterPro Domains	5.23E-08	IPR015615	32	5
Transforming growth factor beta, conserved site	InterPro Domains	5.23E-08	IPR017948	32	5
Transforming growth factor-beta, C-terminal	InterPro Domains	6.11E-08	IPR001839	37	5
Transforming growth factor beta like domain	Pfam	8.79E-08	PF00019	38	5
Chondrogenesis	UniProt Keywords	3.80E-07	KW-0891	16	4
Chondrogenesis, and Repulsive guidance molecule	STRING Clusters	6.39E-07	CL:21350	16	4
Positive regulation of pathway-restricted smad protein phosphorylation	GO Biological Process	2.15E-06	GO:0010862	49	5
SMAD protein signal transduction	GO Biological Process	2.58E-06	GO:0060395	59	5
Transforming growth factor-beta (TGF-beta) family	SMART Domains	3.43E-06	SM00204	29	4
Growth factor	UniProt Keywords	6.02E-06	KW-0339	130	5
Hippo signaling pathway	KEGG Pathways	6.59E-06	hsa04390	153	5
BMP signaling pathway	GO Biological Process	9.69E-06	GO:0030509	90	5

Positive regulation of cartilage development	GO Biological Process	1.63E-05	GO:0061036	32	4
Regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	GO Biological Process	1.63E-05	GO:0090092	244	6
BMP signaling pathway involved in heart development, and Spondylolisthesis	STRING Clusters	2.07E-05	CL:21354	7	3
Cytokine	UniProt Keywords	2.28E-05	KW-0202	186	5
Molecules associated with elastic fibres	Reactome Pathways	2.80E-05	HSA-2129379	38	4
Cell proliferation involved in kidney development	GO Biological Process	8.37E-05	GO:0072111	10	3
Cytokine-cytokine receptor interaction	KEGG Pathways	8.56E-05	hsa04060	282	5
Positive regulation of neuron differentiation	GO Biological Process	9.62E-05	GO:0045666	377	6
BMP receptor complex	COMPARTMENTS	0.00012	GOCC:0070724	9	3
Mostly uncharacterized, incl. protein of unknown function (duf2781), and transmembrane protein 182	STRING Clusters	0.00014	CL:29129	16	3
Growth factor activity	GO Molecular Function	0.00016	GO:0008083	161	5
BMP receptor binding	GO Molecular Function	0.00017	GO:0070700	12	3
Mesenchymal cell proliferation	GO Biological Process	2.00E-04	GO:0010463	16	3
Osteogenesis	UniProt Keywords	0.00032	KW-0892	35	3
Cytokine activity	GO Molecular Function	0.00033	GO:0005125	233	5
Receptor ligand activity	GO Molecular Function	0.00033	GO:0048018	490	6
Differentiation of white and brown adipocyte	WikiPathways	5.00E-04	WP2895	25	3

Regulation of osteoblast differentiation	GO Biological Process	0.00068	GO:0045667	117	4
Odontogenesis	GO Biological Process	0.00071	GO:0042476	120	4
Regulation of animal organ formation	GO Biological Process	0.0015	GO:0003156	38	3
Positive regulation of bone mineralization	GO Biological Process	0.0016	GO:0030501	40	3
Regulation of heart morphogenesis	GO Biological Process	0.0018	GO:2000826	42	3
Endocardial cushion development	GO Biological Process	0.002	GO:0003197	44	3
BMP signaling pathway involved in heart induction	GO Biological Process	0.0021	GO:0003130	3	2
Positive regulation of epithelial to mesenchymal transition	GO Biological Process	0.0021	GO:0010718	47	3
Regulation of chondrocyte differentiation	GO Biological Process	0.0021	GO:0032330	51	3
Cartilage development	GO Biological Process	0.0021	GO:0051216	171	4
Negative regulation of prostatic bud formation	GO Biological Process	0.0021	GO:0060686	4	2
Negative regulation of glomerular mesangial cell proliferation	GO Biological Process	0.0021	GO:0072125	3	2
Mesenchymal cell proliferation involved in ureteric bud development	GO Biological Process	0.0021	GO:0072138	3	2
Regulation of cardiocyte differentiation	GO Biological Process	0.0021	GO:1905207	49	3
Positive regulation of cardiac neural crest cell migration involved in outflow tract morphogenesis	GO Biological Process	0.0021	GO:1905312	3	2

Positive regulation of osteoblast differentiation	GO Biological Process	0.0024	GO:0045669	60	3
Skeletal system development	GO Biological Process	0.003	GO:0001501	499	5
Regulation of branching involved in prostate gland morphogenesis	GO Biological Process	0.003	GO:0060687	6	2
Development of ureteric collection system	WikiPathways	0.003	WP5053	60	3
Cleavage on pair of basic residues	UniProt Keywords	0.0037	KW-0165	278	4
Protein of unknown function (DUF2781)	Pfam	0.0047	PF10914	3	2
Odontogenesis of dentin-containing tooth	GO Biological Process	0.0048	GO:0042475	81	3
Ossification	GO Biological Process	0.0049	GO:0001503	265	4
Metanephros development	GO Biological Process	0.0049	GO:0001656	84	3
Generation of neurons	GO Biological Process	0.0049	GO:0048699	1551	7
Ureteric bud development	GO Biological Process	0.005	GO:0001657	86	3
Kidney development	GO Biological Process	0.005	GO:0001822	271	4
Negative regulation of mesenchymal cell apoptotic process	GO Biological Process	0.0051	GO:2001054	10	2
Mixed, incl. domain of unknown function duf4592, and protein of unknown function (duf2781)	STRING Clusters	0.0051	CL:29131	6	2
Regulation of bmp signaling pathway	GO Biological Process	0.0052	GO:0030510	90	3
Positive regulation of animal organ morphogenesis	GO Biological Process	0.0052	GO:0110110	89	3

Embryonic skeletal joint morphogenesis	GO Biological Process	0.0055	GO:0060272	11	2
Nervous system development	GO Biological Process	0.0057	GO:0007399	2371	8
Telencephalon regionalization	GO Biological Process	0.0068	GO:0021978	13	2
Pathway-restricted smad protein phosphorylation	GO Biological Process	0.0068	GO:0060389	13	2
Secreted	UniProt Keywords	0.007	KW-0964	1818	7
Positive regulation of multicellular organismal process	GO Biological Process	0.008	GO:0051240	1770	7
Regulation of odontogenesis of dentin-containing tooth	GO Biological Process	0.0091	GO:0042487	16	2
EXPERA domain	InterPro Domains	0.0096	IPR033118	5	2
Negative regulation of cell differentiation	GO Biological Process	0.0102	GO:0045596	728	5
Embryonic limb morphogenesis	GO Biological Process	0.0105	GO:0030326	127	3
Regulation of cell differentiation	GO Biological Process	0.0105	GO:0045595	1874	7
Nephron development	GO Biological Process	0.0105	GO:0072006	126	3
Kidney mesenchyme development	GO Biological Process	0.0105	GO:0072074	18	2
Spondylolisthesis	DISEASES	0.0107	DOID:6682	2	2
Branching morphogenesis of an epithelial tube	GO Biological Process	0.0111	GO:0048754	131	3
Positive regulation of chondrocyte differentiation	GO Biological Process	0.012	GO:0032332	20	2

Pericardium development	GO Biological Process	0.012	GO:0060039	20	2
BMP binding	GO Molecular Function	0.0133	GO:0036122	12	2
Co-receptor binding	GO Molecular Function	0.0138	GO:0039706	13	2
Negative regulation of chondrocyte differentiation	GO Biological Process	0.0151	GO:0032331	23	2
Mesenchymal cell differentiation	GO Biological Process	0.0159	GO:0048762	152	3
Dwarfism	UniProt Keywords	0.0169	KW-0242	170	3
Microphthalmia	UniProt Keywords	0.0169	KW-1013	28	2
Pattern specification process	GO Biological Process	0.0173	GO:0007389	432	4
Cardiac muscle tissue development	GO Biological Process	0.0175	GO:0048738	160	3
Pharyngeal system development	GO Biological Process	0.0175	GO:0060037	26	2
Positive regulation of p38mapk cascade	GO Biological Process	0.0175	GO:1900745	26	2
Negative regulation of myoblast differentiation	GO Biological Process	0.0183	GO:0045662	27	2
Regulation of smad protein signal transduction	GO Biological Process	0.0183	GO:0060390	27	2
Adipogenesis	WikiPathways	0.0188	WP236	130	3
DAN domain	Pfam	0.0192	PF03045	9	2
Brachydactyly	HPO	0.0256	HP:0001156	313	4
Short middle phalanx of finger	HPO	0.0256	HP:0005819	39	3
Medially deviated second toe	HPO	0.0256	HP:0008096	3	2

Triangular shaped middle phalanx of the 5th finger	HPO	0.0256	HP:0009182	3	2
Type A2 brachydactyly	HPO	0.0256	HP:0009372	3	2
Ulnar deviation of the 2nd finger	HPO	0.0256	HP:0009464	6	2
Radial deviation of the 2nd finger	HPO	0.0256	HP:0009467	7	2
Short 2nd finger	HPO	0.0256	HP:0009536	11	2
Aplasia/Hypoplasia of the middle phalanx of the 2nd finger	HPO	0.0256	HP:0009568	7	2
Triangular shaped middle phalanx of the 2nd finger	HPO	0.0256	HP:0009575	3	2
Short 2nd metacarpal	HPO	0.0256	HP:0010038	3	2
Facial asymmetry	HPO	0.0258	HP:0000324	116	3
Short middle phalanx of the 5th finger	HPO	0.0258	HP:0004220	16	2
Endocardial cushion morphogenesis	GO Biological Process	0.0262	GO:0003203	34	2
Positive regulation of bmp signaling pathway	GO Biological Process	0.0262	GO:0030513	34	2
Oligodendrocyte specification and differentiation, leading to myelin components for CNS	WikiPathways	0.0277	WP4304	30	2
FGFR3 signaling in chondrocyte proliferation and terminal differentiation	WikiPathways	0.0277	WP4767	27	2
Animal organ morphogenesis	GO Biological Process	0.0278	GO:0009887	967	5
Regulation of cardiac muscle cell differentiation	GO Biological Process	0.0283	GO:2000725	36	2
Hindlimb morphogenesis	GO Biological Process	0.0296	GO:0035137	37	2
Central nervous system development	GO Biological Process	0.0298	GO:0007417	988	5

Heart development	GO Biological Process	0.0298	GO:0007507	522	4
Embryo development	GO Biological Process	0.0309	GO:0009790	1002	5
Regulation of multicellular organismal process	GO Biological Process	0.0309	GO:0051239	3227	8
Cellular response to organic cyclic compound	GO Biological Process	0.0319	GO:0071407	537	4
Glandular epithelial cell differentiation	GO Biological Process	0.0323	GO:0002067	40	2
Negative regulation of mitotic nuclear division	GO Biological Process	0.0323	GO:0045839	40	2
Pituitary gland development	GO Biological Process	0.0345	GO:0021983	42	2
Branching involved in ureteric bud morphogenesis	GO Biological Process	0.0375	GO:0001658	44	2
Tarsal synostosis	HPO	0.0425	HP:0008368	25	2
Negative regulation of striated muscle tissue development	GO Biological Process	0.0466	GO:0045843	50	2
Heart development	WikiPathways	0.0468	WP1591	44	2
Pluripotent stem cell differentiation pathway	WikiPathways	0.0474	WP2848	48	2
Heart morphogenesis	GO Biological Process	0.0487	GO:0003007	251	3
Heart valve morphogenesis	GO Biological Process	0.0493	GO:0003179	52	2
Regulation of pri-mirna transcription by rna polymerase ii	GO Biological Process	0.0493	GO:1902893	52	2

Table S4. Pathways enriched in primary human intestinal myofibroblasts exposed to MFGE8 from normal tissue compared to Crohn's disease stricture tissue

Differentially regulated genes from normal compared to Crohn's disease stricture fibroblasts form the basis for this analysis.

Description	Category	FDR.value	Term.name	Term_Size	Itersection_Size
Glycoprotein	UniProt Keywords	1.76E-11	KW-0325	4349	87
Signal	UniProt Keywords	1.30E-09	KW-0732	3233	69
Cell junction	GO Cellular Component	1.08E-07	GO:0030054	2075	51
Synapse	GO Cellular Component	1.08E-07	GO:0045202	1351	40
Biological adhesion	GO Biological Process	1.40E-07	GO:0022610	931	34
Cell adhesion	UniProt Keywords	2.44E-07	KW-0130	474	22
Cell adhesion	GO Biological Process	2.69E-07	GO:0007155	925	33
Regulation of biological quality	GO Biological Process	3.70E-07	GO:0065008	4042	76
Focal adhesion	KEGG Pathways	4.08E-07	hsa04510	198	15
Neuron differentiation	GO Biological Process	1.51E-06	GO:0030182	1019	33
Secreted	UniProt Keywords	4.89E-06	KW-0964	1818	42
Focal adhesion	WikiPathways	6.06E-06	WP306	196	14
System development	GO Biological Process	1.48E-05	GO:0048731	4426	76
Regulation of cellular component movement	GO Biological Process	1.48E-05	GO:0051270	1009	31
Regulation of cell migration	GO Biological Process	2.03E-05	GO:0030334	865	28
Generation of neurons	GO Biological Process	2.03E-05	GO:0048699	1551	39
Cell junction	UniProt Keywords	2.08E-05	KW-0965	801	25
Cell junction assembly	GO Biological Process	2.15E-05	GO:0034329	280	16
Synapse organization	GO Biological Process	2.23E-05	GO:0050808	283	16
Intrinsic component of plasma membrane	GO Cellular Component	2.46E-05	GO:0031226	1703	40
Positive regulation of developmental process	GO Biological Process	2.54E-05	GO:0051094	1389	36
Regulation of locomotion	GO Biological Process	3.41E-05	GO:0040012	969	29
Regulation of developmental process	GO Biological Process	3.41E-05	GO:0050793	2648	53
Regulation of localization	GO Biological Process	3.44E-05	GO:0032879	2740	54
Synapse assembly	GO Biological Process	4.16E-05	GO:0007416	96	10
Cell junction organization	GO Biological Process	4.16E-05	GO:0034330	493	20
Regulation of synapse organization	GO Biological Process	4.16E-05	GO:0050807	228	14

Anatomical structure morphogenesis	GO Biological Process	4.29E-05	GO:0009653	2165	46
Neuron development	GO Biological Process	4.49E-05	GO:0048666	827	26
Nervous system development	GO Biological Process	7.22E-05	GO:0007399	2371	48
Positive regulation of cell migration	GO Biological Process	7.26E-05	GO:0030335	522	20
Integral component of plasma membrane	GO Cellular Component	0.00014	GO:0005887	1623	37
Multicellular organism development	GO Biological Process	0.00015	GO:0007275	5023	78
Extracellular region	GO Cellular Component	0.00017	GO:0005576	4166	68
Cell-cell adhesion	GO Biological Process	0.00018	GO:0098609	505	19
Regulation of anatomical structure morphogenesis	GO Biological Process	0.00019	GO:0022603	1095	29
Extracellular matrix	GO Cellular Component	2.00E-04	GO:0031012	527	19
Postsynapse	GO Cellular Component	2.00E-04	GO:0098794	643	21
Neuron projection development	GO Biological Process	0.00021	GO:0031175	680	22
Cell junction	COMPARTMENTS	0.00023	GOCC:0030054	1045	29
Intrinsic component of plasma membrane	COMPARTMENTS	0.00023	GOCC:0031226	841	26
Regulation of multicellular organismal process	GO Biological Process	0.00023	GO:0051239	3227	57
Regulation of cell junction assembly	GO Biological Process	0.00023	GO:1901888	199	12
Integral component of plasma membrane	COMPARTMENTS	0.00025	GOCC:0005887	782	24
Intrinsic component of membrane	COMPARTMENTS	0.00025	GOCC:0031224	1552	36
Integral component of membrane	COMPARTMENTS	0.00029	GOCC:0016021	1456	34
Mesenchyme	TISSUES	0.00032	BTO:0001393	10	5
Regulation of cell adhesion	GO Biological Process	0.00041	GO:0030155	712	22
System process	GO Biological Process	0.00047	GO:0003008	1942	40
Regulation of multicellular organismal development	GO Biological Process	0.00047	GO:2000026	2096	42
Skeletal system development	GO Biological Process	0.00048	GO:0001501	499	18
Anatomical structure development	GO Biological Process	5.00E-04	GO:0048856	5402	80
Protein measurement	HPO	0.00052	EFO:0004747	5855	89
Proteoglycans in cancer	KEGG Pathways	0.00057	hsa05205	196	11
Extracellular region	COMPARTMENTS	0.00061	GOCC:0005576	2035	41

Disulfide bond	UniProt Keywords	0.00068	KW-1015	3304	55
Positive regulation of cell differentiation	GO Biological Process	0.00071	GO:0045597	993	26
Postsynaptic membrane	GO Cellular Component	0.00071	GO:0045211	281	13
Plasma membrane region	GO Cellular Component	0.00071	GO:0098590	1219	29
Multicellular organismal process	GO Biological Process	0.00072	GO:0032501	6933	95
Signaling by Receptor Tyrosine Kinases	Reactome Pathways	0.00074	HSA-9006934	502	19
Circulatory system process	GO Biological Process	0.00075	GO:0003013	415	16
Negative regulation of cell death	GO Biological Process	0.00075	GO:0060548	999	26
Synaptic membrane	GO Cellular Component	8.00E-04	GO:0097060	387	15
Endocrine gland cancer	DISEASES	0.00081	DOID:170	84	9
Collagen-containing extracellular matrix	GO Cellular Component	0.00095	GO:0062023	396	15
Asymmetric synapse	GO Cellular Component	0.00099	GO:0032279	350	14
Negative regulation of apoptotic process	GO Biological Process	0.0011	GO:0043066	893	24
Animal organ morphogenesis	GO Biological Process	0.0013	GO:0009887	967	25
Cell morphogenesis	GO Biological Process	0.0014	GO:0000902	726	21
Regulation of protein phosphorylation	GO Biological Process	0.0014	GO:0001932	1459	32
Neuron projection morphogenesis	GO Biological Process	0.0014	GO:0048812	495	17
Regulation of cell morphogenesis	GO Biological Process	0.0015	GO:0022604	498	17
EGFR tyrosine kinase inhibitor resistance	KEGG Pathways	0.0015	hsa01521	78	7
PI3K-Akt signaling pathway	WikiPathways	0.0015	WP4172	336	14
Regulation of nitric oxide mediated signal transduction	GO Biological Process	0.0016	GO:0010749	10	4
Positive regulation of multicellular organismal process	GO Biological Process	0.0016	GO:0051240	1770	36
Postsynaptic specialization	GO Cellular Component	0.0016	GO:0099572	369	14
Cell membrane	UniProt Keywords	0.0016	KW-1003	3246	53
Focal adhesion: PI3K-Akt-mTOR-signaling pathway	WikiPathways	0.0016	WP3932	302	13
Synapse	COMPARTMENTS	0.0019	GOCC:0045202	499	17
Liver carcinoma	DISEASES	0.0019	DOID:686	15	5

Regulation of cell differentiation	GO Biological Process	0.0021	GO:0045595	1874	37
Regulation of nervous system development	GO Biological Process	0.0021	GO:0051960	942	24
Rap1 signaling pathway	KEGG Pathways	0.0023	hsa04015	202	10
PI3K-Akt signaling pathway	KEGG Pathways	0.0023	hsa04151	350	13
Regulation of synapse assembly	GO Biological Process	0.0024	GO:0051963	106	8
Chemical synaptic transmission	GO Biological Process	0.0025	GO:0007268	418	15
Positive regulation of protein autophosphorylation	GO Biological Process	0.0025	GO:0031954	28	5
Integral component of postsynaptic membrane	GO Cellular Component	0.0026	GO:0099055	119	8
Synapse	UniProt Keywords	0.0026	KW-0770	456	15
Postsynaptic density	GO Cellular Component	0.0028	GO:0014069	344	13
Carcinoma	DISEASES	0.0029	DOID:305	275	13
Tissue development	GO Biological Process	0.003	GO:0009888	1760	35
EGFR tyrosine kinase inhibitor resistance	WikiPathways	0.0032	WP4806	83	7
Regulation of cell death	GO Biological Process	0.0034	GO:0010941	1696	34
Regulation of mapk cascade	GO Biological Process	0.0035	GO:0043408	725	20
Positive regulation of nervous system development	GO Biological Process	0.0036	GO:0051962	547	17
Intrinsic component of synaptic membrane	GO Cellular Component	0.0038	GO:0099240	169	9
Extracellular matrix	UniProt Keywords	0.0038	KW-0272	265	11
Glutamatergic synapse	GO Cellular Component	0.0039	GO:0098978	361	13
Plasma membrane	GO Cellular Component	0.0042	GO:0005886	5314	74
Response to reactive oxygen species	GO Biological Process	0.0045	GO:0000302	198	10
Animal organ development	GO Biological Process	0.0045	GO:0048513	3197	52
Cell periphery	GO Cellular Component	0.0046	GO:0071944	5432	75
HIF-1 signaling pathway	KEGG Pathways	0.0046	hsa04066	106	7
Blood circulation	GO Biological Process	0.0048	GO:0008015	394	14
Positive regulation of phosphatidylinositol 3-kinase signaling	GO Biological Process	0.0048	GO:0014068	87	7
Regulation of phosphatidylinositol 3-kinase signaling	GO Biological Process	0.0052	GO:0014066	123	8

Postsynaptic cell membrane	UniProt Keywords	0.0055	KW-0628	144	8
Regulation of cell development	GO Biological Process	0.0059	GO:0060284	956	23
Regulation of leukocyte migration	GO Biological Process	0.0063	GO:0002685	209	10
Regulation of smooth muscle cell migration	GO Biological Process	0.0064	GO:0014910	62	6
Adherens junction	COMPARTMENTS	0.0068	GOCC:0005912	86	7
Positive regulation of nitric oxide mediated signal transduction	GO Biological Process	0.0068	GO:0010750	5	3
Regulation of cell-substrate adhesion	GO Biological Process	0.0068	GO:0010810	212	10
Locomotion	GO Biological Process	0.0068	GO:0040011	1251	27
Embryonic skeletal system development	GO Biological Process	0.0068	GO:0048706	130	8
Positive regulation of intracellular signal transduction	GO Biological Process	0.0068	GO:1902533	1041	24
Positive regulation of glucose metabolic process	GO Biological Process	0.0069	GO:0010907	38	5
Regulation of apoptotic process	GO Biological Process	0.0069	GO:0042981	1550	31
Cell development	GO Biological Process	0.0069	GO:0048468	1629	32
Positive regulation of response to stimulus	GO Biological Process	0.0069	GO:0048584	2257	40
Cell type cancer	DISEASES	0.0072	DOID:0050687	406	15
Disease of cellular proliferation	DISEASES	0.0072	DOID:14566	1012	25
Positive regulation of smooth muscle cell migration	GO Biological Process	0.0074	GO:0014911	39	5
Cancer	DISEASES	0.0074	DOID:162	895	23
Regulation of cell growth	GO Biological Process	0.0078	GO:0001558	423	14
Regulation of smooth muscle cell proliferation	GO Biological Process	0.008	GO:0048660	136	8
Vital signs	HPO	0.0081	EFO:0004303	1265	30
Self reported educational attainment	HPO	0.0081	EFO:0004784	1006	26
Mathematical ability	HPO	0.0081	EFO:0004875	731	22
Systolic blood pressure	HPO	0.0081	EFO:0006335	873	24
Blood protein measurement	HPO	0.0081	EFO:0007937	1815	38
Phenotypic abnormality	HPO	0.0081	HP:0000118	5134	77

Robo4 and VEGF signaling pathways crosstalk	WikiPathways	0.0083	WP3943	6	3
Positive regulation of mapk cascade	GO Biological Process	0.0085	GO:0043410	543	16
Extracellular matrix	COMPARTMENTS	0.0086	GOCC:0031012	253	11
Positive regulation of cell junction assembly	GO Biological Process	0.0093	GO:1901890	102	7
Regulation of immune system process	GO Biological Process	0.0097	GO:0002682	1514	30
Regulation of postsynaptic membrane potential	GO Biological Process	0.0101	GO:0060078	104	7
Regulation of protein localization to plasma membrane	GO Biological Process	0.0101	GO:1903076	104	7
Colon cancer	DISEASES	0.0103	DOID:219	14	4
Hepatocellular carcinoma	DISEASES	0.0103	DOID:684	14	4
Vascular process in circulatory system	GO Biological Process	0.0104	GO:0003018	185	9
Cell differentiation	GO Biological Process	0.0104	GO:0030154	3702	56
Regulation of membrane potential	GO Biological Process	0.0104	GO:0042391	440	14
Regulation of cellular component organization	GO Biological Process	0.0104	GO:0051128	2402	41
Positive regulation of protein phosphorylation	GO Biological Process	0.0107	GO:0001934	1019	23
Cell motility	GO Biological Process	0.0107	GO:0048870	1018	23
Regulation of axonogenesis	GO Biological Process	0.0107	GO:0050770	187	9
Receptor complex	COMPARTMENTS	0.0114	GOCC:0043235	420	14
Angiogenesis	WikiPathways	0.0115	WP1539	24	4
Aryl hydrocarbon receptor Netpath	WikiPathways	0.0115	WP2586	46	5
Caloric restriction and aging	WikiPathways	0.0115	WP4191	8	3
Cell projection organization	GO Biological Process	0.0117	GO:0030030	1170	25
Embryonic organ development	GO Biological Process	0.0117	GO:0048568	448	14
Regulation of transport	GO Biological Process	0.0117	GO:0051049	1776	33
Regulation of cellular localization	GO Biological Process	0.0117	GO:0060341	1027	23
Chemical synaptic transmission, postsynaptic	GO Biological Process	0.0117	GO:0099565	109	7
Eye photoreceptor cell differentiation	GO Biological Process	0.012	GO:0001754	46	5

Negative regulation of leukocyte migration	GO Biological Process	0.012	GO:0002686	46	5
Cell migration	GO Biological Process	0.012	GO:0016477	896	21
Bone resorption	GO Biological Process	0.012	GO:0045453	23	4
Positive regulation of epithelial cell proliferation	GO Biological Process	0.012	GO:0050679	192	9
Eye morphogenesis	GO Biological Process	0.0126	GO:0048592	151	8
Regulation of protein modification process	GO Biological Process	0.0127	GO:0031399	1870	34
Regulation of erk1 and erk2 cascade	GO Biological Process	0.0128	GO:0070372	292	11
Fluid shear stress and atherosclerosis	KEGG Pathways	0.0131	hsa05418	130	7
Cellular response to oxidative stress	GO Biological Process	0.0133	GO:0034599	244	10
Synaptic membrane	COMPARTMENTS	0.0135	GOCC:0097060	139	8
Regulation of protein localization to membrane	GO Biological Process	0.0138	GO:1905475	198	9
Developmental protein	UniProt Keywords	0.014	KW-0217	953	21
Heparin-binding	UniProt Keywords	0.014	KW-0358	88	6
Plasma membrane bounded cell projection organization	GO Biological Process	0.0141	GO:0120036	1122	24
Clear cell renal cell carcinoma pathways	WikiPathways	0.0144	WP4018	85	6
Cardiovascular measurement	HPO	0.0146	EFO:0004298	2382	43
Lipid or lipoprotein measurement	HPO	0.0146	EFO:0005105	2150	40
Cardiovascular disease biomarker measurement	HPO	0.0146	EFO:0005278	2232	41
Prostate cancer	KEGG Pathways	0.0149	hsa05215	96	6
Blood vessel morphogenesis	GO Biological Process	0.0157	GO:0048514	410	13
Cellular response to organonitrogen compound	GO Biological Process	0.0158	GO:0071417	590	16
Plasma membrane	COMPARTMENTS	0.0161	GOCC:0005886	3531	54
Tube morphogenesis	GO Biological Process	0.0162	GO:0035239	656	17
Regulation of system process	GO Biological Process	0.0162	GO:0044057	592	16
Homeostasis of number of cells	GO Biological Process	0.0162	GO:0048872	204	9
Positive regulation of cellular component organization	GO Biological Process	0.0162	GO:0051130	1209	25

Positive regulation of blood vessel endothelial cell proliferation involved in sprouting angiogenesis	GO Biological Process	0.0162	GO:1903589	9	3
Needle	TISSUES	0.0164	BTO:0000917	4	3
Oxidation	UniProt Keywords	0.0166	KW-0558	32	4
Postsynaptic specialization membrane	GO Cellular Component	0.0168	GO:0099634	126	7
Cadherin cytoplasmic C-terminal	Pfam	0.0168	PF16492	42	6
Regulation of cell-matrix adhesion	GO Biological Process	0.0171	GO:0001952	120	7
Hepatobiliary system cancer	DISEASES	0.0177	DOID:0080355	63	6
Camera-type eye morphogenesis	GO Biological Process	0.0178	GO:0048593	121	7
Regulation of cell morphogenesis involved in differentiation	GO Biological Process	0.018	GO:0010769	309	11
Regulation of protein localization	GO Biological Process	0.018	GO:0032880	934	21
Regulation of neuron differentiation	GO Biological Process	0.018	GO:0045664	665	17
Positive regulation of erk1 and erk2 cascade	GO Biological Process	0.018	GO:0070374	209	9
Cell-cell adhesion via plasma-membrane adhesion molecules	GO Biological Process	0.018	GO:0098742	257	10
Eye development	GO Biological Process	0.0181	GO:0001654	365	12
Homophilic cell adhesion via plasma membrane adhesion molecules	GO Biological Process	0.0181	GO:0007156	164	8
Axon development	GO Biological Process	0.0181	GO:0061564	421	13
Positive regulation of cell adhesion	GO Biological Process	0.0184	GO:0045785	423	13
Homeobox protein, antennapedia type, conserved site	InterPro Domains	0.019	IPR001827	20	5
Cadherin, cytoplasmic C-terminal domain	InterPro Domains	0.019	IPR032455	40	6
Platelet alpha granule	GO Cellular Component	0.0191	GO:0031091	91	6
Membrane raft	GO Cellular Component	0.0191	GO:0045121	324	11
Blood pressure	HPO	0.0192	EFO:0004325	1139	26
Lipid measurement	HPO	0.0192	EFO:0004529	1959	37
Positive regulation of phosphate metabolic process	GO Biological Process	0.0203	GO:0045937	1164	24

Positive regulation of smooth muscle cell proliferation	GO Biological Process	0.0203	GO:0048661	88	6
Sensory organ morphogenesis	GO Biological Process	0.0203	GO:0090596	264	10
Postsynapse organization	GO Biological Process	0.0203	GO:0099173	88	6
Phospholipase D signaling pathway	KEGG Pathways	0.0207	hsa04072	147	7
Negative regulation of protein metabolic process	GO Biological Process	0.0208	GO:0051248	1096	23
Positive regulation of cell growth	GO Biological Process	0.0215	GO:0030307	171	8
Positive regulation of cyclin-dependent protein serine/threonine kinase activity	GO Biological Process	0.0217	GO:0045737	30	4
Positive regulation of cell development	GO Biological Process	0.0218	GO:0010720	556	15
Positive regulation of oxidoreductase activity	GO Biological Process	0.0218	GO:0051353	57	5
Response to fatty acid	GO Biological Process	0.0226	GO:0070542	91	6
Integrin binding	GO Molecular Function	0.0232	GO:0005178	147	9
Glycosaminoglycan binding	GO Molecular Function	0.0232	GO:0005539	240	11
Heparin binding	GO Molecular Function	0.0232	GO:0008201	172	10
Blood vessel development	GO Biological Process	0.0238	GO:0001568	500	14
Vascular disease	DISEASES	0.0238	DOID:178	223	10
Cell periphery	COMPARTMENTS	0.0244	GOCC:0071944	3688	55
Axonogenesis	GO Biological Process	0.0247	GO:0007409	384	12
Cell morphogenesis involved in differentiation	GO Biological Process	0.0251	GO:0000904	566	15
Bladder cancer	KEGG Pathways	0.0255	hsa05219	41	4
Positive regulation of signal transduction	GO Biological Process	0.0256	GO:0009967	1654	30
Activation of protein kinase b activity	GO Biological Process	0.0256	GO:0032148	32	4
Peptidyl-tyrosine autophosphorylation	GO Biological Process	0.0256	GO:0038083	32	4
Regulation of cellular protein localization	GO Biological Process	0.0256	GO:1903827	568	15
Insulin receptor complex	COMPARTMENTS	0.0259	GOCC:0005899	9	3
Tubulin complex	COMPARTMENTS	0.0259	GOCC:0045298	9	3
Negative regulation of lipid metabolic process	GO Biological Process	0.0261	GO:0045833	95	6

Negative regulation of cell adhesion	GO Biological Process	0.0274	GO:0007162	280	10
Regulation of actin cytoskeleton	KEGG Pathways	0.0275	hsa04810	209	8
Regulation of signaling	GO Biological Process	0.0276	GO:0023051	3553	52
Response to oxidative stress	GO Biological Process	0.0283	GO:0006979	393	12
Negative regulation of cell-substrate adhesion	GO Biological Process	0.0283	GO:0010812	62	5
Brain measurement	HPO	0.0283	EFO:0004464	1177	26
Extracellular matrix organization	GO Biological Process	0.0293	GO:0030198	338	11
Tissue regeneration	GO Biological Process	0.0293	GO:0042246	63	5
Regulation of kinase activity	GO Biological Process	0.0293	GO:0043549	918	20
Negative regulation of cellular process	GO Biological Process	0.0293	GO:0048523	4874	66
Regulation of epithelial cell proliferation	GO Biological Process	0.0293	GO:0050678	339	11
Regulation of presynapse assembly	GO Biological Process	0.0293	GO:1905606	34	4
Tube development	GO Biological Process	0.0296	GO:0035295	851	19
Human papillomavirus infection	KEGG Pathways	0.0303	hsa05165	325	10
Regulation of protein serine/threonine kinase activity	GO Biological Process	0.0306	GO:0071900	521	14
RAC1/PAK1/p38/MMP2 pathway	WikiPathways	0.0331	WP3303	67	5
Physico-chemical features and toxicity-associated pathways	WikiPathways	0.0331	WP3680	66	5
Disease of mental health	DISEASES	0.0334	DOID:150	689	18
Positive regulation of synapse assembly	GO Biological Process	0.0341	GO:0051965	66	5
Skeletal system morphogenesis	GO Biological Process	0.0347	GO:0048705	240	9
Regulation of cell communication	GO Biological Process	0.0353	GO:0010646	3514	51
Endothelial cell migration	GO Biological Process	0.0356	GO:0043542	67	5
Response to wounding	GO Biological Process	0.0358	GO:0009611	532	14
Negative regulation of small molecule metabolic process	GO Biological Process	0.0359	GO:0062014	104	6
Positive regulation of epithelial cell migration	GO Biological Process	0.0372	GO:0010634	147	7
Circulatory system development	GO Biological Process	0.0372	GO:0072359	872	19

Hormone metabolic process	GO Biological Process	0.0375	GO:0042445	194	8
Ras signaling pathway	KEGG Pathways	0.0377	hsa04014	226	8
Negative regulation of cell-matrix adhesion	GO Biological Process	0.0386	GO:0001953	38	4
Protein kinase b signaling	GO Biological Process	0.0386	GO:0043491	38	4
Positive regulation of glucose import	GO Biological Process	0.0386	GO:0046326	38	4
Platelet alpha granule	COMPARTMENTS	0.039	GOCC:0031091	91	6
Bladder cancer	WikiPathways	0.0393	WP2828	40	4
ECM-receptor interaction	KEGG Pathways	0.0398	hsa04512	88	5
Regulation of postsynapse organization	GO Biological Process	0.04	GO:0099175	107	6
Amplification and expansion of oncogenic pathways as metastatic traits	WikiPathways	0.0401	WP3678	17	3
Positive regulation of dna biosynthetic process	GO Biological Process	0.0408	GO:2000573	70	5
Plasma membrane raft	GO Cellular Component	0.0408	GO:0044853	109	6
Positive regulation of protein modification process	GO Biological Process	0.041	GO:0031401	1252	24
Regulation of protein kinase activity	GO Biological Process	0.0411	GO:0045859	812	18
Cellular response to chemical stimulus	GO Biological Process	0.0415	GO:0070887	2919	44
Regulation of growth	GO Biological Process	0.0416	GO:0040008	676	16
Cell-cell contact zone	GO Cellular Component	0.0416	GO:0044291	72	5
Positive regulation of glycogen biosynthetic process	GO Biological Process	0.0417	GO:0045725	16	3
Positive regulation of lipid metabolic process	GO Biological Process	0.042	GO:0045834	152	7
Positive regulation of dna metabolic process	GO Biological Process	0.0426	GO:0051054	200	8
Regulation of cell shape	GO Biological Process	0.0432	GO:0008360	153	7
TGF-beta signaling in thyroid cells for epithelial-mesenchymal transition	WikiPathways	0.0432	WP3859	18	3
Extracellular vesicles in the crosstalk of cardiac cells	WikiPathways	0.0432	WP4300	18	3
Genes controlling nephrogenesis	WikiPathways	0.0432	WP4823	44	4

Regulation of transferase activity	GO Biological Process	0.0437	GO:0051338	1036	21
Cell-cell junction	GO Cellular Component	0.0438	GO:0005911	490	13
GABA-ergic synapse	GO Cellular Component	0.0438	GO:0098982	74	5
Integral component of postsynaptic specialization membrane	GO Cellular Component	0.0448	GO:0099060	75	5
Mechanoregulation and pathology of YAP/TAZ via Hippo and non-Hippo mechanisms	WikiPathways	0.0451	WP4534	46	4
Positive regulation of cell communication	GO Biological Process	0.0453	GO:0010647	1823	31
Regulation of oxidoreductase activity	GO Biological Process	0.0453	GO:0051341	111	6
Positive regulation of sprouting angiogenesis	GO Biological Process	0.0462	GO:1903672	41	4
Regulation of plasma membrane bounded cell projection organization	GO Biological Process	0.0467	GO:0120035	687	16
Regulation of cellular component biogenesis	GO Biological Process	0.0472	GO:0044087	971	20
Positive regulation of signaling	GO Biological Process	0.0474	GO:0023056	1831	31
Regulation of neurogenesis	GO Biological Process	0.0474	GO:0050767	828	18
Myeloid leukocyte differentiation	GO Biological Process	0.0476	GO:0002573	113	6
Regeneration	GO Biological Process	0.0476	GO:0031099	157	7
Cell population proliferation	GO Biological Process	0.0479	GO:0008283	493	13
Osteoclast differentiation	GO Biological Process	0.0479	GO:0030316	42	4
Positive regulation of kinase activity	GO Biological Process	0.0479	GO:0033674	624	15
Regulation of transcription from rna polymerase ii promoter in response to iron	GO Biological Process	0.0479	GO:0034395	3	2
Regulation of peptidyl-tyrosine phosphorylation	GO Biological Process	0.0479	GO:0050730	258	9
Diterpenoid metabolic process	GO Biological Process	0.0482	GO:0016101	114	6
Regulation of plasma lipoprotein particle levels	GO Biological Process	0.0482	GO:0097006	75	5
Regulation of molecular function	GO Biological Process	0.0485	GO:0065009	4913	65
Angiogenesis	GO Biological Process	0.0486	GO:0001525	315	10

Regulation of cellular protein metabolic process	GO Biological Process	0.0486	GO:0032268	2693	41
Collagen binding	GO Molecular Function	0.0488	GO:0005518	68	6
Cellular response to oxygen-containing compound	GO Biological Process	0.0497	GO:1901701	1055	21

Table S5. Primers used for quantitative and qualitative polymerase chain reaction

Gene	Forward primer	Reverse primer
HSP90AB1	CGAAGTTGGACAGTGGTAAAGAG	TGCCCAATCATGGAGATGTCT
UBC	ATTTGGGTCGCGGTTCTTG	TGCCTTGACATTCTCGATGGT
B2M	GAGGCTATCCAGCGTACTCCA	CGGCAGGCATACTCATCTTTT
GUSB	GTCTGCGGCATTTTGTCGG	CACACGATGGCATAGGAATGG
ACTB	GTTGCTATCCAGGCTGTG	TGATCTTGATCTTCATTGTG
GAPDH	AACTTTGGTATCGTGGAAGGAC	CAGTAGAGGCAGGGATGATGTT
HPRT1	ACCAGTCAACAGGGGACATAA	CTTCGTGGGGTCCTTTTCACC
TFRC	ACCATTGTCATATACCCGGTTCA	CAATAGCCCAAGTAGCCAATCAT
PPIA	CCCACCGTGTTCTTCGACATT	GGACCCGTATGCTTTAGGATGA
RPLP0	AGCCCAGAACACTGGTCTC;ACTCA	GGATTTCAATGGTGCC
RPL13A	GCCATCGTGGCTAAACAGGTA	GTTGGTGTTCATCCGCTTGC
MFGE8	CCTGCCACAACGGTGGTTTAT	GCGATCTGTGAGTTGGCAATGT
ACTA2	AAGAGGAATCCTGACCCTGAA	TGGTGATGATGCCATGTTCT
FN1	TTCTAAGATTTGGTTTGGGATCAAT	TCTTGGTTGGCTGCATATGC
COL1A1	CACACGTCTCGGTCATGGTA	AAGAGGAAGGCCAAGTCGAG
COL3A1	TCCCACTATTATTTTGGCACAACA	TCATCGCAGAGAACGACGGATCC
ITGAV	AATGTAATGATGAGCTTGGTGGAGA	AGGTGACATTGAGATGGGTAGTGG
ITGB3	GAGGTCATCCCTGGCCTCAA	CTGGCAGGCACAGTCACAATC
ITGB5	AGCGGCGACACACTAGGA	ATCCGTCCCGCAGCACT

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