

1 **IL-1-driven stromal-neutrophil interaction in deep ulcers defines a pathotype of therapy
2 non-responsive inflammatory bowel disease**

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62 **Abstract**

63 Current inflammatory bowel disease (IBD) therapies are ineffective in a high proportion of
64 patients. Combining bulk and single-cell transcriptomics, quantitative histopathology, and *in*
65 *situ* localisation, we describe heterogeneity of the tissular inflammatory response in IBD
66 treatment failure. Among inflammatory pathotypes, we found high neutrophil infiltration,
67 activation of fibroblasts, and vascular remodelling at sites of deep ulceration was a feature of
68 non-response to several anti-inflammatory therapies. Activated fibroblasts in the ulcer bed
69 display neutrophil chemoattractant properties that are IL-1R- but not TNF-dependent. The
70 identification of distinct, localised, tissular pathotypes associated with treatment non- response
71 will aid precision targeting of current therapeutics and provide a biological rationale for IL-1
72 signalling blockade in ulcerating disease.

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75 **Keywords:** inflammatory, bowel, therapy, non-response, anti-TNF, IL-1, stroma, neutrophil,
76 single-cell, fibroblast, colitis, Crohn's, ulceration, pathotype, heterogeneity, lymphoid,
77 aggregate

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86 **Introduction**

87 Inflammatory bowel diseases (IBDs) are a heterogeneous group of disorders characterised by
88 inflammation throughout the gastrointestinal tract. The aetiology involves maladaptation
89 between the host and its intestinal microbiota, a dialogue controlled by genetic and
90 environmental factors ¹. The complex multi-factorial nature of IBD is partly reflected in its
91 clinical phenotypes, encompassing both Crohn's disease (CD) and ulcerative colitis (UC), and
92 in a range of microscopic features such as granulomas, lymphoid aggregates, crypt
93 abscesses, and ulcers ^{2,3}. Treatments for IBD include general immunosuppressants (such as
94 corticosteroids), immunomodulators (such as thiopurines), or biologics that target specific
95 inflammatory mediators ⁴. Amongst the latter, tumor necrosis factor α (TNF) targeting is most
96 common ⁵ but alternate approaches, such as blockade of leukocyte homing to the gut (anti-
97 integrin $\alpha 4\beta 7$ (vedolizumab)) are increasingly used ⁶. Although anti-TNF therapy has
98 revolutionised the treatment of IBD, identifying the patients who will respond remains a major
99 challenge. Up to 40% of IBD patients are primary non-responders and for a substantial fraction
100 of initial responders, treatment will later lose efficacy ⁷⁻⁹.

101 Our previous work identified high expression of the IL-6 family member Oncostatin M (OSM),
102 and its receptor OSMR, in the inflamed intestine of IBD patients as associated with non-
103 response to anti-TNF therapy ¹⁰. Notably, OSM produced by leukocytes signals primarily into
104 stromal cells such as fibroblasts and endothelial cells. Subsequent bulk and single-cell
105 transcriptomic studies have associated cell subsets of fibroblasts, inflammatory mononuclear
106 phagocytes (MNP), neutrophils, and pathogenic T- and plasma cells with therapy non-
107 response in both UC and CD ¹¹⁻¹⁷. However, these studies have not identified the processes
108 underlying quantitative changes in cellular ecology nor how they affect treatment response. It
109 is also not known whether the cellular and molecular determinants of treatment response are
110 uniform across non-responsive patients or if several different tissular pathologies promote
111 therapy failure through distinct mechanisms. Similarly, little is known about the mechanistic
112 determinants of therapy response that are shared/unique to individual drugs. Further

113 understanding in these areas is crucial to design personalised treatment regimens and new
114 therapeutics for individuals that do not respond to current options.

115 In this study we dissected the cellular and molecular landscape of inflamed tissue in IBD
116 patients by integrating whole-tissue and single-cell gene expression profiling with quantitative
117 *in situ* analyses and functional *ex vivo* assays. We then explored how individual signatures of
118 tissue inflammation associate with non-response to specific treatments. Transcriptomic
119 changes were found to reflect changes in the tissular response and characterised by distinct
120 histological features. Most notably we identified a pathotype in a subset of patients that was
121 associated with non-response across several current IBD therapeutics. Tissues from those
122 patients are characterised by a high abundance of tissue neutrophils, the activation of a
123 neutrophil-attractant program in fibroblasts, (peri-) vascular cell expansion, and enhanced IL-
124 1 signalling at sites of deep ulceration. These functional definitions of disease provide a basis
125 for rational targeting of existing medications and a novel mechanistic avenue to target
126 inflammation in non-responsive patients displaying ulceration with fibroblast and neutrophil
127 remodelling.

128 **Results**

129 **Identification of gene co-expression signatures of cellular ecology in inflamed IBD**
130 **tissue**

131 The surgical removal of inflamed tissue becomes a therapeutic option in IBD when medical
132 therapies have failed. Using such tissues as our discovery cohort, we examined gene
133 expression profiles in difficult-to-treat IBD. From the 31 IBD patients (n=8 UC, n=22 CD and
134 n=1 IBDu) from which samples were collected, only 20% were treatment-naïve (i.e. had a
135 resection as primary therapy for localised disease), while 48% had received two or more
136 different medications before the time of surgery (Supplementary Table 1). Amongst the n=41
137 tissue samples from these patients, n=15 were classified as (macroscopically) uninflamed,
138 including n=7 samples for which paired uninflamed/inflamed tissue was available. We

139 additionally used unaffected, non-tumour tissue collected from colorectal cancer patients
140 undergoing surgery as non-IBD controls for comparison (n=39). Bulk RNA sequencing
141 (RNAseq) was used to generate whole tissue gene expression profiles across all samples
142 (n=41 IBD and n=39 non-IBD; ‘discovery cohort’).

143 To identify sets of genes reflective of distinct biological processes, we applied weighted gene
144 correlation network analysis (WGCNA) to cluster co-expressed genes in an unbiased manner
145 across all tissue samples. This identified 38 modules of highly co-expressed genes (M1-M38)
146 (Supplementary Table 2). We correlated the expression (module eigengene) of these modules
147 with sample characteristics, clinical phenotypes and histologic (microscopic) inflammation
148 (Nancy Index¹⁸); 28 modules were significantly associated with at least one of these measures
149 (Figure 1A). Modules were found to have dichotomous associations with traits. About half of
150 the modules had significant positive correlations with histologic inflammation, whilst the others
151 had significant negative associations (Figure 1A). Fewer and less strong correlations were
152 observed between module expression levels and other metadata, such as the intestinal
153 sampling site or IBD subtype (CD or UC), and overall these measures displayed correlations
154 in a similar direction to histologic inflammation. Age appeared to have similar associations as
155 inflammation but this was an artefact of the older nature of the non-IBD samples used as
156 controls. In a paired analyses of only inflamed and uninflamed IBD tissue samples from the
157 same patients (n=7), the difference in expression of a module between tissue pairs remained
158 highly correlated with the module’s association with histologic inflammation (Nancy Index)
159 (R=0.8, P<0.001, Extended Data Figure 1A), confirming that these co-expression modules
160 reflected inflammatory processes.

161 To determine whether the gene co-expression patterns we detected reflected changes in the
162 cell type composition of patient tissues, we applied *in silico* cell type deconvolution analysis to
163 the RNAseq data of our discovery cohort (*xCell*,¹⁹). Correlating predicted cell type scores with
164 module expression (eigengenes) (Extended Data Figure 1B), modules positively correlated
165 with histologic inflammation (Figure 1A) were associated with signatures of stromal cells (e.g.,

166 fibroblasts), mononuclear phagocytes (e.g., M2 macrophages), B-lymphocytes and plasma
167 cells, T-lymphocytes (e.g., CD8+ T-cells), and granulocytes (e.g., neutrophils). Modules
168 negatively correlated with histologic inflammation were predicted to reflect epithelial cells,
169 smooth muscle cells and M1 macrophages. These results suggest that the co-expression
170 patterns that we observed to be associated with inflammation were, at least in part, being
171 driven by differences in the cellular composition of the inflamed tissues.

172 **Co-expression signatures of inflammation predict patient response to IBD treatments**

173 Given previous associations between the expression of individual genes and cell types with
174 therapy response in IBD, we aimed to determine if our inflammation-associated gene co-
175 expression signatures represented biological features relevant to treatment outcomes. We
176 projected all of the modules onto whole tissue gene expression data derived from prospective
177 studies of response to anti-TNF, corticosteroid, or anti-integrin therapy ^{15,20,21}. At least 79% of
178 the genes within each module could be identified in the three datasets, enabling accurate
179 quantification of the modules within them (Extended Data Figure 1C and Supplementary Table
180 3). The expression of 15 modules was significantly (adjusted p<0.05) higher in non-responders
181 to anti-TNF prior to treatment (n=61 total patients in the study). Seven modules were
182 significantly higher in non-responders (and one significantly lower) in the corticosteroid study
183 (n=206 patients) and two modules were higher in non-response to anti-integrin therapy (n=20
184 patients) (Supplementary Table 4).

185 Strikingly, across all three therapy-response datasets, each involving different therapeutics,
186 modules M4 and M5 were consistently amongst the strongest associations with non-response
187 in pre-treatment samples (Supplementary Table 4 and Figures 1B, 1C and 1D). This overall
188 trend of increased expression in non-responders was significant in meta-analyses of both M4
189 (p=0.0025, standardised mean difference (SMD)=0.87, 95%CI=0.31-1.44) and M5 (p=0.0123,
190 SMD = 0.88, 95%CI=0.19-1.58) across the different treatments.

191 To determine if the associations with non-response are uniform across the genes in modules
192 M4 and M5 or driven by a small number of highly predictive genes, we compared the ability of
193 all genes individually to predict response to anti-TNF and corticosteroid therapy. This again
194 revealed that genes from modules M4 and M5 were amongst the top predictors of non-
195 response to both anti-TNF and corticosteroid therapy relative to those in other modules (Figure
196 1E, Figure 1F, Supplementary Table 5). Thus, M4 and M5 reflect a coordinated shift in the
197 expression of all their constituent genes in relation to therapy non-response. Overall, M4 and
198 M5 were consistently the top predictors of non-response across multiple IBD medications.

199 **Co-expression modules linked with therapy non-response represent distinct
200 histopathologic features**

201 As well as predicting therapy response, modules M4 and M5 also demonstrated the strongest
202 correlation with histologic inflammation in the discovery cohort, as defined by the Nancy score
203 ¹⁸ (Figure 1A). Using an additional clinical cohort of Oxford UC patients (Supplementary Table
204 6), we confirmed that the Nancy score is higher in non-responders to anti-TNF therapy before
205 the start of treatment (Figure 2A). Interestingly, this was not true for the UCEIS (an endoscopic
206 score of mucosal inflammation) or other clinical or endoscopic measures (Extended Data
207 Figure 2A).

208 On this basis we postulated that the gene co-expression patterns in the dataset, which we
209 previously linked to changes in cellular composition, might also reflect the manifestation of
210 histopathologic features in patient tissues. To explore this, we quantified established
211 histopathologic features of IBD on H&E sections of resected patient tissue (Extended Data
212 Figure 2B). First, we examined the correlation of histopathologic features with each other
213 (Extended Data Figure 2C). The only strong positive correlations observed were between
214 cryptitis/crypt abscess and architectural distortion/ goblet cell depletion; as well as several
215 associations with granulomas, although the latter estimates were based on very few cases
216 where both granulomas and the other features were observed. We then looked for correlations
217 between the expression of the co-expression modules and histologic features scored from

218 tissue where both were available (n=36). Several nominally significant associations were
219 observed between modules and various features (Figure 2B); however, only positive
220 correlations between M4/ulceration and M6/lymphoid aggregates remained significant after
221 adjusting for multiple testing (P adjusted < 0.05, Figure 5A). Notably, the relation of these two
222 inflammation-associated modules was almost orthogonal, each correlating only with one of
223 the features (Figure 2C). Despite not reaching significance after correction, M5 – also highly
224 correlated with the Nancy score (Figure 1A) – correlated strongly with both ulceration and
225 cryptitis/crypt abscesses (Figure 2C). We also confirmed the associations of M4, M5 with
226 ulceration in an independent paediatric cohort (n=172) containing inflamed tissues of both UC
227 and CD patients {Haberman, 2014 #722; Loberman-Nachum, 2019 #1093} (Extended Data
228 Figure 2D). In this dataset, 11% of all patients with IBD showed high M4/M5 tissue expression
229 (Extended Data Figure 2E). Similar to our dataset, M6 expression was not significantly
230 different by ulceration status in the paediatric cohort, although we noted that overall M6
231 expression was also much lower in these biopsy samples (see discussion).

232 The almost orthogonal relation of M4/M5/ulceration with M6/lymphoid aggregates suggested
233 these may represent distinct underlying inflammatory processes that may be more or less
234 dominant in a given patient's tissue. To investigate this, we grouped patients by unsupervised
235 clustering on module M4/M5/M6 expression to determine the relative proportion of samples
236 belonging to these groups. This yielded four groups: M4/M5 high expression (21.7% of
237 patients), M6 high expression, M5 only high expression and M4/M5/M6 low (each 26.1% of
238 patients) (Figure 2D). We then plotted the expression of cytokines and therapeutic targets
239 reliably detected in our discovery cohort across these groups (Figure 2E). The M4/M5 high
240 group displayed significantly increased expression of *IL1B* compared to the rest of the patients
241 (Figure 2E, red). However, neither *ITGA4/ITGB7* (targeted by anti-integrin), *N3RC1* (targeted
242 by corticosteroids) nor *TNF* (targeted by anti-TNF) were increased in the tissue of these
243 patients (Figure 2E). By contrast, high expression of module M6 was linked to increased levels
244 of *ITGA4* and *N3RC1*, as well as *CCL19*, *CCL21* and *CXCL13* but not *TNF* (Figure 2E, blue).

245 Patients high in M5 expression only did not demonstrate significant changes in
246 cytokine/therapeutic target signatures (Figure 2E, orange). These results suggest that patient
247 responses to specific treatments might be determined by which inflammatory pathology
248 predominates at the tissue level. M4/M5-high tissues did not show any increase in current
249 therapeutic targets and these modules were associated with non-response to all therapies
250 tested; whereas M6-high tissues only showed no increase of *TNF* expression in the tissue,
251 which corroborated our previous association analyses where it was only associated with non-
252 response to anti-TNF (Supplementary Table 4).

253 The quantification of histological features confirmed that an increased expression of both M4
254 and M5 is linked to the presence of deep ulcerations and M5 to cryptitis/crypt abscesses.
255 Whereas other inflammatory features were instead correlated with alternative co-expression
256 patterns, as in the case of lymphoid aggregates and the M6 module. Patients with ulceration
257 and high M4/M5 expression showed no significant up-regulation of genes targeted by the
258 current medications, but an increase in *IL1B*, warranting further exploration of the mechanisms
259 underlying this signature.

260 **High expression of modules M4 and M5 reflects neutrophil infiltrates, activated
261 fibroblasts and epithelial cell loss**

262 We performed a more detailed exploration of the changes in cellular composition and
263 activation state that produce the M4 and M5 co-expression module signature. Our *in silico* cell
264 type deconvolution revealed that M4 and M5 were predominantly associated with stromal
265 cells, such as fibroblasts, and granulocytes, such as neutrophils (Extended Data Figure 1B).
266 We confirmed this by projecting modules M4 and M5 onto single-cell transcriptomic datasets
267 derived from inflamed and non-inflamed CD {Martin, 2019 #567} and UC patient tissue ¹². This
268 showed that the module M4 likely reflected the presence of “activated/inflammatory
269 fibroblasts”, whereas module M5 reflected “myeloid cells/inflammatory monocytes” (Figures
270 3A and 3B).

271 Given that cell type deconvolution correlated neutrophil scores with M5, but the single-cell
272 datasets (which did not capture neutrophils) correlated monocytes/macrophages with M5
273 genes, we aimed to identify genes within M4 and M5 enriched in either of these cell types, as
274 well as in stromal cells. We FACS-sorted well-defined hematopoietic and non-hematopoietic
275 cell subsets from the intestinal tissue of non-IBD and IBD patients and measured the
276 expression of selected M4/M5 genes by qPCR (see Extended Data Figure 3A for gating
277 strategy). Several M4/M5 genes were highly expressed in CD16^{hi} neutrophils and
278 PDPN+THY1+ stromal cells (Extended Data Figure 3B). This was confirmed by targeted
279 RNAseq from neutrophils, stromal cells and mononuclear phagocytes (MNP) (see Extended
280 Data Figure 3C for gating strategy), which were bulk-sorted from inflamed endoscopic biopsies
281 of n=13 IBD patients (UC and CD, Figure 3C, see Supplementary Table 7 for patient cohort
282 details). We first carried out pathway analysis to assign function to all the genes (including
283 those not in M4 and M5) enriched in either cell type (see Supplementary Table 8 for differential
284 gene expression analysis). As expected, this demonstrated that neutrophils were enriched in
285 anti-microbial and tissue-toxic granule biology when compared to MNPs that were mostly
286 defined by genes belonging to the antigen presentation pathway (Extended Data Figure 3D).
287 Stromal cells were enriched in many genes assigned to extracellular matrix pathways
288 (Extended Data Figure 3D). Of all differentially expressed genes between the cell types, n=39,
289 n=31 and n=4 of all genes contained in M4/M5 (n=110) were significantly enriched in stromal
290 cells, neutrophils and MNPs, respectively (Supplementary Table 8, Figure 3C). Compared to
291 both neutrophils and MNPs, sorted stromal cells were enriched in transcripts for components
292 (COL7A1) and remodelling enzymes (MMP1/3, ADAMTS7/14) of the extracellular matrix
293 (ECM), markers of activated fibroblasts (THY1, PDPN, FAP), as well as for ligands of the
294 chemokine receptors CXCR1/CXCR2 identified as enriched in neutrophils (CXCL5/6). Genes
295 encoding major neutrophil chemokine receptors (CXCR1/2), subunits of the antimicrobial
296 peptide calprotectin (S100A8/A9), receptors for IgG immunoglobulin constant regions
297 (FCGR3B) and the cytokine OSM, which we previously linked to non-response in IBD ¹⁰, were
298 enriched in neutrophils. Amongst the four genes enriched in MNPs, CD300E is a marker of

299 activated monocytes ²², whereas *LAMP3* has been described as indicative of mature dendritic
300 cells ²³. The enrichment of many M4 and M5 genes in sorted neutrophils explained the high
301 correlation of modules M4 and M5 with the Nancy index (Figure 1A), which is weighted by the
302 abundance of neutrophils for scoring ¹⁸.

303 Since differences in whole tissue gene expression signatures could be driven by both changes
304 in the transcriptional profiles within cell-types and/or overall changes in cell-type composition,
305 we used flow cytometry to test whether the number of neutrophils and fibroblasts correlated
306 with M4 and M5 tissue expression (see Extended Data Figures 3E for classification of tissues
307 by M4/M5 expression). The percentage of neutrophils was significantly increased (up to 10
308 fold) in M4/M5 high tissues while the percentage of stromal cells remained unchanged (Figure
309 3D). Additionally, epithelial cells were significantly decreased in M4/M5 high tissues (Figure
310 3D). We also observed non-significant trends for an increase of MNP and endothelial cells, as
311 well as a trend for decreased eosinophils with high M4/M5 expression (Figure 3D). Whilst
312 these trends may become significant with an increased number of samples, we noted that
313 neutrophils accounted for up to 38% of the total live cells in the M4/M5 intermediate and high
314 group, whilst the percentage of MNPs was much lower (<5%) (Figure 3D). Additionally, we
315 found that the M4/M5 genes significantly enriched in neutrophils and stromal cells, but not
316 MNPs, demonstrated highest predictive power for non-response to anti-TNF and
317 corticosteroids (Figure 3E).

318 FACS counts can be biased by tissue digestion methods, so we also quantified the presence
319 of neutrophils, stromal cells and MNPs *in situ* by immunohistochemical staining of resected
320 formalin-fixed, paraffin-embedded (FFPE) inflamed tissue from IBD patients (Figure 3F).
321 Again, IBD tissues with high expression of M4 and M5 in whole tissue (see Extended Data
322 Figure 3F for classification) demonstrated a higher percentage of Neutrophil Elastase- (NE)
323 and Calprotectin- (S100A8/A9) positive cells, but not PDPN-positive stromal cells or CD68+
324 MNPs, in inflamed tissues (Figure 3G). This further confirmed that M4/M5 high tissue harbours
325 an increased number of neutrophils which stain positive for NE and S100A8/A9.

326 High M4/M5 expression in the whole tissue thus reflects ulceration characterised by a
327 dominance of neutrophil infiltration, expression of genes characteristic of activated fibroblasts,
328 and the loss of epithelial cells.

329 **M4/M5 gene expression is associated with neutrophil-attracting fibroblasts and**
330 **endothelial and perivascular cell expansion**

331 M4/M5 high patients are characterised by a high abundance of neutrophils but the modules
332 also contain many genes indicative of activated stromal cells (Figure 3C). Furthermore, whilst
333 the number of PDPN+ stromal cells was not increased, we observed an increased overall
334 staining intensity of PDPN in M4/M5 high patients (Figure 3F). We therefore hypothesised that
335 the stromal signatures in M4 and M5 arise from altered activation states (including the
336 upregulation of PDPN) and/or changes of cellular composition within the stromal compartment
337 that correlate with the infiltration of neutrophils. To dissect this relationship, we applied single-
338 cell sequencing to EPCAM-CD45- intestinal stromal cells from endoscopic biopsies of
339 inflamed UC patients (n=7) and healthy donors (n=4) (see supplementary Table 7 for patient
340 cohort details), and compared tissues with low, intermediate and high M4/M5 expression
341 (Figure 4A+B and Extended Data Figure 4A). As expected, tissues from all healthy donors
342 were M4/M5 low, as well as the tissue from one IBD patient with a low histological inflammation
343 score (Nancy score=1). We used Harmony to integrate all single-cell datasets and account for
344 inter-patient and inter-sequencing batch effects ²⁴. Overall, 6 stromal clusters were obtained,
345 which we assigned to endothelial cells (ACKR1+CD34+), pericytes (NOTCH3+MCAM+),
346 myofibroblasts (MYH11^{hi}ACTG2+) and three clusters of fibroblasts:
347 PDGFRA^{high}PDPN^{low}SOX6+ (“PDGFRA+”) fibroblasts, PDGFRA^{low}PDPN^{low}ABCA8+
348 (“ABCA8+”) fibroblasts and CD90^{hi}PDPN^{hi}PDGFRA+ABCA8+FAP+ “inflammatory”
349 fibroblasts, based on the top differentially expressed markers and previously described
350 annotations ^{11,12,25} (Figure 4C and Supplementary Table 9). PDPN was expressed by
351 myofibroblasts and all three fibroblast clusters, with highest expression found in inflammatory

352 fibroblasts. *THY1* (CD90) was highly expressed in pericytes and inflammatory fibroblasts and
353 expressed at lower levels in ABCA8+ fibroblasts (Figure 4C).

354 Next, we developed a panel of antibodies for *in situ* analysis of intestinal tissue to confirm that
355 the clusters of stromal cells detected by transcriptomics represented spatially separated cell
356 subsets. The panel comprised anti-*THY1*, anti-*PDPN*, anti-*PDGFRA* and anti-*ABCA8* to
357 localise the different subsets of fibroblasts (Figure 4D and Extended Data Figure 4B). Anti-
358 *THY1* and anti-*MCAM* were used to localise pericytes, and anti-*PECAM1* (CD31) to localise
359 endothelial cells (Figure 4E and Extended Data Figure 4C). In uninflamed large (colon) and
360 small (ileum) intestinal tissue, high *PDGFRA* staining was observed in sub-epithelial
361 fibroblasts, which also stained low for *PDPN* (Figure 4D and Extended Data Figure 4B). By
362 contrast, *ABCA8* stained a distinct fibroblast population residing in the intestinal lamina propria
363 (Figure 4D and Extended Data Figure 4B). Highest *PDPN* staining was found on lymphatic
364 endothelial cells (*PECAM1*+ and *PDPN*+, Figure 3E and Extended Data Figure 4C). By
365 contrast, *THY1* formed a gradient of staining intensity from the perivascular niche toward the
366 lamina propria (as recently described in ²⁶), being expressed by both ABCA8+fibroblasts and
367 *MCAM*+ pericytes, as well as cells in the muscular layer of the submucosa (Figures 3D,E and
368 Extended Data Figure 4B, C).

369 We then characterised how the identified stromal compartments differed across IBD patient
370 tissues with either low, intermediate or high M4/M5 expression. In the single-cell dataset, the
371 percentage of inflammatory fibroblasts, pericytes and endothelial cells was increased in the
372 M4/M5 intermediate and high patient groups at the expense of ABCA8+ and PDGFRA+
373 fibroblasts (Figures 4A+B, Extended Data Figure 4D). FACS analysis verified that *PECAM1*+

374 endothelial cell and *PDPN*+*FAP*+ inflammatory fibroblast frequencies were increased within
375 the stromal compartment in inflamed tissue, compared to uninflamed adjacent tissue
376 (Extended Data Figure 4E).

377 To see which of those clusters contributed most to M4/M5 expression, we projected the genes
378 contained in the M4/M5 modules onto our scRNAseq data. Notably, the highest expression of

379 M4 was detected in the inflammatory fibroblast cluster, suggesting the emergence of this cell
380 cluster as an underlying process in M4/M5 high IBD patient tissue (Figure 5A). Within M4/M5
381 high tissues, neutrophil-targeting CXCR1/CXCR2 ligands CXCL1, CXCL2, CXCL3, CXCL5,
382 CXCL6 and CXCL8 were significantly higher in inflammatory fibroblasts in comparison to other
383 clusters (Figure 4B and Supplementary Table 10). We also identified several genes indicating
384 extracellular matrix remodelling (MMP1, MMP3, MMP13) and previously identified markers
385 associated with inflammatory fibroblasts (IL11, IL24, FAP) as higher in inflammatory
386 fibroblasts compared to other stromal cells (Supplementary Table 10). Within the cluster of
387 inflammatory fibroblasts, *PDPN*, *FAP*, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6 and CXCL8
388 were also significantly increased in M4/M5 high compared to M4/M5 low and intermediate
389 tissues, whereas *ABCA8* expression was downregulated (Supplementary Table 11).
390 Nevertheless, ABCA8 fibroblasts and PDGFRA fibroblasts both still expressed the above-
391 mentioned chemokines in the M4/M5 intermediate and high groups (Figure 5B), raising the
392 possibility that the inflammatory fibroblast cluster represents an activation state of ABCA8
393 fibroblasts or/and PDGFRA fibroblasts. Indeed, trajectory (pseudotime) analysis indicated that
394 inflammatory fibroblasts may represent a transcriptomic state in between ABCA8+ and
395 PDGFRA+ fibroblasts (Extended Data Figure 4F) and could potentially arise from either
396 population.

397 We confirmed these findings at the protein level, where we found that areas of increased
398 *PDPN* and *THY1* staining were also characterised by a reduced staining of *ABCA8* and
399 *PDGFRA* on fibroblasts (Extended Data Figure 4G). Similarly, we verified that an increased
400 neutrophil presence is associated with more intense *PDPN* staining and the expansion of the
401 vascular compartment (*THY1*, *CD31*), by staining for these markers in different IBD tissues
402 with various grades of neutrophil infiltrates and epithelial damage (Figure 5C). In line with the
403 neutrophil-attracting signature of inflammatory fibroblasts, tissues with dense neutrophil
404 infiltrates (NE+ cells) exhibited the highest level of *PDPN* on fibroblasts, particularly in areas
405 of profound epithelial cell loss (i.e., ulceration) (Figure 5C). This was associated with the

406 expansion of THY1+ perivascular cells and blood endothelial vessels
407 (PECAM1+THY1+PDPN-), while lymphatic endothelial cells (PDPN+PECAM1+) were mostly
408 absent in areas of neutrophil presence and deep ulceration (Figure 5C). Furthermore,
409 immunofluorescent localisation revealed that in particular PDPN+ fibroblasts that co-
410 expressed FAP (magenta) are located in areas of NE+ neutrophil influx (Figure 5D).

411 Dissection of the changes within the stromal compartment revealed that the neutrophil
412 infiltrates observed in M4/M5 high patients are associated with the activation of a neutrophil-
413 chemoattractant program in PDPN+FAP+ inflammatory fibroblasts, as well as with
414 angiogenesis and perivascular niche expansion.

415 **Activated inflammatory fibroblasts drive neutrophil recruitment through IL-1R
416 signalling with high levels of IL-1 β at sites of ulceration**

417 To identify potential novel therapeutics targets in the M4/M5 high non-responsive phenotype,
418 we aimed to identify upstream cytokine signalling pathway(s) controlling the observed
419 activation of the neutrophil-attractant program in inflammatory fibroblasts. We culture-
420 expanded primary stromal cell lines (n=33) from surgically resected intestinal tissue of IBD
421 patients and stimulated them with a panel of cytokines associated with IBD ⁴. Of the cytokines
422 assessed, only the NF- κ B activators IL-1 β and TNF- α , but not IL-6 or OSM, were capable of
423 inducing the expression of CXCL5 in primary stromal cell lines after 3 hours of stimulation
424 (Extended Data Figure 5A). Furthermore, RNA sequencing showed that IL-1 β and TNF- α
425 strongly and significantly induced gene expression of all neutrophil-tropic CXCR1 and CXCR2
426 ligands in fibroblasts, namely CXCL1, CXCL2, CXCL3, CXCL5, CXCL6 and CXCL8 (Extended
427 Data Figure 5B). In addition, both cytokines induced the inflammatory fibroblast markers
428 PDPN and FAP (Extended Data Figure 5B). Although TNF- α and IL-1 β both induced a
429 chemokine response, the latter was 100-fold more potent (Extended Data Figures 5B + C).
430 To confirm that the IL-1 signalling pathway is the functionally relevant one for inducing the
431 inflammatory fibroblast phenotype in patients, we developed an *ex vivo* assay using surgically

432 resected tissue from IBD patients. Briefly, we produced conditioned media (CM) from single-
433 cell suspensions of enzymatically digested intestinal tissue. When applied to cultured intestinal
434 fibroblasts, this CM was capable of inducing a robust chemoattractant program (Figure 6A).
435 To determine upstream cytokines driving this response, we blocked IL-1 signalling with the IL-
436 1 receptor (IL-1R) antagonist anakinra (Kineret) or TNF signalling with the anti-TNF agent
437 adalimumab (Humira) in CM. Strikingly, only IL-1R, but not TNF, signalling blockade was able
438 to reduce fibroblast activation in this assay (Figure 6A). These findings demonstrated that
439 soluble mediators contained in gut-resident cell populations of inflamed IBD tissue activate
440 the neutrophil-attracting fibroblast program and that this response is IL-1R but not TNF-
441 dependent. Furthermore, single-cell sequencing showed that inflammatory fibroblasts from
442 M4/M5-high IBD patient tissue were the cell population which demonstrated the strongest IL-
443 1 response pattern (Figure 6B, see Supplementary Table 12 for IL-1 gene expression
444 response), suggesting that this pathway may be associated with the poor therapy response
445 observed in these patients. In line with this, inflammatory fibroblasts and ABCA8 fibroblasts
446 demonstrated the highest fold changes of IL1-receptor (*IL1R1*) expression in M4/M5 high IBD
447 patients (Extended Data Figure 5D). By contrast, TNF receptors (*TNFR1* and *TNFR2*) did not
448 demonstrate this trend (Extended Data Figure 5D). Consistent with the predominant role of IL-
449 1 in these patients, module M5 demonstrated a high enrichment of genes assigned to the
450 inflammasome pathway (Extended Data Figure 5E). Finally, immunohistochemical staining
451 revealed that IL-1 β is localised specifically to the ulcer bed and granulation tissue (Figure 6C,
452 top panel), but not uninflamed tissue or tissues where lymphoid aggregates dominate (Figure
453 6D, top panel). Areas of intense IL-1 β labelling also demonstrated intense staining of FAP
454 (Figures 6C and D, bottom panels), suggesting that IL-1R signalling in the ulcer bed is driving
455 the inflammatory fibroblast program characterised by FAP expression.

456 Overall, these results identify IL-1R signalling as a key driver of the inflammatory
457 fibroblast/neutrophil recruitment phenotype that is observed in IBD tissues with the high

458 M4/M5 pathotype, which is in turn associated with non-response to multiple therapies currently
459 in use.

460 **Discussion**

461 Here we integrated transcriptomics, cellular profiling, histopathology and functional assays to
462 identify new, distinct, inflammatory pathotypes associated with therapy non-response in IBD.
463 Non-response to multiple current therapies was associated with a pathotype defined by two
464 gene expression modules that represented IL-1R-dependent inflammatory fibroblast
465 activation, neutrophil accumulation and (peri-) vascular niche expansion at sites of epithelial
466 depletion and deep ulceration. We also identified an additional pathotype associated with an
467 increased presence of lymphoid aggregates that was only linked to patient response to anti-
468 TNF. Combined, these results highlight the existence of distinct pathotypes within the
469 heterogeneous cellular landscape of inflamed tissues in treatment-refractory IBD that are
470 associated with specific treatment outcomes. This provides a novel platform for personalised,
471 precision targeting of existing medications and novel therapeutic targets where current options
472 fail.

473 Our results highlight neutrophils as a major component of the M4/M5 signature associated
474 with multiple therapy non-response in IBD. In other studies, analysis of tissue-level expression
475 signatures suggested a link between neutrophils and therapy non-response in IBD^{14,15}. Here
476 we have extended those studies by mapping gene expression signatures to intestinal
477 neutrophils isolated from IBD lesions and localising those cells to distinct tissue niches in the
478 inflamed intestine. It is also notable that a dominant neutrophil contribution to the biology of
479 anti-TNF therapy resistance is missing from previous single cell sequencing studies as
480 neutrophils were not analysed^{12 11}. It is not known whether neutrophil accumulation is a cause
481 or a consequence of the chronic tissue damage at sites of tissue ulceration. However, there
482 is evidence that neutrophils can contribute to chronic inflammation through production of
483 extracellular traps (NETs) and the liberation of reactive-oxygen species (ROS)²⁷. We found

484 neutrophils are also the major source of *OSM* expression, a cytokine previously associated
485 functionally with non-response to anti-TNF therapy in IBD¹⁰.

486 The accumulation of neutrophils, activation of fibroblasts and vascular remodelling in response
487 to epithelial damage observed in treatment-refractory IBD lesions is reminiscent of wound
488 healing mechanisms²⁸. It is tempting to speculate that in a subset of non-responsive IBD
489 patients such a chronic wound is a result of an unsuccessful attempt to rebuild the epithelial
490 barrier. Without proper resolution, that process becomes pathogenic, analogous to the
491 concept of a “wound that does not heal” that emerged from the cancer field²⁹. Our single cell
492 RNA sequencing analysis of the stromal compartment identified PDPN and FAP as two
493 markers of fibroblast activation that allowed us to localise inflammatory fibroblasts around
494 ulcers and in proximity to neutrophils. We hypothesise that, rather than being a specialised
495 fibroblast subset, inflammatory fibroblasts may represent an activation state of either ABCA8
496 fibroblasts residing in the lamina propria of the intestine, or subepithelial PDGFRA fibroblasts.
497 The origin of inflammatory fibroblasts may dependent on the site where damage occurs, i.e.
498 at the epithelial lining layer or deeper into the lamina propria. The very specific localisation of
499 IL-1 β in the ulcer bed in proximity to areas of epithelial cell damage suggests disruption of the
500 epithelial cell barrier may be a primary event. Danger-associated molecular patterns (DAMPs)
501 released by necrotic epithelial cells could also lead to the activation of inflammasome
502 pathways and consequently the release of IL-1 β . Indeed, several genes associated with an
503 inflammasome signature were found in module M5 of our discovery cohort supporting the idea
504 that inflammasome activation is an upstream event. Early responders to damage at the barrier
505 may also include resident MNP, that can produce excessive IL-1 β and IL-23, particularly in
506 the context of IL-10 pathway deficiency¹⁶. IL-1R-mediated fibroblast activation leads to the
507 expression of neutrophil-attracting chemokines amongst other inflammatory mediators.
508 Neutrophils are then recruited in high numbers to the site of damage, further contributing to
509 the production of IL-1 β in the ulcer bed. The alarmin IL-1 α may similarly contribute to the
510 activation of fibroblasts and initiation of colitis^{30 31}, and can be released by necrotic epithelial

511 cells in IBD ³². Further studies are required to establish if the IL-1R-driven activation of
512 inflammatory fibroblasts identified here is dominated by IL-1 α or IL-1 β signalling or both. We
513 did not find IL-18 to be increased in the tissue of M4/M5-high patients.

514 Currently, sub-categories of IBD are classified by high level phenotypic observations. A lack
515 of knowledge about the molecular pathotypes in IBD means that therapies are currently not
516 prescribed based on the underlying biologic processes they target and therefore often fail. A
517 number of recent studies have tried to address the challenge of therapy non-response by
518 analysing the cellular and molecular network in treatment-refractory IBD. Whilst several genes
519 found in our M4/M5 modules have been previously associated with non-response to anti-TNF
520 therapy or corticosteroids ^{10-12,14-17,20}, none of those studies addressed the heterogeneity of
521 molecular inflammatory phenotypes in IBD. By relating molecular signatures (modules) to
522 histologic features, we were able establish this link and identified at least two distinct
523 pathotypes with important implications for patient stratification for therapeutic targeting. In
524 addition to patients with high tissue expression of M4/M5 and substantial ulceration (22/11%
525 of patients in the discovery/early-onset cohorts), we also identified patients with high M6 tissue
526 expression (26% of patients in the discovery cohort) that is associated with increased lymphoid
527 aggregates; the high tissue expression of CCL19/CCL21/CXCL13 also suggests that this
528 pathotype reflects the presence of fibroblastic reticular-like cells ²⁵, as opposed to the
529 inflammatory fibroblast phenotype detected in M4/M5 high tissues. The expression of M6 was
530 very low in the early-onset cohort of paediatric UC and CD. This may reflect the different nature
531 of the samples analysed in the two studies. The latter used endoscopic punch biopsies
532 (mucosa), as opposed to full thickness (mucosa/muscularis/submucosa) samples from
533 surgical specimens in our discovery cohort. Although present in the mucosa, lymphoid
534 aggregates are more prominent in submucosal regions. This requires consideration when
535 interpreting lymphoid tissue signals from endoscopic biopsies of the gut. Tissues high in M6
536 showed elevated expression NR3C1 and ITGA4, but not TNF. This is consistent with our
537 findings that M6 is predictive of non-response to anti-TNF, but not of non-response to

538 corticosteroid or anti-integrin, suggesting that such M6 high patients may benefit from
539 vedolizumab or corticosteroids.

540 By contrast patients with UC or CD whose tissues show a high M4/M5 signature and ulceration
541 express high amounts of *IL1B* but not *NR3C1*, *ITGA4* or *TNF* suggesting that subgroup may
542 benefit from blocking IL-1R instead of TNF, to target the neutrophil-attractant program in
543 fibroblasts. Indeed, TNF has been shown to promote mucosal healing ³³ and therefore may
544 be deleterious in patients with deep ulceration that require wound healing. Genetic defects in
545 the IL-1 pathway have been linked to anti-TNF non-response ³⁴ and the principle of
546 ameliorating acute intestinal inflammation by blockade of IL-1 signalling has been
547 demonstrated in several pre-clinical models ³⁵⁻³⁷. In case studies of Mendelian disease-like
548 IBD (MD-IBD) with IL-10 deficiency, the blockade of IL-1 signalling has been successfully
549 applied to treat intestinal inflammation ^{38,39}, providing proof of concept. Surprisingly, larger
550 scale studies of IL-1 blockade in polygenic IBD patient cohorts are lacking, although trials in
551 acute severe ulcerative colitis are in progress ⁴⁰. Future trials may benefit from stratifying
552 participants for inclusion based on the observations presented here. By dissecting IBD patient
553 heterogeneity at a cellular and molecular level, we provide a rationale for targeting
554 therapeutics to the underlying pathologies, based on histologic features and molecular
555 signatures rather than high-level phenotypic diagnoses.

556 Our discovery cohort of surgical resection samples from patients with UC or CD highlights the
557 heterogeneity of inflammatory lesions in this difficult-to-treat patient group. This data is just a
558 snap-shot and does not inform on the evolution and dynamics of these distinct pathotypes.
559 However, the presence of M4/M5 signature high patients before treatment in a number of
560 prospective cohorts suggests that deep ulceration and high M4/M5 signature can occur
561 independently of therapy failure. Our study does not address whether lymphoid aggregates
562 and ulceration are independent processes or connected states. Notably, the presence of
563 M4/M5 and M6 is not mutually exclusive, and a small number of tissues exhibited both
564 ulceration and lymphoid aggregates. Further understanding of the natural history of these

565 distinct pathotypes and their relationship to disease dynamics will require longitudinal
566 analyses.

567 In summary, our combinatorial approach, integrating data across biological levels, identifies
568 new tissue IBD pathotypes that are defined by different molecular, cellular and
569 histopathologic features that are linked to patient responses to current therapeutics. These
570 stratifications provide a basis for personalised targeting of existing medicines and indicate that
571 IL-1 signalling blockade may benefit those individuals with deep ulceration who do not respond
572 to current therapeutics. This may improve treatment trajectories for patients with IBD, both by
573 hastening administration of appropriate interventions and providing a novel mechanism to
574 target in an area of current unmet clinical need.

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621 conflict of interest.

622 **Figure 1. Identification of gene co-expression signatures of inflammation associated**
623 **with patient non-response to multiple different IBD therapies** A) Pearson correlation
624 between module eigengenes and clinical and histologic metadata in inflamed and CRC
625 derived tissues within the discovery cohort; all modules/features with at least one significant
626 association shown, bordered squares indicate significant correlations (FDR p<0.05). B-D)
627 Module M4 and M5 expression (eigengene value) in non-responders and responders before
628 the start of corticosteroid (B ¹⁵), anti-TNF (C+D ^{20,21}) or monthly anti-integrin therapy (D ²¹)
629 (Mann-Whitney U, FDR adjusted P-values, post-hoc to ANOVA comparisons across the
630 various treatment regimens in the Arjis 2018 study in the case of D). E) Performance (AUROC)
631 of individual genes for predicting non-response to corticosteroid (y-axis) and anti-TNF (x-axis)
632 therapy; genes contained in M4 and M5 are labelled and highlighted by turquoise and orange
633 datapoints respectively. F) Violin plots showing the rank of genes based on their predictive
634 power (area-under-the-receiver-operator-curve, AUROC) for response to both anti-TNF and
635 corticosteroid therapy, comparing all modules as detected in the WGCNA analysis. Combined
636 ranks represent the sum of each gene's ranks in the separate corticosteroid and anti-TNF
637 analyses (their ranks on the x and y axes in (E)).

638 **Figure 2. Co-expression modules linked with therapy non-response represent distinct**
639 **histopathologic features**

640 A) Nancy histologic scores in non-responders to anti-TNF therapy before the start of treatment
641 (horizontal bars indicate geometric mean, Mann-Whitney U test P values given). B) Heatmap
642 of correlations between module eigengene expression and histological features quantified
643 across tissues from IBD patients in the discovery cohort. Nominally significant associations
644 (p<0.05) are indicated by borders and FDR significant (FDR p<0.05) associations are

645 indicated by dots. C) Scatter plots showing eigengene expression of M4, M5 and M6 versus
646 selected quantified histologic features in tissue samples from IBD patients of the discovery
647 cohort. D) Classification of M4/M5 high, M5 only high, M6 high and M4/M5/M6 low patients in
648 the discovery cohort, based on hierarchical clustering of module eigengene values from
649 inflamed tissue samples. E) Normalised expression (tpm) of cytokine and therapeutic target
650 genes that were reliably (in >50% of samples) detected in the discovery cohort. The
651 expression of these genes is compared in the M4/M5 high (red), M5 only high (orange), M6
652 high (blue) or M4/M5/M6 low tissues (bottom panel). Horizontal lines indicate the median and
653 p-values (Wilcoxon signed rank test, adjusted for multiple testing) for each comparison are
654 given if significant ($P < 0.05$).

655 **Figure 3. High expression of modules M4 and M5 reflects neutrophil infiltrates,**
656 **activated fibroblasts and epithelial cell loss.**

657 A+B) Module M4 and M5 expression in cell clusters detected by scRNASeq in UC ¹² and CD
658 ¹¹ patient tissue. C) Heatmap of the expression (TPM values, z-score, Manhattan distance
659 clustering) levels of all genes contained within M4 and M5 in THY1+PDPN+ stromal cells,
660 CD16^{hi} neutrophils, and CD14+HLA-DR+/- MNPs, FACS-sorted from inflamed IBD patient
661 tissue. The genes are ordered by their log fold-change of significant enrichment (P adjusted <
662 0.05) in either cell type. D) FACS cell type percentages in tissue isolates from IBD patients,
663 classified into low (white), intermediate (orange) or high (red) expression of M4/M5 (see
664 Extended Data Figure 3E). Pie-charts show medians across samples and boxplots individual
665 samples. (*) in pie charts indicates cell population percentages significantly different between
666 groups, post-hoc 2-way ANOVA adjusted P -values are given, if significant). E) Violin plots
667 showing the rank of genes based on their predictive power (AUROC) for response to both anti-
668 TNF and corticosteroid therapy, comparing genes significantly enriched in neutrophils, stromal
669 cells, MNPs or neither. Combined ranks represent the sum of each genes ranks in the
670 separate corticosteroid and anti-TNF analyses. F) Illustrative IHC staining (DAB, counterstain
671 hematoxylin) of Podoplanin (PDPN), neutrophil-elastase (NE), calprotectin (S100A8/A9) or

672 CD68 in serial sections of IBD patient tissue classified as low, intermediate or high for M4/M5
673 whole tissue gene expression (see Extended Data Figure 3F). G) Automated quantification
674 (% positively stained cells of total cells detected in inflamed areas) of IHC stainings as shown
675 in F); each staining was performed on inflamed tissue sections with low (n=17), intermediate
676 (n=13) and high (n=12) M4/M5 whole tissue gene expression (see Extended Data Figure 3F);
677 post-hoc 2-way ANOVA adjusted P-values are given, where significant.

678 **Figure 4. Stromal architecture of the large and small intestine in health and disease.**
679 A) UMAP of stromal clusters identified by Harmony in stromal compartments FACS-sorted
680 from healthy donor and IBD patient tissue with low, intermediate and high M4/M5 whole tissue
681 gene expression (see Extended Data Figure 4A). B) Proportion (% of total stromal cells) of the
682 cell type clusters in A in the M4/M5 low, intermediate and high tissue. C) Heatmap of selected
683 markers of each of the cellular cluster as in A, as identified by Harmony; Expression values
684 are normalised log2 fold changes (Wald statistic $\frac{\beta_g}{\sigma_g}$) from DESeq2 analyses. D)
685 Immunofluorescent staining of THY1 (Blue), Podoplanin (PDPN, Green), ABCA8 (red) and
686 PDGFRA (yellow) to visualise the localisation of fibroblast subsets in resected tissue from IBD
687 patients (uninflamed areas). E) Immunofluorescent staining of THY1 (Blue), Podoplanin
688 (PDPN, Green), PECAM1 (Red) and MCAM (orange) to visualise the localisation of vascular
689 (endothelial) and perivascular cells (uninflamed areas). PDGFRA: PDGFRA+ fibroblasts,
690 ABCA8+ : ABCA8+ Fibroblasts, BEC, blood endothelial cells, LEC : lymphatic endothelial
691 cells.

692 **Figure 5. M4/M5 gene expression is associated with neutrophil-attracting fibroblasts**
693 **and endothelial and perivascular cell expansion.** A) UMAP of stromal single-cell
694 profiles showing the different stromal clusters as in Figure 4A for comparison (top panel), and
695 the expression level of M4 (middle panel) / M5 (bottom panel) genes in these clusters (as in
696 Figure 3A). B) Heatmap showing normalised gene expression of the top differentially
697 expressed genes between M4/M5 expression levels within each cell cluster. Expression

698 values are normalised log2 fold changes (Wald statistic $\frac{\beta_g}{\sigma_g}$) from DESeq2 analyses (see STAR
699 Methods). C) Staining of NE or PECAM1 (red), THY1 (blue) and PDPN (green) in IBD patient
700 tissues with varying grades of neutrophil infiltration. D) Staining of NE (green), FAP (blue) and
701 PDPN (red) in paired inflamed (deep ulcer) and uninflamed IBD tissue.

702 **Figure 6. Activated inflammatory fibroblasts drive neutrophil recruitment through IL-1R**
703 **signalling with high levels of IL-1 β at sites of ulceration.** A) Ccd18-co fibroblasts were
704 stimulated for 3 h with either mock control or conditioned media produced from IBD patient
705 tissue digests (CM), without pre-treatment (vehicle = PBS), or pre-incubated with IL-1Ra
706 (anakinra) or anti-TNF (adalimumab). Adjusted P-values are shown if significant ($p<0.05$),
707 Friedman test for paired samples. B) Projection of the IL-1 cytokine stimulation response of
708 Ccd18-co fibroblasts onto stromal cell clusters detected by scRNASeq (see Figure 3A). Score
709 was computed as mean z-score of IL-1 upregulated genes. C) IHC stainings of IL-1 β or FAP
710 (DAB, counterstain hematoxylin) in inflamed tissue sections of IBD patients with prominent
711 ulceration and/or granulation tissue. * indicates non-specific staining of erythrocytes or
712 platelets in vessels. D) Stainings as in C), but in inflamed sections of IBD patients with
713 dominant lymphoid aggregates.

714 **Online Methods**

715 ***Patient cohorts and ethics***

716 Patients eligible for inclusion in the discovery cohort were identified by screening surgical
717 programs at Oxford University Hospitals. Samples were obtained from patients undergoing
718 surgical resection of affected tissue for ulcerative colitis (UC), Crohn's disease (CD) or
719 colorectal cancer (CRC) (used as non-IBD controls). All tissue samples included in the study
720 were classified by pathological examination as either macroscopically active inflamed or
721 uninflamed. Additional samples were also obtained from CD and UC patients or from healthy
722 individuals by biopsy. All patients gave informed consent and collection was approved by NHS
723 National Research Ethics Service under the research ethics committee references IBD

724 09/H1204/30 and 11/YH/0020 for IBD or GI 16/YH/0247 for CRC samples and gut biopsies
725 from healthy individuals. Samples were immediately placed on ice (RPMI1640 medium) and
726 processed within 3 hours. All patients gave informed consent and data was fully anonymised
727 prior to analyses. For replication of prospective findings in the discovery cohort, public
728 datasets were used that were derived from endoscopic tissue samples of IBD patients ^{15,20,21,41}
729 (GSE16879, GSE73661, GSE109142, GSE57945).

730 **Isolation of cells from tissue and blood samples**

731 After removing external muscle and adipose layers, and removing bulk epithelial cells by
732 repeated washes in PBS containing antibiotics (Penicillin-streptomycin, amphotericin B,
733 gentamicin, ciprofloxacin) and 5mM Ethylendiaminetetraacetic acid (EDTA, Sigma Aldrich),
734 tissue from surgical resections was minced using surgical scissors. In the case of endoscopic
735 biopsies, the epithelial wash was omitted. Minced tissue was subjected to multiple rounds of
736 digestion in RPMI1640 medium containing 5% fetal bovine serum (FBS), 5mM HEPES,
737 antibiotics as above, and 1mg/ml Collagenase A and DNase I (all from Sigma Aldrich). After
738 30 minutes, digestion supernatant containing cells was taken off, filtered through a cell
739 strainer, spun down and resuspended in 10ml of PBS containing 5% BSA and 5mM EDTA.
740 Remaining tissue was then topped up with fresh digestion medium until no more cells were
741 liberated from the tissue.

742 **Primary culture expansion and conditioned media production**

743 Primary stromal cell lines were expanded by plating the single-cell suspension of tissue
744 digests onto plastic cell culture vessels and expanding the adherent fraction (>95% CD45-
745 EPCAM-CD31- cells, not shown) in RPMI1640 (with 20%FCS, antibiotics, 5mM HEPES)
746 (Sigma). Primary cell lines were used for assays between passage number 7 and 15. For the
747 production of conditioned media, sorted cell populations were plated at 1.000.000 cells/ml in
748 cell culture dishes and RPMI1640 containing 5%FCS (LifeTechnologies), antibiotics, and 5mM

749 HEPES for 16 hours. After that, supernatants were aspirated, spun down to remove cells, and
750 frozen at -80°C until further use.

751 **Fluorescence-activated cell sorting (FACS) and analysis**

752 Single-cell suspensions obtained from tissue digests were stained for FACS analysis or sorting
753 with antibodies (all from Biolegend, except anti-Pdpn: clone NZ-1.3 from eBioscience) in PBS
754 with 5% BSA and 5mM EDTA for 20 minutes on ice. After washing in the same buffer, cells
755 were analysed (LSRII or Fortessa X20) or sorted (Aria III, 100um nozzle).

756 ***Ex vivo* assay of IBD patient conditioned media transferred onto fibroblasts**

757 For the stimulation of stromal cells, either Ccd18-Co colonic fibroblasts (ATCC #CRL-1459)
758 or primary stromal cell lines (isolated as above) were plated at 20000 cells/well in a 48-well
759 plate. Plated cells were starved for 72 hours in culture medium without FCS, before stimulation
760 with cytokines or conditioned media (pre-diluted 1:3 in starving medium) for 3 hours at 37°C.
761 For blockade experiments, recombinant cytokines in starving medium or conditioned media
762 were pre-incubated with 2mg/ml Anakinra (Kineret) or Adalimumab (Humira) for 1 hour at RT
763 (shaking) before stimulation of cells. After 3 hours, supernatants were taken off and cells lysed
764 directly in appropriate RNA lysis buffer.

765 **Isolation of RNA from tissue samples and cell populations**

766 Endoscopic punch biopsies or dissected tissue pieces from surgical resections were stored in
767 RNAlater (Qiagen) upon collection until further processing. Tissue was homogenised using
768 the soft tissue homogenizing CK14 kit (Precellys, Stretton Scientific 03961) in 300µl of RLT
769 lysis buffer (Qiagen) and 20µM DTT (Sigma). RNA was isolated using the Qiagen Mini kit with
770 a DNA digestion step (Qiagen). Bulk-sorted cell populations and cultured cells were directly
771 lysed in RNA lysis buffer, followed by RNA isolation with the according kits and on-column
772 DNase treatment.

773 **Sequencing of RNA from whole tissue and sorted cell populations**

774 Sequencing libraries were prepared using either the QuantSeq 3' mRNA-Seq FWD Library
775 Prep Kit (Lexogen) for whole tissue samples or the Smart-seq2 protocol ⁴² for bulk and cultured
776 cell populations (with our own in-house indexing primers). Libraries were sequenced using an
777 Illumina HiSeq4000 with 75bp paired-end sequencing ⁴³. For qPCR analysis, 15 to 250ng of
778 RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit
779 (Applied Biosystems) and qPCR performed using Precision Fast qPCR mastermix with ROX
780 at a lower level, 12.8mL (Primer design, Precision FAST-LR), and Taqman probes (Life
781 Technologies).

782 Bulk RNA sequencing data were analysed using the bulk processing aspect of
783 pipeline_srnaseq.py (<https://github.com/sansomlab/scseq>). Data quality was assessed using
784 pipeline_readqc.py (<https://github.com/cqat-developers/cqat-flow>). Sequenced reads were
785 aligned to the human genome GRCh38 using Hisat2 (version 2.1.0) ⁴⁴ using a reference index
786 built from the GRCm38 release of the mouse genome and known splice sites extracted from
787 Ensembl version 91 annotations (using the hisat2_extract_splice_sites.py tool). A two-pass
788 mapping strategy was used to discover novel splice sites (with the additional parameters: --
789 dta and --score-min L,0.0,-0.2). Mapped reads were counted using featureCounts (Subread
790 version 1.6.3; Ensembl version 91 annotations; with default parameters) ⁴⁵. Salmon v0.9.1
791 was used to calculate TPM values ⁴⁶ using a quasi-index (built with Ensembl version 91
792 annotations and k=31) and gc bias correction (parameter “--gcBias”). For heatmap
793 visualisations of gene expression levels, z-scores of TPM values and Manhattan distances
794 were calculated within the *heatmap2* package in R. Differential expression analyses were
795 performed using DESeq2 (v1.26.0)⁴⁷. Raw data will be deposited in GEO.

796 Pathway enrichment analysis for groups of genes associated with cell types was carried out
797 by the *enrichGO* function from the *clusterProfiler* package in R ⁴⁸. “Cellular component” GO
798 annotation terms were used as pathways.

799 **Identification and quantification of gene co-expression modules in discovery data**

800 To reduce dimensionality within the dataset, an unbiased approach was used to collapse
801 genes with similar expression patterns in the discovery RNAseq dataset. Normalised (TPM)
802 counts were considered for all genes across all samples, including both inflamed and
803 uninflamed tissues from the IBD patients and the samples from the CRC controls. These were
804 filtered to remove genes with zero counts in over half of the samples and log transformed
805 following the addition of a pseudo count. Transformed counts were then used to define
806 modules of correlated genes using the weighted gene co-expression network analysis
807 approach (WGCNA) in R ⁴⁹. In brief, this process calculates pair-wise Pearson correlation
808 estimates between all genes. These are then raised to the power of a soft-threshold, in this
809 case raising correlation coefficients to the power of 9, which magnifies the differences between
810 large and small correlations. Finally, the network of these amplified correlations (where each
811 gene is a node and each edge is a correlation) is used to generate a topological overlap matrix
812 (TOM). This represents the similarity of expression patterns between a given pair of genes in
813 the data set, similar to the correlation matrix, but taking into account their shared correlation
814 with other genes. Finally, hierarchical clustering of the TOM is used to assign genes into
815 modules based on their co-expression pattern. The *pickSoftThreshold* function was used to
816 identify 9 as an appropriate soft-threshold. The *blockwiseModules* function was then used with
817 this threshold to automatically carry out the aforementioned process and assign genes to
818 modules. Parameters for the function were as follows, a minimum module size of 30 genes, a
819 mergeCutHeight of 0.1, reassignThreshold of 0, and using a signed network.

820 The resultant module definitions were quantified using the eigengene approach within
821 WGCNA. An eigengene is a quantitative representation of the expression of a module as a
822 whole and is derived from the first component of a principle components analysis restricted to
823 the expression data of just the genes in the module. Eigengenes for the modules defined in
824 the resection data were calculated using the *moduleEigengenes* function.

825 Correlations between clinical and metadata measures and module eigengenes were assessed
826 using Pearson correlations with p-values estimated using the *corPValueStudent* function and

827 adjusted for multiple testing. Benjamini-Hochberg correction using the *p.adjust* function was
828 used for all analyses with adjusted p-values. This was carried out on the inflamed IBD tissue
829 samples and CRC tissue samples combined and also on the inflamed IBD tissue samples
830 alone. Eigengenes were also compared between paired inflamed and uninflamed tissues
831 sections using a t-test, adjusting for multiple testing across modules.

832 Cell type composition scores were estimated for each resection sample using the
833 *xCellAnalysis* function from the xCell package ¹⁹. Correlations between module eigengenes
834 and the derived cell type scores were visualized for all cell types scored in over 25% of
835 samples and used to infer the cell types represented by modules within the whole tissue data
836 (discovery cohort).

837 **Quantifying module associations with clinical variables in replication datasets**

838 Publicly available RNAseq (^{15,41} (GSE57945, GSE109142)) or microarray data ^{20,21}
839 (GSE16879, GSE12251) were downloaded from the NCBI gene expression omnibus. These
840 were pre-existing enumerated gene counts in the case of the RNAseq datasets and raw array
841 data in the case of the microarray sets. The latter were processed and normalised to gene
842 counts using the *rma* function from the affy package ⁵⁰, summing values for probes associated
843 with the same gene symbol. Across all datasets, gene symbol annotations were used to map
844 the genes to the module assignments generated from the discovery resection tissue dataset,
845 dropping genes that were not observed in the replication dataset under consideration. The
846 percentage of genes missing from the original module definitions was recorded but was
847 generally low across all datasets. Mapped module assignments were then used to generate
848 eigengenes from the replication expression datasets using the *moduleEigengenes* function.
849 Correlations between clinical metadata and eigengenes in replication datasets was performed
850 using Pearson correlations as for the discovery dataset. In the case of the paediatric cohort
851 data (GSE57945), Mann-Whitney U tests were used to compare the modules between
852 patients scored as ulcerated or not in metadata and hierarchical clustering used to group
853 patients based on M4,5, and M6 expression as for the discovery cohort.

854 Differences in pre-treatment module eigengene values between responders and non-
855 responders in prospective studies were assessed using Mann–Whitney *U* tests, adjusting p-
856 values for testing of multiple modules within each dataset. In the Haberman et al. 2019 study
857 we only considered patients on corticosteroid therapy, combining both patients that received
858 oral and intravenous administration. In the case of the Arjis 2018 study, which tested multiple
859 different therapies and treatment regimens, we used ANOVA to identify any differences
860 between responders and non-responders across all combinations adjusting for regimen and
861 used post-hoc Mann-Whitney *U* tests to identify individual treatment regimens where modules
862 were significantly different by response.

863 Meta-analysis of the expression of the M4 and M5 modules across responders and non-
864 responders in the various replication datasets was carried out using the meta package in R⁵¹.
865 The anti-TNF response data was used from the Arijs 2008 and 2018 papers and the
866 corticosteroid response data was from the Haberman et al. study. Only the 4 week treatment
867 condition was included from the anti-intergrin data from Arijs 2018, as this was the only one
868 that proved significantly different for either M4 or M5. A random effects meta-analysis was
869 carried out comparing standardised mean differences between patient groups using the exact
870 Hedges estimate.

871 In the prospective cohorts, the predictive value of the expression of single genes for response
872 to treatment was assessed using a simple logistic regression where response was the
873 outcome and gene expression the sole predictor. Modelling was carried out for all genes also
874 observed in the discovery cohort, for each of the prospective studies, using the *glm* function
875 in R. The predictive ability of each gene in each dataset was summarised as the area under
876 the curve (AUC) of a receiver operating characteristic (ROC) curve. AUC values for each gene
877 were generated by applying the *roc* function from the pROC package to predictions generated
878 from the logistic regression models. The relative predictive power of genes within modules of
879 interest was compared by summing the rank of genes (based on their AUC value) across
880 datasets and comparing these cumulative ranks between modules.

881 **Pathological scoring of histology using the Nancy index**

882 Formalin-fixed paraffin-embedded (FFPE) and hematoxylin & eosin (H&E)-stained tissue
883 sections of IBD patients were scored according to the Nancy index, based on criteria reported
884 in ¹⁸.

885 **Immunohistochemistry and quantitative histopathology**

886 Tissue specimens were either fixed for 48 hours in 4% neutral-buffered formalin (Sigma) and
887 embedded in paraffin ("FFPE"), or fixed for 24 hours in 2% PFA in phosphate buffer containing
888 L-lysine and Sodium periodate and frozen in OCT (Sigma) after soaking in 30% sucrose for
889 48 hours ("OCT"). Freshly cut, dewaxed, and rehydrated FFPE sections (5µm) were subjected
890 to heat-induced antigen retrieval by boiling in Target Retrieval Solution (Dako, pH=6, for all
891 stainings except neutrophil elastase) for 15minutes (microwave). This was followed by 15
892 minutes of blocking in Bloxall solution (Vector Labs), 60minutes blocking in 5%BSA/TBST with
893 5% serum of the secondary antibody species (Sigma), and 15minutes of blocking in avidin
894 followed by biotin solution (Vector Labs). All steps were performed at ambient temperature.
895 Tissue sections were incubated with primary antibodies in 5%BSA/TBST overnight (>16
896 hours) at 4°C. Following incubation, biotinylated or HRP/AP-conjugated secondary antibodies
897 were applied for 2 hours (RT) in 5% BSA/TBST. For biotinylated secondary antibodies, AB
898 complex (Vector Labs) was incubated for another hour in TBST (RT). Chromogenic stains
899 were developed by applying DAB HRP substrate solution (Vector Labs) and counterstained
900 for 5minutes in Hematoxylin solution (Sigma). Slides were then dehydrated and mounted in
901 DPX (Sigma) mounting medium.

902 Whole section imaging of chromogenic sections was performed on a NanoZoomer S210 digital
903 slide scanner (Hamamatsu). Slide scans of all stains can be made available upon request.
904 Scanned tissue sections, stained using DAB immunohistochemistry, were analysed using
905 Indica Labs HALO® image analysis platform. A consultant gastrointestinal pathologist
906 manually annotated each slide, dividing the mucosa into normal and inflamed. The tissue was

907 scored using Indica Labs analysis modules CytoNuclear v2.0.5, detecting DAB positive and
908 negative cells in inflamed areas. Pathologic features (ulceration/granulation tissue,
909 granulomas, crypt abscess/cryptitis, lymphoid aggregates and architectural distortion/mucin
910 depletion) were manually annotated by a consultant pathologist with a special interest in
911 gastrointestinal pathology. The area of each annotated feature was automatically calculated
912 by the HALO software. Nuclei (cells) in areas of interested and the whole tissue section were
913 detected and counted using Indica Labs – CytoNuclear v2.0.9 analysis module. Scores (%)
914 were normalised to the number of nuclei that were found within a pathological feature over the
915 total number of nuclei detected in the whole tissue section. These normalised counts were
916 used to investigate Pearson correlations between features and correlations with module
917 eigengenes.

918 10µM thick OCT sections were incubated in blocking buffer (PBS1X, 5% Goat serum, 2% FCS
919 and human FcBlock (Miltenyi) with primary antibodies overnight at 4°C. The next day AF488
920 Donkey anti Rat, AF647 Donkey anti Goat, AF555 Donkey anti Rabbit or strepatividin-AF568
921 were applied for 1h at RT in blocking buffer. Finally, nuclei were stained with Hoechst 28332
922 (Life Technologies) for 15min at RT in blocking buffer and then mounted in ProlongGold
923 mounting medium (Life Technologies) prior to imaging with the spectral detector of a Zeiss
924 confocal LSM 880 microscope.

925 **Preparation of cells for single-cell RNA sequencing**

926 Four pairs of biopsies were pooled, minced and frozen in 1mL of CryoStor® CS10 (StemCell
927 Technologies) at -80°C then transferred in LN₂ within 24 hours. Single-cell suspensions from
928 these endoscopic biopsies were then prepared by thawing, washing and subsequent mincing
929 of the tissue using surgical scissors. Minced tissue was then subjected to rounds of digestion
930 in RPM-1640 medium (Sigma) containing 5% FBS (Life Technologies), 5mM HEPES (Sigma),
931 antibiotics as above, and Liberase TL with DNase I (Sigma). After 30 minutes, digestion
932 supernatant was taken off, filtered through a cell strainer, spun down, and resuspended in
933 10ml of PBS containing 5% BSA and 5mM EDTA. Remaining tissue was then topped up with

934 fresh digestion medium until no more cells were liberated from the tissue. Cells were then
935 stained and FACS-sorted, as described above for live EPCAM-CD45- cells, before being taken
936 for microfluidic partitioning (see below).

937 **10x library preparation, sequencing, and data analysis**

938 Single-cell RNAseq data was generated from disaggregated intestinal tissue sorted for Sorted
939 CD45-EPCAM- stromal cells. Viable cells were subjected to a standard droplet single-cell
940 cDNA library preparation protocol. The experimental details to generate cDNA libraries are
941 described in a separate manuscript
942 (<https://www.biorxiv.org/content/10.1101/2021.01.11.426253v1>). We demultiplexed FASTQ
943 files for each 10X library using the Cell Ranger (v3.1.0) mkfastq function ⁵². We then mapped
944 reads to the GRCh38 human genome reference using Kallisto ⁵³ (v0.46.0) and quantified gene
945 by cell-barcode UMI matrices with Bustools (<https://github.com/BUSTools/bustools>) (v0.39.0).
946 For quantification, we used gene annotations provided by Gencode ⁵⁴ (release 33), keeping
947 only protein coding genes and collapsing Ensembl transcripts to unique HGNC approved gene
948 symbols.

949 We filtered for potentially empty droplets and damaged cells by excluding droplets with fewer
950 than 500 unique genes and libraries with greater than 20% of reads assigned to mitochondrial
951 genes. We pooled the resulting high-quality cells from each 10X library into a single cell by
952 gene UMI matrix. We normalized for read depth with the standard logCP10K normalization
953 procedure for gene g and cell i :

$$954 \quad \log CP10K_{gi} = \log\left(1 + 10^4 \times \frac{UMI_{gi}}{\sum_h UMI_{hi}}\right)$$

955 We performed PCA analysis on the top 2000 most variable genes, identified with the VST
956 method implemented in the Seurat ⁵⁵ R package. For PCA, we z-scored each variable gene
957 and computed the top 30 eigenvectors and singular values with the truncated SVD procedure,
958 implemented in the RSpectra (<https://github.com/yixuan/RSpectra>) R package. We defined

959 PCA cell embeddings by scaling eigenvectors by their respective singular values. To account
960 for potential batch effects in the PCA embeddings, we modelled and removed the effect of
961 10X library as identified using the Harmony algorithm. For Harmony ²⁴, we set the cluster
962 diversity penalty parameter θ to 0.5 and used default values for all other parameters. We
963 evaluated the effect of library mixing before and after Harmony using the Local Inverse
964 Simpson's Index (LISI), described in the Harmony manuscript ²⁴. We evaluated the
965 significance of the LISI change with a t-test, with degrees of freedom equal to the number of
966 libraries minus 1. To visualize the cells in 2 dimensions, we input the Harmonized PCs into
967 the UMAP (arXiv:1802:03426 [stat.ML]) algorithm.

968 **Identification of marker genes within single-cell RNA sequencing**

969 We performed joint clustering analysis on all scRNASeq libraries using the cells' Harmonized
970 PCA embeddings. With the 30-nearest neighbour graph, we computed the unweighted shared
971 nearest neighbour (SNN) graph, and truncated SNN similarity values below 1/15 to zero. We
972 then performed Louvain clustering, based on the R/C++ implementation from Seurat, at
973 resolution=0.3, resulting in 8 clusters. We identified upregulated marker genes in each cluster
974 using pseudobulk differential expression with negative binomial regression, implemented in
975 the DESeq2 R package. For pseudobulk analysis, we collapsed cells from the same donor
976 and cluster into one pseudobulk sample, summing the UMI counts from each cell. We then
977 performed differential expression analysis on these pseudobulk samples, with the design $y \sim$
978 $1 + cluster$. This design assigns each gene an intercept term (i.e. mean expression), a
979 multiplicative offset for each cluster. We addressed the degeneracy of the design matrix by
980 assigning a Gaussian prior distribution to the cluster effects (DESeq2 parameter
981 β Prior=TRUE). The full results for this differential expression analysis are reported in
982 Supplementary Table 9.

983 **Differential expression analysis of single-cell data by inflammatory status**

984 We performed differential expression to associate genes with inflammation status within each
985 single-cell cluster. We used DESeq2 on the pseudobulk samples described above, this time
986 analysing each cluster separately with the design $y \sim 1 + InflamStatus$. We treated
987 InflamStatus as a random effect (DESeq2 parameter $\betaPrior=TRUE$) and recovered a mean
988 multiplicative offset for each of the three inflammatory status categories.

989 **Single-cell gene set enrichment scoring**

990 Single-cell gene-set enrichment scores were computed for WGCNA modules and cytokine
991 stimulation signatures using the same strategy. For each gene in the gene set, we computed
992 Z scores (mean centred and unit variance scaled) of logCP10K normalized expression across
993 all cells. Then we summed the Z-scores of genes in the gene set to compute a single gene
994 set score for each cell. This procedure is summarized in the formula below, used to compute
995 the score $S_{G,i}$ for geneset G and cell i using normalized expression y_{gi} , gene mean μ_g , and
996 gene standard deviation σ_g .

997

$$score_{G,i} = \sum_{g \in G} (y_{gi} - \mu_g) / \sigma_g$$

998 **Single-cell trajectory analysis**

999 We performed trajectory using the principal curve method, implemented in the prncurve R
1000 package (<https://www.jstor.org/stable/2289936>). We fit a principal curve to all fibroblasts by
1001 inputting harmonized UMAP coordinates into the principal_curve function. This mapped
1002 fibroblasts to a non-linear, one dimensional space and assigned each cell a unique position,
1003 from 0 to 100, along this trajectory. To directly visualize the abundance of each cluster along
1004 the trajectory, we plotted the relative density of each cluster along the trajectory. In these
1005 density plots, ABCA8⁺ fibroblasts grouped towards the beginning (position=32) of the
1006 trajectory, PDPN⁺ fibroblasts in the middle (position=59), and PDGFRA⁺ fibroblasts towards
1007 the end (position=82). This distribution along the trajectory is also reflected by the canonical
1008 markers of these populations. To visualize this, we discretized pseudotime by binning into 100

1009 uniform-density windows, chosen so that each window has the same number of cells. We then
1010 plotted the scaled gene expression values of ABCA8, PDPN, and PDGFRA, summarized by
1011 mean expression (point) and 95% confidence interval (line).

1012 **Data and Code availability**

1013 RNA sequencing data will be made accessible via GEO (bulk) and ImmPort (single-cell), and
1014 all analysis code will be made available through a GitHub repository at
1015 <https://github.com/microbialman/IBDTherapyResponsePaper>.

1016 **Extended Data**

1017 **Extended Data Figure 1.** A) Scatterplot of the module expression difference between
1018 inflamed and uninflamed tissues paired from the same patients versus the correlation of the
1019 module with the Nancy score across all IBD and non-IBD tissues. Points highlighted with a
1020 diamond indicate a significant difference in paired t-test between inflamed/uninflamed tissue
1021 (FDR $p<0.1$). B) Heatmap of module eigengene – cell type correlations; cell types were
1022 deconvoluted from whole tissue expression data using *xCell*. Modules highlighted in bold
1023 were found to be associated with histologic inflammation. C) Percentage of genes within each
1024 module that were detectable in the publicly available datasets. Bars show mean and standard
1025 error across all modules for each dataset.^{15,20,21}.

1026 **Extended Data Figure 2.** A) Clinical and endoscopic measures in responders and non-
1027 responders to anti-TNF therapy before the start of treatment (horizontal bars indicate
1028 geometric mean, Wilcoxon signed rank test P values are given). B) Representative images of
1029 the various pathological features quantified on H&E histology of resected tissue from IBD
1030 patients. C) Correlation plot of histological features, quantified as the % of nuclei within the
1031 feature area relative to the nuclei with the total section area. Numbers and colours in upper
1032 right corner indicate the Pearson correlation coefficient; histograms on diagonal show the
1033 value distribution of the features within IBD patient tissues; scatter plots in the lower left corner
1034 show the individual datapoints. D) Violin plots of eigengene expression of M4, M5 and M6 in

1035 inflamed tissues of IBD patients with or without deep ulceration observed in a replication cohort
1036 of paediatric CD and UC (n=172). E) Classification of M4/M5 high and M4/M5 low patients in
1037 the paediatric replication cohort, based on hierarchical clustering on module eigengene
1038 values.

1039 **Extended Data Figure 3.** A) Gating strategy for FACS sorting of hematopoietic and non-
1040 hematopoietic cell populations from non-IBD and IBD patient tissue. B) Normalised gene
1041 expression (qPCR, relative to *RPLP0* expression) of selected genes from M4 and M5 in cell
1042 populations sorted as in A. C) FACS-gating strategy for sorting of neutrophils, stromal cells
1043 and MNPs from tissue samples of IBD patients. D) Gene set enrichment analysis using Gene
1044 Ontology (GO) Cellular Components pathway terms, based on all genes significantly enriched
1045 (p adjusted <0.05, $|\log_2 \text{fold change}| > 2$) in either neutrophils MNPs or stromal cells
1046 (Supplementary Table 8). E+F) Heatmaps of whole tissue gene expression of selected genes
1047 that are representative (=highly correlative) of M4 and M5 expression (qPCR, z-score
1048 transformed gene expression values); unsupervised clustering (Manhattan) distinguishes
1049 subgrouping into M4/M5 low, intermediate and high samples; the box-plots on the right show
1050 the eigenvalues of all detected genes on a per patient basis. The respective heatmaps refer
1051 to tissue samples used for FACS analysis (E) and IHC analysis (F) as shown in Figures 3D,
1052 F and G.

1053 **Extended Data Figure 4.** A) Heatmap of whole tissue gene expression of selected genes that
1054 are representative of (highly correlated with) M4 and M5 expression (qPCR, z-score
1055 transformed gene expression values); unsupervised clustering (Manhattan) groups samples
1056 into M4/M5 low, intermediate and high from the set of IBD patients whose samples were
1057 profiled by single cell RNA sequencing; the box-plots on the right show the eigenvalues of all
1058 detected genes on a per patient basis. B) Immunofluorescent staining of ABCA8 (red),
1059 PDGFRA (yellow), THY1 (blue), Podoplanin (PDPN, green) and nuclei (Hoechst, grey) in
1060 ileum and colon of resected tissue from IBD patients (not inflamed). C) Immunostaining
1061 of PECAM1 (red), MCAM (orange) THY1 (blue), Podoplanin (PDPN, green) and nuclei

1062 (Hoechst, grey) in ileum and colonic resected tissue from IBD patients (not inflamed). D) Box
1063 plot showing the proportion of the cell types in M4/M5 low, intermediate and high groups, as
1064 detected by scRNASeq. E) FACS analysis of live stromal cells (CD45-, EPCAM-) in resected
1065 tissue from an IBD patient (adjacent not inflamed and inflamed tissue). Gates for endothelial
1066 cells (PECAM1+), Pericytes (THY+, PDPN-), ABCA8+fibroblasts (THY1 high, PDGFRa low),
1067 PDGFRa+ fibroblasts (PDGFRa high , THY1 low) and inflammatory fibroblasts (FAP+) are
1068 shown. F) Pseudotime analysis of ABCA8+, PDGFRa+ and inflammatory fibroblasts in the
1069 single-cell dataset. Cell densities (top row) or canonical markers (bottom) are shown along the
1070 trajectory, binned to 100 uniform-density windows (each window has the same number of
1071 cells). G) Representative immunofluorescent stainings of PDGFRa (yellow) and ABCA (red)
1072 staining on fibroblasts in paired inflamed and uninflamed samples of the same IBD patient.

1073 **Extended Data Figure 5.** A) Primary fibroblast cell lines (n=33) culture-expanded from
1074 resected IBD patient tissue and stimulated for 3h with recombinant cytokines (adjusted P-
1075 values are shown where significantly different ($p < 0.05$) compared to unstimulated, Kruskall-
1076 Wallis test). B) RNAseq analysis (Salmon log2-transformed TPM values, z-score, see STAR
1077 Methods) of cultured intestinal fibroblast cell line Ccd18-co, stimulated with either TNF- α
1078 (100ng/ml) or IL-1 β (0.01ng.ml) for 3 hours (* P adjusted < 0.05 from DESeq2 differential gene
1079 expression analysis (see STAR Methods)). C) Dose-response of IL-1 β and TNF- α stimulated
1080 Ccd18co fibroblasts for gene expression fold change (FC) of CXLC8 over unstimulated,
1081 measured by qPCR. D) Pseudo-bulk expression fold changes (relative to M4/M5 low groups)
1082 of *ILR1* and *TNFR1* (see Supplementary Table 10) within the cellular clusters detected as in
1083 Figure 3A, across patients with either low, intermediate or high M4/M5 whole tissue
1084 expression. E) Gene set enrichment analysis of all modules detected in the discovery cohort
1085 for genes assigned to inflammasome pathways (GO:0061702).

1086 **Supplementary Table 1. Clinical characteristics of the Oxford IBD patient discovery**
1087 **cohort used in this study.** Samples from the discovery cohort consist of surgically removed
1088 tissue of CD and UC patients (=IBD), as well as surgically removed normal tissue adjacent to

1089 colorectal tumours (= non-IBD). IBD, inflammatory bowel disease; CD, Crohn's Disease; UC,
1090 Ulcerative colitis; IQR, interquartile range; n/a, not applicable.

1091 **Supplementary Table 2. Detailed WGCNA analysis results.** Gene IDs and gene names of
1092 genes contained in the detected modules (modules_genes). Gene set enrichment analysis
1093 (fgsea) results of top 10 pathways upregulated in the detected modules (module GO analysis).
1094 Correlation strength (_Cor) and adjusted significance (_Pvalue) for correlation of individual
1095 modules with metadata traits across the inflamed IBD and CRC tissue samples (module-trait
1096 correlations).

1097 **Supplementary Table 3. Replication of modules defined in the discovery cohort in other
1098 datasets.** Replication of the modules identified in the discovery cohort in publicly available
1099 datasets of IBD whole tissue gene expression.

1100 **Supplementary Table 4. Differential expression of replication set modules in relation to
1101 therapy-response.** Significance test (Wilcoxon signed rank test) results for difference in
1102 module (eigengene value) expression between responders and non-responders to anti-TNF
1103 ²⁰, corticosteroid ¹⁵ and anti-integrin ²¹ therapy, before the start of treatment.

1104 **Supplementary Table 5. Predictive power of individual genes for therapy non-response.**
1105 Ranking of genes contained in all modules and detected in given dataset by area-under-the-
1106 curve (AUC) to predict non-response to anti-TNF ²⁰, corticosteroid ¹⁵ and anti-integrin ²¹
1107 therapy. Combined (summed) ranks for both anti-TNF and corticosteroid response are also
1108 shown.

1109 **Supplementary Table 6. Clinical characteristics of the Oxford UC patient cohort of
1110 response to anti-TNF therapy.** Response to therapy in this UC patient cohort was defined
1111 as stopping anti-TNF therapy (Infliximab or Adalimumab) within 12 months of start, for reason
1112 of non-response (patients that stopped therapy for convenience, switch to biosimilar, or
1113 intolerance were not considered). Nancy histologic scores and UCEIS endoscopic scores, as
1114 well as the other characteristics, within 3 months before the start of anti-TNF therapy are

1115 shown. UC, Ulcerative colitis; IQR, interquartile range; UCEIS, Ulcerative Colitis Endoscopic
1116 Index of Severity.

1117 **Supplementary Table 7. Clinical characteristics of the IBD patients used for RNAseq**
1118 **and FACS analysis.** Clinical characteristics of the IBD patient cohorts used for the
1119 transcriptomic and FACS analysis. UC, Ulcerative colitis; IQR, interquartile range; UCEIS,
1120 Ulcerative Colitis Endoscopic Index of Severity.

1121 **Supplementary Table 8. Differential gene expression between neutrophils, stromal cells**
1122 **and mononuclear phagocytes FACS-sorted from IBD patient tissue.** List of all significant
1123 (adjusted P value < 0.05, $|\log_{2}\text{foldchange}| > 2$) differentially expressed genes between
1124 neutrophils, stromal cells and MNPs sorted from the intestine of IBD patients. The standard
1125 DESeq2 outputs are reported.

1126 **Supplementary Table 9. Differential gene expression between stromal cell clusters**
1127 **detected through scRNAseq.** Differential expression was performed to associate gene
1128 expression with stromal clusters comparing each cluster to all others irrespective of M4/M5
1129 expression status. The standard DESeq2 outputs are reported.

1130 **Supplementary Table 10. Differential gene expression between stromal cell clusters**
1131 **within M4/M5 high tissues only.** Differential expression was performed to associate gene
1132 expression with stromal clusters comparing each cluster to all others clusters within M4/M5
1133 tissues only. The standard DESeq2 outputs are reported.

1134 **Supplementary Table 11. Differential gene expression between inflammation states and**
1135 **clusters detected through scRNAseq.** For each gene (column feature), gene expression
1136 was associated with the tissue sample's overall inflammatory status (column
1137 inflammatory_status), separately within each stromal cluster (column cell_type). The
1138 remaining columns are standard outputs of DESeq2.

1139 **Supplementary Table 12. Differentially expressed genes in Ccd18-Co fibroblasts upon**
1140 **IL-1 β stimulation.** List of all significant (adjusted P value < 0.05) differentially expressed

1141 genes in Ccd18-co fibroblasts after 3hour stimulations with IL-1 β (0.01 ng/ml). The standard
1142 DESeq2 outputs are reported. log2FoldChange is the IL-1 β -specific fold change over
1143 unstimulated condition.

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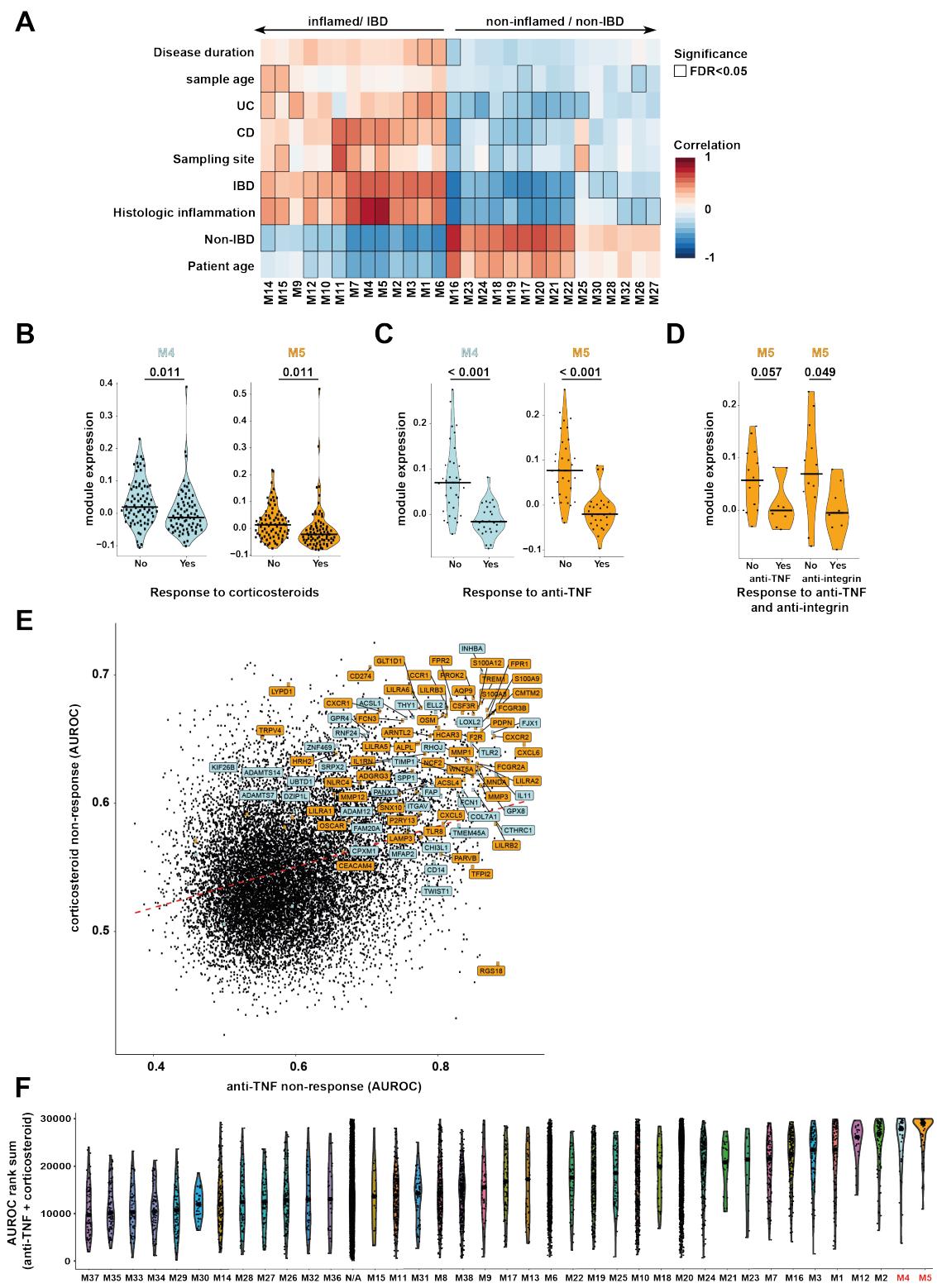


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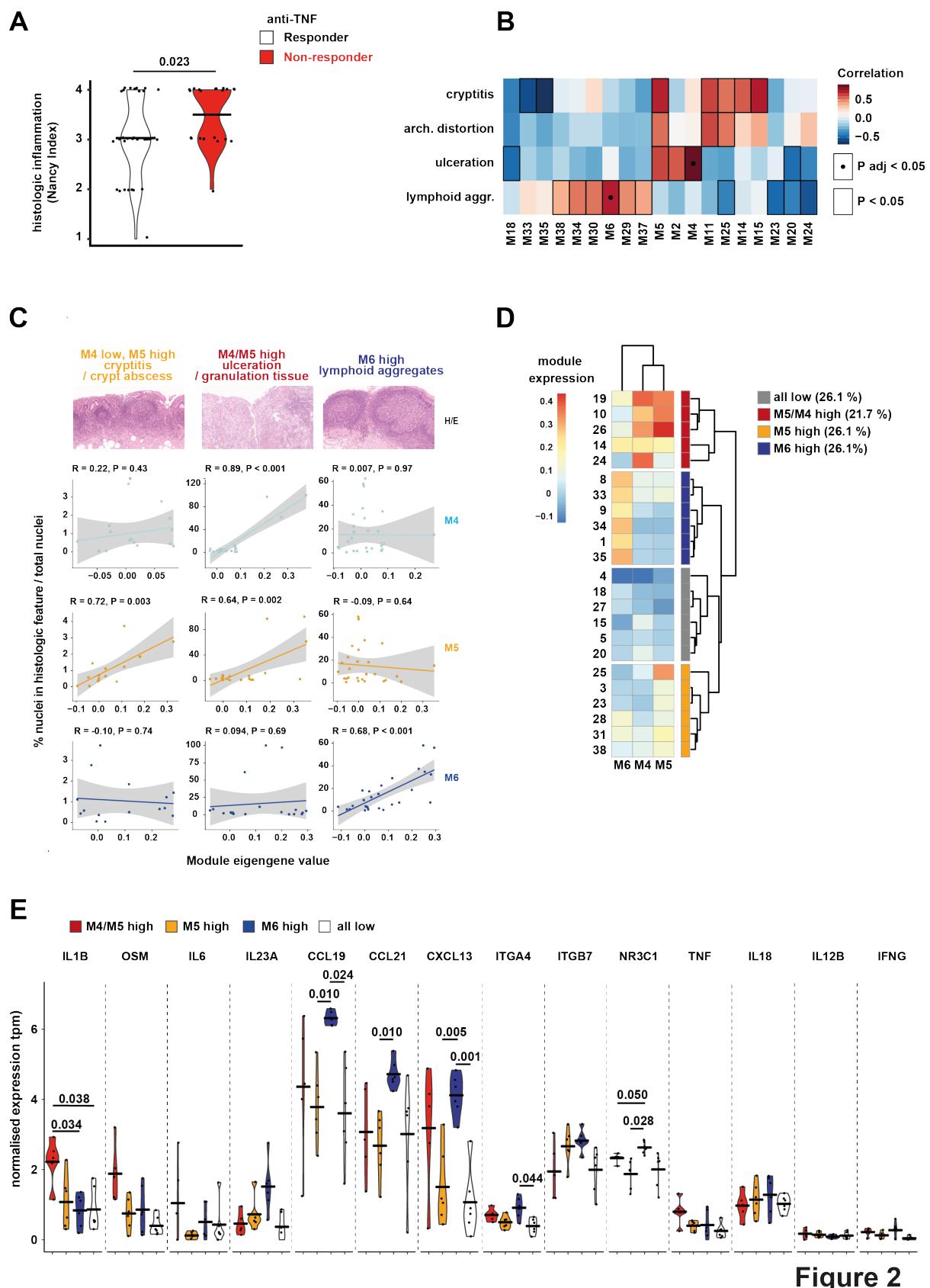


Figure 2

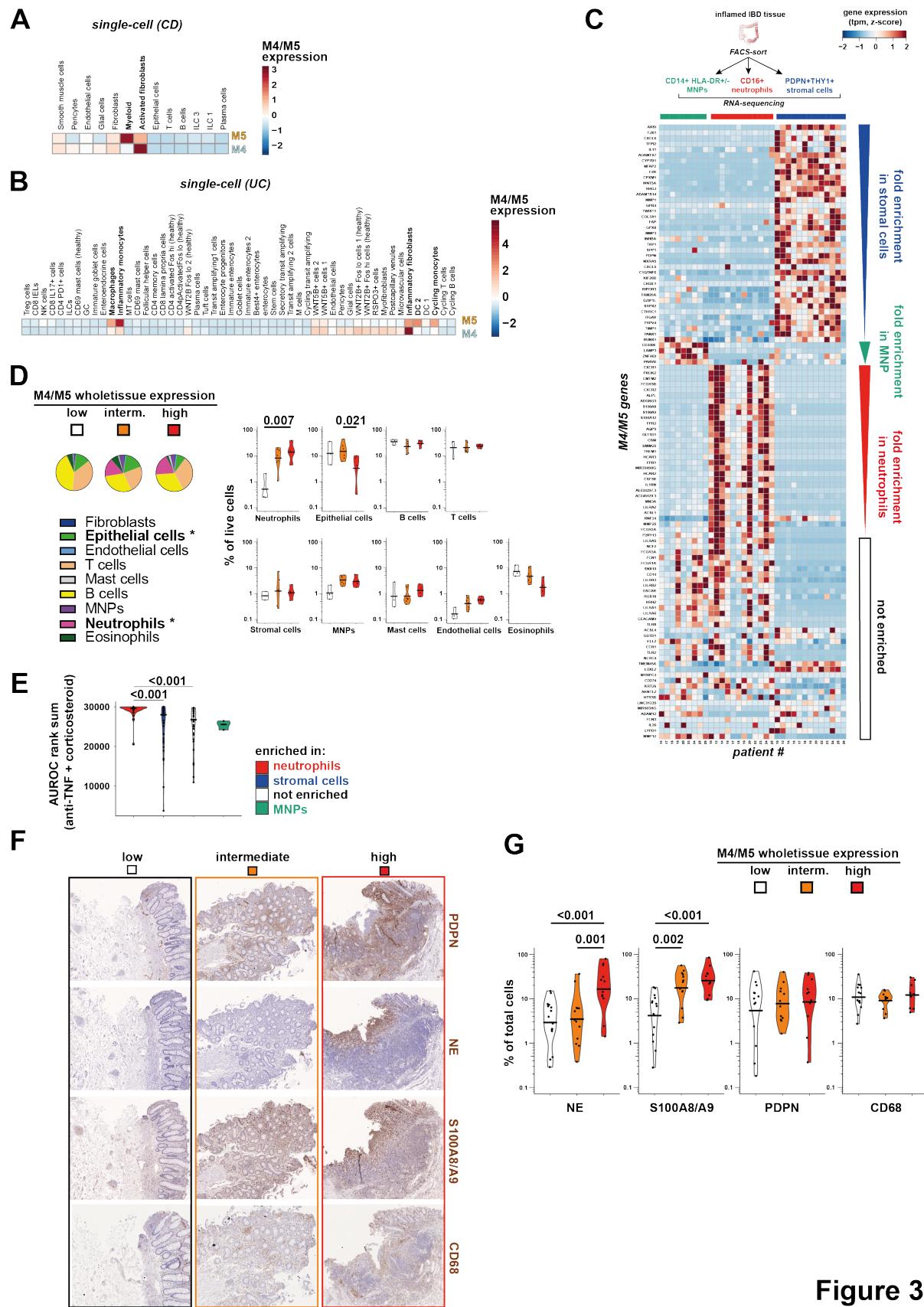


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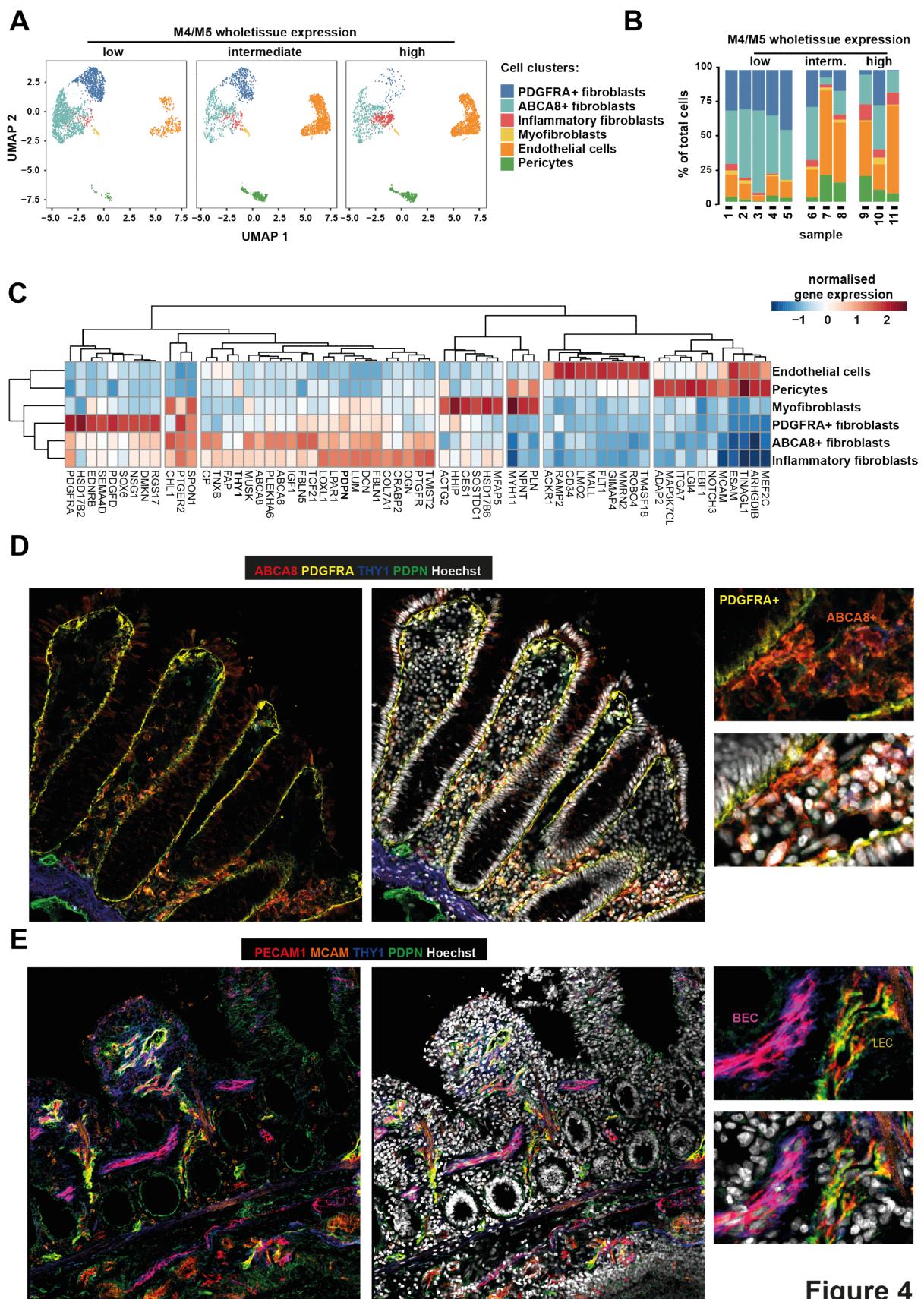


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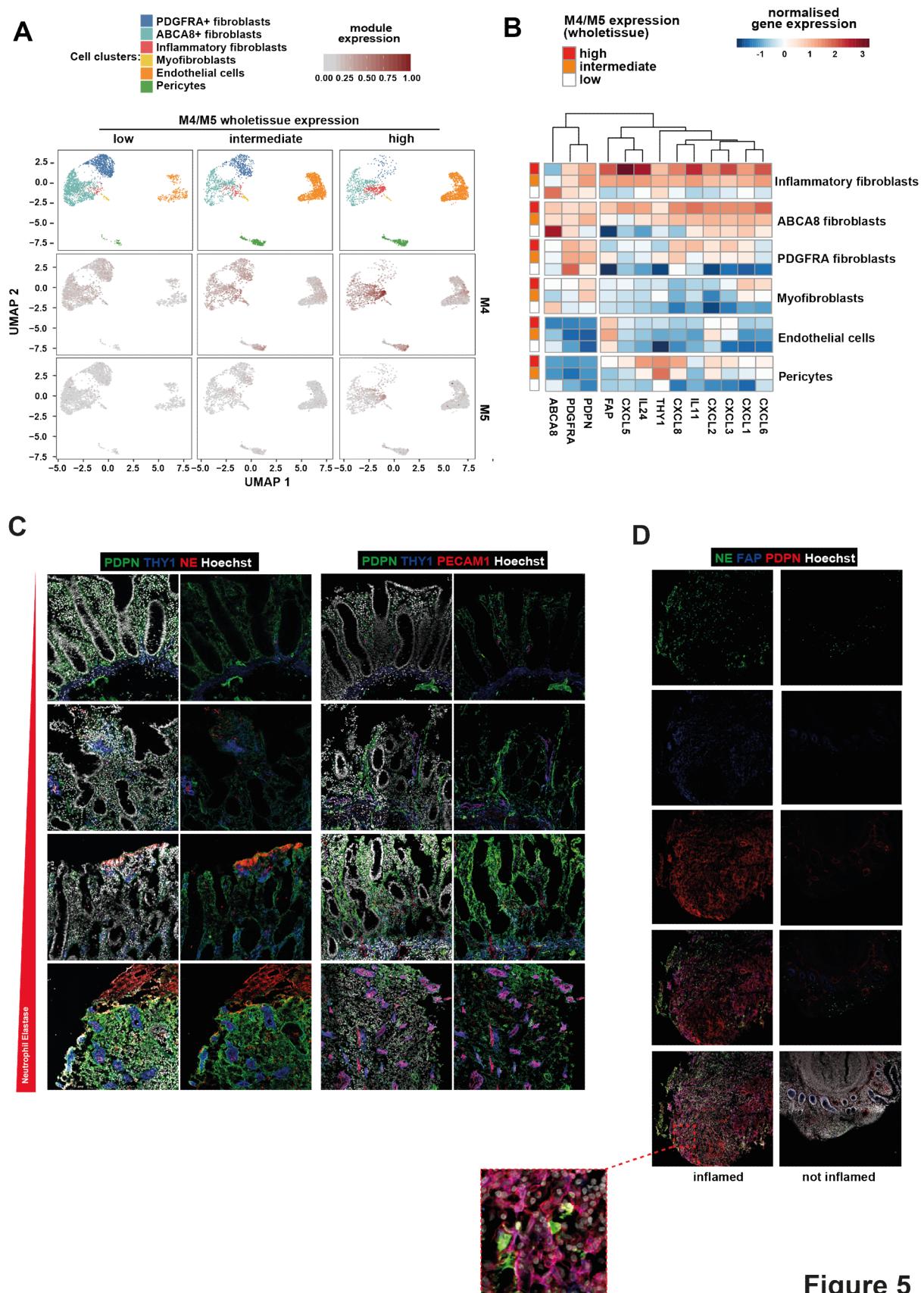


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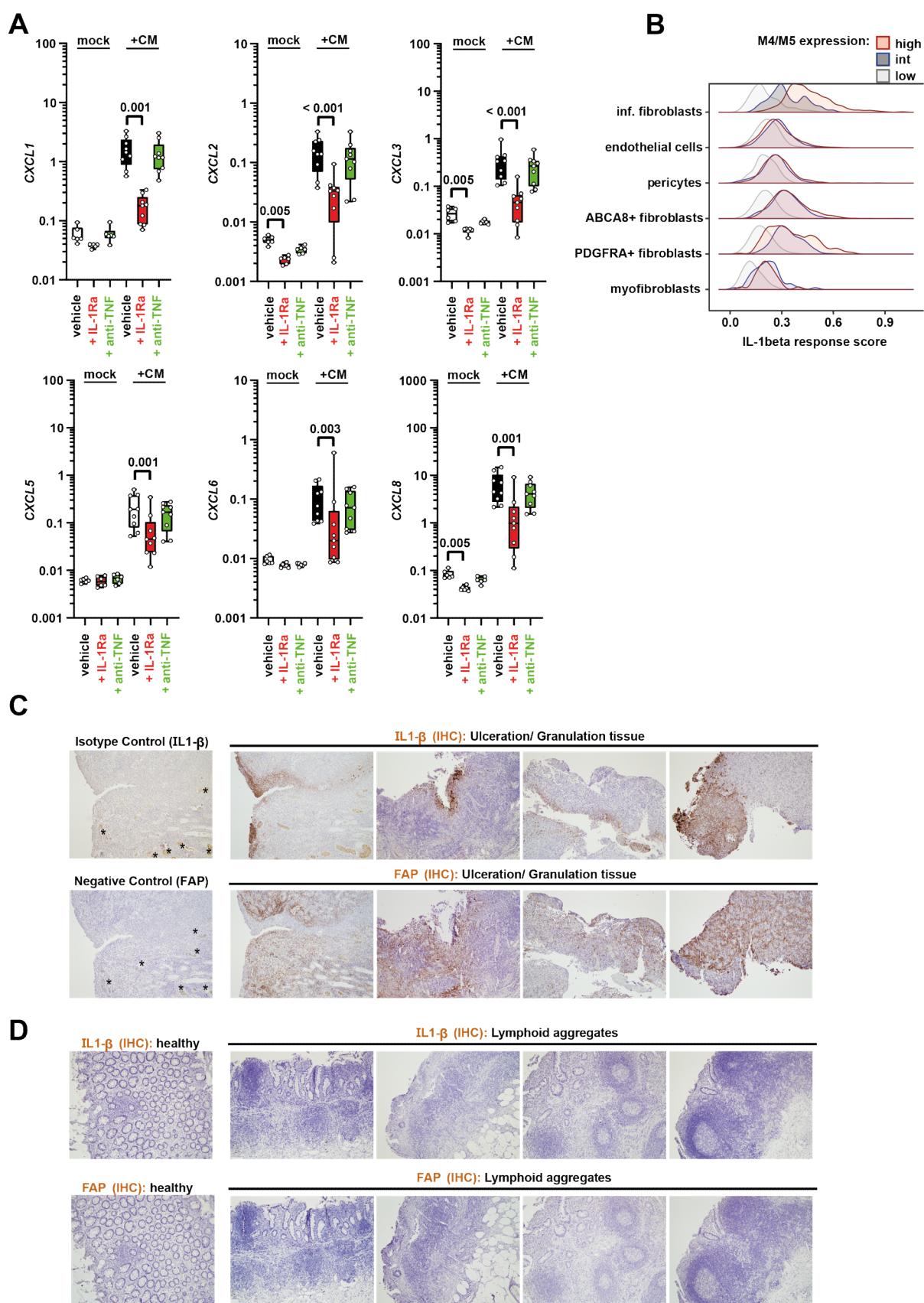
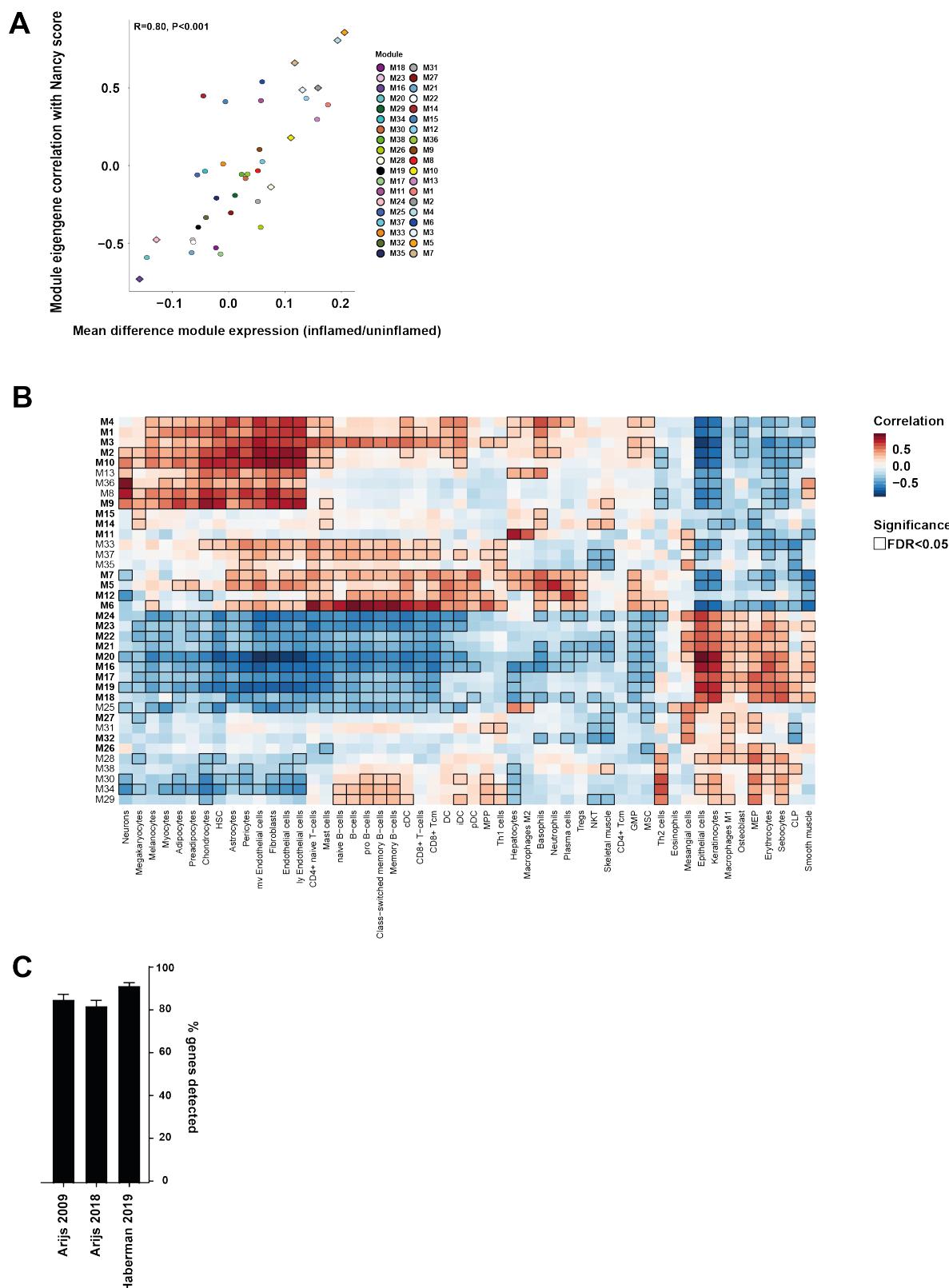
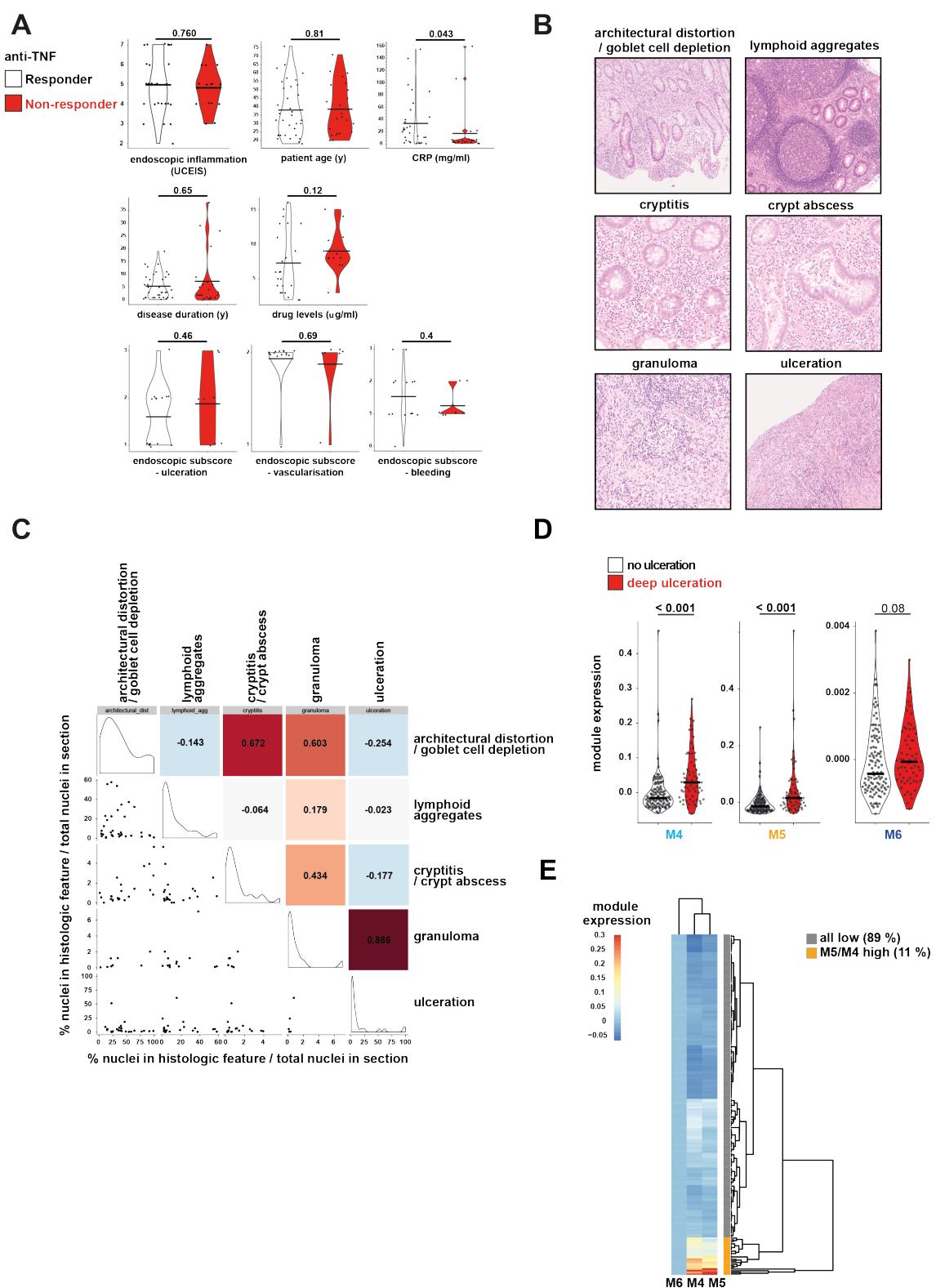


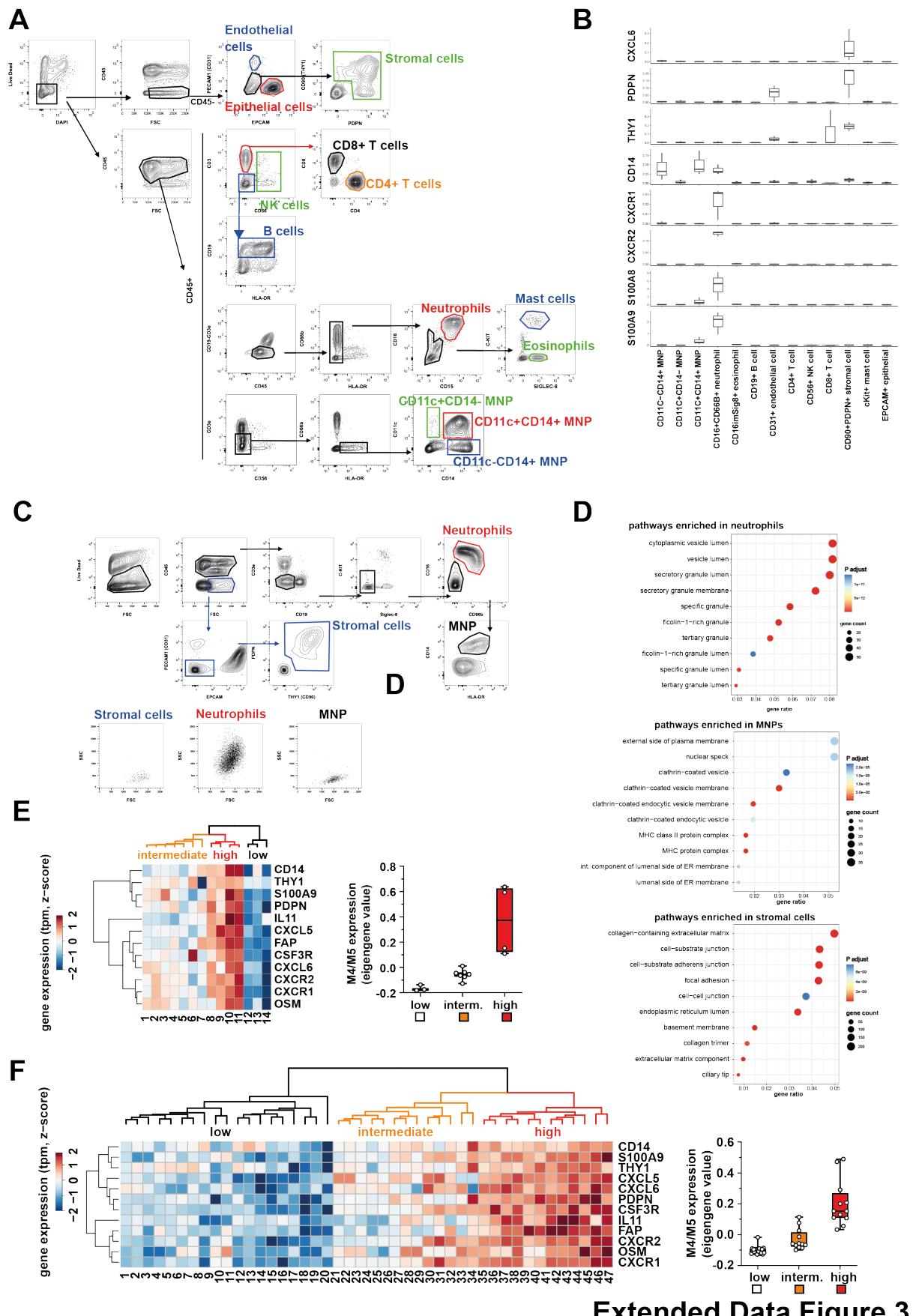
Figure 6



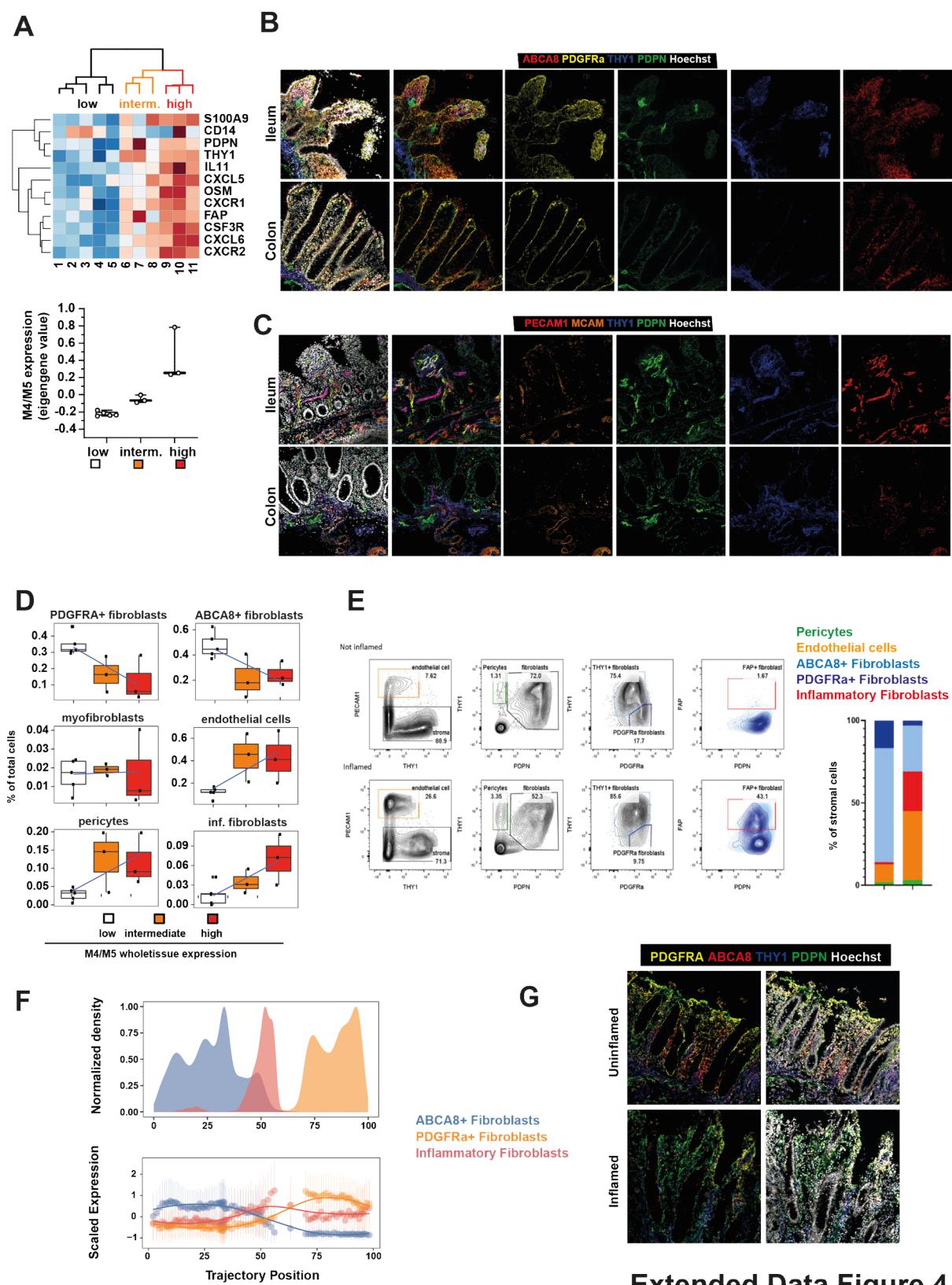
Extended Data Figure 1



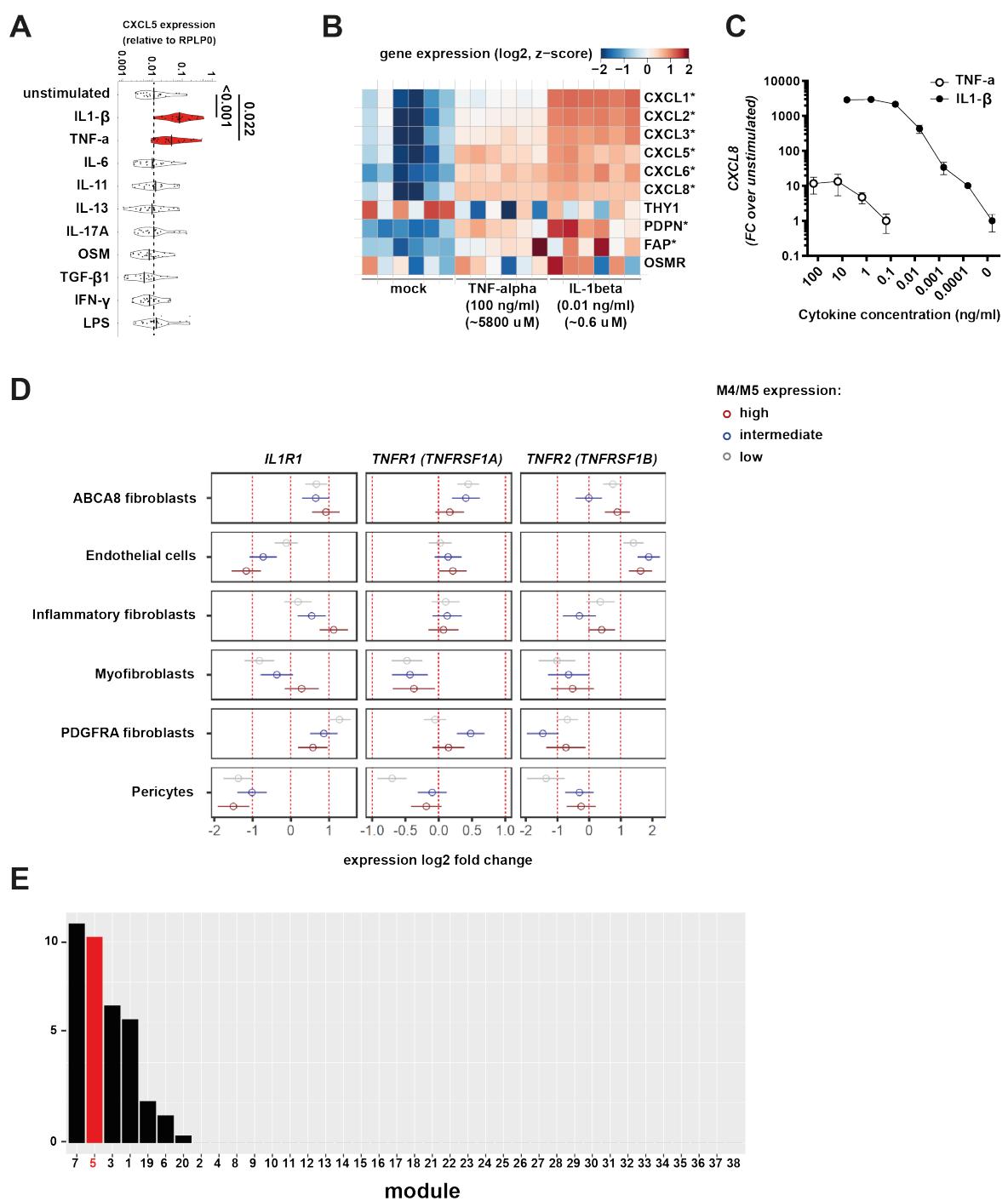
Extended Data Figure 2



Extended Data Figure 3



Extended Data Figure 4



Extended Data Figure 5

