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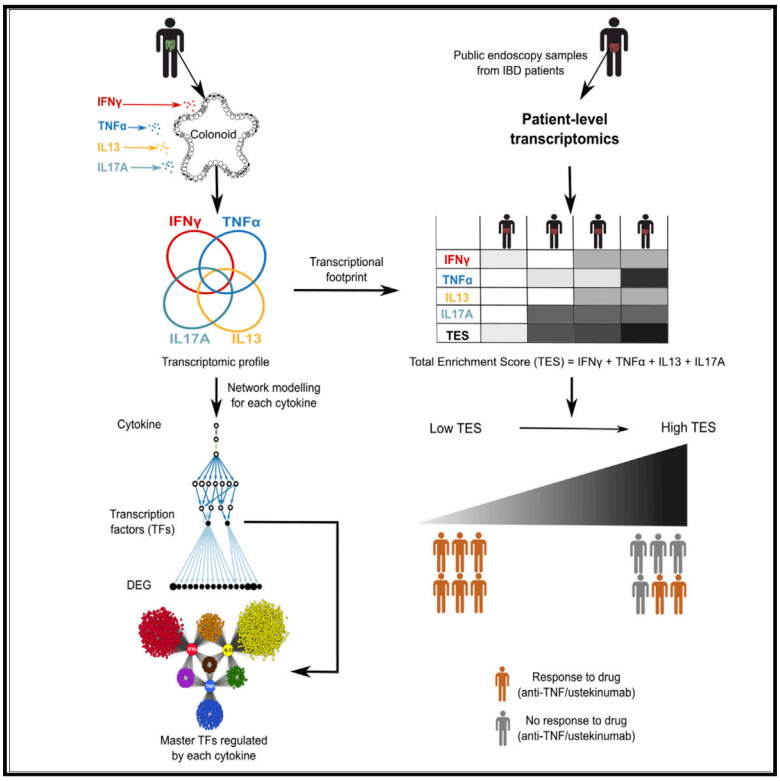
Cytokine responsive networks in human colonic epithelial organoids unveil a molecular classification of inflammatory bowel disease

Graphical abstract



Highlights

- Cytokine-regulated transcriptional programs stratify mucosal responses in IBD
- Patients cluster by a gradient of cytokine-regulated program activation
- Multiple program activation associates with treatment refractory disease

Authors

Polychronis Pavlidis, Anastasia Tsakmaki, Agatha Treveil, ..., Tamas Korcsmaros, Gavin Bewick, Nick Powell

Correspondence

npowell@ic.ac.uk

In brief

Pavlidis et al. mapped the transcriptional landscape of human colonoids in response to interferon gamma, interleukin-13 and -17A and tumor necrosis factor alpha. Using these cytokine transcriptional programs to interrogate mucosal transcriptional profiles of patients with inflammatory bowel disease, they describe a molecular stratification of inflammation with prognostic value.

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Cytokine responsive networks in human colonic epithelial organoids unveil a molecular classification of inflammatory bowel disease

Polychronis Pavlidis,[1,2] Anastasia Tsakmaki,[3] Agatha Treveil,[4,5] Katherine Li,[6] Domenico Cozzetto,[8] Feifei Yang,[6] Umar Niazi,[7] Bu Hussain Hayee,[2] Mansoor Saqi,[7] Joshua Friedman,[6] Tamas Korcsmaros,[8] Gavin Bewick,[3] and Nick Powell[8,9,*]

[1]Centre for Inflammation Biology and Cancer Immunology, King's College London, London, UK
[2]School of Immunology and Microbial Sciences, King's College London, London, UK
[3]Diabetes Research Group, School of Life Course Sciences, Faculty of Life Science and Medicine, King's College London, London, UK
[4]Earlham Institute, Norwich Research Park, Norwich, UK
[5]Quadram Institute Bioscience, Norwich Research Park, Norwich, UK
[6]Janssen Research and Development, 1400 McKean Road, Spring House, PA 19477, USA
[7]Translational Bioinformatics, National Institute for Health Research Biomedical Centre, Guy's and St Thomas' NHS Foundation Trust and King's College London, London, UK
[8]Division of Digestive Diseases, Faculty of Medicine, Imperial College London, London, UK
[9]Lead contact
*Correspondence: npowell@ic.ac.uk
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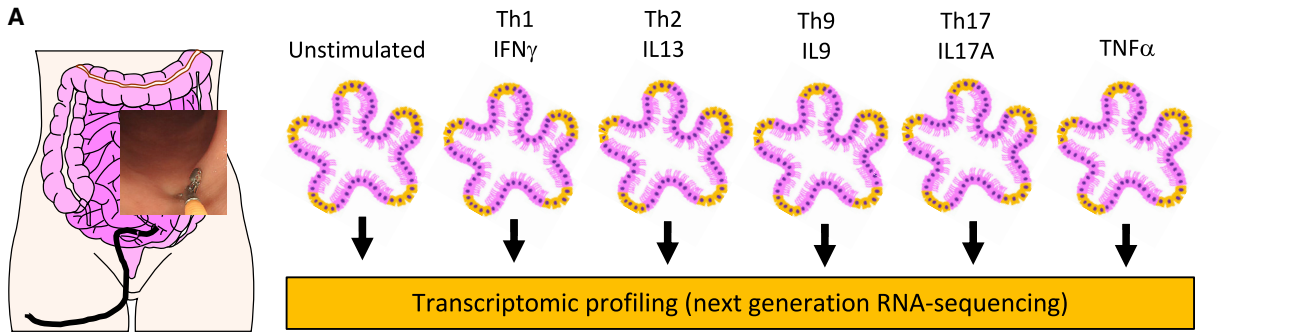
SUMMARY

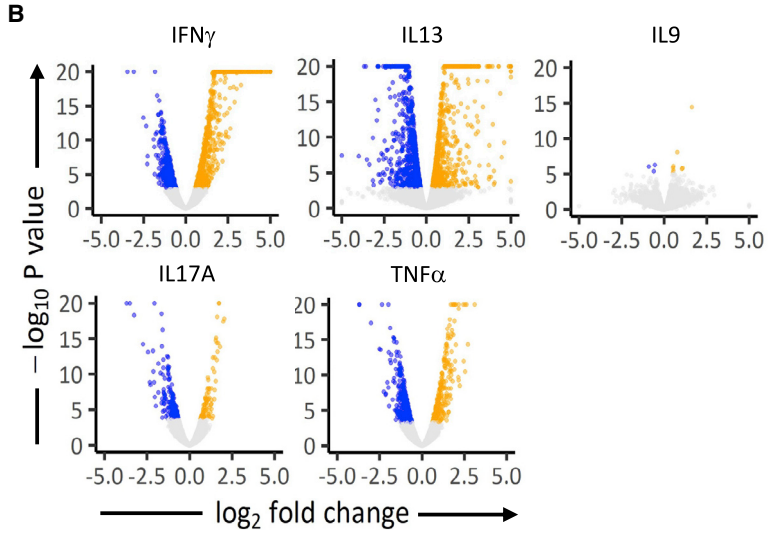
Interactions between the epithelium and the immune system are critical in the pathogenesis of inflammatory bowel disease (IBD). In this study, we mapped the transcriptional landscape of human colonic epithelial organoids in response to different cytokines responsible for mediating canonical mucosal immune responses. By profiling the transcriptome of human colonic organoids treated with the canonical cytokines interferon gamma, interleukin-13, -17A, and tumor necrosis factor alpha with next-generation sequencing, we unveil shared and distinct regulation patterns of epithelial function by different cytokines. An integrative analysis of cytokine responses in diseased tissue from patients with IBD (n = 1,009) reveals a molecular classification of mucosal inflammation defined by gradients of cytokine-responsive transcriptional signatures. Our systems biology approach detected signaling bottlenecks in cytokine-responsive networks and highlighted their translational potential as theragnostic targets in intestinal inflammation.

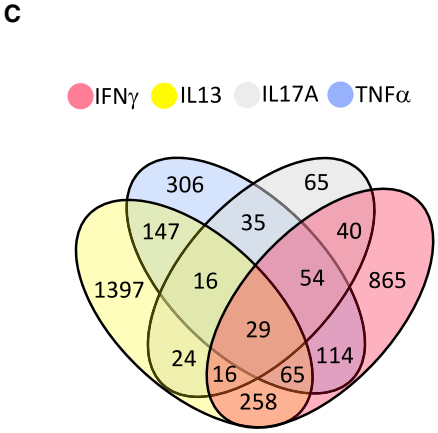
INTRODUCTION

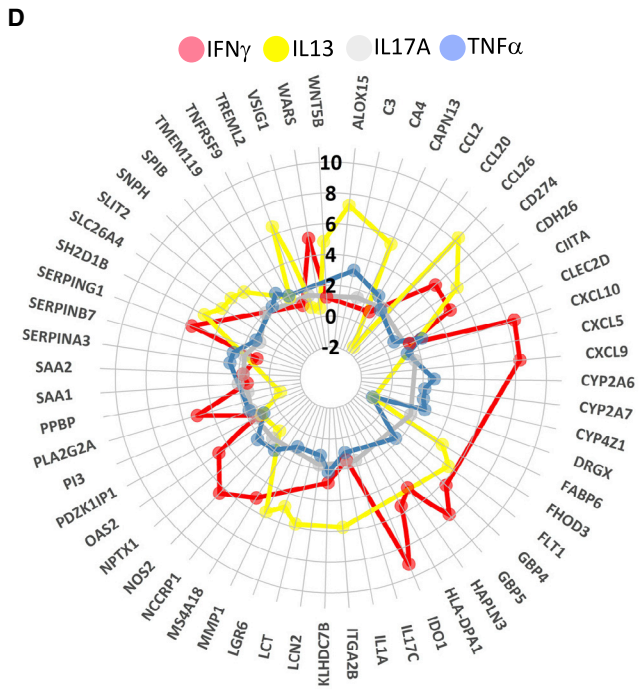
Cytokines play a key role driving inflammation and tissue injury in the gut, and biological therapies targeting them, or the cells that produce them, have revolutionized treatment paradigms in inflammatory bowel disease (IBD). However, many patients fail to achieve sustained remission irrespective of which biologic agent is used. This "ceiling effect" is observed with anti-tumor necrosis factor (TNF), vedolizumab (targeting the gut homing integrin $\alpha4\beta7$), and ustekinumab (targeting interleukin [IL]-12p40) (Cholapranee et al., 2017; Feagan et al., 2013, 2016; Neurath, 2014; Sandborn et al., 2013; Sands et al., 2019).

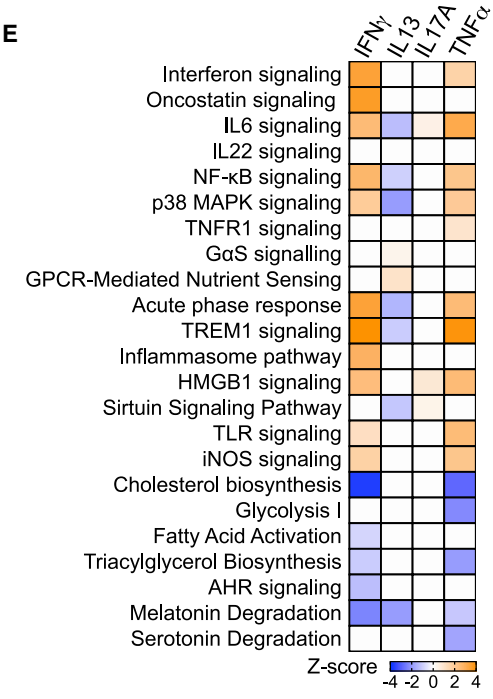
The intestinal epithelium plays an essential role in the maintenance and loss of intestinal homeostasis, and dysregulation of microbial sensing, autophagy, and the unfolded protein response are mechanistically implicated in impaired barrier function and IBD etiology (Cadwell et al., 2008; Kaser et al., 2008; Wehkamp et al., 2005). Epithelial function is directly regulated by mucosal immune activity and cytokines produced by tissue resident lymphocytes. For example, cytokines such as TNF-α, IL-22, and IL-9, which are excessively produced in IBD, drive epithelial-specific pathological processes, including ER stress, apoptosis, and impaired barrier function, triggering colitis in preclinical models (Garrett et al., 2007; Gerlach et al., 2014; Neurath, 2014). Activation of different adaptive immune responses has been reported in diseased tissue, including Th1/Th17 responses in both ulcerative colitis (UC) and Crohn's disease (CD) and Th2/Th9 responses in UC (Gerlach et al., 2014; Nguyen et al., 2015; Rovedatti et al., 2009). Little is known about how these qualitatively different arms of host immunity differentially regulate epithelial function in IBD. The development of three-dimensional epithelial organoids derived from tissue-specific stem cells, characterized by their close structural and functional resemblance to the tissue of origin, have provided a more physiological model system to study immune-epithelial interactions (Fujii et al., 2015; Sato et al., 2009, 2011). In this study, we have adopted an integrative systems biology approach using human organoids to understand the immunological landscape of diseased tissue of patients with IBD by defining the transcriptional topography of canonical cytokine-responsive pathways in colonic epithelial











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tissue and how these cytokines' regulatory footprints relate to disease features in large IBD cohorts.

RESULTS

Mapping the transcriptional landscape of the immune-epithelial interactome

To probe the immune-epithelial interactome, we treated human colonic organoids ("colonoids") with key cytokines responsible for mediating canonical immune responses, including interferon-gamma (IFNγ; characterizing the Th1 response), IL-13 (Th2), IL-9 (Th9), IL-17A (Th17), and TNF-α (as a pro-inflammatory control), and then mapped the transcriptome using next-generation RNA sequencing (RNA-seq) (Figures 1A, 1B, and S1). Collectively, these cytokines induced differential expression of 3,431 genes (Figure 1C). Many of the most highly upregulated differentially expressed genes (DEGs) were consistent with their recognized function in other tissues (Figures 1D and S1). Classical IFN-stimulated genes (ISGs) were highly upregulated by IFNγ, including *IDO*, *NOS2*, and the chemokines *CXCL10* and *CXCL9*, which contribute to Th1 T cell recruitment, activation, and differentiation (Dufour et al., 2002; Groom et al., 2012). IL-13 upregulated transcripts encoding the "eotaxin" family of eosinophil-active chemokines (*CCL26* and *CCL24*), typical of a type 2 response (Kitaura et al., 1999; Matsukura et al., 2001). IL-13 strongly upregulated other genes implicated in aberrant type 2 immunity and barrier function, including *ALOX15* and *CDH26* (Forno et al., 2019). IL-17A-regulated transcripts included *SAA1*, *SAA2*, and the neutrophil chemokine *CXCL5*, as well as *NFKBIZ* and *ZC3H12A* (Nanki et al., 2020). IL-9 had a negligible impact on the colonic epithelial transcriptome (Figure 1B). The transcriptional program regulated by IL-13 was the most distinct, with fewer than a third of IL-13-induced DEGs shared with other cytokines (Figure 1C). Conversely, >75% of IL-17A-regulated transcripts were additionally regulated by other cytokines, indicating redundancy in IL-17A control of epithelial function. Pairwise correlation analysis of DEGs identified a positive correlation between the TNF-α-, IL-17A-, and IFNγ-regulated programs (Figure S1).

There was substantial overlap among the biological processes predicted to be regulated by cytokine-responsive DEGs (Figures 1E and S2). By far the greatest magnitude of shared process activation was observed between IFNγ and TNF-α. Overlapping processes common to multiple cytokines included leukocyte trafficking, innate immunity (TREM1 and HMGB1 signaling), and bacterial sensing. Intriguingly, IL-13 was predicted to activate different processes, such as G protein-mediated nutrient sensing, enteroendocrine signaling, and pathways regulating smooth muscle activity.

A gradient of cytokine-responsive transcriptional signatures stratifies patients with IBD into distinct molecular phenotypes

To determine whether cytokine-responsive transcriptional programs were enriched in diseased tissue of patients with IBD, we probed the transcriptome of colonic biopsies from patients with active UC (n = 634) and active colonic CD (cCD; n = 126). These patients were recruited to the ustekinumab phase III clinical trials program (UNIFI study in UC, n = 550 and UNITI2 for CD, n = 126) (Feagan et al., 2016; Sands et al., 2019) and to PROgECT (n = 84), a phase 2a clinical trial of golimumab (anti-TNF) in UC (Telesco et al., 2018) (patient demographics: Table S1). To determine whether transcripts regulated by individual canonical cytokines were enriched in whole biopsy specimens, we performed gene set variation analysis (GSVA) (Haenzelmann et al., 2013). This algorithm determines the relative enrichment of a user-defined gene list across a large number of heterogeneous samples. Using this method, we defined the transcriptional "footprints" or "signatures" of individual cytokines by selecting the top 50 most highly upregulated transcripts induced by each cytokine in our colonic organoid experiment (Table S2). We observed significant enrichment of IFNγ-, IL-13-, IL-17A-, and TNF-α-responsive transcriptional signatures in whole colonic biopsies from patients with active UC compared with healthy control subjects (Figure 2A). IFNγ- and IL-17A-responsive signatures were also significantly enriched in colonic biopsies from patients with cCD (Figure 2A). A similar pattern of enrichment was observed by interrogating transcriptomic profiles of colonic epithelial layers isolated from patients with active, colonic IBD (E-MTAB_5464; Figure S3A), revealing a concordance in disease-associated enrichment of cytokine-regulated transcriptional responses between the epithelial compartment and the whole biopsy.

Although our analysis demonstrates significant enrichment of cytokine-responsive transcriptional signatures in diseased tissue of patients with IBD at the population level, the distribution of enrichment was heterogeneous for all cytokines. Since IBD is linked to selective expansion of Th1 (CD), Th2 (UC), and Th17 (both UC and CD) effector T cell responses (Heller et al., 2005; Neurath et al., 2002; Rovedatti et al., 2009), we reasoned that individual patients might segregate into discrete immunophenotypes based on selective enrichment of different canonical cytokine-responsive signatures. Consistent with individual patients mounting qualitatively and quantitatively different effector T cell responses in the colon, unsupervised hierarchical clustering demonstrated the existence of discrete subgroups of patients clustering according to preferential

Figure 1. Mapping the transcriptional landscape of the immune-epithelial interactome with human colonic organoids (colonoids)

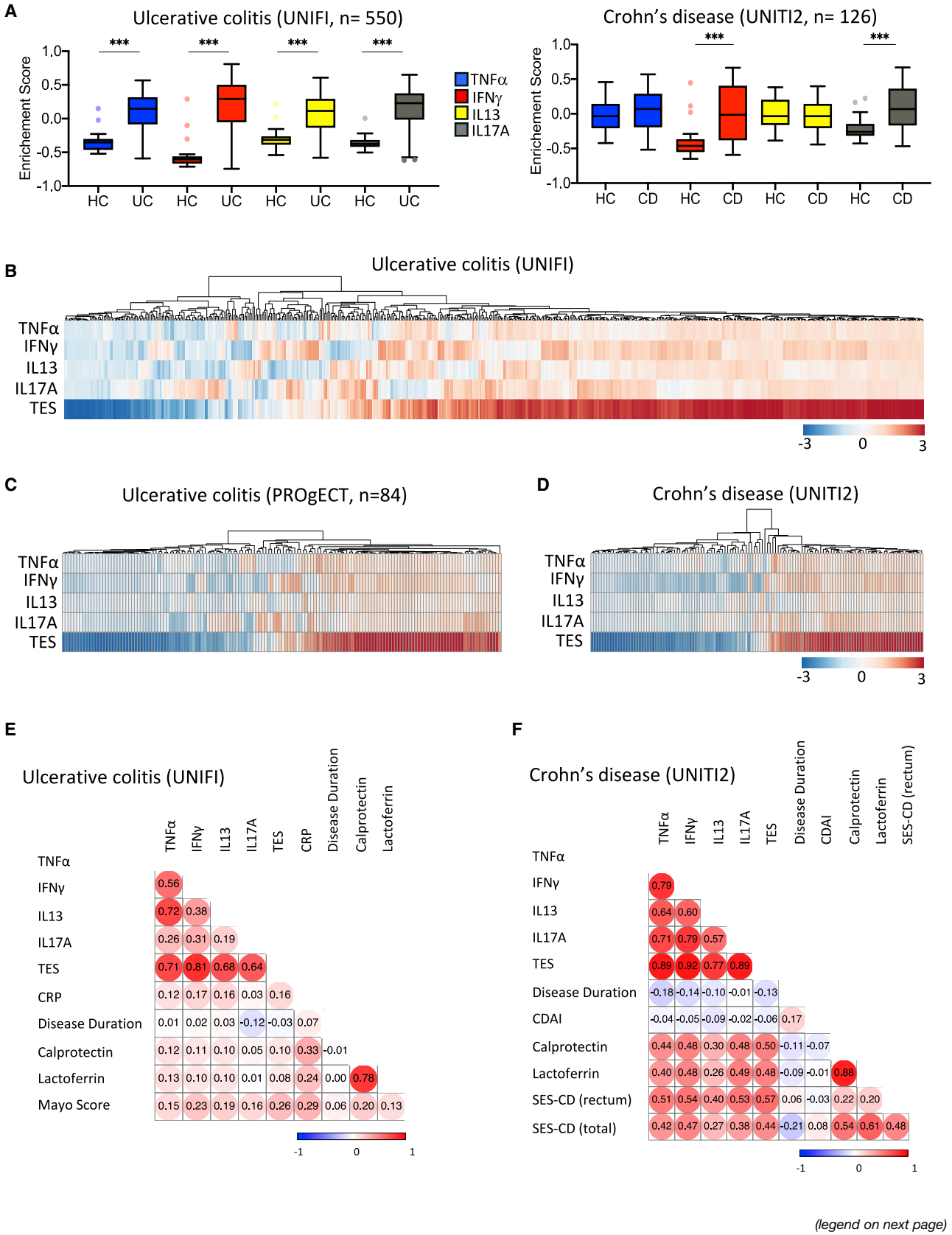
(A) Human colonoids were generated to investigate the immune-epithelial interactome. Crypts were isolated from colonic biopsies taken from four healthy adults undergoing routine colonoscopy, and colonoids were generated and cultured for 24 h with different cytokines. RNA was extracted and transcriptomic profiling performed using next-generation sequencing.

(B) Volcano plots of differentially expressed genes (DEGs) for each canonical cytokine.

(C) Top upregulated transcripts by canonical cytokines in human colonoids (n = 4, biological replicates for TNF-α, IFNγ, and IL-17A and n = 3 for IL-9 and IL-13). The top 15 most highly expressed genes are depicted for each cytokine (log2 fold change).

(D) Overlap of DEGs across canonical cytokine transcriptional programs.

(E) Regulation of key immunological and metabolic pathways by canonical cytokines in human colonoids.



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enrichment of particular cytokine-responsive transcriptional signatures in the colon of patients with UC (Figure 2B). Unexpectedly, instead of separating into immunophenotypes based on predominance of individual pathways, patients differentiated according to an overall gradient of enrichment of cytokine-responsive transcriptional signatures. Some patients had simultaneous activation of all cytokine-responsive transcriptional signatures, whereas others had relatively minimal activation. This gradient could be quantified in individual patients by calculating a total enrichment score (TES), combining the enrichment score for all four cytokines (Figure 2B). These findings were replicated in additional independent cohorts of patients with UC (PROgeCT, GEO: GSE109142 and GSE16879) (Figures 2C, S3B, and S3C). while a similar picture also emerged in cCD (UNITI2: Figure 2D; GEO: GSE16879: Figure S3D).

Considering the molecular overlap across cytokine-regulated programs, we decided to replicate this analysis in UNIFI and UNITI2 by using as signatures the unique, upregulated features of each cytokine-regulated transcriptional program. Reassuringly, a similar pattern was observed, and both patients with UC and cCD separated according to a gradient of cytokine-responsive transcripts (Figure S4), providing further confidence that our observation is associated with the simultaneous activation of multiple cytokine-regulated pathways rather than a core response common to all four cytokines.

We extensively tested the relationship of individual cytokine-responsive transcriptional signatures, and the combined TES, with disease characteristics, including disease activity and severity indices (Figures 2E and 2F). Importantly, our UC cohort of 550 patients was homogeneous for key objective measures of disease activity, such as endoscopic severity of disease (all patients had a Mayo endoscopy subscore of 2 or 3). In keeping with the existence of a gradient of cytokine-responsive transcriptional signatures in the population, there was significant correlation in the magnitude of enrichment of individual cytokines with other cytokines. However, there was only weak correlation with any of the cytokine enrichment scores, or the TES with any of the clinical features, including total Mayo score, CRP, or disease duration (Figure 2E). Similar findings were observed in a large publicly accessible dataset with no clear association between cytokine enrichment scores and biomarkers of disease activity/severity, including fecal calprotectin (fCAL) and histology scores (GEO: GSE109142; Figures S3E and S3F). Taken together, these data are consistent with a molecular stratification of colonic IBD based on a gradient of enrichment of immune response transcripts.

Harnessing cytokine-responsive transcriptional signatures as a precision medicine tool to predict response to IBD therapies

We then investigated whether this molecular stratification of patients with active colitis might herald differences in patient outcomes, as we considered the possibility that simultaneous activation of multiple immune response pathways in individual patients could potentially explain resistance to biological therapies where individual cytokines are selectively targeted.

Associating the enrichment score prior to therapy commencement with the response seen at the end of therapy induction, we found that in patients with cCD, the TES of cytokine-responsive transcriptional signatures was significantly higher in non-responders to infliximab (anti-TNF-α) compared with responders (Figure 3A). Moreover, stratification of this cohort according to the magnitude of enrichment of cytokine-responsive transcripts in biopsies sampled at baseline predicted their subsequent response to infliximab (Figure 3B). Another way of considering molecular phenotype of tissue would be to stratify patients according to the number of canonical cytokine-responsive transcriptional signatures activated in individual patients across the cohort. Strikingly, none of the patients with CD with activation of multiple different cytokine-responsive transcriptional signatures (3–4 pathways) responded to infliximab, whereas all of the patients responded in whom there was minimal activation of cytokine signatures (none of cytokine-responsive pathways achieving the threshold for activation; Figure 3C). The receiver operator characteristic curve (ROC) for TES demonstrated an area under the curve of 95%, with 95% confidence intervals (CIs) of 86 and 100 ($p = 0.0013$).

A similar picture emerged when we analyzed responses to ustekinumab. In baseline biopsies, there was a highly significant difference in TES in patients with cCD who would go on to achieve mucosal healing compared with those not achieving mucosal healing (Figure 3E). Similar to our observations with infliximab, patients with a high TES, or with activation of multiple cytokine-responsive pathways, were very unlikely to respond to subsequent treatment with ustekinumab, whereas patients with a low TES, or with one or fewer pathways activated, had an approximately 4-fold increased response rate (Figures 3F and 3G). The ROC for TES demonstrated an area under the curve of 78%, with 95% CI(61,93), $p = 0.0107$).

Molecular profiling based on the magnitude of enrichment of cytokine-responsive transcriptional signatures in colonic tissue of patients with active UC could also predict patient trajectories, although the predictive power was less differentiating than in cCD. Clinical outcomes for patients with high

Figure 2. A gradient of cytokine-responsive transcriptional signatures stratifies patients with IBD into distinct molecular phenotypes

(A) Activation of cytokine-responsive transcriptional signatures in whole colonic biopsies of patients with UC (UNIFI trial, n = 550) and colonic CD (UNITI2 trial, n = 126) (demographics shown in Table S1). Enrichment of cytokine-responsive transcriptional signatures was evaluated in the transcriptome of colonic biopsies using gene set variation analysis. A score of +1 suggests that all transcripts are upregulated, while a score of −1 suggests that all transcripts are downregulated (showing median and interquartile range [IQR], Mann Whitney test, ***$p < 0.001$).

(B, C, and D) Gradient of cytokine-responsive transcriptional signature activation in IBD. Each column represents a single patient. The sum of all four scores per subject is also depicted as the total enrichment score (TES). Columns have been clustered by Euclidean distance (method: average, tree ordering: original, figure generated with ClustVis). Cohorts: UNITI2 (n = 126), UNIFI (UC, n = 550), and PROgECT (UC, n = 84).

(E and F) Cytokine-responsive transcriptional signature association to clinical indices of human colonic inflammation in UC (UNIFI cohort, n = 550) and cCD (UNITI2 cohort, n = 126). All clinical data were collected prospectively as part of the trials' protocol (Feagan et al., 2016; Sands et al., 2019; Telesco et al., 2018).

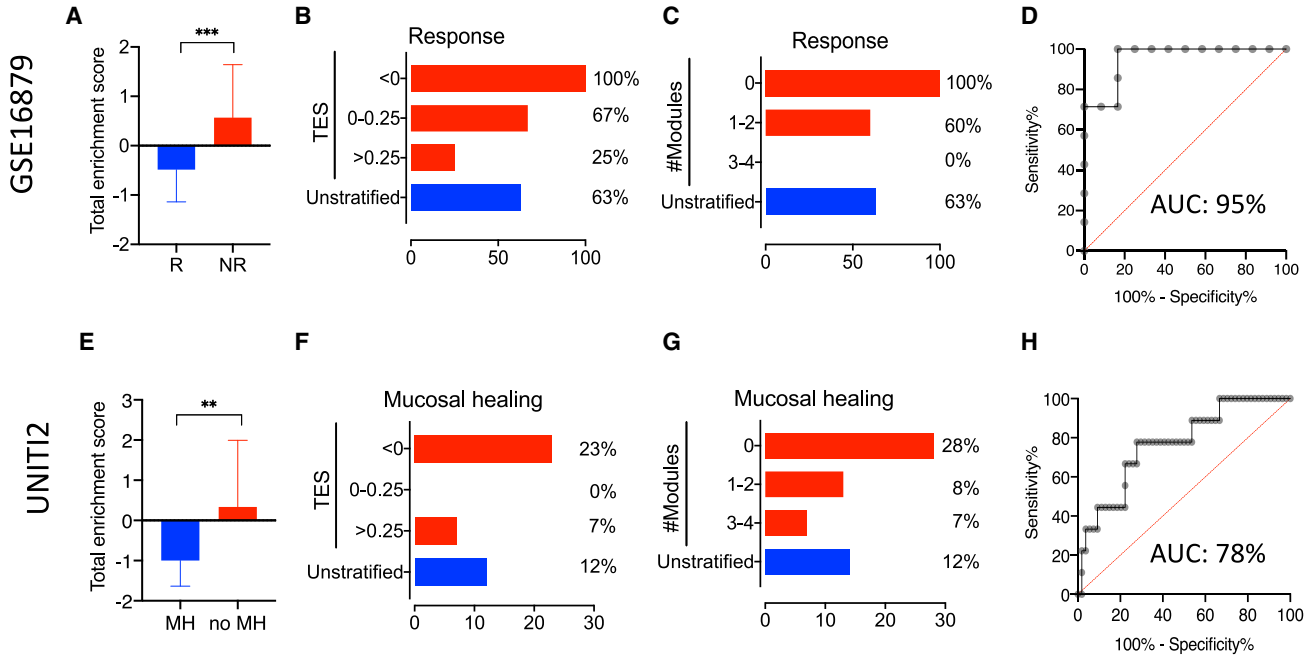


Figure 3. Harnessing cytokine-responsive transcriptional signatures as a precision medicine tool to predict response to IBD therapies

(A) Patients with colonic Crohn's disease [cCD] who do not respond (non-responders [NR]) to infliximab (anti-TNF) have higher TESs at baseline, prior to treatment commencement (Mann Whitney, ***$p < 0.001$).

(B) Response rates to infliximab in patients with cCD based on TES measured at baseline.

(C) Response rates to infliximab in patients with cCD based on the number of cytokine modules found enriched at baseline.

(D) Receiver operator characteristic curve for predicting response to infliximab based on baseline TES. Data analyzed from previously reposited dataset (GEO: GSE16879, n = 19) (Arijs et al., 2009a, 2009b). Response was defined as the composite of clinical symptoms and endoscopic improvement assessed after induction.

(E) Patients with cCD who do not respond to ustekinumab (anti-IL-23p40) have higher TES at baseline, prior to treatment commencement (Mann Whitney, **$p<0.01$).

(F) Response rates to ustekinumab in patients with cCD based on TES measured at baseline.

(G) Response rates to ustekinumab in patients with cCD based on the number of cytokine modules found enriched at baseline.

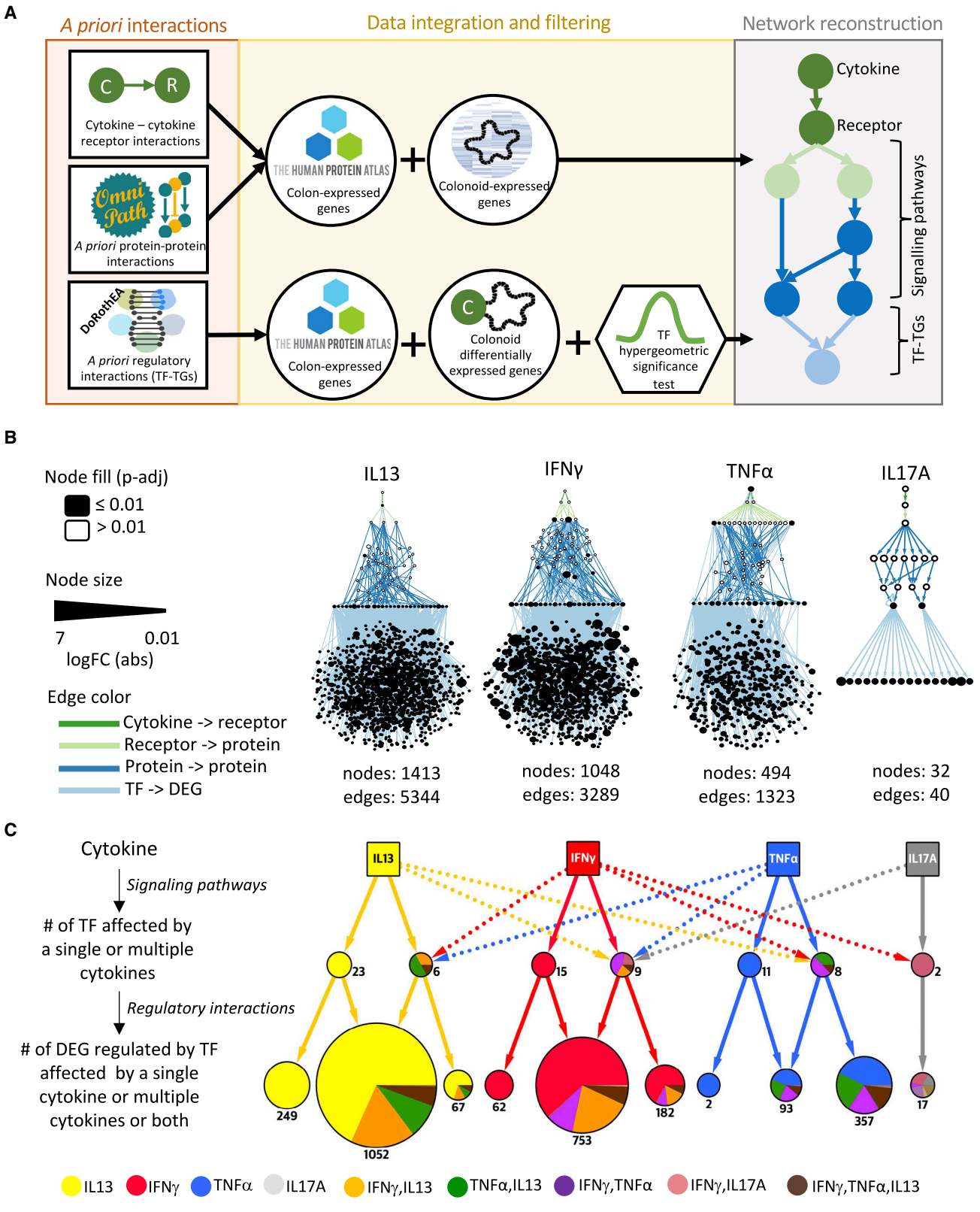
(H) Receiver operator characteristic curve for predicting response to ustekinumab based on baseline TES. Data from the UNITI2 study (n = 126) including patients who previously did not respond to immunomodulators but were naive to biological treatments (Feagan et al., 2016). Mucosal healing [MH] was defined as the complete absence of mucosal ulcerations in any ileocolonic segment among subjects who presented with ulceration in at least one ileocolonic segment at baseline.

enrichment scores and/or activation of multiple cytokine-responsive transcriptional pathways were consistently worse than outcomes of patients with low TESs or with one or fewer pathways activated following treatment with anti-TNF (infliximab, GEO: GSE16879, Figures S5A, or golimumab, PROgECT, Figure S5B) or ustekinumab (UNIFI, Figure S5C).

Taken together, our data highlight that gradients of cytokine-regulated transcriptional responses in diseased colonic tissue of patients with IBD provide a measurement of "molecular severity" of colonic inflammation, which could be harnessed to predict response to anti-cytokine therapy.

Reconstructing cytokine-responsive signaling maps in human colonoids

Considering that non-response to anti-cytokine therapies targeting a single cytokine is associated with the simultaneous activation of transcriptional pathways regulated by multiple canonical cytokines, we used a network biology approach to probe cytokine-driven signaling pathways aiming to identify shared and distinct wiring connections that may be functionally relevant. The first step in this process was to construct signaling maps for each cytokine encompassing the molecular wiring from receptor activation to the observed changes in DEG utilizing published databases containing experimentally verified molecular interactions and integrating them with the observed DEGs (Figure 4A). The generated maps contained directed and signed interactions (i.e., direction and effect for each interaction), allowing us to map the wiring and the direction of signal for each of the four cytokines to the observed transcriptional changes (Figure 4B). As expected from the DEG analysis, the IL-17A-responsive pathway was the smallest (Figure 1B; Table S3), and only two transcription factors (TFs) were predicted to significantly mediate these effects. On the other hand, the IL-13-responsive pathway was the largest network with the largest number of DEGs and corresponding TFs (Figure 4B). Only 27.8% of the molecules in the IL-13-responsive pathway were also present in one or more other cytokine pathways.



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The next step was to compare the cytokine-specific signaling maps and identify shared and distinct characteristics. We found cytokine-specific TFs for all cytokines except for IL-17A, which affects only two TFs, both also affected by IFNγ. We also found that 21%, 37.5%, and 42% of the TFs affected by IL-13, IFNγ, or TNF-α are shared, respectively, indicating a substantial overlap among the downstream regulators of these cytokines. This analysis also allowed us to identify those DEGs that are regulated by cytokine-specific TFs, shared TFs, or both (Figure 4C). This distinction is relevant to understand which TFs could interfere with the transcriptional program of another cytokine. We found that the majority of the DEGs regulated by IL-13-specific or IFNγ-specific TFs (77% and 75.5%, respectively, corresponding to 1,052 and 753 genes) can also be regulated by multiple other TFs, which are affected by TNF-α. This indicates that the majority of IL-13 and IFNγ DEGs are regulated by a combined signal of cytokine-specific and cytokine-shared TFs. Contrary to this, only the 20.6% of the DEGs regulated by TNF-α-specific TFs can also be regulated by other, shared TFs (Figure 4C).

Cytokine-responsive signaling pathways converge at key TFs

Our integrated systems biology approach identified 12 TFs being affected by more than one cytokine (Figure 5A). There were four TFs shared between the IFNγ and TNF-α maps (E2F TF [E2F1], signal transducer and activator of transcription 5A [STAT5A], CCAAT enhancer binding protein alpha [CEBPA], and thyroid hormone receptor alpha [THRA]). Three TFs were shared between IL-13 and TNF-α (sterol regulatory element binding protein 1 [SREBF1], Krüppel like factor 2 [KLF2], and proto-oncogene c-fos-FOS), two between IL-13 and IFNγ (IFN regulatory factor 1 [IRF1] and IRF8), and two between IL-17A and IFNγ (E2F2 and Myc associated zing finger protein [MAZ]). The protein C-ets-1 (ETS1) was the only TF found to be regulated by more than two cytokines (TNF-α, IFNγ, and IL-13). Only three out of the 12 TFs (ETS1, CEBPA, FOS) regulated the majority of all the DEGs in colonoids (Figure 5B), and 83% of the cytokine-specific DEGs (i.e., DEGs affected by only one cytokine) were regulated by at least one shared TF.

Translational relevance of the identified TFs in intestinal inflammation

To test the functional relevance of the 12 shared TFs identified by our integrated systems biology approach, we explored their expression in the colon of patients with and without IBD. Principal-component analysis demonstrated that patients with active colonic inflammation could be completely differentiated from control patients based on the expression of these 12 TFs (GEO: GSE16879; Figure 6A). The expression of the TF was variable in colonic tissue. *ETS1*, *IRF1*, *IRF8*, *STAT5A*, *FOS*, and *KLF2* were mostly upregulated, whereas *SREBF1*, *THRA*, *E2F2*, *E2F1*, *CEBPA*, and *MAZ* were mostly downregulated, in colonic mucosa of patients with IBD (Figure S6). Unsupervised hierarchical clustering demonstrated that the expression of these key TFs tended to cluster with the expression of the canonical cytokines TNF-α, IFNγ, IL-17A, and their receptors (Figure S6).

Since simultaneous enrichment of multiple cytokine-regulated transcriptional programs predicted poor response to anti-cytokine therapies in IBD, we tested the hypothesis that the key signaling bottlenecks predicted by our systems biology experiment would also influence response. As proof of principle, we performed a multivariate logistic regression using ETS1, FOS, and CEBPA in a model to predict response to anti-TNF in IBD. This analysis identified ETS1 as the single predictive TF (odds ratio: 0.09, 95% CI(0.01,0.47), p = 0.01) (Table S4). Non-responders had significantly higher expression of *ETS1* prior to commencement of infliximab (Figure 6B), with an area under the curve (AUC)-predicting response of 0.82, 95%CI(0.69, 0.96), p = 0.0003 (Figure 6C). In agreement with our proposed signaling model, patients with the highest expression of *ETS1* had a higher expression of TNF-α and IFNγ compared with patients with low ETS1 expression (Figure 6D), consistent with the notion of increased induction of these core transcriptional regulators when multiple cytokine pathways are activated.

While the higher expression of ETS1 is associated with the activation of its upstream upregulating cytokines in inflamed colonic tissue, the wiring of the signal is not fully shared. Based on our integrated analysis in colonoids, TNF-α and IFNγ regulate ETS1 expression via separate routes (Figure 6E). Also, the downstream transcriptional program regulated by ETS1, defined based on ETS1-DEG connections, is not shared fully across the cytokines, with only 82 DEGs being common across the TNF-α and IFNγ signaling maps (Figure 6F). Nevertheless, upregulation of ETS1 by either of these two cytokines leads to the activation of key pathways associated with IBD pathogenesis and non-response to anti-TNF therapy (Figure 6G). These observations reinforce further our observation that activation of multiple canonical cytokine-regulated pathways drive resistance to therapy by shared and unique pathways. Expression of *ETS1* predicted response to ustekinumab only weakly (AUC: 0.5838, 95% CI(0.51, 0.66), p = 0.03).

DISCUSSION

This is a comprehensive analysis of the transcriptomic landscape of the cytokine-mediated immune-epithelial interactome, mapping how canonical cytokines regulate shared and distinct patterns of gene expression in the human colonic

Figure 4. Reconstructing cytokine-responsive signaling maps in human colonoids using a systems biology approach

(A) Workflow of causal network reconstruction for each cytokine. Three *a priori* molecular interaction collections are used: cytokine interactions with membrane receptors were manually curated, protein-protein interactions (PPIs; for signaling pathways) were obtained from OmniPath, and transcription factor-target gene interactions were obtained from DoRothEA. All interactions were filtered to include only protein-coding genes expressed in the colon based on the Human Protein Atlas dataset. TF, transcription factor; TF-TG, transcription factor-target gene interactions.

(B) Causal network maps of cytokine-specific signaling and regulatory interactions. Arrows represent direction of signal transduction. Although some nodes are present in multiple levels of the network, they are only displayed once in first occurring level (nearest to the cytokine) (adjusted p value [p adj] < 0.01).

(C) Shared and distinct characteristics of cytokine causal networks.

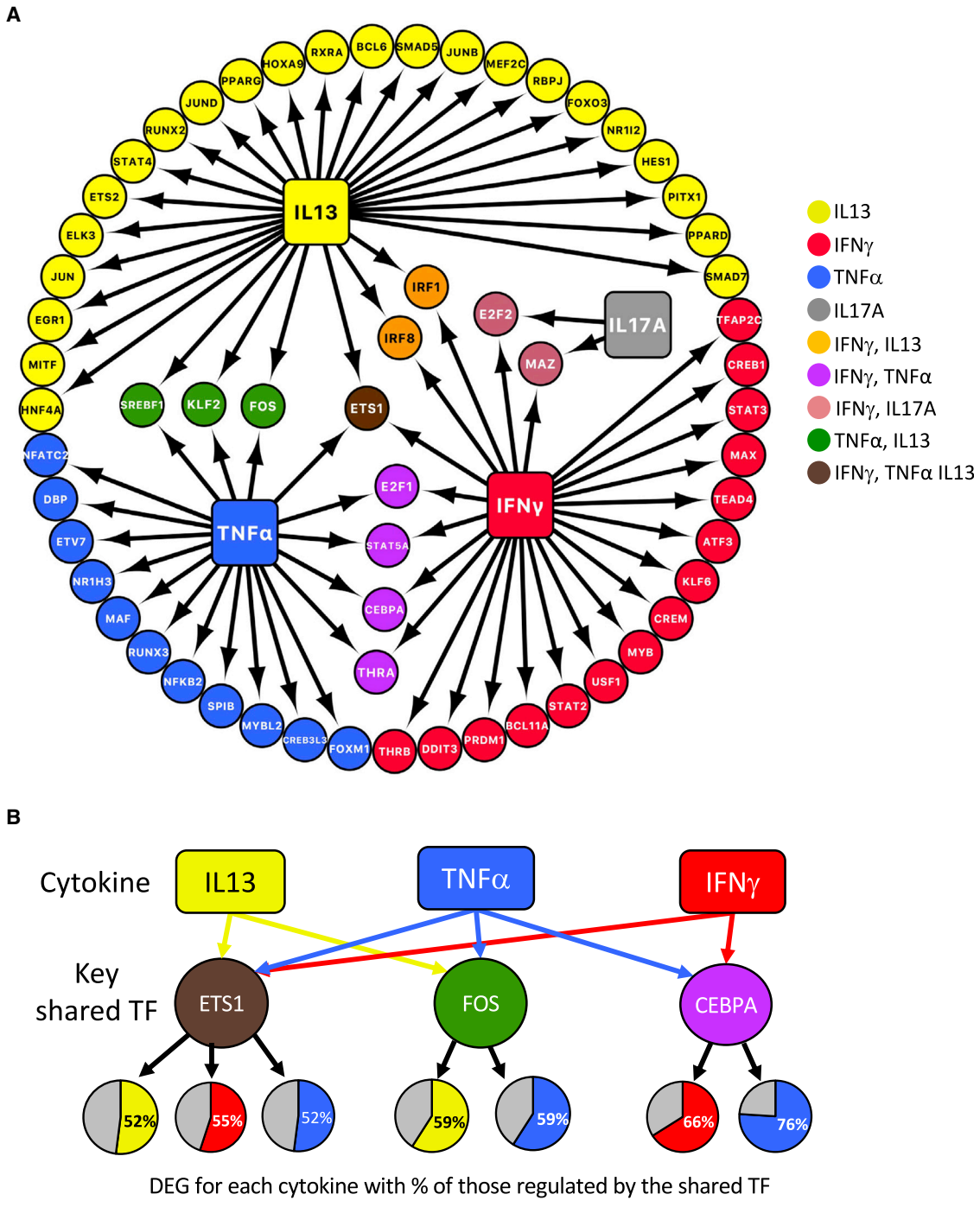

Figure 5. Cytokine-responsive signaling pathways converge at key transcription factors

(A) Unique and complimentary pathways of transcription factor activation in cytokine causal networks. Magnification of the cytokine-regulated causal networks showcasing the signaling route from receptor engagement to transcription factor in human colonoids.

(B) ETS1, FOS, and CEBPA are the three transcription factors (TFs) regulating the majority of DEGs in canonical cytokine causal networks.

epithelium. We show that a molecular stratification of colonic inflammation based on gradients of enrichment of cytokine-responsive modules can be harnessed as a precision medicine tool to predict patient trajectories in IBD, including differentiating patients according to their likelihood of responding to anti-cytokine therapies. An integrated systems biology approach allowed us to visualize the complex network of cytokine responsive signaling and identify key converging TFs,

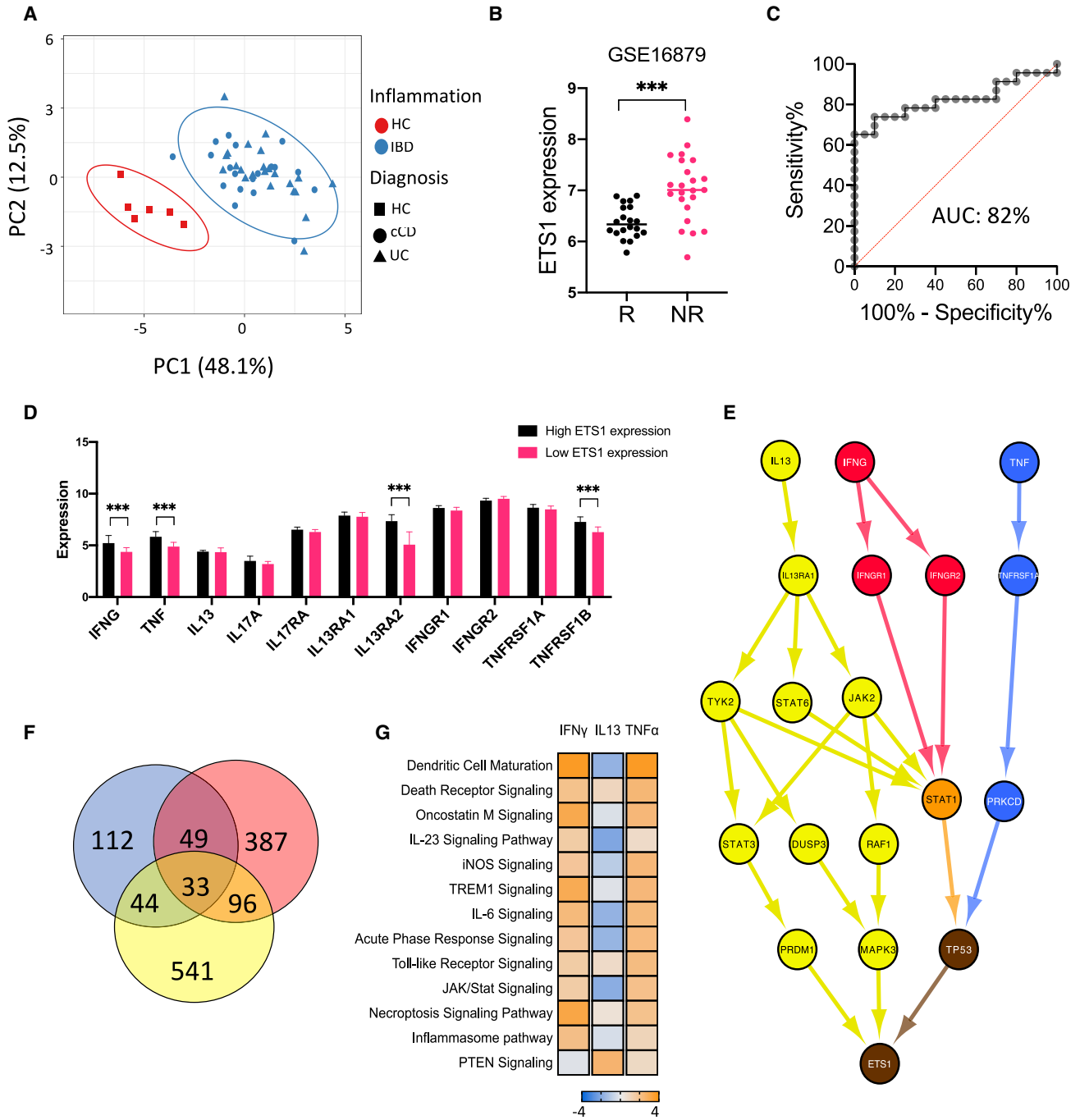

Figure 6. ETS1, a key regulator of the cytokine signaling superhighway in intestinal inflammation

(A) ETS1, CEBPA, and FOS separate perfectly inflamed and non-inflamed colonic tissue. Principal-component analysis of transcriptomic profiles of patients with IBD and controls from a previously published, reposited dataset including healthy controls [HC], patients with colonic Crohn's disease [cCD] and ulcerative colitis [UC] (GEO: GSE16879, n = 49) (Arijs et al., 2009a, 2009b).

(B) ETS1 predicts non-response to anti-TNF in IBD. Expression measured in biopsies of patients with cCD and ulcerative colitis prior to commencing infliximab (GEO: GSE16879, n = 43, Mann Whitney, ***p<0.001).

(C) Receiver operator characteristic curve for predicting response to infliximab induction in IBD based on ETS1 baseline expression in colonic biopsy (GEO: GSE16879, n = 43).

(D) Expression of canonical cytokines and their receptors in patients stratified based on ETS1 expression. High ETS1 expression: high quartile; low ETS1 expression: low quartile (GEO: GSE16879, n = 43,2way ANOVA with Sidak's multiple comparison test, ***adjusted p<0.001).

(legend continued on next page)

which mediate downstream effects and shape outcomes of clinical relevance.

Treatment of colonoids with canonical cytokines allowed us to compare the transcriptional response of cytokines in human colonic epithelial cells. The pathways associated with the cytokine specific transcriptional responses unveil marked similarities in the biological effects of IFNγ and TNF-α and the potentially contrasting effect of IL-13. Conversely, IL-9 showed almost no impact on the colonoids, suggesting that it does not significantly affect epithelial cells at the given concentration or in the absence of prior inflammatory insult (Gerlach et al., 2014).

Epithelial cytokine responses allowed us to quantify the activation of these responses in both epithelial layers and whole biopsies of patients with IBD. We demonstrated this using multiple datasets across several cohorts of patients with IBD and observed simultaneous enrichment of multiple cytokine pathways in a substantial number of patients. On the one hand, these observations potentially challenge current T cell lineage identity paradigms, cast doubt on the notion that individual patients can be separated into immunophenotypes based on expansion of particular effector responses, and suggest that dividing CD and UC in to Th1 and Th2 diseases is an oversimplification (Fuss et al., 1996; Heller et al., 2005; Monteleone et al., 1997). Although Th17 cell enrichment in IBD has expanded this paradigm (Annunziato et al., 2007), it is now recognized that ''polyfunctional'' T cell responses are observed in inflammatory diseases, where individual T cells co-produce multiple cytokines, including IFNγ, IL-17A, and TNF-α (Harrington et al., 2005; Kobayashi et al., 2008; Langrish et al., 2005; Park et al., 2005).

Multiple cytokine targeting biological therapies have been tested over the last 20 years in IBD, including anti-TNF-α (Rutgeerts et al., 2005; Targan et al., 1997), anti-IL-13 (Reinisch et al., 2015), anti-IFNγ (Hommes et al., 2006), anti-IL-17A (Hueber et al., 2012), and, more recently, the anti-IL-12/IL-23 (IL-12p40) monoclonal antibody ustekinumab (Feagan et al., 2016; Sands et al., 2019). Our findings illustrate that simultaneous activation of multiple cytokine-regulated pathways and the observed overlap and complementarity in downstream effector pathways regulated in intestinal epithelial cells may well explain the success or failure of some of these anti-cytokine approaches. Our data support the hypothesis that patients can be stratified according to the molecular complexity of inflammatory circuits in diseased tissue. Even in patients with comparable degrees of macroscopic colonic inflammation, or clinical severity of disease, we could differentiate patients according to gradients of cytokine-responsive transcript enrichment, which could in turn predict how patients would subsequently respond to anti-cytokine therapy.

To further probe similarities and differences between cytokine responses, we employed an integrative systems biology approach to reconstruct causal networks. These highlighted the complementarity and redundancy in immune-epithelial responses. Furthermore, network comparisons identified three TFs driving the majority of the regulatory interactions shared between multiple networks, forming potential molecular bottlenecks. Unsurprisingly, all of these TF have documented associations with immunomodulation, with many of them known to regulate and/or be regulated by cytokines or IBD. For example, ETS1 appears to play a role in cytokine and chemokine gene regulation in lymphoid and endothelial cells (Russell and Garrett-Sinha, 2010) and has been previously identified as an IBD susceptibility locus (de Lange et al., 2017; Ellinghaus et al., 2016; Reshef et al., 2018). A genetic region involving the CEBPA gene was identified as susceptibility locus for early onset IBD (Imielinski et al., 2009). Both TFs appear to contribute to enterocyte differentiation and may be associated with clinical phenotypes of IBD (Treveil et al., 2020). The TF FOS has been shown to play an integral part in the regulation of an inflammatory gene module in macrophages (Najjar et al., 2016).

Cross validating our systems biology approach with independent, whole-tissue transcriptomic data from IBD cohorts, we identified *ETS1* as a key regulator of immune-epithelial interactions. Corroborating the observation that ETS1 expression receives signals from different canonical cytokines with known pathogenic roles in IBD (IFNγ, TNF-α). We also found that higher expression of ETS1 at the tissue level was associated with non-response to infliximab. The simultaneous activation of an upstream regulator (such as IFNγ) that is not targeted by anti-TNF treatment, which regulatei, similar pathogenic pathways with TNF-α, via shared transcription factor induction (e.g. ETS1) provides further evidence that single anti-cytokine treatment approaches may not be sufficient in this context. The weaker performance of ETS1 expression to predict response to ustekinumab is not surprising, as IL-23 and IL-12 are key upstream regulators for both TNF-α and IFNγ production (Langrish et al., 2005). It is possible that ETS1 may play a more complex role in treatment of refractory disease. For example, a higher expression of *ETS1* was associated with higher expression of the TNF receptor as well as TNF. Further mechanistic work can shed more light on the role of this TF in mediating cytokine responses in the epithelium.

Our study has strengths and potential limitations. While we interrogated multiple, large, and independent IBD cohorts with rich phenotypic data of high quality, the cytokine signatures were generated utilizing organoids developed from four adult donors. In addition, these signatures may not be specific for the epithelial compartment but could represent cytokine responses in other cells found in the human colonic mucosa. Reassuringly, the enrichment pattern seen in whole biopsies was also found in colonic epithelial layers isolated from patients with IBD, providing confidence to our findings. If the described cytokine-regulated changes were shared with non-epithelial cells, this would not necessarily represent a limitation of this study. On the contrary, it would suggest that epithelial organoids are well placed to model an individual's immune responses. Afterall, the intestinal epithelium is not a bystander in IBD pathogenesis but is likely an immunologically active participant (Hampe et al., 2007; Kaser et al., 2008; Wehkamp et al., 2005).

(E) Complimentary pathways leading to ETS1 regulation in canonical cytokine causal networks.
(F) Shared and unique features in the ETS1-regulated transcriptional program between canonical cytokines.
(G) Pathway analysis of the ETS1 transcriptional program in canonical cytokines.

Importantly, our observation that patients can be stratified based on a gradient of cytokine program enrichment held true for two complimentary definitions used, a more ''functional'' one based on top upregulated transcripts and a more ''statistical'' one based on unique features. This provides additional reassurance that the proposed stratification does not only reflect the burden of inflammation but also the magnitude of activation of cytokine regulated programs. Complimenting these insights, our systems biology approach provides an additional layer of information on key signaling bottlenecks, which are then validated *in silico* in independent cohorts. The value of such an approach is evident by the identification of ETS1 as a potential biomarker of infliximab non-response, a finding which was not borne out previously using traditional bioinformatic approaches dependent only on DEG analyses (Arijs et al., 2009a). In this study, we focused on possible paths between cytokine receptors and DEGs considering the expression of path members. Potential additional statistical assessment can also be applied, such as the significant shortest path approach (Pepe, 2020), to identify known signaling pathways affected by a cytokine. Instead of looking at *a priori* pathways, we reconstructed possible shortest (i.e., most probable) paths to capture the differences of how each cytokine is affecting the measured DEG.

In conclusion, in this study, we mapped the transcriptional landscapes shaped by canonical cytokines in human colonic epithelial cells and unveiled a more granular view of epithelial responses in chronic intestinal inflammation. We showcase the potential of this resource as a precision medicine tool that can be harnessed to identify functionally important, therapeutically targetable pathogenic pathways.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Human subjects
- METHOD DETAILS
  - Human colonoid culture
  - RNA extraction
  - Library preparation and sequencing
  - Gene expression quantification and differential expression analysis
  - Reconstruction of cytokine-specific causal networks
  - Functional analysis
  - Gene set variation analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2022.111439.

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AUTHOR CONTRIBUTIONS

P.P. conceptualized the study, designed experiments, analyzed data, and wrote the manuscript; A.T. performed experiments and contributed to manuscript preparation; A.T., K.L., D.C., F.Y., U.N., M.S., J.F., T.K., and G.B. performed analysis and contributed to manuscript preparation; organoid analysis was performed in the G.B. lab; systems biology analysis was performed in the T.K. lab; G.B., M.S., K.L., J.F., and T.K. provided scientific and analytical input; N.P. conceptualized the study, designed experiments, supervised the project, analyzed data, and wrote the manuscript.

DECLARATION OF INTERESTS

K.L., F.Y., and J.F. are/were employees of Janssen Pharmaceuticals.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human intestinal tissue for colonoids	King's College Hospital	NRES/IRAS id: 15/LO/1998
Human intestinal biopsies for whole transcriptome profiling (microarray)	Janssen Pharmaceuticals	UNITI2, NCT01369342 UNIFI, NCT02407236 PROgECT, NCT01988961
Chemicals, peptides, and recombinant proteins		
Advanced DMEM/F-12	Gibco	12634010
GlutaMAX	Gibco	11574466
HEPES	Gibco	15630049
Penicillin/Streptomycin	Sigma-Aldrich	P4333
Wnt3a conditioned medium	In-house production	-
R-spondin 1 conditioned medium	In-house production	-
Recombinant murine Noggin	Peprotech	250-38-100
B27 Supplement	Gibco	17504044
N2 Supplement	Gibco	17502048
N-Acetylcysteine	Sigma-Aldrich	A9165
EGF Recombinant Mouse Protein	Gibco	PMG8041
Nicotinamide	Sigma-Aldrich	N0636
Gastrin	Sigma-Aldrich	G9145
A 83-01	Bio-Techne	2939
SB-202190	Sigma-Aldrich	S7067
Y-27632	Sigma-Aldrich	Y0503
CHIR99021	Sigma-Aldrich	SML1046
EDTA	Invitrogen	15575-038
Matrigel	Corning	356231
Cell Recovery Solution	Corning	354253
RNase-Free DNase Set	Qiagen	79254
Recombinant human IL17A	Bio-Techne	7955-IL
Recombinant human TNFα	Bio-Techne	210-TA
Recombinant human IFNγ	Bio-Techne	285-IF
Recombinant human IL13	Bio-Techne	213-ILB
Recombinant human IL9	Bio-Techne	209-ILB
Critical commercial assays		
RNeasy Mini Kit	Qiagen	74104
Qubit RNA BR assay kit	Thermo Fisher Scientific	Q10210
Bioanalyzer RNA 6000 Nano Kit	Agilent	5067-1511
NEBNext® UltraTM RNA Library Prep Kit for Illumina®	New England Biolabs	E7530L
HG U133 PM array	Affymetrix	N/A
Deposited data		
Raw sequencing data (from cytokine-treated human colonoids	This study	GSE190634 GSE190705
UNITI 2 study	N/A	GSE207022
UNIFI study	N/A	GSE206285
PROgECT	N/A	GSE212849

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
GSE16879	Ref (Arijs et al., 2009a, 2009b)	GSE168789
GSE109142	Ref (Haberman et al., 2018)	GSE109142
Experimental models: Cell lines		
HEK293T-HA-Rspol-Fc cell line	Dr Calvin Kuo	N/A
L-Wnt3A cell line	Dr Hans Clever	N/A
Human colonic organoids	This study	N/A
Software and algorithms		
TopHat (v2.0.12)	Ref (Kim et al., 2013)	http://ccb.jhu.edu/software/tophat/index.shtml
HTSeq (v0.6.1)	Ref (Anders et al., 2015)	https://htseq.readthedocs.io/en/master/
QIAGEN Ingenuity Pathway Analysis (IPA)	Qiagen (Kramer et al., 2014)	N/A
DoRothEA v2	Ref (Garcia-Alonso et al., 2019)	N/A
R	Ref (Team, 2013)	N/A
Stan	Ref (Carpenter et al., 2017)	https://bioconductor.org/packages/release/bioc/html/STAN.html
Cytoscape (v3.7.1)	Ref (Shannon et al., 2003)	https://cytoscape.org/
R package ReactomePA	Ref (Jassal et al., 2019; Yu and He, 2016)	https://bioconductor.org/packages/release/bioc/html/ReactomePA.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Nick Powell (npowell@ic.ac.uk). Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jane Doe (janedoe@qwerty.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All sequencing data of this study are available at the Gene Expression Omnibus (GEO).

- RNA-seq and microarray data have been deposited at Gene Expression Omnibus (GEO) and are publicly available as of the date of publication. Accession numbers are listed in the key resources table. This paper also analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human subjects

Human colonic crypts for colonoid generation, were isolated from serial colonic biopsies (x2 ascending colon, x2 transverse, x2 descending, x2 rectosigmoid) taken from six adult individuals (median age: 48, range [33,67], female:3), without past medical history or regular medication who attended for routine colonoscopy in view of abdominal symptoms without a diagnosis of IBD and did not have macroscopic or microscopic evidence of inflammation. All patients provided informed consent (NRES/IRAS id: 15/LO/1998).

The UNITI 2 and UNIFI trials were randomized placebo-controlled phase 3 clinical trials evaluating the efficacy and safety of ustekinumab in antiTNF naive Crohn's disease patients (UNITI2, NCT01369342) and ulcerative colitis (UNIFI, NCT02407236) (Sands et al., 2019). Colonic biopsies were sampled at defined time points prior to treatment and were immediately transferred to RNALater (Qiagen) and stored at −80°C prior to RNA extraction. Whole genome transcriptomics were performed on the Affymetrix HG U133 PM array. Clinical data was recorded prospectively according to the trial protocol. In UNITI2 mucosal healing was defined as the complete absence of mucosal ulcerations in any ileocolonic segment among subjects who presented with ulceration in at least one ileocolonic segment at baseline. In UNIFI mucosal healing was defined as by lack of ulceration (Mayo 0 or 1) and histological criteria (defined as neutrophil infiltration in <5% of crypts, no crypt destruction, and no erosions, ulcerations, or granulation tissue).

Gene expression or enrichment data presented is based on all patients receiving ustekinumab regardless of dose (130 mg and 6 mg/kg) or placebo. Receiver operator curve analysis was performed in those patients treated with ustekinumab only with available outcome data.

The PROgECT study (NCT01988961) was a biomarker discovery study testing the value of a pre-defined gene set in predicting response to golimumab therapy in patients with UC (Telesco et al., 2018). Mucosal healing was defined as lack of ulceration (Mayo 0 or 1) and histological criteria (a grade of 0 or 1 on the Geboes histological scale).

In addition, the following reposited datasets of IBD cohorts were analysed: GSE168789 (Arijs et al., 2009a, 2009b) and GSE109142 (Haberman et al., 2018).

METHOD DETAILS

Human colonoid culture

Establishment of human colonoids was performed as previously described by Fujii et al.(Fujii et al., 2015). The crypts were cultured in growth medium containing advanced Dulbecco's modified Eagle's medium/F12, penicillin/streptomycin (100 units/mL), 10 mM HEPES, 2 mM Glutamax, supplements N2 (1x) and B27 (1x), 50 ng/mL mouse epidermal growth factor (all from Life Gibco), 1 mM N-acetylcysteine (Sigma-Aldrich), 50% v/v Wnt3a conditioned medium, 10% v/v R-spondin-1 conditioned medium, 100 ng/mL murine recombinant noggin protein (Peprotech),10 nM gastrin (Sigma-Aldrich), 500 nM A83-01 (Bio-techne), 10 μM SB202190 (Sigma-Aldrich) and 10 mM Nicotinamide (Sigma-Aldrich). 10 μM Y-27632 (Sigma-Aldrich) was added to the culture medium for the initial 3 days. Medium was changed every 2 days. Differentiation towards a mature epithelium in human colonoids was achieved with reduction of Wnt3a to 15% v/v and withdraw of SB202190 and nicotinamide for 5–7 days. During the last 24 h in differentiation medium human colonoids were treated with human recombinant IL17A (50 ng/mL), TNFα (10 ng/mL), IFNγ (20 ng/mL), IL13 (10 ng/mL) and IL9 (10 ng/mL). Cytokine concentrations were determined based on previous in house experience and published literature.

RNA extraction

Total RNA was isolated from human colonoids (released from Matrigel with Cell Recovery solution (Corning)) using RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. On-column DNase digestion was performed for removing any residual genomic DNA (Qiagen). RNA was quantified using Qubit 2.0 Fluorometer in combination with Qubit RNA BR assay kit (Thermo Fisher Scientific) and quality checked using the Bioanalyzer RNA 6000 Nano Kit (Agilent). Bioanalyzer analysis revealed excellent quality for RNA extracted from colonoids (RIN score>9).

Library preparation and sequencing

Quality control, mRNA enrichment and cDNA library preparation were performed by the Novogene Co. Ltd. All RNA sequencing libraries were constructed by using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB) according to the manufacturer's instructions and sequenced on an Illumina HiSeq platform.

Gene expression quantification and differential expression analysis

Fastq files were firstly processed with in-house Perl scripts to discard reads with adaptor contamination, or at least 10% of uncertain bases (N), or at least 50% of nucleotides with a Phred quality score less than 20. Read pairs were aligned to the human genome (GRCh37/hg19) using TopHat v2.0.12 (Kim et al., 2013). HTSeq v0.6.1 was used to count the read pairs mapped uniquely and concordantly to each gene (Anders et al., 2015). The raw count matrix was screened for genes with low expression levels across all samples (i.e. average count less than 3), and then with an average number of read pairs less than 3 were filtered out normalized following the strategy suggested by Anders et al.(Anders et al., 2015).

Exploratory analysis (EDA) of the normalized data matrix involved plotting low dimensional summaries of each sample, including mean, variance, number of missing data points, principal components and hierarchical clustering plots. The aim of this EDA is to identify potential outliers, assess balance and overlap with respect to pre-treatment variables (covariates) and identify clustering in the data that is not accounted for by the experimental design (batch effects) - in order to control for it at the modelling level.

The unit of analysis (response variable) is the expression count for each gene which is modelled as a negative binomial variable. In addition to the treatment variable, we account for the structure in the data with covariates that include: repeated measurements from subjects, and clustering detected in the data on EDA. We use a varying intercepts hierarchical modelling framework to model the expression for each gene using the model described in the figure/equation (Gelman et al., 2013; Kruschke, 2010; Team, 2013) implemented in R (Team, 2013) and Stan (Carpenter et al., 2017). Model fit checks were performed using posterior predictive simulations to compare replicated datasets to the actual data.

$$\mu_i = \beta_0 + \beta_{t[i]} + \beta_{p[i]} + \beta_{c[i]}$$
$$y_i \sim \textit{Negative Binomial}(\exp(\mu_i), \varphi)$$
$$\beta_{[t]} \sim \textit{Normal}(0, \sigma_1)$$
$$\beta_{[p]} \sim \textit{Normal}(0, \sigma_2)$$
$$\beta_{[c]} \sim \textit{Normal}(0, \sigma_3)$$

where:

t = number of groups in Treatment
p = number of subjects
c = number of clusters
i = number of observations
y = gene expression count
φ = overdispersion parameter
σ_1 = between Treatment standard deviation
σ_2 = between subject standard deviation
σ_3 = between cluster standard deviation

Gene counts were modelled as a negative binomial variable dependent on cytokine treatment as well as covariates accounting for repeated measurements from the same donor and additional sample similarities detected by PCA and hierarchical clustering. The quality of the estimated statistical model was assessed through posterior predictive simulations that compare replicated datasets to the actual data. The output p-values were corrected for multiple testing with the Benjamini and Hochberg method (Benjamini et al., 2001). Pathway analysis of DEG lists was performed with Ingenuity Pathway Analysis (IPA, Qiagen) (Kramer et al., 2014).

Reconstruction of cytokine-specific causal networks

Cytokine-specific causal networks were generated for each cytokine using the organoid transcriptomics data by custom Python scripts. The networks consist of four separate levels. The cytokine and receptor levels were constructed using manually curated information on published cytokine receptors. To better represent the known biology of IL13 signaling, connections between IL13RA1 and IL2RG or IL4R were removed. Whilst these proteins do form complexes together, the downstream signaling from IL2RG and IL4R is not initiated by IL13 binding (Hershey, 2003). The signaling level of the networks connects cytokine receptors to transcription factors through the shortest possible paths of protein-protein interactions (PPIs). All paths consist of three or less intermediary signaling mediators, making a total of six or less steps between the cytokine and the differentially expressed genes. All proteins of the signaling level, including the cytokine receptors, are expressed in the organoid transcriptomics data for the relevant cytokine (FPKM >0 in at least 2 replicates) and in the colon dataset from the Human Protein Atlas (Uhlen et al., 2015). Signaling connections between the proteins were obtained from the OmniPath database (v0.7.111) (Turei et al., 2016). The transcriptional regulation level of the networks was generated by identifying and filtering TF known to regulate the differentially expressed genes previously detected (FDR < 0.01). TF – target interactions with confidence levels in the range from A to D were obtained from DoRothEA v2 (Garcia-Alonso et al., 2019). All differentially expressed genes and TFs are expressed in the colon dataset from the Human Protein Atlas. In addition, to reduce the size of the network and to focus on the most important regulatory interactions, TF were filtered using two further criteria. Firstly, only TF exhibiting differential expression (FDR < 0.01) were included. Secondly, we filtered TF for their influence in the network by carrying out a hypergeometric significance test on any TF with degree $\geq$ 5 to determine if the proportion of connected nodes which are differentially expressed is higher than in the whole TF-TG network. Any TF with p value < 0.05 following Benjamini-Hochberg correction were deemed significant. ID conversion was carried out using Uniprot Swissprot to Ensembl id (GRCh38.p12) conversion, downloaded from Biomart on 11/4/19 (Smedley et al., 2015). All networks were visualized using Cytoscape (v3.7.1) (Shannon et al., 2003). Venn diagrams were generated using the Python package Venn (v0.1.3). The code used to generate the cytokine-specific causal networks has been deposited on GitHub (https://github.com/korcsmarosgroup/colonoid_cytokines, https://doi.org/10.5281/zenodo.5714816).

Functional analysis

Functional enrichment of gene lists was carried out against Reactome pathways using the R package ReactomePA (Jassal et al., 2019). Pathways were considered significantly affected when FDR < 0.1. A separate pathway analysis using the Ingenuity database was also performed that allowed the calculation of z-scores providing information on the directionality and magnitude of pathway activation.

Gene set variation analysis

To test the activation of each of the cytokine regulated transcriptional programs we used gene set variation analysis (GSVA) (Haenzelmann et al., 2013) to probe whole transcriptional profiles of previously reposited datasets and the dataset generated in the context of the ustekinumab and golimumab trials programs.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical details of experiments can be found in the figure legends and the results section including the sample number, information on definition of centre and dispersion and type of statistical tests used to associate or compare groups. No test to assess for a normal distribution of continuous variables was undertaken. Comparison of enrichment scores or relative expression of single features between groups was performed with non parametric tests (i.e. Mann Whitney U test for two groups or Kruskal-Wallis test for more than two groups). Non parametric correlation was used to associate continuous clinical variables with enrichment scores. Statistics and graph plotting was performed with GraphPad Prism version 8.4.3.