(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
09 May 2019 (09.05.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/087200 A1

(51) International Patent Classification:
C12Q 1/686 (2018.01) *G01N 33/53* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/IL2018/051187

(22) International Filing Date:
06 November 2018 (06.11.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/581,960 06 November 2017 (06.11.2017) US

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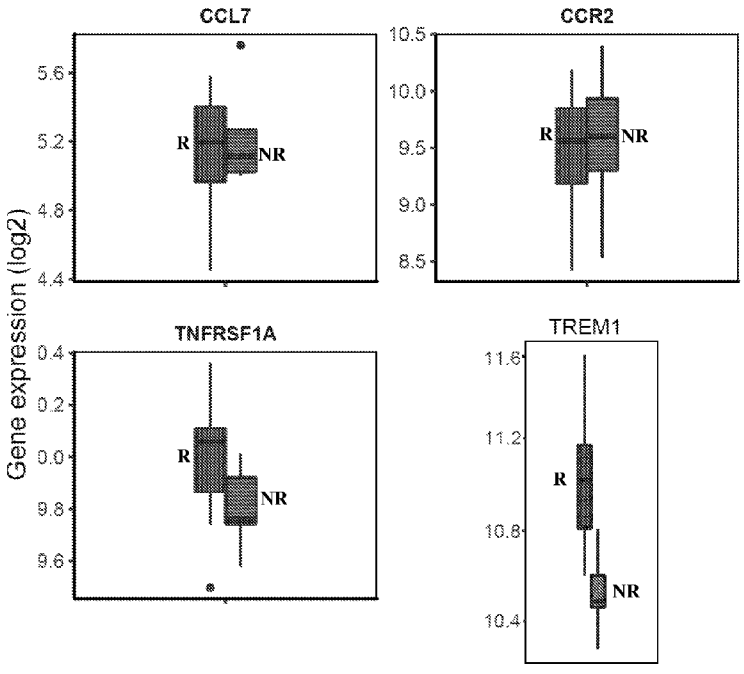
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

(54) Title: PROGNOSTIC METHODS FOR ANTI-TNFA TREATMENT

Figure 5A



(57) Abstract: Methods of determining suitability of a subject to be treated with anti-TNFα therapy comprising measuring blood TREM1 levels or intestinal plasma cell number, intestinal inflammatory macrophage number or intestinal CCL7 or CCR2 levels are provided. Kits for doing same are also provided.

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GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

PROGNOSTIC METHODS FOR ANTI-TNFA TREATMENT

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/581,960, filed November 6, 2017, the contents of which are all incorporated herein by reference in their entirety.

FIELD OF INVENTION

[002] The present invention is in the field of anti-TNFα therapy diagnostics.

BACKGROUND OF THE INVENTION

[003] Inflammatory Bowel Diseases (IBDs) consist primarily of ulcerative colitis (UC) and Crohn's disease (CD) and are prevalent in the industrialized world with increasing incidence in both western and developing countries. IBD is characterized by chronic intestinal inflammation, driven both by the innate and adaptive immune systems, although their pathogenesis is not completely understood. The advent of anti-TNFα therapy was a major breakthrough for IBD treatment and was shown to be effective for treating various forms of disease.

[004] However, anti-TNFα therapy remains suboptimal for several reasons. Treatment choice and administration is empiric and based on data obtained from the "average patient" in clinical trials. This practice is associated with insufficient remission rates, that result from primary non-response (PNR) (20-40% in clinical trials; 10-20% in real life cohorts), and from loss of response commonly due to immunogenicity and increased anti-TNFα clearance in 13-24% of patients at 12 months. Beyond that, the treatment is associated with adverse side effects, is expensive and each therapeutic attempt requires waiting the anticipated time for response during which the disease is active, and damage accumulates. Taken together, there is an urgent unmet need for predicting response prior to treatment initiation to reduce healthcare costs and avoid unnecessary treatment.

[005] Several attempts have been made to define a baseline signature of anti-TNF response in IBD patients using genetics, microbiome, and gene expression data. Yet, no predictive biomarker is in clinical practice. Thus, finding predictive biomarkers of non-response prior to commencing anti-TNFα therapy is of high-value.

SUMMARY OF THE INVENTION

[006] The present invention provides methods of determining suitability of a subject to be treated with anti-TNFα therapy comprising measuring blood TREM1 levels or intestinal plasma cell number, intestinal inflammatory macrophage number or intestinal CCL7 or CCR2 levels. Kits for doing same are also provided.

[007] According to a first aspect, there is provided a method of determining suitability of a subject in need thereof to be treated with anti-Tumor Necrosis Factor Alpha (TNFα) therapy, the method comprising:

a. obtaining a blood sample from the subject;

b. measuring expression levels of Triggering Receptor Expressed on Myeloid cells 1 (TREM1) in the blood sample; and

c. determining the suitability of the subject for anti-TNFα therapy according to the expression levels of TREM1, wherein expression below a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy and expression at or above the predetermined threshold indicates the subject is suitable for the anti-TNFα therapy;

thereby determining suitability of a subject to be treated with anti-TNFα therapy.

[008] According to another aspect, there is provided a method of determining suitability of a subject in need thereof to be treated with anti-Tumor Necrosis Factor Alpha (TNFα) therapy, the method comprising

a. obtaining an intestinal biopsy from the subject;

b. measuring in the intestinal biopsy at least one of:

i. plasma cell number;

ii. inflammatory macrophage; and

iii. expression levels of CCL7, CCR2 or both;

c. determining the suitability of the subject for anti-TNFα therapy according to the plasma cell or inflammatory macrophage number, wherein a number of plasma cells, a number of inflammatory macrophages or expression levels CCL7, CCR2 or both above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy and a number of plasma cells, a number of inflammatory macrophages or expression levels CCL7, CCR2 or both below the predetermined threshold indicates the subject is suitable for the anti-TNFα therapy;

thereby determining suitability of a subject to be treated with anti-TNFα therapy.

[009] According to some embodiments, the blood sample is a peripheral blood sample.

[010] According to some embodiments, a number of plasma cells above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy and a number of plasma cells below the predetermined threshold indicates the subject is suitable for the anti-TNFα therapy.

[011] According to some embodiments, expression levels of CCL7 and CCR2 above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy and expression levels of CCL7 and CCR2 below the predetermined threshold indicates the subject is suitable for the anti-TNFα therapy.

[012] According to some embodiments, the method further comprises measuring in an intestinal biopsy from the subject expression levels of TREM1, wherein intestinal expression levels of TREM1 above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα

therapy and intestinal expression levels of TREM1 below the predetermined threshold indicates the subject is suitable for the anti-TNFα therapy.

[013] According to some embodiments, the subject suffers from inflammation. According to some embodiments, the subject suffers from an autoimmune disease. According to some embodiments, the autoimmune disease is selected from inflammatory bowel disease (IBD) and rheumatoid arthritis (RA). According to some embodiments, the IBD comprises colitis, ulcerative colitis, and Crohn's disease.

[014] According to some embodiments, the anti-TNFα therapy comprises administration of an anti-TNFα antibody. According to some embodiments, the anti-TNFα antibody is selected from infliximab and adalimumab.

[015] According to some embodiments, the method further comprises administering the anti-TNFα therapy to the subject indicated to be suitable for the anti-TNFα therapy. According to some embodiments, the method further comprises administering to the subject indicated to be unsuitable for the anti-TNFα therapy an increased dose of the anti-TNFα therapy above the standard dose. According to some embodiments, the method further comprises administering a non-anti-TNFα anti-inflammation therapy to the subject indicated to be unsuitable for anti-TNFα therapy. According to some embodiments, the non-anti-TNFα anti-inflammation therapy comprises blocking at least one of CCR2, CCR5 and CXCR3.

[016] According to some embodiments, the predetermined threshold is determined from blood levels of TREM1, intestinal numbers of plasma cells or inflammatory macrophages or intestinal expression of CCL7, CCR2 or both in subjects that responded to anti-TNFα therapy, wherein the blood levels, the intestinal numbers and the intestinal expression are from before the subjects received the anti-TNFα therapy.

[017] According to some embodiments, measuring plasma cell number comprises measuring the number of cells positive for surface expression of CD138.

[018] According to some embodiments, measuring expression levels comprises measuring mRNA levels, protein levels or both.

[019] According to some embodiments, measuring expression levels comprises contacting the blood sample or intestinal biopsy with a molecule that detects TREM1, CCL7, or CCR2 mRNA or protein, and wherein the molecule is connected to an artificial solid support.

[020] According to another aspect, there is provided a use of a TREM1 detecting agent for determining serum TREM1 levels in a subject and determining suitability of the subject for treatment with an anti-TNFα therapy.

[021] According to some embodiments, the detecting agent is an anti-TREM1 antibody or a nucleic acid molecule that selectively binds and hybridizes to TREM1 mRNA.

[022] According to another aspect, there is provided a kit comprising a plurality of molecules selected from:

a. a TREM1 detecting molecule;

b. a CCL7 detecting molecule;

c. a CCR2 detecting molecule;

d. a plasma cell detecting molecule; and

e. an inflammatory macrophage detecting molecule.

[023] Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[024] **Figure 1A: Predictive gene signatures from previous gene expression differential analysis.** Heatmaps showing the gene expression of the 6 previously reported predictive gene signatures in their respective discovery cohort(s). The top strip annotates responders (r) and non-responder (n) samples. Panel title and subtitle indicate the corresponding gene signature and

cohort names respectively. The heat map goes from high in responder t o high in non-responder. The row annotation indicates the group in which each gene was up-regulated. All but 4 genes (all from IRRAT) were up-regulated in non-responders.

[025] **Figure 1B: Characteristic sorted cell type expression of gene signatures of anti-TNFα response reported from heterogeneous tissue biopsy show contributions from distinct immune cell subsets.** Analysis of the immune contribution of 109 unique signature genes mapped to a compendium of sorted cell expression profiles, spanning 17 immune cell subpopulations as well as colon tissue samples. Shown are the contributions (x-axis), i.e. number of genes assigned, for 8 major cell lineages (y-axis), highlighted into resting and activated/memory subpopulations. Lineages are ordered by decreasing total contribution, with most signature genes are expressed in the myeloid, B and T-cell lineages (68%, 13% and 19% of the genes respectively). 85% of signature genes are expressed in low abundance in bulk healthy colon.

[026] **Figure 2A. Computational deconvolution of cell subset proportions identifies higher proportions of inflammatory macrophages and plasma cells in non-responders.** Estimated immune cell subset proportions in the UC cohorts. Boxplot shows the baseline estimated proportions of each immune cell subset in each cohort for responders (left) and non-responders (right) to anti-TNFα therapy. Only cell types with at least 75% non-zero values are shown.

[027] **Figures 2B-D. Meta-analysis of computationally deconvolved cell subset proportions identifies consistently higher proportions of inflammatory macrophages and plasma cells in non-responders.** (**2B**) Plasma cell and macrophage log2 proportion fold change between response groups, across three cohorts (p-value ≤ 0.05 by Wilcoxon Rank Sum are shown for significantly higher proportions in non-responders; no significant change is also shown). (**2C-D**) Cellular signature abundance decreases while differences persist after anti-TNFα treatment. Deconvolution derived frequencies of inflammatory macrophages (**2C**) and plasma cells (**2D**) pre and post- anti-TNFα treatment (x-axis) are shown.

[028] **Figure 2E. Multi-cohort analysis of estimated cell proportions identifies consistent baseline differences in inflammatory macrophages and plasma cells.** Each panel shows estimated group proportion differences (pseudo median) and 95% confidence interval for a given cell subset, across all discovery cohorts. Missing data is due to cell type/cohort pairs not included

in the meta-analysis due to too many zero estimated proportions. The x-axis represents the log2 proportion fold change (i.e. log2(Responders/Non-Responders)). Statistical significance was calculated using Wilcoxon rank sum test (nominal p-value ≤ 0.05) and is shown for significantly higher proportions in non-responders (NR) and responders (R) respectively. NS indicates non-significant differences.

[029] **Figure 2F. Differences in cellular biomarkers increase between response groups following treatment.** Deconvolution derived estimates of plasma cells and inflammatory macrophages proportions in responders and non-responder groups in the UC-A and CDc cohorts. Both cell subsets are significantly lower in responders in both cohorts p-values<0.01 BH-adjusted). Strikingly, inflammatory macrophages were undetectable (zero values) in all responders, except for two patients from the CDc cohort. R=responders, NR=non-responders.

[030] **Figures 3A-C. Abundance of plasma cells and macrophage subtypes in biopsies of IBD patients predicts anti-TNFα treatment outcome.** (3**A-C**) ROC curves and AUC computed in the (**3A**) CDc, (**3B**) UC-A and (**3C**) UC-B cohorts from the estimated proportions of inflammatory macrophages and plasma cells or combined, with 89%,88% and 95% AUCs respectively. The x and y axis represent the specificity (true negative fraction) and sensitivity (true positive fraction) respectively.

[031] **Figure 3D. Adjusting gene expression for abundances of inflammatory macrophages and plasma cells significantly breaks the association between gene signatures and treatment response status.** Heatmaps similar to those in Figure 1A, showing the association between the signatures scores UC-A (left), UC-B (middle) and CDc (right) in their respective cohorts, generated from the original gene expression data (top row) and after adjustment for estimated abundances of inflammatory macrophages and plasma cells (bottom row).

[032] **Figures 3E-M. Differences in cell subset is the driving force of the reported gene signatures for predicting anti-TNFα non-response from baseline.** (**3E**) Predictive power of reported gene signatures drops after correction for variations in cellular biomarkers. ROC curves for each original gene signature score in its respective discovery cohort UC-A, UC-B, and CDc on the original data (left) and the data following adjustment for variations in the abundances of both cellular biomarkers (right). Respective AUCs are reduced from 93%, 97% and 100% on the original data, to 57%, 68% and 79% on the adjusted data. (**3F**) The drop in AUC due to cell

subset proportions differences is significant. AUC was calculated for each gene signature score in their respective discovery cohort. Densities represent AUC null distributions obtained by adjusting gene expression data with random proportions, in each cohort (panels). Solid line shows achieved AUC in original (unadjusted) data, dashed line indicates AUC obtained after adjustment for estimated proportions of inflammatory and plasma cells. (**3G-H**) (**3G**) Plasma cells were immunostained with CD138, in an independent set of IBD biopsies. Example staining slides showing visual differences between responders and non-responders these populations. CD138+ plasma cells are colored in brown, showing a clear increased staining in non-responsive patients. (**3H**) ROC curve showing the predictive power of plasma cell and inflammatory macrophages proportions as quantified by a pathologist categorical score (solid line, AUC = 71% and 67% respectively) and a quantitative score for plasma cells (dashed line, AUC = 81%). (**3I**) Boxplots of plasma cell quantitative score in the preliminary cohort (left panel, n=20) and main cohort (right panel, n=52). P values < 0.001 for both cohorts (by Student T-test). (**3J-K**) Distribution of responding and non-responding patients in the preliminary cohort (top panels) and main cohort (bottom panels) as shown by patient proportions and patient counts in each score. (**3J**) Histograms of pathologist plasma cell score, (**3K**) Histograms of inflammation severity scores as determined by an expert pathologist. (**3L**) ROC curve showing analysis of a cohort of 52 IBD patients collected from two medical centers whose biopsies were stained by CD138+ IHC staining. Plasma cell abundance classifies non-response at baseline (AUC = 71% and 74% by the pathologist and quantitative scores respectively). (**3M**) The predictive power increases when restricting to highly inflamed tissues according to the pathologist score (AUC = 82% and 84% by the pathologist and quantitative scores respectively).

[033] **Figures 4A-G. Adjusting samples for cell subset variation unmasks upregulated pathways in biopsies of anti-TNF non-responders.** (**4A**) Meta-analysis of non-response associated biological pathways by GSEA on cell subset adjusted expression data supports up-regulation of TLR2/4, IL-6 and B cell receptor signaling, as well as other inflammatory pathways. (**4B**) Venn diagram showing overlap of cellular biomarker-adjusted differentially expressed pathways across cohorts identified by GSEA. The fifteen pathways differentially expressed in all three cohorts were all upregulated in non-responders. (**4C**) Venn diagrams showing overlap of differentially expressed genes across cohorts. Upregulated genes (left), downregulated (right). (**4D**) GSEA enrichment score are driven by consistent leading-edge genes across cohorts. Curves

show GSEA enrichment scores for the 3 pathways (rows) that have the most consistent leading-edge genes across cohorts (>25% of their genes in leading-edge in all cohorts). (**4E**) Top enriched IPA network identified from meta-analysis of cell subset adjusted gene expression data. The CCL7-CCR2 axis is upregulated in non-responders (**4F**) Boxplots showing the expression of TNFα and its receptors, TNFR1 and TNFR2 in the three biopsy cohorts in the original unadjusted data. (**4G**) Box plots showing the adjusted expression of TNFα and its receptors, TNFR1 and TNFR2 in the three biopsy cohorts.

[034] **Figures 5A-B. Trem1 expression in blood predicts anti-TNF non-response at baseline in Crohn's patients.** (**5A**) Boxplots showing TREM1, CCL7, CCR2, and TNFR1 mRNA expression as measured in whole blood of 29 responding and non-responding CD patients, prior to initiation of infliximab therapy. (**5B**) ROC curve of classifier of anti-TNF response at baseline based on TREM1 expression in whole blood.

[035] **Figures 6A-C. Trem1 expression in blood predicts anti-TNF non-response at baseline in rheumatoid arthritis patients.** (**6A-C**) Boxplots showing TREM1 mRNA expression before (left plot) and after adjustment for cell frequency of PCs and inflammatory macrophages (right plot) in whole blood of RA patients from (**6A**) GEO12051 and (**6B**) GSE58795, prior to initiation of infliximab therapy and from (**6C**) GEO33377 prior to initiation of infliximab and adalimumab.

DETAILED DESCRIPTION OF THE INVENTION

[036] The present invention provides methods and kits for determining suitability for treatment with an anti-TNFα therapy, and also for treatment. Means and methods for identifying altered abundance of plasma cells and inflammatory macrophages in pretreatment intestinal biopsies of anti-TNFα responders versus non-responders are also provided. The present invention provides means and methods for identifying altered expression of certain genes in the blood wherein the altered expression is predictive of non- responsiveness to anti-TNFα therapy in patients suffering from inflammation.

[037] The invention is based on the surprising finding that measuring blood levels of TREM1 alone can accurately predict if a subject will be responsive to anti-TNFα therapy. This finding is

highly surprising in that elevated levels of blood TREM1 are actually indicative of a subject who is responsive. TREM1 levels in biopsies from the intestines were found to have the inverse relationship, i.e. increased levels of TREM1 were indicative of a subject who is non-responsive. The invention is further based on the unexpected finding that elevated levels of CCR2, CCL7 plasma cell number and inflammatory macrophage number in intestine biopsies could each independently accurately predict that a subject would not be responsive to anti-TNFα therapy. Thus, several new and highly accurate methods of determining subject suitability for anti-TNFα therapy are provided.

[038] By a first aspect, there is provided a method of determining suitability of a subject in need thereof to be treated with anti-Tumor Necrosis Factor Alpha (TNFα) therapy, the method comprising:

a. obtaining a sample from the subject;

b. measuring an anti-TNFα therapy predicative factor in the sample;

c. determining the suitability of the subject for anti-TNFα therapy according to the predictive factor, wherein a value of the predicative factor above/below a predetermined threshold indicates that the subject is suitable for anti-TNFα therapy, and a value of the predicative factor on the other side of the threshold indicates the subject is unsuitable for the anti-TNFα therapy;

thereby determining suitability of the subject to be treated with anti-TNFα therapy.

[039] TNFα is a well characterized cytokine, and therapies that target TNFα are well known in the art. In some embodiments, the anti-TNFα therapy comprises reduction of TNFα expression. In some embodiments, anti- TNFα therapy comprises inhibition of TNFα function. In some embodiments, the anti-TNFα therapy comprises reduced TNFα protein, and/or mRNA expression. In some embodiments, anti-TNFα therapy comprises administration of an anti-TNFα antibody. In some embodiments, the antibody is an antigen binding fragment of an antibody. In some embodiments, anti-TNFα therapy comprises reducing TNFα mRNA expression. In some embodiments, anti-TNFα therapy comprises administering a nucleic acid molecule that binds to TNFα mRNA and inhibits its translation or leads to its degradation. In some embodiments, the

nucleic acid molecule is a siRNA, miRNA or the like. In some embodiments, anti-TNFα therapy comprises administering a TNFα antagonist.

[040] As used herein, the term "antagonists" refer to substances which inhibit or neutralize the biologic activity of TNFα. Such antagonists accomplish this effect in a variety of ways. One class of antagonists will bind to the gene product protein with sufficient affinity and specificity to neutralize the biologic effects of the protein. Included in this class of molecules are antibodies and antibody fragments (such as, for example, F(ab) or $F(ab')_2$molecules). Another class of antagonists comprises fragments of the gene product protein, muteins or small organic molecules, i.e., peptidomimetics, that will bind to the cognate binding partners or ligands of the gene product, thereby inhibiting the biologic activity of the specific interaction of the gene product with its cognate ligand or receptor. The TNFα antagonist can be of any of these classes as long as it is a substance that inhibits a biological activity of the gene product.

[041] Antagonists include antibodies directed to one or more regions of the gene product protein or fragments thereof, antibodies directed to the cognate ligand or receptor, and partial peptides of the gene product or its cognate ligand which inhibit a biological activity of the gene product. Another class of antagonists includes siRNAs, shRNAs, antisense molecules and DNAzymes targeting the gene sequence as known in the art are disclosed herein.

[042] In some embodiments, the TNFα antagonist is an anti-TNFα antibody. In some embodiments, the anti-TNFα antibody is selected from infliximab, golimumab, Enbrel, certolizumab pegol, and adalimumab. In some embodiments, the anti-TNFα antibody is selected from infliximab and adalimumab. In some embodiments, the anti-TNFα antibody is infliximab. In some embodiments, the anti-TNFα antibody is adalimumab.

[043] In some embodiments, the subject suffers from inflammation. In some embodiments, the subject suffers from an inflammatory disease. In some embodiments, the subject suffers from a disease characterized by inflammation. In some embodiments, the subject suffers from a disease that can be treated with anti-TNFα therapy. In some embodiments, the subject suffers from a disease that can be treated with ana anti-TNFαntibody. In some embodiments, the subject suffers from autoimmune inflammation. In some embodiments, the subject suffers from an autoimmune disease. In some embodiments, the subject suffers from an autoimmune inflammatory disease. In some embodiments, the autoimmune inflammatory disease is selected from inflammatory

bowel disease (IBD) and rheumatoid arthritis (RA). In some embodiments, the subject suffers from a disease selected from IBD, RA, psoriatic arthritis, ankylosing spondylitis, chronic psoriasis, hidradentisis suppurativa, and juvenile idiopathic arthritis. In some embodiments, the subject suffers from a disease selected from IBD, and RA. In some embodiments, the subject suffers from arthritis. In some embodiments, the subject suffers from RA.

[044] In some embodiments, the subject suffers from inflammatory bowel disease (IBD). In some embodiments, IBD comprises colitis, ulcerative colitis and Crohn's disease. In some embodiments, IBD comprises colitis, ulcerative colitis, Behcet's disease and Crohn's disease. In some embodiments, the IBD is colitis. In some embodiments, the IBD is ulcerative colitis. In some embodiments, the IBD is Crohn's disease. In some embodiments, the anti-TNFα therapy is an anti-IBD therapy. In some embodiments, the IBD is characterized by increased TNFα expression. In some embodiments, the increased TNFα expression is in the intestines.

[045] In some embodiments, the sample is a blood sample. In some embodiments, the blood sample is a peripheral blood sample. In some embodiments, the blood sample is an intestinal blood sample. In some embodiments, the blood sample is from a clinical blood draw. In some embodiments, the sample is an intestinal sample. In some embodiments, the intestinal sample is an intestinal biopsy. In some embodiments, the intestine is the small intestine. In some embodiments, the intestine is the large intestine. In some embodiments, the biopsy is acquired during a colonoscopy. In some embodiments, the sample includes cells. In some embodiments, the sample includes protein. In some embodiments, the sample includes nucleic acids. In some embodiments, the sample includes mRNA.

[046] As used herein, the term "anti-TNFα therapy predicative factor" refers to a measurable biological readout that is predictive of whether a subject will respond to anti-TNFα therapy. In some embodiments, the predicative factor is Triggering Receptor Expressed on Myeloid cells 1 (TREM1) expression. In some embodiments, the predicative factor is Chemokine (C-C motif) ligand 7 (CCL7) expression. In some embodiments, the predicative factor is C-C chemokine receptor type 2 (CCR2) expression. In some embodiments, the predicative factor is CCL7 and CCR2 expression. In some embodiments, the predicative factor is CCL7 and/or CCR2 expression and TREM1 expression. In some embodiments, the predicative factor is plasma cell (PC) number. In some embodiments, the predictive factor is inflammatory macrophage (IM) number. In some

embodiments, the predicative factor is selected from TREM1 expression, CCL7 expression, CCR2 expression, PC number, IM number and a combination thereof.

[047] In some embodiments, TREM1 is serum TREM1. In some embodiments, TREM1 is soluble TREM1. In some embodiments, TREM1 is membranal TREM1. In some embodiments, the serum TREM1 is soluble TREM1, membranal TREM1 or both. In some embodiments, TREM1 is a variant of TREM1.

[048] In some embodiments, TREM1 expression is measured in a blood sample. In some embodiments, TREM1 expression is measured in an intestinal sample. In some embodiments, CCR2, CCL7, PC number, and/or IM number is measured in an intestinal sample. In some embodiments, TREM1 expression is measured in blood to determine suitability of a subject suffering from a non-intestinal disease. In some embodiments, the non-intestinal disease is RA.

[049] In some embodiments, measuring comprises measuring mRNA levels. In some embodiments, measuring comprises measuring protein levels. In some embodiments, measuring comprises counting cells. In some embodiments, measuring comprises automated cell counting. In some embodiments, measuring comprises PCR of a target mRNA. In some embodiments, measuring comprises hybridization with a nucleic acid-based array (for example, a microarray). In some embodiments, measuring comprises immuno-detection. In some embodiments, the immuno-detection is immunohistochemistry. In some embodiments, the immuno-detection is ELISA. In some embodiments, immuno-detection is immunoblotting. In some embodiments, immuno-detection of PC cells comprises detection of CD138. In some embodiments, measuring PC number comprises measuring the number of cells positive for surface expression of CD138. In some embodiments, immuno-detection of IM cells comprises detection of at least one of CD86, CD80, CD68, MHCII, IL-1R, TLR2, TLR4, iNOS and SOCS3. In some embodiments, a combination of markers is immuno-detected. In some embodiments, measuring IM number comprises measuring the number of cells positive for surface expression of CD68 and CD86.

[050] In some embodiments, measuring expression levels comprises contacting the sample with a molecule that detects the predicative factor. In some embodiments, the molecule that detects the predicative factor is connected to an artificial solid support. In some embodiments, the molecule that detects the predicative factor is an artificial molecule. In some embodiments, the molecule that detects the predicative factor is an engineered molecule. In some embodiments, the

sample is processed before measuring. In some embodiments, the processing comprises extracting protein. In some embodiments, the processing comprises extracting nucleic acids. In some embodiments, the processing comprises purifying the extracted protein and/or nucleic acid molecule. In some embodiments, the processing comprises embedding tissue. In some embodiments, the tissue is a biopsy. In some embodiments, the embedding is paraffin embedding. In some embodiments, the detecting comprises contacting the molecule that detects the predicative factor with a second detecting molecule. In some embodiments, the second detecting molecule is a fluorescent molecule. In some embodiments, the second detecting molecule is a colored molecule. Detection of protein, cells and/or mRNA is well known in the art and some examples of such are provided hereinbelow. Any method of such detection however may be employed for the performance of the invention disclosed herein.

[051] In some embodiments, the predetermined threshold is determined from healthy subjects. In some embodiments, the predetermined threshold is determined from subjects suffering from IBD. In some embodiments, the predetermined threshold is determined from subjects suffering from IBD but respond to therapy. In some embodiments, the predetermined threshold is determined from subjects suffering from IBD but do respond to therapy. In some embodiments, the predetermined threshold is the highest or lowest level/number from subjects that respond to therapy. In some embodiments, the predetermined threshold is the highest or lowest levels/number from subjects that do not respond to therapy. In some embodiments, the predetermined threshold is the level/number that best distinguishes subjects that respond and don't respond to therapy. In some embodiments, the predetermined threshold is from subject naïve to anti-TNFα therapy. In some embodiments, the predetermined threshold is baseline level/number from subjects before receiving therapy.

[052] In some embodiments, TREM1 expression is measured in a blood sample and suitability of the subject to be treated with anti-TNFα therapy is determined according to the blood TREM1 levels. In some embodiments, blood TREM1 expression below a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy. In some embodiments, blood TREM1 expression above a predetermined threshold indicates the subject is suitable for the anti-TNFα therapy. Expression at or about at the threshold may mean the subject is either suitable or unsuitable for anti-TNFα therapy and may be determined by a physician. In some embodiments, expression at or about at the threshold indicates the subject is suitable for the anti-TNFα therapy.

[053] In some embodiments, TREM1 expression is measured in an intestinal biopsy and suitability of the subject to be treated with anti-TNFα therapy is determined according to the biopsy TREM1 levels. In some embodiments, biopsy TREM1 expression above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy. In some embodiments, biopsy TREM1 expression below a predetermined threshold indicates the subject is suitable for the anti-TNFα therapy. Expression at or about at the threshold may mean the subject is either suitable or unsuitable for anti-TNFα therapy and may be determined by a physician. In some embodiments, expression at or about at the threshold indicates the subject is suitable for the anti-TNFα therapy.

[054] In some embodiments, intestinal expression or cell numbers are used for determining suitability of subjects suffering from an intestinal disease. In some embodiments, the intestinal disease is IBD.

[055] In some embodiments, CCR2 expression is measured in an intestinal biopsy and suitability of the subject to be treated with anti-TNFα therapy is determined according to the biopsy CCR2 levels. In some embodiments, biopsy CCR2 expression above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy. In some embodiments, biopsy CCR2 expression below a predetermined threshold indicates the subject is suitable for the anti-TNFα therapy. Expression at or about at the threshold may mean the subject is either suitable or unsuitable for anti-TNFα therapy and may be determined by a physician. In some embodiments, expression at or about at the threshold indicates the subject is suitable for the anti-TNFα therapy.

[056] In some embodiments, CCL7 expression is measured in an intestinal biopsy and suitability of the subject to be treated with anti-TNFα therapy is determined according to the biopsy CCL7 levels. In some embodiments, biopsy CCL7 expression above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy. In some embodiments, biopsy CCL7 expression below a predetermined threshold indicates the subject is suitable for the anti-TNFα therapy. Expression at or about at the threshold may mean the subject is either suitable or unsuitable for anti-TNFα therapy and may be determined by a physician. In some embodiments, expression at or about at the threshold indicates the subject is suitable for the anti-TNFα therapy.

[057] In some embodiments, PC number is measured in an intestinal biopsy and suitability of the subject to be treated with anti-TNFα therapy is determined according to the number of PCs in the biopsy. In some embodiments, biopsy PC number above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy. In some embodiments, biopsy PC number below a predetermined threshold indicates the subject is suitable for the anti-TNFα therapy. Expression at or about at the threshold may mean the subject is either suitable or unsuitable for anti-TNFα therapy and may be determined by a physician. In some embodiments, expression at or about at the threshold indicates the subject is suitable for the anti-TNFα therapy.

[058] In some embodiments, IM number is measured in an intestinal biopsy and suitability of the subject to be treated with anti-TNFα therapy is determined according to the number of IMs in the biopsy. In some embodiments, biopsy IM number above a predetermined threshold indicates the subject is unsuitable for the anti-TNFα therapy. In some embodiments, biopsy IM number below a predetermined threshold indicates the subject is suitable for the anti-TNFα therapy. Expression at or about at the threshold may mean the subject is either suitable or unsuitable for anti-TNFα therapy and may be determined by a physician. In some embodiments, expression at or about at the threshold indicates the subject is suitable for the anti-TNFα therapy.

[059] In some embodiments, PC or IM number is measured. In some embodiments, PC and IM number is measured. In some embodiments, both numbers must be below the predetermined threshold for the subject to be suitable. In some embodiments, at least one of the numbers must be below the threshold for the subject to be suitable. In some embodiments, CCL7 or CCR2 is measure. In some embodiments, CCL7 and CCR2 is measured. In some embodiments, both expression levels must be below the predetermined threshold for the subject to be suitable. In some embodiments, at least one of the expression levels must be below the threshold for the subject to be suitable.

[060] In some embodiments, a combination of the predicative factors described herein is measured. In some embodiments, all the measured factors must indicate a subject is suitable for the subject to be determined to be suitable. In some embodiments, at least a plurality of factors must indicate a subject is suitable for the subject to be determined to be suitable. In some embodiments, blood TREM1 and at least one other predicative factor is measured and at least

blood TREM1 and one other predicative factor must indicate a subject is suitable for the subject to be determined to be suitable.

[061] In some embodiments, the factors listed herein are the only factors measured. In some embodiments, the measuring is devoid of measuring any other factors. In some embodiments, only TREM1, CCR2 and/or CCL7 levels are measured. In some embodiments, only TREM1, CCR2 and/or CCL7 levels and the levels of a control gene or protein are measured. In some embodiments, only CCR2 and/or CCL7 levels and the levels of another gene from the CCR2/CCL7 axis are measured. In some embodiments, genes/proteins from the CCR2/CCL7 axis are selected from the group consisting of: AIM2, AP2B1, ARAF, BAG2, BCAT1, CCNF, DDX25, DESI2, DIABLO, EPHB2, ERK1/2, GPC1, HAVCR2, HOMER1, IgG, IL19, IL12 (complex), IL4I1, IL7R, Interferon alpha, Jnk, MYO6, P38 MAPK, PIK3CB, RIPK1, RNF13, S1PR1, SOX17, STAT2, TCR, TRIO, TSPYL2, and WNK2. In some embodiments, genes/proteins selected from AIM2, AP2B1, ARAF, BAG2, BCAT1, CCNF, DDX25, DESI2, DIABLO, EPHB2, ERK1/2, GPC1, HAVCR2, HOMER1, IgG, IL19, IL12 (complex), IL4I1, IL7R, Interferon alpha, Jnk, MYO6, P38 MAPK, PIK3CB, RIPK1, RNF13, S1PR1, SOX17, STAT2, TCR, TRIO, TSPYL2, and WNK2 are also measured. In some embodiments, expression of any one of Sox17, RNF13, TSPYL2, S1PR1, BAG2, HOMER1, IL4I1, AIM2, STAT2, BCAT1, HAVCR2, BCAT1, and IL7R above the predetermined threshold indicates the subject is unsuitable for anti-TNFα therapy. In some embodiments, expression of any one of WNK2, DESI2, GPC1, IL19, DIABLO, DDX25, ARAF, RIPK1, TRIO, CCNF, EPHB2, MYO6, PIK3CB and AP2B1 below the predetermined threshold indicate the subject is unsuitable for anti-TNFα therapy. In some embodiments, a predicative factor is combined with the expression levels of at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 genes from the above list of genes/proteins for determined suitability of the subject for anti-TNFα therapy. Each possibility represents a separate embodiment of the invention.

[062] Control, or housekeeping genes and proteins are well known in the art and may be used for normalization of expression levels in sample. Examples of control genes/proteins include, but are not limited to B-actin, GAPDH, and ribosomal components. In some embodiments, the expression levels are normalized to cell types percentage in the biopsy.

[063] In one embodiment, significantly high plasma cell numbers in anti-TNFα non-responders were validated in IBD patients naive to anti-TNFα therapy. In one embodiment, significantly is at least 10% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 20% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 30% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 40% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 50% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 75% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 100% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 150% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 200% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 300% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 350% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 400% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 450% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 500% higher plasma cell numbers in anti-TNFα non-responders.

[064] In some embodiments, the above the threshold is at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% above the threshold. Each possibility represents a separate embodiment of the invention. In some embodiments, the below the threshold is at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% below the threshold. Each possibility represents a separate embodiment of the invention.

[065] In one embodiment, provided herein a method for identifying an IBD patient non-responding to anti-TNFα therapy, comprising, analyzing intestinal specimen from the IBD patient, wherein significantly high plasma cell numbers in the intestinal specimen compared to a threshold value of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative for IBD non-responding to anti- TNFα therapy, thereby identifying an IBD patient non-responding to anti-TNFα therapy. In one embodiment, intestinal specimen comprises intestinal biopsy.

[066] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to an IBD patient, comprising obtaining from the IBD patient an intestinal biopsy and analyzing the intestinal biopsy for plasma cell number and/or abundance, wherein significantly high plasma cell numbers in the intestinal specimen compared to a threshold value of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti- TNFα therapy, is indicative that the anti-TNFα therapy is ineffective, thereby determining the effectiveness of an anti-TNFα therapy to an IBD patient.

[067] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to an IBD patient, comprising obtaining from the IBD patient an intestinal biopsy and analyzing the intestinal biopsy for plasma cell number and/or abundance, wherein a higher plasma cell numbers in the intestinal specimen compared to the range of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is ineffective, thereby determining the effectiveness of an anti- TNFα therapy to an IBD patient.

[068] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to an IBD patient, comprising obtaining from the IBD patient an intestinal biopsy and analyzing the intestinal biopsy for plasma cell number and/or abundance, wherein plasma cell numbers in the intestinal specimen below a threshold value of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is effective, thereby determining the effectiveness of an anti-TNFα therapy to an IBD patient.

[069] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to an IBD patient, comprising obtaining from the IBD patient an intestinal biopsy and analyzing the intestinal biopsy for plasma cell number and/or abundance, wherein plasma cell numbers in the intestinal specimen within a range of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is effective, thereby determining the effectiveness of an anti-TNFα therapy to an IBD patient.

[070] In one embodiment, provided herein the use of an intestinal biopsy showing plasma cells within the biopsy for method determining the effectiveness of an anti-TNFα therapy to an IBD

patient, wherein plasma cell numbers in the intestinal specimen within a range of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is effective, thereby determining the effectiveness of an anti- TNFα therapy to an IBD patient. In one embodiment, provided herein the use of an intestinal biopsy showing plasma cells within the biopsy for method determining the effectiveness of an anti-TNFα therapy to an IBD patient, wherein plasma cell numbers in the intestinal specimen beyond a range of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is ineffective, thereby determining the effectiveness of an anti-TNFα therapy to an IBD patient. In one embodiment, provided herein the use of an intestinal biopsy showing plasma cells within the biopsy for method determining the effectiveness of an anti-TNFα therapy to an IBD patient, wherein plasma cell numbers in the intestinal specimen higher than the threshold values for number of plasma cell numbers in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti- TNFα therapy is ineffective, thereby determining the effectiveness of an anti-TNFα therapy to an IBD patient.

[071] In one embodiment, intestinal biopsy showing plasma cells is an intestinal biopsy stained for plasma cells. In one embodiment, intestinal biopsy showing plasma cells is an intestinal biopsy wherein the number of plasma cells can be quantified.

[072] In one embodiment, analyzing the intestinal biopsy for plasma cell number and/or abundance is staining an intestinal biopsy and identifying plasma cells. In one embodiment, analyzing the intestinal biopsy for plasma cell number and/or abundance is treating an intestinal biopsy with immunohistochemical reagent for identifying plasma cells. In one embodiment, staining or immunohistochemical reagents for identifying plasma cells are known to a person of skill in the art. In one embodiment, determining, analyzing and/or staining is providing means for counting the number of plasma cell within an intestinal specimen.

[073] In one embodiment, "threshold value of plasma cell numbers" is indicative to the upper threshold of cell numbers or abundance of cells in an IBD patient responding to an anti- TNFα therapy.

[074] In one embodiment, "a range of plasma cell numbers in control intestinal specimen" is indicative to the range of cell numbers or abundance in an IBD patient responding to an anti-

TNFα therapy. In one embodiment, "cell numbers or abundance" include the concentration of cells. In one embodiment, "cells" are plasma cells. In one embodiment, "cell number" is plasma cell number. In one embodiment, intestinal specimen derived from IBD patient responding to anti- TNFα therapy is the mean and/or median values of a plurality of specimens as described herein. In one embodiment, "IBD non-responding to anti-TNFα therapy" is a patient afflicted with IBD wherein anti-TNFα therapy has little or no therapeutic effect. In one embodiment, "IBD non-responding to anti-TNFα therapy" is a patient afflicted with IBD wherein anti- TNFα therapy has insufficient therapeutic effect. In one embodiment, the abundance, concentration and/or the total number of plasma cells within an intestinal biopsy is indicative for the efficiency of anti-TNFα therapy in an IBD patient. In one embodiment, an intestinal biopsy of an IBD patient as described herein is assessed as described herein, prior to determining the actual treatment or prior to prescribing anti-TNFα therapy. In one embodiment, an intestinal biopsy of an IBD patient as described herein is assessed as described herein, upon failure of anti-TNFα therapy. In one embodiment, an intestinal biopsy of an IBD patient as described herein is assessed as described herein, during anti-TNFα therapy. In one embodiment, plasma cells comprise macrophages. In one embodiment, plasma cells comprise inflammatory macrophages.

[075] The present invention provides means for identifying altered abundance of plasma cells and inflammatory macrophages in pretreatment intestinal biopsies of anti-TNFα responders versus non-responders.

[076] In one embodiment, significantly high plasma cell numbers in anti-TNFα non-responders were validated in IBD patients naive to anti-TNFα therapy. In one embodiment, significantly is at least 10% higher plasma cell numbers in anti-TNFα non-responders. In one embodiment, significantly is at least 20% higher plasma cell numbers.

[077] Pathway analysis of the cell-adjusted differentially expressed genes in biopsy between responders and non-responders suggests an upregulation of the TREM1 and CCR2-CCL7 axes in non-responders.

[078] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to an IBD patient, comprising obtaining from the IBD patient an intestinal biopsy and analyzing the intestinal biopsy for the expression TREM1 and/or CCR2-CCL7, wherein an elevated expression of TREM1 and/or CCR2-CCL7 beyond a threshold value of

TREM1 and/or CCR2-CCL7 expression in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is ineffective, thereby determining the effectiveness of an anti- TNFα therapy to an IBD patient.

[079] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to an IBD patient, comprising obtaining from the IBD patient an intestinal biopsy and analyzing the intestinal biopsy for the expression TREM1 and/or CCR2-CCL7, wherein an expression of TREM1 and/or CCR2-CCL7 beyond the range of expression of TREM1 and/or CCR2-CCL7 expression in control intestinal specimen derived from IBD patient responding to anti- TNFα therapy, is indicative that the anti-TNFα therapy is ineffective, thereby determining the effectiveness of an anti- TNFα therapy to an IBD patient.

[080] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to an IBD patient, comprising obtaining from the IBD patient an intestinal biopsy and analyzing the intestinal biopsy for the expression TREM1 and/or CCR2-CCL7, wherein an elevated expression of TREM1 and/or CCR2-CCL7 beyond a threshold value of TREM1 and/or CCR2-CCL7 expression in control intestinal specimen derived from IBD patient responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is ineffective, thereby determining the effectiveness of an anti-TNFα therapy to an IBD patient.

[081] In one embodiment, provided herein a method for determining the effectiveness of an anti-TNFα therapy to a patient suffering from inflammation, comprising obtaining from the patient a blood sample and measuring the expression of TREM1, wherein an expression of TREM1 below the range or threshold of expression of TREM1 expression in control blood specimen derived from patients responding to anti-TNFα therapy, is indicative that the anti-TNFα therapy is ineffective, thereby determining the effectiveness of an anti-TNFα therapy to a patient suffering from inflammation. In one embodiment, analyzing the expression is quantifying the expression, measuring the expression and/or assessing the expression. In one embodiment, a blood sample is a peripheral blood sample.

[082] In one embodiment, plasma cell numbers are quantified by staining the intestinal biopsy by IHC staining. In one embodiment, the intestinal biopsy is a paraffin-embedded intestinal biopsy. In one embodiment, the intestinal biopsy is obtained during colonoscopy.

[083] In some embodiments, the method of the invention further comprises administering the anti-TNFα therapy to a subject indicated to be suitable for the anti-TNFα therapy. In some embodiments, the method of the invention further comprises administering to a subject indicated to be unsuitable for the anti-TNFα therapy an increased dose of the anti-TNFα therapy. In some embodiments, the increased dose is above the standard dose. In some embodiments, increased dose is increased over the doses delivered in the studies whose data was compiled in the analyses described herein. In some embodiments, the increased dose is increased by at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%. Each possibility represents a separate embodiment of the invention. In some embodiments, the method of the invention further comprises administering a non-anti-TNFα anti-inflammation therapy to a subject suffering from inflammation and indicated to be unsuitable for anti-TNFα therapy. In some embodiments, the non-anti-TNFα anti-inflammation therapy is an anti-IBD therapy. In some embodiments, the non-anti-TNFα anti-inflammation therapy is an anti-RA therapy. As the subject has now been determined to be unsuitable for anti-TNFα therapy an alternative inflammation therapy is indicated and may be administered. Examples of inflammation therapies that are not anti-TNFα therapies include, but are not limited to, steroids, NSAIDs, disease-modifying antirheumatic drugs, azathioprine, 4-aminosalicylic acid and derivatives thereof, anti-IL-23 therapy, anti-IL-17therapy, JAK inhibitors, anti-IL-6R therapy, methotrexate, thiopurines and fecal microbiota transplant. In some embodiments, the non-anti-TNFα anti-IBD therapy comprises blocking at least one of CCR2, CCR5 and CXCR3. In some embodiments, the non-anti-TNFα anti-IBD therapy comprises administering a CCR2 and/or CCL7 antagonist. In some embodiments, the non-anti-TNFα anti-inflammation therapy comprises blocking at least one of CCR2, CCR5 and CXCR3. In some embodiments, the non-anti-TNFα anti-inflammation therapy comprises administering a CCR2 and/or CCL7 antagonist.

[084] By another aspect, there is provided a use of a TREM1 detecting agent for determining serum TREM1 levels in a subject and determining suitability of the subject for treatment with an anti-TNFα therapy. In some embodiments, the TREM1 detecting agent is a serum TREM1 detecting agent. In some embodiments, the detecting agent is an anti-TREM1 antibody. In some embodiments, the detecting agent is a nucleic acid molecule that selectively binds and hybridizes to TREM1 mRNA or a TREM1 gene product. In some embodiments, the detecting agent is primers for PCR amplification of TREM1 or a portion thereof. In some embodiments, the

detecting agent is a nucleic acid probe for TREM1 or a portion thereof. In some embodiments, the detecting agent is part of a chip, bead or array. The terms “array” or “microarray” or “biochip” or “chip” as used herein refer to articles of manufacture or devices comprising an immobilized target elements, each target element comprising a “clone,” “feature,” “spot” or defined area comprising a particular composition, such as a biological molecule, e.g., a nucleic acid molecule or polypeptide, immobilized to a solid surface.

[085] By another aspect, there is provided a kit comprising a detecting agent for detecting at least one predicative factor disclosed herein. In some embodiments, the kit comprises a detecting agent for TREM1, a detecting agent for CCR2, a detecting agent for CCL7, a detecting agent for PCs, a detecting agent for IMs and combinations thereof. In some embodiments, the agents are labeled as for prognostic assessment of responsiveness to anti-TNFα therapy. In some embodiments, the kit comprises antibodies against TREM1, CCR2, CCL7, CD138, and/or CD86 and CD68. Each possibility represents a separate embodiment of the invention. In some embodiments, the kit comprises nucleic acid molecules for measuring TREM1, CCR2, CCL7 or another gene from the CCR2/CCL7 axis. In some embodiments, TREM1 is serum TREM1. In some embodiments, TREM1 is blood TREM1. Nucleic acid sequences of the genes and amino acid sequences of the proteins provided herein can be found on numerous websites known to those skilled in the art, including pubmed.org, ncbi.nlm.nih.gov/genbank, and uniport.org among others.

[086] As used herein, the term "about" when combined with a value refers to plus and minus 10% of the reference value. For example, a length of about 1000 nanometers (nm) refers to a length of 1000 nm+- 100 nm.

[087] It is noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polynucleotide" includes a plurality of such polynucleotides and reference to "the polypeptide" includes reference to one or more polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

[088] In those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

[089] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[090] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

[091] Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

[092] Generally, the nomenclature used herein, and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example,

"Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference. Other general references are provided throughout this document.

MATERIALS AND METHODS

Public IBD and RA gene expression data and reported gene signatures

[093] Colon biopsy gene expression data and patient Infliximab (IFX, an anti-TNFα agent) responsiveness was obtained from published studies (Arijs et al., Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis, Gut 2009, and Arijs, et al., Mucosal gene signatures to predict response to infliximab in patient with ulcerative colitis, Gut 2009) available in Gene Expression Omnibus (GEO) and consist of two cohorts of UC patients (UC-A and UC-B) and one CD patient cohort (CDc). In addition, six reported signatures predictive of IFX response consisting in total of 109 unique genes were gathered from (Arijs et al., IBID 2009; Arijs et al., Predictive value of epithelial gene expression profiles for response to infliximab in Crohn's disease, Inflamm. Bowel Dis., 2010; WO2010/044952; Halloran et al., Molecular patterns in human ulcerative colitis and correlation with response to infliximab, Inflamm. Bowel Dis., 2014) (Table 1, Table 3). Blood gene expression data from RA patients was from GEO12051, GEO33377 and GSE58795.

[094] **Table 1. Colon biopsy discovery cohorts *(in-silico)*- IBD predictive gene signatures and patient cohorts used in the immune contribution analysis and cell type meta-analysis respectively.**

*Signature / Cohort**	*Genes in signature*	*IBD type*	*Response***		*GEO dataset*	Associated original publication
			R	*NR*		
UC-A	20	UC	8	16	GSE14580	[10]
UC-B UC-B-knn IRRAT	20 19 29	UC	12	10	GSE12251	[14,16,17]
UC-AB^	53	UC	8 12	16 10	GSE14580 GSE12251	[10]
CDc	20	CD	12	7	GSE16879	[15]

* (Details of the previously reported gene signatures, grouped according to the cohort in which they were identified to be predictive of IFX response). Underlined names being used as cohort names.

** R/NR- Responder/Non-responder number in each cohort

^ Signature defined from overlap between cohort UC-A and UC-B

[095] **Table 3. Previously reported gene signatures predictive of response to anti-TNFα.** List of the gene signatures used in the analysis shown in Figure 2. For each signature, we provide its member genes and their respective annotations.

Signature	ENTREZID	Symbol	Signature	ENTREZID	Symbol
UC_A	3598	IL13RA2	UC_B	366	AQP9
UC_A	942	CD86	UC_B	7097	TLR2
UC_A	2908	NR3C1	UC_B	200315	APOBEC3A
UC_A	25907	TMEM158	UC_B	2357	FPR1
UC_A	4982	TNFRSF11B	UC_B	3772	KCNJ15
UC_A	8490	RGS5	UC_B	2210	FCGR1B
UC_A	969	CD69	UC_B	604	BCL6
UC_A	23111	SPG20	UC_B	728	C5AR1
UC_A	3488	IGFBP5	UC_B	2710	GK
UC_A	7474	WNT5A	UC_B	8843	HCAR3
UC_A	29940	DSE	UC_B	3579	CXCR2
UC_A	2182	ACSL4	UC_B	57214	CEMIP
UC_A	6781	STC1	UC_B_knn	54210	TREM1
UC_A	9839	ZEB2	UC_B_knn	5008	OSM
UC_A	6372	CXCL6	UC_B_knn	50486	G0S2
UC_A	5937	RBMS1	UC_B_knn	5142	PDE4B
UC_A	1910	EDNRB	UC_B_knn	604	BCL6
UC_A	4319	MMP10	UC_B_knn	9586	CREB5
UC_A	23266	ADGRL2	UC_B_knn	3553	IL1B
UC_A	30817	ADGRE2	UC_B_knn	1441	CSF3R
UC_AB	2182	ACSL4	UC_B_knn	7097	TLR2
UC_AB	586	BCAT1	UC_B_knn	728	C5AR1
UC_AB	6351	CCL4	UC_B_knn	2219	FCN1
UC_AB	50856	CLEC4A	UC_B_knn	353514	LILRA5
UC_AB	1303	COL12A1	UC_B_knn	3772	KCNJ15
UC_AB	1439	CSF2RB	UC_B_knn	2268	FGR
UC_AB	6374	CXCL5	UC_B_knn	11024	LILRA1
UC_AB	6372	CXCL6	UC_B_knn	2713	GK3P
UC_AB	116496	FAM129A	UC_B_knn	8843	HCAR3
UC_AB	2207	FCER1G	UC_B_knn	2358	FPR2
UC_AB	2212	FCGR2A	UC_B_knn	10135	NAMPT
UC_AB	2252	FGF7	CDc	4332	MNDA
UC_AB	169792	GLIS3	CDc	6283	S100A12
UC_AB	3488	IGFBP5	CDc	3598	IL13RA2
UC_AB	10261	IGSF6	CDc	3589	IL11
UC_AB	3589	IL11	CDc	2213	FCGR2B
UC_AB	3598	IL13RA2	CDc	9536	PTGES
UC_AB	3569	IL6	CDc	4688	NCF2
UC_AB	3575	IL7R	CDc	10288	LILRB2
UC_AB	3624	INHBA	CDc	3569	IL6
UC_AB	51088	KLHL5	CDc	7130	TNFAIP6

UC_AB	3937	LCP2	CDc	117289	TAGAP
UC_AB	10859	LILRB1	CDc	6515	SLC2A3
UC_AB	10288	LILRB2	CDc	2219	FCN1
UC_AB	23643	LY96	CDc	6279	S100A8
UC_AB	79772	MCTP1	CDc	60675	PROK2
UC_AB	4311	MME	CDc	2357	FPR1
UC_AB	4314	MMP3	CDc	597	BCL2A1
UC_AB	5069	PAPPA	CDc	50486	G0S2
UC_AB	5142	PDE4B	CDc	929	CD14
UC_AB	51050	PI15	CDc	6280	S100A9
UC_AB	51334	PRR16	IRRAT	8754	ADAM9
UC_AB	5743	PTGS2	IRRAT	9510	ADAMTS1
UC_AB	64407	RGS18	IRRAT	9590	AKAP12
UC_AB	8490	RGS5	IRRAT	1004	CDH6
UC_AB	6280	S100A9	IRRAT	1520	CTSS
UC_AB	64092	SAMSN1	IRRAT	1958	EGR1
UC_AB	6401	SELE	IRRAT	2123	EVI2A
UC_AB	5054	SERPINE1	IRRAT	2214	FCGR3A
UC_AB	6515	SLC2A3	IRRAT	2353	FOS
UC_AB	29887	SNX10	IRRAT	3690	ITGB3
UC_AB	9021	SOCS3	IRRAT	3694	ITGB6
UC_AB	5552	SRGN	IRRAT	3934	LCN2
UC_AB	6781	STC1	IRRAT	4057	LTF
UC_AB	117289	TAGAP	IRRAT	84465	MEGF11
UC_AB	7035	TFPI	IRRAT	64332	NFKBIZ
UC_AB	7980	TFPI2	IRRAT	4837	NNMT
UC_AB	7096	TLR1	IRRAT	10562	OLFM4
UC_AB	3371	TNC	IRRAT	9180	OSMR
UC_AB	4982	TNFRSF11B	IRRAT	51050	PI15
UC_AB	7474	WNT5A	IRRAT	5788	PTPRC
UC_AB	30817	ADGRE2	IRRAT	5806	PTX3
UC_AB	23266	ADGRL2	IRRAT	5918	RARRES1
UC_B	4982	TNFRSF11B	IRRAT	6279	S100A8
UC_B	6781	STC1	IRRAT	12	SERPINA3
UC_B	1441	CSF3R	IRRAT	6590	SLPI
UC_B	3553	IL1B	IRRAT	6648	SOD2
UC_B	5648	MASP1	IRRAT	1462	VCAN
UC_B	5743	PTGS2	IRRAT	169693	TMEM252
UC_B	50486	G0S2	IRRAT	4541	ND6
UC_B	5008	OSM			

[096] For initial assessment of cell-type expression pattern of these signature genes a combined cell type gene expression matrix was created from 130 sorted cell-type samples from IRIS and the Human body index (GSE7307), each dataset was normalized, batch corrected and standardized by z-score. Signature genes were enumerated to cell-types and lineages by assigning the expression of each gene to the top three most expressing subpopulations (Table 4).

[097] **Table 4. Compendium of sorted cell expression profiles. Immune contribution to signature gene expression.** Results of the preliminary analysis that derived overall cellular origin of all previously reported signature genes.

SYMBOL	CD 8	CD 4	CD4 m	Ba	CD4 ma	NK	CD PC	4a	Nka	B	8a	mDC	C	mDCa	Bm	Mo	Ma	N	L.B	L.C	L.M	L.N	L.NK	L.T
IL13RA2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	1	0	0	2	0	0
CD86	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	1	0	0	0
NR3C1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	2	0
TMEM158	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	1	0	0	0	1	0	0	3
TNFRSF11 B	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	3	2	0	1	3	0	0
RGS5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	3	2	1	0	3	0	0
CD69	0	0	3	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1
SPG20	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	2	3	0	1	2	0	0
IGFBP5	0	0	0	0	0	0	3	0	0	0	0	0	1	0	0	0	0	2	3	1	0	2	0	0
WNT5A	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0
DSE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0
ACSL4	0	0	0	0	0	0	0	3	0	0	0	0	0	2	0	0	1	0	0	0	1	0	0	3
STC1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2	0	0	1	0	0
ZEB2	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	0	1	0	0	0
CXCL6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	2	3	0	1	2	0	0
RBMS1	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	3	0	0	0	3	2	1
EDNRB	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	3	1	2	0	3	0	0
MMP10	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	2	0	0	0	2	0	0	1
ADGRL2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	3	1	2	0	3	0	0
ADGRE2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0
BCAT1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	2
CCL4	0	0	0	0	0	0	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1
CLEC4A	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	3	0	0	1	3	0	0
CSF2RB	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	2	1	0	0
CXCL5	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	1	0	0	0
FAM129A	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	2	1	0	0
FCER1G	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0	0	1	0	3	0
FCGR2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0

FGF7	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2	0	0	1	0	0	0	0	0	0	0
IGSF6	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
IL11	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2	0	0	0	1	0	0	0	0	0	0
IL6	0	0	0	2	0	0	0	0	0	0	3	0	0	1	0	2	0	1	0	0	0	0	0	0	0
IL7R	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
INHBA	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	3
KLHL5	0	0	0	1	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	1
LCP2	0	0	0	0	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
LILRB1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	1	3	0	0	0	0	0	0
LILRB2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	1	3	0	0	0	0	0	0
LY96	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	2	1	0	0	0	0	0	0	0
MCTP1	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
MME	0	0	0	0	0	0	0	2	0	0	0	0	0	1	2	0	0	1	0	0	0	0	0	0	0
MMP3	0	0	0	0	0	0	0	0	0	0	0	2	0	3	1	2	0	3	1	0	0	0	0	0	0
PAPPA	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2	0	0	1	0	0	0	0	0	0	0
PDE4B	0	0	3	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3
PI15	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2	0	0	1	0	0	0	0	0	0	0
PRR16	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	2	1	0	0	0	0	0	0	0
PTGS2	0	0	0	0	0	3	0	0	0	0	0	0	0	1	2	0	0	1	2	0	0	0	0	0	3
S100A9	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	2	1	0	0	0	0	0	0
SAMSN1	0	0	0	3	0	0	2	0	0	0	1	0	0	0	0	3	0	1	0	0	0	0	0	0	2
SELE	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	1	0	0	2	0	0	0	0	0	0
SERPINE1	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	1	0	0	0	0	0	0	3
SLC2A3	0	0	0	0	0	0	3	0	0	0	0	0	0	2	1	0	0	2	1	0	0	0	0	0	3
SNX10	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	2	0	0	0	0	0	0
SOCS3	0	0	3	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	2	0	0	0	0	0	3
SRGN	0	0	0	0	0	0	0	0	0	2	0	0	3	0	0	0	0	1	0	0	0	3	1	0	2
TFPI	0	0	0	0	0	0	0	0	0	0	3	1	0	0	2	0	0	3	1	0	0	1	0	0	0
TFPI2	0	0	0	1	0	0	0	0	0	0	0	3	0	0	2	0	1	0	2	0	0	2	0	0	0
TLR1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0	1	0	0
TNC	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	2	3	1	0	2	0	0	0	0
CSF3R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	2	1	0	0	0	0
IL1B	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	1	0	0	0	0	2

MASP1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	2	0	0	1	0	0
G0S2	0	0	0	0	0	0	0	0	0	0	2	0	0	3	1	0	0	2	1	0	0	0
OSM	0	0	0	0	3	0	0	2	0	0	0	0	0	1	0	0	0	1	0	2	0	3
AQP9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	2	0	0	0
TLR2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	1	3	0	0	0
FPR1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	2	1	0	0	0
KCNJ15	0	0	0	0	0	0	0	0	0	0	0	3	2	0	1	3	0	2	1	0	0	0
FCGR1B	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	1	3	0	0	0
BCL6	0	0	0	0	0	0	0	0	0	3	0	0	0	2	1	0	0	2	1	0	0	0
C5AR1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0	0
GK	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	2	0	0	0
HCAR3	0	0	0	0	0	0	0	0	0	2	0	0	0	3	1	0	0	3	1	0	0	2
CXCR2	0	0	0	0	0	3	0	0	0	0	2	0	0	0	1	0	0	2	1	3	0	0
CEMIP	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	3	2	0	1	3	0	0
TREM1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0	0
FCN1	0	3	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	1	2	0	0	3
LILRA5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	1	3	0	0	0
FGR	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	1	3	0	0	0
LILRA1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	1	3	0	0	0
GK3P	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2
FPR2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0	0
NAMPT	0	0	0	0	3	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0	3
MNDA	0	0	0	0	0	0	0	0	0	3	0	0	0	2	0	1	0	0	2	1	0	0
S100A12	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	2	1	0	0
FCGR2B	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	2	0	1	0	0	0
PTGES	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3	1	2	0	3	1	0	0
NCF2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	2	1	0	0
TNFAIP6	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	3	0	0	1	3	0	0
S100A8	0	0	0	0	0	3	0	0	0	0	0	0	0	2	0	1	0	0	2	1	3	0
BCL2A1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1	2	0	0	1	2	0	3
CD14	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	1	3	0	0
ADAMTS1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	1	0
AKAP12	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	1	3	0	0	0

CDH6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2	0	0	1	0	0
CTSS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	1	2	0	0
EGR1	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	3	0	0	0	1	3	0	0	2
EVI2A	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	3	0	1	0	0	3	1	0	2
FOS	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2	0	1	0	3	2	1	0	0
ITGB3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	2	0	1	0	0	0
LCN2	0	0	0	0	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	3	0	0	1	0
LTF	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1	3
NNMT	0	0	0	0	0	0	3	0	0	0	0	0	1	0	0	0	0	2	3	1	0	2	0	0
OSMR	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	2	3	1	0	2	0	0
PTPRC	3	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	2	3
PTX3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	3
RARRES1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	3	0	0	0	1	2	0	0	0
SERPINA3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	0	0	2	0	0
VCAN	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	3	1	0	0	0

The following abbreviations are used: CD4m, CD4 memory cells; Ba, B cells activated; CD4ma, CD4 memory cells activated; NK, Natural Killer cells; PC, Plasma Cells; CD4a, CD4 cells activated; NKa, Natural Killer cells activated; B, B cells; CD8a, CD8 cells activated; mDC, myeloid Dendritic Cells; C, Colon; mDCa, myeloid Dendritic Cells activated; Bm, B memory cells; Mo, Monocytes; Ma, Macrophages; N, Neutrophils; L.B, B cell lineage; L.C, Colon lineage; L.M, Myeloid lineage; L.N, Neutrophil lineage; L.NK, Natural Killer lineage; L.T, T cell lineage.

Computational deconvolution and meta-analysis

[098] To estimate cell subset proportions, a linear regression framework was used, in which individual samples were regressed based on a characteristic expression of marker genes expressed in 17 cell-types (See Supplementary Table S4 of the priority document US provisional application 62/581960 and of Gaujoux et al., 2018, Cell-centered meta-analysis reveals baseline predictors of anti-TNFa non-response in biopsy and blood of patients with IBD, *Gut*, April 4). The resulting output of this procedure was an estimated frequency of each cell subset in each sample. These were then re-scaled into proportions, asinh-transformed and compared between groups within each cohort using Wilcoxon rank sum test. Meta-analysis across three cohorts was performed using Fisher's combined probability test and corrected for multiple comparisons. Cell types having nominal p-values ≤ 0.05 in at least 2 cohorts and a combined FDR ≤ 0.05 were selected for further analysis. ROC analyses were performed by scoring individuals based on the mean gene expression scores per signature, whereas for cellular biomarkers we used estimated proportions for individual cell subsets or the average standardized proportions for combined signatures.

Patients in the validation cohorts

[099] Analyses was performed of archival slides of IBD patients in two cohorts: First, in a preliminary cohort 20 biopsies from 16 IBD patients (8 Crohn's Disease, 7 Ulcerative Colitis, 1 IBDU, 3 patients had more than one biopsy) treated at the gastroenterology department of the Rambam Health Care Campus (RHCC) were included. Patient characteristics are represented in Table 2. Clinical response was defined by the attending physician as clinical and/or endoscopic improvement of IBD-related symptoms coupled with a decision to continue IFX therapy, at least 14 weeks after treatment initiation. Non-response was defined by lack of improvement or aggravation of clinical or endoscopic presentation or disease symptoms coupled with therapy change. Colonic biopsies were obtained during endoscopy performed prior to first IFX treatment. Biopsies were taken from inflamed and/or uninflamed areas of the colon or ileum.

[0100] Second, in a primary validation cohort, biopsies from 61 IBD patient from RHCC and Tel-Aviv Sourasky Medical Center (TASMC) were obtained. For this cohort, a decision algorithm was used to determine response (Materials and Methods).

[0101] For blood validation by gene expression analysis, whole blood was obtained at baseline from 28 IBD patients treated with IFX in PaxGene tubes. Response was defined using the same algorithm. RNA was extracted and assayed using Affymetrix Clariom D chips.

[0102] **Table 2. Clinical and demographic characteristics of patients included in the study**

Characteristics	IFX non-responders*	IFX responders*
Cohort validation 1 - biopsy IHC		
Biopsy number	8	12
IBD type (CD/UC/IBDU)	3/4/1	8/4/0
Analyzed biopsy (normal/inflamed)	3/5	3/9
Gender (M/F)	3/5	3/9
Age (years)	40.3±12.5	47.2±19.1
Disease duration (years)	12.5±4.3	17±4.2
Age at diagnosis (A1/A2/A3/NA)^	1/3/0/4	0/4/4/4
Location (L1/L2/L3/NA) for CD^^	0/1/1/1	0/3/3/2
Location (E1/E2/E3/NA) for UC^^^	0/1/2/1	0/0/3/1
Behavior (B1/B2/B3/NA) for CD^^^^	0/0/2/1	2/0/1/5
Perianal disease (yes/no)	3/5	1/11
Biopsy location**	2/0/1/1/2/0/2	3/2/2/1/2/0/2
Concurrent therapy (steroids/thiopurine/antibiotics)***	1/2/0	2/1/0
Past anti-TNFα (Adalimumab) exposure (yes/no)	2/6	1/11

Time range prior to treatment (month)	0 (0-3)	7 (0-93)
Cohort validation 2 - biopsy IHC		
Medical center (RHHC/TASMC)	6/17	15/14
Patient number	23	29
IBD type (CD/UC)	9/14	20/9
Analyzed biopsy (normal/inflamed)	1/22	2/27
Gender (M/F)	11/12	12/17
Age (years)	44.3±11.7	34±13
Disease duration (years)	14.8±9.6	9.6±7.8
Age at diagnosis (A1/A2/A3)^	3/13/7	4/22/3
Location (L1/L2/L3) for CD^^	0/3/6	0/9/11
Location (E1/E2/E3) for UC^^^	0/7/7	0/3/6
Behavior (B1/B2/B3) for CD^^^^	7/0/2	16/4/0
Perianal disease (yes/no)	3/20	10/19
Biopsy location**	2/0/6/9/5/1	2/0/3/5/0/4
Concurrent therapy (steroids/thiopurine/antibiotics)***	10/5/2	13/12/2
Past anti-TNFα (Adalimumab) exposure (yes/no)	1/22	0/29
Time range prior to treatment (median month)	1 (0-3)	2 (0-10)
Cohort 3 - blood gene expression		
Patient number	5	17
IBD type (CD/UC)	5/0	17/0
Gender (M/F)	2/3	11/6
Age (years)	40±14.9	36.1±13.6

Disease duration (years)	10.4±9	8.5±8.9
Age at diagnosis (A1/A2/A3)^	2/2/1	1/13/3
Location (L1/L2/L3)^^	1/1/3	8/1/8
Behavior (B1/B2/B3)^^^^	1/2/2	5/7/5
Perianal disease (yes/no)	3/2	3/14
Concurrent therapy (steroids/thiopurine/antibiotics)	2/5/0	9/11/0
HBI****	7.2±8.6 (0-22)	3.6±3.5 (0-13)
Calprotectin****	1765 (30-1800)	604 (30-1800)
CRP****	24.8±29.5 (3.1-83)	17.8±29.3 (1.6-126)

* According to response classification as detailed in "Methods"

** Ascending colon/transverse colon/descending colon/sigmoid/rectum/non-informed colonic segment/small intestine

*** For cohorts 1-2- at the time when biopsy was taken. For cohort 3- concurrent with IFX

**** At baseline

^ Montreal classification - Age <16 years/17–40 years/>40 years.

^^ Montreal classification - Ileal/colonic/ileocolonic

^^^ Montreal classification - Proctitis/left-side/pancolitis

^^^^ Montreal classification- Inflammatory/structuring/penetrating

Immuno-histochemistry (IHC)

[0103] Formalin-fixed slides of paraffin-embedded colon or ileum tissues, sectioned at 4 µm, were immunostained for the expression of plasma cells (CD138+, obtained from Serotec, clone B-A38, dilution 1:250) in both cohorts and for inflammatory macrophages (CD68+ and CD86+ from Abcam, clone EP1158Y, 1:100) in the preliminary cohort. Slides were deparaffinized and

Polink-1 HRP Broad Spectrum DAB Detection Kit (GBI labs) was used for detection according to the manufacturer's instructions.

Staining data analysis

[0104] Slides were interpreted by an expert pathologist blind to response attributes. A specific cell abundance categorical index between 0 and 3 was determined by the pathologist for plasma cells and inflammatory macrophages. A minimal number of cells was scored as '0', the highest abundance which was seen within all slides was scored as '3'. In addition, slides were coded and scanned by an automatic slide scanner 250 Flash. Intestinal crypts, muscles and lymphatic follicles were excluded in each tissue to evaluate cell proportions in the stroma only. Whole tissue area quantification was performed using ImagePro Premier software V.9.3. CD138+ staining Area was determined with the same color cut-off for all patients, and the ratio between CD138+ staining and the whole-tissue area (in pixels) was evaluated by a researcher blind to response attributes.

Predictive gene signatures

[0105] 6 reported gene signatures were gathered from their respective original publication (Table 1) and mapped to official gene symbols using annotation package *org.Hs.eg.db* (version 3.3.0). Altogether, the signatures counted 126 unique genes, and were named according to the patient cohort from which they were derived (Table 3). Briefly, signatures UC-A and UC-B were defined as the top 20 differentially expressed genes found in two independent cohorts of UC patients, which were labelled A and B as per the original publication (Kaplan, The global burden of IBD: from 2015 to 2025, Nat. Rev. Gastroenterol Hepatol., 2015); Signature UC-AB was defined as the overlap between all differentially expressed genes found in the same UC cohorts A and B, and comprised a total of 53 unique genes (Kaplan, IBID); Signature UC-B-knn was also derived from UC cohort B, but using a different methodology based on a k-nearest-neighbor classifier; Signature CDc was identified in CD patients from colon biopsies (Targan et al., A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N. Engl. J. Med., 2997) (cohort CDc). The remaining signature named IRRAT was taken from the kidney transplant study (Ben-Horin et al., Optimizing anti-TNF treatments in inflammatory bowel disease, Autoimmun. Rev., 2014).

Cell type expression pattern of predictive gene signatures

[0106] CEL files from sorted cell type samples from IRIS (GSE22886) and the Human body index (GSE7307) were normalized separately using frma. In GSE7307, the profiles from all immune cells (32 profiles from monocyte, T cell and B cell lineages) and colon tissues (2 profiles) were extracted. A combined cell type gene expression matrix was created, it was corrected for dataset of origin effects using Combat, and subsequently probe-sets were averaged into genes. This resulted in the creation of an expression matrix of 130 expression profiles (Table 5), which were standardized using z-scores and averaged into major cell lineages split into resting and activation/memory state.

[0107] **Table 5. Compendium of sorted cell expression profiles.** Description of the GEO data used to compute the immune contribution to previously reported signature genes

GSM	GSE	Title	GSM	GSE	Title
GSM565269	GSE22886	CD8Tcell-N0-1	GSM565334	GSE22886	Monocyte-Day0-5
GSM565270	GSE22886	CD8Tcell-N0-2	GSM565335	GSE22886	Monocyte-Day0-6
GSM565271	GSE22886	CD8Tcell-N0-3	GSM565336	GSE22886	Monocyte-Day0-7
GSM565272	GSE22886	CD8Tcell-N0-4	GSM565337	GSE22886	Monocyte-Day0-8
GSM565273	GSE22886	CD4Tcell-N0-1	GSM565338	GSE22886	Monocyte-Day0-9
GSM565274	GSE22886	CD4Tcell-N0-2	GSM565339	GSE22886	Monocyte-Day0-10
GSM565275	GSE22886	CD4Tcell-N0-3	GSM565340	GSE22886	Monocyte-Day0-11
GSM565276	GSE22886	CD4Tcell-Th1-restimulated12hour-1	GSM565341	GSE22886	Monocyte-Day0-12
GSM565277	GSE22886	CD4Tcell-Th1-restimulated12hour-2	GSM565342	GSE22886	Monocyte-Day1-1
GSM565278	GSE22886	CD4Tcell-Th1-restimulated48hour-1	GSM565343	GSE22886	Monocyte-Day1-2
GSM565279	GSE22886	CD4Tcell-Th1-restimulated48hour-2	GSM565344	GSE22886	Monocyte-Day1-3
GSM565280	GSE22886	CD4Tcell-Th1-restimulated48hour-3	GSM565345	GSE22886	Monocyte-Day1-4
GSM565281	GSE22886	CD4Tcell-Th2-restimulated12hour-1	GSM565346	GSE22886	Monocyte-Day1-5

GSM565282	GSE22886	CD4Tcell-Th2-restimulated12hour-2	GSM565347	GSE22886	Monocyte-Day1-6
GSM565283	GSE22886	CD4Tcell-Th2-restimulated12hour-3	GSM565348	GSE22886	Monocyte-Day1-7
GSM565284	GSE22886	CD4Tcell-Th2-restimulated48hour-1	GSM565349	GSE22886	Monocyte-Day1-8
GSM565285	GSE22886	CD4Tcell-Th2-restimulated48hour-2	GSM565350	GSE22886	Monocyte-Day1-9
GSM565286	GSE22886	CD4Tcell-Th2-restimulated48hour-3	GSM565351	GSE22886	Monocyte-Day1-10
GSM565287	GSE22886	MemoryTcell-RO-unactivated-1	GSM565352	GSE22886	Monocyte-Day1-11
GSM565288	GSE22886	MemoryTcell-RO-unactivated-2	GSM565353	GSE22886	Monocyte-Day1-12
GSM565289	GSE22886	MemoryTcell-RO-unactivated-3	GSM565354	GSE22886	Monocyte-Day7-1
GSM565290	GSE22886	MemoryTcell-RO-activated-1	GSM565355	GSE22886	Monocyte-Day7-2
GSM565291	GSE22886	MemoryTcell-RO-activated-2	GSM565356	GSE22886	Monocyte-Day7-3
GSM565292	GSE22886	MemoryTcell-RO-activated-3	GSM565357	GSE22886	Monocyte-Day7-4
GSM565293	GSE22886	NKcell-control-1	GSM565358	GSE22886	Monocyte-Day7-5
GSM565294	GSE22886	NKcell-control-2	GSM565359	GSE22886	Monocyte-Day7-6
GSM565295	GSE22886	NKcell-control-3	GSM565360	GSE22886	Monocyte-Day7-7
GSM565296	GSE22886	NKcell-control-4	GSM565361	GSE22886	Monocyte-Day7-8
GSM565297	GSE22886	NKcell-IL2stimulated-1	GSM565362	GSE22886	Monocyte-Day7-9
GSM565298	GSE22886	NKcell-IL2stimulated-2	GSM565363	GSE22886	Monocyte-Day7-10
GSM565299	GSE22886	NKcell-IL2stimulated-3	GSM565364	GSE22886	Monocyte-Day7-11
GSM565300	GSE22886	NKcell-IL2stimulated-4	GSM565365	GSE22886	Monocyte-Day7-12
GSM565301	GSE22886	NKcell-IL2stimulated-5	GSM565366	GSE22886	DendriticCell-Control-1
GSM565302	GSE22886	NKcell-IL15stimulated-1	GSM565367	GSE22886	DendriticCell-Control-2
GSM565303	GSE22886	NKcell-IL15stimulated-2	GSM565368	GSE22886	DendriticCell-Control-3

GSM565304	GSE22886	NKcell-IL15stimulated-3	GSM565369	GSE22886	DendriticCell-Control-4
GSM565305	GSE22886	NKcell-IL15stimulated-4	GSM565370	GSE22886	DendriticCell-Control-5
GSM565306	GSE22886	NKcell-IL15stimulated-5	GSM565371	GSE22886	DendriticCell-Control-6
GSM565307	GSE22886	NKcell-IL15stimulated-6	GSM565372	GSE22886	DendriticCell-LPSstimulated-1
GSM565308	GSE22886	Bcell-naïve-1	GSM565373	GSE22886	DendriticCell-LPSstimulated-2
GSM565309	GSE22886	Bcell-naïve-2	GSM565374	GSE22886	DendriticCell-LPSstimulated-3
GSM565310	GSE22886	Bcell-naïve-3	GSM565375	GSE22886	DendriticCell-LPSstimulated-4
GSM565311	GSE22886	Bcell-naïve-4	GSM565376	GSE22886	DendriticCell-LPSstimulated-5
GSM565312	GSE22886	Bcell-naïve-5	GSM565377	GSE22886	DendriticCell-LPSstimulated-6
GSM565313	GSE22886	Bcell-naïve-6	GSM565378	GSE22886	Neutrophil-Resting-1
GSM565314	GSE22886	Bcell-naïve-7	GSM565379	GSE22886	Neutrophil-Resting-2
GSM565315	GSE22886	Bcell-Memory_IgG_IgA-1	GSM565380	GSE22886	Neutrophil-Resting-3
GSM565316	GSE22886	Bcell-Memory_IgG_IgA-2	GSM565381	GSE22886	Neutrophil-Resting-4
GSM565317	GSE22886	Bcell-Memory_IgG_IgA-3	GSM565382	GSE22886	Neutrophil-Resting-5
GSM565318	GSE22886	Bcell-Memory_IgG_IgA-4	GSM176285	GSE7307	CD8+ 1 Resting
GSM565319	GSE22886	Bcell-Memory_IgM-1	GSM176286	GSE7307	CD8+ AntiCD3+AntiCD28 1 Activated
GSM565320	GSE22886	Bcell-Memory_IgM-2	GSM176283	GSE7307	CD4+ 1 Resting
GSM565321	GSE22886	Bcell-Memory_IgM-3	GSM176284	GSE7307	CD4+ AntiCD3+AntiCD28 1 Activated
GSM565322	GSE22886	Bcell-Memory_IgM-4	GSM176123	GSE7307	Colon Cecum 10 Normal
GSM565323	GSE22886	PlasmaCell-FromPBMC-1	GSM176009	GSE7307	Monocytes Resting 30h 2 Resting

GSM565324	GSE22886	PlasmaCell-FromPBMC-2	GSM176010	GSE7307	Monocytes LPS+Ifng 30h 2 Activated
GSM565325	GSE22886	PlasmaCell-FromPBMC-3	GSM176003	GSE7307	Monocytes Resting 30h 1 Resting
GSM565326	GSE22886	PlasmaCell-FromBoneMarrow-1	GSM176004	GSE7307	Monocytes LPS+Ifng 30h 1 Activated
GSM565327	GSE22886	PlasmaCell-FromBoneMarrow-2	GSM176007	GSE7307	B Cells Resting 30h 2 Resting
GSM565328	GSE22886	PlasmaCell-FromBoneMarrow-3	GSM176008	GSE7307	B Cells AntiCD40+Il4 30H 2 Activated
GSM565329	GSE22886	PlasmaCell-FromBoneMarrow-4	GSM176001	GSE7307	B Cells Resting 30h 1 Resting
GSM565330	GSE22886	Monocyte-Day0-1	GSM176002	GSE7307	B Cells AntiCD40+Il4 30H 1 Activated
GSM565331	GSE22886	Monocyte-Day0-2	GSM175984	GSE7307	Colon Cecum 3 Normal
GSM565332	GSE22886	Monocyte-Day0-3	GSM175983	GSE7307	Colon Cecum 1 Normal
GSM565333	GSE22886	Monocyte-Day0-4	GSM175905	GSE7307	Colon BD Normal

[0108] Previously, others assign signature genes to the most likely contributing cell subpopulations they were detected from in the samples; here each gene was assigned to the three most expressing cell subsets and how many unique genes were assigned to each of 8 major functional cell lineages was counted (Table 4). Since enrichment of cell type expression was looked for, the analysis was restricted to the 122 signatures genes up-regulated in non-responders, of which 109 genes could be mapped to probe-sets in the sorted cell compendium data.

IBD cohorts' gene expression data

[0109] The gene expression data for each IBD cohort used in the deconvolution meta-analysis were obtained from 3 GEO datasets: UC-A from GSE14580, UC-B form GSE12251 and CDc from GSE16879. These datasets contain biopsy gene expression profiles generated from 2 cohorts of UC patients (Cohort *A* and *B* in GSE14580 and GSE12251 respectively), and 1 cohort of CD patients (part of GSE16879). They were originally designed for the discovery of gene

signatures that can predict, at baseline, if a patient is likely to respond to an anti-TNFα treatment (Infliximab). In terms of signatures, all signatures were identified from baseline gene expression differential analysis between responders and non-responders to Infliximab treatment in the same set of 3 IBD cohorts of UC (cohorts UC-A and UC-B) or CD (cohort CDc) patients, exception being the IRRAT signature which, subsequent to the study it originated with, was found to correlate with anti-TNFα response at baseline in the UC-B cohort. In addition, blood gene expression data of IBD patients for whom endoscopic activity was available was obtained (GSE107865, Gaujoux et al., Cell-centred meta-analysis reveals baseline predictors of anti-TNFα non-response in biopsy and blood of patients with IBD, Gut, 2018) in which we assessed the relation of the CCL7-CCR2-TREM1 axis to monitor disease activity.

Signature scores and ROC analysis

[0110] ROC analyses were performed on signature expression scores that summarize, for each sample, the expression level of all the genes in a predictive gene set. Given a gene expression dataset (including data adjusted for proportion variations) and a gene signature/set, the signature score S_j for sample j was computed as:

$$S_j = \frac{1}{n} * \sum_1^n d_i * \log2(g_i)$$

where g_i is the expression level of the i-th gene of the signature in sample j, and d_i is the sign of the difference between its mean expression in non-responders and responders. For adjusted data where negative expression values occurred, we shifted the data by $\tilde{g}_i = g_i - m_s + 1$, where m_s is the minimum expression value amongst the signature genes. ROC curve analysis of cellular biomarkers was computed either directly on estimated proportions for individual cell subsets or on the average standardized proportions (centered, unit-variance) for combined signatures. AUC values were computed using the R package pROC.

[0111] To assess whether the observed drops in AUC could result from the reduction in degrees of freedom incurred by the adjustment procedure itself, the data was repeatedly adjusted with random pairs of cell subset proportions and compared the derived "random" AUCs with the ones obtained using actual estimated proportions (**Fig. 3F**). This showed that all observed AUC

differences were statistically significant (all p-values≤0.018). The adjusted gene expression datasets were also visualized in order according to their respective signature score data, which confirmed that the association between signature scores and treatment response status was lost after adjustment.

Response classification by a decision algorithm

[0112] In patients with CD, clinical remission was defined as cessation of diarrhea and abdominal cramping or, in the cases of patients with fistulas, cessation of fistula drainage and complete closure of all draining fistulas at week 14, coupled with a decision of the treating physician to continue IFX therapy at the current dosing and schedule. Partial response was defined as a reduction in the amount of diarrhea and abdominal cramping, or, in the case of fistula patients, a decrease in the drainage, size, or number of fistulas at last follow-up. In patients with UC, clinical remission was defined as cessation of diarrhea, rectal bleeding, and abdominal cramping at week 14 as indicated in the patient's chart by the treating physician, coupled with a decision of the treating physician to continue IFX therapy at the current dosing and schedule, whereas partial response was defined as a reduction in the amount of diarrhea, rectal bleeding, and abdominal cramping. Outcomes not meeting one of the above definitions were classified as non-response.

[0113] To further stratify the response, patients deemed as partial responders were allocated to a decision tree following these steps: failure to withdraw steroid treatment at week 14 was deemed as therapeutic failure. In patients not treated with steroids, a substantial decrease (>50%) in biomarker dynamics (fecal calprotectin if available and serum CRP when calprotectin was not available), as an indicator of response to treatment. For subjects who were not steroid dependent and exhibited no substantial biomarker dynamics, response was defined according to the clinical state at week 26.

[0114] IFX levels and antibodies to IFX (ATI) measurements were available for 28 of the patients. After reviewing these patients, the responders for which 2 subsequent measurements of IFX level < 3 (μg/ml) were observed prior to week 26 were excluded, assuming their response status was less likely to be IFX related, as were non-responders with measurements of ATI level > 15 (μg/mL), assuming they had secondary loss of response, not related to susceptibility to TNFα blockade. These criteria left 29 responders and 23 non-responders from the two centers.

Immuno-histochemistry markers

[0115] Plasma cell frequencies were examined by CD138+ IHC staining. For inflammatory macrophages, in-silico deconvolution analysis herein relied on a gene expression signature of monocyte derived macrophages bearing typical macrophage morophology and phagocytic activity. Given the disease context, this suggested a bias towards inflammatory macrophage phenotype (M1), as such, the expert pathologist performing the IHC, assessed the co-expression of the CD68 and CD86 as well as cell morphology, as these markers are co-expressed by monocytes and CD86 also in other cell subsets (e.g B and T). Specifically, to account for morphological differences between macrophages and monocytes, mononuclear cells showing broad cytoplasm and oval nucleus were considered as "inflammatory macrophages", while CD68 and CD86-positive monocytes were ignored.

Example 1: Gene expression signatures for anti-TNFα non-response show contributions from distinct immune cell subsets

[0116] Reported gene expression signatures of anti-TNFα response from biopsy show an enrichment for broad immune response categories but have not directly been implicated with particular immune cell subsets. The likely cellular origin of expression of 109 genes extracted from six different reported signatures associated with baseline anti-TNFα non-response was checked (Table 1, Table 3, **Fig. 1A**). To do so, we analyzed the relative expression pattern of these genes across a compendium of assembled profiles from sorted immune cell subsets and normal bulk colon tissue biopsies available in the public domain.

[0117] Clustering the expression profiles of these signature genes across sorted cell subsets suggested that three distinct lineages contribute to non-response to anti-TNFα treatment (**Fig. 1B**): First, myeloid lineage cell subsets expressing 70% of signature genes; second, B-cell lineage cell subsets in which 30% of signature genes were expressed; third, T and NK cells' genes, which together comprised 30% of genes in the collective signature (Table 4). Of note, only 15% of signature genes were denoted as highly expressed in the bulk colon samples and of these, the majority were also noted to be highly expressed in the B-cell lineage. Taken together, the majority of anti-TNF response signature genes are more highly expressed by immune cell subsets, with non-overlapping gene set contributions stemming from myeloid and B-lineage associated cell

subsets. This further suggested that resident or infiltrating leukocyte populations within biopsy tissues could constitute a good baseline predictor of non-response.

Example 2: Meta-analysis identifies cell type proportion differences between response groups at baseline and following treatment

[0118] Bulk gene expression measurements of a tissue may be strongly confounded by variation in cell subsets. Given the distinct immune cell subset associated genes in anti-TNFα response gene signatures, it was hypothesized that the signature-identified genes may be due to variations in cell subset proportions between individuals. To test this hypothesis, a computational gene expression deconvolution approach was used to estimate the relative composition of 17 immune cell subsets in each sample of three publicly available IBD cohorts (Table 1, cohorts UC-A, UC-B and CDc, 65 samples total), from which the previously reported predictive gene signatures were individually derived (Table 4). Differences in cellular proportions between anti-TNFα therapy responders and non-responders were investigated (**Fig. 2A**, Table 6). To ensure robustness of the downstream analyses, only those cell types for which at least 75% of the samples had non-zero estimated proportions were considered and a meta-analysis of cellular proportion differences across all three cohorts was performed by combining p-values for cell subset differences between response groups across cohorts. The analysis identified two cell subsets, inflammatory macrophages and plasma cells, which were significantly different in at least two out of the three discovery cohorts (nominal p-value$\leq$0.05) and passed a combined false discovery rate of 5% (**Fig. 2B, E**). The proportions of both cell subsets were significantly higher in non-responders than in responders. Repeating the same analysis in a second time point, available for UC-A and CDc for 4-6 weeks post-treatment, a significant decrease in both cell subset abundances was observed, which was independent of response in UC, but statistically significant only in responders in CD (FDR$\leq$0.05, **Fig. 2C-D**). Importantly, both cell subset proportions were still significantly higher in non-responders post-treatment (FDR$\leq$0.05, **Fig. 2F**). Overall, this showed that the differences observed at baseline in inflammatory macrophages and plasma cell abundances were maintained after treatment initiation, and that successful response to anti-TNFα was associated with a sharp decrease in inflammatory macrophage and plasma cell abundances.

[0119] **Table 6: Estimated immune cell subset proportions.** Estimated proportions in all cohorts at baseline and after treatment. The following abbreviations are used: B, Baseline; PT, Post-Treatment; R, Responder; NR, Non-Responder.

Cohort	GSM	Time	R/NR	CD4	CD8	CD8a	BalgM	BmlgG	BmlgM	PC	NK	Nka	Mo	IMa	mDC	mDCa	N	Ba
UC-B	GSM307886	B	R	0.00	0.06	0.00	0.00	0.00	0.10	0.19	0.00	0.04	0.10	0.08	0.00	0.30	0.13	
UC-B	GSM307887	B	NR	0.00	0.02	0.01	0.00	0.00	0.08	0.24	0.02	0.01	0.09	0.08	0.07	0.26	0.10	
UC-B	GSM307888	B	R	0.00	0.02	0.00	0.03	0.00	0.33	0.29	0.00	0.00	0.08	0.05	0.00	0.15	0.07	
UC-B	GSM307889	B	R	0.00	0.04	0.00	0.00	0.00	0.06	0.31	0.01	0.00	0.09	0.08	0.03	0.26	0.12	
UC-B	GSM307890	B	R	0.00	0.00	0.00	0.00	0.00	0.07	0.46	0.00	0.00	0.04	0.11	0.05	0.16	0.10	
UC-B	GSM307891	B	NR	0.00	0.00	0.00	0.00	0.00	0.12	0.26	0.01	0.00	0.07	0.15	0.04	0.13	0.22	
UC-B	GSM307893	B	NR	0.00	0.00	0.00	0.00	0.00	0.14	0.28	0.02	0.01	0.08	0.10	0.04	0.21	0.13	
UC-B	GSM307894	B	R	0.00	0.05	0.02	0.00	0.00	0.02	0.20	0.00	0.07	0.09	0.09	0.05	0.28	0.12	
UC-B	GSM307895	B	R	0.00	0.03	0.00	0.00	0.00	0.18	0.29	0.01	0.00	0.06	0.03	0.01	0.27	0.12	
UC-B	GSM307896	B	R	0.07	0.02	0.03	0.00	0.00	0.01	0.08	0.03	0.04	0.17	0.00	0.08	0.30	0.17	
UC-B	GSM307897	B	R	0.00	0.05	0.02	0.07	0.00	0.14	0.14	0.01	0.01	0.09	0.07	0.08	0.21	0.10	
UC-B	GSM307898	B	NR	0.00	0.00	0.00	0.00	0.00	0.19	0.30	0.00	0.00	0.06	0.17	0.06	0.10	0.10	
UC-B	GSM307899	B	R	0.00	0.03	0.00	0.00	0.00	0.20	0.37	0.00	0.00	0.10	0.08	0.01	0.17	0.05	
UC-B	GSM307900	B	R	0.00	0.04	0.00	0.00	0.00	0.04	0.31	0.01	0.00	0.09	0.10	0.05	0.26	0.10	
UC-B	GSM307901	B	NR	0.00	0.00	0.00	0.00	0.00	0.05	0.26	0.00	0.00	0.04	0.22	0.07	0.14	0.22	
UC-B	GSM307902	B	NR	0.00	0.00	0.00	0.00	0.00	0.08	0.23	0.01	0.00	0.06	0.16	0.03	0.14	0.28	
UC-B	GSM307903	B	NR	0.00	0.03	0.00	0.04	0.10	0.07	0.23	0.01	0.01	0.05	0.05	0.03	0.27	0.10	
UC-B	GSM307904	B	NR	0.00	0.00	0.00	0.00	0.00	0.07	0.28	0.01	0.00	0.08	0.12	0.04	0.19	0.20	
UC-B	GSM307905	B	NR	0.00	0.02	0.00	0.00	0.00	0.11	0.28	0.00	0.03	0.08	0.08	0.02	0.24	0.13	
UC-B	GSM307906	B	R	0.00	0.00	0.00	0.00	0.00	0.08	0.37	0.01	0.00	0.07	0.10	0.06	0.18	0.12	
UC-B	GSM307907	B	R	0.05	0.00	0.10	0.00	0.00	0.06	0.12	0.02	0.00	0.17	0.02	0.08	0.23	0.15	
UC-B	GSM307908	B	NR	0.00	0.01	0.00	0.00	0.00	0.04	0.30	0.02	0.00	0.06	0.18	0.00	0.25	0.14	
UC-A	GSM364633	B	R	0.01	0.07	0.07	0.01	0.00	0.00	0.08	0.00	0.13	0.18	0.01	0.00	0.36	0.10	
UC-A	GSM364634	B	R	0.01	0.03	0.00	0.00	0.00	0.01	0.15	0.00	0.14	0.13	0.04	0.00	0.33	0.16	
UC-A	GSM364635	B	R	0.01	0.02	0.07	0.00	0.00	0.00	0.04	0.00	0.14	0.04	0.09	0.00	0.36	0.22	
UC-A	GSM364636	B	R	0.04	0.01	0.10	0.00	0.00	0.00	0.04	0.00	0.14	0.07	0.08	0.00	0.34	0.19	
UC-A	GSM364637	B	R	0.00	0.03	0.03	0.00	0.00	0.06	0.15	0.00	0.13	0.05	0.13	0.00	0.29	0.13	
UC-A	GSM364638	B	R	0.00	0.03	0.04	0.03	0.00	0.09	0.14	0.00	0.10	0.09	0.07	0.00	0.29	0.12	
UC-A	GSM364639	B	R	0.00	0.07	0.04	0.11	0.06	0.01	0.10	0.01	0.02	0.13	0.09	0.00	0.29	0.08	

UC-A	GSM364640	B	R	0.00	0.03	0.07	0.06	0.10	0.04	0.08	0.00	0.10	0.11	0.03	0.00	0.27	0.12
UC-A	GSM364641	B	NR	0.00	0.02	0.00	0.00	0.00	0.01	0.23	0.00	0.11	0.07	0.11	0.00	0.28	0.18
UC-A	GSM364642	B	NR	0.00	0.01	0.06	0.02	0.00	0.09	0.11	0.00	0.07	0.11	0.07	0.00	0.32	0.15
UC-A	GSM364643	B	NR	0.00	0.00	0.03	0.00	0.00	0.21	0.26	0.00	0.04	0.05	0.10	0.00	0.20	0.11
UC-A	GSM364644	B	NR	0.00	0.02	0.01	0.00	0.00	0.03	0.29	0.00	0.10	0.08	0.08	0.00	0.27	0.12
UC-A	GSM364645	B	NR	0.00	0.03	0.03	0.00	0.00	0.00	0.04	0.00	0.16	0.03	0.12	0.00	0.39	0.20
UC-A	GSM364646	B	NR	0.00	0.00	0.00	0.00	0.00	0.22	0.42	0.01	0.00	0.07	0.08	0.02	0.13	0.05
UC-A	GSM364647	B	NR	0.03	0.00	0.10	0.01	0.00	0.02	0.14	0.00	0.07	0.12	0.19	0.00	0.23	0.09
UC-A	GSM364648	B	NR	0.01	0.04	0.10	0.00	0.00	0.00	0.08	0.00	0.07	0.11	0.08	0.00	0.38	0.12
UC-A	GSM364649	B	NR	0.00	0.00	0.00	0.02	0.00	0.23	0.22	0.01	0.02	0.07	0.14	0.02	0.14	0.12
UC-A	GSM364650	B	NR	0.00	0.00	0.02	0.00	0.00	0.13	0.15	0.01	0.02	0.11	0.17	0.01	0.22	0.16
UC-A	GSM364651	B	NR	0.00	0.00	0.00	0.00	0.00	0.11	0.44	0.02	0.00	0.13	0.07	0.03	0.16	0.05
UC-A	GSM364652	B	NR	0.00	0.02	0.01	0.07	0.28	0.00	0.11	0.00	0.07	0.06	0.07	0.00	0.19	0.11
UC-A	GSM364653	B	NR	0.00	0.03	0.01	0.00	0.00	0.00	0.10	0.00	0.11	0.11	0.12	0.00	0.35	0.18
UC-A	GSM364654	B	NR	0.00	0.00	0.04	0.00	0.00	0.10	0.18	0.00	0.08	0.10	0.12	0.00	0.25	0.13
UC-A	GSM364655	B	NR	0.01	0.06	0.07	0.02	0.00	0.09	0.11	0.00	0.09	0.10	0.10	0.00	0.23	0.12
UC-A	GSM364656	B	NR	0.00	0.02	0.01	0.00	0.00	0.17	0.29	0.00	0.06	0.06	0.13	0.02	0.14	0.10
CDc	GSM423010	B	R	0.00	0.04	0.00	0.14	0.10	0.09	0.04	0.00	0.06	0.07	0.01	0.00	0.25	0.20
CDc	GSM423012	B	R	0.00	0.05	0.06	0.10	0.00	0.18	0.14	0.00	0.06	0.15	0.03	0.01	0.18	0.05
CDc	GSM423013	B	R	0.01	0.04	0.08	0.02	0.00	0.00	0.05	0.00	0.13	0.11	0.05	0.00	0.42	0.09
CDc	GSM423015	B	R	0.00	0.05	0.03	0.00	0.00	0.00	0.07	0.00	0.22	0.08	0.04	0.00	0.34	0.16
CDc	GSM423017	B	R	0.02	0.00	0.07	0.05	0.00	0.05	0.08	0.02	0.04	0.14	0.00	0.00	0.29	0.23
CDc	GSM423019	B	R	0.00	0.04	0.08	0.06	0.00	0.00	0.05	0.00	0.18	0.06	0.09	0.00	0.34	0.10
CDc	GSM423021	B	R	0.02	0.01	0.11	0.03	0.00	0.00	0.06	0.00	0.07	0.11	0.05	0.00	0.38	0.16
CDc	GSM423023	B	R	0.01	0.06	0.03	0.00	0.00	0.00	0.06	0.00	0.21	0.06	0.03	0.00	0.40	0.15
CDc	GSM423025	B	R	0.02	0.01	0.11	0.03	0.00	0.00	0.06	0.00	0.09	0.14	0.05	0.00	0.34	0.15
CDc	GSM423027	B	R	0.02	0.06	0.05	0.04	0.00	0.03	0.08	0.00	0.13	0.11	0.02	0.00	0.35	0.10
CDc	GSM423029	B	R	0.01	0.06	0.05	0.00	0.00	0.13	0.16	0.01	0.05	0.17	0.02	0.00	0.26	0.08
CDc	GSM423031	B	R	0.00	0.00	0.09	0.08	0.00	0.00	0.05	0.00	0.07	0.13	0.07	0.00	0.31	0.20
CDc	GSM423033	B	NR	0.00	0.01	0.06	0.05	0.00	0.04	0.10	0.00	0.08	0.11	0.08	0.00	0.31	0.18

CDc	GSM423035	B	NR	0.00	0.00	0.00	0.00	0.11	0.26	0.02	0.00	0.15	0.08	0.05	0.19	0.14			
CDc	GSM423037	B	NR	0.00	0.01	0.03	0.01	0.00	0.08	0.08	0.00	0.11	0.07	0.09	0.00	0.36	0.15		
CDc	GSM423039	B	NR	0.00	0.08	0.06	0.04	0.09	0.00	0.09	0.00	0.14	0.08	0.05	0.00	0.30	0.07		
CDc	GSM423041	B	NR	0.00	0.02	0.00	0.00	0.00	0.03	0.22	0.00	0.13	0.08	0.14	0.00	0.24	0.14		
CDc	GSM423043	B	NR	0.00	0.00	0.00	0.00	0.00	0.20	0.26	0.00	0.06	0.05	0.10	0.00	0.20	0.13		
CDc	GSM423045	B	NR	0.00	0.04	0.07	0.00	0.00	0.06	0.08	0.00	0.11	0.11	0.08	0.00	0.39	0.06		
UC-A	GSM422963	PT	R	0.05	0.03	0.14	0.02	0.00	0.00	0.05	0.00	0.10	0.16	0.00	0.00	0.36	0.09	0.00	
UC-A	GSM422965	PT	R	0.06	0.05	0.09	0.00	0.00	0.06	0.06	0.00	0.10	0.17	0.00	0.00	0.28	0.14	0.00	
UC-A	GSM422967	PT	R	0.05	0.01	0.09	0.02	0.00	0.00	0.04	0.00	0.10	0.11	0.00	0.00	0.41	0.17	0.00	
UC-A	GSM422969	PT	R	0.07	0.07	0.10	0.05	0.00	0.00	0.05	0.02	0.07	0.16	0.00	0.00	0.33	0.07	0.00	
UC-A	GSM422971	PT	R	0.06	0.04	0.07	0.04	0.00	0.00	0.04	0.00	0.12	0.15	0.00	0.00	0.31	0.17	0.00	
UC-A	GSM422973	PT	R	0.20	0.00	0.15	0.02	0.00	0.00	0.05	0.01	0.06	0.22	0.00	0.00	0.19	0.11	0.00	
UC-A	GSM422975	PT	R	0.09	0.06	0.10	0.04	0.00	0.00	0.07	0.03	0.02	0.16	0.00	0.00	0.31	0.12	0.01	
UC-A	GSM422977	PT	R	0.12	0.00	0.14	0.00	0.00	0.07	0.06	0.01	0.04	0.15	0.00	0.00	0.25	0.14	0.02	
UC-A	GSM422979	PT	NR	0.13	0.00	0.13	0.03	0.00	0.00	0.03	0.00	0.06	0.14	0.02	0.00	0.26	0.21	0.00	
UC-A	GSM422981	PT	NR	0.01	0.01	0.07	0.00	0.00	0.07	0.15	0.00	0.06	0.14	0.13	0.00	0.27	0.10	0.00	
UC-A	GSM422983	PT	NR	0.00	0.02	0.04	0.05	0.00	0.06	0.13	0.00	0.08	0.07	0.05	0.00	0.31	0.17	0.00	
UC-A	GSM422985	PT	NR	0.01	0.03	0.07	0.01	0.00	0.02	0.18	0.00	0.13	0.06	0.15	0.00	0.24	0.11	0.00	
UC-A	GSM422987	PT	NR	0.01	0.05	0.03	0.00	0.00	0.00	0.04	0.00	0.19	0.07	0.02	0.00	0.41	0.18	0.00	
UC-A	GSM422989	PT	NR	0.04	0.01	0.08	0.01	0.00	0.01	0.13	0.00	0.14	0.06	0.11	0.00	0.27	0.12	0.00	
UC-A	GSM422991	PT	NR	0.00	0.03	0.11	0.02	0.00	0.00	0.09	0.00	0.11	0.13	0.12	0.00	0.27	0.11	0.00	
UC-A	GSM422993	PT	NR	0.00	0.00	0.07	0.00	0.00	0.10	0.17	0.02	0.02	0.12	0.11	0.05	0.21	0.14	0.00	
UC-A	GSM422995	PT	NR	0.00	0.02	0.01	0.05	0.00	0.19	0.19	0.00	0.06	0.08	0.12	0.01	0.19	0.09	0.00	
UC-A	GSM422997	PT	NR	0.00	0.00	0.02	0.00	0.00	0.04	0.12	0.00	0.08	0.10	0.13	0.00	0.30	0.20	0.00	
UC-A	GSM422999	PT	NR	0.13	0.05	0.04	0.02	0.00	0.00	0.06	0.00	0.12	0.13	0.00	0.00	0.37	0.08	0.00	
UC-A	GSM423001	PT	NR	0.08	0.00	0.11	0.04	0.00	0.03	0.06	0.00	0.08	0.13	0.00	0.00	0.33	0.16	0.00	
UC-A	GSM423003	PT	NR	0.05	0.01	0.11	0.03	0.00	0.00	0.04	0.01	0.06	0.14	0.00	0.00	0.25	0.31	0.00	
UC-A	GSM423005	PT	NR	0.00	0.01	0.04	0.02	0.00	0.14	0.16	0.00	0.07	0.08	0.15	0.00	0.23	0.09	0.00	
UC-A	GSM423007	PT	NR	0.01	0.09	0.06	0.10	0.10	0.03	0.08	0.00	0.07	0.11	0.04	0.00	0.20	0.09	0.00	
UC-A	GSM423009	PT	NR	0.00	0.03	0.00	0.00	0.00	0.17	0.33	0.00	0.06	0.08	0.07	0.00	0.19	0.08	0.00	

CDc	GSM423011	PT	R	0.06	0.05	0.03	0.00		0.00	0.06	0.00	0.13	0.12	0.00	0.00	0.36	0.19	0.00
CDc	GSM423014	PT	R	0.05	0.00	0.14	0.03		0.00	0.05	0.00	0.07	0.09	0.00	0.00	0.40	0.17	0.00
CDc	GSM423016	PT	R	0.03	0.04	0.07	0.03		0.00	0.05	0.00	0.12	0.13	0.00	0.00	0.37	0.16	0.00
CDc	GSM423018	PT	R	0.05	0.00	0.10	0.01		0.00	0.03	0.01	0.07	0.14	0.00	0.00	0.31	0.28	0.00
CDc	GSM423020	PT	R	0.05	0.04	0.12	0.01		0.00	0.05	0.00	0.15	0.19	0.00	0.00	0.22	0.16	0.00
CDc	GSM423022	PT	R	0.15	0.00	0.22	0.12		0.00	0.04	0.02	0.00	0.16	0.00	0.00	0.21	0.09	0.00
CDc	GSM423024	PT	R	0.01	0.07	0.10	0.06		0.01	0.08	0.00	0.11	0.17	0.01	0.00	0.28	0.11	0.00
CDc	GSM423026	PT	R	0.08	0.00	0.18	0.04		0.00	0.03	0.00	0.08	0.09	0.00	0.00	0.37	0.13	0.00
CDc	GSM423028	PT	R	0.04	0.07	0.05	0.03		0.00	0.04	0.00	0.15	0.10	0.00	0.00	0.39	0.15	0.00
CDc	GSM423030	PT	R	0.00	0.04	0.06	0.01		0.09	0.14	0.00	0.10	0.12	0.09	0.00	0.25	0.12	0.00
CDc	GSM423032	PT	R	0.05	0.00	0.12	0.09		0.00	0.04	0.01	0.04	0.15	0.00	0.00	0.22	0.28	0.00
CDc	GSM423034	PT	NR	0.00	0.00	0.04	0.03		0.06	0.08	0.00	0.04	0.10	0.09	0.00	0.34	0.20	0.00
CDc	GSM423036	PT	NR	0.00	0.00	0.02	0.00		0.07	0.15	0.02	0.04	0.12	0.13	0.03	0.23	0.18	0.00
CDc	GSM423038	PT	NR	0.00	0.01	0.05	0.01		0.00	0.05	0.00	0.13	0.07	0.07	0.00	0.33	0.28	0.00
CDc	GSM423040	PT	NR	0.02	0.07	0.04	0.01		0.02	0.09	0.00	0.16	0.11	0.02	0.00	0.38	0.08	0.00
CDc	GSM423042	PT	NR	0.00	0.00	0.00	0.00		0.09	0.29	0.01	0.00	0.16	0.13	0.01	0.20	0.12	0.00
CDc	GSM423044	PT	NR	0.00	0.02	0.00	0.00		0.22	0.25	0.00	0.07	0.07	0.08	0.00	0.20	0.09	0.00
CDc	GSM423046	PT	NR	0.03	0.01	0.16	0.06		0.00	0.06	0.01	0.08	0.14	0.03	0.00	0.32	0.10	0.00

Example 3: Baseline plasma cell proportions are predictive of anti-TNFα non-response

[0120] The significant differences observed in immune cell subset proportions between response groups suggested they may serve as clinically feasible predictive biomarkers of non-response. To test this, each subset's predictive power was assessed in the training data, and a mean AUC of 83% and 71% respectively was observed for the estimated proportion of inflammatory macrophages and plasma cells predicting non-response (**Fig. 3A-C** for CDc, UC-A and UC-B respectively, Table 7, inflammatory macrophages and plasma cells AUC: (77%, 79%), (82%,45%) and (89%,88%) for UC-A, UC-B and CDc respectively). Combining the two cell subset proportions into a single score (average of standardized proportions) an increase in predictive power was observed over each individual biomarker in the UC-A and CDc cohorts, achieving AUCs of 90% and 95% respectively (**Fig. 3A-C**), whereas UC-B, where tissue samples included both normal and inflamed biopsies, did not yield an improved combined score. Conversely, adjusting the expression data of each cohort for variations in the cellular biomarker proportions, showed a significant decrease (a mean drop of 32%) in the ability of gene signature scores to discriminate responders from non-responders (**Fig. 3D-F**). These results suggest that the predictive power of reported gene signatures is largely based on cell subset proportion differences, whose increase in colon biopsies on non-responders may serve for predictive purposes.

[0121] **Table 7: Results of the cell type proportion meta-analysis**

Cell type	UC-A	UC-B	CDc	Fstat	F p-value	Combined FDR
Inflammatory macrophages	0.03823	0.00896	0.00373	27.14098	0.00014	**0.00150**
Plasma cells	0.02297	0.72234	0.00524	18.70073	0.00470	**0.02585**
T CD8	0.01305	-	0.29055	11.14911	0.02494	0.09144
mDC activated	0.02297	0.14024	0.38446	13.38779	0.03727	0.10251
T CD8 activated	0.14977	-	0.05679	9.53412	0.04905	0.10791
Monocyte	0.61009	0.02058	0.22684	11.72218	0.06846	0.11604
NK activated	0.01555	-	0.90180	8.53459	0.07385	0.11604
Neutrophil	0.74172	0.04257	0.48242	8.36864	0.21232	0.29194
B Mem IgM	0.14702	0.82120	-	4.22839	0.37598	0.44933
NK		0.40849	-	1.79060	0.40849	0.44933
mDC		0.81728	-	0.40355	0.81728	0.81728

[0122] Next, an independent set of 20 paraffin embedded colon biopsies was analyzed. The biopsies were collected as part of standard clinical care from IBD patients for which anti-TNFα response status was available. These were stained for inflammatory macrophages and for plasma cells using IHC staining and assessed for cell type abundance using a discrete scoring (low/medium/high) performed by an expert pathologist who was blind to patient response status (see Materials and Methods). In agreement with the computational analysis, both plasma cells and inflammatory macrophages were observed as associated with non-response to therapy (**Fig. 3G-H**, plasma cell AUC = 71%,81% by pathologist and quantification respectively, inflammatory macrophages AUC=67% by pathologist), while total macrophages abundance was not predictive (AUC 48%).

[0123] For clinical feasibility, a biomarker must exhibit reproducibility and ease of use. Plasma cells have a unique morphology and can be sufficiently defined by a single marker, CD138. Therefore, plasma cells were focused on a second cohort consisting of 61 patients from two medical centers was collected (35 responders and 26 non-responders, Table 2). Plasma cell frequencies were examined by CD138+ IHC staining and again a significantly higher frequency of plasma cells in non-responders as evaluated by an expert pathologist and by automated quantification was observed (p=0.02 and 0.0005 by Student's T-test, **Fig. 3I-J**). This difference enabled predicting non-response class at baseline (**Fig. 3L**, 71% and 74% AUC, n=52, Materials and Methods), which was further increased when the analyses was restricted only to highly inflamed tissues (**Fig. 3M**, AUC = 82% and 84% by an expert pathologist and quantitatively, n= 20, p=0.005/0.002 by Student T-test, **Fig. 3K**). Taken together, the computational deconvolution predictions and external validations confirmed that pretreatment plasma cell abundance are associated with non-response to anti-TNF treatment and suggested that relevant immune pathways should be further studied to understand non-response pathophysiology.

Example 4: A dysregulated gene network masked by cell proportion variation

[0124] Given the differential abundance of inflammatory macrophages and plasma cells between the two response groups, it was reasoned that underlying biological signals may be masked by this difference. To characterize the biological processes associated with non-response that cannot be explained by cell subsets differences, pathways and genes differentially expressed were searched for while adjusting each individual's gene expression profile for variations in the two

cell subsets (see Materials and Methods). Fifteen pathways that were dysregulated across all cohorts (FDR=0.05) were found using Gene Set Enrichment Analysis (GSEA), all of which were up-regulated in non-responders and related to immune signaling and inflammation (**Fig. 4A-C**,). Most prominent was the association of non-response with the MyD88 deficiency (which impairs Toll Like Receptors (TLR2/4) function), Interleukin-6 signaling, and antigen activation of B Cell Receptor, which had a substantial fraction of genes in the leading edge (64%, 30% and 26% of genes respectively (**Fig. 4D.**)).

[0125] At the gene level, 166 differentially expressed genes (DEG) were identified in at least two cohorts across post cellular biomarker adjustment (62 up-regulated and 104 down-regulated, p-value ≤ 0.05, Table 8). This set of genes was analyzed using Ingenuity Pathway Analysis (IPA), in particular, searching for links to the predictive cellular biomarkers. One of the top most enriched networks included 28 genes, including the ligand-receptor pair CCL7-CCR2 which was found to be upregulated in non-responders (**Fig. 4E**, Table 9). The CCL7-CCR2 axis has been associated with inflammation and upregulation in IBD. In the mucosa of IBD patients, CCL7 is produced by inflammatory lymphocytes, including plasma cells, whereas CCR2 is expressed primarily on monocytes, and mediates their recruitment to inflamed tissues.

[0126] Interestingly, IPA analysis identified Triggering Receptor Expressed on Myeloid cells 1 (TREM1) as an upstream regulator of six of the adjustment-derived DEG, including CCL7. TREM1 is expressed on myeloid lineage cells including monocytes and macrophages, has well-documented pro-inflammatory functions, and its blockade has shown promising results in attenuation of symptoms in IBD models. Meta-analysis of TREM1, CCL7 and CCR2 gene expression across the public data biopsy cohorts showed all these genes to be consistently up-regulated in the non-responder group in the original measured data (meta-FDR<0.037). To find the role of TREM1 pathway in anti-TNFα mechanism we searched for TNF-related genes and found TNFα and TNFR2 as up-regulated in non-responders in the original data as well (**Fig. 4F**), probably due to TREM-1 activation via synergism with TLR signaling, which leads to TNFα secretion from the inflammatory macrophages. Unlike CCL7 and CCR2, their expression difference post-adjustment was lost (**Fig. 4G**). Taken together, these results suggest that the plasma cells may be responsible for the recruitment of inflammatory macrophages to the inflamed area, which ultimately impact response potential.

[0127] **Table 8: List of differentially expressed genes when adjusting for inflammatory macrophage and plasma cell proportions.**

Symbol	Avr. Fc	Pval	Cohort with Significance	Symbol	Avr. Fc	Pval	Cohort with Significance
NRXN2	-3.459	0.012	UC-B, UC-A	RSPH6A	-2.444	0.029	UC-B, UC-A
IL7R	3.454	0.021	UC-A, CDc	CDKL1	-2.653	0.021	UC-B, CDc
TNFAIP6	3.697	0.019	UC-B, CDc	PCDHB18P	-2.214	0.039	UC-B, UC-A
IL4I1	3.624	0.011	UC-A, CDc	HMG20A	-2.423	0.025	UC-B, UC-A
UBAP1L	-2.947	0.009	UC-A, CDc	CHSY1	2.237	0.039	UC-A, CDc
FARSA	-3.118	0.014	UC-B, CDc	IL19	-2.621	0.028	UC-B, CDc
ZNF781	-2.937	0.009	UC-B, UC-A	STAT2	2.435	0.029	UC-B, CDc
WDR70	-3.045	0.007	UC-B, CDc	CHST15	2.444	0.029	UC-B, CDc
HAVCR2	2.881	0.015	UC-B, CDc	LDLRAD3	2.335	0.032	UC-B, CDc
MATK	-3.153	0.007	UC-B, CDc	ZNF33B	-2.509	0.023	UC-A, CDc
BCL7B	-3.297	0.012	UC-B, CDc	ACBD3	-2.556	0.021	UC-B, CDc
CCL7	3.303	0.005	UC-B, CDc	DNAJB4	2.602	0.019	UC-B, CDc
RIMBP2	-2.805	0.011	UC-B, UC-A	SSC5D	-2.383	0.029	UC-B, UC-A
PCAT19	3.160	0.006	UC-B, CDc	ERCC6	-2.424	0.026	UC-A, CDc
SNIP1	-2.641	0.023	UC-B, UC-A	TUBA4B	-2.614	0.023	UC-A, CDc
MYO6	-2.918	0.010	UC-A, CDc	WNT5A	2.371	0.032	UC-B, CDc
LANCL2	-3.239	0.007	UC-B, CDc	TMEM95	-2.575	0.021	UC-B, UC-A
AIM2	2.948	0.015	UC-B, CDc	MORC4	-2.544	0.021	UC-B, CDc
FLJ37201	-2.366	0.028	UC-B, UC-A	CD93	2.282	0.036	UC-B, CDc
SLC38A2	2.647	0.018	UC-A, CDc	AAED1	2.450	0.028	UC-A, CDc
FMNL3	3.067	0.014	UC-B, CDc	KCNK6	-2.321	0.032	UC-A, CDc
ATPAF2	-2.591	0.022	UC-A, CDc	MACROD1	-2.346	0.033	UC-B, UC-A
RNLS	-2.977	0.012	UC-B, CDc	PRSS22	-2.380	0.030	UC-B, CDc
HIVEP1	3.047	0.011	UC-B, CDc	KNG1	-2.461	0.025	UC-A, CDc
SKA2	-3.049	0.023	UC-B, CDc	FAM204A	-2.574	0.022	UC-B, CDc
MAP2K6	-3.046	0.008	UC-B, CDc	MEX3D	-2.289	0.033	UC-B, UC-A
HSD17B14	-2.416	0.030	UC-B, UC-A	FECH	-2.379	0.029	UC-A, CDc
LOC440434	-2.587	0.022	UC-A, CDc	LOC100507389	-2.442	0.024	UC-B, UC-A
GFPT2	2.693	0.018	UC-B, CDc	VTI1A	-2.389	0.028	UC-B, UC-A
BCAT1	2.893	0.020	UC-B, CDc	DESI2	-2.553	0.020	UC-B, CDc
CCDC88A	2.725	0.019	UC-B, CDc	NUP210P1	-2.416	0.027	UC-B, UC-A
SNORD8	-2.774	0.013	UC-B, UC-A	GPC1	-2.285	0.035	UC-B, UC-A
MLLT3	-2.942	0.009	UC-B, CDc	TSPYL2	2.446	0.024	UC-B, UC-A
CKAP2L	-2.756	0.018	UC-B, CDc	COG2	-2.448	0.026	UC-B, CDc
TPCN1	-2.407	0.029	UC-B, UC-A	MLKL	2.318	0.032	UC-B, UC-A

WNK2	-2.741	0.024	UC-A, CDc	RBM15B	-2.386	0.031	UC-B, CDc
CCR2	2.981	0.010	UC-B, CDc	TMEM177	-2.294	0.036	UC-B, CDc
HCN2	-2.951	0.014	UC-A, CDc	MDGA2	-2.401	0.030	UC-B, UC-A
CALHM2	2.831	0.023	UC-B, CDc	IL13RA2	2.373	0.029	UC-A, CDc
FLJ31715	2.922	0.009	UC-B, UC-A	DDX25	-2.444	0.025	UC-B, UC-A
ADAMTS12	2.521	0.022	UC-B, CDc	PSG6	-2.450	0.025	UC-B, UC-A
GIMAP8	2.934	0.018	UC-B, CDc	C1orf204	-2.431	0.025	UC-B, UC-A
TPP1	2.959	0.009	UC-B, CDc	FTH1P5	2.399	0.028	UC-A, CDc
PLK4	-2.414	0.028	UC-B, CDc	CPXM2	2.327	0.033	UC-B, CDc
SARS2	-2.595	0.017	UC-B, UC-A	DIABLO	-2.337	0.034	UC-B, CDc
CNIH3	-2.894	0.009	UC-B, UC-A	S1PR1	2.432	0.026	UC-B, CDc
AP2B1	-2.160	0.043	UC-B, UC-A, CDc	DCAF4L1	2.458	0.025	UC-B, CDc
BORCS8	-2.584	0.022	UC-A, CDc	ENTPD1-AS1	-2.380	0.029	UC-A, CDc
RBFA	-2.810	0.026	UC-B, CDc	RIPK1	-2.449	0.025	UC-A, CDc
LRRC8C	2.346	0.033	UC-B, CDc	CTHRC1	2.432	0.028	UC-B, CDc
HOXA11-AS	-2.819	0.017	UC-B, CDc	KCNQ1	-2.318	0.035	UC-B, CDc
CCNF	-2.293	0.035	UC-B, CDc	ZSWIM5	-2.246	0.039	UC-A, CDc
ANPEP	2.780	0.015	UC-B, CDc	C6orf47	2.356	0.030	UC-B, UC-A
FCN1	2.882	0.020	UC-B, CDc	HEATR4	-2.400	0.029	UC-B, CDc
STC1	2.514	0.029	UC-B, CDc	BSPRY	-2.331	0.032	UC-B, CDc
HOOK2	-2.837	0.019	UC-A, CDc	LRRC57	-2.117	0.047	UC-B, UC-A
ZFR	2.688	0.020	UC-A, CDc	TDRD3	-2.402	0.030	UC-A, CDc
RNF13	2.166	0.044	UC-A, CDc	PSG7	-2.226	0.040	UC-B, CDc
SEPT11	-2.529	0.022	UC-B, CDc	GLIPR1	2.227	0.040	UC-B, CDc
MIR4435-2HG	2.468	0.024	UC-B, CDc	DRAM1	2.415	0.028	UC-A, CDc
BIK	-2.769	0.014	UC-B, CDc	IKBIP	2.424	0.029	UC-B, CDc
HOMER1	2.394	0.032	UC-B, CDc	DNAH9	-2.144	0.045	UC-B, UC-A
CLEC4F	-2.701	0.021	UC-A, CDc	CRIP2	2.248	0.038	UC-B, CDc
RAB15	-2.571	0.020	UC-B, CDc	TRIO	-2.341	0.032	UC-A, CDc
SOX17	2.810	0.016	UC-B, CDc	CDC42BPG	-2.307	0.034	UC-A, CDc
SCMH1	-2.675	0.018	UC-B, UC-A	OLFML2B	2.326	0.033	UC-B, CDc
LLGL2	-2.654	0.024	UC-B, CDc	APMAP	2.373	0.032	UC-B, CDc
FAM64A	-2.651	0.017	UC-B, CDc	ADGRF5	2.270	0.038	UC-B, CDc
SERPINH1	2.623	0.020	UC-B, CDc	ALOX12	-2.379	0.029	UC-A, CDc
DMD	2.529	0.025	UC-A, CDc	ARTN	-2.261	0.037	UC-A, CDc
SLC1A3	2.546	0.025	UC-B, CDc	TM6SF1	2.375	0.030	UC-B, CDc
ARAF	-2.645	0.016	UC-B, UC-A	CPEB1	-2.293	0.034	UC-B, UC-A
KCNJ8	2.737	0.027	UC-B, CDc	MAP10	-2.191	0.041	UC-B, UC-A
C9orf85	-2.487	0.022	UC-B, UC-A	B3GALT1	-2.275	0.036	UC-A, CDc

JOSD2	-2.726	0.014	UC-B, UC-A	KIF4A	-2.268	0.038	UC-B, CDc
ZNF440	-2.541	0.024	UC-B, CDc	LOC100289473	-2.238	0.037	UC-B, UC-A
SLC16A6	2.425	0.030	UC-B, CDc	BAG2	2.297	0.035	UC-B, CDc
MFSD13A	-2.314	0.036	UC-B, CDc	MTF1	2.210	0.041	UC-A, CDc
EPHB2	-2.550	0.021	UC-B, CDc	PIK3CB	-2.246	0.039	UC-B, CDc
GLA	2.645	0.019	UC-B, CDc	ZNF367	-2.218	0.041	UC-B, CDc
HOXD13	-2.594	0.023	UC-B, CDc	EVI2B	2.263	0.037	UC-B, CDc
LOC100505920	-2.348	0.031	UC-B, UC-A	SOX12	-2.188	0.042	UC-A, CDc
LOC101928191	-2.596	0.019	UC-B, UC-A	KLHDC7A	-2.158	0.045	UC-B, CDc

[0128] **Table 9: Results from IPA analysis: common regulators, enriched biofunctions and networks**

Focus Molecules	Top Diseases and Functions	Molecules in Network
28	Cell-To-Cell Signaling and Interaction, Cellular Movement, Hematological System Development and Function	AIM2, AP2B1, ARAF, BAG2, BCAT1, CCL7, CCNF, CCR2, DDX25, DESI2, DIABLO, EPHB2, ERK1/2, GPC1, HAVCR2, HOMER1, IgG, IL19, IL12 (complex), IL4I1, IL7R, Interferon alpha, Jnk, MYO6, P38 MAPK, PIK3CB, RIPK1, RNF13, S1PR1, SOX17, STAT2, TCR, TRIO, TSPYL2, WNK2
22	Cell Morphology, Auditory and Vestibular System Development and Function, Embryonic Development	ADGRF5, Akt, ALOX12, ARTN, ATP1B2, CCDC88A, CD93, Cg, CHRNA10, Creb, CRIP2, CTHRC1, DMD, ERK, GIMAP8, GLA, Histone h3, IL13RA2, KCNC1, LANCL2, MAP2K6, MLKL, MLLT3, NFkB (complex), PLK4, SERPINH1, SLC16A6, SOCS4, SOX12, STC1, SYNM, TBC1D4, TMOD4, TNFAIP6, WNT5A
17	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	ADAMTS12, BAG2, CCND1, Chil3/Chil4, CHST15, CLEC4F, CPEB1, DNAJB4, EGFR, FECH, H1FX, HPX, HSD17B14, IL6, KIF20A, KIF4A, KNG1, LLGL2, MFSD13A, MORC4, NRXN2, PPARD, RAG2, SLC10A1, Slco1a1, SNIP1, SNW1, SOX2, SOX4, SOX17, STK3, TCF3, THRAP3, U2AF2, ZNF367
14	Cell-To-Cell Signaling and Interaction, Hematological System Development and Function, Immune Cell Trafficking	BCL7B, BIK, Ccl9, CDH13, CLEC4E, CNIH3, CNR1, CPEB1, CRLF2, FAM204A, GNAI3, GRIA2, GRIP1, HAVCR2, HIVEP1, HOMER2, HOOK2, HOXD13, Ige, IL13, LRRC8C, LRRC8D, LYN, MATK, NT5E, OLFML2B, PAX6, PCM1, RNASE2, SEPT11, SLC1A3, SLPI, TIMP4, TNF, TREM1

12	Cardiovascular Disease, Organismal Injury and Abnormalities, Skeletal and Muscular Disorders	AHR, AREG, CAMP, CCNF, CKAP2L, CRYAB, CTSS, EDN1, ESR1, FAM64A, FCN1, FHL2, FMNL3, GFPT2, KCNK6, LDLRAD3, LMNB1, MACROD1, MMP12, Mt1, MYH7, NUPR1, PDGF BB, RCE1, REL, RSAD2, SKA2, SRF, TAGLN, TOB1, UNC119, VCL, VTI1A, ZFP36, ZFR
10	Cancer, Organismal Injury and Abnormalities, Respiratory Disease	ACBD3, ACSL5, ANPEP, APLP2, APOA1, CHSY1, COL4A1, CYFIP2, DMTF1, DRAM1, GATM, GLIPR1, HMG20A, HMG20B, IKBIP, KCNJ8, KDM1A, KRAS, LAMP2, MAP4K4, MCAM, MMP10, NDRG2, PDE4B, PFKM, PRC1, RAPGEF3, RCOR1, SCMH1, SKIL, Sos, TGFBR2, TNFAIP2, TP53, TPP1
10	Cardiac Arrythmia, Cardiovascular Disease, Organismal Injury and Abnormalities	ANXA1, AR, ATP2A2, B3GALT1, CDC42BPG, CLTC, CYP2C8, ENPP2, ERCC6, EVI2B, FARSA, FOXP3, Hbb-b2, HCN1, HCN2, KCNE1B, KCNQ1, MBP, MTF1, MTOR, MYH6, PLAT, Pln, PSEN1, RORC, SLC38A2, SND1, SULT1E1, TCF7L2, TFIIH, THRA, TOP1, TPCN1, TUBB3, XRCC6
1	Developmental Disorder, Endocrine System Disorders, Gastrointestinal Disease	HOXA11-AS, SBDS
1	Cell Morphology, Cellular Assembly and Organization, Nervous System Development and Function	AP3D1, HEATR4
1	Embryonic Development, Reproductive System Development and Function, Developmental Disorder	PPRC1, TM6SF1
1	Cellular Development, Cellular Growth and Proliferation, Developmental Disorder	CPXM2, OTX2
1	Behavior, Cancer, Cell Morphology	FGFR1, RAB15
1	Behavior, Cellular Assembly and Organization, Hereditary Disorder	CACNA1A, Calmodulin, RIMBP2
1	Behavior, Cancer, Cell Morphology	ADCYAP1, DNAH9, PIH1D3, RFX3
1	Developmental Disorder, Hereditary Disorder, Metabolic Disease	COG1, COG2, COG3, COG5

Example 5: TREM1 expression is a predictive biomarker in blood

[0129] From the point of view of clinical application, prognostic tests performed on blood samples are non-invasive and hence of high value. Whether these findings could lead towards cellular or molecular biomarkers in blood samples was investigated. To do so, whole blood gene expression from a cohort of 28 CD patients prior to anti-TNFα therapy, whose subsequent

response status has been determined similarly to the primary biopsy validation cohort criteria was profiled. The TREM1-CCL7-CCR2 axis was focused on and the genes were tested in a hypothesis directed manner only. Of these, TREM1 was the only gene differentially expressed between responders and non-responders (down-regulated in non-responders, adjusted p-value = 0.007, **Fig. 5A**) in blood, and notably showed a very high prediction accuracy (**Fig. 5B**, AUC = 94%). Importantly, TREM1 was overall highly expressed (average log2-expression $\geq$ 11.9), providing further confidence in the measured signal. In addition, it was observed that TREM-1 and CCR2 gene expression levels in blood were correlated with endoscopic activity in an additional cohort of patients with UC (see Materials and Methods), further supporting monitoring of the axis in blood as an important clinical non-invasive biomarker and their potential for reproducibility as a clinical non-invasive biomarker of anti-TNFα response status at baseline.

Example 6: TREM1 expression is predictive in rheumatoid arthritis

[0130] Anti- TNFα therapy is also used to treat other forms of autoimmune inflammation besides IBD. Rheumatoid arthritis (RA) is also treated with therapeutic anti-TNFα antibodies, including Infliximab. As was done for IBD, blood expression data from RA patients before anti-TNFα therapy was collected from the GEO, and the expression of TREM1 was examined in eventual responders and non-responders. The first cohort examined (GEO12051) contained 37 subjects that were responsive to IFX and seven that were not. Microarray analysis of blood expression of TREM1 found higher levels of TREM1 mRNA in responders both before and after adjustment for plasma cell and inflammatory macrophage number (**Fig. 6A**), as had been seen for IBD subjects. Similar results were observed with a second cohort (GSE58795, 23 responders and 7 non-responders) (**Fig. 6B**). Lastly, a mixed cohort of subjects (GEO33377) treated with IFX and another anti-TNFα antibody, Adalimumab, was examined. Once again responders (18 subjects) had elevated blood TREM1 levels as compared to non-responders (24) (**Fig. 6C**).

[0131] During IFX therapy, circulating monocytes undergo apoptosis, and a shift towards proresolving macrophages occurs in the lamina propria, yielding reduced inflammation, tissue repair and remission. This analysis post-treatment suggests this process occurs to a lesser extent in non-responding individuals implying that the immune milieu observed in patients lies along a continuous immune-phenotypic inflammatory space, in which non-responders represent an extreme. If a non-responsive profile is merely a more exacerbated form of inflammation, then an

increased drug dose may bring effective disease remission. In support of this, patients with acute severe colitis showed increased efficacy when treated with higher drug doses.

[0132] Conversely, non-responders may represent a phenotypic niche which is difficult to resolve therapeutically via anti-TNFα treatments, in which case based on baseline detection of non-response one may elect an alternative therapeutic. These findings suggest that blocking the mechanism that draws increased numbers of plasma cells and inflammatory macrophages to intestinal mucosa would be of benefit. Indeed, experiments in mice showed that blocking CCR2, simultaneously with CCR5 and CXCR3, prevents experimental colitis.

[0133] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

Claims

1. A method of determining suitability of a subject in need thereof to be treated with anti-Tumor Necrosis Factor Alpha (TNFα) therapy, the method comprising:
    a. obtaining a blood sample from said subject;
    b. measuring expression levels of Triggering Receptor Expressed on Myeloid cells 1 (TREM1) in said blood sample; and
    c. determining said suitability of said subject for anti-TNFα therapy according to said expression levels of TREM1, wherein expression below a predetermined threshold indicates said subject is unsuitable for said anti-TNFα therapy and expression at or above said predetermined threshold indicates said subject is suitable for said anti-TNFα therapy;

    thereby determining suitability of a subject to be treated with anti-TNFα therapy.
2. The method of claim 1, wherein said blood sample is a peripheral blood sample.
3. A method of determining suitability of a subject in need thereof to be treated with anti-Tumor Necrosis Factor Alpha (TNFα) therapy, the method comprising
    a. obtaining an intestinal biopsy from said subject;
    b. measuring in said intestinal biopsy at least one of:
        i. plasma cell number;
        ii. inflammatory macrophage; and
        iii. expression levels of CCL7, CCR2 or both;
    c. determining said suitability of said subject for anti-TNFα therapy according to said plasma cell or inflammatory macrophage number, wherein a number of plasma cells, a number of inflammatory macrophages or expression levels CCL7, CCR2 or

both above a predetermined threshold indicates said subject is unsuitable for said anti-TNFα therapy and a number of plasma cells, a number of inflammatory macrophages or expression levels CCL7, CCR2 or both below said predetermined threshold indicates said subject is suitable for said anti-TNFα therapy;

thereby determining suitability of a subject to be treated with anti-TNFα therapy.

4. The method of claim 3, wherein a number of plasma cells above a predetermined threshold indicates said subject is unsuitable for said anti-TNFα therapy and a number of plasma cells below said predetermined threshold indicates said subject is suitable for said anti-TNFα therapy.
5. The method of claim 3, wherein expression levels of CCL7 and CCR2 above a predetermined threshold indicates said subject is unsuitable for said anti-TNFα therapy and expression levels of CCL7 and CCR2 below said predetermined threshold indicates said subject is suitable for said anti-TNFα therapy.
6. The method of any one of claims 1 to 5, further comprising measuring in an intestinal biopsy from said subject expression levels of TREM1, wherein intestinal expression levels of TREM1 above a predetermined threshold indicates said subject is unsuitable for said anti-TNFα therapy and intestinal expression levels of TREM1 below said predetermined threshold indicates said subject is suitable for said anti-TNFα therapy.
7. The method of any one of claims 1-6, wherein said subject suffers from inflammation.
8. The method of claim, 7 wherein said subject suffers from an autoimmune disease.
9. The method of claim 8, wherein said autoimmune disease is selected from inflammatory bowel disease (IBD) and rheumatoid arthritis (RA).

10. The method of claim 9, wherein said IBD comprises colitis, ulcerative colitis, and Crohn's disease.
11. The method of any one of claims 1 to 10, wherein said anti-TNFα therapy comprises administration of an anti-TNFα antibody.
12. The method of claim 11, wherein said anti-TNFα antibody is selected from infliximab and adalimumab.
13. The method of any one of claims 1 to 12, further comprising administering said anti-TNFα therapy to said subject indicated to be suitable for said anti-TNFα therapy.
14. The method of any one of claims 1 to 13, further comprising administering to said subject indicated to be unsuitable for said anti-TNFα therapy an increased dose of said anti-TNFα therapy above the standard dose.
15. The method of any one of claims 7 to 14, further comprising administering a non-anti-TNFα anti-inflammation therapy to said subject indicated to be unsuitable for anti-TNFα therapy.
16. The method of claim 15, wherein said non-anti-TNFα anti-inflammation therapy comprises blocking at least one of CCR2, CCR5 and CXCR3.
17. The method of any one of claims 1 to 16, wherein said predetermined threshold is determined from blood levels of TREM1, intestinal numbers of plasma cells or inflammatory macrophages or intestinal expression of CCL7, CCR2 or both in subjects that responded to anti-TNFα therapy, wherein said blood levels, said intestinal numbers and said intestinal expression are from before said subjects received said anti-TNFα therapy.

18. The method of any one of claims 3 to 17, wherein measuring plasma cell number comprises measuring the number of cells positive for surface expression of CD138.
19. The method of any one of claims 1 to 18, wherein measuring expression levels comprises measuring mRNA levels, protein levels or both.
20. The method of any one of claims 1 to 19, wherein said measuring expression levels comprises contacting said blood sample or intestinal biopsy with a molecule that detects TREM1, CCL7, or CCR2 mRNA or protein, and wherein said molecule is connected to an artificial solid support.
21. Use of a TREM1 detecting agent for determining serum TREM1 levels in a subject and determining suitability of said subject for treatment with an anti-TNFα therapy.
22. The use of claim 21, wherein said detecting agent is an anti-TREM1 antibody or a nucleic acid molecule that selectively binds and hybridizes to TREM1 mRNA.
23. A kit comprising a plurality of molecules selected from:
    a. a TREM1 detecting molecule;
    b. a CCL7 detecting molecule;
    c. a CCR2 detecting molecule;
    d. a plasma cell detecting molecule; and
    e. an inflammatory macrophage detecting molecule.

Figure 1A

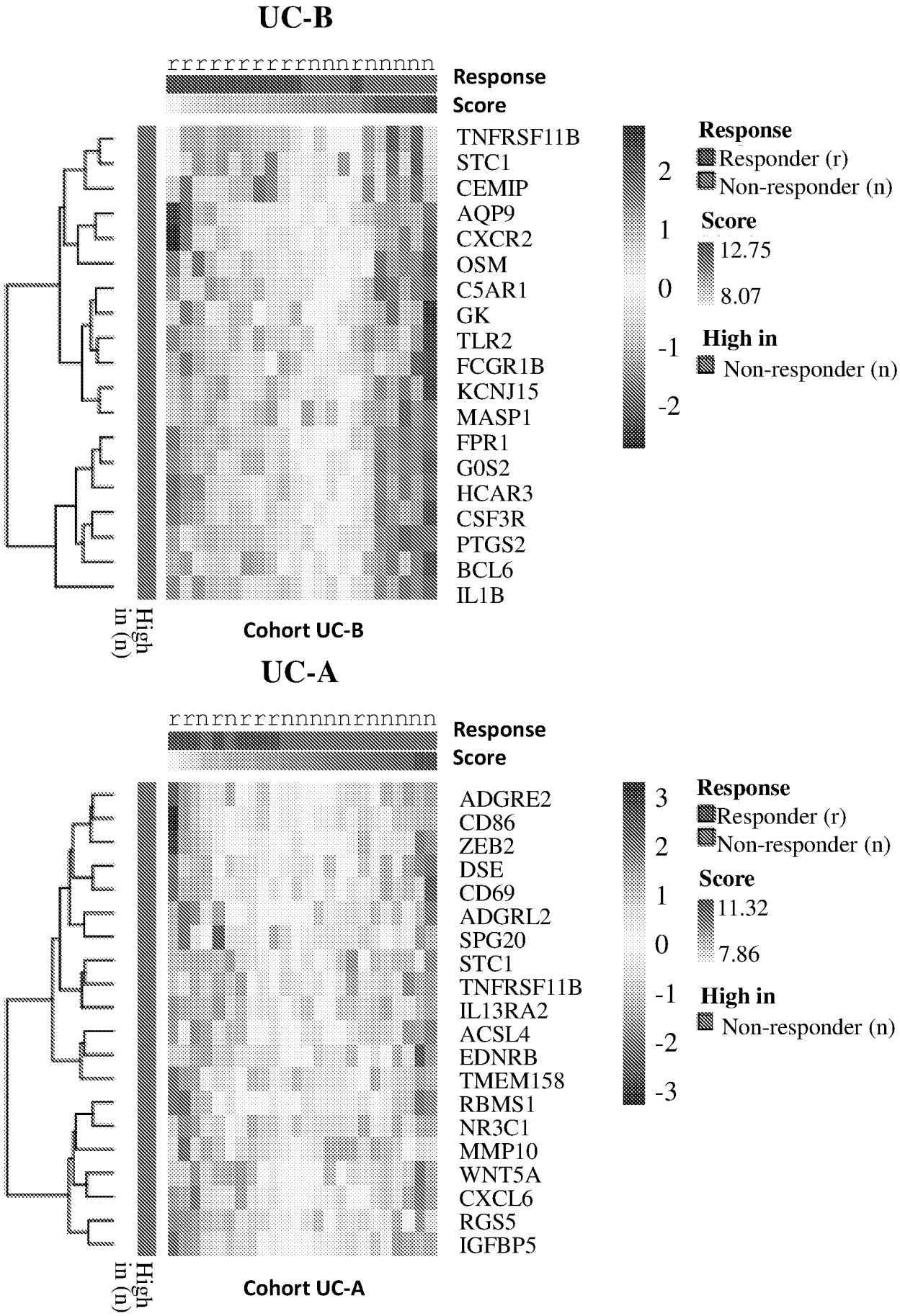

UC-B
rrrrrrrrrrnnnrnnnnn
Response
Score
TNFRSF11B
STC1
CEMIP
AQP9
CXCR2
OSM
C5AR1
GK
TLR2
FCGR1B
KCNJ15
MASP1
FPR1
G0S2
HCAR3
CSF3R
PTGS2
BCL6
IL1B
2
1
0
-1
-2
Response
Responder (r)
Non-responder (n)
Score
12.75
8.07
High in
Non-responder (n)
High in (n)
Cohort UC-B
UC-A
rrnrnrrrnnnnnrnnnnn
Response
Score
ADGRE2
CD86
ZEB2
DSE
CD69
ADGRL2
SPG20
STC1
TNFRSF11B
IL13RA2
ACSL4
EDNRB
TMEM158
RBMS1
NR3C1
MMP10
WNT5A
CXCL6
RGS5
IGFBP5
3
2
1
0
-1
-2
-3
Response
Responder (r)
Non-responder (n)
Score
11.32
7.86
High in
Non-responder (n)
High in (n)
Cohort UC-A

Figure 1A continued

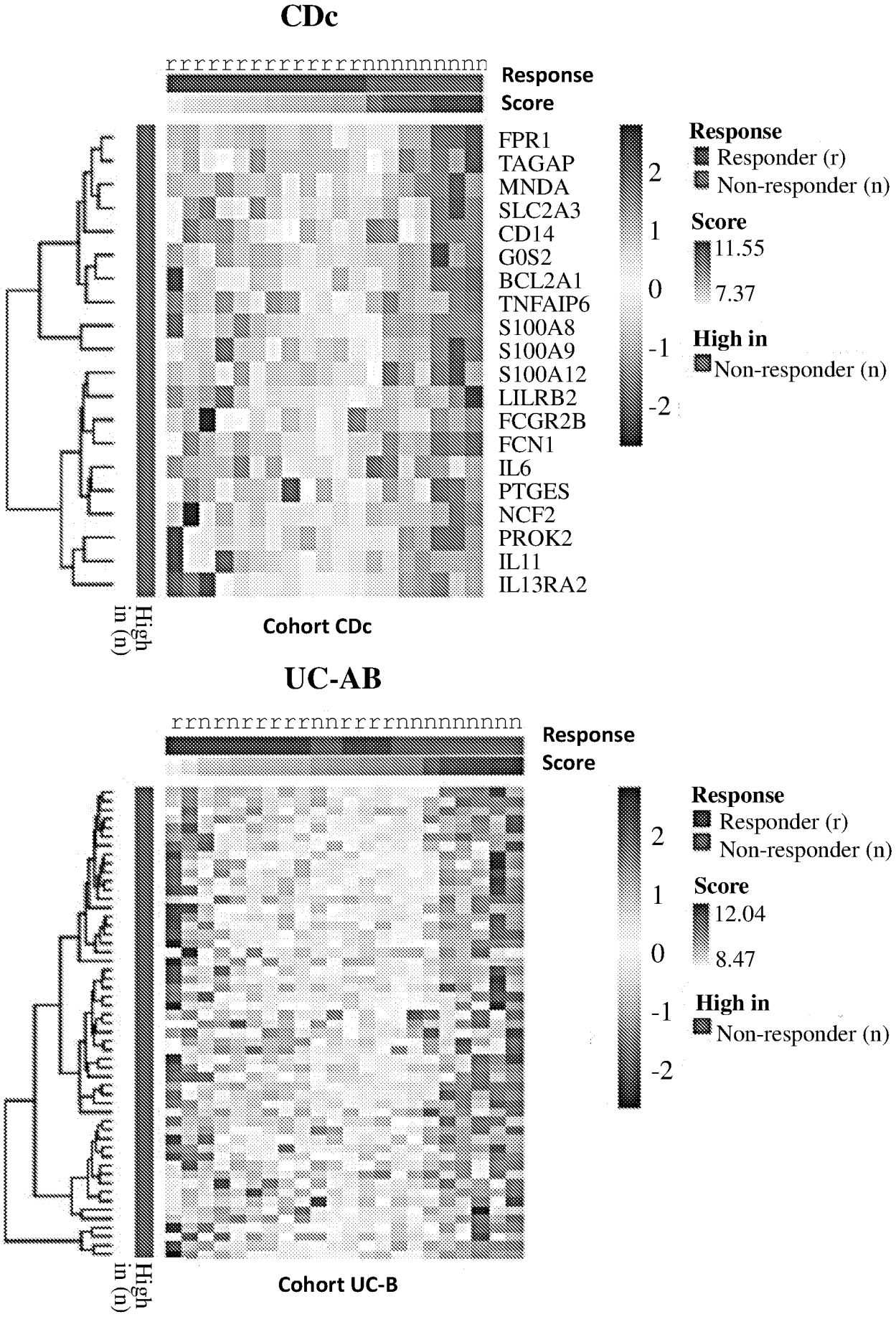


Figure 1A continued

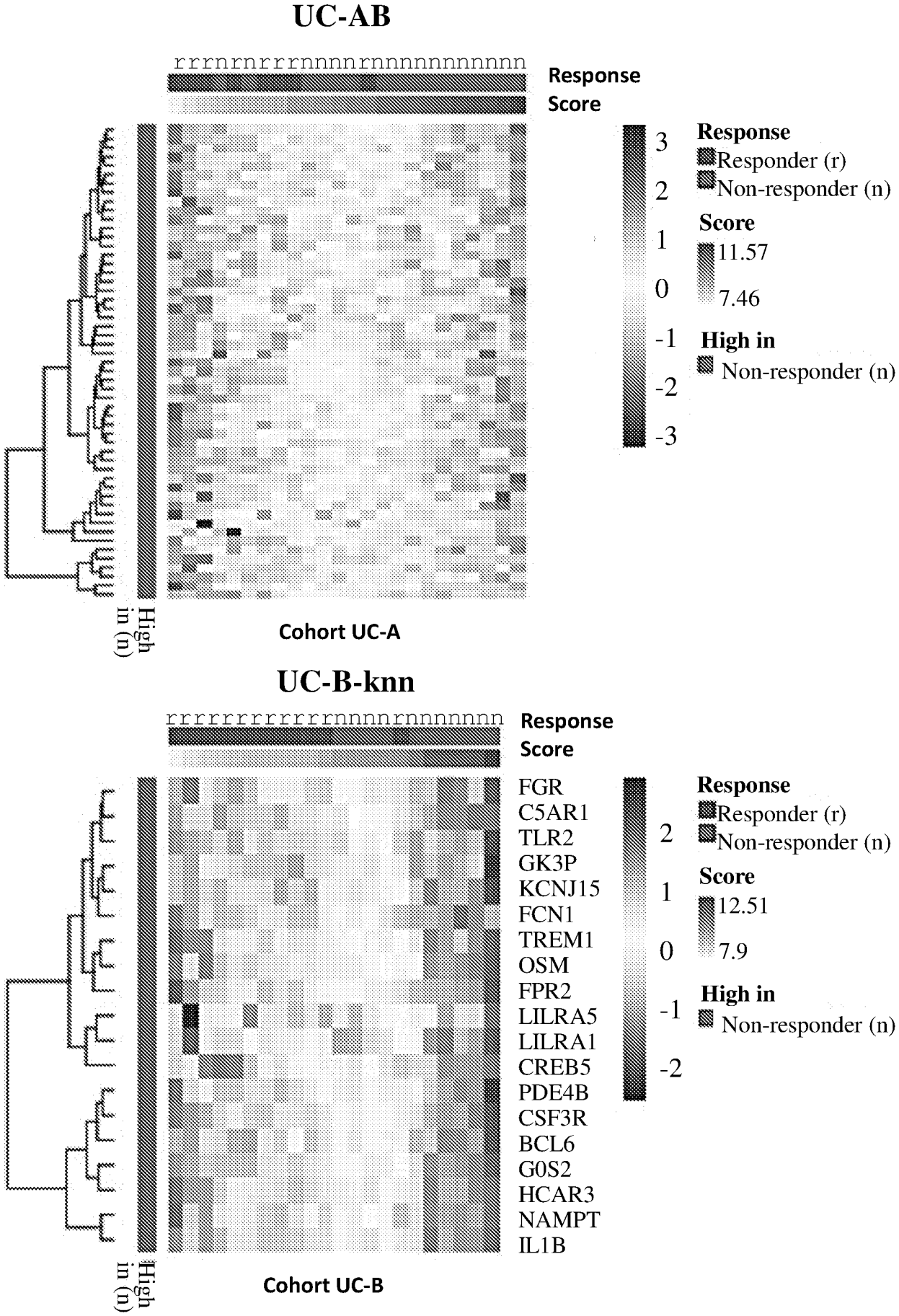
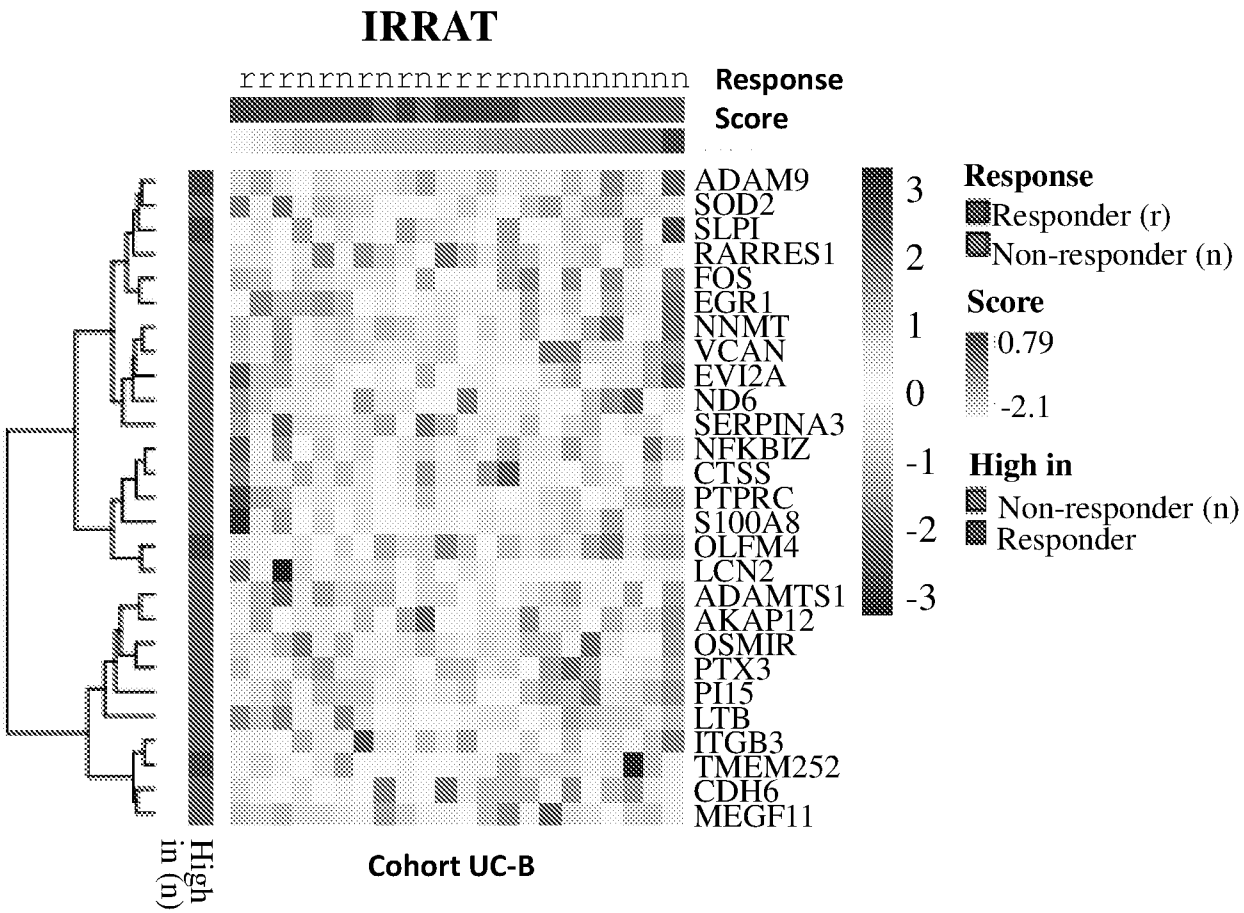


Figure 1A continued



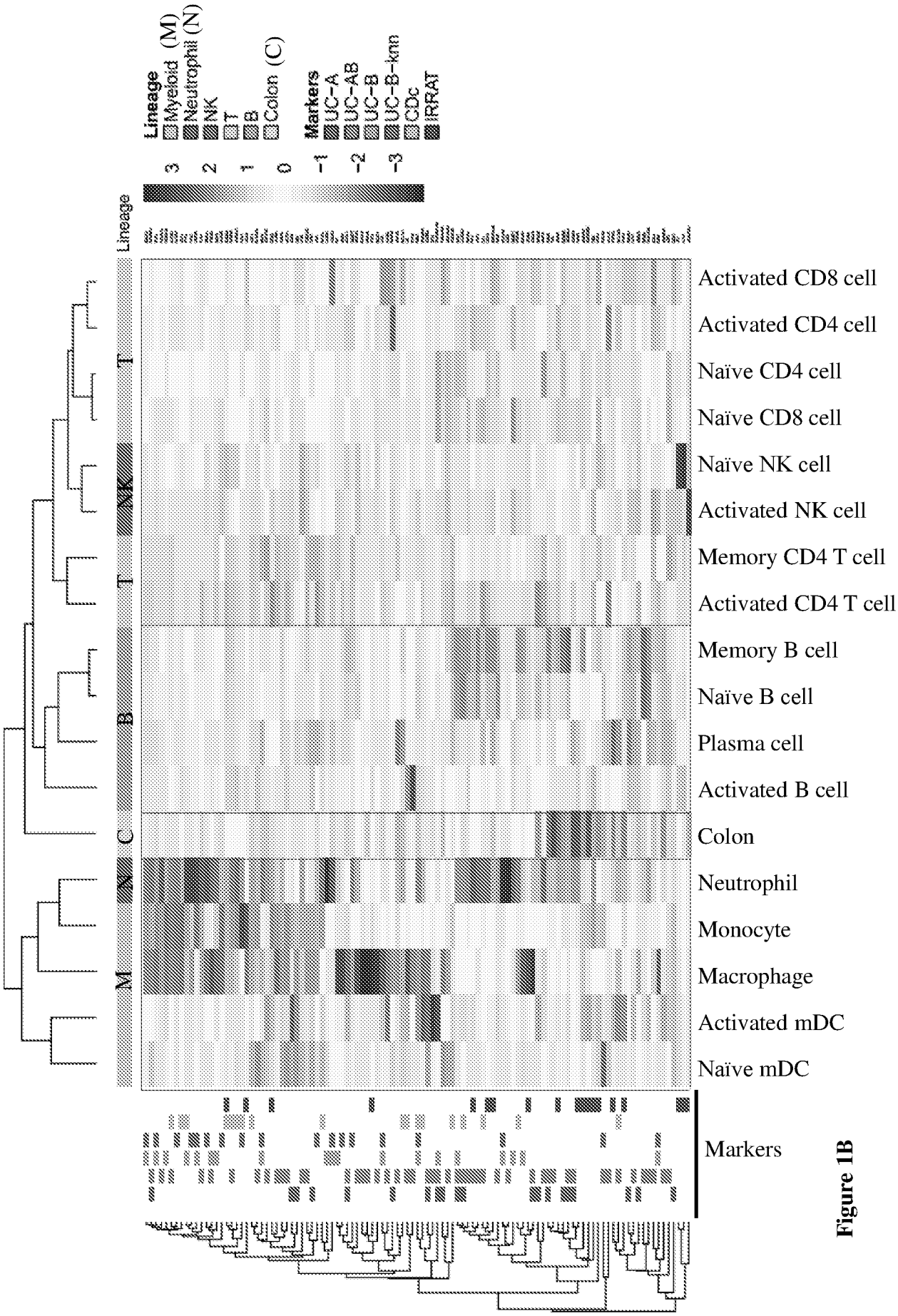

Lineage
Myeloid (M)
Neutrophil (N)
NK
T
B
Colon (C)
Markers
UC-A
UC-AB
UC-B
UC-B-knn
CDc
IRRAT
3
2
1
0
−1
−2
−3
Lineage
T
NK
T
B
C
N
M
Activated CD8 cell
Activated CD4 cell
Naïve CD4 cell
Naïve CD8 cell
Naïve NK cell
Activated NK cell
Memory CD4 T cell
Activated CD4 T cell
Memory B cell
Naïve B cell
Plasma cell
Activated B cell
Colon
Neutrophil
Monocyte
Macrophage
Activated mDC
Naïve mDC
Markers

Figure 1B

Figure 2A

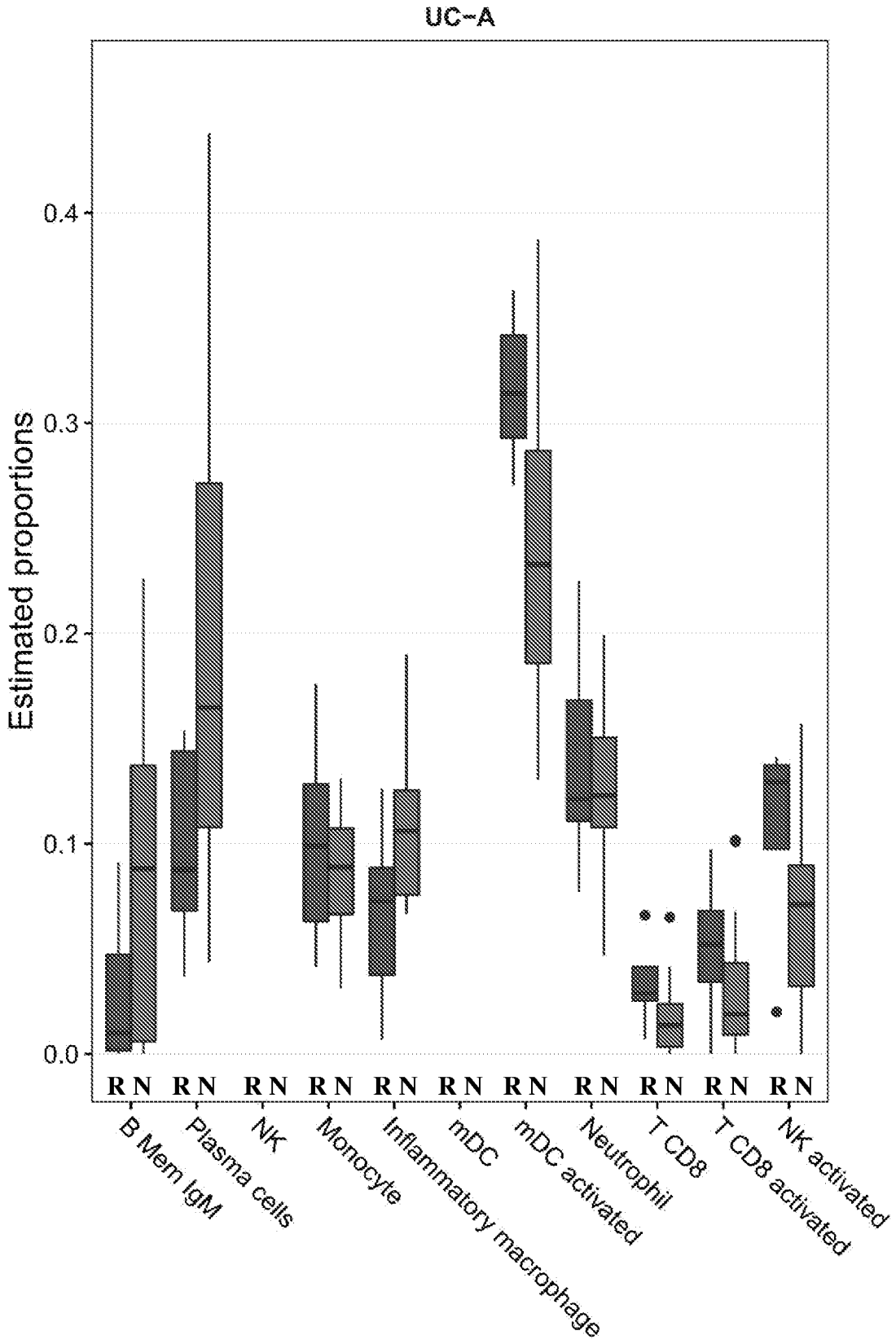

UC-A
Estimated proportions
0.0
0.1
0.2
0.3
0.4
R N R N R N R N R N R N R N R N R N R N R N
B Mem IgM
Plasma cells
NK
Monocyte
Inflammatory macrophage
mDC
mDC activated
Neutrophil
T CD8
T CD8 activated
NK activated

Figure 2A continued

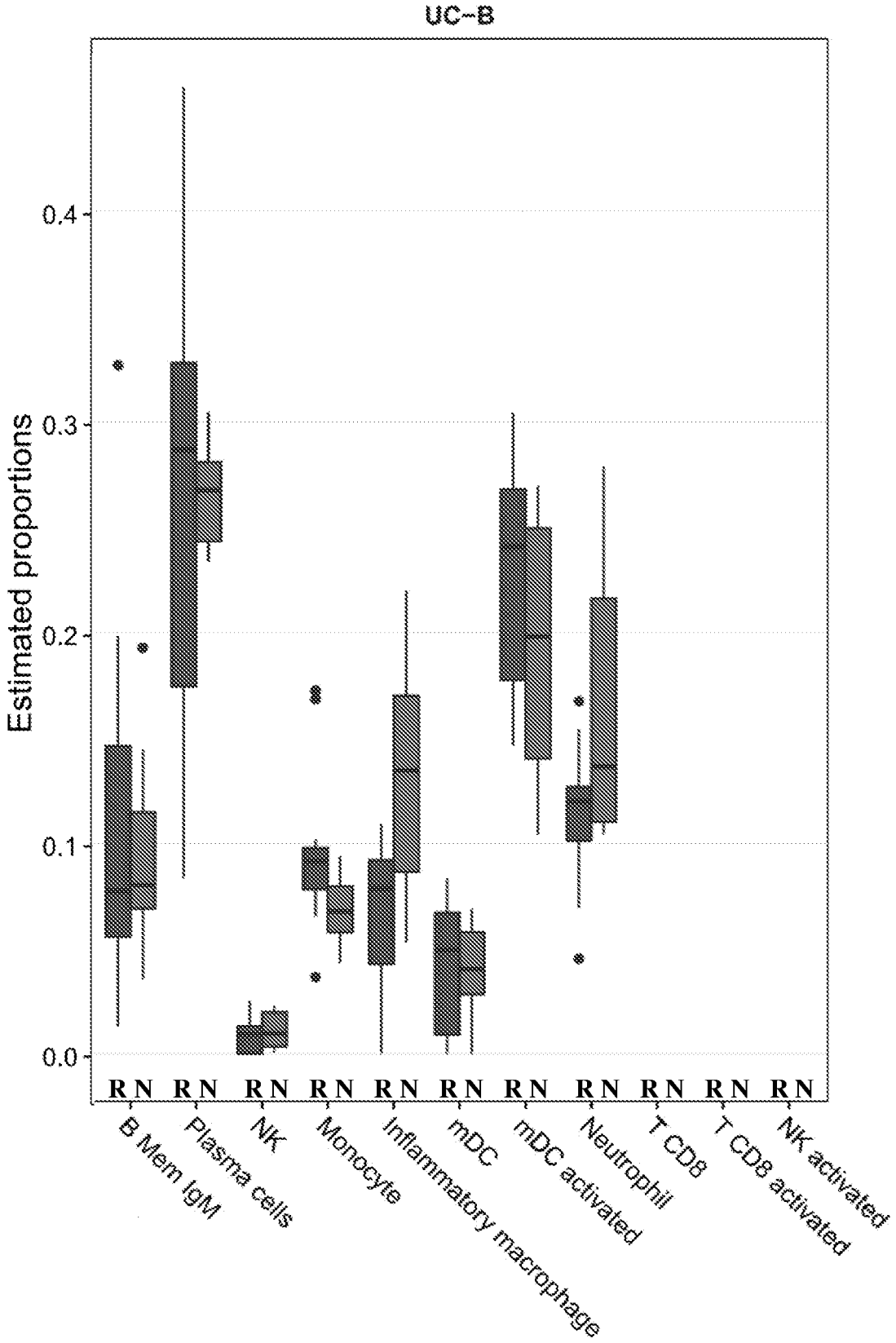


Figure 2A continued

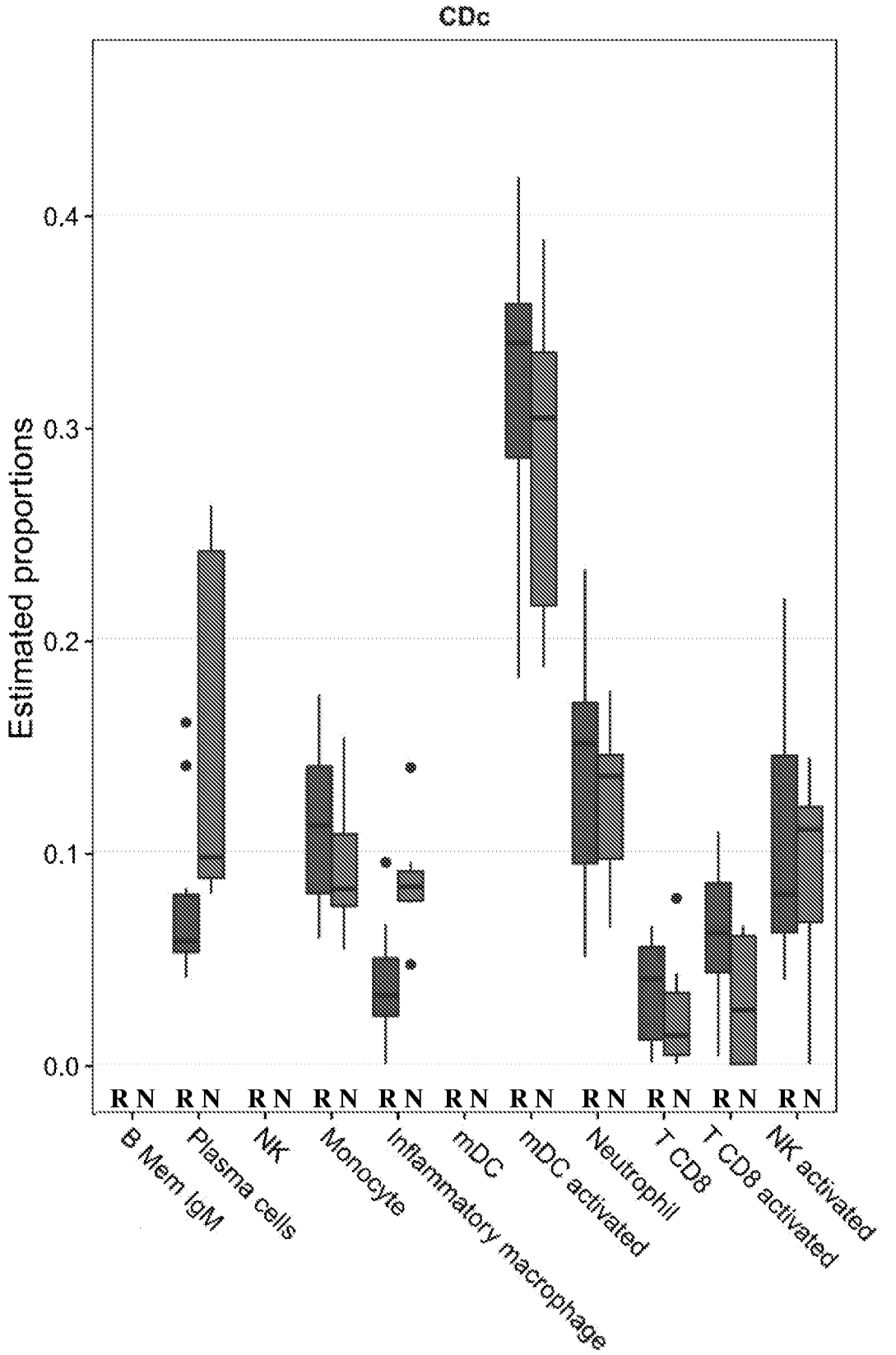


Figure 2B

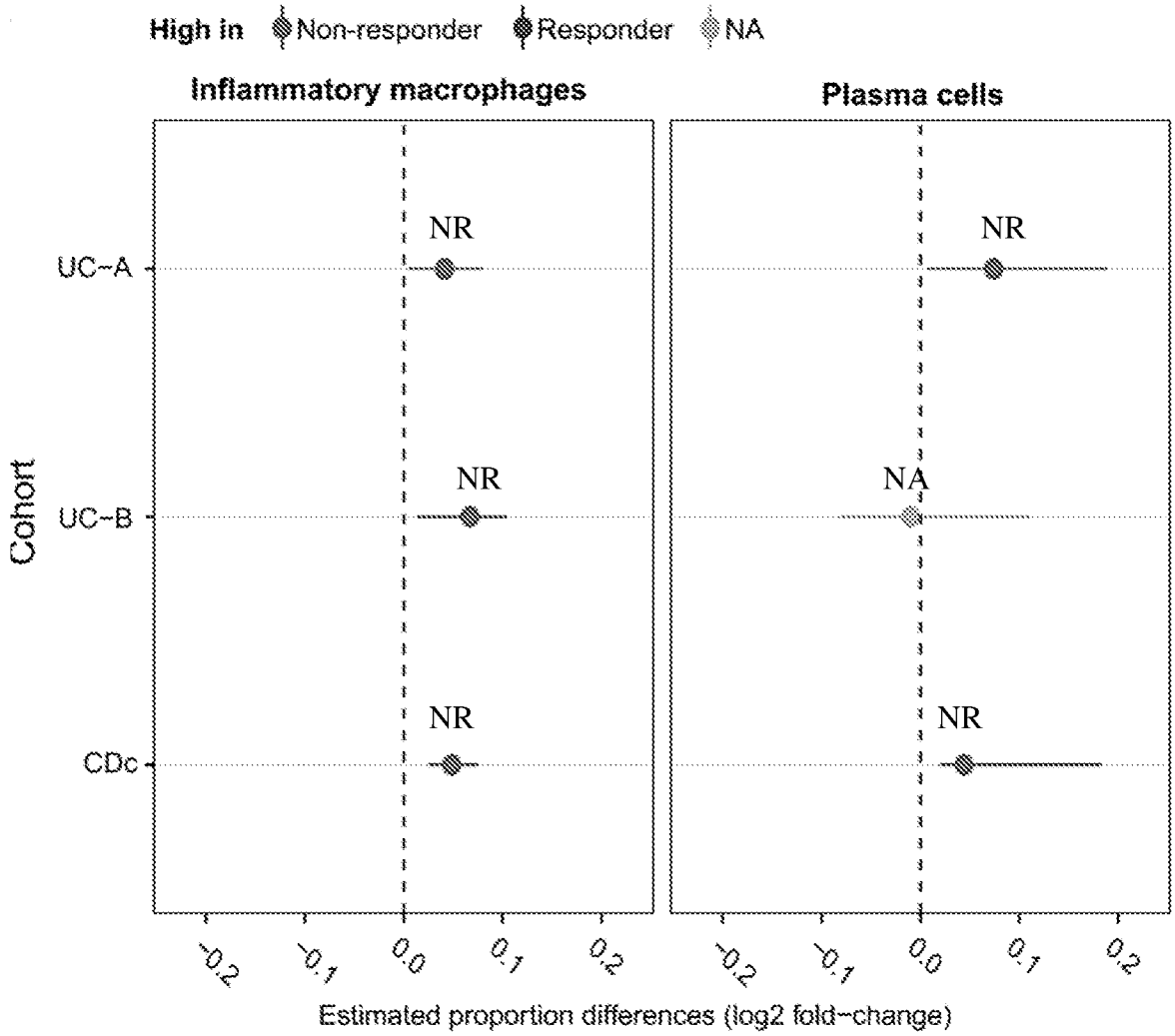

High in
Non-responder
Responder
NA
Inflammatory macrophages
Plasma cells
Cohort
UC~A
UC~B
CDc
NR
NR
NR
NR
NA
NR
-0.2
-0.1
0.0
0.1
0.2
-0.2
-0.1
0.0
0.1
0.2
Estimated proportion differences (log2 fold-change)

Figure 2C

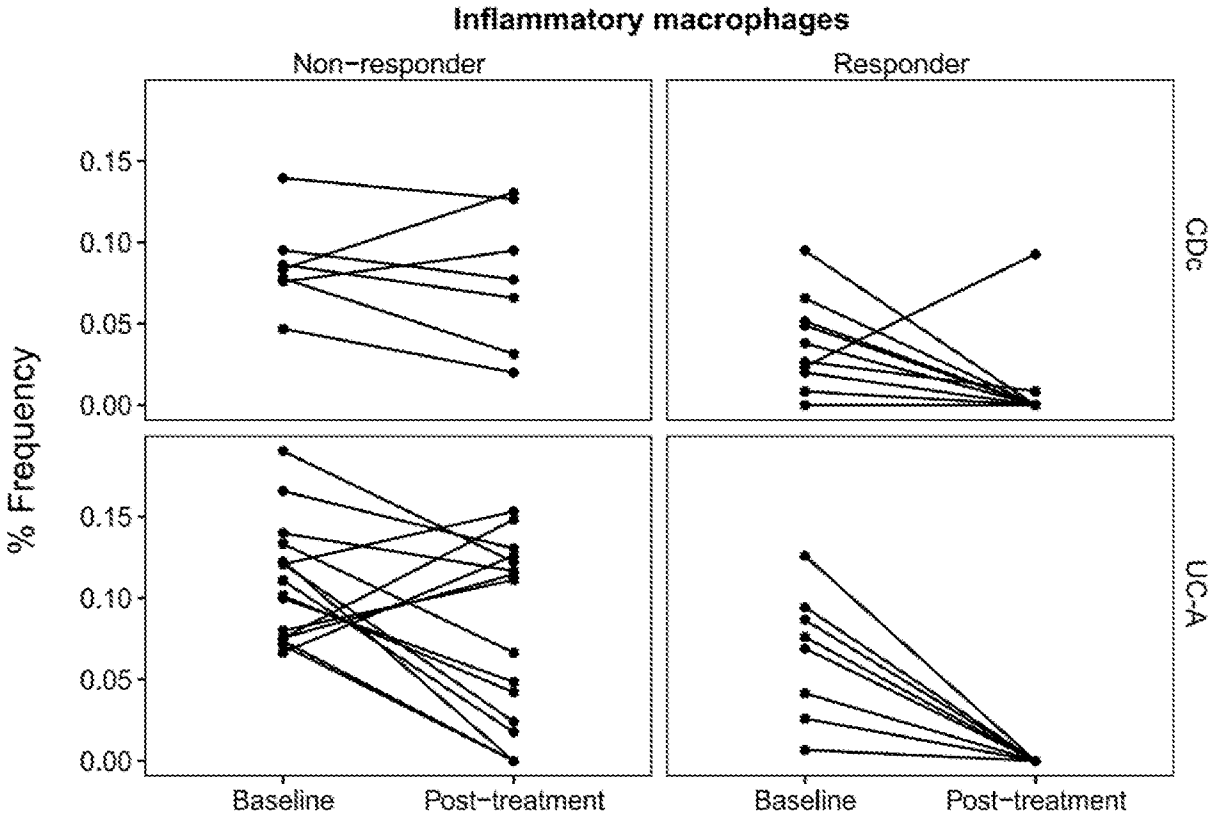
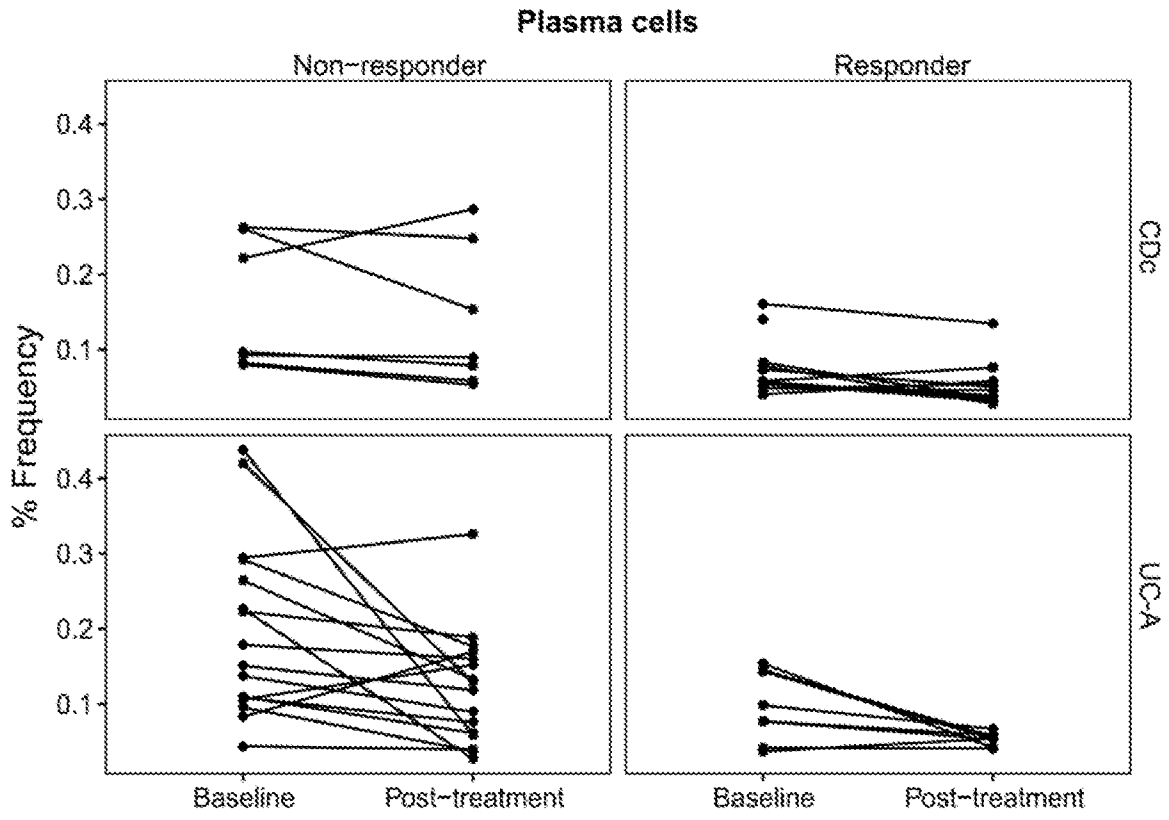


Figure 2D



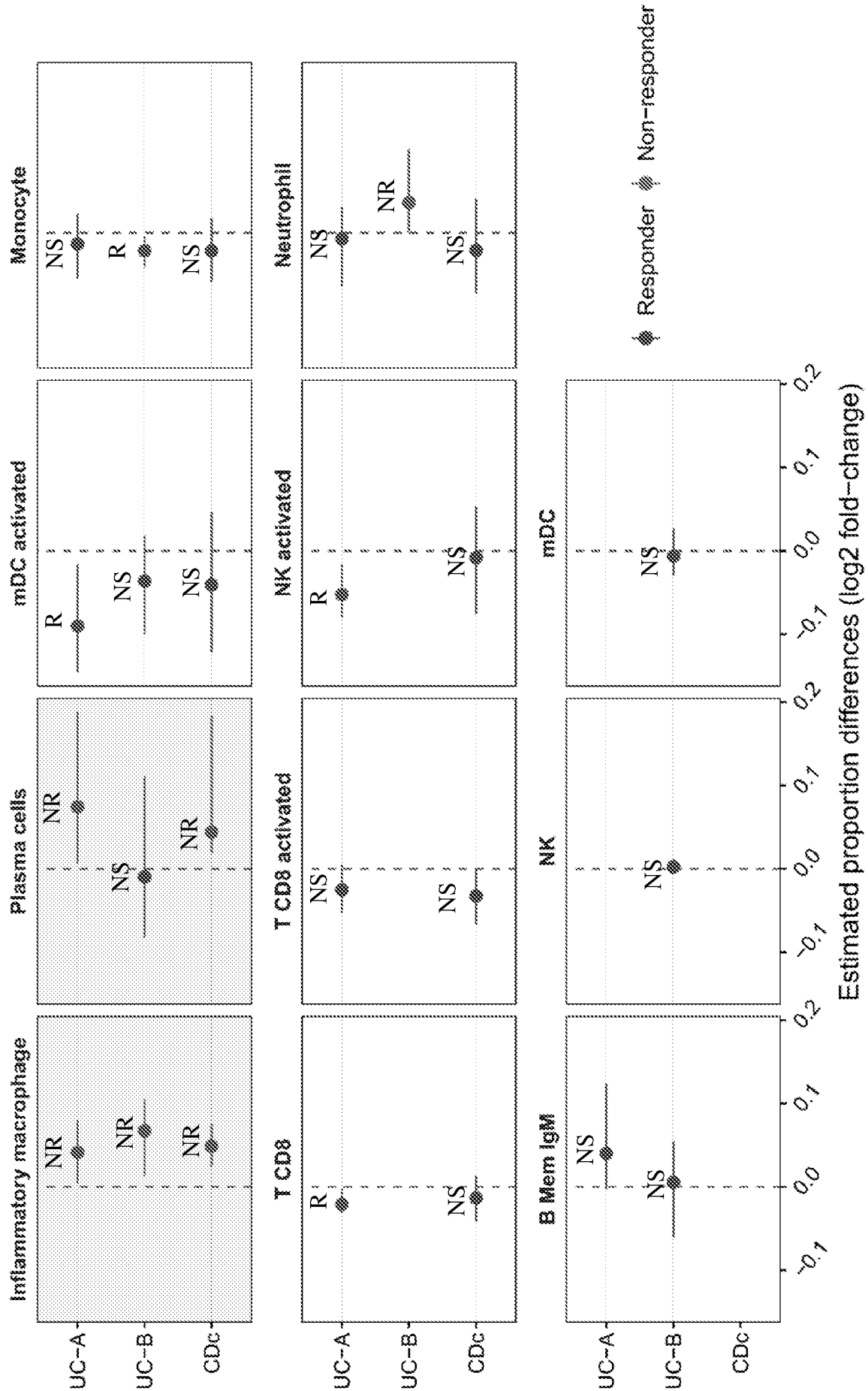

Inflammatory macrophage
Plasma cells
mDC activated
Monocyte
T CD8
T CD8 activated
NK activated
Neutrophil
B Mem IgM
NK
mDC
UC−A
UC−B
CDc
NR
NS
R
Responder
Non−responder
−0.1
0.0
0.1
0.2
Estimated proportion differences (log2 fold−change)

Figure 2E

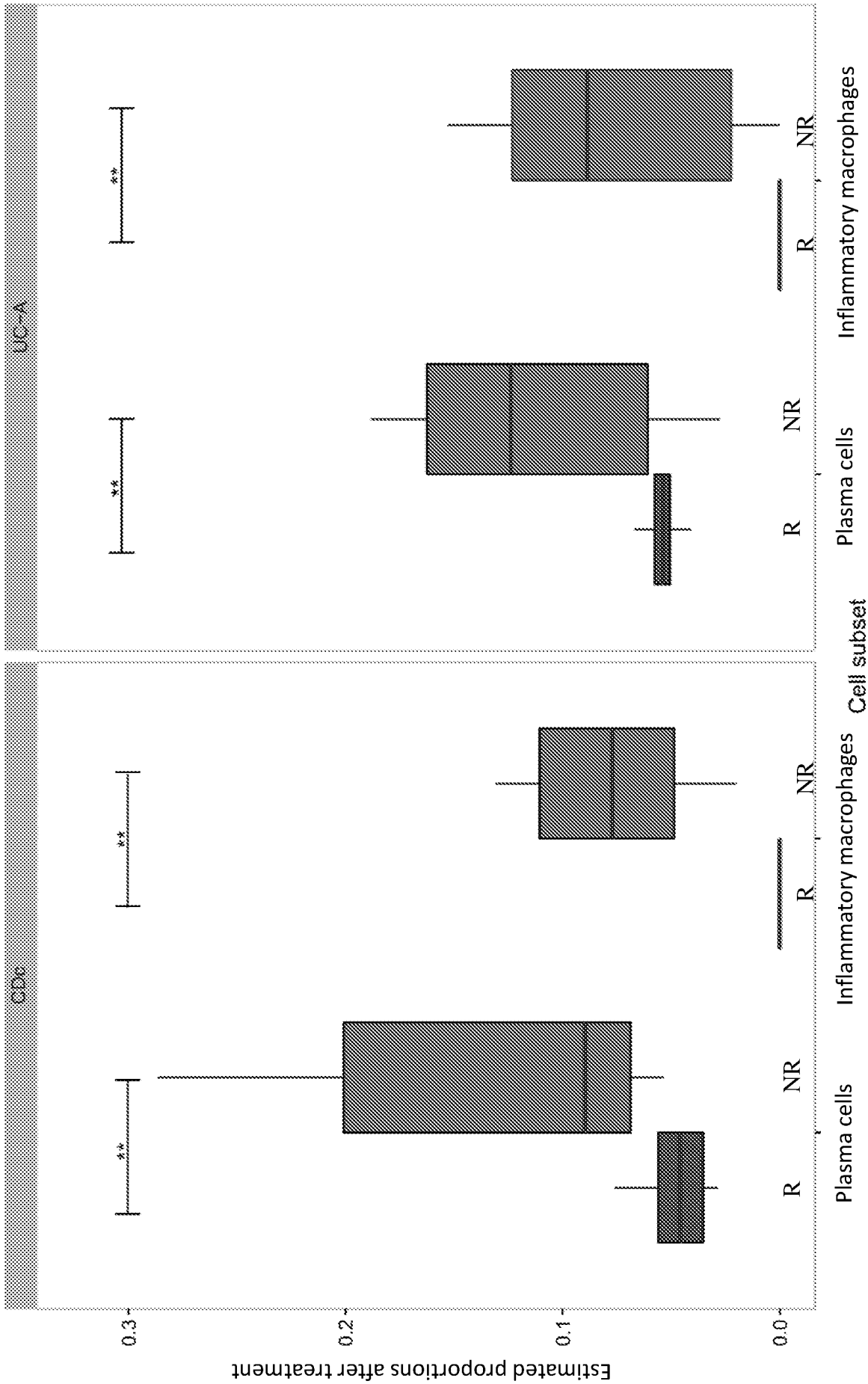

CDr
UC-A
Estimated proportions after treatment
0.0
0.1
0.2
0.3
**
**
**
**
R
NR
R
NR
R
NR
R
NR
Plasma cells
Inflammatory macrophages
Plasma cells
Inflammatory macrophages
Cell subset

Figure 2F

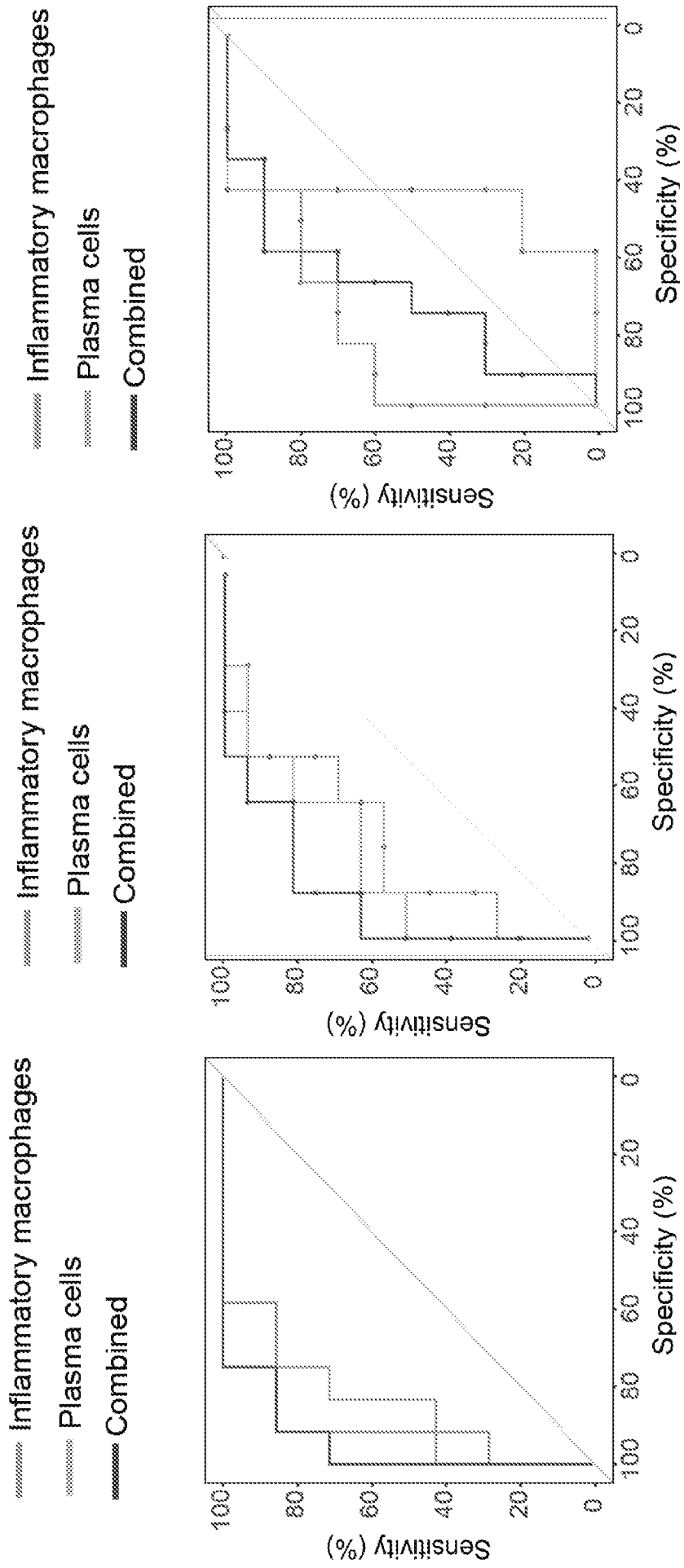


Figure 3A

Figure 3B

Figure 3C

Figure 3D

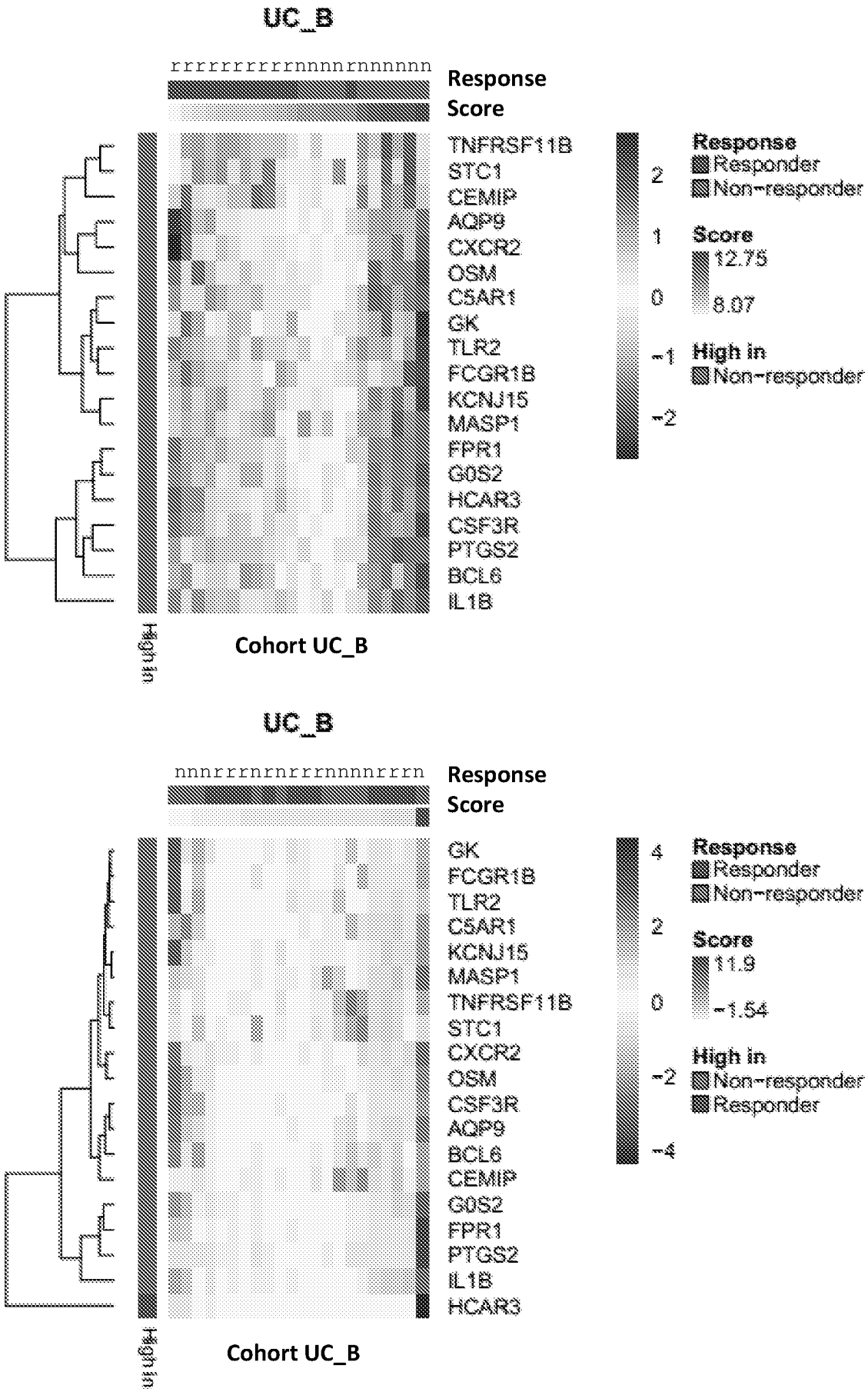

UC_B
rrrrrrrrrrnnnnrnnnnnn
Response
Score
TNFRSF11B
STC1
CEMIP
AQP9
CXCR2
OSM
C5AR1
GK
TLR2
FCGR1B
KCNJ15
MASP1
FPR1
G0S2
HCAR3
CSF3R
PTGS2
BCL6
IL1B
High in
Cohort UC_B
2
1
0
-1
-2
Response
Responder
Non-responder
Score
12.75
8.07
High in
Non-responder
UC_B
nnnrrrnrnrrrnnnnrrrn
Response
Score
GK
FCGR1B
TLR2
C5AR1
KCNJ15
MASP1
TNFRSF11B
STC1
CXCR2
OSM
CSF3R
AQP9
BCL6
CEMIP
G0S2
FPR1
PTGS2
IL1B
HCAR3
High in
Cohort UC_B
4
2
0
-2
-4
Response
Responder
Non-responder
Score
11.9
-1.54
High in
Non-responder
Responder

Figure 3D Continued

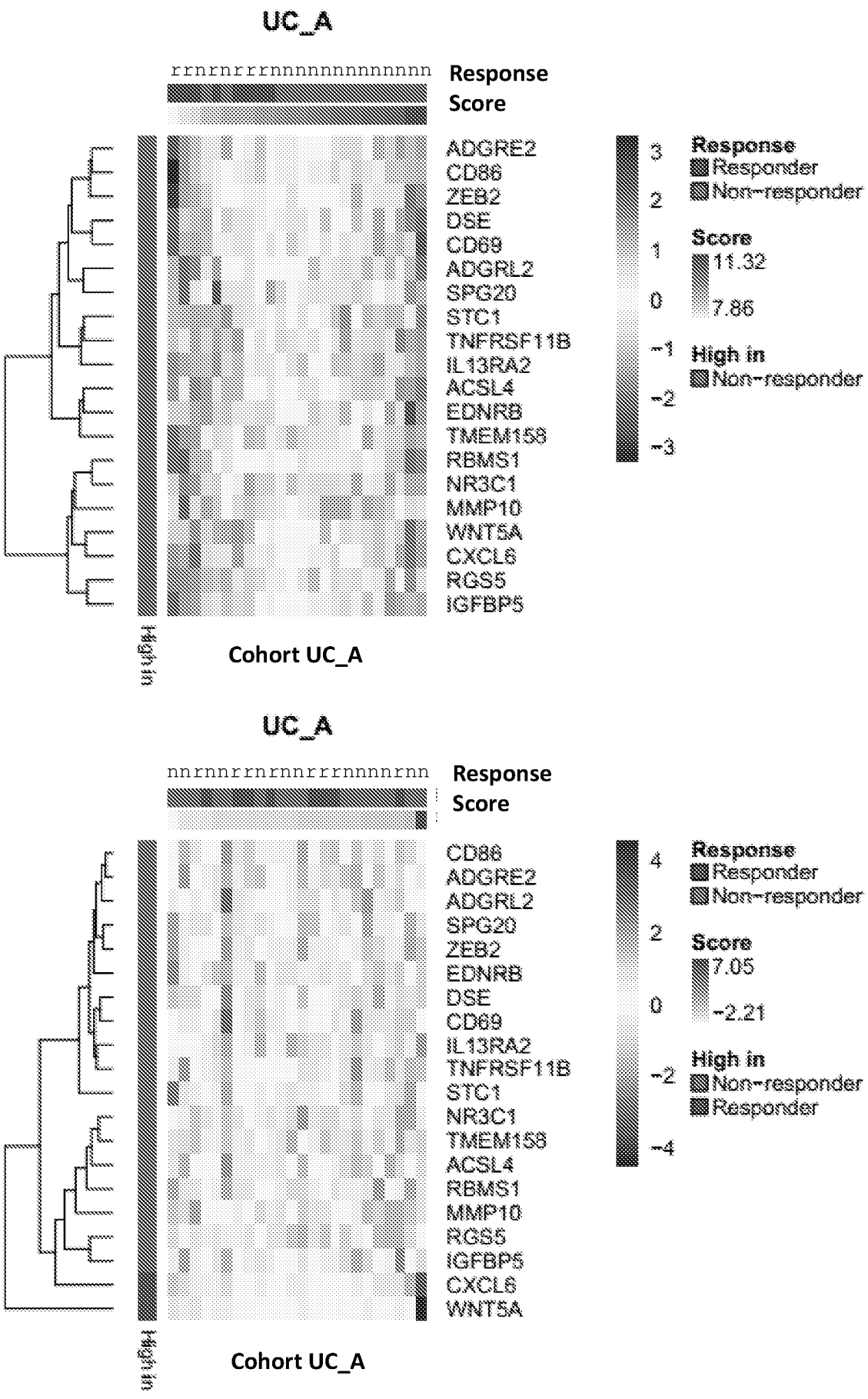
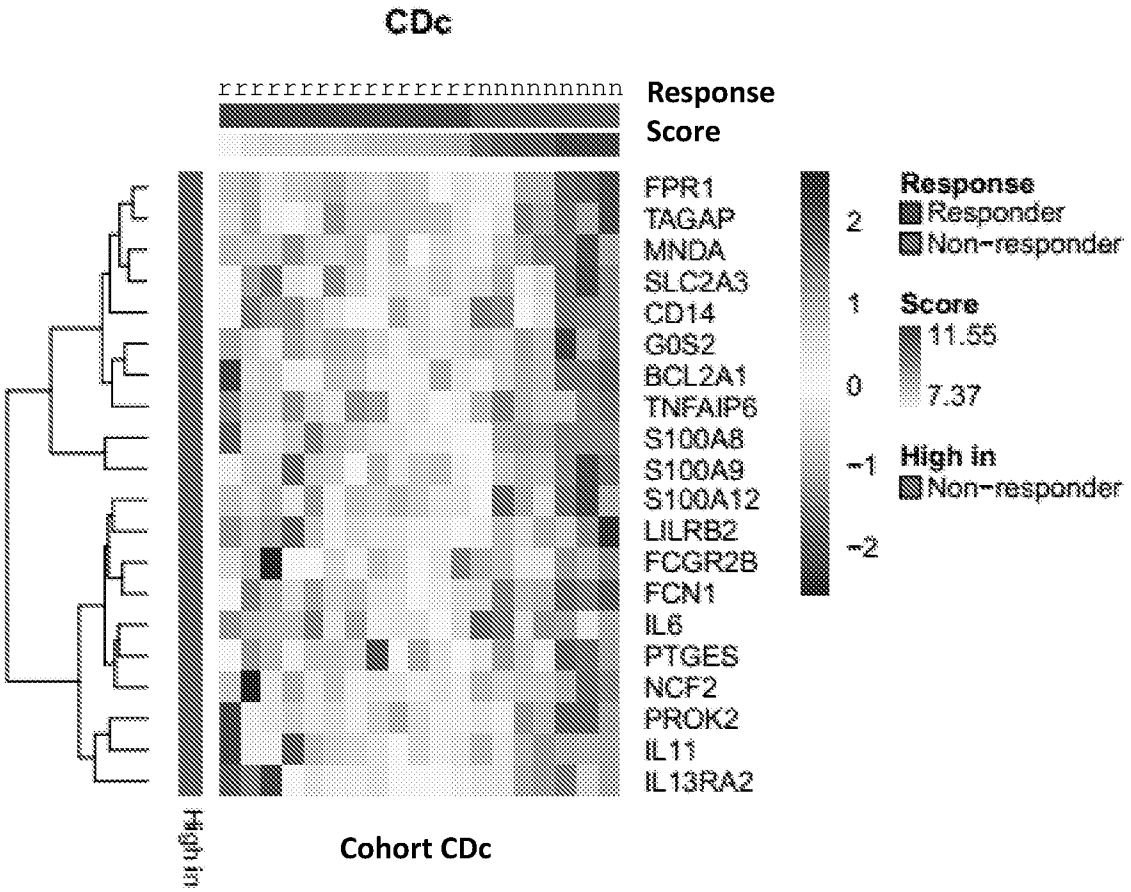
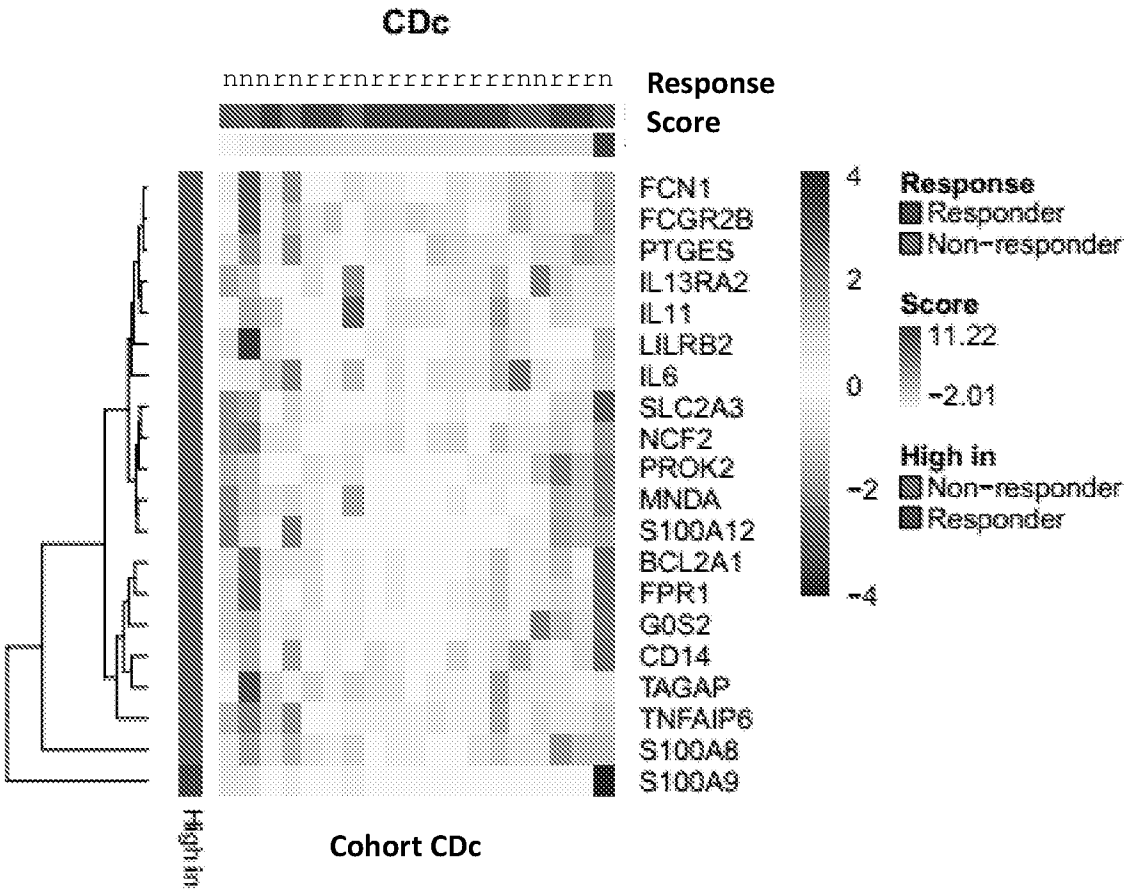


Figure 3D Continued





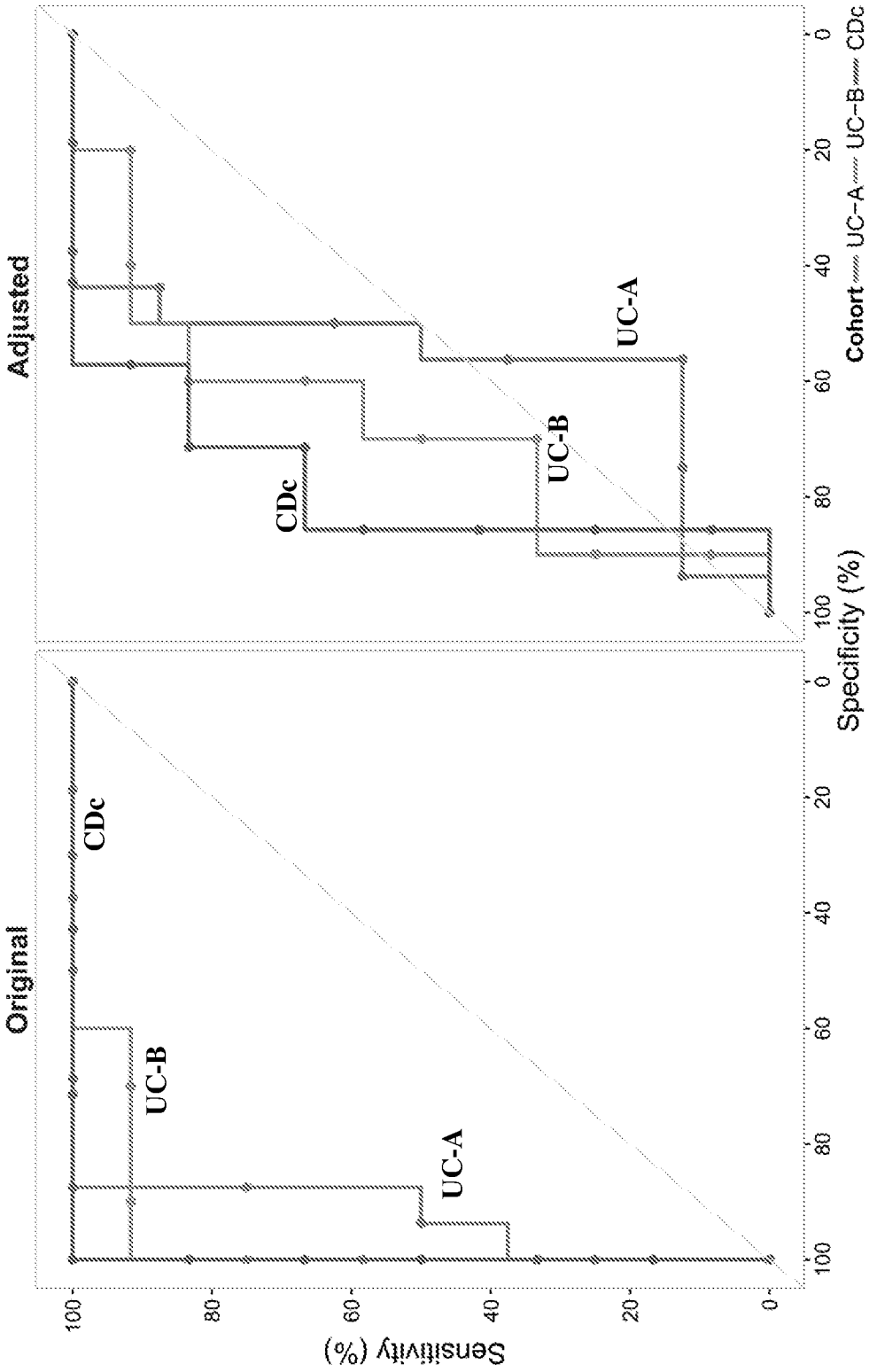

Original
Adjusted
CDc
UC-B
UC-A
Sensitivity (%)
Specificity (%)
Cohort UC-A UC-B CDc
0
20
40
60
80
100

Figure 3E

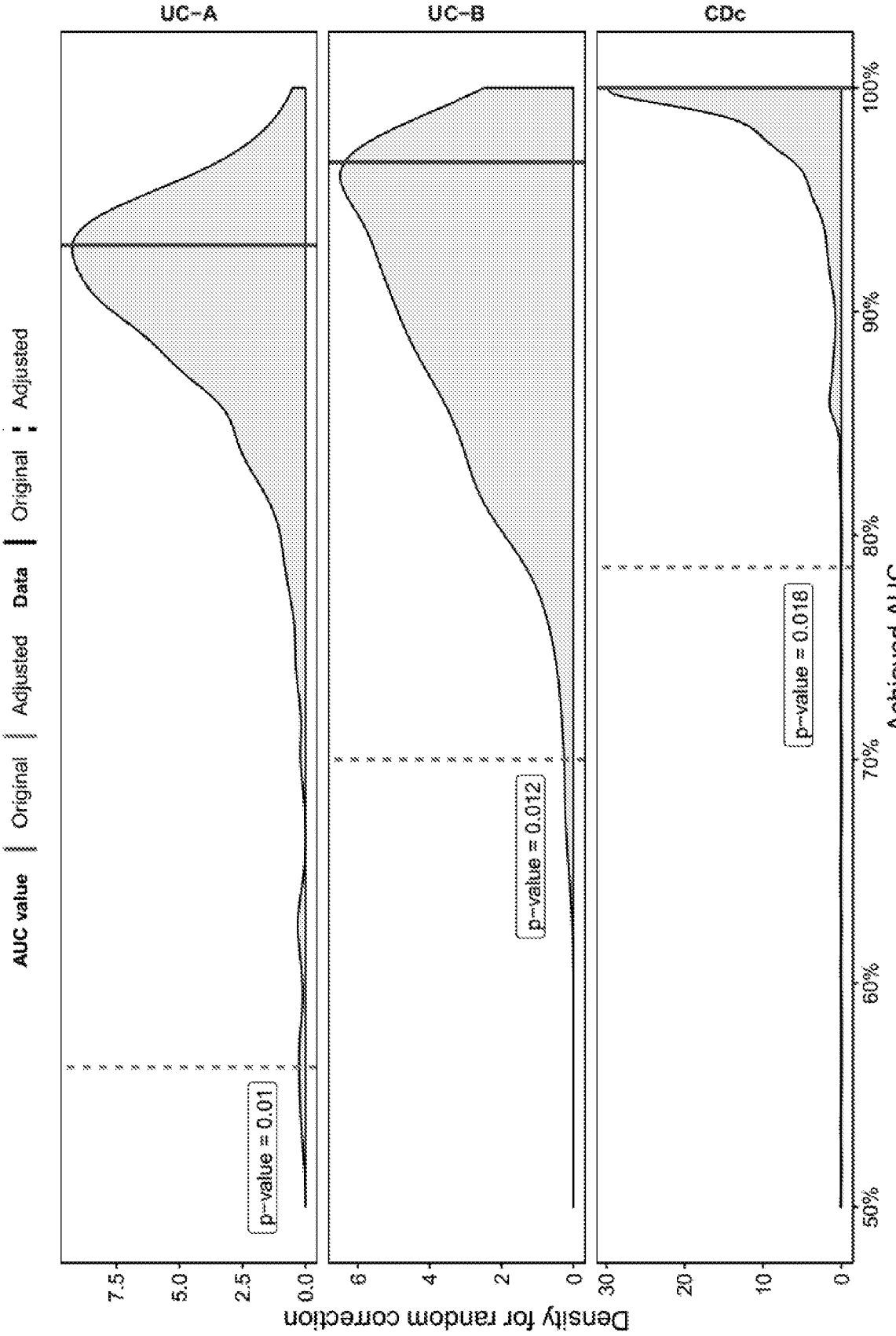

UC-A
UC-B
CDc
AUC value
Original
Adjusted
Data
Original
Adjusted
p-value = 0.01
p-value = 0.012
p-value = 0.018
Density for random correction
Achieved AUC
50%
60%
70%
80%
90%
100%

Figure 3F

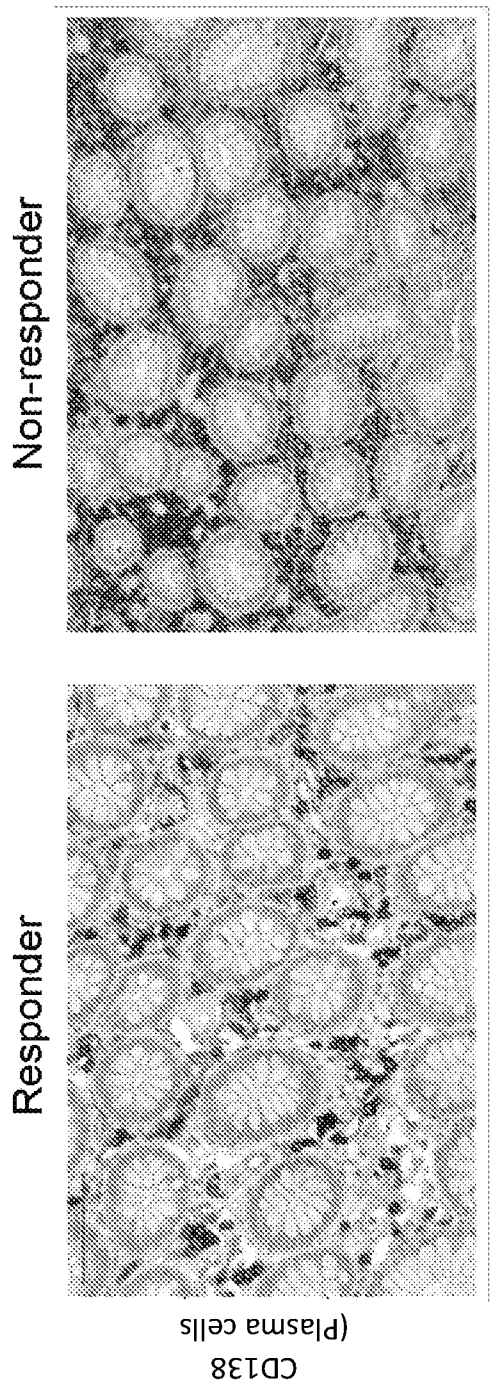


Figure 3G

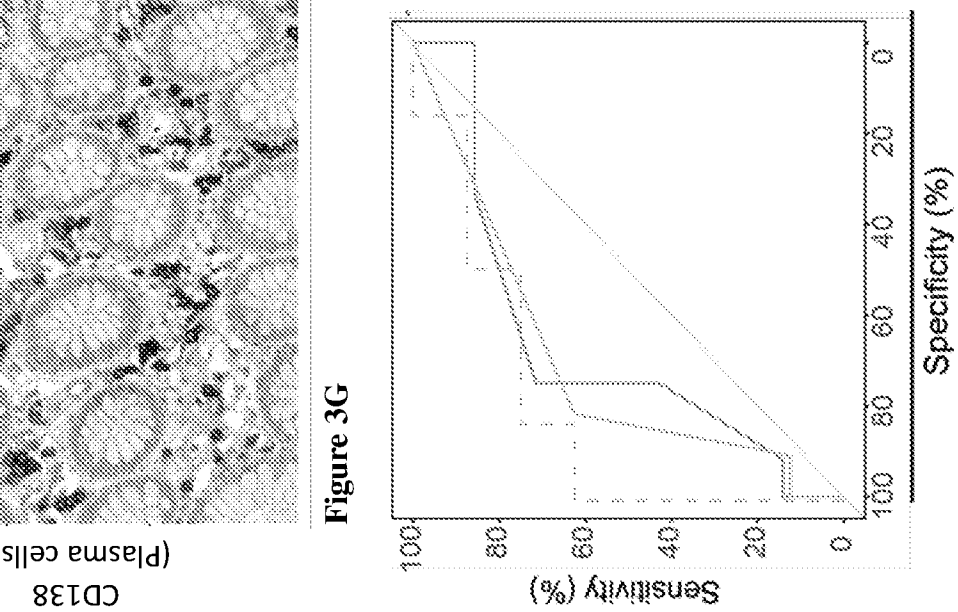


Figure 3H

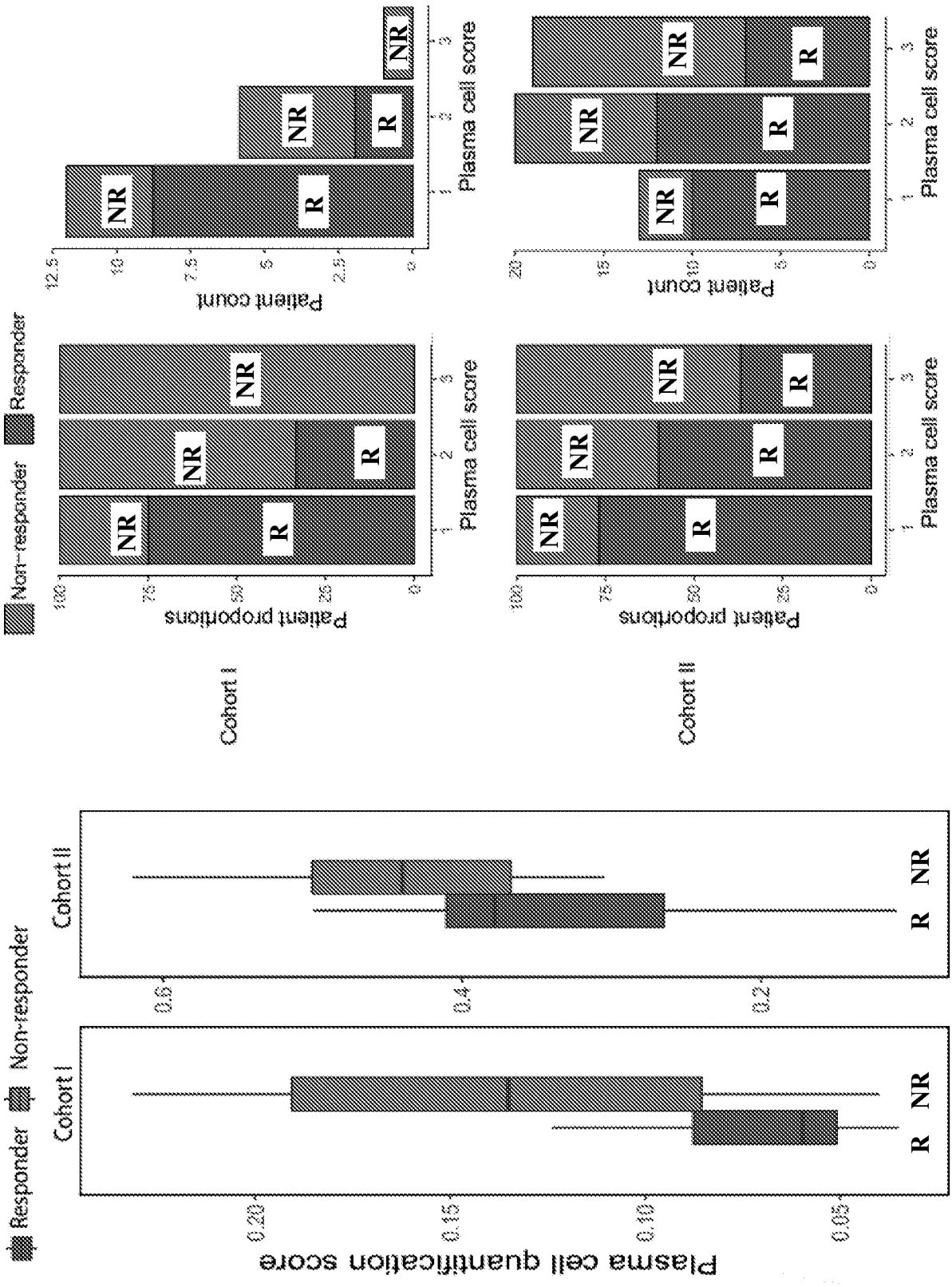
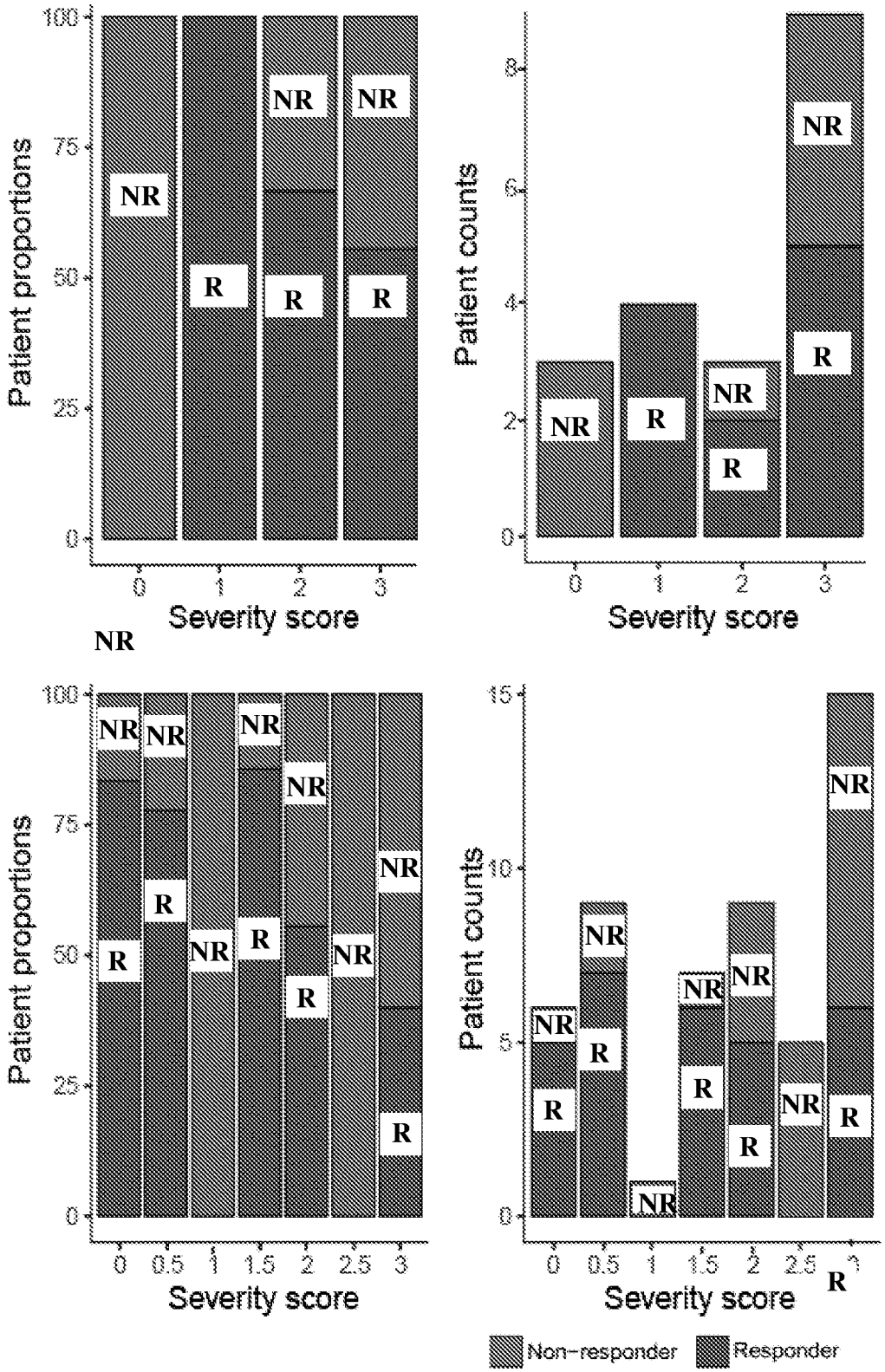


Figure 3I

Figure 3J

Figure 3K


Patient proportions
Patient counts
Severity score
NR
R
Non-responder
Responder

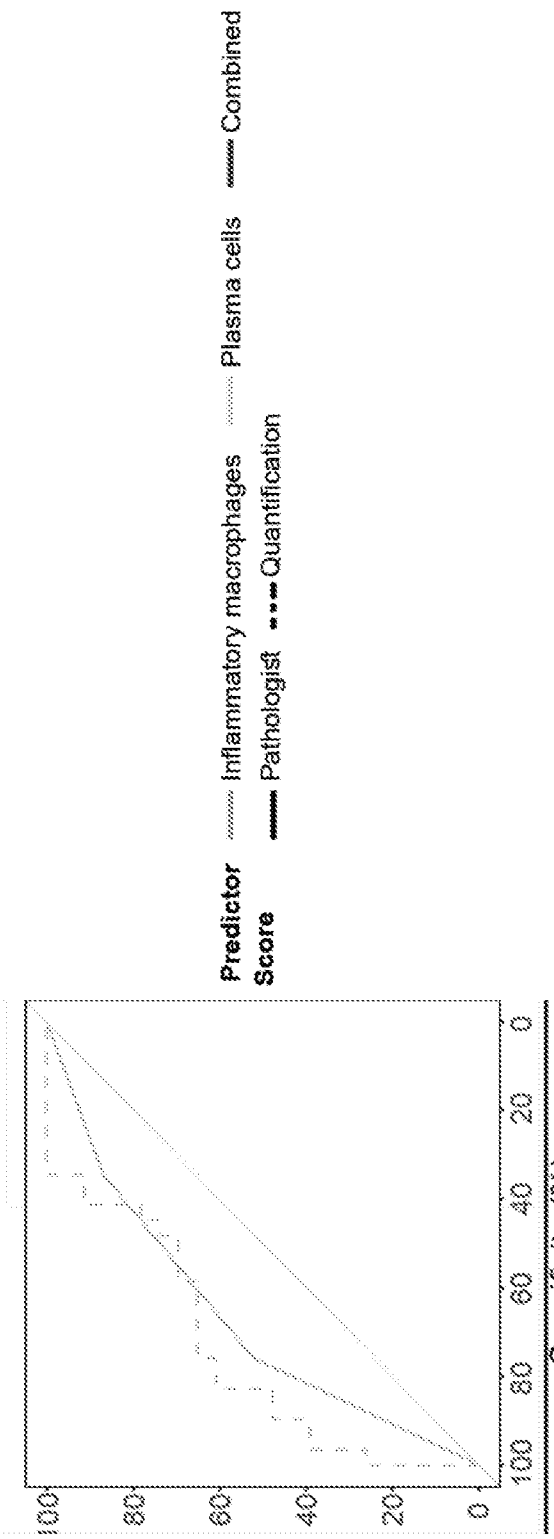


Figure 3L

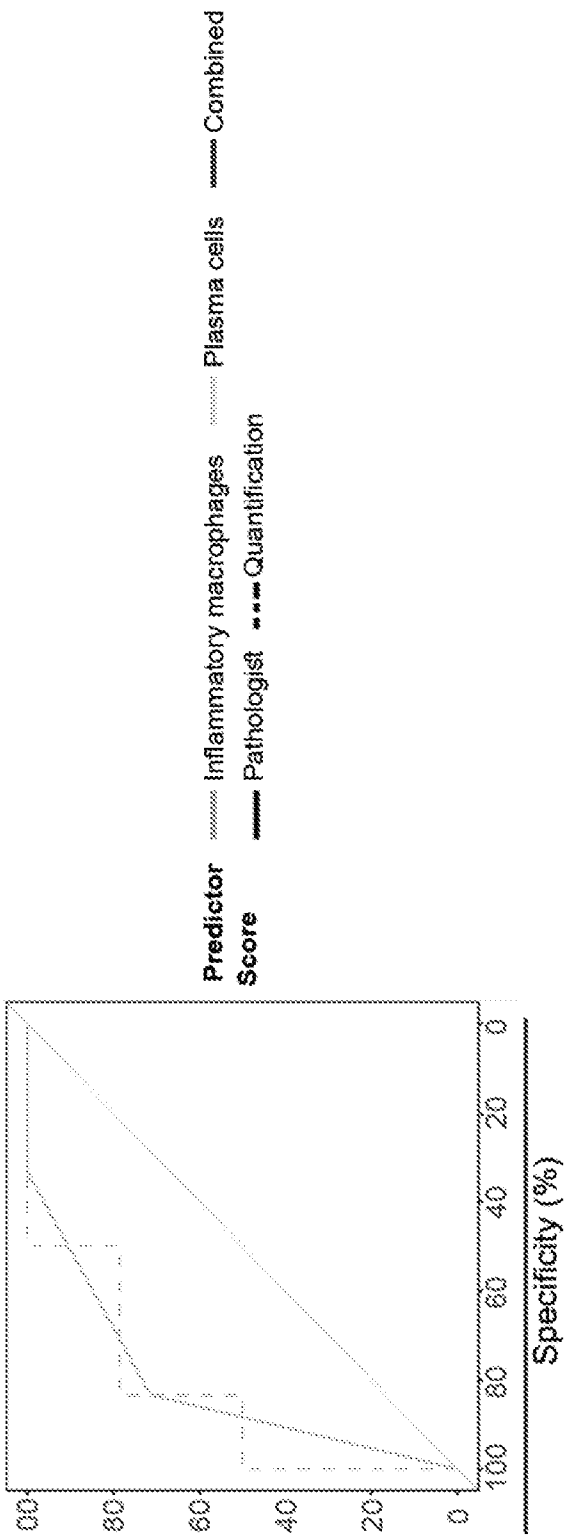


Figure 3M

Figure 4A

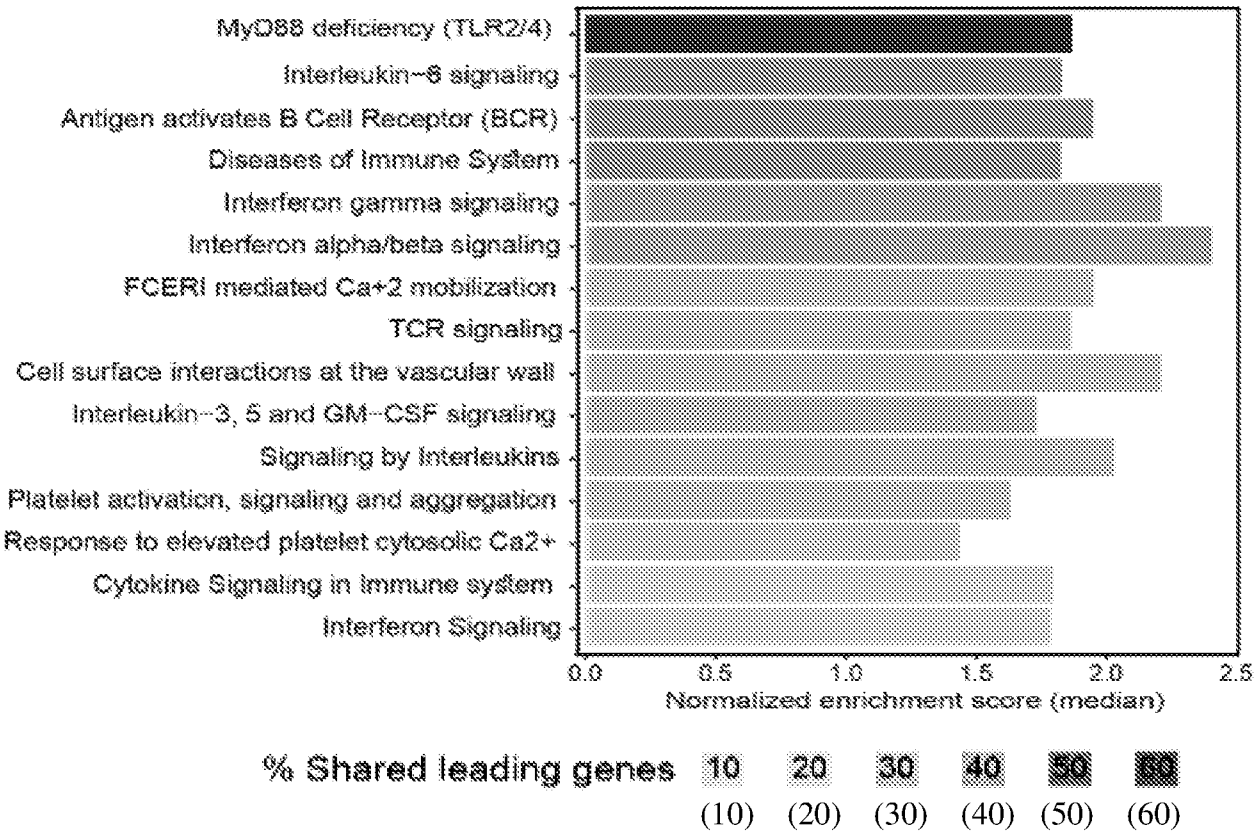
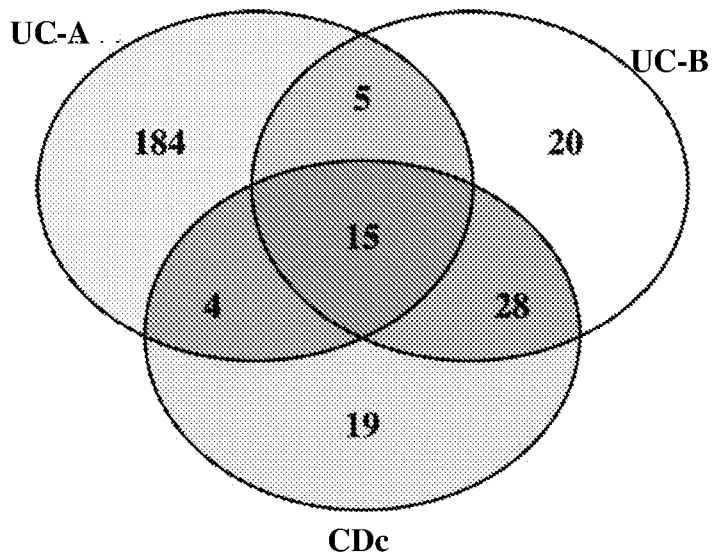


Figure 4B



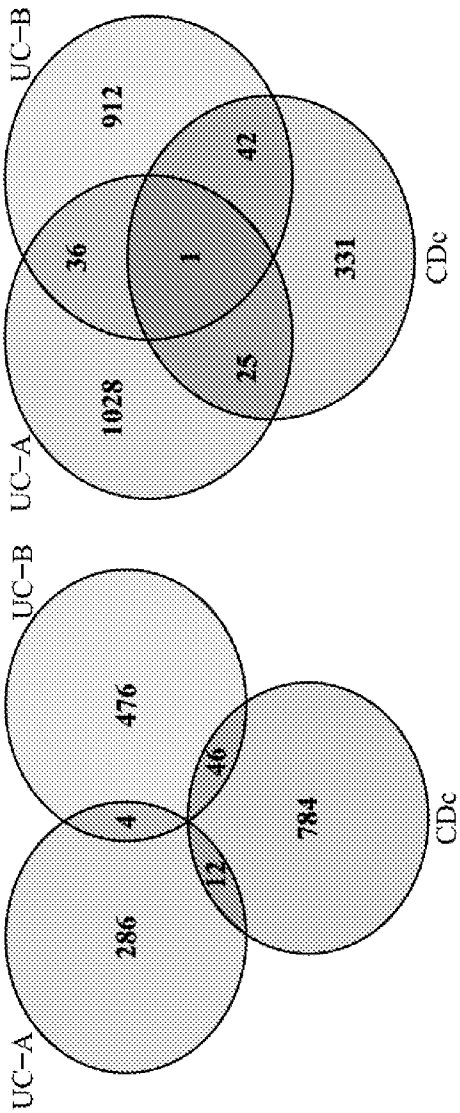


Figure 4C

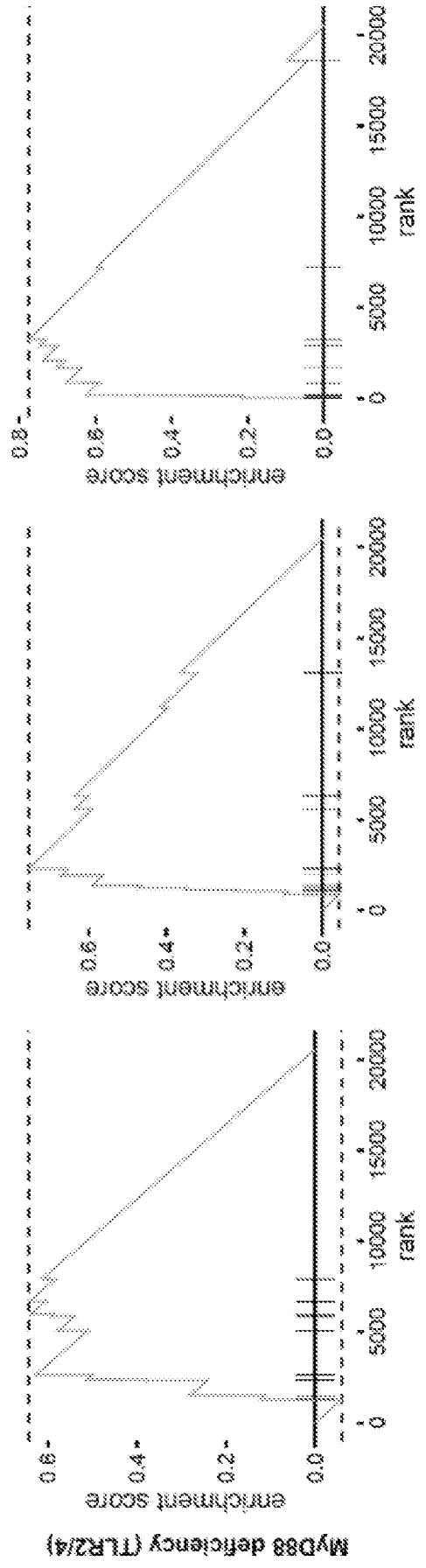


Figure 4D

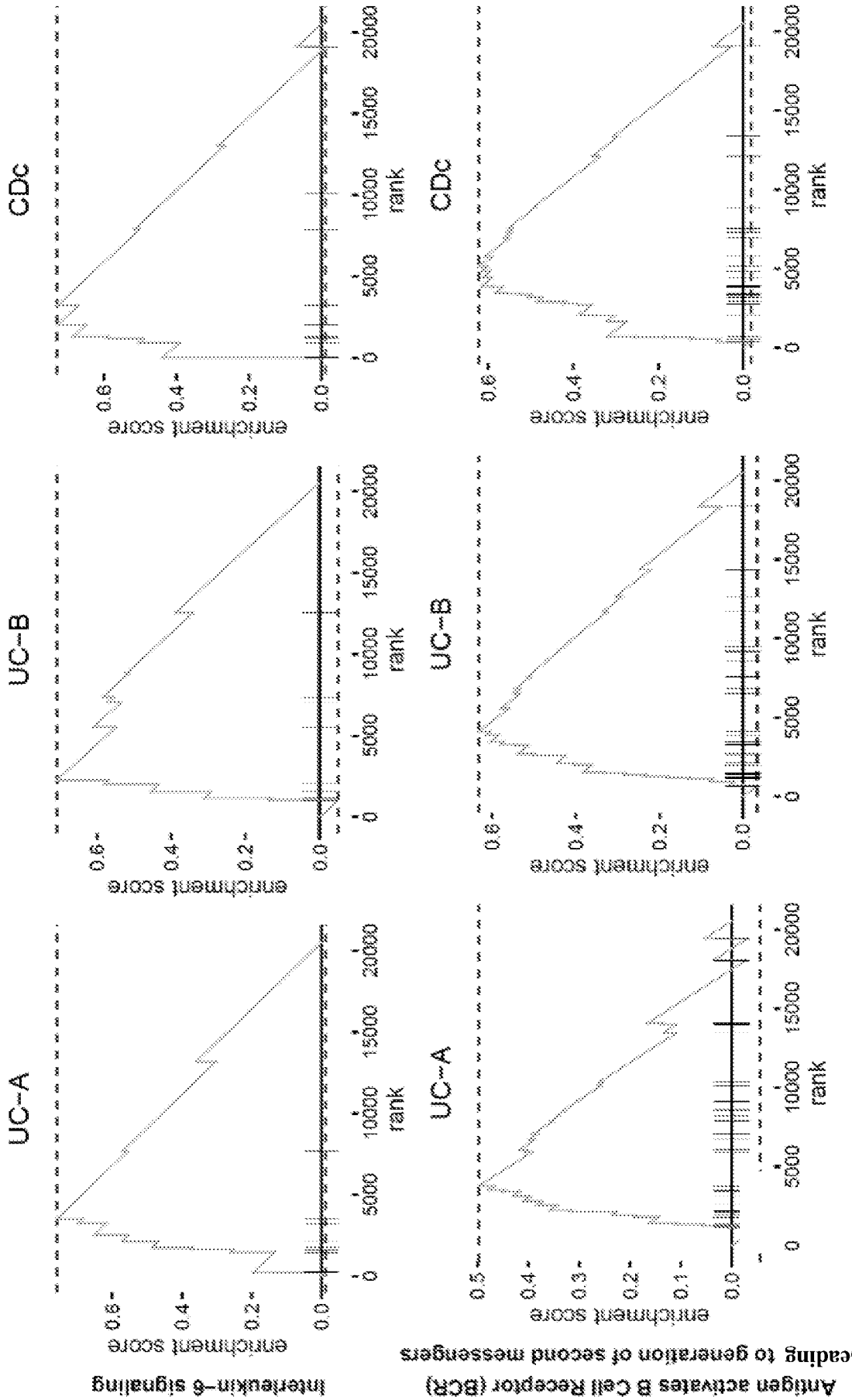
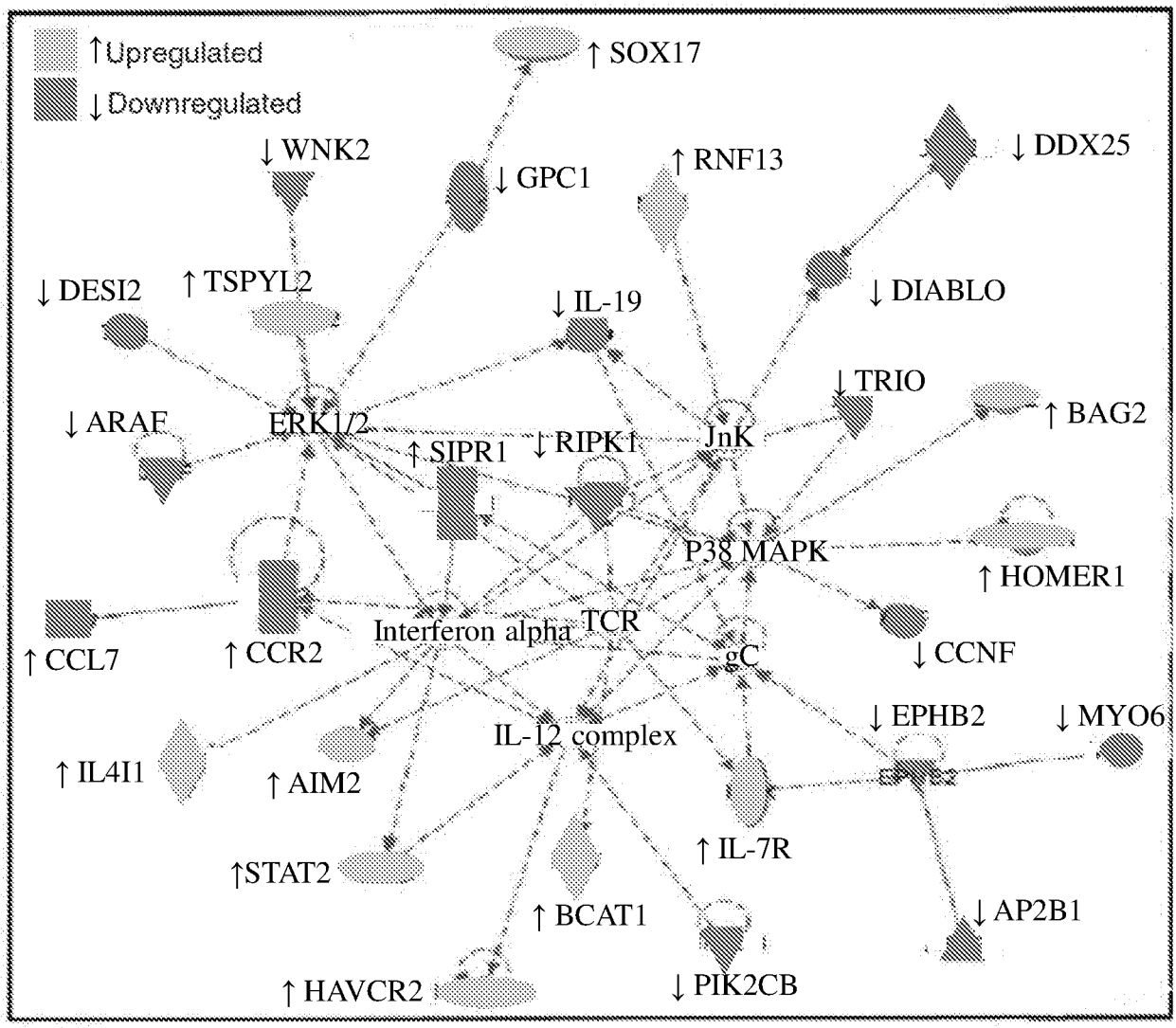


Figure 4D Continued

Figure 4E


↑Upregulated
↓Downregulated
↑ SOX17
↓ WNK2
↓ GPC1
↑ RNF13
↓ DDX25
↓ DESI2
↑ TSPYL2
↓ IL-19
↓ DIABLO
↓ TRIO
↓ ARAF
ERK1/2
↑ SIPR1
↓ RIPK1
JnK
↑ BAG2
P38 MAPK
↑ HOMER1
↑ CCL7
↑ CCR2
Interferon alpha
TCR
gC
↓ CCNF
↓ EPHB2
↓ MYO6
IL-12 complex
↑ IL4I1
↑ AIM2
↑ IL-7R
↑STAT2
↑ BCAT1
↓ AP2B1
↑ HAVCR2
↓ PIK2CB

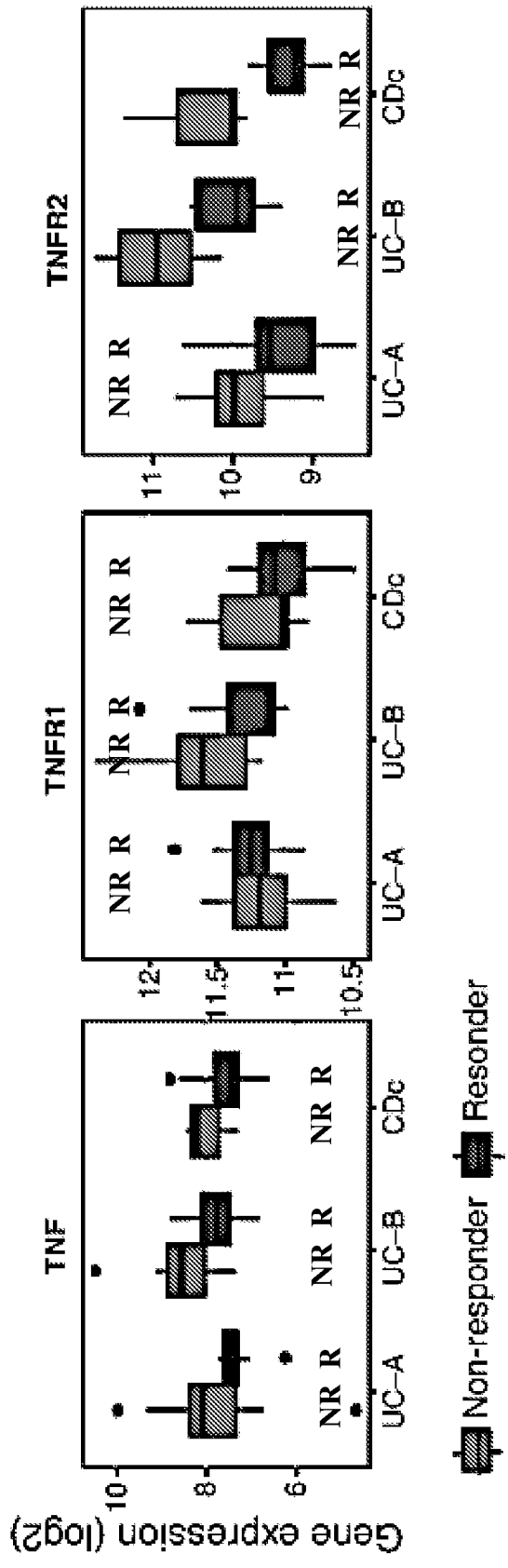


Figure 4F

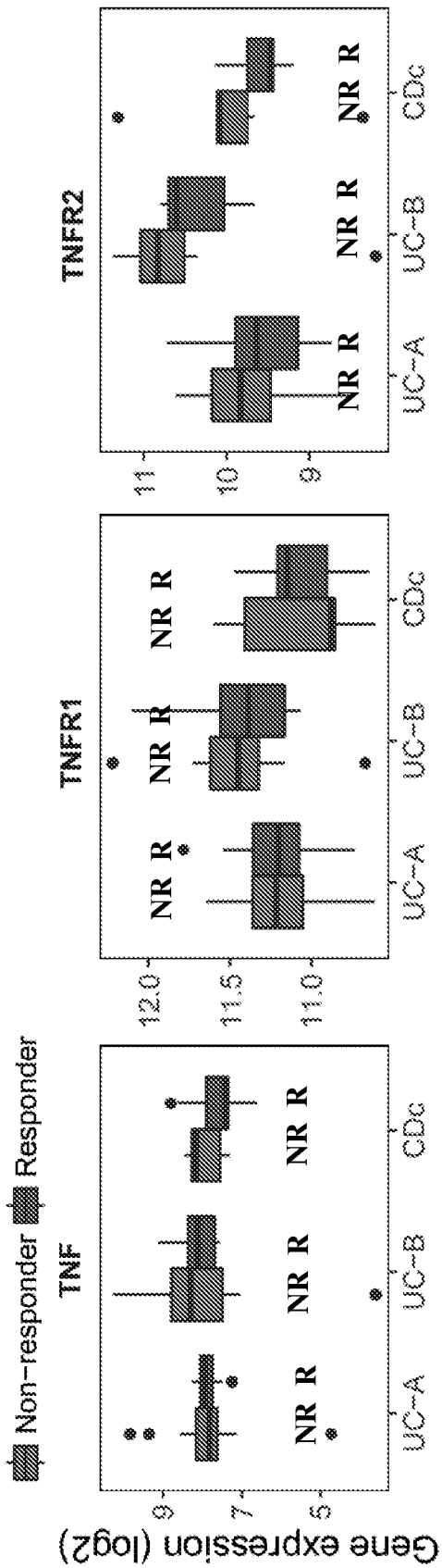


Figure 4G

Figure 5A

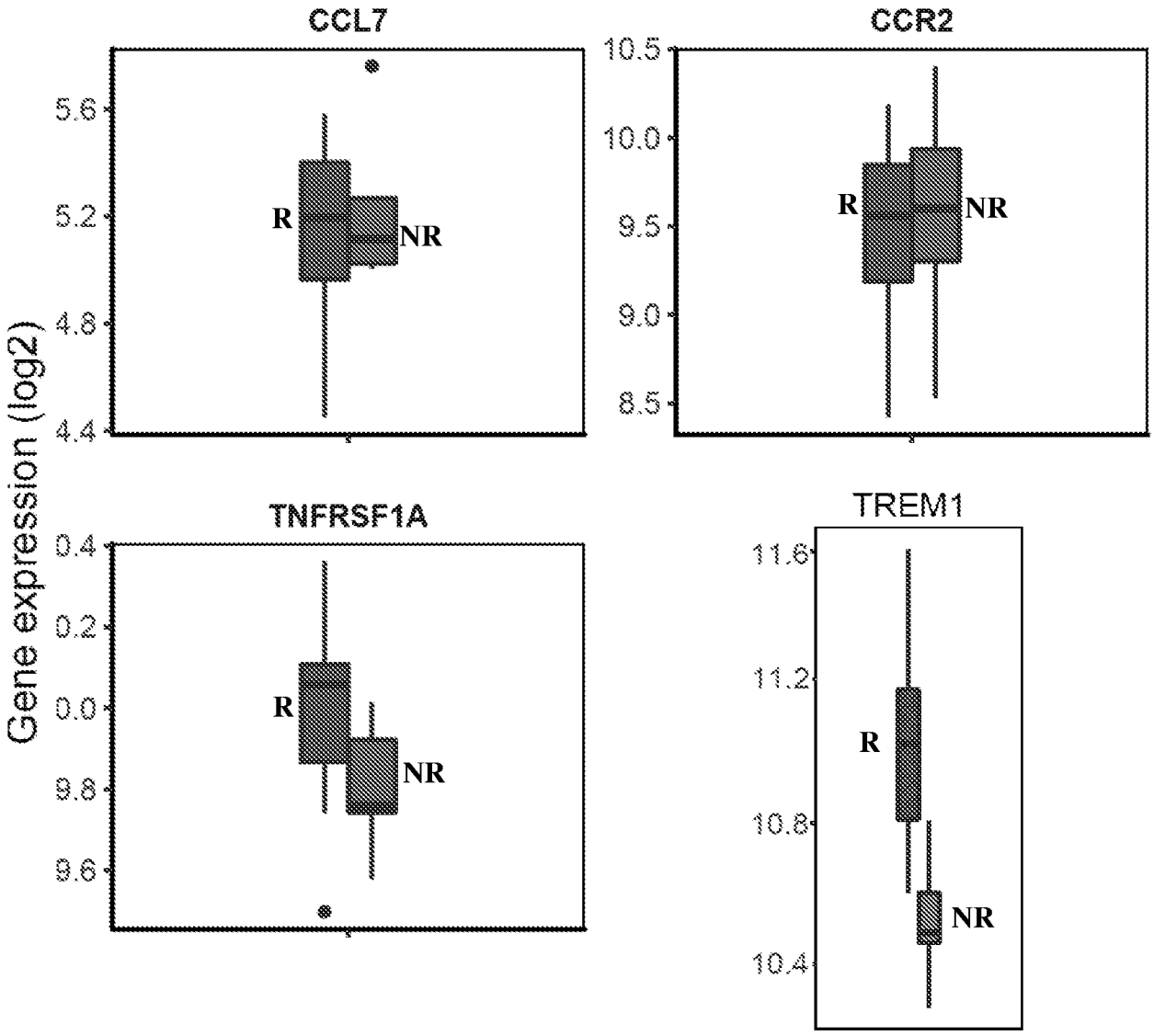
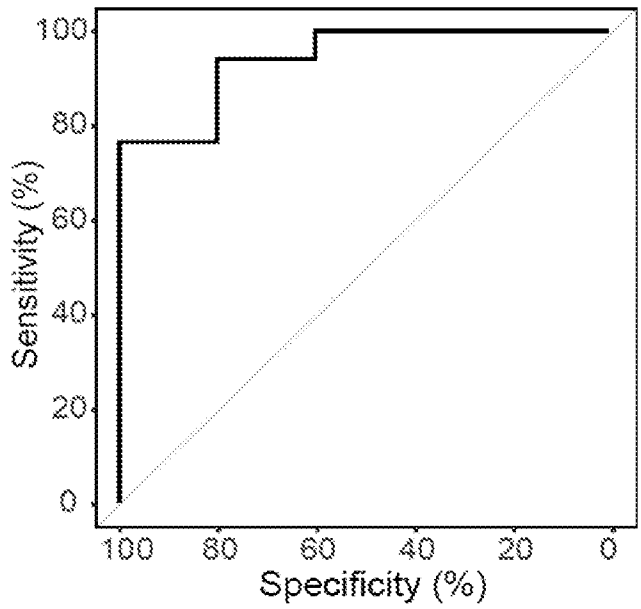


Figure 5B



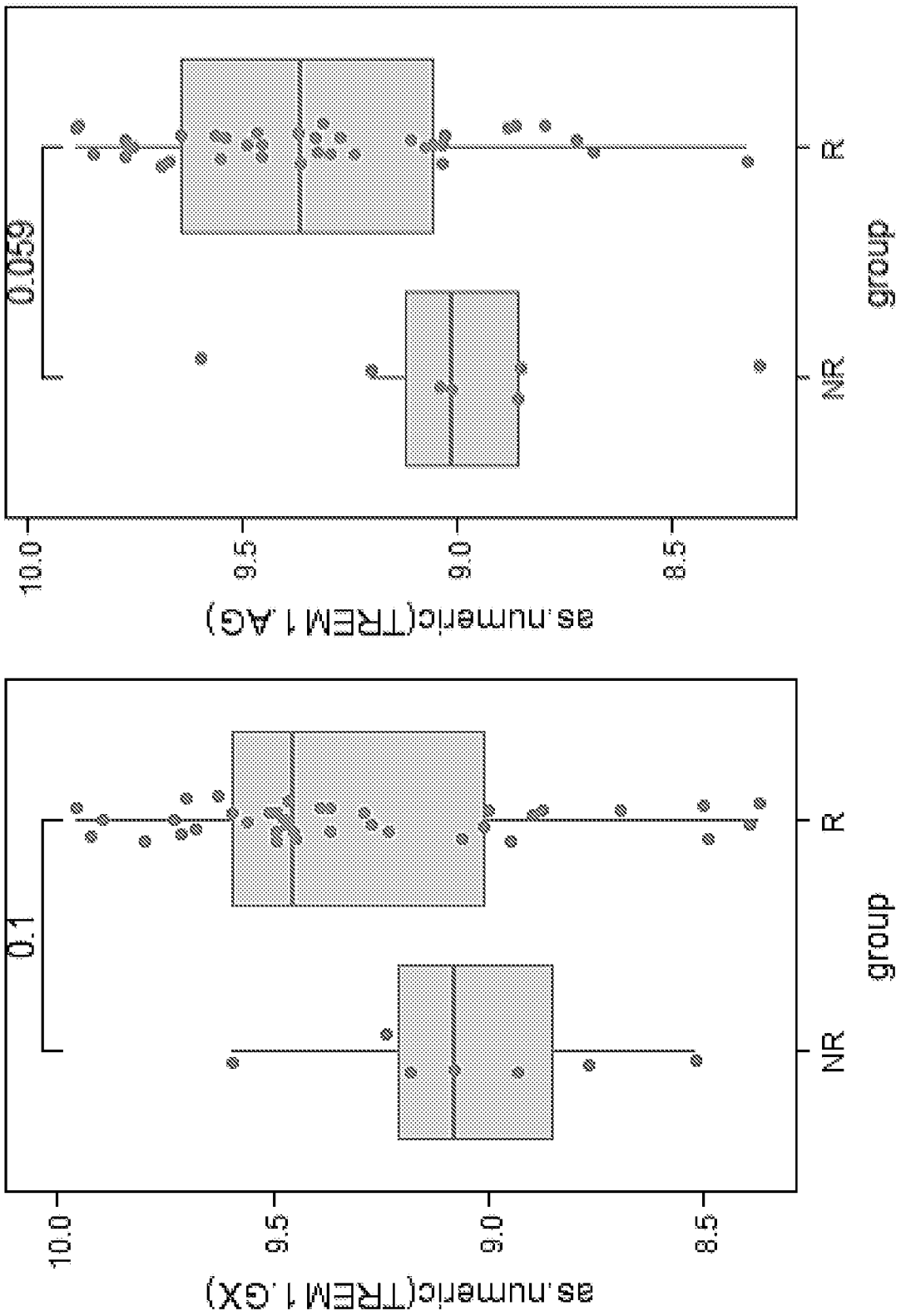

0.059
10.0
9.5
9.0
8.5
as.numeric(TREM1.AG)
NR
R
group
0.1
10.0
9.5
9.0
8.5
as.numeric(TREM1.GX)
NR
R
group

Figure 6A

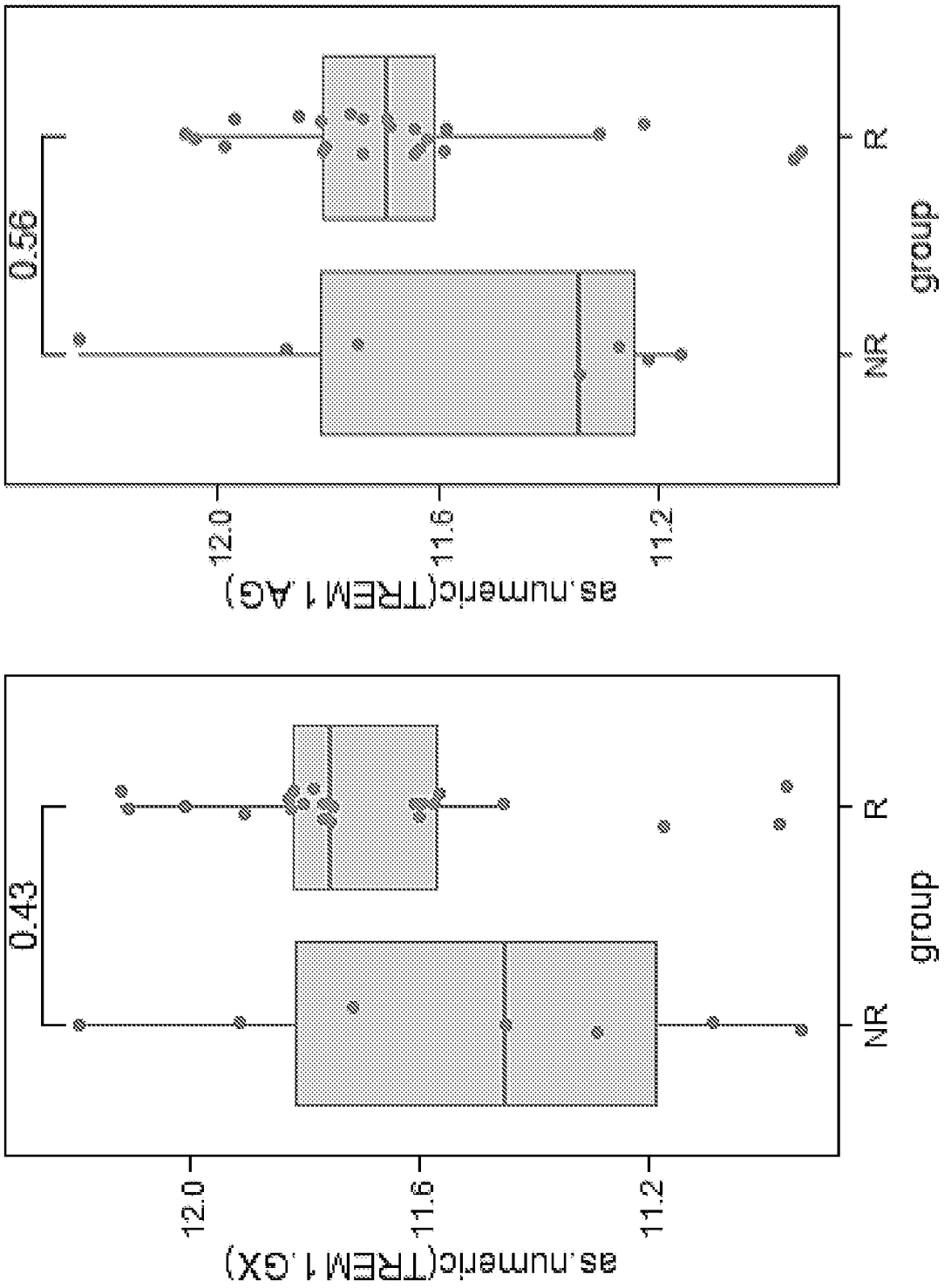

0.56
12.0
11.6
11.2
as.numeric(TREM1.AG)
NR
R
group
0.43
12.0
11.6
11.2
as.numeric(TREM1.GX)
NR
R
group

Figure 6B

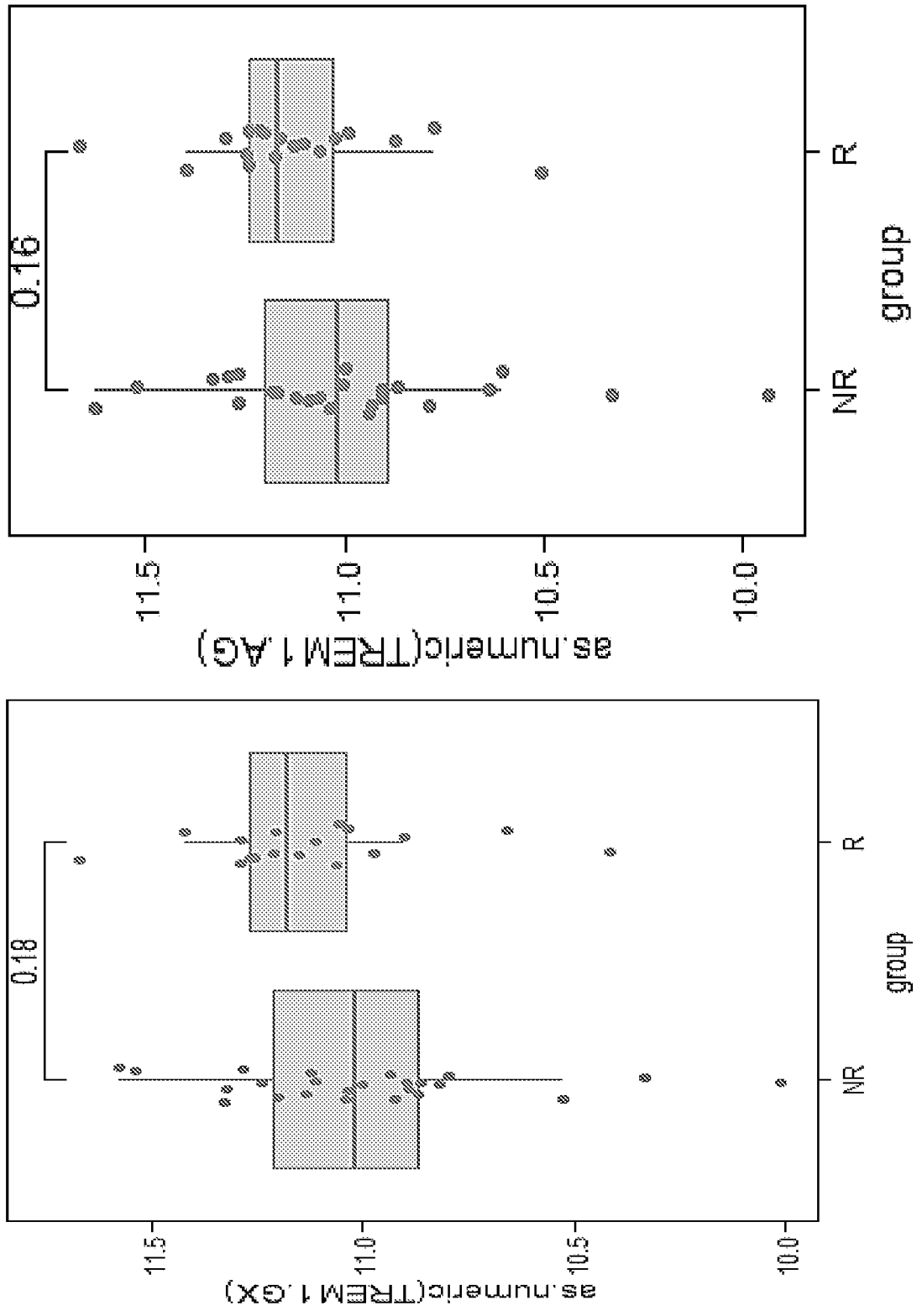

0.16
11.5
11.0
10.5
10.0
as.numeric(TREM1.AG)
NR
R
group
0.18
11.5
11.0
10.5
10.0
as.numeric(TREM1.GX)
NR
R
group

Figure 6C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2018/051187

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2019.01) C12Q 1/686, A61K 39/395, G01N 33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (2019.01) C12Q 1/686, A61K 39/395, G01N 33/53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Databases consulted: PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, Google Patents, Google Scholar
Search terms used: anti-TNFa determine test trem1 ccl7 ccr2 therapy treatment inflammation sutable

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016179469 A1 ABBVIE Inc. 10 Nov 2016 (2016/11/10) page 2 lines 10-28, page 5 lines 15-18,24-30, page 6 line 22, page 31 lines 1-16, page 39 lines 18-22 and figure 23	1,2,6-13,19-22
A	US 2008247955 A1 KUAI et al. 09 Oct 2008 (2008/10/09) Whole document	1-23
A	US 2009017472 A1 STUHLMULLER et al. 15 Jan 2009 (2009/01/15) Whole document	1-23
A	US 2015184244 A1 LI et al. 02 Jul 2015 (2015/07/02) Whole document	1-23

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 Feb 2019	26 Feb 2019

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	POUNY Yehonathan Telephone No. 972-2-5651634

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2018/051187

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2016179469 A1	10 Nov 2016	WO 2016179469 A1	10 Nov 2016
US 2008247955 A1	09 Oct 2008	US 2008247955 A1	09 Oct 2008
		AU 2008205538 A1	24 Jul 2008
		BR PI0806680 A2	16 Jun 2015
		CA 2675583 A1	24 Jul 2008
		CN 101687916 A	31 Mar 2010
		EP 2121750 A2	25 Nov 2009
		JP 2010516678 A	20 May 2010
		MX 2009007368 A	16 Jul 2009
		WO 2008088849 A2	24 Jul 2008
		WO 2008088849 A3	18 Sep 2008
		WO 2008088849 A9	09 Apr 2009
US 2009017472 A1	15 Jan 2009	US 2009017472 A1	15 Jan 2009
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