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Mapping atopic dermatitis and anti-IL-22 response signatures to Type 2-low severe neutrophilic asthma

--Manuscript Draft--

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Abstract:	Background: Transcriptomic changes in patients who respond clinically to biological therapies may identify responses in other tissues or diseases. Objective: To determine whether a disease signature identified in atopic dermatitis (AD) is seen in adults with severe asthma (SA) and whether a transcriptomic signature for AD patients who respond clinically to anti-IL-22 (Fezakinumab, FZ) is enriched in SA. Methods: An AD disease signature was obtained from analysis of differentially expressed genes (DEGs) between AD lesional and non-lesional skin biopsies. DEGs from lesional skin from therapeutic super-responders before and after 12 weeks FZ treatment defined the FZ-response signature. Gene Set Variation Analysis (GSVA) was used to produce enrichment scores (ES) of AD and FZ-response signatures in the U-BIOPRED asthma cohort. Results: The AD disease signature (112 up-regulated genes) encompassing inflammatory, T-cell, Th2 and Th17/Th22 pathways was enriched in the blood and sputum of asthmatics with increasing severity. Asthmatics with sputum neutrophilia and mixed granulocyte phenotypes were the most enriched ($p<0.05$). The FZ-response signature (296 down-regulated genes) was enriched in asthmatic blood ($p<0.05$) and particularly in neutrophilic and mixed granulocytic sputum ($p<0.05$). These data were confirmed in sputum of the ADEPT (Airway Disease Endotyping for Personalized Therapeutics) cohort. IL-22 mRNA across tissues did not correlate with FZ-response ES, but this response signature correlated with Th22/IL-22 pathways. Conclusions: The FZ-response signature in AD identifies severe neutrophilic asthmatics as potential responders to FZ therapy. This approach will help identify patients for future asthma clinical trials of drugs used successfully in other chronic diseases

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Airways Disease
11-03-2021

Dear Professor Ballas,

Re: manuscript JACI-D-20-02105

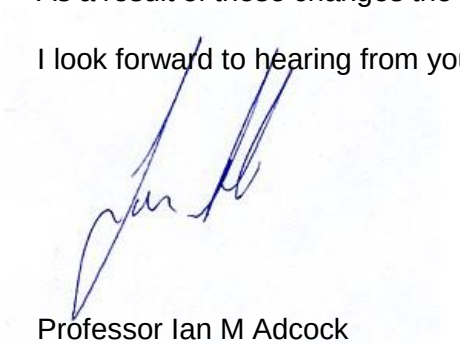
Please find attached a revised version of our manuscript entitled '**Mapping atopic dermatitis and anti-IL-22 response signatures to Type 2-low severe neutrophilic asthma**' by Yusef Badi and colleagues.

We have provided a point by point response to the Reviewers comments and questions. We thank the Reviewers very much for their insightful comments that have improved the manuscript. In particular,

- We have provided a rationale, namely the atopic march, linking AD with asthma within the introduction as requested by Reviewer 1.
- As requested we have also provided new analysis of the data to determine whether the enrichment of the FZ-DOWN signature related to neutrophilia, was affected by skin-specific genes or were impacted by asthma treatment. We can confirm that these do not affect our results.
- Clarified issues around the definition of AD 'super-responder', asthmatic TAC phenotyping, the derivation of the Th22/IL-22 gene signature and the differences between the various data sets in Supplementary Tables 1-3.
- Found a significant correlation between the FZ-DOWN signature enrichment score and the AD SCORAD score after treatment indicating that the FZ-DOWN enrichment score could be used to predict responder-non-responder subjects in AD as well as predicting potential responders in asthma.
- Toned down and modified the conclusions according to the Reviewers' requests.

As a result of these changes the manuscript is now over the word limit for which we apologise.

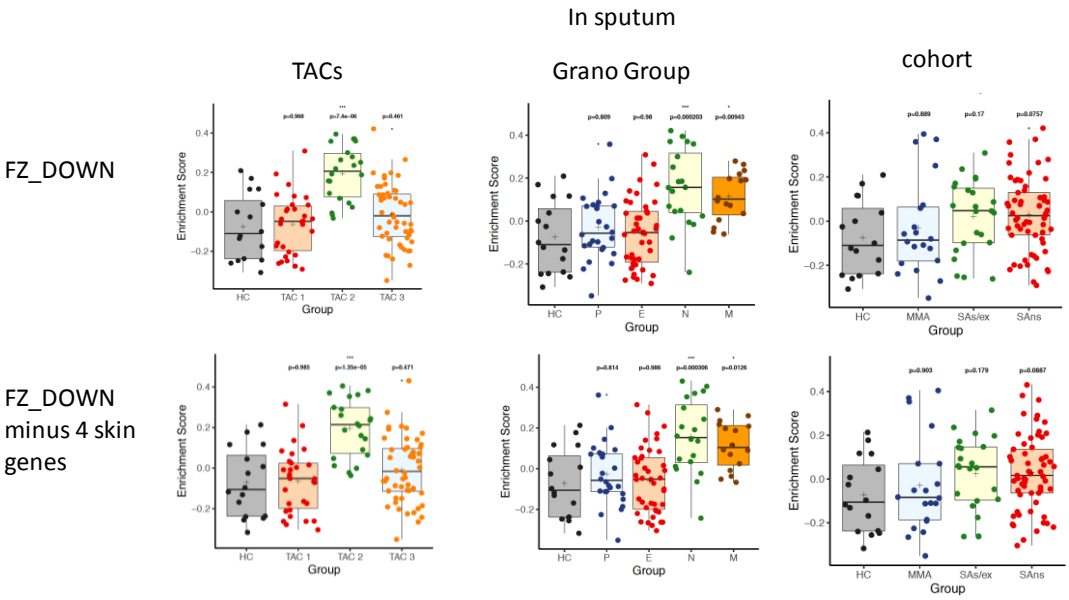
I look forward to hearing from you in the near future,



Professor Ian M Adcock
(on behalf of all authors)

Point-by-point rebuttal
Reviewer 1
The concept that there is overlap in the gene expression signatures of two distinct inflammatory diseases is not unexpected. The rationale for comparing AD with severe asthma could be better justified in the introduction.
Response: We thank the Reviewer for his/her comment. We have added a few sentences regarding the justification of the comparison between AD and asthma namely, the atopic march to the introduction (line 225 of the revised marked manuscript). The added sentences read 'The atopic march is a term used to describe the progression of allegic disease from the early presence of atopic dermatitis, food allergies and rhinitis through to asthma (Davidson WF et al., J Allergy Clin Immunol. 2019 Mar;143(3):894-913). A recent *in silico* analysis of the protein interaction networks in these diseases identified the presence of pathways contributing to the allergic multimorbidity of these diseases (Aguilar D et al., PLoS One. 2017 Jun 9;12(6):e0179125)'.

The cellular composition of skin biopsies, blood, and sputum are highly heterogeneous. Ideally, genes that are highly expressed in skin and not expressed in blood/sputum should be filtered out of the analysis to increase the precision of these studies. For example, the enrichment of the AD-UP signature (112 genes) in blood and sputum TAC2 was much stronger than the results for FZ-DOWN (296 genes), even though 74/112 genes overlapped between the AD-UP and FZ-DOWN signatures, suggesting that the FZ-DOWN signature is contaminated with irrelevant genes.
Response: We thank the Reviewer for their comment. We appreciate the comment regarding the potential confounding effect of 'irrelevant' genes but feel that it is difficult to state what are 'irrelevant' within the FZ signature that may be translated across disease particularly in light of the *in silico* analysis mentioned in the response above. However, we take on board the Reviewers comment and utilised the skin-specific gene data set identified previously (Edqvist PH et al., Expression of human skin-specific genes defined by transcriptomics and antibody-based profiling. J Histochem Cytochem. 2015 Feb;63(2):129-41). The top 30 skin DEGS from Table 2 identified no overlapping genes with the FZ-DOWN signature but Supplementary Table 3 gave a list of 268 skin-enriched genes that were 5-fold more enriched/enhanced in skin than other organs. Only 4 genes intersected between the skin genes and the FZ-DOWN signature - WFDC12, TYR, S1PR5, LYPD5. Edqvist and colleagues also identified only 4 genes that overlapped between the skin and lung epidermis (Figure 2A from their manuscript). Removal of these 4 genes from the FZ-DOWN signature made little difference to the analysis. These



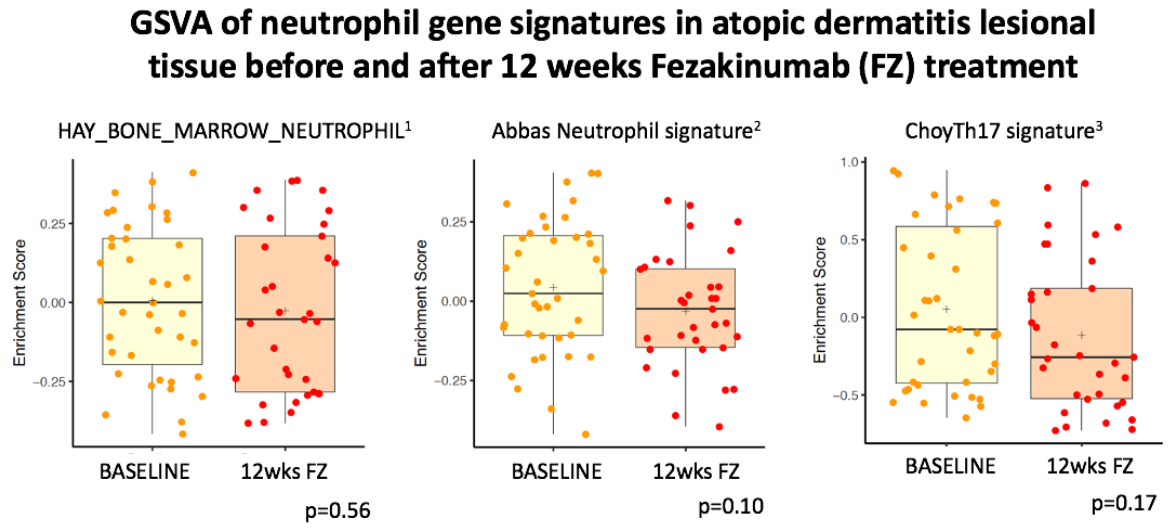
data are shown below and now included as Supplementary Figure 4 with a reference to the impact of the skin-genes made in the text (line 385 of the revised marked manuscript).

Differential gene expression studies in skin, blood, and sputum are potentially confounded by variations in cellular composition. To address this issue, the authors could employ CIBERSORT or a related method to infer the cellular composition of the samples and adjust the analysis for this variation. In the absence of this analysis, the enrichment scores could be more related to the presence or absence of neutrophils, rather than upregulation of specific inflammatory pathways in severe asthma patients. Perhaps this explains why the AD-UP signature correlated with TAC2 but not severe asthma in sputum, which was very different to the findings in blood?

Response: The Reviewer raises an interesting point. Rather than use CIBERSORT, with have used other gene signatures to infer neutrophil levels in the AD lesional biopsies before and after treatment. We used 2 available neutrophil signatures (Hay SB et al., The Human Cell Atlas bone marrow single-cell interactive web portal. Exp Hematol. 2018 Dec;68:51-61; Abbas AR et al., Immune response in silico (IRIS): immune-specific genes identified from a compendium of microarray expression data. Genes Immun. 2005 Jun;6(4):319-31) and the David Choy Th17 signature (Choy DF et al., TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med. 2015 Aug 19;7(301):301ra129) that consists mainly of neutrophil chemoattractant genes for (CXCL1, CXCL2, CXCL3, CXCL8 and CSF3).

We observed a high correlation between AD disease signature ES and neutrophil signature ES indicating that the disease signatures reflected tissue neutrophilia. In particular Pearson's correlation between the AD-UP signature ($p<2.2x10^{-16}$, r=0.754) and the MADAD-UP signature ($p<2.2x10^{-16}$, r=0.684) were very significantly correlated with the IRIS neutrophil signature. In addition, the AD-UP signature ($p=4.479x10^{-7}$, r=0.441) and the MADAD-UP signature ($p=4.304x10^{-7}$, r=0.442) was also significantly correlated with the Human Cell Atlas neutrophil signature.

However, neutrophil levels in the skin are not significantly reduced after FZ treatment (see Fig below and new Supplementary Figure 5). This suggests that despite neutrophil genes contributing to the AD disease signature and some neutrophil genes being present in the FZ response signature, the FZ response phenomenon is unlikely to be driven by neutrophil levels. These data are included in the revised marked manuscript at line 389).



Whilst the disease signature for AD-UP and FZ-DOWN overlaps with severe neutrophilic asthma, it is not clear from the data as presented if the overlap is sufficient to attenuate the pathogenesis of asthma. For example, IL-22 could play a minor or redundant role in these patients. It would be interesting to find out what proportion of a disease signature for severe neutrophilic asthma is accounted for by the AD-UP/FZ-DOWN signature. It would also be interesting to know if a computational drug repurposing analysis based on a signature for severe asthma would identify IL-22 as a highly ranked molecular driver of disease.

Response: We thank the Reviewer for this important comment. We have examined the intersection of genes between the genesets that characterise the TAC2 (predominantly neutrophilic asthma) and FZ-DOWN signatures and find only 3 genes – CASP4, KCNJ15 and SAMSN1. Despite there being little overlap in the gene signature composition we show a good correlation between the TAC2 signature and the FZ-DOWN signature ($p=2.2 \times 10^{-16}$, r=0.784). In addition, we were unable to identify a specific IL-22 signalling pathway in KEGG 2019 and running the FZ-DOWN and TAC genes through EnrichR (https://maayanlab.cloud/Enrichr) identified a non-significant link with TNFAIP3 in the TAC2 signature to the IL-17 pathway. These data are summarise in the revised marked manuscript (line 379).
The response to any monoclonal antibody should be greatest in patients with high expression of that target. We do report that sputum IL-22 protein expression was significantly correlated with the FZ-DOWN ES (p=0.0360, r=0.133) and that sputum IL-22 protein was significantly enriched in patients with TAC2 asthma compared to TAC1 asthma (p=0.0112). Furthermore, IL-22 protein in sputum also significantly correlated with FZ-DOWN ES in nasal brushings for all subjects (p=0.0443, r=0.423). These data together suggest that we are indeed selecting the 'correct' subjects that are more likely to respond to anti-IL-22 therapies.

The lack of correlation between IL-22 gene expression and the FZ-DOWN signature could be due to some other factor driving these responses.
Response: We understand the concern of the Reviewer since an anti-IL-22 antibody should be effective in patients where IL-22 levels are high and drive disease. The lack of correlation between the FZ-DOWN signature and the expression of the IL-22 gene in sputum may reflect the different cell types in the sputum sample and these cells regulate IL-22 gene and protein expression. However, the correlation observed between the FZ-DOWN gene signature ES and the signature of genes downstream to IL-22 (Th22 and IL-22 signature) ES across all lung samples [sputum ($p=4.31 \times 10^{-14}$, r=0.656), bronchial brushings (p<2.2x10-16, r=0.753) and nasal brushings ($p=8.53 \times 10^{-13}$, r=0.755,)] and between sputum FZ-DOWN and sputum IL-22 protein (p=0.0360, r=0.133) gives confidence that we are identifying potential responders. We have moved the current figure showing the correlation with IL-22 and the FZ-DOWN signature (currently Supplementary Figure 5E) as a separate panel in the revised Figure 5. We also emphasise that sputum IL-22 protein is enriched in TAC2 subjects compared with patients with TAC1 asthma (p=0.0112). This is stated in the revised marked manuscript (line 475).

The data from the independent validation cohort (ADEPT) were only borderline significant. Again, the data could simply reflect the presence of neutrophils.
Response: We agree with the Reviewer that the results were borderline significant in a smaller replication cohort but they were significant. We also examined the correlation between sputum neutrophils and the FZ-DOWN signature in the ADEPT cohort and found no significant correlation (% segmented neutrophils; p=0.911, r=0.0186). This is now stated in the revised marked manuscript (line 405).

No data were presented to determine if the findings were impacted by the use of medications?
Response: In a linear model (LM) of FZ-DOWN ES and medication usage, adjusted for age, gender and BMI, we found no significant association between FZ-DOWN signature ES and OCS use in U-BIOPRED asthmatic patients (p=0.702). However, we did find a significant association between FZ-DOWN signature ES and LABA use (p=0.0243) where FZ-DOWN ES was elevated in the twice daily LABA use group (reflecting severity of disease, LM estimate=0.112) and least in the group not taking LABA at all (mildest subjects, LM estimate=-0.117). These data are now included in the results of the revised marked manuscript (line 423).

Reviewer 2

The investigators use the terminology Th22 and IL-22 pathways in their analysis. Can they state in the text, to help the reader, what the pathways are in the Th22/IL-22 gene signature? How do they know it is a Th22 signature? And not IL-22 from Th17 cells? This is a small point but needs clarification.

Response: Thank you for the comment. The IL-22/Th22 gene list is composed of the following genes AHR, CALML5, CCR10, FLG, IL22, IL32, KRT1, KRT10, LOR, S100A7, S100A8, S100A9, S100P, SERPINB1, SERPINB4 and S100A12. While the signature includes IL22 and S100As markers that can be produced by both Th22 and Th17 cells, it also includes Th22 cell specific markers such as CCR10 (Brunner PM et al., Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. J Allergy Clin Immunol. 2019 Jan;143(1):142-154).

We used EnrichR to examine the key pathways associated with these 16 genes. The top pathway hit in the Elsevier Pathway Collection was IL22 Induces Keratinocyte Proliferation in Psoriasis (Adj p value = 1.04×10^{-11}) and the top hit in Reactome was Interleukin-19, 20, 22, 24 Homo sapiens R-HSA-8854691 (p=0.00718) although this did not reach significance at the adjusted p level. The Interleukin-22 soluble receptor signalling pathway was also a hit in BioPlanet 2019 although only at the raw p levels (p=0.0104). In contrast, the top significant pathway in KEGG was IL-17 signaling pathway (adj p=0.00261) whilst other significant pathways were cytokine-cytokine receptor interaction (Adj p=0.0382) and Amoebiasis (Adj p=0.0405).

We have included a statement to this effect in the Results section (line 470) and included this genelist in Supplementary Table 1 of the revised manuscript.

Supp' table. 1. This is difficult to read and interpret. Can the authors group the genes up or down regulated into some kind of function/category (e.g. table 2) e.g., cytokines, chemokines, receptor, host defence, innate immunity etc. This needs to be done to significantly enhance readability and understand outcomes. Or the authors could better explained table 1 relationship to Tables 2 and 3. Also are all the significantly up or down regulated genes described in these tables?

Response: We apologise to the Reviewer about the difficulty of reading Supplementary Table 1. The format used is standard for bioinformatics papers as it is just for utility – sharing the specific genes used in analysis. It just lists all of the genes of the signatures used in the analysis: the AD disease signatures and the FZ-UP and FZ-DOWN signatures. All the gene lists used are presented in this Table. Supplementary Table 2 is a list of all the pathways that the FZ-DOWN gene signature corresponds to and highlights the importance of immune pathways. Supplementary Table 3 is a list of all the pathways that the FZ-UP gene signature corresponds to. None of these FZ-UP pathways were significantly enriched and are mostly skin-related. We have added text in the Results section to better explain the relationship between the data presented in these 3 Supplementary Tables. This text now reads 'In summary, Supplemental Table 1 provides a list of all the gene signatures used in this analysis including the sets of genes up-regulated (AD-UP) or down-regulated (AD-DOWN) in AD whilst Supplemental Table 2 provides a list of all the pathways that the FZ-DOWN gene signature corresponds to and highlights the importance of immune pathways. Supplementary Table 3 is a list of all the pathways that relate to the FZ-UP gene signature. None of these pathways was significantly enriched and are mostly skin-related' at line 359 of the revised marked manuscript.

Reviewer 3

Comment 1. I agree with the authors that the predictive value of the "super responder" signature for FZ treatment in asthma cannot be estimated on the basis of the presented results; it has to be validated empirically and compared to other theragnostic parameters (line 514ff). Hence, the statement in line 148 of the abstract should be tuned down.

Response: We thank the Reviewer for their comment and have toned down the statement regarding the predictive value of the signatures in the Abstract. This now reads 'The FZ-response signature in AD identifies severe neutrophilic asthmatics as potential responders to FZ therapy. This approach will help identify patients for future asthma clinical trials of drugs used successfully in other chronic diseases'.

Comment 2. Line 502ff. In my opinion, the lack of association of the FZ-DOWN signature with IL-22 mRNA in severe asthma is not a limitation rather, I consider it an important result of the study. It indicates differences between AD and asthma that may be important. In the skin, the signature is closely linked to IL-22 expression, in the airways it is not. Is this not a strength of the explorative approach that it may discover common properties beyond the prior expectations? However, I do consider it a limitation that the super-response signature in AD was not derived directly from the patients with the best clinical improvement but from a proxy - namely the basal levels of IL-22 mRNA (see also comment 3).

Response: We thank the Reviewer for their comment. However, the AD super-responders were selected for analysis as they had both a positive clinical response and they also had a unique strong molecular response, inferred from a decreased AD disease signature ES, to FZ as stated in the text. In subsequent analysis, it was evident that these patients were also those that had the highest levels of IL-22 mRNA within the skin biopsies.

To ensure that we do not over-interpret the data we have emphasised this within the Discussion section indicating the study limitations. We have tempered this by adding the corollary that clinical efficacy alone would not necessarily have provided evidence for molecular engagement at the biopsy site and provide a molecular signature to overlay on the asthma patient data (line 571 of marked revised manuscript).

We have changed the Results section to highlight the fact that we did see a significant correlation between the FZ-DOWN signature and IL-22 protein expression in sputum (Revised Fig 5 and line 475 of revised marked manuscript) and that the levels of IL-22 protein in sputum were significantly higher in the TAC2 patients compared to the TAC1 patients. The original focus on the high correlation between the Th22/IL-22 signature and the FZ-DOWN signature in nasal and bronchial brushings was to indicate the possibility of a nasal brushing or scrape being useful in predicting a potential responder phenotype.

Comment 3. I find it difficult to follow the author's strategy for defining the "FZ super-response" signature (lines 241ff). Apparently, AD responders were selected on the basis of basal IL-22 expression rather than directly according to their clinical response. What do the authors mean to say by "gene signature indicative of a super-response to FZ" (line 418 ff)? Were all these patients indeed clinical super-responders (line 506)? If this is not known and the FZ response was defined on the basis of a proxy rather than the clinical outcome, the term "super-response" should be avoided, as it may raise too great expectations. This limitation of the study then needs to be clearly stated.

Response: We apologise for the lack of clarity on this issue. The definition was taken from the original Brunner and colleagues paper (Brunner PM et al., Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. J Allergy Clin Immunol. 2019 Jan;143(1):142-154) and we feel that this published definition should be adhered to.

The term 'Super responder' doesn't mean clinically super responding, but refers to the category of patients who had the best clinical response and a unique and strong molecular response to FZ in the AD lesions. These were subsequently identified as AD patients with the high levels of lesional IL-22 mRNA compared to clinical responders with low levels of lesional IL-22 mRNA at baseline. This has been clarified in the text (line 336 of the revised marked version).

Comment 4. FZ-DOWN was defined by the changes in the transcriptomes in response to FZ treatment. As far as I understand it, the authors' strategy was to search for enrichment of this signature in asthma implying that the biggest changes during treatment are observed in individuals with the highest expression at baseline. But was this the case? Was the FZ-DOWN signature at baseline most enriched in those AD patients that had the greatest clinically benefit from the treatment (line 338)?

Response: Again, we apologise to the Reviewer for the lack of clarity here. The FZ-DOWN signature was derived from AD patients with the best clinical response and the best molecular response to FZ after 12 weeks. This signature represents the list of genes that were up-regulated in AD lesions and most significantly down-regulated following treatment. In the Brunner study (Brunner PM et al., Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. J Allergy Clin Immunol. 2019 Jan;143(1):142-154), the super-responders were the patients with the highest baseline lesional IL-22 mRNA levels.

The Reviewer raises an interesting point as to whether the FZ-DOWN signature would also be a good predictor of response in AD, which would provide additional evidence for the cross-disease utility of enriching a disease population for potential FZ responders. We examined the ES of FZ DOWN at baseline sample in the AD patients. This was significantly (p=0.0496, adjusted for age and gender) positively associated with the AD SCORAD score after treatment. We have added a statement to this effect in the revised marked manuscript (line 355).

MINOR COMMENTS:

5. How precisely were the patients with severe asthma divided into subgroups according to their FZ-DOWN profile?

Response: We described within the data analysis section within the methods that all asthmatics were grouped into predicted responders or predicted non-responders according to their enrichment score of the FZ-DOWN signature. This has been emphasised in the text of the revised marked manuscript (line 413).

"The enrichment score of the FZ-DOWN signature in sputum was used to categorise SA patients as being predicted-responders (PRs) (n=26, ES ≥+0.1) or predicted non-responders (PNRs) (n=18, ES ≤-0.1) whilst filtering out patients with an undirected ES (>+0.1 and >-0.1), MMAs and HCs".

6. Line 129: I do not understand the first sentence in the abstract.

Response: This sentence means that transcriptomic signatures derived from the tissue of patients who respond clinically to biological therapies may be used to identify responses to the same biological treatments in other tissues or diseases. Due to the word limitations in the Abstract this was condensed to "Transcriptomic changes in patients who respond clinically to biological therapies may identify responses in other tissues or diseases".

7. Line 272 - Please explain the TAC groups.

Response: TAC groups are 3 novel phenotypes of asthma defined from the sputum transcriptomics of U-BIOPRED patients published in 2017 (Kuo S et al., ERJ 2017). They did a differential expression between sputum transcriptomics of subjects with eosinophilic inflammation against those with non eosinophilic inflammation. They then clustered on this to reveal 3 groups of asthma called TAC1, 2 and 3. In summary: TAC1 contains patients with a high enrichment for the Woodruff Th2-high gene signature with a very high sputum eosinophilia. The TAC2 is characterised by inflammasome-associated pathways and high sputum neutrophilia whilst TAC3 is associated with high levels of macrophages and a mainly paucigranulocytic phenotype. We have added a statement regarding this in the revised marked manuscript (line 281) to avoid any confusion.

8. Line 311 - please explain the MADAD-UP pooled signature

Response: The MADAD-UP signature is a consensus disease signature of the pathologically upregulated genes which characterise atopic dermatitis across several studies (Ewald DA et al., Meta-analysis derived atopic dermatitis (MADAD) transcriptome defines a robust AD signature highlighting

the involvement of atherosclerosis and lipid metabolism pathways. BMC Med Genomics. 2015;8(60)) – this has now been clarified in the text of the revised marked manuscript (line 325).

9. Line 323 - what do the authors mean by "this response"? Please also compare comments 3 and 4.
Response: We apologise for the lack of clarity regarding this. The text (line 340 in the revised marked manuscript) has now been changed to read 'the transcriptomic changes seen in patients with a high clinical and transcriptomic response was used to generate the FZ-super-responder signature'.

Editorial office comments:
Remove the "Running Head" section. It is not needed
Response: Removed as requested.

Change the heading "Materials and Methods" to "Methods"
Response: The Heading has been changed as requested.

Your revision must include the following items: (1) point-by-point responses to the Editor and reviewer comments, (2) a marked copy of your revision showing the changes made, and (3) a clean (unmarked) copy of your revised manuscript. If your manuscript has any figures, tables, or Online Repository material in separate files, please be sure these are included in the revision as well. For further information regarding formatting of these elements, please consult the Guidelines for Submitting a Revision (found on the Editorial Manager homepage by clicking on Instructions for Authors). To avoid a delay in a final decision on your manuscript, please follow these instructions carefully.

Mapping atopic dermatitis and anti-IL-22 response signatures to Type 2-low severe neutrophilic asthma

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Conflict of Interest statement.

JH Riley, S Bates and S Worsley are employees and shareholders of GlaxoSmithKline. Dr. Uddin reports he is an employee of AstraZeneca and holds shares in the company. Dr. Knowles reports being a former employee of GlaxoSmithkline. Dr. Singer reports personal fees from Vertex Pharmaceuticals Switzerland, personal fees from Novartis Pharma Switzerland , outside the submitted work. Dr. Fowler reports personal fees from AstraZeneca, grants and

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Abstract

Background: Transcriptomic changes in patients who respond clinically to biological therapies may identify responses in other tissues or diseases ~~Transcriptomic signatures of patients who respond clinically to biological therapies may identify responses in other tissues or diseases~~.

Objective: To determine whether a disease signature identified in atopic dermatitis (AD) is seen in adults with severe asthma (SA) and whether a transcriptomic signature for AD patients who respond clinically to anti-IL-22 (Fezakinumab, FZ) is enriched in SA.

Methods: An AD disease signature was obtained from analysis of differentially expressed genes (DEGs) between AD lesional and non-lesional skin biopsies. DEGs from lesional skin from therapeutic super-responders before and after 12 weeks FZ treatment defined the FZ-response signature. Gene Set Variation Analysis (GSVA) was used to produce enrichment scores (ES) of AD and FZ-response signatures in the U-BIOPRED asthma cohort.

Results: The AD disease signature (112 up-regulated genes) encompassing inflammatory, T-cell, Th2 and Th17/Th22 pathways was enriched in the blood and sputum of asthmatics with increasing severity. Asthmatics with sputum neutrophilia and mixed granulocyte phenotypes were the most enriched ($p<0.05$). The FZ-response signature (296 down-regulated genes) was enriched in asthmatic blood ($p<0.05$) and particularly in neutrophilic and mixed granulocytic sputum ($p<0.05$). These data were confirmed in sputum of the ADEPT (Airway Disease Endotyping for Personalized Therapeutics) cohort. IL-22 mRNA across tissues did not correlate with FZ-response ES, but this response signature correlated with Th22/IL-22 pathways.

Conclusions: The FZ-response signature in AD identifies severe neutrophilic asthmatics as potential responders to FZ therapy. This approach will help identify patients for future asthma clinical trials of drugs used successfully in other chronic diseases ~~The FZ-response signature in AD identifies severe neutrophilic asthmatics most likely to respond to FZ therapy. This approach will help identify patients for future asthma clinical trials of drugs used successfully in other chronic diseases~~.

Abstract word count: 249~~50~~

Clinical implications

Identification of transcriptomic drug-response signatures in the target tissue of one chronic immune disease may be utilised in another disease to stratify subjects for subsequent clinical trials or treatment.

Capsule Summary:

We used a signature defined by clinical and transcriptomic super-responders to Fezakinumab in atopic dermatitis to identify severe neutrophilic asthmatics as subjects most suitable for testing the efficacy of the drug in asthmatics.

Key words: Anti-IL-22 antibody, atopic dermatitis, gene set variation analysis, IL-22, severe asthma.

Abbreviations:

AD	Atopic dermatitis
ADEPT	Airway Disease Endotyping for Personalized Therapeutics
ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
ES	Enrichment score
FC	Fold-change
FDR	False discovery rate
FeNO	Fractional exhaled nitric oxide
FZ	Fezakinumab
HC	Healthy control
ILC	innate lymphoid cell
LS	Lesional
MADAD	meta-analysis derived atopic dermatitis
MMA	Mild-moderate asthma
NL	Non-lesional
PNR	Potential non-responder
PR	Potential responder
SA	Severe asthma

190	SAs/ex	Severe asthmatic smoker/ex-smoker
191	T2	Type 2
192	TAC	Transcriptome-Associated Cluster
193	U-BIOPRED	Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes
194	DEGS	Differentially expressed genes
195	GSVA	Gene Set Variation Analysis
196		

Introduction

Asthma is phenotyped according to clinical treatable traits and physiological markers including eosinophilic and non-eosinophilic phenotypes (1,2). The Type 2 (T2) inflammatory phenotype characterised by high expression of an interleukin (IL)-13 stimulated bronchial epithelial cell signature (3,4) and elevated urinary leukotriene (LT)E_4 (5), is a molecular phenotype characterised by high eosinophilic inflammation. However, the molecular phenotypes of non-T2 inflammation remain unclear although one phenotype has been characterised by inflammasome, tumour necrosis factor (TNF)α and interferon (IFN) pathway activation associated with neutrophilic asthma (3,6,7). An IL-17 phenotype characterised by neutrophilic inflammation has also been described (8).

IL-22 belongs to the IL-10 cytokine family and is produced by T helper (Th)17 and Th22 cells, $\gamma\delta$-T cells and Type 3 innate lymphoid cells (ILCs) as well as neutrophils (9). Elevated bronchoalveolar lavage (BAL) (10) and serum IL-22 levels (11,12) in patients with severe asthma has been reported. Neutrophil-high asthmatics show an upregulated presence of bronchial and nasal cells staining positive for IL-22 expression(13,14). IL-22 suppresses IFN-γ-induced pro-inflammatory mediator expression by human bronchial epithelial cells (10) indicating a potential protective role in asthma, but IL-22 also enhances the proliferation and migration of human airway smooth muscle (ASM) cells which may induce airway wall remodelling (15,16). This suggests that IL-22 could play a role in certain endotypes of asthma.

IL-22 is implicated in other chronic inflammatory diseases including atopic dermatitis (AD), a closely-related condition to asthma, often preceding it, in the atopic march (17). Epicutaneous sensitization in mice promotes the generation of antigen-specific IL-22-producing T cells leading to airway inflammation and airway hyperresponsiveness following allergen challenge (18). This suggests that IL-22 may be important in the atopic march. The anti-IL-22 monoclonal antibody, fezakinumab (FZ), improves AD clinical scores (19) whilst AD patients with high baseline IL-22 expression showed the greatest clinical response with down-regulation of transcriptomic features associated with immune pathways involved in T-cell and dendritic cell activation (20).

The atopic march is a term used to describe the progression of allergic disease from the early presence of atopic dermatitis, food allergies and rhinitis through to asthma (21). A recent *in silico* analysis of the protein interaction networks in these diseases identified the presence of pathways contributing to the allergic multimorbidity of these diseases (22). We

hypothesised that a gene signature from AD patients who respond to fezakinumab will be up-regulated in other chronic inflammatory diseases such as asthma. Furthermore, analysis of these 'responder signatures' will select patients most likely to respond to fezakinimab. We analysed differentially expressed genes (DEGs) in eczematous skin lesions of IL-22 high responders between baseline and after 12 weeks of FZ treatment in order to obtain a FZ-response signature. This FZ signature was used to probe the transcriptomes of the lungs and blood of the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) asthma cohort to identify features of asthmatic subjects who may respond to FZ. The results were validated in the independent Airway Disease Endotyping for Personalized Therapeutics (ADEPT) cohort.

~~Materials and~~ Methods (word count=709)

Determination of AD disease and anti-IL-22 responsive signature

Full details of AD patient demographics, samples, transcriptomic analyses and clinical response (NCT01941537) are provided elsewhere (20). The AD disease signature was defined by DEGs identified between eczematous or lesional (LS) skin and non-lesional (NL) skin samples with a fold-change (FC) ≥2 or ≤-2 and a false discovery rate (FDR) ≤0.05 for the whole AD cohort. We also used a composite AD signature derived by comparing the lesional and non-lesional skin transcriptome from 4 microarray studies (MADAD, meta-analysis derived AD)(23).

We defined a FZ treatment response signature by analysis of the LS biopsy data of AD patients at baseline and after 12 weeks of FZ treatment to identify DEGs (FC ≥2 or ≤-2 and FDR<0.05) (20). Patients with high levels of IL-22 mRNA in lesional tissue at baseline had the greatest response to FZ at both the clinical and transcriptomic level. We used DEGS from the IL-22^{high} AD patients to derive a FZ 'super responder' signature (20)(**Supplementary Table 1**).

Asthma cohorts

The U-BIOPRED cohort consists of severe non-smoking asthma (SAn); smokers and ex-smokers with severe asthma (SAs/ex); mild/moderate non-smoking asthmatics (MMA) and healthy non-smoking controls (HC) (24). Expression profiling was performed on RNA extracted from blood cells, sputum cells, epithelial brushings and bronchial biopsies (8)(24). Clinical characteristics and sputum and blood proteomic (SomaLogic) metadata are stored within TransMART as part of the eTRIKs project (25). For validation, the ADEPT cohort (NCT01274507) was analysed (26).

Protein and other assays

The SOMAscan proteomic assay of 1129 analytes was performed on sputum supernatants (SomaLogic, Boulder, CO, USA) (3). The fraction of exhaled nitric oxide (FeNO) was measured online using an electrochemical analyser (NIOX MINO; Aerocrine, Solna, Sweden) at an expiratory flow rate of 50ml/s according to ATS/ERS guidelines (27). Serum IgE was measured using the Thermo Fisher (Uppsala, Sweden) CAP system. Biomarker and sputum and urinary eicosanoid data were generated by multiplex analysis and mass spectrometry (5).

Data analysis

Analysis was performed in R version 3.5.0 (28). Gene set variation analysis (GSVA) was run using the R Bioconductor GSVA package (29) to calculate sample-wise enrichment scores (ES). The ES for AD disease, FZ response and immunological pathway signatures was calculated for each subject across the U-BIOPRED sample compartments. We used a linear model adjusted for age and gender and used the least squares means (30) with the Tukey p-value adjustment method for comparisons of families of estimates (4 for cohort, 5 for granulocyte subtype, and 4 for Transcriptome-Associated Cluster (TAC) group (3)) to analyse the ES differences between groups. Differential expression between sputum transcriptomics of subjects with eosinophilic inflammation against those with non eosinophilic inflammation and subsequent clustering revealed 3 groups. TAC1 contains patients with a high enrichment for the Woodruff Th2-high gene signature with a very high sputum eosinophilia. The TAC2 is characterised by inflammasome-associated pathways and high sputum neutrophilia whilst TAC3 is associated with high levels of macrophages and a mainly paucigranulocytic phenotype (3). Visualization of the distribution of ES was performed with the ggplot2 R package (31). The GSVA signatures are listed in **Supplementary Table 1**.

The FZ response signature in U-BIOPRED sputum subjects was used to categorise SA patients as being predicted-responders (PRs) (n=26, ES ≥+0.1) or predicted non-responders (PNRs) (n=18, ES ≤-0.1) whilst filtering out patients with undirected ES (>+0.1 and >-0.1), MMAs and HCs. All categorical variables were analysed using Fisher's exact test. A T-test was used for continuous clinical variables with normal distribution (Shapiro-Wilk test p-value >0.05), whilst the Wilcoxon rank sum test with continuity correction was used for variables with a skewed distribution.

Differential gene (for all PRs and PNRs) and protein (for those PRs and PNRs with proteomics data) expression analysis was performed using limma 3.38.3 (32) for linear model fitting for each gene or protein. Empirical Bayes moderation of standard errors was used to produce tables of significant DEGs and proteins. P-values were adjusted with the Benjamini–Hochberg False Discovery Rate (FDR-BH) procedure (33). Age and gender were not confounding variables. Significantly up and downregulated genes were determined by a log2 fold change of ≥1 or ≤-1 and an FDR-BH adjusted p≤0.05. Pathway enrichment analysis was

303 performed using ReactomePA (34), utilising the human Reactome ontology (35) with p-value
304 FDR-BH adjustment and cut-off of 0.05.
305

Results

AD signature in asthma

We defined an AD disease signature (**Supplementary Table 1**) according to whether DEGs were significantly up- (112 DEGS, AD-UP) or down-regulated (29 DEGs, AD-DOWN) between lesional and non-lesional skin with a fold-change (FC) ≥2 or ≤-2 and an FDR≤0.05 for the whole AD cohort. T-cell, Th2, Th17/Th22 and general inflammatory genes were up-regulated in the AD-UP signature whereas AD-DOWN reflected lipid pathways and pathways associated with dysregulated dermal epithelial function (20).

This signature was applied to blood (**Fig 1A**) and sputum (**Fig 1B**) of the U-BIOPRED cohort. The AD-UP signature ES trended with severity: significantly enriched in the blood of severe, but not MMA, asthmatics irrespective of smoking status (**Fig 1A**). A similar trend was seen in the sputum of severe asthmatics (**Fig 1B**). When compared by sputum TACs (3) there was an enrichment of the AD-UP signature in sputum from TAC2 (adj.p=$2.87x10^{-6}$) subjects (**Fig 1C**) compared to healthy controls. Assessment based on sputum granulocytes further highlighted the greater enrichment of the AD-UP score in granulocytic asthma (**Fig 1D**) with a greater ES in neutrophilic (adj.p=$6.83x10^{-5}$) and mixed granulocytic (adj.p=0.0005) asthma compared to healthy controls. The enrichment of the AD lesion signature in asthma reflects a composite of the cells within blood and sputum.

We confirmed the appropriateness of the AD-UP signature by using the previously defined MADAD-UP pooled signature (**Fig 1E-H**). The MADAD-UP signature is a consensus disease signature of the pathologically upregulated genes which characterise atopic dermatitis across several studies (23). The overlap between the AD-UP and MADAD-UP gene signatures consisted of 84 genes. This signature was enriched in both blood (**Fig 1E**) and sputum (**Fig 1F**) of severe asthmatics irrespective of smoking status, mirroring results seen in AD-UP blood. Classifying asthmatics according to sputum molecular phenotype or to sputum granulocytes also demonstrated enrichment of the MADAD-UP signature in TAC2 (**Fig 1G**) and neutrophilic/mixed granulocytic subjects (**Fig 1H**). Overall, the AD disease signature was enriched in severe neutrophilic asthma.

Derivation of an FZ super-responder signature in AD

The FZ treatment super-response was defined by those subjects with a good clinical response who also had a good transcriptomic response comparing lesional biopsies at

baseline and after 12 weeks FZ treatment in AD patients to identify the significant DEGs (FC≥2 or ≤-2 and FDR<0.05)(20). The highest clinical and transcriptomic effect was seen in baseline IL-22^{high} lesional tissue and the transcriptomic changes seen in patients with a high clinical and transcriptomic response was used to generate the FZ-super-responder signature.

We identified 417 DEGs (121 up- and 296 or down-regulated by FZ) in lesional AD skin tissue biopsies from patients with the greatest clinical response to FZ at 12 weeks (**Supplementary Table 1**). This FZ-response signature (FZ-DOWN) represents a key proportion of the AD-UP disease signatures. In particular, the AD-UP signature (112 genes) had 74 genes overlapping with the FZ-DOWN (296 genes, 25%) whilst the MADAD-UP signature (405 genes) had 196 genes overlapping with the FZ-DOWN signature (48.4%). A strong correlation existed between the AD-UP and FZ-DOWN ES in asthmatic sputum (R^2=0.8326, p=2.2x10^{-16}) (**Supplementary Fig 1A**) and between MADAD-UP and FZ-DOWN (R^2=0.9156, p=2.2x10^{-16}) (**Supplementary Fig 1B**). The FZ-DOWN signature included pathways associated with general inflammation, T-cell, Th2 and Th17/Th22 activation (**Supplementary Fig 2, Supplementary Table 2**), which are all up-regulated within the AD disease signatures. No pathways were significantly associated with FZ-UP genes although relaxing the FDR threshold identified pathways associated with epidermal signalling (**Supplementary Fig 3, Supplementary Table 3**), which justifies the focus on the FZ-DOWN signature. To test whether the FZ-DOWN signature predicted the response in AD patients, we examined the ES of FZ DOWN in lesional AD baseline samples (20). This was significantly (p=0.0496, adjusted for age and gender) positively associated with the AD SCORAD score after treatment.

In summary, **Supplemental Table 1** provides a list of all the gene signatures used in this analysis including the sets of genes up-regulated (AD-UP) or down-regulated (AD-DOWN) in AD whilst **Supplemental Table 2** provides a list of all the pathways that the FZ-DOWN gene signature corresponds to and highlights the importance of immune pathways. **Supplementary Table 3** is a list of all the pathways that relate to the FZ-UP gene signature. None of these pathways was significantly enriched and are mostly skin-related.

Enrichment of the FZ super-responder signature from AD in U-BIOPRED

The FZ-DOWN signature was significantly enriched in the blood of U-BIOPRED severe asthmatics (adj.p<0.05) (**Fig 2A**) despite the wide variability in ES scores, which may reflect the different types of immune cells found in blood and lesional tissue. The skin contains a

mixture of epithelial cell-like and immune cells but the enrichment observed in blood may indicate detection of the immune components.

The FZ-DOWN signature was significantly enriched in the blood of TAC2 patients (adj.p=0.015, **Fig 2B**). The response in blood when subjects were stratified according to sputum granulocytes was variable and although there was a trend towards enrichment in asthma subtypes, this did not reach significance (**Fig 2C**). There was a greater degree of enrichment in sputum samples compared to blood (compare Fig 3A-C with Fig 3D-F). The ES for FZ-DOWN had a stepwise association with severity and was highly enriched TAC2 patients (adj.p=0.002, **Fig 2E**), and neutrophilic (adj.p=0.0002, **Fig 2F**) and mixed granulocytic (adj.p=0.0098, **Fig 2F**) asthma compared with healthy controls. The good correlation between the TAC2 signature and the FZ-DOWN signature in sputum ($p<2.2x10^{-16}$, r=0.784) was not due to overlapping signatures as only 3 genes were common between the two genesets – CASP4, KCNJ15 and SAMSN1. Importantly, we were able to show that the AD-UP and MADAD-UP (**Fig 3A**) and the FZ-DOWN (**Fig 3B**) signatures were also enriched within the sputum neutrophilic (adj.p<0.05) and mixed granulocytic patients within the ADEPT cohort (**Fig 3A-B**).

To ensure against a confounding effect of tissue heterogeneity we removed the 4 skin-specific genes identified by comparing the FZ-DOWN signature with a published skin transcriptomic profile (36). There were 4 overlapping genes (WFDC12, TYR, S1PR5, LYPD5) and removal of these 4 genes from the FZ-DOWN signature had minimal effect on the analysis (**Supplementary Fig 4**).

Since the FZ-DOWN signature was associated with neutrophilic asthma we checked whether this and the AD disease signatures correlated with 3 neutrophil signatures from the Human Cell Atlas (37), an immune cell gene-signature database (38) and a Th17 signature (39) that consists of genes for neutrophil chemoattractants (CXCL1, CXCL2, CXCL3, CXCL8 and CFS3). We observed a high correlation between FZ-DOWN ES and neutrophil signature ES and also AD disease signature ES and neutrophil signature ES, indicating that the disease signatures reflected tissue neutrophilia. In particular Pearson's correlation between the FZ-DOWN ($p=1.25 x10^{-9}$, r=0.519), AD-UP ($p<2.2x10^{-16}$, r=0.754) and the MADAD-UP ($p<2.2x10^{-16}$, r=0.684) signatures were very significantly correlated with the immune cell database neutrophil signature. In addition, the FZ-DOWN ($p<2.2x10^{-16}$, r= 0.691), AD-UP ($p=4.479x10^{-7}$, r=0.441) and the MADAD-UP ($p=4.304x10^{-7}$, r=0.442) signatures were also significantly correlated with the Human Cell Atlas neutrophil signature.

However, neutrophil levels in the skin were not significantly reduced after FZ treatment (**Supplementary Fig 5**) which suggests that despite neutrophil genes contributing to the AD disease signature and some neutrophil genes being present in the FZ response signature, the FZ response

phenomenon is unlikely to be driven by neutrophil levels alone. This corroborates with a positive but non-significant correlation between sputum neutrophils and sputum IL-22 protein in U-BIOPRED subjects (p=0.0699, r=0.184). We also examined the correlation between sputum neutrophils and the FZ-DOWN signature in the validation ADEPT cohort and found no significant correlation (% segmented neutrophils; p=0.911, r=0.0186).

Clinical features of predicted responders and non-responders in U-BIOPRED

We next examined whether the FZ-DOWN signature was associated with a specific subset of SA patients as the most clinically relevant group. Highly-enriched patients (PRs) were compared with those least-enriched (PNRs) for the FZ-DOWN signature (**Supplementary Fig 6, Supplementary Table 4**). The enrichment score of the FZ-DOWN signature in sputum was used to categorise SA patients as being predicted-responders (PRs) (n=26, ES ≥+0.1) or predicted non-responders (PNRs) (n=18, ES ≤-0.1) whilst filtering out patients with an undirected ES (>+0.1 and >-0.1), MMAs and HCs. The clinical comparison revealed that PRs had more frequent LABA use and significantly elevated sputum neutrophils and lower sputum eosinophils and macrophages in addition to lower IgE levels in contrast to PNRs (**Table 1**). Furthermore, PRs had lower levels of plasma eotaxin-3 and serum IL-13 biomarkers as measured by Luminex or MSD analysis. PRs also had elevated sputum levels of 11-dehydro-TXB_2, 5-HETE and LTB_4 (p=0.0526) but lower LTE_4 reflecting the neutrophilic and low eosinophilic nature of the PR population (**Table 2**).

In a linear model (LM) of asthmatic sputum FZ-DOWN ES and medication usage, corrected for age, gender and BMI, we found no significant association between FZ-DOWN ES and OCS use (p=0.702). However, we did find a significant association between FZ-DOWN ES and LABA use (p=0.0243) where FZ-DOWN ES was elevated in the twice daily LABA use group (reflecting severity of disease, LM estimate=0.112) and least in the group not taking LABA at all (mildest subjects, LM estimate=-0.117).

DEGs between Predicted Responder (PR) and Predicted Non-Responder (PNR) severe asthmatics

We performed DEG analysis between PR and PNR patients and identified 431 up and 19 down sputum DEGs which were significant with a log2 FC of over 1 or below -1 respectively. These are reported in **Supplementary Table 5**. ReactomePA pathway analysis on the up DEGs

indicates a strong neutrophilic component with neutrophil degranulation, cytokine and chemokine receptor and Toll-like receptor (TLR) signalling as well as IL-10 and IFN pathways being highly enriched in PR subjects (**Fig 4, Supplementary Table 6**). The IL-33 receptor (IL1RL1, ST2) was greatly downregulated in the PR group.

Sputum proteomic enrichment of FZ-DOWN signature

We then selected PRs and PNRs who had SomaLogic sputum proteomics data available (n=32) (**Supplementary Table 7**)**.** Differential protein analysis on the sputum SomaLogic data confirmed a strong neutrophilic component (**Table 3**). Significantly upregulated sputum proteins included the neutrophil modulator Sialic acid-binding immunoglobulin-type lectins 9 (siglec-9), the neutrophil serine proteases cathepsin G and azurocidin involved in neutrophil degranulation and microbial killing, B7_H2 which is a costimulatory ligand for CD28, IL-6 which is involved in neutrophilic asthma and increased differentiation of Th17 cells and Oxidized Low Density Lipoprotein Receptor 1 (OLR1) which is involved in tissue remodelling. These proteins together with the enhanced expression of neutrophil degranulation products implicate neutrophil activation as being a key component of asthmatic subjects who are highly enriched for the FZ-DOWN signature.

FZ-DOWN signature markers in blood

We then selected PRs and PNRs who had blood proteomics data available (n=42) (**Supplementary Table 8**)**.** Differential protein analysis on blood SomaLogic data (**Supplementary Table 9**) defined potential FZ responders from non-responders as possessing lower blood IgE and a trend towards elevated expression of the neutrophil modulator siglec-9 and I-TAC as seen in the sputum proteomics analysis.

~~Sputum~~IL-22 pathway and protein correlates with FZ-DOWN enrichment

In AD skin (20), IL-22 gene expression alone predicts the response to FZ. However, IL-22 gene expression was not enriched in blood (**Supplementary Fig 7A**) or sputum according to asthma severity (**Supplementary Fig 7B**) or in TAC2 asthmatics (**Supplementary Fig 7C**). There was no correlation between FZ-DOWN and IL-22 gene expression in blood (**Supplementary Fig 7D**), sputum **(Supplementary Fig 7E**), bronchial brushings (**Supplementary Fig 7F**) or nasal brushings (**Supplementary Fig 7G**).

In contrast, the ES of the Th22/IL-22 signature was significantly correlated with FZ-DOWN ES in asthmatic sputum ($p=4.31x10^{-14}$, r=0.656)(**Fig 5A**), bronchial brushings ($p<2.2x10^{-16}$, r=0.753)(**Fig 5B**), nasal brushings ($p=8.53x10^{-13}$, r=0.755)(**Fig 5C**) and blood ($p=5.06x10^{-6}$, r=0.223). The Th22/IL-22 signature (**Supplementary Table 1**) consists of 16 genes including IL-22 itself and the Th22-specific marker CCR10 (20). Pathway analysis identified several significantly enriched pathways including 'IL22 Induces Keratinocyte Proliferation in Psoriasis', 'Interleukin-19, 20, 22, 24 Homo sapiens R-HSA-8854691' and 'IL-the 17 signaling pathway'.

Importantly, sputum IL-22 protein was significantly enriched in patients with TAC2 asthma compared to those with TAC1 asthma (p=0.0112) and there was a significant correlation between sputum IL-22 protein expression and the FZ-DOWN ES when controlled for age, sex and BMI (p=0.0360, r=0.133)(**Fig 5D**). IL-22 protein in sputum also significantly correlated with FZ-DOWN ES in nasal brushings for all subjects (p=0.0443, r=0.423).

DISCUSSION

We demonstrate that an AD disease signature was enriched in severe neutrophilic asthma in both the U-BIOPRED and ADEPT asthma cohorts and that these subjects were also highly enriched for a gene signature indicative of a super-response to FZ. Pathway analysis indicated that the AD-UP disease signature and the FZ-DOWN-response signature were a composite of Th1, Th2, Th17, Th22 and general inflammatory processes and that sputum proteins linked with a potential FZ response in asthma were associated with neutrophil recruitment and activation. The FZ super-response signature did not correlate with IL-22 gene expression itself although there was a good correlation with the Th22/IL-22 gene signature in nasal and bronchial brushings. Sputum IL-22 protein correlated significantly with FZ-DOWN. Re-purposing transcriptomic data that defines a treatment response across therapeutic areas may aid the stratification of patients for future clinical trials.

Early transcriptomic analysis of skin samples from psoriasis and AD subjects identified neutrophil chemoattractant genes as being highly expressed in both AD and psoriatic skin lesions (40). Furthermore, neutrophil elastase staining is elevated in lesional compared with non-lesion skin in AD patients but to a much lesser extent than seen in patients with psoriasis. This enhanced neutrophilia in AD may reflect concurrent infection with Staphylococcus aureus infection (41). Enhanced neutrophilia may reflect an enhanced Th1/Th17 drive.

The Th2/Th22 pathway is the major pathway in AD as recently confirmed using single cell RNA-sequencing (42). This is seen across all age-groups, however, an enrichment of Th1/Th17 genes is seen in lesional compared to non-lesional skin in adults (43). Indeed, the usual Th2/Th22 drive in AD is skewed towards a Th1/Th17 phenotype with increasing age (44) and severity of disease. For example, enhanced Th1/Th17 mediator expression is reported in the blood of AD patients with severe but not mild disease (45). Importantly, there was a good correlation between Th2/Th22/Th1/Th17 gene and protein expression profiles in lesional and non-lesional AD samples (46).

Severe asthmatic PRs to FZ had neutrophilic or mixed granulocytic asthma, poor lung function and a low asthma quality of life despite frequent LABA use. These subjects also had lower serum IgE levels but with relatively greater atopic disposition, in contrast to subjects with T2 eosinophilic asthma (≥300 cells/µl), suggesting that an anti-IL-22 intervention may be targeted to non-T2 asthmatics with low IgE as opposed to those with a high IgE neutrophilic phenotype (14,47). Gender, BMI and age did not affect the enrichment of the FZ response

signature. Comparison of biomarkers between PRs and PNRs indicated that PR subjects had elevated levels of 11-dehydro-TXB_2, 5-HETE and LTB_4 although the latter did not quite reach significance. Leukotrienes are formed via a 5-LOX dependent process in which arachidonic acid is converted to the unstable epoxide intermediate LTA_4, which can then be converted by either LTC_4 synthase (LTC_4S) to form the cysteinyl-leukotrienes or via LTA_4-hydrolase (LTA_4H) to form LTB_4. Neutrophils have known LTA_4H activity and sputum neutrophils have been previously reported to produce LTB_4 (48). Accordingly, the elevated sputum LTB_4 levels in combination with the lower LTE_4 levels among PR subjects collectively point towards a specific elevation of LTA_4H activity within these neutrophilic subjects, which further support a non-T2 phenotype (49).

We have previously defined asthmatics according to their sputum molecular phenotypes (3). The FZ-DOWN signature was enriched in TAC2 patients that suggests that FZ may be useful for T2-low severe neutrophilic asthmatics. Pathway analysis of the potential FZ responders versus non-responders highlighted the importance of neutrophil degranulation products along with signalling downstream of TLRs, cytokine/chemokines including neutrophil-associated mediators and chemoattractant receptors such as CXCL10, CXCL11, CXCR1 and CXCR2, suggesting an activated neutrophil phenotype. Although previously-defined pathways such as the NLRP3 inflammasome within TAC2 were not specifically enriched in the FZ predicted responder versus non-responder subjects, factors associated with inflammasome activation including IL-1α and IL-1RAP are present (50).

At the cellular level, a significant increase in the percentage of airway neutrophils (75.5% *vs* 35.6%) and a significant decrease in the percentage of airway macrophages (18.9% *vs* 32.5%) in the FZ predicted responders group were observed. Macrophages phagocytose apoptotic neutrophils and contribute to inflammation resolution. It is interesting to speculate whether a reduced number of airway macrophages observed could adversely impede neutrophil clearance, thus promoting the elevated levels of airway neutrophils in this endotype of asthma. Defects in neutrophil apoptosis and/or clearance leading to airway neutrophilia have previously been reported in a small cohort of severe atopic asthmatics with a low-eosinophilic phenotype (≤3% sputum eosinophils) (51).

We have previously shown that GM-CSF/CSF2RB- and IFN-activated macrophages as well as lower enrichment of eosinophils were associated with childhood asthma (52). The AD disease signature indicates that AD, although generally seen as a T2-dominant disease, also

has different degrees of non-T2 driving pathways including Th1, Th17, Th22 and inflammatory pathways (20). Both GM-CSF and IFN pathways were also enriched within the FZ predicted responder population and interestingly, the potential responder-non-responder pathways also indicated the enrichment of IL-10 signalling which is involved in the suppression of IL-5 and GM-CSF expression and eosinophil apoptosis (53). These pathways may also represent therapeutic targets in these SA patients.

The AD-DOWN signature is not enriched in asthmatic peripheral blood but shows some enrichment in airway samples. This signature includes lipid pathways and pathways associated with dysregulated dermal epithelial function that indicates remodelling of epithelial tissues is more prevalent in severe neutrophilic asthma airways. These pathways are also up-regulated by FZ which suggests that FZ may also impact upon asthmatic airway epithelial cell barrier function.

IL-22 possesses potential pro- and anti-inflammatory roles in asthma (11,15,16). In mouse models of allergic sensitisation and challenge, IL-22 attenuates established Th2 cell-mediated allergic inflammation *in vivo* (11,54). However, IL-22 promotes allergic inflammation in similar mouse models at the onset of allergic asthma (11,18), supporting the view that IL-22 may be involved in the atopic march (17). While data from mouse models suggest that anti-IL-22 may be efficacious in early onset allergic asthma, our analysis would indicate that IL-22 might have a pathogenic role in those with neutrophilic inflammation with lower IgE levels.

In our analysis, IL-22 mRNA expression did not correlate with FZ response signatures in blood, sputum, nasal and bronchial brushings whereas there was a significant correlation with sputum IL-22 protein and with the Th22/IL-22 gene signature in sputum, bronchial and nasal brushings. This may reflect the local expression of IL-22 protein in the airways which is not detected at the mRNA level or is not observed due to lack of proteomics data for bronchial and nasal brushings. However, the up-regulation of the FZ-DOWN signature does indicate a significant impact of IL-22 on downstream signalling.

This study has several strengths and also some limitations. We derived a gene signature from skin lesions of AD subjects (AD-UP) and also from patients with a good clinical response and a clear transcriptomic response to FZ after 12 weeks of treatment (FZ-DOWN) to provide evidence for target engagement in the lesional tissue. We utilised the large data-rich U-BIOPRED cohort to define subsets of patients who are more likely to respond to FZ and

validated this in a separate cohort of severe asthmatics. Importantly, we were able to demonstrate markers of high enrichment of this response signature in nasal brushings and peripheral blood. However, we do not have evidence that the changes seen in the lesional skin of AD patients with FZ also occur in the airways of asthmatics. Animal models of severe neutrophilic or mixed granulocytic asthma may be used to address this issue. In asthma, baseline levels of IL-22 mRNA did not correlate with FZ-DOWN signature as predicted from the AD data. This suggests that additional mechanisms may occur in the asthmatic airway compared to the skin. These mechanisms may be linked since there is a strong correlation between the Th22/IL-22 and FZ-DOWN signatures. The good correlation of both IL-22 sputum protein abundance and Th22/IL-22 signature ES with the FZ-DOWN signature ES within nasal brushings indicates a potential alternative readily accessible approach for identifying possible responder populations. Although this data was validated in a separate SA cohort we have not measured the stability of the FZ-response signature over time and whether this changes with T2-directed biologics.

This novel approach of molecularly characterising clinical super-responders to an antibody drug in one disease followed by probing other disease databases may be a more effective way of identifying predicted-responders at the endotypes level compared to looking at drug-target levels alone. By exploiting pre-existing databases and clinical trial data, this approach could lead to a reduction in drug development time and in research costs. The greatest enrichment of the FZ PR signature was observed in severe neutrophilic asthmatics. Furthermore, we found that blood and sputum gene expression and the expression of several proteins in sputum can predict asthmatics with a high enrichment of a FZ response signature in the airway. This stratification process will need validation in a controlled clinical trial, while at the same time examining the long-term efficacy and side-effect profile of FZ in endotypes of severe asthma.

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Table 1. Clinical differences of predicted responders versus non-responders to Fezikinumab in U-BIOPRED.

Characteristic	FZ Predicted Responders	FZ Predicted Non-Responders	p value
Total (n)	26	18	
Age (years)	51.8 (12.7)	55.3 (14)	NS
BMI	28 (4.65)	26.3 (3.39)	NS
Gender: Female (n)	16	9	NS
Severe asthma, non-smokers (n)	18	13	NS
Severe asthma, smokers/ex-smokers (n)	8	5	NS
Severe exacerbation in previous year	2.27 (2.38)	1.72 (2.02)	NS
Nasal polyps (n)	8	6	NS
Eczema (n)	8	7	NS
Allergic rhinitis (n)	9	4	NS
Non-allergic rhinitis (n)	5	3	NS
Gastro-esophageal reflux (n)	12	7	NS
Hay fever (n)	11	5	NS
Positive atopic status (n)	11	6	NS
ACQ5 score	2.44 (1.23)	1.79 (1.31)	NS
AQLQ score	4.35 (1.22)	4.98 (1.41)	NS
HADS score	12.3 (8.16)	10.5 (8.91)	NS
SNOT score	31.2 (18.2)	22.6 (10.8)	NS
FEV1 (% predicted)	63.7 (24.3)	67.2 (17.5)	NS
FVC (% predicted)	86.9 (20.4)	95.3 (17.4)	NS
FEV1/FVC	59.3 (13.1)	57.2 (8.79)	NS
FeNO (ppb)	35 (33)	54.7 (46.9)	NS
Serum IgE (IU/L)	204 (358)	332 (294)	0.02
Blood eosinophil (/10-9L)	0.277 (0.155)	0.401 (0.305)	NS
Blood neutrophil (/10-9L)	4.93 (1.93)	5.41 (2.44)	NS
Blood lymphocyte (/10-9L)	2.12 (0.936)	2.1 (0.9)	NS
Blood monocyte (/10-9L)	0.634 (0.278)	0.581 (0.222)	NS
Sputum neutrophils (%)	75.7 (16.6)	35.6 (18)	2.26E-08
Sputum eosinophils (%)	3.9 (5.55)	30.4 (26.5)	0.0009
Sputum lymphocyte (%)	1.5 (1.6)	1.46 (1.26)	NS
Sputum macrophage (%)	18.9 (14.4)	32.5 (20.8)	0.025
Sputum mast cell (%)	0.0346 (0.087)	0.0333 (0.101)	NS
Oral corticosteroid use daily (n)	12	9	NS
LABA use twice a day (n)	12	2	0.039

ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; BMI: Body Mass Index; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; FeNO: Fractional exhaled nitric oxide; HADS: Hospital Anxiety and Depression Scale; ICS: Inhaled

775 corticosteroids; LABA: long-acting beta agonist; SNOT: SinoNasal Outcome Test. Data shown
776 as mean (Standard Deviation).
777
778

779 **Table 2.** Molecular marker differences of Predicted Responders versus Predicted Non-
780 Responders to Fezakinumab in the U-BIOPRED severe asthma patients.
781

Biomarker	FZ Predicted Responders	FZ Predicted Non-Responders	p value
α1 microglobulin (pg/ml) Luminex (serum)	6120 (2210)	7500 (2390)	NS
C5a (pg/ml) Luminex (serum)	50.8 (33.4)	38.9 (21.6)	NS
CD30 (pg/ml) Luminex (serum)	38.9 (17.1)	42.5 (14.7)	NS
CD40L (pg/ml) Luminex (serum)	4420 (1990)	5210 (2300)	NS
DPPIV (pg/ml) Luminex (serum)	98500 (48100)	91500 (24300)	NS
Galectin 3 (pg/ml) Luminex (serum)	5550 (2050)	5770 (1470)	NS
IL-18 (pg/ml) Luminex (serum)	247 (152)	234 (73.8)	NS
IL-1α (pg/ml) Luminex (serum)	35.5 (9.95)	36.2 (6.12)	NS
IL-6Rα (pg/ml) Luminex (serum)	10600 (2450)	10900 (2020)	NS
LBP (pg/ml) Luminex (serum)	2110000 (891000)	1820000 (668000)	NS
Lumican (pg/ml) Luminex (serum)	131000 (37000)	136000 (25300)	NS
MCP4 (pg/ml) Luminex (serum)	142 (44.7)	168 (71.2)	NS
MMP3 (pg/ml) Luminex (serum)	21400 (18300)	24500 (17900)	NS
RAGE (pg/ml) Luminex (serum)	1260 (414)	1320 (382)	NS
Serpin E1 (pg/ml) Luminex (serum)	95000 (30400)	97600 (19900)	NS
SHBG (pg/ml) Luminex (serum)	3640000 (2840000)	4780000 (4670000)	NS
CCL17 (pg/ml) MSD (plasma)	77.5 (70.8)	134 (120)	NS
CCL22 (pg/ml) MSD (plasma)	796 (316)	866 (218)	NS
EOTAXIN (pg/ml) MSD (plasma)	118 (60.1)	140 (67.6)	NS
EOTAXIN3 (pg/ml) MSD (plasma)	15 (15.4)	72.4 (130)	0.00097
IFNγ (pg/ml) MSD (plasma)	12.2 (12.8)	7.44 (6.18)	NS
IL-6 (pg/ml) MSD (plasma)	1.21 (1.01)	0.804 (0.335)	NS
IL-8 (pg/ml) MSD (plasma)	6.02 (9.75)	3.78 (1.86)	NS
IP10 (pg/ml) MSD (plasma)	386 (250)	305 (183)	NS
MCP1 (pg/ml) MSD (plasma)	117 (36.8)	119 (38.4)	NS
MIP1β (pg/ml) MSD (plasma)	56.1 (18.1)	63.5 (29.8)	NS
TNFα (pg/ml) MSD (plasma)	1.84 (0.483)	1.94 (0.632)	NS
CCL18 (pg/ml) IMPACT serum	169 (63.3)	228 (106)	NS
IL-13 (pg/ml) IMPACT serum	0.608 (0.494)	0.942 (0.384)	0.0074
IL-17A (pg/ml) SINGULEX serum	0.58 (0.381)	0.455 (0.258)	NS
Periostin (ng/ml) ELECSYS serum	51.2 (19.5)	54.2 (16.5)	NS
hCRP (mg/L)	6.29 (11)	1.69 (1.33)	NS
11-dehydroTXB$_2$ (ng/ml) urine	13.9 (8.96)	14.2 (10.5)	NS

2,3 dinor-11β PGF2α (ng/ml) urine	73.8 (30.9)	94.8 (86)	NS
2,3 dinor 8isoPGF2α (ng/ml) urine	244 (137)	296 (319)	NS
2,3 dinor TXB_2 (ng/ml) urine	68.1 (46.6)	52.2 (41.3)	NS
8,12 isoPGF2α (ng/ml) urine	386 (240)	430 (408)	NS
8 isoPGF2α (ng/ml) urine	29.3 (11.8)	32.3 (19.6)	NS
LTE_4 (ng/ml) urine	9.28 (8.35)	10 (6.13)	NS
PGE2 (ng/ml) urine	20.2 (23.2)	18.5 (14.8)	NS
PGF2α (ng/ml) urine	132 (102)	130 (75.1)	NS
Tetranor PGDM (ng/ml) urine	299 (115)	305 (257)	NS
tetranorPGEM (ng/ml) urine	1180 (1190)	1030 (539)	NS
11 dehydroTXB_2 (pg/mL) sputum	231 (283)	63.4 (23.9)	0.00438
12-HETE (pg/mL) sputum	1470 (1300)	1980 (1410)	NS
15-HETE (pg/mL) sputum	4490 (6900)	7180 (9130)	NS
5-HETE (pg/mL) sputum	1570 (1500)	964 (1630)	0.0322
6-ketoPGF1α (pg/mL) sputum	58.6 (27.1)	53.7 (23.6)	NS
LTB_4 (pg/mL) sputum	801 (756)	774 (1540)	NS
LTE_4 (pg/mL) sputum	319 (372)	763 (1030)	0.0312
PGD2 (pg/mL) sputum	269 (317)	174 (159)	NS
PGE2 (pg/mL) sputum	390 (363)	202 (135)	NS
Tetranor PGDM (pg/mL) sputum	66 (57.4)	54.8 (51.9)	NS
Tetranor PGEM (pg/mL) sputum	76.3 (49.1)	67.6 (53.3)	NS

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Table 3. Top and bottom 20 differentially expressed sputum proteins that differentiate U-BIOPRED asthmatic FZ predicted responders (PRs) from predicted non-responders (PNRs) defined from asthma sputum GSVA FZ response signature ES which had sputum proteomic data available (see Supplementary Table 7, see Supplementary Figure 4). Genes are ranked according to log_2 fold change.

Upregulated

Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
Siglec_9	2.04	4.11	0.0066	0.1928
Hemoglobin	1.98	3.96	0.01807	0.2210
PSA1	1.75	3.37	0.01409	0.2059
Cathepsin_G	1.70	3.25	0.00048	0.1368
Carbonic_anhydrase_I	1.45	2.74	0.00081	0.1370
SRCN1	1.43	2.70	0.00702	0.1928
Azurocidin	1.40	2.65	0.00251	0.1661
PLCG1	1.30	2.46	0.01068	0.2059
resistin	1.29	2.45	0.09375	0.3715
Factor_I	1.28	2.44	0.14821	0.4261
IL_6	1.28	2.43	0.00390	0.1869
B7_H2	1.19	2.29	0.04214	0.2830
Ferritin	1.18	2.27	0.0127	0.2059
IP_10	1.15	2.23	0.01319	0.2059
Elastase	1.08	2.12	8.53E-05	0.0959
Transferrin	1.06	2.09	0.16571	0.4476
OLR1	1.02	2.03	0.00309	0.1735
I_TAC	0.99	1.99	0.04652	0.2875
Granzyme_B	0.99	1.98	0.03350	0.2599
Esterase_D	0.97	1.96	0.06937	0.338

Downregulated

Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
a2_Antiplasmin	-1.28	0.40	0.02414	0.2441
Fucosyltransferase_3	-1.29	0.40	0.28234	0.5445
PCSK9	-1.29	0.40	0.00876	0.2017
CATZ	-1.29	0.40	0.04350	0.2830
Kininogen_HMW	-1.37	0.38	0.08360	0.3495
IGFBP_4	-1.37	0.38	0.03957	0.2800
Cathepsin_B	-1.38	0.38	0.00473	0.1869
Phosphoglycerate_mutase_1	-1.46	0.36	0.0435	0.2830
Histone_H2A_z	-1.51	0.34	0.00838	0.2007
FETUB	-1.52	0.34	0.05755	0.3169
Clusterin	-1.55	0.34	0.00521	0.1892

Plasminogen	-1.56	0.33	0.00596	0.1928
amyloid_precursor_protein	-1.60	0.32	0.0251	0.2441
PCI	-1.62	0.32	0.01463	0.2079
Integrin_aVb5	-1.62	0.32	0.00678	0.1928
PTHrP	-1.65	0.31	0.00122	0.1370
CD39	-1.72	0.30	0.00039	0.1368
MIS	-1.73	0.30	0.00130	0.1370
PAPP_A	-1.76	0.29	0.01554	0.2079
Antithrombin_III	-2.31	0.20	0.00490	0.1869

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Figure legends

Figure 1. Gene Set Variation Analysis (GSVA) showing enrichment scores (ES) of gene signatures derived from genes up-regulated (UP) in lesional versus non-lesional tissue from atopic dermatitis (AD). Disease signatures are derived from either the Brunner paper (AD-UP, **A-D**) or from an AD meta-analysis-derived AD (MADAD, **E-H**). The ES for these signatures in U-BIOPRED blood (**A, E**) and sputum (**B-D** and **F-H**) according to severity (**A, B, E, F**), Transcriptome-Associated Cluster (TAC) (**C, G**) and sputum granulocyte subtype (**D, H**). Between group adjusted p values are provided compared to HC values. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). Abbreviations: HC; Healthy Control, MMA; Mild-Moderate Asthma. SAs/ex; Severe Asthma smoker/ex-smoker. SAns; Severe Asthma non-smoker, P; pauci-granulocytic, E; eosinophilic, N; neutrophilic and M; Mixed.

Figure 2. Gene Set Variation Analysis (GSVA) showing enrichment scores (ES) of Fezakinumab (FZ) treatment response signatures of downregulated genes (FZ-DOWN) in lesion versus non-lesional tissue from atopic dermatitis (AD). ES for AD-UP signatures are given for U-BIOPRED blood (**A-C**) and sputum (**D-F**) according to asthma severity (**A, D**), Transcriptome-Associated Clusters (TAC) (**B, E**) and granulocyte subtype (**C, F**). Between group adjusted p values are provided compared to HC values. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). Abbreviations: HC; Healthy Control, MMA; Mild-Moderate Asthma. SAs/ex; Severe Asthma smoker/ex-smoker. SAns; Severe Asthma non-smoker, P; Pauci-granulocytic, E; Eosinophilic, N; Neutrophilic and M; Mixed.

Figure 3. Gene Set Variation Analysis (GSVA) showing enrichment scores (ES) of gene signatures derived from genes up-regulated (UP) in lesion versus non-lesional tissue from atopic dermatitis (AD) in the ADEPT (Airway Disease Endotyping for Personalized Therapeutics) cohort by granulocytic subtype. Disease signatures are derived from either the Brunner paper (AD-UP, **A, upper panel**) or from an AD meta-analysis-derived AD (MADAD, **A, lower panel**). ES of the Fezakinumab (FZ)-DOWN signature obtained from lesional versus non-lesion tissue after 12 weeks treatment (**B**). Between group adjusted p values are provided compared to HC values. *p<0.05. Abbreviations: HC; Healthy Control, P; Pauci-granulocytic, E; Eosinophilic, N; Neutrophilic and M; Mixed.

Figure 4. Protein pathway analysis using ReactomePA of differentially-expressed genes (false discovery rate, FDR<0.05) that distinguish asthmatic patients highly-enriched (Predicted Responders, PRs) for the Fezakinumab (FZ)-response signature (FZ-DOWN) from those poorly-enriched (Predicted Non-Responders, PNRs) for this signature.

Figure 5. Correlation of the transcriptomic enrichment score (ES) of the signature of genes down-regulated by Fezakinumab (FZ) treatment (FZ-DOWN) in lesional samples from atopic dermatitis patients against the ES of the Th22/IL-22 pathway genes in (A) sputum (B) bronchial brushings and (C) nasal brushings of asthmatic subjects and against sputum IL-22 protein abundance in the sputum of asthmatic subjects (D). The correlation for sputum IL-22 protein was controlled for age, gender and body mass index.

Mapping atopic dermatitis and anti-IL-22 response signatures to Type 2-low severe neutrophilic asthma

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Conflict of Interest statement.
JH Riley, S Bates and S Worsley are employees and shareholders of GlaxoSmithKline. Dr. Uddin reports he is an employee of AstraZeneca and holds shares in the company. Dr. Knowles reports being a former employee of GlaxoSmithkline. Dr. Singer reports personal fees from Vertex Pharmaceuticals Switzerland, personal fees from Novartis Pharma Switzerland , outside the submitted work. Dr. Fowler reports personal fees from AstraZeneca, grants and personal fees from Boehringer Ingelheim, personal fees from Chiesi, personal fees from GSK,

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Abstract

Background: Transcriptomic changes in patients who respond clinically to biological therapies may identify responses in other tissues or diseases.

Objective: To determine whether a disease signature identified in atopic dermatitis (AD) is seen in adults with severe asthma (SA) and whether a transcriptomic signature for AD patients who respond clinically to anti-IL-22 (Fezakinumab, FZ) is enriched in SA.

Methods: An AD disease signature was obtained from analysis of differentially expressed genes (DEGs) between AD lesional and non-lesional skin biopsies. DEGs from lesional skin from therapeutic super-responders before and after 12 weeks FZ treatment defined the FZ-response signature. Gene Set Variation Analysis (GSVA) was used to produce enrichment scores (ES) of AD and FZ-response signatures in the U-BIOPRED asthma cohort.

Results: The AD disease signature (112 up-regulated genes) encompassing inflammatory, T-cell, Th2 and Th17/Th22 pathways was enriched in the blood and sputum of asthmatics with increasing severity. Asthmatics with sputum neutrophilia and mixed granulocyte phenotypes were the most enriched ($p<0.05$). The FZ-response signature (296 down-regulated genes) was enriched in asthmatic blood ($p<0.05$) and particularly in neutrophilic and mixed granulocytic sputum ($p<0.05$). These data were confirmed in sputum of the ADEPT (Airway Disease Endotyping for Personalized Therapeutics) cohort. IL-22 mRNA across tissues did not correlate with FZ-response ES, but this response signature correlated with Th22/IL-22 pathways.

Conclusions: The FZ-response signature in AD identifies severe neutrophilic asthmatics as potential responders to FZ therapy. This approach will help identify patients for future asthma clinical trials of drugs used successfully in other chronic diseases

Abstract word count: 249

Clinical implications

Identification of transcriptomic drug-response signatures in the target tissue of one chronic immune disease may be utilised in another disease to stratify subjects for subsequent clinical trials or treatment.

Capsule Summary:

We used a signature defined by clinical and transcriptomic super-responders to Fezakinumab in atopic dermatitis to identify severe neutrophilic asthmatics as subjects most suitable for testing the efficacy of the drug in asthmatics.

Key words: Anti-IL-22 antibody, atopic dermatitis, gene set variation analysis, IL-22, severe asthma.

Abbreviations:

AD	Atopic dermatitis
ADEPT	Airway Disease Endotyping for Personalized Therapeutics
ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
ES	Enrichment score
FC	Fold-change
FDR	False discovery rate
FeNO	Fractional exhaled nitric oxide
FZ	Fezakinumab
HC	Healthy control
ILC	innate lymphoid cell
LS	Lesional
MADAD	meta-analysis derived atopic dermatitis
MMA	Mild-moderate asthma
NL	Non-lesional
PNR	Potential non-responder
PR	Potential responder
SA	Severe asthma

185	SAs/ex	Severe asthmatic smoker/ex-smoker
186	T2	Type 2
187	TAC	Transcriptome-Associated Cluster
188	U-BIOPRED	Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes
189	DEGS	Differentially expressed genes
190	GSVA	Gene Set Variation Analysis
191		

Introduction

Asthma is phenotyped according to clinical treatable traits and physiological markers including eosinophilic and non-eosinophilic phenotypes (1,2). The Type 2 (T2) inflammatory phenotype characterised by high expression of an interleukin (IL)-13 stimulated bronchial epithelial cell signature (3,4) and elevated urinary leukotriene (LT)E_4 (5), is a molecular phenotype characterised by high eosinophilic inflammation. However, the molecular phenotypes of non-T2 inflammation remain unclear although one phenotype has been characterised by inflammasome, tumour necrosis factor (TNF)α and interferon (IFN) pathway activation associated with neutrophilic asthma (3,6,7). An IL-17 phenotype characterised by neutrophilic inflammation has also been described (8).

IL-22 belongs to the IL-10 cytokine family and is produced by T helper (Th)17 and Th22 cells, $\gamma\delta$-T cells and Type 3 innate lymphoid cells (ILCs) as well as neutrophils (9). Elevated bronchoalveolar lavage (BAL) (10) and serum IL-22 levels (11,12) in patients with severe asthma has been reported. Neutrophil-high asthmatics show an upregulated presence of bronchial and nasal cells staining positive for IL-22 expression(13,14). IL-22 suppresses IFN-γ-induced pro-inflammatory mediator expression by human bronchial epithelial cells (10) indicating a potential protective role in asthma, but IL-22 also enhances the proliferation and migration of human airway smooth muscle (ASM) cells which may induce airway wall remodelling (15,16). This suggests that IL-22 could play a role in certain endotypes of asthma.

IL-22 is implicated in other chronic inflammatory diseases including atopic dermatitis (AD), a closely-related condition to asthma, often preceding it, in the atopic march (17). Epicutaneous sensitization in mice promotes the generation of antigen-specific IL-22-producing T cells leading to airway inflammation and airway hyperresponsiveness following allergen challenge (18). This suggests that IL-22 may be important in the atopic march. The anti-IL-22 monoclonal antibody, fezakinumab (FZ), improves AD clinical scores (19) whilst AD patients with high baseline IL-22 expression showed the greatest clinical response with down-regulation of transcriptomic features associated with immune pathways involved in T-cell and dendritic cell activation (20).

The atopic march is a term used to describe the progression of allergic disease from the early presence of atopic dermatitis, food allergies and rhinitis through to asthma (21). A recent *in silico* analysis of the protein interaction networks in these diseases identified the presence of pathways contributing to the allergic multimorbidity of these diseases (22). We

hypothesised that a gene signature from AD patients who respond to fezakinumab will be up-regulated in other chronic inflammatory diseases such as asthma. Furthermore, analysis of these 'responder signatures' will select patients most likely to respond to fezakinimab. We analysed differentially expressed genes (DEGs) in eczematous skin lesions of IL-22 high responders between baseline and after 12 weeks of FZ treatment in order to obtain a FZ-response signature. This FZ signature was used to probe the transcriptomes of the lungs and blood of the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) asthma cohort to identify features of asthmatic subjects who may respond to FZ. The results were validated in the independent Airway Disease Endotyping for Personalized Therapeutics (ADEPT) cohort.

Methods (word count=709)

Determination of AD disease and anti-IL-22 responsive signature

Full details of AD patient demographics, samples, transcriptomic analyses and clinical response (NCT01941537) are provided elsewhere (20). The AD disease signature was defined by DEGs identified between eczematous or lesional (LS) skin and non-lesional (NL) skin samples with a fold-change (FC) ≥2 or ≤-2 and a false discovery rate (FDR) ≤0.05 for the whole AD cohort. We also used a composite AD signature derived by comparing the lesional and non-lesional skin transcriptome from 4 microarray studies (MADAD, meta-analysis derived AD)(23).

We defined a FZ treatment response signature by analysis of the LS biopsy data of AD patients at baseline and after 12 weeks of FZ treatment to identify DEGs (FC ≥2 or ≤-2 and FDR<0.05) (20). Patients with high levels of IL-22 mRNA in lesional tissue at baseline had the greatest response to FZ at both the clinical and transcriptomic level. We used DEGS from the IL-22^{high} AD patients to derive a FZ 'super responder' signature (20)(**Supplementary Table 1**).

Asthma cohorts

The U-BIOPRED cohort consists of severe non-smoking asthma (SAn); smokers and ex-smokers with severe asthma (SAs/ex); mild/moderate non-smoking asthmatics (MMA) and healthy non-smoking controls (HC) (24). Expression profiling was performed on RNA extracted from blood cells, sputum cells, epithelial brushings and bronchial biopsies (8)(24). Clinical characteristics and sputum and blood proteomic (SomaLogic) metadata are stored within TransMART as part of the eTRIKs project (25). For validation, the ADEPT cohort (NCT01274507) was analysed (26).

Protein and other assays

The SOMAscan proteomic assay of 1129 analytes was performed on sputum supernatants (SomaLogic, Boulder, CO, USA) (3). The fraction of exhaled nitric oxide (FeNO) was measured online using an electrochemical analyser (NIOX MINO; Aerocrine, Solna, Sweden) at an expiratory flow rate of 50ml/s according to ATS/ERS guidelines (27). Serum IgE was measured using the Thermo Fisher (Uppsala, Sweden) CAP system. Biomarker and sputum and urinary eicosanoid data were generated by multiplex analysis and mass spectrometry (5).

Data analysis

Analysis was performed in R version 3.5.0 (28). Gene set variation analysis (GSVA) was run using the R Bioconductor GSVA package (29) to calculate sample-wise enrichment scores (ES). The ES for AD disease, FZ response and immunological pathway signatures was calculated for each subject across the U-BIOPRED sample compartments. We used a linear model adjusted for age and gender and used the least squares means (30) with the Tukey p-value adjustment method for comparisons of families of estimates (4 for cohort, 5 for granulocyte subtype, and 4 for Transcriptome-Associated Cluster (TAC) group (3)) to analyse the ES differences between groups. Differential expression between sputum transcriptomics of subjects with eosinophilic inflammation against those with non eosinophilic inflammation and subsequent clustering revealed 3 groups. TAC1 contains patients with a high enrichment for the Woodruff Th2-high gene signature with a very high sputum eosinophilia. The TAC2 is characterised by inflammasome-associated pathways and high sputum neutrophilia whilst TAC3 is associated with high levels of macrophages and a mainly paucigranulocytic phenotype (3). Visualization of the distribution of ES was performed with the ggplot2 R package (31). The GSVA signatures are listed in **Supplementary Table 1**.

The FZ response signature in U-BIOPRED sputum subjects was used to categorise SA patients as being predicted-responders (PRs) (n=26, ES ≥+0.1) or predicted non-responders (PNRs) (n=18, ES ≤-0.1) whilst filtering out patients with undirected ES (>+0.1 and >-0.1), MMAs and HCs. All categorical variables were analysed using Fisher's exact test. A T-test was used for continuous clinical variables with normal distribution (Shapiro-Wilk test p-value >0.05), whilst the Wilcoxon rank sum test with continuity correction was used for variables with a skewed distribution.

Differential gene (for all PRs and PNRs) and protein (for those PRs and PNRs with proteomics data) expression analysis was performed using limma 3.38.3 (32) for linear model fitting for each gene or protein. Empirical Bayes moderation of standard errors was used to produce tables of significant DEGs and proteins. P-values were adjusted with the Benjamini–Hochberg False Discovery Rate (FDR-BH) procedure (33). Age and gender were not confounding variables. Significantly up and downregulated genes were determined by a log2 fold change of ≥1 or ≤-1 and an FDR-BH adjusted p≤0.05. Pathway enrichment analysis was

298 performed using ReactomePA (34), utilising the human Reactome ontology (35) with p-value
299 FDR-BH adjustment and cut-off of 0.05.
300

Results

AD signature in asthma

We defined an AD disease signature (**Supplementary Table 1**) according to whether DEGs were significantly up- (112 DEGS, AD-UP) or down-regulated (29 DEGs, AD-DOWN) between lesional and non-lesional skin with a fold-change (FC) ≥2 or ≤-2 and an FDR≤0.05 for the whole AD cohort. T-cell, Th2, Th17/Th22 and general inflammatory genes were up-regulated in the AD-UP signature whereas AD-DOWN reflected lipid pathways and pathways associated with dysregulated dermal epithelial function (20).

This signature was applied to blood (**Fig 1A**) and sputum (**Fig 1B**) of the U-BIOPRED cohort. The AD-UP signature ES trended with severity: significantly enriched in the blood of severe, but not MMA, asthmatics irrespective of smoking status (**Fig 1A**). A similar trend was seen in the sputum of severe asthmatics (**Fig 1B**). When compared by sputum TACs (3) there was an enrichment of the AD-UP signature in sputum from TAC2 (adj.p=$2.87x10^{-6}$) subjects (**Fig 1C**) compared to healthy controls. Assessment based on sputum granulocytes further highlighted the greater enrichment of the AD-UP score in granulocytic asthma (**Fig 1D**) with a greater ES in neutrophilic (adj.p=$6.83x10^{-5}$) and mixed granulocytic (adj.p=0.0005) asthma compared to healthy controls. The enrichment of the AD lesion signature in asthma reflects a composite of the cells within blood and sputum.

We confirmed the appropriateness of the AD-UP signature by using the previously defined MADAD-UP pooled signature (**Fig 1E-H**). The MADAD-UP signature is a consensus disease signature of the pathologically upregulated genes which characterise atopic dermatitis across several studies (23). The overlap between the AD-UP and MADAD-UP gene signatures consisted of 84 genes. This signature was enriched in both blood (**Fig 1E**) and sputum (**Fig 1F**) of severe asthmatics irrespective of smoking status, mirroring results seen in AD-UP blood. Classifying asthmatics according to sputum molecular phenotype or to sputum granulocytes also demonstrated enrichment of the MADAD-UP signature in TAC2 (**Fig 1G**) and neutrophilic/mixed granulocytic subjects (**Fig 1H**). Overall, the AD disease signature was enriched in severe neutrophilic asthma.

Derivation of an FZ super-responder signature in AD

The FZ treatment super-response was defined by those subjects with a good clinical response who also had a good transcriptomic response comparing lesional biopsies at

baseline and after 12 weeks FZ treatment in AD patients to identify the significant DEGs (FC≥2 or ≤-2 and FDR<0.05)(20). The highest clinical and transcriptomic effect was seen in baseline IL-22^{high} lesional tissue and the transcriptomic changes seen in patients with a high clinical and transcriptomic response was used to generate the FZ-super-responder signature.

We identified 417 DEGs (121 up- and 296 or down-regulated by FZ) in lesional AD skin tissue biopsies from patients with the greatest clinical response to FZ at 12 weeks (**Supplementary Table 1**). This FZ-response signature (FZ-DOWN) represents a key proportion of the AD-UP disease signatures. In particular, the AD-UP signature (112 genes) had 74 genes overlapping with the FZ-DOWN (296 genes, 25%) whilst the MADAD-UP signature (405 genes) had 196 genes overlapping with the FZ-DOWN signature (48.4%). A strong correlation existed between the AD-UP and FZ-DOWN ES in asthmatic sputum (R^2=0.8326, p=2.2x10^{-16}) (**Supplementary Fig 1A**) and between MADAD-UP and FZ-DOWN (R^2=0.9156, p=2.2x10^{-16}) (**Supplementary Fig 1B**). The FZ-DOWN signature included pathways associated with general inflammation, T-cell, Th2 and Th17/Th22 activation (**Supplementary Fig 2, Supplementary Table 2**), which are all up-regulated within the AD disease signatures. No pathways were significantly associated with FZ-UP genes although relaxing the FDR threshold identified pathways associated with epidermal signalling (**Supplementary Fig 3, Supplementary Table 3**), which justifies the focus on the FZ-DOWN signature. To test whether the FZ-DOWN signature predicted the response in AD patients, we examined the ES of FZ DOWN in lesional AD baseline samples (20). This was significantly (p=0.0496, adjusted for age and gender) positively associated with the AD SCORAD score after treatment.

In summary, **Supplemental Table 1** provides a list of all the gene signatures used in this analysis including the sets of genes up-regulated (AD-UP) or down-regulated (AD-DOWN) in AD whilst **Supplemental Table 2** provides a list of all the pathways that the FZ-DOWN gene signature corresponds to and highlights the importance of immune pathways. **Supplementary Table 3** is a list of all the pathways that relate to the FZ-UP gene signature. None of these pathways was significantly enriched and are mostly skin-related.

Enrichment of the FZ super-responder signature from AD in U-BIOPRED

The FZ-DOWN signature was significantly enriched in the blood of U-BIOPRED severe asthmatics (adj.p<0.05) (**Fig 2A**) despite the wide variability in ES scores, which may reflect the different types of immune cells found in blood and lesional tissue. The skin contains a

mixture of epithelial cell-like and immune cells but the enrichment observed in blood may indicate detection of the immune components.

The FZ-DOWN signature was significantly enriched in the blood of TAC2 patients (adj.p=0.015, **Fig 2B**). The response in blood when subjects were stratified according to sputum granulocytes was variable and although there was a trend towards enrichment in asthma subtypes, this did not reach significance (**Fig 2C**). There was a greater degree of enrichment in sputum samples compared to blood (compare Fig 3A-C with Fig 3D-F). The ES for FZ-DOWN had a stepwise association with severity and was highly enriched TAC2 patients (adj.p=0.002, **Fig 2E**), and neutrophilic (adj.p=0.0002, **Fig 2F**) and mixed granulocytic (adj.p=0.0098, **Fig 2F**) asthma compared with healthy controls. The good correlation between the TAC2 signature and the FZ-DOWN signature in sputum ($p<2.2x10^{-16}$, r=0.784) was not due to overlapping signatures as only 3 genes were common between the two genesets – CASP4, KCNJ15 and SAMSN1. Importantly, we were able to show that the AD-UP and MADAD-UP (**Fig 3A**) and the FZ-DOWN (**Fig 3B**) signatures were also enriched within the sputum neutrophilic (adj.p<0.05) and mixed granulocytic patients within the ADEPT cohort (**Fig 3A-B**).

To ensure against a confounding effect of tissue heterogeneity we removed the 4 skin-specific genes identified by comparing the FZ-DOWN signature with a published skin transcriptomic profile (36). There were 4 overlapping genes (WFDC12, TYR, S1PR5, LYPD5) and removal of these 4 genes from the FZ-DOWN signature had minimal effect on the analysis (**Supplementary Fig 4**).

Since the FZ-DOWN signature was associated with neutrophilic asthma we checked whether this and the AD disease signatures correlated with 3 neutrophil signatures from the Human Cell Atlas (37), an immune cell gene-signature database (38) and a Th17 signature (39) that consists of genes for neutrophil chemoattractants (CXCL1, CXCL2, CXCL3, CXCL8 and CFS3). We observed a high correlation between FZ-DOWN ES and neutrophil signature ES and also AD disease signature ES and neutrophil signature ES, indicating that the disease signatures reflected tissue neutrophilia. In particular Pearson's correlation between the FZ-DOWN ($p=1.25 x10^{-9}$, r=0.519), AD-UP ($p<2.2x10^{-16}$, r=0.754) and the MADAD-UP ($p<2.2x10^{-16}$, r=0.684) signatures were very significantly correlated with the immune cell database neutrophil signature. In addition, the FZ-DOWN ($p<2.2x10^{-16}$, r= 0.691), AD-UP ($p=4.479x10^{-7}$, r=0.441) and the MADAD-UP ($p=4.304x10^{-7}$, r=0.442) signatures were also significantly correlated with the Human Cell Atlas neutrophil signature.

However, neutrophil levels in the skin were not significantly reduced after FZ treatment (**Supplementary Fig 5**) which suggests that despite neutrophil genes contributing to the AD disease signature and some neutrophil genes being present in the FZ response signature, the FZ response

phenomenon is unlikely to be driven by neutrophil levels alone. This corroborates with a positive but non-significant correlation between sputum neutrophils and sputum IL-22 protein in U-BIOPRED subjects (p=0.0699, r=0.184). We also examined the correlation between sputum neutrophils and the FZ-DOWN signature in the validation ADEPT cohort and found no significant correlation (% segmented neutrophils; p=0.911, r=0.0186).

Clinical features of predicted responders and non-responders in U-BIOPRED

We next examined whether the FZ-DOWN signature was associated with a specific subset of SA patients as the most clinically relevant group. Highly-enriched patients (PRs) were compared with those least-enriched (PNRs) for the FZ-DOWN signature (**Supplementary Fig 6, Supplementary Table 4**). The enrichment score of the FZ-DOWN signature in sputum was used to categorise SA patients as being predicted-responders (PRs) (n=26, ES ≥+0.1) or predicted non-responders (PNRs) (n=18, ES ≤-0.1) whilst filtering out patients with an undirected ES (>+0.1 and >-0.1), MMAs and HCs. The clinical comparison revealed that PRs had more frequent LABA use and significantly elevated sputum neutrophils and lower sputum eosinophils and macrophages in addition to lower IgE levels in contrast to PNRs (**Table 1**). Furthermore, PRs had lower levels of plasma eotaxin-3 and serum IL-13 biomarkers as measured by Luminex or MSD analysis. PRs also had elevated sputum levels of 11-dehydro-TXB_2, 5-HETE and LTB_4 (p=0.0526) but lower LTE_4 reflecting the neutrophilic and low eosinophilic nature of the PR population (**Table 2**).

In a linear model (LM) of asthmatic sputum FZ-DOWN ES and medication usage, corrected for age, gender and BMI, we found no significant association between FZ-DOWN ES and OCS use (p=0.702). However, we did find a significant association between FZ-DOWN ES and LABA use (p=0.0243) where FZ-DOWN ES was elevated in the twice daily LABA use group (reflecting severity of disease, LM estimate=0.112) and least in the group not taking LABA at all (mildest subjects, LM estimate=-0.117).

DEGs between Predicted Responder (PR) and Predicted Non-Responder (PNR) severe asthmatics

We performed DEG analysis between PR and PNR patients and identified 431 up and 19 down sputum DEGs which were significant with a log2 FC of over 1 or below -1 respectively. These are reported in **Supplementary Table 5**. ReactomePA pathway analysis on the up DEGs

indicates a strong neutrophilic component with neutrophil degranulation, cytokine and chemokine receptor and Toll-like receptor (TLR) signalling as well as IL-10 and IFN pathways being highly enriched in PR subjects (**Fig 4, Supplementary Table 6**). The IL-33 receptor (IL1RL1, ST2) was greatly downregulated in the PR group.

Sputum proteomic enrichment of FZ-DOWN signature

We then selected PRs and PNRs who had SomaLogic sputum proteomics data available (n=32) (**Supplementary Table 7**)**.** Differential protein analysis on the sputum SomaLogic data confirmed a strong neutrophilic component (**Table 3**). Significantly upregulated sputum proteins included the neutrophil modulator Sialic acid-binding immunoglobulin-type lectins 9 (siglec-9), the neutrophil serine proteases cathepsin G and azurocidin involved in neutrophil degranulation and microbial killing, B7_H2 which is a costimulatory ligand for CD28, IL-6 which is involved in neutrophilic asthma and increased differentiation of Th17 cells and Oxidized Low Density Lipoprotein Receptor 1 (OLR1) which is involved in tissue remodelling. These proteins together with the enhanced expression of neutrophil degranulation products implicate neutrophil activation as being a key component of asthmatic subjects who are highly enriched for the FZ-DOWN signature.

FZ-DOWN signature markers in blood

We then selected PRs and PNRs who had blood proteomics data available (n=42) (**Supplementary Table 8**)**.** Differential protein analysis on blood SomaLogic data (**Supplementary Table 9**) defined potential FZ responders from non-responders as possessing lower blood IgE and a trend towards elevated expression of the neutrophil modulator siglec-9 and I-TAC as seen in the sputum proteomics analysis.

IL-22 pathway and protein correlates with FZ-DOWN enrichment

In AD skin (20), IL-22 gene expression alone predicts the response to FZ. However, IL-22 gene expression was not enriched in blood (**Supplementary Fig 7A**) or sputum according to asthma severity (**Supplementary Fig 7B**) or in TAC2 asthmatics (**Supplementary Fig 7C**). There was no correlation between FZ-DOWN and IL-22 gene expression in blood (**Supplementary Fig 7D**), sputum **(Supplementary Fig 7E**), bronchial brushings (**Supplementary Fig 7F**) or nasal brushings (**Supplementary Fig 7G**).

In contrast, the ES of the Th22/IL-22 signature was significantly correlated with FZ-DOWN ES in asthmatic sputum ($p=4.31x10^{-14}$, r=0.656)(**Fig 5A**), bronchial brushings ($p<2.2x10^{-16}$, r=0.753)(**Fig 5B**), nasal brushings ($p=8.53x10^{-13}$, r=0.755)(**Fig 5C**) and blood ($p=5.06x10^{-6}$, r=0.223). The Th22/IL-22 signature (**Supplementary Table 1**) consists of 16 genes including IL-22 itself and the Th22-specific marker CCR10 (20). Pathway analysis identified several significantly enriched pathways including 'IL22 Induces Keratinocyte Proliferation in Psoriasis', 'Interleukin-19, 20, 22, 24 Homo sapiens R-HSA-8854691' and 'IL-the 17 signaling pathway'.

Importantly, sputum IL-22 protein was significantly enriched in patients with TAC2 asthma compared to those with TAC1 asthma (p=0.0112) and there was a significant correlation between sputum IL-22 protein expression and the FZ-DOWN ES when controlled for age, sex and BMI (p=0.0360, r=0.133)(**Fig 5D**). IL-22 protein in sputum also significantly correlated with FZ-DOWN ES in nasal brushings for all subjects (p=0.0443, r=0.423).

DISCUSSION

We demonstrate that an AD disease signature was enriched in severe neutrophilic asthma in both the U-BIOPRED and ADEPT asthma cohorts and that these subjects were also highly enriched for a gene signature indicative of a super-response to FZ. Pathway analysis indicated that the AD-UP disease signature and the FZ-DOWN-response signature were a composite of Th1, Th2, Th17, Th22 and general inflammatory processes and that sputum proteins linked with a potential FZ response in asthma were associated with neutrophil recruitment and activation. The FZ super-response signature did not correlate with IL-22 gene expression itself although there was a good correlation with the Th22/IL-22 gene signature in nasal and bronchial brushings. Sputum IL-22 protein correlated significantly with FZ-DOWN. Re-purposing transcriptomic data that defines a treatment response across therapeutic areas may aid the stratification of patients for future clinical trials.

Early transcriptomic analysis of skin samples from psoriasis and AD subjects identified neutrophil chemoattractant genes as being highly expressed in both AD and psoriatic skin lesions (40). Furthermore, neutrophil elastase staining is elevated in lesional compared with non-lesion skin in AD patients but to a much lesser extent than seen in patients with psoriasis. This enhanced neutrophilia in AD may reflect concurrent infection with Staphylococcus aureus infection (41). Enhanced neutrophilia may reflect an enhanced Th1/Th17 drive.

The Th2/Th22 pathway is the major pathway in AD as recently confirmed using single cell RNA-sequencing (42). This is seen across all age-groups, however, an enrichment of Th1/Th17 genes is seen in lesional compared to non-lesional skin in adults (43). Indeed, the usual Th2/Th22 drive in AD is skewed towards a Th1/Th17 phenotype with increasing age (44) and severity of disease. For example, enhanced Th1/Th17 mediator expression is reported in the blood of AD patients with severe but not mild disease (45). Importantly, there was a good correlation between Th2/Th22/Th1/Th17 gene and protein expression profiles in lesional and non-lesional AD samples (46).

Severe asthmatic PRs to FZ had neutrophilic or mixed granulocytic asthma, poor lung function and a low asthma quality of life despite frequent LABA use. These subjects also had lower serum IgE levels but with relatively greater atopic disposition, in contrast to subjects with T2 eosinophilic asthma (≥300 cells/µl), suggesting that an anti-IL-22 intervention may be targeted to non-T2 asthmatics with low IgE as opposed to those with a high IgE neutrophilic phenotype (14,47). Gender, BMI and age did not affect the enrichment of the FZ response

signature. Comparison of biomarkers between PRs and PNRs indicated that PR subjects had elevated levels of 11-dehydro-TXB_2, 5-HETE and LTB_4 although the latter did not quite reach significance. Leukotrienes are formed via a 5-LOX dependent process in which arachidonic acid is converted to the unstable epoxide intermediate LTA_4, which can then be converted by either LTC_4 synthase (LTC_4S) to form the cysteinyl-leukotrienes or via LTA_4-hydrolase (LTA_4H) to form LTB_4. Neutrophils have known LTA_4H activity and sputum neutrophils have been previously reported to produce LTB_4 (48). Accordingly, the elevated sputum LTB_4 levels in combination with the lower LTE_4 levels among PR subjects collectively point towards a specific elevation of LTA_4H activity within these neutrophilic subjects, which further support a non-T2 phenotype (49).

We have previously defined asthmatics according to their sputum molecular phenotypes (3). The FZ-DOWN signature was enriched in TAC2 patients that suggests that FZ may be useful for T2-low severe neutrophilic asthmatics. Pathway analysis of the potential FZ responders versus non-responders highlighted the importance of neutrophil degranulation products along with signalling downstream of TLRs, cytokine/chemokines including neutrophil-associated mediators and chemoattractant receptors such as CXCL10, CXCL11, CXCR1 and CXCR2, suggesting an activated neutrophil phenotype. Although previously-defined pathways such as the NLRP3 inflammasome within TAC2 were not specifically enriched in the FZ predicted responder versus non-responder subjects, factors associated with inflammasome activation including IL-1α and IL-1RAP are present (50).

At the cellular level, a significant increase in the percentage of airway neutrophils (75.5% *vs* 35.6%) and a significant decrease in the percentage of airway macrophages (18.9% *vs* 32.5%) in the FZ predicted responders group were observed. Macrophages phagocytose apoptotic neutrophils and contribute to inflammation resolution. It is interesting to speculate whether a reduced number of airway macrophages observed could adversely impede neutrophil clearance, thus promoting the elevated levels of airway neutrophils in this endotype of asthma. Defects in neutrophil apoptosis and/or clearance leading to airway neutrophilia have previously been reported in a small cohort of severe atopic asthmatics with a low-eosinophilic phenotype (≤3% sputum eosinophils) (51).

We have previously shown that GM-CSF/CSF2RB- and IFN-activated macrophages as well as lower enrichment of eosinophils were associated with childhood asthma (52). The AD disease signature indicates that AD, although generally seen as a T2-dominant disease, also

has different degrees of non-T2 driving pathways including Th1, Th17, Th22 and inflammatory pathways (20). Both GM-CSF and IFN pathways were also enriched within the FZ predicted responder population and interestingly, the potential responder-non-responder pathways also indicated the enrichment of IL-10 signalling which is involved in the suppression of IL-5 and GM-CSF expression and eosinophil apoptosis (53). These pathways may also represent therapeutic targets in these SA patients.

The AD-DOWN signature is not enriched in asthmatic peripheral blood but shows some enrichment in airway samples. This signature includes lipid pathways and pathways associated with dysregulated dermal epithelial function that indicates remodelling of epithelial tissues is more prevalent in severe neutrophilic asthma airways. These pathways are also up-regulated by FZ which suggests that FZ may also impact upon asthmatic airway epithelial cell barrier function.

IL-22 possesses potential pro- and anti-inflammatory roles in asthma (11,15,16). In mouse models of allergic sensitisation and challenge, IL-22 attenuates established Th2 cell-mediated allergic inflammation *in vivo* (11,54). However, IL-22 promotes allergic inflammation in similar mouse models at the onset of allergic asthma (11,18), supporting the view that IL-22 may be involved in the atopic march (17). While data from mouse models suggest that anti-IL-22 may be efficacious in early onset allergic asthma, our analysis would indicate that IL-22 might have a pathogenic role in those with neutrophilic inflammation with lower IgE levels.

In our analysis, IL-22 mRNA expression did not correlate with FZ response signatures in blood, sputum, nasal and bronchial brushings whereas there was a significant correlation with sputum IL-22 protein and with the Th22/IL-22 gene signature in sputum, bronchial and nasal brushings. This may reflect the local expression of IL-22 protein in the airways which is not detected at the mRNA level or is not observed due to lack of proteomics data for bronchial and nasal brushings. However, the up-regulation of the FZ-DOWN signature does indicate a significant impact of IL-22 on downstream signalling.

This study has several strengths and also some limitations. We derived a gene signature from skin lesions of AD subjects (AD-UP) and also from patients with a good clinical response and a clear transcriptomic response to FZ after 12 weeks of treatment (FZ-DOWN) to provide evidence for target engagement in the lesional tissue. We utilised the large data-rich U-BIOPRED cohort to define subsets of patients who are more likely to respond to FZ and

validated this in a separate cohort of severe asthmatics. Importantly, we were able to demonstrate markers of high enrichment of this response signature in nasal brushings and peripheral blood. However, we do not have evidence that the changes seen in the lesional skin of AD patients with FZ also occur in the airways of asthmatics. Animal models of severe neutrophilic or mixed granulocytic asthma may be used to address this issue. In asthma, baseline levels of IL-22 mRNA did not correlate with FZ-DOWN signature as predicted from the AD data. This suggests that additional mechanisms may occur in the asthmatic airway compared to the skin. These mechanisms may be linked since there is a strong correlation between the Th22/IL-22 and FZ-DOWN signatures. The good correlation of both IL-22 sputum protein abundance and Th22/IL-22 signature ES with the FZ-DOWN signature ES within nasal brushings indicates a potential alternative readily accessible approach for identifying possible responder populations. Although this data was validated in a separate SA cohort we have not measured the stability of the FZ-response signature over time and whether this changes with T2-directed biologics.

This novel approach of molecularly characterising clinical super-responders to an antibody drug in one disease followed by probing other disease databases may be a more effective way of identifying predicted-responders at the endotypes level compared to looking at drug-target levels alone. By exploiting pre-existing databases and clinical trial data, this approach could lead to a reduction in drug development time and in research costs. The greatest enrichment of the FZ PR signature was observed in severe neutrophilic asthmatics. Furthermore, we found that blood and sputum gene expression and the expression of several proteins in sputum can predict asthmatics with a high enrichment of a FZ response signature in the airway. This stratification process will need validation in a controlled clinical trial, while at the same time examining the long-term efficacy and side-effect profile of FZ in endotypes of severe asthma.

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Table 1. Clinical differences of predicted responders versus non-responders to Fezikinumab in U-BIOPRED.

Characteristic	FZ Predicted Responders	FZ Predicted Non-Responders	p value
Total (n)	26	18	
Age (years)	51.8 (12.7)	55.3 (14)	NS
BMI	28 (4.65)	26.3 (3.39)	NS
Gender: Female (n)	16	9	NS
Severe asthma, non-smokers (n)	18	13	NS
Severe asthma, smokers/ex-smokers (n)	8	5	NS
Severe exacerbation in previous year	2.27 (2.38)	1.72 (2.02)	NS
Nasal polyps (n)	8	6	NS
Eczema (n)	8	7	NS
Allergic rhinitis (n)	9	4	NS
Non-allergic rhinitis (n)	5	3	NS
Gastro-esophageal reflux (n)	12	7	NS
Hay fever (n)	11	5	NS
Positive atopic status (n)	11	6	NS
ACQ5 score	2.44 (1.23)	1.79 (1.31)	NS
AQLQ score	4.35 (1.22)	4.98 (1.41)	NS
HADS score	12.3 (8.16)	10.5 (8.91)	NS
SNOT score	31.2 (18.2)	22.6 (10.8)	NS
FEV1 (% predicted)	63.7 (24.3)	67.2 (17.5)	NS
FVC (% predicted)	86.9 (20.4)	95.3 (17.4)	NS
FEV1/FVC	59.3 (13.1)	57.2 (8.79)	NS
FeNO (ppb)	35 (33)	54.7 (46.9)	NS
Serum IgE (IU/L)	204 (358)	332 (294)	0.02
Blood eosinophil (/10-9L)	0.277 (0.155)	0.401 (0.305)	NS
Blood neutrophil (/10-9L)	4.93 (1.93)	5.41 (2.44)	NS
Blood lymphocyte (/10-9L)	2.12 (0.936)	2.1 (0.9)	NS
Blood monocyte (/10-9L)	0.634 (0.278)	0.581 (0.222)	NS
Sputum neutrophils (%)	75.7 (16.6)	35.6 (18)	2.26E-08
Sputum eosinophils (%)	3.9 (5.55)	30.4 (26.5)	0.0009
Sputum lymphocyte (%)	1.5 (1.6)	1.46 (1.26)	NS
Sputum macrophage (%)	18.9 (14.4)	32.5 (20.8)	0.025
Sputum mast cell (%)	0.0346 (0.087)	0.0333 (0.101)	NS
Oral corticosteroid use daily (n)	12	9	NS
LABA use twice a day (n)	12	2	0.039

ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; BMI: Body Mass Index; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; FeNO: Fractional exhaled nitric oxide; HADS: Hospital Anxiety and Depression Scale; ICS: Inhaled

770 corticosteroids; LABA: long-acting beta agonist; SNOT: SinoNasal Outcome Test. Data shown
771 as mean (Standard Deviation).
772
773

774 **Table 2.** Molecular marker differences of Predicted Responders versus Predicted Non-
775 Responders to Fezakinumab in the U-BIOPRED severe asthma patients.
776

Biomarker	FZ Predicted Responders	FZ Predicted Non-Responders	p value
α1 microglobulin (pg/ml) Luminex (serum)	6120 (2210)	7500 (2390)	NS
C5a (pg/ml) Luminex (serum)	50.8 (33.4)	38.9 (21.6)	NS
CD30 (pg/ml) Luminex (serum)	38.9 (17.1)	42.5 (14.7)	NS
CD40L (pg/ml) Luminex (serum)	4420 (1990)	5210 (2300)	NS
DPPIV (pg/ml) Luminex (serum)	98500 (48100)	91500 (24300)	NS
Galectin 3 (pg/ml) Luminex (serum)	5550 (2050)	5770 (1470)	NS
IL-18 (pg/ml) Luminex (serum)	247 (152)	234 (73.8)	NS
IL-1α (pg/ml) Luminex (serum)	35.5 (9.95)	36.2 (6.12)	NS
IL-6Rα (pg/ml) Luminex (serum)	10600 (2450)	10900 (2020)	NS
LBP (pg/ml) Luminex (serum)	2110000 (891000)	1820000 (668000)	NS
Lumican (pg/ml) Luminex (serum)	131000 (37000)	136000 (25300)	NS
MCP4 (pg/ml) Luminex (serum)	142 (44.7)	168 (71.2)	NS
MMP3 (pg/ml) Luminex (serum)	21400 (18300)	24500 (17900)	NS
RAGE (pg/ml) Luminex (serum)	1260 (414)	1320 (382)	NS
Serpin E1 (pg/ml) Luminex (serum)	95000 (30400)	97600 (19900)	NS
SHBG (pg/ml) Luminex (serum)	3640000 (2840000)	4780000 (4670000)	NS
CCL17 (pg/ml) MSD (plasma)	77.5 (70.8)	134 (120)	NS
CCL22 (pg/ml) MSD (plasma)	796 (316)	866 (218)	NS
EOTAXIN (pg/ml) MSD (plasma)	118 (60.1)	140 (67.6)	NS
EOTAXIN3 (pg/ml) MSD (plasma)	15 (15.4)	72.4 (130)	0.00097
IFNγ (pg/ml) MSD (plasma)	12.2 (12.8)	7.44 (6.18)	NS
IL-6 (pg/ml) MSD (plasma)	1.21 (1.01)	0.804 (0.335)	NS
IL-8 (pg/ml) MSD (plasma)	6.02 (9.75)	3.78 (1.86)	NS
IP10 (pg/ml) MSD (plasma)	386 (250)	305 (183)	NS
MCP1 (pg/ml) MSD (plasma)	117 (36.8)	119 (38.4)	NS
MIP1β (pg/ml) MSD (plasma)	56.1 (18.1)	63.5 (29.8)	NS
TNFα (pg/ml) MSD (plasma)	1.84 (0.483)	1.94 (0.632)	NS
CCL18 (pg/ml) IMPACT serum	169 (63.3)	228 (106)	NS
IL-13 (pg/ml) IMPACT serum	0.608 (0.494)	0.942 (0.384)	0.0074
IL-17A (pg/ml) SINGULEX serum	0.58 (0.381)	0.455 (0.258)	NS
Periostin (ng/ml) ELECSYS serum	51.2 (19.5)	54.2 (16.5)	NS
hCRP (mg/L)	6.29 (11)	1.69 (1.33)	NS
11-dehydroTXB$_2$ (ng/ml) urine	13.9 (8.96)	14.2 (10.5)	NS

2,3 dinor-11β PGF2α (ng/ml) urine	73.8 (30.9)	94.8 (86)	NS
2,3 dinor 8isoPGF2α (ng/ml) urine	244 (137)	296 (319)	NS
2,3 dinor TXB_2 (ng/ml) urine	68.1 (46.6)	52.2 (41.3)	NS
8,12 isoPGF2α (ng/ml) urine	386 (240)	430 (408)	NS
8 isoPGF2α (ng/ml) urine	29.3 (11.8)	32.3 (19.6)	NS
LTE_4 (ng/ml) urine	9.28 (8.35)	10 (6.13)	NS
PGE2 (ng/ml) urine	20.2 (23.2)	18.5 (14.8)	NS
PGF2α (ng/ml) urine	132 (102)	130 (75.1)	NS
Tetranor PGDM (ng/ml) urine	299 (115)	305 (257)	NS
tetranorPGEM (ng/ml) urine	1180 (1190)	1030 (539)	NS
11 dehydroTXB_2 (pg/mL) sputum	231 (283)	63.4 (23.9)	0.00438
12-HETE (pg/mL) sputum	1470 (1300)	1980 (1410)	NS
15-HETE (pg/mL) sputum	4490 (6900)	7180 (9130)	NS
5-HETE (pg/mL) sputum	1570 (1500)	964 (1630)	0.0322
6-ketoPGF1α (pg/mL) sputum	58.6 (27.1)	53.7 (23.6)	NS
LTB_4 (pg/mL) sputum	801 (756)	774 (1540)	NS
LTE_4 (pg/mL) sputum	319 (372)	763 (1030)	0.0312
PGD2 (pg/mL) sputum	269 (317)	174 (159)	NS
PGE2 (pg/mL) sputum	390 (363)	202 (135)	NS
Tetranor PGDM (pg/mL) sputum	66 (57.4)	54.8 (51.9)	NS
Tetranor PGEM (pg/mL) sputum	76.3 (49.1)	67.6 (53.3)	NS

777

Table 3. Top and bottom 20 differentially expressed sputum proteins that differentiate U-BIOPRED asthmatic FZ predicted responders (PRs) from predicted non-responders (PNRs) defined from asthma sputum GSVA FZ response signature ES which had sputum proteomic data available (see Supplementary Table 7, see Supplementary Figure 4). Genes are ranked according to log_2 fold change.

Upregulated

Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
Siglec_9	2.04	4.11	0.0066	0.1928
Hemoglobin	1.98	3.96	0.01807	0.2210
PSA1	1.75	3.37	0.01409	0.2059
Cathepsin_G	1.70	3.25	0.00048	0.1368
Carbonic_anhydrase_I	1.45	2.74	0.00081	0.1370
SRCN1	1.43	2.70	0.00702	0.1928
Azurocidin	1.40	2.65	0.00251	0.1661
PLCG1	1.30	2.46	0.01068	0.2059
resistin	1.29	2.45	0.09375	0.3715
Factor_I	1.28	2.44	0.14821	0.4261
IL_6	1.28	2.43	0.00390	0.1869
B7_H2	1.19	2.29	0.04214	0.2830
Ferritin	1.18	2.27	0.0127	0.2059
IP_10	1.15	2.23	0.01319	0.2059
Elastase	1.08	2.12	8.53E-05	0.0959
Transferrin	1.06	2.09	0.16571	0.4476
OLR1	1.02	2.03	0.00309	0.1735
I_TAC	0.99	1.99	0.04652	0.2875
Granzyme_B	0.99	1.98	0.03350	0.2599
Esterase_D	0.97	1.96	0.06937	0.338

Downregulated

Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
a2_Antiplasmin	-1.28	0.40	0.02414	0.2441
Fucosyltransferase_3	-1.29	0.40	0.28234	0.5445
PCSK9	-1.29	0.40	0.00876	0.2017
CATZ	-1.29	0.40	0.04350	0.2830
Kininogen_HMW	-1.37	0.38	0.08360	0.3495
IGFBP_4	-1.37	0.38	0.03957	0.2800
Cathepsin_B	-1.38	0.38	0.00473	0.1869
Phosphoglycerate_mutase_1	-1.46	0.36	0.0435	0.2830
Histone_H2A_z	-1.51	0.34	0.00838	0.2007
FETUB	-1.52	0.34	0.05755	0.3169
Clusterin	-1.55	0.34	0.00521	0.1892

Plasminogen	-1.56	0.33	0.00596	0.1928
amyloid_precursor_protein	-1.60	0.32	0.0251	0.2441
PCI	-1.62	0.32	0.01463	0.2079
Integrin_aVb5	-1.62	0.32	0.00678	0.1928
PTHrP	-1.65	0.31	0.00122	0.1370
CD39	-1.72	0.30	0.00039	0.1368
MIS	-1.73	0.30	0.00130	0.1370
PAPP_A	-1.76	0.29	0.01554	0.2079
Antithrombin_III	-2.31	0.20	0.00490	0.1869

784

Figure legends

Figure 1. Gene Set Variation Analysis (GSVA) showing enrichment scores (ES) of gene signatures derived from genes up-regulated (UP) in lesional versus non-lesional tissue from atopic dermatitis (AD). Disease signatures are derived from either the Brunner paper (AD-UP, **A-D**) or from an AD meta-analysis-derived AD (MADAD, **E-H**). The ES for these signatures in U-BIOPRED blood (**A, E**) and sputum (**B-D** and **F-H**) according to severity (**A, B, E, F**), Transcriptome-Associated Cluster (TAC) (**C, G**) and sputum granulocyte subtype (**D, H**). Between group adjusted p values are provided compared to HC values. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). Abbreviations: HC; Healthy Control, MMA; Mild-Moderate Asthma. SAs/ex; Severe Asthma smoker/ex-smoker. SAns; Severe Asthma non-smoker, P; pauci-granulocytic, E; eosinophilic, N; neutrophilic and M; Mixed.

Figure 2. Gene Set Variation Analysis (GSVA) showing enrichment scores (ES) of Fezakinumab (FZ) treatment response signatures of downregulated genes (FZ-DOWN) in lesion versus non-lesional tissue from atopic dermatitis (AD). ES for AD-UP signatures are given for U-BIOPRED blood (**A-C**) and sputum (**D-F**) according to asthma severity (**A, D**), Transcriptome-Associated Clusters (TAC) (**B, E**) and granulocyte subtype (**C, F**). Between group adjusted p values are provided compared to HC values. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). Abbreviations: HC; Healthy Control, MMA; Mild-Moderate Asthma. SAs/ex; Severe Asthma smoker/ex-smoker. SAns; Severe Asthma non-smoker, P; Pauci-granulocytic, E; Eosinophilic, N; Neutrophilic and M; Mixed.

Figure 3. Gene Set Variation Analysis (GSVA) showing enrichment scores (ES) of gene signatures derived from genes up-regulated (UP) in lesion versus non-lesional tissue from atopic dermatitis (AD) in the ADEPT (Airway Disease Endotyping for Personalized Therapeutics) cohort by granulocytic subtype. Disease signatures are derived from either the Brunner paper (AD-UP, **A, upper panel**) or from an AD meta-analysis-derived AD (MADAD, **A, lower panel**). ES of the Fezakinumab (FZ)-DOWN signature obtained from lesional versus non-lesion tissue after 12 weeks treatment (**B**). Between group adjusted p values are provided compared to HC values. *p<0.05. Abbreviations: HC; Healthy Control, P; Pauci-granulocytic, E; Eosinophilic, N; Neutrophilic and M; Mixed.

Figure 4. Protein pathway analysis using ReactomePA of differentially-expressed genes (false discovery rate, FDR<0.05) that distinguish asthmatic patients highly-enriched (Predicted Responders, PRs) for the Fezakinumab (FZ)-response signature (FZ-DOWN) from those poorly-enriched (Predicted Non-Responders, PNRs) for this signature.

Figure 5. Correlation of the transcriptomic enrichment score (ES) of the signature of genes down-regulated by Fezakinumab (FZ) treatment (FZ-DOWN) in lesional samples from atopic dermatitis patients against the ES of the Th22/IL-22 pathway genes in (A) sputum (B) bronchial brushings and (C) nasal brushings of asthmatic subjects and against sputum IL-22 protein abundance in the sputum of asthmatic subjects (D). The correlation for sputum IL-22 protein was controlled for age, gender and body mass index.

Figure No.1

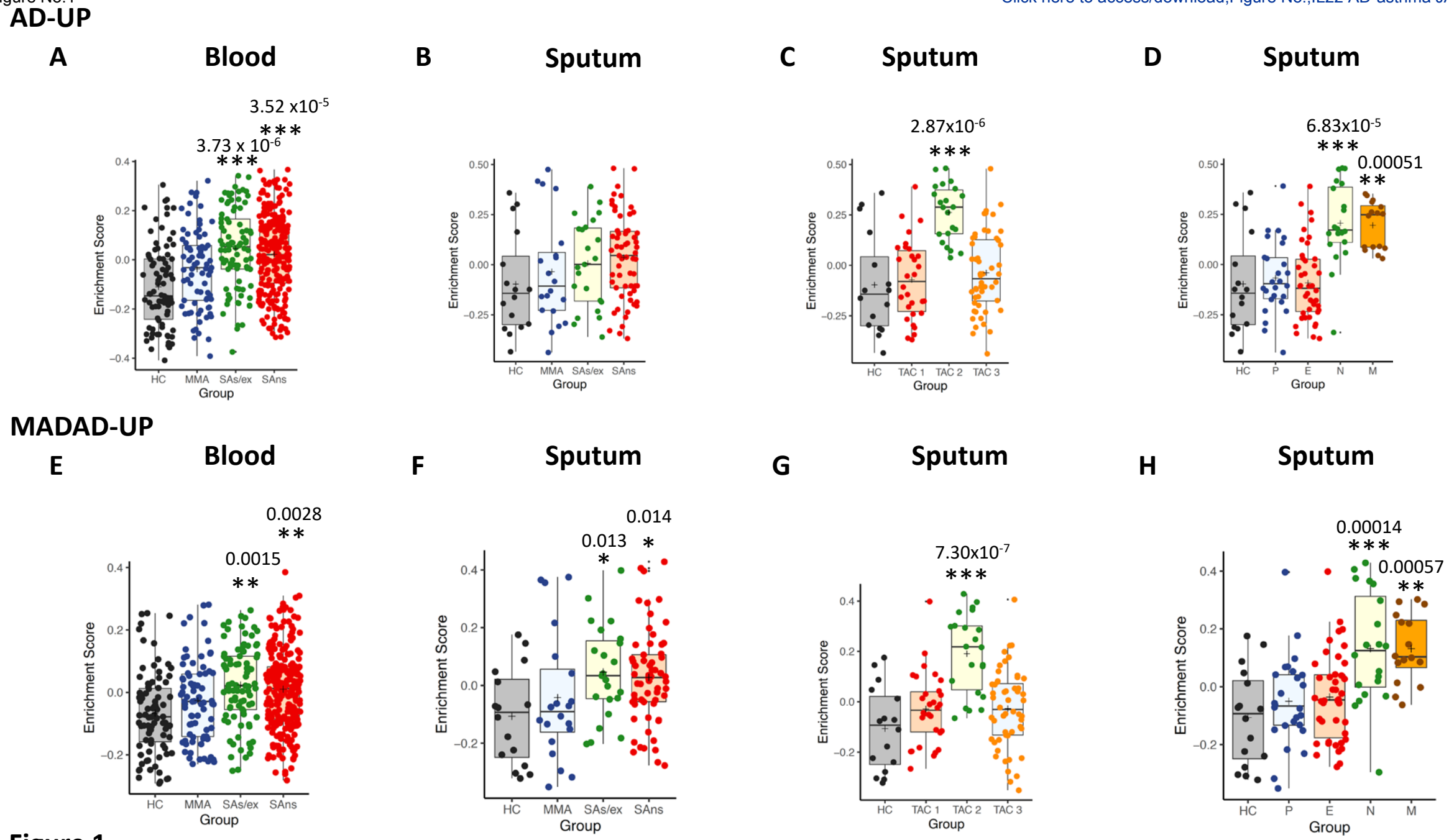

Figure 1

Figure No.2

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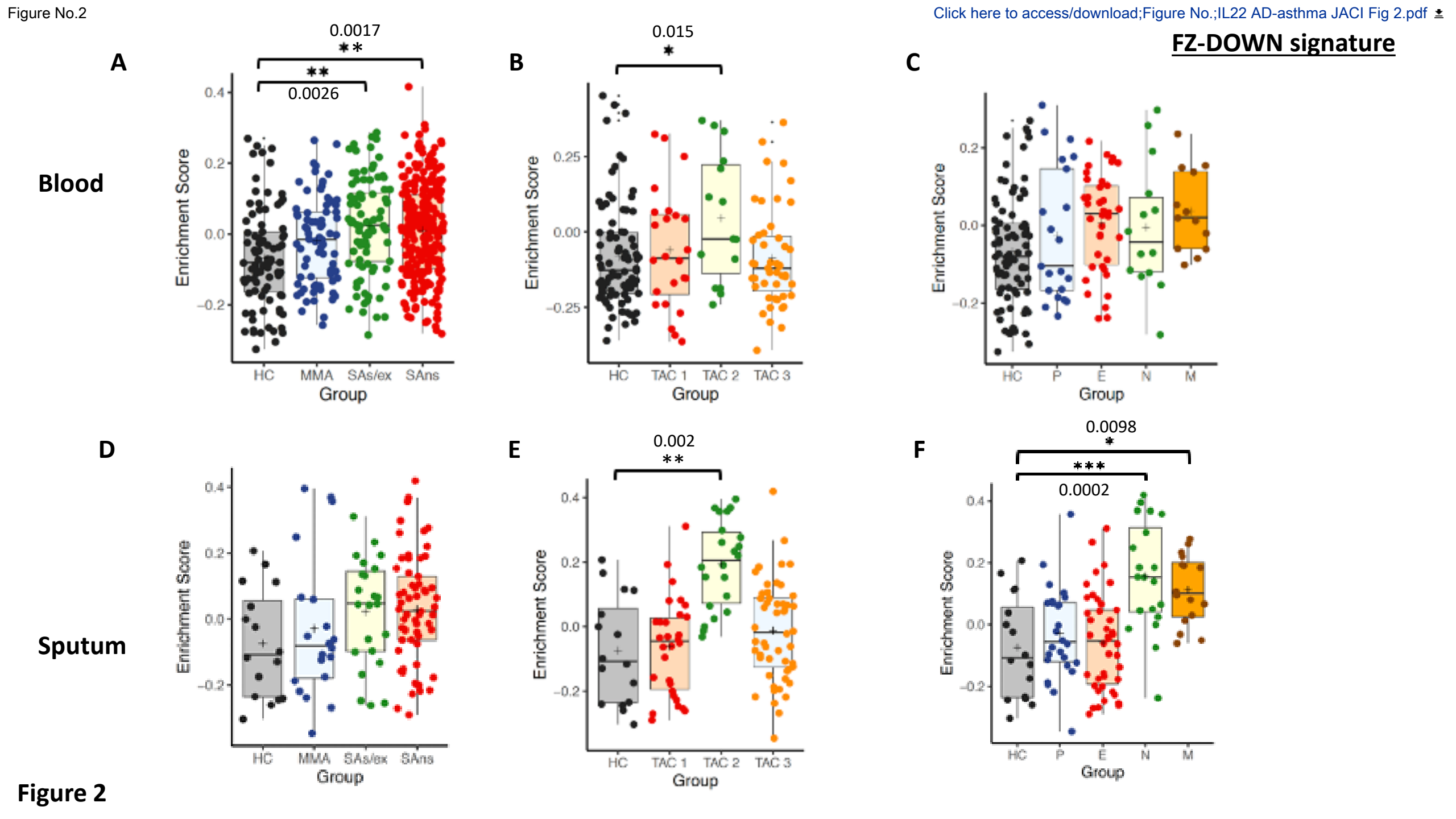

Figure 2

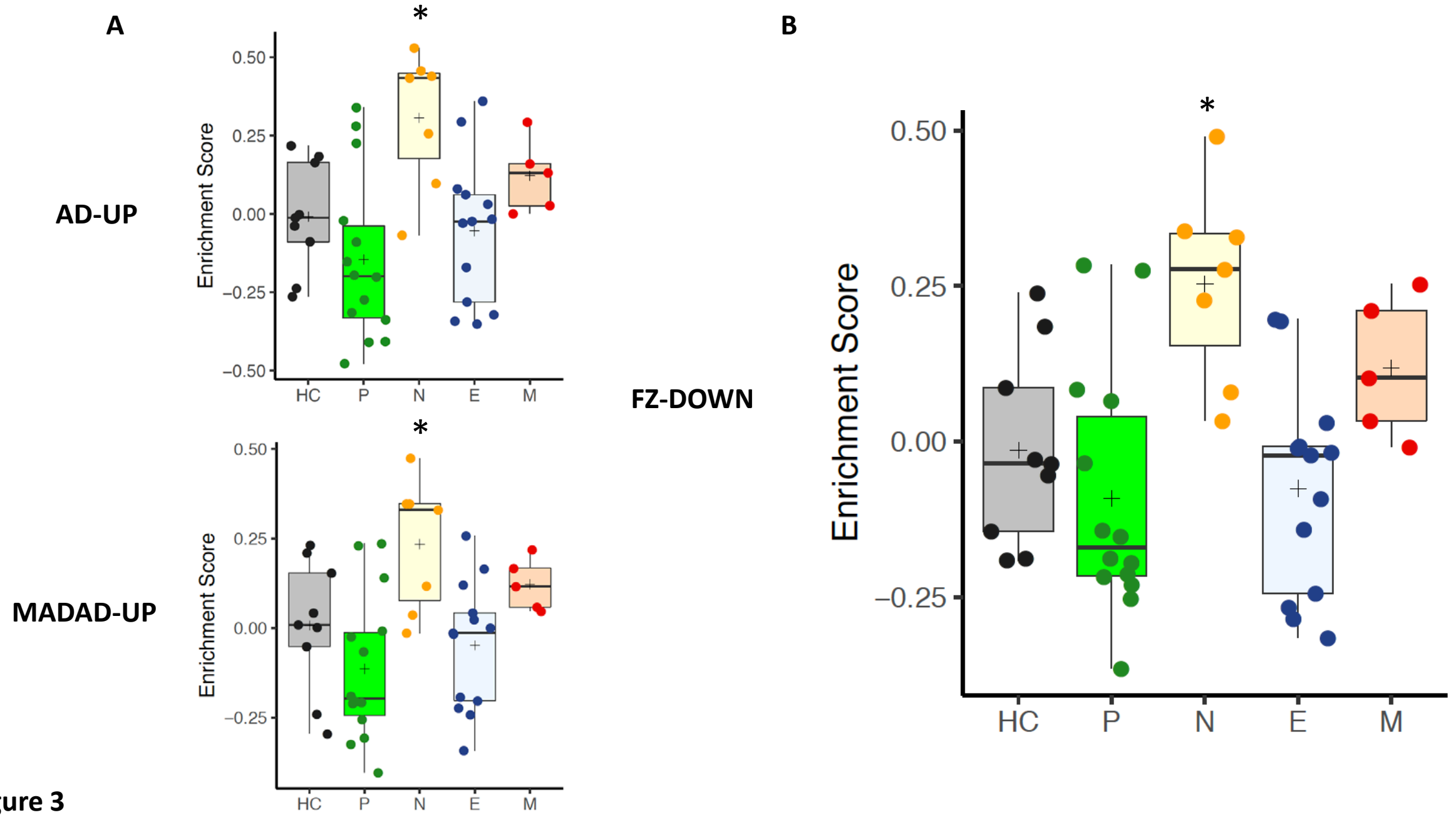

Figure 3

Figure No. 4

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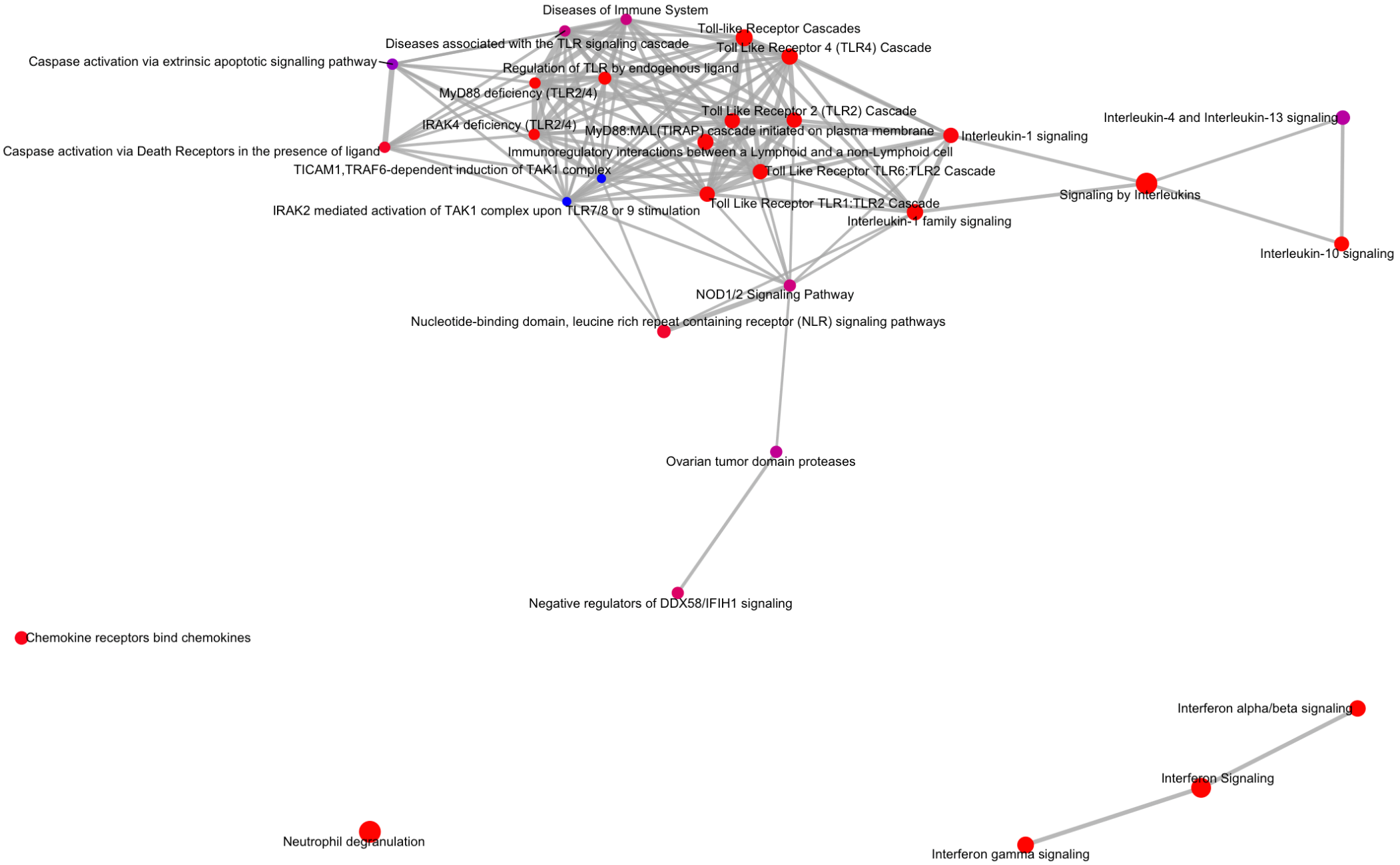
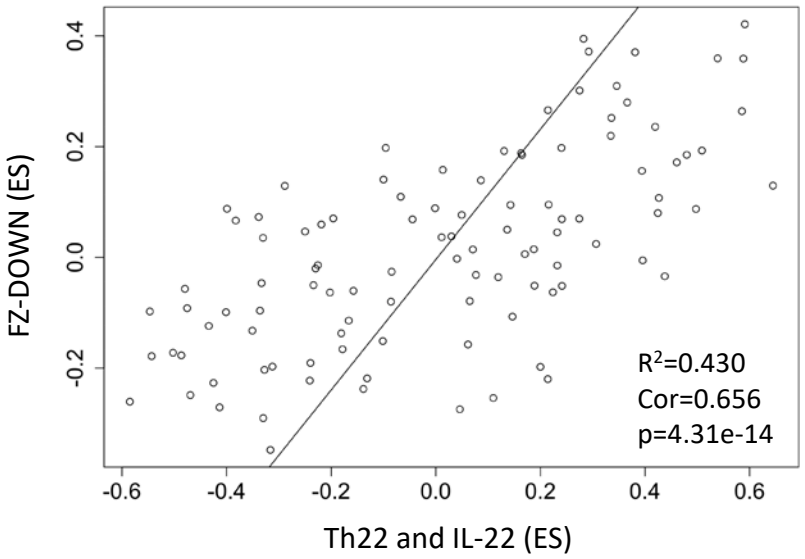


Figure 4

A



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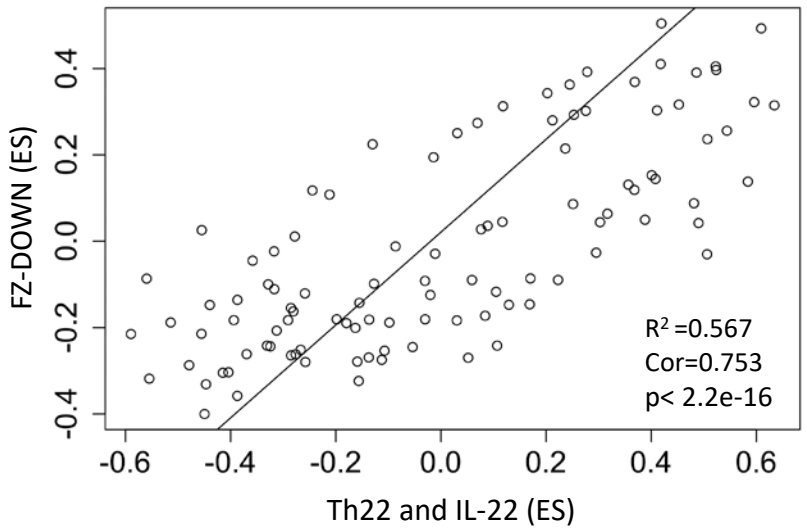

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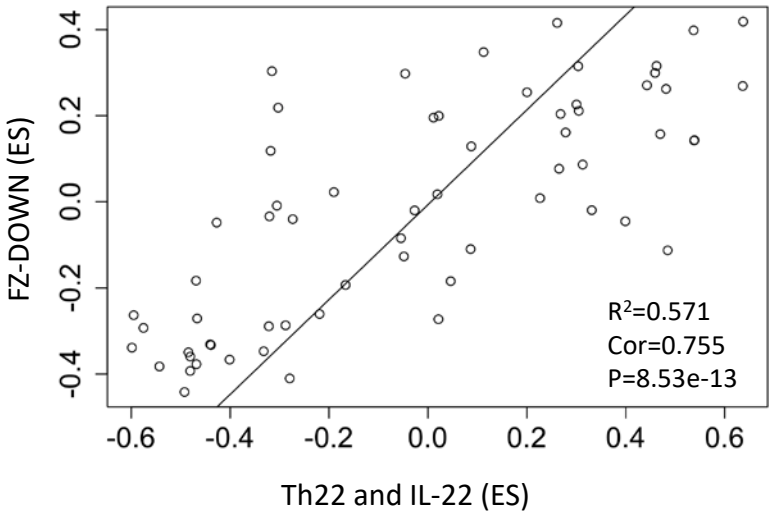

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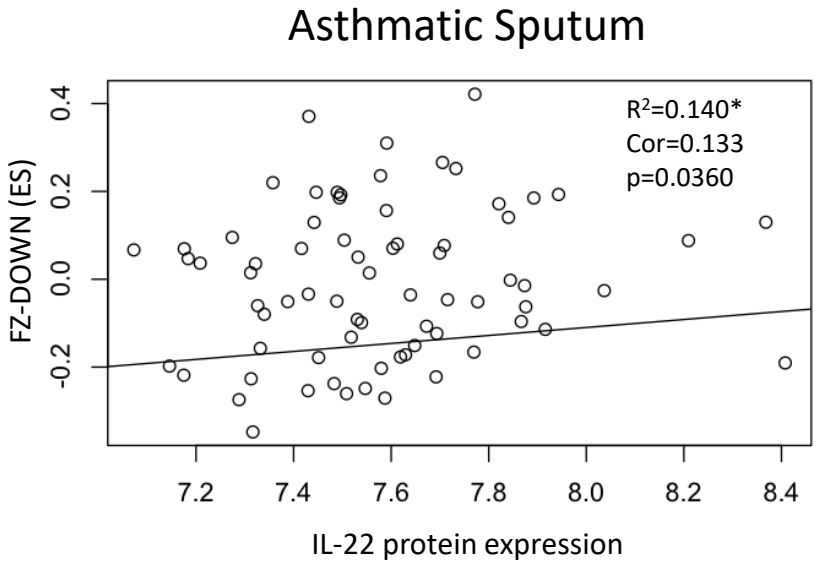

Figure 5

Supplementary data

Mapping atopic dermatitis and anti-IL-22 response signatures to Type 2-low asthma

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Supplementary Table 1. Gene signatures

Signature name	Gene list	Reference
Brunner AD disease signature UP	S100A9 OASL C10orf99 AKR1B10 PRSS53 LINC01094 TEX101 TMPRSS4 SERPINB4 TRIM10 SERPINB3 KRT16 S100A8 CLEC7A KYNU SPRR2C IGFL1 S100A7A PI3 TFEC SERPINB13 EPSTI1 TCN1 FBN2 CCNA2 PTPRC SELL SAMSN1 HAS3 ICOS IL7R GZMB NELL2 CD274 CTLA4 RGS1 MMP12 LGALS2 CXCL2 CD2 DSC2 PI15 LILRB2 CST7 SERPINA1 COL6A5 GPR65 SASH3 RGS18 CXCL1 COL6A6 COL4A4 MMP1 GALNT6 DPY19L1 SPC25 BATF3 OAS2 PLAU STEAP4 RTP4 PLAC8 UBD ICAM1 SLAMF7 BCL2A1 UPP1 ADAM23 ITGAX CLEC4A LAIR2 GNLY CFB CYTIP SNX20 CH25H SAMD9L IL2RG ADAM19 ADAM8 MNDA XCL1 ST8SIA4 IL24 CCL5 XCL2 CD52 SELE CYP27B1 JAML IL15 JAK3 MIR155HG GPRIN3 IFI44 TNFAIP6 PIK3R5 IL13RA2 FAM129C MARK1 ARHGAP9 PRKCQ PLXDC1 RUBCNL TNC CD47 APELA ADAMTS12 CPXM1SPINK6 KLHL6 TDO2	1
Brunner AD disease signature DOWN	AGFG2 IL34 C5orf46 SCIN ARFGEF3 SYNE1 CPEB3 LOC284578 CHPT1 ST6GAL2 GPLD1 PNPLA3 SEMA3E LOC100996902 C1QTNF7 MFSD4A PSORS1C2 MACROD2 SCGB2A1 WIF1 FMO5 ZNF254 FABP7 MYOT FOLR1 NELL1 BTC PHYHIP IL37	1
MADAD UP signature	DEFB4A DEFB4B SERPINB4 S100A9 SERPINB3 MMP1 S100A7A IGFL1 MMP12 AKR1B10 C10orf99 PI3 OASL TMPRSS4 DSC2 GZMB SERPINB13 FOSL1 LCE3D SPRR2D SPRR2B SELE ARNTL2 SPRR3 SPRR1A COL4A4 CLEC7A COL6A5 CXCL10 CCL18 HPSE S100A8 RRM2 IL36G APELA NR4A3 PRSS53 APOBEC3A APOBEC3A_B KRT16 COL6A6 RGS1 EPSTI1 KLHDC7B HAS3 CXCL1 GALNT6 DLGAP5 CD274 CTLA4 CD1B SLAMF7 CEP55 LTF ASPM KIF4A MKI67 SLC2A1 CH25H ZBED2 GPR171 SAMSN1 KIF20A CDCA2 SPRR1B CENPE CXCL8 CCL22 S100A7 BUB1 RTP4 RGS20 NETO2 TRIP13 APOBEC3B CDK1 PKP1 PRKCQ IVL CDKN3 BCL2A1 TYMP ISG20 FCHSD1 IL7R SLC26A9 LGALS2 OAS2 NAPSB MMP9 CASC5 KIAA0101 RAB27A CST7 GPRIN1 TTC39A TGM1 INA VMP1 MIR21 CCL17 BLM NDC80 UGT1A1 UGT1A10 UGT1A8 UGT1A7 UGT1A6 UGT1A5 UGT1A9 UGT1A4 UGT1A3 MIR155HG MIR155 CXCL2 IL13RA2 CD28 CYTIP PRSS27 KLK8 KLK9 ITK NUF2 MPZL2 BIRC5 PI15 HMMR MXD1 HS3ST3A1 PRKCQ-AS1 MIAT ADAM19 GZMA SH3PXD2A-AS1 GPR183 BATF3 CNFN KIF14 SOCS3 AURKA IRF7 LCK NCAPG CENPF WNT5A OAS3 PRR11 PCDH7 MELK CDCA5 MOXD1 CCL26 KYNU MS4A14 SELL LTB KCNJ15 ANGPTL4 TNC CCNB1 STAT1 CCL2 SERPINA1 SASH3 ADAM8 IL36RN RSAD2 SMC4 IFI44 FOSB IRF1 CEMIP CD2 TEX101 TMEM45B F12 CCNB2 UBD GABBR1 C12orf56 PTPN7 ECT2 KLK10 PLAUR SPC25 FAM83A PLAU WWTR1 POLQ C9orf84 FGFBP1 SFT2D2 FPR1 C21orf91 SPTLC2 IL18RAP CCR7 CCL13 DSG3 PTPRC TOP2A KIF2C KIAA1644 SPRR2C CDC20 ASF1B SNX20 LINC01094 UBE2T CXorf65 CD3D TTK LILRB2 CCNA2 CENPA SLC35F6 HERC6 IL12RB1 SLC28A3 HBEGF CLEC10A OAS1 POLR3G IL2RG CDH3 XCL2 XCL1 CCL5 TNFRSF12A KRT6A MIR142 BUB1B NUSAP1 CYP27B1 CACNB4 ADAMDEC1 DIAPH3 SCO2 ADAM10 CD69 TIFAB PML MX1 SLC5A1 SLAMF1 CORO1A C12orf29 C12orf5 SAMD9 UBE2C DNASE1L3 ZC3H12A MIR6732 NEIL3 SLFN5 CKAP2L KRT6B P2RY1 P2RY2 FAS PLXDC1 LRP8 HJURP IL12RB2 PIK3CG	2

	ADAM23 THBD DEFB103B DEFB103A FAM124B FAM26F ITGAX LCN2 NAPG CHEK1 MNDA FOXM1 AIM2 SLAMF8 NUP50 IL4R FAIM3 CXADR ZBED6CL PBK PARVG CA2 GTSE1 DCAF8 GNLY GPSM3 SH3TC1 MND1 PARP9 STK17B KLK13 TAGAP AREG RALGPS2 CPEB2 CENPN CENPW HCK KLRB1 LOC100288860 HAL GK LCP2 01-Mar JAK3 MMP3 DEPDC1B SH2D5 C17orf96 FAM111B TBC1D10C LMNB2 FYB ITGAL CD1E RIT1 DUOXA1 MYO1B PHF19 UBE3C CCNE1 GNA15 KLRK1 KLRC4-KLRK1 IQGAP3 GBP1 SAMD9L KIF18B TFEC PDPN PTAFR PGF SYNCRIP ADAP2 SMOX CFB NOD2 CDT1 IL23A TRBC1 FLVCR2 ELL2 CDK5R1 MFHAS1 STAM2 LYN MMP19 DCTN5 THBS1 TPX2 E2F7 DCANP1 XAF1 CCL8 RELB MCOLN2 CHI3L2 GPR65 DPP4 ICOS ARHGAP9 AMD1 ACPP TRAT1 CCL19 ISG15 NDC1 ACAP1 CTSC PNPT1 PTX3 CD48 CDCA3 TK1 PIK3R5 FPR3 IFI27 TGM3 RAD51AP1 VSNL1 CXCL11 PLAGL2 CDC45 IL27RA MAP4K1 CD6 RASGRP1 SELPLG SNHG3 SNORA73A POC1A THAP2 LAMP3 UBA6 PRDM1 ZC3H12D RNF144B TMC5 PPP2R2C C15orf48	
MADAD DOWN Signature	LMOD1 MYRIP KIAA1324 RORC SLC13A2 FAXDC2 NALCN MEGF10 SEMA3E FAM189A2 LOC100507311 CYP2J2 ZNF471 LINC00663 AQP5 PRKAA2 TRHDE-AS1 GPRC5A CEACAM6 TIMP3 TMC4 FASN AWAT1 SHROOM3 RHPN2 SEMA3B MIR6872 AR SERHL2 SERHL ERBB4 PLCB4 SORBS2 KLRG2 KCNIP2 FGF1 ACVR1C IL20RA SSPN COCH EFHD1 FOXC1 LOC100507557 SYT17 EDAR PIP KRT77 GPRASP1 CA6 TLN2 C1orf95 NSUN7 MOGAT1 NEDD4L SCGB2B2 MAPT ATP6V1B1 CHRM3 CALB2 HSPB6 KRT19 CASQ2 FHL1 COPG2 COPG2IT1 CLIC5 MAP6 PER1 MIR6883 SNCA MUC20 PPARG MIR181A2HG GALNT15 OBP2B OBP2A SH3BGRL2 HRCT1 PPARGC1A CORO2B PSORS1C2 GYG2 GCHFR TRIM2 ACOX2 MRAP SLITRK4 TUSC5 CNKSR2 GPC4 FMO5 SCN7A ADH1B FST C14orf180 CD300LG SCIN MGST1 PLEKHB1 PRB1 STK32A SHANK2 ALDH1A2 LOC101928635 CES1 ATP6V0A4 CKMT2 TG DGAT2 ID4 C2orf40 MFSD4 SCGB1D2 ACADL GSTM5 RNF150 RNF128 LPL RERGL ATP1A2 PDK3 PNPLA3 ABHD12B MIR4454 GPAM C1orf115 MACROD2 FRZB MYBPC1 FA2H PRR4 PRH1-PRR4 VTCN1 SYNE1 BTNL9 TMEM56 PTCSC1 MUC7 ESRRG MYH11 C5orf46 ARFGEF3 HIF3A SGCG PRR15L LGALS12 FAR2 ACOT2 ACOT1 BPY2 PPP1R1B CFTR ALDH1L1 FADS1 MIR1908 KLB YBX2 MSMB ADAMTS9-AS2 01-Mar LGR5 ATP13A5 NR3C2 SGK2 PLEKHA6 AQP7 LOC100509620 LOC101930168 PON3 CUX2 PPP1R1A TMEM132C C1QTNF7 SYN2 ANGPTL7 CIDEA MYEOV ENPP5 ADIPOQ TSPAN8 FABP4 TMEM139 IL37 TF ADRB1 FADS2 RBP4 FGFBP2 LOC284578 C9orf152 FOLR1 HSPB7 MYOC TNMD THRSP PHYHIP GPD1 HAO2 GPIHBP1 CYP4F8 CLDN8 CIDEC SLC14A1 ELOVL3 WDR72 HMGCS2 TIMP4 ZBTB16 PLIN4 SCGB2A1 KANK4 LEP FABP7 PLIN1 GAL KRT79 BTC WIF1 HSD11B1 PM20D1	2
High IL-22 FZ response signature UP	CD300LG FOXC1 CLCNKB HIF3A FOXP2 ADAMTS9-AS2 FBXL13 LOC284578 SORBS2 SNRPN LINC01091 ZNF208 CAPN6 TG FRZB SLC17A7 LMO3 SSPN TRIL NR3C2 GLRB PHYHIP HSD11B1 RUNX1T1 WNT2 GSTM5 GAS2 TPM1 ESRRG KRT7 ADGRL3 CRISP2 GPRC5A SHANK2 ADAM22 ERBB4 TF SPDEF CHRM3 CLDN8 SCAI TSHR	1

	PLA2G5 MUC7 OGN PRR15L FA2H NDNF VTCN1 TGFB2 CNTNAP3P2 IL37 ALDH6A1 KIAA1324 PRND ISYNA1 CRISPLD1 KIAA1549 MMP16 RASD2 ROPN1 FZD8 H19 PPP1R1B LONRF2 PRICKLE1 COL8A1 TMC4 LRRN1 NEDD4L GPC6 WDR72 MYEOV TMEM139 ENPP5 FARP1 PPP1R9A TET1 DACH1 CNTN4 CNKSR2 PRRG3 PLEKHA6 THRSP STK32A C9orf152 B3GALT5 FREM2 KLRG2 ZNF582-AS1 ARFGEF3 PCDH20 SNORD114-3 MIR181A2HG MEGF10 RASSF6 HAND2-AS1 GRIA2 PRDM6 IL34 SLC25A36 GPIHBP1 PRKAA2 SLC26A7 ZNF542P C1QTNF7 HS3ST6 NEGR1 TMEM213 CPEB3 FLG-AS1 TBX18 PTPN14 EBF2 TBXA2R C5orf46 BTC AGFG2 ST6GAL2 PAK3 TMEM56	
High IL-22 FZ response signature DOWN	CLEC7A SAMSN1 SRGN MX1 CFB IRF1 LYN ICAM1 S100A8 RGS1 PLEK S100A9 PI3 IL2RG KYNU IFI6 CXCL1 MMP1 SELL CD52 SASH3 MNDA OAS2 LCP2 LRP8 GPR183 CD3E GZMA TCN1 OAS1 OASL CD2 UBD IL13RA2 SELE XCL1 CCL13 IGSF6 CD28 AKR1B10 TFEC PTPRC DEFB4A LGALS2 SPRR2D CORO1A IFI35 CYTIP SERPINB3 KRT16 GOSR2 CCL19 SH2D1A CST7 GZMB SERPINB4 ICOS PLAUR ITK CERKL PRSS53 XCL2 FAS SERPINB13 OAS3 TMPRSS4 RTP4 IL36G BATF3 ZNF557 TRIM10 CLEC4A CYLD HAS3 RGS18 TEX101 LCE3D IL7R ALYREF MIAT IKZF1 CD274 EPSTI1 JAK3 C10orf99 GALNT6 MIR155HG LINC01094 PRKCQ-AS1 S100A7A MTFR2 LINC01215 CTLA4 TIFAB SLAMF1 IGFL1 RSAD2 ST8SIA4 CCL18 CYP2E1 IL12RB1 WFDC12 PDZK1IP1 ACTR2 UBE3C ABCD3 PLA2G4D NAMPT ITGB2 SYNCRIP TYR MS4A4A LYZ CDKN3 MYRFL FAM111B SH3PXD2A-AS1 LOC100506411 NOP56 SLC2A1 TOP2A PPIF SMC4 LTF BIRC5 GBP1 CCNB2 SERPINA1 CXCL8 SPTLC2 FBN2 CDK1 CCNA2 BUB1B DLGAP5 MPZL2 CXCL9 MMP9 NDC80 FOSL1 INA SAMHD1 UGT1A9 CXCL10 MMP12 CCL5 TTK MELK LCK CENPE CCR1 CHEK1 CCL20 PLAU GNLY DSG3 BCL2A1 CD86 BLM CD8A KLK13 S100A12 S100A7 TGM3 TGM1 UGT1A6 P2RY2 SLC5A1 APOBEC3B ZNF165 CD1B RAB27B UGT1A1 ATP12A CENPF CCL22 CCL17 CYP2C18 UGT1A3 STAT3 RAB27A BUB1 CD24 RRM2 RIT1 SPC25 PRKCQ NAPG TPX2 KCNJ15 RGS20 LILRB2 CXCL11 CHRNA3 CSF2RA BIRC3 TTC39A APOBEC3A IL13RA1 MKI67 VEGFA LCN2 JMJD6 CEBPD CHI3L2 DOCK2 CD3D CASP4 CCL8 FUT3 CCNB1 SOD2 YME1L1 HTR3A TRAT1 TYMP RAB31 CEP55 NCAPG KIF20A PLAC8 RHOF TIGAR PBK SLAMF7 HERC6 HPSE CENPN TMC5 DHRS9 ASPM HS3ST3A1 CARD14 ARNTL2 SPRR2C C21orf91 ANGPTL4 IL26 UGT1A8 GPATCH4 RBM8A PLBD1 DTL NETO2 FLVCR2 EHF XPO5 CDCA3 NUF2 LYAR MCM10 MND1 SNHG12 DEFB103B FAM83D RPS16 TMEM45B CDCA2 FCHSD1 ARHGAP9 PTAFR ZBED6CL RAB7A GPRIN1 NAPSB C17orf96 DDIAS LRG1 DIAPH3 CKAP2L S1PR5 DUOXA2 DSC2 PRSS27 GRHL3 SULF2 KLK8 RNASE7 DENR LEO1 PANX1 RNF144B LYPD5 KLHDC7B KIF14 ULBP2 FAM83A LINC01214 LOC101927972 EPHA1 PPARD SLC26A9 RALGPS2 HBEGF TEAD4 STAT1	1
IL-22/Th22 signature gene list	AHR CALML5 CCR10 FLG IL22 IL32 KRT1 KRT10 LOR S100A7 S100A8 S100A9 S100P SERPINB1 SERPINB4 S100A12	1

Supplementary Table 2. ReactomePA pathway enrichment FZ-DOWN signature. P values adjusted by FDR-BH with cutoff <0.05.

ID	Description	Gene Ratio	Bg Ratio	P value	p. djust	Q value	geneID	Count
R-HSA-6783783	Interleukin-10 signaling	12/212	47/10554	8.90E-11	3.03E-08	2.68E-08	ICAM1/CXCL1/CCL19/CXCL8/CXCL10/CCL5/CCR1/CCL20/CD86/CCL22/STAT3/PTAFR	12
R-HSA-380108	Chemokine receptors bind chemokines	12/212	48/10554	1.16E-10	3.03E-08	2.68E-08	CXCL1/XCL1/CCL13/CCL19/XCL2/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/CXCL11	12
R-HSA-449147	Signaling by Interleukins	33/212	462/10554	1.61E-10	3.03E-08	2.68E-08	LYN/ICAM1/IL2RG/CXCL1/MMP1/IL13RA2/CCL19/IL36G/IL7R/JAK3/IL12RB1/ITGB2/BIRC5/CXCL8/MMP9/CXCL10/CCL5/LCK/CCR1/CCL20/CD86/S100A12/CCL22/STAT3/CSF2RA/IL13RA1/VEGFA/LCN2/CEBPD/SOD2/IL26/PTAFR/STAT1	33
R-HSA-6785807	Interleukin-4 and Interleukin-13 signaling	16/212	108/10554	3.98E-10	5.59E-08	4.95E-08	ICAM1/IL2RG/MMP1/IL13RA2/JAK3/ITGB2/BIRC5/CXCL8/MMP9/CCL22/STAT3/IL13RA1/VEGFA/LCN2/CEBPD/STAT1	16
R-HSA-6798695	Neutrophil degranulation	29/212	479/10554	9.37E-08	1.02E-05	9.04E-06	S100A8/S100A9/CXCL1/SELL/MNDA/TCN1/PTPRC/SERPINB3/PLAUR/ACTR2/ITGB2/LYZ/LTF/SERPINA1/MMP9/PLAU/S100A12/S100A7/RAB27A/LCN2/DOCK2/RAB31/PLAC8/RHOF/HPSE/ARHGAP9/PTAFR/RAB7A/LRG1	29

R-HSA-909733	Interferon alpha/beta signaling	11/212	69/10554	1.08E-07	1.02E-05	9.04E-06	MX1/IRF1/IFI6/OAS2/OAS1/OASL/IFI35/OAS3/RSAD2/SAMHD1/STAT1	11
R-HSA-6803157	Antimicrobial peptides	12/212	97/10554	5.02E-07	4.03E-05	3.57E-05	S100A8/S100A9/PI3/DEFB4A/S100A7A/LYZ/LTF/GNLY/S100A7/LCN2/DEFB103B/RNASE7	12
R-HSA-2514853	Condensation of Prometaphase Chromosomes	5/212	11/10554	1.30E-06	9.17E-05	8.13E-05	SMC4/CCNB2/CDK1/CCNB1/NCAPG	5
R-HSA-2500257	Resolution of Sister Chromatid Cohesion	12/212	124/10554	6.98E-06	0.00043	0.00038	BIRC5/CCNB2/CDK1/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CCNB1/CENPN/NUF2	12
R-HSA-913531	Interferon Signaling	15/212	197/10554	9.82E-06	0.00051	0.00045	MX1/IRF1/ICAM1/IFI6/OAS2/OAS1/OASL/IFI35/OAS3/TRIM10/RSAD2/GBP1/SAMHD1/PTAFR/STAT1	15
R-HSA-388841	Costimulation by the CD28 family	9/212	70/10554	1.01E-05	0.00051	0.00045	LYN/CD3E/CD28/ICOS/CD274/CTLA4/LCK/CD86/CD3D	9
R-HSA-877300	Interferon gamma signaling	10/212	92/10554	1.48E-05	0.00069	0.00061	IRF1/ICAM1/OAS2/OAS1/OASL/OAS3/TRIM10/GBP1/PTAFR/STAT1	10
R-HSA-9020958	Interleukin-21 signaling	4/212	10/10554	3.02E-05	0.00130	0.00115	IL2RG/JAK3/STAT3/STAT1	4
R-HSA-68877	Mitotic Prometaphase	14/212	198/10554	4.46E-05	0.00179	0.00158	SMC4/BIRC5/CCNB2/CDK1/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CCNB1/NCAPG/CENPN/NUF2	14
R-HSA-156588	Glucuronidation	5/212	25/10554	0.00011	0.00424	0.00376	UGT1A9/UGT1A6/UGT1A1/UGT1A3/UGT1A8	5

R-HSA-141424	Amplification of signal from the kinetochores	9/212	96/10554	0.00012	0.00424	0.00376	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2	9
R-HSA-141444	Amplification of signal from unattached kinetochores via a MAD2 inhibitory signal	9/212	96/10554	0.00012	0.00424	0.00376	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2	9
R-HSA-1433557	Signaling by SCF-KIT	6/212	43/10554	0.00020	0.00626	0.00554	LYN/MMP9/LCK/CHEK1/STAT3/STAT1	6
R-HSA-451927	Interleukin-2 family signaling	6/212	44/10554	0.00022	0.00675	0.00598	IL2RG/JAK3/LCK/STAT3/CSF2RA/STAT1	6
R-HSA-69620	Cell Cycle Checkpoints	16/212	293/10554	0.00027	0.00772	0.00684	BIRC5/CCNB2/CDK1/CCNA2/BUB1B/NDC80/CENPE/CHEK1/BLM/CENPF/BUB1/SPC25/CCNB1/CENPN/NUF2/MCM10	16
R-HSA-418594	G alpha (i) signalling events	19/212	396/10554	0.00038	0.0104	0.00920	RGS1/CXCL1/LRP8/GPR183/CCL13/AKR1B10/CCL19/RGS18/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/PRKCQ/RGS20/CXCL11/DHRS9/S1PR5	19
R-HSA-69618	Mitotic Spindle Checkpoint	9/212	112/10554	0.00041	0.0105	0.00932	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2	9
R-HSA-375276	Peptide ligand-binding receptors	12/212	190/10554	0.00044	0.0105	0.00932	CXCL1/XCL1/CCL13/CCL19/XCL2/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/CXCL11	12
R-HSA-5663220	RHO GTPases Activate Formins	10/212	138/10554	0.00045	0.0105	0.00932	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2/DIAPH3	10

R-HSA-202433	Generation of second messenger molecules	5/212	33/10554	0.00046	0.0105	0.00932	LCP2/CD3E/ITK/LCK/CD3D	5
R-HSA-389513	CTLA4 inhibitory signaling	4/212	21/10554	0.00072	0.0157	0.0139	LYN/CTLA4/LCK/CD86	4
R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	16/212	324/10554	0.00082	0.0172	0.0153	CXCL1/GPR183/XCL1/CCL13/CCL19/XCL2/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/P2RY2/CXCL11/PTAFR/S1PR5	16
R-HSA-202427	Phosphorylation of CD3 and TCR zeta chains	4/212	22/10554	0.00087	0.0175	0.0155	CD3E/PTPRC/LCK/CD3D	4
R-HSA-389948	PD-1 signaling	4/212	23/10554	0.00103	0.0201	0.0178	CD3E/CD274/LCK/CD3D	4
R-HSA-6809371	Formation of the cornified envelope	9/212	129/10554	0.00114	0.0215	0.0191	PI3/SPRR2D/KRT16/LCE3D/DSG3/KLK13/TGM1/DSC2/KLK8	9
R-HSA-198933	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	9/212	132/10554	0.00135	0.0245	0.0217	ICAM1/SELL/CD3E/SH2D1A/ITGB2/CD8A/CD1B/CD3D/SLAMF7	9
R-HSA-69273	Cyclin A/B1/B2 associated events during G2/M transition	4/212	25/10554	0.00143	0.0245	0.0217	CCNB2/CDK1/CCNA2/CCNB1	4
R-HSA-8854691	Interleukin-20 family signaling	4/212	25/10554	0.00143	0.0245	0.0217	JAK3/STAT3/IL26/STAT1	4
R-HSA-389359	CD28 dependent Vav1 pathway	3/212	12/10554	0.00153	0.0247	0.0219	CD28/LCK/CD86	3

R-HSA-9020558	Interleukin-2 signaling	3/212	12/10554	0.00153	0.0247	0.0219	IL2RG/JAK3/LCK	3
R-HSA-75035	Chk1/Chk2(Cds1) mediated inactivation of Cyclin B:Cdk1 complex	3/212	13/10554	0.00196	0.0307	0.0272	CDK1/CHEK1/CCNB1	3
R-HSA-162658	Golgi Cisternae Pericentriolar Stack Reorganization	3/212	14/10554	0.00246	0.0365	0.0324	CCNB2/CDK1/CCNB1	3
R-HSA-8983432	Interleukin-15 signaling	3/212	14/10554	0.00246	0.0365	0.0324	IL2RG/JAK3/STAT3	3
R-HSA-202403	TCR signaling	8/212	119/10554	0.00270	0.0390	0.0345	LCP2/CD3E/PTPRC/ITK/LCK/PRKCQ/CD3D/TRAT1	8
R-HSA-2219530	Constitutive Signaling by Aberrant PI3K in Cancer	6/212	71/10554	0.00295	0.0416	0.0368	CD28/ICOS/LCK/CD86/TRAT1/HBEGF	6

Supplementary Table 3. ReactomePA pathway enrichment FZ-UP signature. P values adjusted by FDR-BH with cutoff <0.2.

ID	Description	Gene Ratio	Bg Ratio	P value	p. adjust	Q value	geneID	Count
R-HSA-1250342	PI3K events in ERBB4 signaling	2/60	10/10554	0.00138	0.108	0.102	ERBB4/BTC	2
R-HSA-163125	Post-translational modification: synthesis of GPI-anchored proteins	4/60	92/10554	0.00181	0.108	0.102	PRND/CNTN4/GPIHBP1/NEGR1	4
R-HSA-8847993	ERBB2 Activates PTK6 Signaling	2/60	13/10554	0.00238	0.108	0.102	ERBB4/BTC	2
R-HSA-1250347	SHC1 events in ERBB4 signaling	2/60	14/10554	0.00276	0.108	0.102	ERBB4/BTC	2
R-HSA-200425	Import of palmitoyl-CoA into the mitochondrial matrix	2/60	14/10554	0.00276	0.108	0.102	THRSP/PRKAA2	2
R-HSA-6785631	ERBB2 Regulates Cell Motility	2/60	15/10554	0.00318	0.108	0.102	ERBB4/BTC	2
R-HSA-1963640	GRB2 events in ERBB2 signaling	2/60	16/10554	0.00362	0.108	0.102	ERBB4/BTC	2
R-HSA-1963642	PI3K events in ERBB2 signaling	2/60	16/10554	0.00362	0.108	0.102	ERBB4/BTC	2
R-HSA-9008059	Interleukin-37 signaling	2/60	21/10554	0.00622	0.163	0.153	IL37/PTPN14	2
R-HSA-1250196	SHC1 events in ERBB2 signaling	2/60	22/10554	0.00682	0.163	0.153	ERBB4/BTC	2

Supplementary Table 4. Clinical characteristics of U-BIOPRED asthmatic Predicted Responders (PRs, ES of ≥ +0.1) against predicted non-responders (PNRs, ES of ≤ -0.1) defined from sputum transcriptomic GSVA of the FZ-DOWN signature. See main paper Table 1 for full breakdown.

	Predicted responders	**Predicted non-responders**
n	26	18
Sex		
Male	10	9
Female	16	9
Age mean, yrs	51.8	55.3
Cohort		
Severe Asthma non-smoker (SAns)	18	13
Severe Asthma smoker / ex-smoker (SAs/ex)	8	5

Supplementary Table 5. Top 431 sputum and 19 down transcriptomic DEGS which differentiate U-BIOPRED asthmatic FZ predicted responders (PRs) from predicted non-responders (PNRs). Genes are ranked according to log_2 fold change. All results are significant by FDR adjusted p value.

Upregulated Genes

Gene Symbol	Log_2 Fold Change	FDR-BH adjusted p value
KCNJ2	2.89	1.57E-08
ANXA3	2.87	2.87E-07
CXCL10	2.60	2.72E-05
LRRK2	2.48	1.50E-06
IFIT2	2.44	4.57E-06
GCH1	2.44	1.39E-07
S100A12	2.42	1.05E-06
GRIP1	2.41	2.33E-07
IFIH1	2.37	2.19E-07
HS3ST3B1	2.34	1.29E-08
CXCL11	2.34	5.47E-04
RSAD2	2.34	2.77E-04
GBP5	2.31	2.83E-07
TNFAIP6	2.31	2.60E-08
CALHM6	2.23	8.43E-05
PTGS2	2.21	2.08E-06
LOC105372881	2.17	1.78E-06
TNFSF10	2.15	4.59E-06
IFIT3	2.09	1.59E-04
CCL8	2.08	6.15E-03
FAS	2.06	2.60E-08
DOCK4	2.04	4.55E-06
ISG20	2.03	1.05E-06
KCNJ15	2.01	9.25E-06
STEAP4	2.00	2.52E-06
CLEC4E	1.96	1.50E-06
SELL	1.95	2.74E-06
CXCR2	1.95	7.26E-06
IFITM1	1.95	2.85E-07
GPR84	1.90	1.32E-05
SLC30A4	1.90	1.50E-06
APOBEC3A	1.89	1.85E-06
TNIP3	1.88	3.09E-04
UBD	1.87	1.05E-06
CXCL9	1.86	8.52E-04
TNFRSF10C	1.86	1.13E-03

ACOD1	1.85	3.68E-03
CMPK2	1.82	1.98E-03
AIM2	1.82	1.77E-06
LIMK2	1.81	4.45E-07
PDE4B	1.79	1.99E-06
TIFA	1.78	8.83E-06
VNN3	1.78	3.93E-05
IRAK2	1.77	2.43E-05
IFIT1	1.76	2.43E-03
HIVEP2	1.75	4.52E-06
LINC01270	1.75	7.31E-05
GBP1	1.74	1.17E-06
PROK2	1.73	7.05E-05
WDFY3	1.72	3.96E-07
GJB2	1.71	5.33E-04
LMNB1	1.70	1.07E-06
RAB33B	1.70	6.48E-05
IDO1	1.69	1.35E-03
HPSE	1.69	2.72E-05
N4BP1	1.69	3.77E-06
ZNF200	1.68	7.95E-06
PI3	1.67	6.13E-04
ANKRD22	1.66	4.28E-04
CASP4	1.66	6.41E-08
ALPL	1.64	4.30E-04
SERPINB9	1.64	1.70E-03
GBP4	1.64	6.35E-05
FPR2	1.63	7.13E-06
CXCR1	1.63	2.32E-04
FAM8A1	1.63	1.12E-05
TAGAP	1.63	2.43E-05
BAZ1A	1.63	4.67E-07
ARL5B	1.62	6.74E-06
BMT2	1.61	1.77E-06
LINC00266-1	1.61	4.00E-05
UBE2D1	1.60	1.05E-06
FBXO6	1.60	5.03E-04
MX2	1.59	6.30E-05
P2RY14	1.59	7.08E-04
MGAM	1.58	5.07E-04
LINC01215	1.58	8.85E-04
VNN2	1.58	4.52E-06
TSPAN2	1.57	7.10E-04

ORM1	1.57	3.98E-03
HAL	1.57	9.59E-04
SPATA13	1.57	2.79E-04
MRVI1	1.57	5.33E-04
KLHL15	1.57	1.54E-04
STAT4	1.57	1.86E-04
HERC5	1.56	1.41E-03
CEP83	1.55	9.96E-06
TMEM154	1.55	2.22E-04
FAM129A	1.55	1.68E-06
CMTM2	1.55	2.15E-05
LILRA2	1.55	1.50E-05
SP110	1.54	7.99E-05
CD274	1.54	1.19E-05
KRT23	1.54	7.26E-06
GLT1D1	1.54	1.45E-04
NBN	1.54	8.17E-06
KATNBL1	1.54	4.16E-04
QPCT	1.54	7.14E-07
MANSC1	1.54	1.04E-03
LINC01093	1.53	2.34E-03
ZNF267	1.53	2.33E-07
SMA5	1.53	2.88E-03
IL6ST	1.53	1.96E-05
MSRB1	1.53	6.35E-05
PLXNC1	1.53	8.43E-05
ARHGEF40	1.53	1.42E-04
NMI	1.53	4.14E-07
CCDC71L	1.53	1.42E-04
TLR1	1.52	2.33E-04
PTX3	1.52	2.50E-03
FFAR2	1.52	6.17E-04
BATF	1.52	1.46E-05
TNFSF13B	1.52	7.26E-06
GBP1P1	1.51	2.99E-04
ERV3-2	1.51	1.93E-03
ARFIP1	1.51	5.41E-05
PFKFB3	1.51	5.33E-06
RUBCNL	1.50	1.64E-03
CSGALNACT1	1.50	2.24E-04
IRF1	1.50	2.88E-05
LOC399716	1.50	1.05E-04
NAIP	1.50	1.15E-05

KIAA1551	1.49	9.48E-05
CLEC2B	1.49	4.43E-08
CD177	1.49	2.88E-03
LRG1	1.48	6.00E-05
NLRC5	1.47	4.47E-05
C5orf58	1.47	1.23E-05
LILRA1	1.47	9.06E-04
THAP2	1.46	2.22E-04
CREB5	1.46	4.29E-05
CLEC4D	1.46	1.02E-04
PLEK	1.46	6.41E-08
GALNT3	1.46	6.09E-04
GNG2	1.45	2.83E-04
LOC145474	1.45	5.33E-04
CSF3R	1.45	1.05E-04
MIR29A	1.44	1.51E-04
DGAT2	1.44	1.31E-05
TRIM22	1.43	1.48E-04
CR1	1.43	1.51E-04
GIMAP4	1.43	9.40E-06
C15orf48	1.43	9.25E-06
TMCC3	1.43	5.04E-04
RAPGEF2	1.42	6.41E-05
TANK	1.42	2.10E-05
SORL1	1.41	8.43E-05
BATF2	1.41	1.32E-03
RHOH	1.41	4.14E-04
RNF175	1.41	2.60E-05
HCAR3	1.40	5.99E-05
LOC114224	1.40	5.72E-05
AQP9	1.40	1.30E-06
IFI16	1.40	2.63E-06
LOC254896	1.39	5.47E-04
IL1A	1.38	8.26E-03
ZC3H12D	1.38	8.83E-05
HES1	1.38	2.46E-03
CARD16	1.38	5.24E-06
TOPORS	1.38	2.92E-04
KREMEN1	1.38	1.92E-04
PDP1	1.38	1.42E-03
OSM	1.38	5.72E-05
TREML4	1.38	1.05E-04
CXCL1	1.38	1.92E-04

GTF2IP12	1.37	9.25E-06
CR1L	1.36	1.34E-03
MIR155HG	1.36	4.14E-04
IL18RAP	1.36	7.52E-03
JAK3	1.36	1.21E-04
TNFAIP3	1.36	4.73E-04
NSMAF	1.36	2.20E-04
EIF4E3	1.36	1.27E-05
SGTB	1.36	1.32E-03
MCTP2	1.35	1.10E-02
SLPI	1.35	7.69E-04
IL1B	1.35	1.92E-04
LY96	1.34	3.77E-05
IFITM2	1.34	4.52E-06
ANTXR2	1.33	1.44E-05
SERPINB9P1	1.33	5.19E-03
CASP5	1.32	1.86E-04
SCLT1	1.32	1.72E-03
IFITM3	1.32	3.77E-06
NLRP3	1.32	2.51E-03
EREG	1.32	1.44E-03
P2RY13	1.32	2.73E-03
FAM126B	1.31	3.67E-04
RPGR	1.31	2.29E-04
SNX18	1.31	6.08E-05
PELI2	1.31	4.72E-04
MNDA	1.31	3.93E-05
LINC00528	1.31	9.56E-04
ACAT2	1.30	1.74E-03
PSMB9	1.30	2.09E-05
KCNH7	1.30	9.73E-05
HMGN2P46	1.30	9.87E-03
CLOCK	1.30	4.45E-04
PML	1.29	4.28E-04
IL1R2	1.29	2.93E-04
CYP4F3	1.28	8.08E-03
SNN	1.28	4.67E-07
WTAP	1.28	4.52E-06
SLC7A5	1.28	3.42E-04
IRAK3	1.28	3.67E-03
SLC40A1	1.28	7.56E-03
ADGRE2	1.28	7.95E-06
LRRC70	1.27	3.26E-02

CD48	1.27	1.81E-05
PPP1R3B	1.27	8.77E-03
PIP4P2	1.27	1.27E-03
ADAMDEC1	1.27	1.37E-02
LILRA5	1.27	1.14E-03
ATG3	1.27	4.49E-07
CD8A	1.27	1.22E-05
CSF2RB	1.27	1.05E-06
FCAR	1.26	4.20E-04
GZMB	1.26	8.76E-03
NFKBIZ	1.26	1.05E-06
TRAPPC13	1.26	4.08E-04
CCRL2	1.26	5.40E-04
TRAF1	1.26	9.95E-04
FCGR1B	1.26	3.58E-04
CASP1	1.26	4.35E-05
DYSF	1.25	6.05E-04
SIGLEC5	1.25	8.35E-04
CPD	1.25	8.63E-04
SAMD9	1.25	2.11E-04
BRE-AS1	1.25	1.76E-03
DAPP1	1.25	3.37E-05
C1D	1.25	2.76E-03
TBK1	1.24	1.36E-03
LINC00641	1.24	4.49E-04
LOC100289230	1.24	3.73E-03
POLB	1.24	9.88E-08
SBF2	1.24	1.69E-03
SLC39A8	1.23	7.05E-05
RAB5A	1.23	3.04E-04
PELI1	1.23	1.56E-04
TMEM185B	1.23	7.95E-06
RNF149	1.23	1.27E-05
SAMD9L	1.22	8.40E-04
TLR4	1.22	3.24E-05
USP10	1.22	1.92E-04
XRN1	1.22	1.85E-03
ZC3H12C	1.22	2.84E-03
SERPINB2	1.21	1.40E-02
STAT1	1.21	9.43E-05
PREX1	1.21	3.18E-04
SMCHD1	1.21	6.63E-05
PSMB8-AS1	1.21	8.07E-04

TMEM71	1.21	3.03E-03
OAS3	1.21	8.26E-03
RASSF2	1.21	1.63E-04
S100A8	1.20	2.28E-05
KCNJ2-AS1	1.20	1.56E-03
NFAM1	1.20	8.83E-06
RNF19B	1.20	6.68E-04
CCL5	1.20	1.13E-03
ZNF292	1.20	1.42E-03
BICRAL	1.20	9.02E-05
FLJ32255	1.19	2.89E-04
S100P	1.19	2.03E-03
SLC22A4	1.19	9.43E-05
SCARF1	1.19	2.66E-03
EGR3	1.19	2.67E-03
PLSCR1	1.19	2.03E-07
LAMP3	1.19	1.54E-02
TRIM5	1.19	7.52E-03
HSD17B11	1.19	4.48E-04
GSEC	1.18	1.14E-03
HNRNPH2	1.18	6.36E-05
GBP2	1.18	1.22E-06
LOC100130357	1.18	2.33E-03
CREM	1.18	5.99E-05
S100A9	1.18	9.16E-06
GIMAP8	1.18	2.05E-03
MIR3945HG	1.17	4.73E-04
UBR1	1.17	2.25E-03
LINC01003	1.17	4.15E-03
TCP11L2	1.17	2.36E-03
CNOT11	1.17	1.86E-04
COQ10B	1.17	2.62E-05
PCBP1-AS1	1.16	2.06E-02
ICAM1	1.16	1.74E-06
RNF213	1.16	9.69E-04
IPO11	1.16	5.41E-03
ABHD3	1.15	2.50E-03
RABGAP1L	1.15	9.11E-03
CDC42SE2	1.15	7.86E-05
ST8SIA4	1.15	1.53E-04
NFE2L2	1.15	8.17E-06
SEMA4A	1.15	2.60E-05
PRKCB	1.15	4.20E-03

CEP68	1.15	3.01E-05
RGL4	1.15	9.09E-03
IRF2	1.14	1.35E-05
PARP14	1.14	3.67E-05
RIPOR2	1.14	4.05E-03
GTF2B	1.14	1.20E-04
MAP3K13	1.13	8.25E-04
MARCKS	1.13	4.67E-07
GBP3	1.13	2.88E-02
SP100	1.13	1.26E-04
FGL2	1.13	4.51E-04
TAB2	1.13	1.92E-04
SECTM1	1.13	1.11E-04
ELF2	1.13	2.29E-04
PAK1	1.13	1.72E-03
TCFL5	1.13	6.43E-03
GPR27	1.13	5.34E-04
FPR1	1.13	8.68E-06
DNTTIP2	1.13	3.01E-05
PTEN	1.12	5.67E-04
GZMA	1.12	1.26E-04
LINC00877	1.12	6.10E-04
VAV1	1.12	2.02E-05
TMEM88	1.12	3.85E-04
TLR2	1.12	6.85E-04
CD93	1.11	5.08E-03
BTBD19	1.11	1.14E-03
DDX60L	1.11	1.74E-04
ZBTB21	1.11	3.68E-03
PHF11	1.11	1.44E-04
CHD1	1.11	7.04E-05
PARP8	1.11	4.20E-04
MAK	1.11	9.43E-05
ZNF107	1.11	2.06E-02
TAP1	1.11	8.51E-06
SUSD6	1.10	3.42E-05
CFLAR	1.10	2.60E-05
SNORD89	1.10	1.23E-03
ACSL1	1.10	9.16E-06
BCL10	1.10	4.05E-04
RIN2	1.10	6.61E-03
SLAMF7	1.10	1.05E-02
TECPR2	1.10	2.30E-03

TET3	1.10	1.58E-03
CNTNAP3	1.09	3.94E-02
CHMP2B	1.09	1.35E-03
IDI2-AS1	1.09	2.73E-03
ICAM3	1.09	2.43E-03
EPM2AIP1	1.09	7.35E-03
DTX3L	1.09	5.29E-06
FCN1	1.09	1.42E-03
G0S2	1.09	4.74E-05
SPATA1	1.09	1.23E-05
CHST15	1.08	2.13E-03
TNF	1.08	1.09E-02
SLC15A4	1.08	3.56E-04
HSD11B1-AS1	1.08	3.78E-03
BTNL8	1.08	6.11E-03
TIMP1	1.08	1.21E-04
STX3	1.07	8.73E-03
BCL2A1	1.07	2.85E-07
CDC42EP2	1.07	3.95E-03
PTENP1	1.07	4.54E-04
ERI1	1.07	5.18E-03
AGTPBP1	1.07	9.43E-05
SPAG9	1.07	3.58E-04
GPR65	1.07	5.48E-04
IL1RAP	1.07	2.79E-03
AP1AR	1.06	5.33E-03
LOC441081	1.06	3.57E-03
C16orf54	1.06	7.31E-03
BID	1.06	3.63E-05
NFE2L3	1.06	5.24E-04
TULP2	1.06	1.90E-02
MX1	1.06	6.15E-03
HCG26	1.06	1.52E-03
ZC3HAV1	1.05	6.33E-04
NAF1	1.05	5.17E-03
APOBEC3G	1.05	1.40E-03
C11orf54	1.05	2.76E-03
CHSY1	1.05	1.33E-03
HCK	1.05	4.47E-06
CEACAM1	1.05	5.10E-03
PSTPIP2	1.05	1.72E-03
DLEU2L	1.05	9.65E-03
RNF141	1.05	4.04E-04

PPA1	1.05	3.52E-03
LAP3	1.05	5.84E-03
LOC101928361	1.04	3.60E-03
PPP2R2A	1.04	2.73E-03
CHI3L1	1.04	4.93E-02
PJA2	1.04	2.60E-05
MITD1	1.04	1.29E-04
RALB	1.04	5.21E-05
SMA4	1.04	7.45E-03
BCL3	1.04	1.48E-04
KDM6A	1.04	5.95E-05
LCP2	1.04	7.04E-06
AMPD3	1.04	2.82E-04
PPIF	1.04	3.01E-04
SECISBP2	1.04	1.22E-04
CFP	1.03	3.01E-04
ELOVL5	1.03	1.74E-06
CYSTM1	1.03	1.96E-05
DENND5A	1.03	4.90E-05
ASPRV1	1.03	1.44E-03
IDI1	1.03	1.21E-04
IGSF6	1.03	3.38E-05
MMP25	1.03	5.80E-03
NKG7	1.03	1.48E-04
KDM7A	1.03	1.59E-03
NFE4	1.03	2.35E-02
PHF20L1	1.02	1.33E-04
LYRM1	1.02	1.04E-03
NOD2	1.02	1.64E-03
MIA3	1.02	1.78E-03
GK3P	1.02	1.66E-04
PPP4R2	1.02	3.06E-05
CD55	1.02	8.79E-05
MIER1	1.02	5.94E-05
MLKL	1.02	1.24E-03
PIK3AP1	1.02	1.68E-05
ESCO1	1.01	2.59E-03
TREML2	1.01	1.70E-03
FCGR1A	1.01	3.45E-04
DDX60	1.01	1.34E-02
SRSF12	1.01	2.69E-03
DLGAP1-AS2	1.01	1.45E-03
WASHC2A	1.01	1.69E-03

USP32	1.01	3.95E-03
SPTY2D1	1.01	1.52E-03
ZFX	1.00	5.01E-04
CSRNP1	1.00	2.64E-04
PPP3CA	1.00	3.95E-03
SLAMF1	1.00	4.26E-03
TDP2	1.00	3.68E-03
TLR6	1.00	4.16E-04

Downregulated Genes

Gene Symbol	**Log_2 Fold Change**	**FDR-BH adjusted p value**
TMC6	-1.01	5.61E-05
RNA45SN5	-1.01	1.33E-03
DTX4	-1.01	2.30E-02
TPSAB1	-1.05	4.41E-03
BHLHE41	-1.06	4.39E-02
SPOCD1	-1.08	9.65E-03
FIG4	-1.10	1.58E-02
TGM2	-1.12	4.09E-03
SLC7A8	-1.13	3.43E-04
CD1C	-1.15	3.78E-02
CCL17	-1.22	5.59E-03
HMG20B	-1.25	2.29E-04
PNPLA6	-1.25	1.07E-04
LPL	-1.34	2.59E-02
CHML	-1.43	9.61E-04
TPSB2	-1.51	1.30E-03
PDK4	-1.66	6.45E-03
PRSS33	-1.78	3.12E-02
IL1RL1	-1.91	1.17E-02

Supplementary Table 6. ReactomePA pathway enrichment of the 431 upregulated sputum transcriptomic DEGS which differentiate predicted U-BIOPRED asthmatic FZ predicted responder (PR) from predicted non-responder (PNR) patients (see supplementary Table 5). Pathway enrichment P values adjusted by FDR-BH with cut-off <0.05.

ID	Description	Gene Ratio	BgRatio	P value	P .adjust	Q value	geneID	Count
R-HSA-913531	Interferon Signaling	28/268	197/10554	9.04E-14	6.15E-11	5.45E-11	IFIT2/RSAD2/GBP5/IFIT3/ISG20/IFITM1/IFIT1/GBP1/GBP4/MX2/HERC5/IRF1/TRIM22/EIF4E3/IFITM2/IFITM3/PML/FCGR1B/STAT1/OAS3/TRIM5/GBP2/ICAM1/IRF2/GBP3/SP100/MX1/FCGR1A	28
R-HSA-909733	Interferon alpha/beta signaling	15/268	69/10554	1.32E-10	4.50E-08	3.98E-08	IFIT2/RSAD2/IFIT3/ISG20/IFITM1/IFIT1/MX2/IRF1/IFITM2/IFITM3/STAT1/OAS3/GBP2/IRF2/MX1	15
R-HSA-6798695	Neutrophil degranulation	38/268	479/10554	3.13E-10	7.09E-08	6.28E-08	S100A12/TNFAIP6/SELL/CXCR2/GPR84/HPSE/FPR2/CXCR1/MGAM/ORM1/QPCT/PTX3/CD177/LRG1/CLEC4D/CR1/CXCL1/SLPI/MNDA/FCAR/SIGLEC5/S100A8/NFAM1/S100P/S100A9/FGL2/FPR1/TLR2/CD93/FCN1/SLC15A4/CEACAM1/CHI3L1/AMPD3/CFP/CYSTM1/MMP25/CD55	38
R-HSA-877300	Interferon gamma signaling	16/268	92/10554	1.07E-09	1.82E-07	1.61E-07	GBP5/GBP1/GBP4/IRF1/TRIM22/PML/FCGR1B/STAT1/OAS3/TRIM5/GBP2/ICAM1/IRF2/GBP3/SP100/FCGR1A	16
R-HSA-449147	Signaling by Interleukins	36/268	462/10554	1.57E-09	2.14E-07	1.90E-07	CXCL10/S100A12/PTGS2/IRAK2/LMNB1/STAT4/IL6ST/BATF/CSF3R/IL1A/OSM/CXCL1/IL18RAP/JAK3/IL1B/PELI2/PSMB9/IL1R2/IRAK3/CSF2RB/CASP1/TBK1/PELI1/SERPINB2/STAT1/CCL5/ICAM1/TAB2/FPR1/VAV1/TNF/TIMP1/STX3/IL1RAP/HCK/NOD2	36

R-HSA-6783783	Interleukin-10 signaling	11/268	47/10554	1.79E-08	2.02E-06	1.79E-06	CXCL10/PTGS2/IL1A/CXCL1/IL1B/IL1R2/CCL5/ICAM1/FPR1/TNF/TIMP1	11
R-HSA-5686938	Regulation of TLR by endogenous ligand	7/268	19/10554	2.44E-07	2.37E-05	2.10E-05	TLR1/LY96/TLR4/S100A8/S100A9/TLR2/TLR6	7
R-HSA-168898	Toll-like Receptor Cascades	17/268	155/10554	3.85E-07	3.27E-05	2.90E-05	S100A12/IRAK2/UBE2D1/TLR1/TANK/LY96/PELI2/IRAK3/TBK1/PELI1/TLR4/S100A8/S100A9/TAB2/TLR2/NOD2/TLR6	17
R-HSA-166016	Toll Like Receptor 4 (TLR4) Cascade	15/268	130/10554	9.86E-07	7.45E-05	6.59E-05	S100A12/IRAK2/UBE2D1/TLR1/TANK/LY96/PELI2/IRAK3/TBK1/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	15
R-HSA-5602498	MyD88 deficiency (TLR2/4)	5/268	10/10554	2.31E-06	1.43E-04	1.27E-04	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-446652	Interleukin-1 family signaling	15/268	139/10554	2.32E-06	1.43E-04	1.27E-04	S100A12/IRAK2/IL1A/IL18RAP/IL1B/PELI2/PSMB9/IL1R2/IRAK3/CASP1/TBK1/PELI1/TAB2/IL1RAP/NOD2	15
R-HSA-5603041	IRAK4 deficiency (TLR2/4)	5/268	11/10554	4.15E-06	2.29E-04	2.03E-04	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-166058	MyD88:MAL(TIRAP) cascade initiated on plasma membrane	12/268	95/10554	4.71E-06	2.29E-04	2.03E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12
R-HSA-168188	Toll Like Receptor TLR6:TLR2 Cascade	12/268	95/10554	4.71E-06	2.29E-04	2.03E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12
R-HSA-198933	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	14/268	132/10554	6.21E-06	2.62E-04	2.32E-04	SELL/IFITM1/LILRA2/CLEC2B/LILRA1/TREML4/LILRA5/CD8A/SIGLEC5/ICAM1/SLAMF7/ICAM3/TREML2/FCGR1A	14
R-HSA-168179	Toll Like Receptor TLR1:TLR2 Cascade	12/268	98/10554	6.55E-06	2.62E-04	2.32E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12
R-HSA-181438	Toll Like Receptor 2 (TLR2) Cascade	12/268	98/10554	6.55E-06	2.62E-04	2.32E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12

R-HSA-9020702	Interleukin-1 signaling	12/268	103/10554	1.10E-05	4.16E-04	3.68E-04	S100A12/IRAK2/IL1A/IL1B/PELI2/PSMB9/IL1R2/IRAK3/PELI1/TAB2/IL1RAP/NOD2	12
R-HSA-380108	Chemokine receptors bind chemokines	8/268	48/10554	2.43E-05	8.71E-04	7.72E-04	CXCL10/CXCL11/CXCR2/CXCL9/CXCR1/CXCL1/CCRL2/CCL5	8
R-HSA-140534	Caspase activation via Death Receptors in the presence of ligand	5/268	17/10554	4.90E-05	1.67E-03	1.48E-03	TNFSF10/FAS/LY96/TLR4/CFLAR	5
R-HSA-168643	Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) signaling pathways	8/268	55/10554	6.74E-05	2.18E-03	1.93E-03	AIM2/IRAK2/CASP4/TNFAIP3/NLRP3/CASP1/TAB2/NOD2	8
R-HSA-936440	Negative regulators of DDX58/IFIH1 signaling	6/268	34/10554	1.87E-04	5.79E-03	5.13E-03	IFIH1/UBE2D1/HERC5/NLRC5/TNFAIP3/TBK1	6
R-HSA-168638	NOD1/2 Signaling Pathway	6/268	36/10554	2.60E-04	7.69E-03	6.81E-03	IRAK2/CASP4/TNFAIP3/CASP1/TAB2/NOD2	6
R-HSA-5260271	Diseases of Immune System	5/268	24/10554	2.91E-04	7.91E-03	7.01E-03	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-5602358	Diseases associated with the TLR signaling cascade	5/268	24/10554	2.91E-04	7.91E-03	7.01E-03	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-5689896	Ovarian tumor domain proteases	6/268	38/10554	3.53E-04	9.24E-03	8.18E-03	IFIH1/TNIP3/UBE2D1/TNFAIP3/PTEN/NOD2	6
R-HSA-6785807	Interleukin-4 and Interleukin-13 signaling	10/268	108/10554	4.09E-04	1.03E-02	9.12E-03	PTGS2/BATF/IL1A/OSM/JAK3/IL1B/STAT1/ICAM1/TNF/TIMP1	10
R-HSA-5357769	Caspase activation via extrinsic apoptotic signalling pathway	5/268	27/10554	5.19E-04	1.26E-02	1.12E-02	TNFSF10/FAS/LY96/TLR4/CFLAR	5
R-HSA-9014325	TICAM1,TRAF6-dependent induction of TAK1 complex	4/268	17/10554	7.45E-04	1.69E-02	1.50E-02	IRAK2/LY96/TLR4/TAB2	4

R-HSA-975163	IRAK2 mediated activation of TAK1 complex upon TLR7/8 or 9 stimulation	4/268	17/10554	7.45E-04	1.69E-02	1.50E-02	IRAK2/LY96/TLR4/TAB2	4
R-HSA-168928	DDX58/IFIH1-mediated induction of interferon-alpha/beta	8/268	78/10554	7.85E-04	1.72E-02	1.52E-02	S100A12/IFIH1/UBE2D1/HERC5/NLRC5/TANK/TNFAIP3/TBK1	8
R-HSA-1236975	Antigen processing-Cross presentation	9/268	99/10554	9.04E-04	1.74E-02	1.54E-02	TLR1/LY96/PSMB9/FCGR1B/TLR4/TLR2/TAP1/FCGR1A/TLR6	9
R-HSA-168164	Toll Like Receptor 3 (TLR3) Cascade	9/268	99/10554	9.04E-04	1.74E-02	1.54E-02	S100A12/IRAK2/UBE2D1/TANK/LY96/TBK1/TLR4/TAB2/NOD2	9
R-HSA-73887	Death Receptor Signalling	11/268	141/10554	9.18E-04	1.74E-02	1.54E-02	TNFSF10/FAS/ARHGEF40/TNFAIP3/NSMAF/TRAF1/PREX1/TAB2/VAV1/CFLAR/TNF	11
R-HSA-936964	Activation of IRF3/IRF7 mediated by TBK1/IKK epsilon	4/268	18/10554	9.39E-04	1.74E-02	1.54E-02	TANK/LY96/TBK1/TLR4	4
R-HSA-937072	TRAF6-mediated induction of TAK1 complex within TLR4 complex	4/268	18/10554	9.39E-04	1.74E-02	1.54E-02	IRAK2/LY96/TLR4/TAB2	4
R-HSA-166166	MyD88-independent TLR4 cascade	9/268	100/10554	9.72E-04	1.74E-02	1.54E-02	S100A12/IRAK2/UBE2D1/TANK/LY96/TBK1/TLR4/TAB2/NOD2	9
R-HSA-937061	TRIF(TICAM1)-mediated TLR4 signaling	9/268	100/10554	9.72E-04	1.74E-02	1.54E-02	S100A12/IRAK2/UBE2D1/TANK/LY96/TBK1/TLR4/TAB2/NOD2	9
R-HSA-5213460	RIPK1-mediated regulated necrosis	4/268	20/10554	1.43E-03	2.43E-02	2.15E-02	TNFSF10/FAS/CFLAR/MLKL	4
R-HSA-5218859	Regulated Necrosis	4/268	20/10554	1.43E-03	2.43E-02	2.15E-02	TNFSF10/FAS/CFLAR/MLKL	4
R-HSA-9020958	Interleukin-21 signaling	3/268	10/10554	1.70E-03	2.82E-02	2.50E-02	STAT4/JAK3/STAT1	3
R-HSA-3371378	Regulation by c-FLIP	3/268	11/10554	2.30E-03	3.55E-02	3.14E-02	TNFSF10/FAS/CFLAR	3

R-HSA-5218900	CASP8 activity is inhibited	3/268	11/10554	2.30E-03	3.55E-02	3.14E-02	TNFSF10/FAS/CFLAR	3
R-HSA-69416	Dimerization of procaspase-8	3/268	11/10554	2.30E-03	3.55E-02	3.14E-02	TNFSF10/FAS/CFLAR	3
R-HSA-975138	TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation	8/268	93/10554	2.46E-03	3.72E-02	3.29E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-168181	Toll Like Receptor 7/8 (TLR7/8) Cascade	8/268	94/10554	2.63E-03	3.81E-02	3.37E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-975155	MyD88 dependent cascade initiated on endosome	8/268	94/10554	2.63E-03	3.81E-02	3.37E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-8984722	Interleukin-35 Signalling	3/268	12/10554	3.01E-03	4.26E-02	3.77E-02	STAT4/IL6ST/STAT1	3
R-HSA-5621481	C-type lectin receptors (CLRs)	10/268	142/10554	3.31E-03	4.59E-02	4.07E-02	CLEC4E/UBE2D1/CLEC4D/IL1B/PSMB9/TAB2/PAK1/BCL10/ICAM3/PPP3CA	10
R-HSA-168138	Toll Like Receptor 9 (TLR9) Cascade	8/268	98/10554	3.41E-03	4.64E-02	4.11E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-1169410	Antiviral mechanism by IFN-stimulated genes	7/268	78/10554	3.58E-03	4.77E-02	4.23E-02	IFIT1/MX2/HERC5/EIF4E3/STAT1/OAS3/MX1	7

Supplementary Table 7. Clinical characteristics of U-BIOPRED asthmatic predicted responders and predicted non-responders who have sputum proteomic data in addition to the sputum transcriptomic data from which they were defined.

	Predicted responders	**Predicted non-responders**
n	18	14
Sex		
Male	13	7
Female	5	7
Age mean, years	49.33	56.29
Cohort		
SAns	10	9
SAs/ex	8	5

Supplementary Table 8. Clinical characteristics of U-BIOPRED asthmatic predicted responders and predicted non-responders who have blood proteomic data in addition to the sputum transcriptomic data from which they were defined.

	Predicted responders	**Predicted non-responders**
n	24	18
Sex		
Male	9	9
Female	15	9
Age mean, yrs	53.71	55.28
Cohort		
SAns	17	13
SAs/ex	7	5

Supplementary Table 9. Top and bottom 25 differentially expressed blood proteins that differentiate U-BIOPRED asthmatic FZ predicted responders (PRs) from predicted non-responders (PNRs) defined from asthma sputum GSVA FZ response signature ES which had serum proteomic data available (see Supplementary Table 4). Genes are ranked according to log_2 fold change.

Upregulated

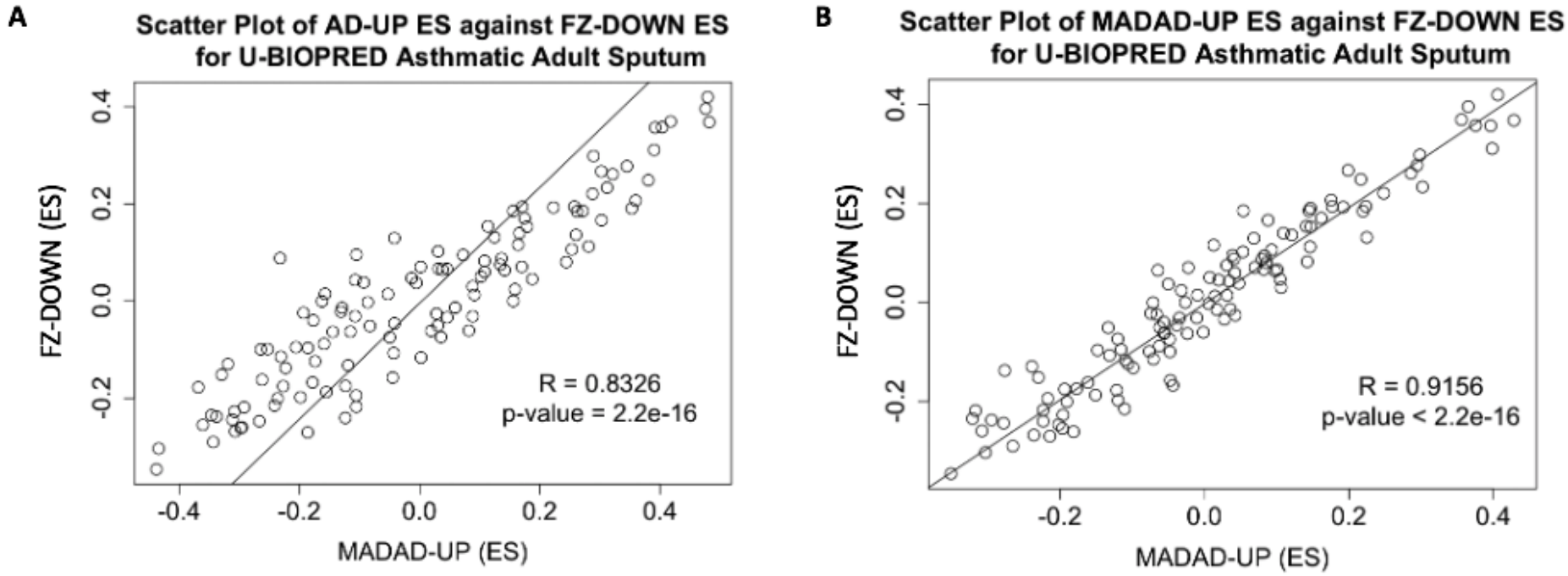
Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
Siglec_9	0.73	1.65	0.05	0.92
ARTS1	0.65	1.57	0.00	0.50
SSRP1	0.61	1.52	0.07	0.92
GSTA3	0.54	1.45	0.02	0.92
TECK	0.52	1.44	0.00	0.72
Glucagon	0.51	1.42	0.03	0.92
SORC2	0.49	1.40	0.14	0.92
b_Endorphin	0.48	1.39	0.05	0.92
GM_CSF	0.47	1.38	0.17	0.92
MICA	0.40	1.32	0.02	0.92
PAK6	0.40	1.32	0.13	0.92
MK08	0.37	1.30	0.03	0.92
vWF	0.37	1.30	0.08	0.92
TSP2	0.37	1.29	0.01	0.92
CRP	0.36	1.29	0.26	0.92
FSH	0.35	1.28	0.45	0.93
TLR2	0.35	1.28	0.28	0.92
C3d	0.34	1.27	0.35	0.92
pTEN	0.34	1.27	0.26	0.92
Aminoacylase_1	0.34	1.26	0.13	0.92
Fas_ligand_soluble	0.33	1.26	0.03	0.92
IL_8	0.33	1.26	0.09	0.92
COMMD7	0.33	1.26	0.12	0.92
I_TAC	0.32	1.25	0.19	0.92
BRF_1	0.32	1.25	0.15	0.92

Downregulated

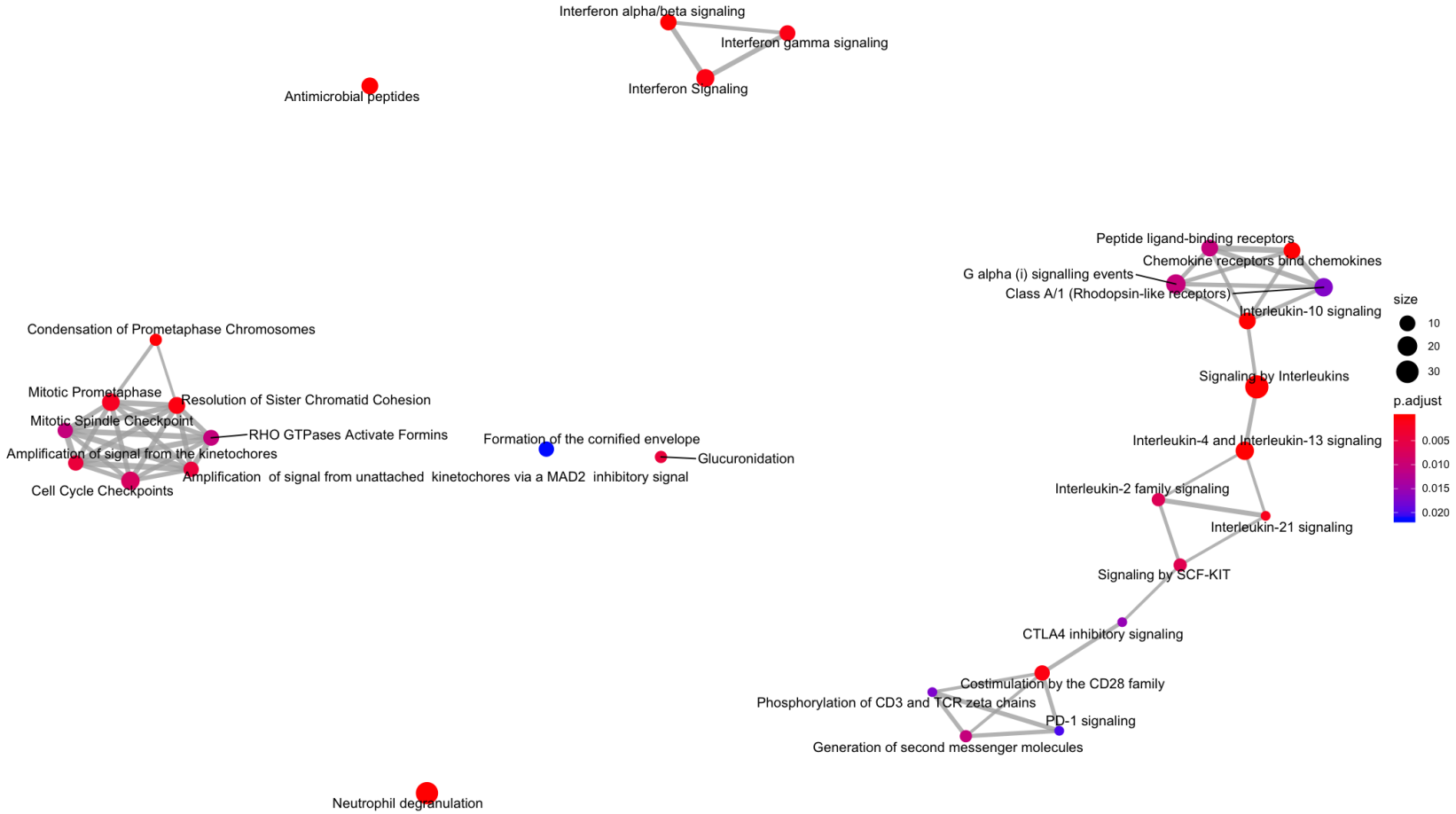
Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
CD5L	-0.23	0.85	0.30	0.92
PSA	-0.24	0.85	0.06	0.92
Carbonic_anhydrase_III	-0.24	0.85	0.19	0.92

PARC	-0.24	0.85	0.16	0.92
Renin	-0.24	0.85	0.24	0.92
MDC	-0.24	0.84	0.13	0.92
BCMA	-0.25	0.84	0.11	0.92
Trypsin	-0.25	0.84	0.08	0.92
BMPER	-0.25	0.84	0.04	0.92
LKHA4	-0.27	0.83	0.11	0.92
MMP_10	-0.27	0.83	0.09	0.92
MIP_3b	-0.28	0.82	0.16	0.92
BSP	-0.29	0.82	0.09	0.92
IgD	-0.31	0.81	0.64	0.96
C3a	-0.32	0.80	0.24	0.92
BLC	-0.35	0.79	0.26	0.92
Chk2	-0.35	0.78	0.33	0.92
PAPP_A	-0.36	0.78	0.10	0.92
Haptoglobin_Mixed_ Type	-0.37	0.77	0.06	0.92
IL_5_Ra	-0.41	0.75	0.01	0.92
NEUREGULIN_1	-0.48	0.72	0.19	0.92
TARC	-0.51	0.70	0.00	0.72
CYTT	-0.53	0.69	0.01	0.92
CYTN	-0.58	0.67	0.00	0.72
IgE	-1.14	0.45	0.06	0.92

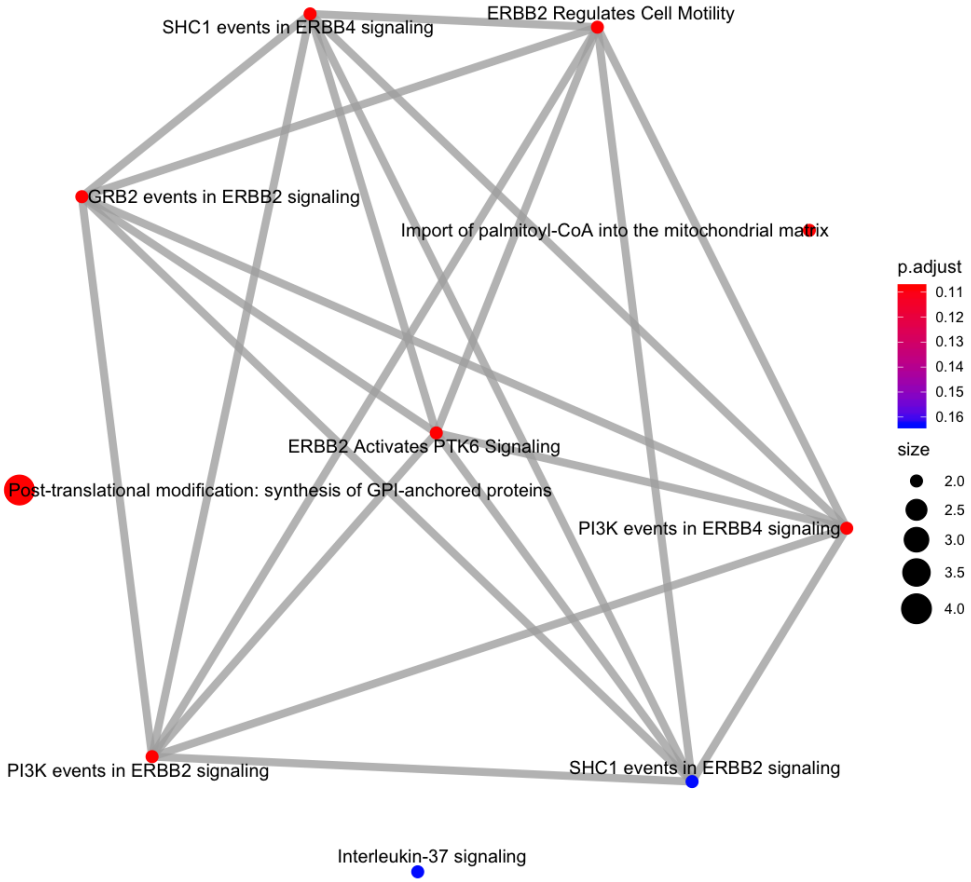
Supplementary Figure 1. Correlation of the enrichment score (ES) of genes down-regulated by fezikinumab (FZ) (FZ-DOWN) in adult asthmatic sputum against (A) the ES of genes up-regulated in lesional compared to non-lesional atopic dermatitis (AD) skin (AD-UP) and (B) the ES of a consensus AD gene signature (MADAD-UP).



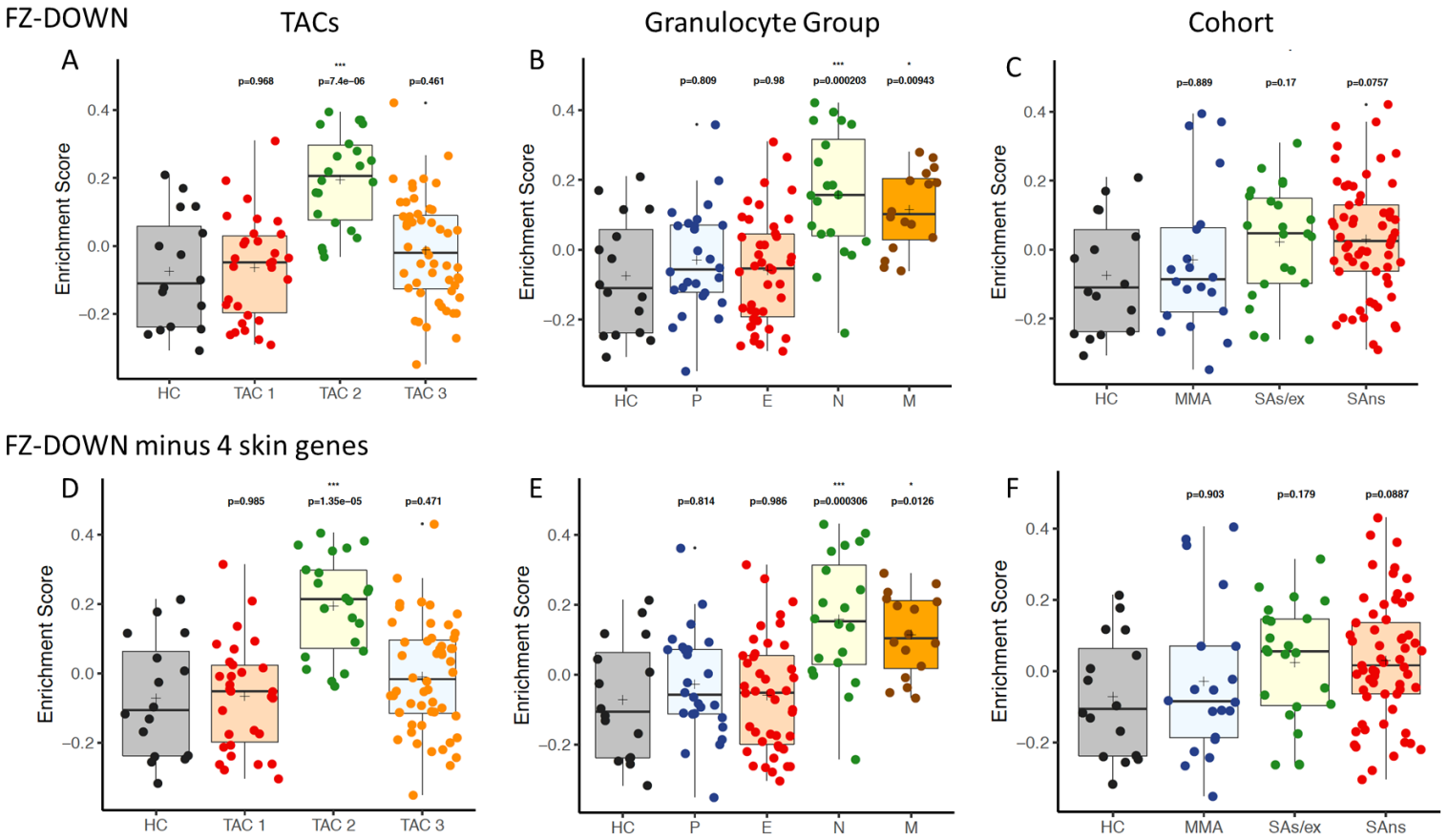
Supplementary Figure 2. Protein pathway analysis of the 40 significantly enriched pathways (false discovery rate, FDR<0.05) for differentially expressed genes down-regulated in atopic dermatitis lesional tissue following 12 weeks Fezakinumab treatment. See **Supplementary Table 8** for more details of these pathways.



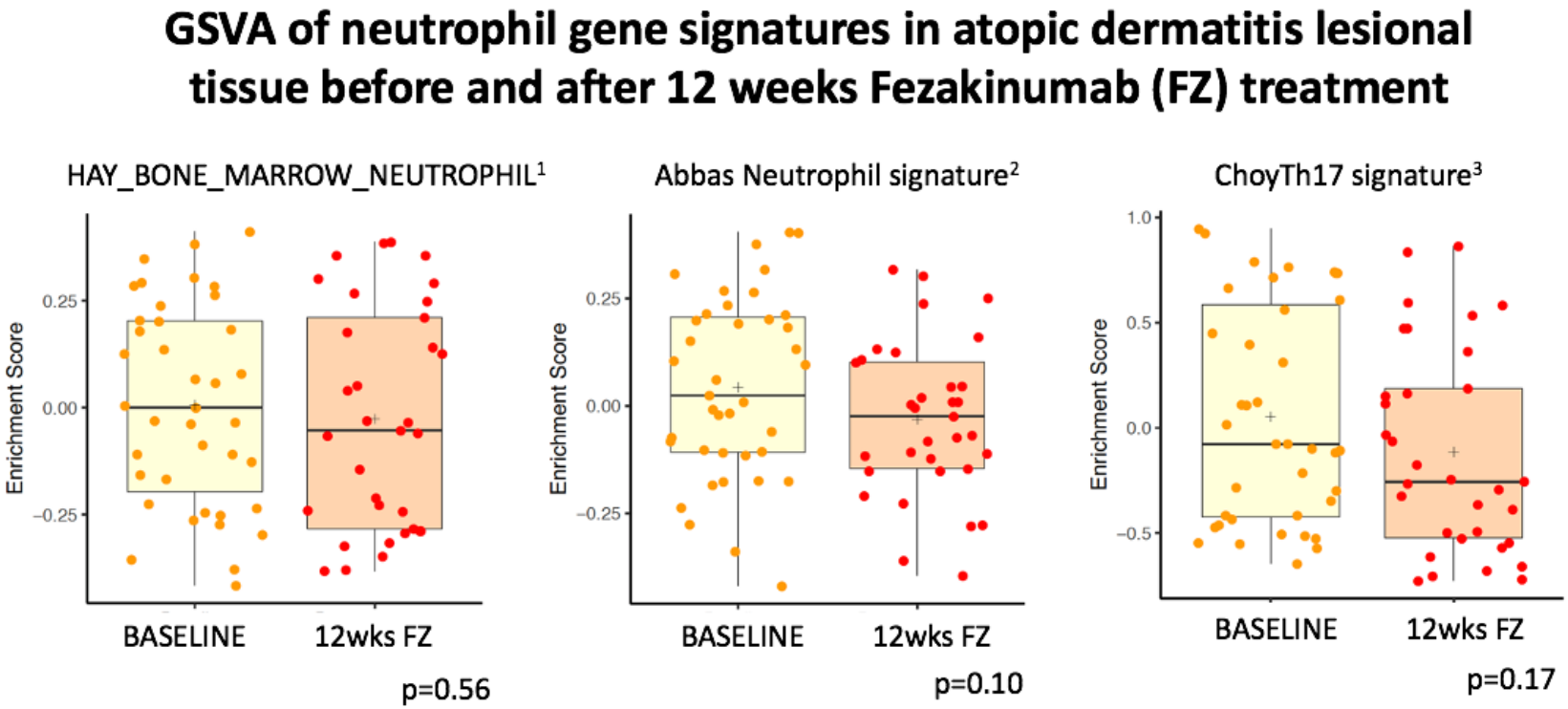
Supplementary Figure 3. Protein pathway analysis at a false discovery rate (FDR)<0.02 for differentially expressed genes up-regulated in atopic dermatitis lesional tissue following 12 weeks Fezakinumab treatment. See **Supplementary Table 9** for more details of these pathways. No pathways were enriched at a FDR<0.05.



Supplementary Figure 4. Minimal effect on the FZ-DOWN signature enrichment scores (A-C) in the sputum of asthmatic and healthy subjects with the removal of 4 skin-specific genes (D-F) when assessed by transcriptome associated cluster (TAC) status (A and D), sputum granulocyte group (B and E) or by asthma severity (C and F). SAns – severe asthma non-smoker; SAs/ex – severe asthma current or ex-smoker; MMA – mild-moderate asthma and HC – healthy control. P - paucigranulocytic; E – eosinophilic; N – neutrophilic and M – mixed granulocytic.



Supplementary Figure 5. Gene set variation analysis (GSVA) show no significant change in enrichment scores for neutrophil signatures in atopic dermatitis skin lesional tissue at baseline and after 12 weeks of Fezikinumab (FZ) treatment. The references from which the neutrophil signatures were obtained are provided beneath the figure.



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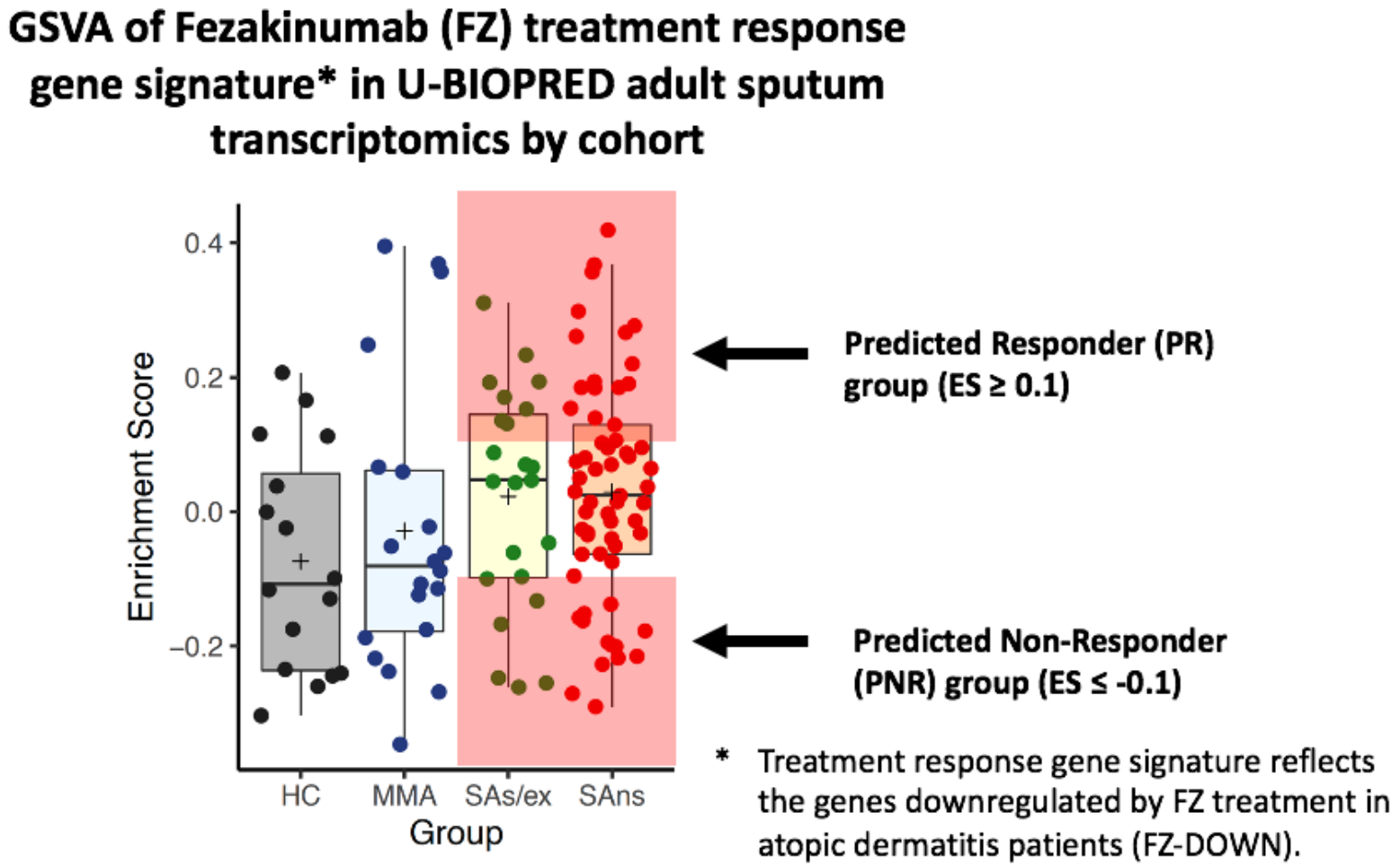
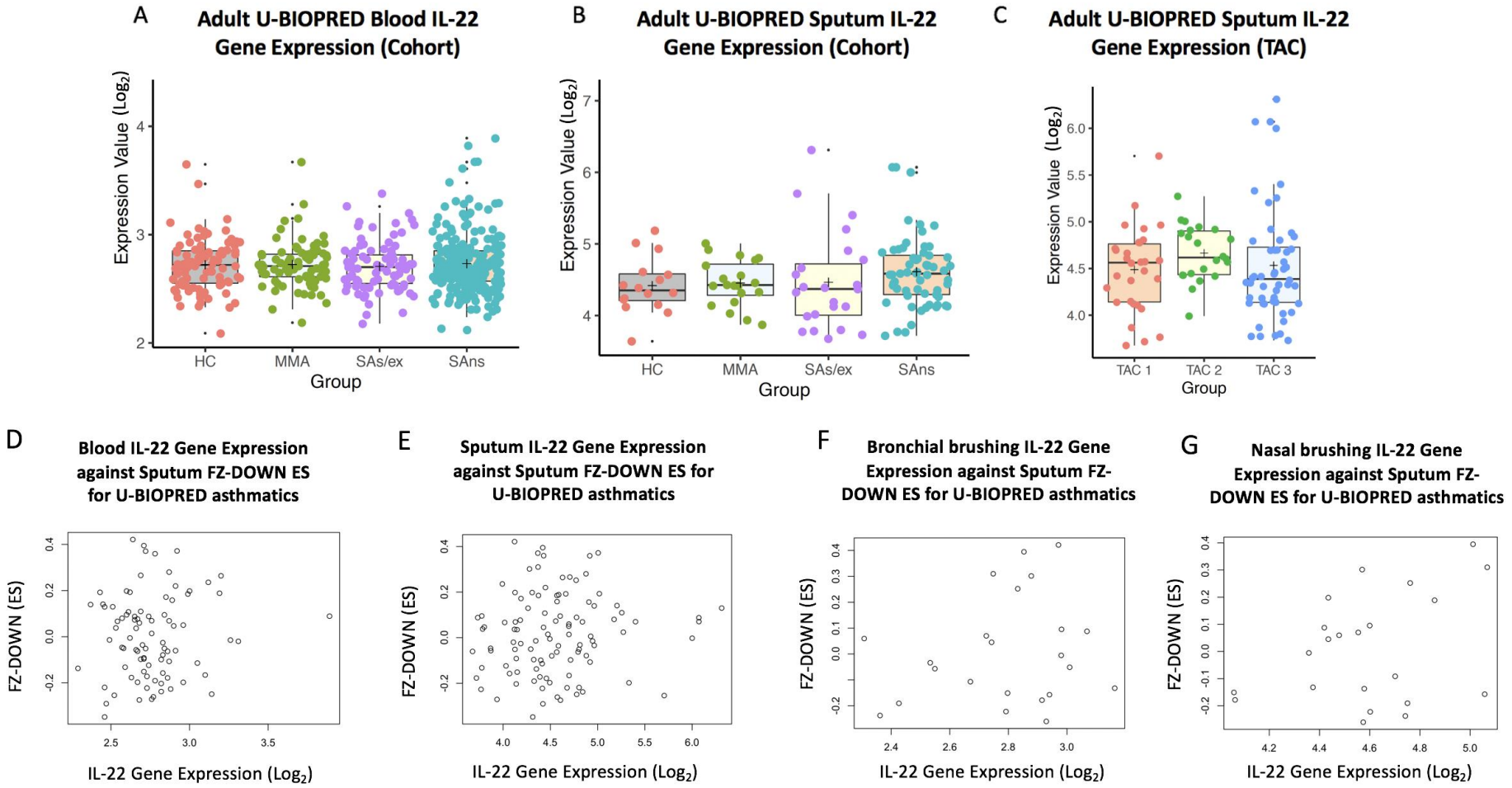
Supplementary Figure 6. Schematic of selection of patients with a high versus low enrichment score (ES) for the gene signature of genes down-regulated by Fezakinumab (FZ) in atopic dermatitis patients. Predicted responders (PRs) were considered as patients most highly enriched (n=26, ES ≥ +0.1) whilst predicted non-responders were defined as those lowly enriched (n=18, ES ≤ -0.1). Clinical and omics variables that defined these patients were then obtained from the U-BIOPRED dataset.



Supplementary Figure 7. IL-22 gene expression in blood (A) and sputum (B, C) is not significantly up-regulated according to asthma severity (B) or transcriptome associated cluster (TAC) status (C). IL-22 gene expression in blood (D), sputum (E), bronchial (F) and nasal (G) brushings does not correlate with the Fezakinumab (FZ)-DOWN signature sputum ES. SAns – severe asthma non-smoker; SAs/ex – severe asthma current or ex-smoker; MMA – mild-moderate asthma and HC – healthy control.



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Supplementary data

Mapping atopic dermatitis and anti-IL-22 response signatures to Type 2-low asthma

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Supplementary Table 1. Gene signatures

Signature name	Gene list	Reference
Brunner AD disease signature UP	S100A9 OASL C10orf99 AKR1B10 PRSS53 LINC01094 TEX101 TMPRSS4 SERPINB4 TRIM10 SERPINB3 KRT16 S100A8 CLEC7A KYNU SPRR2C IGFL1 S100A7A PI3 TFEC SERPINB13 EPSTI1 TCN1 FBN2 CCNA2 PTPRC SELL SAMSN1 HAS3 ICOS IL7R GZMB NELL2 CD274 CTLA4 RGS1 MMP12 LGALS2 CXCL2 CD2 DSC2 PI15 LILRB2 CST7 SERPINA1 COL6A5 GPR65 SASH3 RGS18 CXCL1 COL6A6 COL4A4 MMP1 GALNT6 DPY19L1 SPC25 BATF3 OAS2 PLAU STEAP4 RTP4 PLAC8 UBD ICAM1 SLAMF7 BCL2A1 UPP1 ADAM23 ITGAX CLEC4A LAIR2 GNLY CFB CYTIP SNX20 CH25H SAMD9L IL2RG ADAM19 ADAM8 MNDA XCL1 ST8SIA4 IL24 CCL5 XCL2 CD52 SELE CYP27B1 JAML IL15 JAK3 MIR155HG GPRIN3 IFI44 TNFAIP6 PIK3R5 IL13RA2 FAM129C MARK1 ARHGAP9 PRKCQ PLXDC1 RUBCNL TNC CD47 APELA ADAMTS12 CPXM1SPINK6 KLHL6 TDO2	1
Brunner AD disease signature DOWN	AGFG2 IL34 C5orf46 SCIN ARFGEF3 SYNE1 CPEB3 LOC284578 CHPT1 ST6GAL2 GPLD1 PNPLA3 SEMA3E LOC100996902 C1QTNF7 MFSD4A PSORS1C2 MACROD2 SCGB2A1 WIF1 FMO5 ZNF254 FABP7 MYOT FOLR1 NELL1 BTC PHYHIP IL37	1
MADAD UP signature	DEFB4A DEFB4B SERPINB4 S100A9 SERPINB3 MMP1 S100A7A IGFL1 MMP12 AKR1B10 C10orf99 PI3 OASL TMPRSS4 DSC2 GZMB SERPINB13 FOSL1 LCE3D SPRR2D SPRR2B SELE ARNTL2 SPRR3 SPRR1A COL4A4 CLEC7A COL6A5 CXCL10 CCL18 HPSE S100A8 RRM2 IL36G APELA NR4A3 PRSS53 APOBEC3A APOBEC3A_B KRT16 COL6A6 RGS1 EPSTI1 KLHDC7B HAS3 CXCL1 GALNT6 DLGAP5 CD274 CTLA4 CD1B SLAMF7 CEP55 LTF ASPM KIF4A MKI67 SLC2A1 CH25H ZBED2 GPR171 SAMSN1 KIF20A CDCA2 SPRR1B CENPE CXCL8 CCL22 S100A7 BUB1 RTP4 RGS20 NETO2 TRIP13 APOBEC3B CDK1 PKP1 PRKCQ IVL CDKN3 BCL2A1 TYMP ISG20 FCHSD1 IL7R SLC26A9 LGALS2 OAS2 NAPSB MMP9 CASC5 KIAA0101 RAB27A CST7 GPRIN1 TTC39A TGM1 INA VMP1 MIR21 CCL17 BLM NDC80 UGT1A1 UGT1A10 UGT1A8 UGT1A7 UGT1A6 UGT1A5 UGT1A9 UGT1A4 UGT1A3 MIR155HG MIR155 CXCL2 IL13RA2 CD28 CYTIP PRSS27 KLK8 KLK9 ITK NUF2 MPZL2 BIRC5 PI15 HMMR MXD1 HS3ST3A1 PRKCQ-AS1 MIAT ADAM19 GZMA SH3PXD2A-AS1 GPR183 BATF3 CNFN KIF14 SOCS3 AURKA IRF7 LCK NCAPG CENPF WNT5A OAS3 PRR11 PCDH7 MELK CDCA5 MOXD1 CCL26 KYNU MS4A14 SELL LTB KCNJ15 ANGPTL4 TNC CCNB1 STAT1 CCL2 SERPINA1 SASH3 ADAM8 IL36RN RSAD2 SMC4 IFI44 FOSB IRF1 CEMIP CD2 TEX101 TMEM45B F12 CCNB2 UBD GABBR1 C12orf56 PTPN7 ECT2 KLK10 PLAUR SPC25 FAM83A PLAU WWTR1 POLQ C9orf84 FGFBP1 SFT2D2 FPR1 C21orf91 SPTLC2 IL18RAP CCR7 CCL13 DSG3 PTPRC TOP2A KIF2C KIAA1644 SPRR2C CDC20 ASF1B SNX20 LINC01094 UBE2T CXorf65 CD3D TTK LILRB2 CCNA2 CENPA SLC35F6 HERC6 IL12RB1 SLC28A3 HBEGF CLEC10A OAS1 POLR3G IL2RG CDH3 XCL2 XCL1 CCL5 TNFRSF12A KRT6A MIR142 BUB1B NUSAP1 CYP27B1 CACNB4 ADAMDEC1 DIAPH3 SCO2 ADAM10 CD69 TIFAB PML MX1 SLC5A1 SLAMF1 CORO1A C12orf29 C12orf5 SAMD9 UBE2C DNASE1L3 ZC3H12A MIR6732 NEIL3 SLFN5 CKAP2L KRT6B P2RY1 P2RY2 FAS PLXDC1 LRP8 HJURP IL12RB2 PIK3CG	2

	ADAM23 THBD DEFB103B DEFB103A FAM124B FAM26F ITGAX LCN2 NAPG CHEK1 MNDA FOXM1 AIM2 SLAMF8 NUP50 IL4R FAIM3 CXADR ZBED6CL PBK PARVG CA2 GTSE1 DCAF8 GNLY GPSM3 SH3TC1 MND1 PARP9 STK17B KLK13 TAGAP AREG RALGPS2 CPEB2 CENPN CENPW HCK KLRB1 LOC100288860 HAL GK LCP2 01-Mar JAK3 MMP3 DEPDC1B SH2D5 C17orf96 FAM111B TBC1D10C LMNB2 FYB ITGAL CD1E RIT1 DUOXA1 MYO1B PHF19 UBE3C CCNE1 GNA15 KLRK1 KLRC4-KLRK1 IQGAP3 GBP1 SAMD9L KIF18B TFEC PDPN PTAFR PGF SYNCRIP ADAP2 SMOX CFB NOD2 CDT1 IL23A TRBC1 FLVCR2 ELL2 CDK5R1 MFHAS1 STAM2 LYN MMP19 DCTN5 THBS1 TPX2 E2F7 DCANP1 XAF1 CCL8 RELB MCOLN2 CHI3L2 GPR65 DPP4 ICOS ARHGAP9 AMD1 ACPP TRAT1 CCL19 ISG15 NDC1 ACAP1 CTSC PNPT1 PTX3 CD48 CDCA3 TK1 PIK3R5 FPR3 IFI27 TGM3 RAD51AP1 VSNL1 CXCL11 PLAGL2 CDC45 IL27RA MAP4K1 CD6 RASGRP1 SELPLG SNHG3 SNORA73A POC1A THAP2 LAMP3 UBA6 PRDM1 ZC3H12D RNF144B TMC5 PPP2R2C C15orf48	
MADAD DOWN Signature	LMOD1 MYRIP KIAA1324 RORC SLC13A2 FAXDC2 NALCN MEGF10 SEMA3E FAM189A2 LOC100507311 CYP2J2 ZNF471 LINC00663 AQP5 PRKAA2 TRHDE-AS1 GPRC5A CEACAM6 TIMP3 TMC4 FASN AWAT1 SHROOM3 RHPN2 SEMA3B MIR6872 AR SERHL2 SERHL ERBB4 PLCB4 SORBS2 KLRG2 KCNIP2 FGF1 ACVR1C IL20RA SSPN COCH EFHD1 FOXC1 LOC100507557 SYT17 EDAR PIP KRT77 GPRASP1 CA6 TLN2 C1orf95 NSUN7 MOGAT1 NEDD4L SCGB2B2 MAPT ATP6V1B1 CHRM3 CALB2 HSPB6 KRT19 CASQ2 FHL1 COPG2 COPG2IT1 CLIC5 MAP6 PER1 MIR6883 SNCA MUC20 PPARG MIR181A2HG GALNT15 OBP2B OBP2A SH3BGRL2 HRCT1 PPARGC1A CORO2B PSORS1C2 GYG2 GCHFR TRIM2 ACOX2 MRAP SLITRK4 TUSC5 CNKSR2 GPC4 FMO5 SCN7A ADH1B FST C14orf180 CD300LG SCIN MGST1 PLEKHB1 PRB1 STK32A SHANK2 ALDH1A2 LOC101928635 CES1 ATP6V0A4 CKMT2 TG DGAT2 ID4 C2orf40 MFSD4 SCGB1D2 ACADL GSTM5 RNF150 RNF128 LPL RERGL ATP1A2 PDK3 PNPLA3 ABHD12B MIR4454 GPAM C1orf115 MACROD2 FRZB MYBPC1 FA2H PRR4 PRH1-PRR4 VTCN1 SYNE1 BTNL9 TMEM56 PTCSC1 MUC7 ESRRG MYH11 C5orf46 ARFGEF3 HIF3A SGCG PRR15L LGALS12 FAR2 ACOT2 ACOT1 BPY2 PPP1R1B CFTR ALDH1L1 FADS1 MIR1908 KLB YBX2 MSMB ADAMTS9-AS2 01-Mar LGR5 ATP13A5 NR3C2 SGK2 PLEKHA6 AQP7 LOC100509620 LOC101930168 PON3 CUX2 PPP1R1A TMEM132C C1QTNF7 SYN2 ANGPTL7 CIDEA MYEOV ENPP5 ADIPOQ TSPAN8 FABP4 TMEM139 IL37 TF ADRB1 FADS2 RBP4 FGFBP2 LOC284578 C9orf152 FOLR1 HSPB7 MYOC TNMD THRSP PHYHIP GPD1 HAO2 GPIHBP1 CYP4F8 CLDN8 CIDEC SLC14A1 ELOVL3 WDR72 HMGCS2 TIMP4 ZBTB16 PLIN4 SCGB2A1 KANK4 LEP FABP7 PLIN1 GAL KRT79 BTC WIF1 HSD11B1 PM20D1	2
High IL-22 FZ response signature UP	CD300LG FOXC1 CLCNKB HIF3A FOXP2 ADAMTS9-AS2 FBXL13 LOC284578 SORBS2 SNRPN LINC01091 ZNF208 CAPN6 TG FRZB SLC17A7 LMO3 SSPN TRIL NR3C2 GLRB PHYHIP HSD11B1 RUNX1T1 WNT2 GSTM5 GAS2 TPM1 ESRRG KRT7 ADGRL3 CRISP2 GPRC5A SHANK2 ADAM22 ERBB4 TF SPDEF CHRM3 CLDN8 SCAI TSHR	1

	PLA2G5 MUC7 OGN PRR15L FA2H NDNF VTCN1 TGFB2 CNTNAP3P2 IL37 ALDH6A1 KIAA1324 PRND ISYNA1 CRISPLD1 KIAA1549 MMP16 RASD2 ROPN1 FZD8 H19 PPP1R1B LONRF2 PRICKLE1 COL8A1 TMC4 LRRN1 NEDD4L GPC6 WDR72 MYEOV TMEM139 ENPP5 FARP1 PPP1R9A TET1 DACH1 CNTN4 CNKSR2 PRRG3 PLEKHA6 THRSP STK32A C9orf152 B3GALT5 FREM2 KLRG2 ZNF582-AS1 ARFGEF3 PCDH20 SNORD114-3 MIR181A2HG MEGF10 RASSF6 HAND2-AS1 GRIA2 PRDM6 IL34 SLC25A36 GPIHBP1 PRKAA2 SLC26A7 ZNF542P C1QTNF7 HS3ST6 NEGR1 TMEM213 CPEB3 FLG-AS1 TBX18 PTPN14 EBF2 TBXA2R C5orf46 BTC AGFG2 ST6GAL2 PAK3 TMEM56	
High IL-22 FZ response signature DOWN	CLEC7A SAMSN1 SRGN MX1 CFB IRF1 LYN ICAM1 S100A8 RGS1 PLEK S100A9 PI3 IL2RG KYNU IFI6 CXCL1 MMP1 SELL CD52 SASH3 MNDA OAS2 LCP2 LRP8 GPR183 CD3E GZMA TCN1 OAS1 OASL CD2 UBD IL13RA2 SELE XCL1 CCL13 IGSF6 CD28 AKR1B10 TFEC PTPRC DEFB4A LGALS2 SPRR2D CORO1A IFI35 CYTIP SERPINB3 KRT16 GOSR2 CCL19 SH2D1A CST7 GZMB SERPINB4 ICOS PLAUR ITK CERKL PRSS53 XCL2 FAS SERPINB13 OAS3 TMPRSS4 RTP4 IL36G BATF3 ZNF557 TRIM10 CLEC4A CYLD HAS3 RGS18 TEX101 LCE3D IL7R ALYREF MIAT IKZF1 CD274 EPSTI1 JAK3 C10orf99 GALNT6 MIR155HG LINC01094 PRKCQ-AS1 S100A7A MTFR2 LINC01215 CTLA4 TIFAB SLAMF1 IGFL1 RSAD2 ST8SIA4 CCL18 CYP2E1 IL12RB1 WFDC12 PDZK1IP1 ACTR2 UBE3C ABCD3 PLA2G4D NAMPT ITGB2 SYNCRIP TYR MS4A4A LYZ CDKN3 MYRFL FAM111B SH3PXD2A-AS1 LOC100506411 NOP56 SLC2A1 TOP2A PPIF SMC4 LTF BIRC5 GBP1 CCNB2 SERPINA1 CXCL8 SPTLC2 FBN2 CDK1 CCNA2 BUB1B DLGAP5 MPZL2 CXCL9 MMP9 NDC80 FOSL1 INA SAMHD1 UGT1A9 CXCL10 MMP12 CCL5 TTK MELK LCK CENPE CCR1 CHEK1 CCL20 PLAU GNLY DSG3 BCL2A1 CD86 BLM CD8A KLK13 S100A12 S100A7 TGM3 TGM1 UGT1A6 P2RY2 SLC5A1 APOBEC3B ZNF165 CD1B RAB27B UGT1A1 ATP12A CENPF CCL22 CCL17 CYP2C18 UGT1A3 STAT3 RAB27A BUB1 CD24 RRM2 RIT1 SPC25 PRKCQ NAPG TPX2 KCNJ15 RGS20 LILRB2 CXCL11 CHRNA3 CSF2RA BIRC3 TTC39A APOBEC3A IL13RA1 MKI67 VEGFA LCN2 JMJD6 CEBPD CHI3L2 DOCK2 CD3D CASP4 CCL8 FUT3 CCNB1 SOD2 YME1L1 HTR3A TRAT1 TYMP RAB31 CEP55 NCAPG KIF20A PLAC8 RHOF TIGAR PBK SLAMF7 HERC6 HPSE CENPN TMC5 DHRS9 ASPM HS3ST3A1 CARD14 ARNTL2 SPRR2C C21orf91 ANGPTL4 IL26 UGT1A8 GPATCH4 RBM8A PLBD1 DTL NETO2 FLVCR2 EHF XPO5 CDCA3 NUF2 LYAR MCM10 MND1 SNHG12 DEFB103B FAM83D RPS16 TMEM45B CDCA2 FCHSD1 ARHGAP9 PTAFR ZBED6CL RAB7A GPRIN1 NAPSB C17orf96 DDIAS LRG1 DIAPH3 CKAP2L S1PR5 DUOXA2 DSC2 PRSS27 GRHL3 SULF2 KLK8 RNASE7 DENR LEO1 PANX1 RNF144B LYPD5 KLHDC7B KIF14 ULBP2 FAM83A LINC01214 LOC101927972 EPHA1 PPARD SLC26A9 RALGPS2 HBEGF TEAD4 STAT1	1
IL-22/Th22 signature gene list	AHR CALML5 CCR10 FLG IL22 IL32 KRT1 KRT10 LOR S100A7 S100A8 S100A9 S100P SERPINB1 SERPINB4 S100A12	1

Supplementary Table 2. ReactomePA pathway enrichment FZ-DOWN signature. P values adjusted by FDR-BH with cutoff <0.05.

ID	Description	Gene Ratio	Bg Ratio	P value	p. djust	Q value	geneID	Count
R-HSA-6783783	Interleukin-10 signaling	12/212	47/10554	8.90E-11	3.03E-08	2.68E-08	ICAM1/CXCL1/CCL19/CXCL8/CXCL10/CCL5/CCR1/CCL20/CD86/CCL22/STAT3/PTAFR	12
R-HSA-380108	Chemokine receptors bind chemokines	12/212	48/10554	1.16E-10	3.03E-08	2.68E-08	CXCL1/XCL1/CCL13/CCL19/XCL2/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/CXCL11	12
R-HSA-449147	Signaling by Interleukins	33/212	462/10554	1.61E-10	3.03E-08	2.68E-08	LYN/ICAM1/IL2RG/CXCL1/MMP1/IL13RA2/CCL19/IL36G/IL7R/JAK3/IL12RB1/ITGB2/BIRC5/CXCL8/MMP9/CXCL10/CCL5/LCK/CCR1/CCL20/CD86/S100A12/CCL22/STAT3/CSF2RA/IL13RA1/VEGFA/LCN2/CEBPD/SOD2/IL26/PTAFR/STAT1	33
R-HSA-6785807	Interleukin-4 and Interleukin-13 signaling	16/212	108/10554	3.98E-10	5.59E-08	4.95E-08	ICAM1/IL2RG/MMP1/IL13RA2/JAK3/ITGB2/BIRC5/CXCL8/MMP9/CCL22/STAT3/IL13RA1/VEGFA/LCN2/CEBPD/STAT1	16
R-HSA-6798695	Neutrophil degranulation	29/212	479/10554	9.37E-08	1.02E-05	9.04E-06	S100A8/S100A9/CXCL1/SELL/MNDA/TCN1/PTPRC/SERPINB3/PLAUR/ACTR2/ITGB2/LYZ/LTF/SERPINA1/MMP9/PLAU/S100A12/S100A7/RAB27A/LCN2/DOCK2/RAB31/PLAC8/RHOF/HPSE/ARHGAP9/PTAFR/RAB7A/LRG1	29

R-HSA-909733	Interferon alpha/beta signaling	11/212	69/10554	1.08E-07	1.02E-05	9.04E-06	MX1/IRF1/IFI6/OAS2/OAS1/OASL/IFI35/OAS3/RSAD2/SAMHD1/STAT1	11
R-HSA-6803157	Antimicrobial peptides	12/212	97/10554	5.02E-07	4.03E-05	3.57E-05	S100A8/S100A9/PI3/DEFB4A/S100A7A/LYZ/LTF/GNLY/S100A7/LCN2/DEFB103B/RNASE7	12
R-HSA-2514853	Condensation of Prometaphase Chromosomes	5/212	11/10554	1.30E-06	9.17E-05	8.13E-05	SMC4/CCNB2/CDK1/CCNB1/NCAPG	5
R-HSA-2500257	Resolution of Sister Chromatid Cohesion	12/212	124/10554	6.98E-06	0.00043	0.00038	BIRC5/CCNB2/CDK1/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CCNB1/CENPN/NUF2	12
R-HSA-913531	Interferon Signaling	15/212	197/10554	9.82E-06	0.00051	0.00045	MX1/IRF1/ICAM1/IFI6/OAS2/OAS1/OASL/IFI35/OAS3/TRIM10/RSAD2/GBP1/SAMHD1/PTAFR/STAT1	15
R-HSA-388841	Costimulation by the CD28 family	9/212	70/10554	1.01E-05	0.00051	0.00045	LYN/CD3E/CD28/ICOS/CD274/CTLA4/LCK/CD86/CD3D	9
R-HSA-877300	Interferon gamma signaling	10/212	92/10554	1.48E-05	0.00069	0.00061	IRF1/ICAM1/OAS2/OAS1/OASL/OAS3/TRIM10/GBP1/PTAFR/STAT1	10
R-HSA-9020958	Interleukin-21 signaling	4/212	10/10554	3.02E-05	0.00130	0.00115	IL2RG/JAK3/STAT3/STAT1	4
R-HSA-68877	Mitotic Prometaphase	14/212	198/10554	4.46E-05	0.00179	0.00158	SMC4/BIRC5/CCNB2/CDK1/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CCNB1/NCAPG/CENPN/NUF2	14
R-HSA-156588	Glucuronidation	5/212	25/10554	0.00011	0.00424	0.00376	UGT1A9/UGT1A6/UGT1A1/UGT1A3/UGT1A8	5

R-HSA-141424	Amplification of signal from the kinetochores	9/212	96/10554	0.00012	0.00424	0.00376	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2	9
R-HSA-141444	Amplification of signal from unattached kinetochores via a MAD2 inhibitory signal	9/212	96/10554	0.00012	0.00424	0.00376	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2	9
R-HSA-1433557	Signaling by SCF-KIT	6/212	43/10554	0.00020	0.00626	0.00554	LYN/MMP9/LCK/CHEK1/STAT3/STAT1	6
R-HSA-451927	Interleukin-2 family signaling	6/212	44/10554	0.00022	0.00675	0.00598	IL2RG/JAK3/LCK/STAT3/CSF2RA/STAT1	6
R-HSA-69620	Cell Cycle Checkpoints	16/212	293/10554	0.00027	0.00772	0.00684	BIRC5/CCNB2/CDK1/CCNA2/BUB1B/NDC80/CENPE/CHEK1/BLM/CENPF/BUB1/SPC25/CCNB1/CENPN/NUF2/MCM10	16
R-HSA-418594	G alpha (i) signalling events	19/212	396/10554	0.00038	0.0104	0.00920	RGS1/CXCL1/LRP8/GPR183/CCL13/AKR1B10/CCL19/RGS18/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/PRKCQ/RGS20/CXCL11/DHRS9/S1PR5	19
R-HSA-69618	Mitotic Spindle Checkpoint	9/212	112/10554	0.00041	0.0105	0.00932	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2	9
R-HSA-375276	Peptide ligand-binding receptors	12/212	190/10554	0.00044	0.0105	0.00932	CXCL1/XCL1/CCL13/CCL19/XCL2/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/CXCL11	12
R-HSA-5663220	RHO GTPases Activate Formins	10/212	138/10554	0.00045	0.0105	0.00932	BIRC5/BUB1B/NDC80/CENPE/CENPF/BUB1/SPC25/CENPN/NUF2/DIAPH3	10

R-HSA-202433	Generation of second messenger molecules	5/212	33/10554	0.00046	0.0105	0.00932	LCP2/CD3E/ITK/LCK/CD3D	5
R-HSA-389513	CTLA4 inhibitory signaling	4/212	21/10554	0.00072	0.0157	0.0139	LYN/CTLA4/LCK/CD86	4
R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	16/212	324/10554	0.00082	0.0172	0.0153	CXCL1/GPR183/XCL1/CCL13/CCL19/XCL2/CXCL8/CXCL9/CXCL10/CCL5/CCR1/CCL20/P2RY2/CXCL11/PTAFR/S1PR5	16
R-HSA-202427	Phosphorylation of CD3 and TCR zeta chains	4/212	22/10554	0.00087	0.0175	0.0155	CD3E/PTPRC/LCK/CD3D	4
R-HSA-389948	PD-1 signaling	4/212	23/10554	0.00103	0.0201	0.0178	CD3E/CD274/LCK/CD3D	4
R-HSA-6809371	Formation of the cornified envelope	9/212	129/10554	0.00114	0.0215	0.0191	PI3/SPRR2D/KRT16/LCE3D/DSG3/KLK13/TGM1/DSC2/KLK8	9
R-HSA-198933	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	9/212	132/10554	0.00135	0.0245	0.0217	ICAM1/SELL/CD3E/SH2D1A/ITGB2/CD8A/CD1B/CD3D/SLAMF7	9
R-HSA-69273	Cyclin A/B1/B2 associated events during G2/M transition	4/212	25/10554	0.00143	0.0245	0.0217	CCNB2/CDK1/CCNA2/CCNB1	4
R-HSA-8854691	Interleukin-20 family signaling	4/212	25/10554	0.00143	0.0245	0.0217	JAK3/STAT3/IL26/STAT1	4
R-HSA-389359	CD28 dependent Vav1 pathway	3/212	12/10554	0.00153	0.0247	0.0219	CD28/LCK/CD86	3

R-HSA-9020558	Interleukin-2 signaling	3/212	12/10554	0.00153	0.0247	0.0219	IL2RG/JAK3/LCK	3
R-HSA-75035	Chk1/Chk2(Cds1) mediated inactivation of Cyclin B:Cdk1 complex	3/212	13/10554	0.00196	0.0307	0.0272	CDK1/CHEK1/CCNB1	3
R-HSA-162658	Golgi Cisternae Pericentriolar Stack Reorganization	3/212	14/10554	0.00246	0.0365	0.0324	CCNB2/CDK1/CCNB1	3
R-HSA-8983432	Interleukin-15 signaling	3/212	14/10554	0.00246	0.0365	0.0324	IL2RG/JAK3/STAT3	3
R-HSA-202403	TCR signaling	8/212	119/10554	0.00270	0.0390	0.0345	LCP2/CD3E/PTPRC/ITK/LCK/PRKCQ/CD3D/TRAT1	8
R-HSA-2219530	Constitutive Signaling by Aberrant PI3K in Cancer	6/212	71/10554	0.00295	0.0416	0.0368	CD28/ICOS/LCK/CD86/TRAT1/HBEGF	6

Supplementary Table 3. ReactomePA pathway enrichment FZ-UP signature. P values adjusted by FDR-BH with cutoff <0.2.

ID	Description	Gene Ratio	Bg Ratio	P value	p. adjust	Q value	geneID	Count
R-HSA-1250342	PI3K events in ERBB4 signaling	2/60	10/10554	0.00138	0.108	0.102	ERBB4/BTC	2
R-HSA-163125	Post-translational modification: synthesis of GPI-anchored proteins	4/60	92/10554	0.00181	0.108	0.102	PRND/CNTN4/GPIHBP1/NEGR1	4
R-HSA-8847993	ERBB2 Activates PTK6 Signaling	2/60	13/10554	0.00238	0.108	0.102	ERBB4/BTC	2
R-HSA-1250347	SHC1 events in ERBB4 signaling	2/60	14/10554	0.00276	0.108	0.102	ERBB4/BTC	2
R-HSA-200425	Import of palmitoyl-CoA into the mitochondrial matrix	2/60	14/10554	0.00276	0.108	0.102	THRSP/PRKAA2	2
R-HSA-6785631	ERBB2 Regulates Cell Motility	2/60	15/10554	0.00318	0.108	0.102	ERBB4/BTC	2
R-HSA-1963640	GRB2 events in ERBB2 signaling	2/60	16/10554	0.00362	0.108	0.102	ERBB4/BTC	2
R-HSA-1963642	PI3K events in ERBB2 signaling	2/60	16/10554	0.00362	0.108	0.102	ERBB4/BTC	2
R-HSA-9008059	Interleukin-37 signaling	2/60	21/10554	0.00622	0.163	0.153	IL37/PTPN14	2
R-HSA-1250196	SHC1 events in ERBB2 signaling	2/60	22/10554	0.00682	0.163	0.153	ERBB4/BTC	2

Supplementary Table 4. Clinical characteristics of U-BIOPRED asthmatic Predicted Responders (PRs, ES of ≥ +0.1) against predicted non-responders (PNRs, ES of ≤ -0.1) defined from sputum transcriptomic GSVA of the FZ-DOWN signature. See main paper Table 1 for full breakdown.

	Predicted responders	**Predicted non-responders**
n	26	18
Sex		
Male	10	9
Female	16	9
Age mean, yrs	51.8	55.3
Cohort		
Severe Asthma non-smoker (SAns)	18	13
Severe Asthma smoker / ex-smoker (SAs/ex)	8	5

Supplementary Table 5. Top 431 sputum and 19 down transcriptomic DEGS which differentiate U-BIOPRED asthmatic FZ predicted responders (PRs) from predicted non-responders (PNRs). Genes are ranked according to log_2 fold change. All results are significant by FDR adjusted p value.

Upregulated Genes

Gene Symbol	Log_2 Fold Change	FDR-BH adjusted p value
KCNJ2	2.89	1.57E-08
ANXA3	2.87	2.87E-07
CXCL10	2.60	2.72E-05
LRRK2	2.48	1.50E-06
IFIT2	2.44	4.57E-06
GCH1	2.44	1.39E-07
S100A12	2.42	1.05E-06
GRIP1	2.41	2.33E-07
IFIH1	2.37	2.19E-07
HS3ST3B1	2.34	1.29E-08
CXCL11	2.34	5.47E-04
RSAD2	2.34	2.77E-04
GBP5	2.31	2.83E-07
TNFAIP6	2.31	2.60E-08
CALHM6	2.23	8.43E-05
PTGS2	2.21	2.08E-06
LOC105372881	2.17	1.78E-06
TNFSF10	2.15	4.59E-06
IFIT3	2.09	1.59E-04
CCL8	2.08	6.15E-03
FAS	2.06	2.60E-08
DOCK4	2.04	4.55E-06
ISG20	2.03	1.05E-06
KCNJ15	2.01	9.25E-06
STEAP4	2.00	2.52E-06
CLEC4E	1.96	1.50E-06
SELL	1.95	2.74E-06
CXCR2	1.95	7.26E-06
IFITM1	1.95	2.85E-07
GPR84	1.90	1.32E-05
SLC30A4	1.90	1.50E-06
APOBEC3A	1.89	1.85E-06
TNIP3	1.88	3.09E-04
UBD	1.87	1.05E-06
CXCL9	1.86	8.52E-04
TNFRSF10C	1.86	1.13E-03

ACOD1	1.85	3.68E-03
CMPK2	1.82	1.98E-03
AIM2	1.82	1.77E-06
LIMK2	1.81	4.45E-07
PDE4B	1.79	1.99E-06
TIFA	1.78	8.83E-06
VNN3	1.78	3.93E-05
IRAK2	1.77	2.43E-05
IFIT1	1.76	2.43E-03
HIVEP2	1.75	4.52E-06
LINC01270	1.75	7.31E-05
GBP1	1.74	1.17E-06
PROK2	1.73	7.05E-05
WDFY3	1.72	3.96E-07
GJB2	1.71	5.33E-04
LMNB1	1.70	1.07E-06
RAB33B	1.70	6.48E-05
IDO1	1.69	1.35E-03
HPSE	1.69	2.72E-05
N4BP1	1.69	3.77E-06
ZNF200	1.68	7.95E-06
PI3	1.67	6.13E-04
ANKRD22	1.66	4.28E-04
CASP4	1.66	6.41E-08
ALPL	1.64	4.30E-04
SERPINB9	1.64	1.70E-03
GBP4	1.64	6.35E-05
FPR2	1.63	7.13E-06
CXCR1	1.63	2.32E-04
FAM8A1	1.63	1.12E-05
TAGAP	1.63	2.43E-05
BAZ1A	1.63	4.67E-07
ARL5B	1.62	6.74E-06
BMT2	1.61	1.77E-06
LINC00266-1	1.61	4.00E-05
UBE2D1	1.60	1.05E-06
FBXO6	1.60	5.03E-04
MX2	1.59	6.30E-05
P2RY14	1.59	7.08E-04
MGAM	1.58	5.07E-04
LINC01215	1.58	8.85E-04
VNN2	1.58	4.52E-06
TSPAN2	1.57	7.10E-04

ORM1	1.57	3.98E-03
HAL	1.57	9.59E-04
SPATA13	1.57	2.79E-04
MRVI1	1.57	5.33E-04
KLHL15	1.57	1.54E-04
STAT4	1.57	1.86E-04
HERC5	1.56	1.41E-03
CEP83	1.55	9.96E-06
TMEM154	1.55	2.22E-04
FAM129A	1.55	1.68E-06
CMTM2	1.55	2.15E-05
LILRA2	1.55	1.50E-05
SP110	1.54	7.99E-05
CD274	1.54	1.19E-05
KRT23	1.54	7.26E-06
GLT1D1	1.54	1.45E-04
NBN	1.54	8.17E-06
KATNBL1	1.54	4.16E-04
QPCT	1.54	7.14E-07
MANSC1	1.54	1.04E-03
LINC01093	1.53	2.34E-03
ZNF267	1.53	2.33E-07
SMA5	1.53	2.88E-03
IL6ST	1.53	1.96E-05
MSRB1	1.53	6.35E-05
PLXNC1	1.53	8.43E-05
ARHGEF40	1.53	1.42E-04
NMI	1.53	4.14E-07
CCDC71L	1.53	1.42E-04
TLR1	1.52	2.33E-04
PTX3	1.52	2.50E-03
FFAR2	1.52	6.17E-04
BATF	1.52	1.46E-05
TNFSF13B	1.52	7.26E-06
GBP1P1	1.51	2.99E-04
ERV3-2	1.51	1.93E-03
ARFIP1	1.51	5.41E-05
PFKFB3	1.51	5.33E-06
RUBCNL	1.50	1.64E-03
CSGALNACT1	1.50	2.24E-04
IRF1	1.50	2.88E-05
LOC399716	1.50	1.05E-04
NAIP	1.50	1.15E-05

KIAA1551	1.49	9.48E-05
CLEC2B	1.49	4.43E-08
CD177	1.49	2.88E-03
LRG1	1.48	6.00E-05
NLRC5	1.47	4.47E-05
C5orf58	1.47	1.23E-05
LILRA1	1.47	9.06E-04
THAP2	1.46	2.22E-04
CREB5	1.46	4.29E-05
CLEC4D	1.46	1.02E-04
PLEK	1.46	6.41E-08
GALNT3	1.46	6.09E-04
GNG2	1.45	2.83E-04
LOC145474	1.45	5.33E-04
CSF3R	1.45	1.05E-04
MIR29A	1.44	1.51E-04
DGAT2	1.44	1.31E-05
TRIM22	1.43	1.48E-04
CR1	1.43	1.51E-04
GIMAP4	1.43	9.40E-06
C15orf48	1.43	9.25E-06
TMCC3	1.43	5.04E-04
RAPGEF2	1.42	6.41E-05
TANK	1.42	2.10E-05
SORL1	1.41	8.43E-05
BATF2	1.41	1.32E-03
RHOH	1.41	4.14E-04
RNF175	1.41	2.60E-05
HCAR3	1.40	5.99E-05
LOC114224	1.40	5.72E-05
AQP9	1.40	1.30E-06
IFI16	1.40	2.63E-06
LOC254896	1.39	5.47E-04
IL1A	1.38	8.26E-03
ZC3H12D	1.38	8.83E-05
HES1	1.38	2.46E-03
CARD16	1.38	5.24E-06
TOPORS	1.38	2.92E-04
KREMEN1	1.38	1.92E-04
PDP1	1.38	1.42E-03
OSM	1.38	5.72E-05
TREML4	1.38	1.05E-04
CXCL1	1.38	1.92E-04

GTF2IP12	1.37	9.25E-06
CR1L	1.36	1.34E-03
MIR155HG	1.36	4.14E-04
IL18RAP	1.36	7.52E-03
JAK3	1.36	1.21E-04
TNFAIP3	1.36	4.73E-04
NSMAF	1.36	2.20E-04
EIF4E3	1.36	1.27E-05
SGTB	1.36	1.32E-03
MCTP2	1.35	1.10E-02
SLPI	1.35	7.69E-04
IL1B	1.35	1.92E-04
LY96	1.34	3.77E-05
IFITM2	1.34	4.52E-06
ANTXR2	1.33	1.44E-05
SERPINB9P1	1.33	5.19E-03
CASP5	1.32	1.86E-04
SCLT1	1.32	1.72E-03
IFITM3	1.32	3.77E-06
NLRP3	1.32	2.51E-03
EREG	1.32	1.44E-03
P2RY13	1.32	2.73E-03
FAM126B	1.31	3.67E-04
RPGR	1.31	2.29E-04
SNX18	1.31	6.08E-05
PELI2	1.31	4.72E-04
MNDA	1.31	3.93E-05
LINC00528	1.31	9.56E-04
ACAT2	1.30	1.74E-03
PSMB9	1.30	2.09E-05
KCNH7	1.30	9.73E-05
HMGN2P46	1.30	9.87E-03
CLOCK	1.30	4.45E-04
PML	1.29	4.28E-04
IL1R2	1.29	2.93E-04
CYP4F3	1.28	8.08E-03
SNN	1.28	4.67E-07
WTAP	1.28	4.52E-06
SLC7A5	1.28	3.42E-04
IRAK3	1.28	3.67E-03
SLC40A1	1.28	7.56E-03
ADGRE2	1.28	7.95E-06
LRRC70	1.27	3.26E-02

CD48	1.27	1.81E-05
PPP1R3B	1.27	8.77E-03
PIP4P2	1.27	1.27E-03
ADAMDEC1	1.27	1.37E-02
LILRA5	1.27	1.14E-03
ATG3	1.27	4.49E-07
CD8A	1.27	1.22E-05
CSF2RB	1.27	1.05E-06
FCAR	1.26	4.20E-04
GZMB	1.26	8.76E-03
NFKBIZ	1.26	1.05E-06
TRAPPC13	1.26	4.08E-04
CCRL2	1.26	5.40E-04
TRAF1	1.26	9.95E-04
FCGR1B	1.26	3.58E-04
CASP1	1.26	4.35E-05
DYSF	1.25	6.05E-04
SIGLEC5	1.25	8.35E-04
CPD	1.25	8.63E-04
SAMD9	1.25	2.11E-04
BRE-AS1	1.25	1.76E-03
DAPP1	1.25	3.37E-05
C1D	1.25	2.76E-03
TBK1	1.24	1.36E-03
LINC00641	1.24	4.49E-04
LOC100289230	1.24	3.73E-03
POLB	1.24	9.88E-08
SBF2	1.24	1.69E-03
SLC39A8	1.23	7.05E-05
RAB5A	1.23	3.04E-04
PELI1	1.23	1.56E-04
TMEM185B	1.23	7.95E-06
RNF149	1.23	1.27E-05
SAMD9L	1.22	8.40E-04
TLR4	1.22	3.24E-05
USP10	1.22	1.92E-04
XRN1	1.22	1.85E-03
ZC3H12C	1.22	2.84E-03
SERPINB2	1.21	1.40E-02
STAT1	1.21	9.43E-05
PREX1	1.21	3.18E-04
SMCHD1	1.21	6.63E-05
PSMB8-AS1	1.21	8.07E-04

TMEM71	1.21	3.03E-03
OAS3	1.21	8.26E-03
RASSF2	1.21	1.63E-04
S100A8	1.20	2.28E-05
KCNJ2-AS1	1.20	1.56E-03
NFAM1	1.20	8.83E-06
RNF19B	1.20	6.68E-04
CCL5	1.20	1.13E-03
ZNF292	1.20	1.42E-03
BICRAL	1.20	9.02E-05
FLJ32255	1.19	2.89E-04
S100P	1.19	2.03E-03
SLC22A4	1.19	9.43E-05
SCARF1	1.19	2.66E-03
EGR3	1.19	2.67E-03
PLSCR1	1.19	2.03E-07
LAMP3	1.19	1.54E-02
TRIM5	1.19	7.52E-03
HSD17B11	1.19	4.48E-04
GSEC	1.18	1.14E-03
HNRNPH2	1.18	6.36E-05
GBP2	1.18	1.22E-06
LOC100130357	1.18	2.33E-03
CREM	1.18	5.99E-05
S100A9	1.18	9.16E-06
GIMAP8	1.18	2.05E-03
MIR3945HG	1.17	4.73E-04
UBR1	1.17	2.25E-03
LINC01003	1.17	4.15E-03
TCP11L2	1.17	2.36E-03
CNOT11	1.17	1.86E-04
COQ10B	1.17	2.62E-05
PCBP1-AS1	1.16	2.06E-02
ICAM1	1.16	1.74E-06
RNF213	1.16	9.69E-04
IPO11	1.16	5.41E-03
ABHD3	1.15	2.50E-03
RABGAP1L	1.15	9.11E-03
CDC42SE2	1.15	7.86E-05
ST8SIA4	1.15	1.53E-04
NFE2L2	1.15	8.17E-06
SEMA4A	1.15	2.60E-05
PRKCB	1.15	4.20E-03

CEP68	1.15	3.01E-05
RGL4	1.15	9.09E-03
IRF2	1.14	1.35E-05
PARP14	1.14	3.67E-05
RIPOR2	1.14	4.05E-03
GTF2B	1.14	1.20E-04
MAP3K13	1.13	8.25E-04
MARCKS	1.13	4.67E-07
GBP3	1.13	2.88E-02
SP100	1.13	1.26E-04
FGL2	1.13	4.51E-04
TAB2	1.13	1.92E-04
SECTM1	1.13	1.11E-04
ELF2	1.13	2.29E-04
PAK1	1.13	1.72E-03
TCFL5	1.13	6.43E-03
GPR27	1.13	5.34E-04
FPR1	1.13	8.68E-06
DNTTIP2	1.13	3.01E-05
PTEN	1.12	5.67E-04
GZMA	1.12	1.26E-04
LINC00877	1.12	6.10E-04
VAV1	1.12	2.02E-05
TMEM88	1.12	3.85E-04
TLR2	1.12	6.85E-04
CD93	1.11	5.08E-03
BTBD19	1.11	1.14E-03
DDX60L	1.11	1.74E-04
ZBTB21	1.11	3.68E-03
PHF11	1.11	1.44E-04
CHD1	1.11	7.04E-05
PARP8	1.11	4.20E-04
MAK	1.11	9.43E-05
ZNF107	1.11	2.06E-02
TAP1	1.11	8.51E-06
SUSD6	1.10	3.42E-05
CFLAR	1.10	2.60E-05
SNORD89	1.10	1.23E-03
ACSL1	1.10	9.16E-06
BCL10	1.10	4.05E-04
RIN2	1.10	6.61E-03
SLAMF7	1.10	1.05E-02
TECPR2	1.10	2.30E-03

TET3	1.10	1.58E-03
CNTNAP3	1.09	3.94E-02
CHMP2B	1.09	1.35E-03
IDI2-AS1	1.09	2.73E-03
ICAM3	1.09	2.43E-03
EPM2AIP1	1.09	7.35E-03
DTX3L	1.09	5.29E-06
FCN1	1.09	1.42E-03
G0S2	1.09	4.74E-05
SPATA1	1.09	1.23E-05
CHST15	1.08	2.13E-03
TNF	1.08	1.09E-02
SLC15A4	1.08	3.56E-04
HSD11B1-AS1	1.08	3.78E-03
BTNL8	1.08	6.11E-03
TIMP1	1.08	1.21E-04
STX3	1.07	8.73E-03
BCL2A1	1.07	2.85E-07
CDC42EP2	1.07	3.95E-03
PTENP1	1.07	4.54E-04
ERI1	1.07	5.18E-03
AGTPBP1	1.07	9.43E-05
SPAG9	1.07	3.58E-04
GPR65	1.07	5.48E-04
IL1RAP	1.07	2.79E-03
AP1AR	1.06	5.33E-03
LOC441081	1.06	3.57E-03
C16orf54	1.06	7.31E-03
BID	1.06	3.63E-05
NFE2L3	1.06	5.24E-04
TULP2	1.06	1.90E-02
MX1	1.06	6.15E-03
HCG26	1.06	1.52E-03
ZC3HAV1	1.05	6.33E-04
NAF1	1.05	5.17E-03
APOBEC3G	1.05	1.40E-03
C11orf54	1.05	2.76E-03
CHSY1	1.05	1.33E-03
HCK	1.05	4.47E-06
CEACAM1	1.05	5.10E-03
PSTPIP2	1.05	1.72E-03
DLEU2L	1.05	9.65E-03
RNF141	1.05	4.04E-04

PPA1	1.05	3.52E-03
LAP3	1.05	5.84E-03
LOC101928361	1.04	3.60E-03
PPP2R2A	1.04	2.73E-03
CHI3L1	1.04	4.93E-02
PJA2	1.04	2.60E-05
MITD1	1.04	1.29E-04
RALB	1.04	5.21E-05
SMA4	1.04	7.45E-03
BCL3	1.04	1.48E-04
KDM6A	1.04	5.95E-05
LCP2	1.04	7.04E-06
AMPD3	1.04	2.82E-04
PPIF	1.04	3.01E-04
SECISBP2	1.04	1.22E-04
CFP	1.03	3.01E-04
ELOVL5	1.03	1.74E-06
CYSTM1	1.03	1.96E-05
DENND5A	1.03	4.90E-05
ASPRV1	1.03	1.44E-03
IDI1	1.03	1.21E-04
IGSF6	1.03	3.38E-05
MMP25	1.03	5.80E-03
NKG7	1.03	1.48E-04
KDM7A	1.03	1.59E-03
NFE4	1.03	2.35E-02
PHF20L1	1.02	1.33E-04
LYRM1	1.02	1.04E-03
NOD2	1.02	1.64E-03
MIA3	1.02	1.78E-03
GK3P	1.02	1.66E-04
PPP4R2	1.02	3.06E-05
CD55	1.02	8.79E-05
MIER1	1.02	5.94E-05
MLKL	1.02	1.24E-03
PIK3AP1	1.02	1.68E-05
ESCO1	1.01	2.59E-03
TREML2	1.01	1.70E-03
FCGR1A	1.01	3.45E-04
DDX60	1.01	1.34E-02
SRSF12	1.01	2.69E-03
DLGAP1-AS2	1.01	1.45E-03
WASHC2A	1.01	1.69E-03

USP32	1.01	3.95E-03
SPTY2D1	1.01	1.52E-03
ZFX	1.00	5.01E-04
CSRNP1	1.00	2.64E-04
PPP3CA	1.00	3.95E-03
SLAMF1	1.00	4.26E-03
TDP2	1.00	3.68E-03
TLR6	1.00	4.16E-04

Downregulated Genes

Gene Symbol	**Log_2 Fold Change**	**FDR-BH adjusted p value**
TMC6	-1.01	5.61E-05
RNA45SN5	-1.01	1.33E-03
DTX4	-1.01	2.30E-02
TPSAB1	-1.05	4.41E-03
BHLHE41	-1.06	4.39E-02
SPOCD1	-1.08	9.65E-03
FIG4	-1.10	1.58E-02
TGM2	-1.12	4.09E-03
SLC7A8	-1.13	3.43E-04
CD1C	-1.15	3.78E-02
CCL17	-1.22	5.59E-03
HMG20B	-1.25	2.29E-04
PNPLA6	-1.25	1.07E-04
LPL	-1.34	2.59E-02
CHML	-1.43	9.61E-04
TPSB2	-1.51	1.30E-03
PDK4	-1.66	6.45E-03
PRSS33	-1.78	3.12E-02
IL1RL1	-1.91	1.17E-02

Supplementary Table 6. ReactomePA pathway enrichment of the 431 upregulated sputum transcriptomic DEGS which differentiate predicted U-BIOPRED asthmatic FZ predicted responder (PR) from predicted non-responder (PNR) patients (see supplementary Table 5). Pathway enrichment P values adjusted by FDR-BH with cut-off <0.05.

ID	Description	Gene Ratio	BgRatio	P value	P .adjust	Q value	geneID	Count
R-HSA-913531	Interferon Signaling	28/268	197/10554	9.04E-14	6.15E-11	5.45E-11	IFIT2/RSAD2/GBP5/IFIT3/ISG20/IFITM1/IFIT1/GBP1/GBP4/MX2/HERC5/IRF1/TRIM22/EIF4E3/IFITM2/IFITM3/PML/FCGR1B/STAT1/OAS3/TRIM5/GBP2/ICAM1/IRF2/GBP3/SP100/MX1/FCGR1A	28
R-HSA-909733	Interferon alpha/beta signaling	15/268	69/10554	1.32E-10	4.50E-08	3.98E-08	IFIT2/RSAD2/IFIT3/ISG20/IFITM1/IFIT1/MX2/IRF1/IFITM2/IFITM3/STAT1/OAS3/GBP2/IRF2/MX1	15
R-HSA-6798695	Neutrophil degranulation	38/268	479/10554	3.13E-10	7.09E-08	6.28E-08	S100A12/TNFAIP6/SELL/CXCR2/GPR84/HPSE/FPR2/CXCR1/MGAM/ORM1/QPCT/PTX3/CD177/LRG1/CLEC4D/CR1/CXCL1/SLPI/MNDA/FCAR/SIGLEC5/S100A8/NFAM1/S100P/S100A9/FGL2/FPR1/TLR2/CD93/FCN1/SLC15A4/CEACAM1/CHI3L1/AMPD3/CFP/CYSTM1/MMP25/CD55	38
R-HSA-877300	Interferon gamma signaling	16/268	92/10554	1.07E-09	1.82E-07	1.61E-07	GBP5/GBP1/GBP4/IRF1/TRIM22/PML/FCGR1B/STAT1/OAS3/TRIM5/GBP2/ICAM1/IRF2/GBP3/SP100/FCGR1A	16
R-HSA-449147	Signaling by Interleukins	36/268	462/10554	1.57E-09	2.14E-07	1.90E-07	CXCL10/S100A12/PTGS2/IRAK2/LMNB1/STAT4/IL6ST/BATF/CSF3R/IL1A/OSM/CXCL1/IL18RAP/JAK3/IL1B/PELI2/PSMB9/IL1R2/IRAK3/CSF2RB/CASP1/TBK1/PELI1/SERPINB2/STAT1/CCL5/ICAM1/TAB2/FPR1/VAV1/TNF/TIMP1/STX3/IL1RAP/HCK/NOD2	36

R-HSA-6783783	Interleukin-10 signaling	11/268	47/10554	1.79E-08	2.02E-06	1.79E-06	CXCL10/PTGS2/IL1A/CXCL1/IL1B/IL1R2/CCL5/ICAM1/FPR1/TNF/TIMP1	11
R-HSA-5686938	Regulation of TLR by endogenous ligand	7/268	19/10554	2.44E-07	2.37E-05	2.10E-05	TLR1/LY96/TLR4/S100A8/S100A9/TLR2/TLR6	7
R-HSA-168898	Toll-like Receptor Cascades	17/268	155/10554	3.85E-07	3.27E-05	2.90E-05	S100A12/IRAK2/UBE2D1/TLR1/TANK/LY96/PELI2/IRAK3/TBK1/PELI1/TLR4/S100A8/S100A9/TAB2/TLR2/NOD2/TLR6	17
R-HSA-166016	Toll Like Receptor 4 (TLR4) Cascade	15/268	130/10554	9.86E-07	7.45E-05	6.59E-05	S100A12/IRAK2/UBE2D1/TLR1/TANK/LY96/PELI2/IRAK3/TBK1/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	15
R-HSA-5602498	MyD88 deficiency (TLR2/4)	5/268	10/10554	2.31E-06	1.43E-04	1.27E-04	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-446652	Interleukin-1 family signaling	15/268	139/10554	2.32E-06	1.43E-04	1.27E-04	S100A12/IRAK2/IL1A/IL18RAP/IL1B/PELI2/PSMB9/IL1R2/IRAK3/CASP1/TBK1/PELI1/TAB2/IL1RAP/NOD2	15
R-HSA-5603041	IRAK4 deficiency (TLR2/4)	5/268	11/10554	4.15E-06	2.29E-04	2.03E-04	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-166058	MyD88:MAL(TIRAP) cascade initiated on plasma membrane	12/268	95/10554	4.71E-06	2.29E-04	2.03E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12
R-HSA-168188	Toll Like Receptor TLR6:TLR2 Cascade	12/268	95/10554	4.71E-06	2.29E-04	2.03E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12
R-HSA-198933	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	14/268	132/10554	6.21E-06	2.62E-04	2.32E-04	SELL/IFITM1/LILRA2/CLEC2B/LILRA1/TREML4/LILRA5/CD8A/SIGLEC5/ICAM1/SLAMF7/ICAM3/TREML2/FCGR1A	14
R-HSA-168179	Toll Like Receptor TLR1:TLR2 Cascade	12/268	98/10554	6.55E-06	2.62E-04	2.32E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12
R-HSA-181438	Toll Like Receptor 2 (TLR2) Cascade	12/268	98/10554	6.55E-06	2.62E-04	2.32E-04	S100A12/IRAK2/TLR1/LY96/PELI2/IRAK3/PELI1/TLR4/TAB2/TLR2/NOD2/TLR6	12

R-HSA-9020702	Interleukin-1 signaling	12/268	103/10554	1.10E-05	4.16E-04	3.68E-04	S100A12/IRAK2/IL1A/IL1B/PELI2/PSMB9/IL1R2/IRAK3/PELI1/TAB2/IL1RAP/NOD2	12
R-HSA-380108	Chemokine receptors bind chemokines	8/268	48/10554	2.43E-05	8.71E-04	7.72E-04	CXCL10/CXCL11/CXCR2/CXCL9/CXCR1/CXCL1/CCRL2/CCL5	8
R-HSA-140534	Caspase activation via Death Receptors in the presence of ligand	5/268	17/10554	4.90E-05	1.67E-03	1.48E-03	TNFSF10/FAS/LY96/TLR4/CFLAR	5
R-HSA-168643	Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) signaling pathways	8/268	55/10554	6.74E-05	2.18E-03	1.93E-03	AIM2/IRAK2/CASP4/TNFAIP3/NLRP3/CASP1/TAB2/NOD2	8
R-HSA-936440	Negative regulators of DDX58/IFIH1 signaling	6/268	34/10554	1.87E-04	5.79E-03	5.13E-03	IFIH1/UBE2D1/HERC5/NLRC5/TNFAIP3/TBK1	6
R-HSA-168638	NOD1/2 Signaling Pathway	6/268	36/10554	2.60E-04	7.69E-03	6.81E-03	IRAK2/CASP4/TNFAIP3/CASP1/TAB2/NOD2	6
R-HSA-5260271	Diseases of Immune System	5/268	24/10554	2.91E-04	7.91E-03	7.01E-03	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-5602358	Diseases associated with the TLR signaling cascade	5/268	24/10554	2.91E-04	7.91E-03	7.01E-03	TLR1/LY96/TLR4/TLR2/TLR6	5
R-HSA-5689896	Ovarian tumor domain proteases	6/268	38/10554	3.53E-04	9.24E-03	8.18E-03	IFIH1/TNIP3/UBE2D1/TNFAIP3/PTEN/NOD2	6
R-HSA-6785807	Interleukin-4 and Interleukin-13 signaling	10/268	108/10554	4.09E-04	1.03E-02	9.12E-03	PTGS2/BATF/IL1A/OSM/JAK3/IL1B/STAT1/ICAM1/TNF/TIMP1	10
R-HSA-5357769	Caspase activation via extrinsic apoptotic signalling pathway	5/268	27/10554	5.19E-04	1.26E-02	1.12E-02	TNFSF10/FAS/LY96/TLR4/CFLAR	5
R-HSA-9014325	TICAM1,TRAF6-dependent induction of TAK1 complex	4/268	17/10554	7.45E-04	1.69E-02	1.50E-02	IRAK2/LY96/TLR4/TAB2	4

R-HSA-975163	IRAK2 mediated activation of TAK1 complex upon TLR7/8 or 9 stimulation	4/268	17/10554	7.45E-04	1.69E-02	1.50E-02	IRAK2/LY96/TLR4/TAB2	4
R-HSA-168928	DDX58/IFIH1-mediated induction of interferon-alpha/beta	8/268	78/10554	7.85E-04	1.72E-02	1.52E-02	S100A12/IFIH1/UBE2D1/HERC5/NLRC5/TANK/TNFAIP3/TBK1	8
R-HSA-1236975	Antigen processing-Cross presentation	9/268	99/10554	9.04E-04	1.74E-02	1.54E-02	TLR1/LY96/PSMB9/FCGR1B/TLR4/TLR2/TAP1/FCGR1A/TLR6	9
R-HSA-168164	Toll Like Receptor 3 (TLR3) Cascade	9/268	99/10554	9.04E-04	1.74E-02	1.54E-02	S100A12/IRAK2/UBE2D1/TANK/LY96/TBK1/TLR4/TAB2/NOD2	9
R-HSA-73887	Death Receptor Signalling	11/268	141/10554	9.18E-04	1.74E-02	1.54E-02	TNFSF10/FAS/ARHGEF40/TNFAIP3/NSMAF/TRAF1/PREX1/TAB2/VAV1/CFLAR/TNF	11
R-HSA-936964	Activation of IRF3/IRF7 mediated by TBK1/IKK epsilon	4/268	18/10554	9.39E-04	1.74E-02	1.54E-02	TANK/LY96/TBK1/TLR4	4
R-HSA-937072	TRAF6-mediated induction of TAK1 complex within TLR4 complex	4/268	18/10554	9.39E-04	1.74E-02	1.54E-02	IRAK2/LY96/TLR4/TAB2	4
R-HSA-166166	MyD88-independent TLR4 cascade	9/268	100/10554	9.72E-04	1.74E-02	1.54E-02	S100A12/IRAK2/UBE2D1/TANK/LY96/TBK1/TLR4/TAB2/NOD2	9
R-HSA-937061	TRIF(TICAM1)-mediated TLR4 signaling	9/268	100/10554	9.72E-04	1.74E-02	1.54E-02	S100A12/IRAK2/UBE2D1/TANK/LY96/TBK1/TLR4/TAB2/NOD2	9
R-HSA-5213460	RIPK1-mediated regulated necrosis	4/268	20/10554	1.43E-03	2.43E-02	2.15E-02	TNFSF10/FAS/CFLAR/MLKL	4
R-HSA-5218859	Regulated Necrosis	4/268	20/10554	1.43E-03	2.43E-02	2.15E-02	TNFSF10/FAS/CFLAR/MLKL	4
R-HSA-9020958	Interleukin-21 signaling	3/268	10/10554	1.70E-03	2.82E-02	2.50E-02	STAT4/JAK3/STAT1	3
R-HSA-3371378	Regulation by c-FLIP	3/268	11/10554	2.30E-03	3.55E-02	3.14E-02	TNFSF10/FAS/CFLAR	3

R-HSA-5218900	CASP8 activity is inhibited	3/268	11/10554	2.30E-03	3.55E-02	3.14E-02	TNFSF10/FAS/CFLAR	3
R-HSA-69416	Dimerization of procaspase-8	3/268	11/10554	2.30E-03	3.55E-02	3.14E-02	TNFSF10/FAS/CFLAR	3
R-HSA-975138	TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation	8/268	93/10554	2.46E-03	3.72E-02	3.29E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-168181	Toll Like Receptor 7/8 (TLR7/8) Cascade	8/268	94/10554	2.63E-03	3.81E-02	3.37E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-975155	MyD88 dependent cascade initiated on endosome	8/268	94/10554	2.63E-03	3.81E-02	3.37E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-8984722	Interleukin-35 Signalling	3/268	12/10554	3.01E-03	4.26E-02	3.77E-02	STAT4/IL6ST/STAT1	3
R-HSA-5621481	C-type lectin receptors (CLRs)	10/268	142/10554	3.31E-03	4.59E-02	4.07E-02	CLEC4E/UBE2D1/CLEC4D/IL1B/PSMB9/TAB2/PAK1/BCL10/ICAM3/PPP3CA	10
R-HSA-168138	Toll Like Receptor 9 (TLR9) Cascade	8/268	98/10554	3.41E-03	4.64E-02	4.11E-02	S100A12/IRAK2/LY96/PELI2/PELI1/TLR4/TAB2/NOD2	8
R-HSA-1169410	Antiviral mechanism by IFN-stimulated genes	7/268	78/10554	3.58E-03	4.77E-02	4.23E-02	IFIT1/MX2/HERC5/EIF4E3/STAT1/OAS3/MX1	7

Supplementary Table 7. Clinical characteristics of U-BIOPRED asthmatic predicted responders and predicted non-responders who have sputum proteomic data in addition to the sputum transcriptomic data from which they were defined.

	Predicted responders	**Predicted non-responders**
n	18	14
Sex		
Male	13	7
Female	5	7
Age mean, years	49.33	56.29
Cohort		
SAns	10	9
SAs/ex	8	5

Supplementary Table 8. Clinical characteristics of U-BIOPRED asthmatic predicted responders and predicted non-responders who have blood proteomic data in addition to the sputum transcriptomic data from which they were defined.

	Predicted responders	**Predicted non-responders**
n	24	18
Sex		
Male	9	9
Female	15	9
Age mean, yrs	53.71	55.28
Cohort		
SAns	17	13
SAs/ex	7	5

Supplementary Table 9. Top and bottom 25 differentially expressed blood proteins that differentiate U-BIOPRED asthmatic FZ predicted responders (PRs) from predicted non-responders (PNRs) defined from asthma sputum GSVA FZ response signature ES which had serum proteomic data available (see Supplementary Table 4). Genes are ranked according to log_2 fold change.

Upregulated

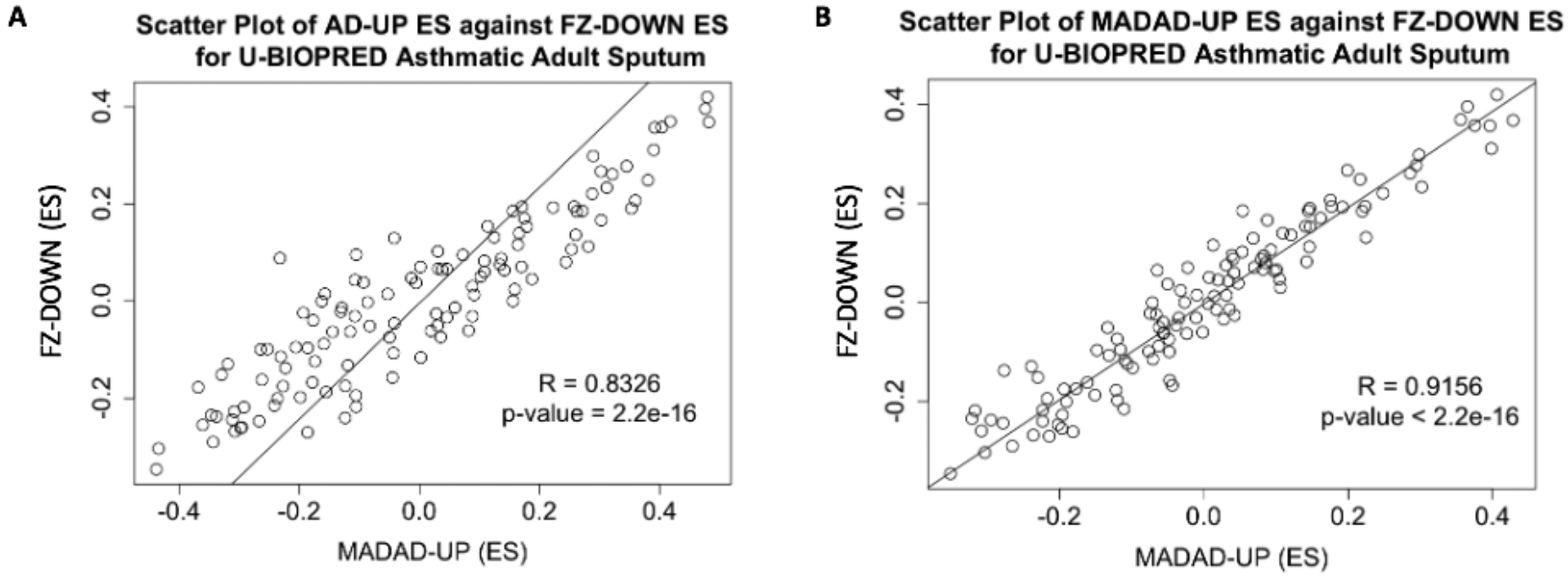
Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
Siglec_9	0.73	1.65	0.05	0.92
ARTS1	0.65	1.57	0.00	0.50
SSRP1	0.61	1.52	0.07	0.92
GSTA3	0.54	1.45	0.02	0.92
TECK	0.52	1.44	0.00	0.72
Glucagon	0.51	1.42	0.03	0.92
SORC2	0.49	1.40	0.14	0.92
b_Endorphin	0.48	1.39	0.05	0.92
GM_CSF	0.47	1.38	0.17	0.92
MICA	0.40	1.32	0.02	0.92
PAK6	0.40	1.32	0.13	0.92
MK08	0.37	1.30	0.03	0.92
vWF	0.37	1.30	0.08	0.92
TSP2	0.37	1.29	0.01	0.92
CRP	0.36	1.29	0.26	0.92
FSH	0.35	1.28	0.45	0.93
TLR2	0.35	1.28	0.28	0.92
C3d	0.34	1.27	0.35	0.92
pTEN	0.34	1.27	0.26	0.92
Aminoacylase_1	0.34	1.26	0.13	0.92
Fas_ligand_soluble	0.33	1.26	0.03	0.92
IL_8	0.33	1.26	0.09	0.92
COMMD7	0.33	1.26	0.12	0.92
I_TAC	0.32	1.25	0.19	0.92
BRF_1	0.32	1.25	0.15	0.92

Downregulated

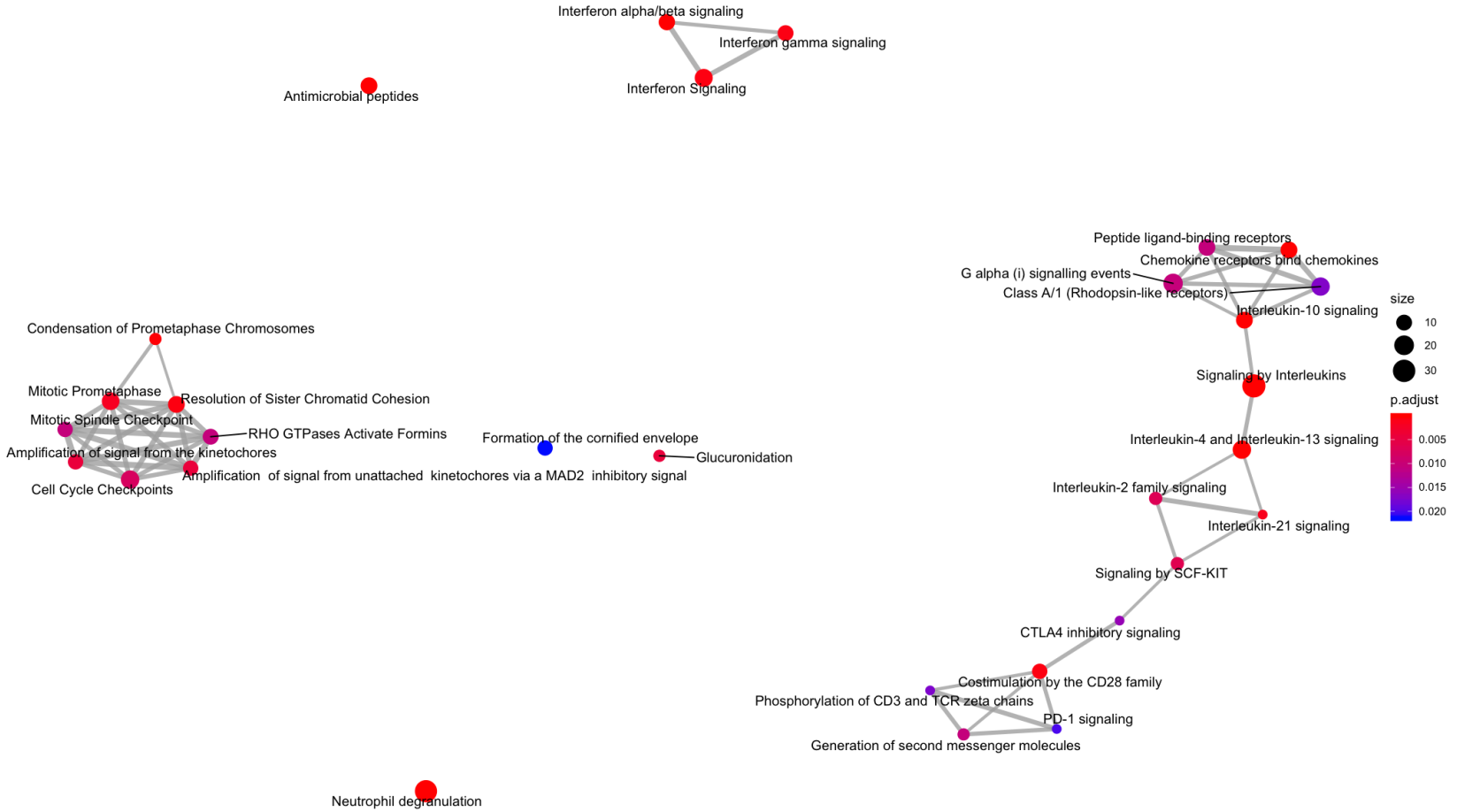
Gene Symbol	Log2 Fold Change	Fold Change	P value	FDR-BH adjusted P value
CD5L	-0.23	0.85	0.30	0.92
PSA	-0.24	0.85	0.06	0.92
Carbonic_anhydrase_III	-0.24	0.85	0.19	0.92

PARC	-0.24	0.85	0.16	0.92
Renin	-0.24	0.85	0.24	0.92
MDC	-0.24	0.84	0.13	0.92
BCMA	-0.25	0.84	0.11	0.92
Trypsin	-0.25	0.84	0.08	0.92
BMPER	-0.25	0.84	0.04	0.92
LKHA4	-0.27	0.83	0.11	0.92
MMP_10	-0.27	0.83	0.09	0.92
MIP_3b	-0.28	0.82	0.16	0.92
BSP	-0.29	0.82	0.09	0.92
IgD	-0.31	0.81	0.64	0.96
C3a	-0.32	0.80	0.24	0.92
BLC	-0.35	0.79	0.26	0.92
Chk2	-0.35	0.78	0.33	0.92
PAPP_A	-0.36	0.78	0.10	0.92
Haptoglobin_Mixed_ Type	-0.37	0.77	0.06	0.92
IL_5_Ra	-0.41	0.75	0.01	0.92
NEUREGULIN_1	-0.48	0.72	0.19	0.92
TARC	-0.51	0.70	0.00	0.72
CYTT	-0.53	0.69	0.01	0.92
CYTN	-0.58	0.67	0.00	0.72
IgE	-1.14	0.45	0.06	0.92

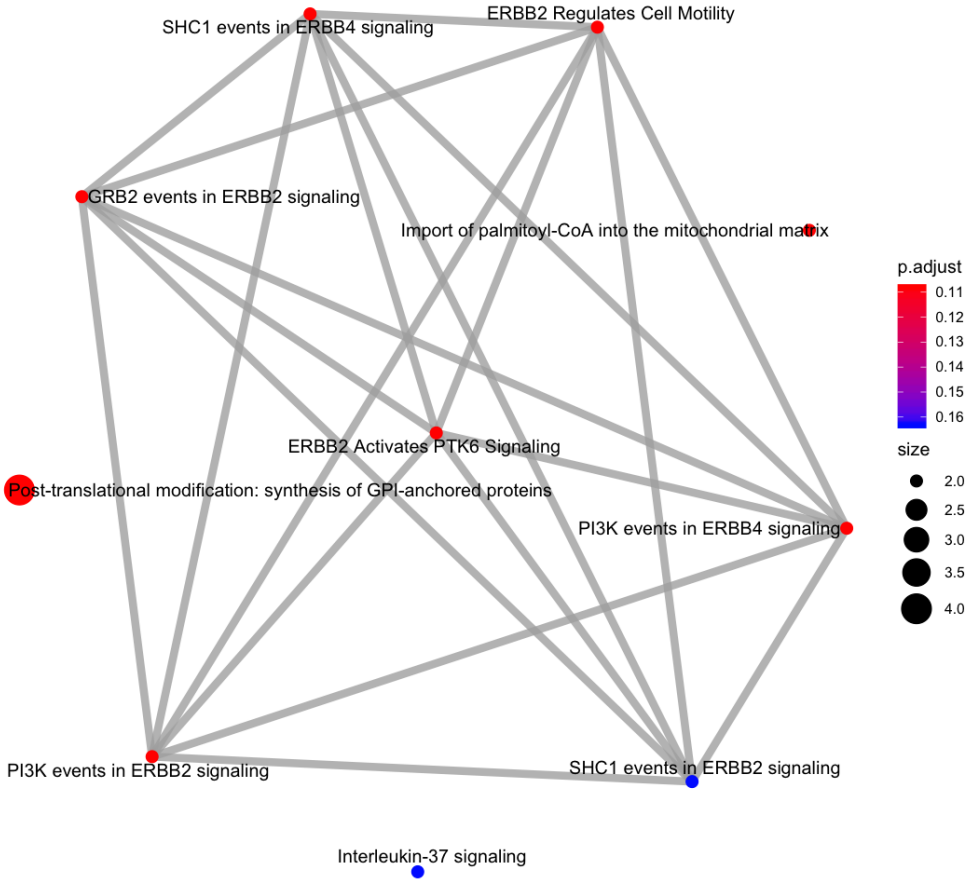
Supplementary Figure 1. Correlation of the enrichment score (ES) of genes down-regulated by fezikinumab (FZ) (FZ-DOWN) in adult asthmatic sputum against (A) the ES of genes up-regulated in lesional compared to non-lesional atopic dermatitis (AD) skin (AD-UP) and (B) the ES of a consensus AD gene signature (MADAD-UP).



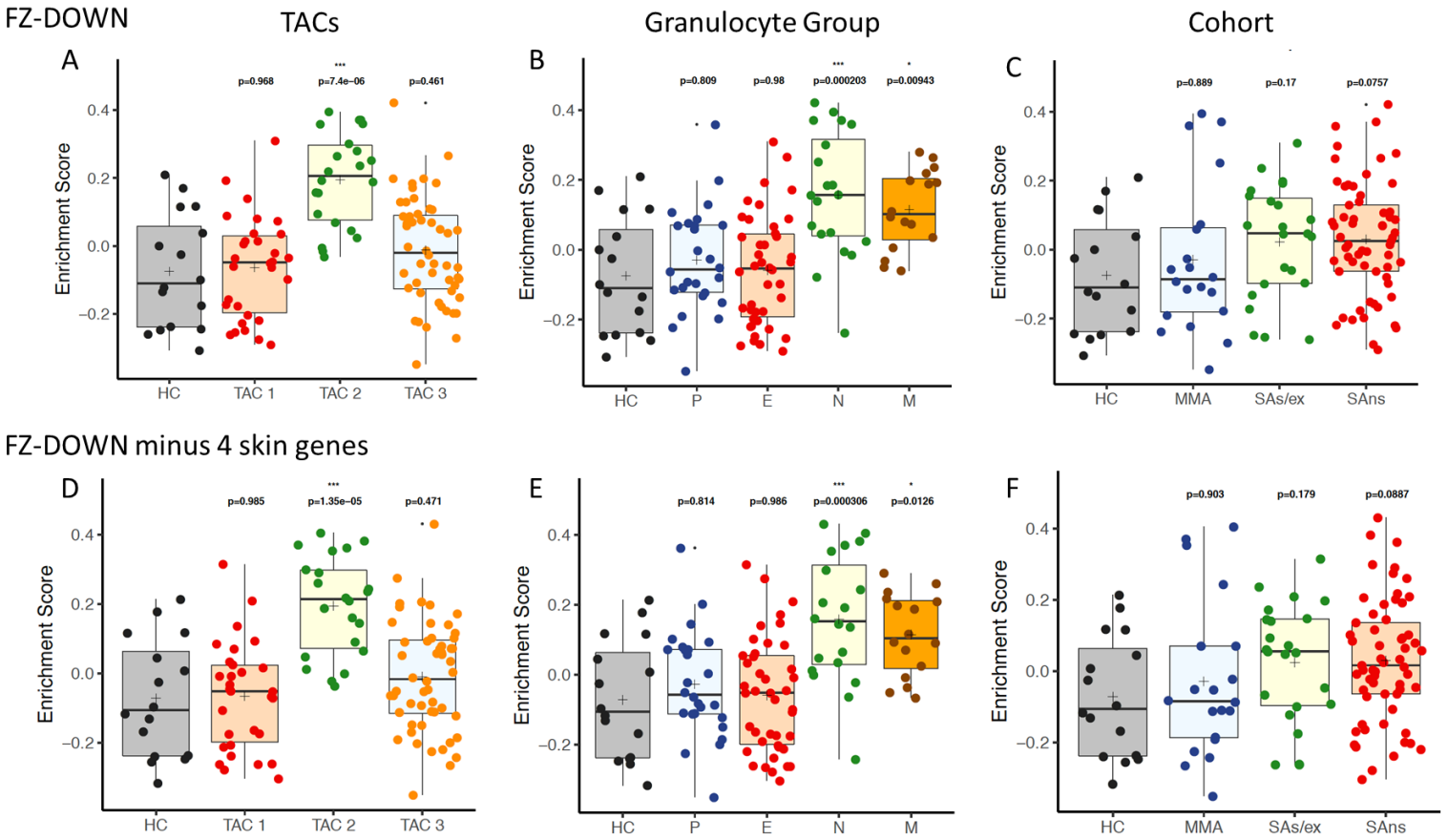
Supplementary Figure 2. Protein pathway analysis of the 40 significantly enriched pathways (false discovery rate, FDR<0.05) for differentially expressed genes down-regulated in atopic dermatitis lesional tissue following 12 weeks Fezakinumab treatment. See **Supplementary Table 8** for more details of these pathways.



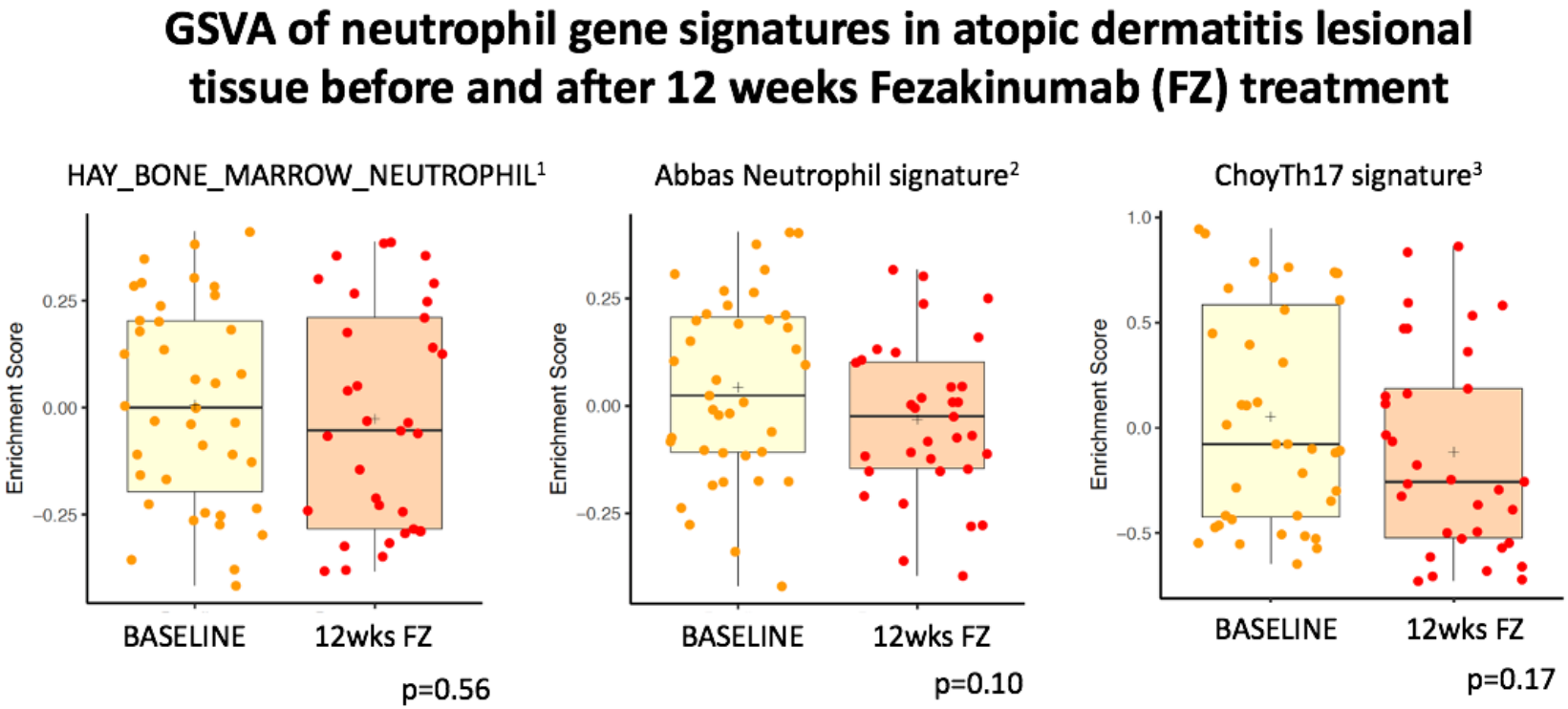
Supplementary Figure 3. Protein pathway analysis at a false discovery rate (FDR)<0.02 for differentially expressed genes up-regulated in atopic dermatitis lesional tissue following 12 weeks Fezakinumab treatment. See **Supplementary Table 9** for more details of these pathways. No pathways were enriched at a FDR<0.05.



Supplementary Figure 4. Minimal effect on the FZ-DOWN signature enrichment scores (A-C) in the sputum of asthmatic and healthy subjects with the removal of 4 skin-specific genes (D-F) when assessed by transcriptome associated cluster (TAC) status (A and D), sputum granulocyte group (B and E) or by asthma severity (C and F). SAns – severe asthma non-smoker; SAs/ex – severe asthma current or ex-smoker; MMA – mild-moderate asthma and HC – healthy control. P - paucigranulocytic; E – eosinophilic; N – neutrophilic and M – mixed granulocytic.



Supplementary Figure 5. Gene set variation analysis (GSVA) show no significant change in enrichment scores for neutrophil signatures in atopic dermatitis skin lesional tissue at baseline and after 12 weeks of Fezikinumab (FZ) treatment. The references from which the neutrophil signatures were obtained are provided beneath the figure.



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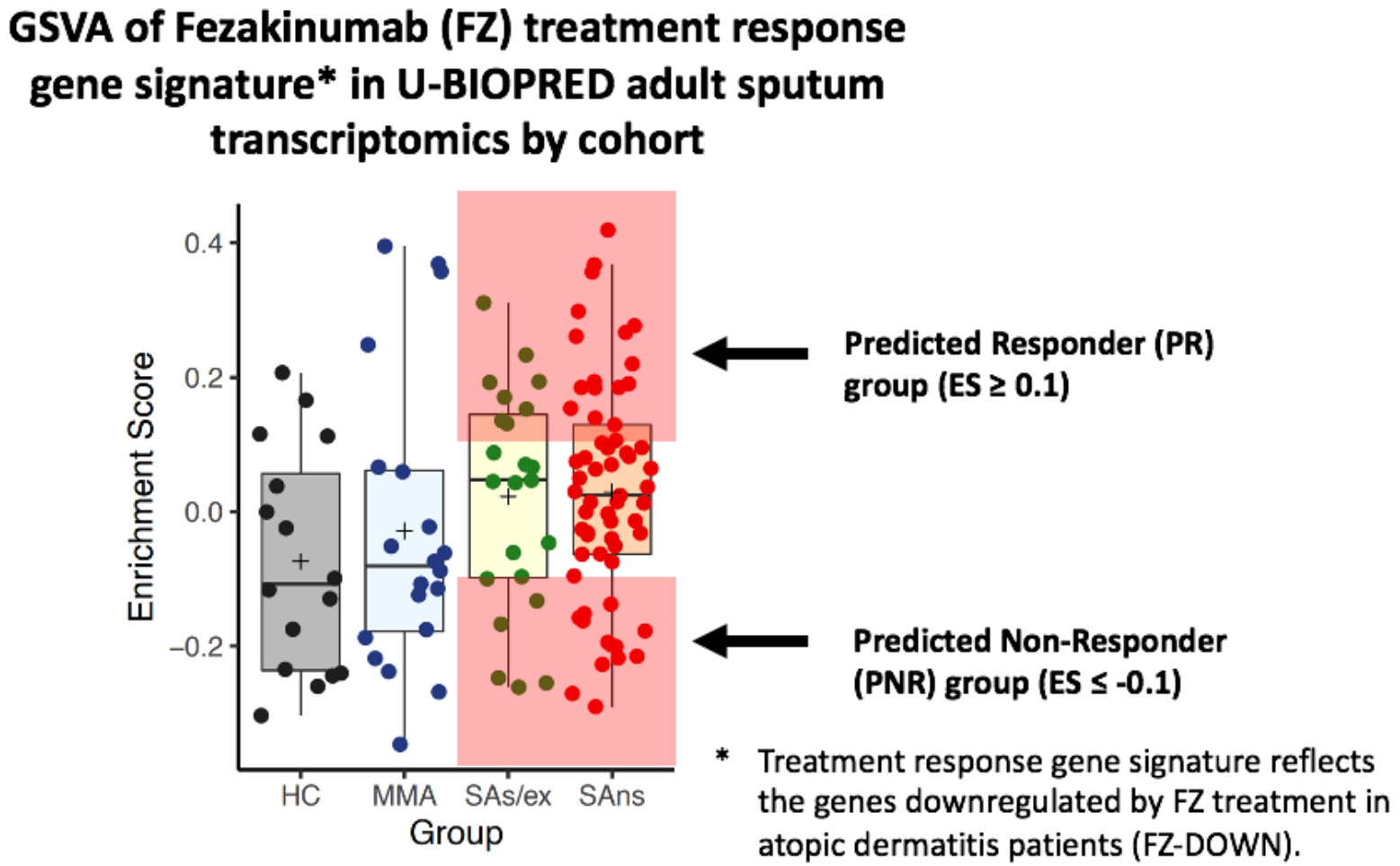
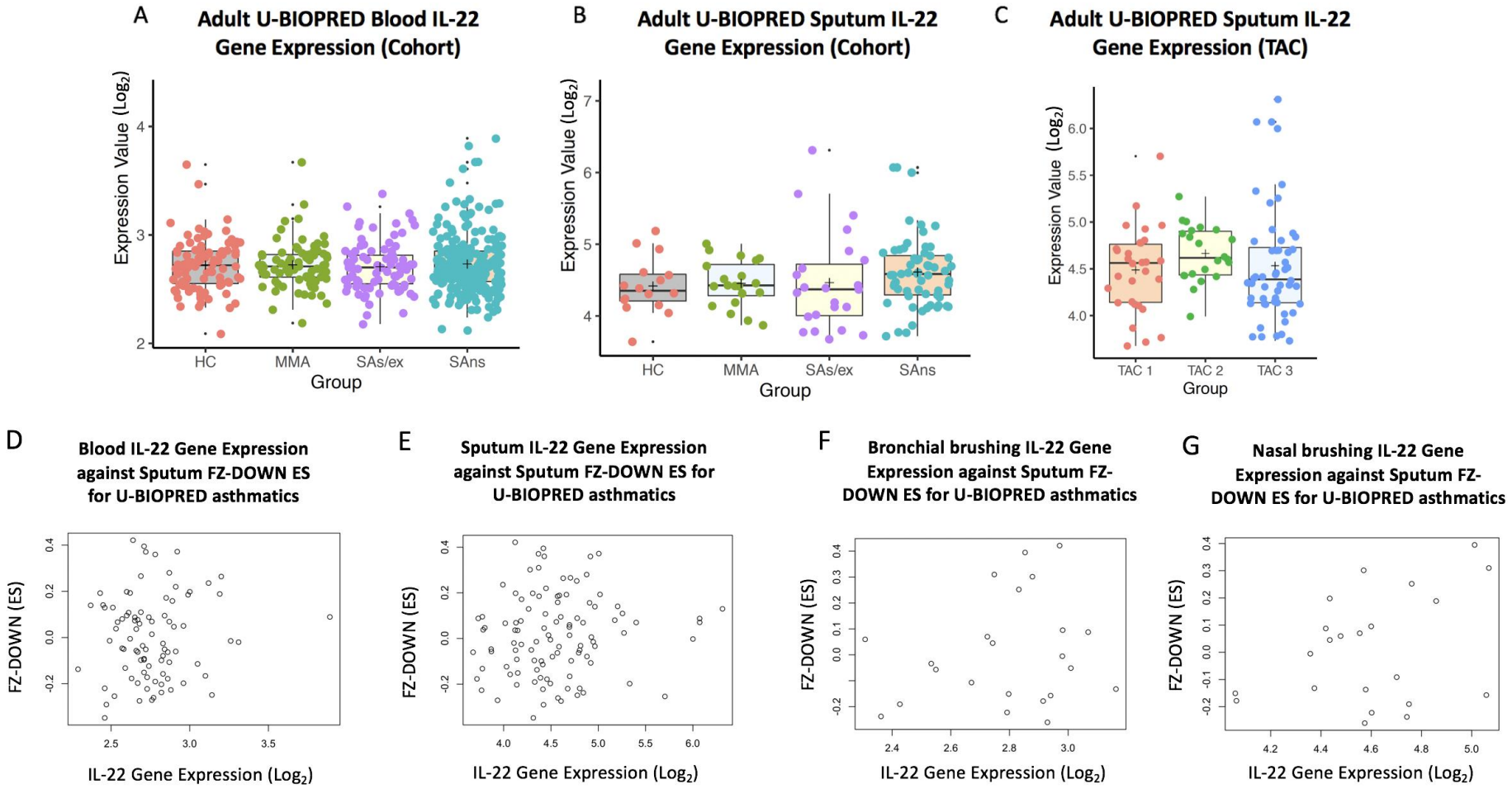
Supplementary Figure 6. Schematic of selection of patients with a high versus low enrichment score (ES) for the gene signature of genes down-regulated by Fezakinumab (FZ) in atopic dermatitis patients. Predicted responders (PRs) were considered as patients most highly enriched (n=26, ES ≥ +0.1) whilst predicted non-responders were defined as those lowly enriched (n=18, ES ≤ -0.1). Clinical and omics variables that defined these patients were then obtained from the U-BIOPRED dataset.



Supplementary Figure 7. IL-22 gene expression in blood (A) and sputum (B, C) is not significantly up-regulated according to asthma severity (B) or transcriptome associated cluster (TAC) status (C). IL-22 gene expression in blood (D), sputum (E), bronchial (F) and nasal (G) brushings does not correlate with the Fezakinumab (FZ)-DOWN signature sputum ES. SAns – severe asthma non-smoker; SAs/ex – severe asthma current or ex-smoker; MMA – mild-moderate asthma and HC – healthy control.



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