-Supplementary Information-

In-depth serum proteomics reveals biomarkers of psoriasis severity and response to traditional Chinese medicine

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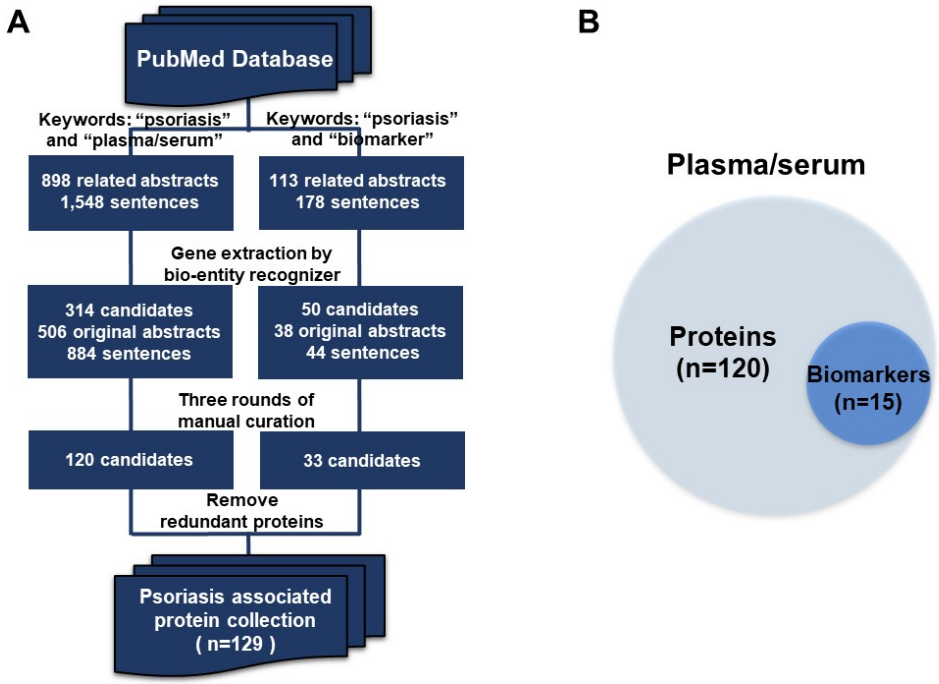
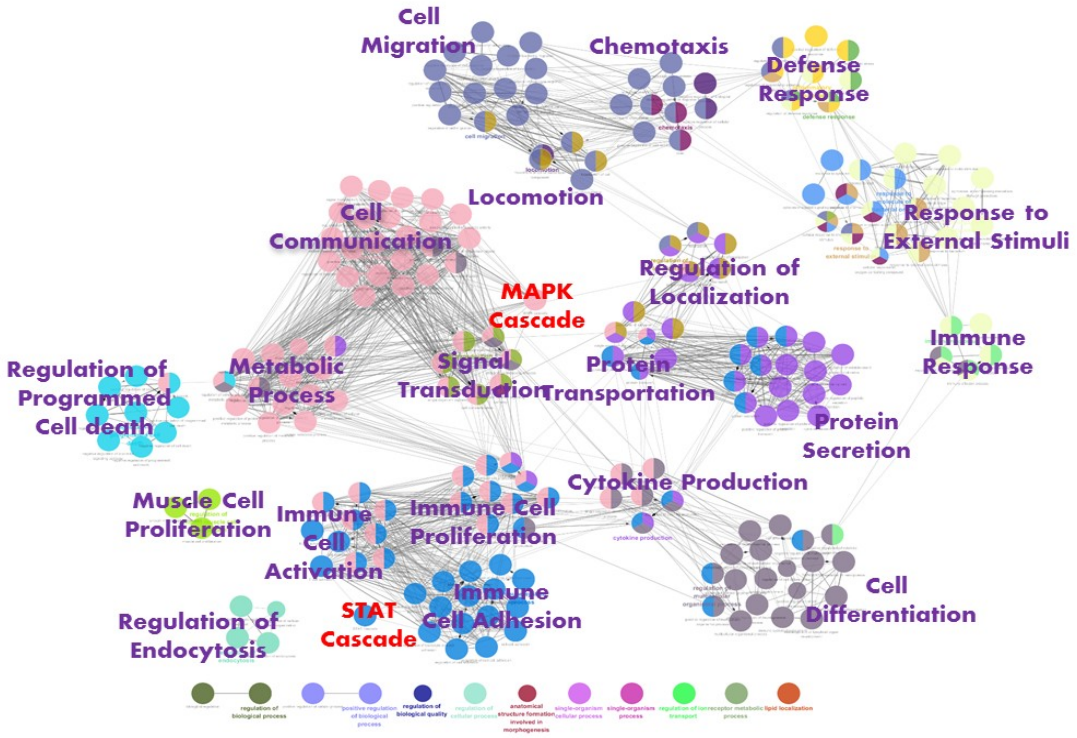
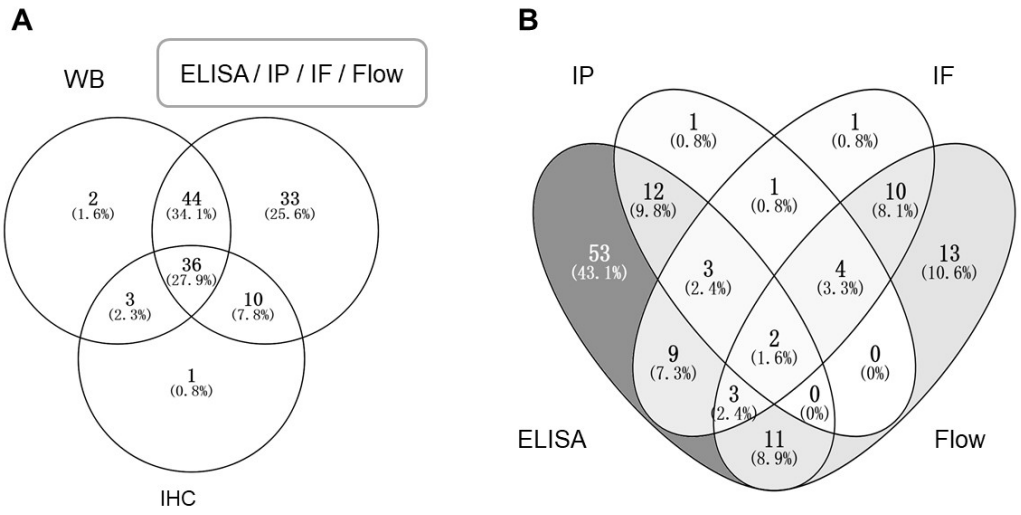
Supplemental Figure S17	Principle component analysis distinguishing responders and non-responders to YinXieLing treatment.



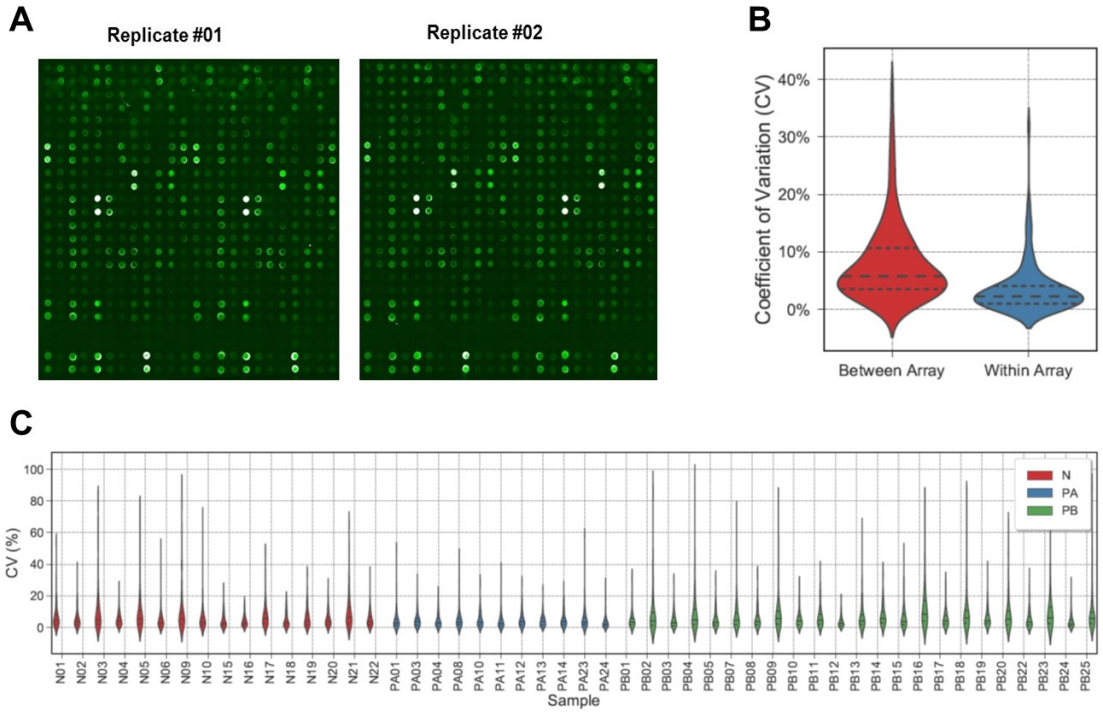
Supplemental Figure S1. Literature mining of psoriasis serum/plasma proteins and biomarkers in PubMed database. (A) Workflow of text mining and manual curation for psoriasis plasma/serum proteins and biomarkers in the PubMed database (https://www.ncbi.nlm.nih.gov/pubmed/); (B) Venn diagram analysis of psoriasis plasma/serum proteins and biomarkers identified by text mining and manual curation (i.e., 120) and those identified in this study as potential biomarkers of psoriasis (i.e., 15).



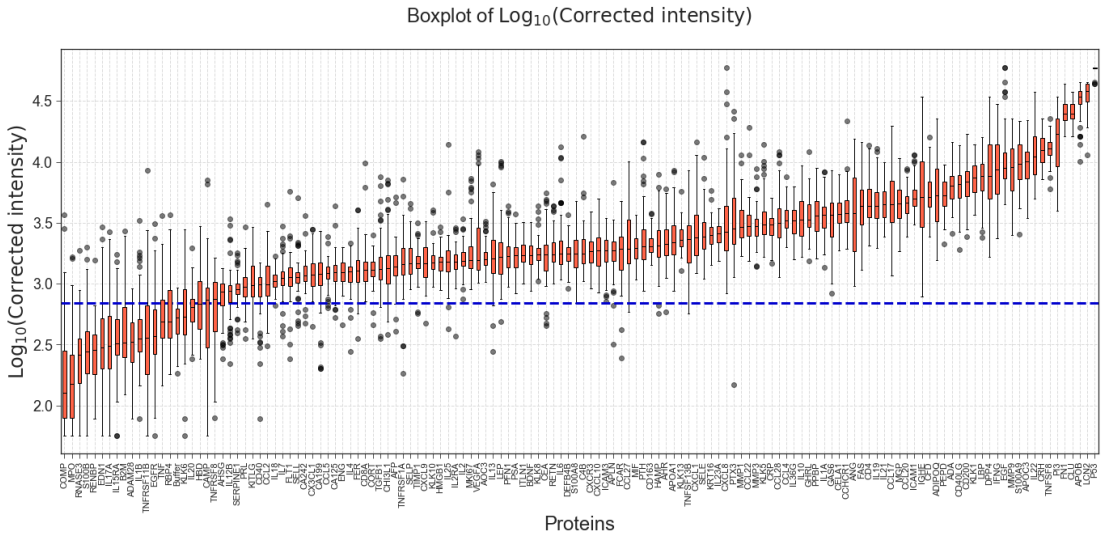
Supplemental Figure S2. Functional analysis of proteins identified by text mining using Cytoscape and ClueGO. A p-value ≤ 0.01 was used for biological process selection.



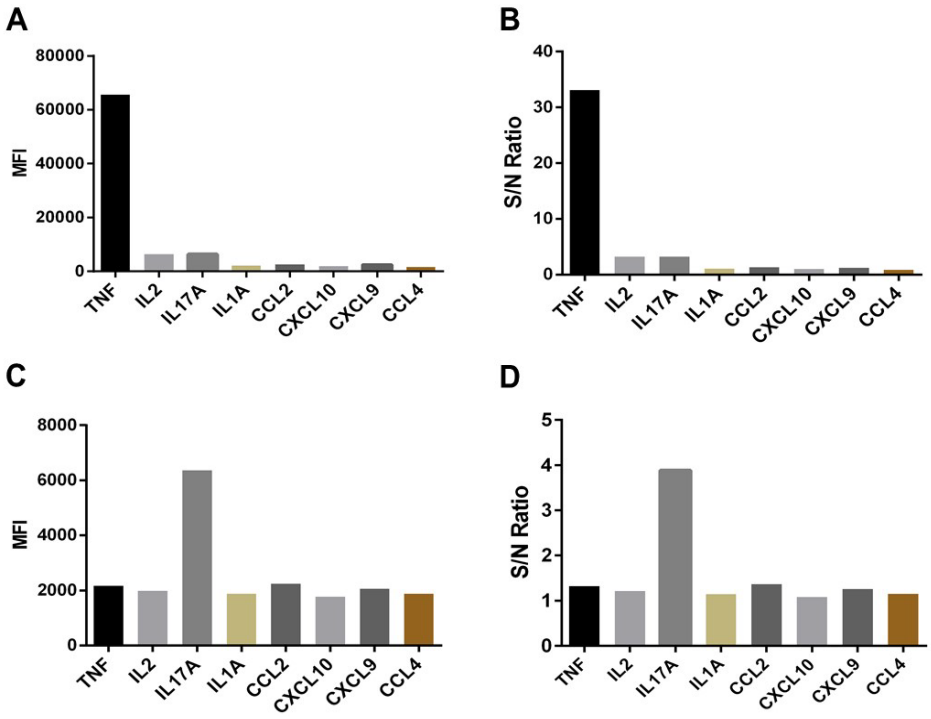
Supplemental Figure S3. Selection of antibodies for fabricating a psoriasis-specific antibody microarray. (A) Venn diagram represents antibodies that have been validated by different methods recognizing conformational epitopes (ELISA, IP, IF and Flow), partial conformational epitopes (IHC) and linear epitopes (WB); (B) Venn diagram analysis of antibodies validated by ELISA, IP, IF and Flow respectively IP, immune-precipitation; IF, Immunofluorescence; Flow, Flow cytometry; IHC, Immunohistochemistry.



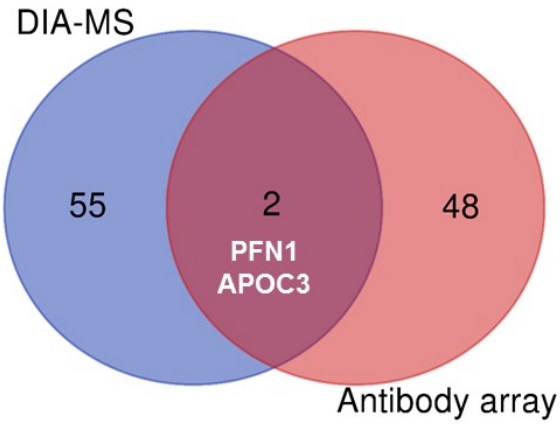
Supplemental Figure S4. Reproducibility of psoriasis antibody microarrays with serum samples. (A) is array images of technical replicates detected via fluorescence; (B) is the distribution of CV(%) within two technical replicate arrays or across duplicate spots within the same array capable of detecting 129 proteins; (C) is the distribution of CV(%) of antibody microarray measurements across 50 serum samples for 129 proteins (Table 1). N, normal/healthy controls; PA, psoriatic arthritis patients after YinXieLing treatment; PS, psoriatic arthritis patients before YinXieLing treatment.



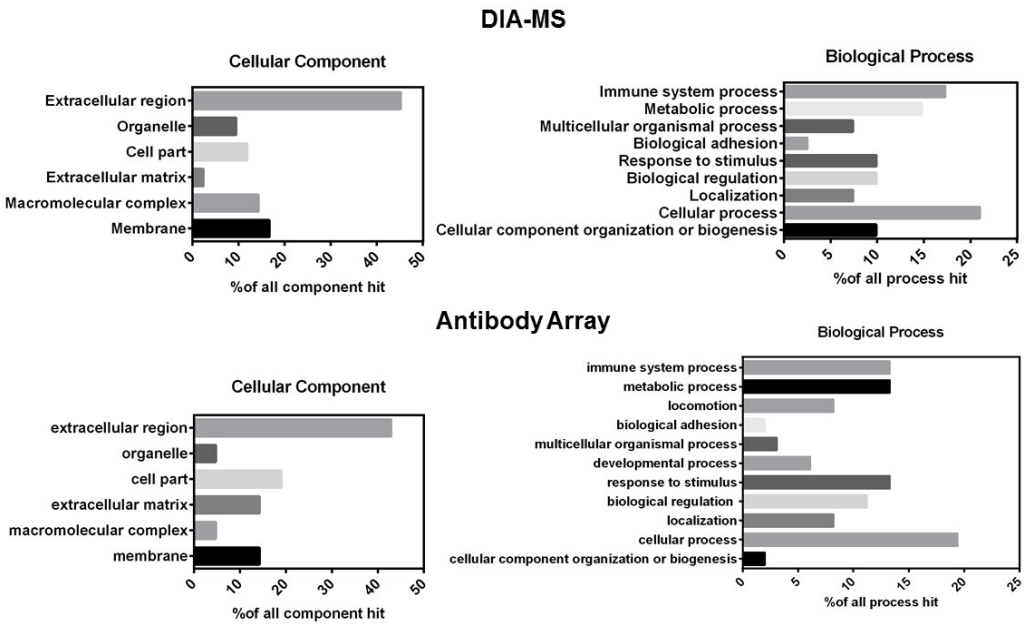
Supplemental Figure S5. Distribution of protein expression in serum samples detected by psoriasis antibody microarrays. The blue line is the threshold, calculated as the mean of normalized intensity of the negative control (PBS buffer) plus twice the standard deviations, for serum proteins that were detected on our psoriasis-specific antibody microarrays using clinical samples.



Supplemental Figure S6. Validation of TNF and IL17A antibodies using protein arrays. (A) Mean of fluorescent intensity (MFI) and (B) signal to noise (S/N) ratio of the anti-TNF antibody binding to TNF protein; (C) Mean of fluorescent intensity (MFI) and (D) signal to noise (S/N) ratio of the anti-IL17A antibody binding to IL17A protein.

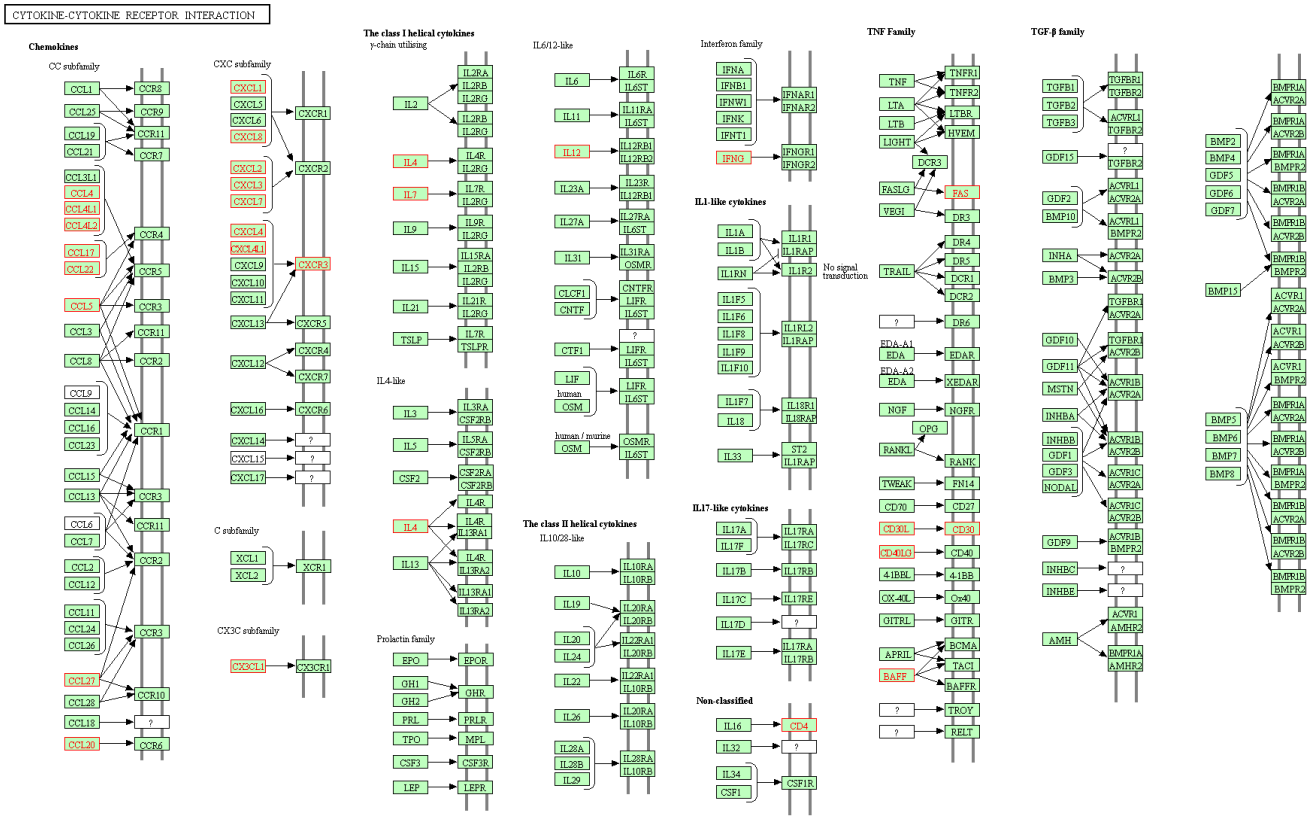


Supplemental Figure S7. Venn diagram analysis of differentially-expressed proteins identified by DIA-MS and psoriasis antibody microarrays. The Venn diagram was created using http://bioinformatics.psb.ugent.be/webtools/Venn/.

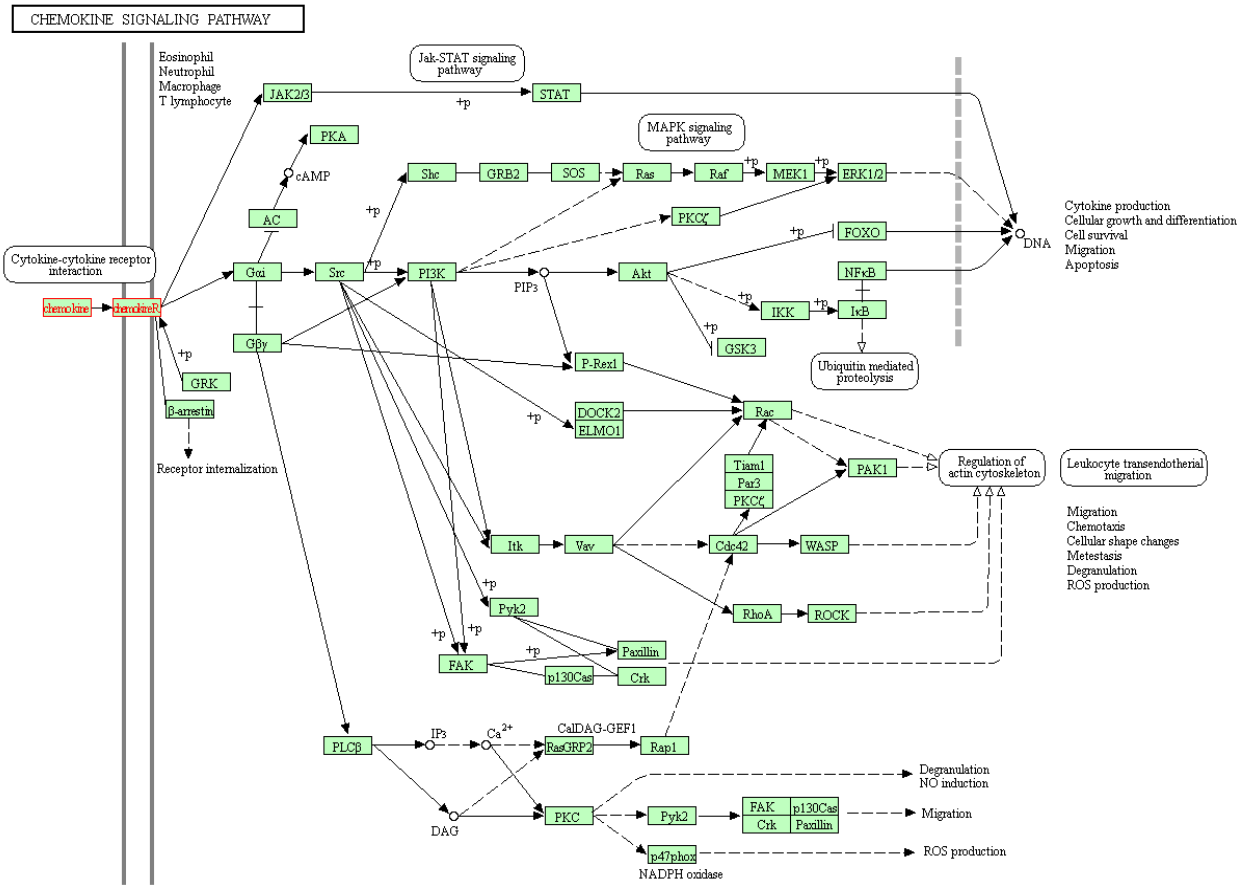


Supplemental Figure S8. Functional GO analysis of psoriasis-associated serum proteins identified with DIA-MS and antibody microarrays. GO analysis was performed using the PANTHER database (http://pantherdb.org/).

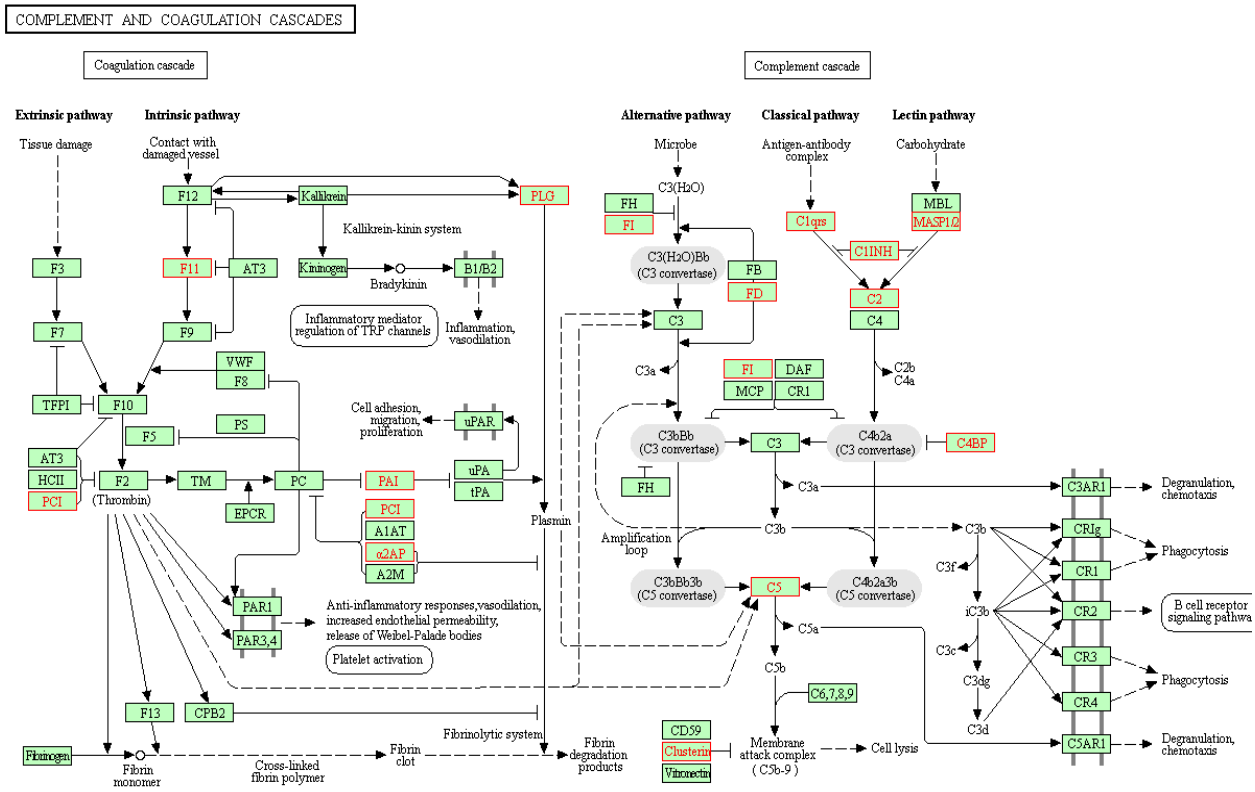
A


CYTOKINE-CYTOKINE RECEPTOR INTERACTION
Chemokines
CC subfamily
CXC subfamily
C subfamily
CX3C subfamily
The class I helical cytokines
γ-chain utilising
IL4-like
Prolactin family
IL6/12-like
The class II helical cytokines
IL10/28-like
Interferon family
IL1-like cytokines
No signal transduction
IL17-like cytokines
Non-classified
TNF Family
TGF-β family

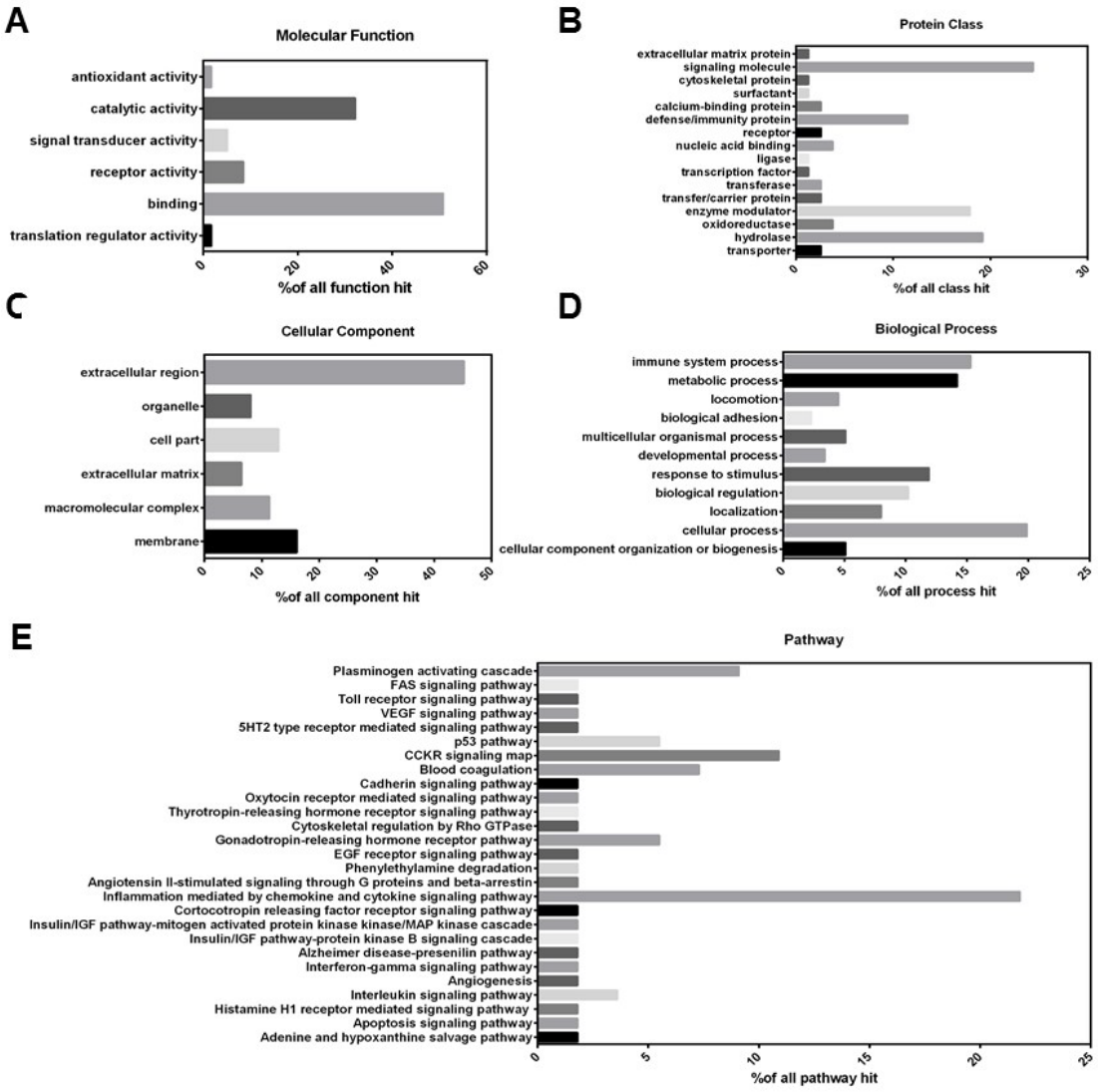
B


CHEMOKINE SIGNALING PATHWAY
Eosinophil
Neutrophil
Macrophage
T lymphocyte
Jak-STAT signaling pathway
MAPK signaling pathway
Cytokine-cytokine receptor interaction
chemokine
chemokineR
Receptor internalization
cAMP
PIP3
DNA
Cytokine production
Cellular growth and differentiation
Cell survival
Migration
Apoptosis
Ubiquitin mediated proteolysis
Regulation of actin cytoskeleton
Leukocyte transendothelial migration
Migration
Chemotaxis
Cellular shape changes
Metastasis
Degranulation
ROS production
IP3
DAG
NADPH oxidase
Degranulation
NO induction
Migration
ROS production

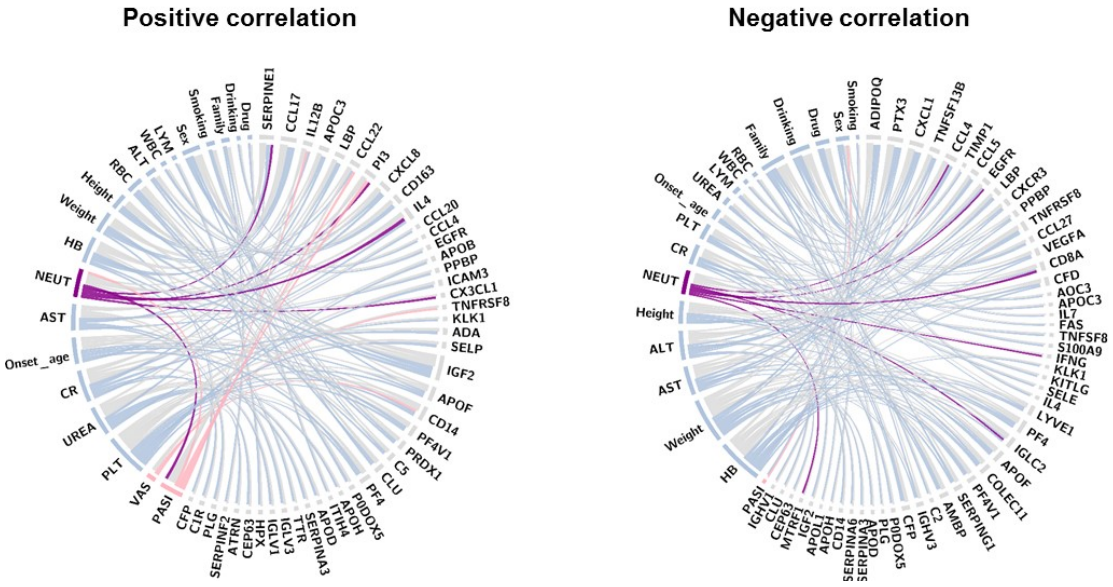
Supplemental Figure S9. Functional GO analysis of psoriasis-associated serum proteins identified with DIA-MS and antibody microarrays (A) Cytokine-cytokine receptor interaction and (B) chemokine signaling pathway of the psoriasis-associated proteins. Pathway analysis was performed using the KEGG database (https://www.genome.jp/kegg/). The red label is the psoriasis-associated serum proteins identified by our in-depth serum proteomics platform.



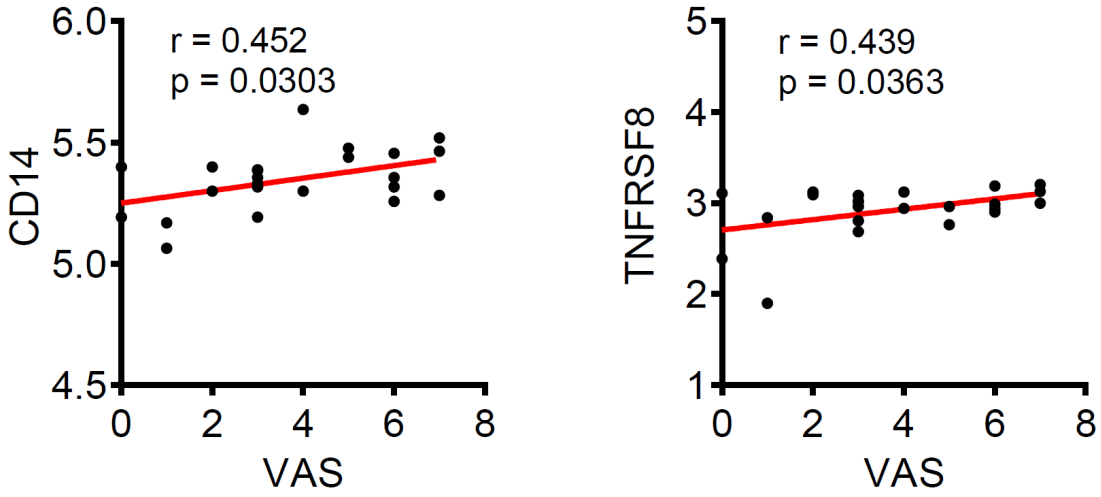
Supplemental Figure S10. KEGG analysis of psoriasis-associated proteins and their coagulation and complement pathways. Pathway analysis was performed using the KEGG database (https://www.genome.jp/kegg/). The red label is the psoriasis-associated serum proteins identified by our in-depth serum proteomics platform.



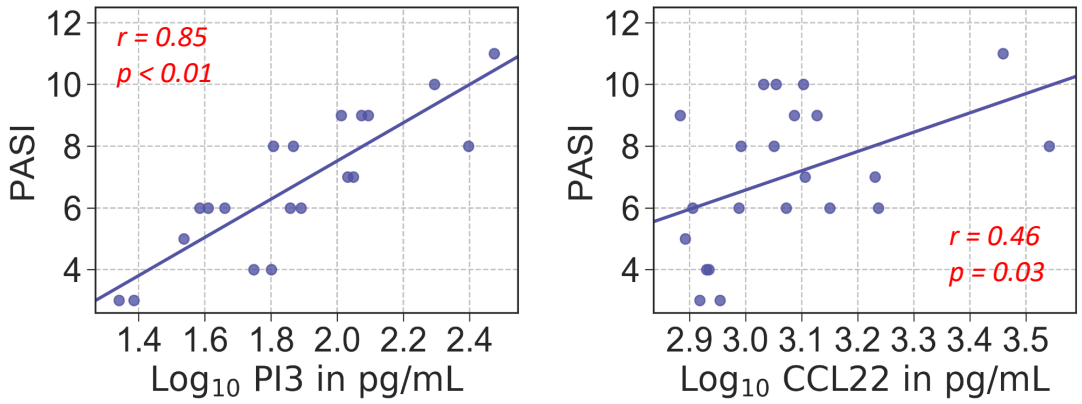
Supplemental Figure S11. Functional annotation of psoriasis-associated proteins using in-depth serum proteomics. (A-E) are the functional annotations of the molecular functions, protein classes, cell components, biological processes and signaling pathways, respectively, of the psoriasis-associated proteins identified by our in-depth serum proteomics platform. GO analysis was performed using the PANTHER database (http://pantherdb.org/).



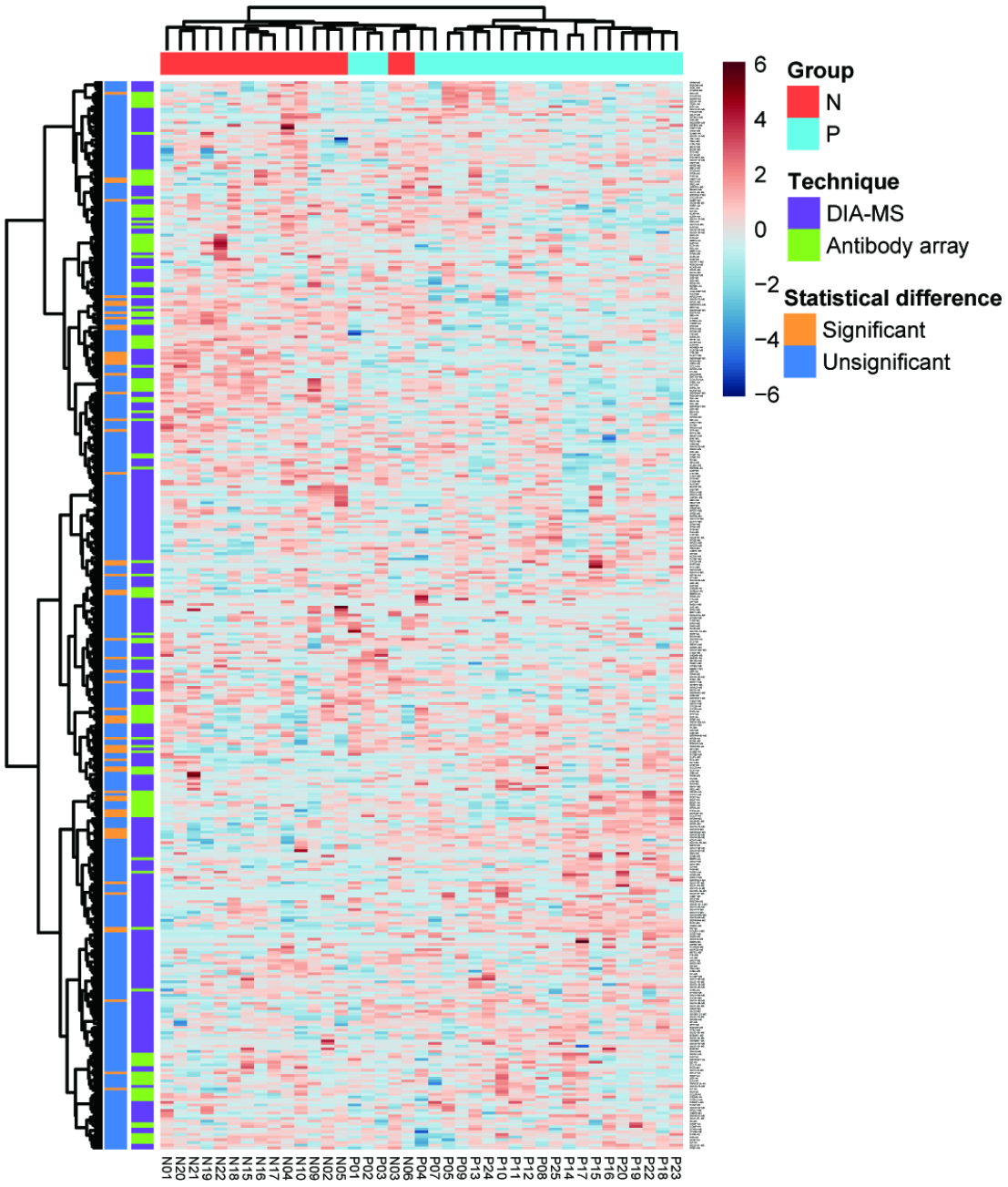
Supplemental Figure S12. Correlation interaction of neutrophils to the serum proteome and psoriasis area and severity index (PASI).



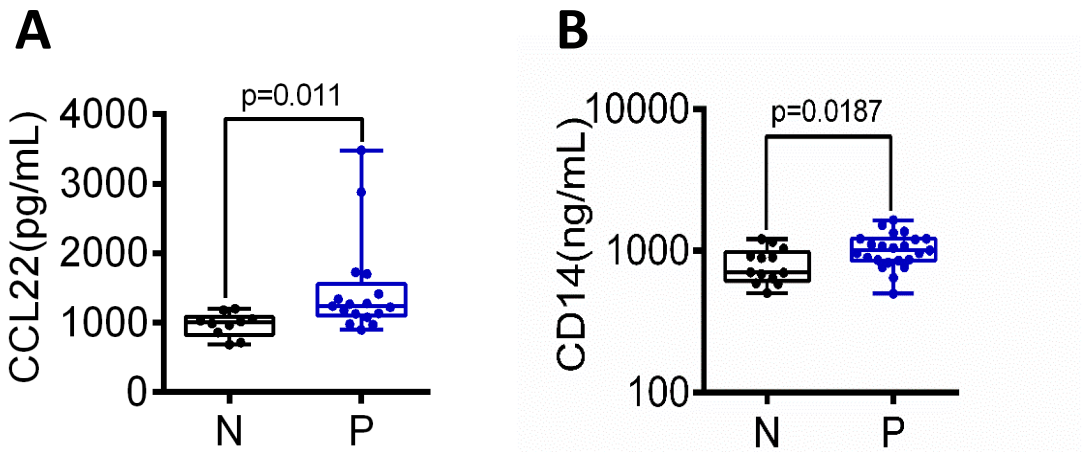
Supplemental Figure S13. Correlation of differentially expressed proteins, CD14 and TNFRSF8, in psoriatic patients with the visual analog scale (VAS).



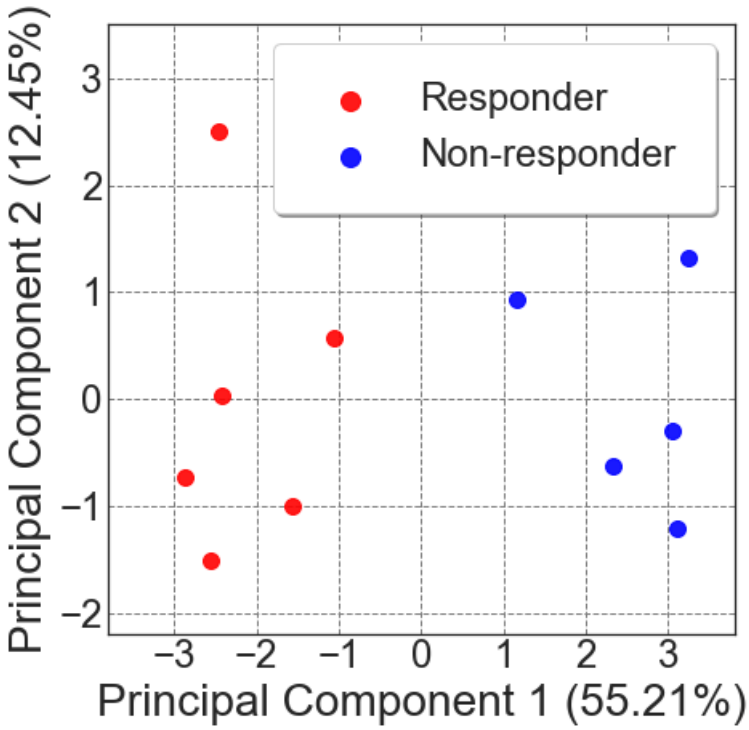
Supplemental Figure S14. Validation of the correlation between PI3 and CCL22 protein expression with the PASI score by ELISA. N, healthy control (n=22); P, psoriasis patients (n=25) (see also Table 1).



Supplemental Figure S15. Non-biased hierarchical clustering analysis of the serum proteome and clinical serum samples. N and P represent healthy control and psoriatic patients, respectively.



Supplemental Figure S16. Validation of differentially expressed proteins in psoriasis, CCL22 and CD14, by ELISA. Protein concentration comparison of (A) CCL22 and (B) CD14 between N and P groups, respectively. N, healthy control (n=22); P, psoriasis patients (n=25) (see also Table 1).



Supplemental Figure S17. Principle component analysis distinguishing responders from non-responders to YinXieLing treatment.