Supplementary Materials

Supplementary Methods

Standardized tissue processing protocol followed in phase II siltuximab trial

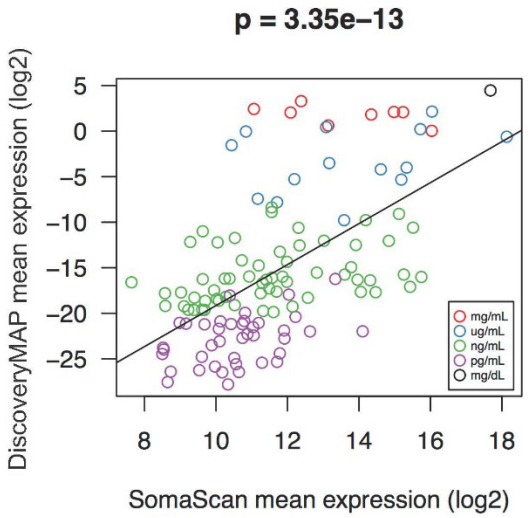
Tissue was stained using the following antibodies: IL-6 (Santa Cruz, SC-7920): polyclonal rabbit IgG, dilution 0.175 ug/ml, incubated overnight, pre-treated with SHIER8, and a Rabbit GBI detection system and pSTAT3 (Cell Signaling, 9131): polyclonal rabbit IgG, dilution 1:250, incubated overnight, pretreated with SHIER7+ enzyme (1:40), and a Rabbit GBI detection system. After staining, slides were dehydrated through an alcohol series to absolute ethanol followed by xylene rinses. Slides were permanently coverslipped with glass coverslips and Cytoseal. Slides were examined under a microscope to assess staining. Positive staining is indicated by the presence of a brown chromogen (DAB-HRP) reaction product. Hematoxylin counterstain provides a blue nuclear stain to assess cell and tissue morphology.

The IHC testing and scoring were performed blinded to the treatment group at QualTek laboratory. Samples were scored as percentage of lymph node cells (0-100% at 10% intervals) and intensity of staining (0-3 with 0 = no staining, 1= low staining, 2=moderate staining, and 3= high staining).

An H-score was determined for each marker for each subcellular localization. The H-score is more representative of the staining of the entire scored areas of interest in the germinal center or mantle zone areas of the lymph node sections. It accounts for the differences between samples with high intensity staining in fewer cells versus low intensity staining in more number of cells. The H-Score (0 to 300 range) was determined by Qualtek laboratory using the following formula, integrating percent positive cells at

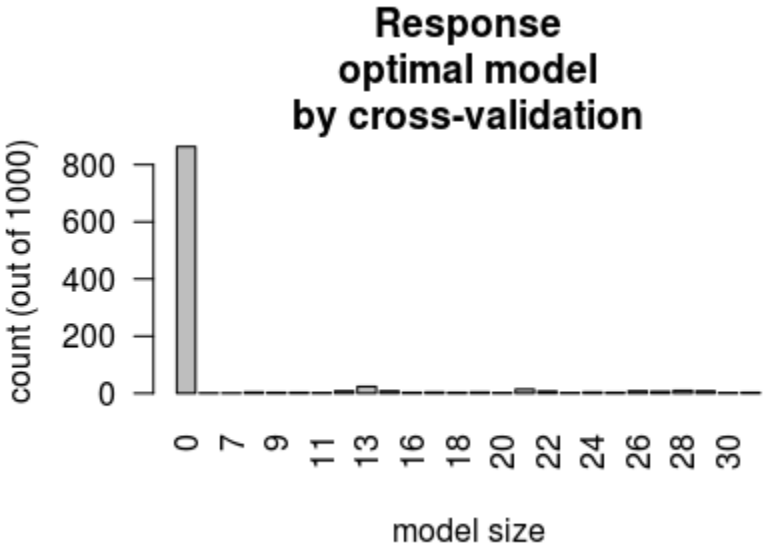
each staining intensity score: H-Score = [(% at <1)*0] + [(% at 1+)*1] + [(% at 2+)*2] + [(% at 3+)*3]. Qualtek Laboratory provided percent positive cells, staining intensity, and H-score for the cytoplasm of IL-6 and the nucleus of p-STAT3 in the germinal center and in the mantle zone and relative score within the interfollicular space for IL-6 only. P-values from a chi-square test for differential expression between responders and non-responders are provided for IL-6 germinal center (H score), mantle zone (H score), and interfollicular space (relative score) as well as pSTAT3 germinal center (H score) and mantle zone (H score).

Fig. S1.



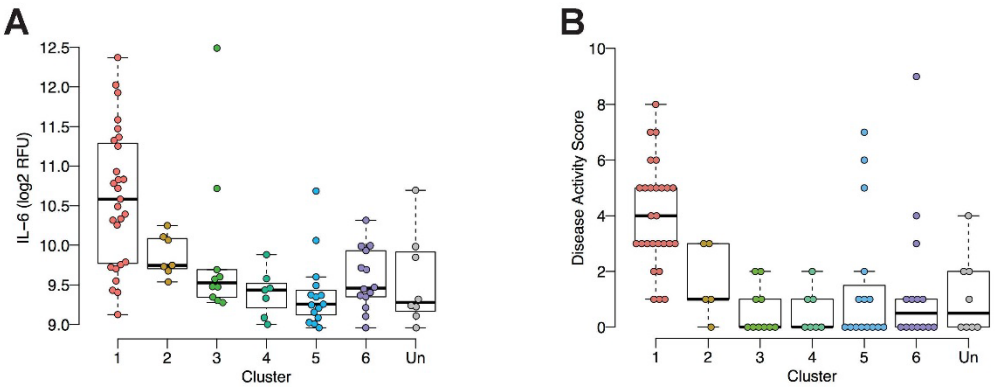
Comparison of analyte levels for those measured by both the Somalogic SOMAscan and RBM DiscoveryMAP platforms. RBM values were converted from the original units (used for coloring) to mg/ml and then log transformed (base 2). Additionally, values below the experimentally determined per-target least detectable dose were truncated to the least detectable dose

Fig. S2.



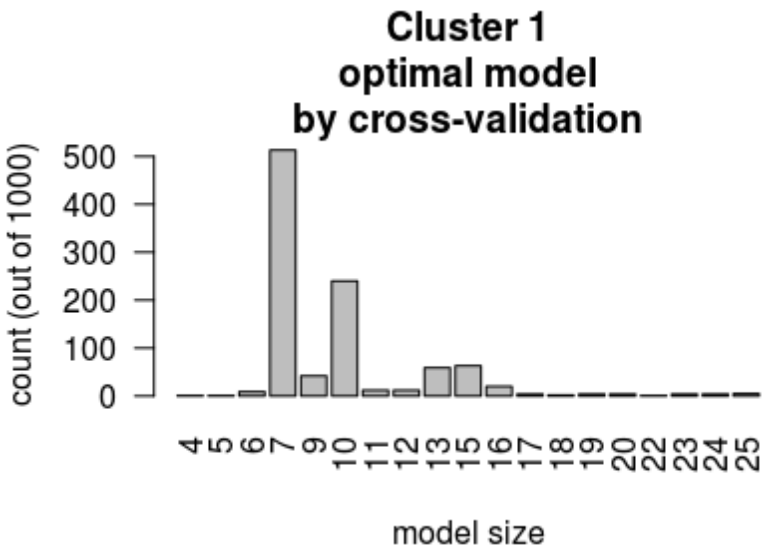
Results from model selection to identify an optimal model that differentiates siltuximab responders from non-responders. An elastic net algorithm and performed 5-fold cross validation was performed 1000 times to determine the fewest proteins present on both discovery and validation platforms that could most effectively predict response in the discovery dataset. The model chosen 863 out of 1000 randomizations had 0 proteins, the model predicting all patients as non-responders, which demonstrates that these data do not support a model that predicts siltuximab responders from the full cohort.

Fig. S3.



IL-6 and disease activity score by iMCD cluster. (A) IL-6 levels by iMCD cluster as measured by SOMAscan. Units are log2(relative fluorescence unit). (B) Disease activity scores by cluster, as measured by a disease activity score, which includes C-reactive protein, hemoglobin, and albumin levels. Box plots show center median, first and third quartile, and whiskers extend to 1.5*interquartile range.

Fig. S4.



Results from model selection to identify an optimal model that most effectively predicts Cluster-1 membership in the discovery dataset. An elastic net algorithm and performed 5-fold cross validation was performed 1000 times to determine the fewest proteins present on both discovery and validation platforms that could most effectively predict Cluster-1 membership in the discovery dataset. The model chosen 513 out of 1000 randomizations had 7 proteins, and the second most frequently chosen model, 240 out of 1000 randomizations, included 10 proteins.

Fig S5.
A.

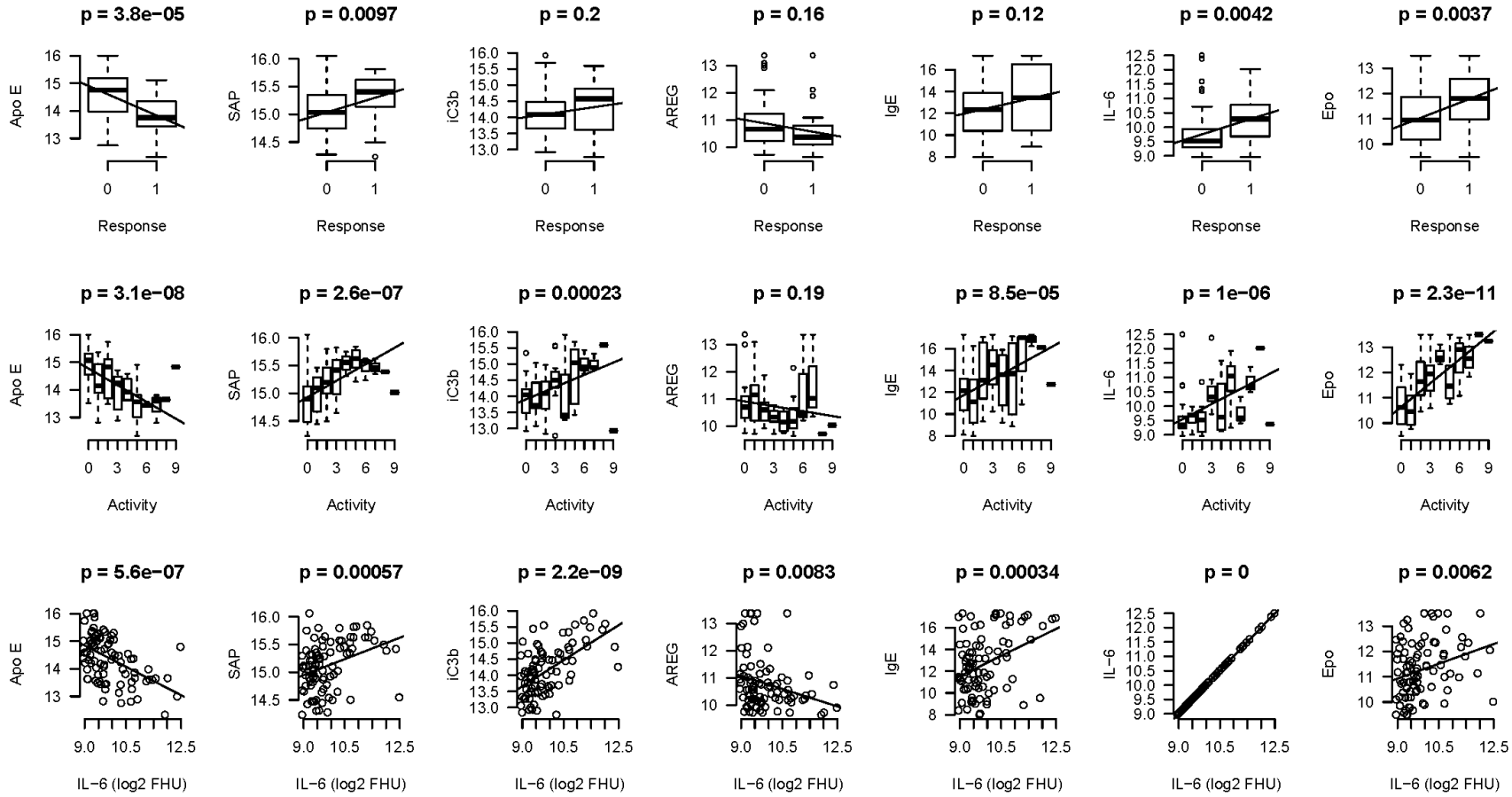
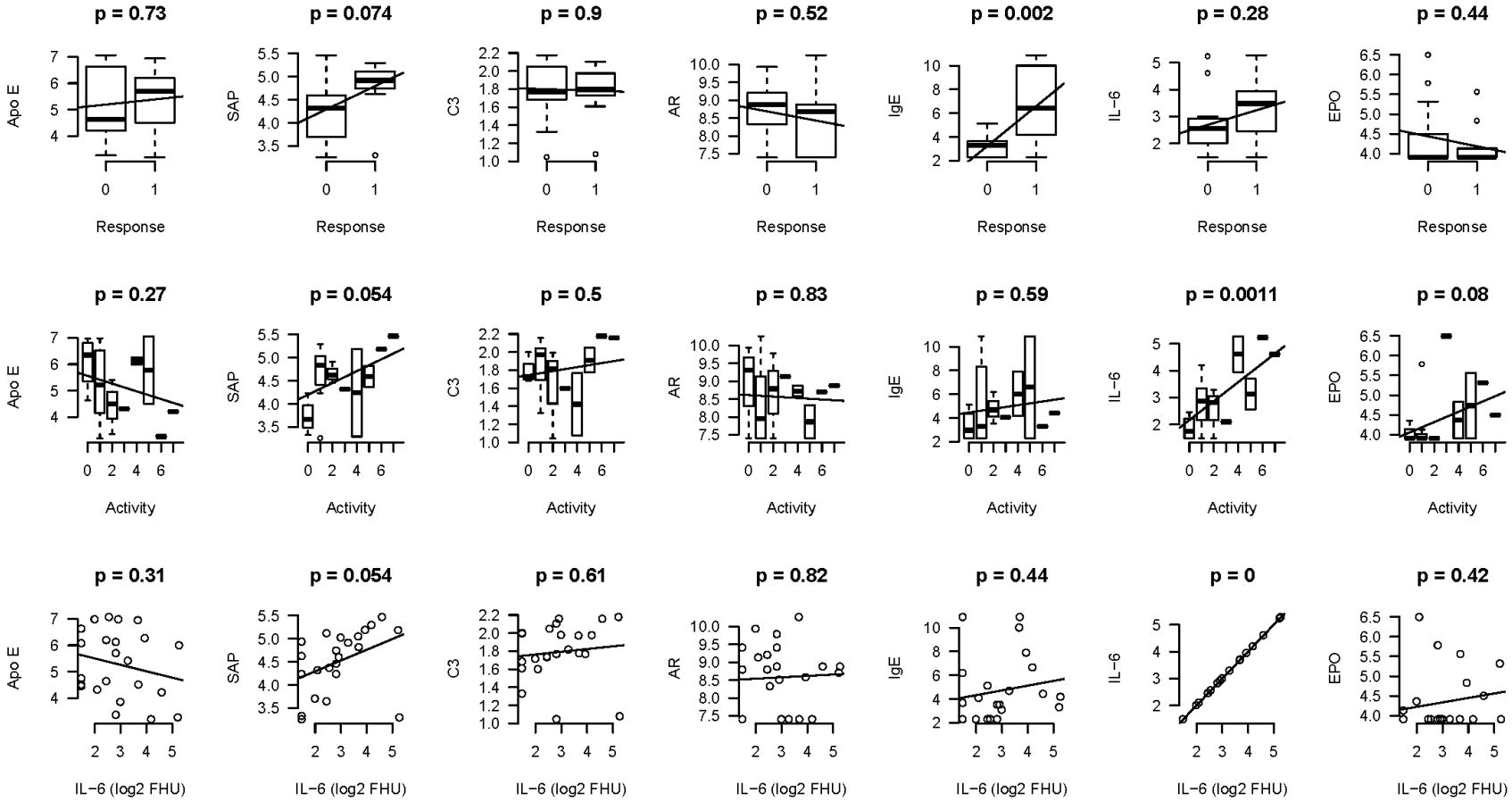
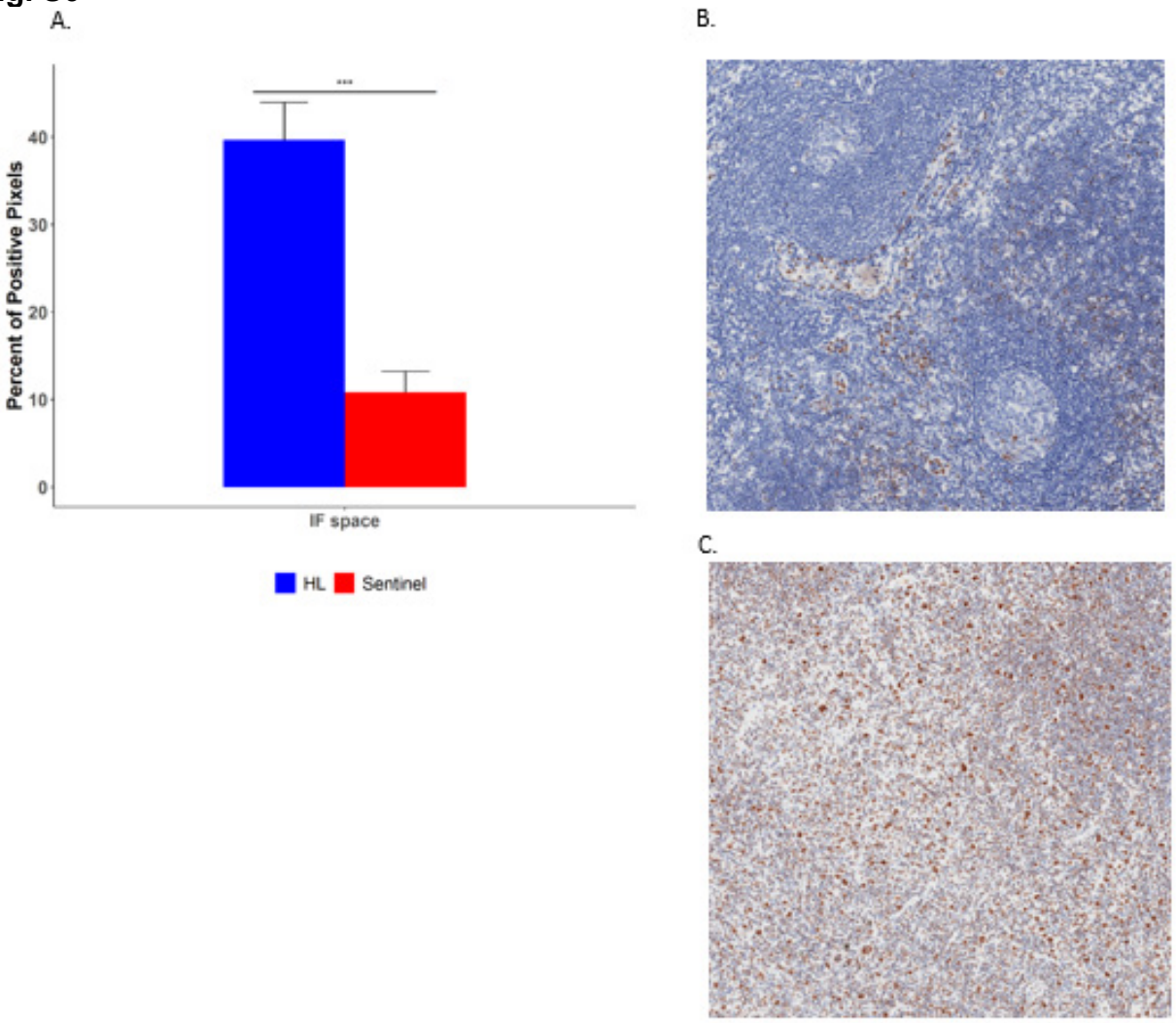

p = 3.8e−05
p = 0.0097
p = 0.2
p = 0.16
p = 0.12
p = 0.0042
p = 0.0037
Apo E
SAP
iC3b
AREG
IgE
IL−6
Epo
Response
p = 3.1e−08
p = 2.6e−07
p = 0.00023
p = 0.19
p = 8.5e−05
p = 1e−06
p = 2.3e−11
Activity
p = 5.6e−07
p = 0.00057
p = 2.2e−09
p = 0.0083
p = 0.00034
p = 0
p = 0.0062
IL−6 (log2 FHU)

Fig. S5
B.



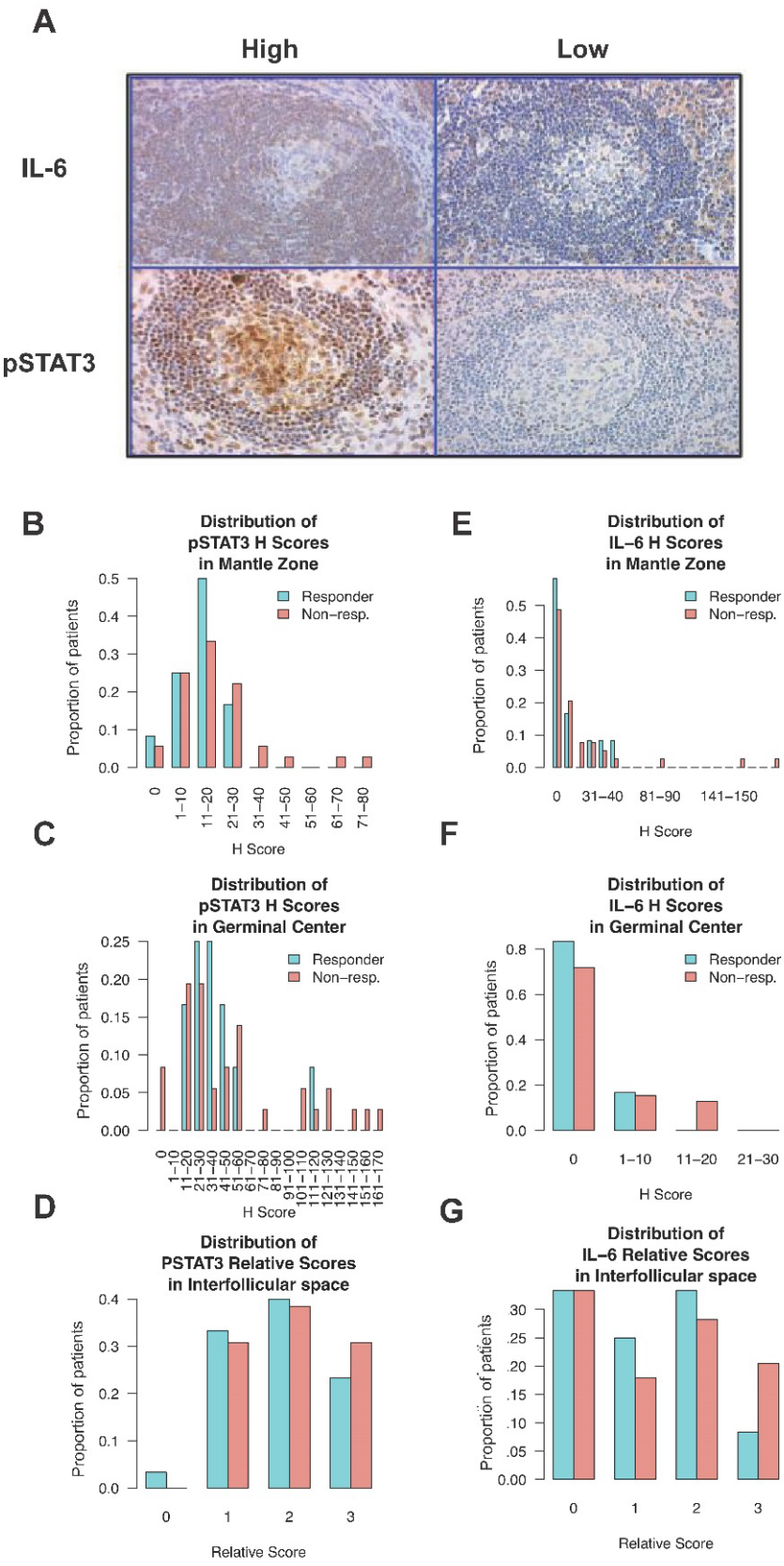
Univariate analysis of each of the 7-proteins included in the Cluster-1 prediction algorithm with each response, disease activity, and serum IL-6 from the (A) discovery cohort and (B) validation cohort.

Fig. S6



The enrichment of the IL-6 JAK-STAT3 pathway in serum proteomics led us to hypothesize that pSTAT3 would be elevated in iMCD compared to normal control. As a positive validation for the assay, we compared HL to normal lymph nodes. (A) HL lymph nodes demonstrated significantly increased pSTAT3 staining in the interfollicular space compared to normal control lymph nodes ($p = 0.0002166$). Representative images of a (B) normal lymph node and a (C) HL lymph node (40X magnification) are provided.

Fig. S7.


A
High
Low
IL-6
pSTAT3
B
Distribution of pSTAT3 H Scores in Mantle Zone
Responder
Non–resp.
Proportion of patients
0.0
0.1
0.2
0.3
0.4
0.5
0
1–10
11–20
21–30
31–40
41–50
51–60
61–70
71–80
H Score
C
Distribution of pSTAT3 H Scores in Germinal Center
Responder
Non–resp.
Proportion of patients
0.00
0.05
0.10
0.15
0.20
0.25
0
1–10
11–20
21–30
31–40
41–50
51–60
61–70
71–80
81–90
91–100
101–110
111–120
121–130
131–140
141–150
151–160
161–170
H Score
D
Distribution of PSTAT3 Relative Scores in Interfollicular space
Proportion of patients
0.0
0.1
0.2
0.3
0.4
0
1
2
3
Relative Score
E
Distribution of IL–6 H Scores in Mantle Zone
Responder
Non–resp.
Proportion of patients
0.0
0.1
0.2
0.3
0.4
0.5
0
31–40
81–90
141–150
H Score
F
Distribution of IL–6 H Scores in Germinal Center
Responder
Non–resp.
Proportion of patients
0.0
0.2
0.4
0.6
0.8
0
1–10
11–20
21–30
H Score
G
Distribution of IL–6 Relative Scores in Interfollicular space
Proportion of patients
0.00
.05
.10
.15
.20
.25
30
0
1
2
3
Relative Score

We evaluated IL-6 and pSTAT3 IHC expression in responders and non-responders in the siltuximab treatment arm of the phase II study to investigate potential differences in the IL-6 JAK STAT3 pathway. (A) Immunohistochemistry for IL-6 and pSTAT3 expression in lymph node tissue from 51 and 48 patients, respectively, in the discovery cohort did not reveal significant differences in expression of IL-6 and pSTAT3 between responders and non-responders. Four representative images of patients with high and low expression of IL-6 and pSTAT3 (40X magnification) are provided. (B-G) A summary of immunohistochemistry scores demonstrating no significant differences in the expression of IL-6 in germinal centers ($p = 0.56$), mantle zone ($p = 0.96$), or interfollicular space ($p = 0.34$) or pSTAT3 in germinal centers ($p = 0.86$), interfollicular space ($p = 1.0$), or mantle zone ($p = 0.98$) in anti-IL-6 responders versus non-responders are provided.

Table S1. Clinical characteristics of patients included in the study.

	Discovery Cohort (Siltuximab Phase II Trial) (n=73)	Discovery Cohort (Real World iMCD patients) (n=15)	Validation Cohort (Siltuximab Phase I Trial) (n=23)
Sex, N(%)			
F	24 (32.9%)	4 (26.7%)	12 (52.2%)
M	49 (67.1%)	11 (73.3%)	11 (47.8%)
Age			
Mean (SD)	52.2 (13.4)	55.7 (13.2)	51.3 (12.1)
Median [Min, Max]	54.0 [26.0, 85.0]	52.0 [34.0, 76.0]	53.00 [24.0, 76.0]
Missing	7 (9.6%)	0 (0%)	0 (0%)
Race, N(%)			
Asian	37 (50.7%)	3 (20.0%)	2 (8.7%)
Black	3 (4.1%)	1 (6.7%)	2 (8.7%)
White	26 (35.6%)	9 (60.0%)	19 (82.6%)
Other	5 (6.8%)	2 (13.3%)	0 (0%)
Missing	2 (2.7%)	0 (0%)	0 (0%)
Response according to CT assessment, N(%)			
Yes	17 (23.3%)	0 (0%)	10 (43.5%)
No	32 (43.8%)	0 (0%)	13 (56.5%)
Not Assessed (NA)	24 (32.9%)	15(100%)	0 (0%)
Disease Activity Score			
Mean (SD)	1.9 (2.2)	3.4 (2.3)	2.2 (2.2)
Median [Min, Max]	1.0 [0.0, 8.0]	3.0 [1.0, 9.0]	1 [0.0, 8.0]
Missing	0 (0.0%)	2 (13.3%)	1 (4.3%)
CRP, mg/L			
Mean (SD)	37.5 (46.9%)	66.1 (71.0)	45.8 (60.9)
Median [Min, Max]	13.2 [0.2, 170.0]	24.8 [4.6, 189.9]	25.5 [1.0, 260.0]
Missing	0 (0%)	1 (6.7%)	0 (0%)
Hemoglobin, g/dL			
Mean (SD)	12.1 (2.5)	10.3 (2.0)	11.9 (2.3)
Median [Min, Max]	12.3 [6.5, 18.1]	10.1 [7.1, 14.4]	12.0 [7.6, 16.6]
Albumin, g/dL			

Mean (SD)	3.5 (0.7)	3.0 (0.7)	3.5 (0.7)
Median [Min, Max]	3.6 [1.5, 4.9]	2.8 [1.5, 4.0]	3.6 [2.2, 4.9]
Missing	0 (0%)	1 (6.7%)	1 (4.3%)
IL-6, pg/mL			
Mean (SD)	0.7 (0.8)	5.3 (6)	16.9 (21.9)
Median [Min, Max]	0.6 [0.0, 4.0]	2.3 [0.5, 17.5]	4.9 [4.9,4.9]
Missing	6 (8.2%)	3 (20.0%)	1 (4.3%)
IgA, mg/dL			
Mean (SD)	371.4 (227.9)	294.4 (198.7)	394.9 (302.8)
Median [Min, Max]	310 [0.0, 1000.0]	284.0 [30.0, 770.0]	297.0 [36.0, 1000.0]
Missing	0 (0%)	5 (33.3%)	0 (0%)
IgG, mg/dL			
Mean (SD)	2674.2 (2201.5)	1683.9 (942.3)	2031.4 (1574.7)
Median [Min, Max]	1670.0 [472.0, 10800.0]	1595.0 [341.0, 3340.0]	1440.0 [464.0, 6202.0]
Missing	0 (0%)	5 (33.3%)	0 (0%)

*Note: One outlier IL-6 value was removed as per the clinical study report for the Siltuximab phase II trial

Table S2. Demographic characteristics of iMCD patients included in pSTAT3 IHC

	pSTAT3 IHC cohort (n=10)
Sex, N(%)	
F	5 (50.0%)
M	5 (50.0%)
Age	
Mean (SD)	52.2 (13.4)
Median [Min, Max]	54.0 [26.0, 85.0]
Missing	7 (9.6%)
Race, N(%)	
American Indian	1 (10.0%)
Asian	1 (10.0%)
White	6 (60.0%)
Other	1 (10.0%)
Missing	1 (10.0%)
CRP, mg/L	
Mean (SD)	44.3 (49.3)
Median [Min, Max]	28.5 [4.0, 116.0]
Missing	6 (60.0%)
Hemoglobin, g/dL	
Mean (SD)	8.0 (2.0)
Median [Min, Max]	7.0 [6.1, 11.3]
Missing	3 (30.0%)
Albumin, g/dL	
Mean (SD)	2.3 (0.8)
Median [Min, Max]	1.95 [1.4, 3.6]
Missing	3 (30.0%)
IgA, mg/dL	
Mean (SD)	160.7 (81.7)
Median [Min, Max]	121 [54.0, 261.0]
Missing	3 (30.0%)
IgG, mg/dL	
Mean (SD)	1560.9 (855.2)

Median [Min, Max]	1372.0 [585.0, 3264.0]
Missing	3 (30.0%)

Table S3.

Statistical tests and results				
iMCD is a heterogeneous disorder compared to related inflammatory and neoplastic disorders				
	Test	Test statistic	Degrees of freedom	P-value
iMCD cluster v Race	Chi-squared	34.229	30	0.2718
iMCD cluster v Site	Chi-squared	53.769	24	0.0004575
iMCD cluster v Sex	Chi-squared	5.8797	6	0.4368
iMCD cluster v Corticosteroids at Baseline	Chi-squared	6.5995	6	0.3595
iMCD cluster v Prednisone at Baseline	Chi-squared	3.7003	6	0.7172
iMCD cluster v Prednisolone at Baseline	Chi-squared	7.021	6	0.3189
iMCD cluster v Hydrocortisone at Baseline	Chi-squared	4.8558	6	0.5624
iMCD cluster v Prior antineoplastics	Fisher exact p	NA	NA	0.4334
iMCD cluster v Prior immunosuppressants	Fisher exact p	NA	NA	0.8408
iMCD cluster v Prior corticosteroids	Fisher exact p	NA	NA	0.7067
Identification of a novel iMCD subgroup with a superior response to siltuximab				
iMCD C1 v Activity (not including unclustered)	F-statistic	37.4	1, 76	3.854e-08
iMCD C1 v Activity (incl. unclustered)	F-statistic	41.48	1, 84	7.062e-09
iMCD C1 v Soma IL-6 (not including unclustered)	F-statistic	38.17	1, 78	2.742e-08
iMCD C1 v Soma IL-6 (including unclustered)	F-statistic	41.88	1, 86	5.709e-09
iMCD C1 v Response (not including unclustered)	F-statistic	11.75	1, 42	0.001376
iMCD C1 v Response (including unclustered)	F-statistic	12.58	1, 42	0.0008944
Validation of a novel subgroup of iMCD with a superior response to siltuximab				
Mean expression Soma v RBM	F-statistic	66.82	1, 121	3.351e-13
C1 score v Response (discovery)	F-statistic	20.67	1, 75	2.051e-05
C1 score vs Activity (discovery)	F-statistic	62.56	1, 88	7.085e-12
C1 score v Clinical IL-6 (discovery)	F-statistic	24.74	1, 85	3.375e-06
C1 score v Soma IL-6 (discovery)	F-statistic	61.89	1, 90	7.656e-12
C1 score v Response (validation)	F-statistic	2.216	1, 21	0.1514
C1 score v Response (validation), one-sided	t-statistic	1.488	21	0.0757
C1 score vs Activity (validation)	F-statistic	1.860	1, 20	0.07765
C1 score vs Activity (validation), one-sided	t-statistic	3.46	21	0.0385
C1 score v IL-6 (validation)	F-statistic	3.118	1, 21	0.09196
C1 score v IL-6 (validation), one-sided	t-statistic	1.766	21	0.0460
Response v predicted response (validation of prior predictive algorithm)	Z-statistic	0.705	21	0.481
Identification of JAK-STAT3 as a candidate driver pathway in siltuximab non-responders				

Siltuximab responders v non responders (IL-6 expression in germinal centers)	Fisher exact p	NA	NA	0.56
Siltuximab responders v non responders (IL-6 expression in mantle zone)	Fisher exact p	NA	NA	0.96
Siltuximab responders v non responders (IL-6 expression in interfollicular space)	Fisher exact p	NA	NA	0.34
Siltuximab responders v non responders (pSTAT3 expression in germinal centers)	Fisher exact p	NA	NA	0.86
Siltuximab responders v non responders (pSTAT3 expression in mantle zone)	Fisher exact p	NA	NA	0.98
Cluster 1 responder v healthy donor (TNFa signaling via NKfB)	Normalized enrichment score*	1.68	NA	p = 0.004 q = 0.090
Cluster 1 responder v healthy donor (Estrogen Response Early)	Normalized enrichment score	1.59	NA	p = 0.013 q = 0.137
Cluster 1 responder v healthy donor (IFN gamma response)	Normalized enrichment score	1.51	NA	p = 0.033 q = 0.149
Cluster 1 responder v healthy donor (Allograft Rejection Signature)	Normalized enrichment score	1.47	NA	p = 0.033 q = 0.167
Cluster 1 responder v healthy donor (IL-6 JAK STAT3 Signaling)	Normalized enrichment score	1.52	NA	p = 0.020 q = 0.184
Non-responder v healthy donor (KRAS Signaling up)	Normalized enrichment score	1.54	NA	p =0.029 q = 0.118
Non-responder v healthy donor (IL-6 JAK STAT3 Signaling)	Normalized enrichment score	1.54	NA	p = 0.031 q = 0.144
Non-responder v healthy donor (TNFa signaling via NFkB)	Normalized enrichment score	1.66	NA	p = 0.006 q = 0.173
Non-responder v healthy donor (Allograft Rejection Signature)	Normalized enrichment score	1.42	NA	p = 0.043 q = 0.177
Non-responder v healthy donor (IL2 STAT5 Signaling)	Normalized enrichment score	1.55	NA	p = 0.018 q = 0.179

*Normalized Enrichment Score = actual Enrichment score / mean (Enrichment scores against all permutations of the dataset). Enrichment score is calculated by calculating a running sum statistic over a ranked list of genes, increasing when a gene is in the gene set and decreasing when not

Table S4: Frequencies of each disease group within each cluster.

	Cluster A	Cluster B	Cluster C	Cluster D	Cluster E	Unclustered
iMCD	5 (5.7)	18 (20.4)	11 (12.5)	22 (25)	20 (22.3)	12 (12.6)
Cluster 1	0	0	0	22 (81.4)	0	5 (18.5)
Cluster 2	0	0	7 (100)	0	0	0
Cluster 3	0	0	0	0	10 (100)	0
Cluster 4	0	0	0	0	7 (100)	0
Cluster 5	0	15 (100)	0	0	0	0
Cluster 6	5 (35.7)	3 (21.4)	0	0	0	6 (42.8)
Unclustered	0	0	4 (50)	0	3 (37.5)	1 (12.5)
Hodgkin lymphoma	0	0	0	19 (95)	0	1 (5)
Rheumatoid arthritis	19 (95)	0	0	0	1 (5)	0
HHV8-associated MCD	17 (85)	2 (10)	0	0	0	1 (5)

Table S5. Example FDA-approved drugs that can inhibit the enriched pathways along with their previously reported uses in iMCD.

Pathway	Drug	Use in iMCD	Response
TNFa signaling via NFkB	Etanercept	1	0/1
	Adalimumab	0	0/0
	Infliximab	0	0/0
	Golimumab	0	0/0
	Certolizumab	0	0/0
IL-6 JAK-STAT3 Signaling	Ruxolitinib	0	0/0
	Tofacitinib	0	0/0
	Upadacitinib	0	0/0
	Fedratinib	0	0/0
IFN gamma response	Emapalumab	0	0/0
IL2 STAT5 Signaling	Basiliximab	0	0/0
IL2 STAT5 Signaling AND Allograft Rejection Signature	Cyclosporin	10	4/7
	Tacrolimus	2	2/2
Allograft Rejection Signature	Sirolimus	3	3/3
KRAS Signaling Up	N/A	N/A	N/A

* 3 not assessable due to adverse event(s)