

Supplementary Materials for **Distinct plasma immune signatures in ME/CFS are present early in the course of illness**

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Supplementary Materials and Methods

Dimensionality reduction methods. Feature selection strategies select subsets of relevant features for use in regression model construction based on the assumption that the data contain many redundant or irrelevant features. Feature extraction transforms highly dimensional input data into a reduced, representative set of features by extracting as much relevant information as possible from the input data. We employed one of the most widely used feature selection techniques, LASSO (least absolute shrinkage and selection operator) (69), and two feature extraction approaches, principal component analysis (PCA) and the partial least square (PLS) method.

LASSO regularizes the least squares by applying the constraint that the L1 norm of the parameter vector is no greater than a given value, whereby increasing the penalty will cause more and more of the parameters to be driven to absolute zero. To avoid overfitting, we selected variables that had non-zero coefficients when the cross-validation deviance reached the minimum plus one standard error of the mean (SEM). Our feature extraction approach began with the most popular orthogonal transformation procedure, PCA, wherein the PC of the original predictors are used as independent variables of the logistic regression model (73). A stepwise selection scheme was used to choose the optimal set of PCs wherein one PC was entered or excluded at each step to achieve the greatest improvement of model performance. Two separate criteria were used to evaluate the improvement in model performance: (1) conditional likelihood-ratio test and (2) cross-validation error rate. Upon arriving at the optimal set of PCs for the logistic regression model, the parameter vector and variance-covariance matrix were used to calculate the odds ratios (OR) and p values of all the original predictor variables, because all the PCs are certain linear combinations of them. If one predictor variable was significant ($p < 0.05$) for both model improvement criteria, that predictor variable was considered a potential candidate for the final logistic regression model.

Because PCA is an unsupervised dimension reduction technique in which no importance is assigned to how each predictive variable relates to the dependent variable, we conducted an additional feature extraction method, PLS, that simultaneously incorporates the response variable into the procedure. Similar methods were then pursued, using PLS latent variables instead of PCs. The same two-model improvement strategy were applied here, wherein a potential candidate for the final logistic regression model independent variable was chosen if it was significant for both criteria.

For the final logistic regression model, a cytokine variable was entered either if it was selected by LASSO or if it was a potential candidate via both the PCA and PLS feature extraction methods. The ORs, their 95% confidence intervals (CIs) and p values of the selected cytokines were calculated accordingly.

Supplementary Tables

Table S1. Comparison of plasma immune analytes in ME/CFS subjects vs. controls.*

Analyte	<i>p</i> value
IL1ra	0.9068
IL1 α	0.5751
IL1 β	0.2609
IL2	0.4518
IL4	0.3296
IL5	0.2423
IL6	0.0401
IL7	0.7758
CXCL8 (IL8)	0.0437
IL10	0.0241
IL12p40	0.0803
IL12p70	0.3737
IL13	0.5806
IL15	0.7576
IL17A	0.0043
IL17F	0.1411
IFN α 2	0.1906
IFN β	0.7508
IFN γ	0.1631
TNF α	0.5123
TNF β	0.0028
CD40L	0.1389
sFasL	0.0129
TRAIL (TNFSF10)	0.0950
CCL2 (MCP1)	0.4500
CCL3 (MIP1 α)	0.0524
CCL4 (MIP1 β)	0.9308
CCL5 (RANTES)	0.7691
CCL7 (MCP3)	0.9036
CCL11 (eotaxin)	0.0858
CXCL1 (GRO α)	0.6067
CXCL5 (ENA78)	0.1643
CXCL9 (MIG)	0.0863
CXCL10 (IP10)	0.0444
TGF α	0.1189
TGF β	0.0694
SCF (SF)	0.2187
CSF1 (MCSF)	0.0235
CSF2 (GMCSF)	0.3184
CSF3 (GCSF)	0.5793
PDGFBB	0.9647
β NGF	0.1838
FGFb	0.9093
HGF	0.6292
VEGFA	0.9068
LIF	0.1466
Resistin	0.1866
Leptin	0.0301
Serpin E1 (PAI1)	0.0909
sICAM1 (CD54)	0.3000
VCAM1 (CD106)	0.7695

* One-way ANOVA/GLM with independent variable of diagnosis (ME/CFS vs. controls)

Table S2. Comparison of plasma immune analytes in ME/CFS subjects vs. controls.*

Analyte	Diagnosis	Sex	Diagnosis x Sex	Age
	<i>p</i> value	<i>p</i> value	<i>p</i> value	<i>p</i> value
IL1ra	0.9175	0.6933	0.6678	0.0558
IL1 α	0.9413	0.7373	0.3884	0.0829
IL1 β	0.5874	0.9190	0.3640	0.1601
IL2	0.7543	0.0838	0.4941	0.1804
IL4	0.7944	0.9429	0.2223	0.0158
IL5	0.3641	0.0727	0.8380	0.4267
IL6	0.0483	0.6017	0.7052	0.0428
IL7	0.7033	0.7754	0.1971	0.2505
CXCL8 (IL8)	0.0947	0.1898	0.8144	0.0047
IL10	0.1106	0.6836	0.4414	0.2619
IL12p40	0.2721	0.9597	0.3602	0.0002
IL12p70	0.5700	0.0663	0.6891	0.9641
IL13	0.3478	0.9500	0.3508	0.0321
IL15	0.9063	0.4405	0.4339	0.4828
IL17A	0.0203	0.9748	0.7014	0.0034
IL17F	0.4386	0.0093	0.3079	0.8692
IFN α 2	0.2912	0.5468	0.8652	0.1741
IFN β	0.9446	0.2998	0.4873	0.0504
IFN γ	0.3834	0.1225	0.4900	0.2584
TNF α	0.7592	0.6926	0.5889	0.0448
TNF β	0.0073	0.9654	0.9248	0.0001
CD40L	0.3436	0.1087	0.4513	0.0394
sFasL	0.0385	0.2258	0.7717	0.0001
TRAIL (TNFSF10)	0.3209	0.8575	0.3306	0.0032
CCL2 (MCP1)	0.8703	0.2498	0.0939	0.4618
CCL3 (MIP1 α)	0.1641	0.2522	0.5142	0.1733
CCL4 (MIP1 β)	0.6983	0.0007	0.4813	0.3767
CCL5 (RANTES)	0.5778	0.5815	0.5429	0.0014
CCL7 (MCP3)	0.8130	0.6848	0.4864	0.1881
CCL11 (eotaxin)	0.1167	0.0121	0.8770	0.0008
CXCL1 (GRO α)	0.3414	0.4070	0.3093	0.3734
CXCL5 (ENA78)	0.4476	<0.0001	0.3531	0.0210
CXCL9 (MIG)	0.2302	0.5377	0.5458	0.5985
CXCL10 (IP10)	0.1505	0.4965	0.5011	0.1518
TGF α	0.1938	0.2441	0.9014	0.0023
TGF β	0.2131	0.0712	0.4980	0.3418
SCF (SF)	0.2181	0.7787	0.7416	0.1228
CSF1 (MCSF)	0.0671	0.4191	0.7182	0.0002
CSF2 (GMCSF)	0.2545	<0.0001	0.6672	0.1394
CSF3 (GCSF)	0.7341	0.3920	0.7782	0.0795
PDGFBB	0.7005	0.0844	0.4040	<0.0001
β NGF	0.3029	0.5960	0.7809	0.4046
FGFb	0.5949	0.8500	0.3757	0.5767
HGF	0.9309	0.0007	0.2640	0.1341
VEGFA	0.7822	0.4449	0.7310	0.4215
LIF	0.4132	0.0569	0.3673	0.4916
Resistin	0.1509	0.4838	0.5869	<0.0001
Leptin	0.0195	<0.0001	0.6649	0.8029
Serpin E1 (PAI1)	0.2248	0.4853	0.5865	0.0308
sICAM1 (CD54)	0.3357	0.0131	0.9554	0.0057
VCAM1 (CD106)	0.7551	0.2373	0.9268	0.0001

* Two-way ANOVA/GLM with independent variables of diagnosis (ME/CFS vs. controls) and sex with age as a continuous covariate

Table S3. Comparison of plasma immune markers in ME/CFS subjects vs. controls.

Analyte	ME/CFS (n=298)	Control (n=348)	ME/CFS vs. Control
	mean (SEM)	mean (SEM)	<i>p</i> value
IL1ra	39.6 (1.3)	40.5 (1.8)	0.9068
IL1 α	7.2 (0.2)	7.4 (0.2)	0.5751
IL1 β	4.5 (0.1)	4.7 (0.1)	0.2609
IL2	4.0 (0.1)	4.1 (0.1)	
IL4	48.7 (0.8)	49.7 (0.7)	0.3296
IL5	30.0 (0.7)	31.1 (0.8)	
IL6	10.2 (0.4)	10.9 (0.3)	0.0401
IL7	15.3 (0.3)	15.4 (0.3)	
CXCL8 (IL8)	3.4 (0.2)	4.0 (0.2)	0.0437
IL10	6.8 (0.2)	7.2 (0.2)	0.0241
IL12p40	5.3 (0.1)	5.6 (0.1)	0.0803
IL12p70	13.7 (0.4)	14.0 (0.3)	
IL13	9.9 (0.2)	9.8 (0.2)	0.5806
IL15	40.6 (1.4)	40.6 (1.1)	
IL17A	18.3 (0.6)	20.6 (0.6)	0.0043
IL17F	1.0 (0.1)	1.6 (0.6)	
IFN α 2	26.1 (1.2)	27.2 (1.0)	0.1906
IFN β	109.7 (13.6)	113.2 (10.7)	
IFN γ	1.1 (0.0)	1.2 (0.0)	0.1631
TNF α	8.4 (0.3)	8.7 (0.4)	0.5123
TNF β	2.4 (0.2)	3.3 (0.2)	0.0028
CD40L	109.6 (5.3)	100.6 (5.3)	0.1389
sFasL	10.2 (0.3)	11.4 (0.3)	0.0129
TRAIL (TNFSF10)	51.9 (7.3)	48.9 (1.6)	0.0950
CCL2 (MCP1)	31.2 (0.5)	31.6 (0.5)	0.4500
CCL3 (MIP1 α)	11.2 (0.6)	12.4 (0.7)	0.0524
CCL4 (MIP1 β)	267.6 (30.0)	241.3 (7.7)	
CCL5 (RANTES)	74.6 (1.6)	79.5 (4.8)	
CCL7 (MCP3)	149.3 (2.7)	149.7 (2.4)	
CCL11 (eotaxin)	31.0 (0.8)	28.7 (0.6)	
CXCL1 (GRO α)	13.2 (0.3)	13.2 (0.3)	
CXCL5 (ENA78)	116.5 (4.4)	126.0 (5.2)	
CXCL9 (MIG)	74.4 (9.4)	74.8 (3.8)	0.0863
CXCL10 (IP10)	40.5 (0.8)	43.5 (1.2)	0.0444
TGF α	2.6 (0.1)	2.8 (0.1)	0.1189
TGF β	36.3 (1.1)	38.8 (1.1)	0.0694
SCF (SF)	10.8 (0.2)	11.1 (0.2)	0.2187
CSF1 (MCSF)	7.1 (0.3)	7.9 (0.3)	0.0235
CSF2 (GMCSF)	38.2 (1.4)	35.7 (1.1)	
CSF3 (GCSF)	25.3 (0.5)	25.8 (0.5)	
PDGFBB	170.7 (10.6)	158.1 (8.8)	0.9647
β NGF	27.5 (0.7)	26.3 (0.5)	
FGFb	11.0 (0.2)	11.0 (0.2)	
HGF	15.2 (0.3)	15.6 (0.4)	
VEGFA	32.2 (1.0)	31.6 (0.6)	
LIF	3.8 (0.1)	4.0 (0.1)	
Resistin	280.7 (8.2)	290.0 (6.5)	0.1866
Leptin	1506.4 (76.4)	1398.4 (96.3)	
Serpin E1 (PAI1)	556.4 (11.8)	555.5 (21.2)	
sICAM1 (CD54)	1932.7 (104.2)	2078.3 (110.5)	0.3000
VCAM1 (CD106)	269.1 (6.3)	281.0 (12.0)	0.7695

* *p* value included for analytes meeting GLM criteria; green cells represent upregulated analytes and red cells represent downregulated analytes (2-group comparisons)

+ t-test with independent variables of diagnosis (ME/CFS vs. controls)

Table S4. Comparison of plasma immune analytes in short vs. long duration ME/CFS subjects vs. controls.*

Analyte	<i>p</i> value
IL1ra	0.0104
IL1 α	0.0067
IL1 β	0.0052
IL2	0.7353
IL4	0.0001
IL5	0.3836
IL6	0.0030
IL7	0.6648
CXCL8 (IL8)	<0.0001
IL10	0.0001
IL12p40	<0.0001
IL12p70	0.2011
IL13	0.0458
IL15	0.7660
IL17A	<0.0001
IL17F	0.1032
IFN α 2	0.0108
IFN β	0.1456
IFN γ	0.0073
TNF α	0.0094
TNF β	<0.0001
CD40L	<0.0001
sFasL	<0.0001
TRAIL (TNFSF10)	<0.0001
CCL2 (MCP1)	0.0059
CCL3 (MIP1 α)	0.0007
CCL4 (MIP1 β)	0.6579
CCL5 (RANTES)	0.0615
CCL7 (MCP3)	0.4299
CCL11 (eotaxin)	0.0557
CXCL1 (GRO α)	0.6696
CXCL5 (ENA78)	0.2779
CXCL9 (MIG)	0.0011
CXCL10 (IP10)	0.0015
TGF α	0.0031
TGF β	0.0332
SCF (SF)	0.0006
CSF1 (MCSF)	<0.0001
CSF2 (GMCSF)	0.2917
CSF3 (GCSF)	0.1037
PDGFBB	0.0003
β NGF	0.3567
FGFb	0.6892
HGF	0.0837
VEGFA	0.9907
LIF	0.3236
Resistin	<0.0001
Leptin	0.0740
Serpin E1 (PAI1)	0.1738
sICAM1 (CD54)	0.0238
VCAM1 (CD106)	0.0612

* One-way ANOVA/GLM with independent variable of diagnosis (short vs. long duration ME/CFS vs. controls)

Table S5. Comparison of plasma immune analytes in short vs. long duration ME/CFS subjects vs. controls.*

Analyte	Diagnosis	Sex	Diagnosis x Sex	Age
	<i>p</i> value	<i>p</i> value	<i>p</i> value	<i>p</i> value
IL1ra	0.1913	0.8262	0.5094	0.1724
IL1 α	0.1270	0.9731	0.4614	0.2485
IL1 β	0.0665	0.9108	0.6050	0.4164
IL2	0.9371	0.1740	0.7216	0.1934
IL4	0.0096	0.9179	0.4317	0.1021
IL5	0.6365	0.1219	0.8689	0.4990
IL6	0.0410	0.2902	0.5550	0.1232
IL7	0.8881	0.8465	0.3836	0.3130
CXCL8 (IL8)	0.0022	0.4767	0.7934	0.0436
IL10	0.0045	0.8667	0.7166	0.6830
IL12p40	0.0015	0.9485	0.5104	0.0048
IL12p70	0.4531	0.1233	0.7969	0.7254
IL13	0.1482	0.8706	0.6381	0.0921
IL15	0.9653	0.5248	0.6670	0.5529
IL17A	0.0004	0.8737	0.7787	0.0357
IL17F	0.2767	0.1254	0.5854	0.8721
IFN α 2	0.0837	0.5465	0.9030	0.4024
IFN β	0.4496	0.4974	0.7663	0.1098
IFN γ	0.0932	0.1994	0.6486	0.5555
TNF α	0.1720	0.5073	0.5716	0.1437
TNF β	0.0010	0.7754	0.8397	0.0017
CD40L	0.0020	0.1523	0.7395	0.2195
sFasL	0.0003	0.4624	0.8347	0.0017
TRAIL (TNFSF10)	0.0073	0.8671	0.5180	0.0307
CCL2 (MCP1)	0.1359	0.2304	0.0978	0.8835
CCL3 (MIP1 α)	0.0240	0.5497	0.6048	0.4676
CCL4 (MIP1 β)	0.8490	0.0538	0.3937	0.2894
CCL5 (RANTES)	0.2083	0.6093	0.7843	0.0059
CCL7 (MCP3)	0.8198	0.6086	0.6324	0.2732
CCL11 (eotaxin)	0.1746	0.0968	0.9480	0.0022
CXCL1 (GRO α)	0.6325	0.4512	0.4809	0.4363
CXCL5 (ENA78)	0.4815	<0.0001	0.3318	0.0335
CXCL9 (MIG)	0.0164	0.6125	0.7497	0.8942
CXCL10 (IP10)	0.0351	0.7020	0.6823	0.3910
TGF α	0.0815	0.2421	0.8045	0.0117
TGF β	0.1380	0.3012	0.7846	0.5668
SCF (SF)	0.0264	0.3547	0.4948	0.3862
CSF1 (MCSF)	0.0012	0.9998	0.4268	0.0040
CSF2 (GMCSF)	0.2081	0.0001	0.5438	0.2293
CSF3 (GCSF)	0.3028	0.6094	0.9592	0.1739
PDGFBB	0.0992	0.1167	0.4564	<0.0001
β NGF	0.5627	0.6857	0.9579	0.4609
FGFb	0.7459	0.9677	0.6589	0.6919
HGF	0.3134	0.0109	0.4923	0.2819
VEGFA	0.9082	0.4245	0.8237	0.4089
LIF	0.7007	0.1274	0.5585	0.4478
Resistin	0.0093	0.4317	0.8176	<0.0001
Leptin	0.0633	<0.0001	0.1511	0.8545
Serpin E1 (PAI1)	0.1604	0.9041	0.6489	0.0163
sICAM1 (CD54)	0.0951	0.0407	0.9456	0.0228
VCAM1 (CD106)	0.2988	0.2958	0.9588	0.0004

* Two-way ANOVA/GLM with independent variables of diagnosis (short vs. long duration ME/CFS vs. controls) and sex with age as a continuous covariate

Table S6. Comparison of plasma immune markers in short vs. long duration ME/CFS vs. controls.

Analyte	Short duration ME/CFS (n=52)	Long duration ME/CFS (n=246)	Control (n=348)	Short vs. long duration ME/CFS	Short duration ME/CFS vs. Control	Long duration ME/CFS vs. Control
	mean (SEM)	mean (SEM)	mean (SEM)	p value* [†]	p value* [†]	p value* [†]
IL1ra	45.2 (2.4)	38.5 (1.4)	40.5 (1.8)	0.0028	0.0105	0.2864
IL1α	8.4 (0.5)	7.0 (0.2)	7.4 (0.2)	0.0017	0.0178	0.1309
IL1β	5.1 (0.2)	4.4 (0.1)	4.7 (0.1)	0.0015	0.0504	0.0440
IL2	4.0 (0.3)	4.0 (0.1)	4.1 (0.1)			
IL4	55.4 (1.7)	47.3 (0.8)	49.7 (0.7)	<0.0001	0.0028	0.0257
IL5	30.9 (1.6)	29.8 (0.8)	31.1 (0.8)			
IL6	12.1 (0.9)	9.8 (0.5)	10.9 (0.3)	0.0105	0.1996	0.0048
IL7	15.7 (0.7)	15.3 (0.4)	15.4 (0.3)			
CXCL8 (IL8)	4.6 (0.3)	3.1 (0.2)	4.0 (0.2)	<0.0001	0.0112	0.0011
IL10	7.9 (0.4)	6.6 (0.2)	7.2 (0.2)	0.0004	0.0644	0.0011
IL12p40	6.5 (0.3)	5.0 (0.1)	5.6 (0.1)	<0.0001	0.0009	0.0012
IL12p70	14.8 (1.0)	13.4 (0.4)	14.0 (0.3)			
IL13	10.9 (0.5)	9.7 (0.2)	9.8 (0.2)	0.0128	0.0198	0.8047
IL15	41.2 (2.6)	40.5 (1.6)	40.6 (1.1)			
IL17A	23.8 (1.6)	17.2 (0.6)	20.6 (0.6)	<0.0001	0.0243	<0.0001
IL17F	0.9 (0.1)	1.0 (0.1)	1.6 (0.6)			
IFNα2	33.1 (3.6)	24.7 (1.2)	27.2 (1.0)	0.0089	0.1052	0.0330
IFNβ	108.3 (11.5)	110.0 (16.3)	113.2 (10.7)			
IFNγ	1.3 (0.1)	1.1 (0.0)	1.2 (0.0)	0.0040	0.1077	0.0262
TNFα	10.0 (0.7)	8.0 (0.4)	8.7 (0.4)	0.0029	0.0261	0.1192
TNFβ	4.0 (0.4)	2.1 (0.2)	3.3 (0.2)	<0.0001	0.0957	<0.0001
CD40L	72.8 (8.5)	117.3 (6.1)	100.6 (5.3)	<0.0001	0.0037	0.0060
sFasL	13.7 (0.7)	9.4 (0.4)	11.4 (0.3)	<0.0001	0.0042	0.0001
TRAIL (TNFSF10)	94.7 (41.0)	42.8 (1.5)	48.9 (1.6)	<0.0001	0.0079	0.0027
CCL2 (MCP1)	34.6 (1.2)	30.4 (0.6)	31.6 (0.5)	0.0025	0.0208	0.0889
CCL3 (MIP1α)	13.2 (0.8)	10.8 (0.7)	12.4 (0.7)	0.0011	0.0688	0.0042
CCL4 (MIP1β)	246.9 (14.6)	272.0 (36.2)	241.3 (7.7)			
CCL5 (RANTES)	83.1 (3.6)	72.8 (1.8)	79.5 (4.8)			
CCL7 (MCP3)	157.1 (7.0)	147.7 (2.9)	149.7 (2.4)			
CCL11 (eotaxin)	26.8 (1.1)	31.9 (0.9)	28.7 (0.6)			
CXCL1 (GROα)	13.5 (0.6)	13.2 (0.3)	13.2 (0.3)			
CXCL5 (ENA78)	113.1 (11.4)	117.3 (4.8)	126.0 (5.2)			
CXCL9 (MIG)	127.9 (52.8)	63.1 (2.2)	74.8 (3.8)	0.0011	0.0668	0.0071
CXCL10 (IP10)	45.0 (1.7)	39.5 (0.9)	43.5 (1.2)	0.0023	0.1435	0.0046
TGFα	3.2 (0.2)	2.5 (0.2)	2.8 (0.1)	0.0036	0.0712	0.0153
TGFβ	40.4 (2.7)	35.4 (1.3)	38.8 (1.1)	0.0666	0.5251	0.0207
SCF (SF)	12.5 (0.6)	10.5 (0.2)	11.1 (0.2)	0.0008	0.0110	0.0185
CSF1 (MCSF)	10.2 (0.9)	6.4 (0.3)	7.9 (0.3)	<0.0001	0.0028	0.0002
CSF2 (GMCSF)	33.1 (2.5)	39.2 (1.6)	35.7 (1.1)			
CSF3 (GCSF)	27.6 (1.2)	24.9 (0.6)	25.8 (0.5)			
PDGFBB	97.8 (13.9)	186.1 (12.3)	158.1 (8.8)	<0.0001	0.0004	0.1789
βNGF	28.5 (3.0)	27.3 (0.5)	26.3 (0.5)			
FGFb	11.4 (0.5)	11.0 (0.2)	11.0 (0.2)			
HGF	16.7 (0.8)	14.9 (0.4)	15.6 (0.4)			
VEGFA	31.5 (1.3)	32.3 (1.2)	31.6 (0.6)			
LIF	3.9 (0.2)	3.8 (0.1)	4.0 (0.1)			
Resistin	335.2 (13.0)	269.2 (9.4)	290.0 (6.5)	<0.0001	0.0024	0.0087
Leptin	1600.6 (192.0)	1486.5 (83.3)	1398.4 (96.3)			
Serpin E1 (PAI1)	540.2 (31.9)	559.9 (12.6)	555.5 (21.2)			
sICAM1 (CD54)	2560.0 (362.3)	1800.1 (98.7)	2078.3 (110.5)	0.0122	0.1175	0.0684
VCAM1 (CD106)	314.5 (17.3)	259.6 (6.6)	281.0 (12.0)			

* p value included for analytes meeting GLM criteria; green cells represent upregulated analytes and red cells represent downregulated analytes (2-group comparisons)

[†] t-test with independent variable of diagnosis (short vs. long duration ME/CFS, short duration ME/CFS vs. controls, long duration ME/CFS vs. controls)

Table S7. Spearman correlations of levels of plasma immune markers with duration of illness in ME/CFS.*

Analyte	Correlation Coefficient	<i>p</i> value
IL1ra	-0.183	0.001539
IL1 α	-0.138	0.016906
IL1 β	-0.164	0.004619
IL2	0.04	0.488784
IL4	-0.232	0.000051
IL5	-0.018	0.759259
IL6	-0.115	0.046946
IL7	-0.027	0.641715
CXCL8 (IL8)	-0.236	0.000038
IL10	-0.188	0.001127
IL12p40	-0.286	0.000001
IL12p70	-0.061	0.297387
IL13	-0.133	0.022093
IL15	-0.015	0.797466
IL17A	-0.217	0.000155
IL17F	-0.056	0.333938
IFN α 2	-0.111	0.054611
IFN β	-0.045	0.435424
IFN γ	-0.143	0.013186
TNF α	-0.156	0.006910
TNF β	-0.276	0.000001
CD40L	0.197	0.000637
sFasL	-0.257	0.000007
TRAIL (TNFSF10)	-0.261	0.000005
CCL2 (MCP1)	-0.146	0.011542
CCL3 (MIP1 α)	-0.138	0.016923
CCL4 (MIP1 β)	-0.021	0.721205
CCL5 (RANTES)	-0.134	0.021111
CCL7 (MCP3)	-0.047	0.420360
CCL11 (eotaxin)	0.096	0.098306
CXCL1 (GRO α)	-0.068	0.244968
CXCL5 (ENA78)	-0.011	0.853600
CXCL9 (MIG)	-0.135	0.019609
CXCL10 (IP10)	-0.123	0.033956
TGF α	-0.173	0.002768
TGF β	-0.085	0.145443
SCF (SF)	-0.202	0.000439
CSF1 (MCSF)	-0.261	0.000005
CSF2 (GMCSF)	0.102	0.077525
CSF3 (GCSF)	-0.113	0.051063
PDGFBB	0.174	0.002615
β NGF	0.035	0.551497
FGFb	-0.059	0.313315
HGF	-0.119	0.040546
VEGFA	-0.019	0.741025
LIF	-0.027	0.644775
Resistin	-0.258	0.000006
Leptin	-0.049	0.399718
Serpin E1 (PAI1)	0.006	0.913159
sICAM1 (CD54)	-0.196	0.000659
VCAM1 (CD106)	-0.184	0.001421

* green cells represent positive correlations with $p < 0.05$; red cells represent inverse correlations with $p < 0.05$

Table S8. Feature selection data for logistic regression predicting short vs. long duration ME/CFS.

Variable	PCA LR		PCA CV LR		PLS LR		PLS CV LR		LASSO
	OR	p value	OR	p value	OR	p value	OR	p value	
Age	0.029	0.000	0.041	0.000	0.097	0.009	0.074	0.000	-2.140082812
Sex	0.712	0.428	0.774	0.514	0.394	0.056	0.786	0.534	0
IL1ra	0.826	0.541	0.364	0.345	3.268	0.399	1.514	0.565	0
IL1 α	1.403	0.079	1.228	0.494	29.422	0.000	1.477	0.459	0
IL1 β	15.175	0.005	1.024	0.929	0.010	0.000	0.272	0.492	0
IL2	11.198	0.000	1.868	0.329	5.460	0.001	0.612	0.623	0
IL4	0.157	0.373	1.121	0.555	0.435	0.187	0.682	0.767	0
IL5	0.000	0.000	1.169	0.513	0.001	0.000	1.247	0.889	0
IL6	8.767	0.155	0.456	0.386	0.462	0.002	0.715	0.894	0
IL7	0.048	0.166	0.692	0.397	3.953	0.000	5.652	0.213	0
CXCL8 (IL8)	11.730	0.000	1.008	0.963	4.561	0.000	2.115	0.694	0
IL10	1.212	0.210	0.545	0.502	6.032	0.000	1.580	0.816	0
IL12p40	1.241	0.914	1.103	0.795	8.682	0.000	2.250	0.402	1.05083007
IL12p70	0.000	0.001	0.082	0.041	0.000	0.000	0.035	0.012	0
IL13	3.580	0.027	1.019	0.924	15.500	0.000	3.730	0.575	0
IL15	0.000	0.042	0.815	0.469	0.136	0.002	1.183	0.919	0
IL17A	0.000	0.093	1.157	0.235	2.026	0.000	0.975	0.993	1.124991176
IL17F	0.000	0.001	2.101	0.158	0.000	0.000	0.221	0.343	0
IFN α 2	12.276	0.006	1.924	0.348	4.769	0.000	23.358	0.005	0
IFN β	2.993	0.574	1.052	0.954	0.000	0.000	0.085	0.350	0
IFN γ	20.874	0.011	11.525	0.001	50.430	0.000	33.856	0.000	0
TNF α	0.000	0.000	0.092	0.006	0.000	0.000	0.011	0.019	0
TNF β	0.002	0.004	0.992	0.963	0.000	0.000	0.474	0.371	0
CD40L	5.765	0.005	3.351	0.339	0.569	0.525	2.617	0.253	0
sFasL	3.554	0.271	0.842	0.481	1.813	0.002	0.901	0.876	0.439220737
TRAIL (TNFSF10)	1.959	0.129	0.941	0.855	12.404	0.000	8.527	0.761	0
CCL2 (MCP1)	80.695	0.000	0.793	0.690	16.755	0.000	4.601	0.222	0
CCL3 (MIP1 α)	0.016	0.272	0.692	0.370	20.611	0.034	2.124	0.500	0
CCL4 (MIP1 β)	5.500	0.163	0.295	0.309	0.001	0.000	0.153	0.118	0
CCL5 (RANTES)	9.538	0.000	0.846	0.851	33.594	0.000	1.936	0.497	0
CCL7 (MCP3)	4.012	0.020	1.285	0.705	1.677	0.439	6.059	0.022	0
CCL11 (eotaxin)	0.002	0.000	0.849	0.776	0.005	0.000	0.125	0.008	-1.179
CXCL1 (GRO α)	1.960	0.674	2.349	0.481	7.914	0.011	3.023	0.207	0
CXCL5 (ENA78)	0.129	0.030	0.523	0.375	0.466	0.394	1.557	0.079	0
CXCL9 (MIG)	17.549	0.001	0.859	0.692	19.802	0.000	0.823	0.978	0
CXCL10 (IP10)	5.784	0.084	1.091	0.867	0.072	0.002	0.241	0.024	0
TGF α	49.667	0.016	1.387	0.345	4.879	0.007	1.696	0.772	0
TGF β	0.022	0.036	1.149	0.801	0.077	0.000	0.583	0.534	0
SCF (SF)	1.652	0.245	1.142	0.815	0.931	0.827	0.613	0.299	0
CSF1 (MCSF)	13.256	0.045	1.437	0.473	3.297	0.000	2.403	0.787	1.412636997
CSF2 (GMCSF)	16.208	0.051	0.428	0.193	0.012	0.000	0.375	0.141	-1.336685279
CSF3 (GCSF)	0.285	0.564	0.932	0.933	0.017	0.000	0.389	0.174	0
PDGFBB	0.003	0.000	0.750	0.626	0.063	0.000	0.693	0.612	-0.540375323
β NGF	0.394	0.253	1.115	0.682	8.505	0.000	21.938	0.000	0
FGFb	0.027	0.120	1.238	0.755	0.012	0.000	0.207	0.176	0
HGF	0.019	0.000	0.627	0.484	0.134	0.000	0.218	0.003	0
VEGFA	14.938	0.002	0.224	0.178	9.058	0.083	2.113	0.655	0
LIF	2.214	0.015	1.140	0.688	0.233	0.071	0.295	0.258	0
Resistin	2.217	0.685	2.850	0.431	31.180	0.000	12.428	0.028	0
Leptin	0.046	0.069	0.511	0.449	0.012	0.000	0.068	0.001	0
Serpin E1 (PAI1)	0.896	0.843	0.989	0.984	0.740	0.631	0.473	0.463	0
sICAM1 (CD54)	0.507	0.456	1.340	0.462	0.299	0.226	1.368	0.569	0
VCAM1 (CD106)	1.637	0.361	1.694	0.403	1.906	0.434	6.387	0.021	0

Key: CV, cross-validation; LASSO, least absolute shrinkage and selection operator; LR, logistic regression; ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome; OR, odds ratio; PCA, principal component analysis; PLS, partial least square. Yellow highlights indicate cytokines (“features”) that met either LASSO or both the PCA and PLS criteria for inclusion in the final logistic regression model