Supplemental Table 1. Study subject co-morbidities and family disease frequency.

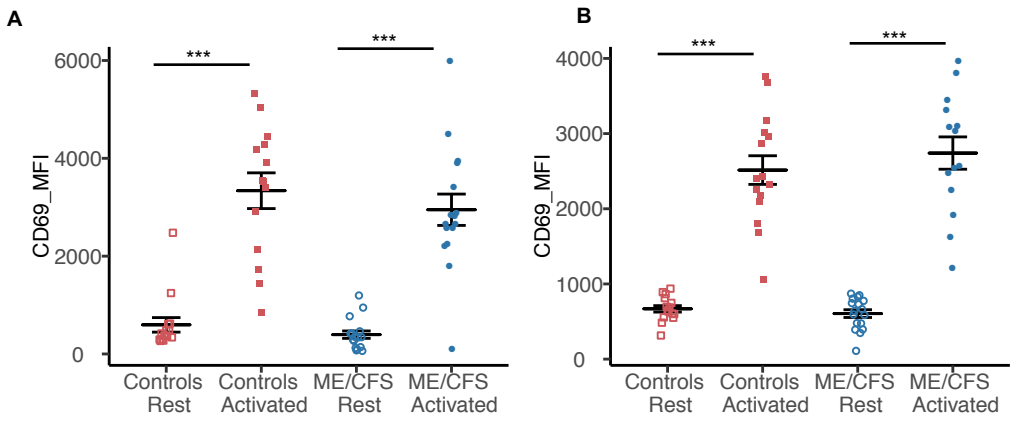
		ME/CFS (n=53)	Healthy Controls (n=45)	p-value
Gastrointestinal	Irritable Bowel Syndrome	23/53 (43.4%)	3/45 (6.7%)	p<0.001
	Ulcerative Colitis	5/53 (9.4%)	0/45 (0%)	0.1
	Crohn's Disease	0/53 (0%)	1/45 (2.2%)	0.9
	Gastric Reflux	16/53 (30.2%)	4/45 (8.9%)	0.02
	Esophagitis	9/53 (17%)	0/45 (0%)	0.01
	Hiatal Hernia	11/53 (20.8%)	0/45 (0%)	0.003
	Frequent lack of appetite	16/53 (30.2%)	0/45 (0%)	p<0.001
	Frequent abdominal pain	20/53 (37.7%)	3/45 (6.7%)	p<0.001
Disease Frequency	Cancer	13/53 (24.5%)	4/44 (9.1%)	0.08
	Fibromyalgia	3/53 (5.7%)	0/45 (0%)	0.3
	Multiple Sclerosis	0/53 (0%)	0/45 (0%)	1
	Sjogren's Syndrome	1/53 (1.9%)	0/45 (0%)	1
	Graves' Disease	1/53 (1.9%)	0/45 (0%)	1
	Rheumatoid Arthritis	2/53 (3.8%)	0/45 (0%)	0.55
	Hashimoto's Disease	1/53 (1.9%)	0/45 (0%)	1
	Systemic Lupus Erythematosus	1/53 (1.9%)	0/45 (0%)	1
	Type I Diabetes	0/53 (0%)	0/45 (0%)	1
	Small Fiber Neuropathy	2/53 (3.8%)	0/45 (0%)	0.55
	Sarcoidosis	0/53 (0%)	0/45 (0%)	1
	Multiple Chemical Sensitivity	2/53 (3.8%)	0/45 (0%)	0.55
	Allergies	39/53 (73.6%)	22/45 (48.9%)	0.02
Family Disease Frequency	Fibromyalgia	9/53 (17%)	5/45 (11.1%)	0.59
	Multiple Sclerosis	5/53 (9.4%)	2/45 (4.4%)	0.57
	Sjogren's Syndrome	5/53 (9.4%)	1/45 (2.2%)	0.29
	Graves' Disease	2/53 (3.8%)	1/45 (2.2%)	1
	Rheumatoid Arthritis	20/53 (37.7%)	6/45 (13.3%)	0.01
	Hashimoto's Disease	4/53 (7.5%)	3/45 (6.7%)	1
	Systemic Lupus Erythematosus	7/53 (13.2%)	1/45 (2.2%)	0.11
	Type I Diabetes	13/53 (24.5%)	2/45 (4.4%)	0.01
	Lymphoma	8/53 (15.1%)	1/45 (2.2%)	0.06
	Leukemia	10/53 (18.9%)	3/45 (6.7%)	0.14
	Small Fiber Neuropathy	0/53 (0%)	2/45 (4.4%)	0.4
	Sarcoidosis	2/53 (3.8%)	1/45 (2.2%)	1
	Multiple Chemical Sensitivity	2/53 (3.8%)	1/45 (2.2%)	1
	Thyroid cancer	3/53 (5.7%)	0/45 (0%)	0.3

Supplemental Table 2. Comparison of plasma cytokines in ME/CFS patients and healthy controls.

Cytokine	Controls (n=37) Mean (SEM)	ME/CFS (n=36) Mean (SEM)	Unadjusted p-value	FDR-adjusted p-value (q-value)
FGFb	58.9 (2.7)	55.1 (2.1)	0.22	0.89
CTACK (CCL27)	478.9 (71.7)	426.6 (24.2)	0.26	0.89
Eotaxin (CCL11)	25.0 (2.0)	26.4 (1.9)	0.49	0.89
G-CSF	18.9 (4.1)	14.2 (1.5)	0.59	0.89
GM-CSF	0.9 (0.1)	0.6 (0.1)	0.08	0.89
GROα	370.3 (39.0)	341.8 (38.4)	0.68	0.89
HGF	232.6 (18.7)	213.9 (9.0)	0.83	0.91
IFN-α2	3.9 (0.3)	3.4 (0.3)	0.42	0.89
IFNγ	4.9 (0.5)	4.3 (0.2)	0.76	0.89
IL-10	1.8 (0.2)	1.6 (0.2)	0.73	0.89
IL-12p70	2.3 (0.2)	2.2 (0.2)	0.79	0.89
IL-12p40	29.3 (2.9)	26.8 (2.7)	0.52	0.89
IL-13	1.5 (0.1)	1.5 (0.1)	0.74	0.89
IL-16	229.6 (87.9)	73.0 (10.1)	0.66	0.89
IL-17	6.0 (0.4)	5.9 (0.3)	0.77	0.89
IL-18	38.1 (2.4)	33.5 (2.5)	0.15	0.89
IL-1α	13.8 (1.5)	13.5 (1.6)	0.74	0.89
IL-1β	6.7 (0.4)	5.8 (0.3)	0.12	0.89
IL-1rα	201.3 (54.0)	119.8 (9.7)	0.56	0.89
IL-2	2.3 (0.1)	2.2 (0.2)	0.58	0.89
IL-2rα	66.5 (4.2)	69.3 (4.6)	0.71	0.89
IL-3	0.3 (0.0)	0.3 (0.0)	0.97	0.97
IL-4	0.5 (0.0)	0.5 (0.0)	0.78	0.89
IL-6	1.5 (0.2)	1.3 (0.1)	0.66	0.89
IL-7	74.6 (4.6)	72.3 (4.5)	0.73	0.89
IL-8	7.1 (0.7)	5.7 (0.3)	0.23	0.89
IL-9	69.4 (5.8)	64.2 (4.3)	0.88	0.92
IP-10 (CXCL10)	408.5 (79.1)	302.1 (55.9)	0.63	0.89
LIF	17.2 (3.5)	13.9 (2.0)	0.53	0.89
M-CSF	22.6 (1.9)	21.7 (1.9)	0.72	0.89
MCP-1 (CCL2)	11.4 (2.2)	11.6 (0.9)	0.06	0.89
MCP-3	1.4 (0.1)	1.3 (0.1)	0.25	0.89
MIF	1,124.3 (266.7)	791.5 (74.3)	0.75	0.89
MIG (CXCL9)	173.9 (45.0)	119.1 (25.0)	0.17	0.89
MIP-1α (CCL3)	2.4 (0.2)	2.1 (0.1)	0.18	0.89
MIP-1β (CCL4)	57.4 (3.0)	53.1 (1.2)	0.50	0.89
PDGF-BB	1,660.8 (234.4)	1,230.4 (90.0)	0.29	0.89
RANTES	4,173.3 (494.8)	3,714.6 (319.5)	0.91	0.93
SCF	55.7 (3.1)	60.6 (4.2)	0.53	0.89
SCGF-β	17,876.9 (1,521.3)	18,840.9 (1,868.3)	0.35	0.89
SDF-α	261.5 (16.8)	251.2 (12.6)	0.86	0.92
TNFα	16.1 (1.3)	14.2 (0.7)	0.54	0.89
TRAIL	39.1 (1.1)	38.4 (1.1)	0.46	0.89
VEGF	26.6 (2.7)	27.2 (3.5)	0.76	0.89

Supplemental Table 3. Antibodies and dyes used for confocal microscopy and flow cytometry analysis.

Dye/Target	Clone	Fluorophore	Catalog #	Company
Mitotracker Green	NA	NA	M7514	Thermo Fisher Scientific
Mitotracker Red CMXRos	NA	NA	M7512	Thermo Fisher Scientific
Hoechst 33342	NA	NA	H21492	Thermo Fisher Scientific
eBioscience™ Fixable Viability Dye eFluor™ 506	NA	eFluor 506	65-0866-14	Thermo Fisher Scientific
CD69	FN50	PE-Cy5	555532	BD Biosciences
CD69	FN50	PE-Cy5	310908	BioLegend
CD8	RPA-T8	APC-Cy7	557760	BD Biosciences
CD4	RPA-T4	APC-Cy7	557871	BD Biosciences
CD3	HIT3a	BV605	564712	BD Biosciences
CD3	UCHT1	Alexa 700	56-0038-42	Thermo Fisher Scientific
GLUT1	202915	APC	FAB1418A	R&D Biosystems



Supplemental Figure 1. CD4+ and CD8+ T cells increase expression of early activation marker CD69 after overnight stimulation. (A) CD4+ T cell CD69 MFI at rest and after overnight activation (n=15/14/17/16). **(B)** CD8+ T cell CD69 MFI at rest and after overnight activation (n= 15/15/17/14). Error bars represent mean ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, Dunn's test with multiple testing correction (false discovery rate).