

Supplementary Appendix

For

Dysregulated NK cell PLC γ 2 Signaling and Function in Juvenile Dermatomyositis

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Table S1. Surface and intracellular antibodies for mass cytometry.¹

Antigen	Elemental	Clone
CD45	089Y	HI30
CCR6	141Pr	G034E3
CD19	142Nd	HIB19
CD45RA	143Nd	HI100
p-PLCy2	144Nd	K86-689.37
CD4	145Nd	RPA-T4
IgD	146Nd	IA6-2
CD11c	147Sm	Bu15
CD14	148Nd	RMO52
CD127	149Sm	A019D5
p-STAT5	150Nd	47
CD123	151Eu	6H6
p-AKT	152Sm	D9E
p-STAT1	153Eu	58D6
p-Itk/Btk	154Sm	24a/BTK
CD27	155Gd	L128
CXCR3	156Gd	G025H7
p-STAT3	158Gd	4/P-Stat3
p-MAPKAPK2	159Tb	27B7
CD69	160Gd	FN50
Ki-67	161Dy	B56
p-LCK	162Dy	4/LCK-Y505
p-JAK2 ¹	163Dy	D4A8
IκBa	164Dy	L35A5
CD45RO	165Ho	UCHL1
p-NFKB	166Er	K10-95.12.50
p-ERK	167Er	D13.14.4E
CD8	168Er	SK1
CD25	169Tm	2A3
CD3	170Er	UCHT1
p-Syk/ZAP70	171Yb	17a
IgM	172Yb	MHM-88
HLA-DR	173Yb	L243
p-STAT4	174Yb	38/p-Stat4
PD-1	175Lu	EH12.2H7
CD56	176Yb	NCAM16.2
CD16	209Bi	3G8

¹ p-JAK2 was not included in some specimens due to issues with reagent availability

Table S2. Significant p-values using 2 tailed Welch's t-test and MHC. *Denotes significant by Bonferroni correction ($\alpha_{adj}=0.05/897$). All others are significant by Benjamini-Hochberg FDR multiple hypothesis correction.

name	tval	pval	FDRthresh
NK cells p-PLC γ 2 unst*	-7.852986436	2.42584E-08	5.57414E-05
NK cells p-PLC γ 2 15 min*	-7.498464205	1.50352E-07	0.000111483
NK cells p-PLC γ 2 3 min*	-7.260336613	1.81878E-07	0.000167224
Non-classical monocytes p-PLC γ 2 unst*	-6.475463858	3.28929E-07	0.000222965
Non-classical monocytes p-PLC γ 2 3 min*	-5.907332717	1.76436E-06	0.000278707
mDCs p-PLC γ 2 unst*	-5.534029597	4.55974E-06	0.000334448
Non-classical monocytes pMAPKAPK2 15 min*	-5.093395629	1.55108E-05	0.00039019
Non-classical monocytes p-PLC γ 2 15 min*	-4.931659373	2.50224E-05	0.000445931
Classical monocytes p-ERK 15 min*	-4.771229485	3.86315E-05	0.000501672
Naive B cells p-STAT3 unst	-4.581093204	0.000114761	0.000557414
Non-classical monocytes p-NFkB 15 min	-4.402050767	0.000115162	0.000613155
Unswitched memory B cells p-PLC γ 2 15 min	-4.60841832	0.000134452	0.000668896
Naive B cells p-AKT 15 min	-4.324920957	0.000140748	0.000724638
Naive B cells p- PLC γ 2 unst	-4.465519349	0.000155212	0.000780379
Classical monocytes p-NFkB 15 min	-4.385934405	0.000182175	0.00083612
Naive B cells p-AKT 3 min	-4.18625037	0.000216521	0.000891862
Non-classical monocytes p-AKT 15 min	-4.319755417	0.000216603	0.000947603
Classical monocytes p- PLC γ 2 3 min	-4.175200378	0.000216783	0.001003344
Naive B cells p-AKT unst	-4.165542806	0.00022982	0.001059086
Classical monocytes Ikb α unst	-4.216688736	0.000242932	0.001114827
NK cells pMAPKAPK2 15 min	-4.432499128	0.000246073	0.001170569
Classical monocytes p-AKT 15 min	-4.221088834	0.000261133	0.00122631
Classical monocytes Ikb α 3 min	-4.128551449	0.000291522	0.001282051
Classical monocytes p- PLC γ 2 unst	-4.051880176	0.00030337	0.001337793
Classical monocytes p-AKT 3 min	-4.017485092	0.000401034	0.001393534
Classical monocytes p-ZAP70/SYK unst	-4.052172809	0.000413522	0.001449275
Class-switched memory B cells p-STAT3 unst	-4.021879424	0.000429642	0.001505017
NK cells pMAPKAPK2 3 min	-4.177410213	0.000446839	0.001560758
Classical monocytes p-STAT5 3 min	-3.91696179	0.000463162	0.001616499
IgM memory B cells p- PLC γ 2 unst	-4.081576067	0.000497738	0.001672241
CD8 CM T cells p-AKT 15 min	-3.898795411	0.000532513	0.001727982
mDCs p-AKT 15 min	-4.00689282	0.000553302	0.001783724
Classical monocytes p-STAT5 unst	-3.869090839	0.000581397	0.001839465
pDCs p-PLC γ 2 15 min	-4.033481198	0.000608125	0.001895206
IgM memory B cells p-PLC γ 2 15 min	-4.031382903	0.000643085	0.001950948
CD8 CM T cells p-AKT 3 min	-3.848868811	0.000664293	0.002006689
mDCs p-PLC γ 2 15 min	-3.727960689	0.00075426	0.00206243

Non-classical monocytes p-AKT 3 min	-3.790249617	0.00077987	0.002118172
Non-classical monocytes p-STAT1 3 min	-3.713451586	0.000806289	0.002173913
Non-classical monocytes pMAPKAPK2 3 min	-3.772895964	0.000843054	0.002229654
Non-classical monocytes p-ERK 15 min	-3.693105014	0.000893942	0.002285396
NK cells pMAPKAPK2 unst	-3.824242602	0.000897015	0.002341137
Classical monocytes p-AKT unst	-3.725288925	0.00094697	0.002396878
IgM memory B cells p-PLC γ 2 3 min	-3.859116855	0.001001104	0.00245262
pDCs p-PLC γ 2 unst	-3.751242004	0.001023663	0.002508361
mDCs p-PLC γ 2 3 min	-3.570848826	0.001149672	0.002564103
Naive B cells p-PLC γ 2 3 min	-3.642749714	0.001229433	0.002619844
Naive CD8 T cells p-ERK 15 min	-3.675820485	0.001271397	0.002675585
mDCs p-AKT unst	-3.654776711	0.00128198	0.002731327
Unswitched memory B cells p-PLC γ 2 unst	-3.743624532	0.00129909	0.002787068
CD8 EM T cells I κ Ba unst	-3.633263879	0.001311013	0.002842809
CD8 CM T cells p-AKT unst	-3.594127595	0.001362115	0.002898551
Non-classical monocytes pMAPKAPK2 unst	-3.657639336	0.001391583	0.002954292
Non-classical monocytes p-ZAP70/SYK 3 min	-3.573470136	0.001414836	0.003010033
CD8 CM T cells p-NF κ B 3 min	-3.603965427	0.001436343	0.003065775
Class-switched memory B cells p-AKT unst	-3.508707981	0.001445751	0.003121516
Class-switched memory B cells p-AKT 3 min	-3.476047577	0.00148754	0.003177258
CD8 TEMRA p-AKT 15 min	-3.503207678	0.001498708	0.003232999
Classical monocytes p-NF κ B unst	-3.55602452	0.001503956	0.00328874
Non-classical monocytes p-NF κ B unst	-3.600974645	0.001597665	0.003344482
Classical monocytes p-NF κ B 3 min	-3.534940025	0.001623767	0.003400223
NK cells p-AKT 15 min	-3.522490015	0.001749898	0.003455964
Naive B cells p-PLC γ 2 15 min	-3.463834188	0.001795491	0.003511706
Class-switched memory B cells p-PLC γ 2 unst	-3.622862181	0.001817119	0.003567447
Non-classical monocytes p-NF κ B 3 min	-3.430234587	0.001982258	0.003623188
TH17/22 p-PLC γ 2 3 min	-3.380269726	0.002049574	0.00367893
Naive CD8 T cells p-AKT 15 min	-3.388441856	0.002100108	0.003734671
Classical monocytes pMAPKAPK2 15 min	-3.343633827	0.00219741	0.003790412
TFH p-AKT unst	-3.3742471	0.002297207	0.003846154
Naive CD8 T cells p-ERK 3 min	-3.374354435	0.002386375	0.003901895
mDCs p-AKT 3 min	-3.398881571	0.002403988	0.003957637
NK cells p-AKT 3 min	-3.387935699	0.002427461	0.004013378
Class-switched memory B cells p-AKT 15 min	-3.288670837	0.002478457	0.004069119
CD4 CM p-AKT 3 min	-3.336454037	0.002524976	0.004124861
CD8 EM T cells I κ Ba 3 min	-3.316517198	0.002576971	0.004180602
mDCs p-STAT5 unst	-3.283645486	0.002616175	0.004236343
NK cells p-STAT3 unst	3.332834793	0.002716647	0.004292085
CD4 EM p-PLC γ 2 3 min	-3.279326492	0.002748886	0.004347826

CD4 CM p-AKT unst	-3.291346836	0.002836356	0.004403567
IgM memory B cells p-AKT unst	-3.231259371	0.002856454	0.004459309
Naive CD8 T cells p-ERK unst	-3.332624156	0.002857043	0.00451505
Unswitched memory B cells p-PLCγ2 3 min	-3.348006256	0.00291983	0.004570792
Naive CD8 T cells p-PLCγ2 unst	-3.2931526	0.002921574	0.004626533
pDCs p-PLCγ2 3 min	-3.376797972	0.003049141	0.004682274
IgM memory B cells p-AKT 3 min	-3.204208034	0.003070744	0.004738016
Class-switched memory B cells p-PLCγ2 15 min	-3.395494296	0.003085244	0.004793757
Naive CD8 T cells p-AKT 3 min	-3.239428869	0.003107065	0.004849498
pDCs p-NFκB unst	-3.292382589	0.003177485	0.00490524
CD8 CM T cells p-NFκB 15 min	-3.221865275	0.003218725	0.004960981
Unswitched memory B cells pMAPKAPK2 15 min	-3.404283166	0.003294037	0.005016722
Naive CD8 T cells p-PLCγ2 15 min	-3.262248851	0.003343216	0.005072464
Tregs p-AKT 3 min	-3.199021407	0.003370795	0.005128205
Class-switched memory B cells p-PLCγ2 3 min	-3.342794512	0.003400203	0.005183946
CD8 CM T cells p-NFκB unst	-3.295327918	0.003463346	0.005239688
IgM memory B cells p-ZAP70/SYK unst	-3.155224622	0.003500603	0.005295429
Naive CD8 T cells p-PLCγ2 3 min	-3.244108017	0.003511206	0.005351171
Naive CD4 T cells p-PLCγ2 3 min	-3.257447901	0.003535796	0.005406912
CD4 CM p-PLCγ2 3 min	-3.191298426	0.00355494	0.005462653
Naive CD8 T cells p-STAT4 3 min	-3.16759611	0.003749432	0.005518395
CD4 CM p-AKT 15 min	-3.155078427	0.003778482	0.005574136
Naive CD4 T cells p-AKT 3 min	-3.173456488	0.003883861	0.005629877
Naive CD8 T cells p-LCK 15 min	-3.107778253	0.004005701	0.005685619
IgM memory B cells p-AKT 15 min	-3.104200254	0.004063048	0.00574136
CD4 CM IκBa 3 min	-3.179637231	0.004126611	0.005797101
TH1 p-AKT 3 min	-3.133980329	0.004170055	0.005852843
CD4 TEMRA p-ZAP70/SYK 3 min	-3.084840822	0.004209115	0.005908584
CD8 CM T cells IκBa 3 min	-3.172215067	0.004265661	0.005964326
Class-switched memory B cells p-STAT3 15 min	-3.079361952	0.004288836	0.006020067
NK cells p-AKT unst	-3.136855295	0.004559049	0.006075808
CD4 TEMRA pMAPKAPK2 unst	-3.066632579	0.004562753	0.00613155
CD4 TEMRA p-ERK 3 min	-3.050840446	0.00456971	0.006187291
Naive CD8 T cells IκBa 3 min	-3.161824418	0.004774268	0.006243032
TFH p-AKT 15 min	-3.063074407	0.004842181	0.006298774
TH2 p-PLCγ2 3 min	-3.083410753	0.004848321	0.006354515
TFH p-AKT 3 min	-3.076026225	0.004898365	0.006410256
CD8 CM T cells IκBa unst	-3.141189278	0.004912292	0.006465998
Tregs p-AKT unst	-3.041373313	0.005080226	0.006521739
Non-classical monocytes p-STAT1 15 min	-3.015180288	0.005368756	0.00657748
Naive CD8 T cells p-AKT unst	-3.015932582	0.005409759	0.006633222

CD8 TEMRA p-AKT 3 min	-2.985277009	0.005494042	0.006688963
CD4 EM p-AKT unst	-3.056428529	0.005533822	0.006744705
mDCs p-ERK 15 min	-3.044660179	0.005613143	0.006800446
TH1 p-AKT 15 min	-3.000354694	0.005903431	0.006856187
TH1 p-AKT unst	-2.978988325	0.005996179	0.006911929
CD8 CM T cells p-ERK 3 min	-2.998182731	0.006206259	0.00696767
CD8 EM T cells p-AKT 15 min	-2.936492295	0.006240829	0.007023411
Tregs p-AKT 15 min	-2.958468378	0.006240873	0.007079153
TH1 IκBa 3 min	-3.02117059	0.00639121	0.007134894
NK T cells p-ZAP70/SYK 15 min	3.024677793	0.006412514	0.007190635
CD4 EM p-AKT 3 min	-2.955508607	0.006447829	0.007246377
IgM memory B cells p-STAT3 unst	-2.910286202	0.006523654	0.007302118
CD4 CM IκBa unst	-2.99732679	0.006607945	0.00735786
Naive CD4 T cells p-PLCγ2 15 min	-2.948283458	0.006625331	0.007413601
CD8 TEMRA IκBa 3 min	-2.979820568	0.007080528	0.007469342
Class-switched memory B cells p-STAT3 3 min	-2.913304778	0.007088316	0.007525084
Naive CD8 T cells p-STAT4 15 min	-2.89216144	0.007203813	0.007580825
Unswitched memory B cells p-AKT 3 min	-2.868796619	0.007238945	0.007636566
Naive CD8 T cells IκBa 15 min	-2.973565525	0.007443768	0.007692308
IgM memory B cells pMAPKAPK2 15 min	-3.013202668	0.007472737	0.007748049
TH2 p-AKT 3 min	-2.904080312	0.007529999	0.00780379

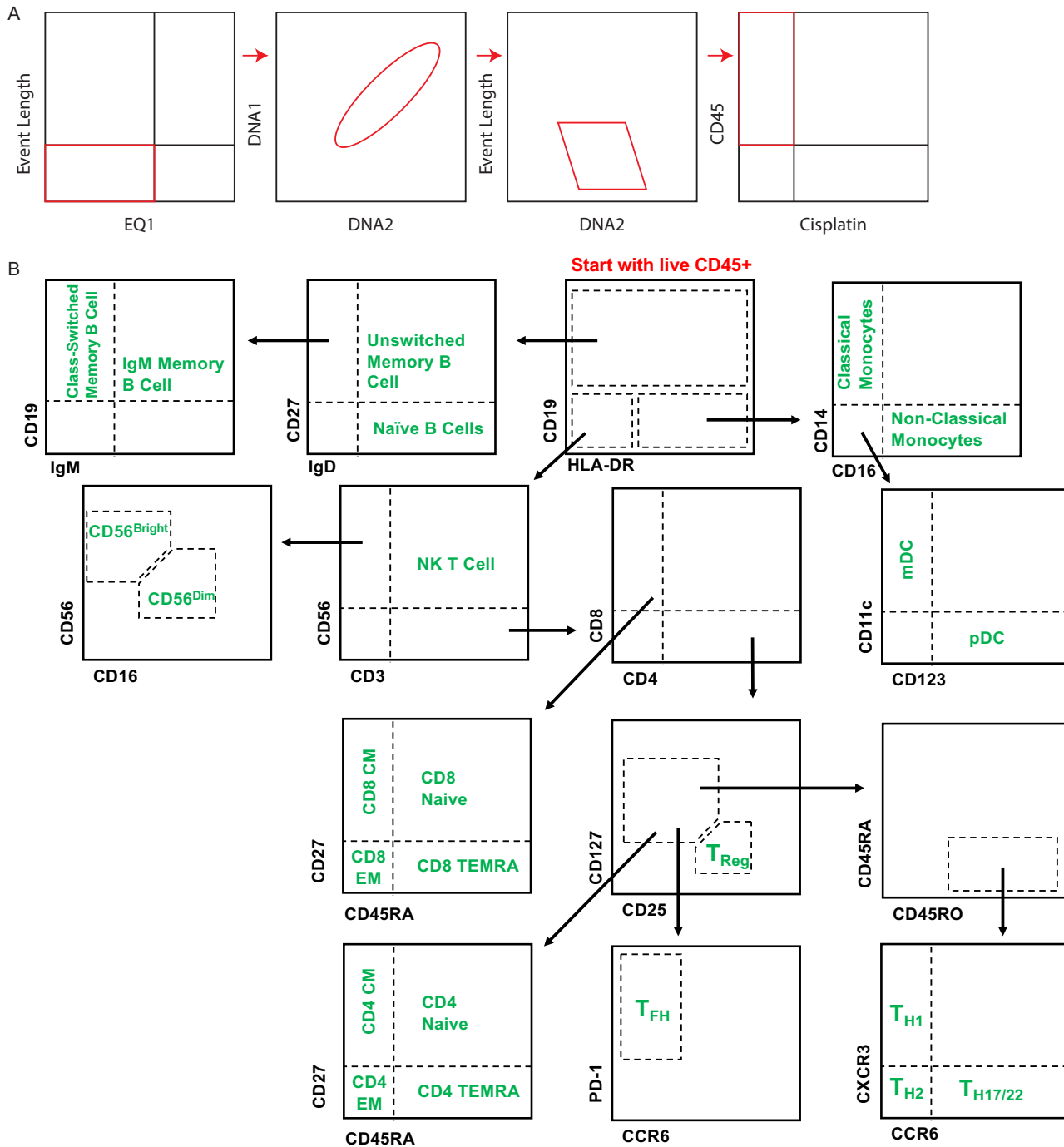


Figure S1. Gating schemes for A) live singlet lymphocytes and B) immune cell subsets.

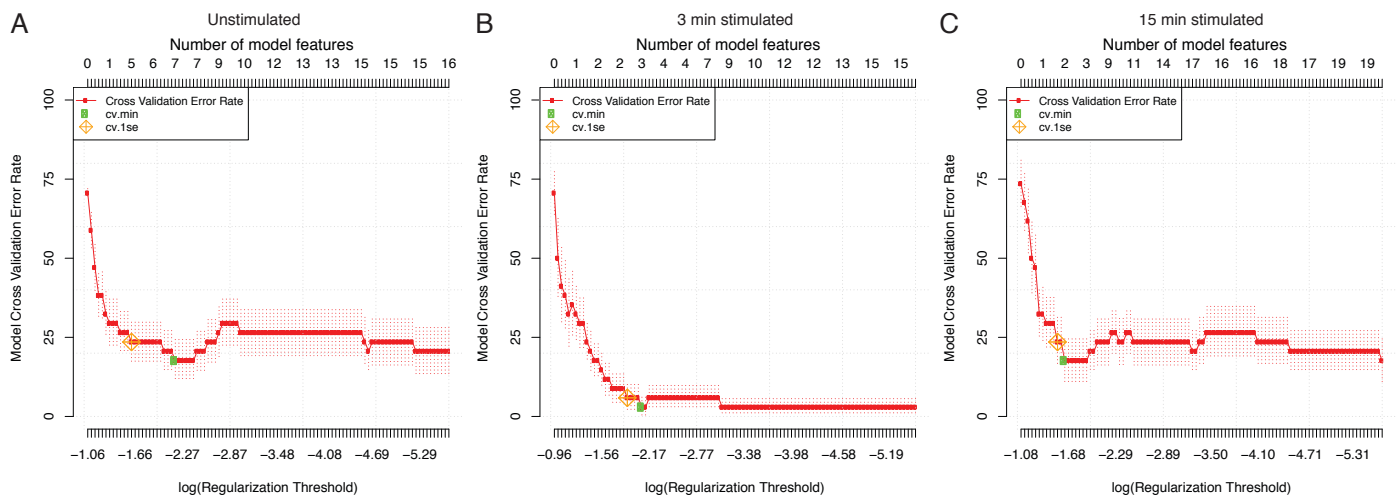


Figure S2. Citrus error plots for LASSO classification models between treatment naïve JDM patients and controls for A) unstimulated samples, B) 3 minute stimulated samples, and C) 15 minute stimulated samples.

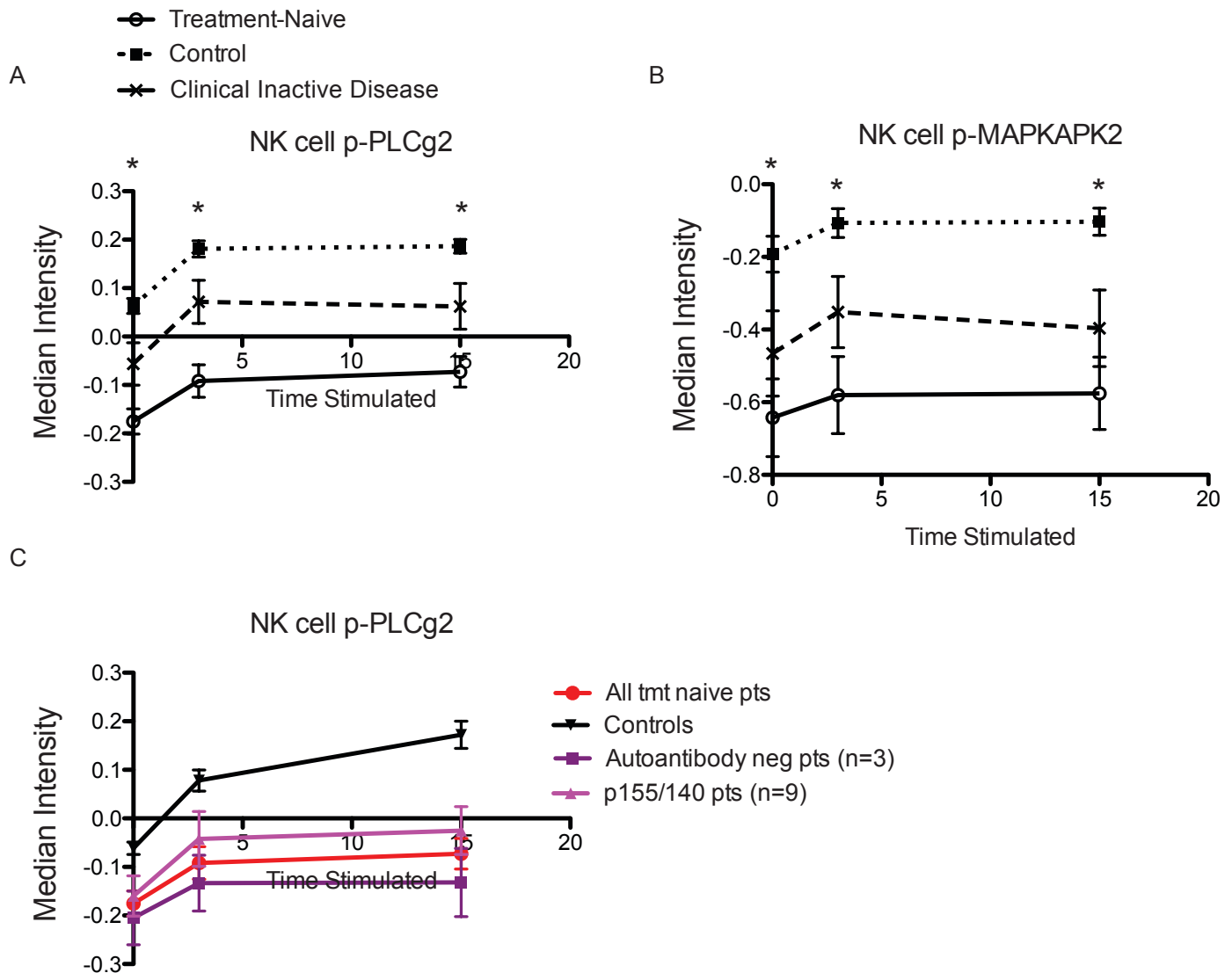


Figure S3. Assessment of p-PLC γ 2 and p-MAPKAPK2 in JDM patients with clinical inactive disease and in relationship to MSA autoantibody status. Data are displayed as the arcsinh ratio of the median intensity of the sample normalized to the run control. A) PLC γ 2 phosphorylation time course in NK cells from JDM treatment-naïve patients (n=17), JDM patients with clinical inactive disease (n=11), and healthy controls (n=17). PLC γ 2 phosphorylation in NK cells from JDM patients with clinical inactive disease is intermediate between that observed in NK cells from JDM treatment-naïve patients and controls. B) MAPKAPK2 phosphorylation time course in NK cells for treatment-naïve patients (n=17), patients with clinical inactive disease (n=11), and controls (n=17). MAPKAPK2 phosphorylation in NK cells from from JDM patients with clinical inactive disease is intermediate between that observed in NK cells from JDM treatment-naïve patients and controls, C) PLC γ 2 phosphorylation in NK cells from treatment-naïve JDM patients (n=17) is compared with two subsets of treatment-naïve JDM patients with p155/140 autoantibodies (n=9) or no MAS antibodies (n=3). Asterisk denote significance between treatment-naïve JDM patients and controls.

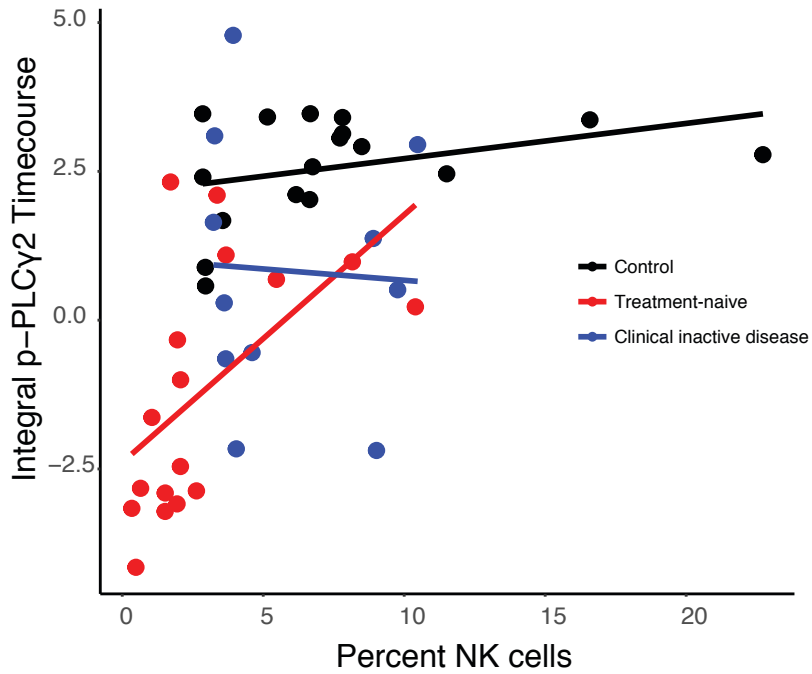


Figure S4. NK cell frequency correlates with PLCγ2 phosphorylation intensity in treatment-naïve JDM patient NK cells. Correlation of integrated p- PLCγ2 time course versus NK cell percentage for JDM patients and controls (treatment naïve patients: $y = -2.39 + 0.42x$, $R = 0.55$, $p = 0.0235$, $n = 17$; clinical inactive disease patients: $y = 1.05 - 0.039x$, $R = -0.051$, $p = 0.88$, $n = 11$; controls: $y = 2.12 + 0.059x$, $R = 0.35$, $p = 0.167$, $n = 17$).

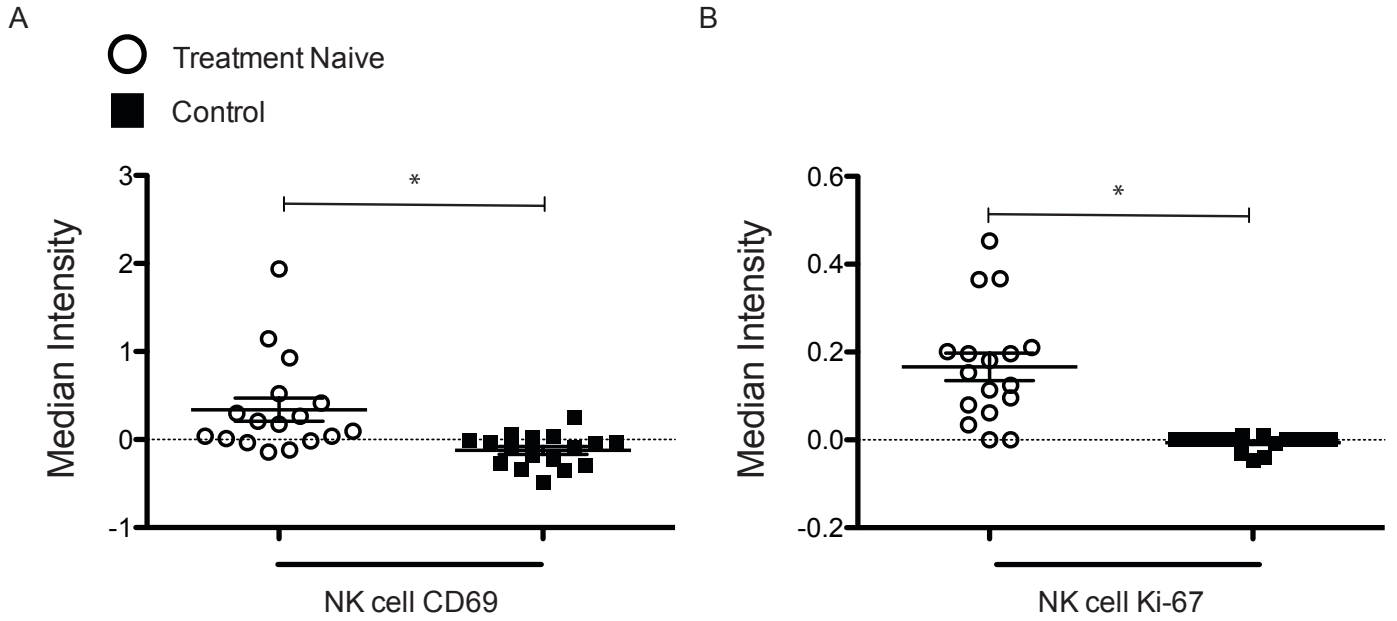


Figure S5. Treatment-naïve JDM patient and control NK cells differ in activation and proliferation, as assessed by CD69 and Ki67 expression levels. Data are displayed as the arcsinh ratio of the median intensity of the sample normalized to the run control. A) Signal intensity of CD69 in treatment naïve JDM patient (n=17) and control (n=17) NK cells ($t=3.327$, $df=32$, $p=0.0022$), B) Signal intensity of Ki-67 in treatment naïve JDM patient (n=17) and control (n=17) NK cells ($t=5.463$, $df=32$, $p<0.0001$).

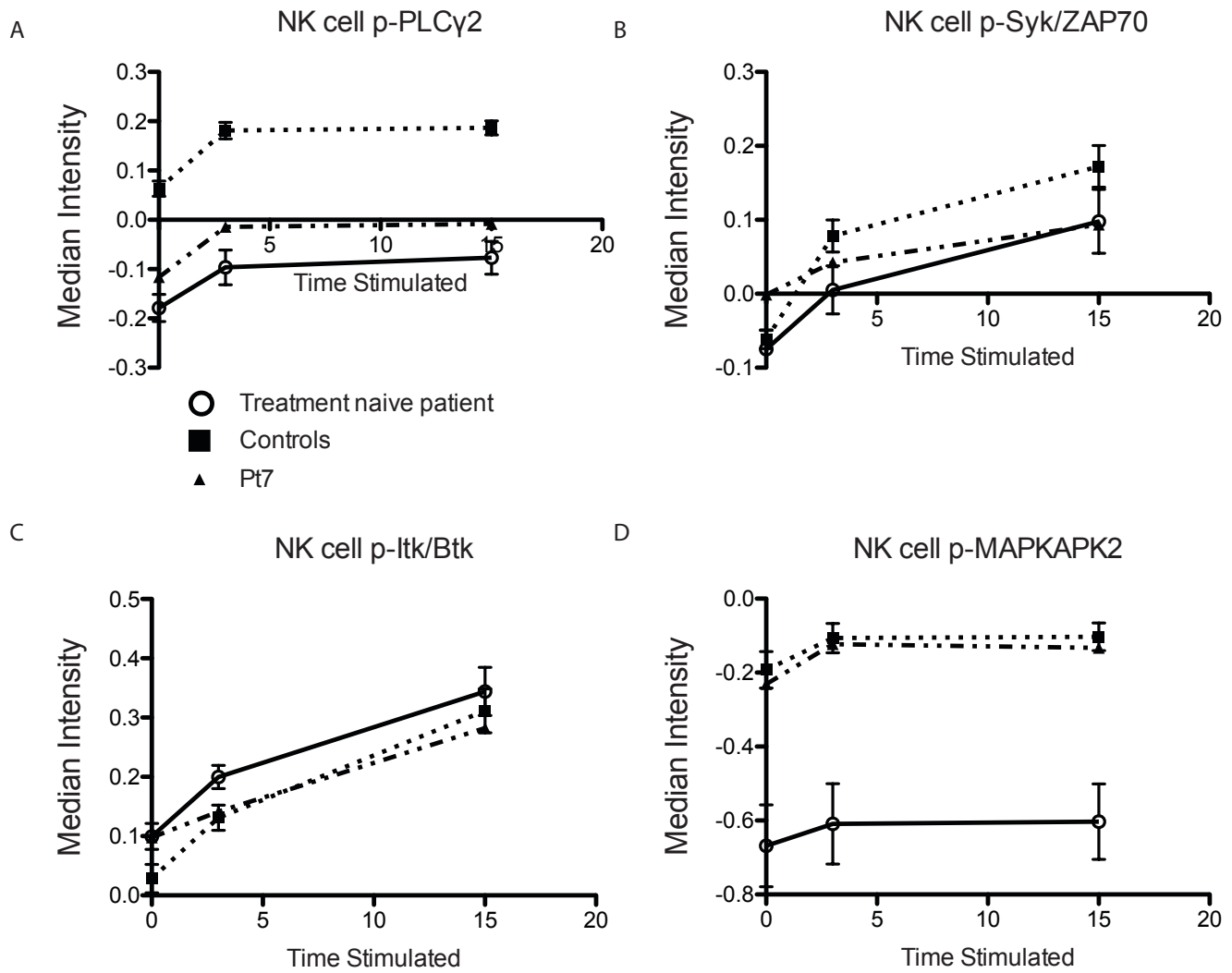


Figure S6. NK cell phosphorylation time courses with patient 7 mapped separately from n=16 other treatment-naïve patients and n=17 matched controls. Data are displayed as the arcsinh ratio of the median intensity of the sample to the run control. A) NK cells PLC γ 2 phosphorylation, B) NK cell Syk/ZAP70 phosphorylation, C) Itk/Btk phosphorylation, and, D) MAPKAPK2 phosphorylation.

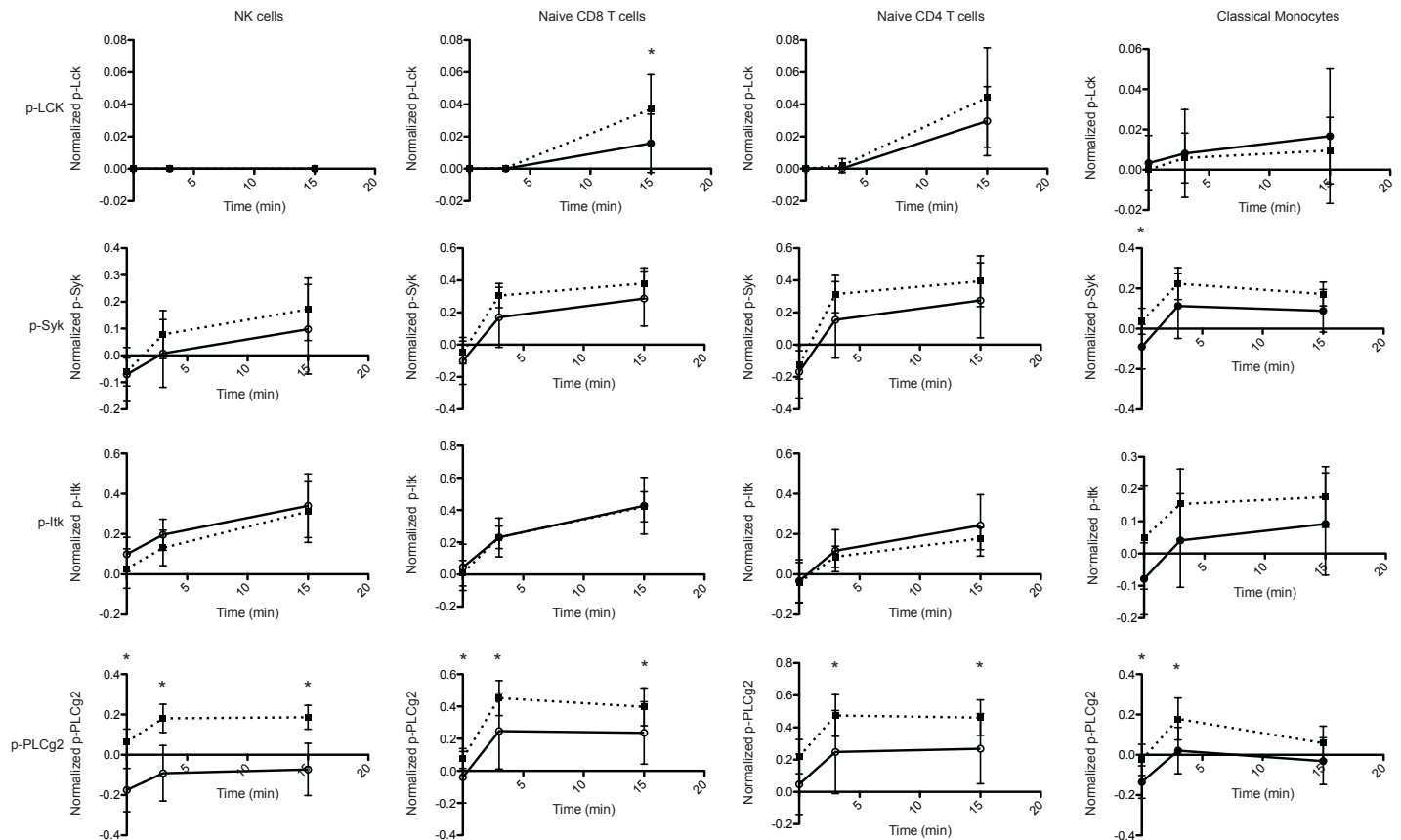


Figure S7. Phosphorylation time courses for manually gated immune cell populations similar to those retained by LASSO in Citrus (n=17 patients, n=17 controls). It should be noted that in contrast to the upregulation of PLCγ2 phosphorylation identified in the stratifying clusters of CD4 and CD8 T cells in the Citrus analysis (Fig 2D), the phosphorylation of PLCγ2 in bulk populations (manually gated) of CD4 and CD8 T cells was decreased in treatment-naïve JDM patients compared to controls (Fig S7). The significance this observation in regard to JDM is unclear since T cells primarily utilize PLCγ1, and no defects in T cell function were observed in mice in which PLCγ2 was knocked out (20, 21). Open circles with solid lines indicate patients. Closed squares with dotted lines indicate controls. Asterick denotes statistically significant differences between treatment-naïve patients and controls at each timepoint.