Supplementary Material:

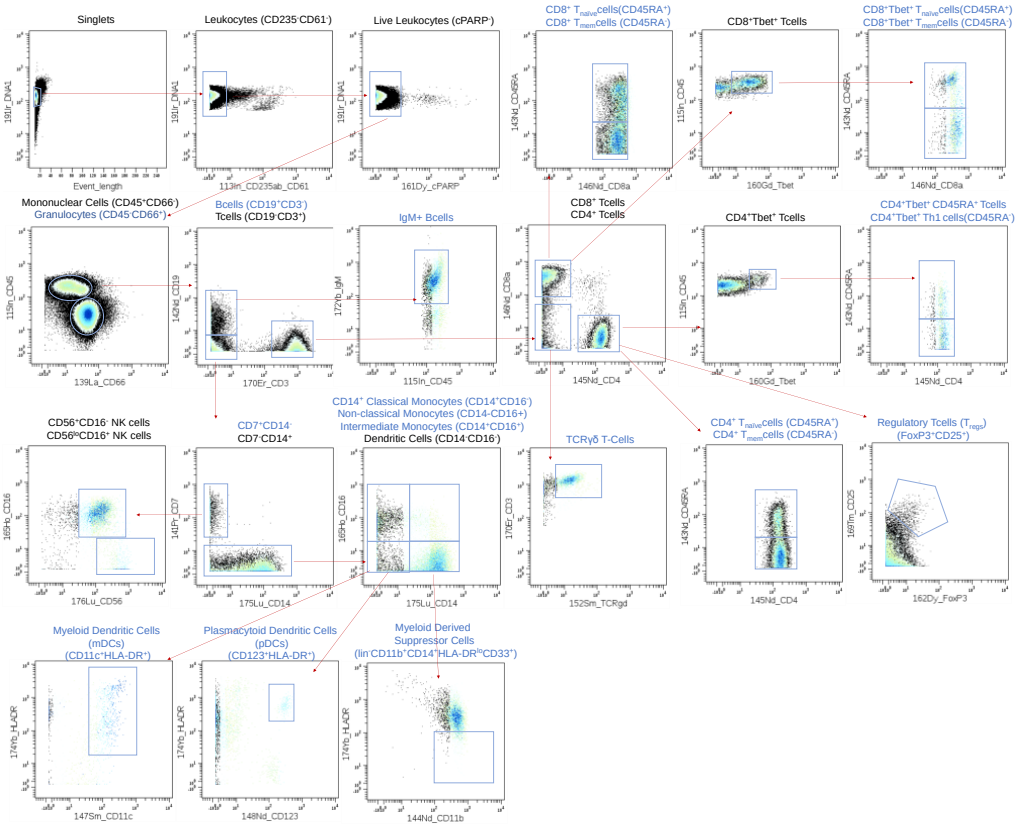

Figure S1. Gating Strategy. Two-dimensional dot plots are shown for a representative patient sample. Gating was performed using Cytobank (www.cytobank.org). Twenty innate and adaptive cell types were manually gated and included in the analysis.

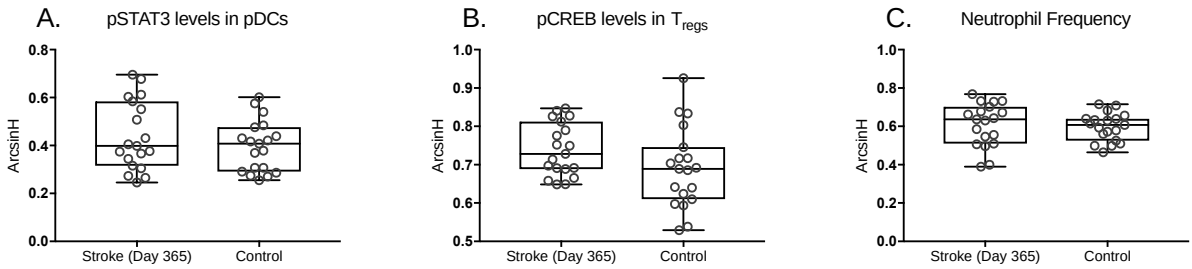

Figure S2. EN components in patients one year after stroke compared to a control cohort. The most prominent immune features of the (**A**) acute phase, (**B**) intermediate phase, and (**C**) late phase models one year after stroke were compared between stroke patients and a sex- and age-matched control cohort. Pre-surgical samples from twenty-four sex- and age-matched patients undergoing primary hip arthroplasty (demographics listed in Supplemental Table S1) were barcoded and stained simultaneously with samples from matched stroke patients, and then analyzed with mass cytometry.

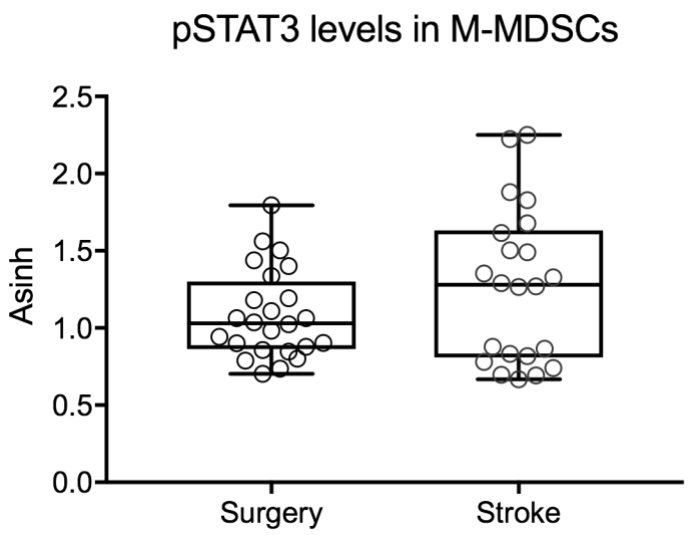

Figure S3. Stroke to Surgery Comparison. pSTAT3 levels in M-MDSCs within 24 hours of stroke is comparable to primary hip arthroplasty (PHA). No difference in arcsinh transformed values of pSTAT3 in M-MDSCs between stroke and surgery cohorts were observed.

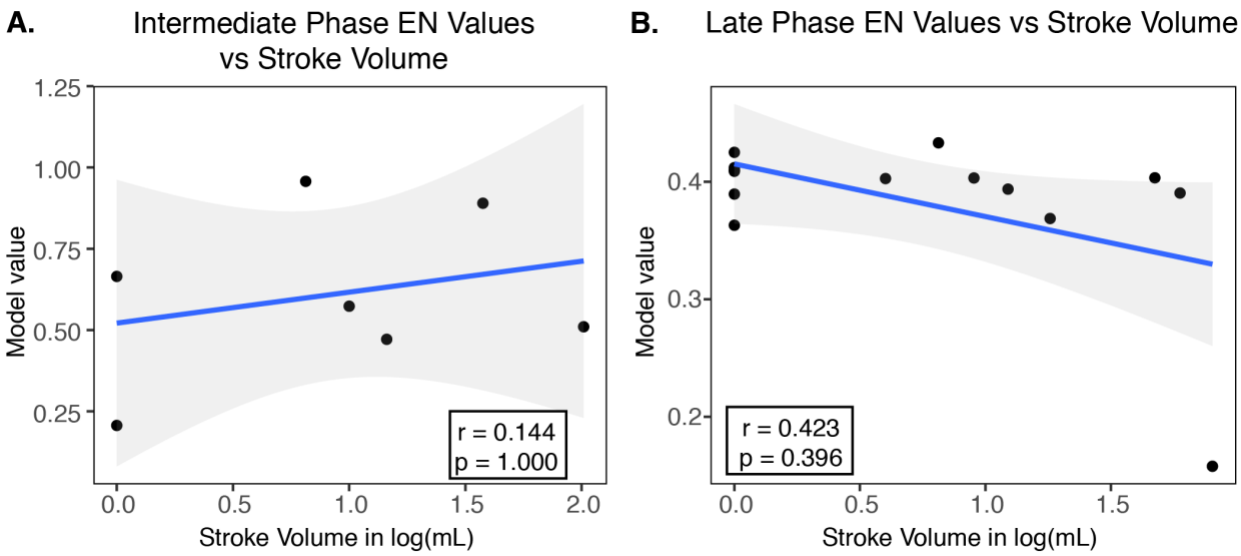

Figure S4. Correlation of EN Values and Stroke Size. EN values of the (**A**) intermediate phase model, and (**B**) late phase model are plotted against stroke volume (in log(mL)). Non-significant correlation (blue regression line) for the intermediate and late phase model were observed. The 95% confidence intervals are shaded in gray. Spearman coefficients and Bonferroni-corrected p-values are depicted in each plot.

Supplemental Table S1. Population characteristics of control cohort

Matched Demographics	
Age (years, mean (SD)) median (min-max)	63.8 ± 13.3 67.5 (33 – 84)
Female sex (%)	10 (41.7%)

Supplemental Table S2. Antibody Panel used for Mass Cytometry Analysis

Antibody	Manufacturer	Atomic Symbol	Atomic Mass	Clone	Comment
Barcode 1	Trace Sciences	Pd	102		Barcode
Barcode 2	Trace Sciences	Pd	104		Barcode
Barcode 3	Trace Sciences	Pd	105		Barcode
Barcode 4	Trace Sciences	Pd	106		Barcode
Barcode 5	Trace Sciences	Pd	108		Barcode
Barcode 6	Trace Sciences	Pd	110		Barcode
CD235ab	Biolegend	In	113	HIR2	Phenotype
CD61	BD	In	113	VI-PL2	Phenotype
CD45	Biolegend	In	115	HI30	Phenotype
CD66	BD	La	139	CD66a-B1.1	Phenotype
CD7	BD	Pr	141	M-T701	Phenotype
CD19	Biolegend	Nd	142	HIB19	Phenotype
CD45RA	Biolegend	Nd	143	HI100	Phenotype
CD11b	Fluidigm	Nd	144	ICRF44	Phenotype
CD4	Fluidigm	Nd	145	RPA-T4	Phenotype
CD8a	Fluidigm	Nd	146	RPA-T8	Phenotype
CD11c	Fluidigm	Sm	147	Bu15	Phenotype
CD123	Biolegend	Nd	148	6H6	Phenotype
pCREB (pS133)	Cell Signaling Technology	Sm	149	87G3	Phenotype
pSTAT5 (pY694)	Fluidigm	Nd	150	47	Function
pp38 (pT180/pY182)	BD	Eu	151	36/p38	Function
TCRγδ	Fluidigm	Sm	152	11F2	Phenotype
pSTAT1 (pY701)	Fluidigm	Eu	153	58D6	Function
pSTAT3 (pY705)	Cell Signaling Technology	Sm	154	M9C6	Function
pS6 (pS235/pS236)	Cell Signaling Technology	Gd	155	D57.2.2E	Function
CD24	Biolegend	Gd	156	ML5	Phenotype
CD38	Biolegend	Gd	157	HIT2	Phenotype
CD33	Fluidigm	Gd	158	WM53	Phenotype
pMAPKAPK2 (pT334)	Fluidigm	Tb	159	27B7	Function
Tbet	Fluidigm	Gd	160	4B10	Function
cPARP	BD	Dy	161	F21-852	Function
FoxP3	Fluidigm	Dy	162	PCH101	Phenotype
IκB	Fluidigm	Dy	164	L35A5	Function

CD16	Fluidigm	Ho	165	3G8	Phenotype
pNFκB (pS529)	Fluidigm	Er	166	K10-895.12.50	Function
pERK1/2 (pT202/pY204)	Fluidigm	Er	167	D13.14.4E	Function
pSTAT6 (pY641)	Fluidigm	Er	168	18	Function
CD25	Biolegend	Tm	169	M-A251	Phenotype
CD3	Fluidigm	Er	170	UCHT1	Phenotype
CD27	BD	Yb	171	M-T271	Phenotype
IgM	Fluidigm	Yb	172	MHM-88	Phenotype
CCR2	Biolegend	Yb	173	K036C2	Phenotype
HLA-DR	Fluidigm	Yb	174	L243	Phenotype
CD14	Fluidigm	Yb	175	M5E2	Phenotype
CD56	BD	Yb	176	NCAM16.2	Phenotype
DNA1	Fluidigm	Ir	191		DNA
DNA2	Fluidigm	Ir	192		DNA

Supplemental Table S3. Features of the Acute Phase Model (Day 2 compared to Day 365). Immune features that comprise the model were examined individually. Univariate p values were obtained using a Wilcoxon Test to compared each feature are Day 2 to Day 365. Elastic Net coefficients are also listed. Features are listed in order of their respective p-values.

Feature	p-value	Coefficient
pDCs_STAT3	0.003133012	0.038549129
cMC_STAT3	0.004932664	0.061372167
MDSCs_STAT3	0.006404096	0.097431805
pDCs Frequency	0.011412334	-0.077135508
Tregs_p38	0.015578886	0.076230168
CD4 Naive T-Cells_STAT3	0.015578886	0.015834997
CD4 Memory T-Cells_p38	0.018111611	0.053694786
mDCs_STAT3	0.019504626	0.000710444
CD4 Naive T-Cells_p38	0.022565919	0.074817895
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_p38	0.026025103	0.001699668
ncMC_IkB	0.064884401	0.060093211
$CD7^{+}CD14^{-}$ Frequency	0.07307725	-0.000723323
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_p38	0.086893527	0.19071907
$Tbet^{+}CD8^{+}CD45RA^{-}$ T-Cells_STAT1	0.086893527	-0.073344311
intMC_IkB	0.091931	0.000221267
Neutrophils_STAT1	0.114404566	-0.015175318
pDCs_ERK	0.120633831	0.001563947
CD4 Memory T-Cells_STAT5	0.140880095	0.000188092
MDSCs Frequency	0.188859351	0.00536173
ncMC Frequency	0.207207814	-0.000660488
CD4 Naive T-Cells_STAT5	0.207207814	0.009348159
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_STAT3	0.207207814	0.000106293
intMC_MAPKAPK2	0.207207814	-0.025039915
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_IkB	0.226785775	0.000115791
CD8 Memory T-Cells_STAT3	0.269721336	0.00414818
$CD7^{+}CD14^{-}$_STAT1	0.281254777	-0.046404005
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_IkB	0.281254777	0.000319841
$CD7^{+}CD14^{-}$_STAT5	0.317792372	-0.026712474

intMC_p38	0.343766483	-0.120822548
Tregs_ERK	0.385134537	0.07345742
intMC Frequency	0.399558376	0.009699213
intMC_STAT5	0.399558376	0.003371477
Neutrophils_MAPKAPK2	0.399558376	-0.014446278
B-Cells_STAT1	0.414295027	-0.000774053
IgM+ B-Cells	0.429341175	0.000186084
ncMC_STAT5	0.429341175	0.055855695
Tregs_S6	0.429341175	0.003275702
ncMC_p38	0.444692966	0.048603638
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_ERK	0.476295895	0.004026225
intMC_CREB	0.509063783	-0.002349003
CD8 Memory T-Cells_STAT5	0.509063783	0.000126256
$Tbet^{+}CD8^{+}CD45RA^{+}$ T-Cells_STAT1	0.509063783	-0.009157675
IgM+ B-Cells_ERK	0.509063783	-0.000715586
Neutrophils_p38	0.525870025	-0.036842249
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_S6	0.525870025	0.031734521
CD8 Naive T-Cells_MAPKAPK2	0.542949072	-8.57E-05
Tregs_MAPKAPK2	0.542949072	0.013092869
mDCs_CREB	0.560293861	-0.000996989
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells Frequency	0.59574995	-3.73E-05
TCRgd T-cells_CREB	0.59574995	-0.001000404
TCRgd T-cells_STAT5	0.59574995	0.000938858
cMC_STAT5	0.59574995	0.028933424
B-Cells_MAPKAPK2	0.613844833	0.031897358
intMC_pSTAT6	0.613844833	0.024428588
B-Cells_STAT5	0.650724095	-0.00165113
$Tbet^{+}CD8^{+}CD45RA^{-}$ T-Cells_MAPKAPK2	0.650724095	-0.009723011
$Tbet^{+}CD8^{+}CD45RA^{+}$ T-Cells_MAPKAPK2	0.688458983	-0.000284972
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_ERK	0.707621985	0.000474064
$Tbet^{+}CD8^{+}CD45RA^{+}$T-Cells_CREB	0.726967778	-0.00907308
IgM+ B-Cells_STAT1	0.726967778	-0.000816286

ncMC_pSTAT6	0.726967778	-0.000916305
ncMC_STAT1	0.746485315	0.00077333
IgM^+ B-Cells_CREB	0.785989639	-0.001316337
CD8 Naive T-Cells_CREB	0.785989639	-0.000249306
$Tbet^+CD4^+CD45RA^+$ T-Cells_CREB	0.785989639	-0.000112572
Neutrophils_NFkB	0.785989639	-0.000402312
CD8 Naive T-Cells Frequency	0.805952757	-0.002680539
$Tbet^+CD4^+CD45RA^+$ T-Cells Frequency	0.846239828	-0.000302871
$CD7^+CD14^-$_CREB	0.846239828	-9.65E-05
CD4 Memory T-Cells_S6	0.846239828	0.002883853
cMC_MAPKAPK2	0.866538653	-0.001467536
pDCs_pSTAT6	0.866538653	0.016482314
Tregs_pSTAT6	0.886923897	1.67E-05
Neutrophils_S6	0.907382582	0.000283116
B-Cells_S6	0.907382582	2.01E-05
TCRgd T-cells Frequency	0.927901509	-0.01123539
B-Cells_CREB	0.927901509	-0.098458215
pDCs_STAT5	0.927901509	0.001733622
MDSCs_p38	0.927901509	-4.98E-06
$CD7^+CD14^-$_MAPKAPK2	0.948467446	0.012440116
MDSCs_MAPKAPK2	0.948467446	-0.003048691
CD8 Naive T-Cells_STAT1	0.969066991	-0.019907989
CD8 Naive T-Cells_pSTAT6	0.989686757	-0.001096702
MDSCs_STAT5	1	0.002683005

Supplemental Table S4: Features of the Intermediate Phase Model (Day 5 compared to Day 365). Immune features that comprise the model were examined individually. Univariate p values were obtained using a Wilcoxon Test to compared each feature are Day 5 to Day 365. Elastic Net coefficients are also listed. Features are listed in order of their respective p-values.

Feature	p-value	Coefficient
Tregs_CREB	2.13E-05	0.059320858
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_CREB	0.000747948	0.046199462
CD4 Memory T-Cells_p38	0.002824567	0.027898062
$Tbet^{+}CD8^{+}CD45RA^{-}$ T-Cells_CREB	0.003578595	0.022315452
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_CREB	0.003578595	0.005834086
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_p38	0.004490727	0.034782437
TCRgd T-cells_CREB	0.004490727	0.000129622
Tregs_STAT3	0.005594406	0.038059203
CD4 Memory T-Cells_CREB	0.018072362	0.005561427
CD8 Memory T-Cells_CREB	0.018072362	0.000680663
Tregs_p38	0.021511098	0.018159412
CD4 Naive T-Cells_STAT3	0.025472788	0.05825626
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_CREB	0.025472788	0.029355302
CD8 Naive T-Cells_CREB	0.03	0.05029409
MDSCs_MAPKAPK2	0.035165704	0.000240364
MDSCs Frequency	0.047622378	0.042954218
Tregs_IkB	0.047622378	0.013221894
CD4 Memory T-Cells_STAT3	0.055041046	0.031831157
MDSCs_ERK	0.063347522	0.001392671
$CD7^{+}CD14^{-}$_STAT5	0.072593493	-0.021543278
TCRgd T-cells_p38	0.072593493	0.008354608
pDCs_STAT3	0.072593493	0.000337684
mDCs_S6	0.082861052	0.010324231
CD8 Memory T-Cells Frequency	0.082861052	-0.005724106
mDCs_MAPKAPK2	0.082861052	-0.000780269
ncMC_IkB	0.094198845	0.029766859
CD4 Naive T-Cells_CREB	0.094198845	0.002244352
$CD7^{+}CD14^{-}$_STAT1	0.106682882	-0.061480772
MDSCs_S6	0.106682882	0.030462202
cMC_p38	0.106682882	-0.008046741

ncMC_p38	0.106682882	0.002465176
Tregs_STAT1	0.106682882	0.000606465
pDCs_p38	0.106682882	0.000488953
CD4 Naive T-Cells_p38	0.106682882	0.000282637
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_p38	0.120364853	0.041404482
Neutrophils_STAT5	0.120364853	-0.005606854
CD4 Memory T-Cells_IkB	0.120364853	6.49E-05
Neutrophils_STAT1	0.151571906	-0.020478172
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_IkB	0.151571906	0.011592994
cMC_MAPKAPK2	0.151571906	-0.006708648
B-Cells_CREB	0.151571906	-0.005238393
intMC_p38	0.151571906	-0.002517416
MDSCs_IkB	0.151571906	5.21E-05
CD4 Naive T-Cells_IkB	0.169206446	0.00051692
Tregs_MAPKAPK2	0.188245667	0.049893203
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells	0.188245667	0.03327968
ncMC Frequency	0.188245667	-0.006547156
cMC_S6	0.188245667	0.000285258
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_IkB	0.208741259	0.003817205
intMC_CREB	0.208741259	-0.00211248
CD8 Naive T-Cells_MAPKAPK2	0.230717543	-0.00234361
$CD7^{+}CD14^{-}$_STAT3	0.254211006	-0.032133338
Tregs_S6	0.254211006	0.013506835
CD8 Naive T-Cells_p38	0.279224688	-0.000325586
CD8 Naive T-Cells_STAT3	0.279224688	0.00021398
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_STAT3	0.305785953	0.032523178
intMC_ERK	0.305785953	0.000742371
Tregs_STAT5	0.305785953	0.000645723
cMC_STAT1	0.333879599	-0.01397357
$Tbet^{+}CD8^{+}CD45RA^{+}$ T-Cells_p38	0.333879599	-3.23E-05
TCRgd T-cells Frequency	0.363505625	-0.013103192
B-Cells_STAT1	0.394639708	-2.22E-05
CD8 Memory T-Cells_MAPKAPK2	0.394639708	-8.22E-06
TCRgd T-cells_STAT1	0.427260566	-0.062700204

CD4 Memory T-Cells_STAT5	0.427260566	0.001007083
mDCs_STAT3	0.427260566	0.000280909
Neutrophils_CREB	0.427260566	-0.000134706
CD4 Naive T-Cells_STAT1	0.427260566	5.68E-05
Neutrophils_p38	0.46131651	-0.013584413
CD4 Naive T-Cells_STAT5	0.46131651	0.001662461
Tregs_ERK	0.496774095	0.017322845
$Tbet^{+}CD8^{+}CD45RA^{-}$ T-Cells Frequency	0.496774095	-0.007929833
pDCs_MAPKAPK2	0.496774095	0.002550779
IgM+ B-Cells_NFkB	0.496774095	0.001545001
Neutrophils_IkB	0.496774095	0.001533564
$Tbet^{+}CD4^{+}CD45RA^{+}$ T-Cells_MAPKAPK2	0.496774095	0.001187826
ncMC_S6	0.496774095	0.000316704
intMC_MAPKAPK2	0.533560353	-0.015670307
pDCs_STAT1	0.533560353	0.003556651
ncMC_ERK	0.533560353	0.001071799
$Tbet^{+}CD8^{+}CD45RA^{-}$ T-Cells_STAT5	0.571611432	0.005429471
CD4 Naive T-Cells Frequency	0.571611432	-0.002673417
ncMC_CREB	0.571611432	0.000318037
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_STAT5	0.610845242	0.022856137
IgM+ B-Cells_STAT5	0.610845242	0.002243329
Tregs Frequency	0.610845242	0.001289879
TCRgd T-cells_STAT5	0.651176649	0.063083984
Neutrophils_NFkB	0.651176649	-0.002815322
B-Cells_p38	0.651176649	-0.000156503
$CD7^{+}CD14^{-}$ Frequency	0.69249924	0.043988235
CD4 Memory T-Cells Frequency	0.69249924	-0.005856135
ncMC_NFkB	0.69249924	1.34E-05
TCRgd T-cells_ERK	0.73471876	-0.003519962
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_STAT1	0.73471876	0.000724772
MDSCs_STAT3	0.73471876	0.000262134
ncMC_STAT3	0.777713591	0.027820707
mDCs Frequency	0.777713591	-0.015398957
$Tbet^{+}CD8^{+}CD45RA^{-}$ T-Cells_STAT1	0.777713591	-0.001761404

Tbet^{+}CD4^{+}CD45RA^{+} T-Cells_STAT5	0.777713591	0.001041067
Tbet^{+}CD8^{+}CD45RA^{+} T-Cells Frequency	0.821365157	0.01045509
TCRgd T-cells_pSTAT6	0.821365157	-0.00309783
CD7^{+}CD14^{-}_S6	0.821365157	-0.002041832
TCRgd T-cells_STAT3	0.821365157	0.001741213
ncMC_MAPKAPK2	0.821365157	-5.94E-05
Neutrophils_S6	0.865551839	0.028926013
IgM+ B-Cells_S6	0.865551839	-0.028793138
B-Cells_S6	0.865551839	-0.026395287
Tbet^{+}CD4^{+}CD45RA^{-} T-Cells_ERK	0.865551839	0.018761154
CD8 Naive T-Cells Frequency	0.865551839	0.015877526
Tbet^{+}CD8^{+}CD45RA^{-} T-Cells_ERK	0.865551839	0.004538424
CD4 Memory T-Cells_MAPKAPK2	0.865551839	0.000497186
intMC Frequency	0.865551839	0.00014669
pDCs_pSTAT6	0.865551839	-0.000130589
B-Cells_pSTAT6	0.910142901	-0.021327861
pDCs Frequency	0.95500152	-0.030818702
Tbet^{+}CD4^{+}CD45RA^{+} T-Cells_STAT3	0.95500152	0.021317923
CD7^{+}CD14^{-}_p38	0.95500152	-0.011736172
intMC_pSTAT6	0.95500152	-0.001650841
ncMC_pSTAT6	0.95500152	2.50E-05
Tregs_pSTAT6	1	0.036735403
Tbet^{+}CD4^{+}CD45RA^{-} T-Cells_pSTAT6	1	0.010366561
Tbet^{+}CD4^{+}CD45RA^{-} T-Cells_MAPKAPK2	1	0.004518351
Tbet^{+}CD4^{+}CD45RA^{-} T-Cells	1	-0.001121399
IgM+ B-Cells_pSTAT6	1	-0.000506729

Supplemental Table S5: Features of the Late Phase Model (Day 90 compared to Day 365). Immune features that comprise the model were examined individually. Univariate p values were obtained using a Wilcoxon Test to compared each feature are Day 90 to Day 365. Elastic Net coefficients are also listed. Features are listed in order of their respective p-values.

Feature	p-value	Coefficient
Neutrophil Frequency	0.113947274	-0.023192096
$Tbet^{+}CD4^{+}CD45RA^{-}$ T-Cells_MAPKAPK2	0.270688695	0.000140469
pDCs_STAT5	0.303720532	0.000739524
pDCs_STAT1	0.321171628	0.005077958
B-Cells_CREB	0.357940817	-0.000272042
IgM+ B-Cells_S6	0.653240186	-0.000108077
IgM+ B-Cells_CREB	0.760104029	-0.000374841

Supplemental Table S6. ΔMoCA and Acute EN Model Value remain significant when controlling for potential confounders

Controlled Variable	Correlation	P-value
Stroke Volume	-0.664	0.026
Left Hemisphere Lesion	-0.706	0.015
Right Hemisphere Lesion	-0.706	0.015
IV-tPA Treatment Only	-0.708	0.015
tPA and IAT Treatment	-0.691	0.019
Fatigue	-0.691	0.018
CES-D	-0.731	0.011
BMI	-0.699	0.017
Age	-0.687	0.020
Gender	-0.691	0.019