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Supplemental material for:

Landscape of coordinated immune responses to H1N1 challenge in humans

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This file includes:

Supplemental Figures: Figures S1 to S10

Supplemental Tables: Tables S1 to S9 (legends only are provided for Tables S1, S3, S6, S8, and S9 with full data available for download online)

Supplemental Figure 1

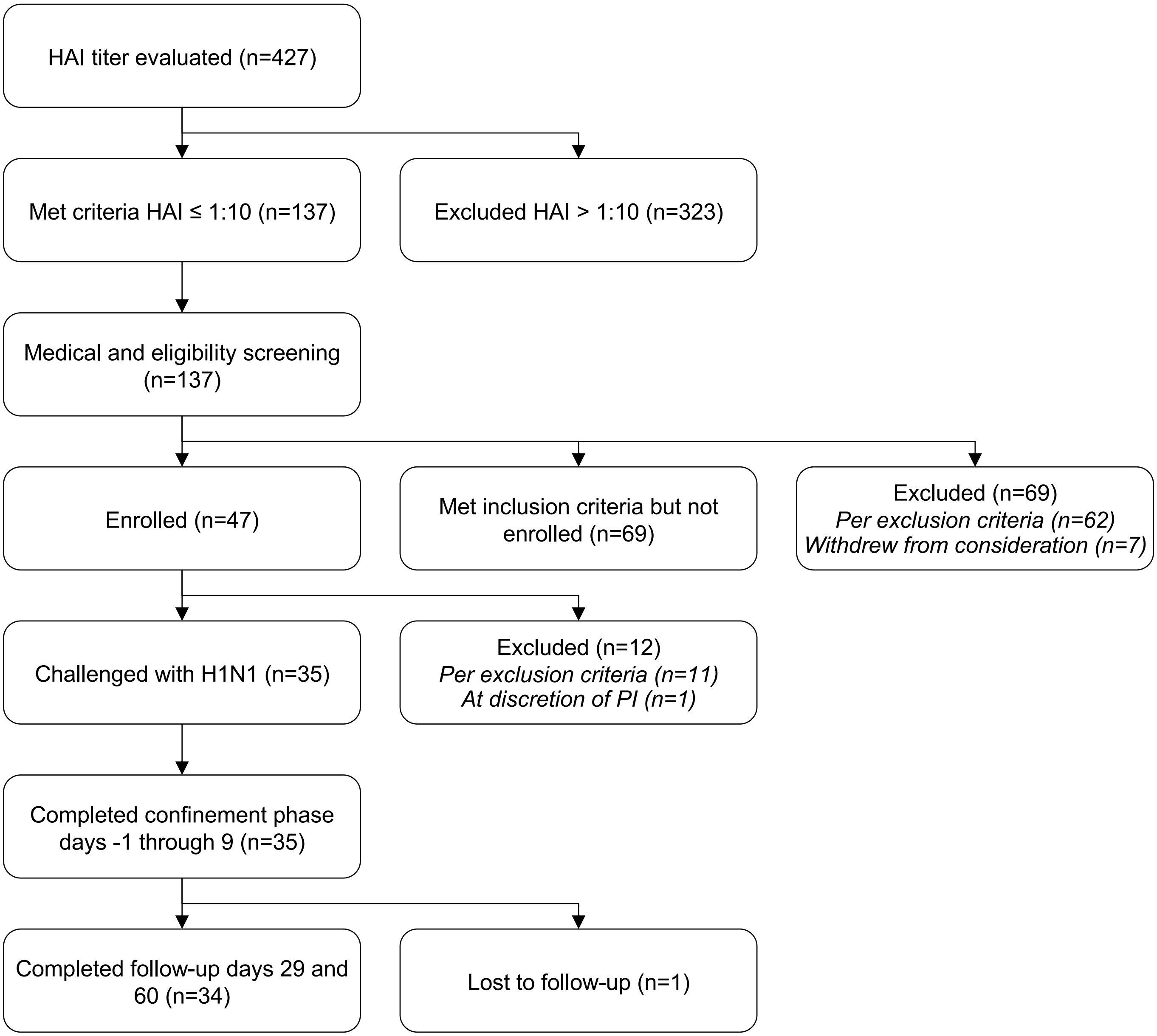


Figure S1. Schematic of study enrollment and completion

Supplemental Figure 2

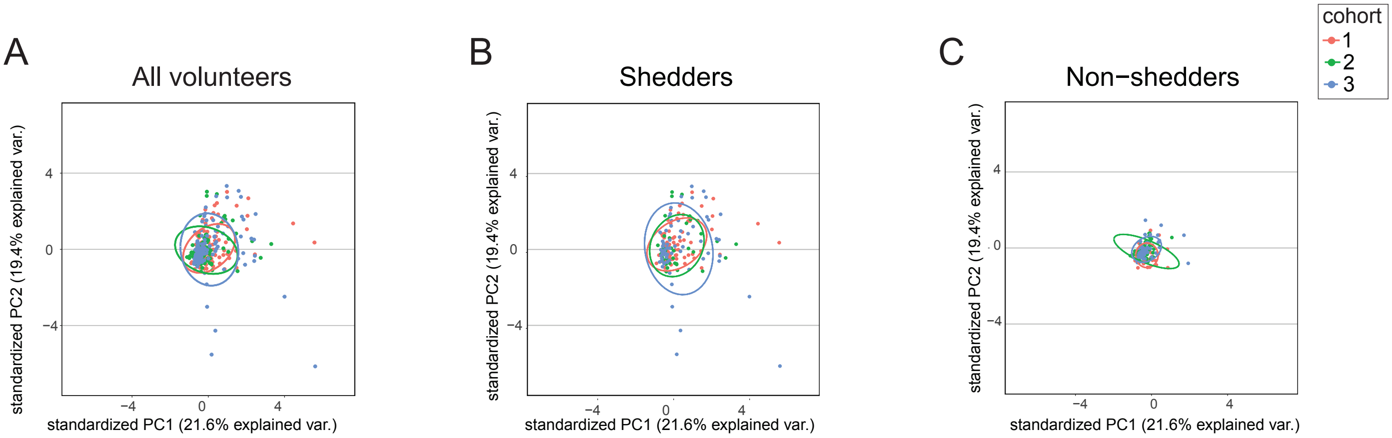


Figure S2. Principle component analysis for potential batch effects between cohorts. (A-C) PCA was applied to all study data. Each dot represents a single volunteer on a given day. Dots are color-coded by cohort: Cohort 1, red; cohort 2, green; cohort 3, blue. A) Data on all volunteers plotted together. B) Data on shedders. C) Data on non-shedders. A-C n=35 volunteers.

Supplemental Figure 3

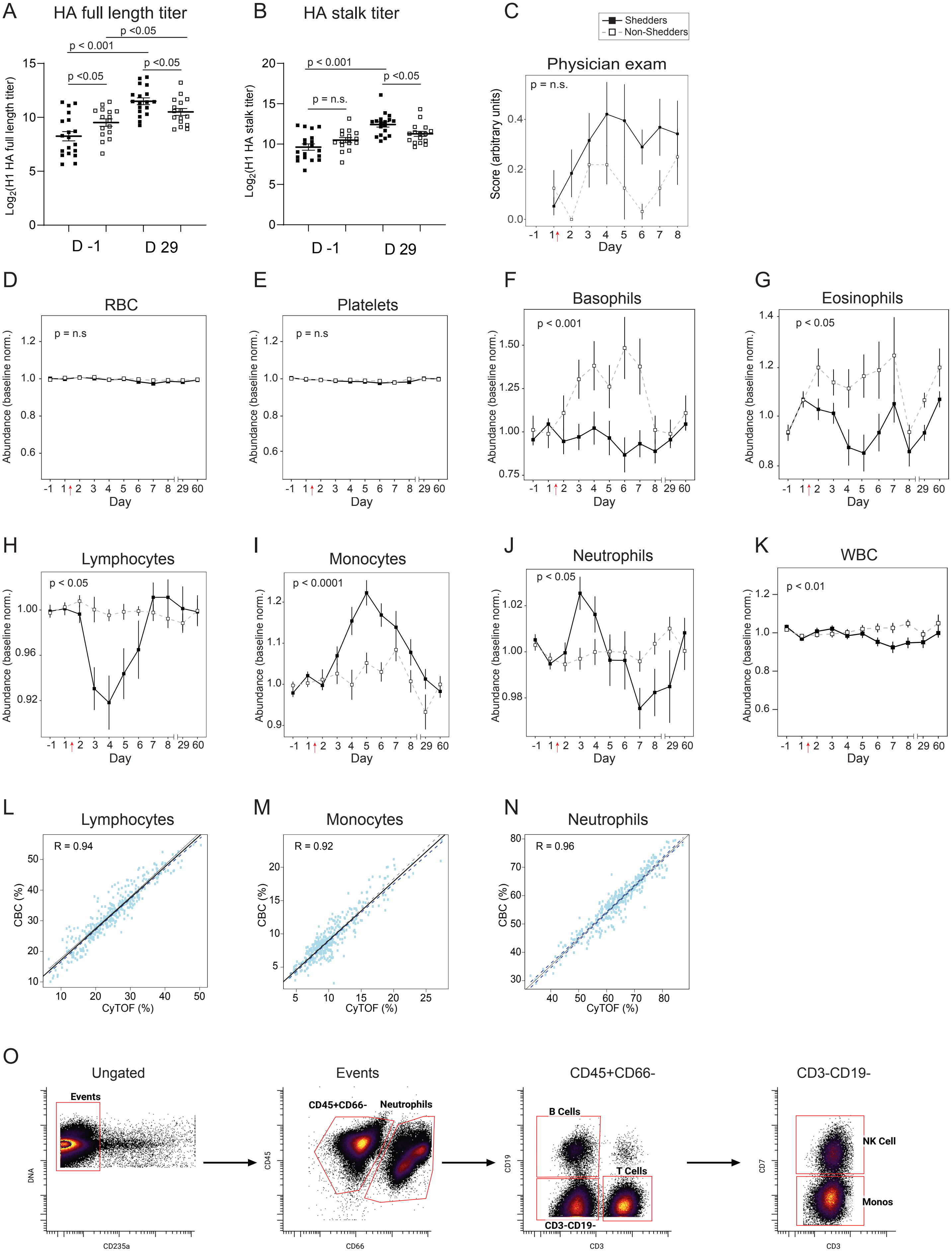
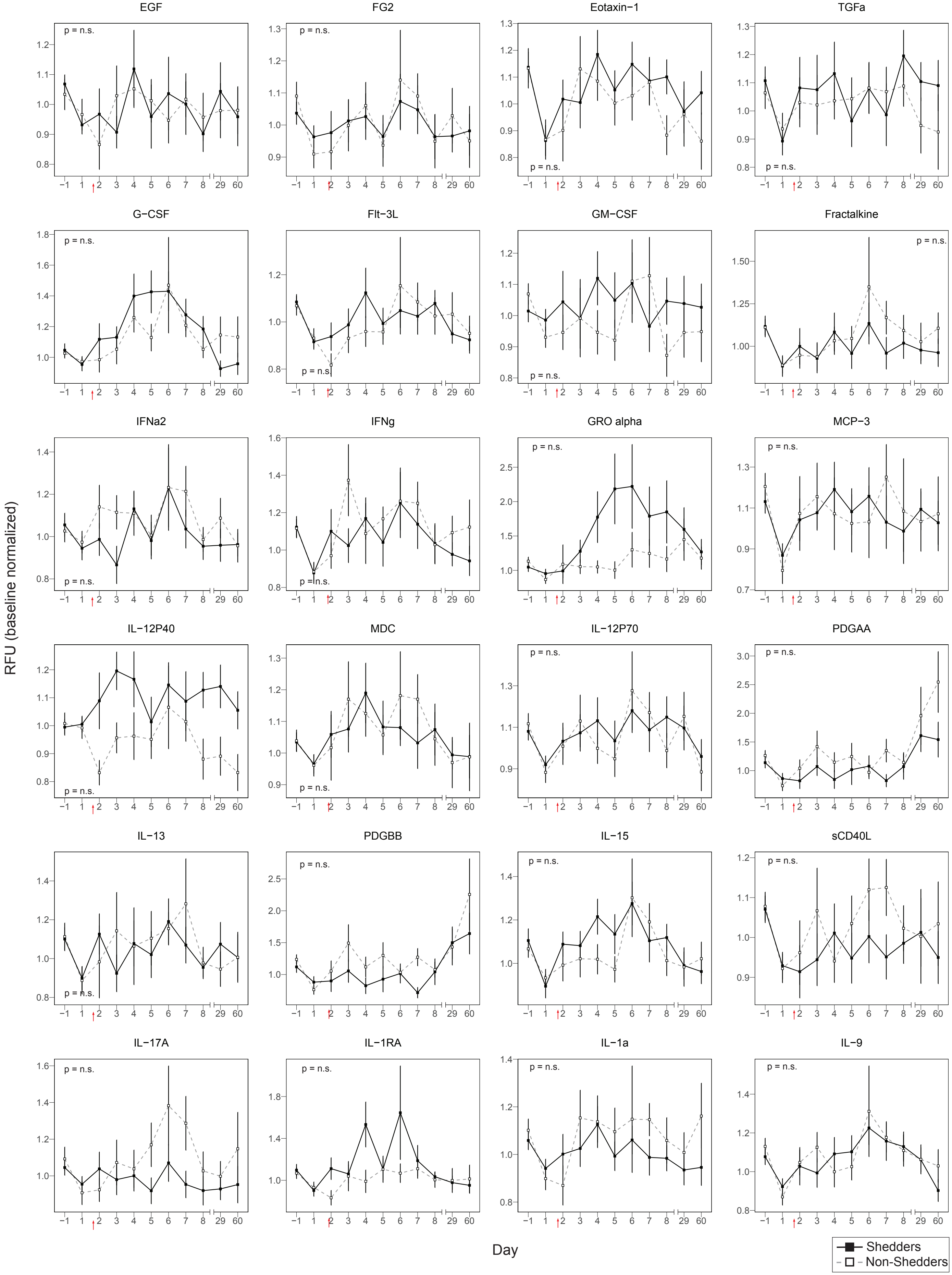
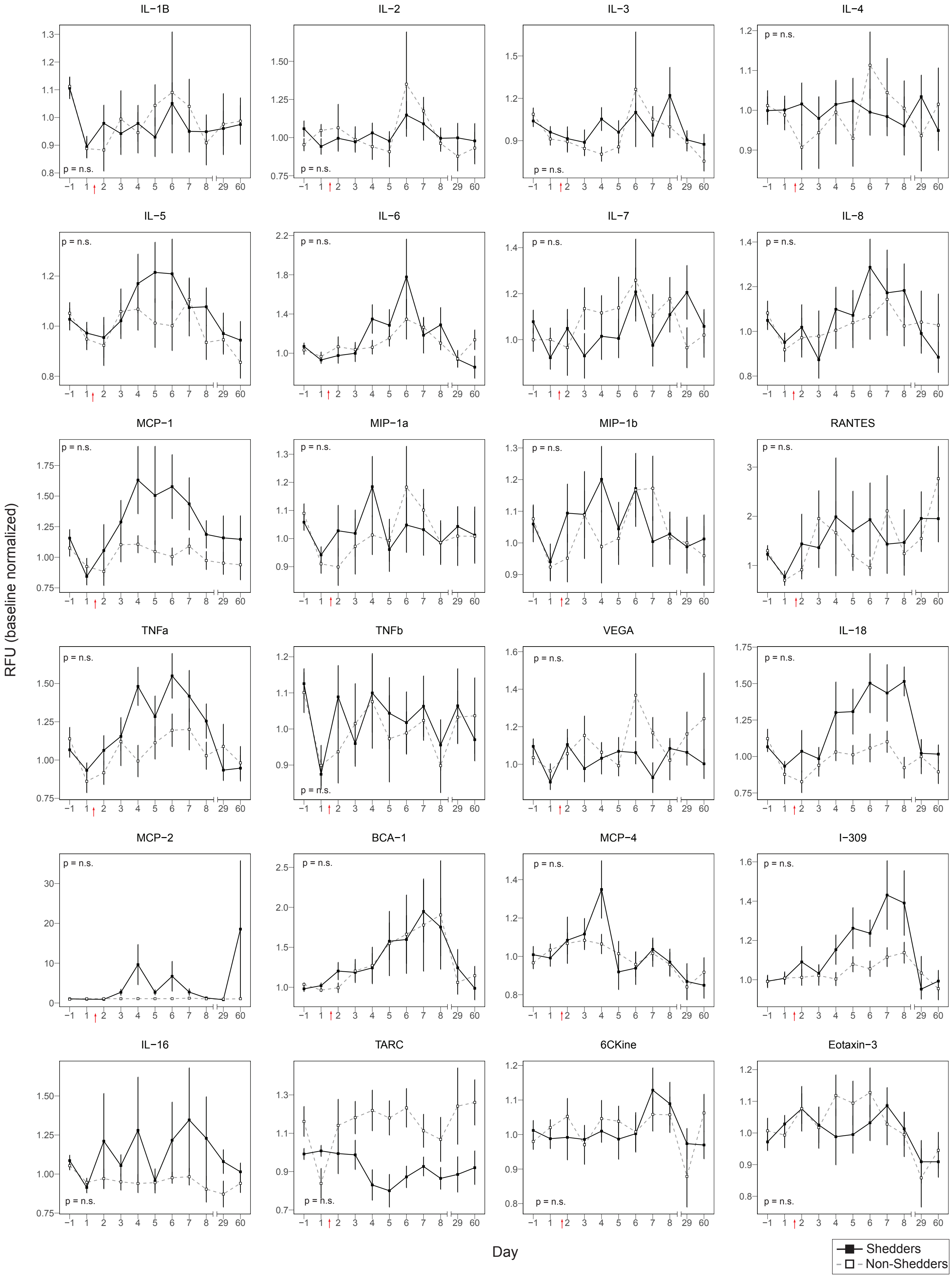


Figure S3. HA ELISA, physician exam, and clinical blood counts with comparison to mass cytometry. (A, B) Titers of antibodies to **A)** full-length HA and **B)** the HA stalk measured by ELISA in plasma at study day -1 and 29 for virus shedders (closed squares) and virus non-shedders (open squares). Horizontal bars indicate mean ± SEM. Statistics: Welch's t-test. **(C)** Mean daily symptom score as determined by physician exam. **(D-K)** Baseline-normalized CBCs values for **D)** red blood cells, **E)** platelets, **F)** basophils, **G)** eosinophils, **H)** lymphocytes, **I)** monocytes, **J)** neutrophils, **K)** white blood cells**.** Data for virus shedders are indicated with closed squares and solid lines, and data for non-shedders are indicated with open squares and dashed lines (mean ± SEM). Statistics: Bonferroni adjusted p-value of the time-shedding interaction term. **(L-N)** Correlations between CBCs and mass cytometry quantifications of **L)** lymphocytes, **M)** monocytes, and **N)** neutrophils in paired blood samples. Statistics: Spearman correlation. A-N n=35 volunteers.

Supplemental Figure 4



Supplemental Figure 4 cont'd



Supplemental Figure 4 cont'd

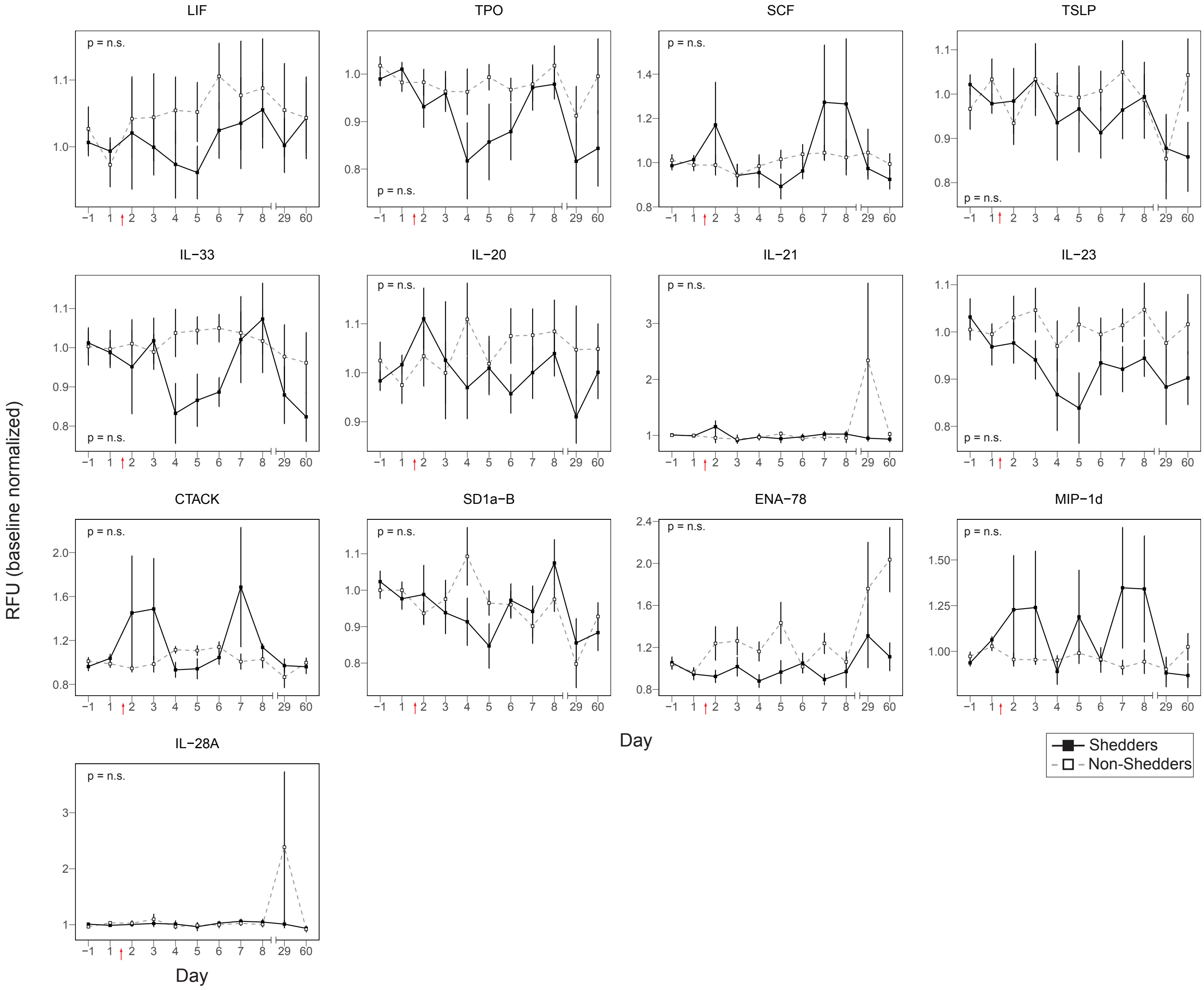


Figure S4. Baseline-normalized plasma cytokine and chemokine levels measured by Luminex assay. Baseline-normalized RFU values are plotted for each day of the study for virus shedders (closed squares, solid lines) and non-shedders (open squares, dashed lines). Values shown are mean ± SEM. Statistics: Bonferroni adjusted p-value of the time-shedding interaction term. n=35 volunteers.

Supplemental Figure 5

Figure S5. Complete mass cytometry gating strategy. Gates used to define all cell populations examined are shown for one representative sample (volunteer 204, day 7).

Supplemental Figure 6

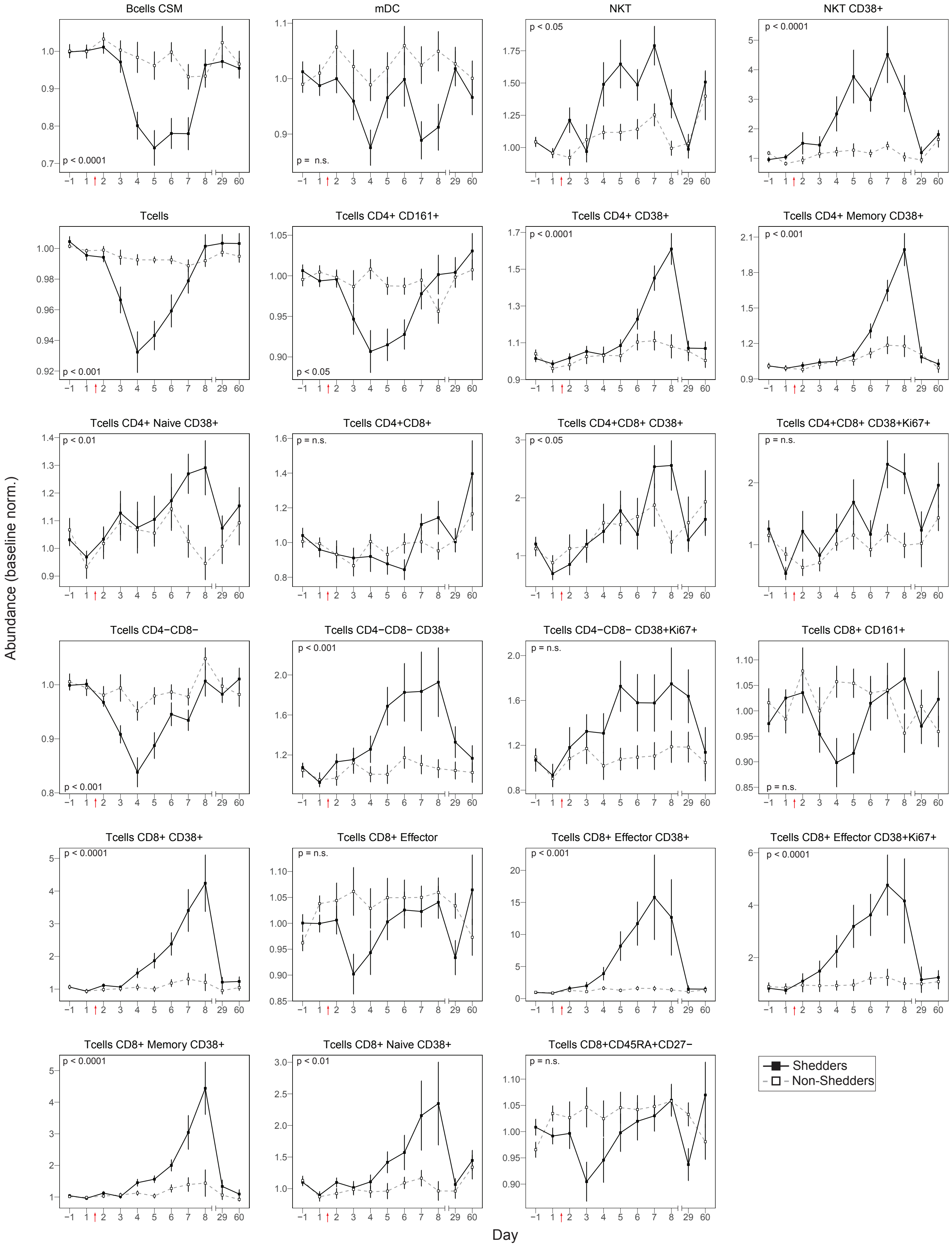
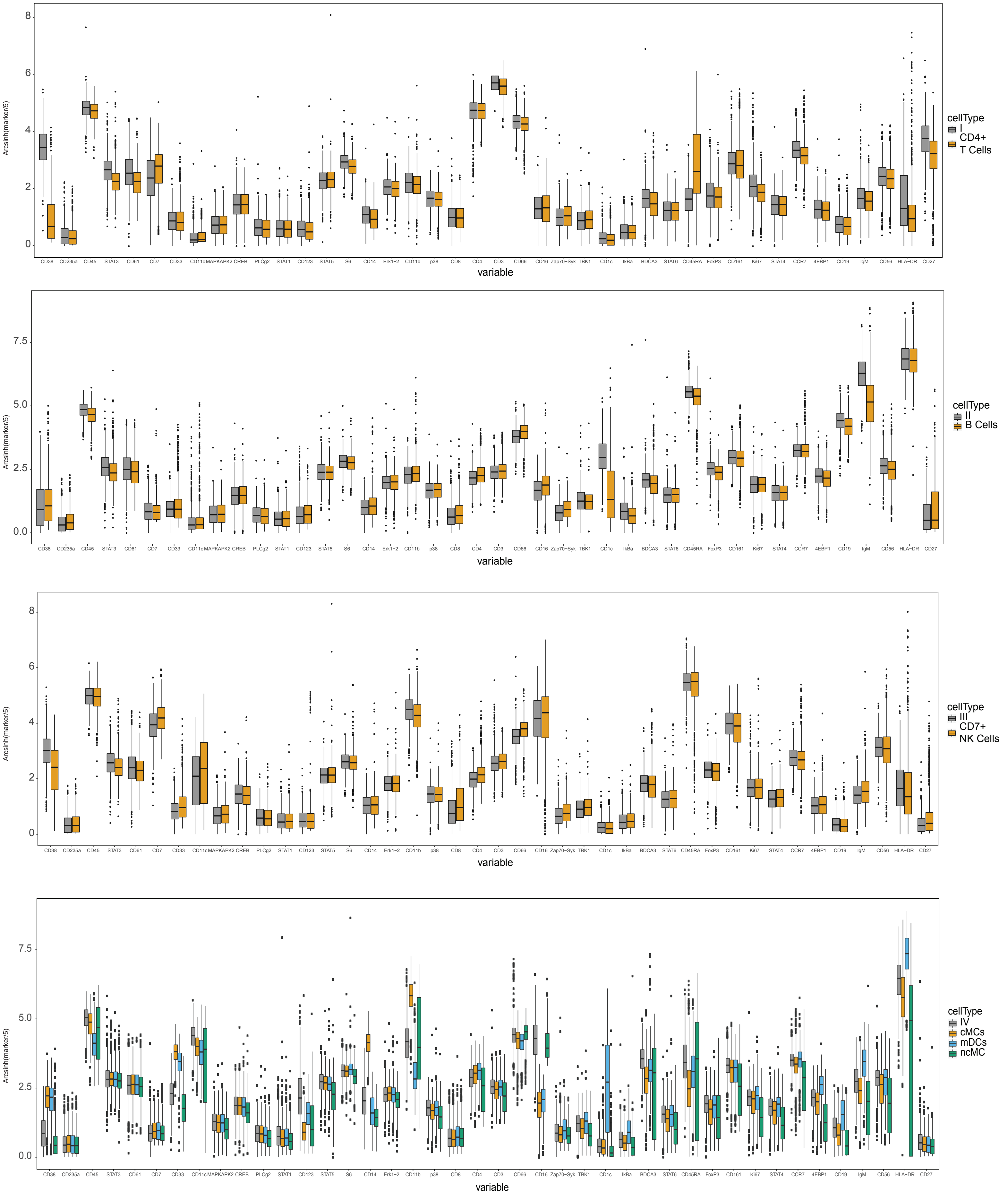


Figure S6. Mass cytometry profiling of adaptive and innate immune cell subsets during H1N1 infection not shown in Figures 3, 4 and 5. Baseline-normalized values (mean ± SEM) for cell abundance of indicated populations. Data for virus shedders are indicated with solid lines and closed squares; data for non-shedders are indicated with dashed lines and open squares. Statistics: Bonferroni adjusted p-value of the time-shedding interaction term, n=35 volunteers.

Supplemental Figure 7



Supplemental Figure 7 Cont'd

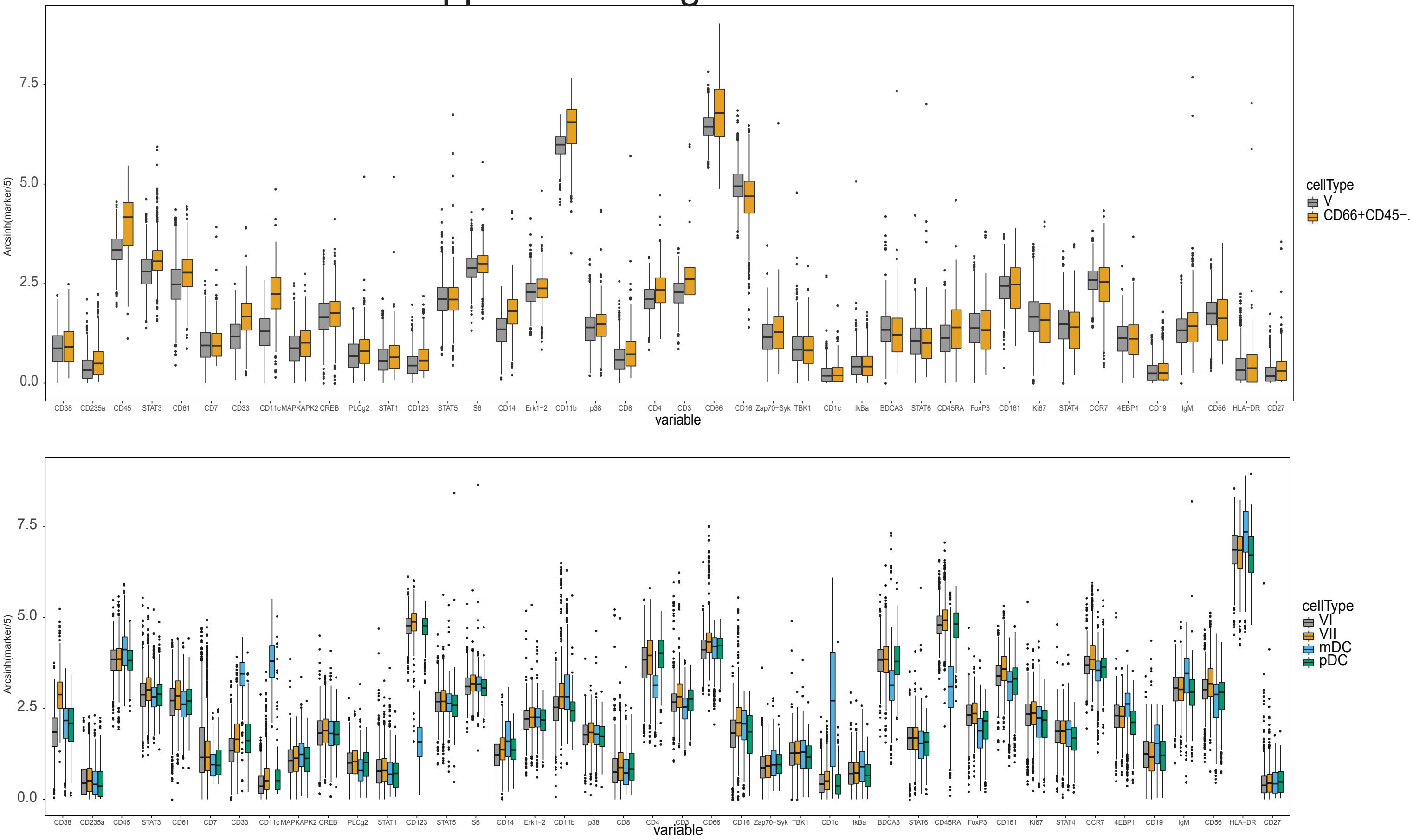


Figure S7. Marker expression on Scaffold clusters indicated in Figure 7. Box plots show expression levels for 42 markers analyzed by mass cytometry for Scaffold clusters indicated in Figure 7 relative to their nearest manually gated landmarks. n=35 volunteers.

Supplemental Figure 8

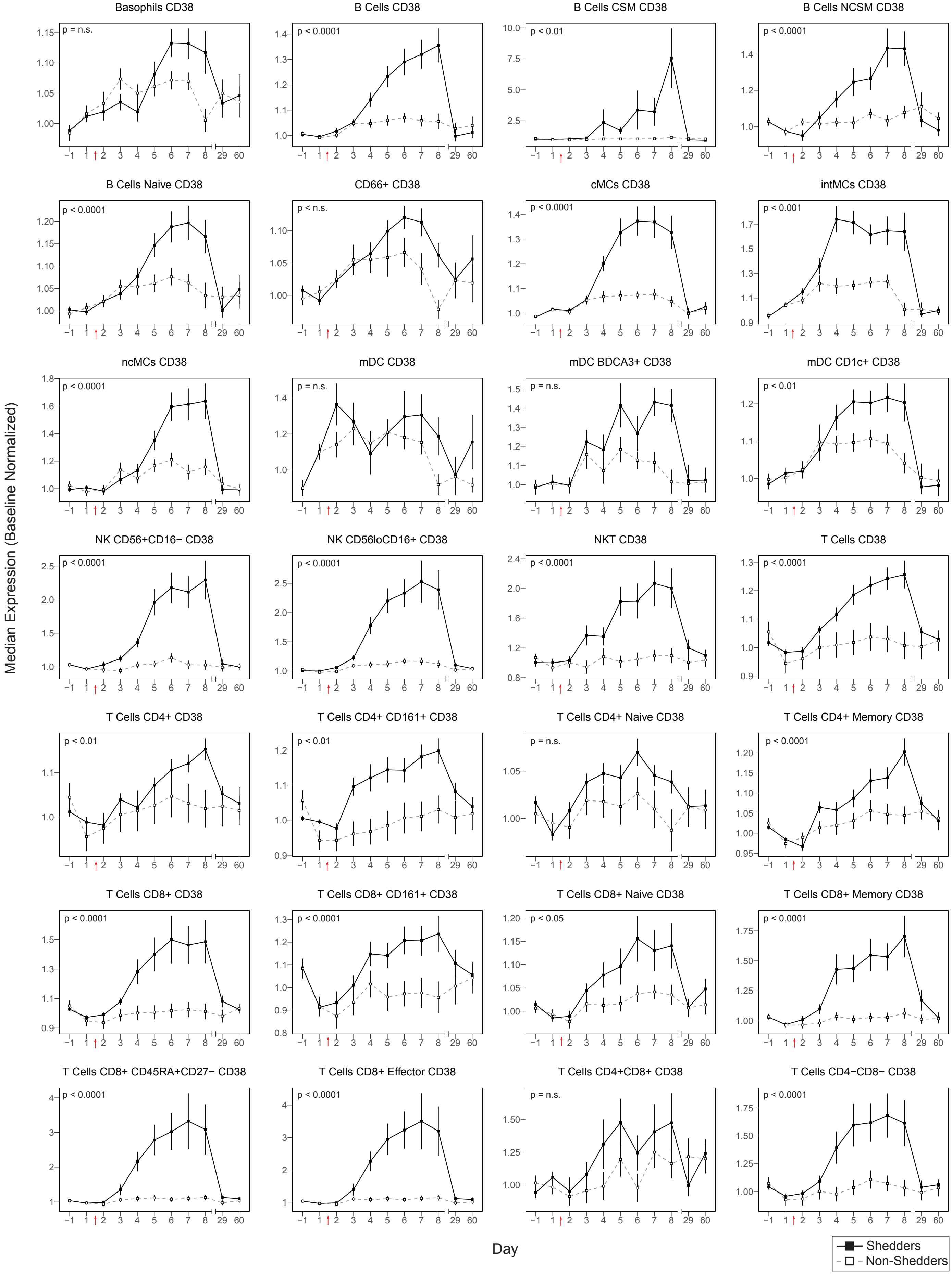


Figure S8. CD38 medians on manually gated cell populations. Line plots for CD38 medians on indicated manually gated populations. Data for virus shedders are indicated with closed squares and solid lines, and data for non-shedders are indicated with open squares and dashed lines. Values shown are baseline normalized (mean ± SEM). Statistics: n=35 volunteers, Bonferroni adjusted p-value of the time-shedding interaction term.

Supplemental Figure 9

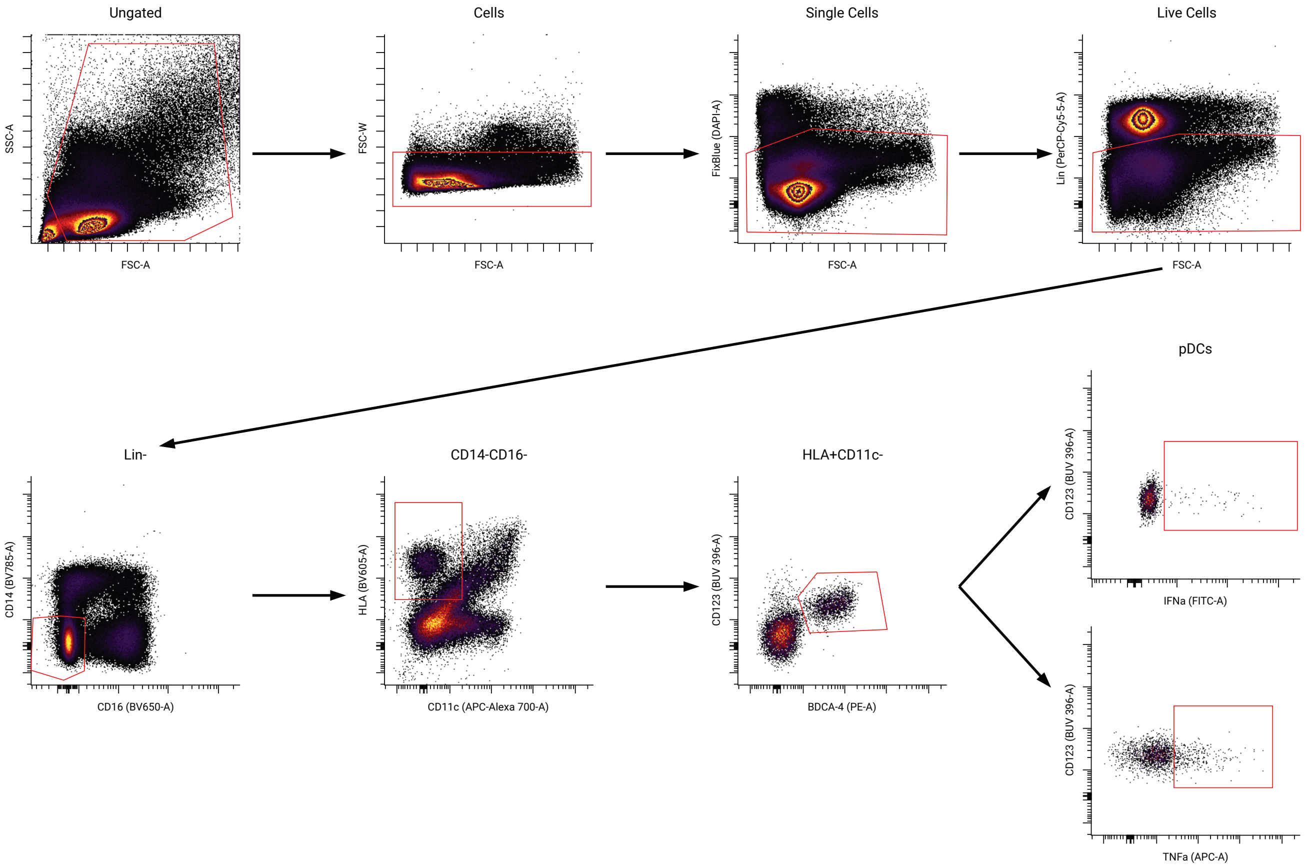


Figure S9. Flow cytometry gating strategy for pDCs. Gates used to define pDCs and intracellular cytokine expression.

Supplemental Figure 10

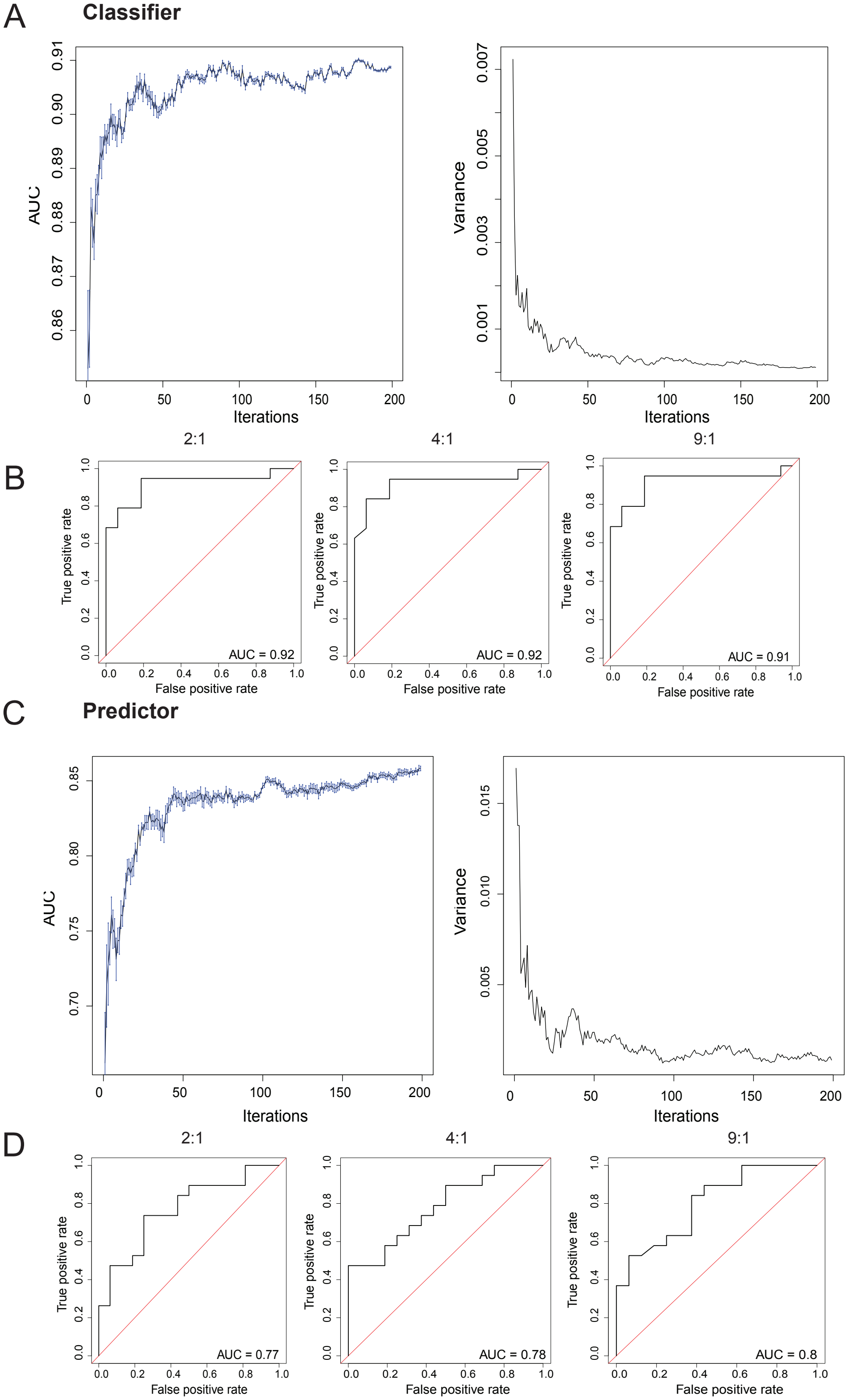


Figure S10. Robustness of Classification and Prediction models. (A) Line plots of the area under the curve (AUC) versus the number of iterations for the classifier (day 6), where iterations are the number of train-test data splits performed (left panel), and variance in AUC versus number of iterations (right panel) (B) ROC curves for different ratios of train-test splits performed for the classifier model. (C) Line plots of the area under the curve (AUC) versus the number of iterations for predictor (day 1), where iterations are the number of train-test data splits performed (left panel), and variance in AUC versus number of iterations (right panel). (D) ROC curves for different ratios of train-test splits performed for the predictor model. A, C mean (black line) ± 2 x variance (for enhanced visibility, blue bars) from 10 repetitions.

Supplemental Table 1

- Please see Table S1 in separate file. –

Table S1. A/California/04/2009 (H1N1) hemagglutination inhibition assay results for the screened vaccine naive population. HAI geometric mean titer (GMT) and age for 437 individuals; none reported being vaccinated for influenza in the 2 years prior to sampling.

Supplemental Table 2

All subjects:

	Inoculum volume	Total inoculum dose ($TCID_{50}$)	Subjects (n)	qRT-PCR positive, n (%)
Cohort 1	250 µL	3.5×10^6	12	8 (67%)
Cohort 2	250 µL	7.0×10^6	11	4 (36%)
Cohort 3	500 µL	7.0×10^6	12	7 (58%)
All cohorts	N/A	N/A	35	19 (54%)
inter-cohort comparison	*N/A*	*N/A*	*N/A*	*p= 0.92*

qRT-PCR negative subjects (non-shedders):

	Seroconversion, n (%)^1	Physician exam (mean)^2	Symptom score card (mean)^3	Viral load (mean)^4
Cohort 1	2 (50%)	1.3	0.1	*N/D*
Cohort 2	5 (71%)	0.6	0.1	*N/D*
Cohort 3	4 (80%)	1.5	0.1	*N/D*
All cohorts	11 (69%)	1.1	0.1	*N/D*
inter-cohort comparison	*p=0.50*	*p=0.74*	*p=0.81*	*N/A*

qRT-PCR positive subjects (shedders):

	Seroconversion, n (%)^1	Physician exam (mean)^2	Symptom score card (mean)^3	Viral load (mean)^4
Cohort 1	4 (50%)	1.5	1.4	5.3
Cohort 2	1 (25%)	1.7	0.6	5.4
Cohort 3	4 (57%)	3.4	1.1	6.5
All cohorts	9 (47%)	2.2	1.1	5.7
inter-cohort comparison	*p=0.71*	*p=0.26*	*p=0.43*	*p= 0.80*

[1] Seroconversion defined as ≥ 4-fold rise in HAI titer at study Day 29 or Day 60 relative to baseline.
[2] Physician exam is area under the curve for mean daily sum of physician exam scores
[3] Symptom score card is area under the curve for mean daily sum of subject symptom score card
[4] Viral load is area under the curve for virus shedding (Log_{10} copies/mL) by qRT-PCR
ND = below lower limit of detection

Table S2. Cohort level summary of symptomology, virus shedding, and seroconversion. Cohort comparisons for symptomology (physician exam, volunteer-reported symptom scorecard), viral shedding (viral load), and seroconversion.

Supplemental Table 3

- Please see Table S3 in separate file. –

Table S3. Complete dataset for symptomology, virus shedding, and seroconversion. Values shown are sum of mean daily values of symptom scorecard (SC_MEAN_SUM), sum of mean daily value of targeted physician exam score (TPE_MEAN_SUM), H1N1 copies/mL based on qPCR (VIRUS), max daily oral temp (Oral Temp), pulse rate, systolic blood pressure, diastolic blood pressure, HAI geometric mean titer (HAI), headless H1 HA stalk ELISA (HA Stalk Titer), full-length H1 HA ELISA (HA Full-length Titer), HA seroconversion Stalk , and HA seroconversion Full-length.

Supplemental Table 4

Antigen	Clone	Label	Vendor and Catalog Number
CD38	HIT2	89	Biolegend 303502
CD235a	HIR2	113	Biolegend 306602
CD45	HI30	115	Biolegend 304002
CD61	VI-PL2	140	Biolegend 336402
CD7	M-T701	141	BD Biosciences 555359
CD33	AC104.3E3	142	Miltenyi (Custom)
CD11c	Bu15	143	Biolegend 337202
CD123	7G3	148	BD Biosciences 554527
CD14	M5E2	151	Biolegend 301802
CD11b	ICRF44	153	Biolegend 301302
CD8	RPA-T8	155	Biolegend 301002
CD4	OKT4	156	Biolegend 317402
CD3	SP34.2	157	BD Biosciences 551916
CD66	YTH71.3	158	Pierce MA1-36189
CD16	3G8	159	Biolegend 302033
CD1c	AD5-8E7	162	Miltenyi (Custom)
BDCA3	1A4	164	BD Biosciences 559780
CD45RA	HI100	166	Biolegend 304102
CD161	HP-G310	168	Biolegend 339902
CCR7	150503	171	R&D MAB197-100
CD19	J3-119	173	Beckman Coulter IM1313
IgM	G20-127	174	BD Biosciences 555780
CD56	NCAM16.2	175	BD Biosciences 559043
HLA-DR	Immu357	176	Beckman Coulter Immu357
CD27	O323	209	Biolegend 302802

Table S4. Mass cytometry surface antibody panel.

Supplemental Table 5

Antigen	**Clone**	**Label**	**Vendor and Catalog Number**
STAT3 pY705	4	139	BD Biosciences 612357
MAPKAPK2 pT334	27B7	144	CST 3007
CREB pS133	87G3	145	CST 9198
PLCγ2 pY759	K86-689.37	146	BD Biosciences (Custom)
STAT1 pY701	4a	147	BD Biosciences 612233
STAT5 pY694	47	149	BD Biosciences 611965
S6 pS235/236	2F9	150	CST 4856
Erk1/2 pT202/Y204	D13.14.4E	152	CST 4370
P38 pT180/Y182	36/p38	154	BD Biosciences 612289
Zap70/Syk pY319/Y352	17a	160	BD Biosciences 612574
TBK1/NAK pS172	D52C2	161	CST 5483
IkBa amino-terminal	L35A5	163	CST 4814
STAT6 pY691	18	165	BD Biosciences 611597
FoxP3	PCH101	167	Thermo Fisher 14-4776-82
Ki67	SolA15	169	Thermo Fisher 14-5698-82
STAT4 pY693	38	170	BD Biosciences 612738
4E-BP1 pT37/46	236B4	172	CST 2855

Table S5. Mass cytometry intracellular antibody panel.

Supplemental Table 6

- Please see Table S6 in separate file. –

Table S6. Scaffold cluster cell abundance. Cell quantities in Scaffold clusters for all volunteers and study days.

Supplemental Table 7

Antigen	Clone	Fluorophore	Vendor and Catalog Number
IFNa	MMHA-11	FITC	PBL Assay Science 21112-3
TNFa	MAb11	APC	Biolegend 502912
CD38	HB-7	FITC	Biolegend 356610
PD-L1	29E.2A3	PE-Cy7	Biolegend 329718
CD80	2D10	BV421	Biolegend 305222
CD86	2331 (FUN-1)	BUV737	BD Biosciences 564428
BDCA4	12C2	PE	Biolegend 354503
CD123	7G3	BUV395	BD Biosciences 564195
HLA-DR	L243	BV605	Biolegend 307640
CD11c	Bu15	Alexa700	Biolegend 337220
CD16	3G8	BV650	Biolegend 302042
CD14	M5E2	BV785	Biolegend 301840
CD66b	G10F5	PerCP-Cy5.5	Biolegend 305108
CD335	9E2	PerCP-Cy5.5	Biolegend 331920
CD20	2H7	PerCP-Cy5.5	Biolegend 302326
CD19	HIB19	PerCP-Cy5.5	Biolegend 302230
CD3	UCHT1	PerCP-Cy5.5	Biolegend 300430

Table S7. Flow cytometry antibody panel.

Supplemental Table 8

- Please see Table S8 in separate file. –

Table S8. All study data used for classification and predictive models. Values for each parameter for each volunteer and study day used to generate classification and predictive models. Headings indicate the category of a feature: 1 cytokine/chemokines (Luminex), 2 complete blood counts (CBC), 3 cell abundance (Cell quantity), and 4 cell activation (CD38) and proliferation (Ki67) (Medians).

Supplemental Table 9

- Please see Table S9 in separate file. –

Table S9. Importance of features for classification and predictive models. Rows indicate feature and feature category: cytokine/chemokines (Luminex), complete blood counts (CBC), cell abundance (Cell quantity), and cell activation (CD38) and proliferation (Ki67) (Medians). Columns indicate the importance of feature for classifier (Day 6) [sort parameter] or predictor (day 1), univariate p-values for differences in features between shedders and non-shedders (with and without FDR-correction), and whether the feature was used to build predictor.