Supplemental information

Distinct immune cell dynamics correlate

with the immunogenicity and reactogenicity

of SARS-CoV-2 mRNA vaccine

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Figure S1
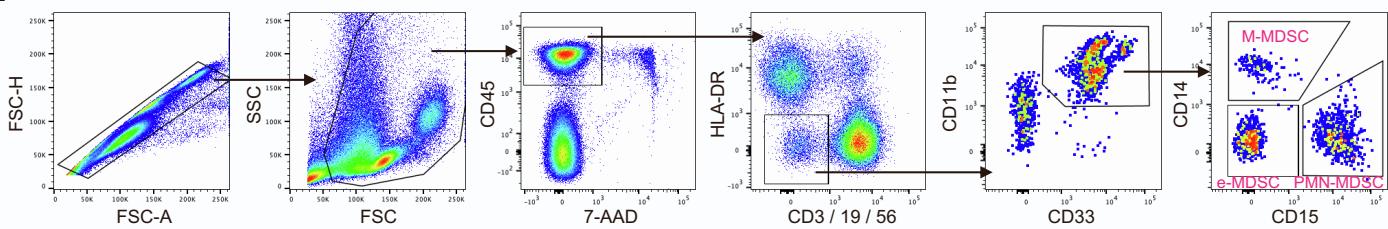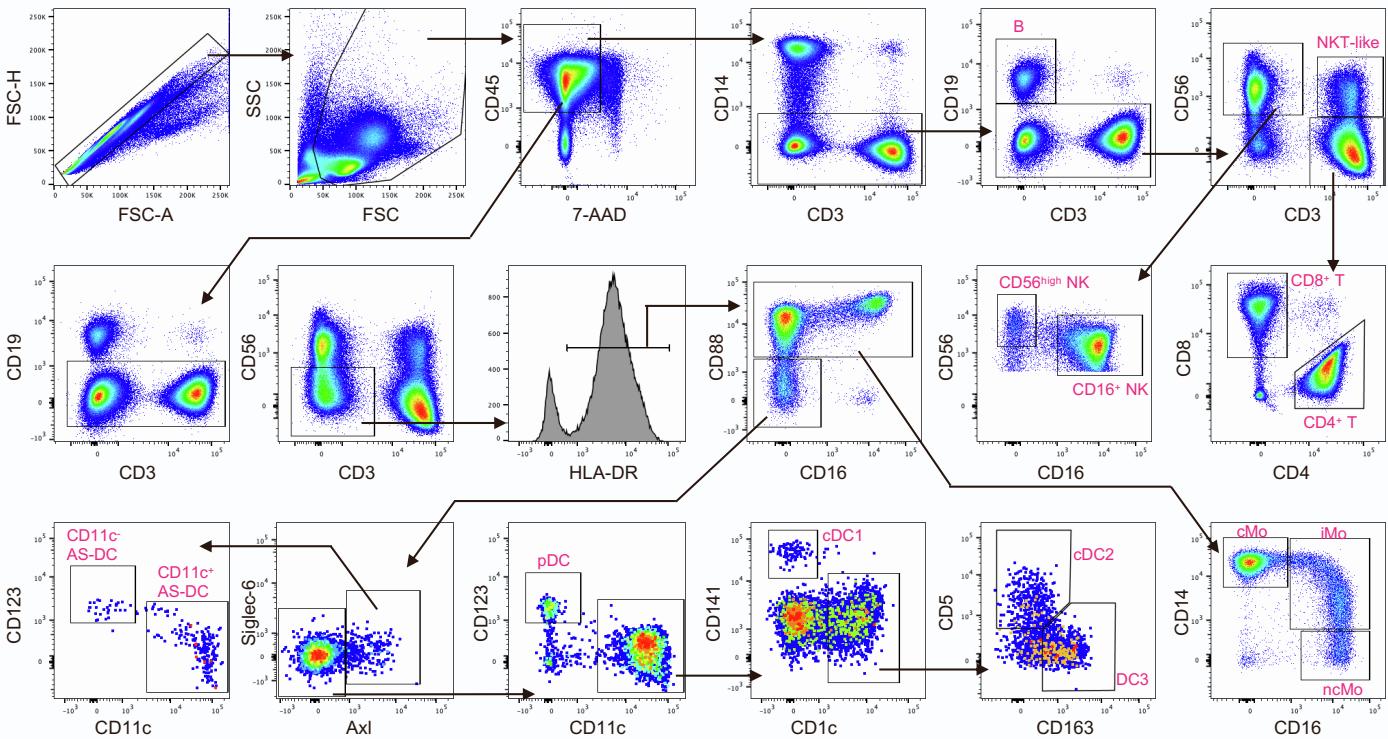
Figure S1. Gating strategy of flow cytometric analysis, Related to Figure 1.

(A) Gating strategy for identifying e-MDSCs, M-MDSCs, and PMN-MDSCs.

(B) Gating strategy for identifying B cells, CD4$^+$ T cells, CD8$^+$ T cells, CD16$^+$ NK cells, CD56^{high} NK cells, NKT-like cells, classical monocytes, non-classical monocytes, intermediate monocytes, cDC1s, cDC2s, DC3s, CD11c$^+$ AS-DCs, CD11c$^-$ AS-DCs, and pDCs.

Figure S2
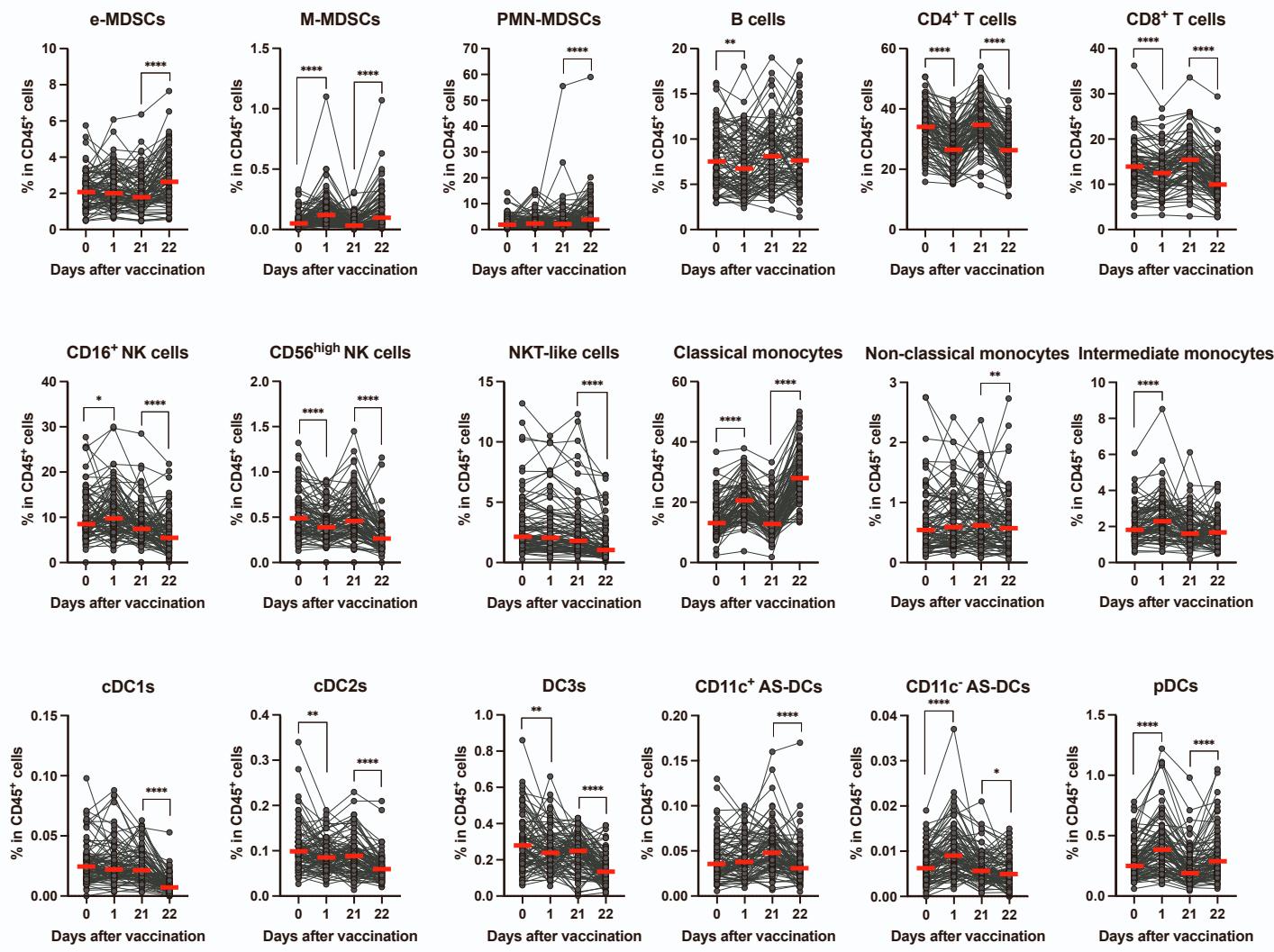
Figure S2. Longitudinal analysis of 18 immune cells frequencies, Related to Figure 1.

Longitudinal plots of cell frequencies at each time point; n = 92. Circles connected with a line represent data from the same individual. Bars represent the median and each dot represents the data for an individual participant. Statistical significance is indicated as follows; *p < 0.05, **p < 0.01, ****p < 0.0001, Wilcoxon test (d0 vs d1, and d21 vs d22).

Figure S3
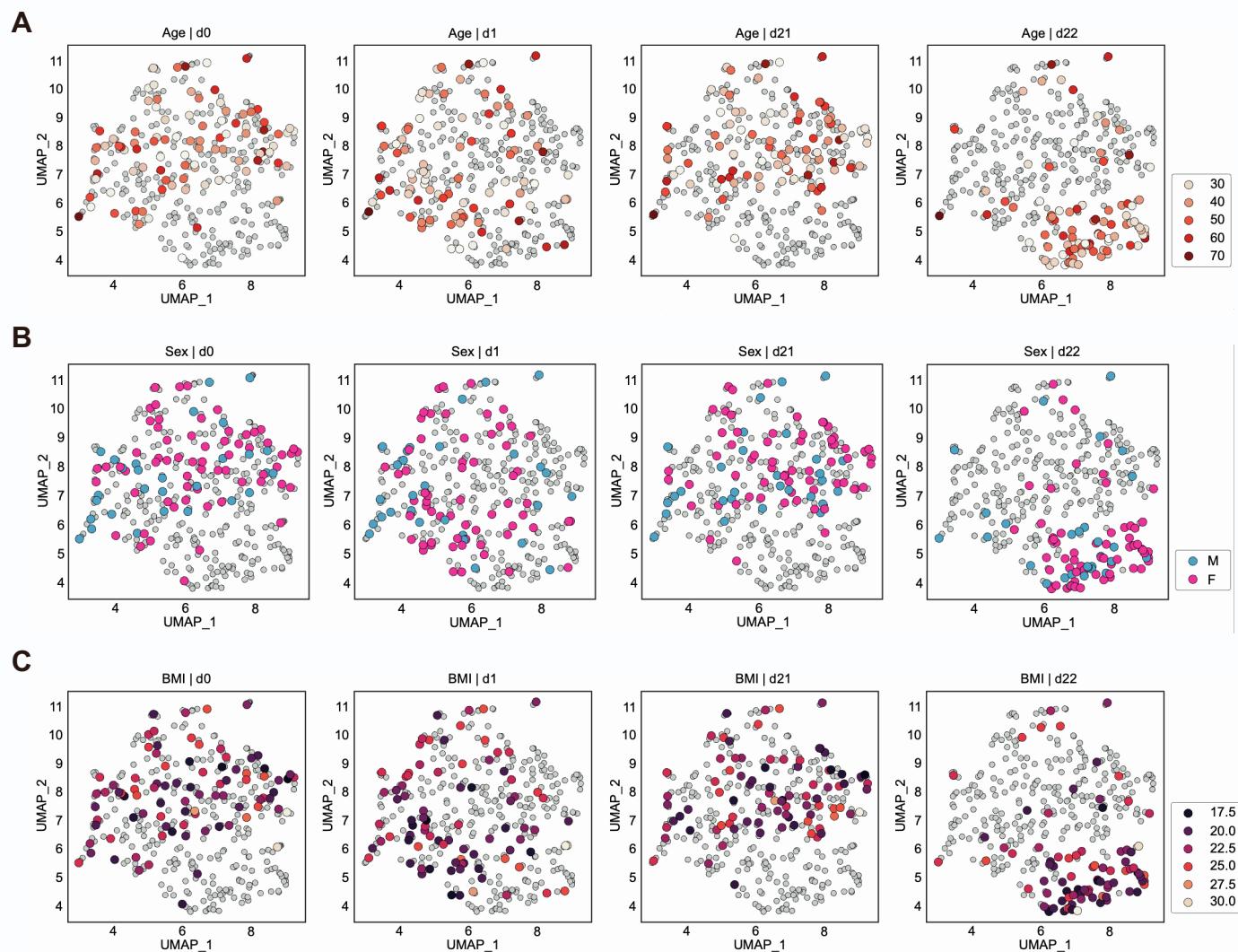
**Figure S3. UMAP of 18 immune cell frequencies among four time points, Related to Figure 1.**

(A–C) UMAPs of 18 immune cell frequencies shown in Fig. 1J. Each dot represents the data for an individual participant at each time point. n = 92. (A) Indicated points are colored by age at each time point. (B) Indicated points are colored by gender at each time point. (C) Indicated points are colored by BMI at each time point.

Figure S4

A

G-CSF | GM-CSF | IFN-γ | IFN-α | IL-1β | IL-2

(plots, y-axis Log₁₀ pg/mL, x-axis d0 d1 d21 d22)

IL-4 | IL-5 | IL-6 | IL-8 | IL-10 | IL-12p70

IL-17F | IP-10 | MCP-1 | MIG | MIP-1α | TNF

B

	1° RBD IgG	1° NT	Pain at injection site	Redness	Swelling	Local	Fever	Fatigue	Headache	Chills	Vomiting	Diarrhea	Muscle pain	Joint pain	Systemic
IL-6	-0.057	-0.041	-0.125	0.044	-0.106	-0.130	0.158	-0.086	0.102	0.053	0.142	0.051	-0.157	0.160	-0.066
IL-8	0.148	0.041	-0.051	0.028	5.4 ×10⁻⁵	-0.022	-0.038	0.005	0.069	0.098	-0.082	-0.062	-0.078	-0.019	-0.050
IL-10	0.118	0.092	-0.091	0.054	-0.035	-0.040	-0.024	-0.038	-0.102	-0.055	-0.024	**0.327**	-0.097	0.070	-0.065
IP-10	0.025	-0.099	-0.019	0.073	0.005	0.036	-0.085	0.053	0.008	0.013	0.065	0.109	-0.077	0.195	0.033

Adjusted FDR
☐ < 0.05

p-value
* < 0.05
** < 0.01
*** < 0.001
**** < 0.0001

Figure S4. Quantification of plasma cytokines/chemokines after the second vaccination, Related to Figures 2 and 3.

(A) Concentration of cytokines in plasma. Circles connected with a line represent data from the same individual. n = 92. Bars represent the median and each dot represents the data for an individual participant. Dotted lines represent the detection limits.

(B) Heat map representation of Spearman correlation matrix between antibody responses / symptom severity scores after primary vaccination and cytokine/chemokine dynamics (d1–d0 post/pre ratios). n = 92.

Statistical significance is indicated as follows; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Wilcoxon test in (A) (d0 vs d1, and d21 vs d22), Spearman's rank-order coefficient test in (B). Spearman's r values are indicated in each cell in (B).

Figure S5

A

	e-MDSCs	M-MDSCs	PMN-MDSCs	B	CD4+ T	CD8+ T	CD16+ NK	CD56high NK	NKT-like	Classical Mo	Non-classical Mo	Intermediate Mo	cDC1	cDC2	DC3	CD11c+ AS-DC	CD11c- AS-DC	pDC
Pain	0.006	0.196	0.175	0.125	-0.039	-0.041	-0.024	-0.099	0.054	0.018	0.008	-0.006	0.130	0.111	0.203	-0.036	0.179	0.150
Redness	-0.046	-0.012	0.064	0.042	-0.073	-0.026	0.047	0.007	-0.020	-0.066	-0.023	-0.019	-0.066	-0.091	-0.162	-0.158	0.096	0.159
Swelling	0.031	-0.089	0.010	-0.107	-0.079	-0.091	0.067	0.022	0.020	-0.036	0.058	0.065	0.062	0.197	0.049	-0.037	0.168	0.193
Local	-0.044	0.101	0.106	0.051	-0.056	-0.044	0.004	-0.078	0.042	-0.042	0.032	0.012	0.101	0.127	0.127	-0.101	0.182	0.200

p-value
* < 0.05
** < 0.01
*** < 0.001
**** < 0.0001

B

	e-MDSCs	M-MDSCs	PMN-MDSCs	B	CD4+ T	CD8+ T	CD16+ NK	CD56high NK	NKT-like	Classical Mo	Non-classical Mo	Intermediate Mo	cDC1	cDC2	DC3	CD11c+ AS-DC	CD11c- AS-DC	pDC
Fever	-0.132	0.116	-0.148	-0.069	-0.148	0.089	0.148	-0.101	-0.136	0.053	0.085	-0.069	0.065	-0.018	-0.091	-0.101	-0.097	0.109
Fatigue	0.027	0.025	0.065	0.062	-0.091	-0.036	0.081	0.067	0.024	-0.090	0.147	-0.028	0.103	6.1×10^{-5}	-0.023	0.010	-0.035	0.072
Headache	* 0.244	0.134	0.142	0.051	-0.033	-0.185	-0.039	0.088	-0.025	0.068	0.040	-0.016	-0.047	0.016	4.1×10^{-5}	0.054	-0.028	0.042
Chills	0.115	-0.077	0.037	0.055	** 0.301	-0.160	* -0.236	0.070	-0.160	-0.019	-0.162	* -0.246	-0.008	0.034	-0.136	-0.172	-0.104	0.110
Vomiting	0.097	0.144	0.172	0.014	0.144	-0.034	-0.120	0.101	-0.120	-0.010	-0.144	-0.168	-0.014	0.128	-0.172	-0.164	-0.030	0.148
Diarrhea	0.124	-0.105	-0.010	-0.065	0.113	0.049	0.010	0.136	-0.022	-0.128	0.089	-0.006	0.116	0.132	-0.049	0.081	0.156	0.144
Muscle pain	-0.074	0.049	-0.044	0.199	-0.030	0.078	0.119	-0.069	0.099	-0.162	0.111	-0.177	-0.080	-0.135	-0.076	-0.111	-0.003	0.052
Joint pain	0.057	0.024	-0.028	0.026	0.132	0.051	-0.030	-0.046	0.002	-0.166	0.037	-0.067	-0.150	0.038	-0.175	-0.158	0.060	0.050
Systemic	0.056	0.052	-0.049	0.146	-0.060	-0.003	0.125	-0.022	0.003	-0.128	0.164	-0.151	-0.040	-0.069	-0.096	-0.114	-0.056	0.086

p-value
* < 0.05
** < 0.01
*** < 0.001
**** < 0.0001

Figure S5. Correlation between cellular dynamics and adverse events after the primary vaccination, Related to Figure 4.

(A) Heat map representation of Spearman correlation matrixes between local symptom severity scores (primary vaccination) and cell dynamics (d1–d0 post/pre ratios). n = 92.

(B) Heat map representations of Spearman correlation matrix between systemic symptom severity scores (primary vaccination) and cell dynamics (d1–d0 post/pre ratios). n = 92. Statistical significance is indicated as follows; *p < 0.05, **p < 0.01, Spearman's rank-order coefficient test. Spearman's r values are indicated in each cell.

Figure S6
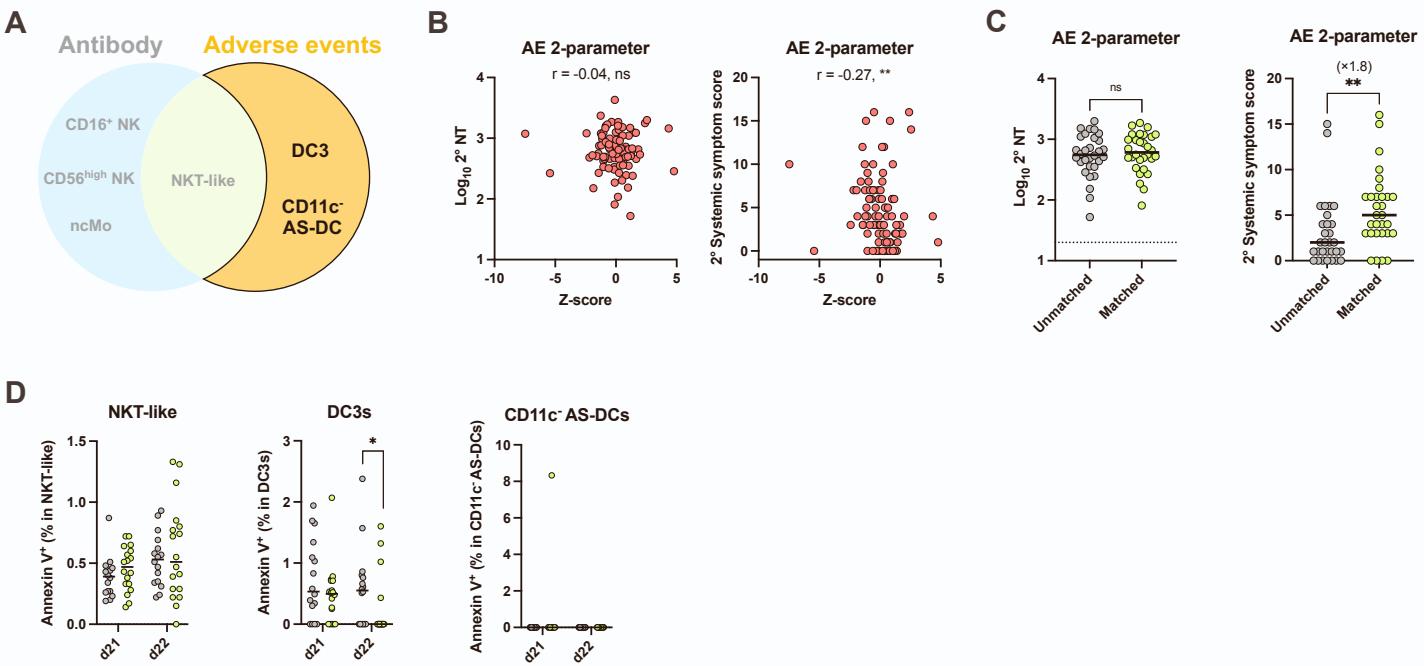
Figure S6. Immune cell dynamics classify a subgroup of vaccinees with severe adverse events, Related to Figure 5.

(A) Scheme of the 2-parameter model specific to adverse events.

(B) Z-score was calculated based on two parameters (i.e., the post/pre ratios of DC3s and CD11c⁻ AS-DCs). Correlation of Z-score and NT titers at day 47–51 and systemic symptom severity scores. n = 92.

(C) Participants were stratified into matched and unmatched group for 2-parameter model specific to adverse events. NT titers and systemic symptom severity scores compared between matched group and unmatched group. Matched group, n = 30; unmatched group, n = 30.

(D) Binding of Annexin V at day 21 and day 22 were compared between AE-related matched group and unmatched group. Matched group, n = 18; unmatched group, n = 16 (AE 3-parameter).

Statistical significance is indicated as follows; **p < 0.01, Mann-Whitney test in (C and D), Spearman's rank-order coefficient test in (B). Spearman's r values are indicated above the plots in (B).

Figure S7
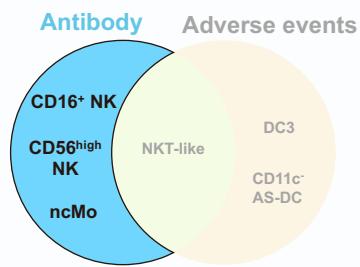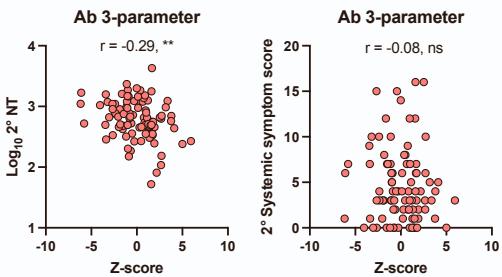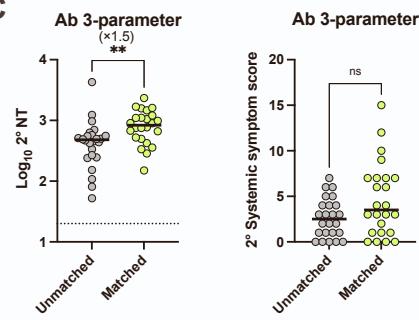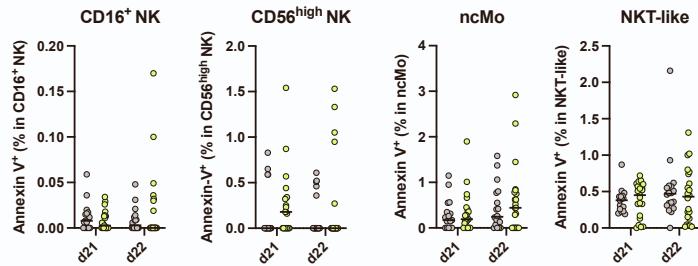
Figure S7. Immune cell dynamics classify a subgroup of vaccinees with high neutralizing antibody responses, Related to Figure 6.

(A) Scheme of the 3-parameter model specific to antibody responses.

(B) Z-score was calculated based on three parameters (i.e., the post/pre ratios of CD16+ NK, CD56high NK, and ncMo). Correlation of Z-score and NT titers at day 47–51 and systemic symptom severity scores. n = 92.

(C) Participants stratified into matched and unmatched group for 3-parameter model specific to antibody responses. NT titers and systemic symptom severity scores compared between matched group and unmatched group. Matched group, n = 24; unmatched group, n = 24.

(D) Binding of Annexin V at day 21 and day 22 were compared between Ab-related matched group and unmatched group. Matched group, n = 21 (d21), n = 22 (d22); unmatched group, n = 17 (d21), n = 18 (d22) (Ab 4-parameter).

Statistical significance is indicated as follows; **p < 0.01, Mann-Whitney test in (C and D), Spearman's rank-order coefficient test in (B). Spearman's r values are indicated above the plots in (B).

Table S1. Criteria of symptom severity score, Related to Figure 1.

Local Symptom	Grade 1	Grade 2	Grade 3	Grade 4
Pain	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization
Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Swelling	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis

Systemic Symptom	Grade 1	Grade 2	Grade 3	Grade 4
Fever	38.0 – 38.4 °C	38.5 – 38.9 °C	39.0 – 40 °C	> 40 °C
Fatigue	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization
Headache	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization
Chills	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization
Vomiting	1 – 2 episodes / 24 hours	> 2 episodes / 24 hours	Requires intravenous hydration	ER visit or hospitalization
Diarrhea	2 – 3 loose stools / 24 hours	4 – 5 loose stools / 24 hours	6 or more loose stools / 24 hours	ER visit or hospitalization
Muscle pain	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization
Joint pain	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization

Table S2. Immune cell phenotypes, Related to Figure 1.

Cell type	Phenotype
e-MDSCs	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^-$CD11b$^+$CD33$^+$CD14$^-$CD15$^-$
M-MDSCs	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^-$CD11b$^+$CD33$^+$CD14$^+$CD15$^-$
PMN-MDSCs	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^-$CD11b$^+$CD33$^+$CD14$^-$CD15$^+$
B cells	CD45$^+$CD14$^-$CD3$^-$CD19$^+$
CD4$^+$ T cells	CD45$^+$CD3$^+$CD4$^+$
CD8$^+$ T cells	CD45$^+$CD3$^+$CD8$^+$
CD16$^+$ NK	CD45$^+$CD14$^-$CD3$^-$CD19$^-$CD56$^+$CD16$^+$
CD56^{high} NK	CD45$^+$CD14$^-$CD3$^-$CD19$^-$CD56^{hi}
NKT-like	CD45$^+$CD14$^-$CD3$^+$CD19$^-$CD56^{hi}
Classical monocyte	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^+$CD14$^+$CD16$^-$
Non-classical monocyte	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^+$CD14$^-$CD16$^+$
Intermediate monocyte	CD45+CD3-CD19$^-$CD56$^-$HLA-DR$^+$CD88$^+$CD14$^+$CD16$^+$
cDC1	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^-$CD16$^-$Siglec-6$^-$Axl$^-$CD11c$^+$CD123$^-$CD1c$^-$CD141$^+$
cDC2	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^-$CD16$^-$Siglec-6$^-$Axl$^-$CD11c$^+$CD123$^-$CD1c$^+$CD141$^-$CD5$^+$CD163$^-$
DC3	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^-$CD16$^-$Siglec-6$^-$Axl$^-$CD11c$^+$CD123$^-$CD1c$^+$CD141$^-$CD5$^-$CD163$^+$
CD11c$^+$ AS-DC	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^-$Siglec-6$^+$Axl$^+$CD11c$^+$CD123$^-$$^{/lo}$
CD11c$^-$ AS-DC	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^-$Siglec-6$^+$Axl$^+$CD11c$^-$CD123$^+$
pDC	CD45$^+$CD3$^-$CD19$^-$CD56$^-$HLA-DR$^+$CD88$^-$CD16$^-$Siglec-6$^-$Axl$^-$CD11c$^-$CD123$^+$