EXTENDED DATA FIGURES & TABLES

Extended Data Table 1 | Characteristics of study participants

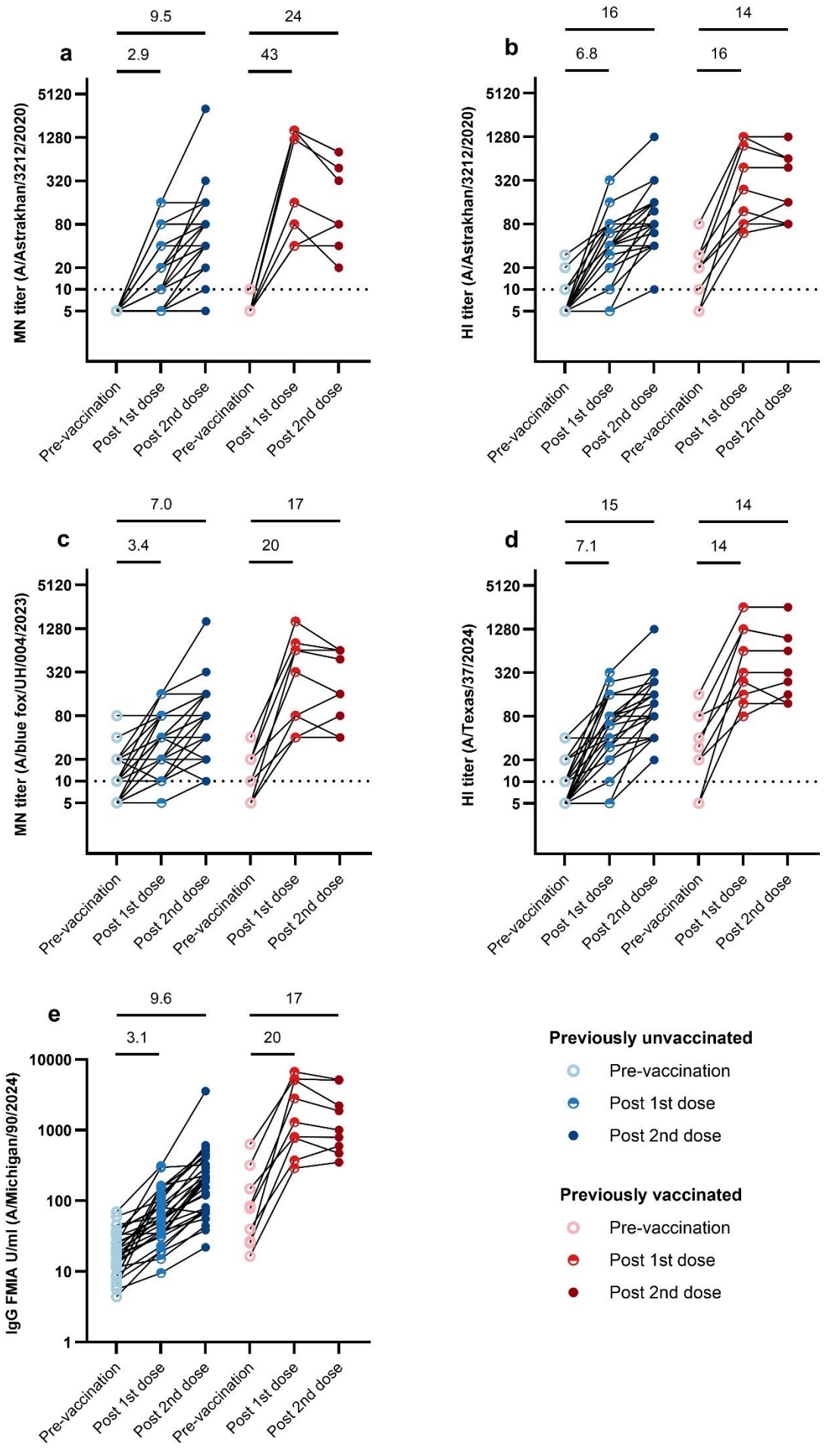
	Samples included in antibody analyses		Samples included in analyses of T cells	
	Previously unvaccinated	**Previously vaccinated**	**Previously unvaccinated**	**Previously vaccinated**
n	30	9	13	5
Sex, n (%) female	24 (80%)	8 (89%)	12 (92%)	4 (80%)
Age (years), median (range)	41 (27–63)	51 (40–61)	35 (27–54)	49 (40–51)
Target group				
Fur farm workers	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Poultry farm workers	3 (10%)	0 (0%)	0 (0%)	0 (0%)
Veterinarians	4 (13%)	0 (0%)	1 (8%)	0 (0%)
Bird ringers	5 (17%)	0 (0%)	0 (0%)	0 (0%)
Laboratory workers	18 (60%)	9 (100%)	12 (92%)	5 (100%)
Dose interval (days), median (range)	28 (20-53)	28 (18-42)	28 (20-53)	28 (18-35)
Number of previous H5 vaccine doses, n (%)				
2	NA	4 (45%)	NA	2 (40%)
4	NA	2 (22%)	NA	1 (20%)
6	NA	3 (33%)	NA	2 (40%)

NA= not applicable

Extended Data Table 2 | Avian influenza vaccines administered in previously vaccinated participants.

	Pre-pandemic A(H5N1), inactivated, AS03-adjuvanted A/Indonesia/5/2005-like split virion vaccine, clade 2.1.3.2 (GlaxoSmithKline)		A(H5N1) inactivated adjuvant-free A/Vietnam/1203/2004-like whole virus vaccine, clade 1 (Baxter)		A(H5N1) inactivated MF59-adjuvanted A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) vaccine, clade 2.2.1 (Novartis)		Zoonotic A(H5N8), inactivated, M59-adjuvanted, A/Astrakhan/3212/2020-like strain (CBER-RG8A) vaccine, (clade 2.3.4.4b) (Seqirus Vaccines)	
	2009		**2011-2012**		**2018**		**2024**	
Participant	1st dose	2nd dose	1st dose	2nd dose	1st dose	2nd dose	1st dose	2nd dose
1					•	•	•	•
2					•	•	•	•
3	•	•	•	•			•	•
4	•	•	•	•	•	•	•	•
5	•	•	•	•	•	•	•	•
6	•	•	•	•	•	•	•	•
7					•	•	•	•
8	•	•			•	•	•	•
9					•	•	•	•

Detailed information and the number of A(H5) vaccine doses given prior to and during this study indicated by year for each participant. Vaccination is indicated by a solid circle (•).

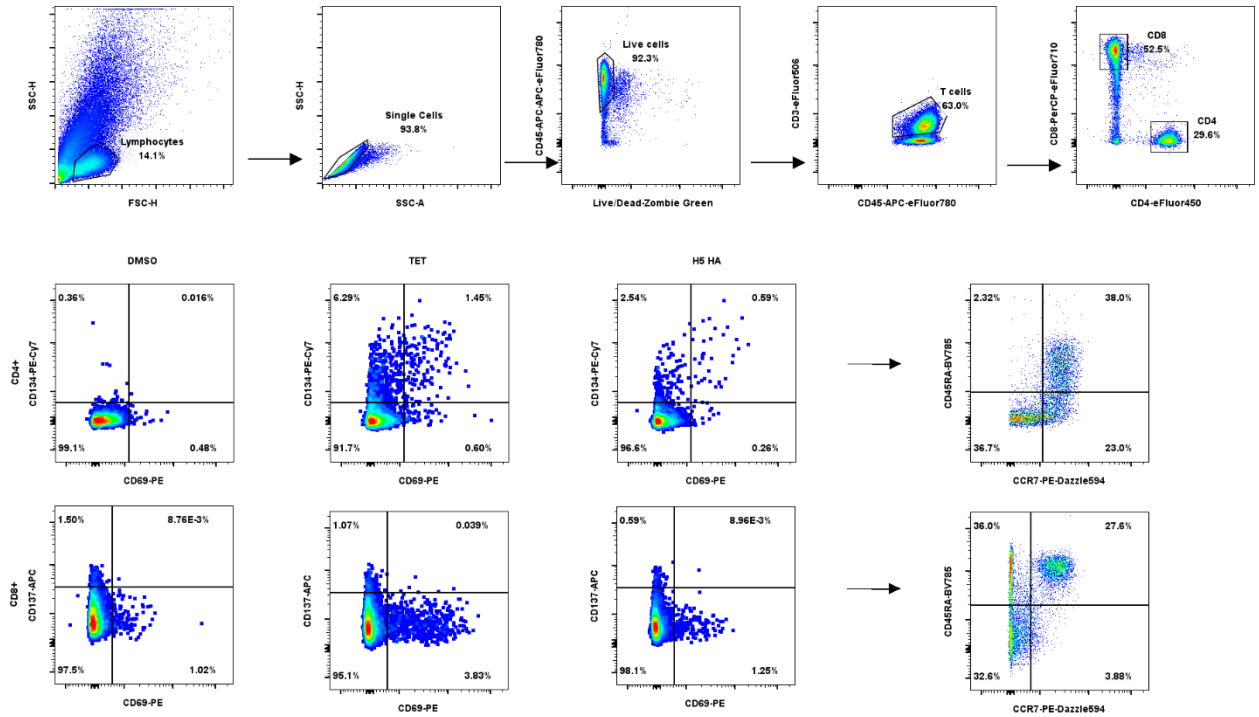

a
9.5
24
2.9
43
MN titer (A/Astrakhan/3212/2020)
5120
1280
320
80
20
10
5
Pre-vaccination
Post 1st dose
Post 2nd dose
Pre-vaccination
Post 1st dose
Post 2nd dose
b
16
14
6.8
16
HI titer (A/Astrakhan/3212/2020)
c
7.0
17
3.4
20
MN titer (A/blue fox/UH/004/2023)
d
15
14
7.1
14
HI titer (A/Texas/37/2024)
e
9.6
17
3.1
20
IgG FMIA U/ml (A/Michigan/90/2024)
10000
1000
100
10
1
Previously unvaccinated
Pre-vaccination
Post 1st dose
Post 2nd dose
Previously vaccinated
Pre-vaccination
Post 1st dose
Post 2nd dose

Extended Data Fig. 1 | Kinetics of vaccine-induced individual antibody responses. a, b, Antibodies targeting the vaccine antigen A(H5N8) A/Astrakhan/3212/2020 were measured using the microneutralization (MN) and the hemagglutination inhibition (HI) assay. c, Antibodies targeting A(H5N1) A/blue fox/UH/004/2023 were measured by the MN assay. d, Antibodies targeting A(H5N1) A/Texas/37/2024 were measured by HI assay. e, IgG antibodies binding to purified A(H5N1) A/Michigan/90/2024 H5 type HA antigen were measured by fluorescent bead-based multiplex immunoassay (FMIA). Individual responses are shown as lines for two groups, A(H5N1) unvaccinated (n=30) and previously vaccinated (n=9), at three time points: pre-vaccination, and three weeks after the first dose and the second dose. The dashed line indicates the positivity threshold. Fold changes in the mean antibody titers before vaccination and after the first dose, as well as before vaccination and after the second dose, represented by lines within the graphs are shown.

Extended Data Table 3 | Viruses and antigens used in the microneutralization (MN) assay, the hemagglutination inhibition (HI) assay, and the fluorescent bead-based multiplex immunoassay (FMIA).

Virus or antigen name	GISAID isolate ID	HA accession number	Subtype	HA clade	Full virus or RG or HA	Passage history	Origin of the virus or HA	Biosafety level handling	Method
A/Astrakhan/3212/2020 CVV	EPI_ISL_13655139	EPI2084527	A(H5N8)	2.3.4.4.b	IDCDC-RG71A	E1M2	The Crick Worldwide Influenza Centre, London (Ex/E1)	BSL2+	MN
A/Astrakhan/3212/2020	EPI_ISL_1038924	EPI1846961	A(H5N8)	2.3.4.4.b	7+1 PR/8 HY	293TM2	Gene synthesis from Proteogenix	BSL2	HI
A/blue fox/UH/004/2023	EPI_ISL_18764855 (E1 passage)	EPI2914899 (E1 passage)	A(H5N1)	2.3.4.4.b	Full virus	M2	Associate Professor Tarja Sironen, University of Helsinki	BSL3	MN
A/Texas/37/2024	EPI_ISL_19027114	EPI3171488	A(H5N1)	2.3.4.4.b	7+1 PR/8 HY	293TM2	Plasmid from Dr. Daniel R. Perez, University of Georgia, US	BSL2	HI
A/Michigan/90/2024	EPI_ISL_19162802	EPI3334182	A(H5N1)	2.3.4.4.b	HA amino acids 1-530	Original	Antigen from Native Antigen Company	BSL1	FMIA

CVV = candidate vaccine virus, HA = hemagglutinin, RG = reverse genetics, PR/8 HY = A/Puerto Rico/8/1934 high yield, M = MDCK.



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29 **Extended Data Fig. 2 | Gating strategy for differentiating blood cell populations after flow cytometry.**
30 Representative gating strategy for $CD4^+$ and $CD8^+$ T cells after peptide pool stimulation. DMSO, tetanus
31 toxoid (TET) and H5 HA peptide pool stimulated cells are shown as representatives.

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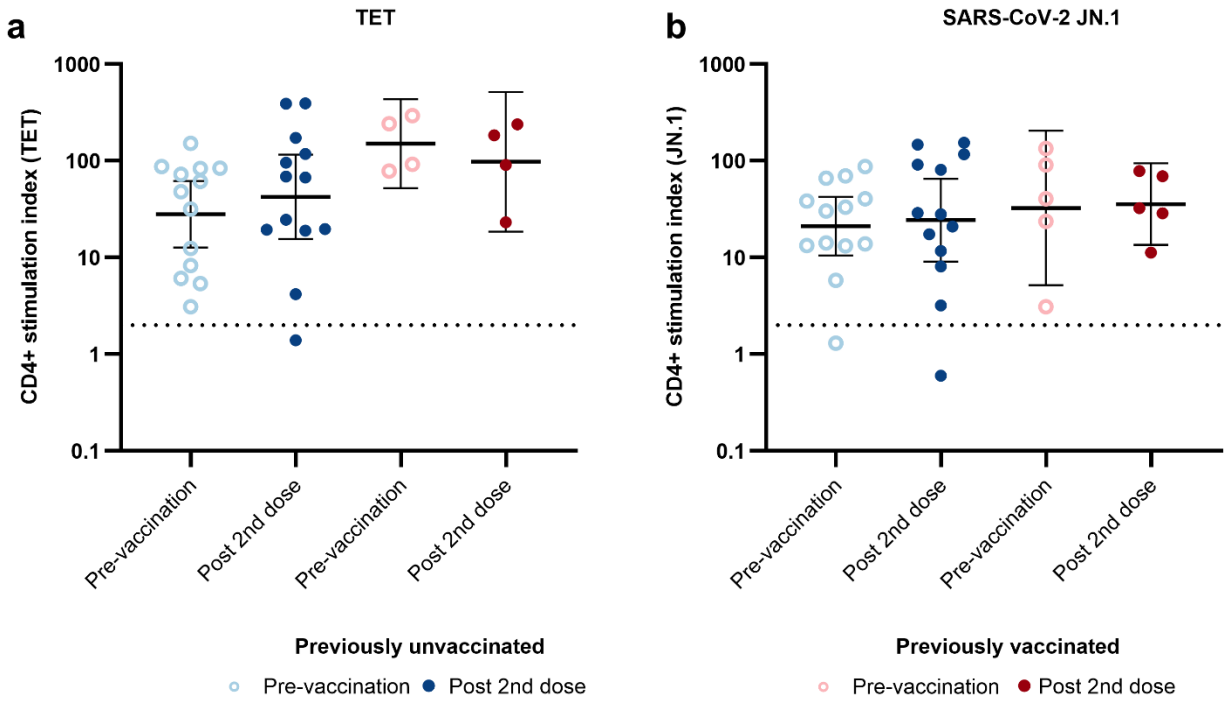
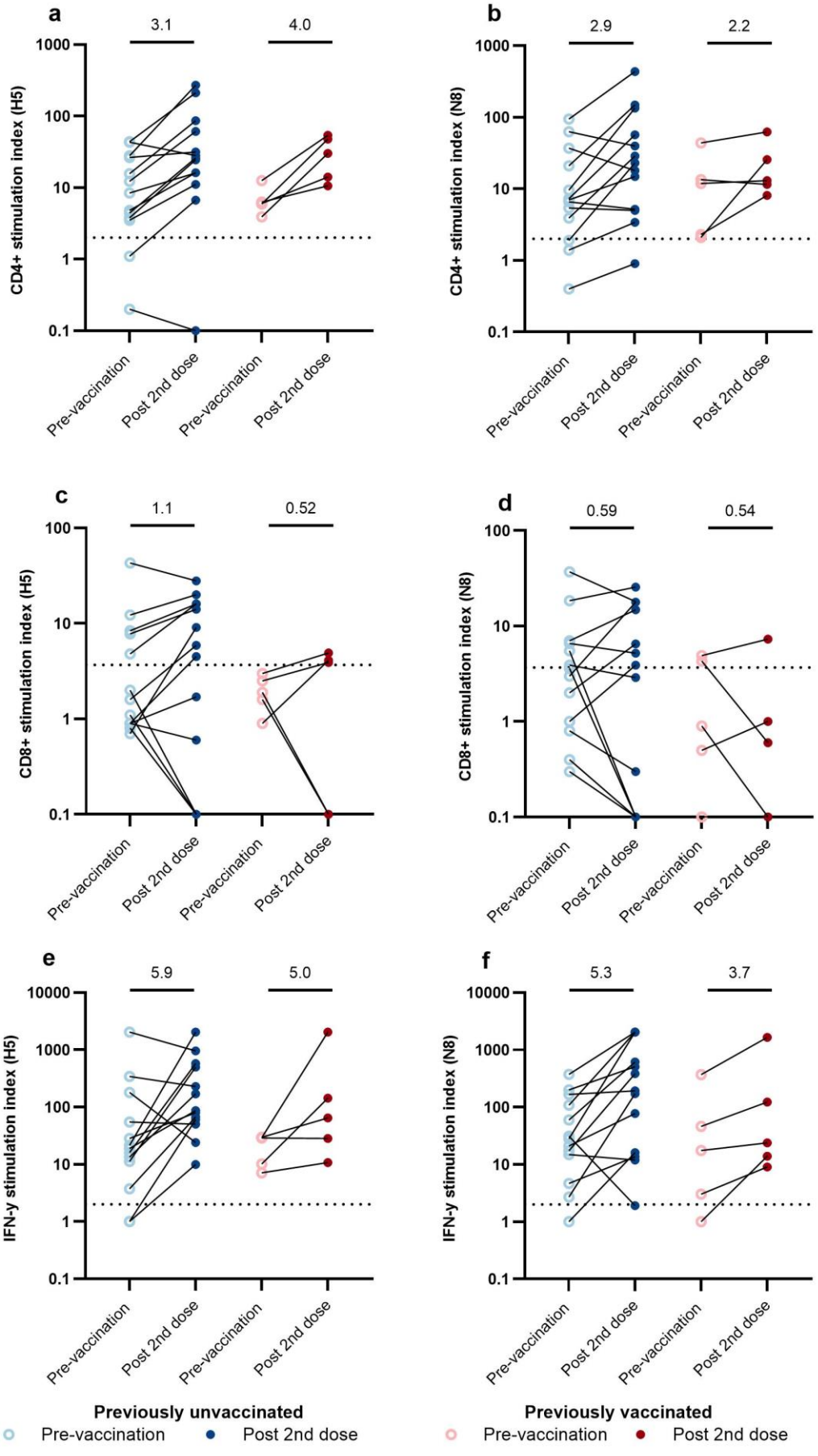
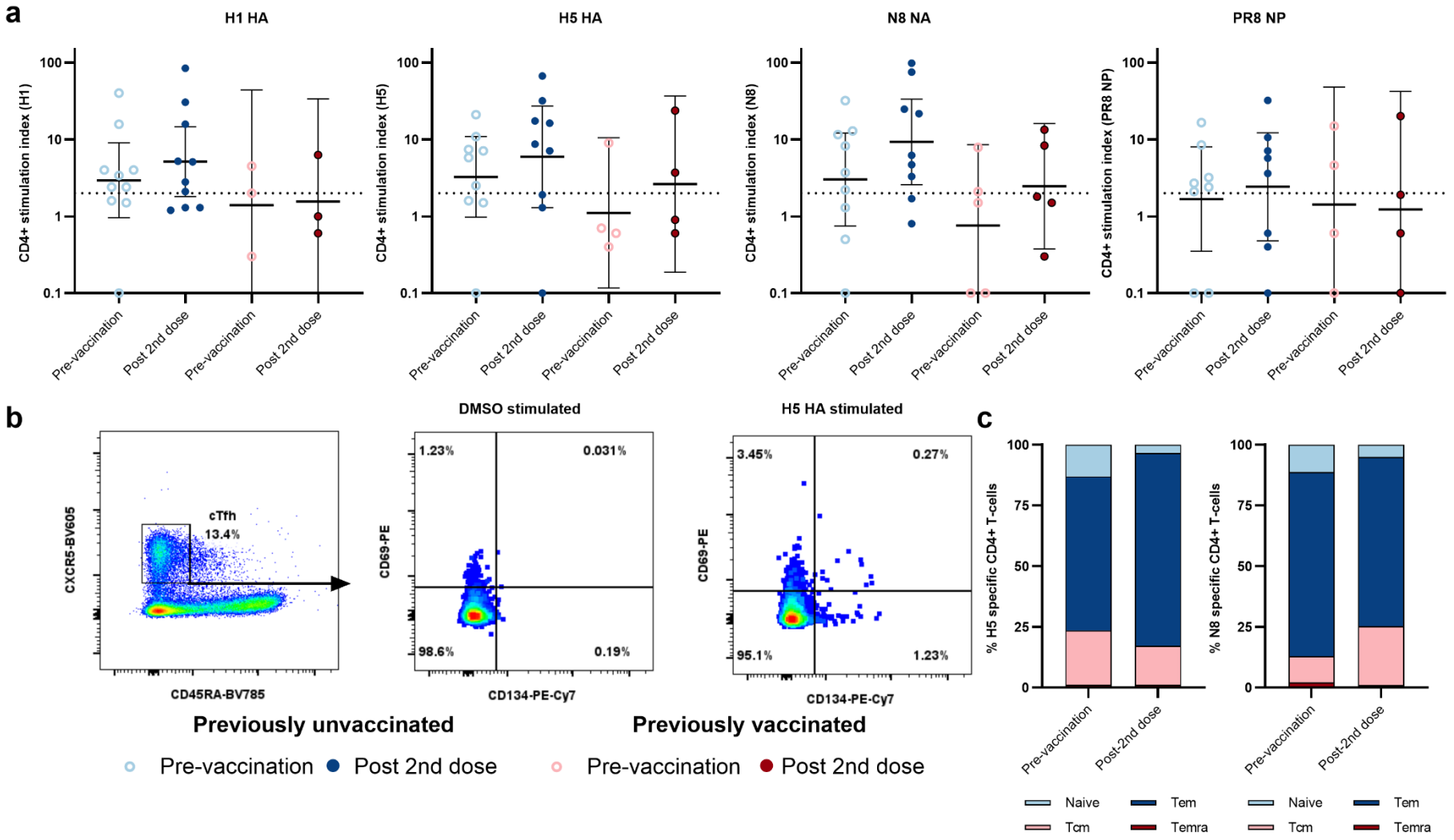


Extended Data Fig. 3 | CD4⁺ and CD8⁺T-cell responses specific to TET and SARS-CoV-2 JN.1. a, Stimulation index fold increases of CD4⁺/CD69+/CD134+ populations in relation to DMSO. b, Stimulation index fold increases of CD8⁺/CD69+/CD134+ populations in relation to DMSO. Blue dots indicate individuals with no previous avian influenza vaccinations, and red dots indicate individuals with previous avian influenza vaccinations. The graphs display geometric mean indices (lines) and 95% confidence intervals (whiskers). Dashed line indicates the cut-off threshold. Statistical significance was determined with Wilcoxon matched-pairs signed rank test. Two-tailed $p<0.05$ is considered a significant difference.


a
3.1
4.0
CD4+ stimulation index (H5)
b
2.9
2.2
CD4+ stimulation index (N8)
c
1.1
0.52
CD8+ stimulation index (H5)
d
0.59
0.54
CD8+ stimulation index (N8)
e
5.9
5.0
IFN-γ stimulation index (H5)
f
5.3
3.7
IFN-γ stimulation index (N8)
Pre-vaccination
Post 2nd dose
Previously unvaccinated
Previously vaccinated

Extended Data Fig. 4 | Kinetics of individual cell-mediated immune responses. a, Activation induced marked based $CD4^+$/CD69+/CD134+ T-cell responses specific to H5 peptide pool stimulation. b, $CD4^+$/CD69+/CD134+ T-cell responses specific to N8 peptide pool stimulation. c, $CD8^+$/CD69+/CD134+ T-cell responses specific to H5 peptide stimulation. d, $CD8^+$/CD69+/CD134+ cell responses specific to N8 peptide stimulation. Stimulation indices of secreted IFN-γ from PBMC supernatants after stimulation with e, H5 and f, N8 peptide pools. Individual responses are shown as lines for two groups, A(H5N1) unvaccinated (n=13) and previously vaccinated (n=5), at two time points: pre-vaccination, and three weeks after the second dose. The dashed line indicates the positivity threshold, which was SI 2. The mean fold changes between the samples before vaccination and after the second dose as shown as number on top of the lines.



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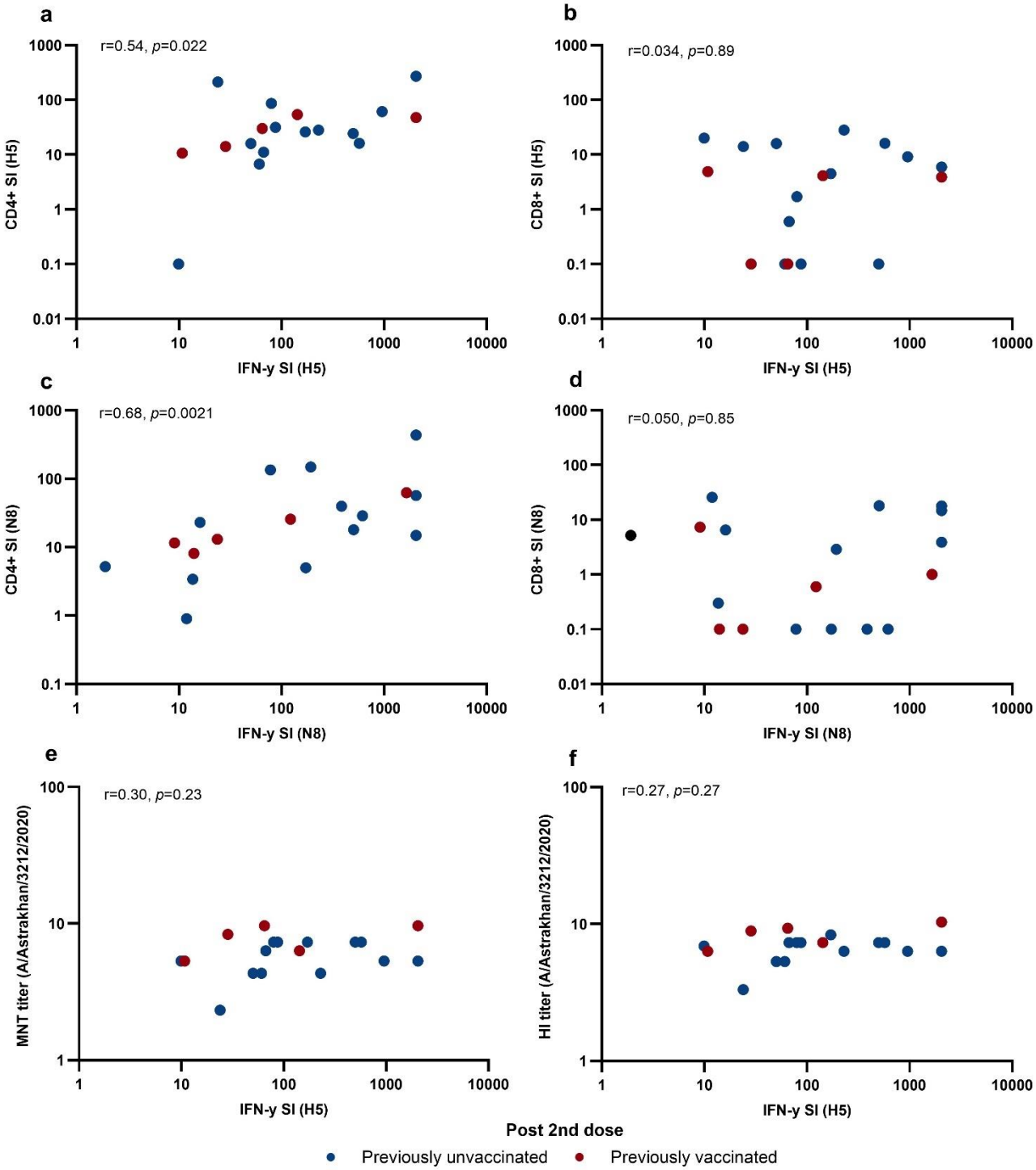
55 **Extended Data Fig. 5 | Activation induced marker analysis of cTfh responses to influenza peptide stimulation, and distribution of influenza antigen-**
56 **specific CD4⁺ T cells to naïve, Tem, Tcm, and Temra subclasses.** a, Gating strategy for identifying $CD4^+/CXCR5^+/CD45RA^-/CD69^+/CD134^+$ follicular T helper cell

(cTfh) populations. b, Stimulation indices of H1, H5, N8 and NP peptide pool stimulated $CD4^+/CXCR5^+/CD45RA^-/CD69^+/CD134^+$ T cell populations in relation to DMSO stimulated cells. c, H5-specific $CD4^+$ T cells and N8-specific $CD4^+$ T cells, displayed as percentages of average respective populations.



Extended Data Figure 6 | Correlation between IFN-γ secretion and CD4⁺/CD8⁺ T-cell responses and antibody titers. Spearman correlation was used to assess the relationship between SI of CD4⁺ and CD8⁺ T cells and IFN-γ SI in response to H5 and N8 peptide pool stimulation. a, CD4⁺ SI (H5) correlated positively with IFN-γ SI (H5), b, CD8⁺ SI (H5) did not show a significant correlation with IFN-γ SI (H5), c, CD4⁺ SI (N8) showed a strong positive correlation with IFN-γ SI (N8), d, CD8⁺ SI (N8) showed no correlation with IFN-γ SI (N8). Correlations between IFN-γ SI (H5 peptide pool stimulated) and antibody titers against A/Astrakhan/3212/202 H5N8 virus were also assessed e, microneutralization (MN) titer, and f,

hemagglutination inhibition (HI) titer, neither of which showed significant correlations. Correlation coefficients (r) and corresponding p values (*p*) are reported in the figure panels.

Extended Data Table 4 | Fluorochorome-antibody panel used in the flow cytometry

Antibody	Fluorochrome	Manufacturer	Cat#
Anti-human CD45 (HI30 clone)	APC-eFluor780	Invitrogen/Life technologies	47-0459-42
Anti-human CD3 (UCHT1 clone)	eFluor506	Invitrogen/Life technologies	69-0038-42
Anti-human CD4 (RPA-T4 clone)	eFluor450	Invitrogen/Life technologies	48-0049-42
Anti-human CD8a (SK1)	PerCP-eFluor710	Invitrogen/Life technologies	46-0087-42
Anti-human CD69 (FN50 clone)	PE	BD Biosciences	555531
Anti-human CD134 (ACT35 clone)	PE/Cyanine7	BioLegend	350012
Anti-human CD137 (4B4-1 clone)	APC	BioLegend	309810
Anti-human CD45RA (HI100 clone)	Brilliant Violet 785	BioLegend	304140
Anti-human CD197 (CCR7) (G043H7 clone)	PE/Dazzle 594	BioLegend	353236
Anti-human CD185 (CXCR5) (J252D4 clone)	Brilliant Violet 605	BioLegend	356930