T-cell and antibody responses in immunocompromised patients with hematologic malignancies indicate strong potential of SARS-CoV-2 mRNA vaccines

Authors

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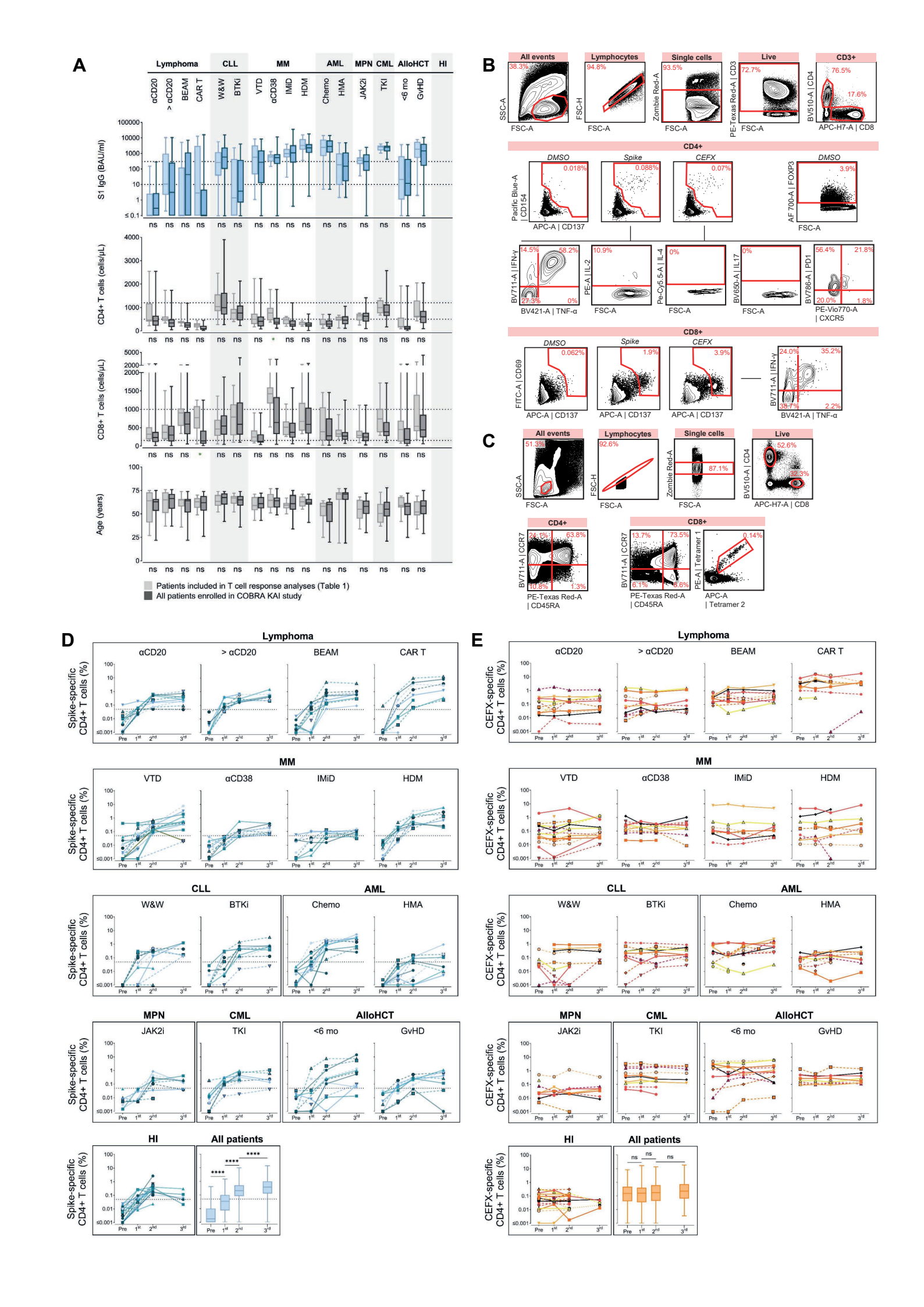
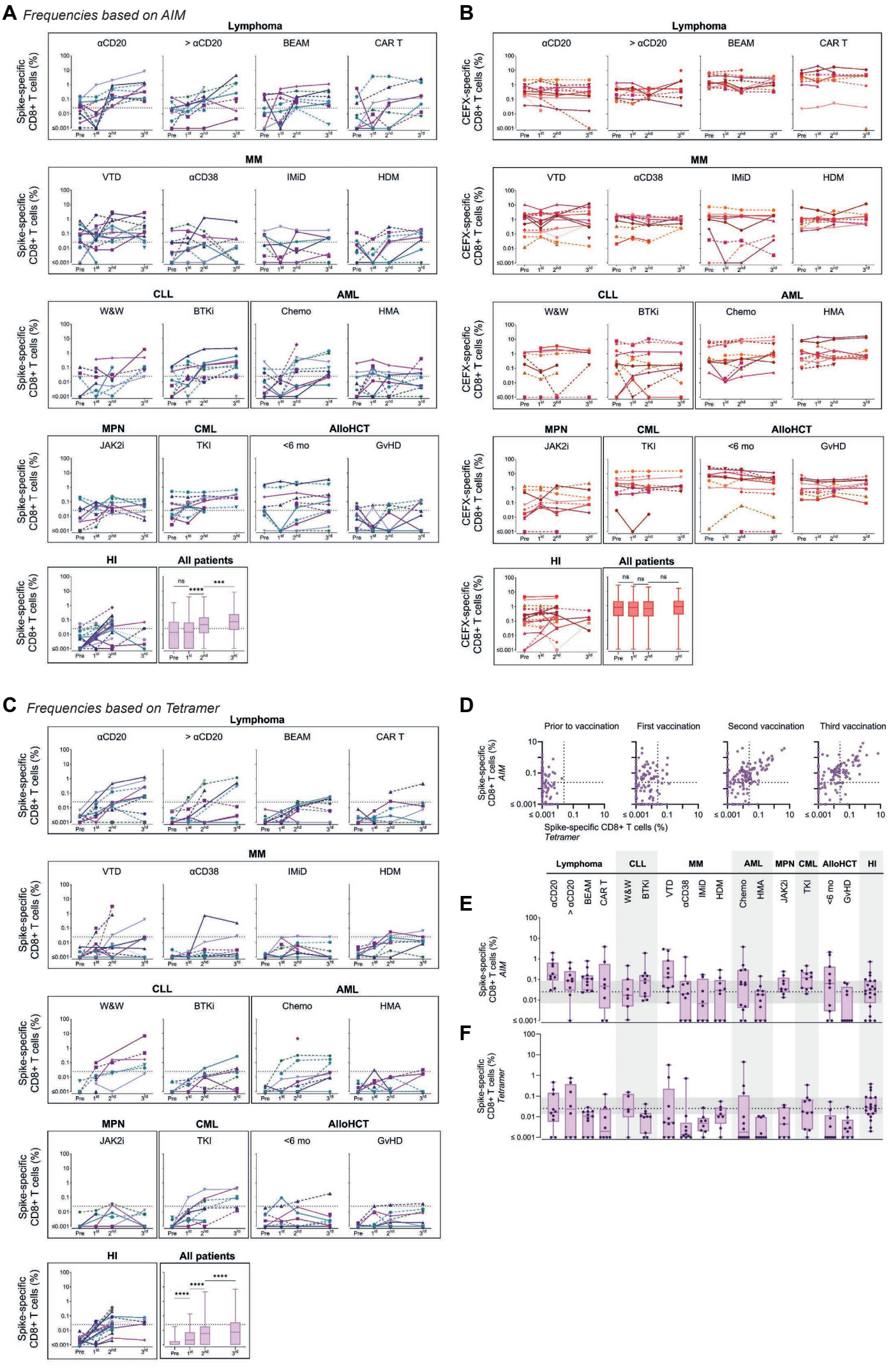

A
Lymphoma
CLL
MM
AML
MPN
CML
AlloHCT
HI
αCD20
> αCD20
BEAM
CAR T
W&W
BTKi
VTD
αCD38
IMiD
HDM
Chemo
HMA
JAK2i
TKI
<6 mo
GvHD
S1 IgG (BAU/ml)
CD4+ T cells (cells/µL)
CD8+ T cells (cells/µL)
Age (years)
Patients included in T cell response analyses (Table 1)
All patients enrolled in COBRA KAI study
B
All events
Lymphocytes
Single cells
Live
CD3+
CD4+
CD8+
DMSO
Spike
CEFX
C
D
E
Spike-specific CD4+ T cells (%)
CEFX-specific CD4+ T cells (%)
Pre
1st
2nd
3rd
HI
All patients

Supplementary figure 1

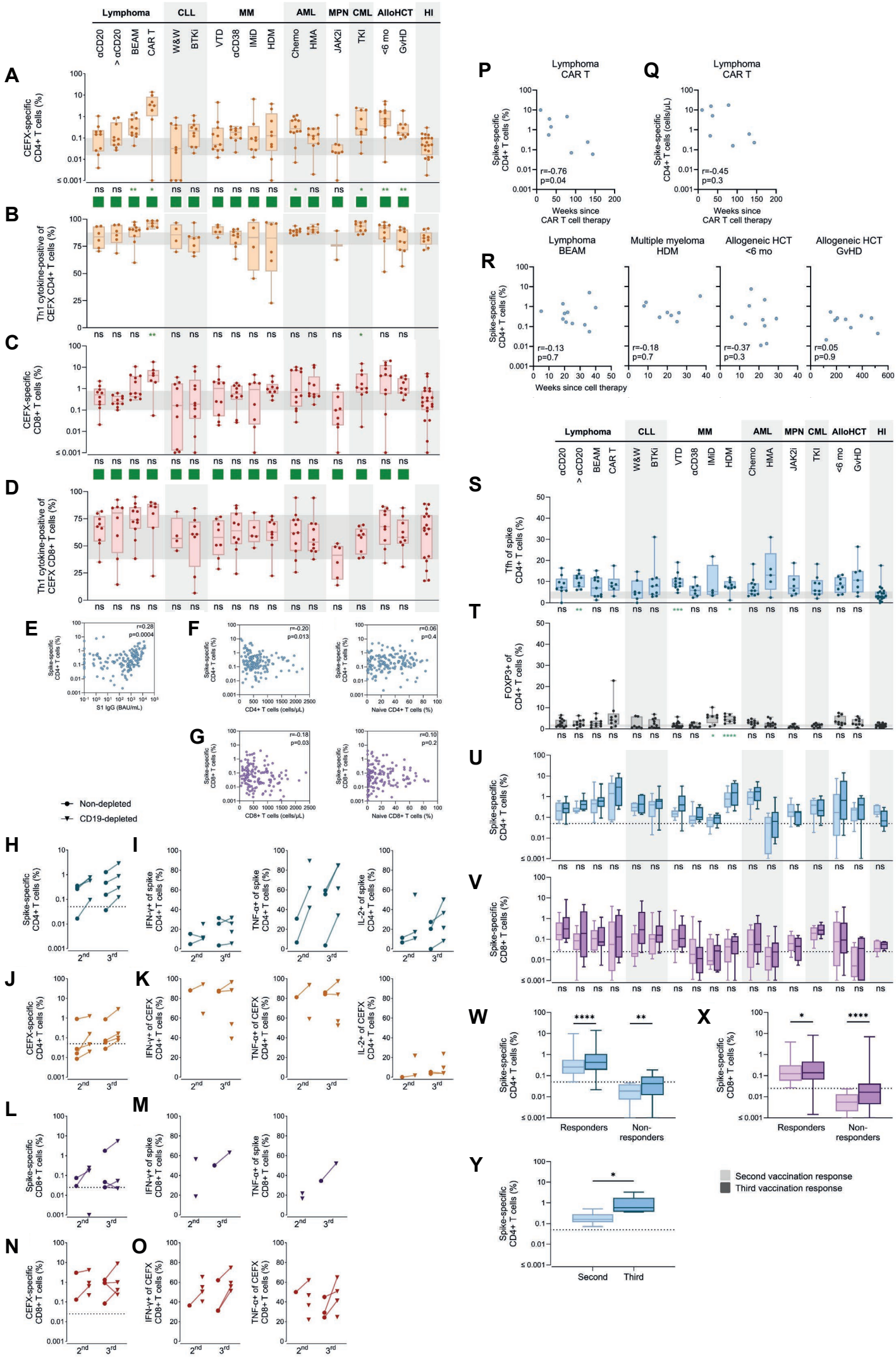
A) Clinical characteristics of patients included in T cell analyses in relation to full COBRA KAI study cohorts. S1 IgG indicates concentration following the second mRNA vaccination. T cells are depicted as numbers per microliter blood. Values are compared to those in HI by Mann-Whitney U tests and significance corrected for multiple testing (times 16) is shown. Last figure shows the age of the patients at start of vaccination. **B**) Representative example of flow cytometry gating strategy for antigen-reactive CD4+ and CD8+ T cells. Samples were measured on a three-laser Aurora (Cytek Biosciences) and analyzed using OMIQ (www.omiq.ai). All events were gated on lymphocytes, single cells, viable cells, CD3-positive, and either CD4- or CD8-positive. Only samples with more than 5,000 events in the CD4+ or CD8+ gates were analyzed. Activation was measured by upregulation of CD137 and CD154 by CD4+ T cells and CD137 and CD69 by CD8+ T cells, compared to DMSO. Response positivity thresholds were set at 0.05% for CD4+ T cells and 0.025% for CD8+ T cells based on results of an independent previous healthy cohort. Analysis of cytokine-producing spike-specific cells (IFN-γ, TNF-α, IL-2, IL-4, IL-17) and spike-specific Tfh cells (CD4+CXCR5+PD-1+) was only performed if the response positivity threshold was met and more than 25 events were measured in the CD154+/CD137+ gate for CD4+ T cells and in the CD137+CD69+ gate for CD8+ T cells. FOXP3+ cells were gated within total CD4+ T cell population in DMSO condition. **C**) Representative example of flow cytometry gating strategy for differentiation status of CD4+ and CD8+ T cells and for spike-specific CD8+ T cells using peptide-HLA tetramer technology. In addition to peptide-stimulation, 2 million unstimulated PBMCs were stained with a fixed pool of peptide-HLA tetramers to detect spike-specific CD8+ T cells. The tetramers consist of 23 peptides that were previously reported spike epitopes with strong (predicted) binding to 8 HLA-types common in The Netherlands. Tetramer staining was combined with antibodies directed against CD4, CCR7, and CD45RA followed by anti-CD8 staining. Samples were measured on a three-laser Aurora (Cytek Biosciences) and analyzed using OMIQ (www.omiq.ai). Only samples with more than 5,000 events in the CD4+ or CD8+ gates were analyzed, whilst 10,000 events were required in the CD8+ gate for the frequency of peptide-HLA tetramer-binding cells. All events were gated on lymphocytes, single cells, viable cells and CD4- or CD8-positive. Subsequently, the percentage of CD4+ or CD8+ T cells that express CCR7/CD45RA was determined. CD8+ T cells were gated on double positive tetramer binding for the detection of spike-specific CD8+ T cells. **D-E**) Kinetics of antigen-specific CD4+ T cell frequencies before and during the three-dose vaccination schedule. PBMCs isolated prior to vaccination (Pre), four weeks after the first vaccination (1^{st}), four weeks after the second vaccination (2^{nd}) and four weeks after the third vaccination (3^{rd}) were incubated with SARS-CoV-2 spike or CEFX peptide pool. Frequencies of CD4+ T cells positive for CD154 or CD137, corrected for DMSO, were plotted over

time. Dotted line indicates response positivity threshold. Each line represents one individual. Ns: $p>0.05$; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$.


A Frequencies based on AIM
B
C Frequencies based on Tetramer
D
E
F
Lymphoma
αCD20
> αCD20
BEAM
CAR T
MM
VTD
αCD38
IMiD
HDM
CLL
W&W
BTKi
AML
Chemo
HMA
MPN
JAK2i
CML
TKI
AlloHCT
<6 mo
GvHD
HI
All patients
Spike-specific CD8+ T cells (%)
CEFX-specific CD8+ T cells (%)
Pre
1st
2nd
3rd
ns
****
***
Prior to vaccination
First vaccination
Second vaccination
Third vaccination
Spike-specific CD8+ T cells (%) AIM
Spike-specific CD8+ T cells (%) Tetramer

Supplementary figure 2

A-B) Kinetics of antigen-specific CD8+ T cell frequencies before and during the three-dose vaccination schedule, determined by AIM (see legend S1B). PBMCs isolated before vaccination (Pre), four weeks after the first vaccination (1^{st}), four weeks after the second vaccination (2^{nd}) and four weeks after the third vaccination (3^{rd}) were incubated with SARS-CoV-2 spike (A, C; purple) or CEFX (B; red) peptide pool. Frequencies of CD8+ T cells positive for CD137 and CD69, corrected for DMSO, were plotted over time. Bottom right figure shows data points of all patients combined. **C**) Frequencies of spike-specific CD8+ T cells as measured by peptide-HLA tetramer technology is shown. PBMCs were, in parallel to AIM assay, incubated with peptide-HLA tetramers consisting of known prevalent epitopes. **D**) Frequency of spike-specific CD8+ T cells measured by activation induced markers (AIM) plotted against frequency of spike-specific CD8+ T cells measured by peptide-HLA tetramer technology. Spike-specific CD8+ T cell frequencies are shown before vaccination and four weeks after the first, second, and third mRNA vaccine dose. **E**) Frequency of spike-specific CD8+ T cells measured by AIM assay four weeks after the second mRNA vaccination. **F**) Frequency of spike-specific CD8+ T cells measured by peptide-HLA tetramer technology four weeks after the second mRNA vaccination. Horizontal grey area shows interquartile range of HI. Dotted line indicates response positivity threshold (0.025%). Each line or dot represents one individual. Difference in frequency after each vaccination was tested by a Wilcoxon matched-pairs Signed-Rank test (ns: $p>0.05$; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$) and correlations were tested using Spearman's correlation.


Lymphoma
CLL
MM
AML
MPN
CML
AlloHCT
HI
αCD20
> αCD20
BEAM
CAR T
W&W
BTKi
VTD
αCD38
IMiD
HDM
Chemo
HMA
JAK2i
TKI
<6 mo
GvHD
A
CEFX-specific CD4+ T cells (%)
B
Th1 cytokine-positive of CEFX CD4+ T cells (%)
C
CEFX-specific CD8+ T cells (%)
D
Th1 cytokine-positive of CEFX CD8+ T cells (%)
E
r=0.28
p=0.0004
S1 IgG (BAU/mL)
F
r=-0.20
p=0.013
CD4+ T cells (cells/µL)
r=0.06
p=0.4
Naive CD4+ T cells (%)
G
r=-0.18
p=0.03
CD8+ T cells (cells/µL)
r=0.10
p=0.2
Naive CD8+ T cells (%)
Non-depleted
CD19-depleted
H
Spike-specific CD4+ T cells (%)
I
IFN-γ+ of spike CD4+ T cells (%)
TNF-α+ of spike CD4+ T cells (%)
IL-2+ of spike CD4+ T cells (%)
J
CEFX-specific CD4+ T cells (%)
K
IFN-γ+ of CEFX CD4+ T cells (%)
TNF-α+ of CEFX CD4+ T cells (%)
IL-2+ of CEFX CD4+ T cells (%)
L
Spike-specific CD8+ T cells (%)
M
IFN-γ+ of spike CD8+ T cells (%)
TNF-α+ of spike CD8+ T cells (%)
N
CEFX-specific CD8+ T cells (%)
O
IFN-γ+ of CEFX CD8+ T cells (%)
TNF-α+ of CEFX CD8+ T cells (%)
2nd
3rd
P
Lymphoma
CAR T
Spike-specific CD4+ T cells (%)
r=-0.76
p=0.04
Weeks since CAR T cell therapy
Q
Lymphoma
CAR T
Spike-specific CD4+ T cells (cells/µL)
r=-0.45
p=0.3
Weeks since CAR T cell therapy
R
Lymphoma
BEAM
Multiple myeloma
HDM
Allogeneic HCT
<6 mo
Allogeneic HCT
GvHD
Spike-specific CD4+ T cells (%)
r=-0.13
p=0.7
r=-0.18
p=0.7
r=-0.37
p=0.3
r=0.05
p=0.9
Weeks since cell therapy
S
Tfh of spike CD4+ T cells (%)
T
FOXP3+ of CD4+ T cells (%)
U
Spike-specific CD4+ T cells (%)
V
Spike-specific CD8+ T cells (%)
W
Spike-specific CD4+ T cells (%)
Responders
Non-responders
X
Spike-specific CD8+ T cells (%)
Responders
Non-responders
Y
Spike-specific CD4+ T cells (%)
Second
Third
Second vaccination response
Third vaccination response

Supplementary figure 3

A-D) Frequency of CEFX-specific T cells and the frequency of cytokine-producing cells after two SARS-CoV-2 mRNA vaccinations. The frequency of CEFX-specific CD4+ (A; orange) or CD8+ (C; red) T cells was determined using AIM assay. In the same staining, the frequency of CEFX-specific CD4+ (B) or CD8+ (D) T cells that produce IFN-γ, TNF-α and/or IL-2 was determined using ICS. Frequency was calculated by subtracting the frequency of cells that do not produce any of these cytokines from 100%. **E-F**) Correlation between spike-specific CD4+ T cell frequencies after two vaccinations and different parameters: S1 IgG concentration after two vaccinations (E), CD4+ T cell numbers in blood at start of vaccination (F; left figure), or naive CD4+ T cell frequencies (CCR7+CD45RA+) at start of vaccination (F; right figure). **G**) Correlation between spike-specific CD8+ T cell frequencies after two vaccinations (left figure) and CD8+ T cell numbers in blood at start of vaccination or naive CD8+ T cell frequencies (CCR7+CD45RA+) at start of vaccination (right figure). **H-O**) Frequencies of spike-specific T cell frequencies, including cytokine production, in CD19-depleted (triangles) and non-depleted (circles) PBMC from patients with CLL. PBMCs from four patients with CLL with B cell counts >5000/µl were randomly selected and depleted from CD19+ cells using QuadroMACS™ (Miltenyi) before incubation with peptides and measurement. Dotted line indicates response positivity threshold. Frequency and cytokine production of spike-specific CD4+ T cells (H-I; blue), CEFX-specific CD4+ T cells (J-K; orange), spike-specific CD8+ (L-M; purple) and CEFX-specific CD8+ T cells (N-O; red) was determined. **P-Q**) Correlation between spike-specific CD4+ T cell frequency (P) or counts (R) and weeks since CAR T cell or HCT infusion. **S**) Frequency follicular helper T cells (Tfh; CXCR5+PD-1+) of spike-specific CD4+ T cells after two mRNA vaccinations **T**) frequency of FOXP3+ CD4+ T cells at start of vaccination. **U-V**) Spike-specific CD4+ (U) or CD8+ (V) T cell frequencies after two (light color) or three (dark color) SARS-CoV-2 mRNA vaccinations. **W-X**) Spike-specific CD4+ (W) or CD8+ (X) T cell frequencies after two (light color) or three (dark color) SARS-CoV-2 mRNA vaccinations from all patients, separated in responders and non-responders based on spike-specific T cell frequencies above (responders) or below (non-responders) the response positivity threshold after two vaccinations. **Y**) Comparison of spike-specific CD4+ T cell frequencies after the second and third vaccination of patients that received an autoHCT between second and third vaccination. For all figures, horizontal grey area corresponds to interquartile range in healthy individuals. Dotted lines indicate response positivity thresholds (0.05% for CD4+ T cells and 0.025% for CD8+ T cells). Each line or dot represents one individual. In panels A-B and S-T, T cell frequencies from each cohort are compared to those in HI by Mann-Whitney U tests. In panels U-Y, T cell frequencies are compared between four weeks after second and third vaccination using a paired t-test. Ns: $p>0.05$; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$. Correction for multiple testing was performed (p-value times 16) for both tests. Correlation was tested using Spearman's correlation test.