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mRNA-1273 vaccinated inflammatory bowel disease patients receiving TNF inhibitors develop broad and robust SARS-CoV-2-specific CD8$^+$ T cell responses

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ABSTRACT

SARS-CoV-2-specific CD8$^+$ T cells recognize conserved viral peptides and in the absence of cross-reactive antibodies form an important line of protection against emerging viral variants as they ameliorate disease severity. SARS-CoV-2 mRNA vaccines induce robust spike-specific antibody and T cell responses in healthy individuals, but their effectiveness in patients with chronic immune-mediated inflammatory disorders (IMIDs) is less well defined. These patients are often treated with systemic immunosuppressants, which may negatively affect vaccine-induced immunity. Indeed, TNF inhibitor (TNFi)-treated inflammatory bowel disease (IBD) patients display reduced ability to maintain SARS-CoV-2 antibody responses post-vaccination, yet the effects on CD8$^+$ T cells remain unclear.

Here, we analyzed the impact of IBD and TNFi treatment on mRNA-1273 vaccine-induced CD8$^+$ T cell responses compared to healthy controls in SARS-CoV-2 experienced and inexperienced patients. CD8$^+$ T cells were analyzed for their ability to recognize 32 SARS-CoV-2-specific epitopes, restricted by 10 common HLA class I allotypes using heterotetramer combinatorial coding. This strategy allowed in-depth *ex vivo* profiling of the vaccine-induced CD8$^+$ T cell responses using phenotypic and activation markers.

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mRNA vaccination of TNFi-treated and untreated IBD patients induced robust spike-specific CD8$^+$ T cell responses with a predominant central memory and activated phenotype, comparable to those in healthy controls. Prominent non-spike-specific CD8$^+$ T cell responses were observed in SARS-CoV-2 experienced donors prior to vaccination. Non-spike-specific CD8$^+$ T cells persisted and spike-specific CD8$^+$ T cells notably expanded after vaccination in these patient cohorts. Our data demonstrate that regardless of TNFi treatment or prior SARS-CoV-2 infection, IBD patients benefit from vaccination by inducing a robust spike-specific CD8$^+$ T cell response.

1. Introduction

Novel vaccine platforms, such as the mRNA-based mRNA-1273 (Moderna) and the BNT162b2 (Pfizer/BioNTech) vaccines, were developed to combat the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Although the vaccines induced robust humoral and cellular immunity against the parental SARS-CoV-2 strain, vaccine-induced antibodies were less effective against emerging SARS-CoV-2 variants [1–3]. In the absence of neutralizing antibodies, CD8$^+$ T cells form an important second line of protection as they remain functional against emerging variants by recognizing conserved viral peptides [4–10] and ameliorate disease severity [11–16]. Previous research has shown that vaccine and infection-induced SARS-CoV-2-specific CD8$^+$ T cells persist long-term in healthy individuals [9,17–21]. However, it is less well established whether immune-mediated inflammatory disorders and their immunosuppressive treatments affect the patient's ability to generate robust CD8$^+$ T cell responses upon vaccination.

Tumor necrosis factor inhibitors (TNFi) are frequently prescribed as a therapy to treat some chronic immune-mediated inflammatory disorders (IMIDs), like inflammatory bowel disease (IBD). Crohn's disease and ulcerative colitis are the main entities of IBD, which are chronic relapsing inflammatory disorders of the gastrointestinal tract. Previously, TNFi treatment was associated with reduced antibody responses after vaccination against viruses, such as hepatitis B, hepatitis A and influenza [22–25]. However, recent studies indicated that SARS-CoV-2 mRNA-vaccinated IBD patients treated with TNFi (IBD-TNFi) induced similar seroconversion rates compared to healthy (non-IBD) controls (HCs). Still, TNFi treatment was associated with a greater decline in the humoral response over time [26–30]. These findings underpin the importance of understanding whether IBD-TNFi patients may benefit from T cell immunity as a second line of defense. Thus far, research on SARS-CoV-2-associated cellular immune response in TNFi-treated patients is inconsistent, as some studies showed reduced cellular responses [31–34], whereas others reported similar or even augmented T cell responses compared to HC [35–39]. Most of these studies used long overlapping SARS-CoV-2 peptides to identify SARS-CoV-2-specific T cells through the production of cytokines. However, these long peptides particularly stimulate CD4$^+$ T cells and are less capable of activating CD8$^+$ T cells [40]. Hence, the effect of TNFi on vaccine-induced SARS-CoV-2-specific CD8$^+$ T cell immunity remains to be elucidated using a different approach.

CD8$^+$ T cells use their T cell receptor (TCR) to recognize viral peptides (p) presented by human leukocyte antigen class I (pHLA-I) complexes, together known as an epitope, on the surface of infected cells. Recognition results in T cell activation, targeted killing of virus-infected cells and memory CD8$^+$ T cell formation [14]. TNF acts directly on T cells as a costimulatory molecule to lower the threshold of TCR signaling and activation [41], resulting in enhanced T cell proliferation, effector functions and cytokine production [41,42]. Additionally, TNF can activate other immune cells, such as dendritic cells, which in turn can boost CD8$^+$ T cell responses [43]. Furthermore, TNF is involved in the downregulation and immune homeostasis of T cell expansion, as the absence of TNF receptors in mice resulted in enhancement of (memory) CD8$^+$ T cells during acute lymphocytic choriomeningitis virus infection [42,44]. Considering the direct and indirect effects of TNF on CD8$^+$ T cell activation, TNFi treatment may affect the generation of robust CD8$^+$ T cells upon mRNA vaccination. Here, we analyzed the impact of IBD and TNFi treatment on mRNA-1273 vaccine-induced SARS-CoV-2-specific CD8$^+$ T cell responses. Heterotetramer combinatorial coding allowed parallel detection of 32 SARS-CoV-2 epitope-specific CD8$^+$ T cell populations, restricted by 10 common HLA class I allotypes. The analyzes was further combined with phenotypic and activation markers to enable in-depth *ex vivo* profiling of vaccine-induced SARS-CoV-2-specific CD8$^+$ T cell responses. Unique to our study is the parallel establishment of the immunodominance (frequency) and phenotype of the SARS-CoV-2 epitope-specific CD8$^+$ T cells in IBD patients. This work shows that, regardless of TNFi-treatment and previous SARS-CoV-2 infection, IBD patients benefit from vaccination through the induction of a robust CD8$^+$ T cell response. Therefore, this study emphasizes the importance of selecting CD8$^+$ T cell epitopes matching prominent HLA-I molecules in the population, for next-generation vaccine designs to provide broad CD8$^+$ T cell-driven protection against current and emerging SARS-CoV-2 variants in both healthy and immunocompromised individuals.

2. Methods

2.1. Study design and sample collection

CD8$^+$ T cell analysis was performed as part of a national prospective observational multicentre cohort study (T2B!) focusing on the vaccine-induced SARS-CoV-2-specific immunity in patients with the autoimmune disease treated with immunomodulatory medications [45]. IBD patients diagnosed with ulcerative colitis or Crohn's disease by a gastroenterologist untreated or receiving TNFi treatment were included. Only TNFi-treated patients who started their Infliximab or Adalimumab treatment at least 6 weeks before first vaccination were included. Healthy individuals, who had no history of an immune-mediated disorder and did not use any form of systemic immunosuppressive therapy were recruited as a healthy control group. Baseline characteristics, including comorbidities, were collected from all study participants (Supplementary Table 1). When vaccines first became available, participants were vaccinated with the mRNA-1273 (Moderna) vaccine with a six-week interval, according to the Dutch national vaccination guidelines. The convalescent control cohort (CCC), consisting of convalescent healthy individuals who were infected during the first wave of the pandemic, was part of our previously study [9] (Supplementary Table 2).

Peripheral blood was collected before first vaccination (T0), and 7–13 days after the second vaccination (T3) (Fig. 1A). Ficoll-Paque separation of heparinized peripheral blood was used to isolate Peripheral blood mononuclear cells (PBMCs), plasma and granulocytes at T0 and T3. PBMCs were cryopreserved in liquid nitrogen. Additional serum and fingerpicks were collected ~28 days after first vaccination (T1), which were used to measure antibody responses. No plasma/serum samples were collected for 5 donors at T1 and for 2 donors at T2 (Supplementary Table 1).

This study was approved by the medical ethical committee (NL74974.018.20 and EudraCT 2021-001102-30, local METC number: 2020_194) and registered at the Dutch Trial Register (Trial ID NL8900). Written informed consent was obtained from all study participants.

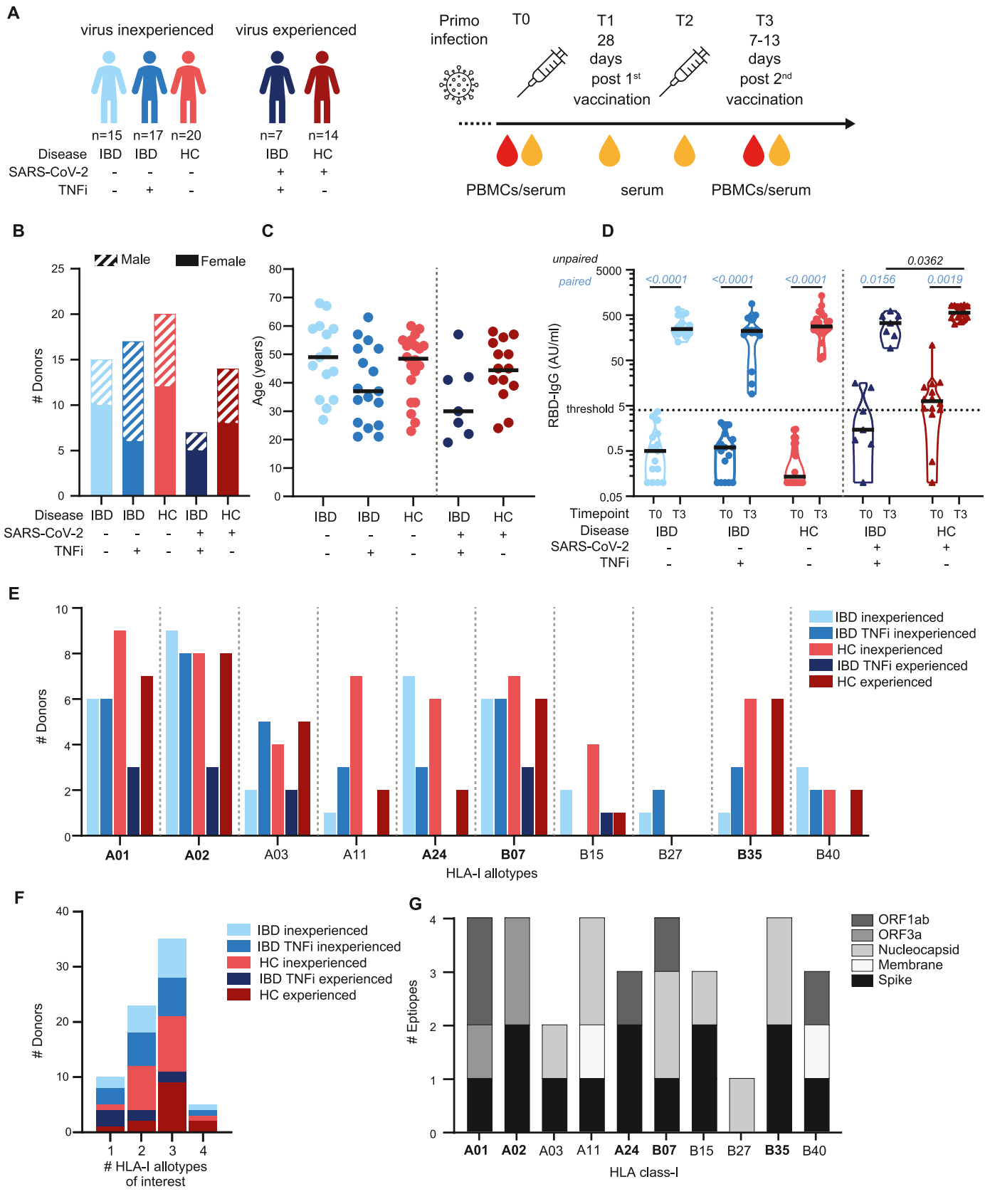

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Fig. 1. IBD and HC vaccination cohort seroconverted and contain distinct HLA-I profiles. (A) Overview IBD and HC vaccination cohort and study design. Two mRNA-1273 vaccine doses were administered to SARS-CoV-2 (in)experienced IBD patients with or without TNF inhibitor (TNFi) treatment and (in)experienced healthy controls (HCs). PBMCs and plasma (red) or serum (yellow) were collected at indicated time points. Distribution sex (B) and age (C) in donor groups. (D) Anti-SARS-CoV-2 RBD-IgG titers measured by ELISA in plasma were stratified per donor group at T0 and T3. Dots represent individual donors. Triangles represent donors with prior SARS-CoV-2 experiences determined previously by RT-PCR, N-bridge and/or RBD-bridge assays, the dotted line indicates the seroconversion threshold. (E) Number of donors with HLA-I alleles of interest per donor group, donors were included based on the expression of at least one of five prominent HLA-I allotypes in bold. (F) Number simultaneously expressed HLA-I allotype of interest across our cohort. (H) Distribution of 32 SARS-CoV-2 epitopes per HLA-I allotype. Statistical analysis in (C) Dunn's multiple comparisons test for inexperienced donors and Mann-Whitney *U* test for experienced donor groups, in (D) Wilcoxon signed-rank test for paired analysis of T0 and T3 (blue) and the Dunn's multiple comparisons test for unpaired analysis between inexperienced donor at T3 (black) and the Mann-Whitney *U* test between experienced donor at T0 or T3 (black). Significant *p* values are provided above the graph.

2.2. HLA typing

Genomic DNA, extracted from the granulocytes using the QIAamp DNA mini kit (Qiagen, Hilden, Germany), was used for HLA class I genotyping through the Department of Immunogenetics Sanquin Diagnostiek B.V. or the Axiom genotyping platform according to the Axiom Propel XPRES 384HT Workflow (Applied Biosystems).

For Axiom genotyping the quality control metrics for each sample, plate and probe set were calculated and genotypes were called using the Axiom Analysis Suite v5.1.1.1 (AxAS) software (Applied Biosystems). The genotype data were converted to a Variant Call Format using AxAS and the second-field resolution HLA class I and class II types were imputed from the genotype data using the Axiom HLA Analysis v1.2.0.38 software (Applied Biosystems), which utilizes the HLA*IMP:02 HLA type imputation model and a multi-population reference panel.

2.3. Serology

Prior SARS-CoV-2 infection was established by RT-PCR of nose and throat swaps and/or by measuring the total Ig levels of receptor binding domain (RBD) at T0 and nucleocapsid (N) at T2 (to catch infections between first and second vaccination) with a sensitive semiquantitative bridging ELISA (RBD or N-bridge assay) as described previously [46] (Supplementary Table 1). Optical density (OD) was normalized for a reference serum pool on every plate. Donors were considered SARS-CoV-2 experienced when RBD normalized OD was $\geq$0.1 and/or N normalized OD $\geq$ 0.14 (Supplementary Table 1).

An additional quantitative anti-RBD IgG ELISA was performed for T0, T1, T2 and T3, as described previously [46–48]. The signals were quantified using a serially diluted calibrator consisting of a reference plasma pool of previously confirmed convalescent coronavirus disease 2019 (COVID-19) patients included on each plate. This calibrator was arbitrarily assigned a value of 100 AU mL-1, seroconversion threshold of IgG was set at 4 AU/mL as determined by using pre-outbreak samples [46,47].

2.4. SARS-CoV-2 epitopes

A set of 32 prominent SARS-CoV-2-specific $CD8^+$ T cell epitopes were selected for heterotetramer combinatorial coding (HTCC; see 2.6) based on previous findings in infected individuals [49–54]. (Supplementary Table 3). Additionally, 2 Cytomegalovirus (CMV), 3 Epstein Barr virus (EBV) and 2 influenza virus (Flu) derived control epitopes were included (Supplementary Table 4). Binding of the 8- to 10-amino acids long peptides (JPT, Berlin, Germany) to their respective HLA-I allotype was confirmed as described previously [9].

Results were compared to those previously published for SARS-CoV-2 convalescent donors (CCC) [9] for overlapping epitopes, except for B15/S_{919}, B15/S_{634}, B35/S_{687} and A24/S_{448} as these were not included in the convalescent control cohort (Supplementary Table 3).

2.5. Generation of combinatorial encoded pHLA-I tetramers

HLA-I complexes with UV-cleavable peptides were generated in-house by the Sanquin Reagents, as described previously [55]. In short recombinant A01, A02, A03, A11, A24, B07, B15, B27, B35 and B40 heavy chains and the B_2M light chain were produced in *Escherichia coli*. pHLA-I complexes were formed by combining heavy chain, light chain and UV-cleavable peptides [56], which were subsequently purified by gel-filtration High-Performance Liquid Chromatography (HPLC). Biotinylated UV-sensitive pHLA-I complexes were stored at −80 °C until use. UV-sensitive pHLA-I complexes were subjected to 366 nm UV light resulting in a UV-mediated exchanges to generate pHLA-I complexes [55]. Tetramers were generated by conjugating eight different fluorescent streptavidin-conjugates (APC, PE, PE-Cy7 (Thermo Fisher), BV421, BV605, BV711, BUV737 (BD bioscience, Vianen, The Netherlands), and BV785 (Biolegend, Amsterdam, The Netherlands)) to the SARS-CoV-2 peptide loaded HLA-I complexes [57]. The UV-exchange and combinatorial coding techniques are patent-protected in Europe, the US and other countries WO 2010/060439 and WO 2006/080837. Simultaneous staining of A03/N_{361} and A11/N_{361} epitopes was omitted due to overlapping peptide sequences, therefore HC355 was only stained with A11/N_{361} even though the donor also encoded A03. Altogether $CD8^+$ T cell analysis was performed for 32 prominent SARS-CoV-2 derived epitopes, with up to 15 SARS-CoV-2-associated epitopes per individual.

2.6. Heterotetramer combinatorial coding (HTCC) flow cytometry

PBMCs were thawed in RPMI 1640 (Life Technologies, cat. 21875034) supplemented with 10% FCS (Bondinco, Alkmaar, The Netherlands), 1% L-glutamine (Sigma), 1% penicillin-streptomycin (Sigma, Zwijndrecht, The Netherlands) and 1:1000 DNase (Worthington Biochemical Corporation, Lakewood, USA, cat. LS002140, 10 mg/mL). Cells were washed in MACS buffer (0.5% BSA and 2 mM EDTA in PBS), after which 4–10 million cells per donor were resuspended in FACS-buffer (0.5% BSA (Sigma, cat. A7030) 0.1% NaN3 in PBS, 0.2 μm filtered (Whatman, Medemblik, The Netherlands). Donors with overlapping HLA-I allotypes were grouped. HLA-I tetramer pools were generated for each group, in the presence of Brilliant Staining Buffer Plus (BD bioscience, cat. 566385). Cells were incubated with the HLA-I tetramer pools for 30 min on ice, after which cells were stained with anti-human CD8 FITC (clone SK1, BD bioscience, cat.345772), anti-human CD3 AF700 (clone UCHT1, BD bioscience, cat. 557943), anti-human CD45RA BUV395 (clone HI1000, BD bioscience, cat. 740298), anti-human CD27 BV510 (clone O323, BD bioscience, cat.751672), anti-human CD95 PE-CF595 (clone DX2, BD bioscience cat. 562395), anti-human PD1 BB700 (clone EH12.1, BD bioscience cat. 566460), anti-human HLA-DR BUV496 (clone L203, BD bioscience cat. 752493), anti-human CD38 BUV805 (clone HB7, BD bioscience cat. 742074 and Near-IR-Dye (Invitrogen, Carlsbad, USA, cat. L10119) for 30 min on ice. Following staining cells were washed twice and fixated with IntraStain (Agilent Dako, Santa Clara, USA, cat. K231111-2) following the manufacturer's instructions. Next, cells were resuspended in FACS buffer for acquisition on the BD FACSymphonyTM A5 with FACSDiva software (BD Biosciences) and analyzed using FlowJo (V10.8.1) (Treestar, Ashland, USA). The detection threshold was set at $\geq$3 double tetramer-positive cells, visually confirmed in the correct location. A threshold of $\geq$9 $tetramer^+CD8^+$ T cells was used for phenotypic characterization, as per our previous studies [9].

2.7. Statistics

SARS-CoV-2 experienced and inexperienced donors were analyzed separately.

Statistics of the assumed nonparametric serology dataset was analyzed in GraphPad Prism (v9.1.1). Paired serology data were tested with the Wilcoxon signed-rank test for unpaired analysis the Dunn's multiple comparisons test was performed on inexperienced donors and the Mann-Whitney *U* test on experienced donors.

Statistics of the assumed nonparametric $CD8^+$ T cell panel was analyzed in R (v4.2.2). In R statistical significance was assessed using paired and unpaired Wilcoxon signed-rank test (wilcox_test function). Multiple comparison analysis was corrected using the Bonferroni-Holm method [58]. Lastly, Spearman's rank-order correlation was computed to assess the relationship between multiple parameters. P-values lower than 0.05 were considered statistically significant.

3. Results

3.1. IBD-TNFi patients display delayed but robust antibody responses upon two SARS-CoV-2 vaccinations

The effect of IBD and TNFi on mRNA vaccine-induced SARS-CoV-2-specific immunity was studied in SARS-CoV-2 inexperienced untreated IBD patients (n = 15), TNFi-treated IBD patients (n = 17) and HCs (n = 20) in addition to SARS-CoV-2 experienced TNFi-treated IBD patients (n = 7) and HCs (n = 14) (Fig. 1A; Supplementary Table 1). SARS-CoV-2 experienced IBD patients without TNFi treatment could not be included as these were underrepresented in the cohort. Blood samples were collected prior to first SARS-CoV-2 mRNA vaccination (T0) and 7–13 days post-second vaccination (T3, median 7 days). Additional serum samples were collected on day 28 post-first vaccination (T1) and prior to second vaccination (T2) (Fig. 1A; Supplementary Table 1).

Female participants were more prevalent in inexperienced IBD (67% female), HCs (60%), experienced IBD-TNFi (71%) and HCs (57%), but not in inexperienced IBD-TNFi (35%) (Fig. 1B). Although there were no significant age differences between the groups, a trend for younger participants was observed for IBD-TNFi treated patients (inexperienced untreated IBD (median 49 years), IBD-TNFi (37 years), HCs (48.5 years), experienced IBD-TNFi (30 years) and HCs (45 years)) (Fig. 1C). The IBD cohort consisted of patients diagnosed with ulcerative colitis and Crohn's disease (Supplementary Fig. 1A) Inexperienced IBD-TNFi patients predominantly received Adalimumab (n = 15) compared to Infliximab (n = 2), whereas experienced IBD-TNFi patients were primarily treated with Infliximab (n = 5) compared to Adalimumab (n = 2) (Supplementary Fig. 1B).

A significant increase in RBD-IgG titer was observed upon second vaccination in all donors (T3). Inexperienced donors displayed comparable RBD-IgG titers following second vaccination, whereas experienced HCs had significantly higher RBD-IgG titers compared to experienced IBD-TNFi patients (Fig. 1D). Significantly lower titers were also observed in inexperienced IBD-TNFi patients compared to the other inexperienced groups, between first and second vaccination (T1 and T2), suggesting that TNFi treatment may delay SARS-CoV-2-specific antibody generation (Supplementary Fig. 1C). Additionally, SARS-CoV-2-specific antibodies were detected in 78% of the experienced HCs, but only in 28% of the experienced IBD-TNFi patients before vaccination (Fig. 1D). This may reflect a more rapid decrease of infection-induced antibodies in IBD-TNFi patients compared to healthy control, although time since infection could not be established for all SARS-CoV-2 experienced donors.

3.2. IBD patients express HLAs previously associated with robust SARS-CoV-2-specific $CD8^+$ T cell immunity in healthy individuals

An in-depth SARS-CoV-2-specific $CD8^+$ T cell analysis requires knowledge of the HLA profile of individuals. Studies in SARS-CoV-2 infected patients identified SARS-CoV-2-derived epitopes, restricted to 10 prominent HLAs, compatible with our assay for analysis, namely HLA-A*01:01 (A01), HLA-A*02:01 (A02), HLA-A*03:01 (A03), HLA-A*11:01 (A11), HLA-A*24:02 (A24), HLA-B*07:02 (B07), HLA-B*15:01 (B15), HLA-B*27:05 (B27), HLA-B*35:01 (B35) and/or HLA-B*40:01 (B40) [9,19,49–52,54,59–61]. Five of these, namely A01, A02, A24, B07 and B35, are particularly prevalent in the Dutch population and/or were associated with dominant SARS-CoV-2-specific $CD8^+$ T cell immunity [9]. Therefore, we aimed to select at least 6 donors expressing at least one of the five high-prevalent HLAs and preferably multiple of the 10 HLA allotypes of interest (Fig. 1E and F; Supplementary Fig. 1D). Due to the small number of SARS-CoV-2 experienced IBD patients, all donors with at least 1 of the 10 HLA-I allotypes of interest were included. Due to the relatively lower prevalence of B35 in the Dutch population this HLA allotype was underrepresented in inexperienced IBD patients and A24 was underrepresented in inexperienced IBD-TNFi patients (Fig. 1E). Overall, 86% of our donors co-expressed multiple HLAs of interest, namely 2 HLA-I (n = 23), 3 (n = 35) or 4 (n = 5) (Fig. 1F). The HLA-I profiles of our donors provide a unique opportunity to establish the SARS-CoV-2-specific $CD8^+$ T cell responses across multiple epitopes in closely HLA-matched patient groups.

3.3. SARS-CoV-2 epitope selection

To study SARS-CoV-2-specific $CD8^+$ T cells, we selected 32 previously identified SARS-CoV-2 derived epitopes [9,49–54,59–66], restricted by the 10 HLA class I (HLA-I) allotypes in our study (Supplementary Table 3). Up to four SARS-CoV-2 epitopes were selected per HLA-I allotype including at least one spike-derived epitope to provide optimal coverage of vaccine-induced responses. However, no spike-derived epitopes were selected for B27, as to the best of our knowledge, no B27 restricted spike-derived epitopes were identified at the start of this study (Fig. 1G). Non-spike SARS-CoV-2 epitopes were selected to study infection-induced responses and potential changes in immunodominance of SARS-CoV-2-specific $CD8^+$ T cell responses upon vaccination in experienced donors. Non-SARS-CoV-2-specific epitopes derived from Cytomegalovirus (CMV), Epstein Barr virus (EBV) and influenza (Flu) were included as controls (Supplementary Fig. 1E; Supplementary Table 4). Overall, a similar number of SARS-CoV-2-derived spike and non-spike $CD8^+$ T cell epitopes were tested across SARS-CoV-2 inexperienced groups, whereas significantly more spike and non-spike-derived epitopes were tested across SARS-CoV-2 experienced HCs compared to experienced IBD-TNFi patients (Supplementary Figs. 1F and G). However, the frequency of tested spike and non-spike-derived epitopes was comparable between all groups (Supplementary Fig. 1H).

3.4. Robust SARS-CoV-2-specific $CD8^+$ T cell frequencies in vaccinated IBD-TNFi patients

Heterotetramer Combinatorial Coding (HTCC) of pHLA-I complexes allowed us to establish the frequency of 15 unique SARS-CoV-2-specific $CD8^+$ T cell populations simultaneously in a single donor directly *ex vivo*. Epitope-specific $CD8^+$ T cells were identified as double-positive for both fluorophores of a specific pHLA complex (Fig. 2A–C; Supplementary Fig. 2).

Combined frequency analysis showed a significant increase of spike-specific $CD8^+$ T cells (Fig. 2C and D) following second vaccination (T3) in all groups. The spike-specific $CD8^+$ T cell response of IBD patients was robust and similar in magnitude to HCs, independent of TNFi treatment and/or previous SARS-CoV-2 infection. Overall, the pre-vaccination (T0) spike and non-spike-specific $CD8^+$ T cell populations in experienced IBD-TNFi patients were similar to those detected in the previously studied convalescent control cohort (CCC) [9]. However, significantly lower spike-specific $CD8^+$ T cell populations were detected in

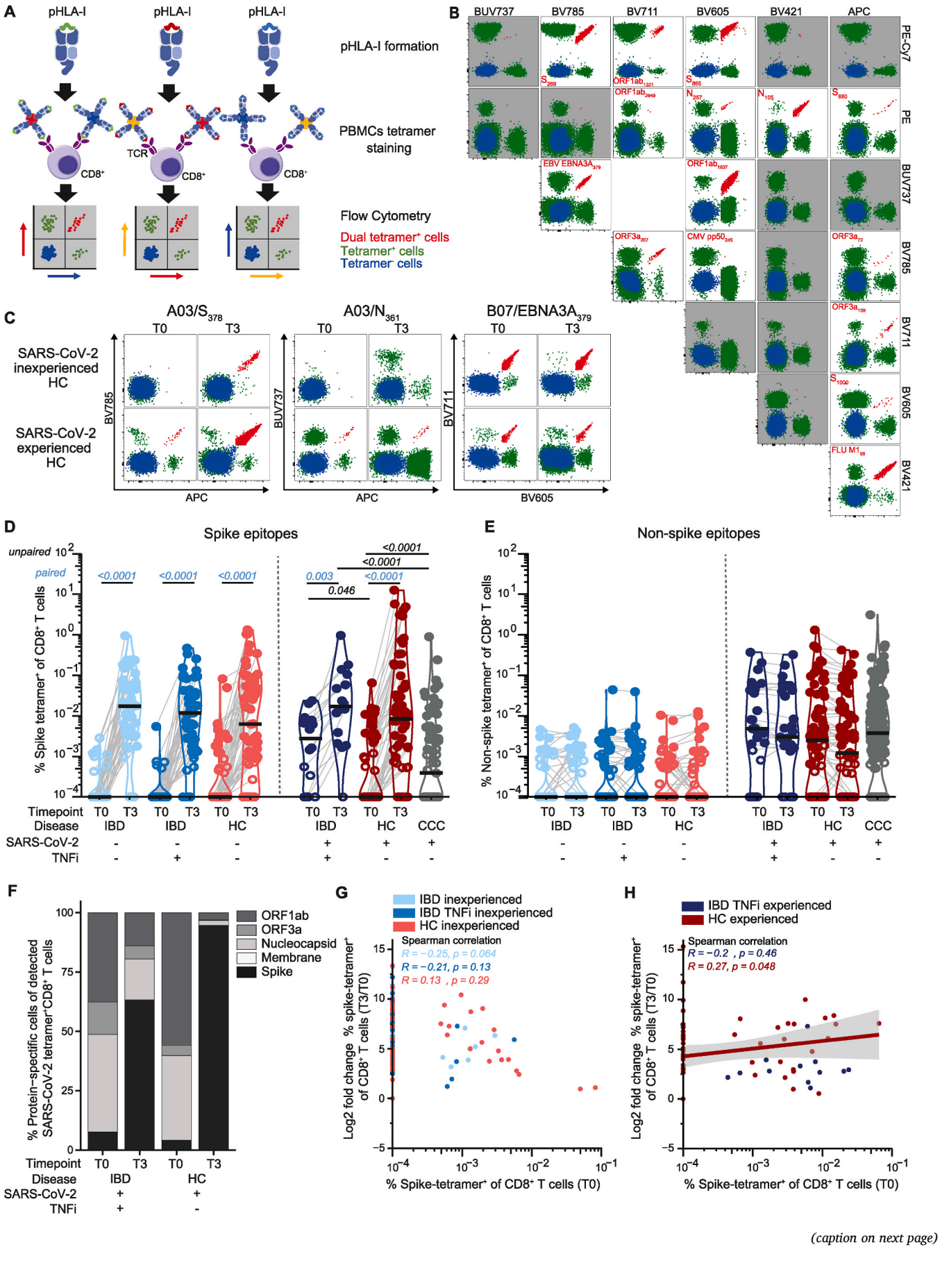

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Fig. 2. Robust induction of spike-specific $CD8^+$ T cells after vaccination of IBD patients independent of treatment or previous infection. (A) Overview of the Heterotetramer Combinatorial Coding (HTCC) approach, adjusted from van den Dijssel et al. [9]. Peptide-loaded HLA-I complexes were conjugated to two different fluorophores to generate the dual-coded tetramers for each pHLA-I combination. PBMCs were stained with these combinatorial encoded tetramers and analyzed using flow cytometry. (B) Representative flow cytometry plots (donor HC483) created by Boolean gating $CD8^+$ T cell populations expressing tetramer fluorophores (see also Supplementary Fig. 2). White plots are epitopes that match the donors' HLA-I profile, grey plots do not. (C) Representative FACS plots of spike ($A03/S_{378}$), non-spike ($A03/N_{361}$) and control ($B07/EBNA3A_{379}$) dual-coding tetramer-positive cells populations on in SARS-CoV-2 inexperienced (donor HC383) and experienced (donor HC498) donors, before (T0) and after (T3) vaccination. Frequency of $tetramer^+CD8^+$ T cells for SARS-CoV-2 spike (D) or non-spike (E) epitopes, each dot represents an epitope-specific population of an individual donor. $Tetramer^+CD8^+$ T cell populations consisting of 3–8 detected cells are indicated by open symbols and were excluded from phenotypic analysis. (D,E) Undetectable frequencies are included on the graph at 0.0001% to show the number of donors tested; these were set at 0% for statistical analysis. (F) Distribution of SARS-CoV-2 viral protein derived-specific $CD8^+$ T cell frequencies in the total SARS-CoV-2-specific $CD8^+$ T cell population. Spearman's rank-order correlation of the frequency of SARS-CoV-2 spike-specific $CD8^+$ T cells at T0 and the Log 2 fold change of SARS-CoV-2 spike-specific $CD8^+$ T cell frequencies between T0 and T3 in SARS-CoV-2 inexperienced (G) and experienced (H) donors. Lines indicate significant correlations ($p \leq 0.05$), the 95% confidence interval is shown in grey. (G,H) Data were right-shifted by 0.0001 to include fold-changes for epitopes that had undetectable precursor frequencies at T0. Statistical analyses for D and E include the paired Wilcoxon signed-rank test (blue) between T0 and T3 within each group. The unpaired Wilcoxon signed-rank test with Bonferroni-Holm's multiple comparison correction (black) was performed for inexperienced and experienced donors, to compare T0 or T3 between donor groups, the convalescent control cohort donor group (CCC) was included in T0 and T3 analysis of experienced donors. Significant *p* values are provided above the graph.

experienced HCs compared to experienced IBD-TNFi prior to vaccination (Fig. 2D and E).

Non-spike (Fig. 2C–E) and non-SARS-CoV-2-specific viral epitope responses remained stable between pre- (T0) and post- (T3) vaccination (Fig. 2C; Supplementary Fig. 3A). Non-spike tetramer-positive populations were prominent in SARS-CoV-2 experienced IBD-TNFi and HCs prior to vaccination (T0; Fig. 2F) but shifted to a spike-dominated SARS-CoV-2-specific immune response post-vaccination (T3) (Fig. 2F; Supplementary Fig. 3B), which was expected since the vaccine only contained the spike protein and was in accordance with previous studies [9,49–53,60,61,66–68]. Noteworthy, spike and non-spike tetramer--positive precursor $CD8^+$ T cell populations, were detected in SARS-CoV-2 inexperienced donors before antigen exposure, albeit at low frequencies (Fig. 2D and E), in line with previous findings [19,59,60,69,70]. A modest positive correlation ($R = 0.27$) between spike-specific $CD8^+$ T cell frequencies at T0 and their Log2fold change from T0 to T3 could be detected in SARS-CoV-2 experienced HCs, but no correlations were found in other groups suggesting that the magnitude of SARS-CoV-2 $CD8^+$ T cell responses is not impacted by precursor frequencies.

Overall, these results demonstrate that the mRNA-1273 vaccine induces robust SARS-CoV-2 spike-specific $CD8^+$ T cell responses in IBD patients with or without TNFi treatment.

3.5. SARS-CoV-2 vaccination induces a broad spike-specific $CD8^+$ T cell response in IBD patients independent of TNFi treatment

The magnitude of the individual SARS-CoV-2 epitope-specific $CD8^+$ T cell populations was compared to assess potential HLA-I-dependent differences between donor groups. Robust increases of spike-specific $CD8^+$ T cell frequencies were observed for studied spike epitopes across all donor groups, except for $A11/S_{529}$, $B15/S_{634}$, $B35/S_{229}$ and $B35/S_{687}$ (Fig. 3A) which were also detected at low frequencies in convalescent donors from our previous study [9]. The post-vaccination frequencies of individual spike-derived epitope-specific $CD8^+$ T cells were mostly similar between inexperienced IBD, IBD-TNFi and HCs and experienced IBD-TNFi and HCs (Fig. 3A), except $A02/S_{1000}$-specific $CD8^+$ T cells which were significantly higher in experienced IBD-TNFi patients compared to HCs (Fig. 3A). $A03/S_{378}$-specific $CD8^+$ T cell populations were detected at lower frequencies in the inexperienced IBD-TNFi patients (0.0239%) compared to IBD (0.5946%) and HCs (0.2404%), albeit not significant (Fig. 3A).

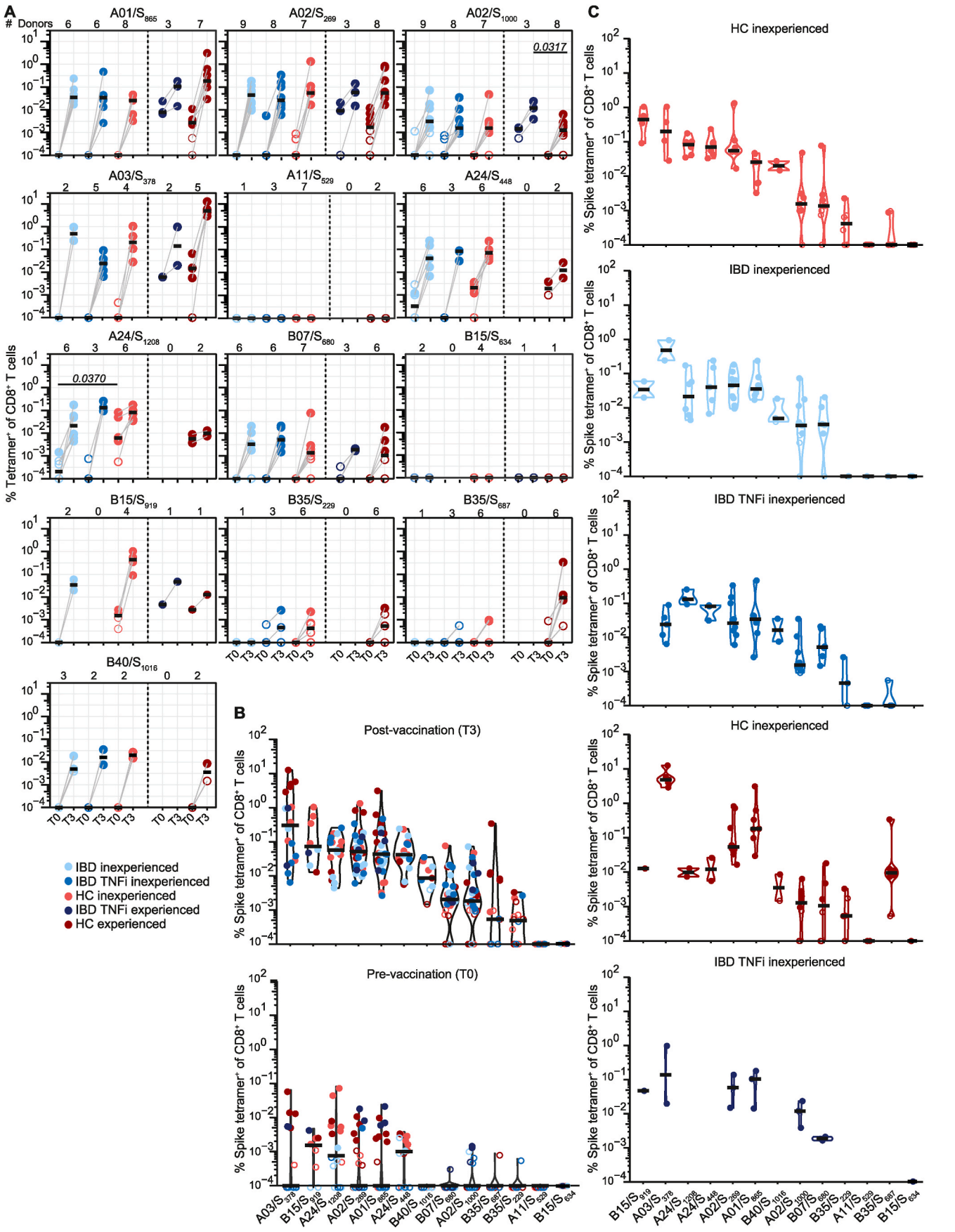
The immunodominance hierarchy of the spike epitope-specific $CD8^+$ T cell frequencies was determined by ordering them based on their median frequency (Fig. 3B). Robust vaccine-induced spike-specific $CD8^+$ T cell frequencies were detected for 7 epitopes in all donors expressing the associated HLA-I allotypes, with the highest response detected for $A03/S_{378}$ ($A03/S_{378}$ 0.3085% > $B15/S_{919}$ 0.0746% > $A24/S_{1208}$ 0.0569% > $A02/S_{269}$ 0.0512% > $A01/S_{865}$ 0.0432% > $A24/S_{448}$ 0.0157% > $B40/S_{1016}$ 0.0085%). Lower $CD8^+$ T cell frequencies were detected for 4 epitopes identified in a portion of the associated HLA-I expressing donors ($B07/S_{680}$ 0.0020%, 24/28 donors > $A02/S_{1000}$ 0.0018%, 30/35 > $B35/S_{687}$ 0.0005%, 9/16 > $B35/S_{229}$ 0.0004%, 10/16). No responses were detected for $A11/S_{529}$ and $B15/S_{634}$ (Fig. 3B). Six out of 20 non-spike-specific $CD8^+$ T cell populations were detected in all HLA-matched SARS-CoV-2 experienced donors at T0 and T3, with the most prominent responses detected against $A01/ORF1ab_{1637}$ (T0 0,1426%; T3 0.0965%), $B07/N_{105}$ (T0 0.0717%; T3 0.0568%) and $B35/N_{325}$ (T0 0.0674%; T3 0.0595%) (Supplementary Fig. 4A). These results corresponded to our previous observation in convalescent donors where $A01/ORF1ab_{1637}$ and $B07/N_{105}$ also had the largest frequencies [9].

To understand whether the vaccine-induced immunodominance patterns were affected by IBD or TNFi treatment, we ordered the spike epitope-specific $CD8^+$ T cell responses according to median frequencies detected in inexperienced HCs (Fig. 3C). The most dominant responses in inexperienced HCs included $B15/S_{919}$ (0.4539%) followed by $A03/S_{378}$ (0.2404%) and $A24/S_{1208}$ (0.0830%). Although these three epitopes were among the most prominent spike-specific $CD8^+$ T cell responses in the inexperienced IBD and IBD-TNFi donors, slight differences in their immunodominance landscape were detected. $A03/S_{378}$-specific $CD8^+$ T cells were most prominent in untreated IBD patients (0.5946%), but less dominant in TNFi-treated IBD patients (0.0239%). $A24/S_{1208}$ dominated the spike-specific immune response in inexperienced IBD-TNFi patients (0.1320%) (Fig. 3C). In contrast, changes in the immunodominance hierarchy of experienced HCs were observed, as the vaccine particularly boosted $A03/S_{378}$ (4.8970%), $A01/S_{865}$ (0.183%) and $B35/S_{687}$ (0.010%), indicating that these epitope-specific $CD8^+$ T cell populations benefited from the previous SARS-CoV-2 infection (Fig. 3C). The low number of experienced IBD-TNFi patients were insufficient to generate conclusive immunodominance hierarchy landscapes.

Together these results suggest that SARS-CoV-2 mRNA-1273 vaccines induce a broad spike-specific $CD8^+$ T cell response in untreated and TNFi-treated IBD patients, similar in frequency and with relatively similar immunodominance landscapes as to those observed in HCs.

3.6. SARS-CoV-2 vaccine-induced spike-specific $CD8^+$ T cells display an activated memory phenotype

Next to frequency, the memory phenotype and activation status of the SARS-CoV-2-specific $CD8^+$ T cells were assessed, by combining their identification through combinatorial encoded tetramers with analysis of phenotypic markers (CD27, CD45RA, CD95) and activation markers (CD38, HLA-DR and PD1) (Fig. 4A), to establish the potency of the vaccine-induced immune response. More specifically, we assessed the frequency of epitope-specific naive (T_{naive}; $CD27^+CD45RA^+CD95^-$),



(caption on next page)

Fig. 3. Immunodominance landscapes of epitope-specific CD8$^+$ T cell frequencies in IBD patients with or without TNFi treatment and healthy controls. (A) Dynamics between donor groups of 13 SARS-CoV-2 spike epitope-specific CD8$^+$ T cell populations. Each dot represents a donor, the number of donors is indicated at the top of the graph. (B) Immunodominance landscape of tetramer$^+$CD8$^+$ T cells for SARS-CoV-2 spike-epitopes of pooled donor groups at T3 (top) and T0 (bottom). Epitopes are ordered based on median frequency at T3. Each dot represents an individual donor expressing the respective HLA-I allotype. (C) Immunodominance hierarchy of SARS-CoV-2 spike-epitope frequencies at T3, epitopes are ordered based on the median frequency of inexperienced healthy controls. Each dot represents an individual donor. Tetramer$^+$CD8$^+$ T cells detected of 3–8 cells within a dual-positive gate are indicated by open symbols, horizontal bars indicate the median. Undetectable frequencies are included on the graph at 0.0001% to show the number of donors tested; these were set at 0% for statistical analysis. The Wilcoxon signed-rank test was performed to compare frequencies at T0 or T3 between inexperienced or experienced donor groups, including Bonferroni-Holm's multiple comparison correction on inexperienced donors. Significant *p* values are provided above the graph.

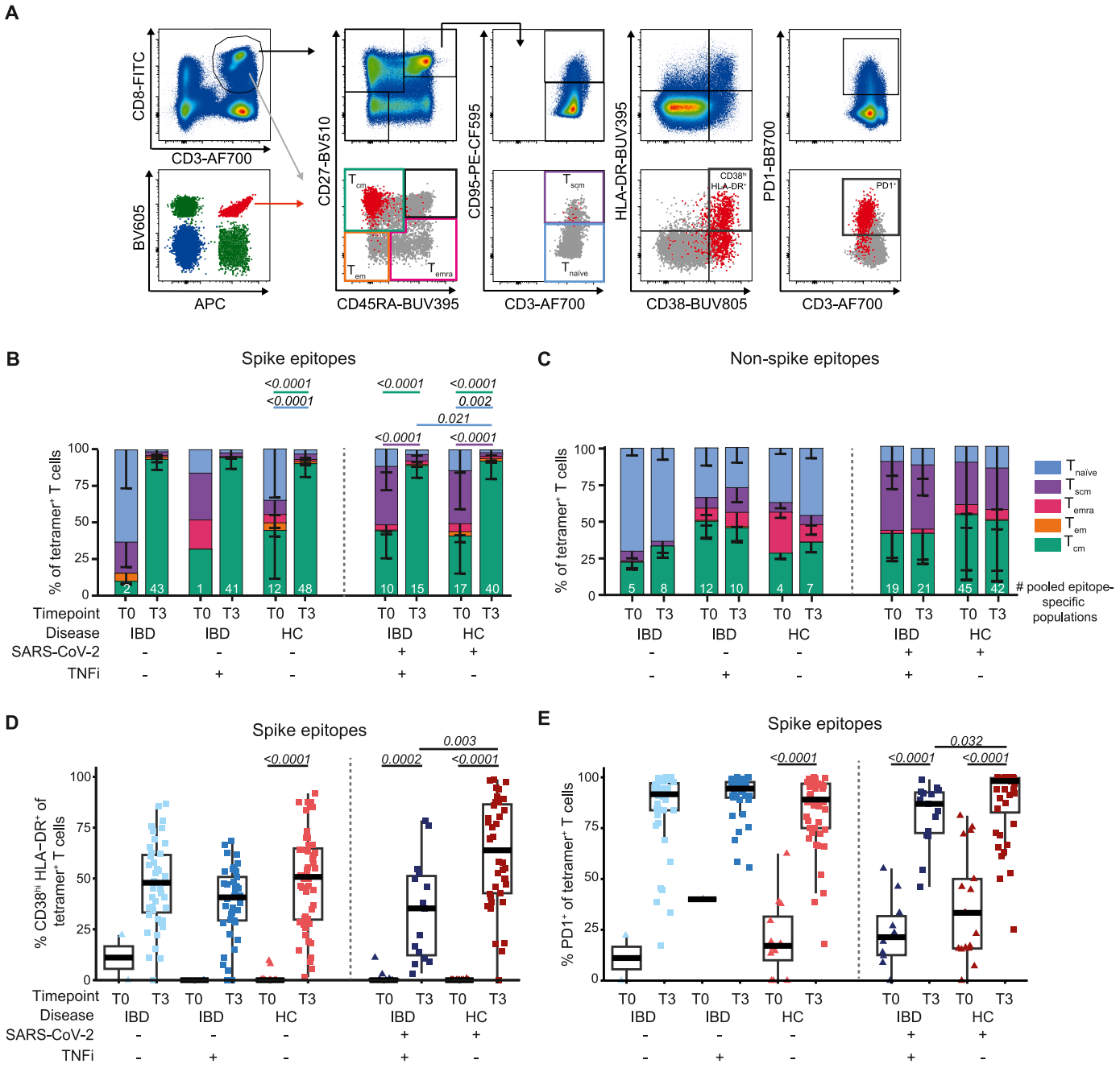


Fig. 4. Vaccine-induced SARS-CoV-2 spike-specific CD8$^+$ T cells display an activated and memory phenotype. (A) Representative FACS gating strategy used to characterize the phenotype profile of SARS-CoV-2 epitope-specific CD8$^+$ T cells. CD27, CD45RA and CD95 were used to identify memory subsets and CD38, HLA-DR and PD1 to assess the activation status of tetramer$^+$CD8$^+$ T cell populations with ≥9 cells. Gates were set based on the total CD8$^+$ T cell population (top panel). The bottom panel displays a combination of total CD8$^+$ T cells (grey dots) with dual-tetramer positive cells (red dots). Mean phenotypic frequencies of spike (B) and non-spike-specific (C) CD8$^+$ T cells pooled per donor group. Error bars represent the standard deviation. The number of epitope-specific populations pooled is indicated at the base of the bar. Median CD38^{hi}HLA-DR$^+$ (D) and PD1$^+$ (E) pooled spike-specific CD8$^+$ T cell frequencies per donor group. (B–E) An unpaired Wilcoxon signed-rank test was performed to compare phenotype populations between T0 and T3 within the donor group or across donor groups (separating inexperienced and experienced groups), including Bonferroni-Holm's multiple comparison correction on inexperienced donors. Significant *p* values are provided above the graph.

central memory (T_{cm}; $CD27^{+}CD45RA^{-}$), effector memory (T_{em}; $CD27^{-}CD45RA^{-}$), CD45RA expressing terminally differentiated effector memory (T_{emra}; $CD27^{-}CD45RA^{+}$), and stem cell memory (T_{scm}; $CD27^{+}CD45RA^{+}CD95^{+}$) $CD8^{+}$ T cell populations. Phenotyping precursor frequencies (T0) was hampered in inexperienced donors due to the low frequencies of tetramer-specific cells, as <9 tetramer $^{+}$ cells were omitted from phenotypic analysis. The populations that could be analyzed displayed a mixed phenotype (Fig. 4B). The prominent increase in spike-specific $CD8^{+}$ T_{cm} cells upon vaccination was comparable between all groups (Fig. 4B; inexperienced IBD 93%, IBD-TNFi 95%, HC 91%; experienced IBD-TNFi 89%, HC 92%). The prominent spike-specific $CD8^{+}$ T_{scm}/T_{cm} phenotype in experienced IBD-TNFi patients and HCs prior to vaccinations (T0) shifted towards a robust T_{cm} cells phenotype post-vaccination (T3), while the $CD8^{+}$ T_{scm} population of non-spike-specific cell remained stable (Fig. 4B and C). The memory phenotype of non-spike-specific, control epitope-specific and total $CD8^{+}$ T cell populations remained stable between pre- and post-vaccination samples across all groups (Fig. 4C, Supplementary Figs. 5A and B).

The activation status of SARS-CoV-2-specific $CD8^{+}$ T cell populations was determined by co-expression of CD38 and HLA-DR or by expression of PD1 (Fig. 4A–D-E). All groups demonstrated a robust vaccine-induced activation of spike-specific $CD8^{+}$ T cells (Fig. 4D and E). In contrast, no substantial changes in the frequency of activated non-spike-specific, control epitope-specific or total $CD8^{+}$ T cell populations were detected after vaccination (Supplementary Figs. 5C–H). Interestingly, vaccination induced significantly higher frequencies of activate spike-specific $CD8^{+}$ T cells ($CD38^{hi}HLA\text{-}DR^{+}$ or $PD1^{+}$) in experienced HCs compared to IBD-TNFi patients. A similar trend was observed for $PD1^{+}$ frequency in the non-spike-specific, control epitope-specific and total $CD8^{+}$ T cell populations between SARS-CoV-2 experienced IBD-TNFi patients and HCs, albeit both not significant. This tendency was not observed in inexperienced donor groups (Supplementary Figs. 5E–H), which may indicate that TNFi hampers the expression of PD1 both on vaccine-boosted $CD8^{+}$ T cells and broader T cell populations of SARS-CoV-2 experienced donors.

Next, we investigated the relationship of spike-specific $CD8^{+}$ T cell frequencies and their activation phenotype, measured by the frequency of $CD38^{hi}HLA\text{-}DR^{+}tetramer^{+}CD8^{+}$ T cells (Supplementary Figs. 6A and B). The frequency of overall spike-specific $CD8^{+}$ T cell populations (T3) positively correlated with the percentage of $CD38^{hi}HLA\text{-}DR^{+}$ T cells in vaccinated inexperienced and experienced HCs. Notability, this correlation was not evident in IBD patients, suggesting that IBD and/or TNFi treatment may hamper the activation of spike-specific $CD8^{+}$ T cells after vaccination despite a substantial rise in frequency.

Together these results demonstrate that vaccination induced a strong T_{cm} phenotype and robust activation of spike-specific $CD8^{+}$ T cell populations, which were not affected by IBD diagnosis and treatment status. However, TNFi treatment may hamper the expression of PD1 in SARS-CoV-2 experienced donors. This indicates that IBD patients with or without TNFi treatment are capable of forming robust memory SARS-CoV-2-specific $CD8^{+}$ T cell populations across a broad range of spike epitopes upon mRNA-1273 vaccination.

4. Discussion

SARS-CoV-2 mRNA vaccines are known to induce robust humoral and cellar immunity in healthy individuals [20,52,71]. However, recent studies demonstrated that immunomodulatory and biological treatment of chronic IMID patients can negatively affect the generation of robust immunity upon SARS-CoV-2 vaccinations [26–30,72]. IBD patients are often treated with TNFi, which was associated with a more rapid decline in vaccine-induced SARS-CoV-2-specific antibodies [26–30]. Our study demonstrated that SARS-Cov-2 mRNA-1273 vaccinated IBD patients, regardless of TNFi treatment and previous SARS-CoV-2 infection status, induced robust and broad SARS-CoV-2 spike-specific $CD8^{+}$ T cell responses. These SARS-CoV-2-specific $CD8^{+}$ T cell populations were comparable to those in healthy controls in terms of magnitude, activation status and ability to establish memory. Robust $CD8^{+}$ T cell responses form an essential line of protection against severe disease upon reinfection, particularly in the context of waning SARS-CoV-2-specific antibodies.

Previous studies compared vaccine-induced SARS-CoV-2-specific T cell responses between TNFi-treated patients and healthy individuals by identifying activated T cells upon stimulation with long overlapping peptide pools, revealing similar [32,35,36], reduced [31,33,34] or augmented [35,37,38] SARS-CoV-2-specific T cell responses between TNFi-treated individuals and HCs [35,37,38]. Of these studies, only two specified the SARS-CoV-2-specific $CD8^{+}$ T cell frequencies in TNFi-treated IBD patients using an activation-induced marker assay (AIM) [35,36]. The differences between those studies could potentially be explained by differences in HLA expression between IBD patients and HCs, as the magnitude of the overall SARS-CoV-2-specific immune response is impacted by the individual's HLA allotype profile [9,51,60,61,66]. The novelty of our study lies in the heterotetramer combinatorial coding which allowed detection and simultaneous phenotyping of up to 15 SARS-CoV-2 epitope-specific $CD8^{+}$ T cell populations in a single donor directly *ex vivo*. This allowed us to reliably compare the magnitude, activation and phenotype profiles across IBD, IBD-TNFi and HCs on an individual epitope level, resulting in the detection of similar frequencies and phenotypes between groups.

Research in mice has shown that TNF enhances $CD8^{+}$ T cell contraction and promotes immune homeostasis after viral clearance [42,44], but does not impact the proliferative renewal of memory $CD8^{+}$ T cells [44]. Consistent with these murine studies, Qui et al. demonstrated that TNFi-treated IBD patients displayed significantly higher $CD4^{+}IFN\gamma^{+}$ and $CD8^{+}IFN\gamma^{+}$ T cells compared to HCs or untreated IBD patients respectively at 94 days post-second mRNA vaccination. This was despite similar IFNγ responses upon restimulating whole blood with SARS-CoV-2 peptide pools at 15 days post-second vaccination, which corroborate our findings on robust memory spike-specific $CD8^{+}$ T cell responses upon vaccination. Importantly, the study by Qui et al. indicated that SARS-CoV-2 vaccine-induced T cell responses are durable in TNFi-treated patients [35].

Prior work demonstrated that $PD1^{+}$ SARS-CoV-2-specific $CD8^{+}$ T cells in HCs during acute infection and/or at a convalescent state were not exhausted and remained functional [73]. Using an antigen-reactive T cell enrichment assay after peptide pool stimulation, Geisen et al. found a significant decrease of $PD1^{+}$ memory T cells in vaccinated TNFi-treated rheumatoid arthritis patients compared to patients treated with other immunomodulatory medications and HCs [32]. Our study did not observe differences in activated spike-specific $CD8^{+}$ T cell frequencies, defined as $CD38^{hi}HLA\text{-}DR^{+}$ and/or $PD1^{+}$, between inexperienced IBD-TNFi, IBD and HC donors post-vaccination. However, SARS-CoV-2 experienced IBD-TNFi patients displayed significantly lower expression of these activation markers compared to experienced HCs post-vaccination, which is in line with the data from Geisen et al. Further studies are needed to understand whether TNFi treatment truly hampers the re-activation of memory populations or whether it has altered the dynamics/timing of the activation.

Previous studies demonstrated that traditional vaccine approaches against other pathogens are hampered in TNFi-treated patients [22–25]. Therefore, our findings support the development of T cell-based mRNA vaccines for other pathogens, such as influenza, to improve vaccine-driven protection in TNFi-treated patients.

To summarize, we showed that TNFi-treated IBD patients could generate robust and broad spike-specific memory $CD8^{+}$ T cell populations upon mRNA vaccination similar to those observed in untreated IBD patients and HCs, which was independent of prior SARS-CoV-2 infections. These findings suggest that TNFi treatment does not impair the establishment of spike-specific $CD8^{+}$ T cell populations in IBD patients. The induced SARS-CoV-2-specific $CD8^{+}$ T cell-mediated immune response helps explain the vaccine-induced protectiveness against viral

infection described in IBD-TNFi patients [74–76], despite the observed decline in humoral immunity [26–30]. The implications of our findings extend beyond the IBD research field, as TNFis are frequently prescribed to treat other chronic immune diseases, such as rheumatoid arthritis, psoriatic arthritis, psoriasis and axial spondylitis [77].

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Declaration of competing interest

No conflict of interest to report.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2024.103175.

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