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## BACKGROUND

PWH with low CD4 count are at high risk for severe and prolonged mpox. Data on immune response and its persistence after MVA-BN vaccination are lacking. We evaluated humoral and cellular responses 3 years after MVA-BN vaccination in people with (PWH) and without HIV (PWoH), according to CD4 count.

## METHODS

Peripheral whole blood was collected 3 years after the primary course of MVA vaccination (single dose in smallpox vaccine-experienced individuals; two doses in naïve individuals) across three centres of the Italian multicentre ICONA cohort. Anti-MPXV IgG and neutralising antibodies (nAb) were measured by immunofluorescence assay and PRNT50. Orthopox (OP)-specific T-cell responses were assessed by ELISpot, as well as by activation-induced marker (AIM) and intracellular cytokine staining (ICS) assays, integrated into a multiparametric flow cytometry panel.

Statistics: Kruskal-Wallis, Dunn's, Mann-Whitney, Wilcoxon tests.

**Table 1. Main characteristics of the study population**

Characteristic	Overall (N=92)	PWH >350 CD4 T cells (N=27)	PWH <350 CD4 T cells (N=14)	People Without HIV (N=51)
<b>Sex at birth</b>				
Male	92 (100%)	27 (100%)	14 (100%)	51 (100%)
<b>Age<sup>2</sup></b>	49 [42;56]	47 [43;54]	58 [52;61]	47 [37;52]
<b>Viral load at vaccination<sup>3</sup> &lt;30 copies/mL</b>	41 (100%)	27 (100%)	14 (100%)	Nd
<b>Previous smallpox vaccination</b>	27 (29.4%)	9 (33.3%)	9 (64.3%)	9 (17.6%)
<b>Route of administration of the primary vaccination cycle</b>				
Subcutaneous	55 (59.8%)	20 (74.1%)	2 (14.3%)	33 (64.7%)
Intradermal	34 (37.0%)	6 (22.2%)	12 (85.7%)	16 (31.4%)
Unknown	3 (3.3%)	1 (3.7%)	0 (0%)	2 (3.9%)

Nd=not determinable; <sup>1</sup>Wilcoxon-Mann-Whitney U test, Chi-squared, Fisher's Exact test

<sup>2</sup>median [Q1;Q2]; <sup>3</sup>among people living with HIV (PWH)

## PLAIN LANGUAGE SUMMARY

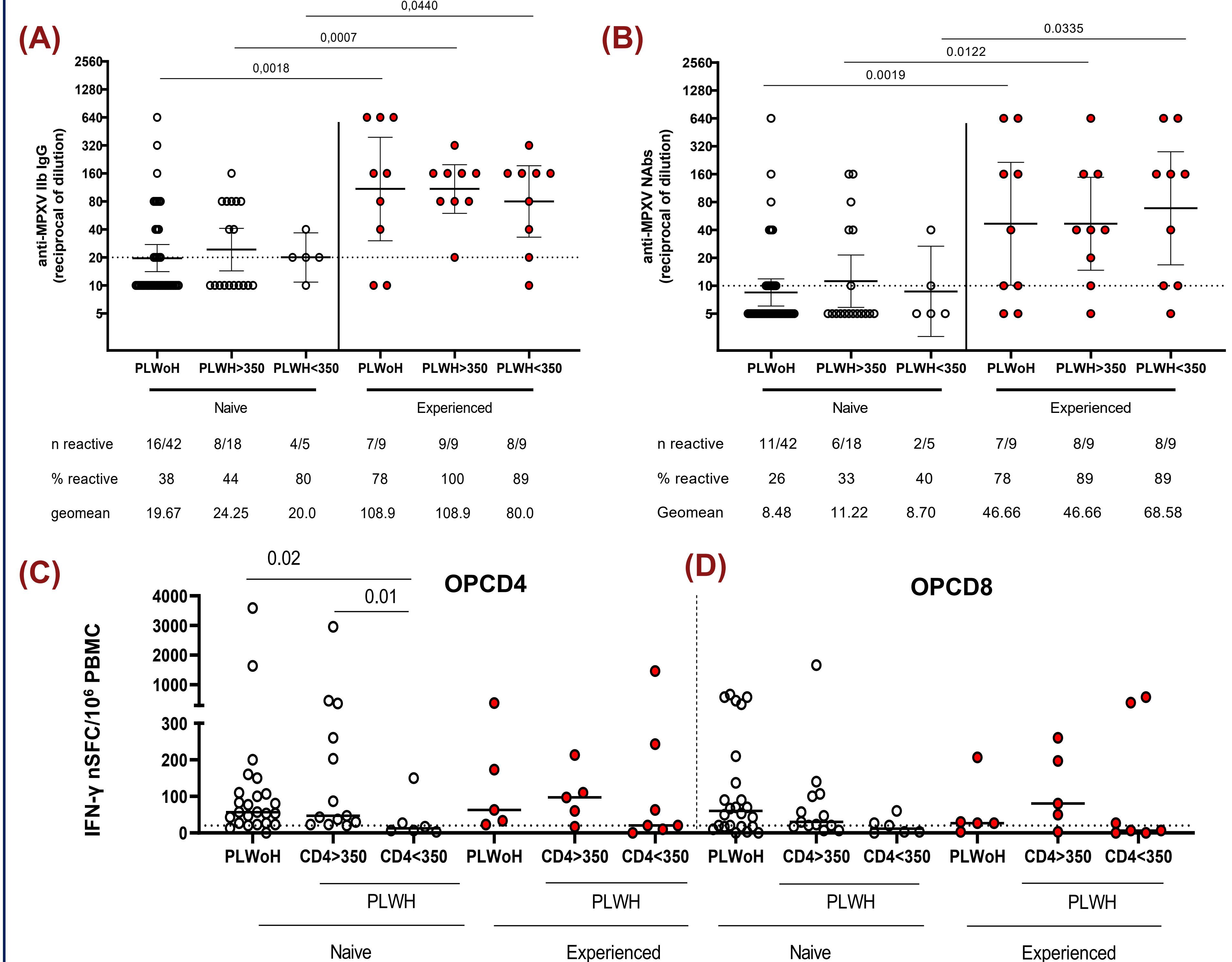
Three years after the mpox vaccine, immune responses were strongest in people previously vaccinated against smallpox, while those without prior smallpox vaccination had low antibodies and people living with HIV with a weaker immune system had weaker T-cell responses.

## Three years after MVA-BN, durable humoral immunity is largely confined to smallpox-primed participants; PWH with CD4<350 exhibit reduced OPX-specific T-cell responses—arguing for boosting to sustain protection.

## RESULTS

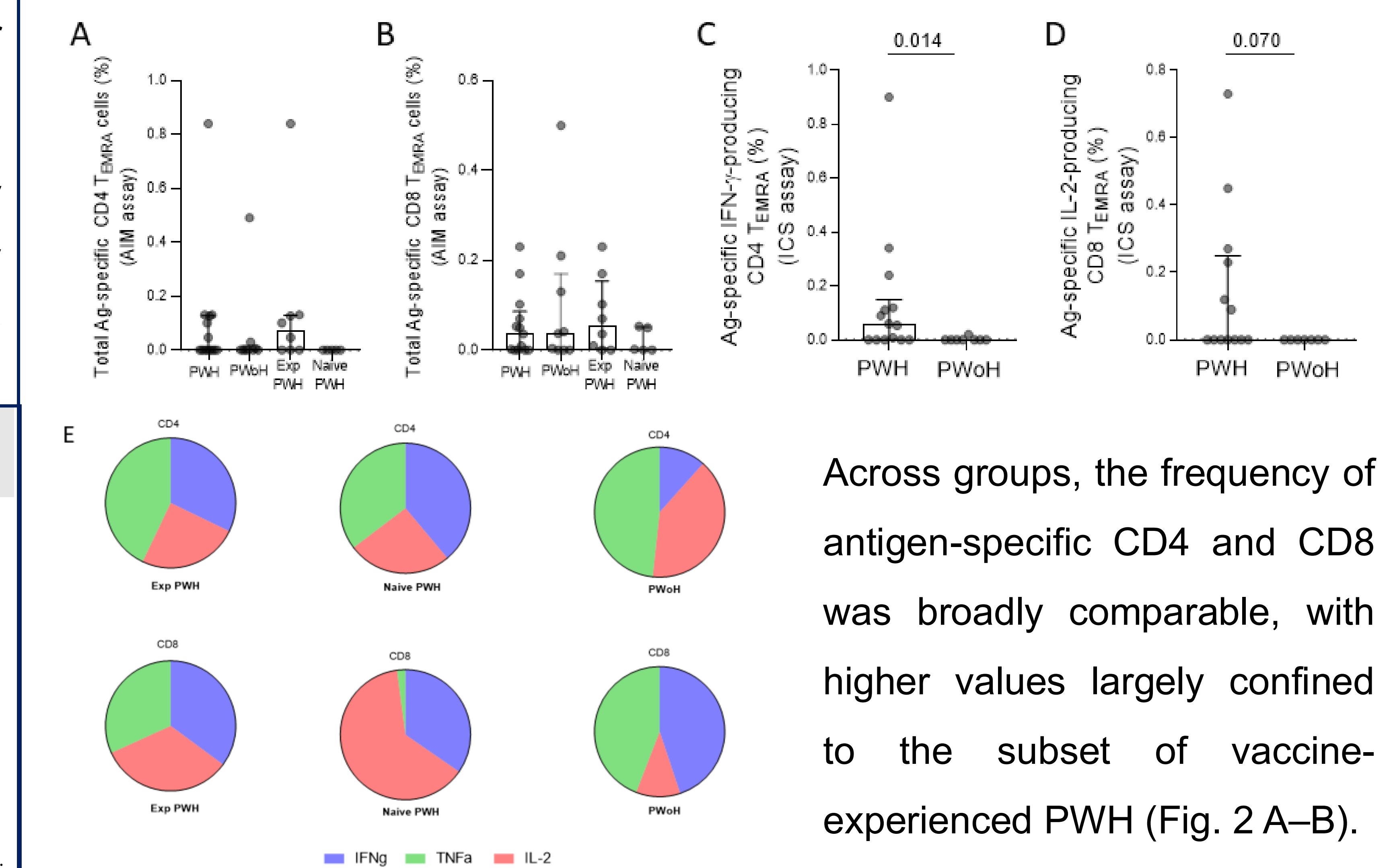
We analysed 92 male participants: 51 PWoH, 27 PWH with CD4 >350 cells/μL, and 14 PWH with CD4 counts <350 cells/μL. Smallpox vaccine-naïve individuals showed lower IgG titers compared to those who were smallpox vaccine-experienced, across all three groups (p = 0.0020, p = 0.0028, p = 0.0495) (Fig. 1A). A similar pattern was observed for nAbs (Fig. 1B). No evidence for a difference emerged in the comparison of HIV strata. Although detectable in all groups, OP-derived CD4 responses were significantly higher in PWoH and PWH with CD4 >350 than in PWH with CD4 <350 (Fig. 1C), with a similar trend in CD8 responses (Fig. 1D).

**FIGURE 1. Evaluation of MPXV-specific IgG (A), neutralising antibodies (nAbs) (B), OPCD4 T-cells (C) and OPCD8 T-cells (D) specific response by ELISpot.**



Dot lines represent the detection limit for IgG (1:20), nAbs (1:10) and median values of T-cell response evaluated in 10 healthy controls matched for age and sex. Geometric mean IgG and nAbs titers (GMT-95% CI) are shown for each time range or study group. Red dots represent smallpox vaccine-experienced participants.

**FIGURE 2. Antigen-specific T-cell responses by AIM/ICS in the subset of PWH with low CD4 (naive and experienced) vs naive PWoH**



Across groups, the frequency of antigen-specific CD4 and CD8 was broadly comparable, with higher values largely confined to the subset of vaccine-experienced PWH (Fig. 2 A–B).

Functionally, PWH showed a higher frequency of IFN-γ-producing CD4 TEMRA (p=0.014) and a non-significant trend toward higher IL-2-producing CD8 TEMRA (p=0.070) versus PWoH. Naïve PWH showed a different Th1 cytokine profile, skewed towards IFN-γ-CD4 and IL-2-CD8 (Fig. 2C–E).

## CONCLUSIONS

Three years after MVA-BN, IgG and nAbs persisted mainly in smallpox-primed individuals, including PWH with advanced immunosuppression. Smallpox-naïve participants showed low residual humoral immunity, supporting the need for a booster dose. Orthopox-specific CD4/CD8 responses remained detectable in PWoH and PWH with CD4>350 but were attenuated in PWH with CD4<350. Overall, vaccine-experienced, immune-competent individuals retained durable cellular memory consistent with long-term protection.

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