# Supplementary Appendix

Supplement to: Kottkamp AC, Samanovic MI, Duerr R, et al. Antibody titers against mpox virus after vaccination. N Engl J Med 2023;389:2299-301. DOI: 10.1056/NEJMc2306239

This appendix has been provided by the authors to give readers additional information about the work.

**Supplementary Appendix**
**Antibody Titers against Mpox Virus after Vaccination**

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Supplement to: Kottkamp AC, Samanovic MI, Mulligan MJ et al. Antibody Titers against Mpox Virus after Vaccination.

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## MATERIALS AND METHODS

### Study Design

The New York City Observational Study of Mpox Immunity (NYC OSMI) is an ongoing observational study of adults with and without HIV receiving MVA-BN (JYNNEOS™) vaccination, or who had mpox disease. All data reported in this study interim report is from non-MPXV-infected, vaccinated individuals. Blood is collected at defined time points, as diagrammed in Supplementary Figure S1. Participants could enroll before or after mpox vaccination, through 365 days post-1st dose. Trial Registration: ClinicalTrials.gov; NCT Number: NCT05654883; https://clinicaltrials.gov/ct2/show/NCT05654883. As of May 15, 2023, 155 adults have provided written consent for enrollment with approval from the NYU Grossman School of Medicine Institutional Review Board (protocol 22-01338). Participant characteristics of individuals who have not been previously infected with mpox (n=145), are summarized in the Supplementary Table S1. Prior vaccination with a live replicating smallpox vaccine was reported by participants (presented in Table S1 as smallpox vaccine received during childhood vs adulthood vs unknown timeframe) and confirmed by examination of the upper arms for scarring, prior military participation, country of birth, and whether the year of vaccination was consistent with active smallpox vaccine distribution. CD4+ T cell counts were obtained from electronic medical records at time of enrollment for HIV+ participants. Mpox vaccination date was confirmed with electronic medical records, and vaccine route was confirmed with electronic medical records when available, or by participant's description of vaccination procedure.

### Blood Sample Processing and Storage

Venous blood was collected by standard phlebotomy. Serum was collected in SST tubes (BD Biosciences) and frozen immediately at -80°C.

## Enzyme-linked Immunosorbent Assay (ELISA)

Direct ELISA was used to quantify H3L IgG antibody titers in participant serum. H3L is an immunodominant membrane antigen on the surface of mature poxvirus particles, including MPVX. It is involved in cell attachment and a direct target of neutralizing antibodies[1]. Ninety-six well plates were coated with 0.5 µg/mL MPXV H3L protein (Sino Biological Inc., 40893-V08H1) diluted in phosphate-buffered saline (PBS) and were then incubated overnight at 4°C. Plates were washed four times with PBS containing 0.05% Tween 20 (Thermo Fisher Scientific) (PBS-T), and blocked with PBS-T containing 5% non-fat milk at room temperature for 1 hour. Serum samples were heat-inactivated at 56°C for 1 hour prior to use. Samples were diluted to a starting concentration of 1:50 then serially diluted 1:3 in blocking solution. The final volume in all wells after dilution was 100 µL. After a 2-hour incubation period at room temperature, plates were washed four times with PBS-T. Horseradish peroxidase (HRP)-conjugated goat-anti-human IgG (Southern BioTech, 2040-05) was diluted in blocking buffer (1:2,000) and added to each well. Plates were incubated for 1 hour at room temperature then washed four times with PBS-T. After developing for 5 min with TMB Peroxidase Substrate 3,3′,5,5′-Tetramethylbenzidine (Thermo Fisher Scientific), the reaction was stopped with 1N hydrochloric acid. The optical density was determined by measuring the absorbance at 450 nm on a Synergy 4 (BioTek) plate reader. We determined the endpoint titer of a sample by the reciprocal of the highest dilution that gives a positive reaction. As a positive reaction cutoff, we used 2x the standard deviation of the mean value of 16 negative control samples. Endpoint titers were calculated by interpolating the cutoff values from a dilution curve with non-linear fit in GraphPad Prism v.9.\5.1. To summarize data collected on individuals, endpoint titers were normalized using replicates of pooled positive control serum samples on each plate to reduce variability between plates. Sera with titers below the limit of detection (50) were scored as 25. Although MPXV H3L protein is an appropriate surrogate, it is not whole virus being used as the ELISA antigen, therefore some immunodominant proteins which will also generate immune responses are being missed.

## Immunofluorescence-based Microneutralization Assay

All MPXV work was conducted in a certified biosafety level 3 (BSL3) facility, in accordance with its Biosafety Manual and Standard Operating Procedures. Investigators were trained for BSL3 work and vaccinated against MPXV. MPXV passage 3 stocks were generated by plaque purification and subsequent sucrose purification over 36% sucrose in TNE buffer in Vero E6 cells (ATCC #CRL-1586) using a seed stock from BEI Resources (NR-58622). Purified MPXV stocks were sequence verified. On the day before infection $1.5x10^4$ Vero E6 cells were plated in each well of a black 96-well assay plate in cell culture media (Dulbecco's Modified Eagle Medium, DMEM, supplemented with penicillin/streptomycin, 2 mM L-glutamine, and 10% heat-inactivated Fetal bovine Serum, FBS) and incubated at 37°C and 5% $CO_2$. Participant serum was 2-fold serially diluted in infection media (DMEM lacking sodium pyruvate supplemented with 2% heat-inactivated FBS) starting at a 10-fold dilution. Prior to infection, serum dilutions were incubated with MPXV (sufficient for MOI ≈ 0.05) for 1 hour at 37°C. Cells were washed with 1x PBS then incubated with serum-virus mixtures for 42 hours at 37°C and 5% $CO_2$. Plates were submerged in 10% formalin for 1 hour at room temperature (RT). Fixed samples were rinsed with water then permeabilized and blocked with 3% Bovine Serum Albumin (BSA) and 0.1% Triton X-100 in PBS for 30 min at RT. Samples were then incubated with a polyclonal rabbit anti-vaccinia virus Lister

strain antibody (Abbexa #abx023200) diluted 1:1,000 in 3% BSA in PBS (blocking buffer) for 1 hour at RT. All plates were washed four times with PBS then stained with 1:2,000 dilution of donkey anti-rabbit AlexaFluor647 secondary (Thermo #A-31573) and 1:4,000 dilution of DAPI in blocking buffer. Following staining, cells were washed four times with 1x PBS with an additional 100 µL of PBS left in each well for imaging and quantification using the BioTek Cytation 7 Cell Imaging Multi-Mode Reader and Gen5 Image Prime software. MPXV neutralizing titers ($IC_{50}$ values) were calculated using GraphPad Prism v.9.5.1 non-linear regression (variable slope with four parameters) with top and bottom constraints (100 and 0, respectively).

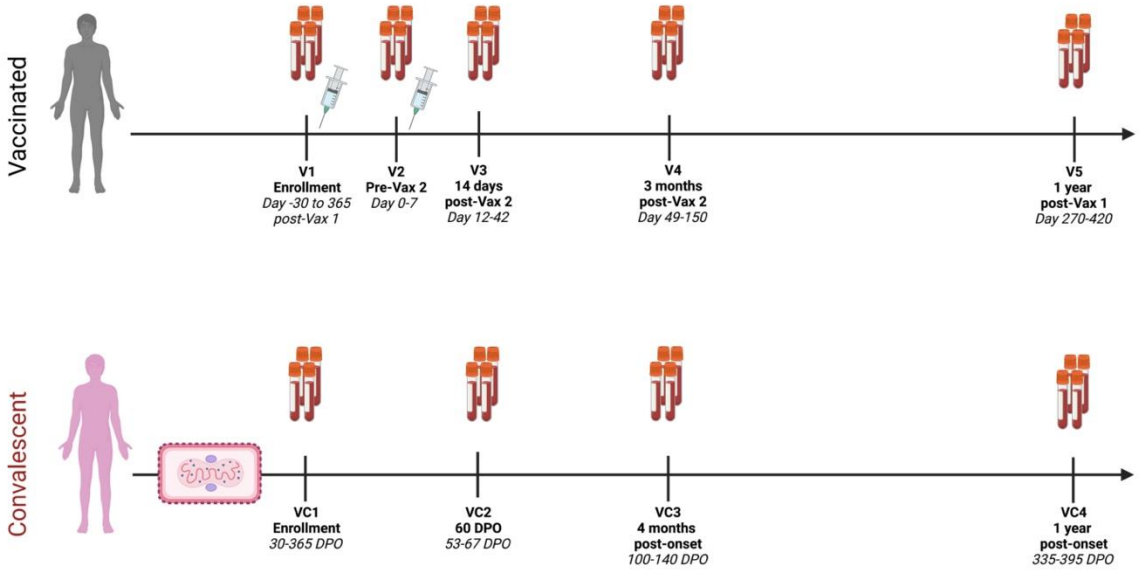
## Statistical Analyses and Visualization

All statistical analyses followed the pre-specified statistical analysis plan within the study protocol and were performed using R v.4.3.1[2]. Figures were generated using GraphPad Prism v.9.5.1 and the ggplot2 and viridis packages in R[2]. To account for within-individual correlation, linear mixed effects models were fit to all repeated measures data. Both random intercept and random slope models, as well as higher order polynomials were tested for goodness of fit. The final model for each analysis was selected by comparing differences in AIC, $R^2$, and residual vs. fitted plots. While maximum likelihood (ML) estimation was used when comparing model fits, all final analyses applied restricted maximum likelihood (REML) estimation. Ordinary least squares regression was used for all other analyses of non-correlated data. Due to the exploratory nature of this observational study, no statistical comparisons of data points between groups were made. Instead point estimates and 95% confidence intervals, or median and interquartile range (IQR) were reported for small sample sizes. Confidence interval widths were not adjusted for multiplicity as all analyses were exploratory. Therefore, formal hypothesis testing was not conducted. Sample size calculations were also not performed prior to the start of this non-randomized, non-interventional, observational study. Outlier analyses were not performed, with the exception of the analyses associated with Figure 1B.

## SUPPLEMENTARY FIGURES

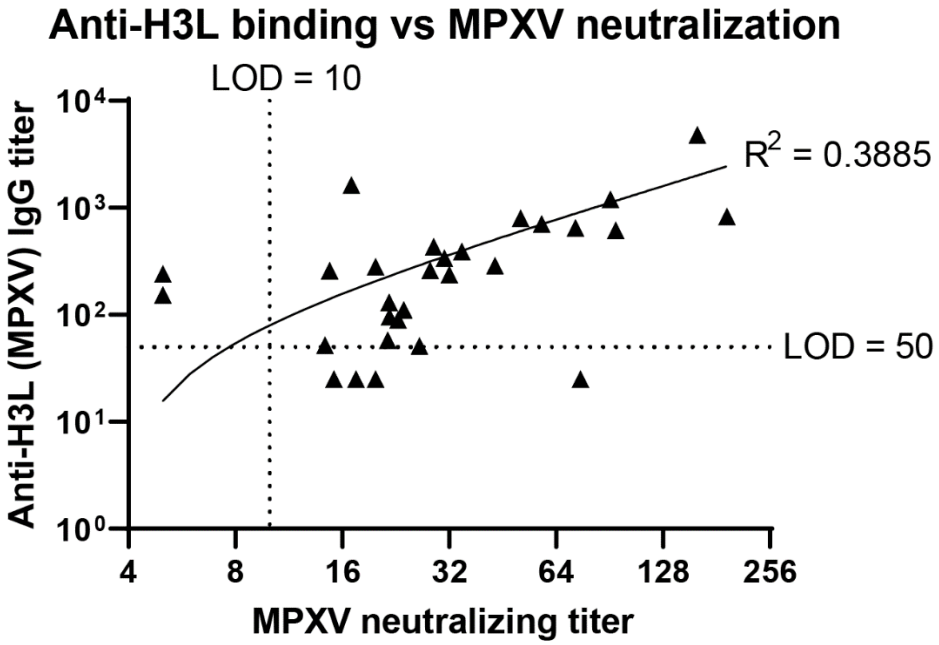
### Supplementary Figure S1. NYC Observational Study of Mpox Immunity (OSMI) Schematic (Year 1).

DPO, Days post-onset of infection. Annual visits for further longevity analyses will also occur in study years 2 and 3. V, visit for vaccinated participants; VC, visit for mpox-convalescent participants.



**Supplementary Figure S2: Correlation of anti-MPXV H3L IgG and MPXV neutralizing titers*.**

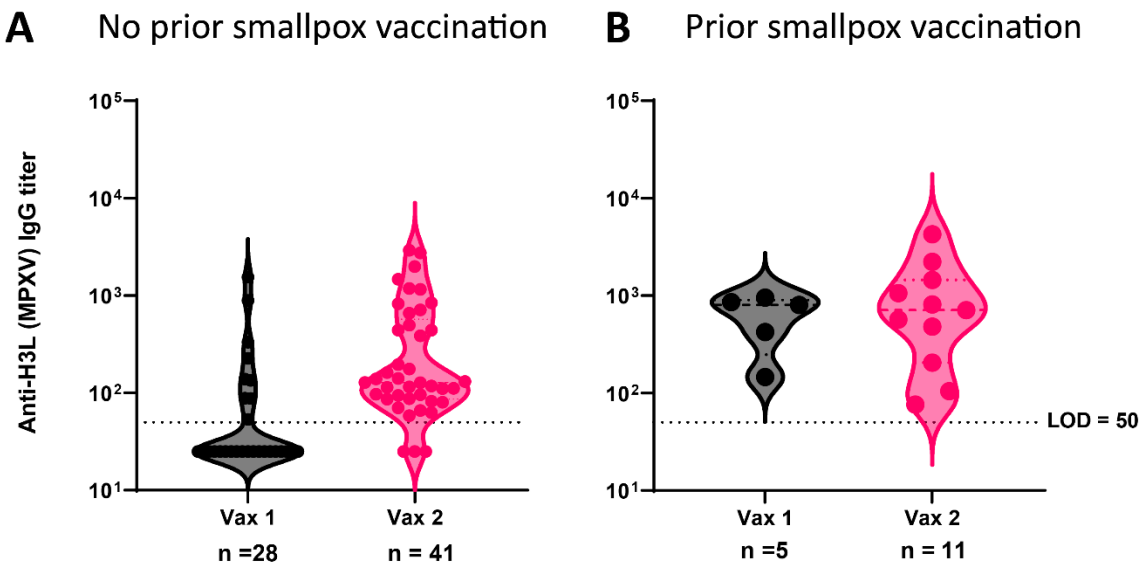
Anti-MPXV H3L IgG in participant sera measured by direct ELISA. MPXV neutralizing titers in participant sera measured by immunofluorescence-based microneutralization assay in BS-C-1 cells. Linear regression and goodness of fit measures calculated in R v.4.3.1. and GraphPad Prism v.9.5.1. LOD, limit of detection. n=29.




**Prior studies on correlation of neutralizing antibodies and protection against orthopoxviruses are limited. One study published in 1972 showed that smallpox contacts with a titer of 1:32 (neutralizing antibodies) or higher did not develop the disease. Another study published in 1975 showed that contacts who developed smallpox were unvaccinated and had neutralizing antibody titers of 10 or less*.[3,4]

**Supplementary Figure S3. Comparison of Anti-MPXV H3L IgG Antibody Titers After One and Two Doses of MVA-BN (JYNNEOS) Vaccine.**

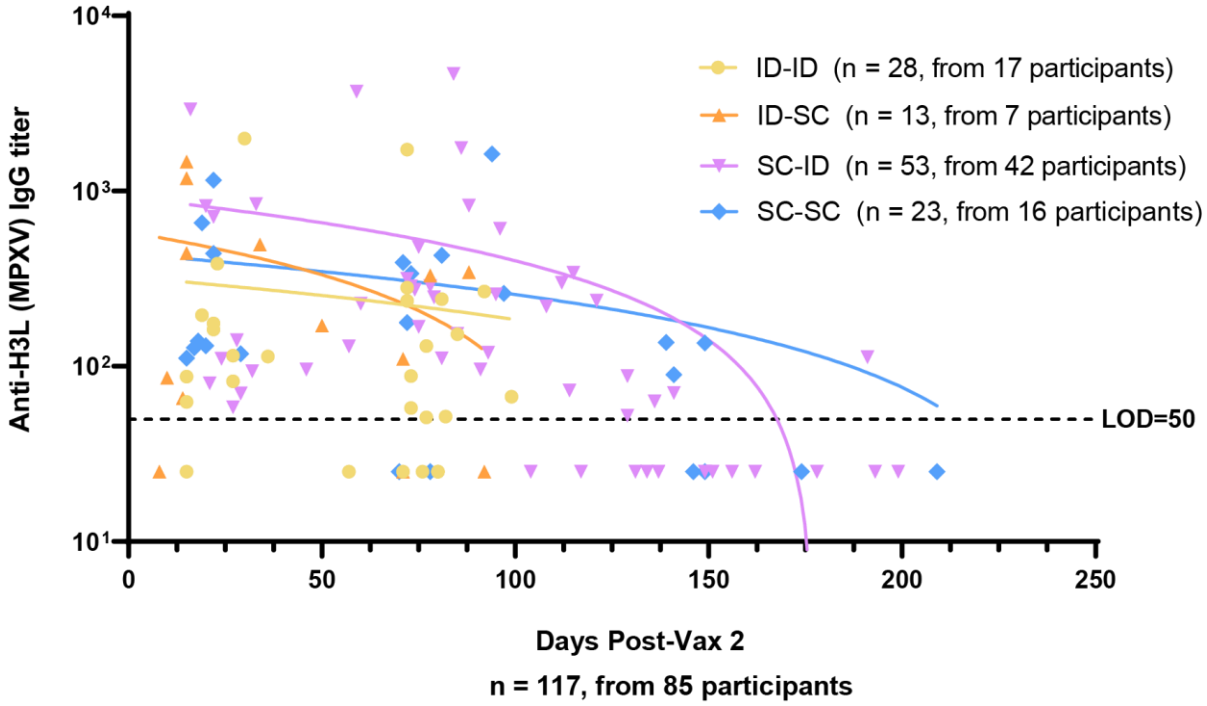
Evaluation of MPXV-specific IgG titers post-first and post-second vaccination, in participants with no prior smallpox vaccination (A) or with prior smallpox vaccination (B). GMT, geometric mean titer; IQR, interquartile range; LOD, limit of detection; Vax 1 or 2, vaccination doses 1 or 2. An analysis of the relationship between Anti-H3L IgG titer and number of days post vaccinations 1 and 2 did not indicate a signal that time since vaccination was a confounder with ELISA titers. Note: confidence intervals have not been adjusted for multiplicity and should not be used for hypothesis testing.



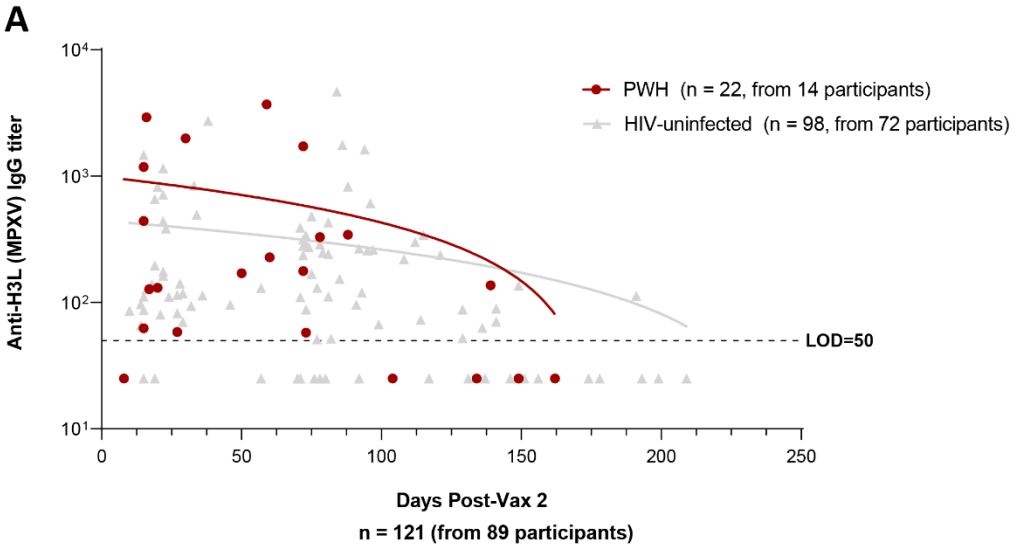

| | | | | |
|---|---|---|---|---|
| **GMT (95% CI)** | 49.55 (31.33, 78.34) | 199.35 (133.54, 297.61) | 526.14 (198.76, 1392.73) | 601.95 (262.26, 1381.59) |
| **Median titer (IQR)** | 25.0 (25.0, 87.88) | 127.9 (87.34, 494.6) | 801.5 (422.3, 857.2) | 710.1 (343.6, 1253.7) |
| **Median days post-Vax 1/2 (IQR)** | 33.0 (29.0, 152.0) | 21.0 (15.0, 27.5) | 95.0 (39.5, 148.0) | 24.5 (18.3, 37.0) |
| **Above LOD** | 32.1% | 92.7% | 100% | 100% |

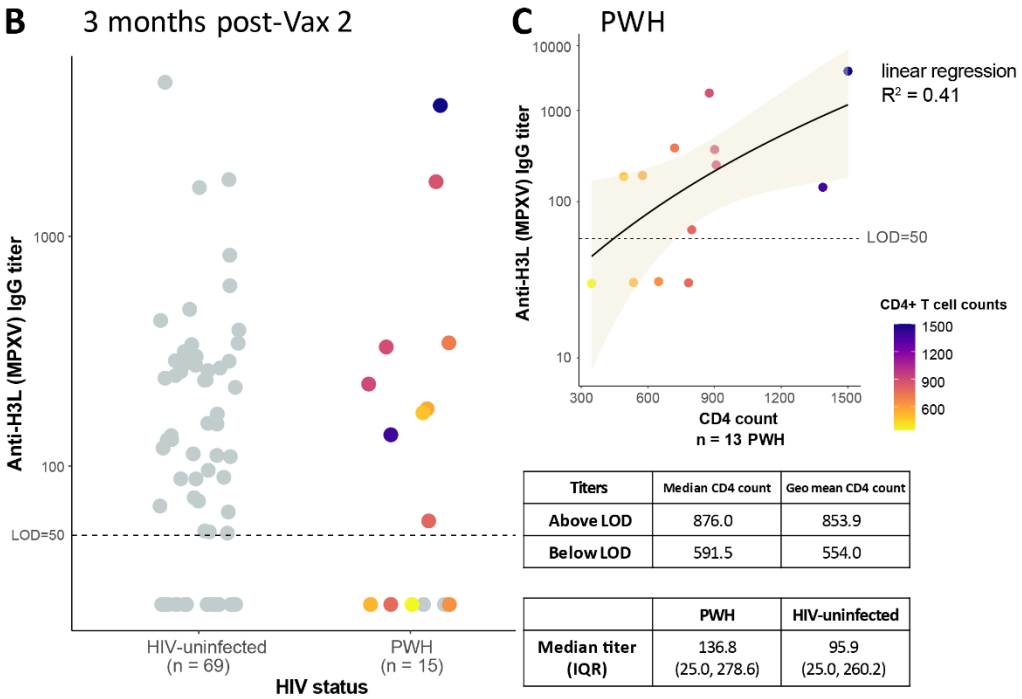
**Supplementary Figure S4. Longevity of Anti-MPXV H3L IgG Titers by Vaccine Administration Route.**

Figure shows linear mixed effects models of MPXV-specific IgG titers (y-axis) vs days post vaccination 2, for types of vaccine administration in individuals with no prior smallpox vaccination. LOD, limit of detection; ID, intradermal; SC, subcutaneous; Vax 2, vaccination dose 2.



**Supplementary Figure S5. Vaccination Induces Comparable MPXV-specific Titers in HIV-uninfected Participants and People with HIV (PWH).**
Panel A shows linear mixed effects models of MPXV-specific IgG titers vs days post-second dose, in HIV-uninfected participants and PWH. Panel B shows a comparison of MPXV-specific IgG titers between PWH and HIV-uninfected individuals (similar as in Figure 1C [right]) where data points in PWH are color-coded by CD4+ T cell counts (CD4 count). Panel C shows a linear regression analysis (with 95% CI) of MPXV-specific IgG titers in PWH over their CD4 counts. The tables below panel C indicate the median IgG titers (PWH vs HIV-uninfected; lower table) and the geometric mean and median CD4 counts for participants whose MPXV H3L IgG titers were above or below the assay limit of detection (PWH only; upper table). LOD, limit of detection**;** GMT, geometric mean titer; PWH, people with HIV; Vax 2, vaccination dose 2.




| Titers | Median CD4 count | Geo mean CD4 count |
|---|---|---|
| Above LOD | 876.0 | 853.9 |
| Below LOD | 591.5 | 554.0 |

| | PWH | HIV-uninfected |
|---|---|---|
| Median titer (IQR) | 136.8 (25.0, 278.6) | 95.9 (25.0, 260.2) |

## SUPPLEMENTARY TABLES

### Supplementary Table S1. Demographic and Clinical Characteristics.

145 NYC Observational Study of Mpox Immunity (OSMI) Vaccinated (Mpox disease-Naive) Participants, as of May 15, 2023.

| Characteristic | Vaccinated (mpox disease-naive) n=145 |
|---|---|
| **Gender — no. (%)*** | |
| Female | 17 (11.7%) |
| Male | 117 (80.7%) |
| Non-binary/gender fluid/gender queer | 11 (7.6%) |
| **LGBTQ+ Status — no. (%)*** | |
| LGBTQ+ | 129 (89.0%) |
| **Prior smallpox vaccination** | |
| no. (%)<br>Received in childhood<br>Received in adulthood<br>Unknown age of vaccination | 29 (20.0%)<br>21 (72.4%)<br>3 (10.3%)<br>5 (17.2%) |
| **Route of MVA-BN (JYNNEOS) vaccination (doses 1 & 2) — no. (%)** | |
| ID-ID | 28 (19.3%) |
| ID-SC | 13 (9.0%) |
| SC-ID | 62 (42.8%) |
| SC-SC | 24 (16.5%) |
| Not Determined[#] | 18 (12.4%) |
| **HIV status** | |
| PWH — no. (%) | 35 (24.1%) |
| CD4+ T cell counts — median; IQR (cells/mm$^3$) | 682; 529-902 |
| **Age** | |
| Age — median ± SD (yr.) | 38 ± 14.7 |
| Age 50 and older — no. (%) | 42 (29.0%) |
| **Race or ethnic group — no. (%)*^** | |
| White | 91 (62.8%) |
| Asian | 16 (11.0%) |

| Characteristic | Vaccinated (mpox disease-naive) n=145 |
|---|---|
| Black or African-American | 14 (9.7%) |
| Native American or Alaska Native | 4 (2.8%) |
| Other | 20 (13.8%) |
| Hispanic or Latino | 34 (24.4%) |

*Gender, race or ethnic group, and LGBTQ+ identification were reported by participants
#As a participant may have elected to not receive a second dose, or if a second dose has not yet occurred, or route of second dose has not yet been reported.
^Responses are not mutually exclusive
IQR, Interquartile range
ID, Intradermal
SC, Subcutaneous
SD, Standard deviation

## Supplementary Table S2: Statistical Results for Figure 1A.

(Top) Frequency of number of longitudinal measurements per individual. (Bottom) Estimates [95% Confidence Intervals] and variance explained after fitting linear mixed effects model with random intercept to data from participants with no prior smallpox vaccination: Dependent Variable – Anti-H3L IgG titer, Independent Variable – days post vaccine 2. Note: confidence intervals have not been adjusted for multiplicity and should not be used for hypothesis testing.

| **Frequency of Longitudinal Measurements** | **Sample size (n = 86)** |
|---|---|
| Individuals with only one measurement at 2 weeks post Vax 2 | 45 |
| Individuals with only one measurement at 3 months post Vax 2 | 5 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 36 |
| **Linear Mixed Effects Model with Random Intercept** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -2.675 [-5.102, -0.263] |
| Intercept | 577.32 [333.23, 821.27] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 523.3 [201.356,688.558] |
| Residual | 491.8 [381.422, 675.982] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.0327 |
| Conditional | 0.546 |

*Note: Marginal $R^2$ is the proportion of variance explained by the fixed effects relative to the overall variance, and conditional $R^2$ is the proportion of variance explained by both fixed and random effects relative to the overall variance.

## Supplementary Table S3: Statistical Results for Figure 1B.

Complete case analysis followed by sensitivity analysis: (Top) Frequency of number of longitudinal measurements per individual. (Bottom) Estimates [95% Confidence Intervals] and variance explained after fitting linear mixed effects model with random intercept to data from participants with prior smallpox vaccination: Dependent Variable – Anti-H3L IgG titer, Independent Variable – days post vaccine 2. Note: confidence intervals have not been adjusted for multiplicity and should not be used for hypothesis testing.

| **COMPLETE CASE ANAYSIS** | |
|---|---|
| **Frequency of Longitudinal Measurements** | **Sample size (n = 26)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 2 |
| Individuals with only one measurement at 3 months post Vax 2 | 16 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 8 |
| **Linear Mixed Effects Model with Random Intercept** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -3.111 [-9.281, 5.474] |
| Intercept | 1811.91 [883.58, 2759.39] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 2042.3 [1533.758, 2732.258] |
| Residual | 363.2 [218.686, 656.057] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.0027 |
| Conditional | 0.9694 |
| **SENSITIVITY ANALYSIS**<br>*Outliers removed at 129 and 155 days* | |
| **Frequency of Longitudinal Measurements** | **Sample size (n = 24)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 2 |
| Individuals with only one measurement at 3 months post Vax 2 | 14 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 8 |
| **Linear Mixed Effects Model with Random Intercept** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -4.894 [-10.976, 1.434] |
| Intercept | 1469.676 [808.177, 2131.719] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 1274.1 [940.125, 1728.04] |
| Residual | 349.4 [215.84, 586.446] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.012 |
| Conditional | 0.931 |

*Note: Marginal $R^2$ is the proportion of variance explained by the fixed effects relative to the overall variance, and conditional $R^2$ is the proportion of variance explained by both fixed and random effects relative to the overall variance.

## Supplementary Table S4: Statistical Results for Figure 1C.

Estimates [95% Confidence Intervals] and variance explained after fitting ordinary linear regression model to data from participants with no prior smallpox vaccination: Dependent Variable – Anti-H3L IgG titer, Independent Variable – days between vaccinations. Note: confidence intervals have not been adjusted for multiplicity and should not be used for hypothesis testing.

| **Fixed Effects** | **Estimate [95% CI]** |
|---|---|
| Time (Days) | 2.753 [-2.165, 7.672] |
| Intercept | 135.014 [-191.298, 461.326] |
| **$R^2$** | **0.0149** |

## Supplementary Table S5: Statistical Results for Figure S2.

Estimates [95% Confidence Intervals] and variance explained after fitting ordinary linear regression model to data from participants with no prior smallpox vaccination: Dependent Variable – Anti-H3L IgG titer, Independent Variable – days between vaccinations. Note: confidence intervals have not been adjusted for multiplicity and should not be used for hypothesis testing.

| **Fixed Effects** | **Estimate [95% CI]** |
|---|---|
| MPXV Neutralizing Titer | 12.84 [6.479, 19.198] |
| Intercept | -48.56 [-438.784, 341.670] |
| **$R^2$** | **0.389** |

## Supplementary Table S6: Statistical Results for Figure S4.

Anti-MPXV H3L IgG titers by vaccine administration route: ID-ID, ID-SC, SC-ID, SC-SC. Estimates [95% Confidence Intervals] and variance explained after fitting linear mixed effects models with random intercept to data from participants with prior smallpox vaccination: Dependent Variable – Anti-H3L IgG titer, Independent Variable – days post vaccine 2. Note: confidence intervals have not been adjusted for multiplicity and should not be used for hypothesis testing.

| **ID-ID** | |
|---|---|
| **Frequency of Longitudinal Measurements** | **Sample size (n = 17)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 0 |
| Individuals with only one measurement at 3 months post Vax 2 | 6 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 11 |
| **Linear Mixed Effects Model with Random Intercept** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -1.124 [-2.532, 0.275] |
| Intercept | 294.353 [71.278, 516.959] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 424.20 [301.269, 604.184] |
| Residual | 88.32 [58.549, 135.85] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.00542 |
| Conditional | 0.9587 |
| **ID-SC** | |
| **Frequency of Longitudinal Measurements** | **Sample size (n = 7)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 1 |
| Individuals with only one measurement at 3 months post Vax 2 | 0 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 6 |
| **Linear Mixed Effects Model with Random Intercept**<br>*Note – issue with model convergence due to small sample size* | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -4.996 [-12.682, 2.690] |
| Intercept | 582.438 [172.041, 992.835] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 0.00 [0.00, INF] |
| Residual | 448.1 [293.152, 640.434] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.1175 |
| Conditional | NA |

| SC-ID | |
|---|---|
| **Frequency of Longitudinal Measurements** | **Sample size (n = 42)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 0 |
| Individuals with only one measurement at 3 months post Vax 2 | 31 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 11 |
| **Linear Mixed Effects Model with Random Intercept**<br>*Note – issue with model convergence* | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -5.180 [-10.017, -0.343] |
| Intercept | 918.062 [402.147, 1433.977] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 0.00 [0.00, INF] |
| Residual | 869.1 [NA, NA] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.078 |
| Conditional | NA |
| **SC-SC** | |
| **Frequency of Longitudinal Measurements** | **Sample size (n = 16)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 1 |
| Individuals with only one measurement at 3 months post Vax 2 | 8 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 7 |
| **Linear Mixed Effects Model with Random Intercept** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -0.6253 [-2.961, 1.476] |
| Intercept | 302.925 [29.405, 577.351] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 322.6 [199.441, 478.368] |
| Residual | 158.8 [97.475, 269.601] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.010 |
| Conditional | 0.807 |

*Note: Marginal $R^2$ is the proportion of variance explained by the fixed effects relative to the overall variance, and conditional $R^2$ is the proportion of variance explained by both fixed and random effects relative to the overall variance.

## Supplementary Table S7: Statistical Results for Figure S5.

For figure S5A: Anti-MPXV H3L IgG titers by HIV status. Estimates [95% Confidence Intervals] and variance explained after fitting linear mixed effects models with random intercept: Dependent Variable – Anti-H3L IgG titer, Independent variable – days post vaccine 2.
For figure S5C: Estimates [95% Confidence Intervals] and variance explained after fitting ordinary linear regression model: Dependent Variable – Anti-H3L IgG titer, Independent Variable – CD4 count. Note: confidence intervals have not been adjusted for multiplicity and should not be used for hypothesis testing.

| **Figure S5A** | |
|---|---|
| **PWH** | |
| **Frequency of Longitudinal Measurements** | **Sample size (n = 14)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 1 |
| Individuals with only one measurement at 3 months post Vax 2 | 5 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 8 |
| **Linear Mixed Effects Model with Random Intercept:** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -5.582 [-13.936, 2.716] |
| Intercept | 1020.639 [268.819, 1774.687] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 729.0 [0.00, 1305.0377] |
| Residual | 752.7 [459.161, 1285.672] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.0627 |
| Conditional | 0.516 |
| **HIV-uninfected** | |
| **Frequency of Longitudinal Measurements** | **Sample size (n = 72)** |
| Individuals with only one measurement at 2 weeks post Vax 2 | 5 |
| Individuals with only one measurement at 3 months post Vax 2 | 41 |
| Individuals with both measurements at 2 weeks and 3 months post Vax 2 | 26 |
| **Linear Mixed Effects Model with Random Intercept:** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| Time (Days) | -1.667 [-3.970, 0.601] |
| Intercept | 443.307 [203.056, 683.460] |
| **Random Effects:** | **SD [95% CI]** |
| Intercept | 508.1 [199.533, 655.024] |
| Residual | 377.0 [282.344, 570.092] |
| **$R^{2*}$** | **Estimate** |
| Marginal | 0.0166 |

| | |
|---|---|
| Conditional | 0.651 |
| **Figure S5C** | |
| **Fixed Effects:** | **Estimate [95% CI]** |
| CD4 count | 2.0465 [0.424, 3.669] |
| Intercept | -1111.032 [-2515.718, 293.655] |
| **$R^2$** | 0.412 |

*Note: Marginal $R^2$ is the proportion of variance explained by the fixed effects relative to the overall variance, and conditional $R^2$ is the proportion of variance explained by both fixed and random effects relative to the overall variance.

## REFERENCES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