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Supplemental information

Monkeypox infection elicits strong antibody and B cell response against A35R and H3L antigens

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Table S1: VPXV donors' data

Donor ID	Age	Sex	Time from vaccination to sample collection (days)
Vpx01	31	Male	39
Vpx02	35	Male	39
Vpx03	44	Male	26
Vpx04	47	Male	26
Vpx05	35	Male	39
Vpx06	34	Male	39

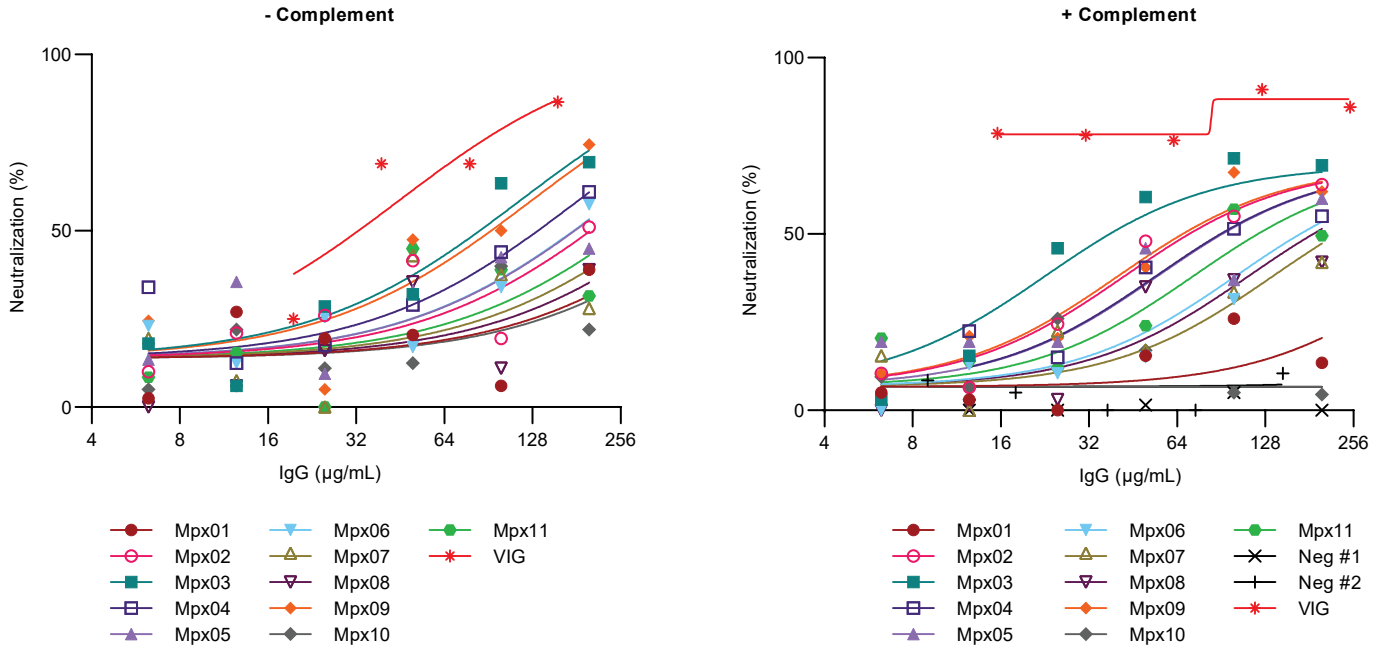

Figure S1: Serum response against VACV in PRNT. Related to Figure 1.
VACV Lister neutralization of infection by PRNT in Vero cells of 6 consecutive dilutions of MPXV purified IgG, starting from 200 µg/mL. Trend lines represent nonlinear regression of each MPXV recoveree (individual donors' symbols are indicated below the graph). VIG serves as a positive control shown in red, while the uninfected non-vaccinated sera (Neg#1 and Neg#2) are in black. Left panel: without complement. Right panel: with complement.

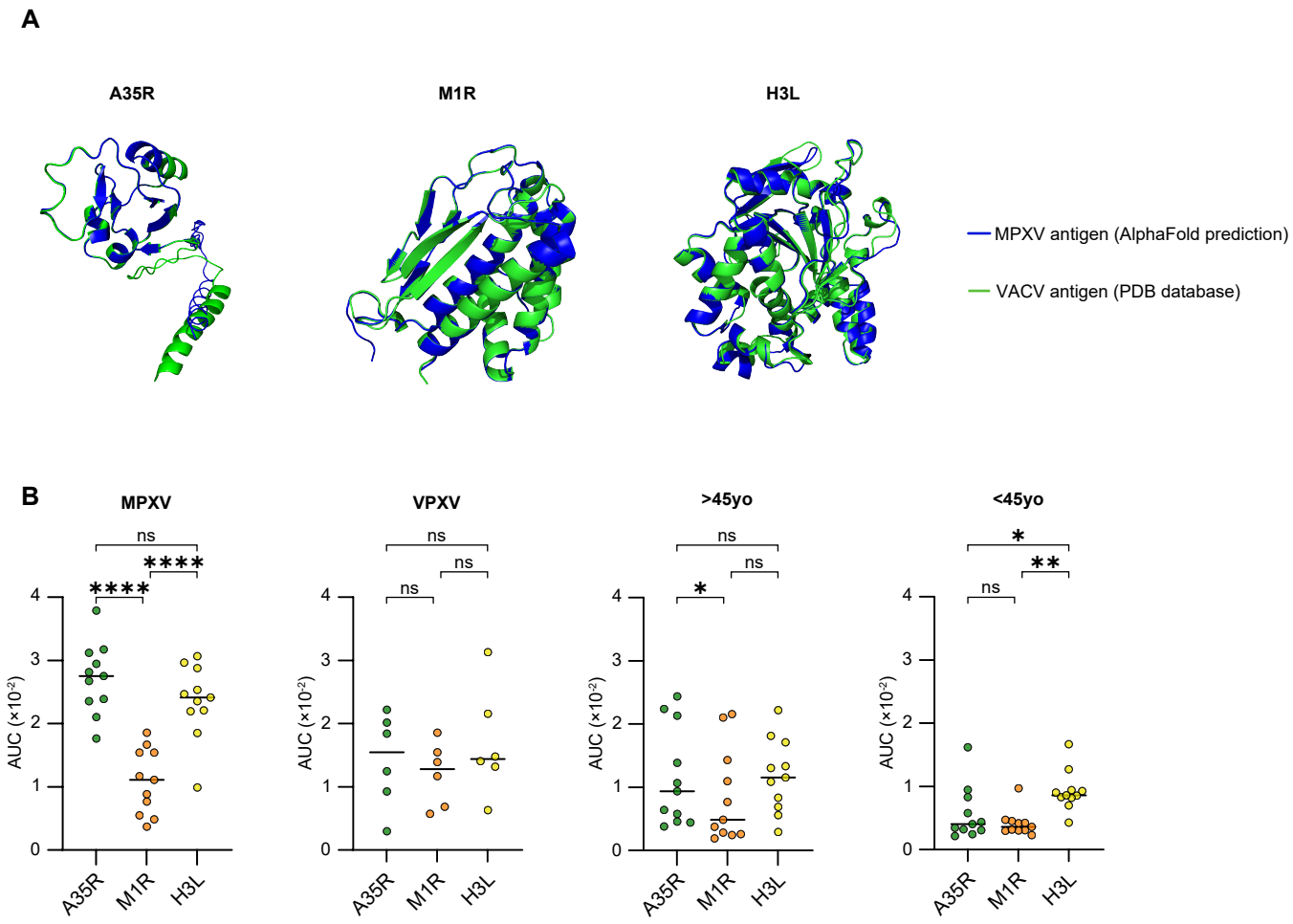

Figure S2: In silico prediction and sera response to recombinantly expressed MPXV antigens. Related to Figure 2. **(A)** In silico 3-dimensional cartoon representation of the recombinantly expressed MPXV antigens. AlphaFold predicted structures of the truncated A35R, M1R and H3L expressed antigens (blue) were superimposed onto the atomic structures of their corresponding truncated VACV homologs (green) as described in Figure 2A. His- and Avi-tags are not shown. Images generated using PyMOL version 2.5.2. **(B)** Serum response by ELISA against A35R (green), M1R (orange) and H3L (yellow). AUC values of MPXV recoverees (n=11), VPXV (n=6), uninfected >45yo (n=11) and uninfected <45yo (n=11) are shown. Statistical analysis was performed using One-way ANOVA. *p<0.05, **p<0.01, ****p<0.0001.

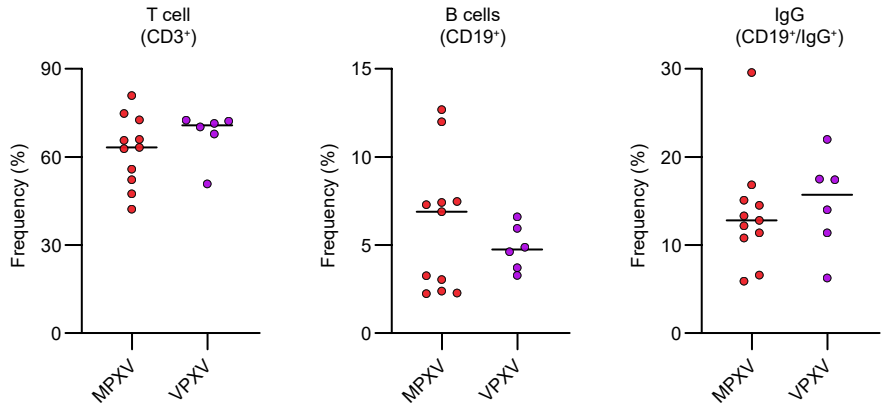

Figure S3: Frequencies of MPXV recoverees' lymphocytes. Related to Figure 3.
Left, middle and right panels: frequency of CD3+, CD19+ and IgG+ B cells (gated from CD19+ population), respectively, from PBMCs of MPXV recoverees (n=11) and VPXV (n=6) as detected by Flow Cytometry.