SUPPLEMENTARY MATERIALS

FUNCTIONAL DELINEATION OF A PROTEIN-MEMBRANE INTERACTION HOTSPOT SITE ON THE HIV-1 NEUTRALIZING ANTIBODY 10E8

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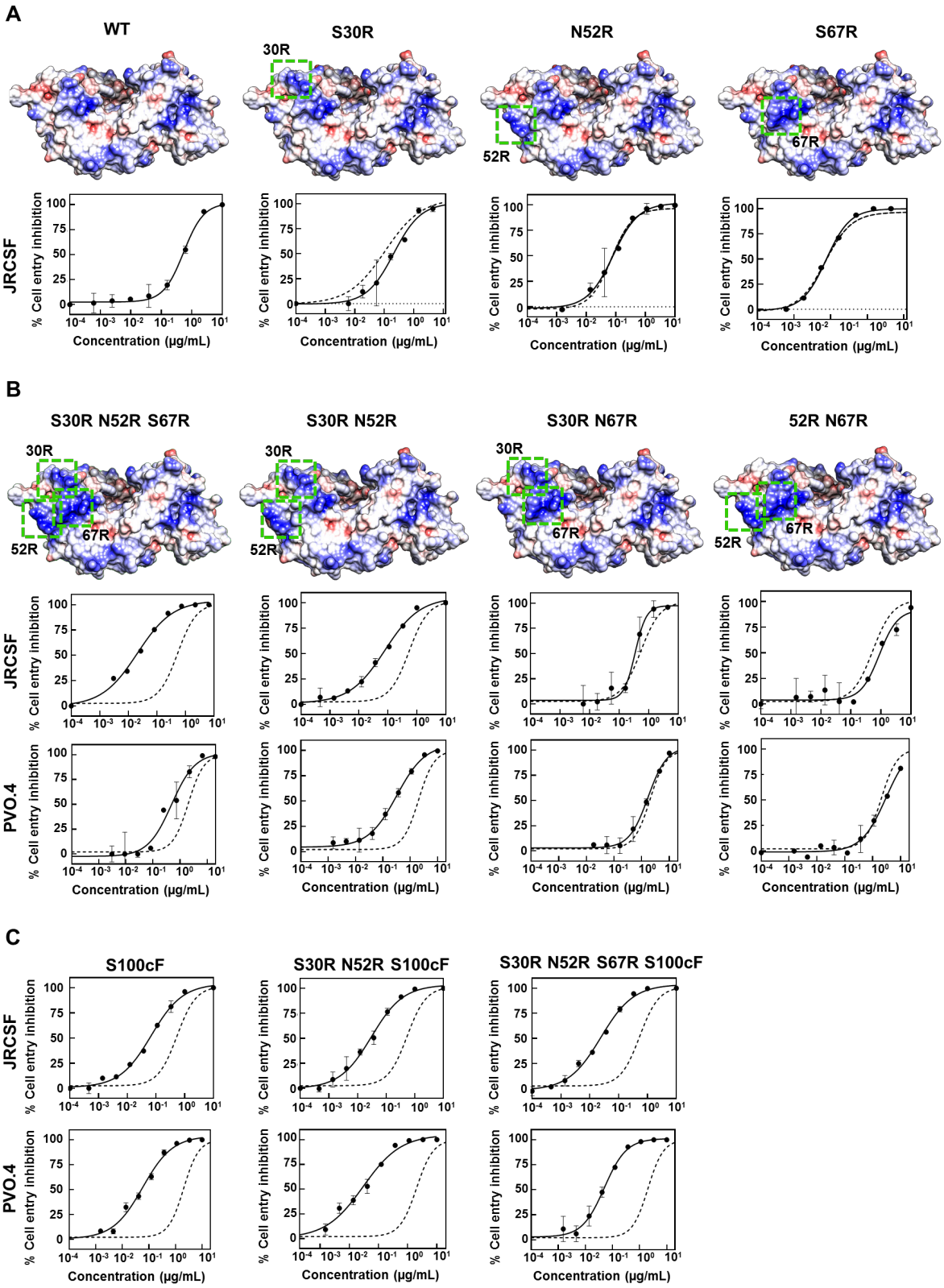
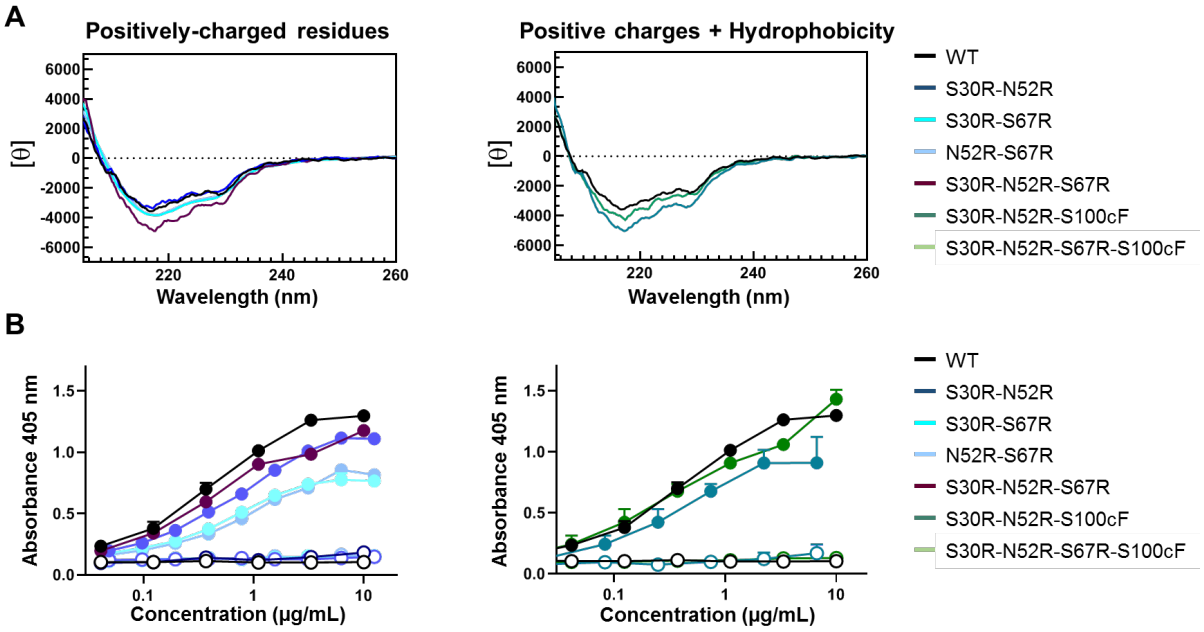
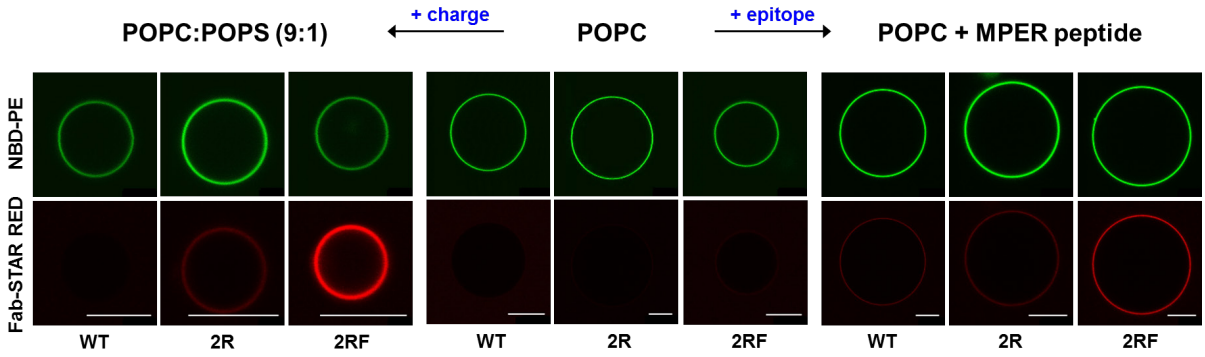

A
WT
S30R
30R
N52R
52R
S67R
67R
JRCSF
% Cell entry inhibition
Concentration (μg/mL)
B
S30R N52R S67R
S30R N52R
S30R N67R
52R N67R
PVO.4
C
S100cF
S30R N52R S100cF
S30R N52R S67R S100cF

Supplementary Figure S1 (previous page): Cell-entry inhibition by 10E8 Fab mutants. Surface density charge representation and neutralization curves of individual (A) and combined (B) Arg mutants. The regions where the mutations are located are highlighted with a green box. C) Neutralization curves of S100cF substitution-containing mutants. 10E8-WT Fab is shown as a dotted line in each plot for comparison.



Supplementary Figure S2: Characterization of 10E8 mutant Fabs. (A) Secondary structure by CD and (B) ELISAs to determine functional binding to $MPER_{671-693}$ epitope peptide (filled symbols) and $_{mut}MPER_{671-693}$ peptide (empty symbols), the latter included as a control for binding specificity.



Supplementary Figure S3: Representative images of POPC vesicle binding experiments in the absence (middle) and presence (right) of epitope, and in vesicles including the anionic phospholipid POPS without epitope (left). Scale bars, 5 μm

Supplementary Table S1: Kinetic parameters of binding of Fab 10E8 variants to MPER-$TMD_{671-709}$ peptide reconstituted in SLBs

Fab	K_D **(M)**	k_a **(M^{-1} s^{-1}))**	k_{off} **(s^{-1})**
WT	$1.0 \cdot 10^{-9} \pm 0.3 \cdot 10^{-9}$	$1.9 \cdot 10^{6} \pm 0.3 \cdot 10^{6}$	$1.9 \cdot 10^{-3} \pm 0.6 \cdot 10^{-3}$
2R	$1.1 \cdot 10^{-9} \pm 0.4 \cdot 10^{-9}$	$1.5 \cdot 10^{6} \pm 0.5 \cdot 10^{6}$	$1.6 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3}$
2RF	$6.3 \cdot 10^{-10} \pm 5 \cdot 10^{-10}$	$7.3 \cdot 10^{5} \pm 6 \cdot 10^{5}$	$5.1 \cdot 10^{-4} \pm 6 \cdot 10^{-4}$