Supporting Information

Calcium ions directly interact with the Ebola virus fusion peptide to promote structure-function changes that enhance infection

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This document contains the supplementary figures for the article listed above, on 12 consecutive pages.

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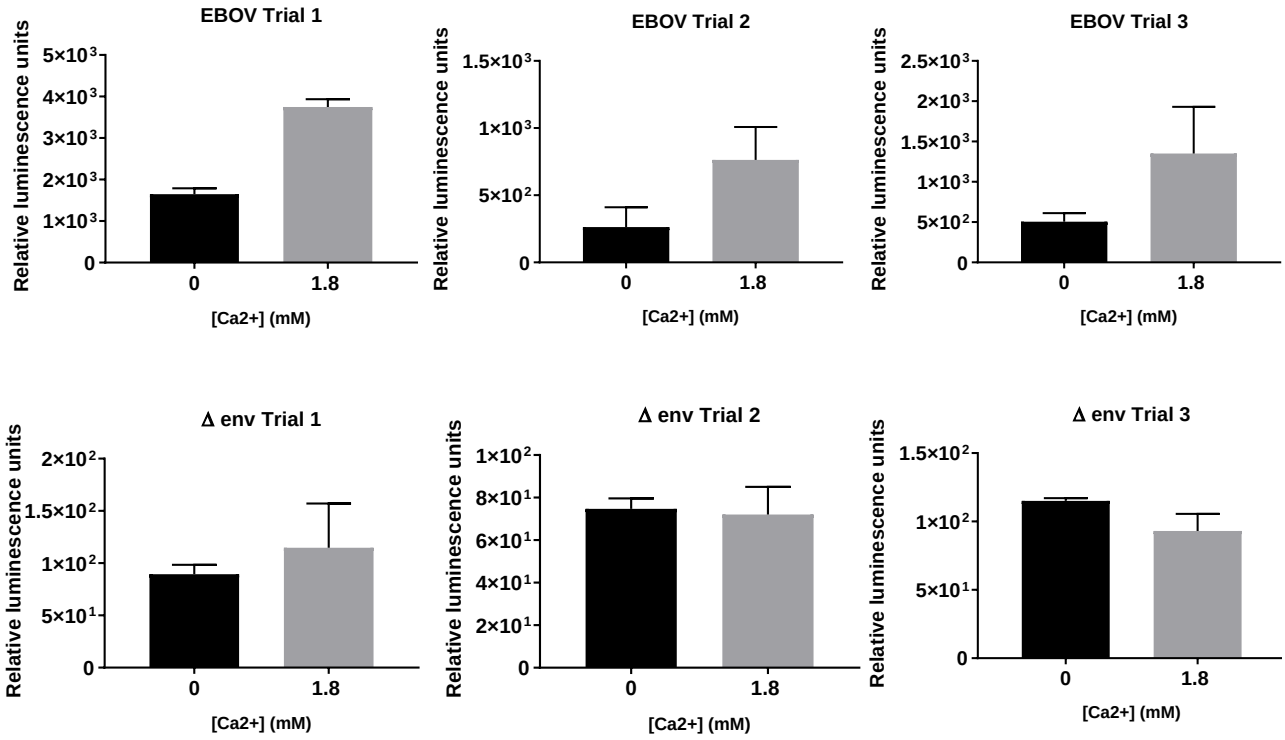
8) Western blot confirming removal of mucin cap from pseudovirus GP
9) Lipid mixing data from pseudovirus/lipid mixture
10) Helical contents (%) vs Ca^{2+} concentration based on CD spectroscopy.

a)



b)

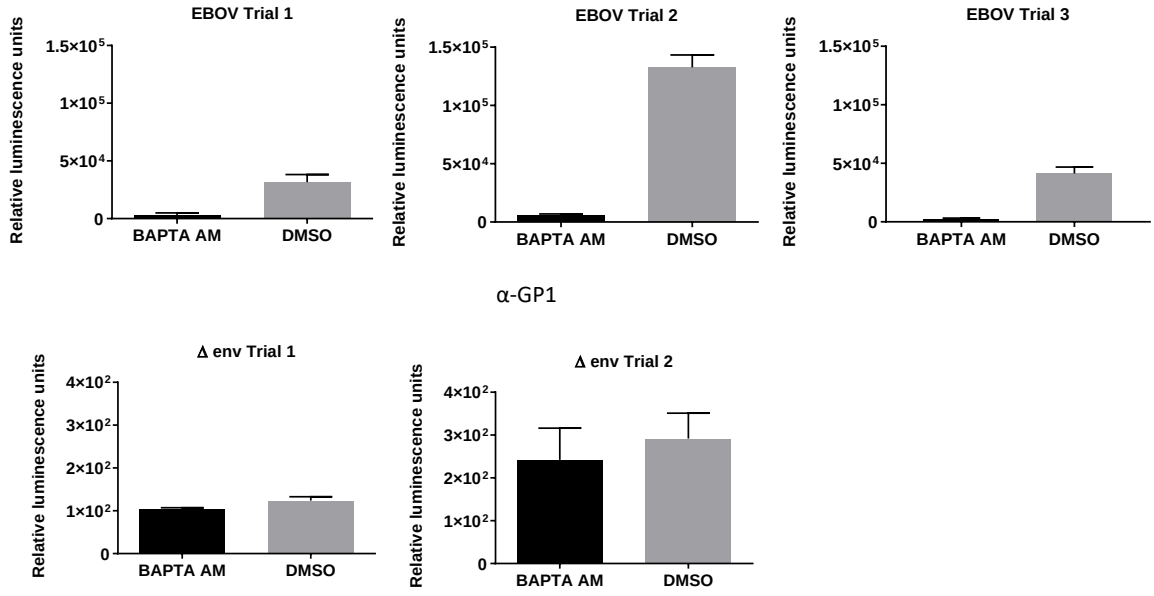


Fig. S1. Raw data of a) extracellular and b) intracellular calcium experiments. Δ env is a pseudotyped virus that lacks a spike protein and thus serves as a non-infectious negative control. It is produced by omitting the spike protein plasmid during transfection of HEK 293T cells for pseudotyped virus production. Error bars represent s.d. among 3 technical replicates. Note the difference in scale between graphs.

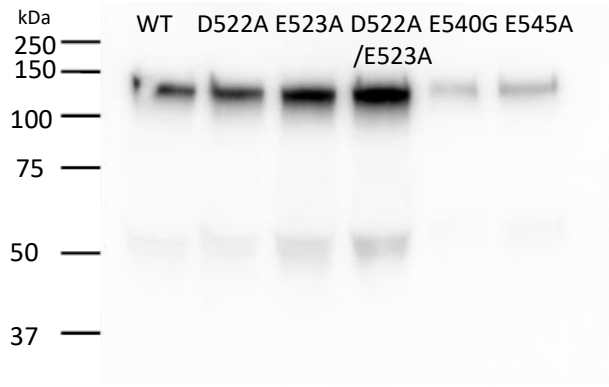


Fig. S2. Western blot confirming incorporation of WT and mutated GP into pseudotyped virus particles. Supernatant from transfected cells was harvested 48 h post-transfection, filtered through a 0.45 μm membrane, and stored at -80°C. After thawing from -80 °C, particles were pelleted at 42000 rpm and resuspended in 30 μL PBS to concentrate the sample prior to blotting.

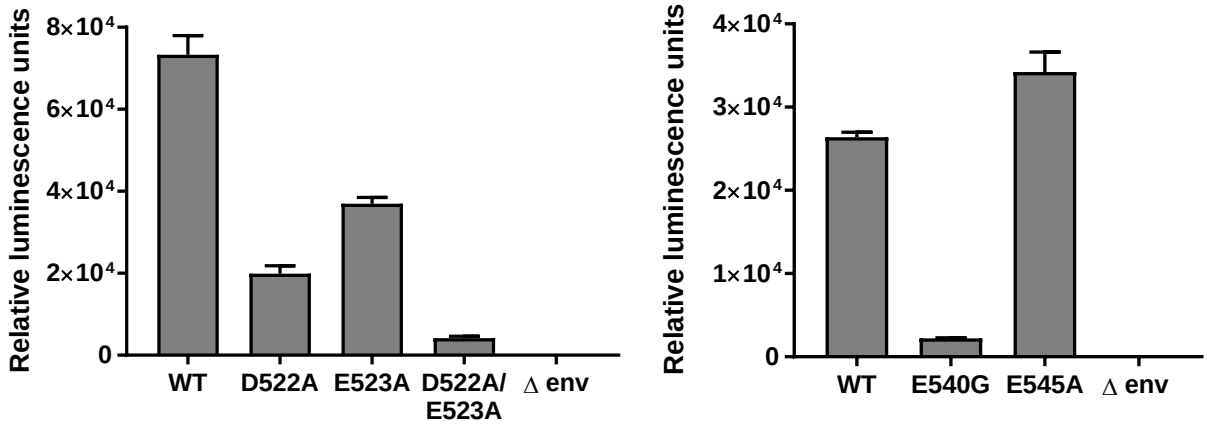


Fig. S3. Infectivity of mutant GP pseudotyped virus and non-infectious control (Δ env). Error bars indicate s.d. among 3 technical replicates from testing the same batch of virus.

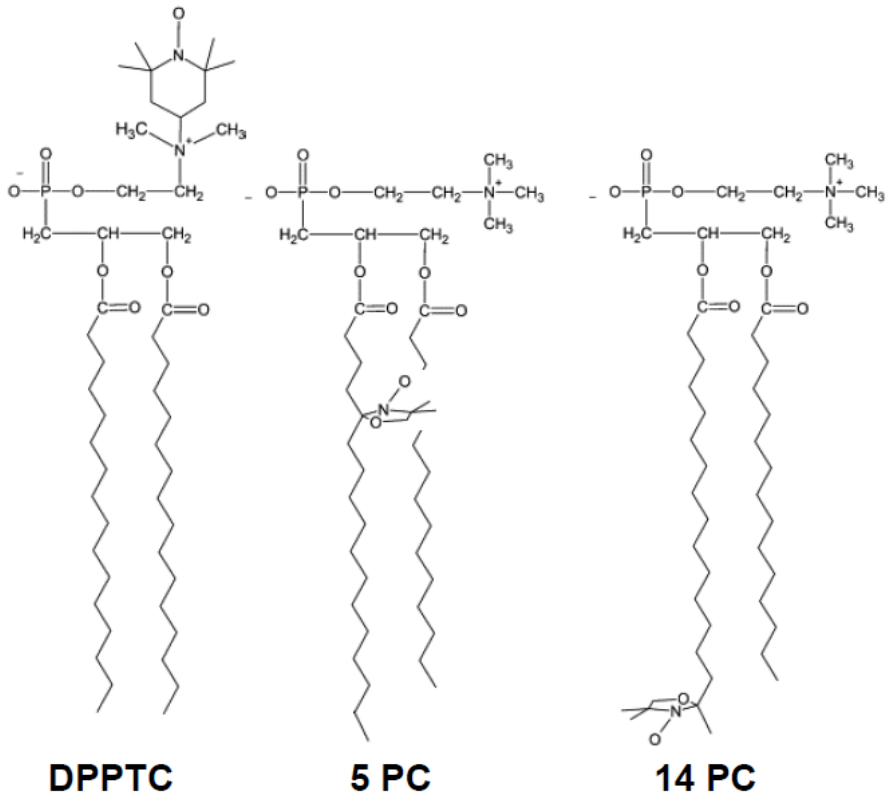


Fig. S4. Structure of spin labeled lipids used in this study.

Long FP 501-560

G S I E G R A Q P K C N P N L H Y W T T Q D E **G A A I G L A**

W I P Y F G P A A E G I Y I E G L M H N Q D G L I C G L R Q

Short FP 524-539

G A A I G L A W I P Y F G P A A

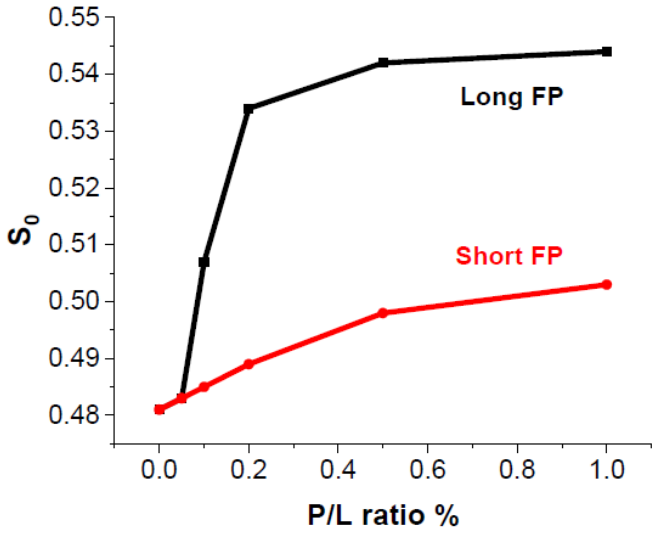


Fig. S5. The long FP has a stronger ordering effect than the short FP. Plot of order parameter (S_0) of DPPTC versus peptide:lipid ratio (P/L ratio) of FP.

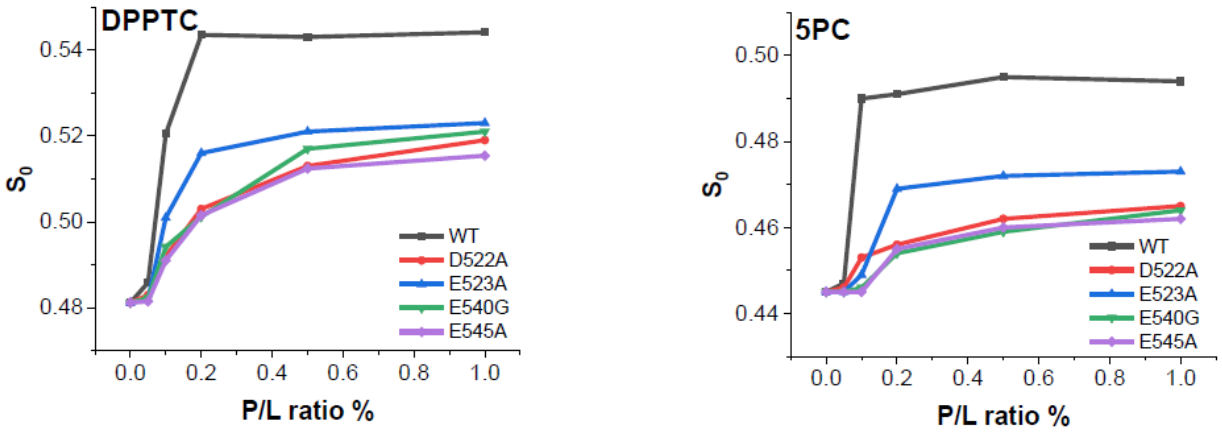


Fig. S6. Comparing the membrane ordering effects of the WT and mutant EBOV FPs on DPPTC (left) and 5PC (right) as a function of peptide:lipid ratio (P/L ratio). Black, WT FP; red, D522A; blue, E523A; green,E540G ; purple, E545A.

Fig S7A

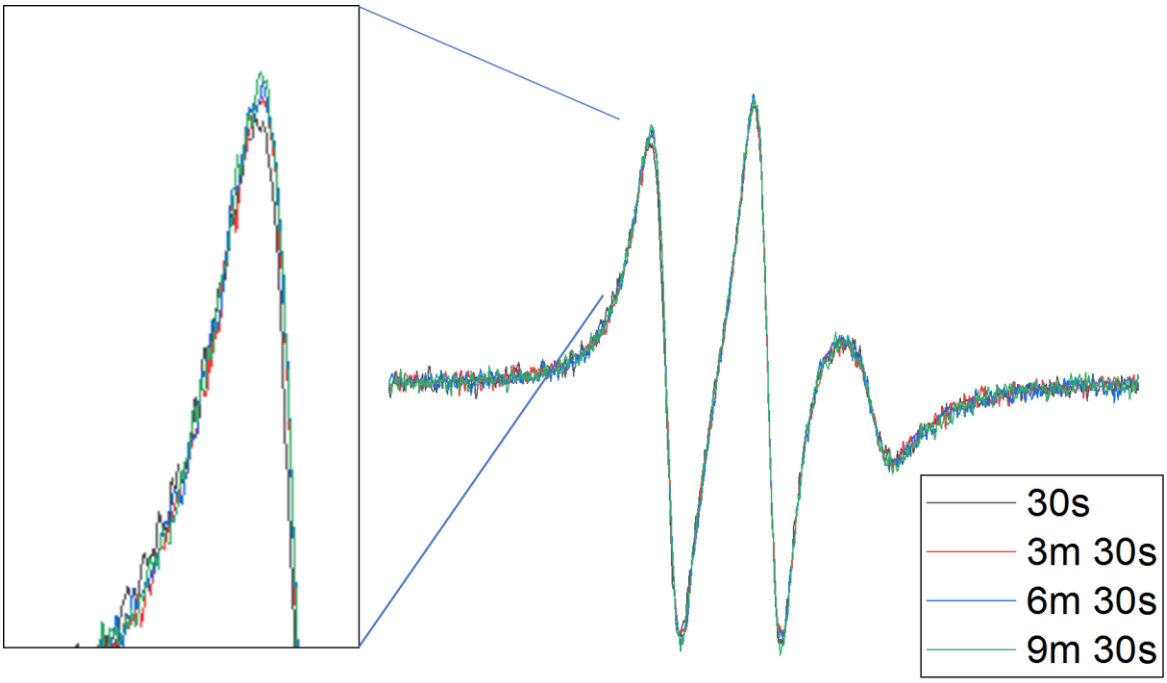


Fig S7B

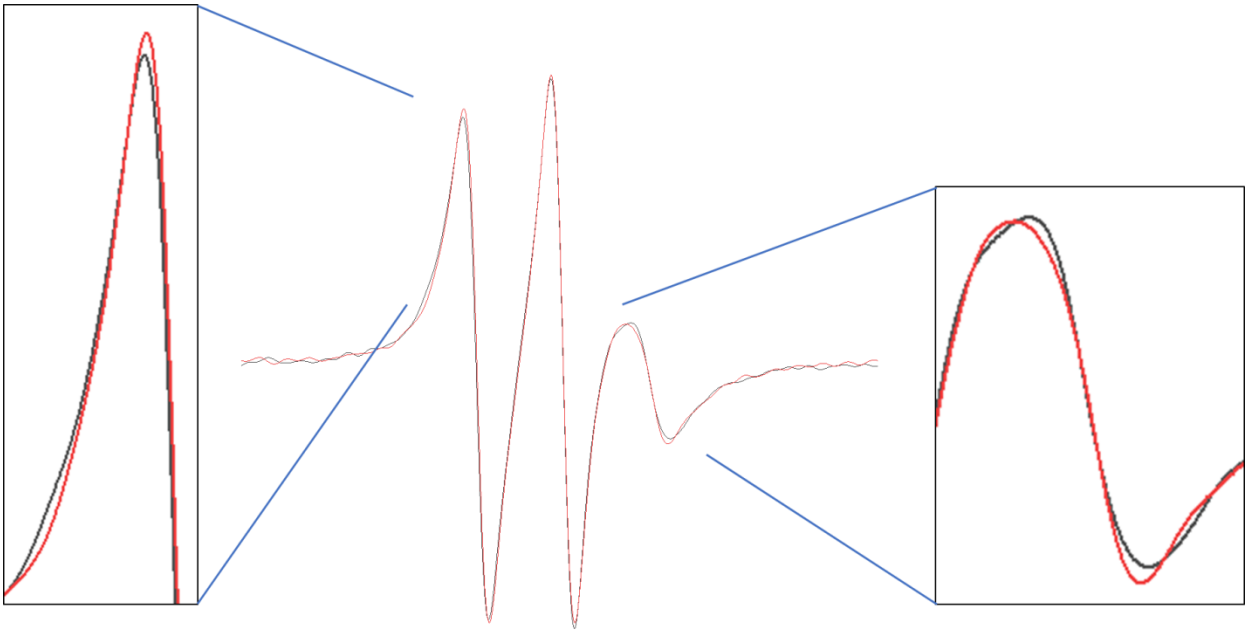


Fig S7C

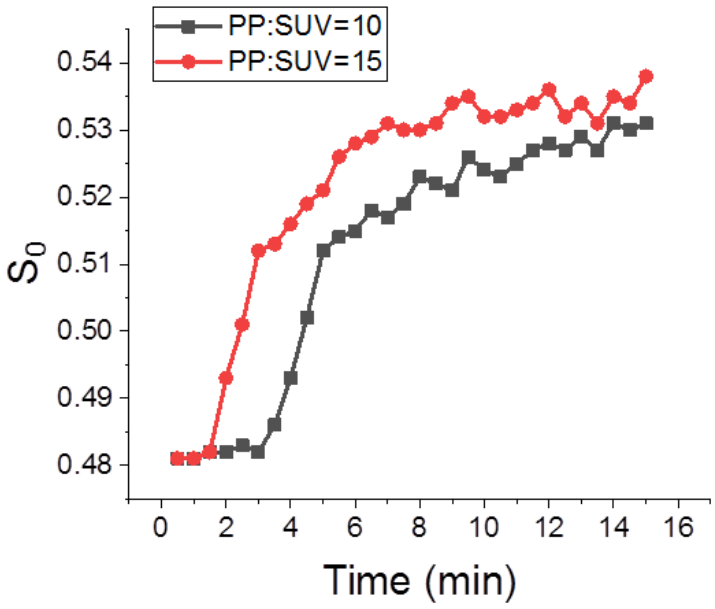


Fig. S7. (A) The shape of ESR curves of DPPTC on SUV change after the activation by acidification in the SUV:PP mixture. Black, collected at 0.5 min, red, at 3.5 min, blue, at 6.5 min, and green at 9.5 min. (B) after Wavelet Denoising, the change is more obvious, black, collected at 0.5 min, red, at 3.5 min. (C) the plot of S0-time, showing the "jump" time is related to the PP:SUV ratio. Black, PP:SUV=10, red, PP:SUV=15.

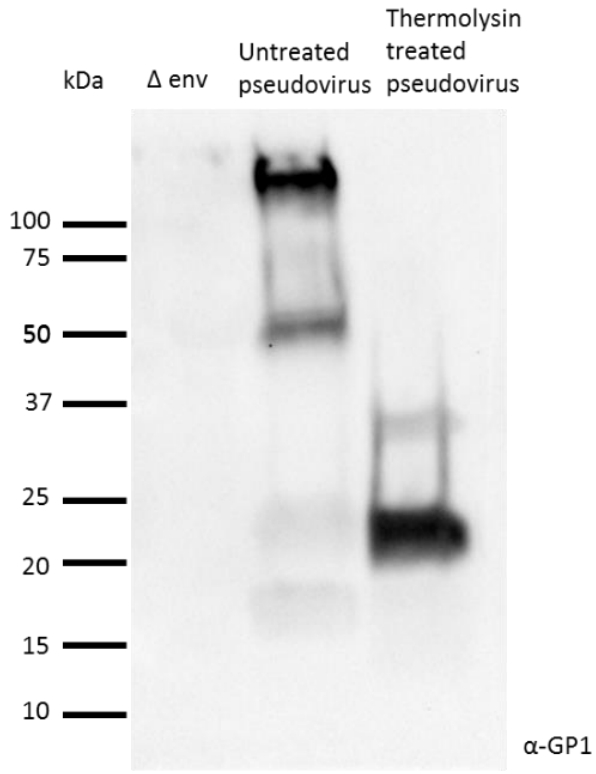


Fig. S8. Treatment with thermolysin removes the glycan cap.

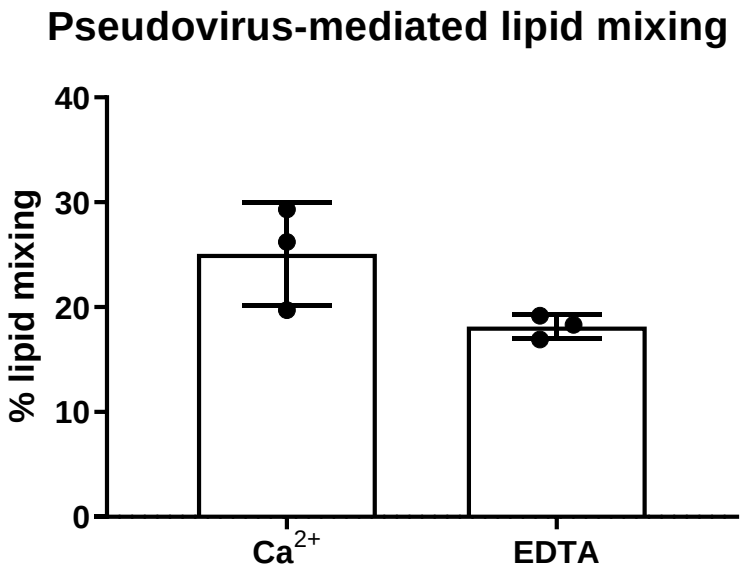


Fig. S9. Lipid mixing mediated by pseudovirus.

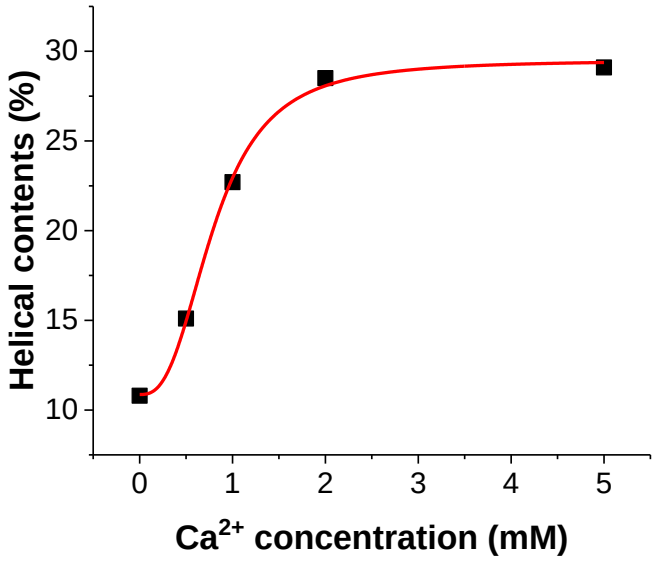


Fig. S10. Helical contents (%) vs. Ca^{2+} concentration based on CD spectroscopy. The red line is a fit to the logistic function equation.