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Supplementary Materials

Cholesterol Modulates the Interaction Between HIV-1 Viral protein R and Membrane

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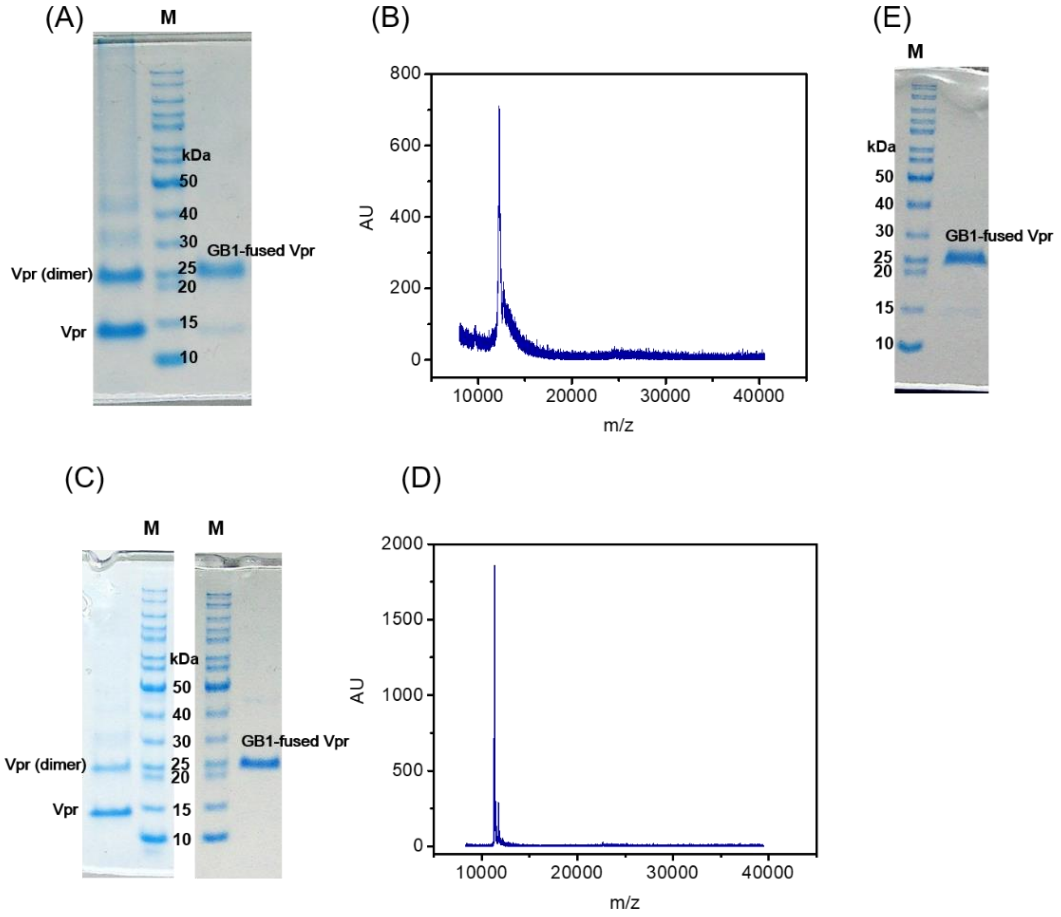

Figure S1. Examples of the characterizations of Vpr proteins. (**A**) The SDS-PAGE characterization of [U-^{2}H,^{13}C,^{15}N] labeled His-tagged GB1-fused Vpr and urea solubilized [U-^{2}H,^{13}C,^{15}N] labeled Vpr. (**B**) MALDI-TOF mass spectrum of [U-^{2}H,^{13}C,^{15}N] labeled Vpr. (observed: 12.37 kDa; theoretical: 12.67 kDa) (**C**) The SDS-PAGE of [1-^{13}C, 99%] Cysteine selective labeled His-tagged GB1-fused Vpr and urea solubilized [1-^{13}C, 99%] Cysteine selective labeled Vpr. (**D**) MALDI-TOF mass spectrum of [1-^{13}C, 99%] Cysteine selective labeled Vpr. (observed: 11.33 kDa; theoretical: 11.33 kDa) (**E**) The SDS-PAGE characterization of natural abundance His-tagged GB1-fused Vpr used for calcein release assay.

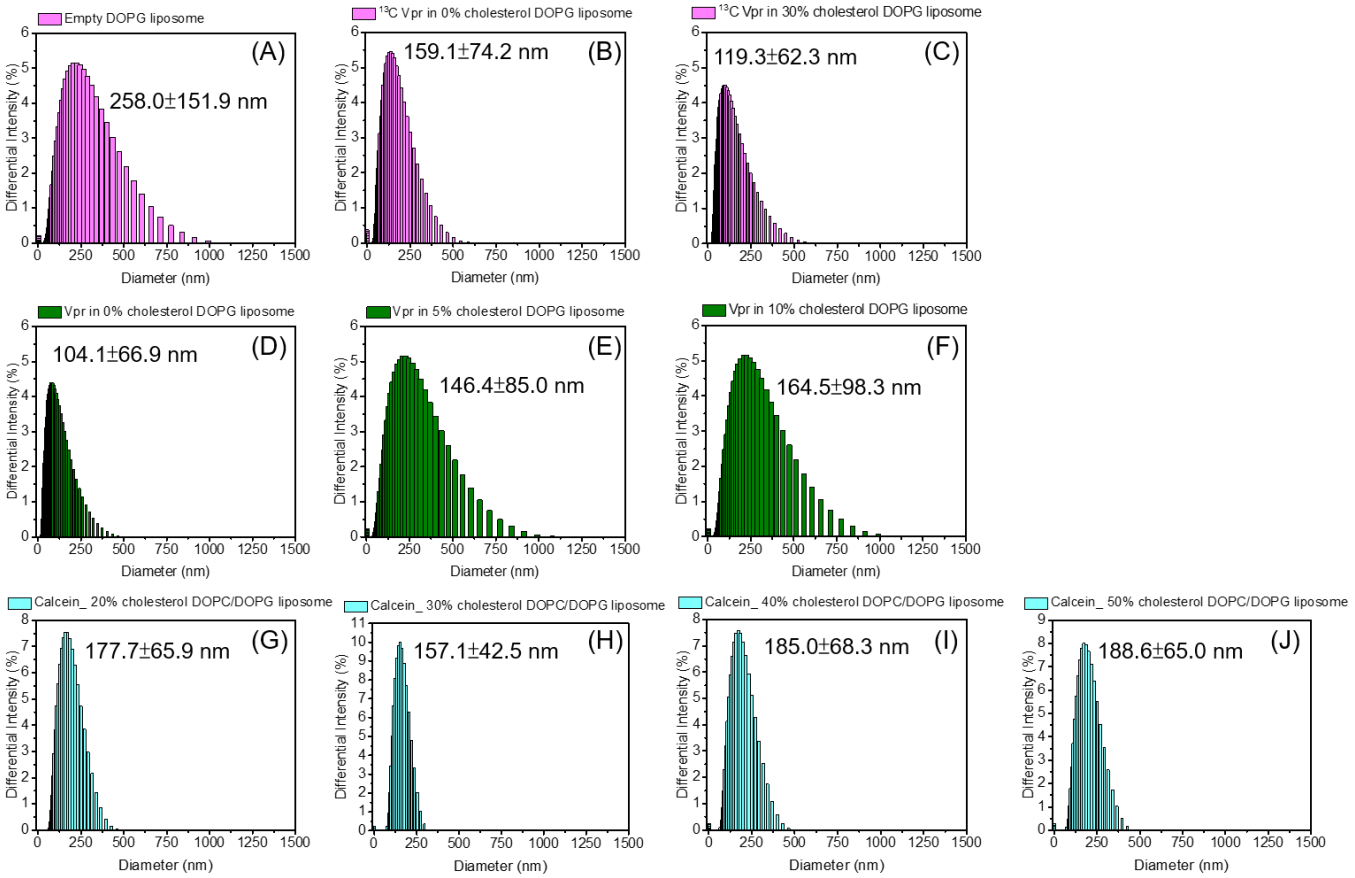

Figure S2. Intensity distribution of liposomes measured by dynamic light scattering. (**A**–**C**) Liposomes used in the ssNMR experiments. (**D**–**F**) Liposomes used in the CD spectra measurements. (**G**–**J**) Liposomes used in the calcein release assay.

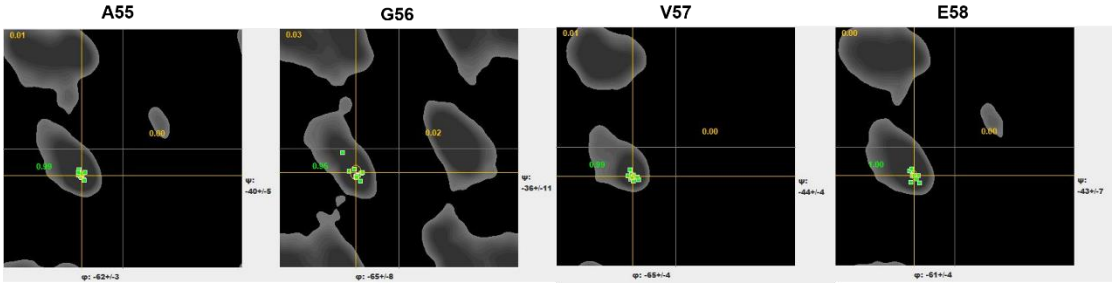

Figure S3. Ramachandran plot of the assigned protein segments from A55 to E58 shows well defined left-handed α-helical structure predicted by Talos+.

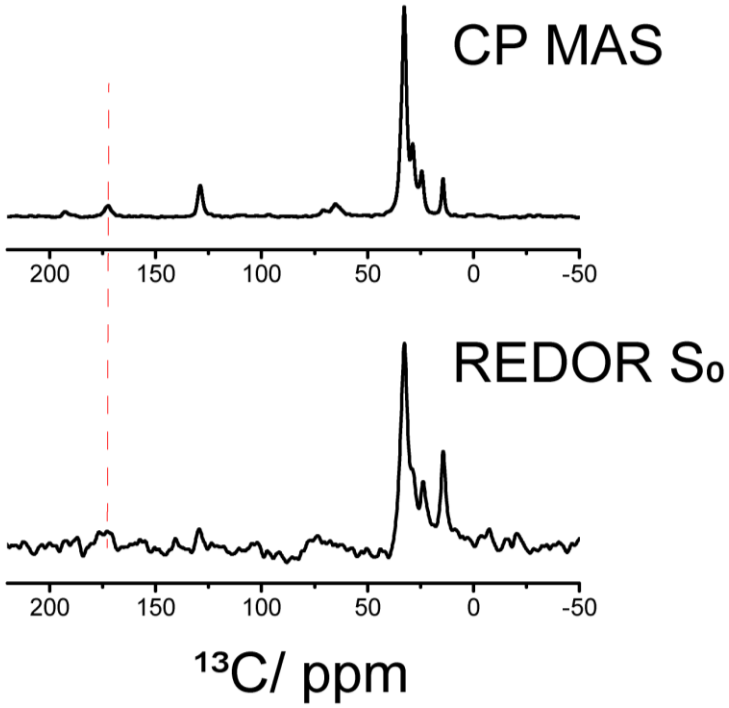

Figure S4. The ^{13}C CPMAS spectrum and $^{13}C\{^{31}P\}$REDOR S_0 spectrum of the DOPG liposome sample. The $^{13}C\{^{31}P\}$REDOR S_0 spectrum was collected with 20 ms evolution time, but without the dephasing pulses turned on, which was the same experimental condition as in Figure 5. It is obvious that the contribution of DOPG lipid to the resonance signal around 170 ppm is trivial.

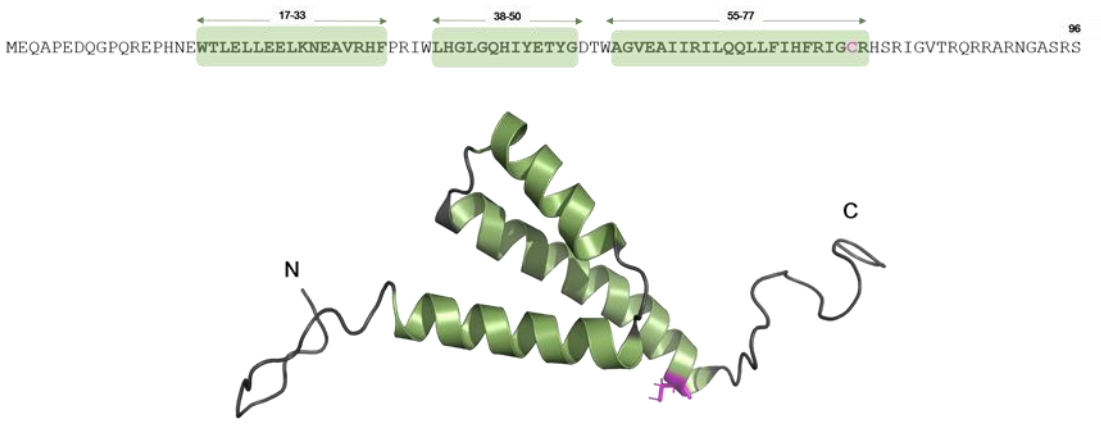

Figure S5. The sequence of Vpr protein and its structure (PDB: 1M8L) with 3 α-helical segments (green) and Cys-76 (magenta) specified.