

Supplemental Information

Binding of the SARS-CoV-2 envelope E protein to human BRD4 is essential for infection

Kendra R. Vann, et al.

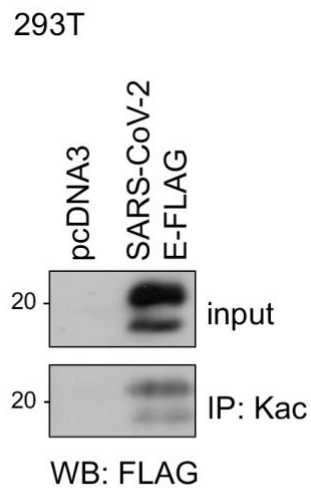


Figure S1. The SARS-CoV-2 E protein is acetylated in 293T cells, Related to Figure 1. Duplicate immunoprecipitation experiment with anti-acetyl-lysine antibodies on whole cell extracts from 293T cells expressing FLAG-tagged SARS-CoV-2 E protein or empty tag (pcDNA3) followed by western blot with FLAG antibody indicates that SARS-CoV-2 E is acetylated *in vivo*.

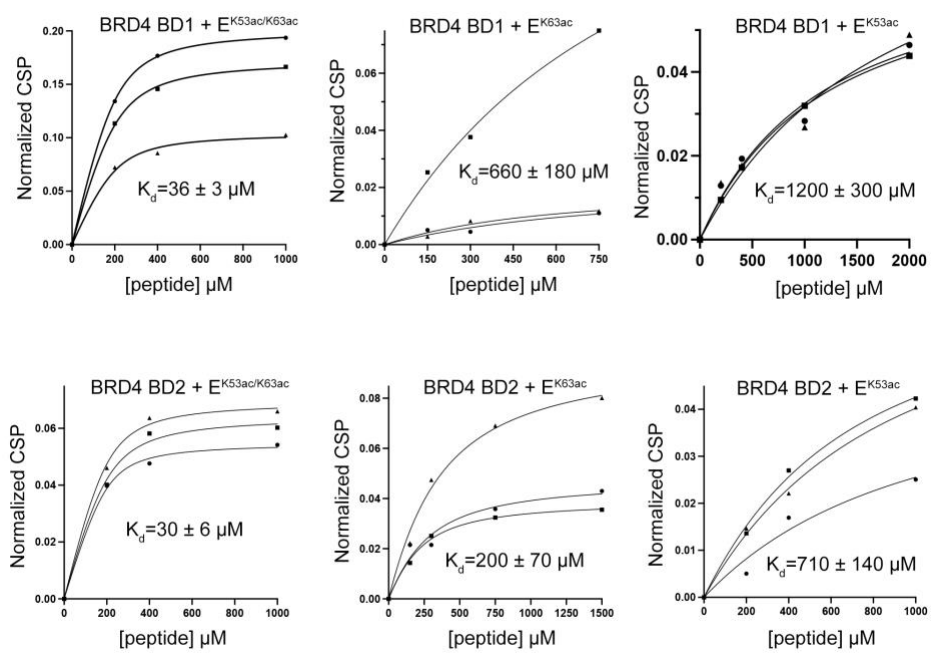


Figure S2. Binding affinities of BRD4 BD1 and BD2 for the indicated acetylated SARS-CoV-2 E peptides, Related to Figure 1. Representative binding curves used to determine K_d values by NMR.

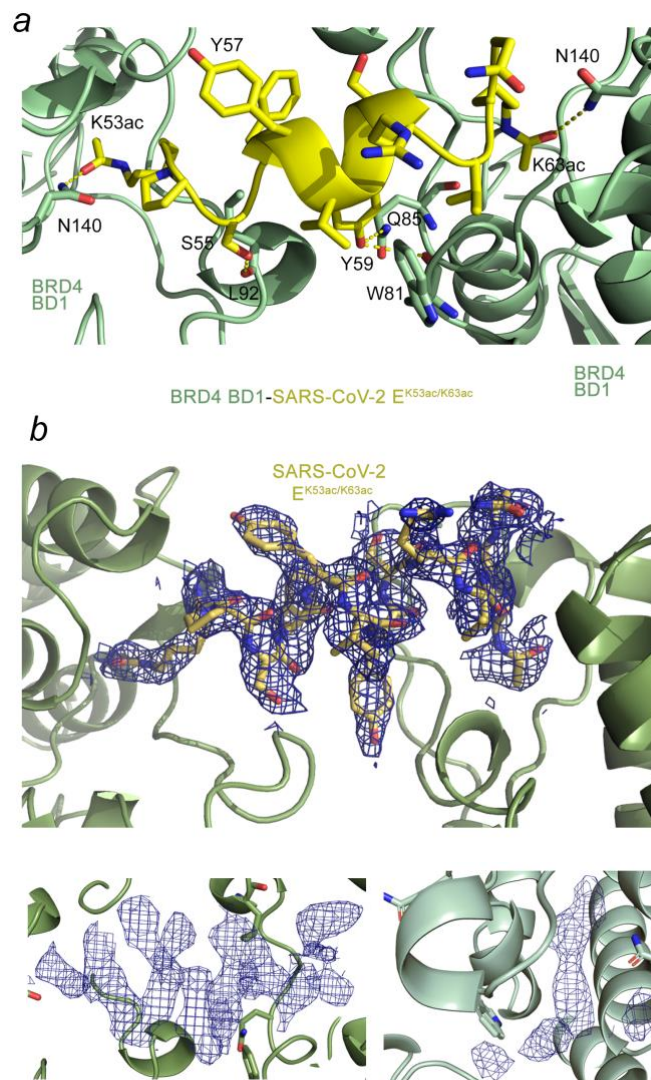


Figure S3. Structure of BRD4 BD1 in complex with SARS-CoV-2 E^{K53ac/K63ac}, Related to Figure 2. (a) A zoom in view of the crystal structure of BRD4 BD1 (green) in complex with the SARS-CoV-2 E^{K53ac/K63ac} peptide (aa 53-64 of SARS-CoV-2 E, yellow). Yellow dashed lines represent hydrogen bonds. (b) 2Fo - Fc electron density map of the SARS-CoV-2 E^{K53ac/K63ac} peptide, contoured at 1 σ with the peptide shown. Below, 2Fo - Fc electron density maps of the SARS-CoV-2 E^{K53ac/K63ac} peptide (left) and SARS-CoV-2 E^{K63ac} peptide (right) without peptides shown.

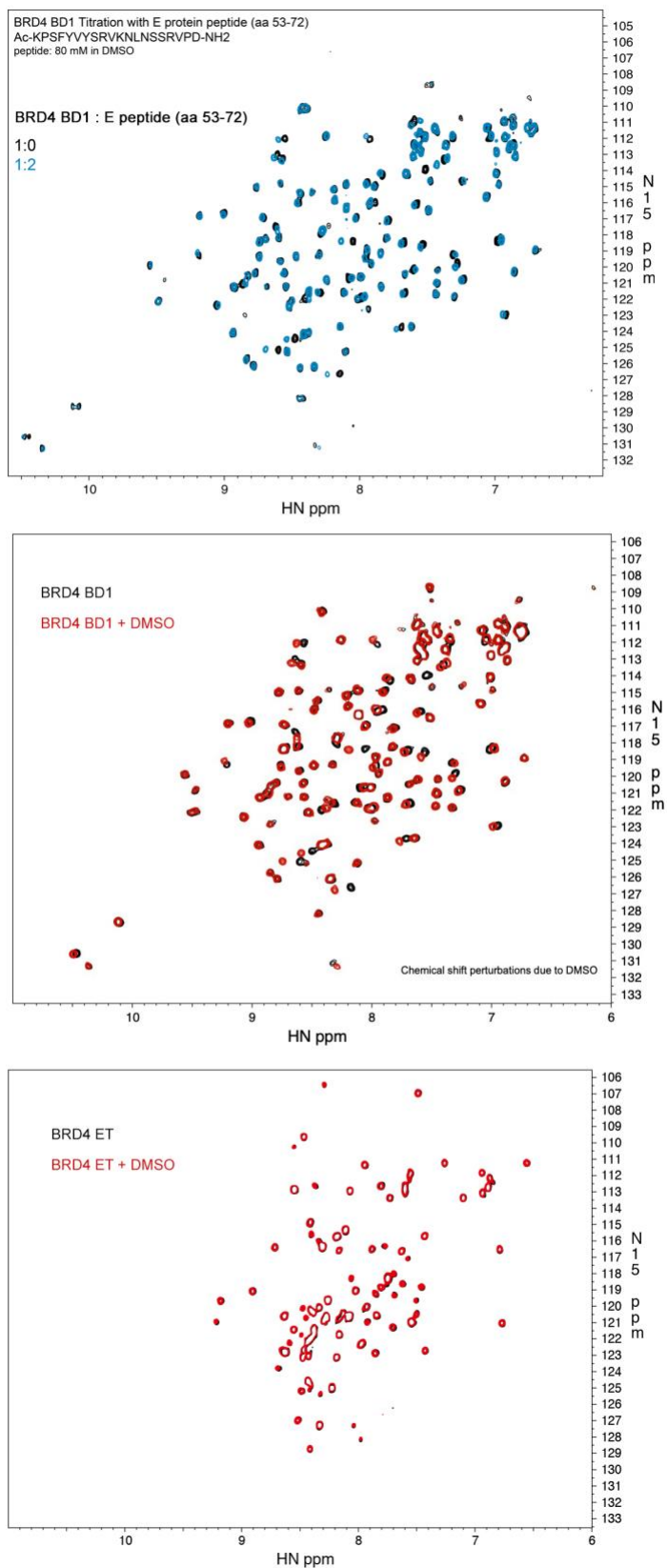
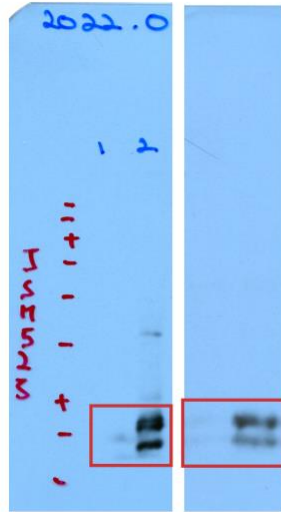
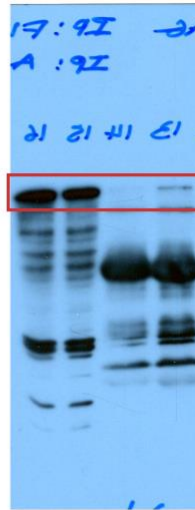


Figure S4. BRD4 BD1 does not recognize non-acetylated SARS-CoV-2 (aa 53-72), Related to STAR methods and Figures 1 and 3. Overlaid ^1H , ^{15}N HSQC spectra of ^{15}N -labeled BRD4 BD1 recorded before and after addition of non-acetylated SARS-CoV-2 E (aa 53-72) peptide (80 mM stock in DMSO) (top) or the same amount of DMSO (middle). Similar CSPs in both experiments are due to binding of DMSO. Likewise, BRD4 BD2 was shown to not recognize non-acetylated SARS-CoV-2 E peptide by Chen et al, 2021, BioRxiv. (bottom) Absence of CSPs in ^1H , ^{15}N HSQC spectra of ^{15}N -labeled BRD4 ET upon addition of DMSO indicates that DMSO does not bind to the ET domain.



Related to Fig. 1b



Related to Fig. 1c

Figure S5. Uncropped western blots, Related to STAR methods and Figure 1.