

Supporting Information

Development of a dendritic cell-targeted vaccine strategy using proximity-induced conjugation

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I. Supplemental Tables

Table S1. Summary of antibodies

Table S2. Summary of amino acid sequences of DC-targeted scFv

II. Supplemental Figures

Figure S1. Optimization of DC-targeted scFv conformations.

Figure S2. Binding affinity analysis of α mCD205 scFv following Fc fusion.

Figure S3. LC-MS analysis of HTB1 (A24pBPA)-OVA and HTB1 (K28pBPA)-OVA.

Figure S4. Comparison of conjugation efficacy of A24 and K28 sites.

Figure S5. α mCD205 affinity detection of HTB1-OVA and HTB1 (A24pBPA)-OVA.

Figure S6. Optimization of photo-reactive PIC (P-PIC) conditions.

Figure S7. Assessment of antigen integrity of HTB1 (A24pBPA)-OVA-Flag following UV exposure.

Figure S8. Enhanced antigen uptake mediated conjugation to α hCD205.

Figure S9. Analysis of CD4⁺ TILs and serum OVA-specific antibody levels.

Figure S10. Schematic illustration of the location and functional epitopes within T3 EER.

Figure S11. LC-MS analysis of HTB1 (A24pBPA)-T3.

Figure S12. Assessment of antigen integrity of HTB1 (A24pPBA)-T3 following UV exposure.

Figure S13. Analysis of crosslinking efficiency and binding affinity of α mCD205-T3_{P-PIC}.

Figure S14. Analysis of crosslinking efficiency and binding affinity analysis of α hCD205-T3_{P-PIC}.

Figure S15. Optimization of chemically-reactive PIC (C-PIC) adaptors.

Figure S16. Synthesis route and characterization of FPheK and verification.

Figure S17. LC-MS analysis of C-PIC products.

Figure S18. Assessment of antigen integrity of MinZ (E25FPheK)-OVA after incubation.

Figure S19. Analysis of antigen uptake and binding affinity of the C-PIC vaccine.

Figure S20. Analysis of crosslink efficiency and binding affinity of the C-PIC vaccine.

Figure S21. Evaluation of the C-PIC vaccine in the B16-OVA therapeutic tumor model.

Figure S22. Serum IL-6 and TNF- α levels following vaccination.

III. Reference

IV. Plasmid Sequences

V. Protein Sequences

I. Supplemental Tables

Table S1 summary of antibodies

Reagent	Source	Identifier
Anti-mouse CD3 ϵ PE	Biolegend	Cat#100308
Anti-mouse CD8a FITC	Biolegend	Cat#100706
Anti-mouse IFN- γ APC	Invitrogen	Cat#17-7311-82
Anti-RAB7 AF647	Abcam	ab198337
Anti-Flag tag AF488	Proteintech	CL488-80010

Table S2. Summary of amino acid sequences of DC-targeting scFv

Name	Sequence
NLDC VL	DIQMTQSPSFLSTSLGNSITITCHASQNIKGWLAWYQQKSGNAPQLLI YKASSLQSGVPSRFSGSGSGTDYIFTISNLQPEDIATYYCQHYQSFPW TFGGGTKLEIKRAA
NLDC VH	EVKLQQSGTEVVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLE WIGWIFPGEGSTEYNEKFKGRATLSVDKSSSTAYMELTRLTSEDSAVY FCARGDYRRYFDLWGQGTTVTVSS
MG38-3 VL	QAVVTQESALTTSPGETVTLTCRSSTGAVTISNYANWVQEKPDHLFTG LIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYNN QFIFGSGTKVTVL
MG38-3 VH	EVQLQQSGPVLVKPGASVKMSCKASGNTFTDSFMHWMKQSHGKSL EWIGIINPYNGGTSYNQKFKGKATLTVDKSSSTAYMELNSLTSEDSAV YYCARNGVRRYYFDYWGQGTTTLTVSS

II. Supplemental Figures

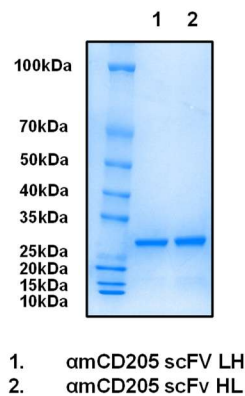
A



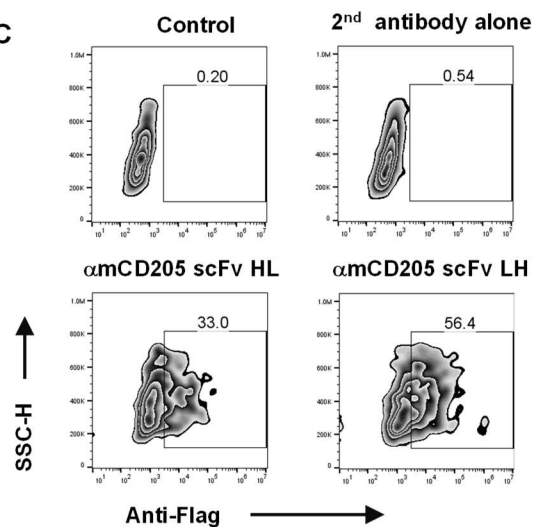
α mCD205 scFv LH

α mCD205 scFv HL

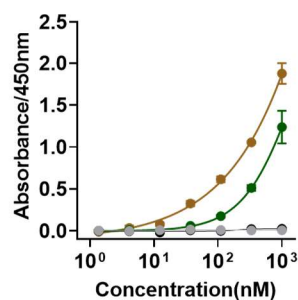
B



C



D



E

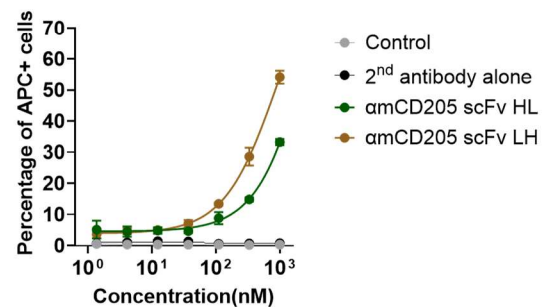


Figure S1. Optimization of DC-targeted scFv conformations.

(A) Schematic illustration of different antibody structures. The Flag tag was added to the C-term of α mCD205 scFv for binding affinity detection. (B) SDS-PAGE analysis of α mCD205-scFv LH and HL configurations, HL denotes the VH-VL orientation, while LH denotes the VL-VH orientation. (C) Representative flow cytometry plots showing the binding of α mCD205-scFv in LH and HL configuration to RAW264.7 cells. (D) Binding curves of α mCD205-scFv in LH and HL configurations to RAW264.7 cells across a concentration gradient (n=3). (E) Cell-based ELISA analysis comparing the binding affinities of different α mCD205 scFv to RAW264.7 cells (n=3).

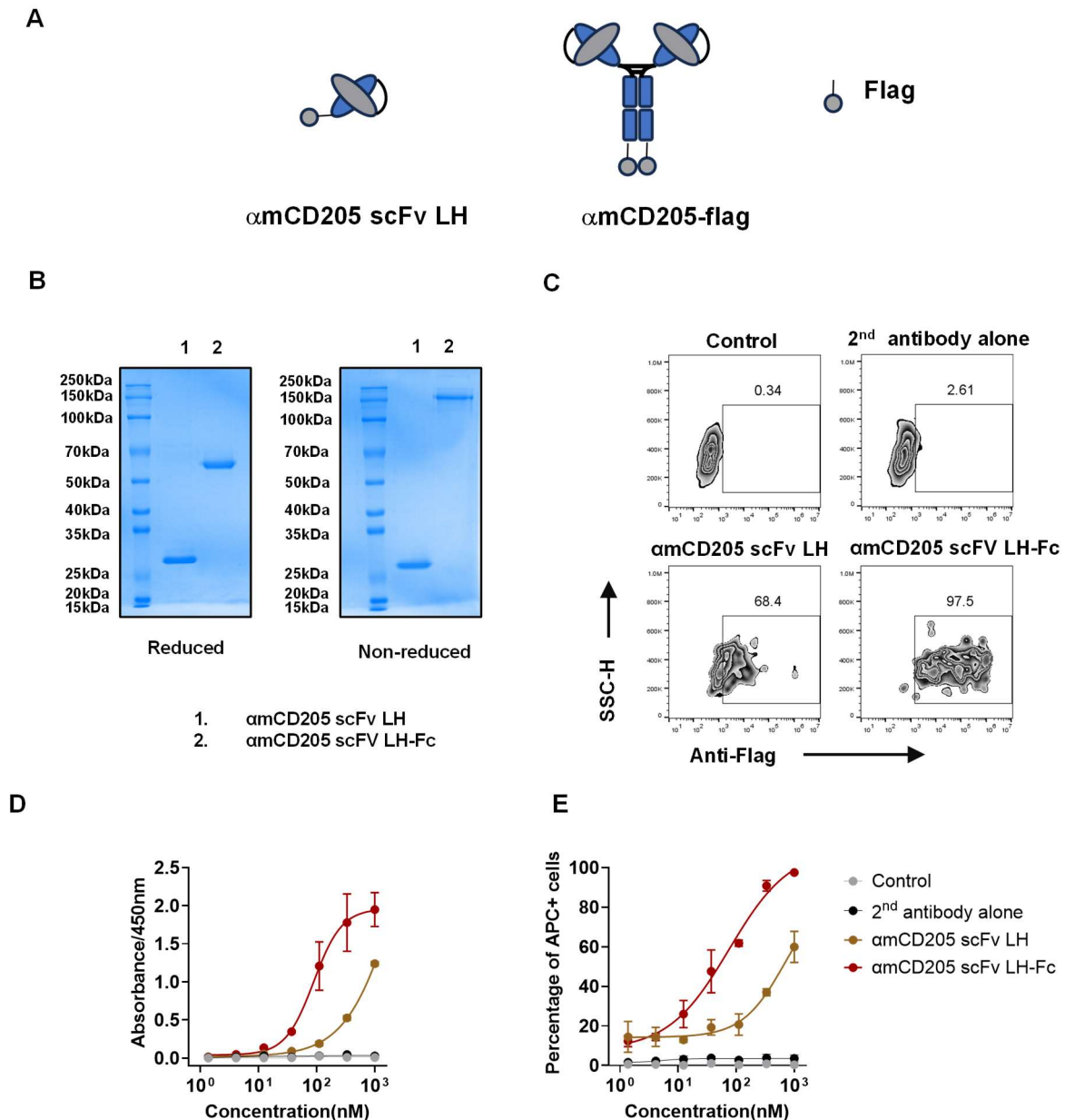
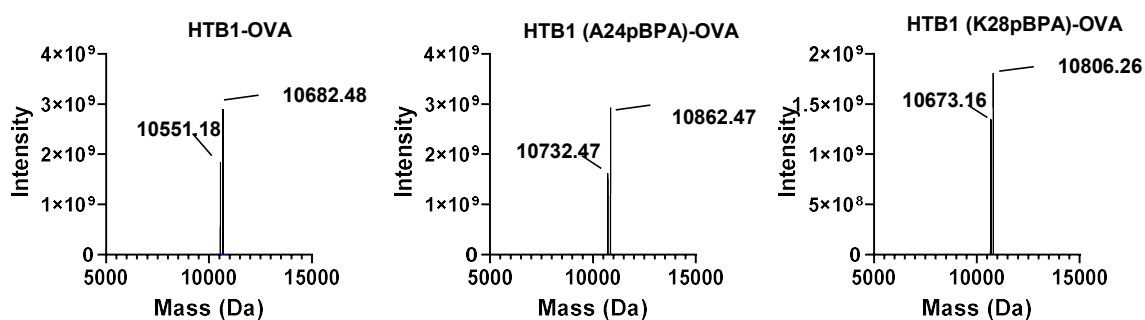


Figure S2. Binding affinity analysis of α mCD205 scFv following Fc fusion.

(A) Schematic illustration of different antibody structures. The Flag tag was added to the C-term of α mCD205 scFv and α mCD205 scFv-Fc for binding affinity detection. (B) SDS-PAGE analysis of α mCD205 scFv LH and α mCD205 scFv LH-Fc under reducing and non-reducing conditions. (C) Representative flow cytometry plots showing the binding of α mCD205-scFv LH and α mCD205-scFv LH-Fc to RAW264.7 cells. (D) Binding curves of α mCD205-scFv LH and α mCD205-scFv LH-Fc to RAW264.7 cells across a concentration gradient (n=3). (E) Cell-based ELISA analysis comparing the binding affinities of the different constructs to RAW264.7 cells (n=3).



Protein	Expected MW/Da	Expected MW-Met/Da	Observed MW/Da
HTB1-OVA	10682.89	10551.7	10682.48/10551.18
HTB1 (A24pBPA)-OVA	10863.19	10732	10862.47/10732.47
HTB1 (K28pBPA)-OVA	10805.99	10674.8	10806.26/10673.16

Figure S3. LC-MS analysis of HTB1 (A24pBPA)-OVA and HTB1 (K28pBPA)-OVA

Proteins were desalted with ddH₂O in an ultrafiltration tube. The solution was then transferred to an LC sample tube, and the molecular weight of each protein was detected using Q Exactive HF-X (Thermo Fisher Scientific). Data analysis was performed using BioPharma Finder 3.2 software. Proteins expressed in *E. coli* often undergo removal of the initiator methionine (Met¹) during post-translational processing, leading to the detection of two distinct molecular weights by LC-MS analysis^{1,2}.

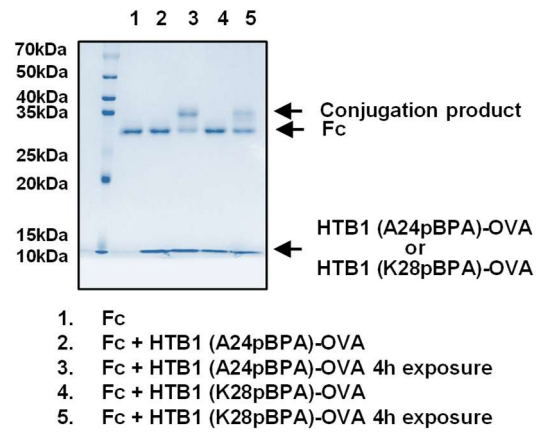
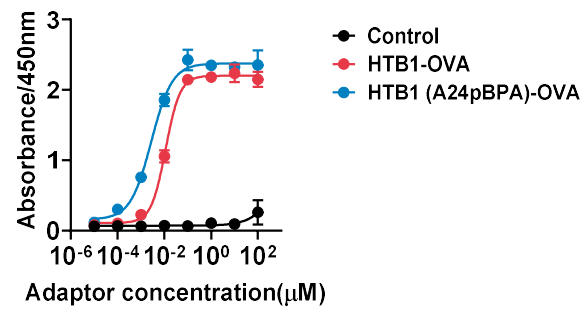


Figure S4. Comparison of conjugation efficacy of A24 and K28 sites.

HTB1 (A24pBPA)-OVA or HTB1 (K28pBPA)-OVA was mixed with glycosylated Fc at a molar ration of 1:4. Next the mixture was placed on ice bath and exposed to 365 nm UV for 4 h. Reducing SDS-PAGE was conducted to analyze the conjugation efficacy.



	EC_{50} (μ M)
HTB1-OVA	0.011
HTB1 (A24pBPA)-OVA	0.002

Figure S5. α mCD205 affinity detection of HTB1-OVA and HTB1 (A24pBPA)-OVA.

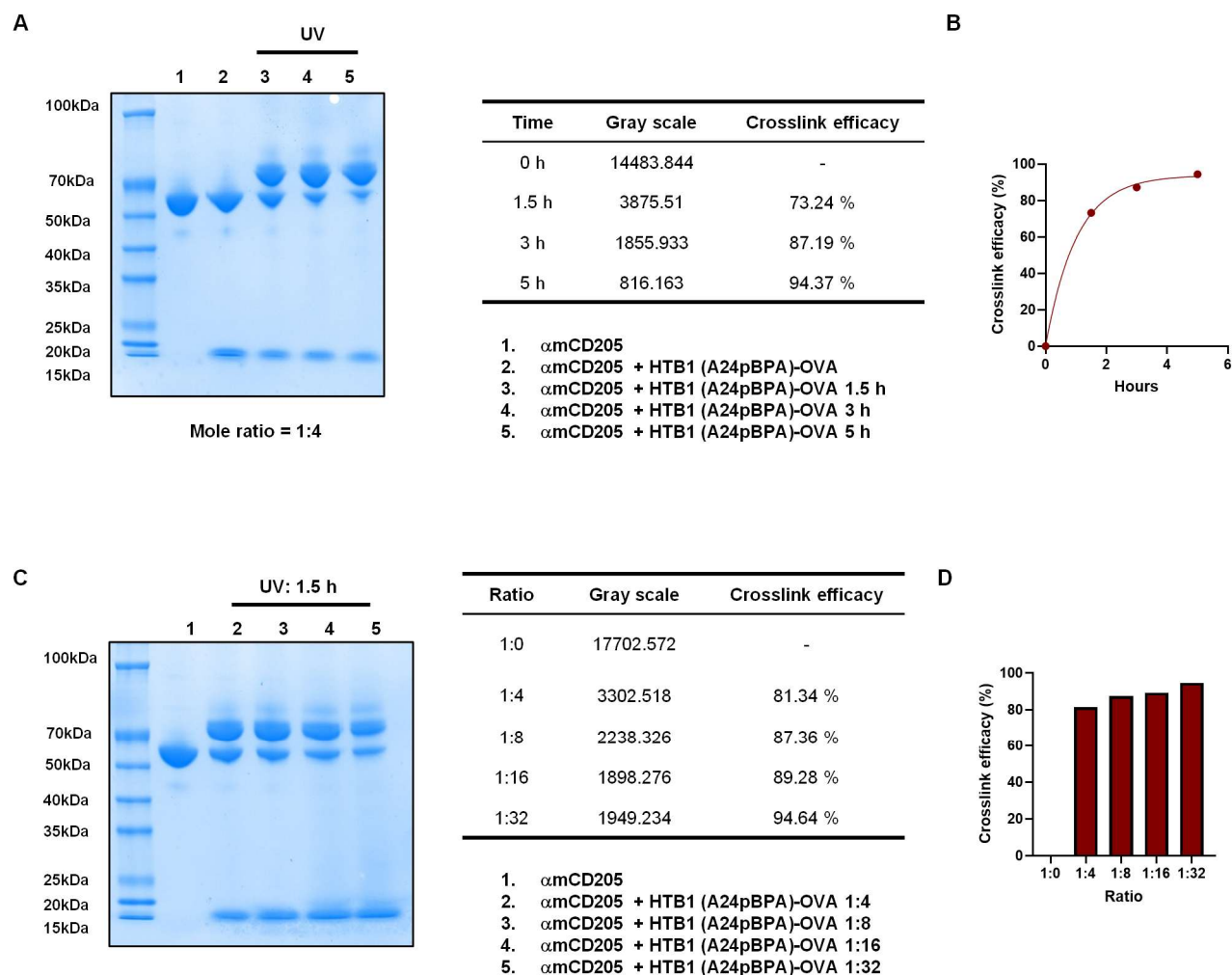
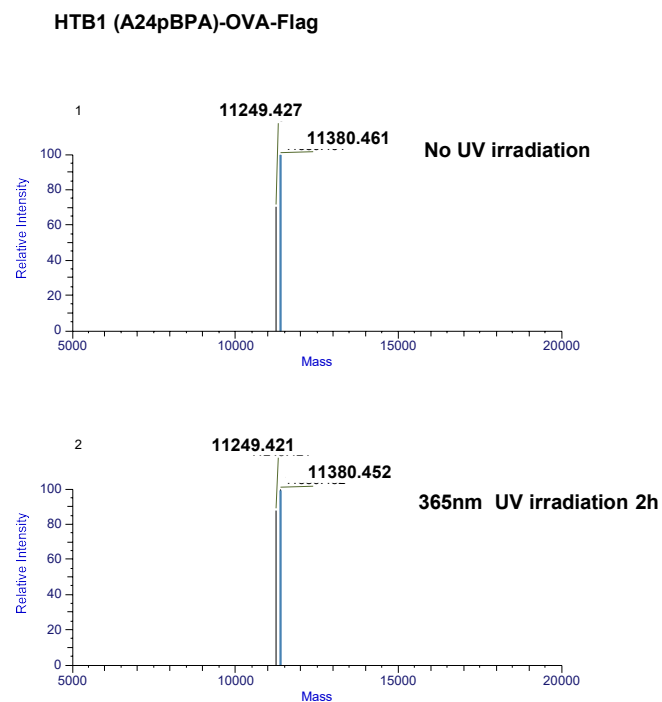


Figure S6. Optimization of photo-reactive PIC (P-PIC) conditions

(A) α mCD205 expressed in eukaryotic cells was mixed with HTB1 (A24pBPA)-OVA at a molar ration of 1:4. The mixture was placed on ice and exposed to 365 nm UV irradiation for varying durations. (B) Summary of crosslinking efficiency at different UV exposure times. (C) α mCD205 expressed in eukaryotic cells was mixed with HTB1 (A24pBPA)-OVA at different molar ratios and exposed to 365 nm UV irradiation for 1.5h. (D) Summary of crosslinking efficiency at different mix ratios. Conjugation efficiency was analyzed by reducing SDS-PAGE.



Protein	Expected MW/Da	Expected MW-Met/Da	Observed MW/Da
HTB1 (A24pBPA)-OVA-Flag No UV irradiation	11387.53	11255.32	11380.461 / 11249.427
HTB1 (A24pBPA)-OVA-Flag 365nm UV irradiation 2h	11387.53	11255.32	11380.452 / 11249.421

Figure S7. Assessment of antigen integrity of HTB1 (A24pBPA)-OVA-Flag following UV exposure. HTB1 (A24pBPA)-OVA-Flag (10 mmol) was exposed to 365 nm UV irradiation for 2h followed by molecular weight analysis by mass spectrometry.

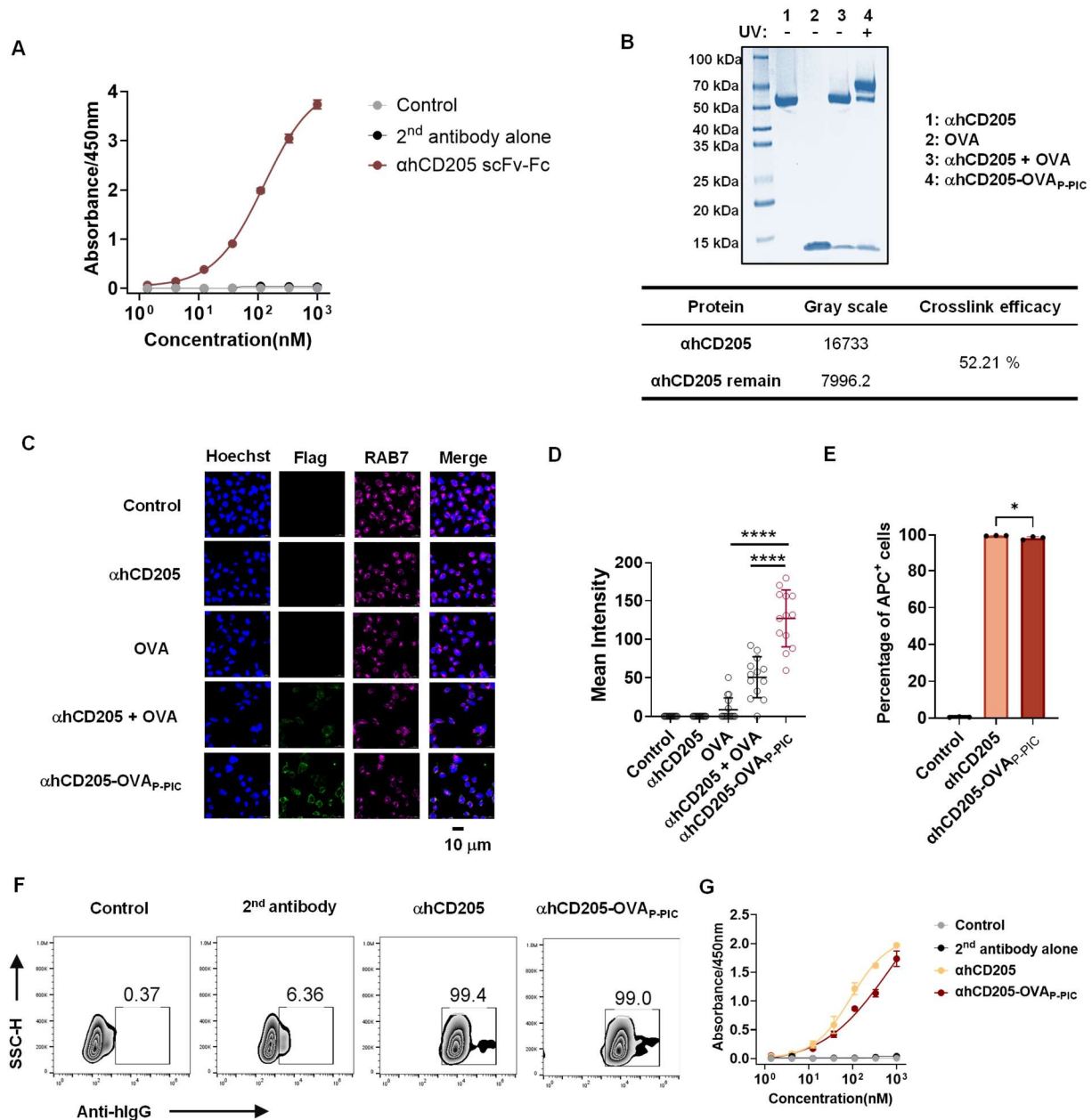


Figure S8. Enhanced antigen uptake mediated by conjugation to αhCD205.

(A) Flow cytometric analysis binding of αhCD205 scFv-Fc to THP-1 cells. (B) Reducing SDS-PAGE analysis of HTB1 (A24pBPA)-OVA conjugated to αhCD205. (C) Representative colocalization images of THP-1 cells incubated with αhCD205, OVA, αhCD205 + OVA, or αhCD205-OVA_{P-PIC}. Antigens were stained with an anti-Flag antibody (green), RAB7 was stained with an anti-RAB7 antibody (pink), and nuclei were counterstained with Hoechst (blue). Scale bars = 20 μm. (D) Quantification of mean integrated fluorescence intensity in the indicated groups (n = 13). (E) Comparison of binding affinity to THP-1 cells between αhCD205 and αhCD205-OVA_{P-PIC}. (F) Representative flow cytometry plots showing the binding of αhCD205 and αhCD205-OVA_{P-PIC} to THP-1 cells. (G) Cell-based ELISA analysis comparing the binding affinities of different constructs to THP-1 cells (n = 3). The data are shown as means ± SDs. Statistical significance was determined using a one-way ANOVA with Tukey's multiple comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

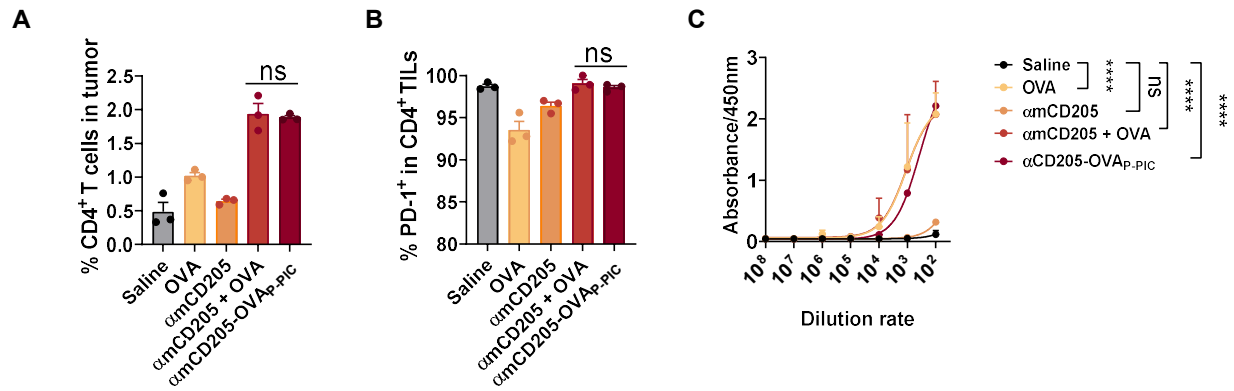


Figure S9. Analysis of CD4⁺ TILs and serum OVA-specific antibody.

(A) Quantification of tumor-infiltrating CD4⁺ T cells in B16-OVA tumor tissues (n = 3). (B) Analysis of PD-1 expression on CD4⁺ TILs in B16-OVA tumor tissues (n = 3). (C) Titration of OVA-specific serum antibodies in the B16-OVA model. Blood samples were collected via the orbital vein, and serum was isolated by centrifugation. Plates coated with ovalbumin were used to detect OVA-specific antibodies, (n = 3). The data are shown as the means ± SDs. Statistical significance was determined using a two-way ANOVA with Tukey's multiple comparisons test for (A-B) and two-way ANOVA with Tukey's multiple comparisons test for (C) (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

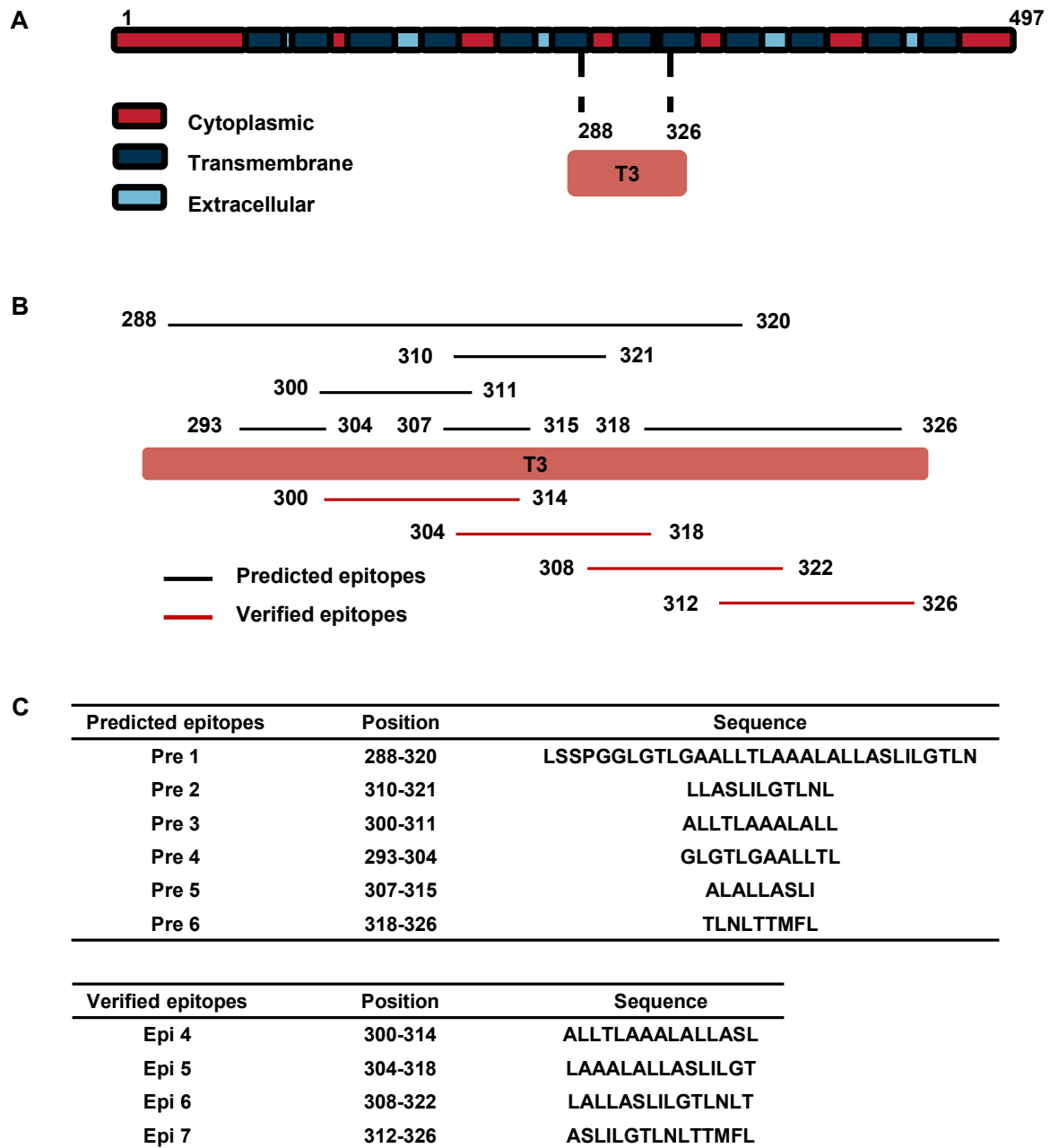
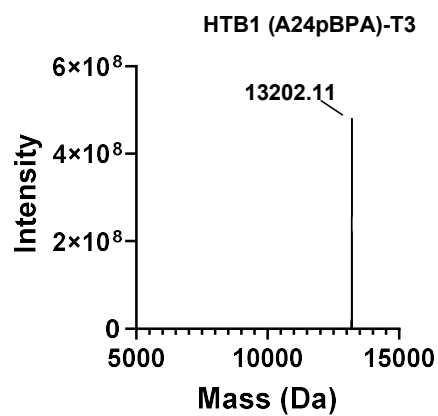


Figure S10. Illustration of the location and functional epitopes within T3 EER.

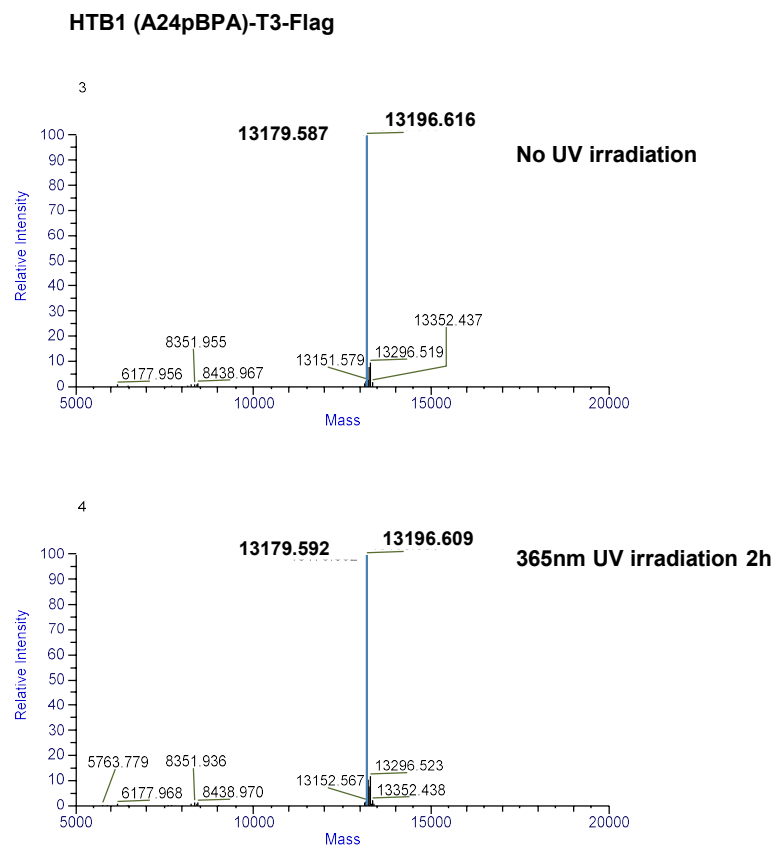
(A) T3 EER location at LMP2A was shown. (B) Predicted epitopes and verified epitopes within T3 were illustrated. (C) Summary of position and sequence of verified epitopes within T3³. Epi means verified epitope; Pre means predicted epitopes.



Protein	Expected MW/Da	Expected MW-Met/Da	Observed MW/Da
HTB1 (A24pBPA)-T3	13204.87	--	13202.11

Figure S11. LC-MS analysis of HTB1 (A24pBPA)-T3.

The observed molecular weight of HTB1(A24pBPA)-T3 was 13202.11 Da, which is consistent with the expected molecular weight of 13202.11 Da.



Protein	Expected MW/Da	Observed MW/Da
HTB1 (A24pBPA)-T3-Flag No UV irradiation	13202.11	13196.616 / 13179.587
HTB1 (A24pBPA)-T3-Flag 365nm UV irradiation 2h	13202.11	13196.609 / 13179.592

Figure S12. Assessment of antigen integrity test of HTB1 (A24pPBA)-T3 after following UV exposure. HTB1 (A24pPBA)-T3 (10 mmol) was exposed to 365 nm UV irradiation for 2 hours and the molecular weight was determined by mass spectrometry.

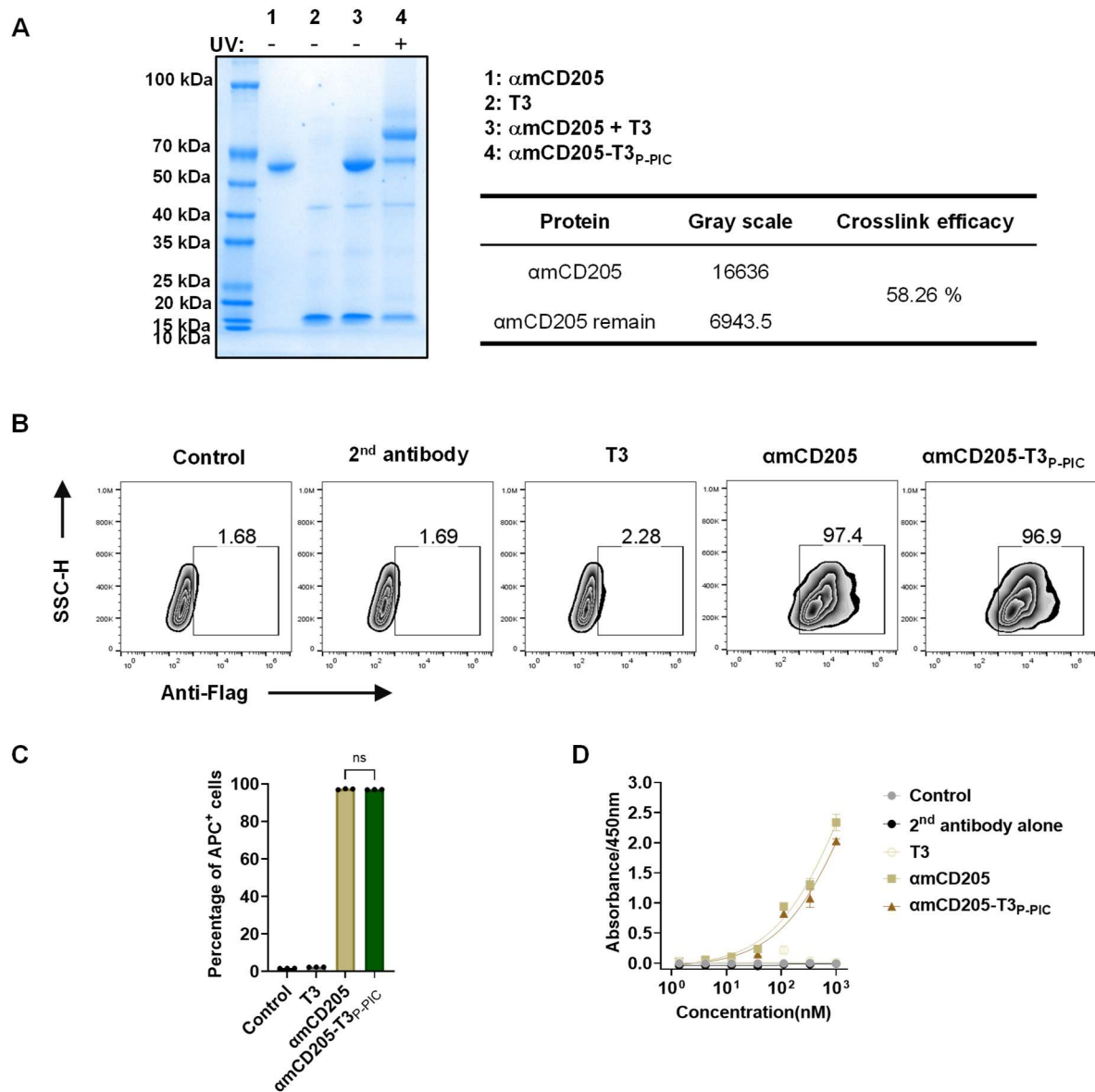


Figure S13. Analysis of crosslinking efficiency and binding affinity of α mCD205-T3_{P-PIC}

(A) Reducing SDS-PAGE analysis of α mCD205 conjugated to HTB1 (A24pBPA)-T3 conjugation.

Crosslinking efficiency was calculated based on band intensity. (B) Representative flow cytometry plots showing the binding of T3, α mCD205 and α mCD205-T3_{P-PIC} to RAW264.7 cells. (C) Quantification of

binding affinity (n = 3). (D) Cell-based ELISA analysis of α mCD205-T3_{P-PIC}, α mCD205 and T3 to

RAW264.7 cells (n = 3). The data are shown as the means $\pm$ SDs. Statistical significance was determined using a one-way ANOVA with Tukey's multiple comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

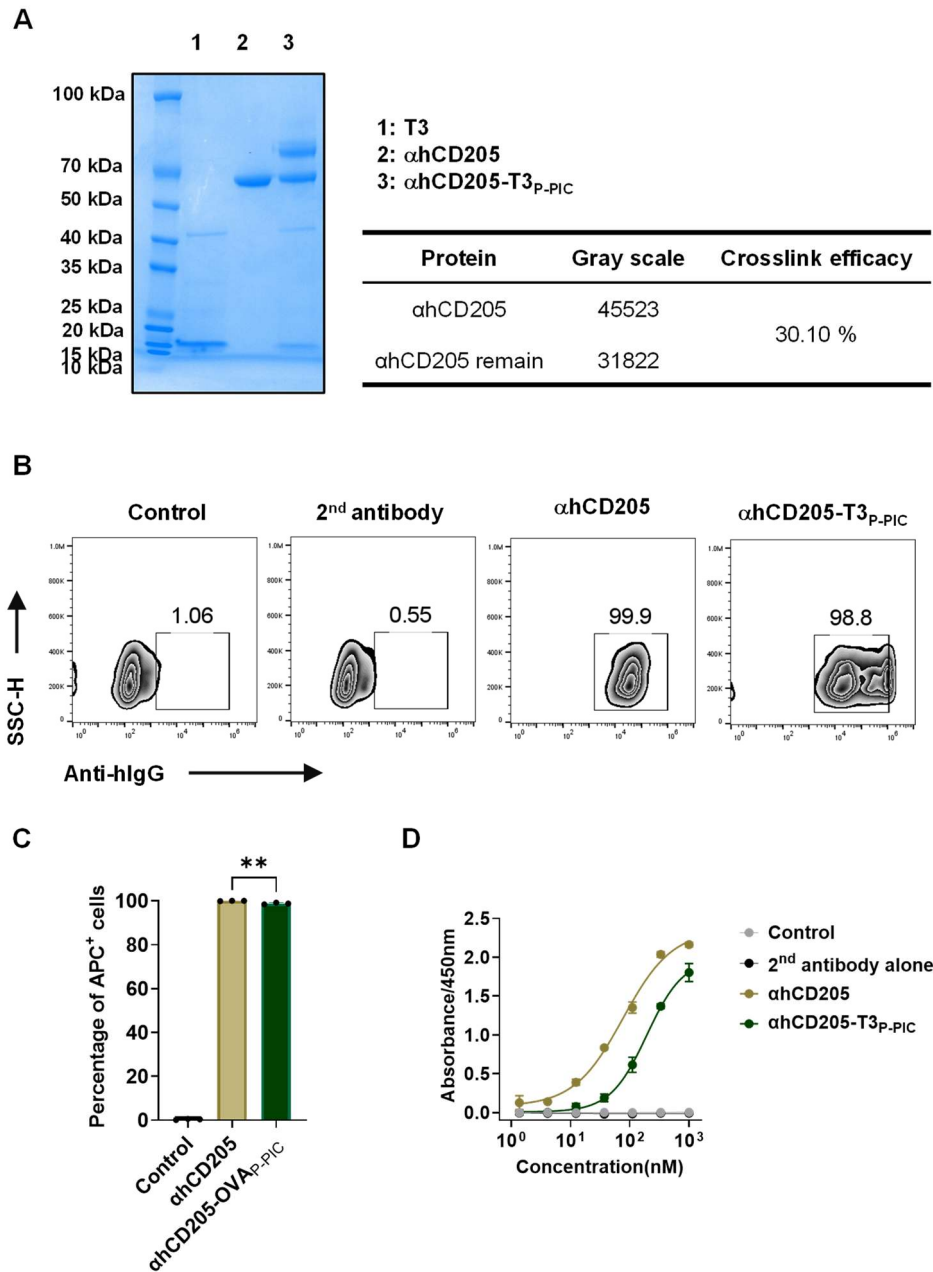


Figure S14. Analysis of crosslinking efficiency and binding affinity of α hCD205-T3_{P-PIC}.

(A) Reducing SDS-PAGE analysis of α hCD205 conjugated to HTB1 (A24pBPA)-T3 conjugation.

Crosslinking efficiency was calculated based on band intensity. (B) Representative flow cytometry plots

showing the binding of α hCD205 and α hCD205-T3_{P-PIC} to THP-1 cells. (C) Quantification of binding

affinity (n = 3). (D) Cell-based ELISA analysis of α hCD205-T3_{P-PIC}, α hCD205 to THP-1 cells (n = 3). The

data are shown as means $\pm$ SDs. Statistical significance was determined using a one-way ANOVA with

Tukey's multiple comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

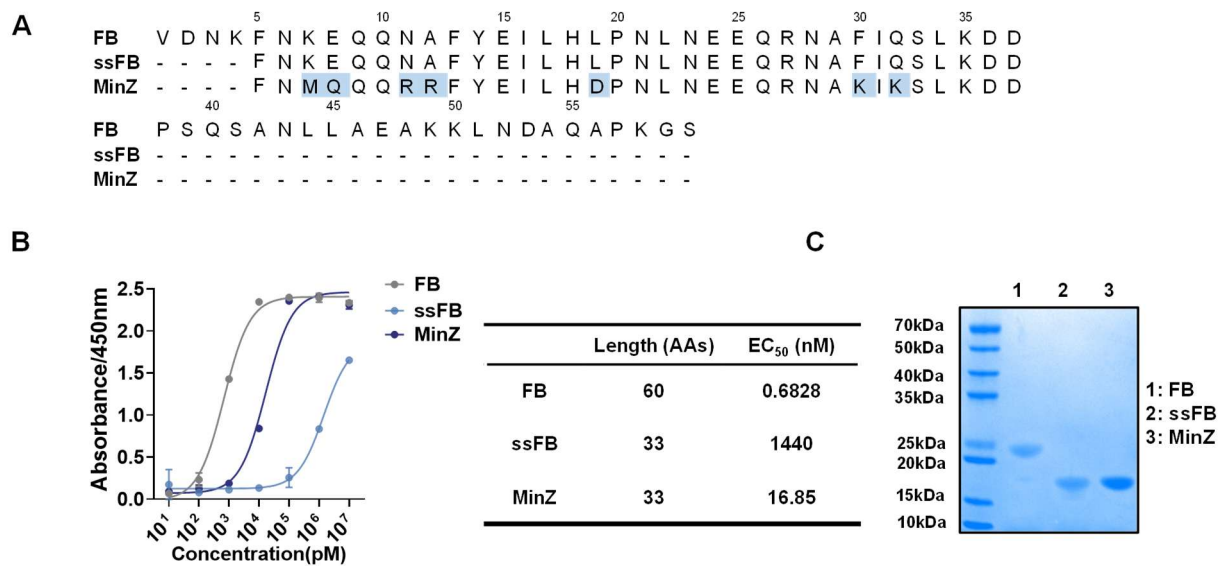


Figure S15. Optimization of chemically-reactive PIC (C-PIC) adaptors.

(A) Amino acid sequence alignment of FB, ssFB and MinZ. (B) Comparison of the affinity to glycosylated Fc between FB, ssFB and MinZ. (C) SDS-PAGE analysis of FB, ssFB and MinZ.

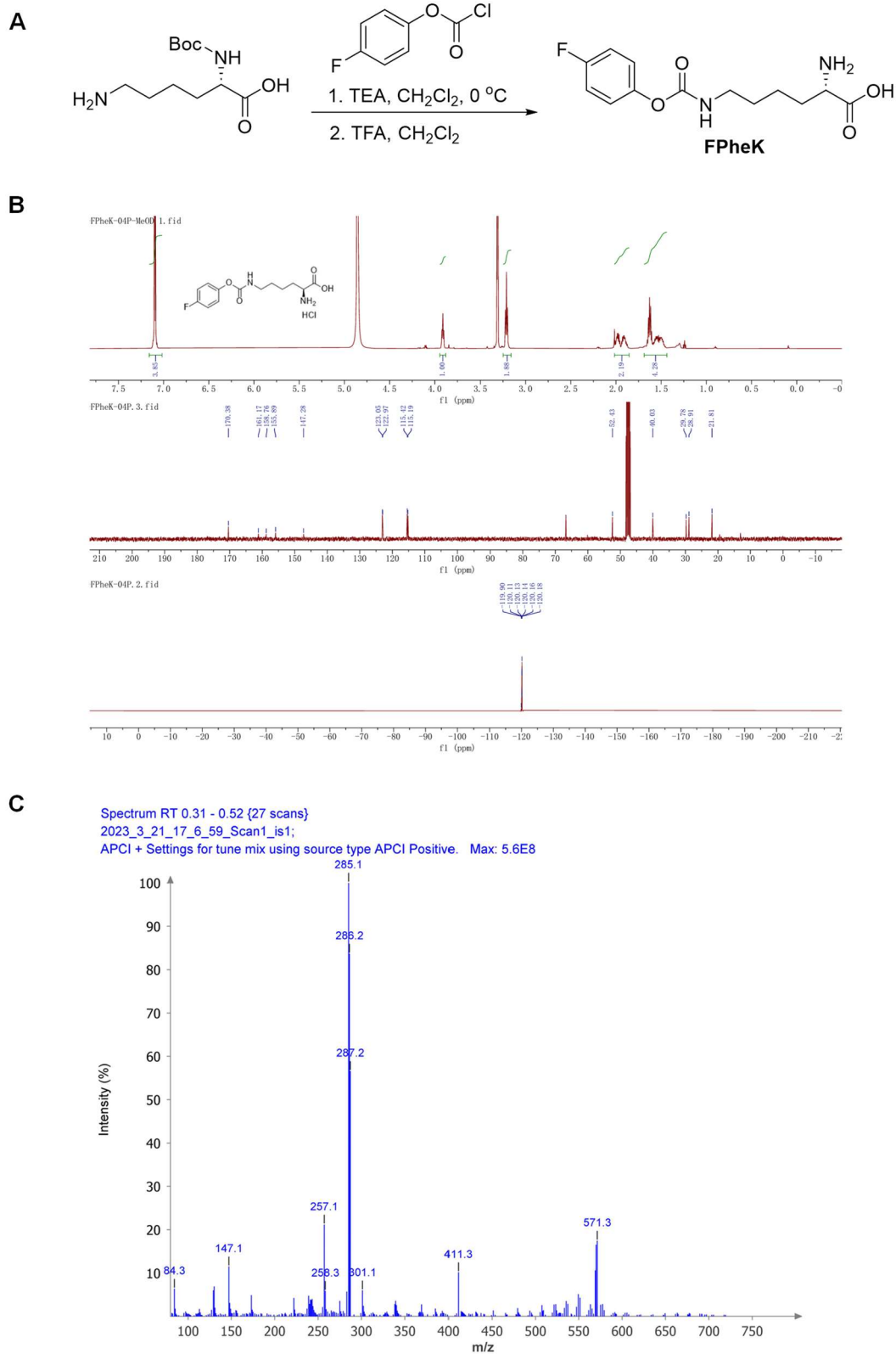
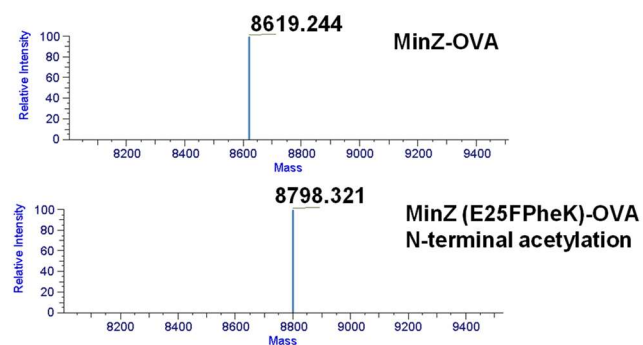


Figure S16. Synthesis route and characterization of FPheK.

(A) Reaction Scheme: 1. 4-fluorophenyl chloroformate, triethylamine, dichloromethane, 0 °C; 2.

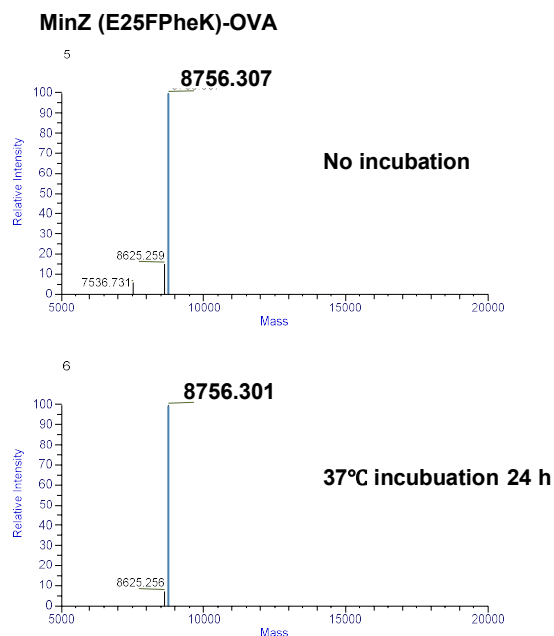
Trifluoroacetic acid, dichloromethane. (B) ^1H , ^{13}C and ^{19}F NMR of FPheK in $\text{MeOH-}d_4$. (C) LC-MS of FPheK (calcd. for $\text{C}_{13}\text{H}_{17}\text{FN}_2\text{O}_4$ $[\text{M}+1]$ 285.1, found 285.1).



Protein	Expected MW/Da	Observed MW/Da
MinZ-OVA	8624.61	8619.24
MinZ (E25FPheK)-OVA	8761.6	8798.32

Figure S17. LC-MS analysis of C-PIC products.

LC-MS analysis of MinZ-OVA and MinZ (E25FPheK)-OVA molecular weights. N-terminal acetylation of FPheK modified protein has been reported previously⁴.



Protein	Expected MW/Da	Observed MW/Da
MinZ (E25FPheK)-OVA No incubation	8755.32	8756.307
MinZ (E25FPheK)-OVA 37°C incubation 24 h	8755.32	8756.301

Figure S18. Assessment of antigen integrity of MinZ (E25FPheK)-OVA after incubation
 HTB1 (E25FPheK)-OVA (10 mmol) was incubated at 37 °C for 24 h and the molecular weight was determined by mass spectrometry.

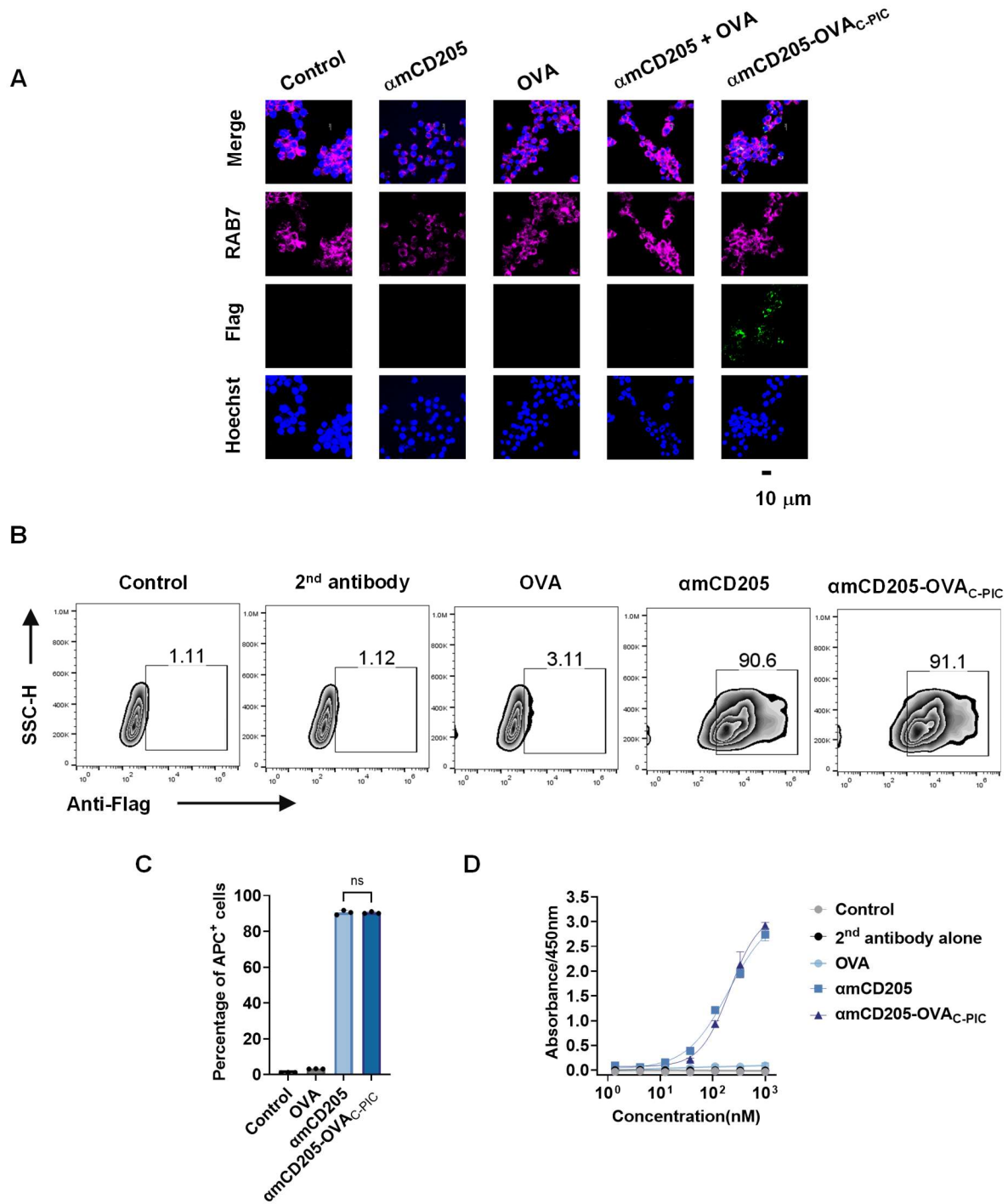


Figure S19. Analysis of antigen uptake and binding affinity of the C-PIC vaccine.

(A) Representative confocal microscopy images of RAW264.7 cells incubated with the C-PIC vaccine, Scale bar = 10 μ m. (B) Representative flow cytometry plots showing the binding of OVA, α mCD205 and α mCD205-OVA_{C-PIC} to RAW264.7 cells. (C) Quantification of binding affinity (n = 3). (D) Cell-based ELISA analysis of α mCD205-OVA_{C-PIC}, and α mCD205 to RAW264.7 cells (n = 3). The data are shown as means $\pm$ SDs. Statistical significance was determined using a one-way ANOVA with Tukey's multiple comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

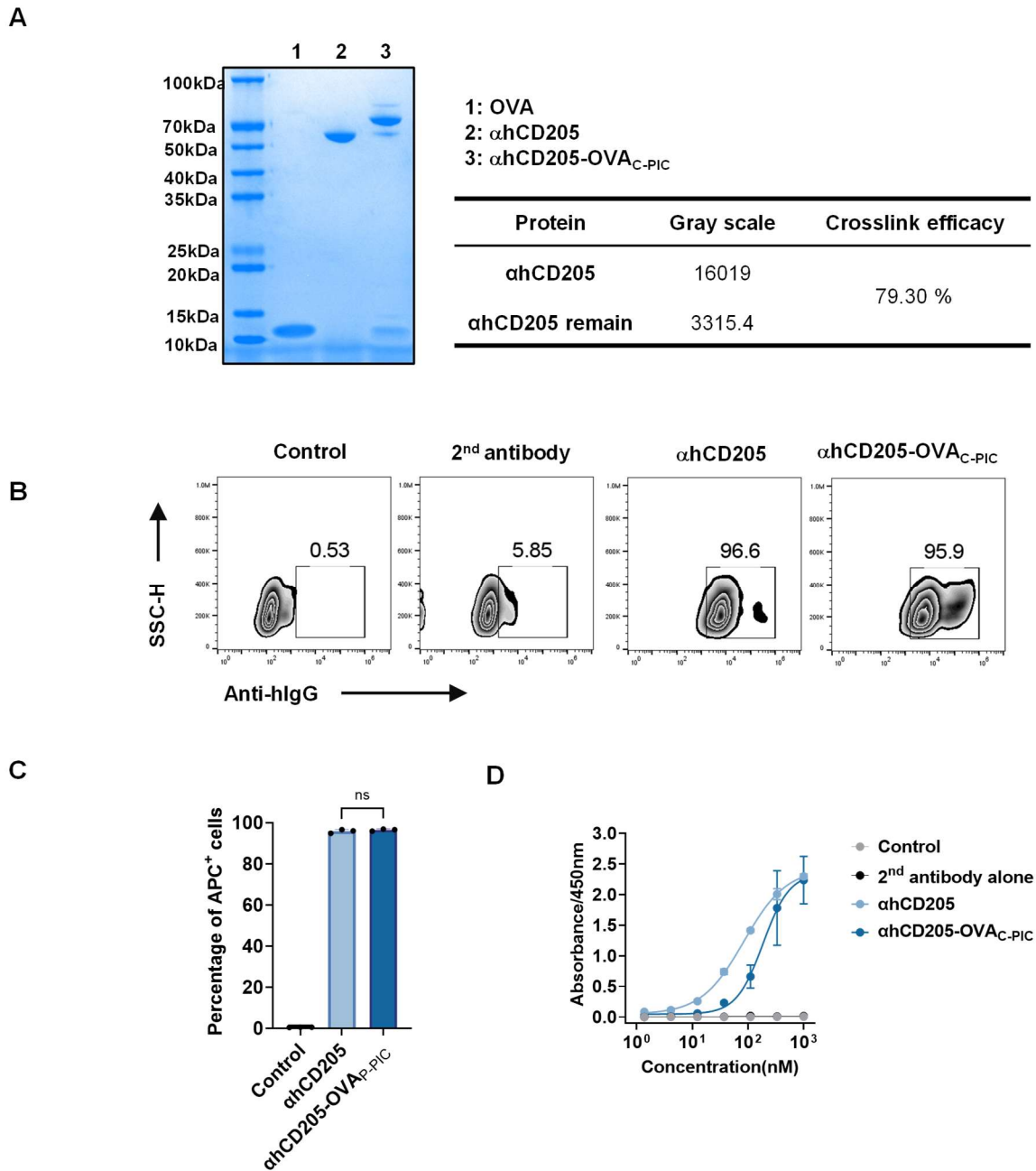


Figure S20. Analysis of crosslink efficiency and binding affinity of the C-PIC vaccine.

(A) Reducing SDS-PAGE analysis of α hCD205 conjugated to MinZ (E25FPheK)-OVA conjugation. (B) Representative flow cytometry plots showing the binding of α hCD205 and α hCD205-OVA_{C-PIC} to THP-1 cells. (C) Quantification of binding affinity (n = 3). (D) Cell-based ELISA analysis of α hCD205-OVA_{C-PIC}, and α hCD205 to THP-1 cells (n = 3). The data was shown as means $\pm$ SDs. Statistical significance was determined using a one-way ANOVA with Tukey's multiple comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

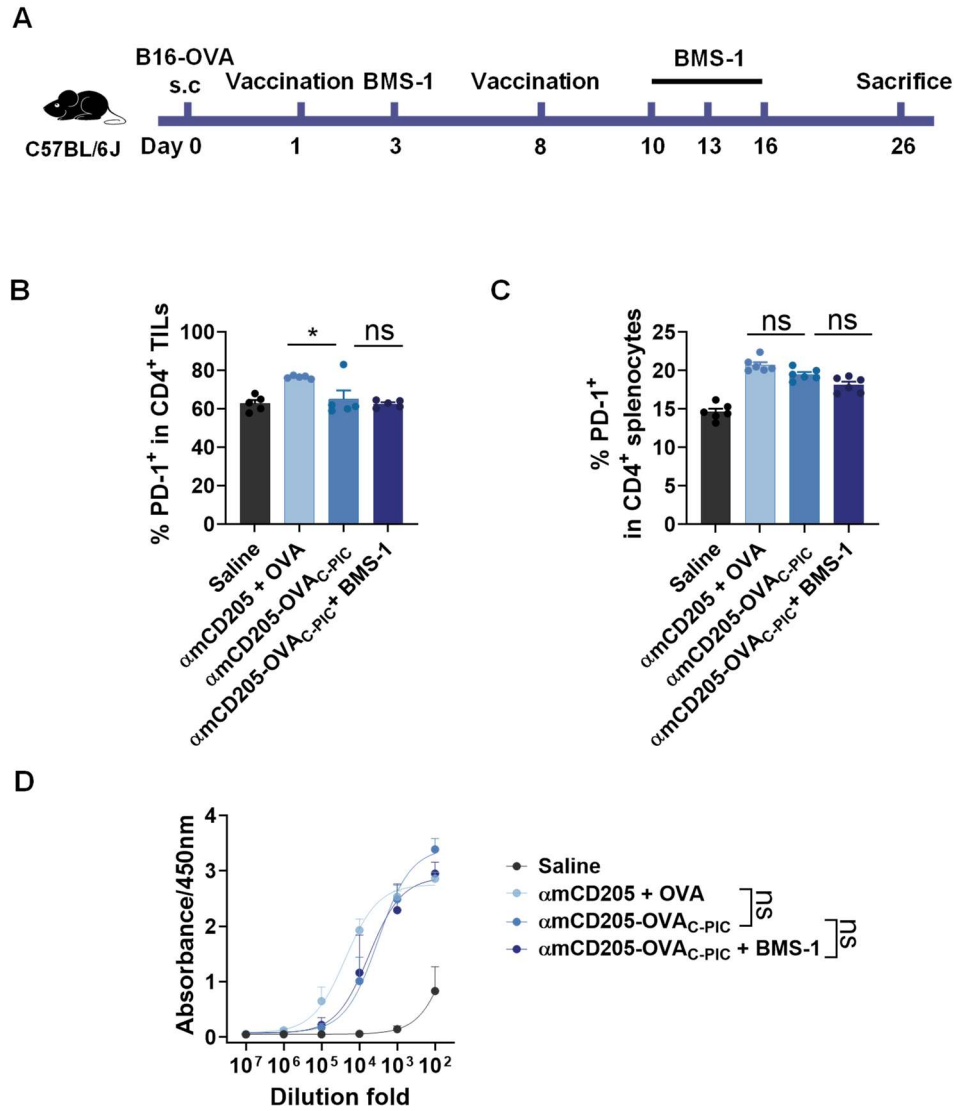


Figure S21. Evaluation of C-PIC vaccine in the B16-OVA therapeutic tumor model

(A) Schematic timeline of C-PIC vaccine administration in the B16-OVA therapeutic model. (B)

Quantification of PD-1 expression on CD4⁺ TILs, (n = 5). (B) Quantification of PD-1 expression on CD4⁺

splenocytes, (n = 6). (D) Serum antibody titration in the C-PIC vaccine therapeutic model. Serum OVA-

specific antibody titration was measured by ELISA, and no significant differences were observed among the

αmCD205 + OVA, αmCD205-OVA_{P-PIC} and αmCD205-OVA_{P-PIC} + BMS-1 groups. The data are shown as

means ± SDs. Statistical significance was determined using a one-way ANOVA with Tukey's multiple

comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

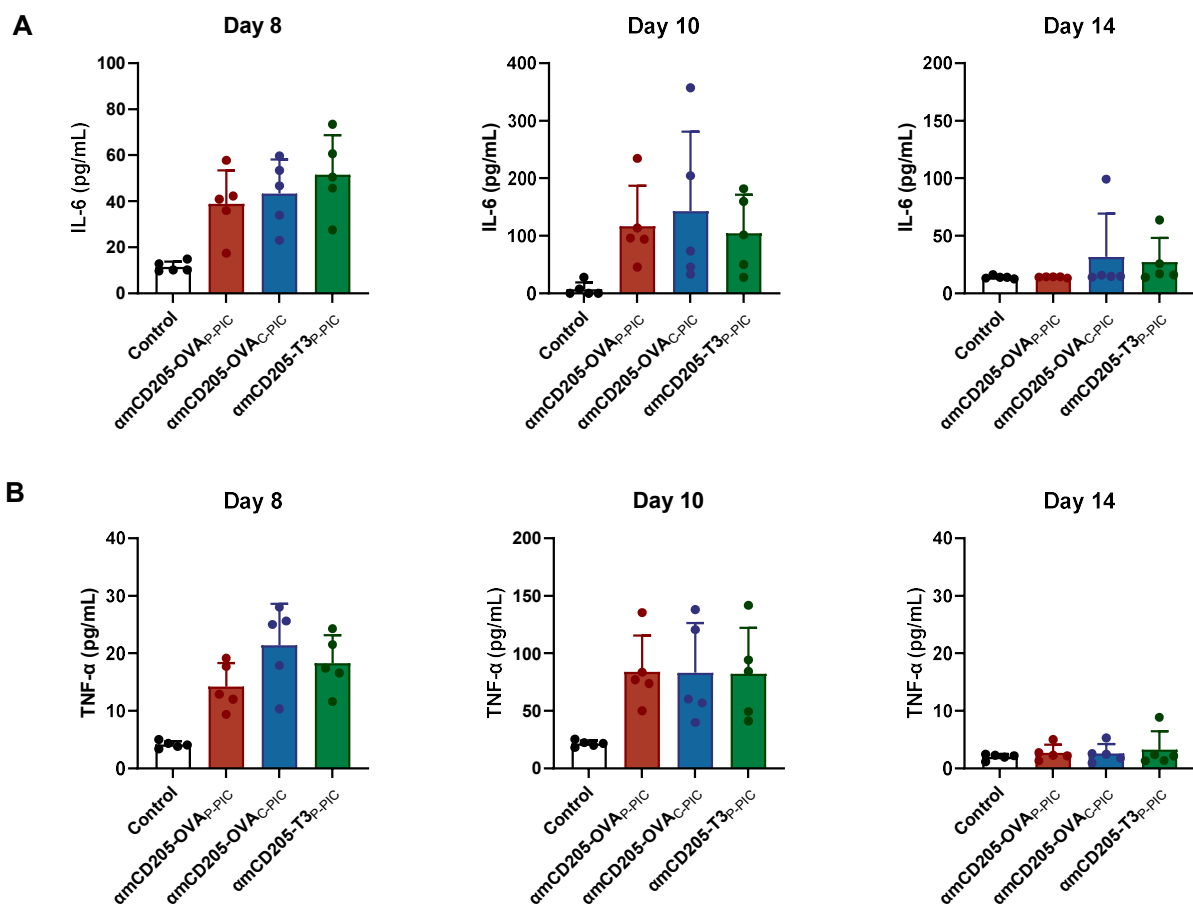


Figure S22. Serum IL-6 and TNF- α levels following vaccination

(A) Serum IL-6 level measured on day 8, 10 and 14 (n = 5). (B) Serum TNF- α level measured on day 8, 10 and 14 (n = 5).

III. References

1. Grünewald, J.; Hunt, G. S.; Dong, L.; Niessen, F.; Wen, B. G.; Tsao, M. L., et al., Mechanistic studies of the immunochemical termination of self-tolerance with unnatural amino acids. *Proc Natl Acad Sci U S A* **2009**, *106* (11), 4337-42.
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4. Xuan, W.; Shao, S.; Schultz, P. G., Protein crosslinking by genetically encoded noncanonical amino acids with reactive aryl carbamate side chains. *Angewandte Chemie (International ed. in English)* **2017**, *56* (18), 5096-100.

IV. Plasmid Sequences

pCAGGS-*am*CD205 scFv LH-His-Flag

5'-GTCGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTTCATTAGTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCC
CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACG
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pCAGGS- α hCD205 scFv LH-Fc

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CTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACC
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pET32a-HTB1 A24TAG-OVA (SIINFEKL)-His

5'-TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGCGCA
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CCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAAGTATATCCGGAT-3'

Red: amber codon TAG for pBPA incorporation.

pET32a-HTB1 K28TAG-OVA (SIINFELK)-His

5'-TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA
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CCTTGGGGCCTCTAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGAT-3'

Red: amber codon TAG for pBPA incorporation.

pET32a-MBP-Thrombin-Flag-HTB1 A24TAG-T3 EER-His

5'-TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA

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TATATCCGGAT-3'

Red: amber codon TAG for pBPA incorporation.

pEVOL-pBPARS

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T-3'

pET22b-MinZ-TrxA-His

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AT-3'

pET22b-MinZ-OVA (SIINFEKL)-His

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pET22b-MinZ -OVA (SIINFELK)-Flag-His

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TGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGAT-3'

pET22b-MinZ E25TAG-OVA (SIINFEKL)-His

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TTTTTTGCTGAAAGGAGGAACTATATCCGGAT-3'

Red: amber codon TAG for pBPA incorporation.

pET22b-MinZ E25TAG-OVA (SIINFEKL)-Flag-His

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Red: amber codon TAG for pBPA incorporation.

pUltra-FPheKRS

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GTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGAC
ATCGTATAACGTTACTGGTTTCACATTC-3'

V. Protein Sequences

α mCD205 scFv LH-His-Flag (Expected MW: 28220.05 Da)

DIQMTQSPSFLSTSLGNSITITCHASQNIKGWLAWYQQKSGNAPQLLIYKASSLQSGVPSRFSGSGSG
TDYIFTISNLQPEDIATYYCQHYQSFPWTFGGGTKLEIKRAAGGGGSGGGGSGGGGSEVKLQQSGTE
VVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIGWIFPGEGSTEYNEKFKGRATLSVDKSSS
TAYMELTRLTSEDSAVYFCARGDYRRYFDLWGQGTTVTVSSHHHHHHHDYKDDDDK

α mCD205 scFv HL-His-Flag (Expected MW: 28220.05 Da)

EVKLQQSGTEVVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIGWIFPGEGSTEYNEKFKG
RATLSVDKSSSTAYMELTRLTSEDSAVYFCARGDYRRYFDLWGQGTTVTVSSGGGGSGGGGSGGGG
GSDIQMTQSPSFLSTSLGNSITITCHASQNIKGWLAWYQQKSGNAPQLLIYKASSLQSGVPSRFSGSG
SGTDYIFTISNLQPEDIATYYCQHYQSFPWTFGGGTKLEIKRAAHHHHHHHDYKDDDDK

α mCD205 scFv LH-Fc (Expected MW: 52460.75 Da)

DIQMTQSPSFLSTSLGNSITITCHASQNIKGWLAWYQQKSGNAPQLLIYKASSLQSGVPSRFSGSGSG
TDYIFTISNLQPEDIATYYCQHYQSFPWTFGGGTKLEIKRAAGGGGSGGGGSGGGGSEVKLQQSGTE
VVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIGWIFPGEGSTEYNEKFKGRATLSVDKSSS
TAYMELTRLTSEDSAVYFCARGDYRRYFDLWGQGTTVTVSSEPKSSDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQ
KSLSLSPGK

α mCD205 scFv LH-Fc-His-Flag (Expected MW: 54278.57 Da)

DIQMTQSPSFLSTSLGNSITITCHASQNIKGWLAWYQQKSGNAPQLLIYKASSLQSGVPSRFSGSGSG
TDYIFTISNLQPEDIATYYCQHYQSFPWTFGGGTKLEIKRAAGGGGSGGGGSGGGGSEVKLQQSGTE
VVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIGWIFPGEGSTEYNEKFKGRATLSVDKSSS
TAYMELTRLTSEDSAVYFCARGDYRRYFDLWGQGTTVTVSSEPKSSDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQ
KSLSLSPGKHHHHHHHDYKDDDDK

α hCD205 scFv LH-Fc (Expected MW: 51549.85 Da)

QAVVTQESALTTSPGETVTLTCRSSTGAVTISNYANWVQEKPDLFTGLIGGTNNRAPGVPARFSGSL
IGDKAALTITGAQTEDEAIYFCALWYNNQFIFGSGTKVTVLGGGGSGGGGSGGGGSEVQLQQSGPV
LVKPGASVKMSCKASGNTFTDSFMHWMKQSHGKSLEWIGIINPYNGGTSYNQKFKGKATLTVDKS
SSTAYMELNSLTSEDSAVYYCARNGVRYYFDYWGQGTTLTVSSEPKSSDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

HTB1-OVA (SIINFEKL)-His (Expected MW: 10551.70 Da)

TFKLIINGKTLKGEITIEAVDAAEAEEKIFKQYANDYGIDGEWYDDATKTFTVTEGGGGSSIINFELT
EWTSSNVMEERKIKEAAAKHHHHHHH

HTB1 A24pBPA-OVA (SIINFEKL)-His (Expected MW: 10732 Da)

TFKLIINGKTLKGEITIEAVDA~~X~~EAEKIFKQYANDYGIDGEWYDDATKTFTVTEGGGGSSIINFELT
EWTSSNVMEERKIKEAAAKHHHHHHH

X: pBPA incorporation at 24th position.

HTB1 K28pBPA-OVA (SIINFEKL)-His (Expected MW: 10647.8 Da)

TFKLIINGKTLKGEITIEAVDAAEAEXIFKQYANDYGIDGEWYDDATKTFTVTEGGGGSSIINFELT
EWTSSNVMEERKIKEAAAKHHHHHHH

X: pBPA incorporation at 28th position.

Flag-HTB1 A24pBPA-T3 EER-His (Expected MW: 13204.87 Da)

PRGSDYKDDDDKSSGMTFKLIINGKTLKGEITIEAVDA~~X~~EAEKIFKQYANDYGIDGEWYDDATKTF
TVTEGGGGSLSSPGGLGTLGAALLTLAAALALLASLILGTLNLTMTFLEAAAKHHHHHHH

X: pBPA incorporation at 24th position.

FB-TrxA-His (Expected MW: 21129.59 Da)

VDNKFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLNDAPKPGSGSTSGS
GKPGSGEGSTKGSDKIIHLTDDSFDTDLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLT
AKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGSGSGHHHHHHH

ssFB-TrxA-His (Expected MW: 18352.51 Da)

FNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDGSTSGSGKPGSGEGSTKGSDKIIHLTDDSFDTDL
KADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGE
VAATKVGALSKGQLKEFLDANLAGSGSGHHHHHH

MinZ-TrxA-His (Expected MW: 18464.70 Da)

FNMQQQRRFYEILHDPNLNEEQRNAKIKSLKDDGSTSGSGKPGSGEGSTKGSDKIIHLTDDSFDTDL
LKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNG
EVAATKVGALSKGQLKEFLDANLAGSGSGHHHHHH

MinZ-OVA (SIINFEKL)-His (Expected MW: 8624.61 Da)

MFNMQQQRRFYEILHDPNLNEEQRNAKIKSLKDDGGGGSSIINFEKLTEWTSSNVMEERKIKEAAA
KHHHHHH

MinZ -OVA (SIINFEKL)-Flag-His (Expected MW: 9149.05 Da)

MFNMQQQRRFYEILHDPNLNEEQRNAKIKSLKDDGGGGSSIINFEKLTEWTSSNVMEERKIKDYKD
DDDKHHHHHH

MinZ E25FPheK-OVA (SIINFEKL)-His (Expected MW: 8761.6 Da)

MFNMQQQRRFYEILHDPNLN~~X~~EQRNAKIKSLKDDGGGGSSIINFEKLTEWTSSNVMEERKIKEAAA
KHHHHHH

X: FPheK incorporation at 25th position.

MinZ E25FPheK-OVA (SIINFEKL)-Flag-His (Expected MW: 9286.04 Da)

MFNMQQQRRFYEILHDPNLN~~X~~EQRNAKIKSLKDDGGGGSSIINFEKLTEWTSSNVMEERKIKDYKD
DDDKHHHHHH

X: FPheK incorporation at 25th position.