

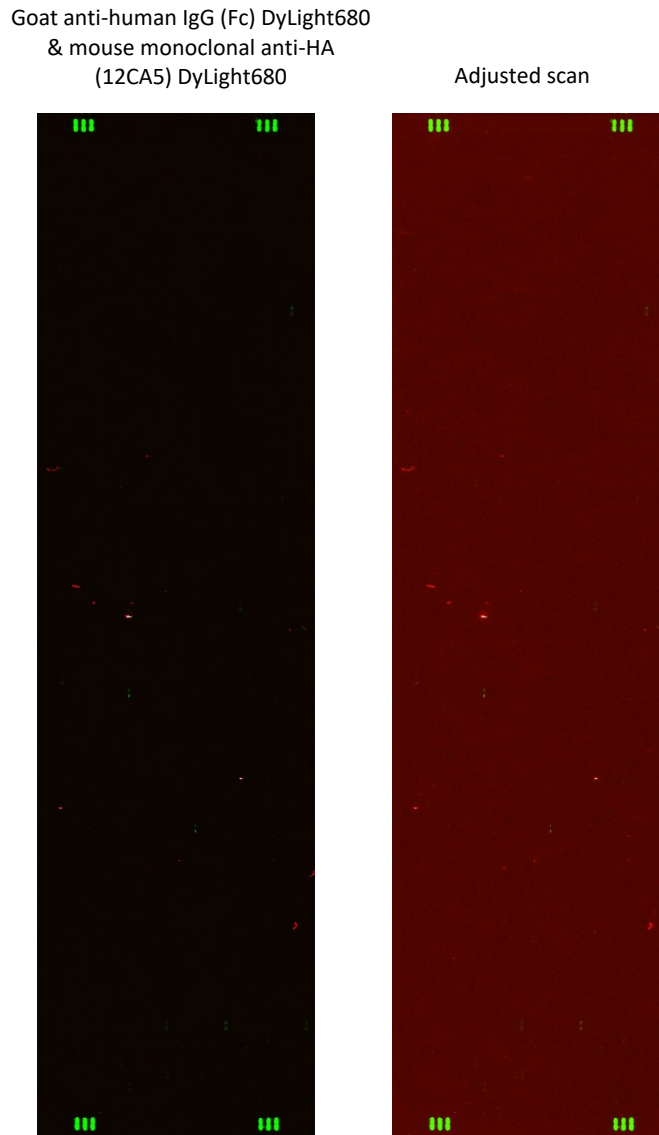


Figure S1: Fluorescence intensities of HEV cyclic peptide microarray slide incubated with control and secondary antibodies.

Slide was incubated with positive control antibody [mouse monoclonal anti-HA (12CA5) DyLight680] and secondary antibody [goat anti-human IgG (Fc) DyLight680]. Right panel shows same image with increased brightness and contrast.

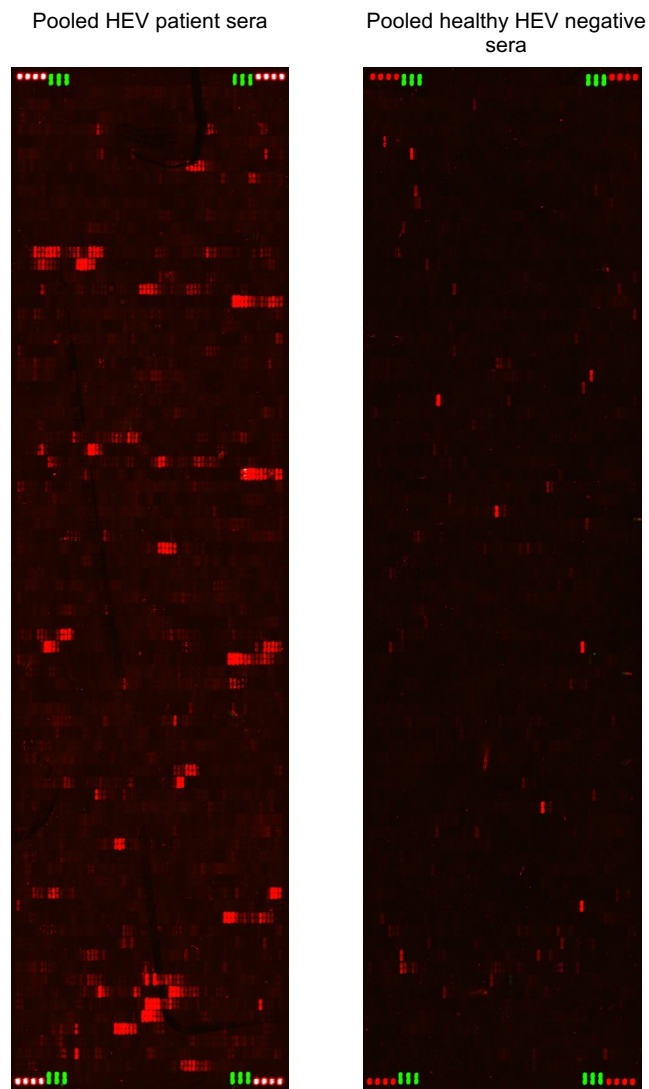


Figure S2: Fluorescence intensities of HEV cyclic peptide microarray slide with test sera.

Left panel was incubated with pooled HEV patient sera and right panel was incubated with pooled healthy HEV negative volunteer sera (1:1000 dilution).

Figure S3

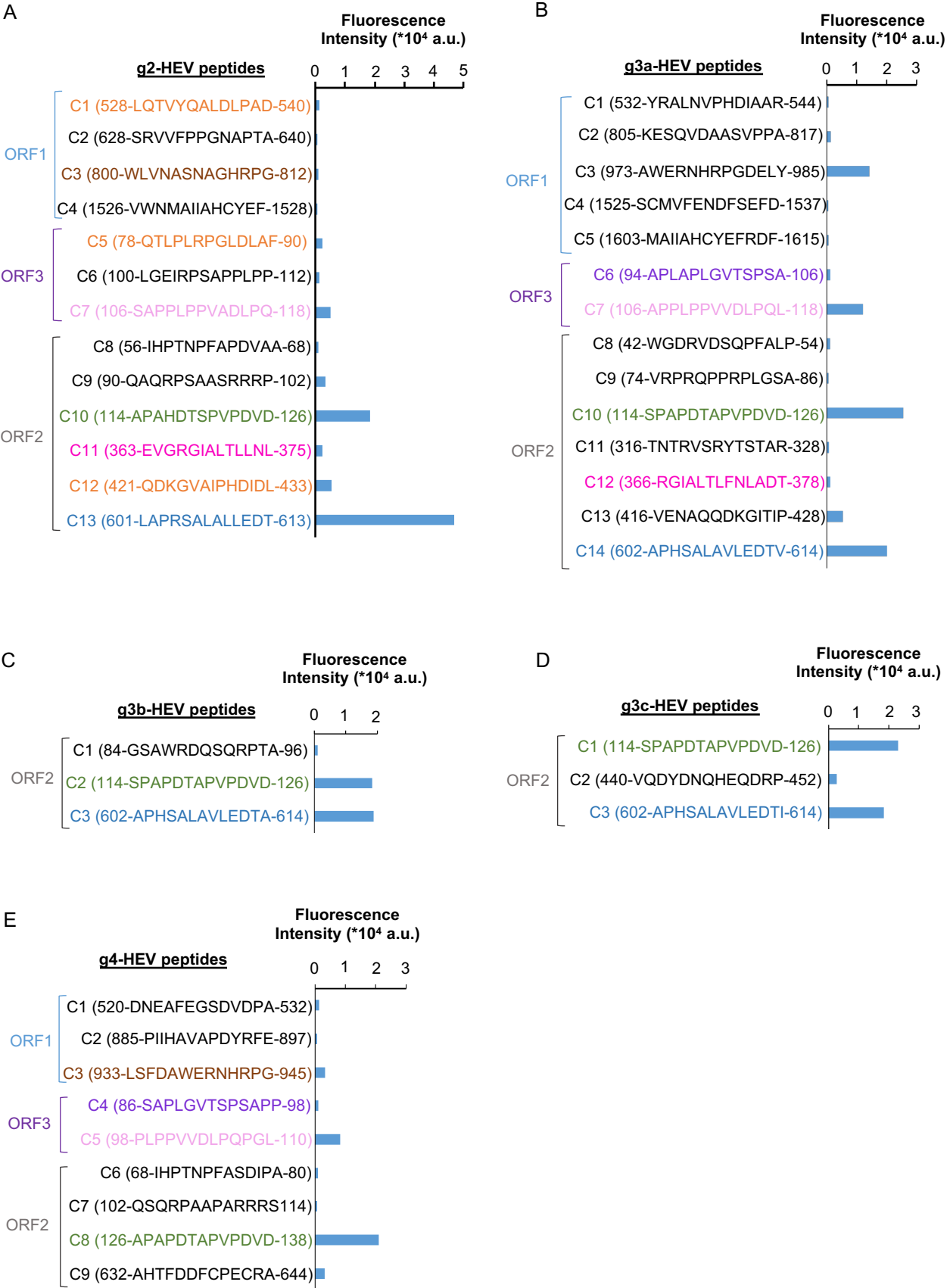


Figure S3: Peptide sequences and intensity plot graph of B-cell epitopes present in g2-, g3a-, g3b-, g3c- and g4- HEV.

Peptide sequences and intensity plot graph of B-cell epitopes present in (A) g2-HEV, (B) g3a-HEV, (C) g3b-HEV, (D) g3c-HEV and (E) g4-HEV. Color coding: Orange denotes epitopes common in g1- and g2-; Brown denotes common epitopes in g1-, g2- and g4-HEV; Lavender denotes common epitopes in g1-, g2-, g3a- and g4-HEV; Pink denotes common epitopes in g1-, g2- and g3-HEV; Green denotes g1-, g2-, g3a-, g3b-, g3c- and g4-HEV; Blue denotes common epitopes in g1-, g2-, g3a-, g3b-, and g3c-HEV; Purple denotes common epitopes in g3a- and g4-HEV.

Figure S4

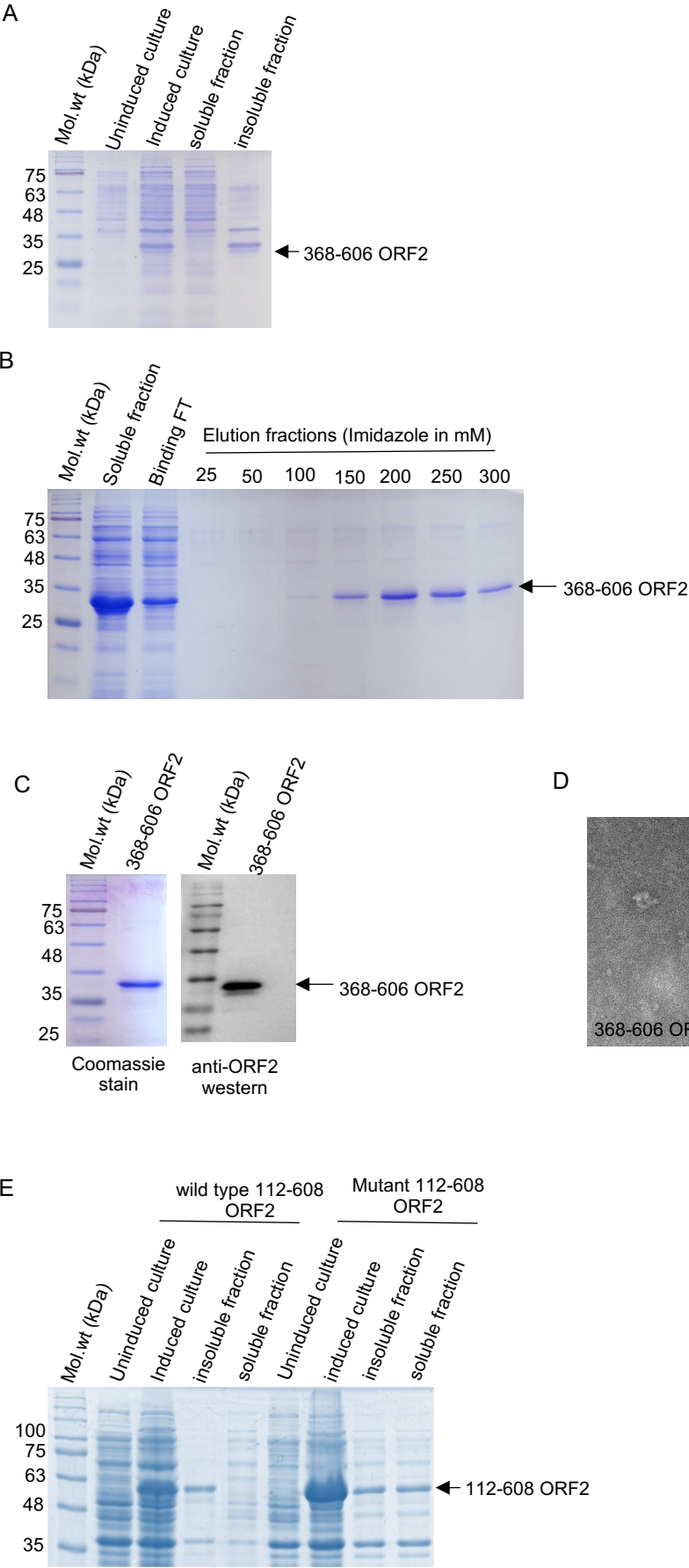


Figure S4: Purification and characterization of HEV 368-606 ORF2 protein.

A) CBB staining of 368-606 ORF2 protein expressed in C41(DE3) cells. B) CBB staining of elution fractions obtained by Ni²⁺-NTA affinity chromatography of 368-606 ORF2 protein purified from the insoluble fraction C) CBB staining and western blot of purified 368-606 ORF2 with anti-ORF2 antibody. D) Transmission electron micrograph of purified 368-606 ORF2 protein (Scale: 50nm). E) CBB staining of wild type and mutant 112-608 ORF2 protein expressed in C41(DE3) cells.

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HEV_G1 1 AVAPAHDTTPVPVDVDSRGAILRRQYNLSTSPLTSSVATGTNLVLYAAPLSPLLPLQDGTNTHIMATEASNYAQYRVARAT 80
HEV_G2 1 AVAPAHDTSPVPVDVDSRGAILRRQYNLSTSPLTSSVASGTNLVLYAAPLNPLLPLQDGTNTHIMATEASNYAQYRVARAT 80
HEV_G3 1 AVSPAPDPTAPVPDVSRRGAILRRQYNLSTSPLTSSVASGTNLVLYAAPLNPLLPLQDGTNTHIMATEASNYAQYRVVRAT 80
HEV_G4 1 AVAPAPDPTAPVPDVSRRGAILRRQYNLSTSPLTSTTATGTNLVLYAAPLSPLLPLQDGTNTHIMATEASNYAQYRVVRAT 80
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HEV_G2 161 TSGLVMLCIHGSPVNSYNTNTPYTGA LGLLDFALELEFRNLTPGNTNTRVSRYSSTARHRLRRGADGTAE LTTTAATRFMK 240
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HEV_G2 241 DLHFTGLNGVGEVGRGIALTLFNLADTLLGGLPTELISSAGGQLFYSRPVVSANGEP TVKLYTSVENAQDQKGIAPHDI 320
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HEV_G4 401 RSLDWSKVTLDGRPLPTTIQQYSKTFEVLPLRGKLSFWEAGTTKAGYPNYNTTASDQIL IENAAGHRVCI STYTTS NLGSG 480
HEV_G7 401 RSLDWTKVTLTGRLPTTIQQYSKTFEVLPLRGKLSFWEAGTTKAGYPNYNTTASDHIL IENAAGHRVAISTYTTS LGAG 480
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HEV_G2 481 PVAISAAVLA PRSALA 497
HEV_G3 481 PASISAVGVLAPHSALA 497
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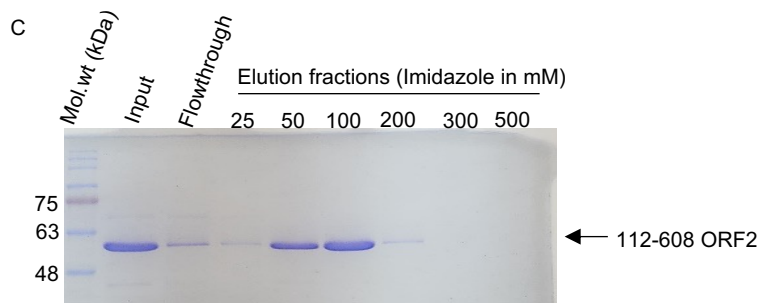
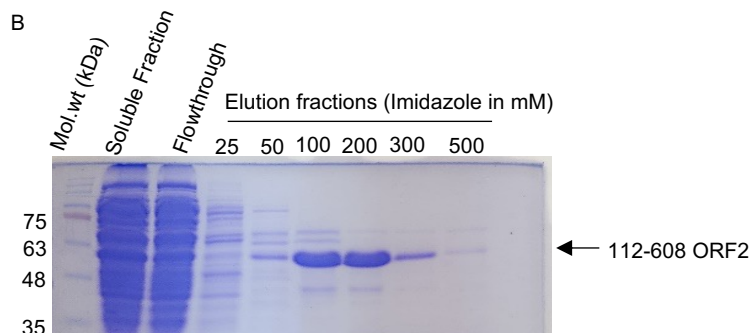


Figure S5: Sequence alignment and purification of HEV 112-608 ORF2 protein.

A) Protein sequence alignment of 112-608aa region of g1, g2, g3, g4 and g7-HEV ORF2.
B) CBB staining of elution fractions obtained by Ni²⁺-NTA affinity chromatography of 112-608 ORF2 protein purified from the soluble fraction C) CBB staining of elution fractions obtained by NTA-Co²⁺ affinity chromatography of elution fractions obtained in Ni²⁺-NTA affinity chromatography.

Figure S6

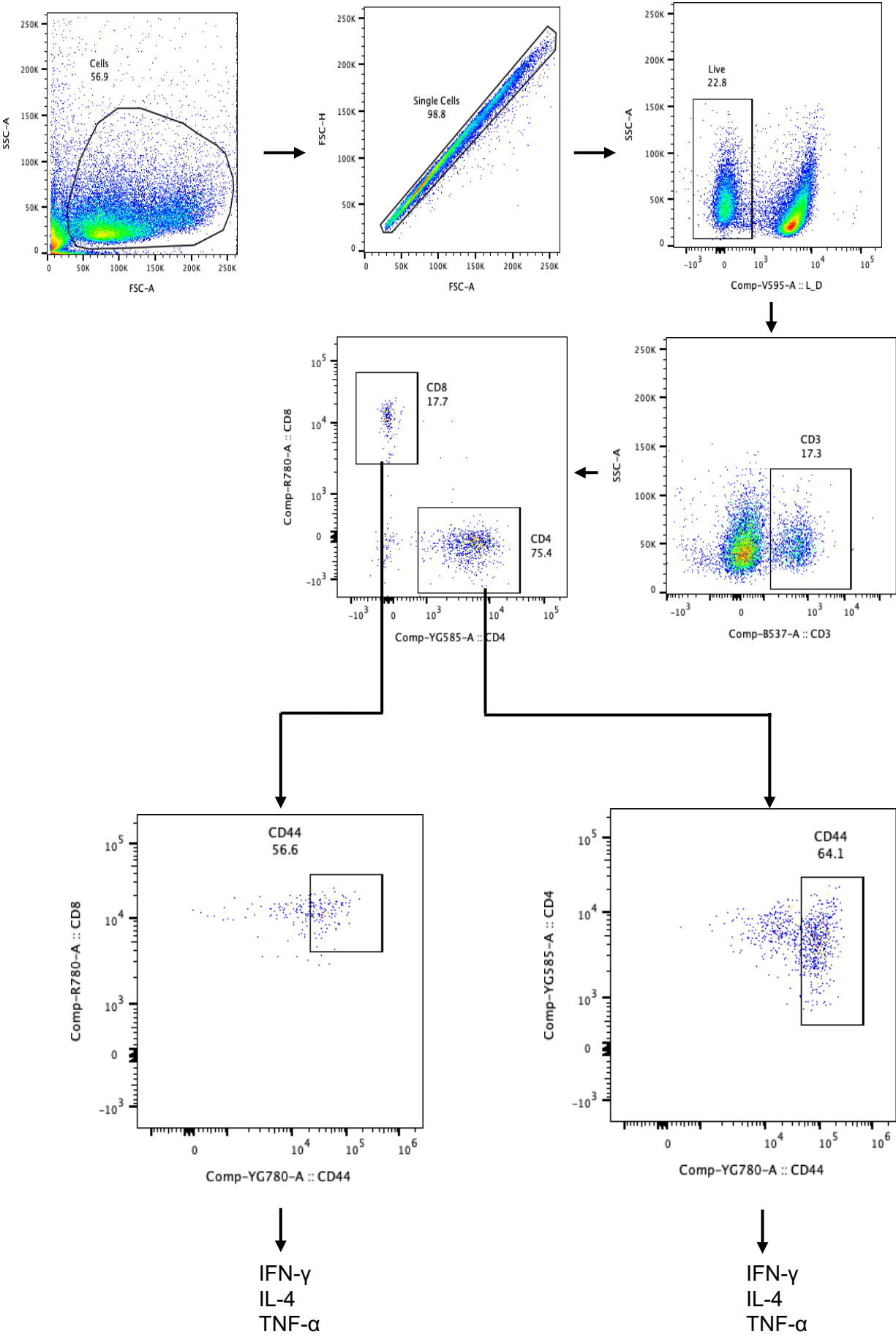


Figure S6: Flow cytometry gating strategy.

Representative FACS plot showing the gating strategy of lymphocytes, single cells, live cells, CD3⁺, CD44⁺CD4⁺ and CD44⁺CD8⁺ T-cells. Subsequent gates were applied to quantify the target cell population expressing IFN- γ , IL-4, and TNF- α .

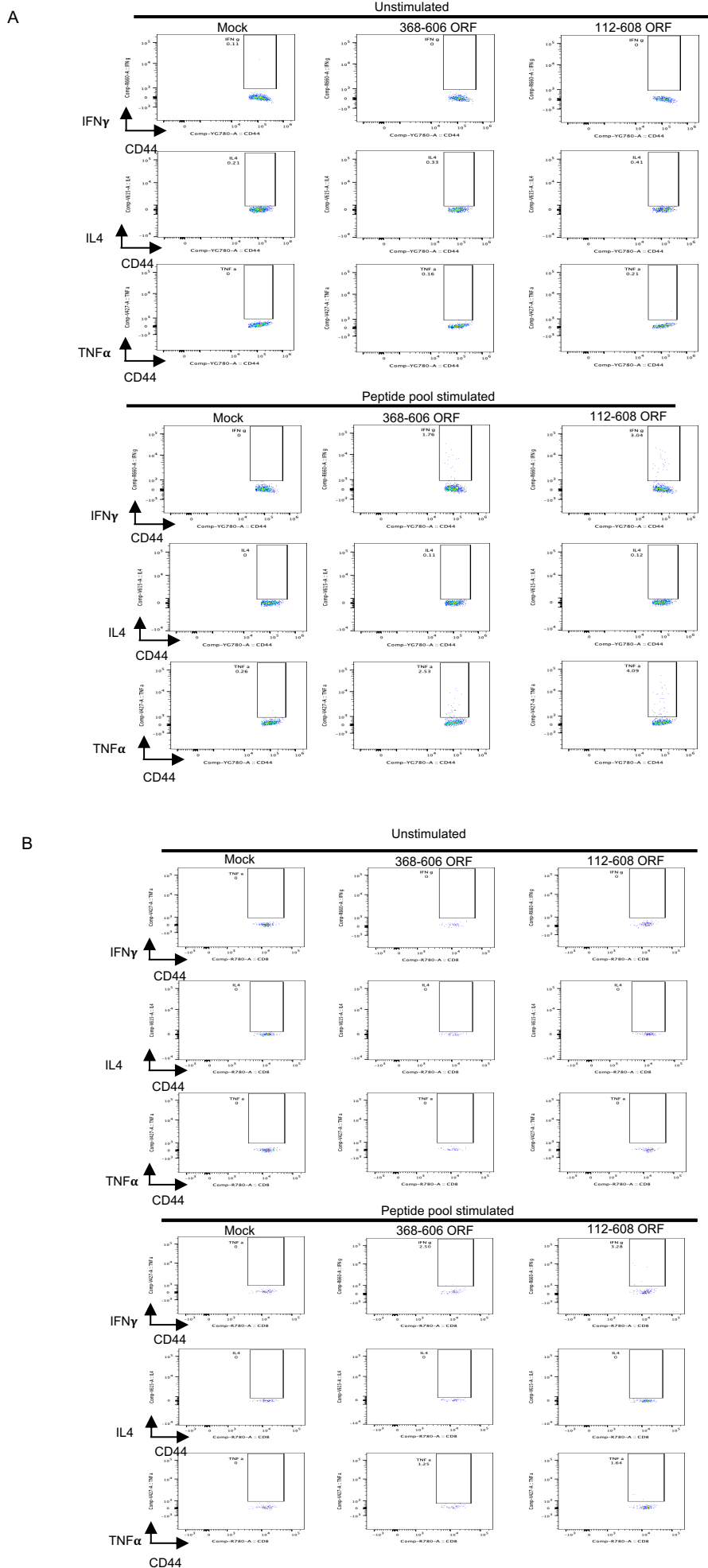


Figure S7: Representative FACS plots showing IFN- γ , IL-4, and TNF- α expression in CD4 and CD8 positive cell population.

A) IFN- γ , IL-4, and TNF- α expression in CD4⁺CD44⁺ T-cell population in unstimulated cells (upper panel) and ORF2 peptide pool stimulated cells (Lower panel). B) IFN- γ , IL-4, and TNF- α expression in CD8⁺CD44⁺ T-cell population in unstimulated cells (upper panel) and ORF2 peptide pool stimulated cells (Lower panel).