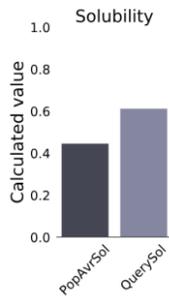
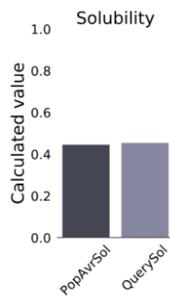
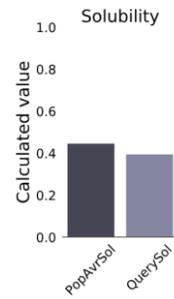


**A****Primary antigen**

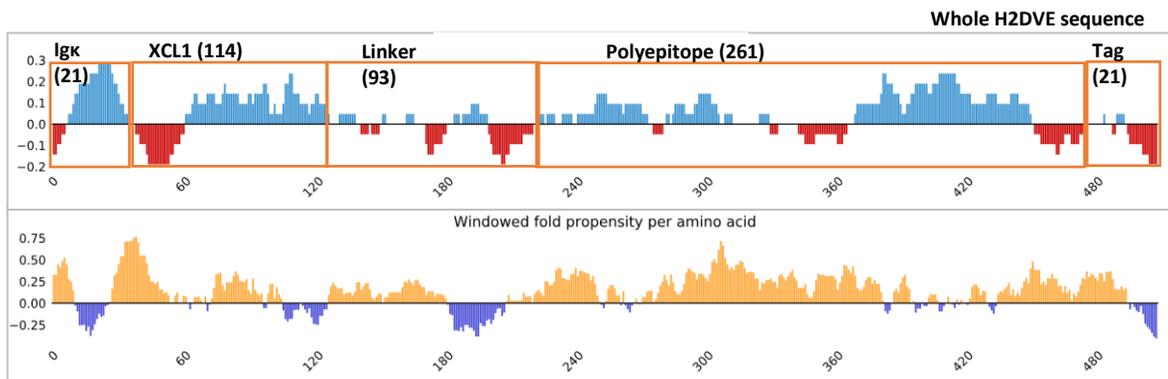
PSS: 0.612

**XCL1 + primary antigen**

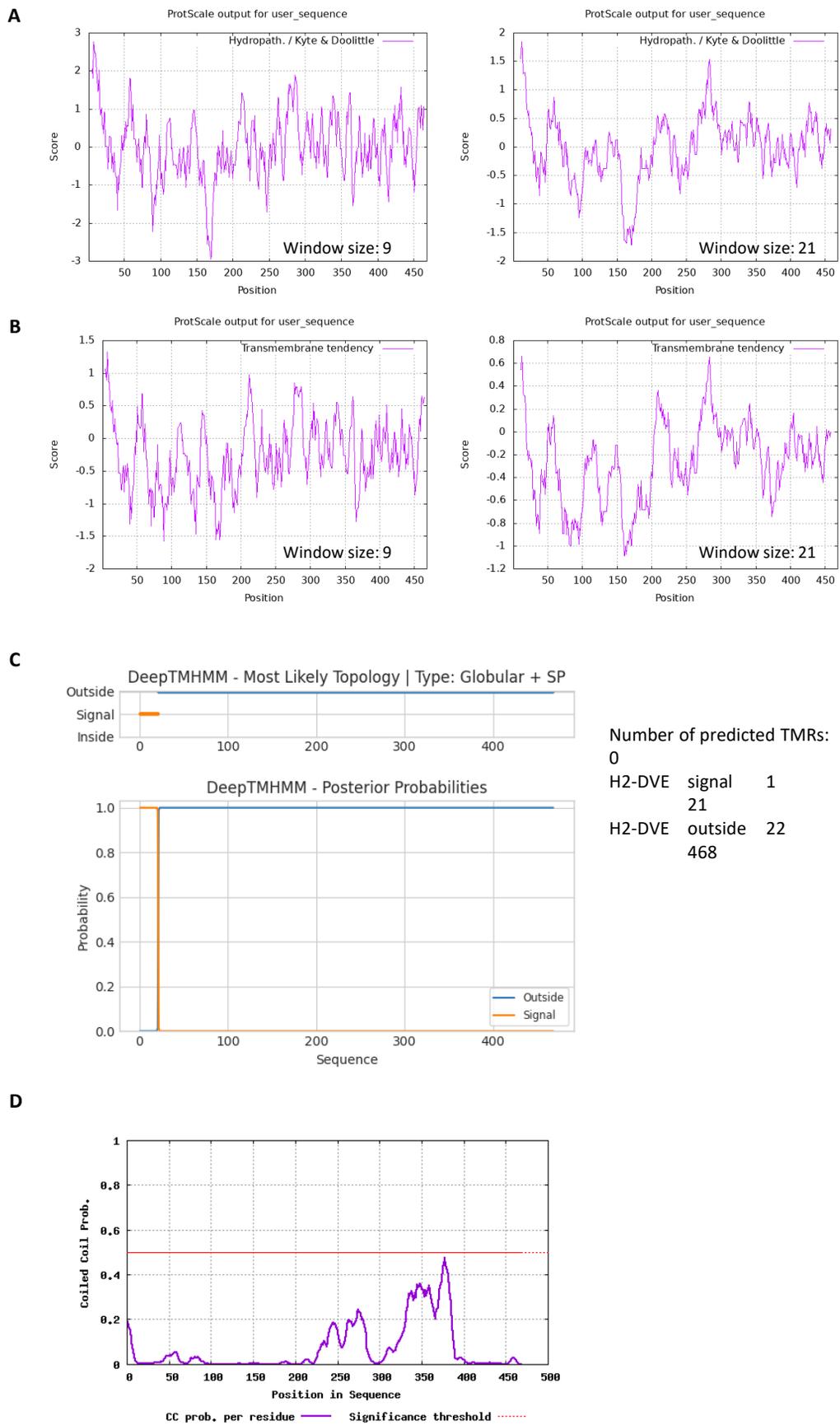
PSS: 0.454

**Whole H2DVE sequence**

PSS: 0.395

**B****Polyepitope**

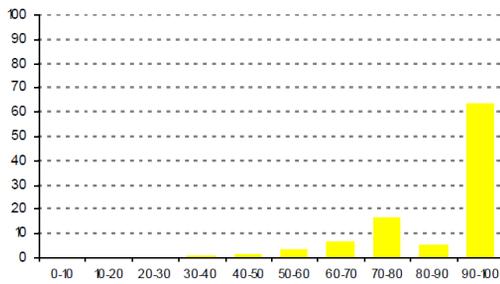
**Figure S1:** Protein-Sol solubility calculation results for the H2DVE recombinant protein. **(A)** PSS scores of three H2DVE segments: primary antigen alone; XCL1 (GenBank: AAA56752.1) fused with primary antigen; and the entire H2DVE sequence. **(B)** Plots depicting windowed fold propensity and windowed net charge for the whole H2DVE sequence, with residues lengths indicated in parentheses. (PSS: predicted scaled solubility)



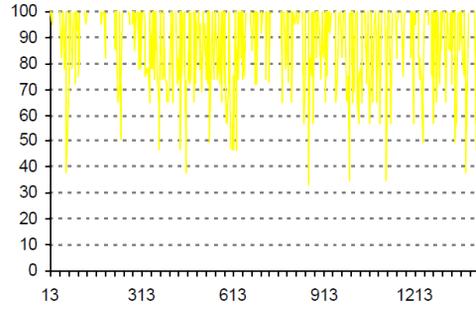
**Figure S2:** H2DVE characterization through various analyses: **(A)** Hydropathicity assessed using the Kyte & Doolittle method on the ExPASy ProtScale server, **(B)** Transmembrane tendency evaluated with window sizes of 9 and 21, considering relative weight variations between window edges and center (100%), **(C)** Transmembrane topology prediction using DeepTMHMM (TMRs: transmembrane regions), and **(D)** Coiled-coil domain prediction.

**A**

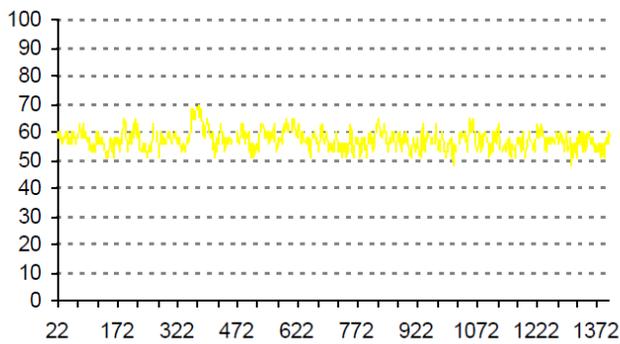
Optimized with GeneOptimizer™

**Codon Quality Distribution**Codon Adaptation Index : **0.87****B**

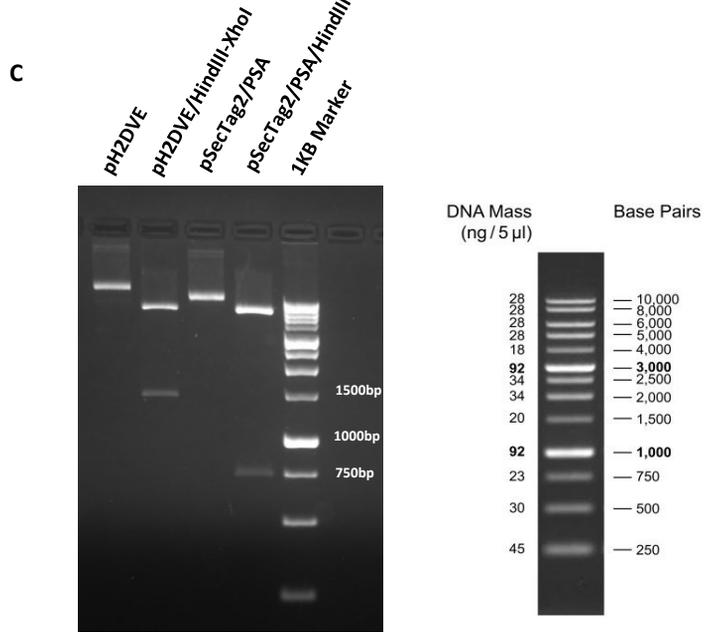
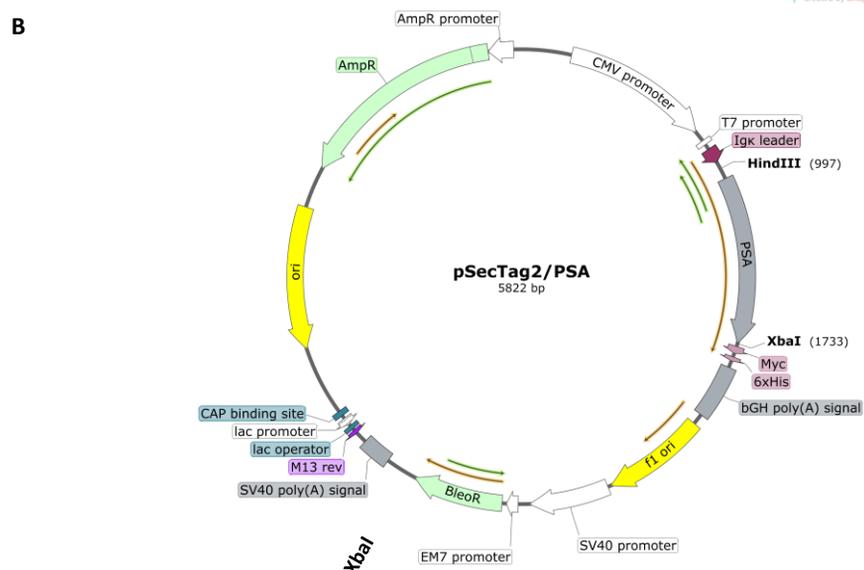
Optimized with GeneOptimizer™

**Codon Quality Plot****C**

Optimized with GeneOptimizer™

**GC Content**Average GC content: **57 %**

**Figure S3:** GeneOptimizer Assisted Sequence Analysis of H2DVE encoding nucleotide sequence. **(A)** The histograms show the percentage of sequence codons which fall into a certain quality class. The quality value of the most frequently used codon for a given amino acid in the desired expressed system is set to 100, the remaining codons are scaled accordingly. **(B)** The plots show the quality of the used codon at the indicated codon position. **(C)** The plot shows the GC content in a 40 bp window centered at the indicated nucleotide position.



**Figure S4:** Plasmid sequence map of **(A)** pH2DVE and **(B)** pSecTag2/PSA, created using SnapGene Viewer software (v7.0). **(C)** Electrophoresed DNA of undigested and double restriction digested products of pH2DVE and pSecTag2/PSA, as indicated, where sequence length was estimated using 1 kb molecular marker (GeneDirex).



**Figure S5:** Plasmid sequence map (pET303/CT-His/H2DVE) of the codon-optimized sequence of H2DVE subcloned into the pET303/CT-His vector, created using SnapGene Viewer software (v7.0).