

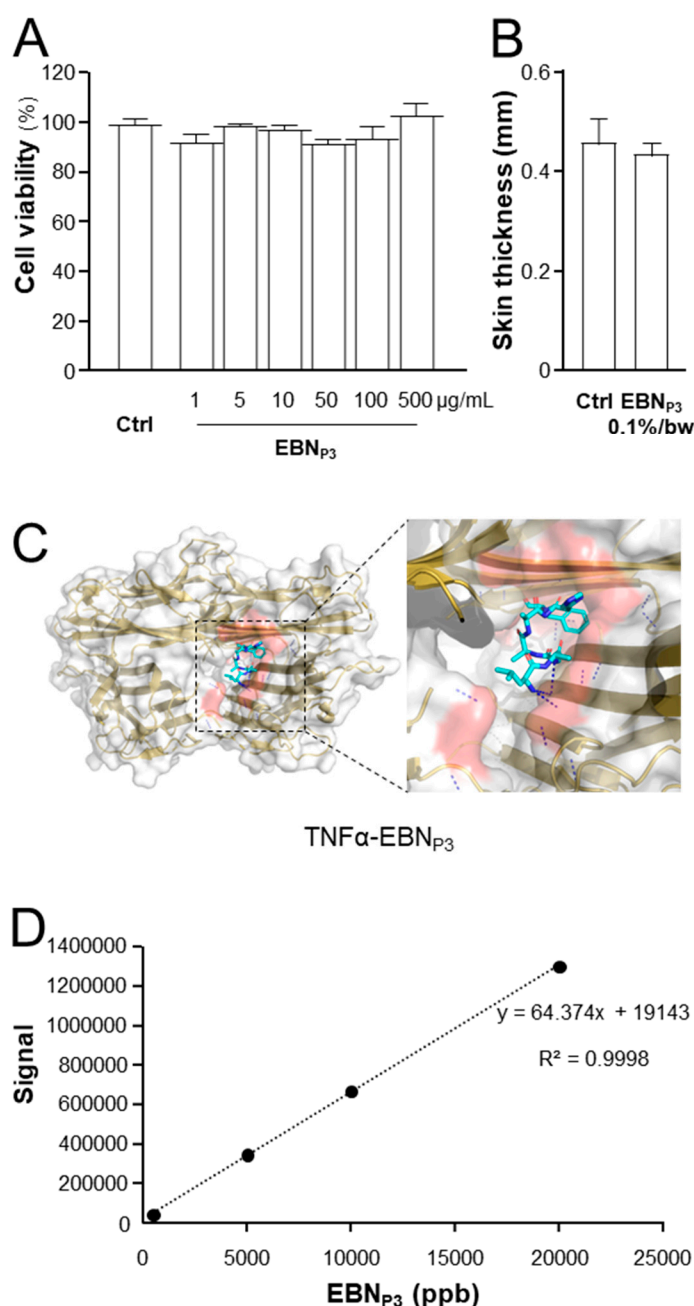


Figure S1. Bioactivity, binding prediction, and quantification of EBN_{p3}. (A) HaCaT cell viability. HaCaT cell viability assessed after 48 hours incubations of 1, 5, 10, 50, 100, and 500 $\mu\text{g/mL}$ of EBN_{p3}. A statistical difference comparing with the control group was analyzed by one-way ANOVA, and presented herein as mean $\pm$ SEM, $n = 3$. (B) Mouse dorsal skin thickness. Skin thickness measured 2 weeks after topical application of 1,000 mg/kg EBN_{p3} on mouse dorsal skin. A statistical difference comparing with the control group was analyzed by one-way ANOVA, and presented herein as mean $\pm$ SEM, $n = 3$. (C) Predicted TNF- α -EBN_{p3} binding model. The X-ray crystal structure of TNF- α (PDB ID: 2AZ5, <https://www.rcsb.org/>), a key pro-inflammatory cytokine in the NF- κ B signaling pathway and the pathology of skin psoriasis, was selected as the target. Peptide-protein docking was employed to explore the binding affinity between EBN_{p3} and TNF- α [20], with potential inhibitory effects and therapeutic applications for skin inflammation. AlphaFold2 colab (<https://colab-research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb>) was used to predict

five highly ranked structures (pLDDT > 60%) for EBN_{P3} [16]. The active sites of TNF- α were predicted using the CASTp server (<http://sts.bioe.uic.edu/castp/index.html?2pk9>), which measured and characterized the cavities, pockets, and channels of the TNF- α protein structure, and predicted the key amino acids binding to potential inhibitors [30]. The solvent-accessible surface area and volume of the TNF- α pocket were predicted to be 2,743.256 Å² and 4,514.068 Å³ by CASTp. The HDOCK server (<http://hdock.phys.hust.edu.cn/>) was used to carry out the docking analysis between EBN_{P3} and TNF- α , with Certolizumab Fab (PDB ID: 5WUV) serving as the positive control. The interactions between polar atoms in the best docked complex were visualized by PyMOL™ 2.5.5, with potential contact areas on TNF- α filled in red and molecular interactions indicated by blue dashed lines. The potential binding interactions between EBN_{P3} and TNF- α were predicted at the Gln61, Tyr119, Leu120, Gly121, Gln149, and Tyr151 active site residues on TNF- α chain A; the Leu57, Tyr59, Ser60, Gln61, Tyr119, Leu120, Gly121, Gly122, and Tyr151 active site residues and Ile58 inactive site residue on TNF- α chain B; the Lys11 active site residue on TNF- α chain C; and the Leu55 and Leu157 active site residues on TNF- α chain D. The most prominent docking score and shortest ligand rmsd values for EBN_{P3} were -203.58 and 87.22 Å, respectively, compared to -256.90 and 86.34 Å for the certolizumab light domain, a commercial TNF- α monoclonal antibody. **(D)** LC-MS/MS quantification of EBN_{P3} in EBN digests. Standard addition calibration curve for EBN_{P3} determined by LC-MS/MS. The curve was constructed by spiking an EBN enzymatic digest with EBN_{P3} standard at concentrations of 500, 5,000, 10,000, and 20,000 ppb, respectively.

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