

## Supplementary Information

### Glucagon-like peptide-1 mimotopes screened from an Fv- antibody library

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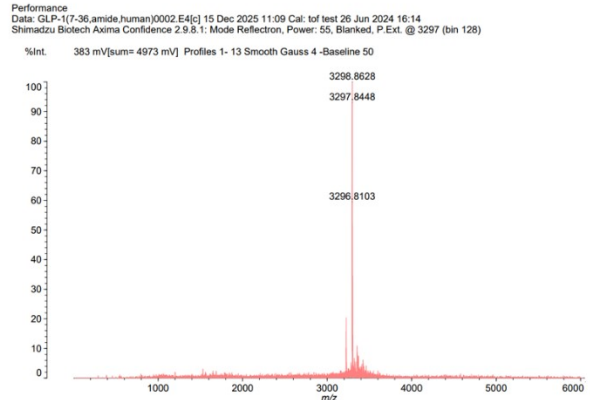
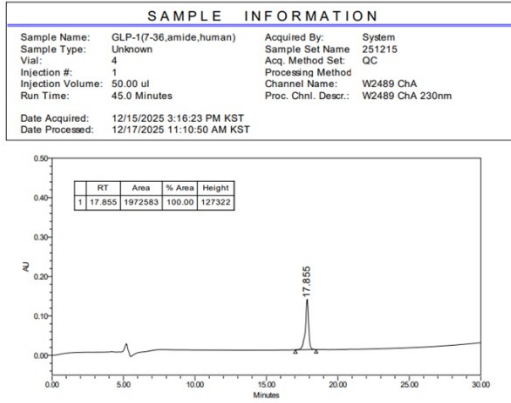
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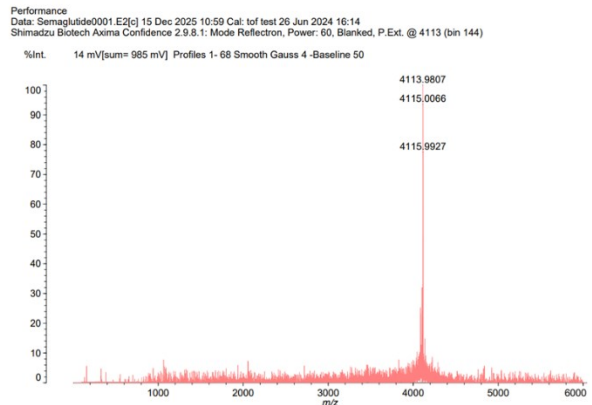
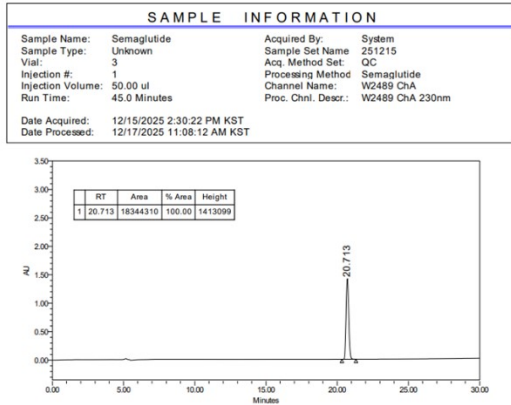
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**Figure S1.** Analytical RP-HPLC and MS characterization of GLP-1R agonist reference peptides. (a) GLP-1 (7–36) amide (human), left: analytical RP-HPLC chromatogram monitored at 230 nm; right: MALDI-TOF MS spectrum. (b) Semaglutide, left: analytical RP-HPLC chromatogram monitored at 230 nm; right: MS spectrum. (c) Tirzepatide, left: analytical RP-HPLC chromatogram monitored at 230 nm; right: MS spectrum.

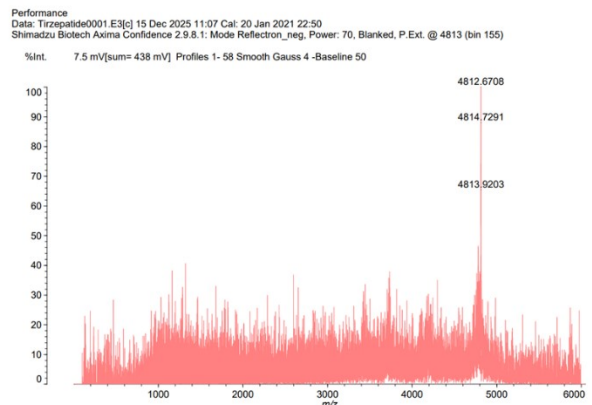
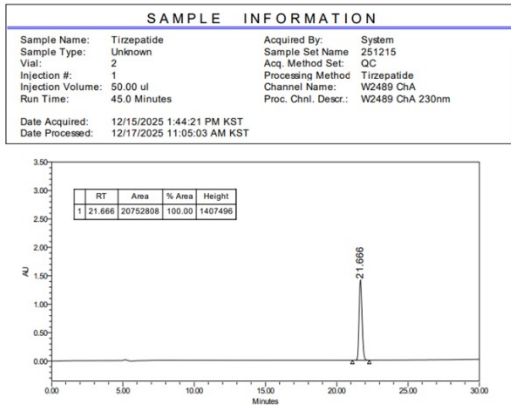
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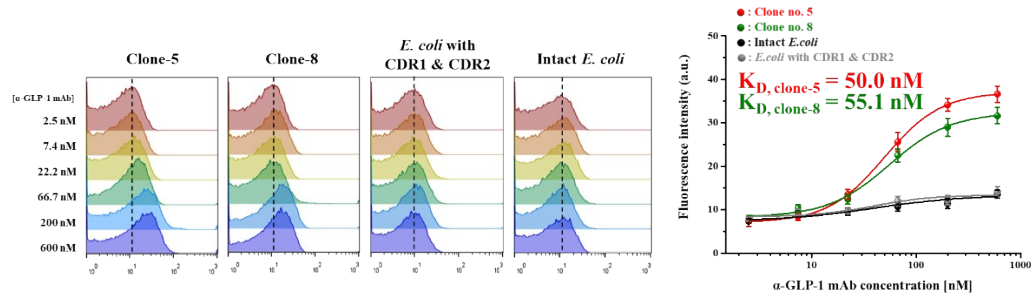
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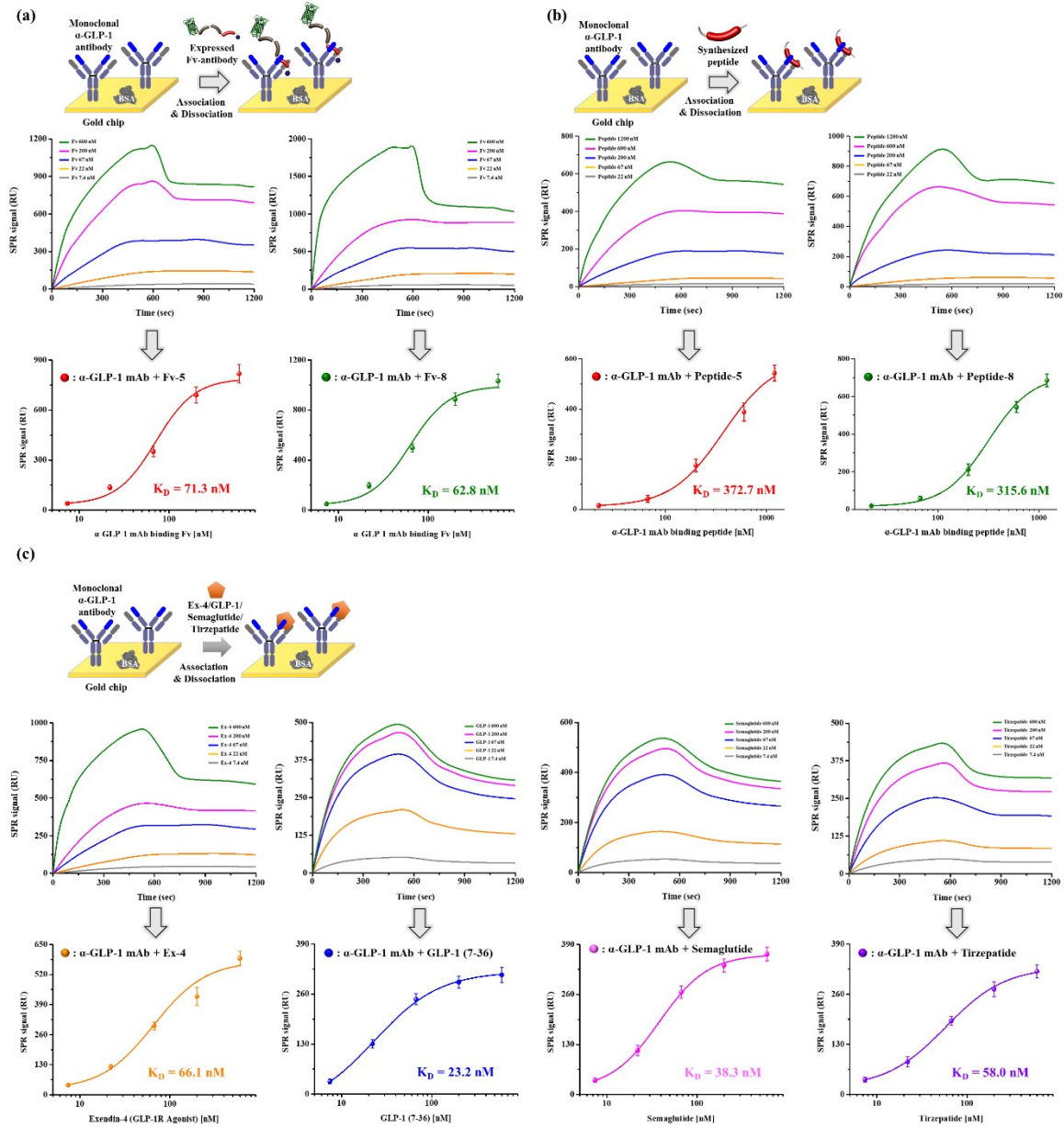
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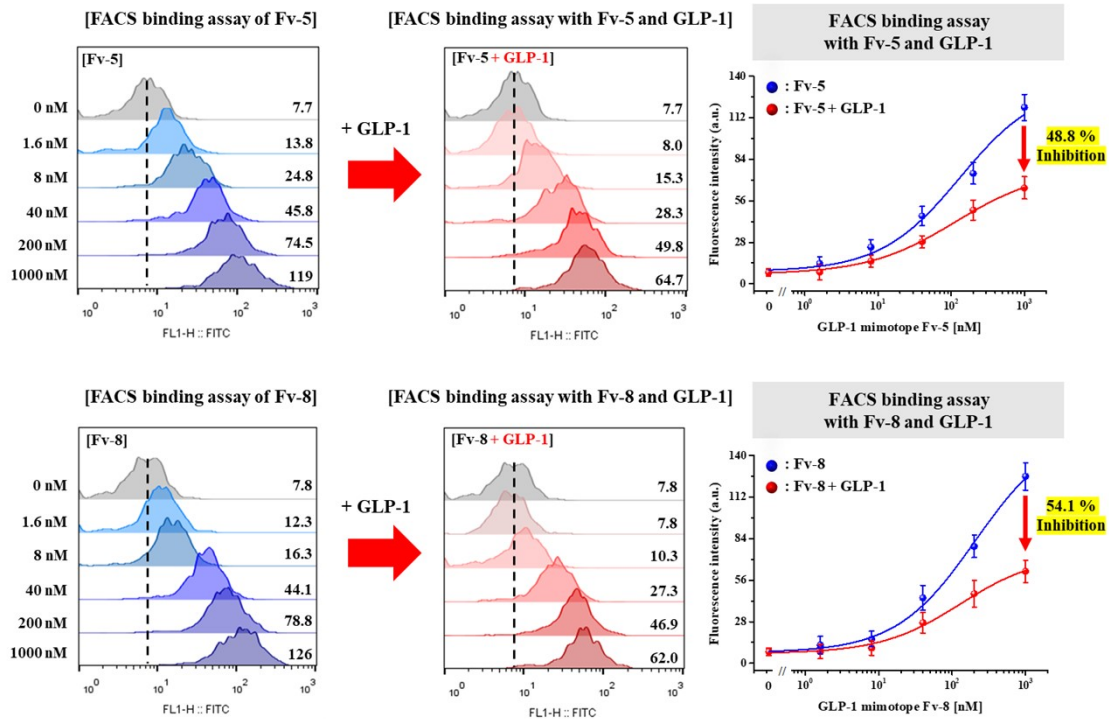
**Figure S2.** The binding affinities of screened clone-5 and clone-8 determined via flow cytometry.



**Figure S3.** SPR-based binding characterization of GLP-1 mimotopes and GLP-1R agonist benchmarks. (a) Binding analysis of expressed Fv-antibodies using an SPR biosensor, followed by  $K_D$  determination based on dose–response curves. (b) Binding analysis of synthesized peptides using an SPR biosensor, followed by  $K_D$  determination based on dose–response curves. (c) Binding analysis of Exendin-4 (Ex-4), GLP-1 (7-36), semaglutide, and tirzepatide using an SPR biosensor, followed by  $K_D$  determination based on dose-response curves.



**Figure S4.** Analysis of competitive binding between Fv mimotopes and GLP-1 in beta-TC-6 cells using FACS. (top: Fv-5; bottom: Fv-8)



**Table S1.** V<sub>H</sub> framework and CDR1–CDR3 amino acid sequences of the synthetic template, Fv-5, and Fv-8.

Region	Amino acid sequence (N → C)		
	Synthetic template	Fv-5	Fv-8
Frame-1	EVQLVESAAEVRPPGASVKITCKASGYDFS	EVQLVESAAEVRPPGASVKITCKASGYDFS	EVQLVESAAEVRPPGASVKITCKASGYDFS
CDR1	TYGIQ	TYGIQ	TYGIQ
Frame-2	WMRQAPGQRPEWLG	WMRQAPGQRPEWLG	WMRQAPGQRPEWLG
CDR2	WIHAGTGGTKYSRKFQG	WIHAGTGGTKYSRKFQG	WIHAGTGGTKYSRKFQG
Frame-3	RITITRDTSAANTVYLDLNSLTSEDVAVYYCAR	RITITRDTSAANTVYLDLNSLTSEDVAVYYCAR	RITITRDTSAANTVYLDLNSLTSEDVAVYYCAR
CDR3	<sup>1</sup> D-K-V-T-V- <sup>6</sup> W-A-C-Q-D- <sup>11</sup> N	<sup>1</sup> G-A-P-G-T- <sup>6</sup> R-L-H-G-D- <sup>11</sup> F	<sup>1</sup> C-S-P-G-G- <sup>6</sup> N-R-V-R-D- <sup>11</sup> F
Frame-4	WGQGTTVTVSS	WGQGTTVTVSS	WGQGTTVTVSS