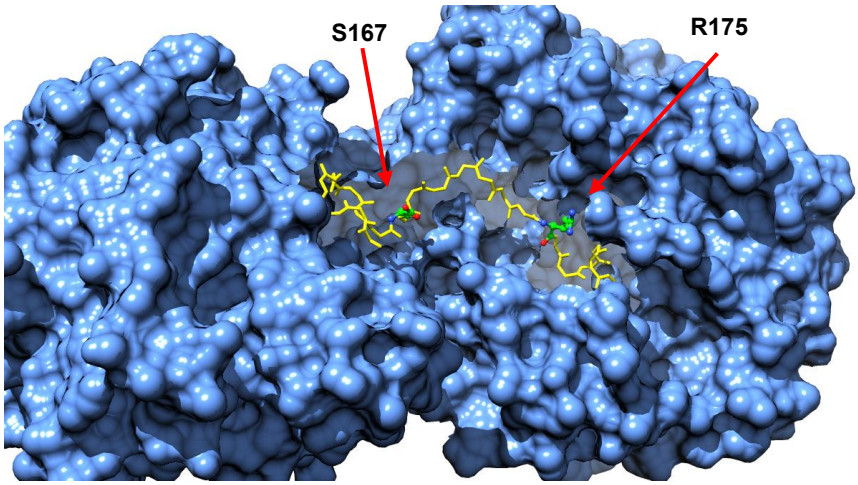
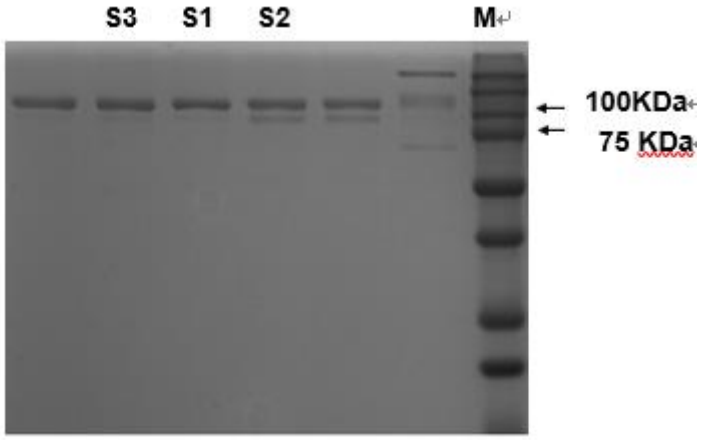
Supplementary



Supplementary Fig. 1 Structure of FGF21 C-terminal tail in complex with β-klotho (PDB:5VAQ). KLB (blue) is shown as surface and FGF21 C-tail (yellow) is shown as backbone stick representations. S167 and R175 (green) are labeled and shown as ball & stick representation.

a



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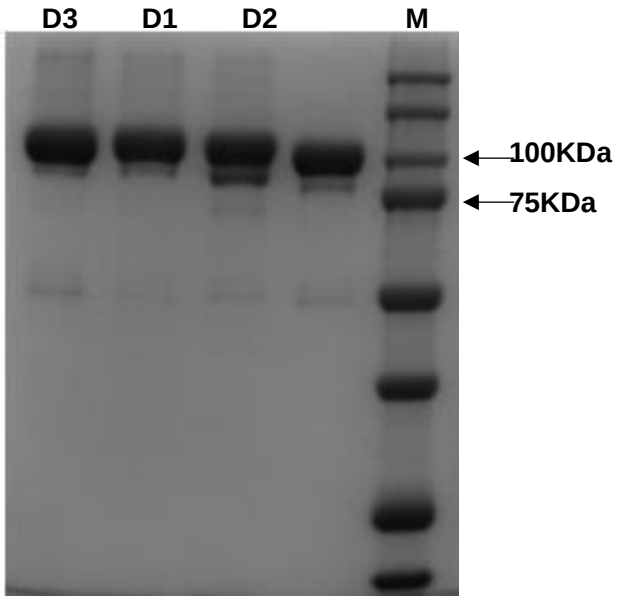

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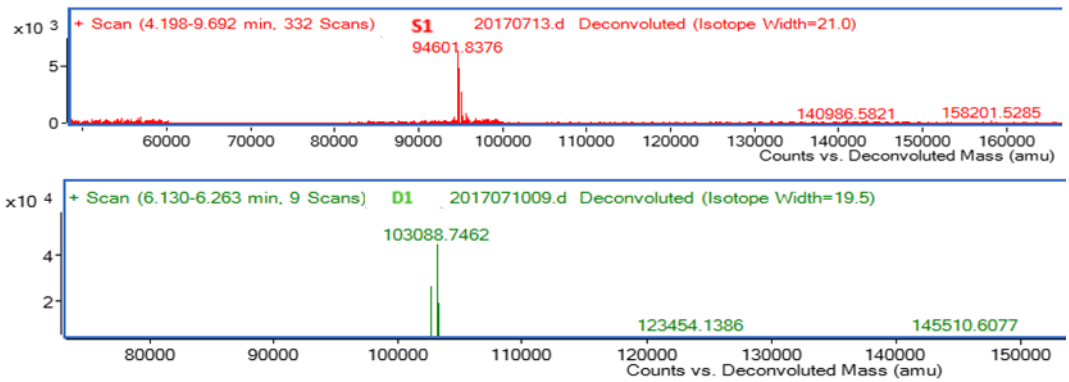
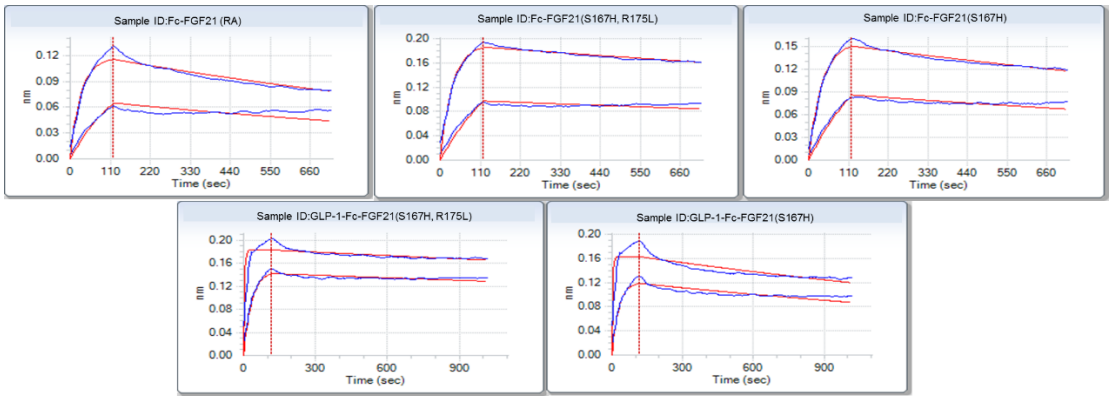
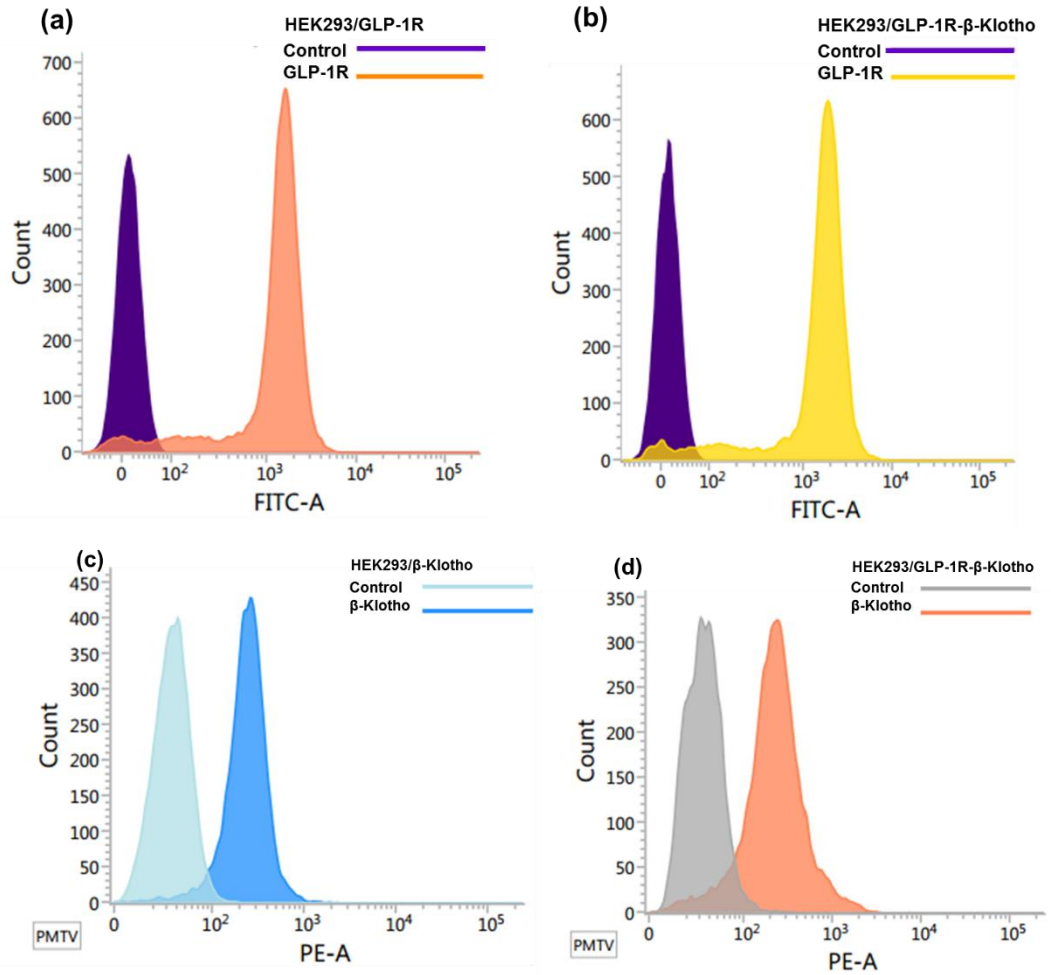


Supplementary Fig. 2 Characterization of FGF21 fusion proteins. a. SDS PAGE of Fc-FGF21 purified by protein A column affinity chromatography. b. SDS PAGE of GLP-1-Fc-FGF21 purified by protein A column affinity chromatography. c. The mass spectrometry

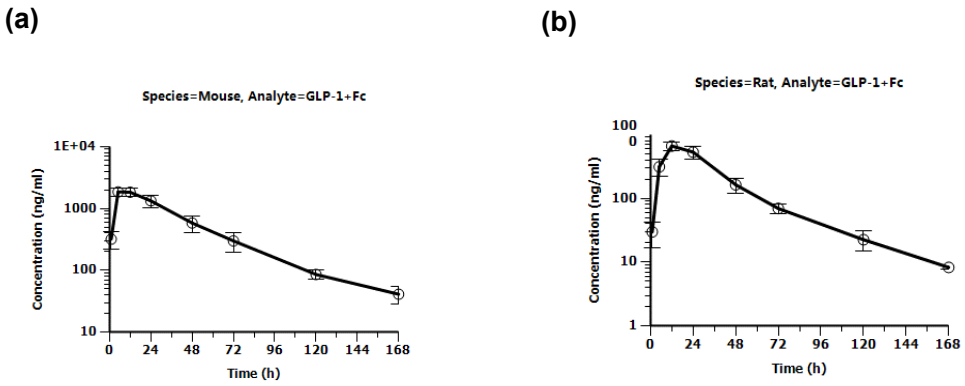
detection diagrams of Fc-FGF21 S1and GLP-1-Fc-FGF21 D1 fusion proteins. The mass spectra are assigned to the corresponding m/z values.



Supplementary Fig. 3 Kinetic analysis of the interaction between FGF21 mutants and β-Klotho. Data were processed and curve fit using a 1:1 binding model.



Supplementary Fig. 4 The receptor expression of HEK293/GLP-1R, HEK293/β-Klotho or HEK293/GLP-1R-β-Klotho were assessed by flow cytometry analysis. (a) Data shown are representative histograms from flow cytometry showing expression for GLP-1R in HEK293/GLP-1R cell line. (b) and for GLP-1R in HEK293/ GLP-1R-β-Klotho cell line. (c) and for β-Klotho in HEK293/β-Klotho cell line. (d) and for β-Klotho in HEK293/ GLP-1R-β-Klotho cell line. Isotype control antibody at the same concentration was used as a negative control.



Supplementary Fig. 5 PK profiles of D1 in mice and rats. (A)Mean plasma concentration-time profiles in mice following single SC administrations at 1 mg/kg. (B) Mean plasma concentration-time profiles in rats following single SC administrations at 0.5 mg/kg. Values presented are Mean ± SD with n = 5 for mice and rats.

Species	Dose (mg/kg)	Route	AUC_{last} (μg*h/mL)	C_{max} (ng/mL)	T_{max} (h)	MRT (h)	$t_{1/2}$ (h)	CL/F (mL/h/kg)	Vz/F (mL/kg)
Mouse	1	SC	82.2±16	2010±190	7.8±3.8	35.5±2.7	30.3±1.6	12.3±2.6	541±140
Rat	0.5	SC	24.9±4.5	659±98	12.0±0.0	31.6±3.3	25.9±2.5	20.2±3.6	746±94

Supplementary Table 4 Pharmacokinetic parameters in C57BL6 mice and Sprague-Dawley rats. Plasma concentrations of D1 were determined with a sandwich ELISA recognizing the N-terminus of the GLP-1 portion and the Fc portion. AUC_{last}, area under the curve from the time of dosing to the last measurable concentration; C_{max}, maximum observed concentration; T_{max}, time to C_{max}; MRT, mean residence time; $t_{1/2}$, terminal half-life; CL/F, total body clearance for extravascular administration; Vz/F, volume of distribution as a function of bioavailability. All values are expressed as (Means ± SD) with n = 5 for mice and rats.

GLP-1	HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGG
FGF21	HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKA LKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRERLLEDGYNVYQSEAHGLP LHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLHMVGASQ GLSPSYAS

Supplementary Table 5 The sequences for GLP-1 and FGF21 in GLP-1-Fc-FGF21 D1.