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# Supplementary Materials for

## Molecular targeting of FATP4 transporter for oral delivery of therapeutic peptide

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**This PDF file includes:**

a

Sequence of Exendin-4 :

**His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Ala-Val-Arg-**
**-Leu-Phe-Ile-Glu-Trp-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH2**

b

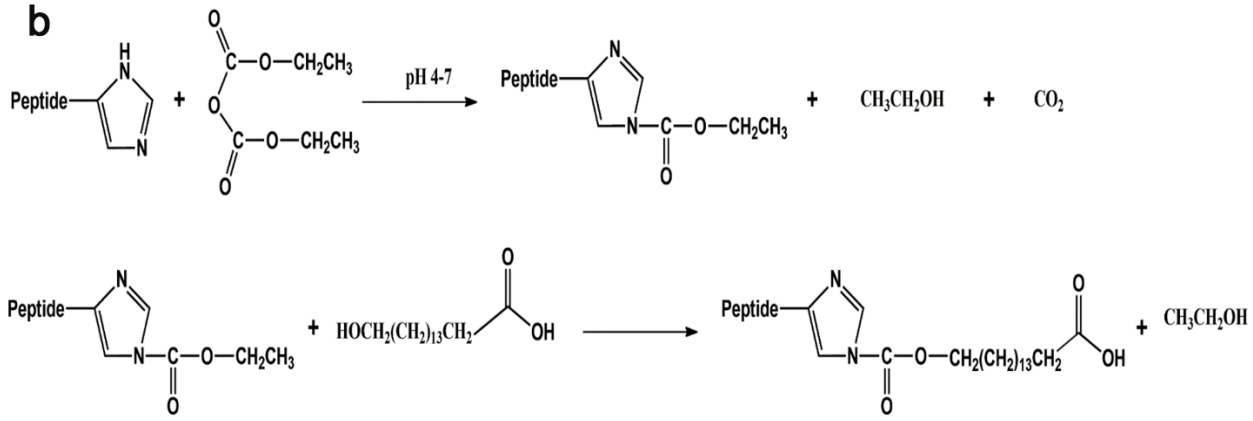


**Fig. S1. Exendin-4 (Ex4) peptide sequence and LCFA-Ex4 synthesis.** a) Amino acid sequence of Ex4. b) Chemical reactions to synthesize LCFA-Ex4.

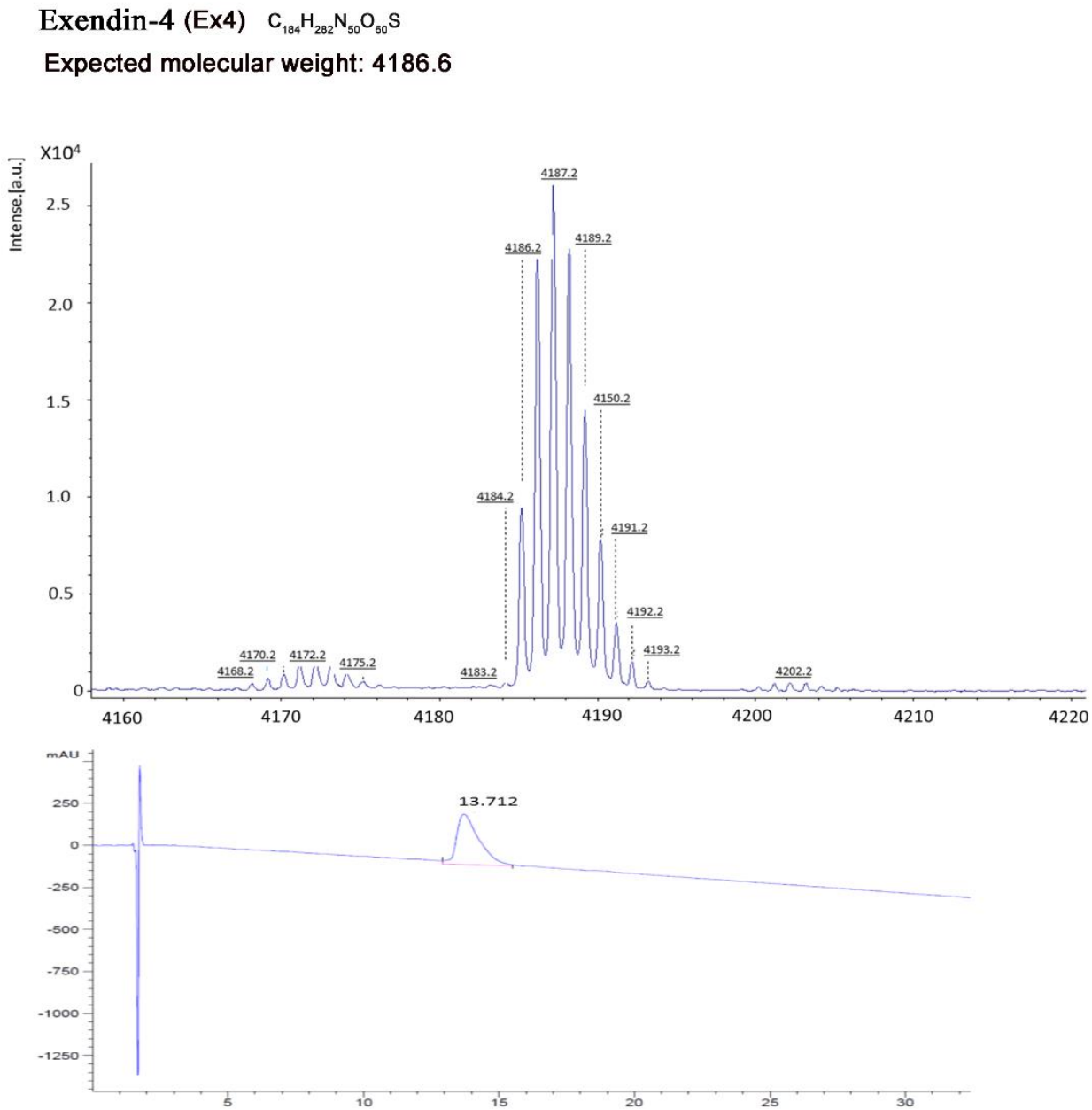


**Fig. S2. MALDI and HPLC spectra of Ex4.** Upper panel: Based on MALDI analysis, molecular weight of Ex4 was 4187.2. Bottom panel: HPLC analysis revealed EX4 had a retention time of 13.712 minutes.

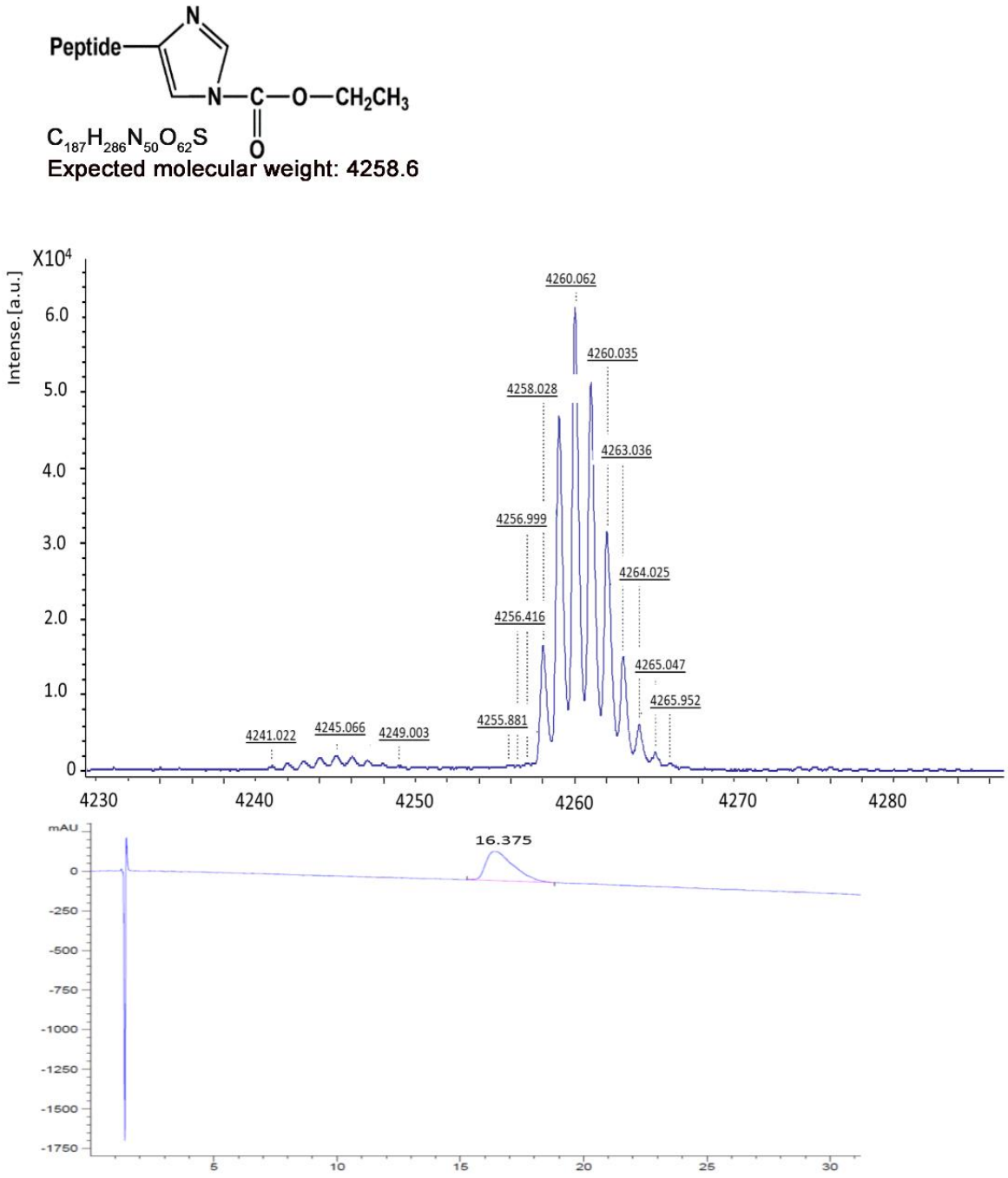


**Fig. S3. MALDI and HPLC spectra of intermediate.** Upper panel: Based on MALDI analysis, molecular weight of the ethyl ester intermediate was 4260.062. Bottom panel: HPLC analysis revealed the intermediate had a retention time of 16.375 minutes.

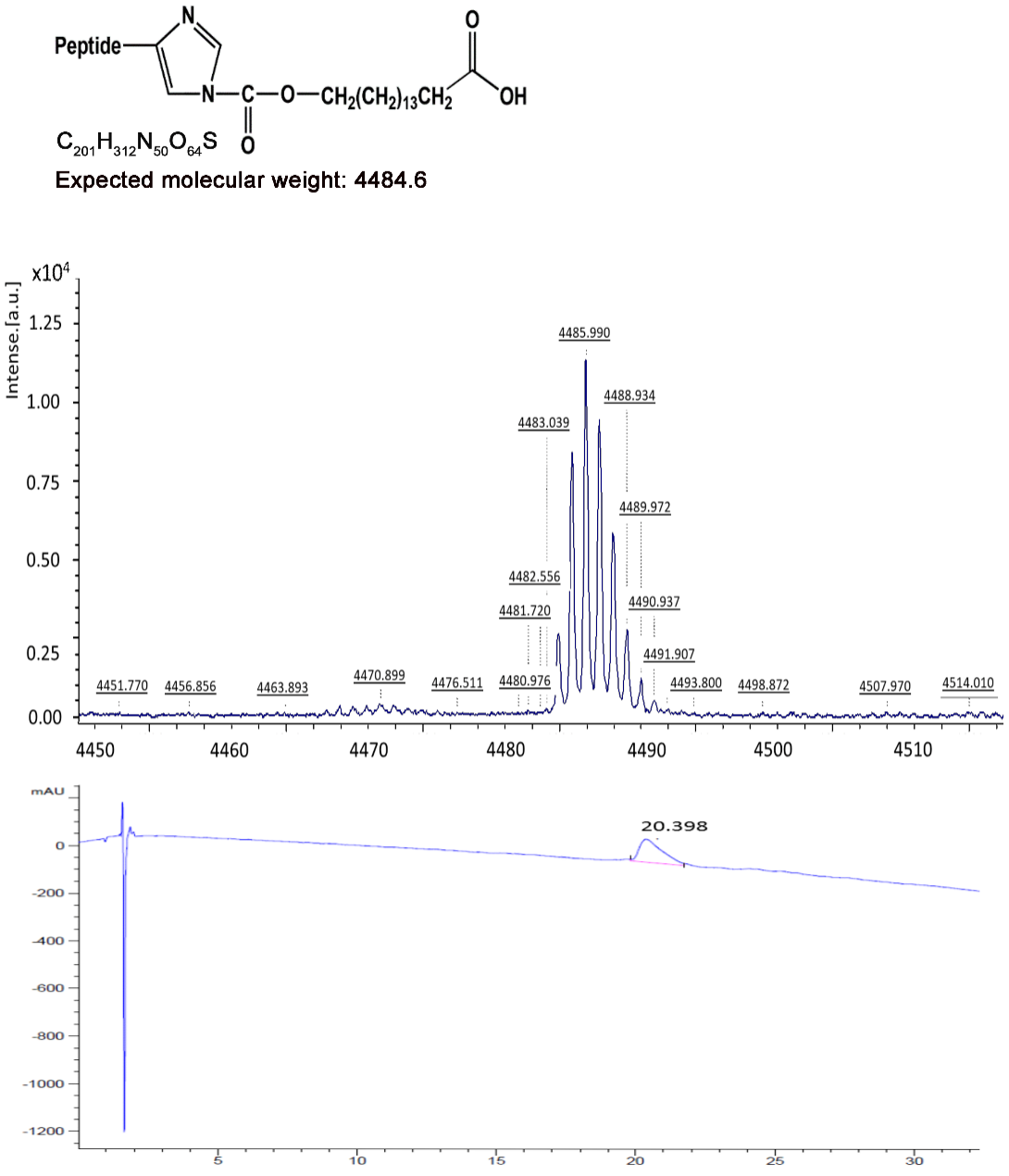


**Fig. S4. MALDI and HPLC spectra of LCFA-Ex4.** Upper panel: Based on MALDI analysis, molecular weight of LCFA-Ex4 was 4485.990. Bottom panel: HPLC analysis revealed LCFA-EX4 had a retention time of 20.398 minutes.

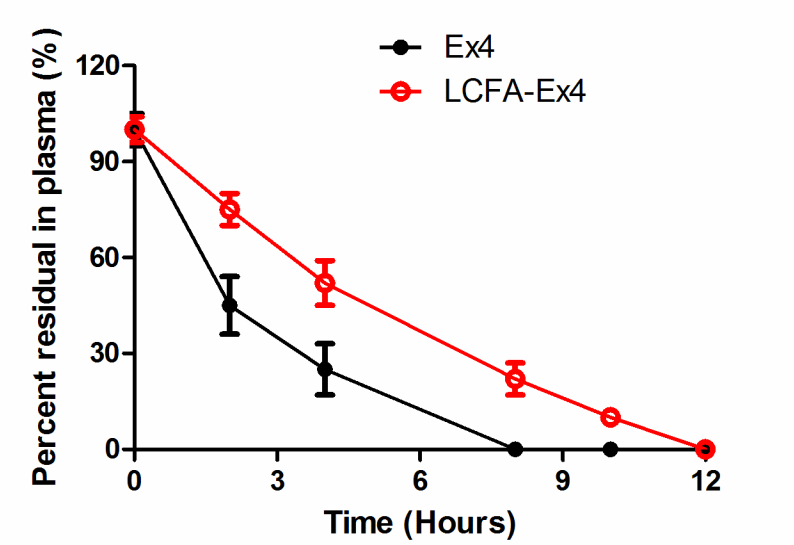


**Fig. S5. Stability of Ex4 and LCFA-Ex4 in murine plasma.** Ex4 and LCFA-Ex4 were incubated with murine plasma at a final concentration of 0.2 mg/mL. During incubation at 37°C, samples were collected at the 0, 2, 4, 8, and 12-hour timepoints, and residual peptides were quantified with HPLC (n=3).

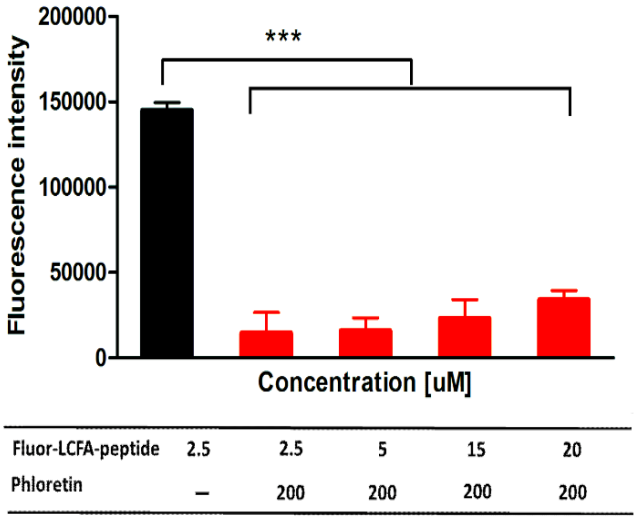


**Fig. S6. Uptake of LCFA-Ex4 is blocked by the FATP4 inhibitor phloretin.** Caco-2 cells were pre-treated for 30 minutes with or without phloretin at 37°C before they were incubated with increasing concentrations of Alexa 594-labeled LCFA-Ex4. Uptake of Alexa 594-labeled LCFA-Ex4 was determined based on fluorescent intensity (n=3). Data are presented as mean ± SEM. ***: $P < 0.001$.

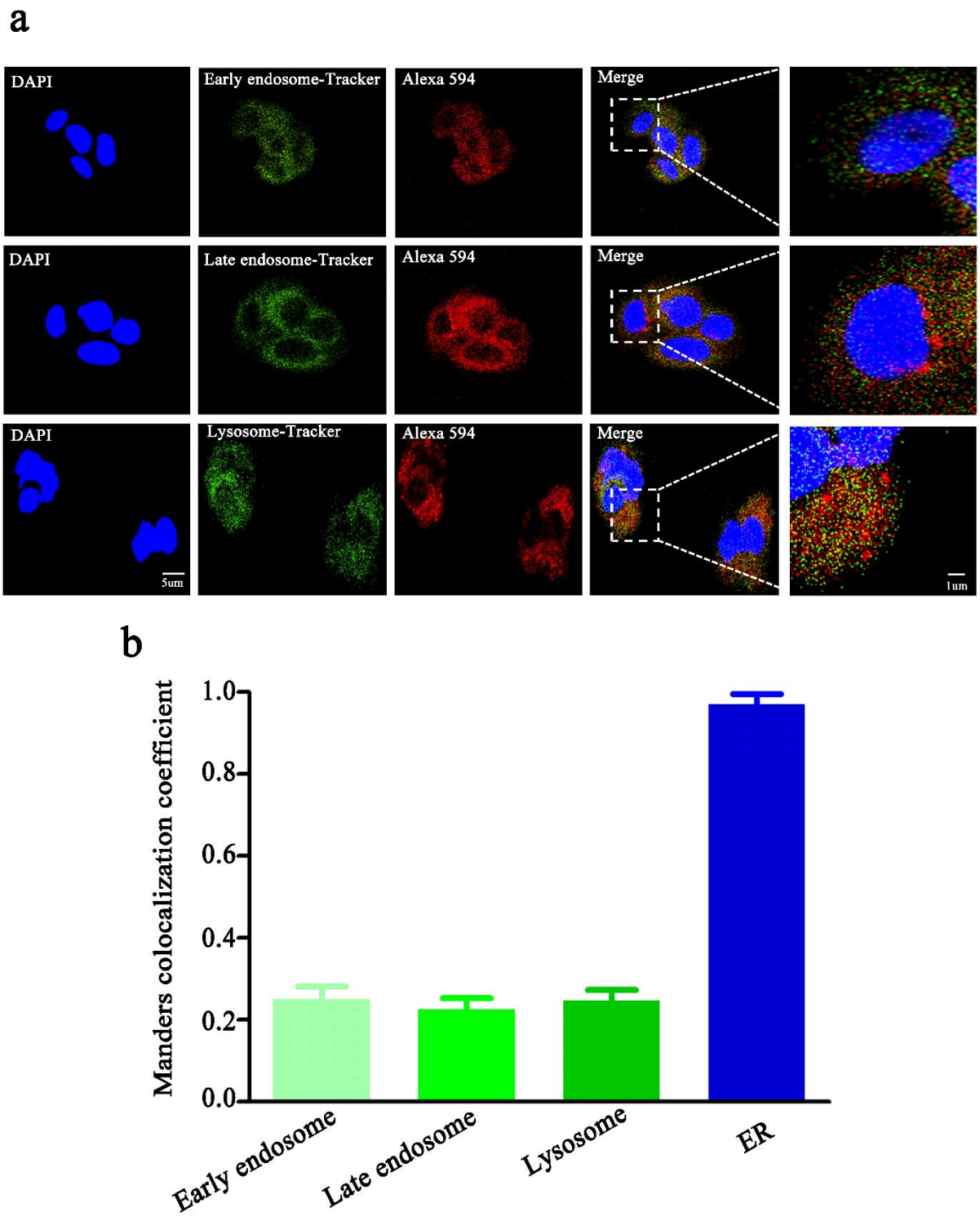


**Fig. S7. Confocal microscopic analysis on intracellular transport of Alexa 594-labeled LCFA.** a) Cells were treated with Alexa 594-labeled LCFA-Ex4 and then stained with early

endosome tracker, later endosome tracker, or lysosomal tracker. Confocal images were taken to colocalize Alexa 594-labeled LCFA-Ex4 with early endosomes, late endosomes, and lysosomes. b) Colocalization analysis of confocal images in a) and Fig. 1i was assessed using the Manders' colocalization coefficient of red versus green. The analysis was performed using ImageJ2 software. Data are presented as mean ± SEM (n=4).

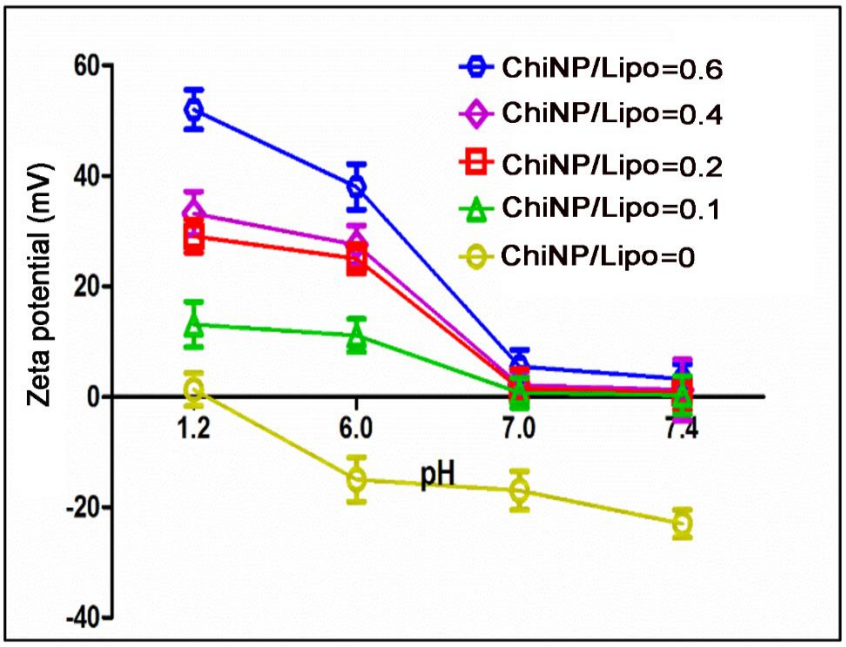


**Fig. S8. Changes in surface charge from ChiNP-lipo under different pH conditions.** Zeta potential values of ChiNP-lipo containing different ChiNP/Lipo ratios at pH 1.2, 6.0, 7.0 and 7.4 (n=3). Surface charge of ChiNP-lipo was close to neutral in pH neutral solutions (pH 7.0 and pH 7.4). Data are presented as mean ± SEM.

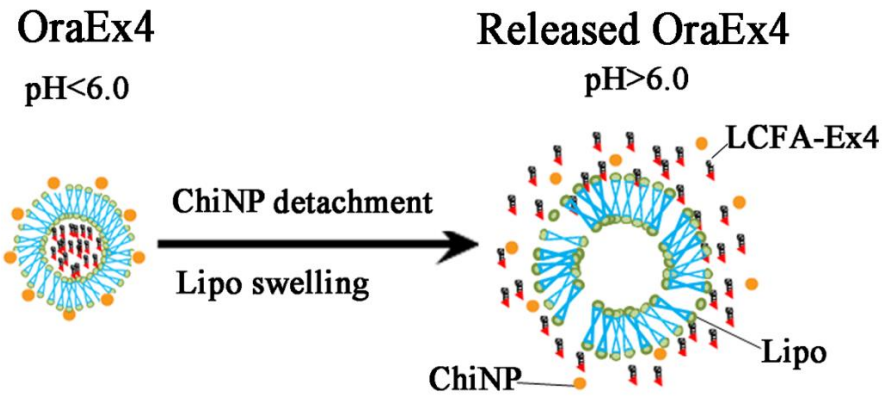


**Fig. S9. Schematic view on detachment of ChiNP from liposome and swelling and disruption of liposome.**

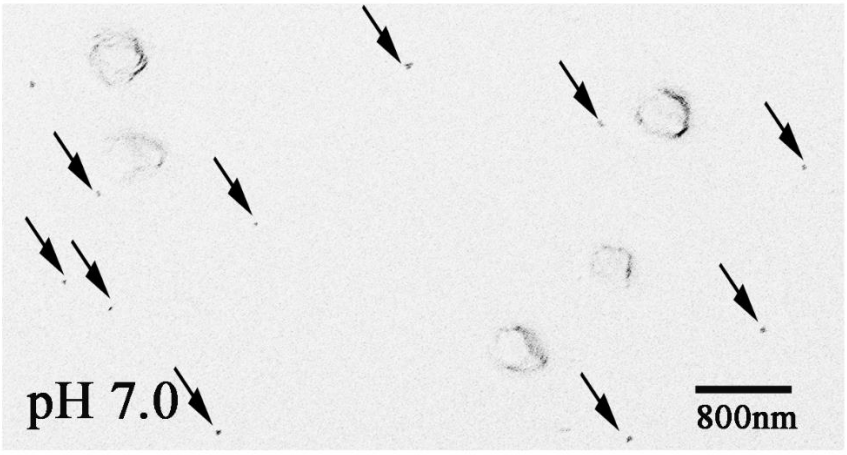


**Fig. S10. TEM image of OraEx4 at pH 7.0.** Black arrows point to detached ChiNP particles from swollen liposomes.

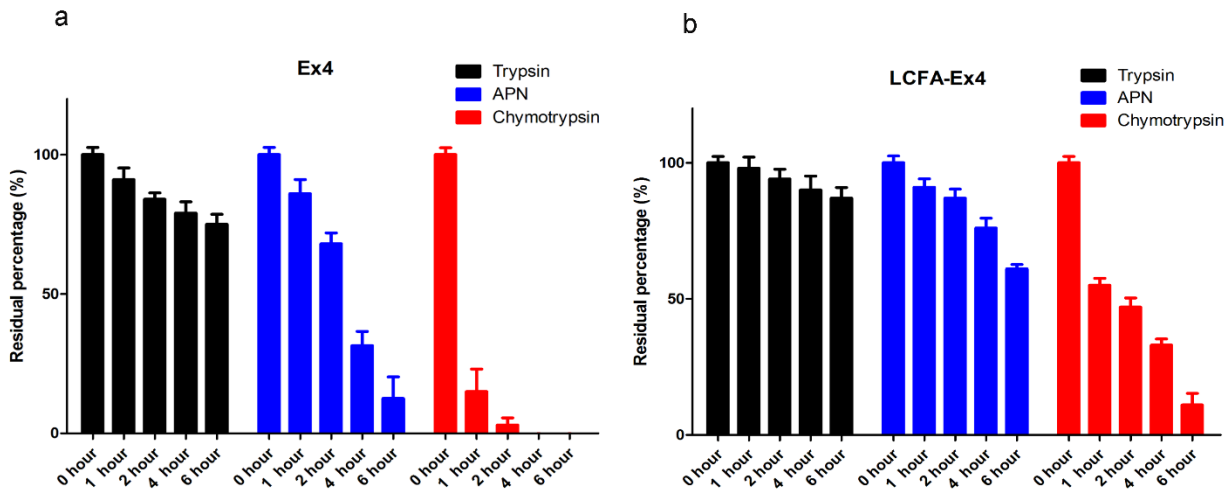


**Fig. S11. Stability of Ex4 and LCFA-Ex4 in intestinal enzyme-containing solutions.** a) Ex4 (100 μg/mL) and b) LCFA-Ex4 (100 μg/mL) were incubated with 0.2 U/mL trypsin, 0.2 U/mL aminopeptidase N (APN) or 0.2 U/mL chymotrypsin. Samples were collected at different timepoints, and residual levels of Ex4 and LCFA-Ex4 were determined and compared to the undigested product (n = 3). Each value represents mean ± SEM.

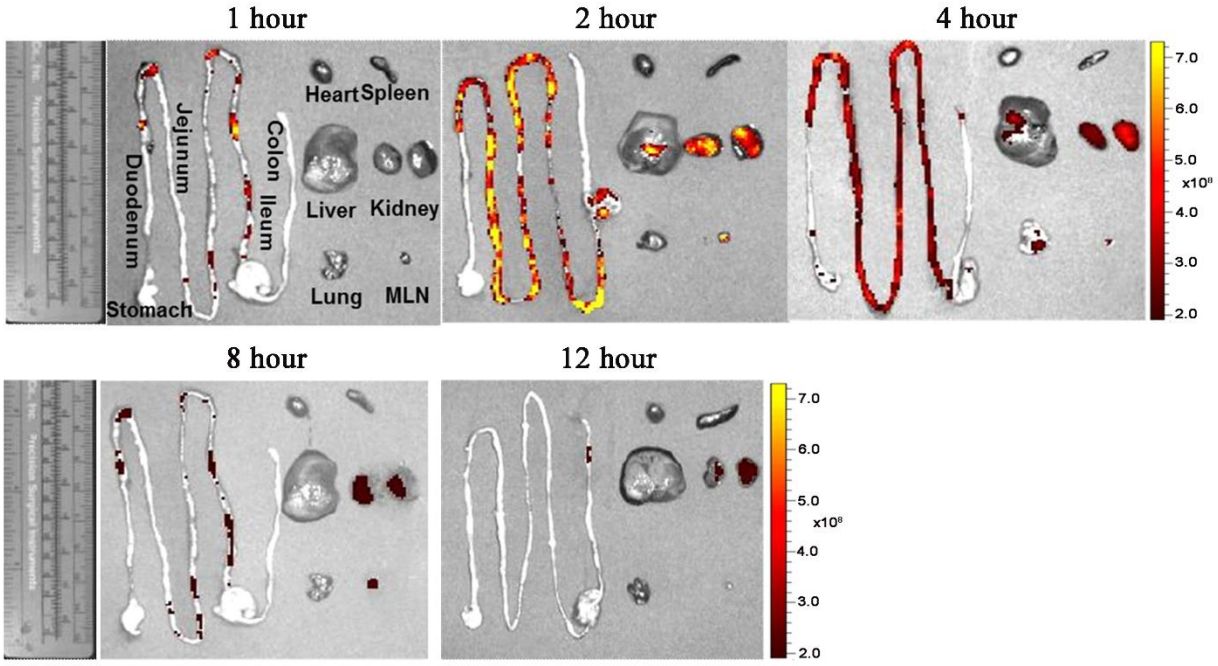


**Fig. S12. Biodistribution of Alexa 594-labeled OraEx4 in major organs after gavage.** Major organs were collected from C57BL/6 mice 1, 2, 4, 8 and 12 hours after administration of OraEx4, and images were captured with an IVIS-200 imaging system. Representative images are shown.

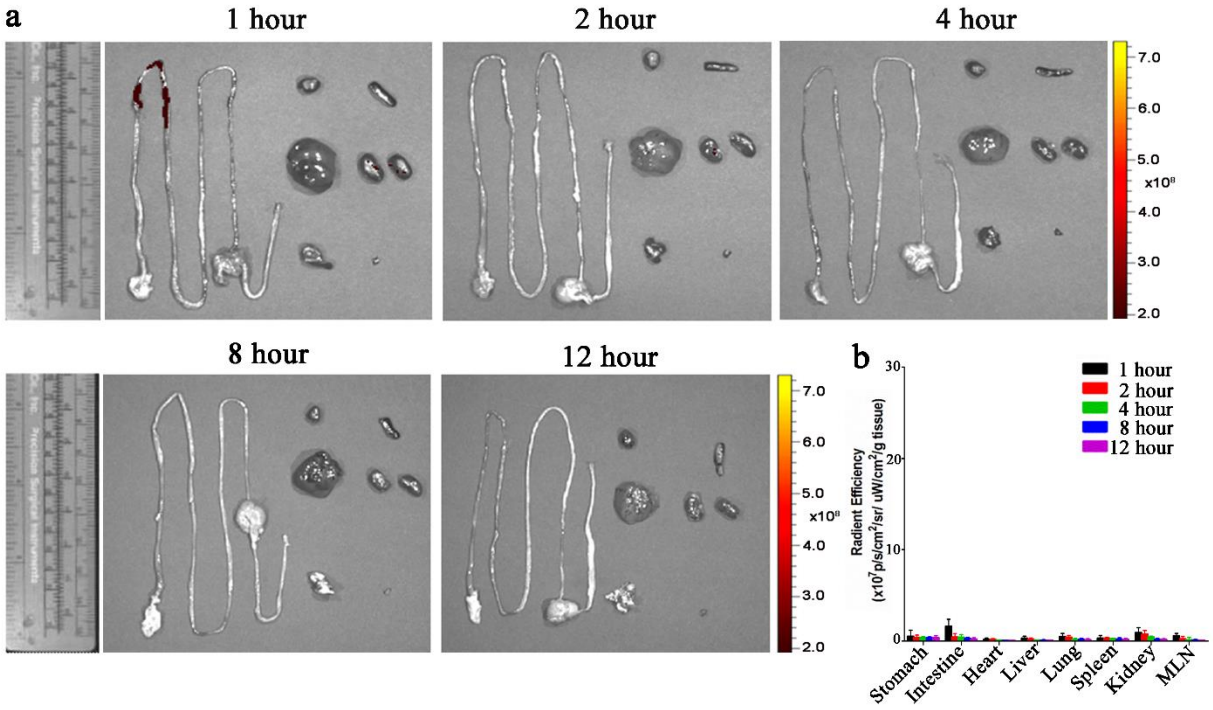


**Fig. S13. Biodistribution of Alexa 594-labeled LCFA-Ex4 after oral gavage.** a) Major organs were collected from C57BL/6 mice 1, 2, 4, 8 or 12 hours after oral administration of Alexa 594-labeled LCFA-Ex4, and images were captured with an IVIS-200 imaging system (n=3). b) Quantification of fluorescence in major organs. All data are normalized to tissue weight. Data are presented as mean ± SEM.

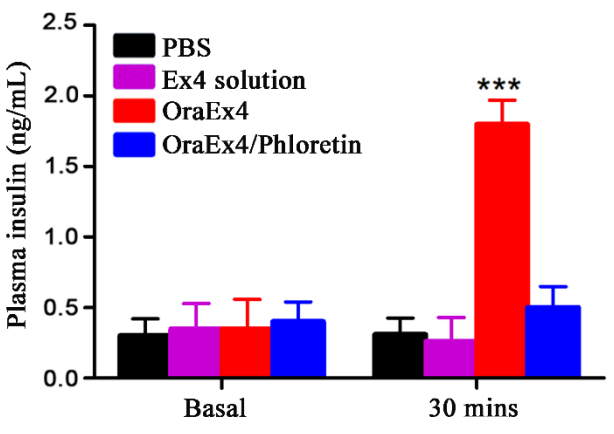


**Fig. S14. Insulin secretion during glucose tolerance test in C57BL/6 mice.** Mice were treated with free Ex4, OraEx4 or OralEx4 plus phloretin (n=6)**.** Blood samples were collected before and 30 minutes after administration of glucose. Plasma insulin levels were measured. Data are presented as mean ± SEM. ***: $P < 0.001$.

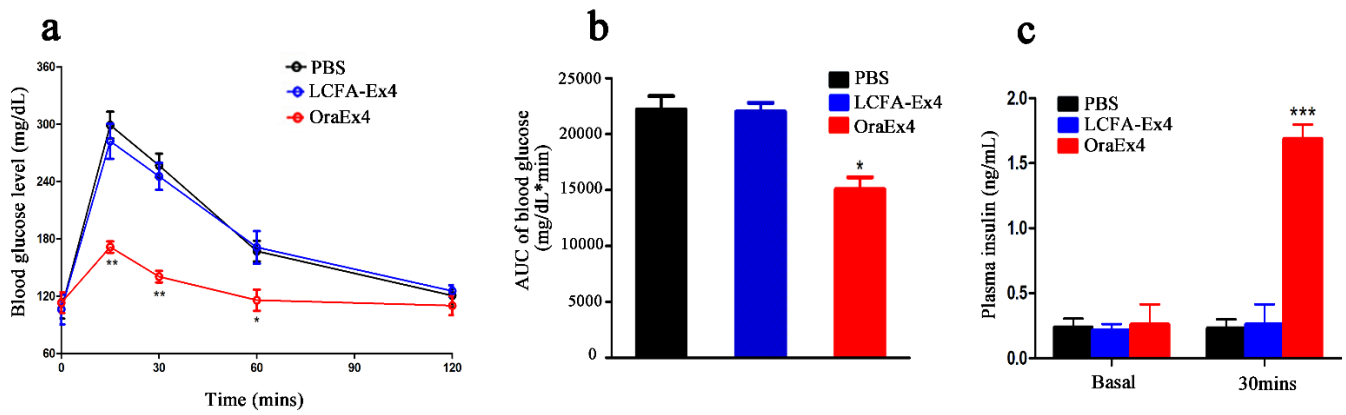


**Fig. S15. IPGGT in C57BL/6 mice treated with oral administration of LCFA-Ex4 or OraEx4.** a) Changes of blood glucose level during the glucose tolerance test (n=6). b) AUC of blood glucose level in a). c) Insulin levels during glucose tolerance test. Data are presented as mean ± SEM. *: $P < 0.05$; **: $P<0.01$; ***: $P < 0.001$.

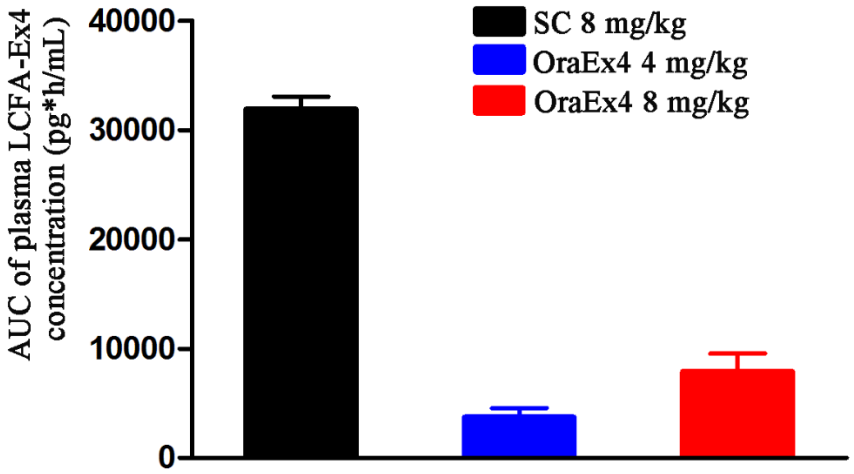


**Fig. S16. Area under the curve of plasma LCFA-Ex4 in C57 BL/6 mice after oral administration of OraEx4.** db/db mice were treated with single dose 4 mg/kg or 8 mg/kg OraEx4 at single dose by oral gavage, or subcutaneous injection of 8 mg/kg LCFA-Ex4 (n=5). Blood samples were collected and plasma LCFA-Ex4 levels were determined. Data are presented as mean ± SEM.

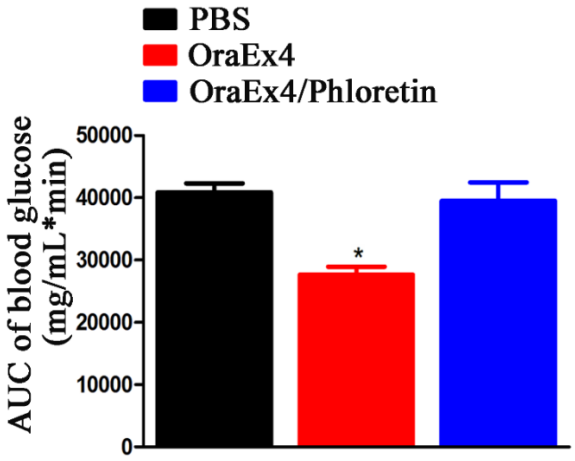


**Fig. S17. Area under the curve of blood glucose during glucose tolerance test in db/db mice treated with OraEx4.** Glucose tolerance test was performed in db/db mice treated with PBS, OraEx4, or OraEx4 plus Phloretin. Data are presented as mean ± SEM (n=5), *P < 0.05.

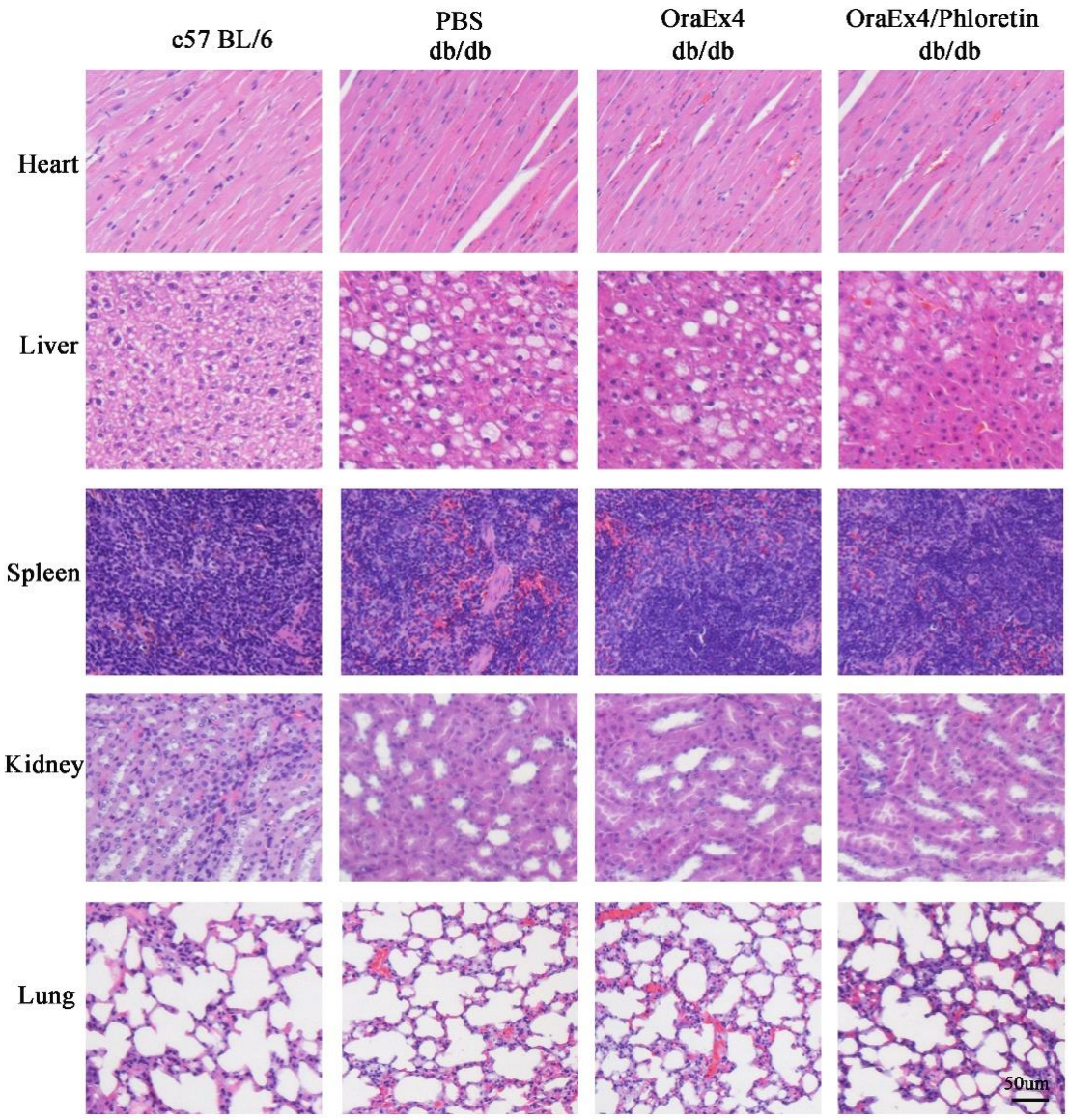


**Fig. S18. Histopathology of major organs in db/db mice after daily treatment with OraEx4.** db/db mice were treated daily with PBS, OraEx4 alone or OraEx4 plus phloretin for 12 days (n=5). Major organs were collected and processed for H&E staining. Tissue blocks were compared to those from corresponding organs in C57BL/6 mice. Representative images are shown.

**Table S1. Physicochemical characterization of OraEx4**

| Ratio of ChiNP/Lipos (w/w) | Particle size[a] (nm) | Zeta potential[b] (mV) | LC[c] (%) | EE[d] (%) |
|---|---|---|---|---|
| ChiNP/Lipos (0:1) | 204±2 | -15.0±4.0 | 4.37±0.1 | 87.30±1.31 |
| ChiNP/Lipos (0.1:1) | 177±2 | 11.1±3.0 | 4.26±0.11 | 85.10±1.1 |
| ChiNP/Lipos (0.2:1) | 107±17 | 25.0±2. | 4.14±0.09 | 82.71±1.89 |
| ChiNP/Lipos (0.4:1) | 80±15 | 27.1±3.5 | 3.80±0.10 | 75.9±2.45 |

[a] Particle size was measured by DLS in ultrapure water. Data represents mean ± SEM (n = 3).

[b] Zeta potential was measured by electrophoretic Light Scattering in PBS. Data represents mean ± SEM (n = 3).

[c] LC: Loading content; [d] EE: Encapsulation efficiency. Data represents mean ± SEM (n = 3)