Liver-targeting oral lipid nanoparticle for CRISPR/Cas9 therapy in metabolic dysfunction-associated steatotic liver disease

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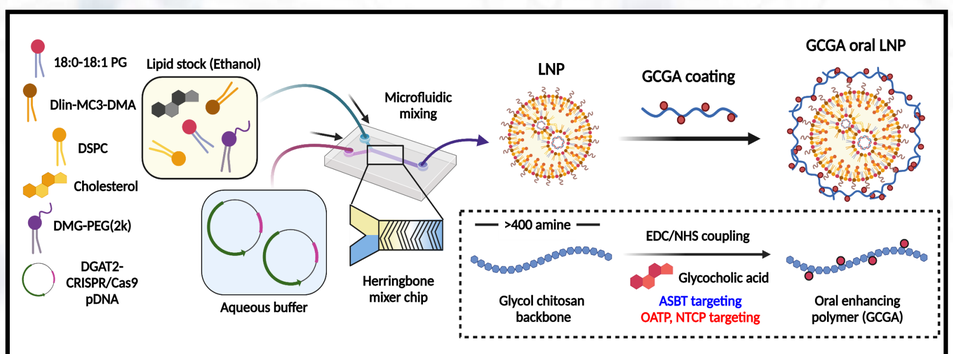
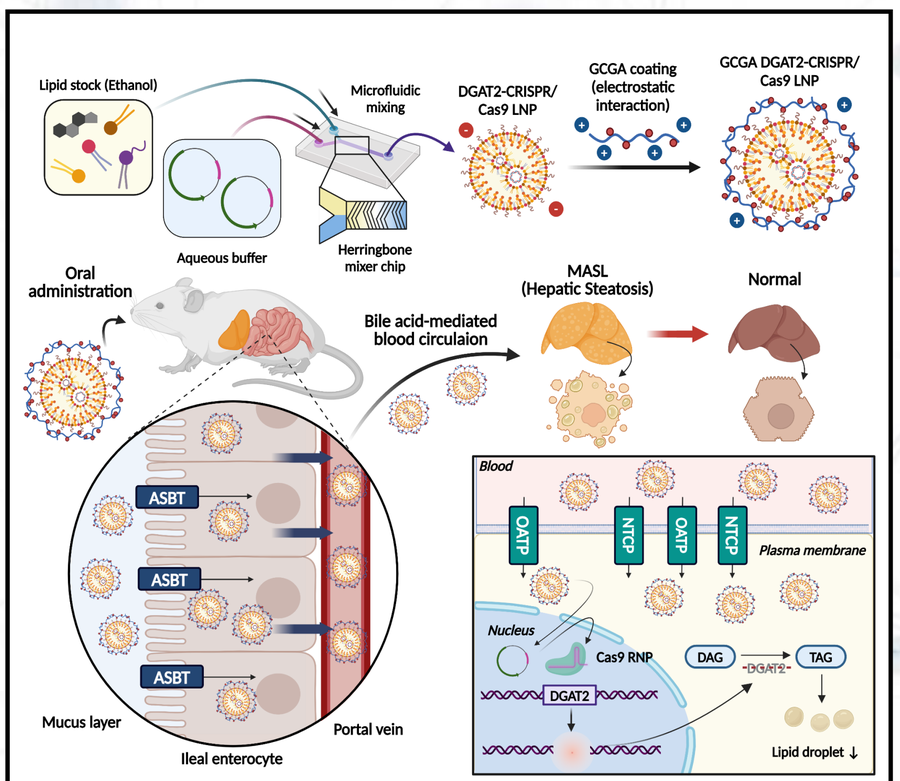
RESEARCH CONCEPT



Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent chronic condition characterized by excessive lipid accumulation in the liver. Gene therapy targeting key metabolic enzymes presents a promising therapeutics, but efficient, non-invasive delivery systems remain a challenge. **Inhibition of DGAT2**, a main enzyme in triglyceride synthesis, **alleviates hepatic steatosis and improve metabolic dysfunction**. In this study, we developed a **liver-targeted oral lipid nanoparticle (LNP)** system. The LNP, conjugated with **glycol chitosan-glycocholic acid (GCGA)**, enhances gastrointestinal stability, mucoadhesion, and **targeted uptake through bile acid transport pathways in the small intestine and liver**. GCGA-coated LNPs exhibited preferential liver accumulation and higher gene expression compared to unmodified LNPs after oral administration. The oral LNPs delivering **CRISPR components for DGAT2 inhibition effectively attenuated hepatic steatosis in MASLD models**. This study addresses the potential of the bile acid-polymer-coated LNP as a scalable, patient-friendly oral gene delivery platform for treatment of MASLD and other liver diseases.

OVERALL SCHEME



CONCLUSION

Liver-targeting oral enhancing polymer(GCGA) was synthesized and optimized for oral LNP

GCGA coated LNP enhances stability under pH and enzymatic conditions in the GI tract

GCGA-LNP uptake via bile acid receptors has been confirmed *in vitro* and *in vivo*

Orally administered GCGA-LNP accumulated in the liver with high gene expression

DGAT2-CRISPR/Cas9 LNP alleviates hepatic steatosis in MASLD models

➢ ***GCGA-LNP exhibits great potential for MASLD treatment and oral gene therapy***

EXPERIMENTAL RESULTS

1. Development of liver-targeting oral enhancer polymer(GCGA) via EDC/NHS chemistry

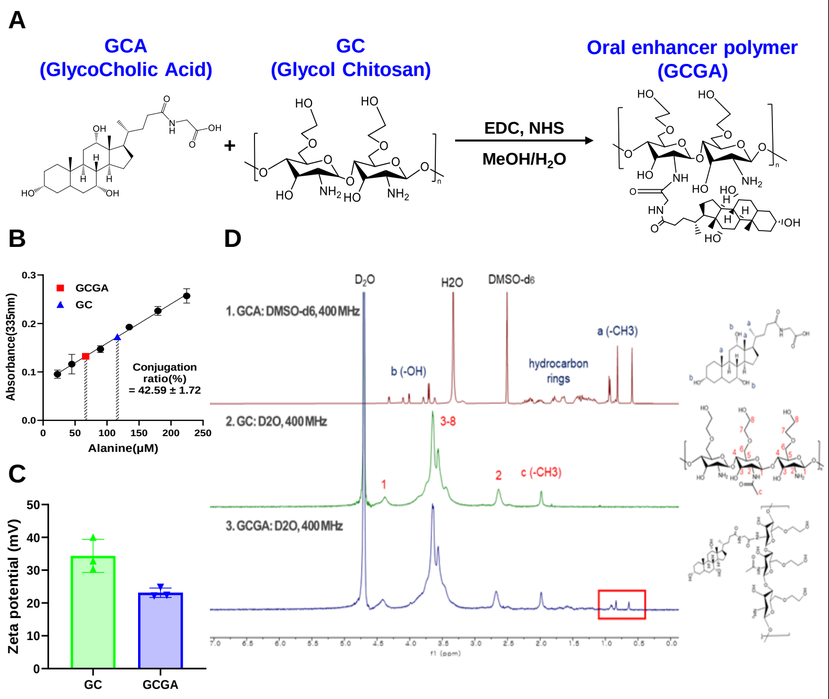


Figure 1. (A) Chemical synthesis scheme of liver-targeting oral enhancer polymer GCGA(Glycol Chitosan-Glycocholic Acid). (B) TNBS assay and (C) zeta potential measurement of glycol chitosan(GC) and GCGA. (D) ^{1}H-NMR analysis of glycocholic acid(GCA), GC, and GCGA polymer.

The oral enhancer polymer GCGA was synthesized, and its conjugation was verified by physicochemical methods.

2. Optimization, characterization and structure analysis of GCGA coated LNP

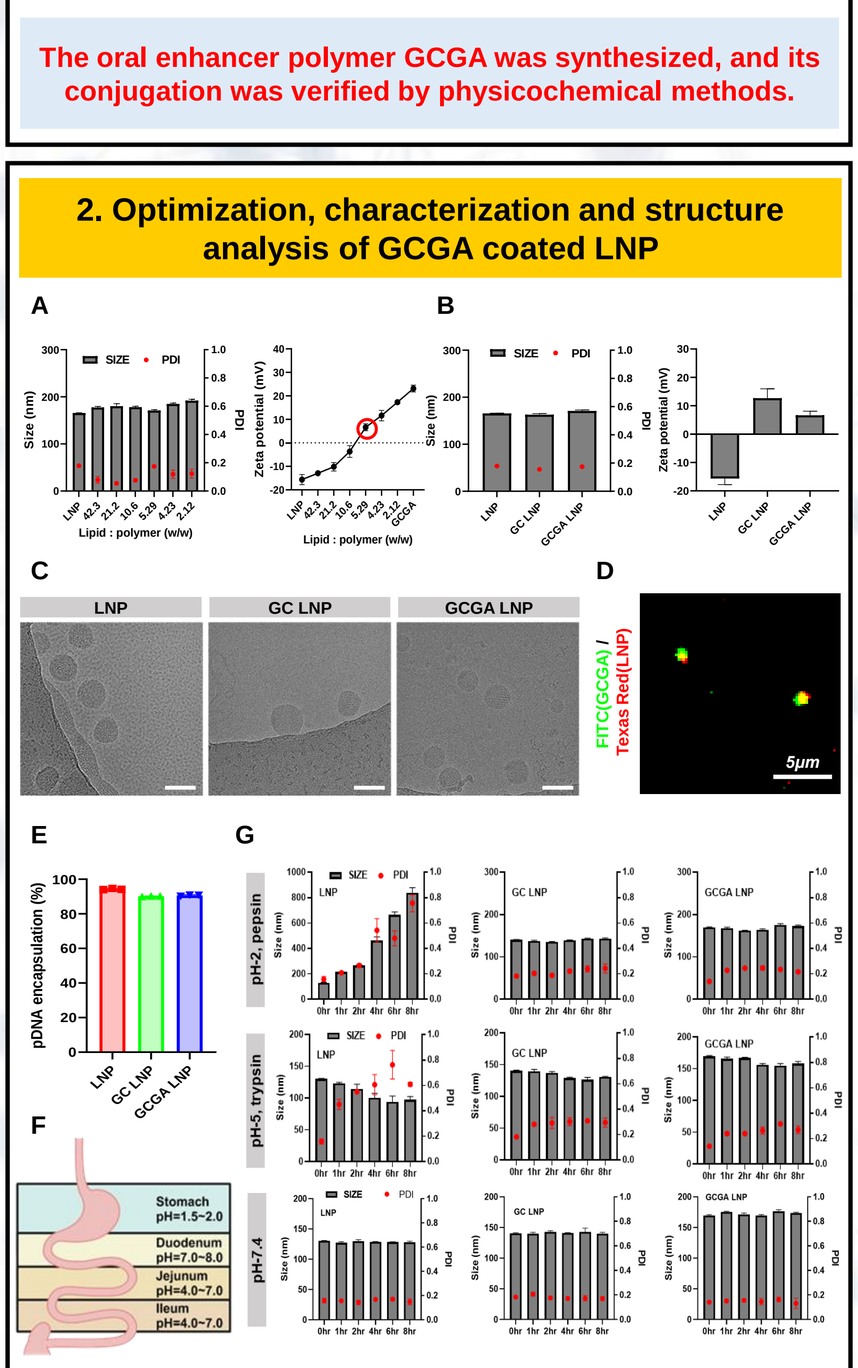


Figure 2. (A) Hydrodynamic size, PDI, and surface zeta potential of GCGA oral LNP for lipid/polymer ratio optimization (n=3). (B) Hydrodynamic size, PDI, and surface zeta potential of LNPs (n=3). (C) Surface morphology analysis by Cryo-TEM (scale bar=100nm). (D) CLSM analysis visualizing co-localization between the polymer and the LNP (scale bar=5μm). (E) pDNA encapsulation efficiency of LNPs by Quant-iT Ribogreen assay (n=3). (F) Illustration describing pH conditions in GI tract. (G) Stability assessment of LNPs against pH and enzymatic degradation under GI conditions.

The optimal GCGA-to-LNP ratio was selected, and its characterization, morphology, and stability were analyzed.

3. Bile acid channel-mediated cellular uptake and transport of GCGA-LNP (*in vitro*)

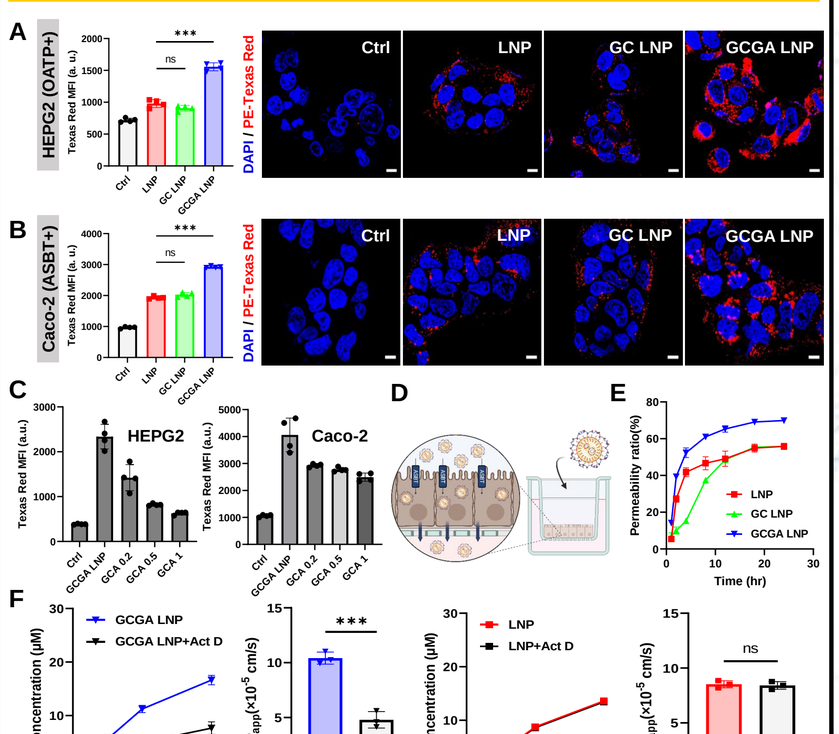


Figure 3. (A,B) FACS and CLSM analysis for cellular uptake of LNPs in HEPG2 and Caco-2 cell lines (n=4, scale bar=10μm). (C) Competitive cellular uptake of GCGA-LNP with co-treatment of free GCA in HEPG2 and Caco-2 cell line (n=4). (D) Schematic illustration of transepithelial permeability experiment by using Caco-2 monolayer in the apical side of transwell chamber. (E) Cumulative transepithelial permeability of FITC-LNPs in a Caco-2 transwell model (n=3). (F) Cumulative transepithelial permeability of FITC-LNPs in a Caco-2 transwell model in the presence of actinomycin D (ASBT inhibitor) (n=3).

Increased *in vitro* cellular uptake and transport of GCGA-LNP were observed via bile acid receptors, which served as the primary pathway.

4. Liver targeting of GCGA-LNP via bile acid transport pathway after oral administration (*in vivo*)

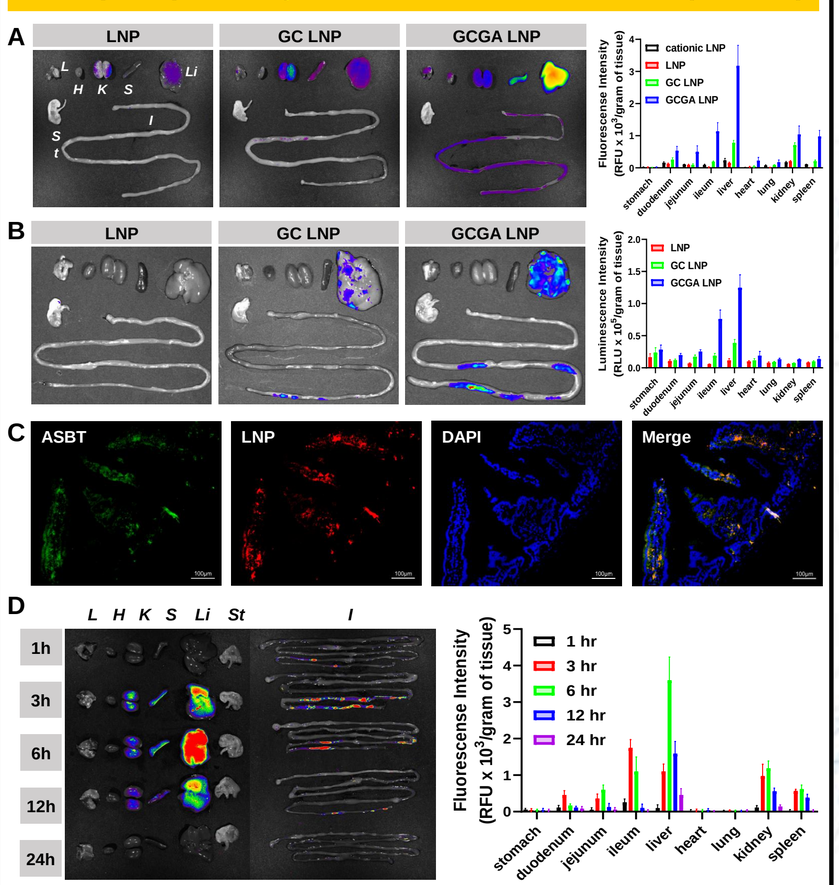


Figure 4. (A) *In vivo* biodistribution of LNPs after oral injection: *ex vivo* fluorescence imaging and quantitative analysis of fluorescence intensity in major organs (L: Lung, H: Heart, K: Kidney, S: Spleen, Li: Liver, St: Stomach, I: Small Intestine) (n=3). (B) *In vivo* luciferase activities of LNPs after oral injection: *ex vivo* luminescence imaging and quantitative analysis of luciferase activity in major organs (n=5). (C) Cross-sectional CLSM images of the ileum 4 hr after oral administration of GCGA-LNP (FITC: ASBT, Cy5.5: LNP) (scale bar=100μm). (D) Time-dependent *in vivo* biodistribution of GCGA LNPs: *ex vivo* fluorescence imaging and quantitative analysis (n=5).

Orally administered GCGA-LNPs exhibited ASBT-mediated uptake, liver targeting, and high hepatic gene expression.

5. Design and selection of a CRISPR/Cas9 guide RNA sequence targeting DGAT2

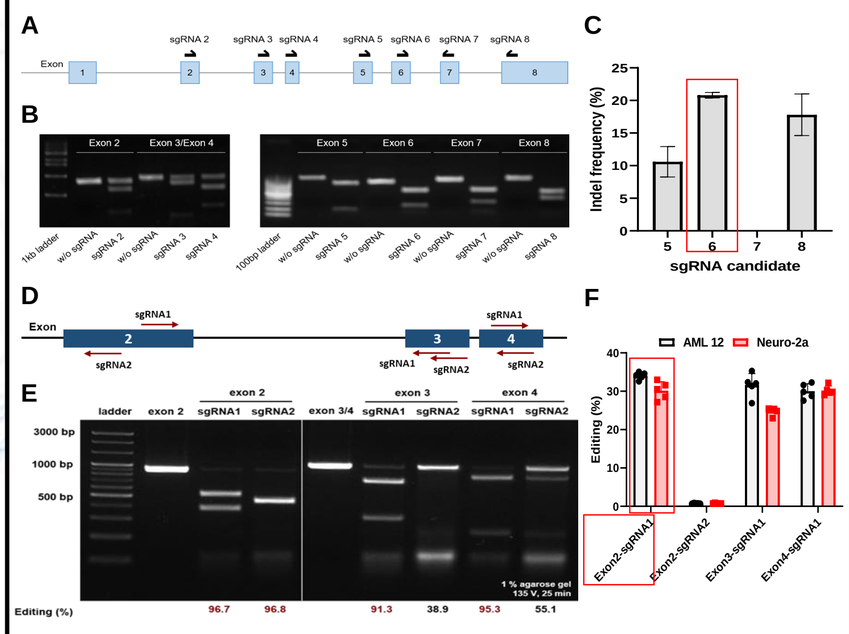


Figure 5. (A,D) Schematic of DGAT2 sgRNA candidates in hepatic cell lines (A: human-HEPG2, D: mouse-AML12). (B,E) In vitro cleavage assay of DGAT2 sgRNA candidates in hepatic cell lines (B: HEPG2, E: AML12). (C,F) NGS analysis of DGAT2 sgRNA candidates in hepatic cell lines (C: HEPG2, F: AML12 and Neuro-2a).

6. Therapeutic efficacy of DGAT2-CRISPR/Cas9 LNP in hepatic steatosis models (*in vitro, in vivo*)

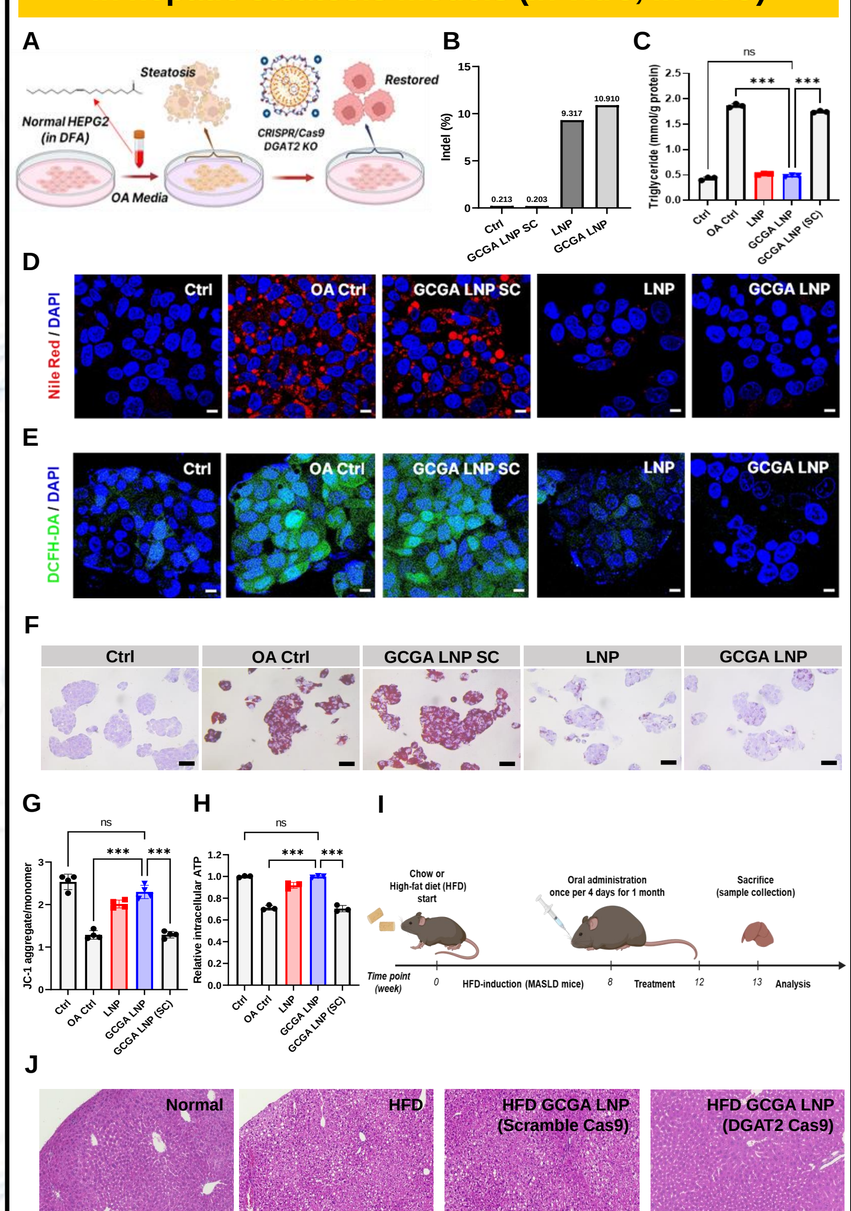


Figure 6. (A) Schematic illustration of establishing *in vitro* hepatic steatosis model and its lipid alleviation by DGAT2-CRISPR/Cas9 therapy. (B) NGS analysis of DGAT2-CRISPR/Cas9 LNP *in vitro* hepatic steatosis model. (C) Triglyceride analysis of *in vitro* hepatic steatosis model (n=3). (D,E) CLSM analysis of *in vitro* hepatic steatosis model for (D) fatty acid and (E) ROS (scale bar=10μm). (F) Oil Red O staining of *in vitro* hepatic steatosis model (scale bar=100μm). (G,H) Analysis on mitochondrial activity of *in vitro* hepatic steatosis model (n=4). (I) Illustration describing *in vivo* MASLD model (HFD) induction and MASLD therapy by oral administration of GCGA DGAT2-CRISPR/Cas9 LNP. (J) H&E staining of the HFD-induced mouse liver to analyze fatty liver phenotypic patterns (scale bar=100μm).

GCGA coated DGAT2-CRISPR/Cas9 LNP effectively alleviates hepatic lipid accumulation in MASLD models.