Supplementary Methods

GALK1 antibody validation

The specificity of GALK1 antibody used for western blot analysis of human muscle samples was validated by CRISPR-Cas9 knockout (KO) in C2C12 myoblasts. The CRISPR-Cas9 guide RNAs and plasmids for knocking out mouse GALK1 were customed designed by the MRC PPU Reagents and Services, the University of Dundee (UK). A full transcript map of the GALK1 locus was constructed, combining data from both NCBI (NC_000017.11 (75751469..75765192) and Ensembl (ENSMUSG00000020766). Analysis confirmed the expression of two transcripts, both of which encode the same protein (NP_000145.1, Q9R0N0), thus KO guides were chosen to target as far upstream as possible within the first coding exon (exon 1, ENSMUSE00000253437). Analysis using a Sanger webtool (https://wge.stemcell.sanger.ac.uk/find_crisprs) highlighted a candidate nickase pair (sense 5'-(g)ccgggccgcgtcaacctcat and antisense 5'- ggctccaaactcctccatga). Complementary oligonucleotides were designed and annealed to generate dsDNA inserts with overhangs compatible to BbsI digested destination vectors according to the Zhang method (Cong et al., 2013). The nickase sense guide insert was subsequently cloned into BbsI digested pBABED P U6 (University of Dundee DU48788), and the antisense nickase guide was cloned into BbsI digested pX335 (Addgene #42335).

C2C12 myoblasts were maintained at 37°C under 5% CO2 in Dulbecco's modified Eagle's medium (31966021, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (SV30160.03, Cytiva, Austria) and 1% Penicillin-Streptomycin (15140122, Thermo Fisher Scientific, Waltham, MA, USA). Myoblasts were seeded at 220,000 cells per well in a 6-well plate and immediately transfected with plasmids (pBABED 1.25 ng and pX335 1.25 ng) using TransIT-X2 (Mirus Bio, Madison, WI, USA). After 24 h transfection, 2 mL of fresh medium containing 3 µg/mL of puromycin was added to each plate for 48 h selection, replacing puromycin medium after 24 h. Following selection, cells were lysed on ice with 100 µL lysis buffer containing 250 mM sucrose, 50 mM Tris-base (pH 7.5), 50 mM sodium fluoride, 10 mM sodium β-glycerophosphate, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM EGTA, 1 mM benzamidine, 1 mM sodium orthovanadate, 1 x complete Mini EDTA-free protease inhibitor cocktail (Roche, Switzerland), 1% Triton X-100, and 100 mM 2-chloroacetamide. Cell lysates were centrifuged at 13,000 rpm for 15 min at 4°C and supernatants were collected. Protein concentrations were quantified using Bradford protein assay. Western blot sample preparation and analysis are described in the main manuscript.

Supplementary Results

Western blotting outcomes are presented in Figures S1 and S2. GALK1 was successfully knocked out in a C2C12 mouse muscle cell line (Figure S1), demonstrating the validity of the antibody for use in skeletal muscle tissue. Full blots of GALK1, GALT, and GALE protein expression in human muscle samples are depicted in Figure S2.

Reference

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013 Feb 15;339(6121):819-23. doi: 10.1126/science.1231143

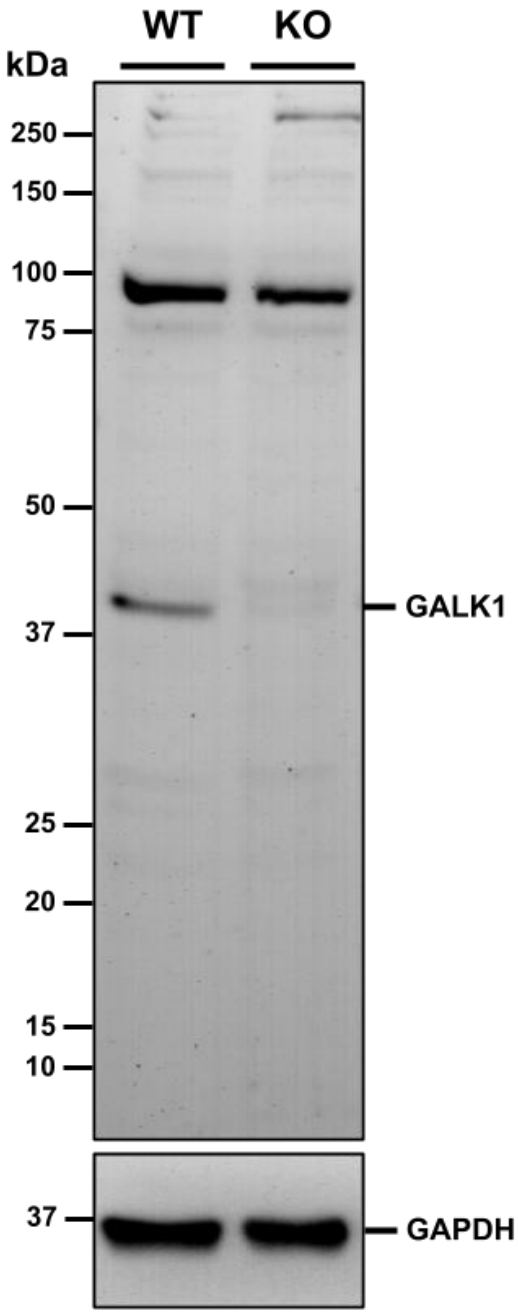

Figure S1. Full western blot images of galactokinase 1 enzyme (GALK1) in wild type (WT) and CRISPR-Cas9 knockout (KO) C2C12 myoblasts. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as loading control.

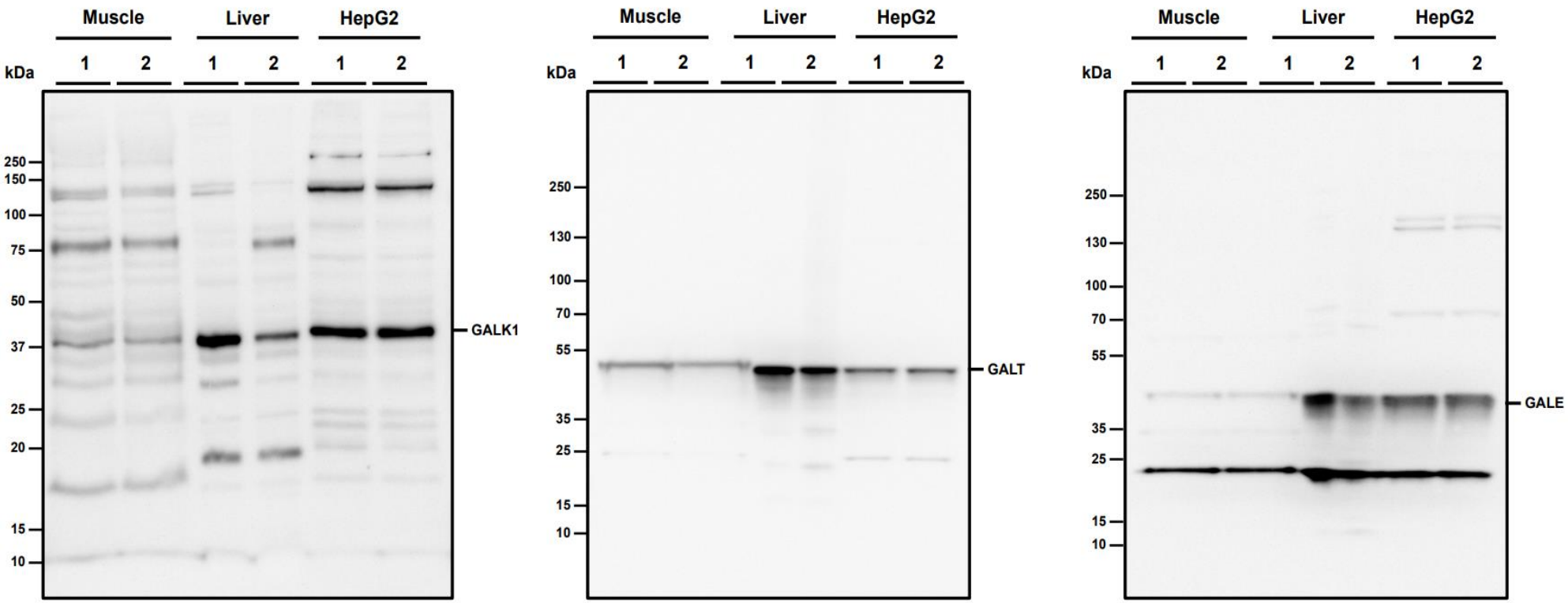

Figure S2. Full western blot images of galactokinase 1 (GALK1 – left panel), UDP-galactose-4-epimerase (GALE – middle panel) and galactose-1-phosphate uridylyltransferase (GALT – right panel) protein expression in human skeletal muscle tissue (60 µg), human liver tissue (10 µg) and HepG2 cells (30 µg).