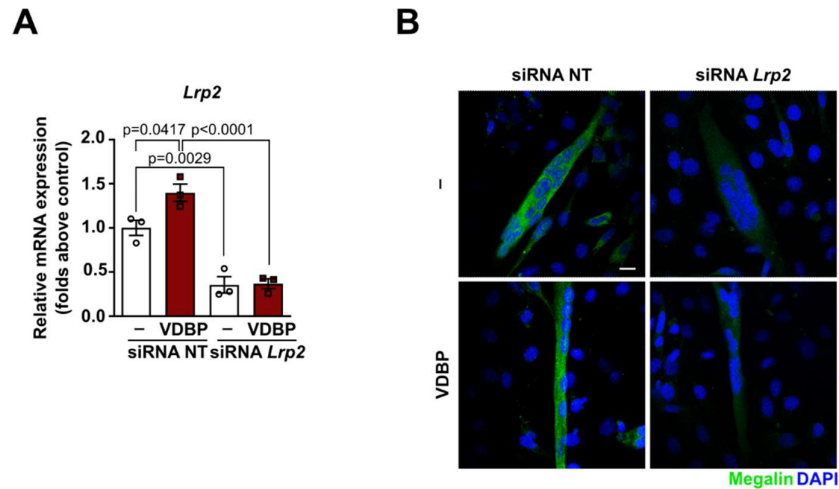
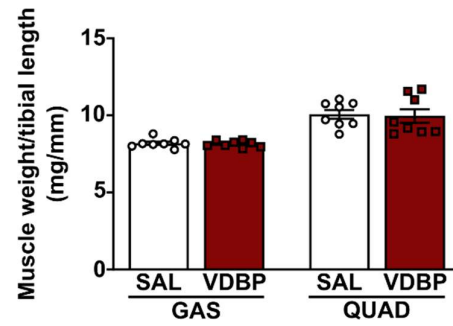


Supplementary information

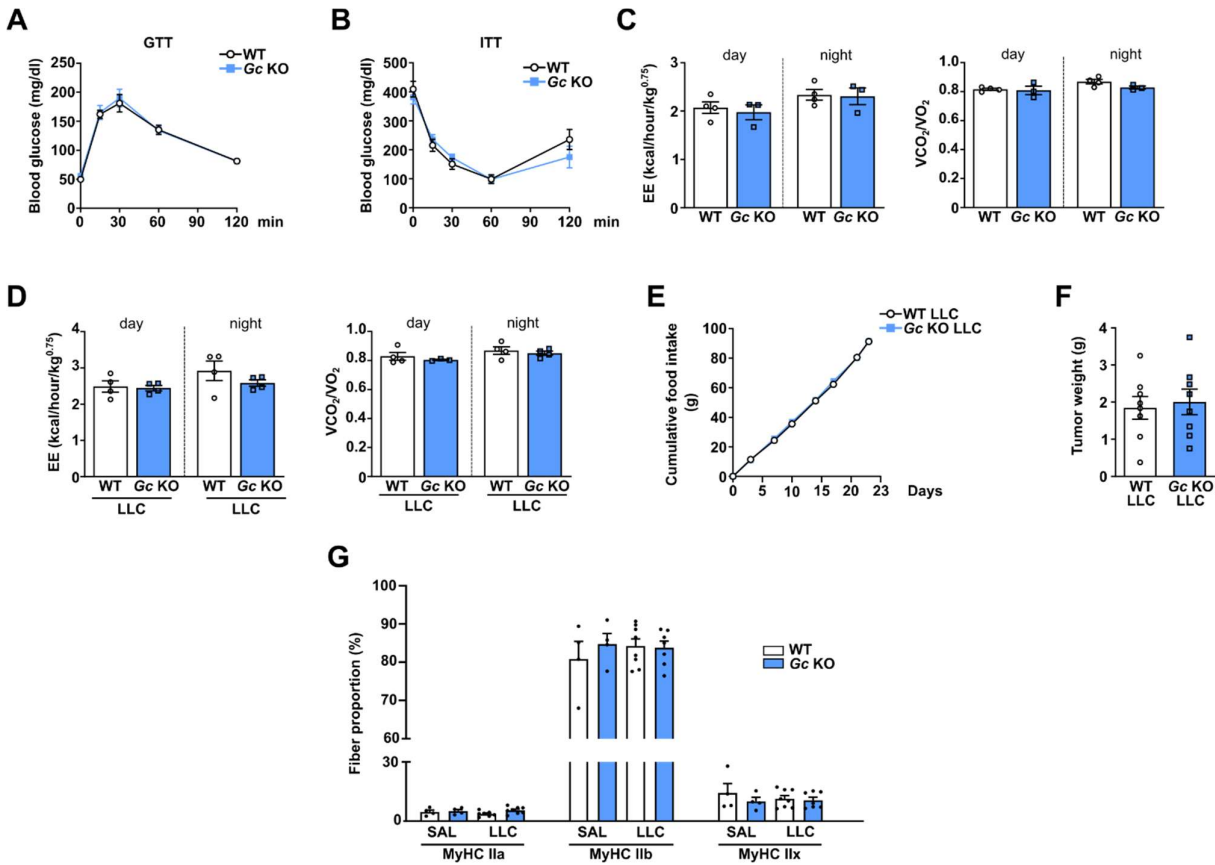
**VITAMIN D BINDING PROTEIN INDUCES SKELETAL MUSCLE ATROPHY
AND CONTRIBUTES TO CANCER-ASSOCIATED MUSCLE WASTING
INDEPENDENTLY OF VITAMIN D STATUS**



Supplementary Figure 2. *Lrp2* silencing validation in C2C12 myotubes. (A-B) C2C12 myotubes were transfected with 50 pmol of non-targeting siRNA (NT) or siRNA targeting megalin (*Lrp2*). 24 h post-silencing, myotubes were treated with 50 nM VDBP for 24h. (A) Megalin expression was assessed by real-time RT-PCR in C2C12 myotubes, using *Gusb* as the housekeeping gene; n=3 biological replicates. (B) Representative images of megalin immunofluorescence; n=3 biological replicates. Scale bar 20 μ m. All treatments were in serum-free medium. Data are presented as means $\pm$ SEM. Statistical analysis: one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



Supplementary Figure 3. Muscle weights of VDBP-injected *Gc* KO mice. Weights of gastrocnemius (GAS) and quadriceps (QUAD) after 8 days of VDBP administration were normalized on tibial length; n=8 for each group. Data are presented as means $\pm$ SEM. Statistical analysis: one-way ANOVA, not significant.



Supplementary Figure 4. Comparison of *Gc* KO vs. WT mice. (A) Glucose tolerance test (GTT) in wild type (WT) and VDBP KO mice; n=6 for each group. (B) Insulin tolerance test (ITT) in WT and VDBP KO mice; n=6 for each group (C) Metabolic parameters of WT and VDBP KO mice were measured for 24 h through metabolic cages. Left, energy expenditure (EE) and right, respiratory quotient (RQ) during day and night. WT n=4; *Gc* KO n=3. (D) Metabolic parameters of LLC-bearing WT and *Gc* KO mice. Left, EE and right, RQ during day and night; n=4 for each group. (E) Cumulative food intake of WT and *Gc* KO LLC-bearing mice. WT LLC and *Gc* KO LLC n=8. (F) Tumor weights at the end of the experiment; n=8 for each group. (G) Proportion of the different myofiber types (MyHC IIa, MyHC IIb, and MyHC IIx) in the gastrocnemius of LLC-bearing and saline-injected (SAL) WT and *Gc* KO mice. WT SAL and *Gc* KO SAL n=4; WT LLC n=7; *Gc* KO LLC n=8. Data are presented as means $\pm$ SEM. Statistical analysis: multiple unpaired two-tailed t-tests were performed with FDR correction using the two-stage step-up method of Benjamini, Krieger and Yekutieli (Q=1%) for (A), (B), and (E); one-way ANOVA followed by Tukey's multiple comparisons test for (G); two-tailed unpaired t-test for (C), (D), and (F), not significant. Source data are provided as a Source Data file.

Supplementary Table 1

Primary antibodies						
Antibody	Host species	Distributor, cat. number	Dilution			
			WB	IF	IP	PLA
Anti- β -actin (AC-15)	mouse, monoclonal	Sigma-Aldrich, #A5441	1:1000			
Anti-DRP1 (8/DLP1)	mouse, monoclonal	BD Biosciences, #611113	1:1000			1:50
Anti-laminin	rabbit, polyclonal	Dako, # Z0097		1:200		
Anti-LC3B	rabbit, polyclonal	Proteintech, #18725-1-AP	1:600	1:50		
Anti-LC3B	mouse, polyclonal	Nanotools, #AB_2943418		1:50		
Anti-LRP2	rabbit, polyclonal	Proteintech, #19700		1:50		
Anti-MyHC sarcomere (MF-20)	mouse, monoclonal	Developmental Studies Hybridoma Bank		1:10		
Anti-MyHC Type IIa (SC-71)	mouse, monoclonal	Developmental Studies Hybridoma Bank		1:100		
Anti-MyHC Type IIb (BF-F3)	mouse, monoclonal	Developmental Studies Hybridoma Bank		1:100		
Anti-neurofilament	chicken, polyclonal	Abcam, ab4680		1:1000		
Anti TOM20	Rabbit, monoclonal	Proteintech, #11802-1-AP		1:50		
Anti-tubulin	mouse, monoclonal	Santa Cruz Biotechnology, #sc-32293	1:1000			
Anti-VDAC	rabbit, polyclonal	Cell Signaling Technology, #4661	1:1000			
Anti-Vinculin	mouse, monoclonal	Proteintech, #66305-2-Ig; 3A11F8	1:20000			
Anti-VDBP	rabbit, polyclonal	Proteintech, #16922-1-AP	1:1000	1:100	1:100	1:100
Secondary antibodies						
Anti-chicken IgY (H+L) Alexa Fluor 546	goat	Thermo Fisher Scientific, #A11040		1:400		
Anti-mouse IgG (H+L) Alexa Fluor 488	donkey	Thermo Fisher Scientific, #A21202		1:400		
Anti-mouse IgG (H+L) Alexa Fluor 546	goat	Thermo Fisher Scientific, #A11003		1:400		
Anti-mouse IgG HRP-labeled	goat	PerkinElmer, #NEF822001EA	1:3000			
Anti-mouse IgG1 Alexa Fluor 488	goat	Thermo Fisher Scientific, #A21121		1:400		
Anti-mouse IgM Alexa Fluor 546	goat	Thermo Fisher Scientific #A21045		1:400		
Anti-rabbit IgG (H+L) Alexa Fluor 405	goat	Thermo Fisher Scientific, #A31556		1:400		
Anti-rabbit IgG (H+L) Alexa Fluor 488	donkey	Thermo Fisher Scientific, #A21206		1:400		
Anti-rabbit IgG HRP-labeled	goat	PerkinElmer, #NEF812001EA	1:3000			

List of antibodies used in this study.

WB: Western blot; IF: immunofluorescence; IP: immunoprecipitation; PLA: proximity ligation assay.