Limb girdle muscular disease caused by *HMGCR* mutation and statin myopathy treatable with mevalonolactone - Supplementary Material

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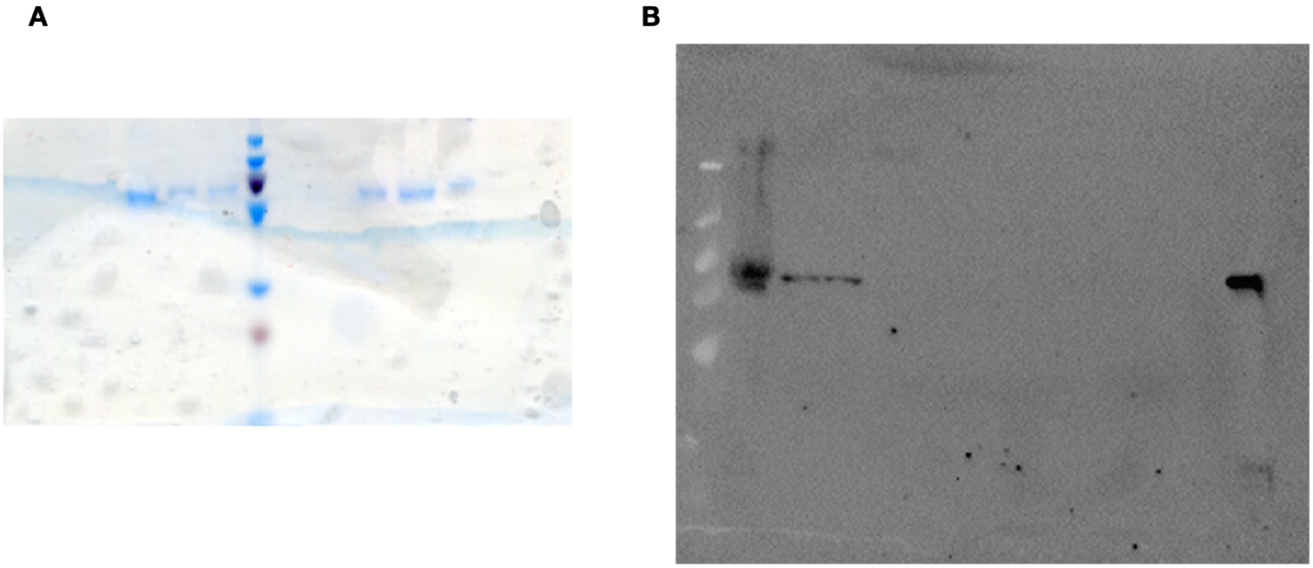
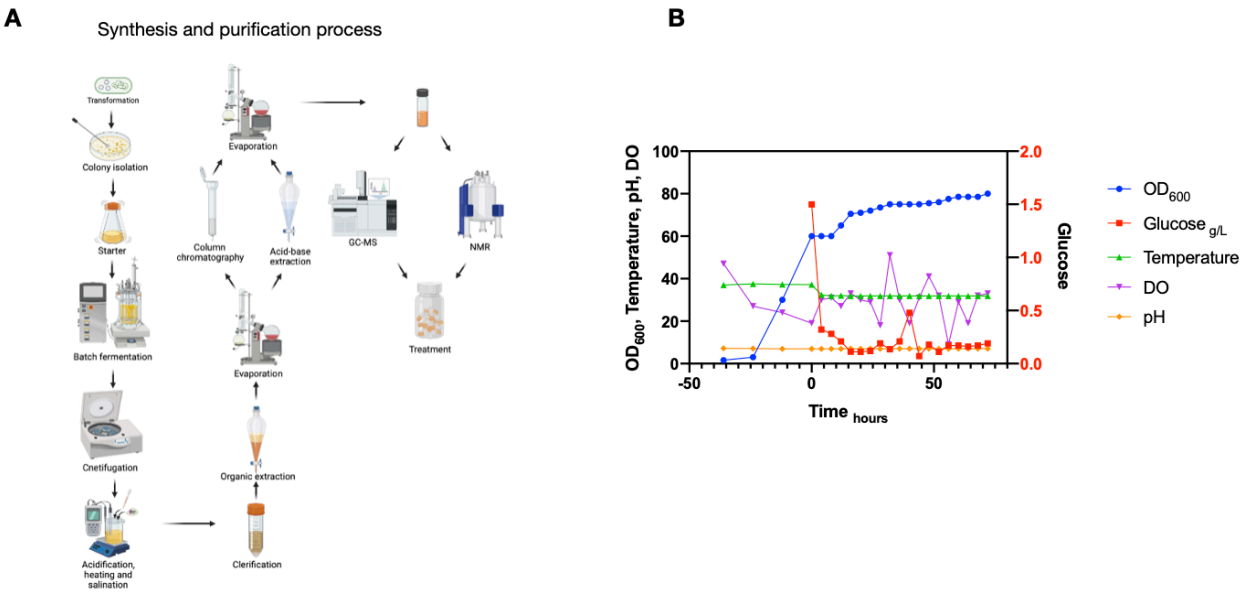
Figure S1: HMG CoA-Reductase purification



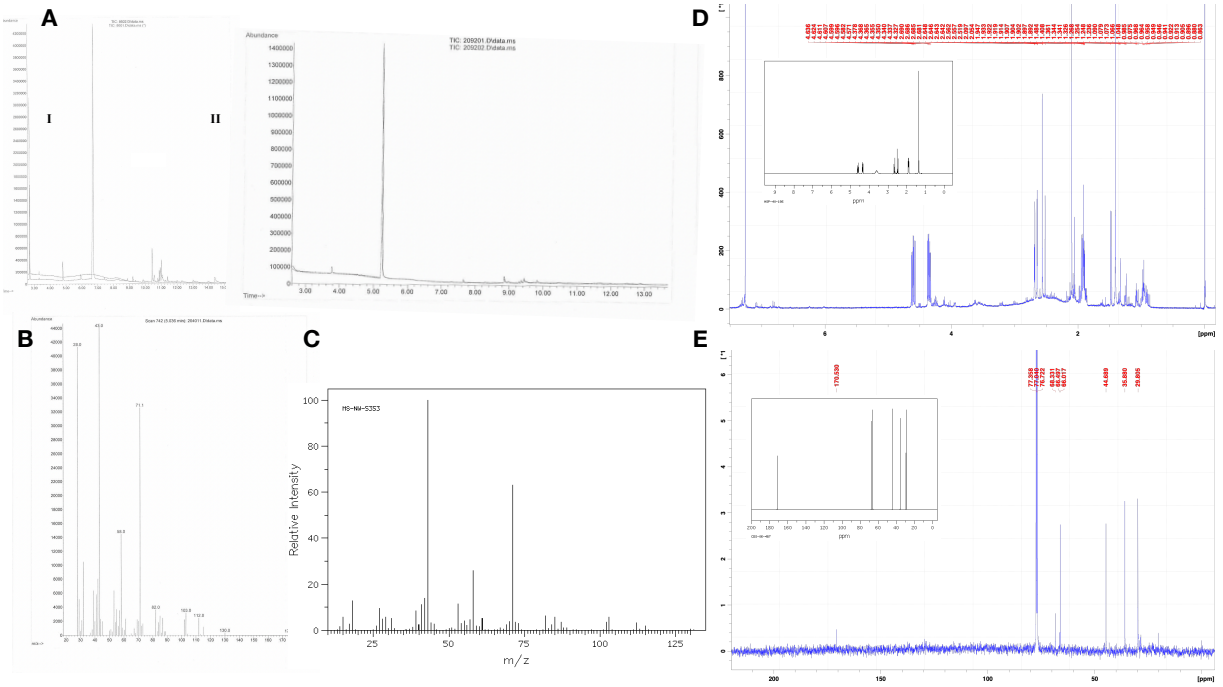
Representative Coomassie staining (A) and western blot of purified HMGCR proteins (B). Left- wildtype protein 0.6mg/ml and 0.06mg/ml, right- mutant protein 0.6mg/mL, stained with rabbit anti-HMGCR antibody.

Figure S2: Mevalonolactone synthesis and purification process



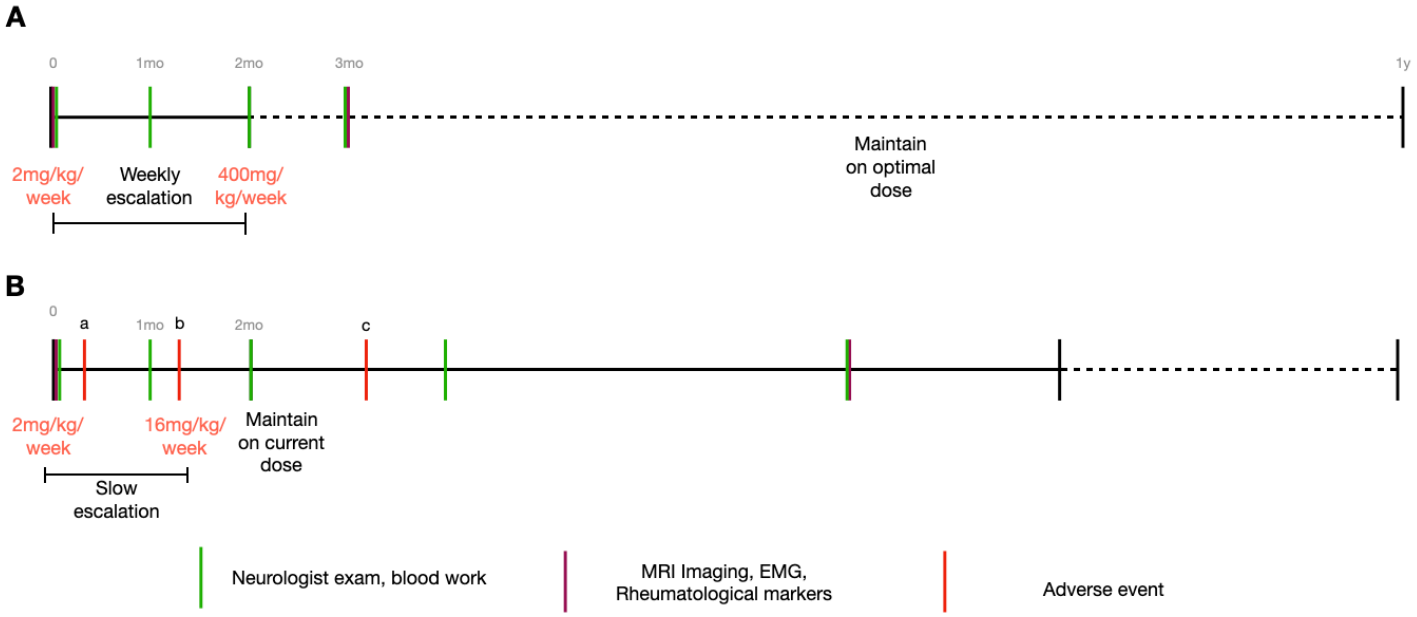
(A)Schematic representation of the synthesis and purification protocol. (B) An exemplary time course of mevalonolactone batch fermentation. Induction with IPTG was performed at time=0.

Figure S3: Mevalonolactone purity assessment



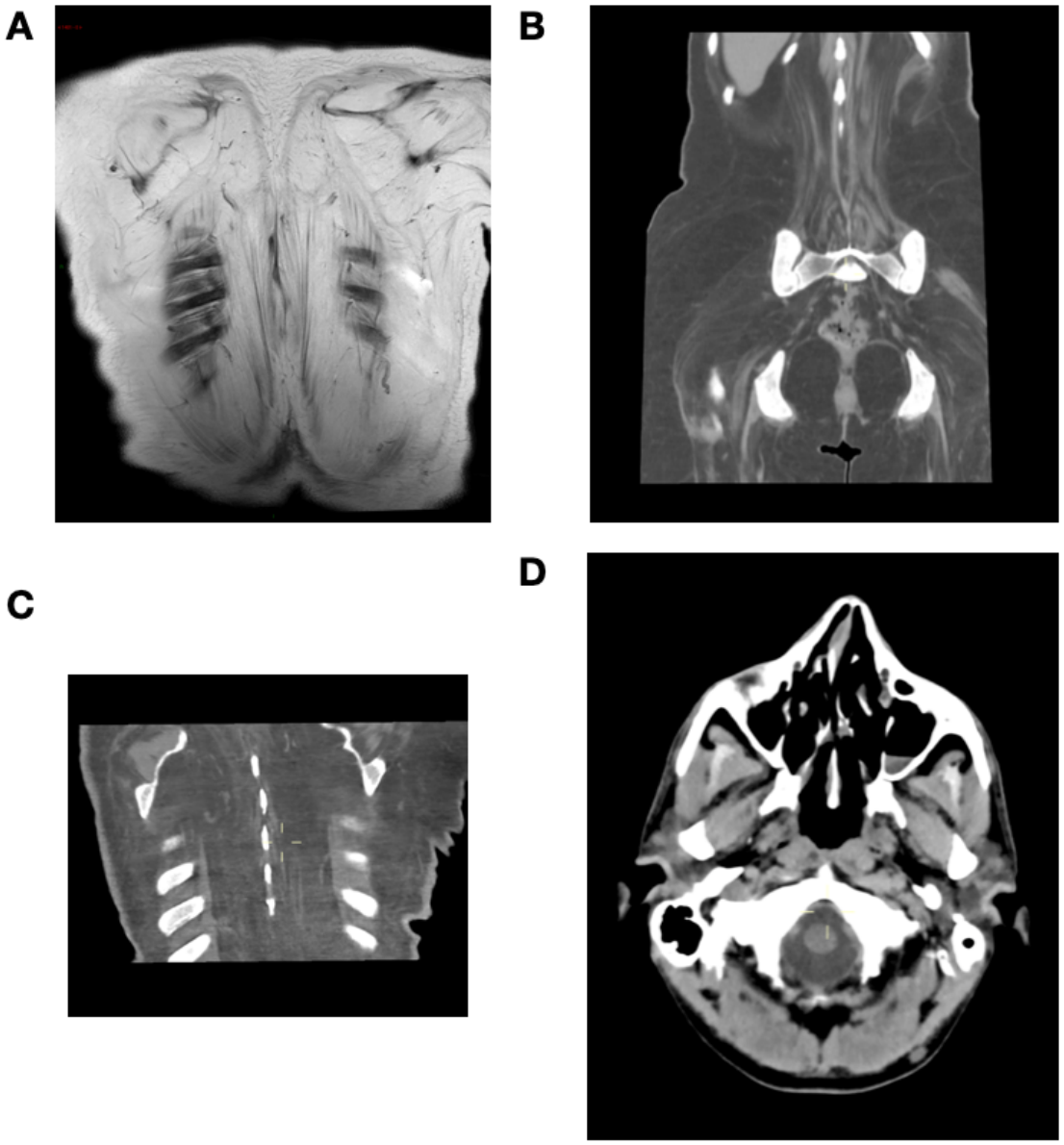
(A) GC-MS chromatogram of a sample produced using batch fermentation. I: original sample and blank reference, II: the same sample after acid-base extraction. (B) Spectra of the 5.036 peak, showing the expected fragments at 43, 71 and 58 m/z. (C) GC-MS reference spectra for mevalonolactone, obtained from AIST SDBS. (D) Observed 1H and (E) 13C-NMR spectra of samples, and expected reference spectra obtained from AIST SDBS inset.

Figure S4: Treatment timeline



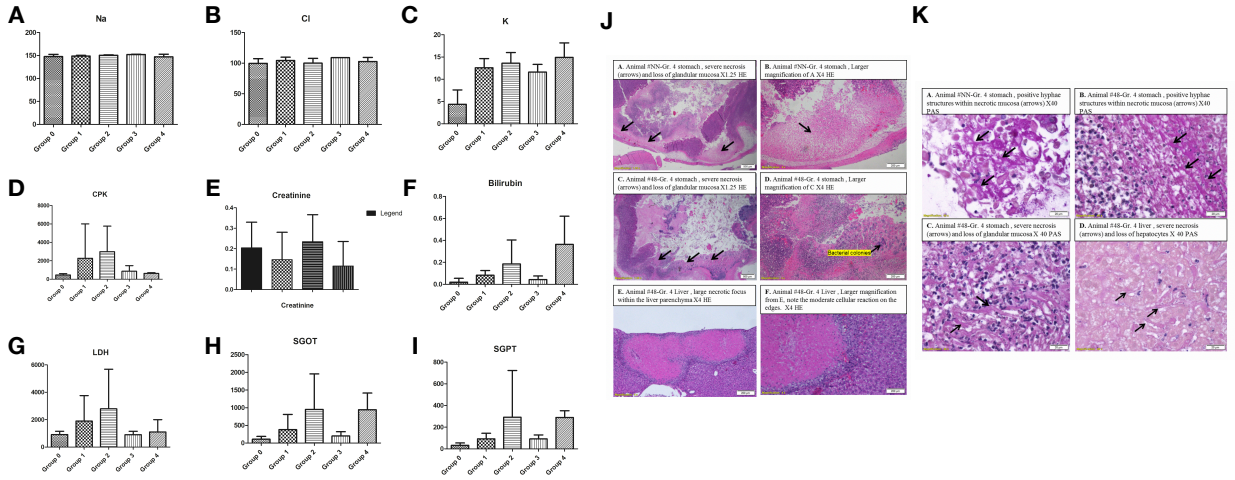
(A) Proposed treatment timeline, lasting one year, with an escalating dose protocol lasting 2 months. (B) Modified protocol, also indicating adverse events- (a) Subjective feeling of swelling in the wrists (no measured difference); (b) Severe headache, resolved overnight; (c) Near syncope.

Figure S5: Radiological features of HMGCR-LGMD



MRI and CT studies of HMGCR-LGMD patients showing atrophy and fatty replacement. (A) T1 MRI demonstrating atrophy of the upper back muscles in patient V:2, age 49. (B) CT scan showing atrophy of the lower back and thigh muscles of patient V:12, age 44. (C) CT scan showing atrophy of the upper back muscles of patient V:5, age 58. (D) CT scan showing sparing of facial, bulbar and neck muscles in patient V:9, age 41.

Figure S6: Mevalonolactone toxicity study in mice



Mice were orally administered either saline (group 1) or 20mg/Kg (group 2), 200mg/Kg (group 3) or 2000mg/Kg (group 4) mevalonolactone daily for 1 week (n=5 each). Mice were then euthanized by CO2 inhalation and blood and tissues were analyzed. Blood was also analyzed from 4 animals prior to initiation of the trial (group 0). (A-I) blood tests of all groups. Notice no significant differences between groups except for potassium levels. This is most probably the result of euthanasia with CO2. (J-K) Abnormal finding in histological studies of 2/5 mice in group 4. These are likely caused by non-related infections, possibly in combination with the corrosive nature of highly concentrated large dose of mevalonolactone.

66 **Table S1: Phenotypic delineation of patients affected with LGMD**

INDIVIDUAL	V:2	V:5	V:8	V:9	V:12	V:13
SEX	F	M	M	M	F	M
AGE AT EXAMINATION	49	58	37	42	51	41
AGE AT ONSET	31	39	24	33	31	34
PROXIMAL STRENGTH- UPPER LIMB	0/5	3/5	5/5	5/5	2/5	3/5
LOWER LIMB	0/5	2/5	5/5	4/5	2/5	4/5
ATROPHY – UPPER LIMB	Marked	Marked	-	-	Evident	Evident
LOWER LIMB	Marked	Marked	-	-	Evident	-
DEEP TENDON REFLEXES	Absent	Diminished	+	+	Diminished	Diminished
PAIN ON EXERTION	+	+	+	+	+	+
AMBULATORY	-	-	+	+	-	+
MOBILITY RESTRICTION	Bedridden	Wheelchair bound	-	-	Wheelchair bound	Uses assistive devices
RESPIRATORY DIFFICULTIES (BY PATIENT REPORT OR RESPIRATORY ACIDOSIS)	+ Ventilated through tracheostomy	+	-	-	+	-
DYSPHAGIA	-	-	-	-	-	-
ECHOCARDIOGRAPHY	Normal	Mild diastolic dysfunction	Normal	Normal	NA	NA
CPK (REFERENCE 20-180 U/L)	167 (14-1466)	1501 (254-9482)	9065 (1856-35761)	477 (271-1039)	542 (77-1428)	3797 (576-31617)

MAXIMAL TROPONIN T (0-14NG/L)	32.06	18.59	NA	64.82	23.39	NA
CREATININE	0.29 (0.08-0.67)	0.42 (0.2-0.64)	0.91 (0.78-0.99)	0.92 (0.82-1.04)	0.28 (0.15-0.6)	0.89 (0.72-1.02)
AST (REFERENCE 0-35 U/L)	34 (12-106)	54 (15-241)	277 (68-905)	23 (19-29)	43 (21-138)	98 (28-566)
ALT (REFERENCE 0-45 U/L)	31 (9-113)	50 (10-199)	322 (43-911)	15 (11-25)	44 (12-173)	80 (21-375)
ALKALINE PHOSPHATASE (REFERENCE 30-120 U/L)	151 (108-331)	109 (78-130)	78 (67-88)	89 (65-107)	100 (72-132)	79 (68-94)
TOTAL CHOLESTEROL AVERAGE (RECOMMENDED <200MG/DL)	146 (127-167)	159 (144-182)	128 (111-158)	136 (79-160)	171 (147-211)	128 (104-137)
TRIGLYCERIDES (RECOMMENDED <150MG/DL)	87 (47-129)	123 (79-230)	95.5 (95-96)	108 (58-160)	149 (55-270)	167 (77-232)
HDL (RECOMMENDED >60MG/DL)	49 (31-65)	49 (43-57)	38 (30-46)	45 (31-50)	55 (30-70)	41 (27-49)
LDL (RECOMMENDED <100MG/DL)	80 (68-99)	87 (79-104)	77 (62-92)	67 (28-81)	82.5 (50-112)	55 (31-71)
VLDL	17 (9-26)	25 (15-46)	19	22 (12-32)	30 (11-154)	33 (15-46)
FASTING BLOOD SUGAR	390	123	127	111	124	155
ANA, RF, C3, C4 ABNORMALITIES	-	-	-	NA	-	NA

ANTI-SM, ANTI JO-1, ANTI-SSA/B, ANCA, AMA	-	NA	NA	NA	-	NA
ANTI-HMGCR AB	-	-	-	-	-	-
ABNORMAL BRAIN IMAGING	-	-	NA	-	-	-
MYOPATHIC CHANGES IN EMG	+	+	NA	NA	+	+
ABNORMAL NCV	-	(+) L4-5 radiculopathy	NA	NA	-	-
MUSCLE BIOPSY-NORMAL DYSTROPHIN, NADH, SDH, COX, ATPASES, ELECTRON MICROSCOPY	+ Mild type-II fiber deficiency	+	NA	NA	+	NA
COMORBIDITIES	Insulin dependent diabetes - onset at age 19	COPD, Diastolic dysfunction, ICRBBB, Lymphocytosis		ICRBBB	Single kidney	

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Table S2: Primer list

Name		Sequence
HMGCR	Forward	GGTACTGCCAGTCAGGCTG
	Forward 2	GATAGGAACGGTGGGTGGTG
	Reverse	TCTTGGTGCAAGCTCCTTGG
	RFLP Reverse	AAAAAAAAAAAAAACCAGGATTATCTTTG CATGCTCCTTGAGCA
HMGCR-cDNA	Forward	ATGTTGTCAAGACTTTTTCGAATGCATG
	Reverse	TCAGGCTGTCTTCTTGGTGCAAG
Cloning and sequencing	EcoRI-FLAG-Nostop-HMGCR_R	GAATTCCTTATCGTCGTCATCCTTGTAAT CTAAGGCTGTCTTCTTGGTGC
	HMGCR-426-BmtI_F	GCTAGCTCATCAGTACTGGTGACACAGG AACC
	HMGCR-426-EcoRI_F	GAATTCTCATCAGTACTGGTGACACAGG AACC
	HMGCR-426-MfeI_F	CAATTGAATCATCAGTACTGGTGACACA GGAACC
	HMGCR_mutation_F1	ATGCTAGATGTTCAAGGAGCATGCAA
	HMGCR_mutation_F2	TTGCAGATGCTAGATGTTCAAGGAG
	HMGCR_mutation_R1	TTGAACATCTAGCATCTGCAAACAGG
	HMGCR_mutation_R2	CTCCTTGAACATCTAGCATCTGCAA
	HMGCR_Seq-R	GGTCAGTGTCACTGTCCCCAC
	HMGCR_Seq1	CCATGTCAGGGGTACGTCAGC
	HMGCR_Seq2	GTAGACGTGAACCTATGCTGGTC
	HMGCR_Seq3	CCAGCACCAATAGAGGCTGC
	NdeI-HMGCR-F	AAACATATGATGTTGTCAAGACTTTTTCG AATGCATG
	XhoI-HMGCR-R	AAAACTCGAGTCAGGCTGTCTTCTTGGT GCAAG
	XhoI-Kozak-HMGCR_F	CTCGAGGCCACCATGTTGTCAAGACTTT TTCGAATGCATG
	HMGCR_Seq_R2	CCACGAGTCATCCCATCTGC
HMGCR sgRNA #1	Forward	CACCGTCTCCCTGGCTACAGATGCT
	Reverse	AAACAGCATCTGTAGCCAGGGAGAC

HMGCR sgRNA #2	Forward	CACCGACACCTAGCATCTGTAGCCA
	Reverse	AAACTGGCTACAGATGCTAGGTGTC
HMGCR KI ssODN	HMGCR_KI_ ssODN	CACACAATTCGGGCAAGCTGCCGGGCA TTTTCCCCAGGATTATCTTTGCATGCTCC TTGAACATCTAGCATCTGTAGCCAGGGA GAGACACAAC

SMN primers		
541C960		GTAATAACCAAATGCAATGTGAA
541C1120		CTACAACACCCTTCTCACAG
R111		AGACTATCAACTTAATTTCTGATCA
Exon2a	Forward	TGTGTGGATTAAGATGACTCTTGG
	Reverse	TGCCTCCACAAAGGATGACATA
Exon3+4	Forward	CCTCCCCACTGATCAAAACGA
	Reverse	GCTACAAAAGTTTCATGGGAGAGC
Exon5	Forward	AGTCTGTTTGACTTCAGGATTTGGT
	Reverse	GGGACTACAAGAGCACTGCAT
Exon7	Forward	AAATGTCTTGTGAAACAAAATGCTT
	RFLP Reverse	AAAAAAAAAAAAAAAAAAAACCTTCCTTC TTTTTGATTTTGTTT
	Reverse	GTCTGCTGGTCTGCCTACTA
Exon1	Forward	GCGAGGCTCTGTCTCAAAACA
	Reverse	GATCGACTTGATGCTGTCCCGA
Exon2b	Forward	GGTGTATGATGCCTTTAAGAGCAGTTT
	Reverse	CTTCTCCCTGCCTTCCATTCACA
Exon6	Forward	CAACATAGCAAGACCTCGTCT
	Reverse	TGCAAGAGTAATTTAAGCCTCAGA
Exon 8	Forward	GTTTAACTGGTGTCCACAGAGG
	Forward 2	TTCGTCAAGCCTCTGGTTCT
	Reverse	CATACACAAAATGCTATGGTGGCA
	Reverse 2	CAAAATATGGGCCAAAGGGCA
Full length	Forward	GTTGGGGGATCAAATATCTTCTAGTGTT
	Reverse	CCCCCACCCCAGTCTTTTACAGATGGT

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Table S3: Plasmid list

Plasmids

Name	Source
pGEX-6P	Cytiva
pGEX-6P-HMGCR-426-888	This article
pMevT	Addgene plasmid #17815[1]
pPalmitoyl-mTurquoise2	Addgene plasmid #36209[2]
pEF.myc.ER-E2-Crimson	Addgene plasmid #38770[3]
px459	Addgene plasmid #62988[4]

Table S4: Solutions and buffers

Name	Composition
0.5M K_2HPO_4 (pH=7.4) stock solution	Dissolve 68.05g K_2HPO_4 and 15.64g NaOH in 900mL DDW, titrate pH and adjust to 1L
Buffer A – bacteria lysis buffer	20mM K_2HPO_4 (pH=7.4) 20mM Tris (pH=7.6) 500mM $(NH_4)_2SO_4$ 1mM EDTA 2mM TCEP 1mM $MgCl_2$ 10% glycerol 0.01% Triton X-100
Buffer B – wash buffer and elution buffer	20mM K_2HPO_4 20mM Tris (pH=8) 200mM $(NH_4)_2SO_4$ 1mM EDTA 2mM TCEP 10% glycerol For elution of GST-fused protein add 10mM reduced glutathione
Buffer C- Ion-exchange low salt buffer	50mM K_2HPO_4 50mM KCl 1mM EDTA 2mM TCEP
Buffer D- Ion-exchange high salt buffer	50mM K_2HPO_4 1M KCl 1mM EDTA 2mM TCEP
Buffer E – HMGCR protein and assay buffer	100mM K_2HPO_4 120mM KCl 1mM EDTA 2mM TCEP

Movie S1: Improvement in muscle function of HMGCR-LGMD patient with mevalonolactone treatment

After 4 months of treatment, patient V:2 is able to fully abduct her arm when laying, an action she was unable to perform for several years prior to treatment

Movie S2: Improvement in muscle function of statin-myopathy murine model with mevalonolactone treatment

Mice treated with mevalonolactone (right) show much greater muscle endurance evaluated by wire-hanging test, as opposed to control mice (left).

Supplemental references

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2. Goedhart, J., Von Stetten, D., Noirclerc-Savoye, M., et al., Structure-guided evolution of cyan fluorescent proteins towards a quantum yield of 93%. *Nat. Commun.*, 2012, **3**.
3. Strack, R. L., Hein, B., Bhattacharyya, D., Hell, S. W., Keenan, R. J., and Glick, B. S., A rapidly maturing far-red derivative of DsRed-Express2 for whole-cell labeling. *Biochemistry*, 2009, **48**, 8279–8281.
4. Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., and Zhang, F., Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.*, 2013, **8**, 2281–2308.