

Supplementary Figures for

LIM and Cysteine-rich Domains 1 (LMCD1) regulates skeletal muscle hypertrophy, calcium handling, and force

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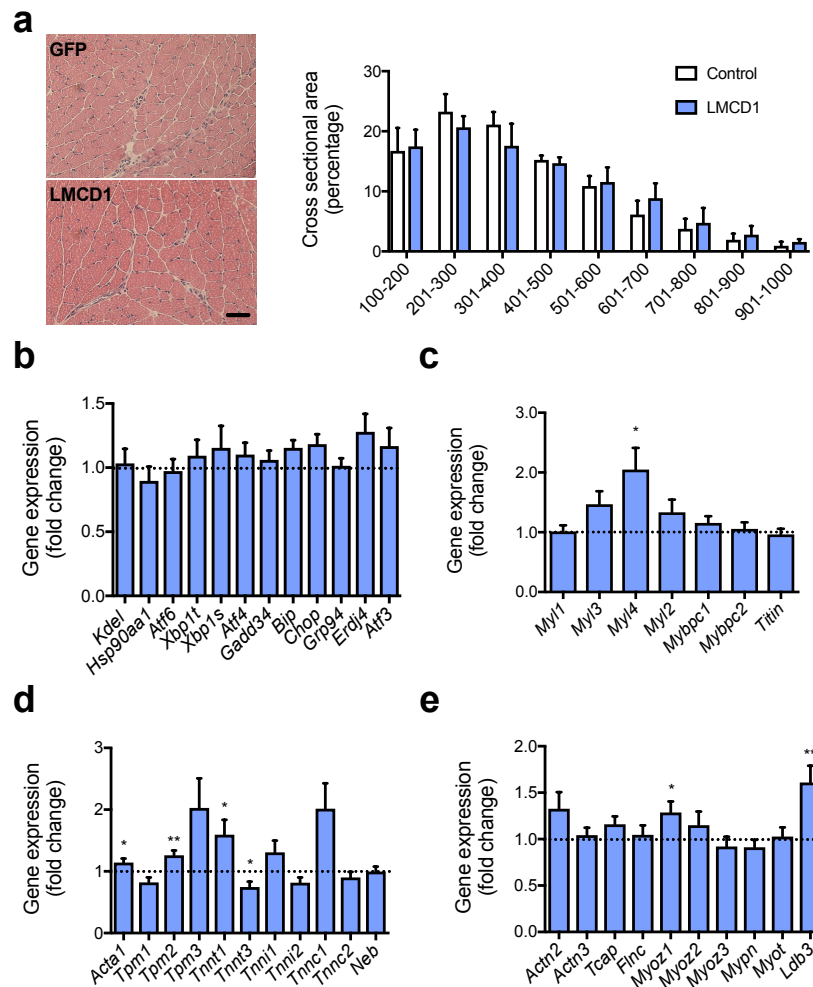


Figure S1. LMCD1 induces small changes in gene expression of genes related with sarcoplasmic reticulum (SR) stress and muscle structural proteins. **(a)** Determination of fiber cross sectional area. Hematoxylin and eosin staining of sections from *gastrocnemius* muscle 7 days after GFP (control) or LMCD1 adenovirus injection and respective percentage of fiber sectional area ($n = 3$). **(b)** Quantitative RT-PCR (qRT-PCR) of genes involved in SR stress in mice treated as in (a) ($n = 6$). **(c)** qRT-PCR of genes involved in the expression of proteins from the thick filaments in *gastrocnemius* muscle in mice treated as in (a) ($n = 6$). **(d)** qRT-PCR of genes involved in the expression of proteins from the thin filaments in *gastrocnemius* muscle in mice treated as in (a) ($n = 6$). **(e)** qRT-PCR of genes involved in the expression of proteins present in the Z-disk in *gastrocnemius* muscle in mice treated as in (a) ($n = 6$). Data is shown as mean $\pm$ SEM and * $p < 0.05$; ** $p < 0.01$. Abbreviations: *Kdel*, (Lys-Asp-Glu-Leu) Endoplasmic Reticulum Protein Retention Receptor 1; *Hsp90aa1*, Heat Shock Protein 90 Alpha Family Class A Member 1; *Atf6*, Activating Transcription Factor 6; *Xbp1t*, X-box binding protein 1 total; *Xbp1s*, X-box binding protein 1 short; *Atf4*, Activating Transcription Factor 4; *Gadd34*, Growth arrest and DNA damage-inducible protein 34; *Bip*, Binding immunoglobulin protein; *Chop*, C/EBP homologous protein; *Grp94*, 94 kDa

glucose- regulated protein; *Erdj4*, Endoplasmic Reticulum DNA J Domain-Containing Protein 4; *Atf3*, Activating Transcription Factor 3; *My11*, Myosin light chain 1; *My13*, Myosin light chain 3; *My14*, Myosin light chain 4; *My12*, Myosin light chain 2; *Mybpc1*, Myosin Binding Protein C 1; *Mybpc2*, Myosin Binding Protein C 2; *Acta1*, Actin, Alpha 1; *Tpm1*, Tropomyosin 1; *Tpm2*, Tropomyosin 2; *Tpm3*, Tropomyosin 3; *Tnnt1*; Troponin T1; *Tnnt3*, Troponin T3; *Tnni1*, Troponin I1; *Tnni2*, Troponin I2; *Tnnc1*, Troponin C1; *Tnnc2*, Troponin C2; *Neb*, Nebulin; *Actn2*, Actinin Alpha 2; *Actn3*, Actinin Alpha 2; *Tcap*, Telethonin; *Flnc*, Filamin C; *Myoz1*, Myozenin 1; *Myoz2*, Myozenin 2; *Myoz3*, Myozenin 3; *Mypn*, Myopalladin; *Myot*, Myotilin; *Ldb3*, LIM Domain Binding 3.

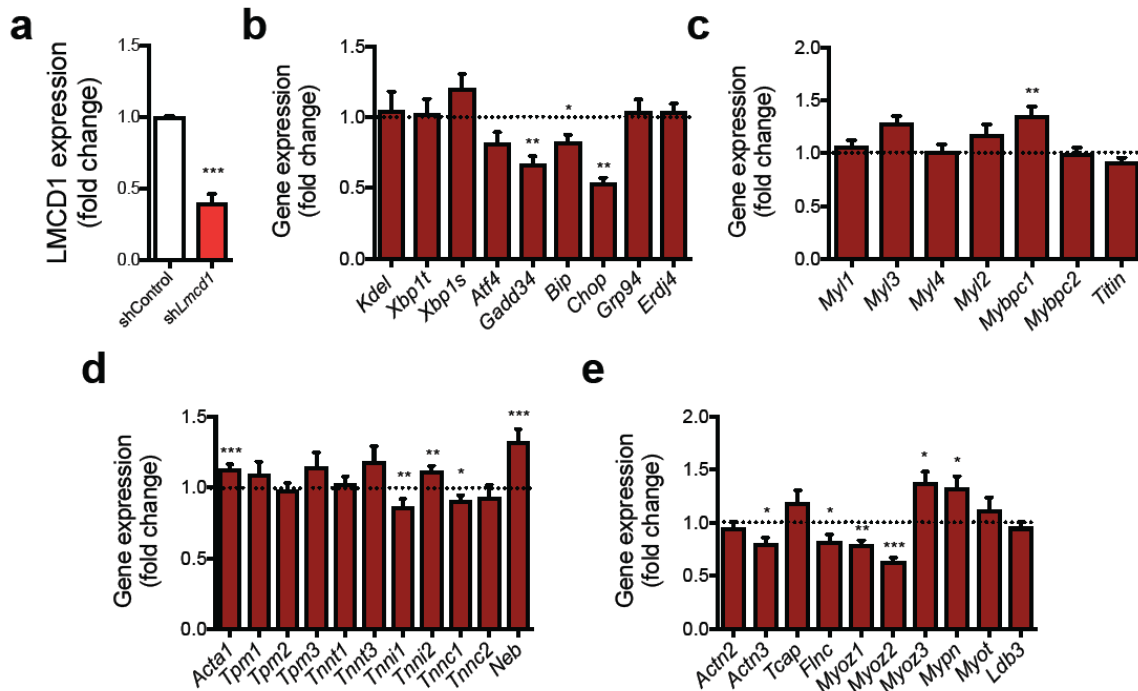


Figure S2. Silencing *Lmcd1* does not change gene expression. **(a)** qRT-PCR for *Lmcd1* in *gastrocnemius* 7 days after intramuscular delivery of a scrambled/control shRNA or a sh*Lmcd1* ($n = 6$). **(b)** qRT-PCR of genes involved in SR stress in differentiated primary mouse myotubes after 2 days transduction of scrambled shRNA or sh*Lmcd1* adenovirus ($n = 8$). **(c)** qRT-PCR of genes involved in the expression of proteins from the thick filaments in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). **(d)** qRT-PCR of genes involved in the expression of proteins from the thin filaments in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). **(e)** qRT-PCR of genes involved in the expression of proteins present in the Z-disk in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). Data is shown as mean $\pm$ SD and * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Abbreviations: *Kdel*, (Lys-Asp-Glu-Leu) Endoplasmic Reticulum Protein Retention Receptor 1; *Hsp90aa1*, Heat Shock Protein 90 Alpha Family Class A Member 1; *Atf6*, Activating Transcription Factor 6; *Xbp1t*, X-box binding protein 1 total; *Xbp1s*, X-box binding protein 1 short; *Atf4*,

Activating Transcription Factor 4; *Gadd34*, Growth arrest and DNA damage-inducible protein 34; *Bip*, Binding immunoglobulin protein; *Chop*, C/EBP homologous protein; *Grp94*, 94 kDa glucose-regulated protein; *Erdj4*, Endoplasmic Reticulum DNA J Domain-Containing Protein 4; *Myl1*, Myosin light chain 1; *Myl3*, Myosin light chain 3; *Myl4*, Myosin light chain 4; *Myl2*, Myosin light chain 2; *Mybpc1*, Myosin Binding Protein C 1; *Mybpc2*, Myosin Binding Protein C 2; *Acta1*, Actin, Alpha 1; *Tpm1*, Tropomyosin 1; *Tpm2*, Tropomyosin 2; *Tpm3*, Tropomyosin 3; *Tnnt1*, Troponin T1; *Tnnt3*, Troponin T3; *Tnni1*, Troponin I1; *Tnni2*, Troponin I2; *Tnnc1*, Troponin C1; *Tnnc2*, Troponin C2; *Neb*, Nebulin; *Actn2*, Actinin Alpha 2; *Actn3*, Actinin Alpha 2; *Tcap*, Telethonin; *Flnc*, Filamin C; *Myoz1*, Myozenin 1; *Myoz2*, Myozenin 2; *Myoz3*, Myozenin 3; *Mypn*, Myopalladin; *Myot*, Myotilin; *Ldb3*, LIM Domain Binding 3.

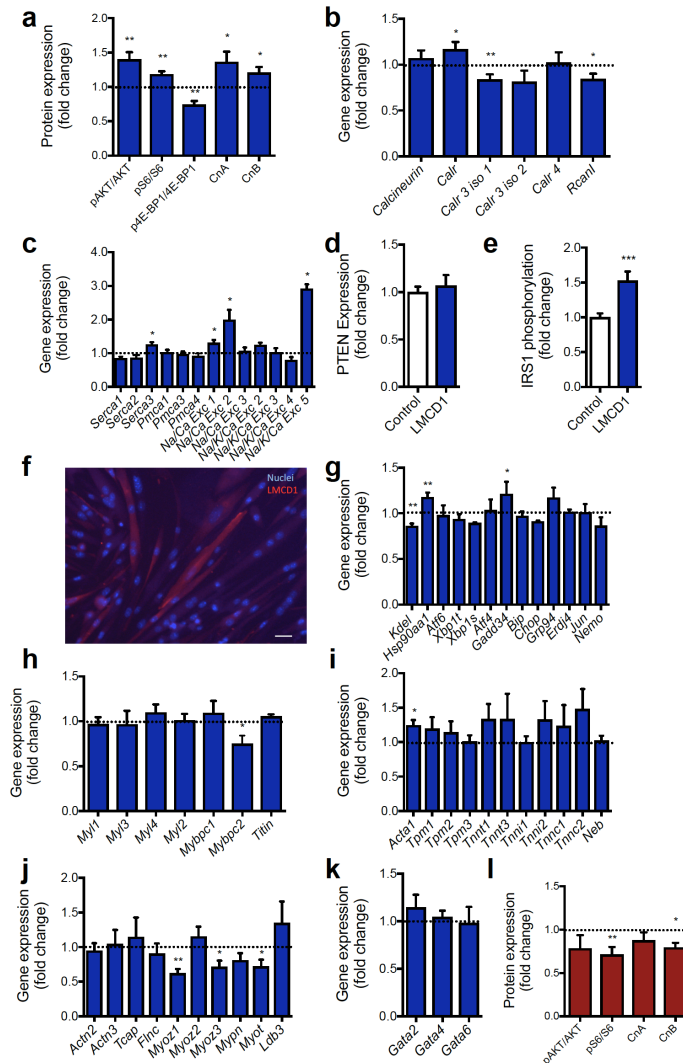


Figure S3. LMCD1 has mainly cytosolic location and induces small changes in gene expression in differentiated primary mouse myotubes. **(a)** Western blot

quantification for AKT Ser⁴⁷³ phosphorylation, S6 Ser²³⁵ phosphorylation, 4E-BP1 Thr³⁷ phosphorylation, calcineurin A and calcineurin B in differentiated primary mouse myotubes after 2 days of transduction with GFP (dotted line) or LMCD1 adenovirus ($n = 8$). **(b)** qRT-PCR of genes involved in the expression of Ca²⁺ related proteins in differentiated primary mouse myotubes treated as in (a) ($n = 8$). **(c)** qRT-PCR of genes involved in the expression of Ca²⁺ channels proteins in differentiated primary mouse myotubes and transduced as in (a) ($n = 8$). **(d)** Western blot quantification for PTEN in differentiated myotubes transduced as in (a) ($n = 6$). **(e)** Western blot quantification for IRS1 Tyr⁶¹² phosphorylation in differentiated myotubes transduced as in (a) ($n = 4$). **(f)** Representative microscopy image of immunocytochemistry against LMCD1 in differentiated primary mouse myotubes. DAPI staining was used to stain nuclei (200 x; $n = 6$). Scale bar = 50 μ m. **(g)** qRT-PCR of genes involved in SR stress in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). **(h)** qRT-PCR of genes involved in the expression of proteins from the thick filaments in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). **(i)** qRT-PCR of genes involved in the expression of proteins from the thin filaments in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). **(j)** qRT-PCR of genes involved in the expression of proteins present in the Z-disk in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). **(k)** qRT-PCR of *Gata* genes in differentiated primary mouse myotubes transduced as in (a) ($n = 8$). **(l)** Western blot quantification for AKT Ser⁴⁷³ phosphorylation, S6 Ser²³⁵ phosphorylation, calcineurin A and calcineurin B in differentiated primary mouse myotubes after 2 days transduction of scrambled shRNA or sh*Lmcd1* adenovirus ($n = 8$). Data is shown as mean $\pm$ SD and * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Abbreviations: AKT, Protein kinase B; 4E-BP1, Eukaryotic translation initiation factor 4E-binding protein 1; CnA, Calcineurin A; CnB, Calcineurin B; *Calr*, Calreticulin; *Rcan1*, Calciopressin 1; *Serca*, Sarco/Endoplasmic Reticulum Ca²⁺-ATPase; *Pmca*, Plasma Membrane Ca²⁺-ATPase; *Kdel*, (Lys-Asp-Glu-Leu) Endoplasmic Reticulum Protein Retention Receptor 1; *Hsp90aa1*, Heat Shock Protein 90 Alpha Family Class A Member 1; *Atf6*, Activating Transcription Factor 6; *Xbp1t*, X-box binding protein 1 total; *Xbp1s*, X-box binding protein 1 short; *Atf4*, Activating Transcription Factor 4; *Gadd34*, Growth arrest and DNA damage-inducible protein 34; *Bip*, Binding immunoglobulin protein; *Chop*, C/EBP homologous protein; *Grp94*, 94 kDa glucose- regulated protein; *Erdj4*, Endoplasmic Reticulum DNA J Domain-Containing Protein 4; *Nemo*, NF- κ B essential modulator; *Myl1*, Myosin light chain 1; *Myl3*, Myosin light chain 3; *Myl4*, Myosin light chain 4; *Myl2*, Myosin light chain 2; *Mybpc1*, Myosin Binding Protein C 1; *Mybpc2*, Myosin Binding Protein C 2; *Acta1*, Actin, Alpha 1; *Tpm1*, Tropomyosin 1; *Tpm2*, Tropomyosin 2; *Tpm3*, Tropomyosin 3; *Tnnt1*, Troponin T1; *Tnnt3*, Troponin T3; *Tnni1*, Troponin I1; *Tnni2*, Troponin I2; *Tnnc1*, Troponin C1; *Tnnc2*, Troponin C2; *Neb*, Nebulin; *Actn2*, Actinin Alpha 2; *Actn3*, Actinin Alpha 2; *Tcap*, Telethonin; *Flnc*, Filamin C; *Myoz1*, Myozenin 1; *Myoz2*, Myozenin 2; *Myoz3*, Myozenin 3; *Mypn*, Myopalladin; *Myot*, Myotilin; *Ldb3*, LIM Domain Binding 3.