Lmo7 is an emerin-binding protein that regulates the transcription of emerin and many other muscle-relevant genes

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X-linked Emery–Dreifuss muscular dystrophy (X-EDMD) is inherited through mutations in emerin, a nuclear membrane protein. Emerin has proposed roles in nuclear architecture and gene regulation, but direct molecular links to disease were unknown. We report that Lim-domain only 7 (Lmo7) binds emerin directly with 125 nM affinity; the C-terminal half of human Lmo7 (hLmo7C) was sufficient to bind emerin in vitro. Lmo7 appeared relevant to EDMD because a deletion that removes Lmo7 (plus eight exons of a neighboring gene) in mice causes dystrophic muscles [Semenova, E., Wang, X., Jablonski, M.M., Levrone, J. and Tilghman, S.M. (2003) An engineered 800 kilobase deletion of Uchl3 and Lmo7 on mouse chromosome 14 causes defects in viability, postnatal growth and degeneration of muscle and retina. *Hum. Mol. Genet.*, 12, 1301–1312]. Lmo7 localizes in the nucleus, cytoplasm and cell surface, particularly adhesion junctions [Ooshio, T., Irie, K., Morimoto, K., Fukuhara, A., Imai, T. and Takai, Y. (2004) Involvement of LMO7 in the association of two cell-cell adhesion molecules, nectin and E-cadherin, through afadin and alpha-actinin in epithelial cells. *J. Biol. Chem.*, 279, 31365–31373]. Our data suggest endogenous Lmo7 is a nucleocytoplasmic shuttling protein, and might also localize at focal adhesions in HeLa cells. Two key results show that Lmo7 regulates emerin gene expression: rat Lmo7 isoforms directly activated a luciferase reporter gene in vivo, and emerin mRNA expression decreased 93% in Lmo7-downregulated HeLa cells. Thus, Lmo7 not only binds emerin protein but is also required for emerin gene transcription. Microarray analysis of Lmo7-downregulated HeLa cells identified over 4200 misregulated genes, including 46 genes important for muscle or heart. Misregulation of 11 genes, including four (CREBBP, NAP1L1, LAP2, RBL2) known to be misregulated in X-EDMD patients and emerin-null mice [Bakay, M., Wang, Z., Melcon, G., Schiltz, L., Xuan, J., Zhao, P., Sartorelli, V., Seo, J., Pegoraro, E., Angelini, C. et al. (2006) Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of Rb-MyoD pathways in muscle regeneration. *Brain*, 129, 996–1013; Melcon, G., Kozlov, S., Cutler, D.A., Sullivan, T., Hernandez, L., Zhao, P., Mitchell, S., Nader, G., Bakay, M., Rottman, J.N. et al. (2006) Loss of emerin at the nuclear envelope disrupts the Rb1/E2F and MyoD pathways during muscle regeneration. *Hum. Mol. Genet.*, 15, 637–651] was confirmed by real-time PCR. Overexpression of wild-type emerin, but not emerin mutant P183H (which causes EDMD and selectively disrupts binding to Lmo7), decreased the expression of CREBBP, NAP1L1 and LAP2, suggesting Lmo7 activity is both EDMD-relevant and inhibited by direct binding to emerin. We conclude that Lmo7 positively regulates many EDMD-relevant genes (including emerin), and is feedback-regulated by binding to emerin.

**INTRODUCTION**

Emery–Dreifuss muscular dystrophy (EDMD) is inherited through mutations in either of two genes: *LMNA*, encoding A-type lamins, and *EMD*, which encodes a nuclear membrane protein named emerin (1). EDMD is characterized by progressive skeletal muscle weakening, contractures of major tendons and potentially fatal cardiac conduction system.
defects (1,2). Emerin is an integral protein of the nuclear inner membrane (3,4). Emerin is present in all human cells tested (5). Emerin belongs to the ‘LEM-domain’ family of nuclear proteins, defined by a ~40-residue-folded domain (the ‘LEM domain’). Other members include LAP2, MAN1, Lem2 and several uncharacterized human genes (6–9). A major shared function of all studied LEM-domain proteins is their binding (via the LEM domain) to a chromatin protein named Barrier-to-Autointegration Factor (BAF) (11). BAF is a small, highly conserved essential protein with direct roles in higher-order chromatin structure, nuclear assembly and gene regulation (12–18). Emerin also binds A-type lamins, which anchor emerin at the nuclear inner membrane (19,20). Together, emerin and lamin A can form stable ternary complexes with other binding partners in vitro (12), with the potential to form a variety of oligomeric protein complexes (3,21).

Emerin is expressed ubiquitously, but EDMD only strikes specific tissues. To explain the tissue specificity of EDMD, emerin was variously proposed to have roles in tissue-specific gene expression, signaling or nuclear structure (2,22,23). Architectural models fail to explain the cardiac conduction phenotype of EDMD, but are consistent with the structural defects (aberrant shape and nuclear envelope herniations) seen in a subset of nuclei from EDMD patients (24) and in a subset of patients with other diseases linked to mutations in LMNA (‘laminopathies’) (19,20,22). Architectural models are supported by evidence that emerin is one of four known pointed-end F-actin capping proteins in the cell (25). On the other hand, growing evidence also supports roles for emerin in gene expression. Emerin binds directly to at least three transcription regulators named GCL (11), Btf (26) and β-catenin (27), and mRNA splicing factor YT521-B (28). Emerin is closely related to LAP2β, a LEM-domain protein that also binds GCL and regulates gene expression in vivo (29,30). An early study suggested at least 60 genes were misregulated in X-EDMD patient fibroblasts (31). A recent large-scale microarray analysis of gene expression in muscle biopsies from EDMD patients revealed at least 45 significantly misregulated genes, including many regulated by Rb and MyoD (32). Interestingly, both proposed roles for emerin (gene regulation and nuclear architecture) might be involved in regulating gene expression in response to mechanical force (33).

To explore the EDMD disease mechanism, we used affinity chromatography to purify novel emerin-binding or emerin-associated proteins from mammalian cell (HeLa) nuclear extracts. We identified several interesting proteins including actin (reported separately) (25) nuclear myosin I (NMI), αII-spectrin and an uncharacterized protein, to be reported separately. Here we focus on Lim domain only 7 protein (Lmo7), the most abundant emerin-binding protein recovered. We report that Lmo7 is a shuttling transcription activator that positively regulates the emerin gene, and is inhibited by binding to emerin.

**RESULTS**

To purify proteins that bind emerin, we covalently attached purified recombinant emerin protein (residues 1–222; which lack the transmembrane domain) to Affi-gel beads as described (see Materials and Methods) (12). BSA was conjugated to Affi-gel beads as a negative control. We then incubated the emerin-beads and BSA-beads each with 50 mg protein from nuclear extracts of HeLa cells (see Materials and Methods). Beads were washed, and bound proteins eluted with SDS-sample buffer, resolved by SDS–PAGE and stained with Coomassie Blue. The most abundant protein recovered was identified by mass spectrometry (data not shown) as Lmo7, a potential transcription factor. Lmo7 is alternatively spliced and expressed in most tissues tested, with high levels in muscle and heart (34,35). Lmo7 localizes in the nucleus and cytoplasm, and also at adherens junctions at the surface of MDCK cells (36,37). Interestingly, an 800 kbp genomic deletion of LMO7 (Lmo7Δ800) causes muscle degeneration in mice (37), with significantly greater penetrance of the dystrophic phenotype and more severe skeletal muscle degeneration and growth retardation than mice null for UCHL3 alone (37). The Lmo7Δ800 mouse phenotype suggested Lmo7 might be directly relevant to muscle and the EDMD disease mechanism.

Lmo7 contains a predicted calponin homology (CH) domain, a putative F-box, a PDZ domain and the LIM domain (Fig. 1A). The CH domain is predicted to bind actin, and the PDZ and LIM domains are each protein–protein interaction domains (36). Many F-box proteins are the substrate recognition components of ubiquitin ligases (38). An alternatively spliced isoform of rat Lmo7a (rLmo7a), named rLmo7b, lacks the F-box, but includes both the PDZ and LIM domains (Fig. 1A). Two related proteins, Lmo2 and Lmo4, localize to the nucleus and regulate gene transcription (39,40). Lmo2 regulates hematopoetic stem cell commitment (39), whereas Lmo4 regulates differentiation, binding directly to other transcription regulators including LDB1 (41), BRCA1 and ctIP (40). These related Lmo-proteins suggested a gene regulatory role for Lmo7. We therefore characterized the Lmo7 protein and its interaction with emerin, and tested the hypothesis that Lmo7 was an emerin-binding transcription regulator.

**Lmo7 binds emerin directly in vitro**

We cloned a cDNA encoding a region of the predicted human Lmo7 protein (GenBank accession no. Q8WW11) that corresponded to the C-terminal half (797 residues) of rLmo7a. This polypeptide (residues 888–1683), termed hLmo7C contained the PDZ and LIM (protein–protein interaction) domains, but lacked the CH and F-box domains (Fig. 1A). The predicted full-length human Lmo7 (hLmo7) is 71.8% identical (77.4% similar) to rLmo7a, and hLmo7C is 73.3% identical (79.2% similar) to the C-terminal half of rLmo7a. Given this high conservation, we hypothesized that rat and human Lmo7 function similarly and used both in the following studies. To test potential direct binding of emerin to Lmo7, we first expressed and purified hLmo7C. Recombinant emerin (2.2 nmol) and hLmo7C (0.56 nmol) were incubated (final concentrations, 11 μM and 2.8 μM, respectively) and then emerin was immunoprecipitated using a monoclonal antibody against emerin (Fig. 1B). Control reactions contained hLmo7C and emerin antibody, but no emerin protein. Most hLmo7C
co-immunoprecipitated with emerin, as detected by immunoblotting for the His-tag on hLmo7C and emerin (Fig. 1B, +Emr, α-His and α-Emr, respectively). hLmo7C did not pellet in the absence of emerin (Fig. 1B, −Emr), but bound beads nonspecifically and was removed by washing (data not shown). Thus, the C-terminal half of Lmo7 was sufficient to bind emerin in vitro. To determine if emerin binds Lmo7 in vivo, HeLa cells were lysed in 50 mM HEPES (pH 7.4), 300 mM NaCl, 0.3% Triton X-100 (see Materials and Methods), and the supernatant was incubated with Protein G beads in the presence (α-emr) or absence (No Ab) of monoclonal antibodies against emerin (Fig. 1C). Endogenous Lmo7 co-immunoprecipitated efficiently with emerin, detected by probing western blots with polyclonal antibodies specific for emerin (serum 2999) or Lmo7 (serum CO5; Fig. 1C, lane 3). Only background signals for Lmo7 and emerin were seen with beads alone (Fig. 1C, lane 5); Lmo7 again bound beads nonspecifically and was depleted during washing. We concluded that Lmo7 binds emerin directly and specifically, both in vitro and in vivo. Interestingly, two Lmo7 bands co-immunoprecipitated with endogenous emerin (Fig. 1C, lane 3); the expected 195 kDa band and a second band at ~210 kDa, which appeared to enrich with emerin. We did not pursue this band, but speculate that it could be a post-translationally-modified or alternatively-spliced form of Lmo7 in HeLa cells.

We next determined the affinity of the emerin-hLmo7C interaction using a microtiter well-binding assay, in which recombinant purified emerin protein (residues 1–222) was immobilized in microtiter wells and incubated with increasing concentrations of 35S-Met-labeled hLmo7C (see Materials and Methods). The affinity of 35S-hLmo7C for emerin in this assay was 125 nM (range 74–198 nM; n = 5; Fig. 2A) with a stoichiometry of 1 mol hLmo7C per mole emerin (data not shown). To map the Lmo7C-binding domain within emerin, we immobilized either purified wild-type emerin or selected emerin mutants bearing clusters of alanine-substitution mutations (‘m’-series; 15, 43), or BSA as the negative control in microtiter wells, and incubated each with 35S-hLmo7C protein. Emerin mutants m24, m145 and m164 bound hLmo7C as well as wild-type emerin (Fig. 2B). Mutants m70, m76, m112, m151, m161, m198 and m206 bound hLmo7C nearly (51–75%) as well as wild-type (Fig. 2B). Mutants m34, m40, m45A, m122, m196 and m214 had reduced binding (25–50% of wild-type; Fig. 2B). Mutants m45E, m175, m179, m192 and m207 had essentially background binding to hLmo7C (Fig. 2B). These results showed that Lmo7 is sensitive to mutations in two regions, previously named Repressor Binding Domain-1 (RBD-1) and RBD-2 (Fig. 2D), which are also required to bind two known transcription repressors GCL and Btf (11,25) and splicing factor YT521-B (27).

Because Lmo7Δ800 mice have dystrophic muscle phenotypes (37), we hypothesized that Lmo7 binding to emerin might be particularly relevant to EDMD. We therefore also tested four EDMD disease-causing mutations in emerin, namely S54F, D95–99, Q133H and P183H, for binding to Lmo7 in vitro. These mutations are potentially revealing in terms of the EDMD disease mechanism. In contrast to most EDMD-causing mutations, which are either nonsense mutations or cause emerin protein to misfold and degrade, emerin proteins bearing these ‘special’ mutations each localize normally at the nuclear envelope, yet behave ‘null’ and cause typical EDMD disease (42,43). The D95–99 mutation, which deletes five residues, was previously shown to have no effect on binding to BAF (44,45) or nesprin-1α (unpublished observations), but disrupts binding to all other partners tested including lamin A, GCL, Btf, YT521-B and actin (12,25,26,28,44,45; unpublished observations). However, the D95–99 mutation had little or no effect on Lmo7 binding (Fig. 2C). Only one EDMD-causing missense mutant, P183H, was deficient in binding hLmo7C (Fig. 2C). All other previously-tested partners including BAF, lamin A, nesprin-1α, actin, YT521-B, MAN1, GCL and Btf bind the P183H mutant normally (12,25,26,28,44,45; unpublished observations).
observations) suggesting this mutation affects Lmo7 selectively. This result strongly supported the hypothesis that Lmo7 is an emerin-binding protein relevant to the EDMD-disease mechanism, and suggested perturbed Lmo7 activity as a specific molecular mechanism for patients with the P183H mutation (see Discussion). However, perturbed Lmo7-binding cannot fully explain the EDMD-disease mechanism, since the other three ‘special’ mutants (S54F, D95–99, Q133H) all bound Lmo7 normally in vitro (Fig. 2C; see Discussion).

**hLmo7C shuttles between the nucleus and cell surface in HeLa cells**

When stained by indirect immunofluorescence with affinity-purified CO5 antibody, endogenous Lmo7 localizes diffusely in the nucleus and cytoplasm, and also at the surface of MDCK and MTD-1A cells (36,46). We saw a similar distribution for endogenous Lmo7 in HeLa cells stained with the CO5 antibody (Fig. 3A, α-Lmo7). To determine whether hLmo7C (which is sufficient to bind emerin) also had targeting information, we transiently transfected HeLa cells with plasmids encoding GFP fused to the N-terminus of hLmo7C (Fig. 3A, GFP-hLmo7C). Cells were fixed 36 h after transfection and viewed by direct fluorescence microscopy. GFP-hLmo7C localized relatively less prominently in the nucleus, and more prominently at the cell surface (Fig. 3A). We concluded that the C-terminal half of Lmo7 can target the cell surface and has some, but not all, information needed to accumulate in the nucleus. GFP-hLmo7C also seemed to influence cell shape; many transfected cells had prominent spikes at the cell surface not seen in untransfected controls (Fig. 3A). The most unexpected finding was that emerin staining at the nuclear envelope was lost in cells that overexpressed GFP-hLmo7C (data not shown; see below).

The dual (nuclear and cell-surface) localizations of endogenous Lmo7 suggested Lmo7 might shuttle between these compartments. Prediction programs identified two putative nuclear export signals in human Lmo7 beginning at residue 118 (PIAGLDNINV) and residue 650 (LGTTVPPISF), and one predicted nuclear localization signal beginning at residue 1189 (LKNLKRR), which is present in hLmo7C. These putative signals, though not yet validated by mutagenesis, were consistent with Lmo7 shuttling. To test for potential shuttling activity, HeLa cells were
Figure 3. Localizations of Lmo7 and hLmo7C and effects of emerin-downregulation. (A) Endogenous Lmo7 and GFP-hLmo7C both localize at the plasma membrane, cytoplasm and nucleus in HeLa cells, as determined by indirect immunofluorescence microscopy with serum CO5 against Lmo7 (α-Lmo7) or direct microscopy of GFP-fluorescence in cells 36 h after transient transfection with pEGFP-hLmo7C (GFP-hLmo7C). Scale bars, 10 μm. (B) Endogenous Lmo7 accumulates in the nucleus when Crm1-dependent nuclear export is inhibited by leptomycin B (LepB). HeLa cells were treated 2 h with either DMSO (control) or 35 nm Leptomycin B in DMSO (LepB/DMSO). Cells were then fixed and stained by indirect immunofluorescence using antibodies against endogenous Lmo7 (serum CO5), emerin and α-tubulin (sc-9104; Santa Cruz, Inc.). (C) Leptomycin B treatment causes nuclear accumulation of Lmo7. HeLa cells were treated 2 h with either DMSO (control) or 35 nm Leptomycin B in DMSO (LepB/DMSO) and fractionated into nuclear and cytosolic fractions. 4 × 10^6 cells (L) and 1 × 10^6 cell equivalents of either the nuclear fraction (N) or cytosolic fraction (C) were resolved by SDS–PAGE and western-blotted with antibodies against Lmo7 (CO5), emerin (2999) or β-tubulin (sc-9104; Santa Cruz, Inc.). (D) GFP-hLmo7C co-localizes with paxillin at focal adhesions. HeLa cells were transiently transfected with pEGFP-C1 or the control, pEGFP-hLmo7C, cultured 36 h, fixed and viewed by indirect immunofluorescence microscopy with antibodies against endogenous paxillin and endogenous emerin plus DAPI to stain DNA. Chevrons indicate selected paxillin-and-Lmo7-positive foci in cells overexpressing GFP-hLmo7C. Scale bars, 10 μm. (E) Steady-state nuclear localization of endogenous Lmo7 is emerin-dependent. HeLa cells were transfected with an siRNA construct to downregulate emerin expression. After 36 h, cells were fixed and double-stained by indirect immunofluorescence using serum 2999 against emerin and either serum CO5 or NO2 against Lmo7, plus DAPI to stain DNA. Upper and lower panels are images of unaffected cells (20% of total) and emerin-downregulated cells (80% of total) from the same experiment. Scale bars, 10 μm.

Endogenous Lmo7 also localizes at the nuclear envelope

The above localizations of Lmo7 were based on the CO5 antibody, raised against residues 1573–1723 of rat Lmo7 (36,46) (Fig. 1). However Takai and colleagues also raised a second antibody, named NO2, against residues 1320–1412 of Lmo7 (Fig. 3). The above localizations of Lmo7 were based on the CO5 antibody, raised against residues 1573–1723 of rat Lmo7 (36,46) (Fig. 1). However Takai and colleagues also raised a second antibody, named NO2, against residues 1320–1412 of Lmo7 (Fig. 3). Indirect immunofluorescence staining of HeLa cells with affinity-purified NO2 antibodies revealed little or no cell surface or cytoplasmic staining, but instead gave strong nuclear envelope staining plus a single bright juxtanuclear dot, presumably the centrosome (Fig. 3E). We raised an independent rabbit serum against the NO2 polypeptide. However, GFP-hLmo7C might reflect (i) an over-expression artifact, or (ii) biologically relevant association with focal adhesions that is exaggerated, possibly because this construct lacks the N-terminal domain.

Cells treated with LepB/DMSO accumulated more Lmo7 in the nucleus, compared with DMSO-treated controls (Fig. 3B). The positive control, paxillin (a shuttling component of focal adhesions), accumulated significantly in the nuclei of LepB-treated cells (Fig. 3B) as expected (51). To quantify Lmo7 shuttling, we treated HeLa cells with LepB/DMSO (+LepB) or DMSO (–LepB) for 2 h, fractionated cells, resolved the nuclear and cytosolic fractions by SDS–PAGE and western-blotted for endogenous Lmo7 (serum CO5), emerin and β-tubulin (Fig. 3C). Fractionation was clean, as shown by the distribution of nuclear and cytoplasmic marker proteins emerin and β-tubulin. Densitometry of these results, normalized to emerin or β-tubulin (data not shown), showed 1.6-fold more Lmo7 in the nuclear fraction and 1.9-fold less Lmo7 in the cytoplasmic fraction of LepB-treated cells (Fig. 3C, +LepB) compared to DMSO-treated controls (Fig. 3C, –LepB). Takai and coworkers previously showed that Lmo7 localizes at cell–cell adhesions, via binding to afadin and α-actinin (36,46). However, HeLa cells do not express cadherins (52) and generally do not form cell–cell adhesions. To determine where GFP-hLmo7C localized at the plasma membrane of HeLa cells, cells were transiently transfected with either GFP or GFP-hLmo7C, incubated for 36 h, fixed and stained by indirect immunofluorescence using antibodies against endogenous paxillin (Fig. 3D). hLmo7C co-localized with paxillin at presumed focal adhesion sites (Fig. 3D, chevrons). However, GFP-hLmo7C also localized at paxillin-negative regions of the plasma membrane (Fig. 3D). Given that endogenous Lmo7 overlapped with, but did not concentrate at, paxillin-positive foci (Fig. 3B), the behavior of GFP-hLmo7C might reflect (i) an over-expression artifact, or (ii) biologically relevant association with focal adhesions that is exaggerated, possibly because this construct lacks the N-terminal domain.

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Emerin-dependent nuclear localization of endogenous Lmo7

Emerin is required for the steady-state nuclear localizations of two other proteins, GCL (54) and β-catenin (27). Given this precedent, and the nuclear envelope localization of some Lmo7 molecules, we hypothesized that emerin might regulate the nuclear localization of Lmo7. To test this hypothesis, we used the siRNA method to downregulate emerin expression in HeLa cells, and localized Lmo7 using antibodies CO5 and NO2 (Fig. 3E). Downregulation was effective, since ~80% of cells had significantly decreased emerin signals at the nuclear envelope by indirect immunofluorescence (Fig. 3E, ‘80%’). Neighboring cells that were unaffected by siRNA treatment, identified by their strong emerin signal at the nuclear envelope, still had diffusely-nuclear or nuclear envelope-localized signals for Lmo7 depending on whether they were stained with CO5 or NO2 antibodies (Fig. 3E, ‘20%’). In contrast, both Lmo7 antibodies showed that emerin-downregulated cells had significantly decreased or undetectable nuclear Lmo7 (Fig. 3E, ‘80%’). Emerin downregulation did not affect Lmo7 protein levels in western blots (data not shown), suggesting emerin somehow promotes the steady-state nuclear localization of Lmo7. These results suggested that emerin, a nuclear inner membrane protein, is required for both the gross nuclear accumulation and nuclear envelope localization of Lmo7 (see Discussion).

Lmo7 regulates gene expression

Since two related other Lmo proteins regulate gene expression (35,38), we hypothesized that Lmo7 might also regulate gene expression. As noted earlier, we also wanted to pursue why emerin protein levels were reduced at the nuclear envelope in cells that overexpressed GFP-hLmo7C. We therefore used an siRNA oligonucleotide specific for Lmo7, or non-specific oligonucleotide as control, to downregulate HeLa cells and then measured protein levels of Lmo7 and emerin, with actin as the loading control, by western blotting (Fig. 4A). Actin levels were unaffected in Lmo7-downregulated cells (Fig. 4A, α-Actin). The siRNA treatment was effective since Lmo7 protein levels decreased 93% in Lmo7-downregulated cells (Fig. 4A, α-Lmo7). Interestingly, emerin protein levels also decreased, by 71%, in Lmo7-downregulated cells (Fig. 4A, α-Emr), suggesting Lmo7 positively regulated emerin expression. To determine if Lmo7 influenced the emerin gene, we used real-time PCR to measure emerin mRNA levels in cells treated with either Lmo7-siRNA or control-siRNA, normalized to mRNA levels of the housekeeping gene G3PDH (Fig. 4B). The Lmo7-downregulated cells had 97% decreased Lmo7 mRNA levels, and 93% decreased emerin mRNA levels (Fig. 4B), demonstrating that Lmo7 is a positive regulator of emerin transcription. However, these experiments did not reveal whether Lmo7 activated transcription directly or indirectly (e.g. by repressing a repressor).

To distinguish between direct and indirect models, full length rLmo7a, rLmo7b and hLmo7C were fused to the DNA-binding domain of Gal4 (DBD-Gal4) and co-transfected into HeLa cells with a luciferase reporter containing five Gal4-binding sites upstream of an SV40 promoter (Gal5SV40-luc) (55). The DBD alone served as the negative control. As positive controls for transcription repression and activation, respectively, we used DBD fused to transcription repressor zTGIF (DBD–zTGIF) (55), or transcription activator dTGIF (DBD–dTGIF) (55). These controls behaved as expected: DBD–zTGIF repressed the luciferase reporter 8.2-fold and DBD–dTGIF activated the luciferase reporter 3-fold, relative to DBD alone (Fig. 4C, DBD). Interestingly, luciferase activity increased 2.2-fold in cells transfected with either DBD–rLmo7a or DBD–rLmo7b (Fig. 4C), demonstrating that rLmo7a and rLmo7b can directly activate transcription in vivo. DBD-hLmo7C had no effect on luciferase reporter activity, suggesting that hLmo7C lacks the transcription activation domain. These findings supported the hypothesis that Lmo7 might directly activate the emerin gene.

The C-terminal half of Lmo7 (hLmo7C) dominantly represses emerin gene expression

Since endogenous Lmo7 was required for emerin gene expression, we were curious why emerin protein apparently disappeared from HeLa cells that overexpressed the
C-terminal construct, hLmo7C (data not shown). To document and examine this question, HeLa cells that transiently overexpressed GFP-hLmo7C specifically reduced emerin at the nuclear envelope. HeLa cells were transiently transfected with pEGFP-hLmo7C, cultured 36 h fixed, permeabilized and stained with DAPI to visualize DNA and double-stained by indirect immunofluorescence using rabbit serum 2999 against emerin and rabbit serum 2804 against the constant region of LAP2. Chevrons indicate nuclear envelopes with reduced emerin signal. Scale bars, 10 μm. (B) Western blot showing reduced emerin protein levels in cells overexpressing GFP-hLmo7C. HeLa cells transfected with pEGFP-C1 or pEGFP-hlo7C were analyzed 36 h post-transfection by immunoblotting using antibodies specific for A-type lamins (NCL-Lamin A), actin (A-5060), emerin (serum 2999), LAP2 (serum 2804) or GFP (sc-9996). Prior to harvesting, cells were treated for 6 h with either DMSO or DMSO containing Leptomycin B (35 nm final concentration) to inhibit Crm1-mediated nuclear export. (C) Emerin mRNA levels are reduced in cells overexpressing GFP-hLmo7C. Real-time PCR analysis of mRNA from cells transfected 36 h with pEGFP-C1 (GFP) or pEGFP-hLmo7C (Lmo7C). Emerin, LAP2β and lamin A mRNA levels were normalized to G3PDH mRNA levels and graphed relative to pEGFP-C1 (GFP) transfected cells. Bars (C and D) indicate SEM.

**Figure 5.** Dominant effects of exogenous hLmo7C on emerin expression. (A) Overexpressed GFP-hLmo7C specifically reduces emerin at the nuclear envelope. HeLa cells were transiently transfected with pEGFP-hLmo7C, cultured 36 h fixed, permeabilized and stained with DAPI to visualize DNA and double-stained by indirect immunofluorescence using rabbit serum 2999 against emerin and rabbit serum 2804 against the constant region of LAP2. Chevrons indicate nuclear envelopes with reduced emerin signal. Scale bars, 10 μm. (B) Western blot showing reduced emerin protein levels in cells overexpressing GFP-hLmo7C. HeLa cells transfected with pEGFP-C1 or pEGFP-hLmo7C were analyzed 36 h post-transfection by immunoblotting using antibodies specific for A-type lamins (NCL-Lamin A), actin (A-5060), emerin (serum 2999), LAP2 (serum 2804) or GFP (sc-9996). Prior to harvesting, cells were treated for 6 h with either DMSO or DMSO containing Leptomycin B (35 nm final concentration) to inhibit Crm1-mediated nuclear export. (C) Emerin mRNA levels are reduced in cells overexpressing GFP-hLmo7C. Real-time PCR analysis of mRNA from cells transfected 36 h with pEGFP-C1 (GFP) or pEGFP-hLmo7C (Lmo7C). Emerin, LAP2β and lamin A mRNA levels were normalized to G3PDH mRNA levels and graphed relative to pEGFP-C1 (GFP) transfected cells. Bars (C and D) indicate SEM.

Because overexpression of either rLmo7a or rLmo7b in mouse C2C12 myoblasts increased emerin mRNA expression 2.5- and 1.8-fold, respectively, whereas hLmo7C reduced emerin mRNA levels 2-fold (data not shown).

**Identification of Lmo7-regulated genes in HeLa cells**

Loss-of-function analysis is the best way to understand function. We therefore used DNA microarray analysis to look for changes in gene expression in HeLa cells siRNA-downregulated for Lmo7. HeLa cells were transfected with the control siRNA or an Lmo7-specific siRNA (n = 3) for 36 h, then RNA was isolated and analyzed using Affymetrix U133 Plus2 chips, which represent the entire human genome. Robust Multichip Average was used to identify genes with significant changes in expression between the control and Lmo7-downregulated cells.

Surprisingly, expression of 4770 genes changed significantly in Lmo7-downregulated cells; of these, 542 were uncharacterized loci that might or might not represent real genes (data not shown). Among the 4228 characterized genes, expression of 1598 increased in Lmo7-downregulated samples, whereas 2630 had decreased expression. Thus, 62% of affected genes were underexpressed in Lmo7-downregulated cells. To assess the potential relevance of Lmo7 to muscular dystrophy, we focused on affected genes with roles in muscle or heart. Interestingly, a large number of such genes were affected in HeLa cells, including 41 muscle-relevant genes (14 upregulated, 29 downregulated) and five cardiac-relevant genes (one upregulated, four downregulated; Table 1).

Cardiac-relevant genes that were misregulated in Lmo7-downregulated HeLa cells included tropomyosin 1a (Tpm1; 1.8–2-fold decrease), the McKusick–Kaufman syndrome gene (MKKS; 1.5-fold decrease), the gene encoding the potassium voltage-gated channel, Isk-related family, member 1-like (Kcne1; 1.4-fold decrease), myocyte enhancing factor 2B (Mef2b; 1.4–1.5-fold decrease) and MEF2D (1.4-fold increase; Table 1). Muscle-relevant genes included several transcriptional regulators of myogenic differentiation (Mef2b, Mef2c and Mef2d, two of which are also important for heart), known regulators of gene expression including Inhibitor of DNA-binding 2 (Id2), Muscleblind-like-1 (Mbnl1), Flightless homolog 1 (Fli1) and Rho-associated coiled-coil containing protein kinase 2 (Rock2), plus the sarcomeric structural protein nebulin (Neb). Expression of Mef2d
We focused on a subset of these genes, plus selected genes previously shown to be misregulated in X-EDMD patients (32), that were also misregulated in Lmo7-downregulated HeLa cells (Table 1). Previous microarray analysis of muscle from X-EDMD patients (32) and decreased an average of 1.6-fold in Lmo7-downregulated HeLa cells (Table 1; LAP2/TMPO). The direction of these changes was consistent with decreased Lmo7 localization, and hence decreased Lmo7-dependent gene activation in the nucleus of emerin-null cells (Fig. 7). These simple comparisons suggest that Lmo7 regulates a striking number of EDMD-relevant genes, plus additional genes that may influence the EDMD phenotype.

To independently validate their misregulation in Lmo7-downregulated HeLa cells, we used real-time PCR to measure mRNA levels of eleven genes (MEF2B, MEF2C, MEF2D, Rbl1, nebulin, CREBBP, PCAF, MBNL1, Id2, NAP1L1, FlI1) in cells downregulated for Lmo7 versus non-specific siRNA control, normalized to G3PDH. Nebulin, MEF2D and Rbl1 were upregulated 3.4-fold, 3.4-fold and 1.6-fold, respectively (Table 1). The other genes were reduced 1.4-fold to 2.5-fold (Table 1). These real-time PCR results confirmed the microarray results and supported the hypothesis that Lmo7 regulates gene expression, including the expression of muscle-relevant and heart-relevant genes.

We noted that Lmo7-downregulation (our results) and loss of emerin (32,56) had opposing effects on the expression of muscle-relevant and heart-relevant genes.

Table 1. Microarray analysis results and RT-PCR confirmation of selected genes

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and nebulin increased 1.4-fold in Lmo7-downregulated cells. The other genes all had 1.4- to 3.8-fold reduced expression in Lmo7-downregulated cells (Table 1). Emerin was not identified in the microarray analysis, but its downregulation was confirmed by real-time PCR analysis of the same RNA samples used in the microarray studies, and in multiple other assays (n ~ 50), using different sets of primers (Fig. 4 and data not shown). We are therefore confident that emerin gene expression is reduced in Lmo7-downregulated cells.

We noted that Lmo7-downregulation (our results) and loss of emerin (32,56) had opposing effects on the expression of muscle-relevant and heart-relevant genes.
in Lmo7-downregulated cells. These findings suggested a simple model in which these genes are activated by Lmo7, and repressed by emerin, potentially through inhibition of Lmo7. This model predicted that overexpression of wild-type emerin might ‘super-repress’ Lmo7-dependent gene expression. To test this model, we transfected either wild-type full-length emerin or emerin mutant P183H, which is selectively deficient in binding to Lmo7 (Fig. 2C), into HeLa cells and measured the expression levels of NAP1L1, CREBBP and LAP2. The control was Rbl1, for which expression increased in both Lmo7-downregulated (Table 1) and emerin-null cells (32). Supporting our model, overexpression of wild-type emerin decreased the expression of NAP1L1, CREBBP and LAP2 by 3.6-fold, 1.6-fold and 1.7-fold, respectively (Fig. 6), whereas Rbl1 expression was unchanged. These results supported the ‘super-repression’ prediction. Importantly, there was no significant change in the expression of NAP1L1, CREBBP, LAP2 or Rbl1 in cells transfected with emerin mutant P183H (Fig. 6), strongly supporting the hypothesis that Lmo7 is inhibited by direct binding to emerin.

DISCUSSION

We found that the putative transcription regulator Lmo7 binds directly to emerin in vitro, associates with emerin in vivo and is a transcription activator in vivo. Notably, Lmo7 is required for emerin gene expression, but also requires emerin to localize efficiently in the nucleus, and is functionally inhibited by binding to emerin, suggesting mutual feedback regulation. We propose that Lmo7 senses the amount of emerin at the nuclear envelope; when emerin is limiting or occupied by other partners, then Lmo7 is free to activate the expression of emerin and other genes (Fig. 7). However, when emerin is available, Lmo7 is bound and its gene expression activity attenuated. We speculate that specific signals might release Lmo7 from the cell surface, favoring its nuclear localization and enhanced expression of emerin and other Lmo7-dependent genes (Fig. 7). The nature of these putative cell surface signals, which might include cell-ECM attachments important for mechanical feedback in muscle cells, or changes in cell–cell adhesion, are discussed subsequently as key questions for future study.

Our findings suggest Lmo7 and emerin are mutual regulators, and that their interactions are directly relevant to muscle and the EDMD disease mechanism. On one hand, the failure of Lmo7 to accumulate at normal levels in emerin-downregulated HeLa nuclei, implies that at least some Lmo7-regulated genes might be misregulated in emerin-null EDMD patients. On the other hand, emerin would no longer be there to inhibit Lmo7 activity. Two previous studies suggest that muscle regeneration pathways controlled in part by MEF2, CREBBP, CDK4/6, PCAF and NAP1L1 are perturbed in EDMD patients and emerin-null mice (32,56). At least three components of this pathway were also perturbed in Lmo7-downregulated HeLa cells—a remarkable finding since HeLa cells are clearly no model for muscle physiology. To understand this finding, and the functional consequences of the proposed mutual regulation between Lmo7 and emerin, we are currently comparing all genes and pathways misregulated in HeLa cells that lack either Lmo7 or emerin, and will extend this to mouse C2C12 myoblasts downregulated for either Lmo7 or emerin, in comparison to the human emerin-null EDMD patient muscle microarray results (32).
Emerin expression requires Lmo7, suggesting Lmo7 activates the emerin gene, but whether Lmo7 binds the emerin promoter directly remains to be tested. Interestingly, emerin regulates Lmo7 both positively and negatively. As a positive regulator, emerin is required for Lmo7 to localize efficiently in the nucleus and reach its target genes. The mechanism of this accumulation is unknown, but we speculate that emerin or emerin-associated protein complexes might regulate either the nuclear export kinetics of Lmo7 or its affinity for intranuclear targets. As a negative regulator, binding to emerin inhibits Lmo7 activity, as shown by the effects of overexpressing either wild-type emerin or an Lmo7-nonbinding mutant, on several Lmo7-dependent genes. How emerin regulates Lmo7-mediated gene expression is an important open question. Potential mechanisms include direct sequestration of Lmo7 at the nuclear envelope (modeled in Fig. 7), or inhibitory posttranslational modifications of Lmo7 mediated by emerin-associated protein complexes. Lmo7, on the other hand, appears to be a strictly positive regulator of emerin, since loss of Lmo7 significantly reduced emerin mRNA levels. For this reason we suggest that the severe dystrophic muscle phenotype of Lmo7Δ800 mice (37) was due, at least in part, to reduced expression of emerin and consequent misregulation of emerin-dependent genes. We predict these mice also suffered perturbed expression of other Lmo7-regulated genes and pathways, including those relevant to muscle physiology identified in our microarray study (Table 1). Direct tests of these predictions are not yet possible, since the Lmo7Δ800 mice also lacked exons of a neighboring gene, uchl3 (37). Complete deletion of uchl3 alone caused a mild dystrophic phenotype in 11% of mice (37), whereas the Lmo7Δ800 mice had 100% penetrance of the dystrophic phenotype and more severe muscle degeneration and growth retardation (37). While strongly suggesting that Lmo7 loss was the major cause of muscular dystrophy, one cannot rule out potential synergistic effects caused by mutations in both genes. A clean Lmo7-null mouse, needed for further study, will be the focus of future experiments.

Patients with the ‘special’ EDMD-causing mutations S54F, Q133H, or P183H have normal or near-normal protein levels and NE localization of emerin. These patients are clinically indistinguishable from emerin-null EDMD patients. It is therefore puzzling that each mutation selectively disrupts a different part(s) in vitro: among at least eight partners tested, the S54F mutation disrupts only Btf (26), Q133H disrupts only MAN1 and actin (25,43), and P183H disrupts only Lmo7 (this report). Future work will test whether these mutations also disrupt (i) an undiscovered disease-relevant partner or (ii) common downstream pathways required for muscle function or regeneration in vivo or (iii) the regulation of emerin in vivo.

**Lmo7 as a proposed signaling molecule**

Takai and colleagues first showed dual localization of Lmo7 in MDCK cells (36). Our evidence further suggests Lmo7 shuttles from the cell surface and cytoplasm to the nucleus. Thus Lmo7 might transduce either ligand-based or mechanical signals to the nucleus. For example, Lmo7 might behave like β-catenin, an adherens junction component that is activated when Wnt, a secreted signaling protein (57), binds the frizzled receptor at the cell surface. Activated Wnt then binds other transcription factors, enters the nucleus and activates transcription (58). Remarkably, β-catenin was recently shown to bind emerin directly, and is proposed to be attenuated by this binding (27). Whether the β-catenin and putative Lmo7-mediated signaling pathways overlap will be an interesting question for the future. A second precedent for the involvement of LEM-domain proteins in regulating signal transduction comes from MAN1, which directly binds regulatory SMADs and thereby inhibits TGF-β signaling during development and in specialized tissues (21,59–63). Interestingly, MAN1 can also bind directly to emerin (45).

The behavior of our dominant hLmo7C construct suggests endogenous Lmo7 might, in addition, transmit mechanical signals from focal adhesions. A subset of plasma membrane-associated hLmo7C co-localized with paxillin at focal adhesions. Focal adhesions are proposed to sense mechanical forces through integrins, which bind ECM molecules (53). Integrins then recruit cytosolic proteins including Focal Adhesion Kinase, p130Cas, vinculin and paxillin (53,64), which in turn activate small GTPases including Rac, Ras and Rho (53,64). Lmo7 might mediate focal adhesion signaling, since cells that overexpressed the C-terminal half of Lmo7 (hLmo7C) had exaggerated numbers of plasma membrane protrusions (Fig. 3A and D). Loss of emerin might disrupt mechanosensitive signaling by both perturbing the nuclear localization of Lmo7 and failing to regulate Lmo7 activity. We propose that as Lmo7 shuttles into the nucleus it ‘senses’ the amount of emerin, or emerin occupancy by other transcription regulators and/or architectural partners (modeled in Fig. 7). In normal cells, positive feedback regulation of emerin and other genes by Lmo7 might be required for tissue-appropriate changes in gene expression in response to changes in mechanical force or cell adhesion (e.g. increased emerin might reinforce architectural partners and thereby mechanically stabilize the nucleus). Elucidating the Lmo7 signaling pathway(s) and determining how emerin regulates Lmo7-mediated transcription are important new directions to understand the EDMD disease mechanism.

**MATERIALS AND METHODS**

**Plasmids and protein purification**

Wild-type pEGFP-emerin was described previously (16) and pEGFP-P183H emerin was constructed using the Quick-change® site-directed mutagenesis kit (Stratagene, Inc.). A cDNA encoding human Lmo7C (corresponding to nucleotides 2283–4671 of predicted full length Lmo7) was cloned into pQE31 (Qiagen, Inc.) and pEGFP-C1, to create His-tagged hLmo7C and GFP-hLmo7C, respectively, using the following primers: forward, 5'-atgcgaactttggcttgcgttctctcc-3'; reverse, 5'-tcataatggtgtcctctcgc-3'.

Recombinant His-hLmo7C was purified per manufacturer specifications from *E. coli* BL21(DE3):pLysS. Plasmids encoding FLAG-rLmo7a, FLAG-rLmo7b, GFP-rLmo7a and GFP-rLmo7b were generous gifts from Y. Takai (Osaka University). The emerin-specific siRNA-targeting vector
was created by ligating DNA oligonucleotides 5'-gtcccgggc tataaatagactctaagagagatgctgtcttgcccctttta-3' and 5'-age taaaaggtcataagtacactctctgatgctctgattgctaggcggg-3', into pSUPER (Oligogene). The plasmids used for luciferase assays (pM-zTGIF, pM-dTGIF, pRL-SV40 and p(Gal);SV40-luc reporter) were described previously (55) and obtained from David Wotton (University of Virginia). Recombinant emerin protein comprising the entire nucleoplasmic domain of emerin (residues 1–222) and lacking the transmembrane domain was expressed in bacteria from pET11c-emerin and purified as described (11).

Affinity purification using emerin-conjugated beads and mass spectrometry

Wild-type emerin residues 1–222 or BSA (negative control) were coupled to Affigel-15 beads (Bio-Rad Laboratories) per manufacturer instructions. Nuclear extracts were prepared from 10¹⁰ HeLa-S3 cells by hypotonic lysis (65). HeLa cells were obtained from the National Cell Culture Center. For affinity purification, 50 mg nuclear extract was incubated 4 h at 4°C with 0.5 mg/ml (0.5 mg) of either bead-coupled protein (either emerin or BSA) in Binding Buffer (BB: 50 mM HEPES, 250 mM NaCl, 0.1% Triton X-100) containing protease inhibitors and 2 mM DTT. Beads were collected by centrifugation at 500g, washed five times with BB and bound proteins were eluted with SDS–PAGE sample buffer. Proteins were identified using a QSTAR/Pulsar mass spectrometer at the Mass Spectrometry/Proteomics Facility at The Johns Hopkins University School of Medicine (www.hopkinsmedicine.org/msf). These results will be reported in full elsewhere (Holaska and Wilson, in preparation).

Binding assays

Co-immunoprecipitation assays were done using equal masses (5 µg) of recombinant hLmo7C and wild-type emerin (residues 1–222), essentially as described (44). Proteins were incubated 2 h at 22°C in PBS with 0.1% Triton X-100 (PBS-T), 2 mM DTT, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10 µg/ml pepstatin A and 1 mM PMSF, and then incubated 4 h with emerin monoclonal antibody NCL-Emerin (Novocastra Laboratories, Ltd) coupled to Protein-A Sepharose. The beads were washed five times with PBS-T, and bound proteins were eluted with SDS-sample buffer, resolved by SDS–PAGE and western blotted with antibodies against either emerin (rabbit serum 2999) (44) or the His-tag (Santa Cruz, Inc.). For the in vivo co-immunoprecipitation assays, HeLa cells were lysed with 50 mM HEPES (pH 7.4), 0.3 M NaCl, 0.3% Triton X-100 (Lysis Buffer, LB). Lysates were incubated with either monoclonal emerin antibodies (NCL-Emerin; Novocastra, Ltd) and Protein G beads or Protein G beads alone, washed five times with LB and bound proteins were eluted in sample buffer. Samples were resolved by SDS–PAGE, transferred to nitrocellulose, and immunoblotted with emerin serum 2999 (1:10 000 dilution) and Lmo7 serum CO5 (1:10 000 dilution). Microtiter well-binding assays were done as described (12). To measure hLmo7C affinity for emerin, 11 pmol of emerin were immobilized in microtiter wells, blocked with BSA and incubated with increasing concentrations (15 nM to 3.8 µM) of 35S-hLmo7C synthesized using the TnT® Quick Coupled Transcription/Translation System per manufacturer instructions (Promega, Corp.). When testing for binding to emerin mutants, subsaturating amounts of 35S-hLmo7C (50 nM) were used, with 15–25 pmol of emerin typically present per well. Wells were not allowed to dry at any time during these assays. In all cases, bound 35S-labeled proteins were extracted with 5% SDS and counted in a scintillation counter.

Transfection and immunofluorescence

HeLa cells were transfected using Mirus LT-1 transfection reagent per manufacturer specifications, using 2 µg plasmid per 35-mm dish. Cells were incubated 36–48 h, fixed with 3.7% formaldehyde, permeabilized with PBS, 0.2% Triton X-100 for 20 min and blocked with 3% BSA in PBS, 0.1% Triton X-100. Antibodies were diluted into PBS, 0.1% Triton X-100 prior to incubation. Either DMSO alone or Leptomycin B in DMSO (final concentrations 35 nM Leptomycin B and 0.3% DMSO) were added to cells 30 h after transfection, then incubated either 2 or 6 h and fixed in 3.7% formaldehyde. Antibodies used for indirect immunofluorescence were rabbit serum 2999 against emerin (1:1000 dilution) (44), the NCL-emerin monoclonal antibody (Novocastra Laboratories, Ltd; 1:200 dilution), rabbit serum 2804 against the constant region of LAP2 (1:1000 dilution) (10), lamin A/C monoclonal antibody (Novocastra Laboratories, Ltd; 1:200 dilution), and affinity-purified rabbit sera CO5 and NO2 (1:10 000 dilution; generous gifts from Y. Takai, Osaka University). For double-staining, transfected cells were incubated sequentially with primary antibodies (1 h each at 22–25°C) and then incubated 1 h at 22–25°C with Cy3-conjugated goat anti-mouse antibodies, Cy5-conjugated goat anti-rabbit antibodies, or both (Jackson Laboratories; 1:250 dilution) and coverslips were mounted onto slides containing one drop of Vectashield (Vector Laboratories, Inc.) anti-fade reagent. Slides were viewed on a Nikon Eclipse E600 microscope and images acquired using a Q-imaging Retiga EXi CCD camera controlled by IPLab v3.9.4 run on a Macintosh G5 computer. GFP fluorescence was viewed directly.

siRNA-mediated downregulation of Lmo7

Cells were transfected with siRNA specific for Lmo7 (16704, Ambion, Inc.) or control siRNA (4611, Ambion, Inc.) using Oligofectamine (Invitrogen). All steps were done at 22–25°C unless otherwise indicated. Each siRNA (12 µl of 20 µM stock) was diluted in 200 µl Opti-MEM (Invitrogen) and incubated for 10 min. In separate tubes, 12 µl Oligofectamine was diluted into 48 µl Opti-MEM (Invitrogen) and incubated for 10 min. The tubes were then mixed, incubated for 40 min and added to HeLa cells in six-well tissue-culture plates containing 2 ml fresh culture medium. Cells were cultured 24 h, then supplemented with fresh media and incubated another 12–24 h prior to resuspension in SDS–PAGE sample buffer. Proteins from equal numbers of cells were resolved by SDS–PAGE and immunoblotted using rabbit serum 2999 against emerin (44) or affinity-purified rabbit CO5 antibodies.
against Lmo7 (36), each at 1:10 000 dilution, or antibodies against actin (A-5060, Sigma-Aldrich, Inc) at 1:2500 dilution.

**Luciferase assays**

Luciferase activity was assayed as described (55). HeLa cells were transfected with 1 ng pRL-SV40 DNA, 2 µg of (GAL)5SV40-luc DNA and 200 ng of either pM, pM-Lmo7a, pM-Lmo7b, pM-hLmo7C, pM-dTGIF or pM-zTGIF DNA. Luciferase activity was measured 36–48 h after transfection using the Dual-Luciferase® Reporter Assay System (Promega, Inc.) in a FLUOstar OPTIMA microplate reader using FLUOstar OPTIMA software v1.32 (BMG LabTech). Firefly luciferase activity was normalized to renilla luciferase, encoded by pRL-SV40.

**Measuring mRNA levels**

HeLa cells were transfected with either pEGFP-C1 or pEGFP-hLmo7C, and RNA was extracted 36 h later, using TRIZol® Reagent (Invitrogen), per manufacturer’s instructions. RNA levels were analyzed by real-time PCR using iQSYBR-Green Supermix (Bio-RAD Laboratories) per manufacturer instructions. Primers used were: emerin, 5'-ctgatggcttgctagagatctccc-3' and 5'-ccaggtatcaagccgcatcc-3'; MEF2C, 5'-cggagaaaaaatccagatctccc-3' and 5'-tgagatgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgct


