

#### DOI: 10.1038/ncb1812

rabbit mouse human	+ MG53: <mark>MSAAPGLUHQELSCPLCLQLFDAP</mark> MG53:MSAAPGLURQELSCPLCLQLFDAP MG53: <u>MSAAPGLUHQELSCPLCLQLFDAP</u>	+ VTAECGHSFCRACLSRVAGE VTAECGHSFCRACLIRVAGE VTAECGHSFCRACLGRVAGE	+ PAADGTVNCPCCQAPTRPQALSTNLQLAR PAADGTVACPCCQAPTRPQALSTNLQLSR PAADGTVLCPCCQAPTRPQALSTNLQLAR	+ LVEGLAQVPQGHCEEHL LVEGLAQVPQGHCEEHL LVEGLAQVPQGHCEEHL	90 90 90
		RING	Intron 2		
DPLS DPLS DPLS	+ IYCEQDRVLVCGVCASLGSHRGHRLLPAAE IYCEQDRTLVCGVCASLGSHRGHRLLPAAE IYCEQDRALVCGVCASLGSHRGHRLLPAAE	AHSRLKTQLPQQKLQLQEAS AQARLKTQLPQQKMQLQEAC AHARLKTQLPQQKMQLQEAC	MRKEKSVAVLEHQUTEVEETVRQFRGAVG MRKEKTVAVLEHQLVEVEETVRQFRGAVG MRKEKSVAVLEHQLVEVEETVRQFRGAVG	+ EQLGKMRVFLAALEGSL EQLGKMRMFLAALESSL EQLGKMRVFLAALEGSL	190 190 190
	B-box2	Intron 3	putative coiled-coil	Intron 5	
DREA DREA DREA	ERVRSEAGVALRRELJGLHSYLEQLROMEK ERVRGDAGVALRRELSSLNSYLEQLROMEK ERVRGEAGVALRRELJSLNSYLEQLROMEK	VLEEVADKPQTEFLMKKCLV VLEEVADKPQTEFLMKFCLV VLEEVADKPQTEFLMKFCLV	TSRLQKILAESPPPARLDIQLPIISDDFK ISRLQKILSESPPPARLDIQLPVISDDFK ISRLQKILAESPPPARLDIQLPIISDDFK	FQVWRKMFRALMPALEE FQVWKKMFRALMPALEE FQVWRKMFRALMPALEE	290 290 290
LTFD LTFD LTFD	PSSAHPSLVVSPTGRRVECSEDKAPPAGED PSSAHPSLVVSSSGRRVECSDDKAPPAGED <u>PSSAHPSLVVS</u> SS <u>GRRVECSEDKAPPAGED</u> PRY	ARQFDKAVAVVAQQULSDGE IRQFDKAVAVVAQQULSQGE RRQFDKAVAVVAHQQLSEGE	HYWEVDVGDKPRWALGVMASEASRRÖRLH HYWEVDVGDKPRWALGVMAADASRRGRLH HYWEVDVGDKPRWALGVI AABAPRRGRLH SPRY	AVPSQGLWLLGLRDGKT AVPSQGLWLLGLRDGKI AVPSQGLWLLGLREGKI	390 390 390
LEAH LEAH LEAH	VEAKEPRALRTPERRPTRLGLYLSFGDGVL VEAKEPRALRTPERPPARIGLYLSFADGVL VEAKEPRALRSPERRPTRIGLYLSFGDGVL	AFYDASDADADELLFAFRER AFYDASNPDVLIPIFSFHER SFYDASDADALVPLFAFHER	LPGPVTPFFDVCWHDKGKNAQPLLLVGFD LPGPVYPIFDVCWHDKGKNAQPLLLVGFB LBRPVYPFFDVCWHDKGKNAQPLLLVGFB	GQEA QECA GAEA	477 477 477

Figure S1 Alignment of amino acid sequence of rabbit, mouse and human MG53. The rabbit and mouse sequences were determined by cDNA cloning, and the human sequence was deduced from the genomic sequence in various databases. Motif sequences predicted in database searches

are underlined. The positions of introns interrupting the protein-coding sequence in the mouse and human genes are indicated with arrowheads. Conserved cystine resides mutated in these studies are indicated by asterisks.



**Figure S2** Generation of MG53 null mice. **a**, Strategy of targeted disruption of MG53 gene. For screening of recombination-positive embryonic stem cells, genomic DNA was digested with Hind III and examined by Southern blot analysis using the indicated probe. **b**, PCR genotyping was carried out using the primer sets detailed in the methods section. Size markers are

indicated in base pairs. **c**, Northern blot analysis of skeletal muscle and heart RNA (10 µg/lane) from MG53 null mice. The probe used was the full-length MG53 cDNA. Size markers are indicated in kilobases. **d**, Western blotting demonstrates the expression pattern of MG53 protein in mouse tissues.



**Figure S3** Eccentric exercise accelerates muscle pathology and decreases muscle performance in *mg53-/-* mice. **a**, When *mg53-/-* and strain matched *wt* mice (7 months old) are run on a downhill slope on a treadmill at 4.4 m/s to acclimate to eccentric exercise , the *mg53-/-* mice show progressive loss of ability to maintain prolonged exercise (at days 8-11).

When mice are challenged to run at 20 m/s there is a significant decrease in the performance of *mg53-/-* mice (at days 12-13). Data presented as means±s.d. (n=3). \**p*<0.05, \*\**p*<0.001. **b**, Mason's Trichrome staining indicates increased fibrosis in *mg53-/-* mice after prolonged eccentric exercise trial.



**Figure S4** Subcellular localization of native MG53 in skeletal muscle. **a**, Immunofluorescence staining of transverse sections from rabbit skeletal muscle with mAb5259. **b**, Electron microscopic observation of the cell periphery (*left*) of EDL skeletal muscle and immunogold-staining (*right*) analysis using MG53 monocolonal antibody mAb5259. PM, plasma membrane; MF, myofibril.



# b



**Figure S5** GFP-MG53 containing vesicles traffic to and fuse with plasma membrane, and participate in membrane resealing in C2C12 cells. **a**, C2C12 myotubes transfected with GFP-MG53 display active vesicle trafficking and fusion with the plasma membrane. Frequent axial movement (*line to solid circle*) of GFP-MG53 labeled particles (*dotted line*) or small vesicles

could be observed (*arrows*). In addition, dynamic fusion of GFP-MG53 vesicles with the sarcolemmal membrane is evident (see also Movie 6). **b**, GFP-MG53 expressed in a C2C12 myotube can rapidly translocate toward sites of massive membrane injury, following scraping of the cell with a microelectrode (see also Movie 8).



Figure S6. GFP-MG53 translocation to acute membrane injury sites in C2C12 myotubes depends on oxidative entry. a, Addition of 2  $\mu$ M thimerosal to the extracellular solution accelerates accumulation of GFP-MG53 at the damage site of a C2C12 myotube following penetration of a microelectrode (*arrow*).

b, The presence of DTT (5 mM) in the bathing solution of C2C12 myotubes abolishes GFP-MG53 accumulation following microelectrode penetration.
c, GFP-C242A localizes to intracellular vesicles and plasma membrane, but does not accumulate at microelectrode injury sites in C2C12 myotubes.



**Figure S7** Chemical cross-linking analyses of MG53. (*a*) WT-MG53, (*b*) C242A-MG53, (*c*) LZM1-MG53, and (*d*) LZM2-MG53 were incubated with various concentrations of formamide (0, 0.2, 1.0, 2.0 mM). After cross-linking, protein samples were separated on SDS-PAGE with DTT present. Polyclonal anti-MG53 was used to detect monomeric (**M**) or dimeric (**D**) forms of recombinant MG53 expressed in HEK293 cells. LZM1-MG53 contains L176A/L183A/L190A mutations in a putative

leucine-zipper motif (LZM) that is conserved in MG53 from different species. LZM2-MG53 contains L212A/L219A/L226A mutations. These cross-linking studies show that C242A-MG53 display normal cross-linking property as the WT-MG53. LZM1A-MG53 displays defective cross-linking properties, whereas LZM2A-MG53 is similar to WT-MG53 and C242A-MG53. Our data suggest that LZM may provide a site necessary for oligomerization of MG53.



**Figure S8** *In vivo* transfection of adult skeletal muscle with GFP-MG53. Whole mount brightfield (*top*) and fluorescent (*bottom*) images of a FDB

muscle at 7 days after transfection with GFP-MG53 using electroporation. Over 80% of muscle fibers display GFP fluorescence after transfection.