SUPPLEMENTARY MATERIAL

TRIM32 is an E3 ubiquitin ligase for dysbindin

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1

Characterisation of the 3293 anti-TRIM32 antibody.

(**A**) TRIM32 specificity. Myc-TRIM2, -TRIM3, -TRIM32, and untagged TRIM32 constructs were transfected into HEK293T cells and crude cell lysate were analysed by western blot. The 3293 antibody only detects untagged TRIM32 and myc-TRIM32 and none of the other TRIMs. α-tubulin was used as a loading control. An identical blot was probed with anti-myc (9E10) to confirm presence of transfected protein in each lane. Western blot analysis of TRIM32 distribution in mouse tissue extracts (**B**) The TRIM32 3293 antibody detects a protein of approximately 72kDa that corresponds to TRIM32 in all tissues. Low levels of TRIM32 in are found in muscle whereas the protein is abundantly synthesised in brain and testis. *Mu*, muscle; *Br*, brain; *He*, heart; *Li*, liver; *Sp*, spleen; *Lu*, lung; *Ki*, kidney; *Te*, testis.

Supplementary Figure 2

Labelling and detection specificities in transfected cells.

(**A**) Confocal microscopy was used to determine the effect of pre-absorption on the labelling specificity of the 3293 polyclonal antibody. COS-7 cells expressing TRIM32-EYFP were labelled with anti-TRIM32 3293 polyclonal antibody with and without pre-absorption with the fusion protein used to generate the anti-TRIM32 antisera as indicated. Pre-absorption completely blocked the detection of TRIM32 with the 3293 polyclonal in cells expressing the TRIM32-EYFP when viewed on the green channel. (**B**) To demonstrate that the conspicuous co-localisation of dysbindin with TRIM32 in COS-7 cells expressing both proteins was not due to bleed through from the different channels, cells were co-transfected with dysbindin (detected with PA3111) and TRIM32-EYFP as indicated. Images collected by confocal microscopy using sequential scans clearly show that the EYFP-TRIM32 aggregates do not bleed-through on to the red channel and *vice versa*. Scale bars are 20μm.

Supplementary Figure 3

TRIM32 expression in guinea pig skeletal muscle and during myoblast differentiation. **(A)** Protein lysates were made of various guinea pig muscles. 100μg of each sample was separated by SDS-PAGE and probed by western blot with the anti-TRIM32 3293 antibody. TRIM32 is expressed at comparable levels in all muscles studied. *Sol*, *soleus*; *Gas*, *gastrocnemius*; *Quad*, *quadriceps*; *EDL*, *extensor digitorum longus*; *TA*, *tibialis anterior*. As a control for 3293, a lysate of COS-7 cells transfected with TRIM32/pCIneo was run alongside the muscle samples. (**B**) Cultured C2C12 myoblasts were induced to differentiate by switching the growth media to DMEM + 2% horse serum (Day 0). Cells lysates were made at various time points throughout myoblast fusion and western blotted. As a positive control for differentiation, the lysates were western blotted and probed with α1CT-FP, which detects a muscle specific isoform of α-dystrobrevin-1 (+vr3), which is induced during myoblast fusion. The lysates were then probed with 3293 (TRIM32), 12G10 (tubulin) and PA3111A (dysbindin). As a control for 3293, a COS-7 lysate expressing TRIM32 was run alongside the samples.

Supplementary Figure 4

Subcellular distribution of TRIM32 and mutants in C2C12 myoblasts. C2C12 cells were transfected with TRIM32-EYFP, D487N-EYFP and P130S-EYFP. Cells were fixed in methanol and analysed by confocal microscopy. Wild-type TRIM32 and P130S form cytoplasmic speckles whereas D487N has a diffuse cytoplasmic staining pattern. TRIM32-EYFP expressing cells were stained with FK1 antibody, which detects polyubiquitinated proteins, shown in red. The merged image shows areas of yellow where TRIM32-EYFP co-localises with the FK1 staining.

Supplementary Figure 5

Wild-type and mutant TRIM32 co-localise in co-transfected cells. COS-7 cells co-transfected with myc-TRIM32 and TRIM32-EYFP, D487N-EYFP or R394H-EYFP were stained with the 9E10 anti-myc monoclonal antibody and were processed for confocal microscopy. Each experiment showed that wild-type myc-TRIM32 co-

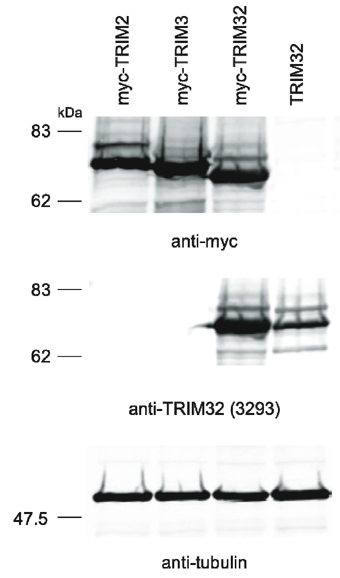
localised with each of the EYFP-tagged constructs. These experiments suggest that the wild type protein is able to recruit the LGMD2H/STM mutants into aggregates.

SUPPLEMENTARY TABLE 1

Half-lives of endogenous TRIM32 and constructs used in transfections. Quantitative western blot was used to determine the half-lives of each protein after new protein synthesis was blocked with cycloheximide. Each experiment was performed in triplicate.

Protein	Half-life (h)
Endogenous TRIM32 (C2C12 myoblast)	33.0
TRIM32 (COS-7 cells)	17.7
D487N (COS-7 cells)	18.5
P130S (COS-7 cells)	20.8
TRIM32 ring mutant (COS-7 cells)	24.5

A



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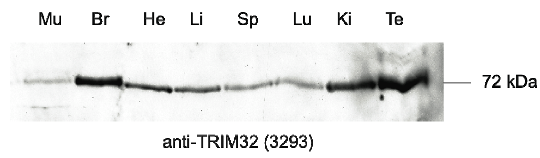
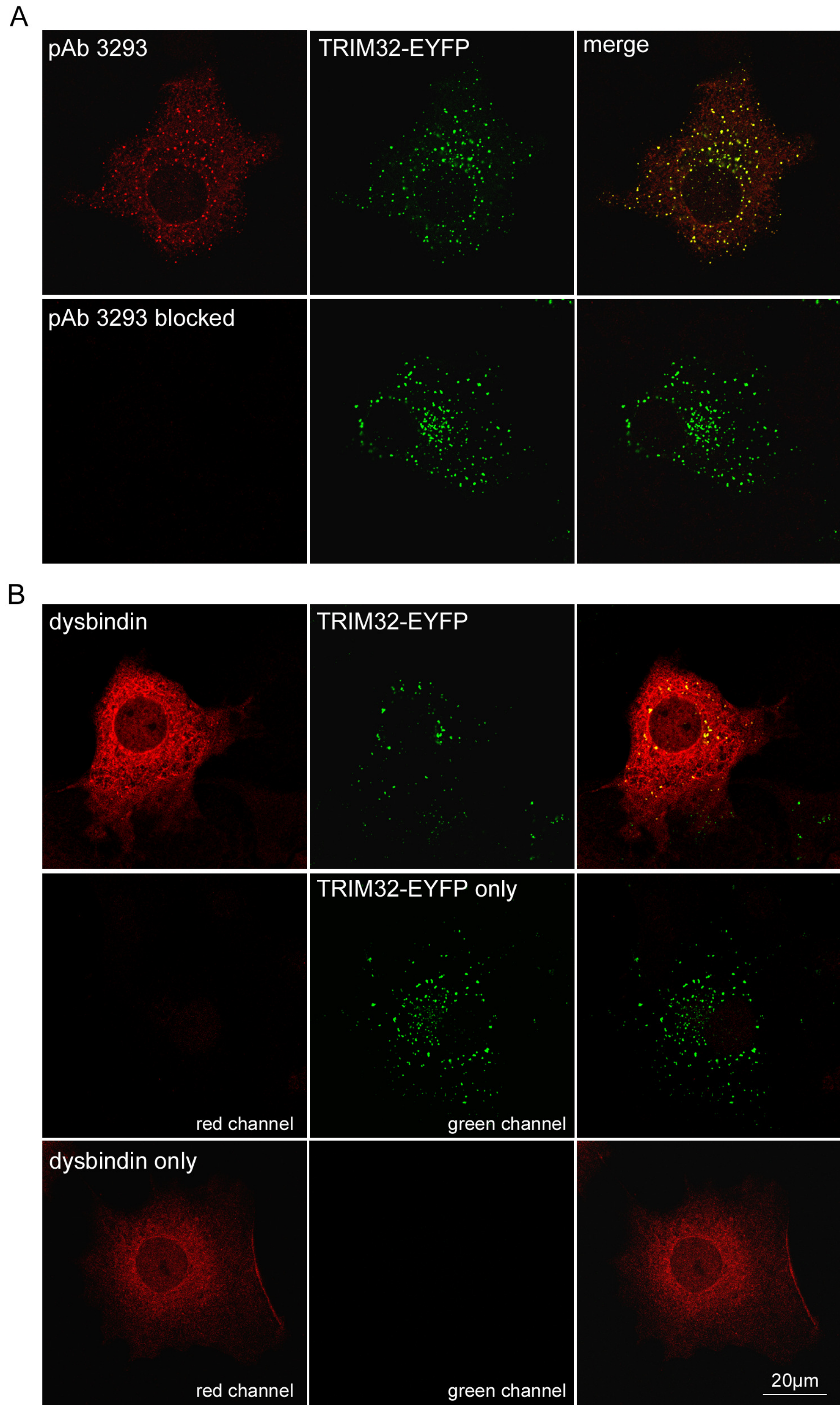
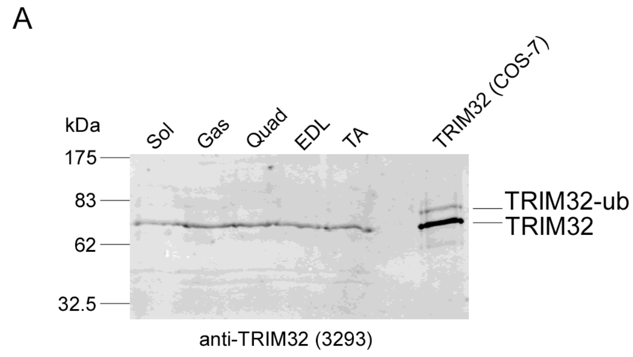
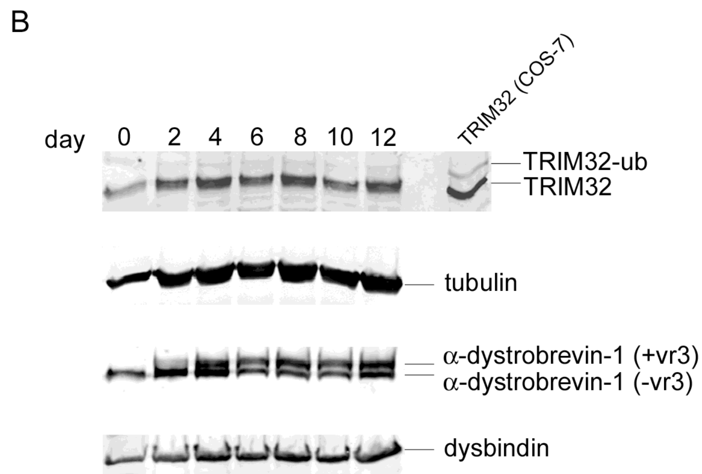


Supplementary Figure 1

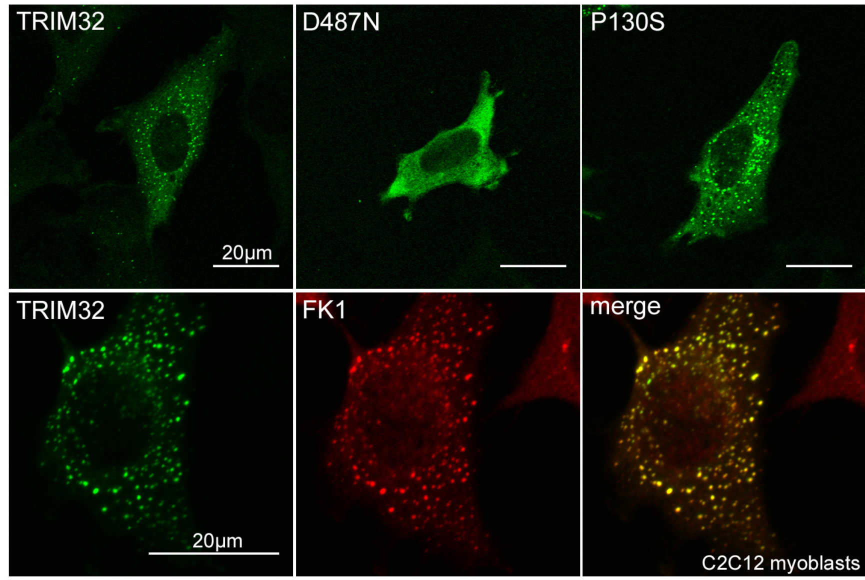


Supplementary Figure 2

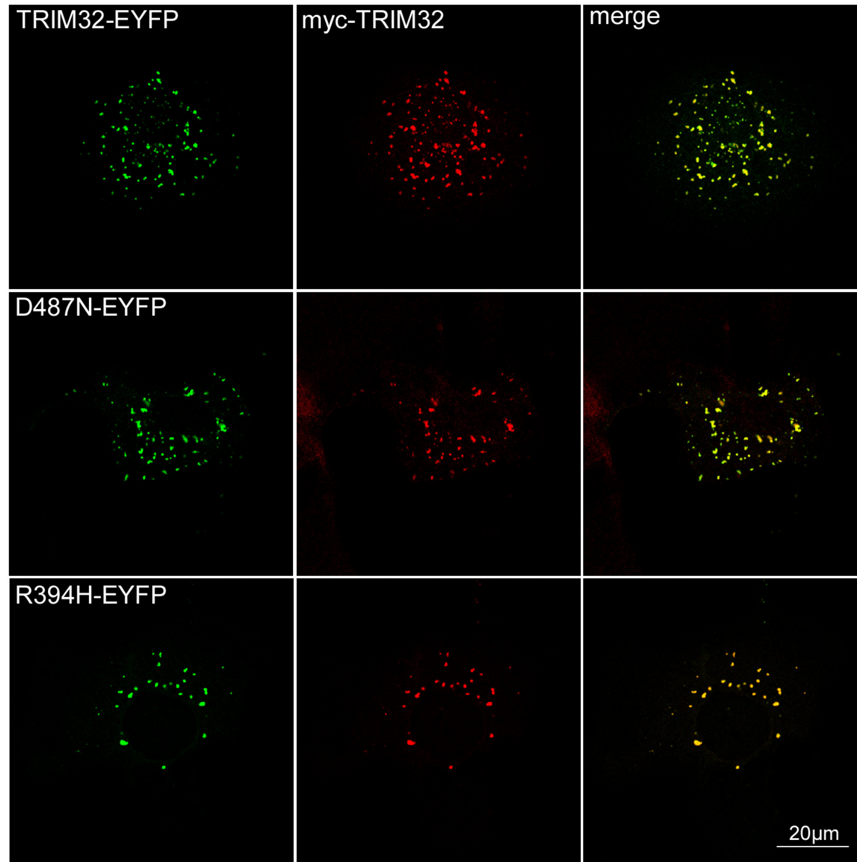




Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5