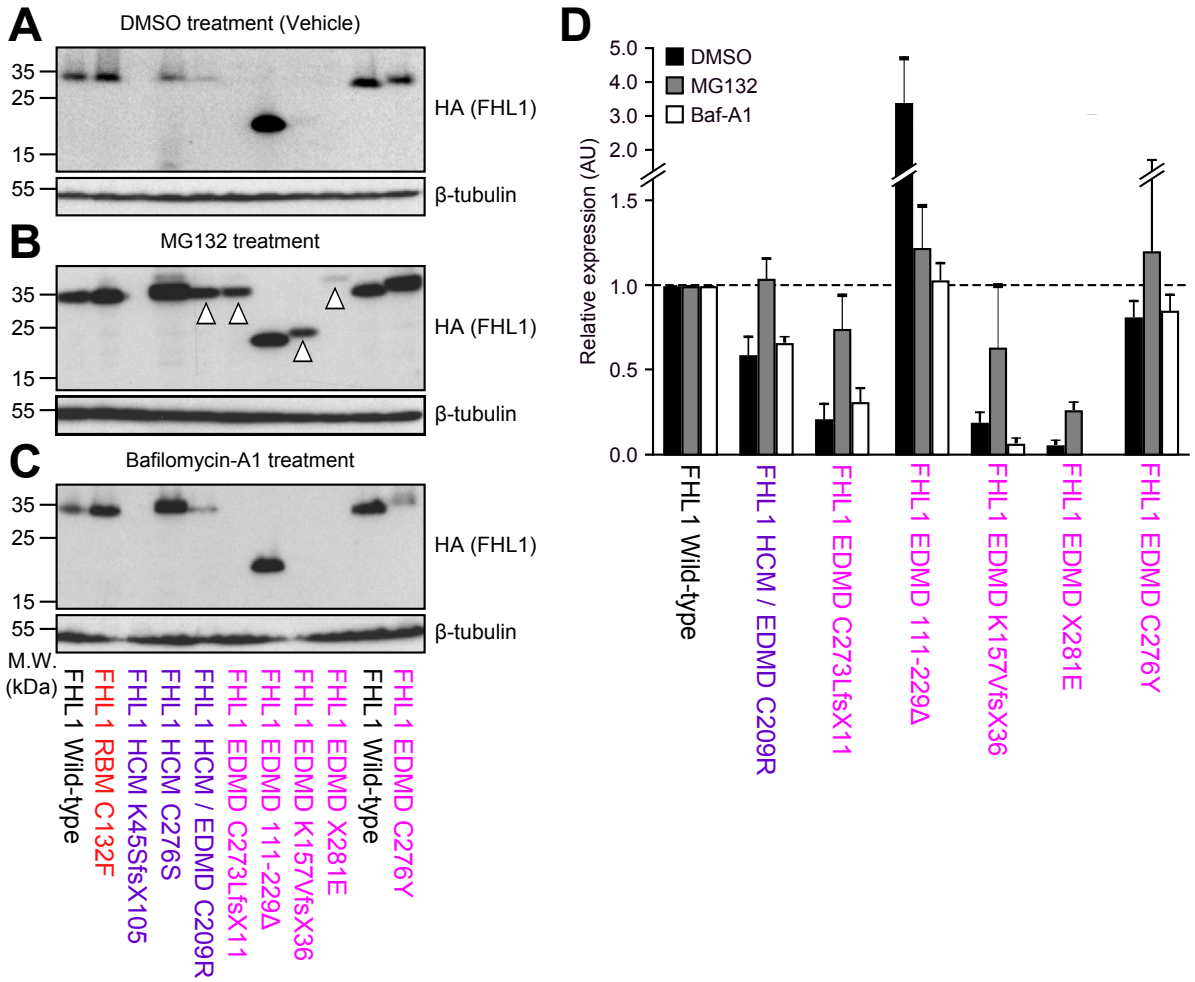

Fig. S1. HCM and EDMD HA-FHL1 mutant expression is partially restored by MG132 inhibition of the proteasome but not Bafilomycin-A1 inhibition of autophagy. C2C12 myoblasts were transiently transfected with 5 µg of DNA encoding HA-tagged wild-type, HCM or EDMD FHL1 mutants and whole cell lysates were immunoblotted with HA and β-tubulin antibodies after 48 h expression (myoblasts). Cells were treated with DMSO vehicle (A), 20 µM MG132 (B) or 5 nM Bafilomycin-A1 (C) for 24 h before harvest. Arrows show mutant HA-FHL1 expression post treatment. (D) HA-FHL1 immunoblots were analysed by densitometry and quantified relative to β-tubulin loading control and represented relative to wild-type HA-FHL1 which is represented as 1. Data represent the mean ± s.e.m. (*n*=2 for MG132 and Bafilomycin-A1 experiments, *n*=3 for DMSO control).

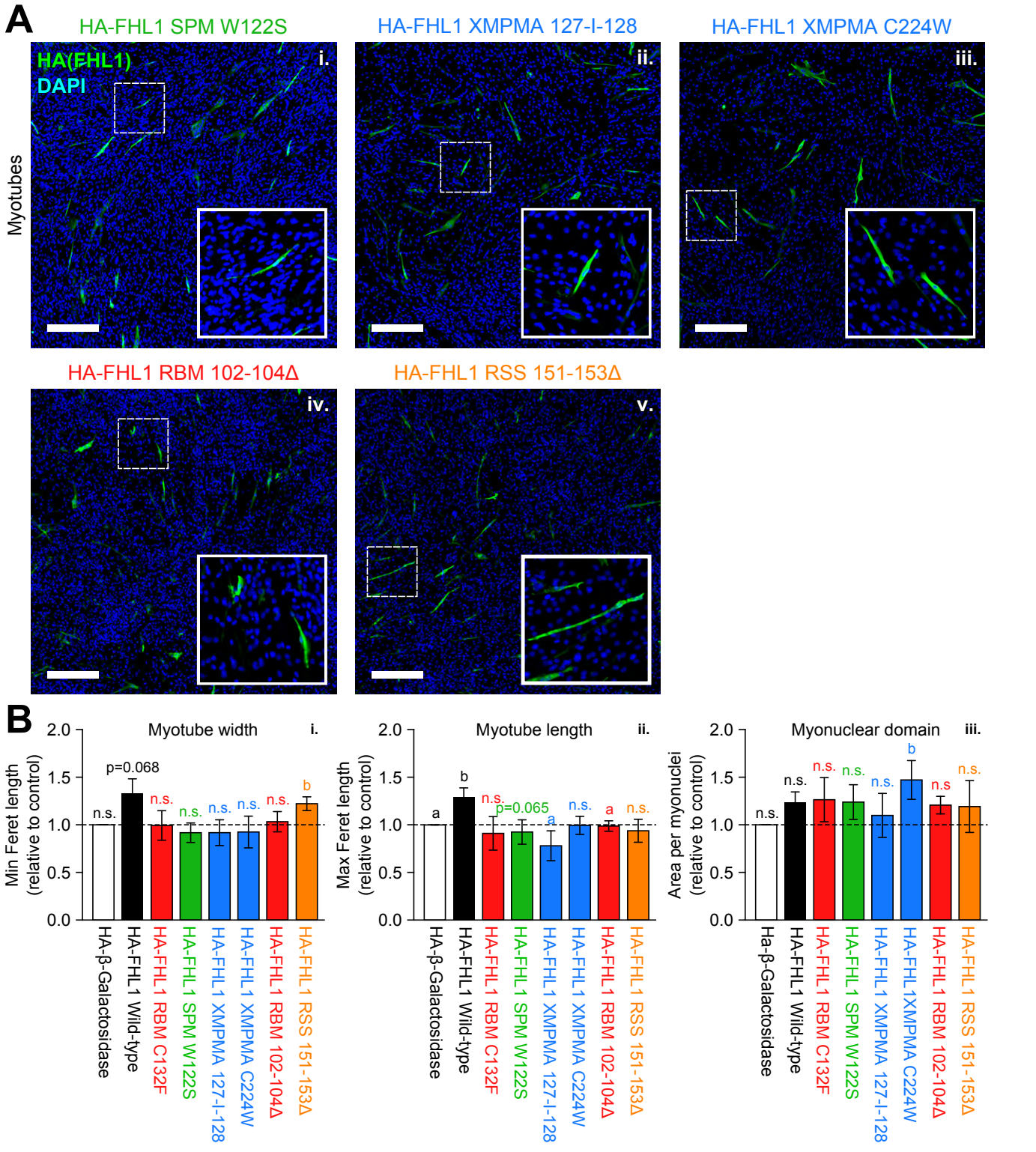

Fig. S2. RBM, SPM and XMPMA but not RSS HA-FHL1 mutants impair myoblast differentiation. Myoblast differentiation was assessed in mutant FHL1 expressing myotubes. (A) C2C12 myoblasts were transiently transfected with HA-FHL1 mutants RBM C132F (shown in Fig. 1Aiii) or RBM 102–104Δ (iv.), SPM W122S (i.), XMPMA 127-I-128 (ii.) or XMPMA C224W (iii.), RSS 151–153Δ (v.), wild-type HA-FHL1 (shown in Fig. 1Aii) or β-galactosidase control (shown in Fig. 1Ai), then differentiated for 72 h. Cells were co-stained with HA (FHL1, green), myosin heavy chain (MHC, not shown) and DAPI (nuclei, blue), then imaged by large tiled fluorescence confocal microscopy. Myotubes were defined by MHC positive staining. Scale bars: 250μm. (B) Transfected myotubes (HA-FHL1 and MHC positive cells) were assessed for myotube width (i.), length (ii.) and myonuclear domain (iii.) relative to β-galactosidase control. See Fig. 7C for myotube area, fusion index and percentage MHC+ cells. At least 100 cells per independent experiment were scored and data represent the mean percentage ± s.e.m. ($n \geq 3$; a=$P<0.05$ compared to wild-type HA-FHL1; b=$P<0.05$ compared to β-galactosidase control; n.s.=not significant).

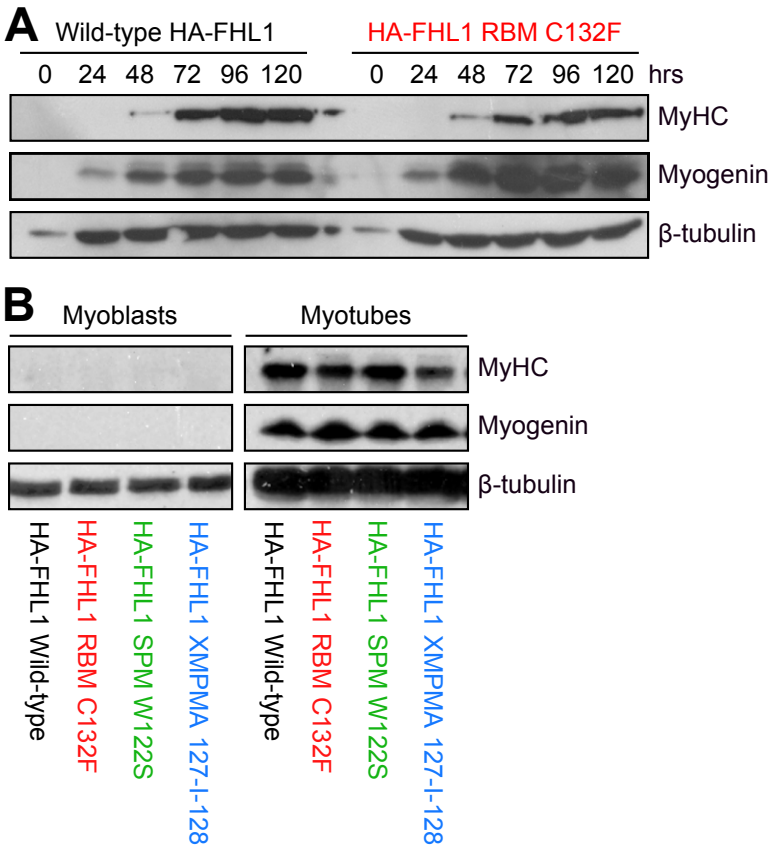

Fig. S3. RBM, SPM and XMPMA HA-FHL1 mutants do not alter expression of myosin heavy chain or myogenin. (A) Myoblast differentiation time-course was assessed in HA-FHL1 RBM C132F expressing myotubes. C2C12 myoblasts were transiently transfected with HA-FHL1 RBM C132F or wild-type HA-FHL1, then differentiated for 120 h. Whole cell lysates were immunoblotted with myosin heavy chain (MHC), myogenin or β-tubulin antibodies at 24, 48, 72, 96 and 120 h differentiation. (B) C2C12 myoblasts were transiently transfected with HA-tagged wild-type FHL1, RBM C132F, SPM W122S or XMPMA 127-I-128 (representative of each myopathy), and whole cell lysates were immunoblotted with MHC, myogenin or β-tubulin antibodies after 24 h expression (myoblasts) and after 72 h differentiation (myotubes).

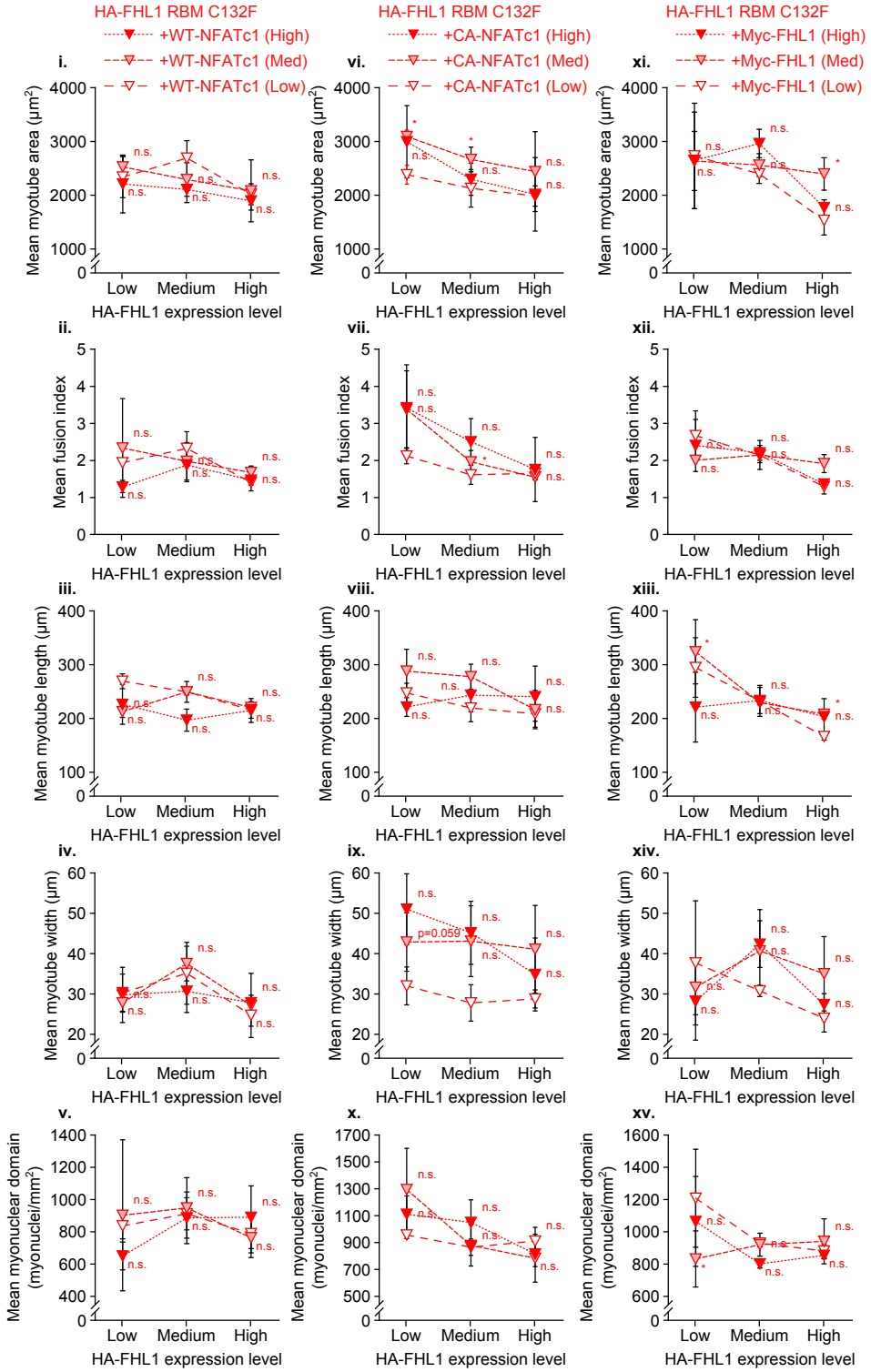

Fig. S4. Impaired differentiation of RBM mutant HA-FHL1 myotubes is rescued by expression of constitutively active NFATc1. C2C12 myoblasts were transiently co-transfected with HA-FHL1 RBM C132F, and wild-type GFP-NFATc1 (i.–v.), constitutively active GFP-NFATc1 (vi.–x.) or Myc-FHL1 (xi.–xv.), then differentiated for 72 h. Cells were co-stained with HA (568 nm), myosin heavy chain (647 nm), and DAPI (405 nm), then imaged by large tiled fluorescence confocal microscopy. Cells were manually traced in ImageJ using cytoplasmic HA-FHL1 and MHC fluorescence. NFAT/Myc expression was blindly measured by GFP fluorescence (488 nm) or Myc staining (488 nm). Each transfected myotube (HA-FHL1+/MHC+) was blindly categorised into low (<15%), medium (15–60%) or high (>60%) HA-FHL1 expression based on 568 nm average fluorescence, and blindly categorised into low (<15%), medium (15–50%) or high (>50%) rescue expression based on 488 nm average fluorescence. Myotube area, fusion index, length, width and myonuclear domain was automatically calculated in ImageJ based on individual cell tracing. At least 50 cells per independent experiment were scored and data represent the mean percentage ± s.e.m. ($n \geq 3$; paired *t*-test, *=$P<0.05$ compared to low level expression of rescue in the same HA-FHL1 RBM C132F expression level; n.s.=not significant).

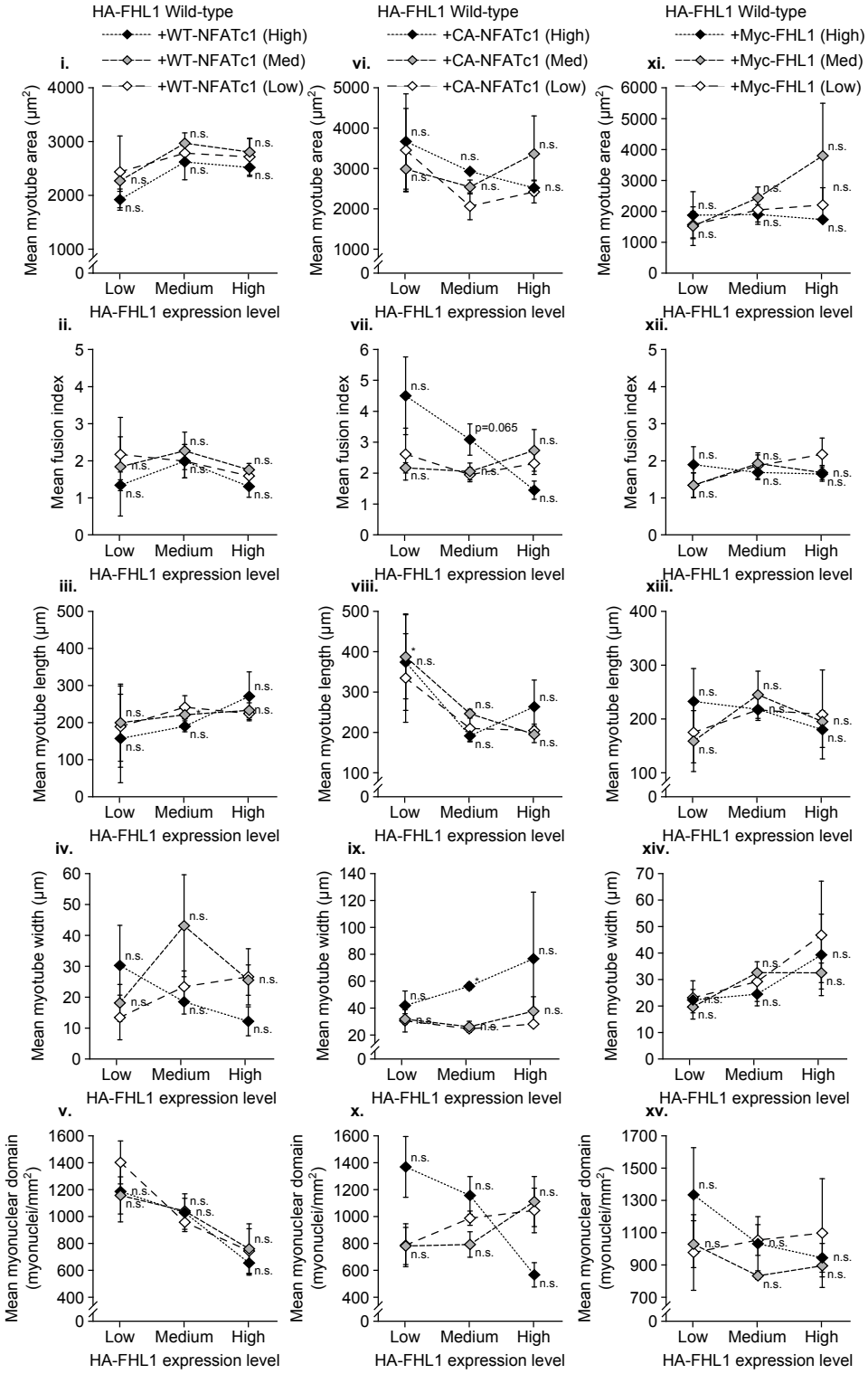

Fig. S5. Co-expression of Myc-FHL1 or GFP-NFAT does not alter differentiation in wild-type HA-FHL1 expressing myoblasts. C2C12 myoblasts were transiently co-transfected with wild-type HA-FHL1, and wild-type GFP-NFATc1 (i.–v.), constitutively active GFP-NFATc1 (vi.–x.) or Myc-FHL1 (xi.–xv.), then differentiated for 72 h. Cells were co-stained with HA (568 nm), Myosin Heavy Chain (647 nm), and DAPI (405 nm), then imaged by large tiled fluorescence confocal microscopy. Cells were manually traced in ImageJ using cytoplasmic HA-FHL1 and MHC fluorescence. NFAT/Myc expression was blindly measured by GFP fluorescence (488 nm) or Myc staining (488 nm). Each transfected myotube (HA-FHL1+/MHC+) was blindly categorised into low (<15%), medium (15–60%) or high (>60%) HA-FHL1 expression based on 568 nm average fluorescence, and blindly categorised into low (<15%), medium (15–50%) or high (>50%) rescue expression based on 488 nm average fluorescence. Myotube area, fusion index, length, width and myonuclear domain was automatically calculated in ImageJ based on individual cell tracing. At least 50 cells per independent experiment were scored and data represent the mean percentage ± s.e.m. ($n \geq 3$; paired *t*-test, *=$P<0.05$ compared to low level expression of rescue in the same wild-type HA-FHL1 expression level; n.s.=not significant).

Table S1. Summary of FHL1 myopathy mutants generated and investigated. Expression of RBM, SPM and XMPMA mutant FHL1 in affected muscle is highly variable however inheritance is generally dominant. HCM and EDMD mutant FHL1 protein expression is decreased/absent and variable penetrance is observed. Representative FHL1 mutants of each disease were investigated in this study: RBM (H123Y, C132F, C153Y, 102–104del), SPM W122S, XMPMA (127-I-128, C224W), RSS (151–153del), HCM (C209R*) and EDMD (C273LfsX11, 111–229Δ, K157VfsX36, X281E, C276Y). *The C209R missense mutation was identified in HCM with 'Emery Dreifuss-like syndrome'.

Download Table S1

Table S2. FHL1 mutation primers used to generate FHL1A myopathy mutants. Mutants representative of RBM, SPM, XMPMA, RSS, HCM and EDMD mutants were generated by PCR mutagenesis. Base-substitution, deletion and insertion mutations were introduced into wild-type FHL1 cDNA (NM_001449.3) and 5′ *Xba*I and 3′ *Xma*I restriction sites were introduced for cloning.

Download Table S2