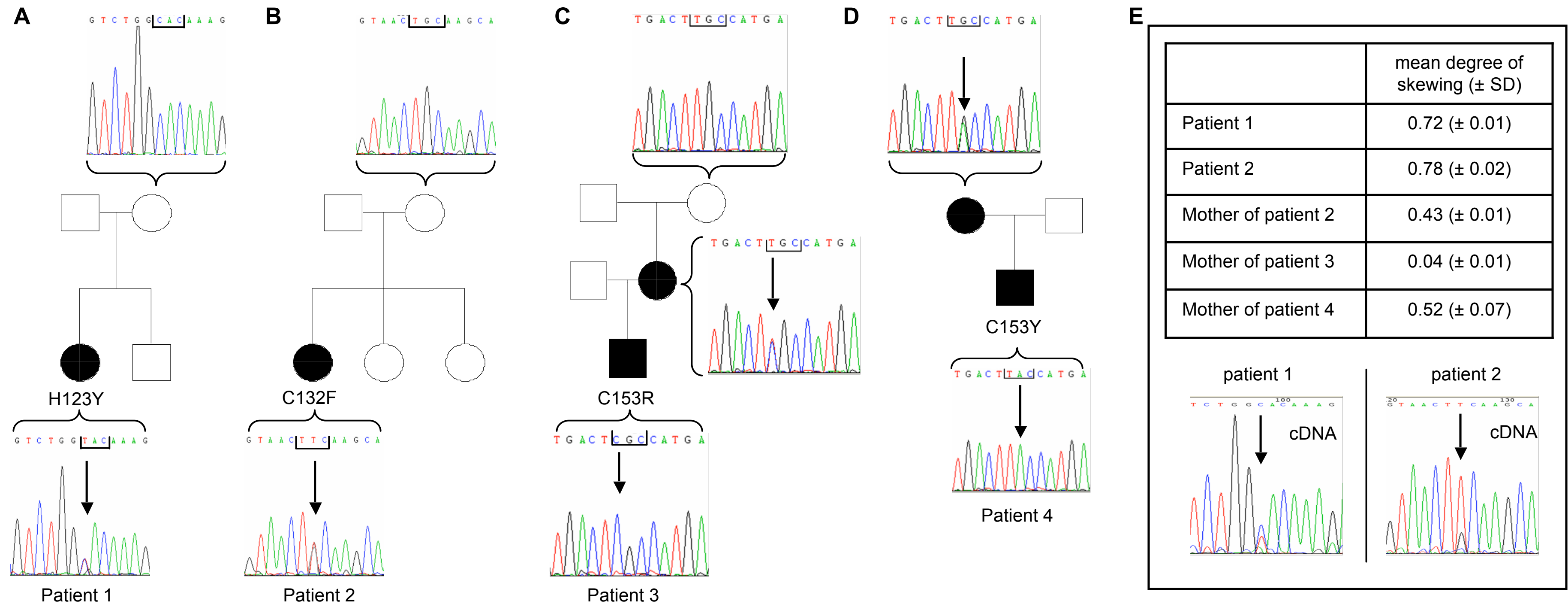
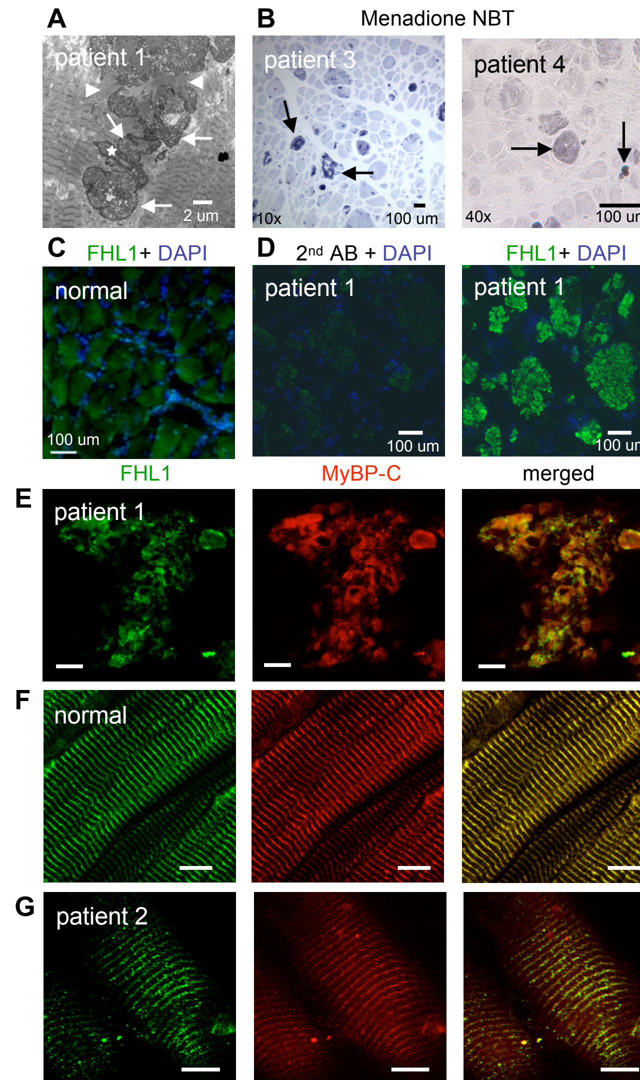
Supplementary Figure 1.



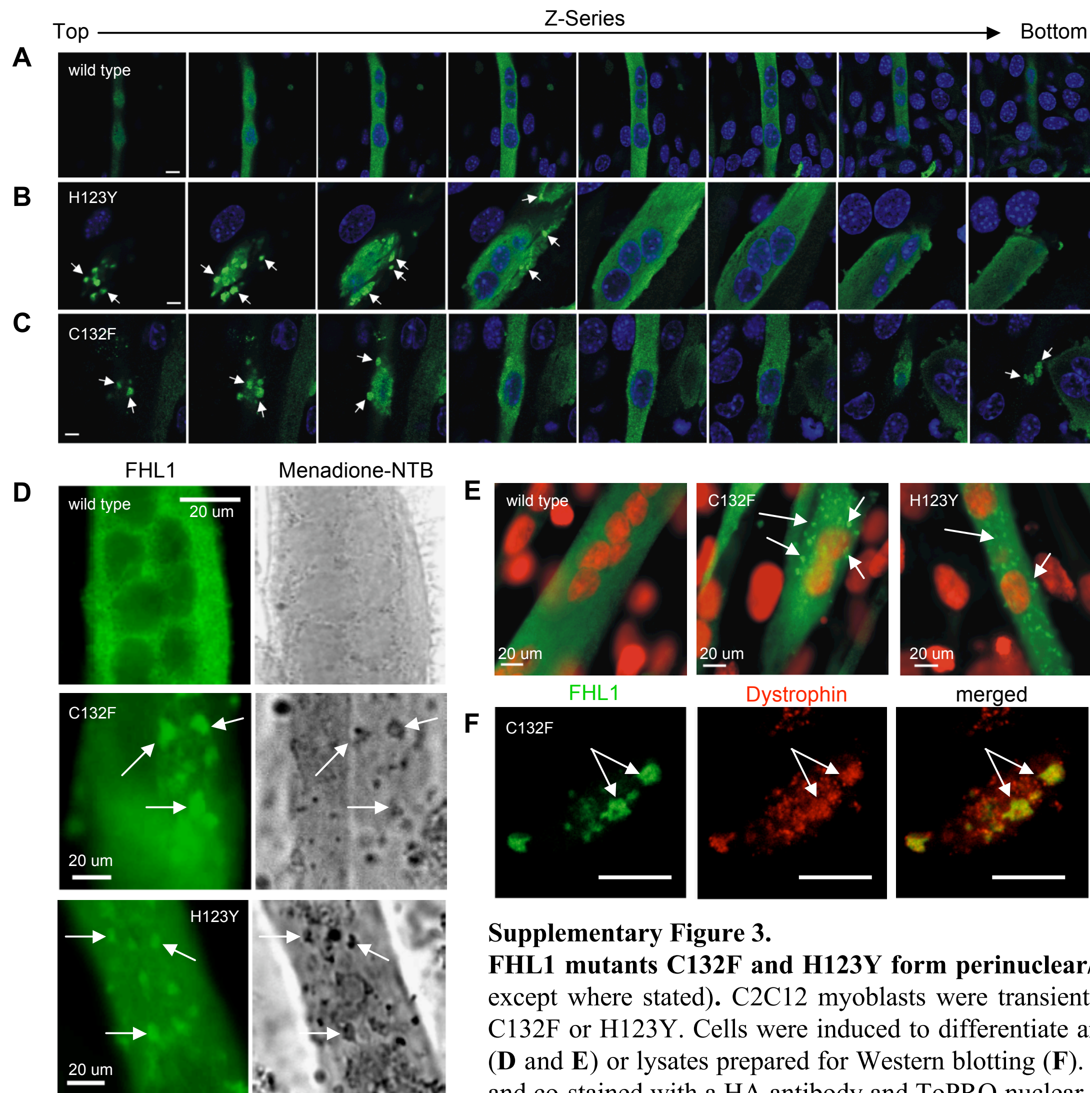
Supplementary Figure 1.
FHL1 mutations and pedigrees of patient families and X inactivation studies. (**A-D**), Family structures for patients 1-4, with mutations indicated and genomic sequence tracings displayed. Family members designated by black symbols carried confirmed mutations, family members designated by open symbols were sequenced and found to be wild type (even if the sequence is not shown). Affected mothers were heterozygote for the mutation. The mutation were de-novo in patients 1 and 2 and in the mother of patient 3. Grandparents for patient 4 were not available. (**E**) Assessment of X-inactivation patterns in female patients, using an assay based on the methylation status near the androgen receptor CAG repeat length polymorphism in leukocyte DNA. Significantly skewed X-inactivation is considered to be present for degrees greater 0.75. There was no significant skewing in the affected mothers. In patient 2 on skeletal muscle RT-PCR the majority of the amplification was from the mutant allele (red T peak, arrow). This patient had a degree of 0.78 (78%) skewed X-inactivation, thus likely preferentially silencing the wild type X chromosome. The degree of skewing in patient 1 does not reach a significant level (0.72), her RT-PCR from muscle suggests insignificantly higher transcription from the wild type allele (blue C peak, arrow).

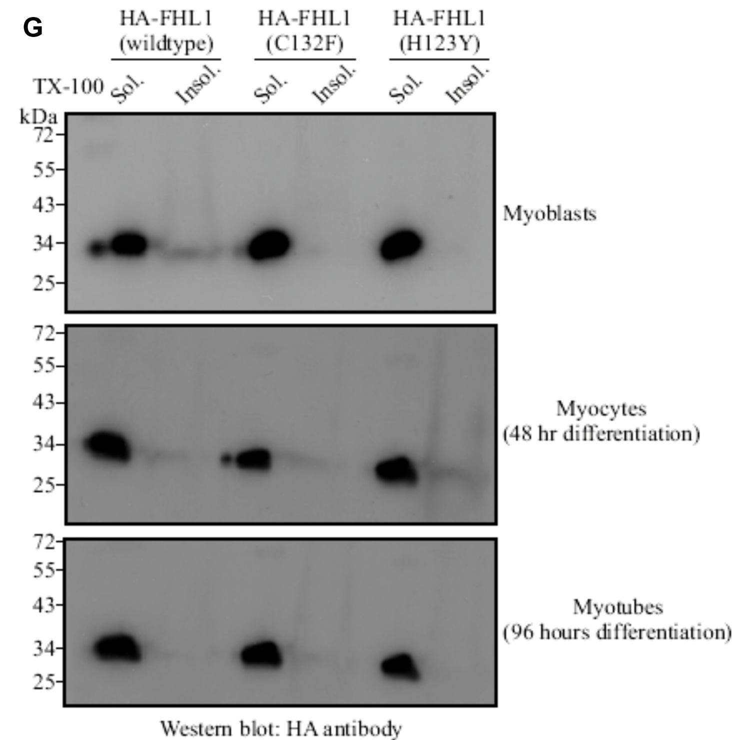
Supplementary Figure 2.



Supplementary Figure 2.

Additional histology (bars = 10 µm, except where stated)**.** (**A**) EM at lower power of biopsy in patient 1 with cytoplasmic inclusions corresponding to reducing bodies (arrows) some of which abut a myonucleus (star), plus likely cytoplasmic bodies (arrow heads) that are interspersed. (**B**) Menadione-nitro-blue-tetrazolium staining of biopsies in patient 3 and 4 demonstrating the presence of reducing bodies (arrows). (**C**) Staining with FHL1 in an additional normal control biopsy without reducing bodies. (**D**) There is no positive staining of the inclusions with secondary antibody (2nd AB) only, indicating specific binding of the primary FHL1 antibody. (**E-G**) The FHL1 interacting protein MyBP-C is also included in the reducing bodies (**E**) but there is no obvious difference in its localization to the contractile apparatus between normal (**F**) and patient muscle (**G**).





Supplementary Figure 3.
FHL1 mutants C132F and H123Y form perinuclear/cytoplasmic inclusions in C2C12 skeletal myotubes (bars = 10 µm, except where stated)**.** C2C12 myoblasts were transiently transfected with either wild type HA-FHL1, or HA-FHL1 mutants C132F or H123Y. Cells were induced to differentiate and either stained for immunofluorescence (**A** and **B**), menadione-NTB (**D** and **E**) or lysates prepared for Western blotting (**F**). For immunofluorescence studies cells were differentiated for 72 hours and co-stained with a HA antibody and ToPRO nuclear stain. Cells were viewed using laser scanning confocal microscopy and images are presented as a Z-series, scanning from the top to the bottom of the cell, to maximize visualization of inclusions (arrows) formed in cells expressing mutant FHL1 H123Y (**B**) or C132F (**c**), but not FHL1 wild type (**A**). (**D** and **E**) cells were stained with menadione-NTB next to FHL1 (**D**) or FHL1 and propidium iodide to detect nuclei (**E**) and viewed using fluorescence microscope. Arrows point out inclusions. (**F**) Inclusions in differentiated C2C12 cells induced by transfection of mutant FHL1 H123Y or C132F include endogenous muscle proteins dystrophin (arrows). (**G**) For Western blot analysis Triton-soluble and –insoluble lysates were prepared at 0 hr (myoblasts), 48 hr (myocytes) and 96 hr (myotube) differentiation, and 25 mg of protein immunoblotted with a HA antibody. Immunoreactive material is detected in the soluble but not in the the insoluble fractions, thus, the mutants are soluble.

Supplementary Table 1.

Protein	Accession number	Patient 1	Patient 2	Number of Peptides
Four and a half LIM domains protein 1	FHL1_HUMAN	yes	yes	2
Tropomyosin 1 alpha chain (Alpha-tropomyosin)	TPM1_HUMAN (+1)	yes	yes	1
Ubiquitin	UBIQ_HUMAN	yes	yes	2
Actin, aortic smooth muscle	ACTA_HUMAN	yes	yes	2
Actin, cytoplasmic 1 (Beta-actin)	ACTB_HUMAN	yes		2
Antigen peptide transporter 2 (APT2)	TAP2_HUMAN		yes	1
Carboxypeptidase B precursor	CPBD_HUMAN			1
Dermcidin precursor (Preproteolysin)	DCD_HUMAN		yes	1
Desmin	DESM_HUMAN	yes		3
Hemoglobin subunit beta	HBB_HUMAN	yes		2
Homeobox protein Hox-A4	HXA4_HUMAN	yes		1
Interleukin-2 receptor beta chain precursor (IL-2 receptor)	IL2RB_HUMAN		yes	1
Kinesin-like protein KIF14	KIF14_HUMAN		yes	1
Microtubule-associated serine/threonine-protein kinase-like	MASTL_HUMAN	yes		1
Myosin light chain 1, skeletal muscle isoform	MLE1_HUMAN	yes		1
Protein S100-A7 (S100 calcium-binding protein A7)	S10A7_HUMAN	yes		1
Putative GTP-binding protein 9	GTPB9_HUMAN	yes		1
X-linked retinitis pigmentosa GTPase regulator-interacting protein 1	RPGR1_HUMAN		yes	1

Summary of protein ID data. All peptides were required to have a probability of greater than 95% and were identified using 2 independent search engines.

Supplementary Table 2.

	GFP positive cells [in %]	Total number of cells counted	Cells with inclusions in %
wild type	202 [17.53%]	1152	0.5%
mutant H123Y	246 [22.12%]	1112	1.98%
mutant C132F	268 [20.41%]	1313	2.01%
mean (± SD)	238.6 (±33.6); [20.02% (±2.32)]	1192.3 (±106.4)	

Transfection efficiency of wild type and FHL1 mutant into COS-7 cell line. Transfection efficiency was calculated by counting the total amount of cells and the GFP positive cells in 14 randomized spots 24 hours after transfection of wild type and FHL1 mutants H123Y and C132F into COS-7 cells. There was nearly equal transfection efficiency in wild type and the two mutants. Cells with GFP-positive inclusion at this stage are indicated as percentage of all cells (SD = standard deviation).