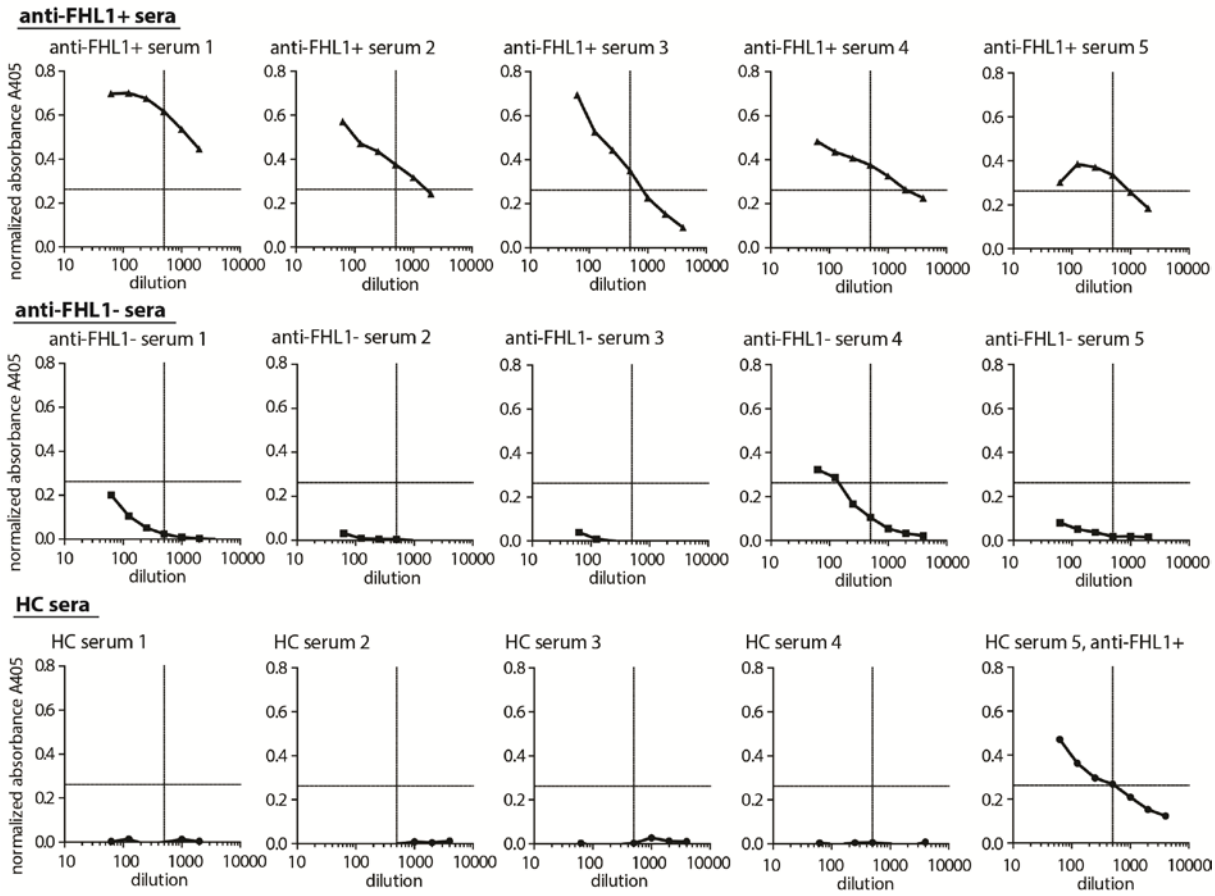
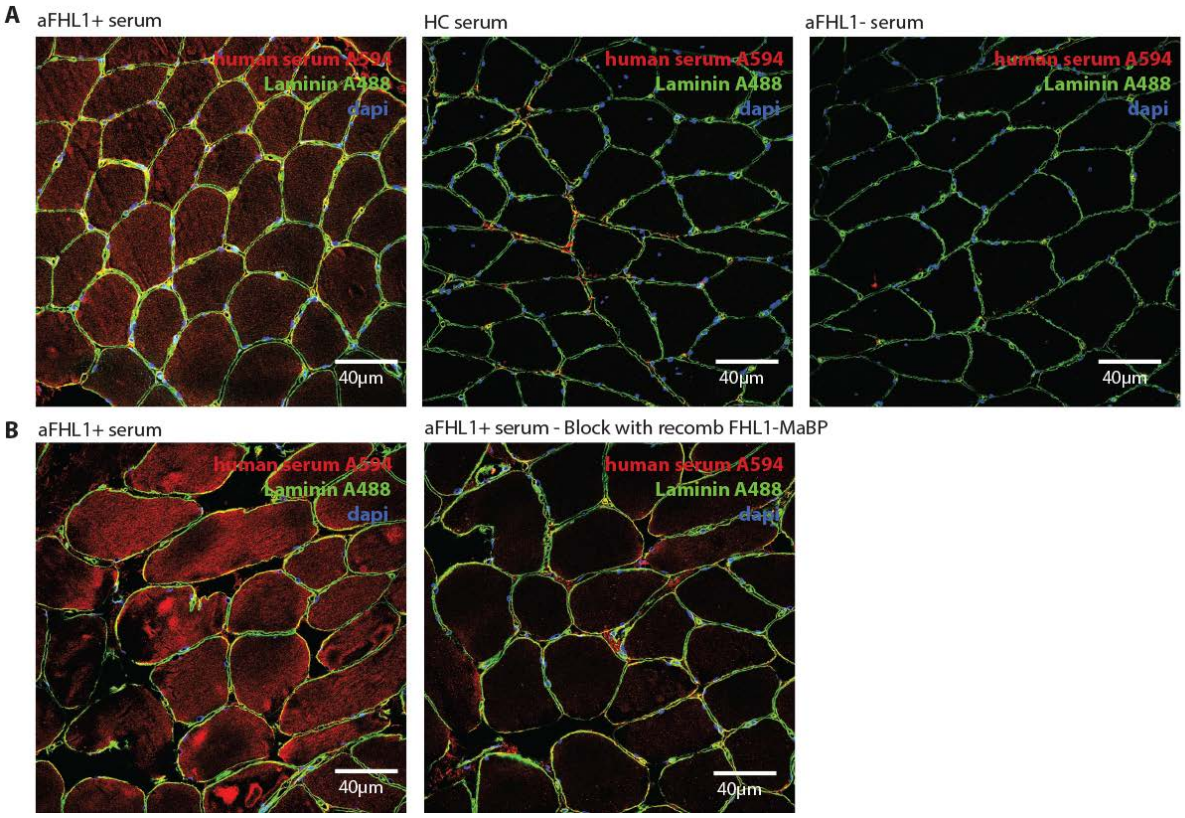
Supplementary Materials:

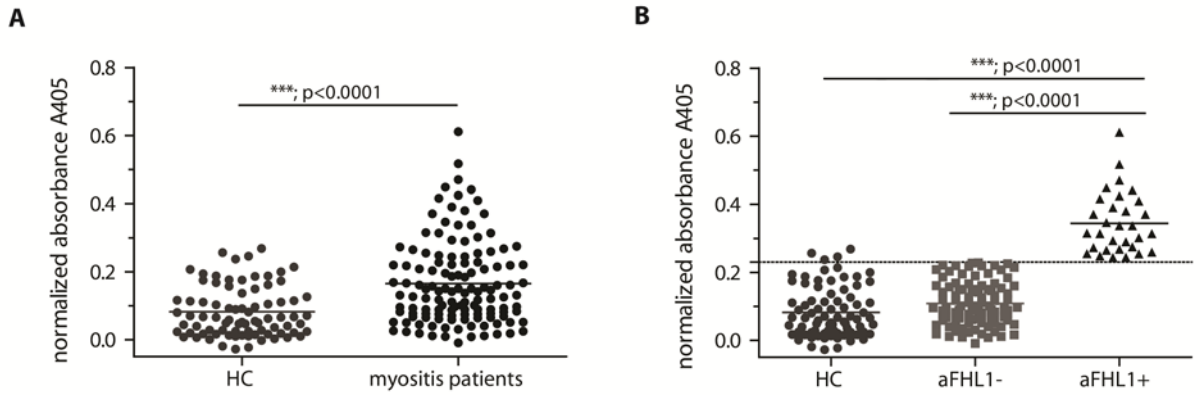
Supplemental Figures



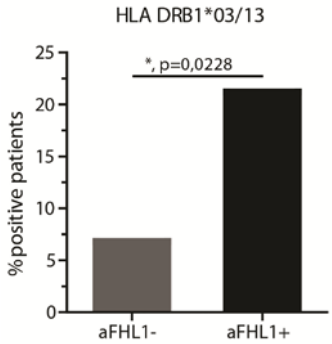
Supplemental Figure 1: Serial dilution ELISA of anti-FHL1$^+$, anti-FHL1$^-$ and HC sera. Sera of 5 anti-FHL1$^+$ IIM patients (upper panel), 5 anti-FHL1$^-$ patients (middle panel) and 5 HCs (lower panel) were analyzed by serial dilution ELISA for reactivity to recombinant FHL1-MaBP fusion protein. Starting dilution was 1:62.5 and sera were serially diluted in 1:2-steps. The horizontal line indicates the cut-off value determined by measuring reactivity of HC sera to FHL1, the vertical line indicates the used dilution for the performed ELISA assays of 1:500. HC serum 5 (lower panel) was one of the 3 anti-FHL1$^+$ healthy individuals and revealed a comparable curve as the displayed anti-FHL1$^+$ myositis sera.



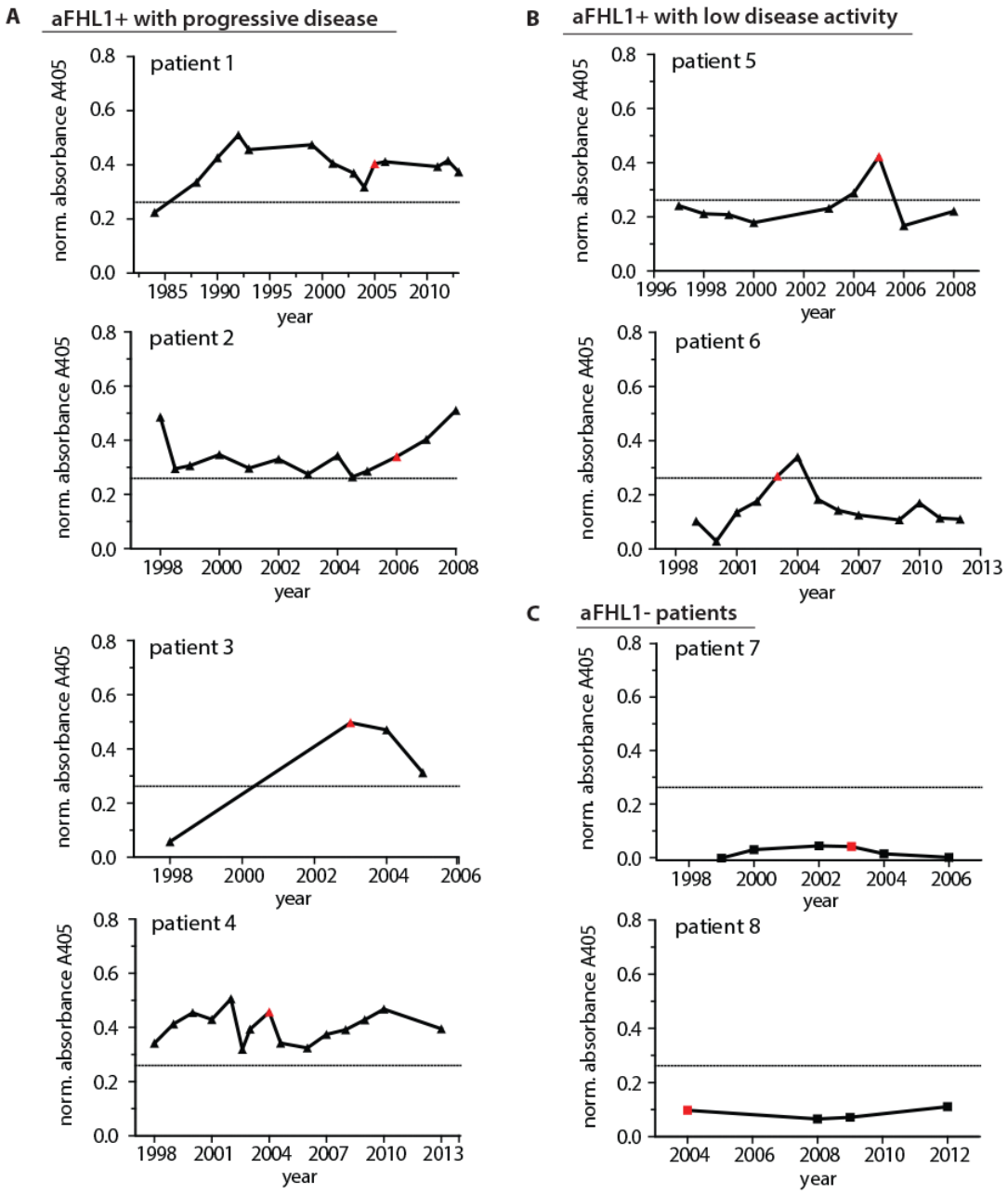
Supplemental Figure 2: Reactivity of anti-FHL1^{+} myositis sera with normal human muscle tissue. (A) HC muscle tissue sections were stained with sera from anti-FHL1^{+} patients (right panel, n=3), from HCs (middle panel, n=3) and from anti-FHL1^{-} patients (left panel, n=3), and analyzed by confocal microscopy. **(B)** Anti-FHL1^{+} sera (n=2) were pre-incubated with recombinant FHL1-MaBP and HC muscle tissue was stained with unblocked or blocked anti-FHL1^{+} serum. Positive reactivity is displayed in red (anti-human IgG secondary antibody coupled to Alexa594), nuclei are visualized by using DAPI (blue); and laminin was stained to visualize the sarcolemma (green; Alexa488). Yellow staining in the sarcolemma indicates positive double staining for FHL1 in the sarcolemma. One representative staining is shown. The white bar indicates 40 µm.



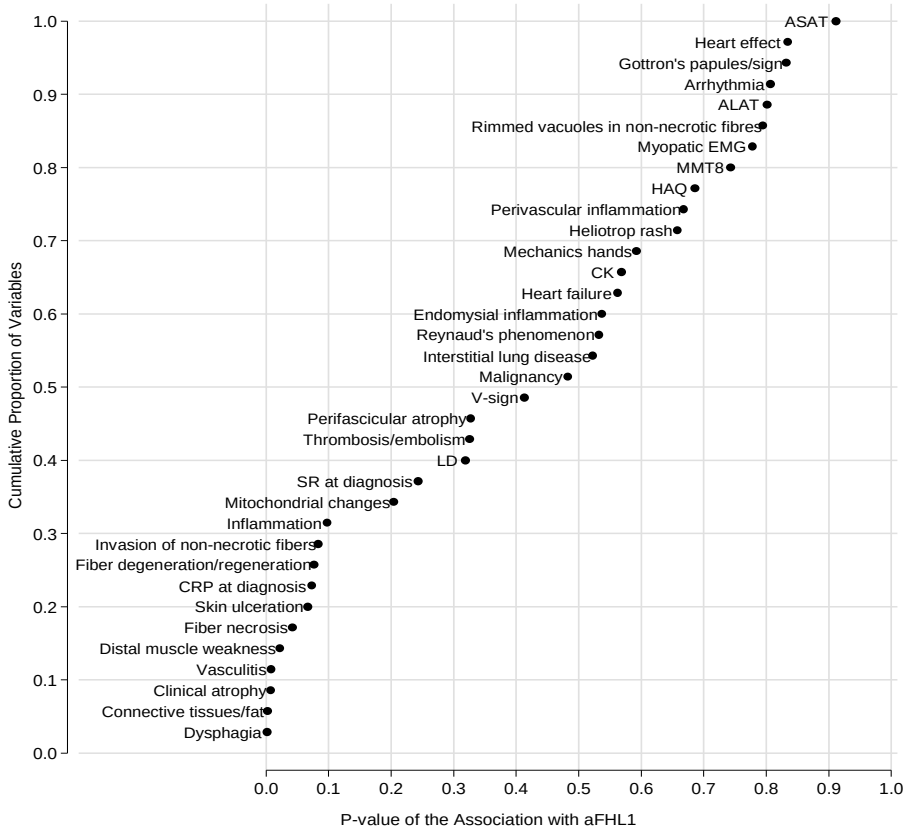
Supplemental Figure 3: Reactivity to FHL1 in IIM patients of a cohort from the Czech Republic. **(A)** Sera of 129 IIM patients from a cohort from Czech Republic were analyzed by ELISA for reactivity to recombinant FHL1-MaBP fusion protein and compared to 81 Czech healthy controls (HC). **(B)** According to the normalized absorbance values of the HCs, a cut-off value (line) was calculated allowing subdivision of the patients into anti-FHL1^{-} and anti-FHL1^{+} patients. Statistical analysis: two-tailed Mann Whitney test. Each data point represents one individual and horizontal bars indicate mean values.



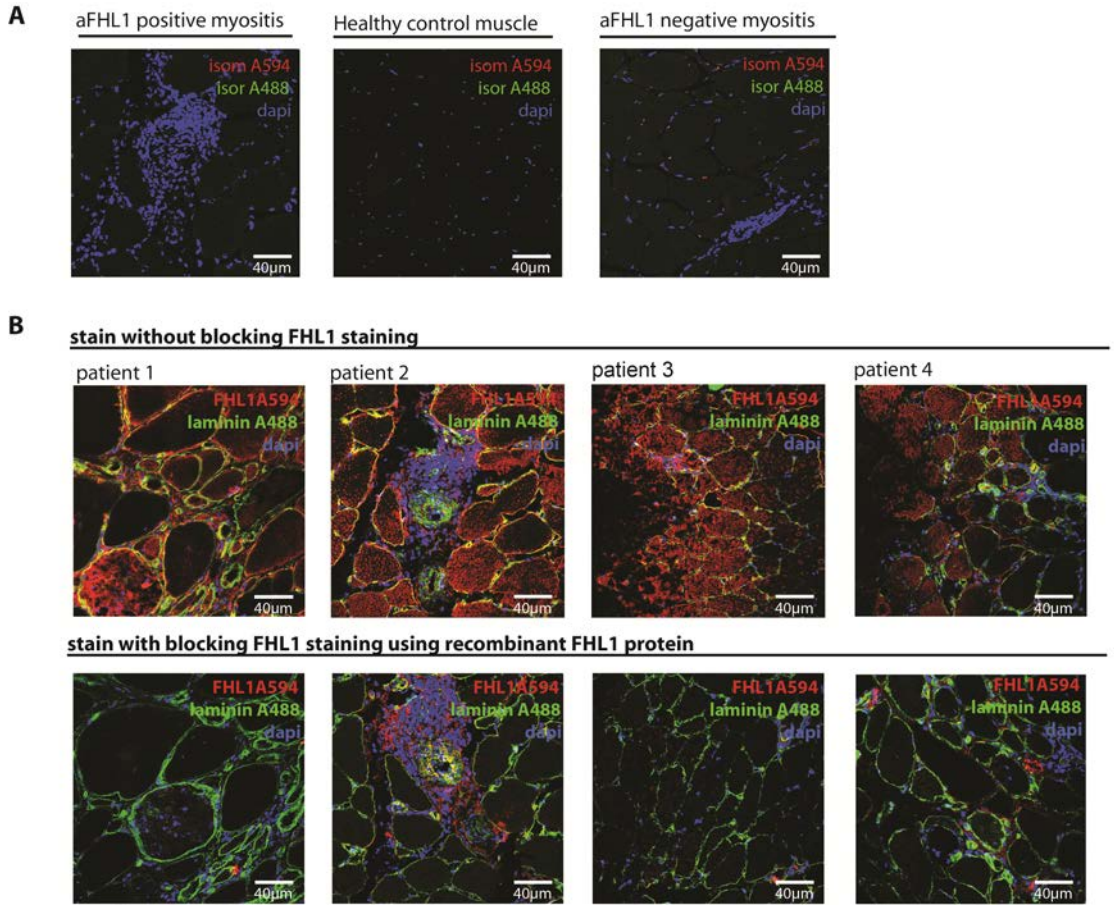
Supplemental Figure 4: The HLA DRB1*03/*13 genotype is more frequent in anti-FHL1^{+} *vs* anti-FHL1^{-} patients. Statistical analysis revealed that anti-FHL1^{+} patients compared to anti-FHL1^{-} patients had a higher incidence of showing HLA DRB1*03/*13 genotype (p= 0,0228; Chi-square test).



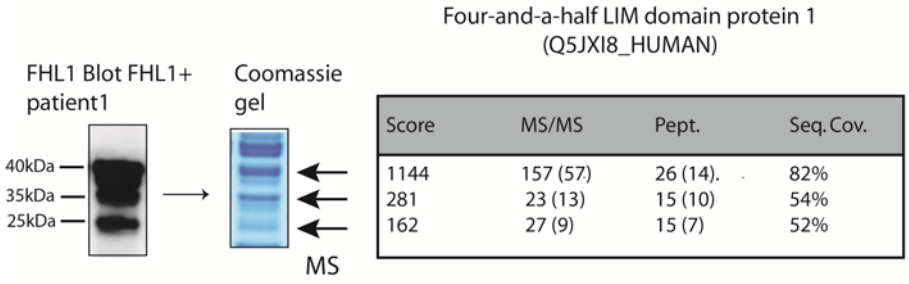
Supplemental Figure 5: Stability of anti-FHL1 reactivity tested by ELISA of longitudinal samples. (A) Longitudinal serum samples of four anti-FHL1^{+} patients with therapy-resistant, progressive disease (score according Figure 2B=4.5-5), **(B)** of two anti-FHL1^{+} patients who improved with immunosuppressive treatment (score=0 and 2, respectively), and **(C)** of two anti-FHL1^{-} patients with stable disease, low disease activity, were analyzed by ELISA for reactivity to FHL1. The horizontal line indicates the cut-off value. The red symbol represents the time point of analysis displayed in Figure 1. Except for patient 3 (time point of diagnosis 2003), the first symbol on each graph indicates the time point of diagnosis.



Supplemental Figure 6: Test for proportion of p-values < 0.01 is equal to 0.1; Swedish cohort of IIM patients. The variables considered are plotted against the p-value they showed when their association with the presence of anti-FHL1 autoantibodies was tested. The proportion of variables with a p-value < 0.10 was about 30%, significantly larger than what expected by mere chance (binomial exact test p-value < .01). The associations for muscle enzymes, CK, LD, ALAT and ASAT, were based on the highest measured level during a patient´s disease history. For the clinical measurements MMT-8 and HAQ, the associations were based on the measurement at the latest visit found in the clinical records or registries. Abbreviations: aFHL1, anti-FHL1 autoantibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LD, lactate dehydrogenase; CK, creatine kinase; HAQ, health assessment questionnaire; MMT-8, manual muscle test-8; EMG, electromyogram; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.



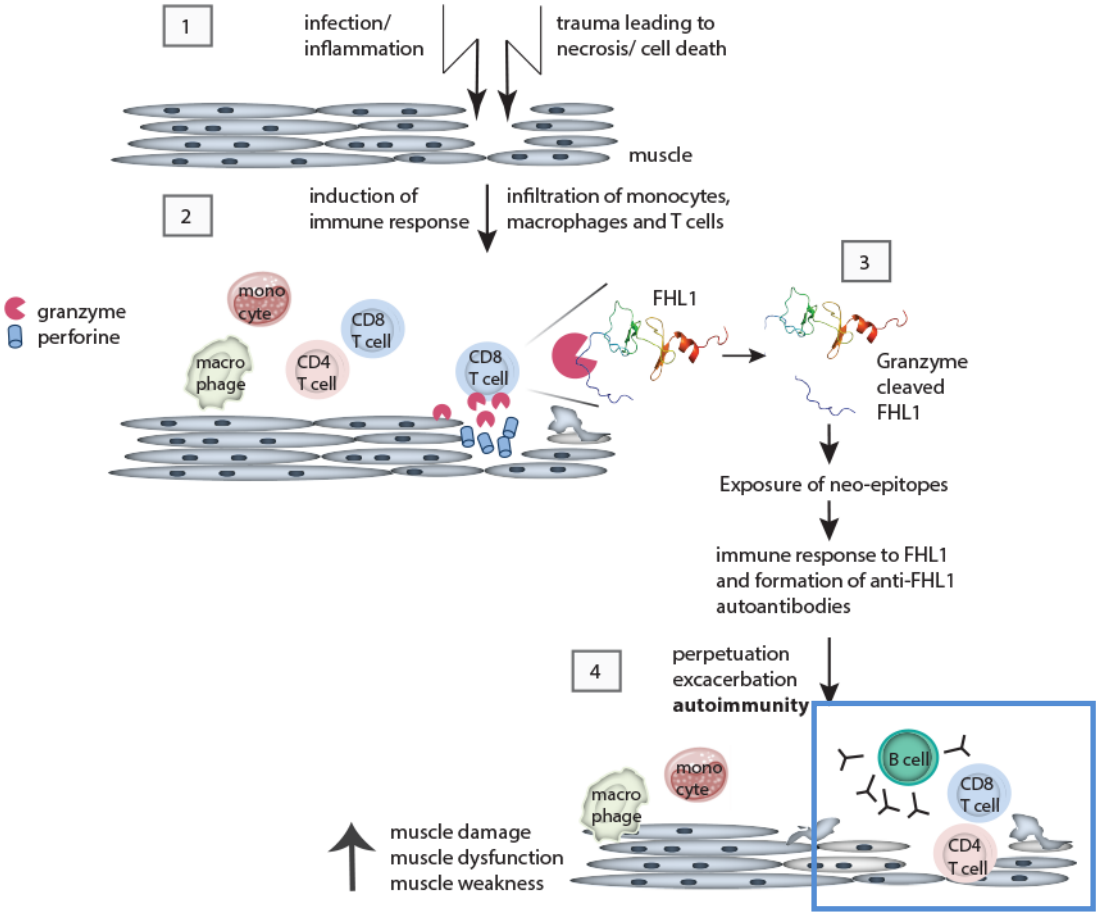
Supplemental Figure 7: FHL1 confocal staining controls. (A) Representative isotype stainings for anti-FHL1^{+}, anti-FHL1^{-} and HC muscle tissue. For monoclonal mouse anti-FHL1 (abcam, ab76912), a mouse IgG2a isotype together with a secondary anti-mouse antibody coupled to Alexa594 was used; control for laminin was a rabbit IgG together with a secondary anti-rabbit antibody coupled to Alexa488. Nuclei are stained with DAPI. Isotype stainings were performed for all samples shown in Fig. 3. **(B)** Monoclonal mouse anti-FHL1 was incubated with or without recombinant FHL1 protein over night at 4ºC. The next day, serial sections of muscle tissue from 4 anti-FHL1^{+} patients were stained with unblocked (upper panel) or blocked (lower panel) anti-FHL1 (secondary anti-mouse coupled to Alexa594) and visualized by confocal microscopy. Laminin was stained in addition (Alexa488) and nuclei are visualized by using DAPI (blue).



Four-and-a-half LIM domain protein 1 (Q5JXI8_HUMAN)

Score	MS/MS	Pept.	Seq. Cov.
1144	157 (57)	26 (14).	82%
281	23 (13)	15 (10)	54%
162	27 (9)	15 (7)	52%

Supplemental Figure 8: Detection of FHL1 in western blot bands of IIM muscle lysates by mass-spectrometry. Bands identified by western blotting to contain FHL1 were excised from a Coomassie stained gel. After reduction, alkylation and tryptic digestion, the peptides were analyzed by Q Exactive MS and searched against the human complete proteome database using Mascot. Left panel shows part of a blot probed by anti-FLH1 antibodies, middle panel shows part of the Coomassie stained gel, and the table lists the protein scores, the number of MS/MS, the number of peptides and the sequence coverage for the FHL1 protein. The non-redundant numbers of MS/MS and peptides are given in parenthesis.



Supplemental Figure 9: Proposed model for initiation of autoimmunity to FHL1. Initial muscle damage occurs through infection and subsequent inflammatory response or trauma leading to necrosis/cell death (1). Pathogen-associated or damage-associated signals initiate infiltration of inflammatory cells and activate professional antigen-presenting cells which induce CD4 and CD8 T cell responses (2). Infiltrating CD8 T cells secrete granzymes and perforin. Granzyme-susceptible autoantigens including FHL1 are cleaved (3), leading to exposure of neo-epitopes and a specific immune response to FHL1 including activation of B cells and formation of anti-FHL1 autoantibodies. The final result is a break of tolerance, perpetuation and exacerbation of the initial immune response and initiation of autoimmunity leading to an increase of muscle damage and progressive muscle dysfunction and weakness (4).

Supplemental Tables

Supplemental Table 1. Characteristics of the patients used for cDNA library screen

Patient	gender	age	diagnosis	Autoantibody status
A	female	55	DM	Negative*
B	female	57	paraneoplastic DM	Negative*
C	male	56	PM	Jo-1, Ro52

Abbreviations: PM, polymyositis; DM, dermatomyositis; * tested for ANA, Jo1, anti-SRP, Ro52, and anti-U1RNP

Supplemental Table 2. HLA haplotypes of the Swedish cohort of IIM patients

HLA DRB1 n (%)	aFHL^{+} (n=33)	aFHL^{-} (n=88)	P value
*01/*01	0 (0.0)	5 (5.7)	0.3214
*01/*03	3 (9.1)	10 (11.4)	1.0000
*01/*04	0 (0.0)	3 (3.4)	0.5613
*01/*08	0 (0.0)	1 (1.1)	1.0000
*01/*11	0 (0.0)	1 (1.1)	1.0000
*01/*13	0 (0.0)	1 (1.1)	1.0000
*01/*15	1 (3.0)	1 (1.1)	0.4727
*01/*16	1 (3.0)	0 (0.0)	0.2727
*03/*03	2 (6.1)	1 (1.1)	0.1803
*03/*04	4 (12.1)	9 (10.2)	0.7490
*03/*07	0 (0.0)	1 (1.1)	1.0000
*03/*08	1 (3.0)	3 (3.4)	1.0000
*03/*09	1 (3.0)	0 (0.0)	0.2727
*03/*10	1 (3.0)	1 (1.1)	0.4727
*03/*11	1 (3.0)	2 (2.3)	1.0000
***03/*13**	**7 (21.2)**	**6 (6.8)**	**0.0228**
*03/*14	1 (3.0)	2 (2.3)	1.0000
*03/*15	1 (3.0)	8 (9.1)	0.4416
*03/*10	0 (0.0)	0 (0.0)	-
*04/*04	1 (3.0)	3 (3.4)	1.0000
*04/*07	2 (6.1)	1 (1.1)	0.1803
*04/*08	0 (0.0)	1 (1.1)	1.0000
*04/*09	1 (3.0)	0 (0.0)	0.2727
*04/*13	1 (3.0)	6 (6.8)	0.6724
*04/*14	1 (3.0)	0 (0.0)	0.2727
*04/*15	0 (0.0)	2 (2.3)	1.0000
*07/*08	0 (0.0)	1 (1.1)	1.0000
*07/*11	0 (0.0)	1 (1.1)	1.0000
*07/*13	0 (0.0)	2 (2.3)	1.0000
*07/*14	0 (0.0)	1 (1.1)	1.0000
*07/*16	0 (0.0)	1 (1.1)	1.0000
*08/*11	0 (0.0)	1 (1.1)	1.0000
*08/*15	1 (3.0)	2 (2.3)	1.0000
*09/*13	0 (0.0)	1 (1.1)	1.0000
*09/*15	0 (0.0)	1 (1.1)	1.0000
*11/*11	1 (3.0)	2 (2.3)	1.0000
*11/*13	0 (0.0)	2 (2.3)	1.0000
*11/*14	1 (3.0)	0 (0.0)	0.2727
*11/*15	0 (0.0)	1 (1.1)	1.0000
*11/*16	0 (0.0)	1 (1.1)	1.0000
*13/*13	0 (0.0)	1 (1.1)	1.0000
*13/*15	0 (0.0)	1 (1.1)	1.0000
*15/*15	0 (0.0)	1 (1.1)	1.0000

Supplemental Table 3. Descriptive characteristics of the Swedish cohort of IIM patients

Variable	aFHL1^{+} (n=33)	aFHL1^{-} (n=99)	Total (n=132)	P value
Gender, n (%) Female Male	 26 (79) 7 (21)	 63 (64) 36 (36)	 89 (67) 43 (33)	0.135
Diagnosis, n (%) PM DM IBM JDM	 19 (58) 10 (30) 3 (9) 1 (3)	 47 (47) 39 (39) 9 (9) 4 (4)	 66 (50) 49 (37) 12 (9) 5 (4)	0.812
Ethnicity, n (%) Caucasian Other	 30 (91) 3 (9)	 95 (96) 4 (4)	 125 (95) 7 (5)	0.366
Insidious progress of disease, n (%)	22 (71)	64 (80)	86 (77)	0.320
Malignancy, n (%)	6 (18)	26 (27)	32 (24)	0.482
Age at first symptom, median (IQR), years	49 (30-57)	49 (39-61)	49 (39-61)	0.604
Age at diagnosis, median (IQR), years	50 (30-57)	50 (40-63)	50 (40-62)	0.652
Age at serum test, median (IQR), years	59 (52-68)	61 (48-67)	60 (49-68)	0.827
Disease duration until serum sampling[a], median (IQR), years	8 (3-13)	5 (1-8)	5 (2-10)	0.058
Disease duration until last clinical evaluation[b], median (IQR), years	12 (8-18)	16 (9-19)	11 (7-17)	0.066
Heredity for neuromuscular diseases, n (%)	0 (0)	2 (4)	2 (2)	1.000
Heredity for inflammatory, rheumatic diseases, n (%)	7 (23)	33 (42)	40 (36)	0.078

[a] Time from diagnosis to serum sampling for anti-FHL1 autoantibody analysis

[b] Time from diagnosis to last clinical evaluation

Abbreviations: PM, polymyositis; DM, dermatomyositis; IBM, inclusion body myositis; JDM, juvenile dermatomyositis; IQR, interquartile range; MMT8, manual muscle test-8; HAQ, health assessment questionnaire

Supplemental Table 4. Clinical and muscle biopsy features of the Swedish cohort of IIM patients

Variable	aFHL1^{+} (n=33)	aFHL1^{-} (n=99)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	P value
Clinical muscle features, n (%)							
Muscle weakness	33 (100)	96 (98)	25.58	100.00	100.00	2.04	1.000
Proximal muscle weakness	31 (100)	93 (97)	25.00	100.00	100.00	3.13	1.000
Distal muscle weakness	***29 (94)***	***68 (74)***	***29.90***	***92.31***	***93.55***	***26.09***	***0.022***
Clinical muscle atrophy	***21 (66)***	***34 (37)***	***38.18***	***83.82***	***65.63***	***62.64***	***0.007***
Myopathic EMG	26 (87)	73 (83)	26.26	78.95	86.67	17.05	0.778
Skin manifestations, n (%)							
Gottron's papules	11 (33)	31 (31)	26.19	75.56	33.33	68.69	0.832
Heliotrope rash	8 (24)	30 (30)	21.05	73.40	24.24	69.70	0.658
Mechanic's hands	7 (22)	15 (16)	31.82	75.25	21.88	83.52	0.592
Raynaud's phenomenon	14 (44)	35 (37)	28.57	76.92	43.75	63.16	0.532
Calcinosis	4 (12)	11 (11)	26.67	75.00	12.12	88.78	1.000
Shawl sign	4 (13)	13 (14)	23.53	74.31	12.50	86.17	1.000
Skin ulceration	4 (12)	3 (3)	57.14	76.61	12.12	96.94	0.067
V-sign	7 (21)	14 (14)	33.33	76.15	21.21	85.57	0.413
Extramuscular involvement, n (%)							
Interstitial lung disease	9 (27)	35 (35)	20.45	72.73	27.27	64.65	0.523
Dysphagia	***28 (85)***	***52 (54)***	***35.00***	***89.80***	***84.85***	***45.83***	***0.002***
Heart involvement overall	11 (33)	37 (37)	22.92	73.81	33.33	62.63	0.835
Arrhythmia	6 (18)	22 (22)	21.43	74.04	18.18	77.78	0.806
Heart failure	3 (9)	14 (14)	17.65	74.34	9.38	85.71	0.562
Myocarditis /cardiomyopathy	1 (3)	4 (4)	20.00	75.63	3.33	95.74	1.000
Thrombosis/embolism	5 (15)	8 (9)	38.46	75.00	15.15	91.30	0.326
Vasculitis	***6 (18)***	***3 (3)***	***66.67***	***77.69***	***18.18***	***96.91***	***0.008***
Arthritis/synovitis	15 (45)	45 (45)	25.00	75.00	45.45	54.55	1.000
Muscle biopsy features, n (%)							
Inflammation	30 (94)	75 (81)	28.57	90.00	93.75	19.35	0.098
Endomysial inflammation	19 (59)	46 (52)	29.23	76.79	59.38	48.31	0.537
Perivascular inflammation	22 (69)	56 (63)	28.21	76.74	68.75	37.08	0.668
MHC class I expression in sarcolemma	16 (80)	47 (81)	25.40	73.33	80.00	18.97	1.000
Muscle fiber necrosis	***7 (26)***	***7 (9)***	***50.00***	***78.49***	***25.93***	***91.25***	***0.042***
Perifascicular atrophy	9 (28)	18 (20)	33.33	76.23	28.13	80.43	0.327
Rimmed vacuoles	5 (16)	17 (18)	22.73	73.53	15.63	81.52	0.795
Invasion of non-necrotic fibers	16 (52)	28 (33)	36.36	79.45	51.61	67.44	0.083
Fiber degeneration / regeneration	24 (80)	52 (62)	31.58	84.21	80.00	38.10	0.077
Increased amount of connective tissue/fat	***12 (44)***	***11 (14)***	***52.17***	***81.93***	***44.44***	***86.08***	***0.002***
Mitochondrial changes	7 (32)	7 (16)	50.00	70.59	31.82	83.72	0.204

Abbreviations: EMG, electromyogram; MHC, major histocompatibility complex

Supplemental Table 5. Clinical and laboratory measures of the Swedish cohort of IIM patients

Variable, median (IQR)	aFHL1^{+} (n=33)	aFHL1^{-} (n=99)	AUC	P value
MMT8 at latest visit	74.5 (64.5-79.5)	75 (63-79)	0.521	0.743
HAQ at latest visit	0.88 (0.44-1.815)	1.25 (0.5-1.75)	0.474	0.686
Highest measured serum ALAT	1.24 (0.9-2.48)	1.31 (0.88-2.52)	0.485	0.801
Highest measured serum ASAT	1.08 (0.88-2.57)	1.44 (0.79-2.65)	0.494	0.911
Highest measured serum CK	12.9 (5.9-50)	17.9 (8.1-52.8)	0.467	0.569
Highest measured serum LD	12.5 (8.65-22.2)	10.95 (7.6-18)	0.559	0.319
CRP at diagnosis, before treatment	7 (5.5-12)	10 (7-38)	0.368	0.073
SR at diagnosis, before treatment	17 (10-35)	22 (13-45.5)	0.428	0.243

Abbreviations: IQR, interquartile range; AUC, area under the receiver operating characteristic curve; MMT8, manual muscle test-8; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CK, creatine kinase; LD, lactate dehydrogenase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate

Supplemental Table 6. Autoantibody data of the Swedish cohort of IIM patients

Autoantibody, n (%)	**aFHL1^{+} (n=33)**	**aFHL1^{-} (n=99)**	**P value**
ANA	23 (77)	43 (61)	0.1202
Jo-1	3 (9)	20 (20)	0.1888
SRP	2 (6)	3 (3)	0.6029
Ro52	14 (42)	31 (32)	0.2589
U1RNP	6 (18)	7 (7)	0.0636
Autoantibody, n (%)	**aFHL1^{+} (n=19)**	**aFHL1^{-} (n=39)**	**P value**
cN-1A	3 (16)	13(33)	0.2174

Abbreviations: ANA, antinuclear antibodies; SRP, signal recognition particle; U1RNP, U1 ribo-nucleoprotein; cN-1A, cytosolic 5'-nucleotidase 1A