Supplementary methods

Human protein microarray analysis

ProtoArray® Human Protein Microarray v. 5.1 from Life Technologies was applied in the study. Human recombinant proteins were produced by transferring human clones into insect cells via baculovirus infection. Each protein is tagged with Glutathione-S-Transferase (GST), which enables high-throughput affinity purification under conditions that retain activity. All purified proteins are printed onto arrays, and the concentration of each protein is determined. Each array contains 9,374 purified human proteins immobilised on glass slides.

Microarray slides were blocked in blocking buffer (50 mM HEPES, 200 mM NaCl, 0.01% Triton X-100, 25% glycerol, 20 mM reduced glutathione, 1.0 mM DTT, 1X Synthetic Block) at 4°C for 1 hour. After blocking, arrays were rinsed once with freshly prepared PBST buffer (1X PBS, 0.1% Tween 20, and 1 X Synthetic Block). Arrays were then probed with a 1:500 dilution of each sample diluted in 5 mL of PBST buffer. Arrays were incubated for 90 minutes at 4 °C in QuadriPERM 4-well trays (Greiner) with gentle agitation. After incubation, slides were washed five times (5 minutes per wash) in 5 ml PBST buffer in 4-well trays. An Alexa Fluor®647-conjugated goat anti-human IgG antibody diluted in 5 ml PBST buffer to a 1.0 g/ml final concentration was added to each array and allowed to incubate with gentle shaking at 4 °C for 90 minutes. After incubation, the secondary antibody was removed, and arrays were washed as described above. Arrays were dried by spinning in a table top centrifuge equipped with a plate rotor at 200x gravity for 2 minutes. Finally, the arrays were scanned using the Tecan fluorescent microarray PowerScanner, and the probe signals were acquired using GenePix Pro 6.0 software (Molecular Devices, Sunnyvale, CA). Life Technologies' proprietary ProtoArray® Prospector software was used to analyse data resulting from protein microarray studies, with the goal of identifying proteins that can be used to statistically differentiate two populations.

Supplementary Table S1. Demographic features and cancer types of CAM patients and cancer controls.

Variables	IIM patients with CAM[a] (n=50)	Cancer controls (n=27)	*p*-value
Sex n(%)			
Male	14 (28.0)	6 (22.2)	0.581
Female	36 (72.0)	21 (77.8)	
Age, median (IQR), years	59 (47, 64)	59 (48, 65)	0.375
Cancer types n(%)			
Urinary genital cancer	23 (46.0)	15 (55.6)	0.315
Gastrointestinal tract cancer	9 (18.0)	8 (29.6)	
Respiratory cancer	7 (14.0)	1 (3.7)	
Hematological cancer	3 (6.0)	1 (3.7)	
Endocrine cancer	8 (16.0)	2 (7.4)	

IIM: idiopathic inflammatory myopathy; IQR: interquartile range; [a]CAM (cancer-associated myositis): the diagnosis of cancer within 3 years before or after the onset of myositis.

Supplementary Table S2. Longitudinal correlations between anti-HSF1 levels and disease activity.

Clinical parameters	β[#]	95% CI	*p*-value
Constitutional VAS	1.5	-0.8-3.7	0.195
Cutaneous VAS	-0.5	-4.1-3.1	0.782
Articular VAS	0.005	-0.1-0.1	0.933
Gastrointestinal VAS	0.1	-0.2-0.4	0.544
Pulmonary VAS	4.7	3.5-5.8	<0.001
Cardiovascular VAS	0.3	-1.0-1.5	0.664
Muscle VAS	4.3	2.3-6.3	<0.001
PGA VAS	2.9	1.1-4.7	0.001

HSF1: heat shock factor 1; VAS: visual analogue scales; PGA: physician global assessment of disease activity; 95% CI: 95% confidence interval.
[#]Generalised estimating equation (GEE) model was applied to analyse the correlations between serum anti-HSF1 levels and disease activity.

Anti-HSF1 ELISA

The 96-well ELISA plates (Thermo Scientific, Roskilde, Denmark) were coated with 200 ng/well of recombinant full-length human HSF1 protein (Abcam, Cambridge, UK), diluted in carbonate buffer (pH 9.6), and incubated overnight at 4°C. Wells were blocked with PBS containing 10%, v/v, horse serum (HS; Gibco, Grand Island, USA), 2%, w/v, sucrose (Solarbio, Beijing, China) for 2 hrs at 37°C. Human serum samples were diluted 1:50 in dilution buffer, which was PBS containing 0.05% Tween-20 and 10% HS, and incubated for 1hr at 37°C. And then, the plates were incubated with a horseradish peroxidase (HRP)-conjugated goat anti-human secondary antibody (Abcam, Cambridge, UK), diluted 1:15000, for 30 mins at room temperature (RT). The absorbance was measured at 450 nm (A_{450}). Similarly, another recombinant HSF1 protein (OriGene, Maryland, USA) was also applied for ELISA to detect anti-HSF1 autoantibodies in our study. All serum samples were examined in duplicate, and anti-HSF1$^+$ and anti-HSF1$^-$ serum measurements on every plate ensured functionality of the assay. Serial dilution ELISA was performed in 4 anti-HSF1$^+$ and 4 anti-HSF1$^-$ serum samples in 1:2 dilution steps to determine the optimal dilution ratio for anti-HSF1 detection. A cut-off value discriminating between anti-HSF1$^+$ and anti-HSF1$^-$ patients was defined as the mean A_{450} plus 2 times the SD of HC group.

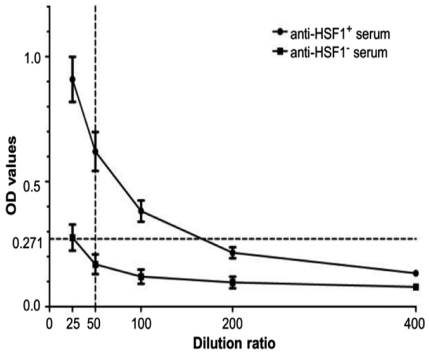
A Blocking ELISA was designed to validate the specificity of anti-HSF1 ELISA results. Serum of 3 anti-HSF1$^+$ IIM patients and 2 anti-HSF1$^-$ healthy controls were diluted and preincubated with the recombinant HSF1 protein (Abcam, Cambridge, UK) overnight at 4°C, and then tested for anti-HSF1 positivity by ELISA.

Immunoblot and dot blot assay

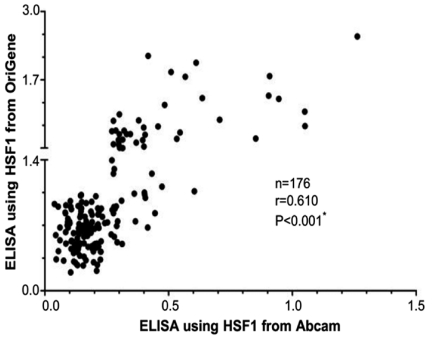
Immunoblot and dot blot were used for testing anti-HSF1 autoantibodies in serum samples from patients and HCs, and were performed as previously described with some modifications (1). Recombinant HSF1 protein (Abcam, Cambridge, UK) was electrophoresed on 10% SDS-polyacrylamide gels with 1ug/lane, and then transferred to nitrocellulose membranes at 200 mA for 1.5 hrs using a Bio-Rad Trans-Blot electrophoretic transfer cell. After blocked using 5% non-fat milk-TBST (TBS-0.05% Tween 20, PH 7.4), the membranes were incubated overnight at 4°C with 1:50 dilution of serum from patients and HCs (20 ul in 980 ul in blocking buffer). Membranes were then incubated at RT for 1 hr with an HRP-conjugated anti-human secondary antibody (Abcam, Cambridge, UK) diluted 1:1000 in blocking buffer. For dot blot assay, recombinant HSF1 protein (Abcam, Cambridge, UK) was applied onto nitrocellulose membranes with 200ng/dot, and blocked with 5% non-fat milk-PBST (PBS-0.1% Tween 20, PH 7.4) at RT for 2 hrs. The membranes were then incubated overnight at 4°C with serum from patients and HCs with a dilution of 1:500 in 2.5% non-fat milk-PBST (PBS-0.1% Tween 20, PH 7.4). Subsequently, the membranes were incubated with an HRP-conjugated anti-human secondary antibody (Abcam, Cambridge, UK) at RT for 1 hr. At last, the membranes were incubated in western blot detection reagent (Thermo Scientific, Roskilde, Denmark), stained bands or dots were visualised with ECL (GE Healthcare, USA).

Reference

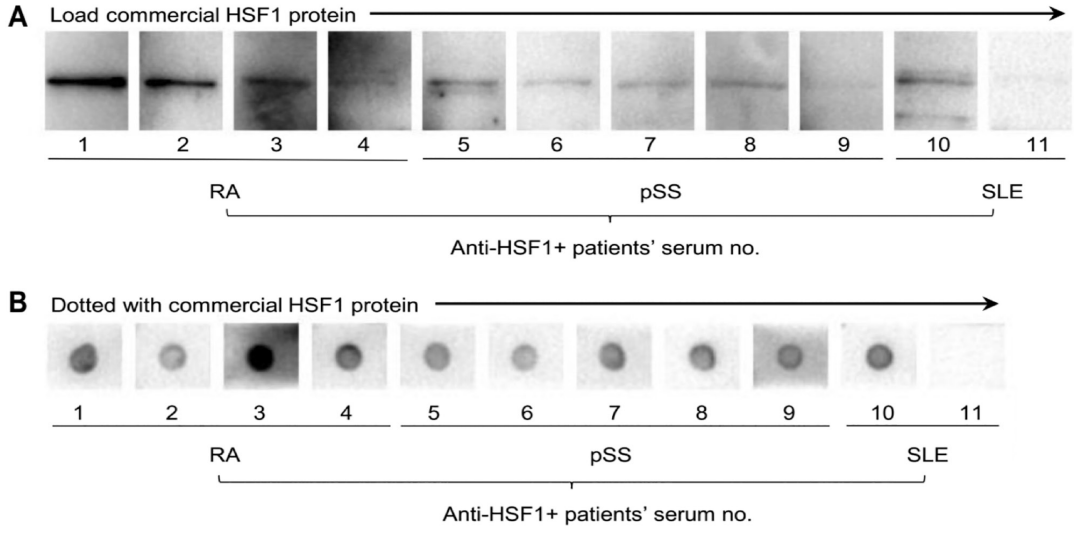
1. KOBAYASHI S, HOSHINO T, HIWASA T *et al*.: Anti-FIRs (PUF60) auto-antibodies are detected in the sera of early-stage colon cancer patients. *Oncotarget* 2016; 13: 82493-503.



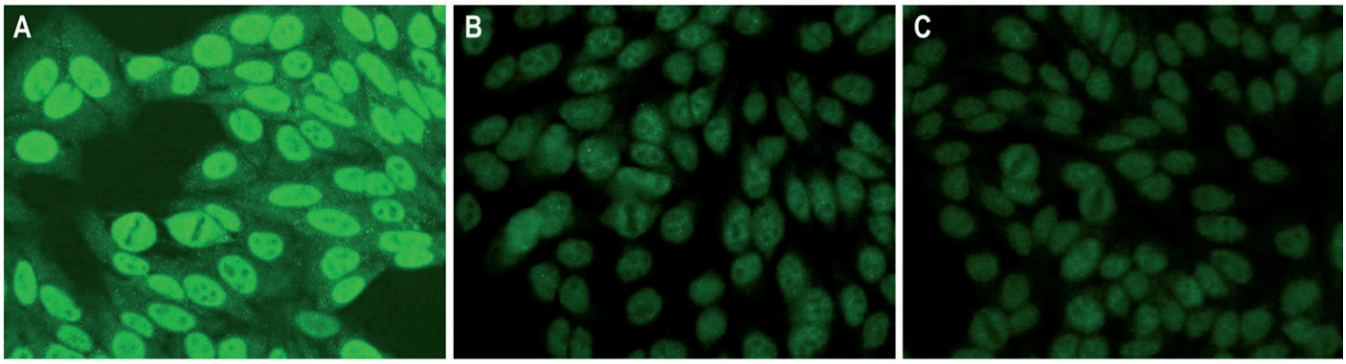
Supplementary Fig. 1. Serial dilution ELISA of anti-HSF1$^+$ and anti-HSF1$^-$ serum. Four anti-HSF1$^+$ and 4 anti-HSF1$^-$ serum samples were analysed by serial dilution ELISA for reactivity to recombinant HSF1 protein (Abcam). Starting dilution was 1:25 and serum were serially diluted in 1:2-steps. The vertical line indicates the used dilution (1:50) for anti-HSF1 detection by ELISA, the horizonal line indicates the cut-off value (0.271) for discriminating anti-HSF1$^+$ serum samples from anti-HSF1$^-$ serum samples. HSF1, heat shock factor 1; ELISA, enzyme-linked immunosorbent assay.



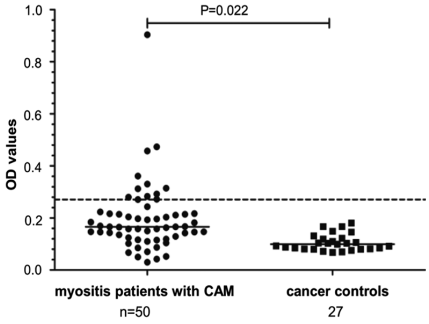
Supplementary Fig. 2. Correlation plots of anti-HSF1 levels obtained by ELISA using two different recombinant HSF1 proteins.
Serum anti-HSF1 levels of 143 IIM patients and 33 HCs detected by ELISA using HSF1 protein of Abcam were strongly correlated with the levels detected by ELISA using HSF1 protein of OriGene (r=0.610, p<0.001). The HSF1 protein of Abcam was derived from E.coli expression system, while the HSF1 protein of OriGene was derived from eukaryotic HEK293 expression system. **p<0.05. HSF1, heat shock factor 1; ELISA, enzyme-linked immunosorbent assay; IIM, idiopathic inflammatory myopathy.



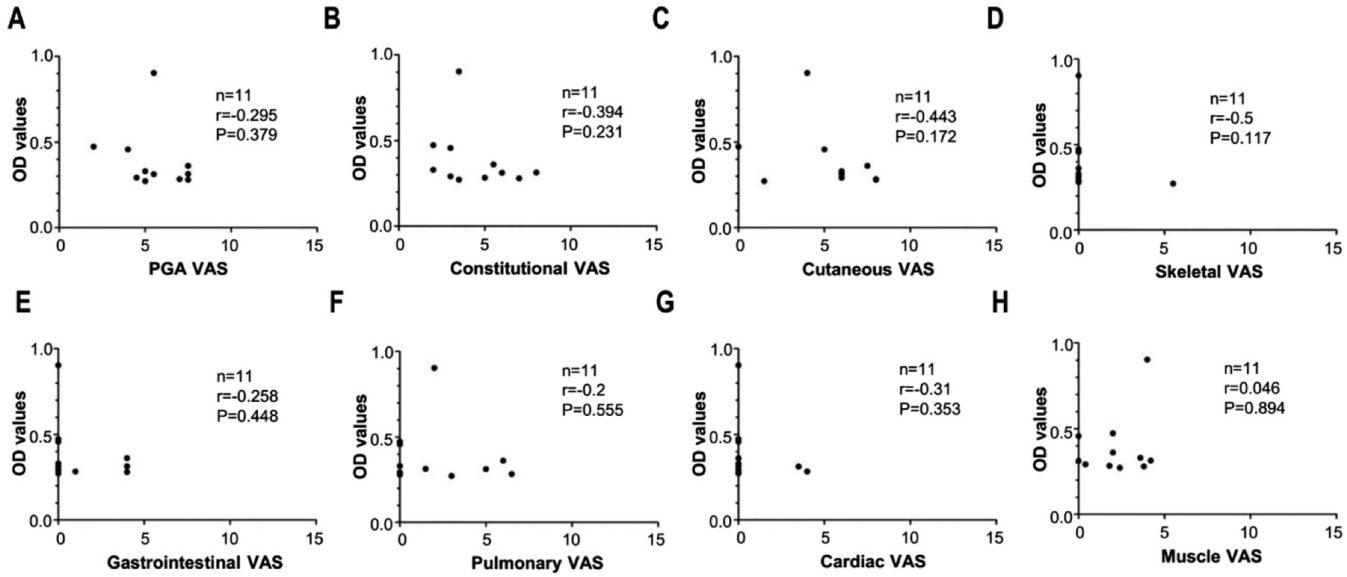
Supplementary Fig. 3. Immunoblot and dot blot assay of anti-HSF1 autoantibodies in disease controls.
A: Immunoblotting using commercial HSF1 protein (Abcam) in 11 anti-HSF1$^+$ patients, including 4 with RA, 5 with pSS, and 2 with SLE.
B: Dot blotting with commercial HSF1 protein (Abcam) in 11 anti-HSF1$^+$ patients, including 4 with RA, 5 with pSS, and 2 with SLE.
HSF1: heat shock factor 1; RA: rheumatoid arthritis; pSS: primary Sjögren's syndrome; SLE: systemic lupus erythematosus.



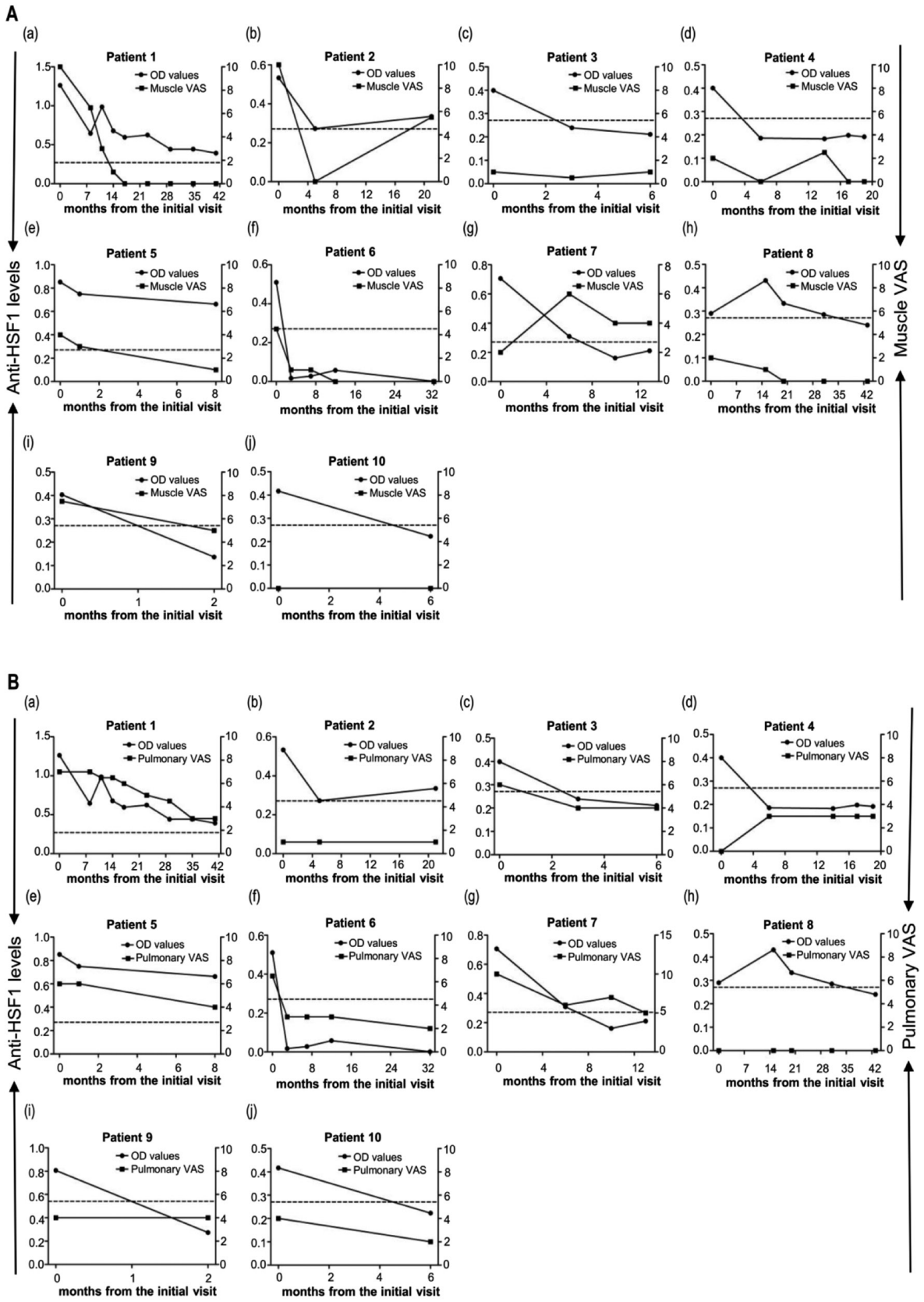
Supplementary Fig. 4. Indirect immunofluorescence assay on HEP2 cells of anti-HSF1$^+$ IIM patients without autoantibodies to currently known myositis autoantigens and ENA.
A: Specked (≥1:160) and Cytoplasmic (≥1:80) pattern.
B: Specked (≥1:40) and Cytoplasmic (≥1:40) pattern.
C: Specked (≥1:40) pattern.
HSF1: heat shock factor 1; IIM: idiopathic inflammatory myopathy; ENA: extractable nuclear antigens.



Supplementary Fig. 5. The prevalence of anti-HSF1 autoantibodies in CAM patients and cancer controls.
Anti-HSF1 autoantibodies could be detected in 22% (11/50) CAM patients, but 0% (0/27) cancer controls (x^2=5.25, p=0.022). The horizonal line indicates the cut-off value (cut-off = mean [A450 HC] + 2×SD=0.271) used for discriminating anti-HSF1$^+$ patients from anti-HSF1$^-$ patients. HSF1: heat shock factor 1; CAM: cancer-associated myositis.



Supplementary Fig. 6. Serum anti-HSF1 levels are not correlated with disease activity in anti-HSF1$^+$ CAM patients.
Spearman correlation analyses in 11 anti-HSF$^+$ CAM patients were used to detect the correlations between anti-HSF1 levels and disease activity indicators of IIM such as PGA (**A**), constitutional (**B**), cutaneous (**C**), skeletal (**D**), gastrointestinal (**E**), pulmonary (**F**), cardiac (**G**) and muscle (**H**) VAS.
HSF1: heat shock factor 1; CAM: cancer-associated myositis; IIM: idiopathic inflammatory myopathy; PGA: physician global assessment of disease activity; VAS: visual analogue scale.



Supplementary Fig. 7. Longitudinal changes in serum anti-HSF1 levels and muscle (**A**)/pulmonary (**B**) VAS over time in 10 anti-HSF1$^+$ IIM patients without cancer.

The horizonal line indicates the cut-off value (cut-off = mean [A450 HC] + 2×SD=0.271) used for discriminating anti-HSF1$^+$ patients from anti-HSF1$^-$ patients.

HSF1: heat shock factor 1; IIM: idiopathic inflammatory myopathy; VAS: visual analogue scales.