Anti-RuvBL1/2

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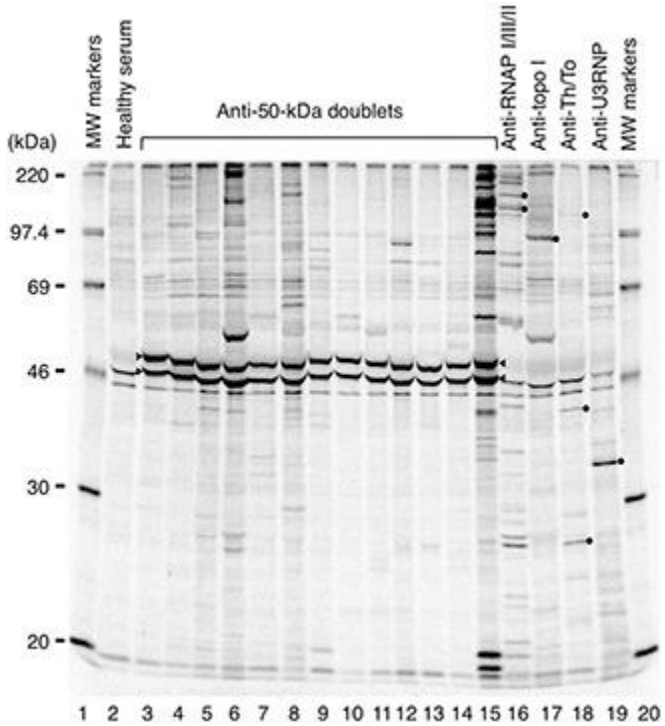
Article	Kaji et al. (2014)	Takahashi et al. (2016)	Pauling et al. (2018)	Nomura et al. (2020)	Landon-Cardinal et al. (2020)
Number of patients	37	1	2	1	2
Detection method	IP with radioactive labeled protein, IP-western blot	IP not further specified	IP with radioactive labeled protein	IP not further specified	IP with radioactive labeled protein
HEp-IFA results	10 nuclear speckled*, 4 nuclear speckled* + cytopl. granular, 27 NA	Nuclear speckled 1/2560	2 negative**	NA	2 nuclear speckled
Clinical phenotype	19 SSc-IIM 18 SSc (13 dcSSc, 5 lcSSc)	1 SSc-IIM	1 SSc-IIM 1 SSc-SjS	1 SSc-IIM	2 SSc-IIM



Detection of autoantibodies reactive with the 50-kd doublets by a protein immunoprecipitation assay.

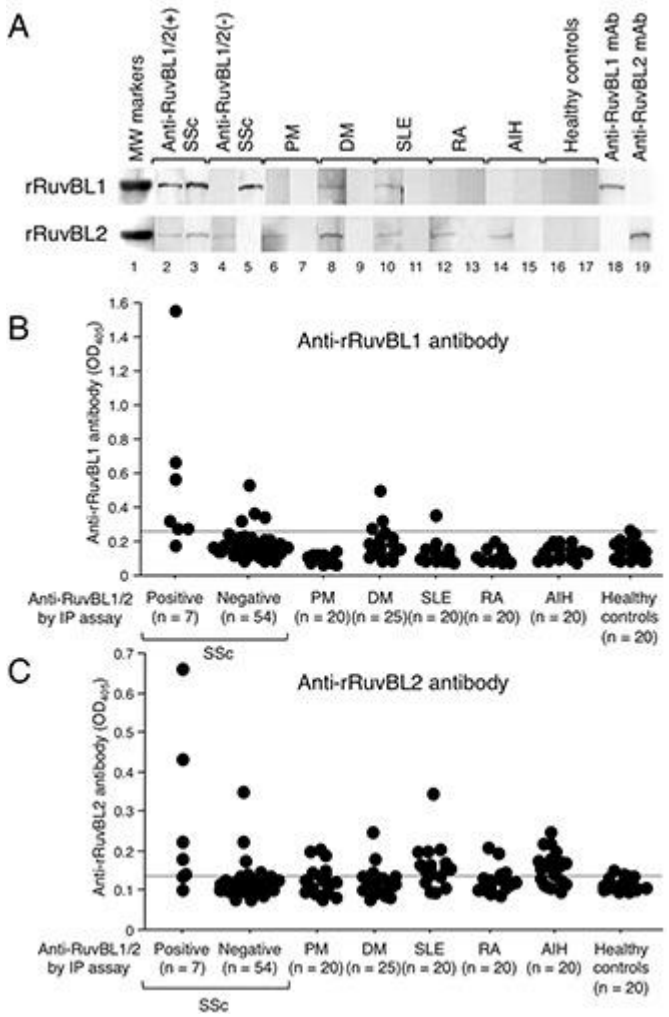
Immunoprecipitates from ^{35}S-methionine–labeled K562 cellular extracts were subjected to 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by autoradiography.

Lanes 1 and 20 = molecular weight (MW) markers; lane 2 = healthy serum; lanes 3–15 = sera from systemic sclerosis (SSc) patients positive for the anti–50-kd doublets (RuvBL1/2) from the Kanazawa cohort (lanes 3–8) and Pittsburgh cohort (lanes 9–15); lane 16 = SSc serum with anti–RNA polymerase I/III/II (anti–RNAP I/III/II); lane 17 = SSc serum with anti–topoisomerase I (anti-topo I); lane 18 = SSc serum with anti-Th/To; lane 19 = SSc serum with anti–U3 RNP.

Arrowheads show the 50-kd doublets.

Dots in sera positive for anti–topo I, anti–RNAP III, anti-Th/To, and anti–U3 RNP show the main target antigens.

Kaji K, et a. Autoantibodies to RuvBL1 and RuvBL2: a novel systemic sclerosis-related antibody associated with diffuse cutaneous and skeletal muscle involvement. Arthritis Care Res (Hoboken). 2014;66:575-84.



Detection of antibodies to recombinant RuvBL1 and RuvBL2 (rRuvBL2 and rRuvBL2).

A, Immunoblots using rRuvBL1 (upper) and rRuvBL2 (lower) as antigens. Systemic sclerosis (SSc) sera positive for anti-RuvBL1/2 antibody by immunoprecipitation (IP) assay (lanes 2 and 3), SSc sera negative for anti-RuvBL1/2 antibody by IP assay (lanes 4 and 5), polymyositis (PM) sera (lanes 6 and 7), dermatomyositis (DM) sera (lanes 8 and 9), systemic lupus erythematosus (SLE) sera (lanes 10 and 11), rheumatoid arthritis (RA) sera (lanes 12 and 13), autoimmune hepatitis (AIH) sera (lanes 14 and 15), healthy control sera (lanes 16 and 17), anti-RuvBL1 monoclonal antibody (mAb; lane 18), and anti-RuvBL2 mAb (lane 19) are shown. Lane 1 indicates molecular weight (MW) markers, and a band corresponds to 75 kd.

B and C, Antibodies to rRuvBL1 and rRuvBL2 measured by enzyme-linked immunosorbent assay in 61 sera from SSc patients, 20 from PM patients, 25 from DM patients, 20 from SLE patients, 20 from RA patients, 20 from AIH patients, and 20 from healthy controls. SSc patients were divided into 2 groups based on the presence or absence of anti-RuvBL1/2 antibodies detected by IP assay. Broken lines show cutoff levels for positivity, which were set at 2 SDs above the mean of healthy controls (0.25 for anti-rRuvBL1 antibody and 0.14 for anti-rRuvBL2 antibody).

OD405 = optical density at 405 nm.

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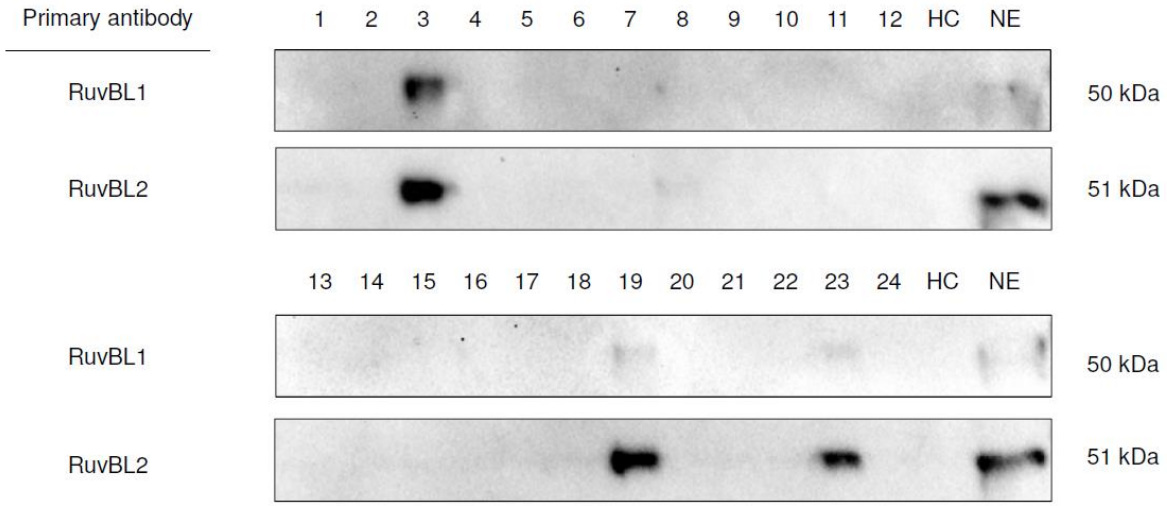
Function RuvBL1/2 complex:

- transcription
- DNA repair
- chromatin remodeling
- small nucleolar RNP assembly



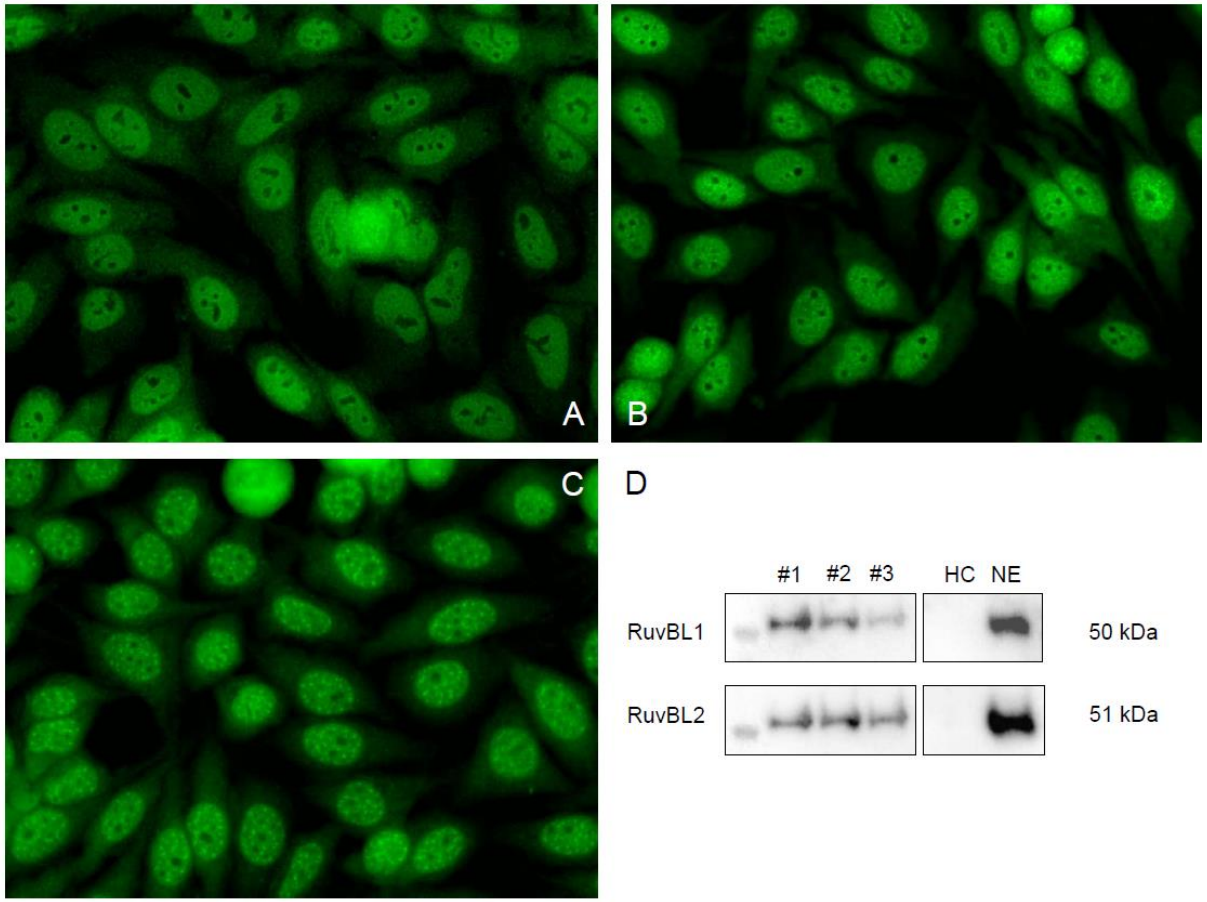
Immunoprecipitation-western blot analysis of 24 patients with SSc, SSc-IIM or IIM with a nuclear speckled pattern on HEp-IIF

HC healthy control, NE HeLa nuclear extract (25 µg).

IP was performed with a HeLa nuclear extract (100 µg) and human serum (1/30 5% non-fat milk/TBS) cross-linked to magnetic beads (Pierce A/G Magnetic Beads, Thermo Fisher Scientific, USA) with 5 mM BS3 (Thermo Fisher Scientific, USA).

Polyclonal rabbit anti-RuvBL1 antibodies (1/2000 in 5% BSA/TBS; OriGene, USA) and anti-RuvBL2 antibodies (1/2000 in 5% BSA/TBS after stripping; OriGene, USA) were used as primary antibody and Veriblot IgG (1/1000 in 5% non-fat milk/TBS; Abcam, UK) as secondary antibody.

Membranes were developed with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, USA), visualized with the Chemidoc XRS+ System (Bio-Rad, USA) and analyzed with ImageLab software (Bio-Rad, USA).



A-C HEp-2 indirect immunofluorescence analysis of the three anti-RuvBL1/2-positive patients, performed with Quanta Lite® ANA at 1/80 dilution, visualized with NOVA View (both Inova, USA)

D Immunoprecipitation-western blot of anti-RuvBL1/2 positive patients. #1-3 anti-RuvBL1/2 positive patients (corresponding to panel A-C respectively), HC healthy control, NE HeLa nuclear extract (25 µg).

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	Case 1	Case 2	Case 3
Sex	M	F	F
Age at diagnosis	52 y	29 y	70 y
2013 EULAR/ACR SSc	Yes	No	Yes
2017 EULAR/ACR IIM	Probable IIM	Probable IIM	No
Cutaneous involvement	Limited SSc	No	Limited SSc
Myopathy	Yes	Yes	Yes
Arthritis	No	No	Yes (PIPs and MCPs)
Raynaud phenomenon	No	Yes	Yes
Digital ulcers or scars	No	No	Yes
Cardiac involvement	Yes (heart failure, arrhythmia)	No	No
GI involvement	No	No	No
Interstitial lung disease*	Yes (fNSIP)	No	No (UIP)
Pulmonary arterial hypertension	No	No	No
Scleroderma renal crisis	No	No	No
Initial manifestation	Myopathy, cutaneous involvement, ILD	Myopathy	Myopathy, arthritis
HEp-2-IIFA pattern(s), maximal titre(s)	Nuclear fine speckled, 1/1280 + cytoplasmic fine speckled, 1/320	Nuclear fine speckled, 1/320 + cytoplasmic dense fine speckled, 1/80	Nuclear fine speckled, 1/320 + cytoplasmic dense fine speckled, 1/80
Response to treatment	Poor (CS + MMF + CYC)	Good (CS + AZA)	Good (CS + MTX)

- Acknowledgments

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  - Ellen De Langhe
  - Steven Vanderschueren
  - Daniel Blockmans
  - Patrick Verschueren