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Supplementary file 1

Supplementary methods

Monoclonal antibodies against newly identified centromere autoantigens

The antigen-binding beads were incubated with a monoclonal antibody, which were cloned from antibody secreting cells in human salivary glands, at 1 μg/ml in staining buffer (phosphate-buffered saline with 2 mM ethylenediaminetetraacetic acid and 0.5% bovine serum albumin). After washing with staining buffer, the beads were stained with APC-conjugated anti-human IgG-Fc antibody. After washing, the beads were analysed by FACSVerse and FlowJo software.

Direct detection of antibody-producing cells in salivary glands

SBP-GFP fusion antigens were expressed by 293T cells and purified with Streptavidin Sepharose High Performance (Cytiva Japan, Tokyo, Japan). The purity of the antigens was assessed by Coomassie Brilliant Blue staining, and the concentration was measured by the Quick Start Bradford Protein Assay (Bio-Rad, CA, USA). Fresh-frozen sections of labial salivary gland samples were incubated with GFP fusion antigens and anti-CD138 antibody (unconjugated, MI15, BioLegend). After washing, the samples were stained with anti-mouse IgG1 antibody (Alexa Fluor 594, goat, Thermo Fisher Scientific). ASCs were semiquantified using an LSM 710 microscope and ZEN 3.0 software (ZEISS, Oberkochen, Germany). The detailed methods were previously described.[17]

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Supplementary Table S1. Diagnostic performance of antigen-binding bead assay with various cut off values

	Cut off values of identified ACAs		
	≦ 1 antibody	≦ 2 antibodies	≦ 3 antibodies
Sensitivity	0.69	0.49	0.41
Specificity	0.82	0.97	0.97
Positive likelihood ratio	3.9	17	14

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Supplementary Table S2. Comparison of the clinical characteristics of SS patients with 2 or more ACAs by antigen-binding bead assay with or without anti-CENP-B antibody by ELISA

	Anti-CENP-B antibody		p value
	Negative n=13	Positive n=11	
Age (y), median (IQR)	63 (41-71)	68 (55-74)	0.21
Female %	92	100	1.00
Disease duration (m), median (IQR)	192 (12-246)	108 (48-144)	0.087
Dry eye %	69	91	0.33
Dry mouth %	100	91	0.46
Extraglandular symptom %	23	0	0.22
sSS %	7.7	36	0.14
Anti-SSA antibody %	92	72	0.30
Anti-SSB antibody %	46	18	0.21
IgG (mg/dL), median (IQR)	2171 (1599-2561)	1582 (1363-1744)	0.13
IgA (mg/dL), median (IQR)	308 (215-713)	219 (196-313)	0.24
IgM (mg/dL), median (IQR)	91 (33-159)	88 (67-167)	1.00
RF (IU/mL), median (IQR)	59 (21-355)	16 (10-32)	0.082

RF, rheumatoid factor; sSS, secondary SS.

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Supplementary Table S3. Comparison of the clinical characteristics of SSc patients with 2 or more ACAs by antigen-binding bead assay with or without anti-CENP-B antibody by ELISA

	Anti-CENP-B antibody		
	Negative	Positive	
	n=8	n=13	p value
Age (y), median (IQR)	59 (42-76)	59 (50-70)	0.97
Female %	75	100	0.13
Disease type			
dcSSc %	62.5	0	<0.01
lcSSc %	37.5	100	<0.01
Disease duration (y), median (IQR)	15 (8.0-22)	9.3 (6.3-14)	0.22
Anti-Topo 1 %	50	8	0.11
Anti-U1RNP %	0	0	1.00
Anti-RNAPIII %	0	0	1.00
IgG (mg/dL), median (IQR)	1544 (1248-1689)	1362 (1016-1490)	0.22
CRP (mg/dL), median (IQR)	0.17 (0.038-0.36)	0.04 (0.015-0.16)	0.18
mRSS, median (IQR)	9 (3-18)	3 (2-4)	0.018
Digital ulcer %	25	8	0.53
Pulmonary hypertension %	0	0	1.00
Interstitial lung disease %	75	31	0.081
Cardiac involvement %	13	8	1.00
Upper GI symptom %	50	38	0.67
Lower GI symptom %	25	0	0.13
Joint involvement %	0	8	1.00

GI, gastrointestinal; mRSS, modified Rodnan total skin thickness score.

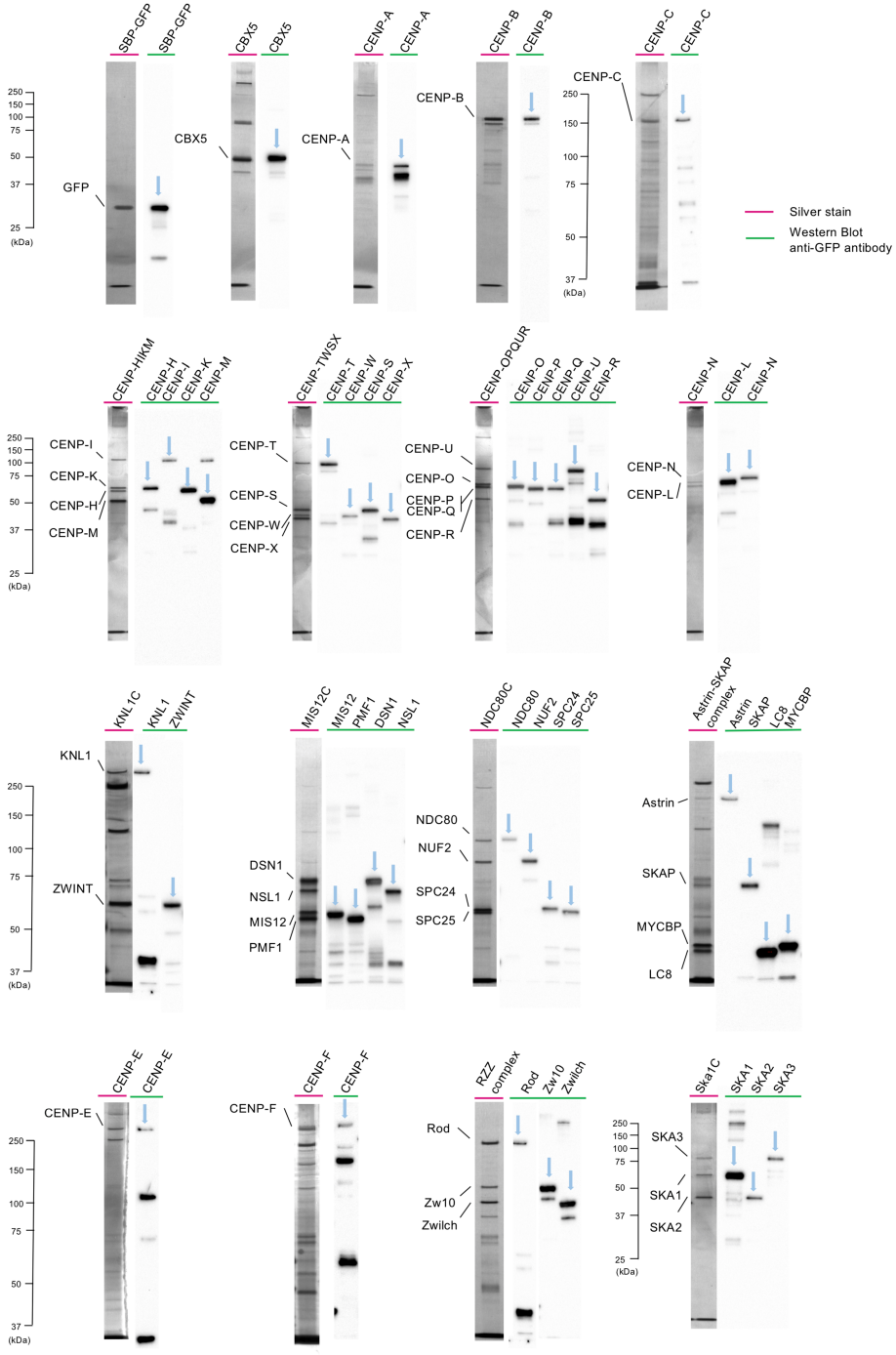
Supplementary Table S4. Comparison of the clinical characteristics of PBC patients with 2 or more ACAs by antigen-binding bead assay with or without anti-CENP-B antibody by ELISA

	Anti-CENP-B antibody		
	Negative	Positive	p value
	n=2	n=3	
Age (y)	59(55-63)	57(57-77)	0.55
Female %	100	100	1.00
Ursodeoxycholic acid dose (mg/day), median (IQR)	150(0-300)	600(0-600)	0.36
Anti-mitochondrial antibody %	100	100	1.00
IgG (mg/dL), median (IQR)	1902 (1841-1964)	945 (934-958)	0.083
Total bilirubin (mg/dL), median (IQR)	0.6 (0.6-0.6)	0.7 (0.7-0.7)	0.046
Aspartate aminotransferase (U/mL), median (IQR)	34 (20-47)	19 (16-25)	0.25
Alanine aminotransferase (U/mL), median (IQR)	32 (10-54)	13 (9-18)	0.56
Alkaline phosphatase (U/mL), median (IQR)	230 (179-281)	312 (215-344)	0.25
γ-glutamyl transferase (U/mL), median (IQR)	95 (95-95)	23 (20-26)	0.18
Albumin (g/dL), median (IQR)	4.0 (3.8-4.1)	4.1 (4.1-4.5)	0.20
Platelet count (10^3/μL), median (IQR)	21 (20-23)	28 (25-29)	0.083

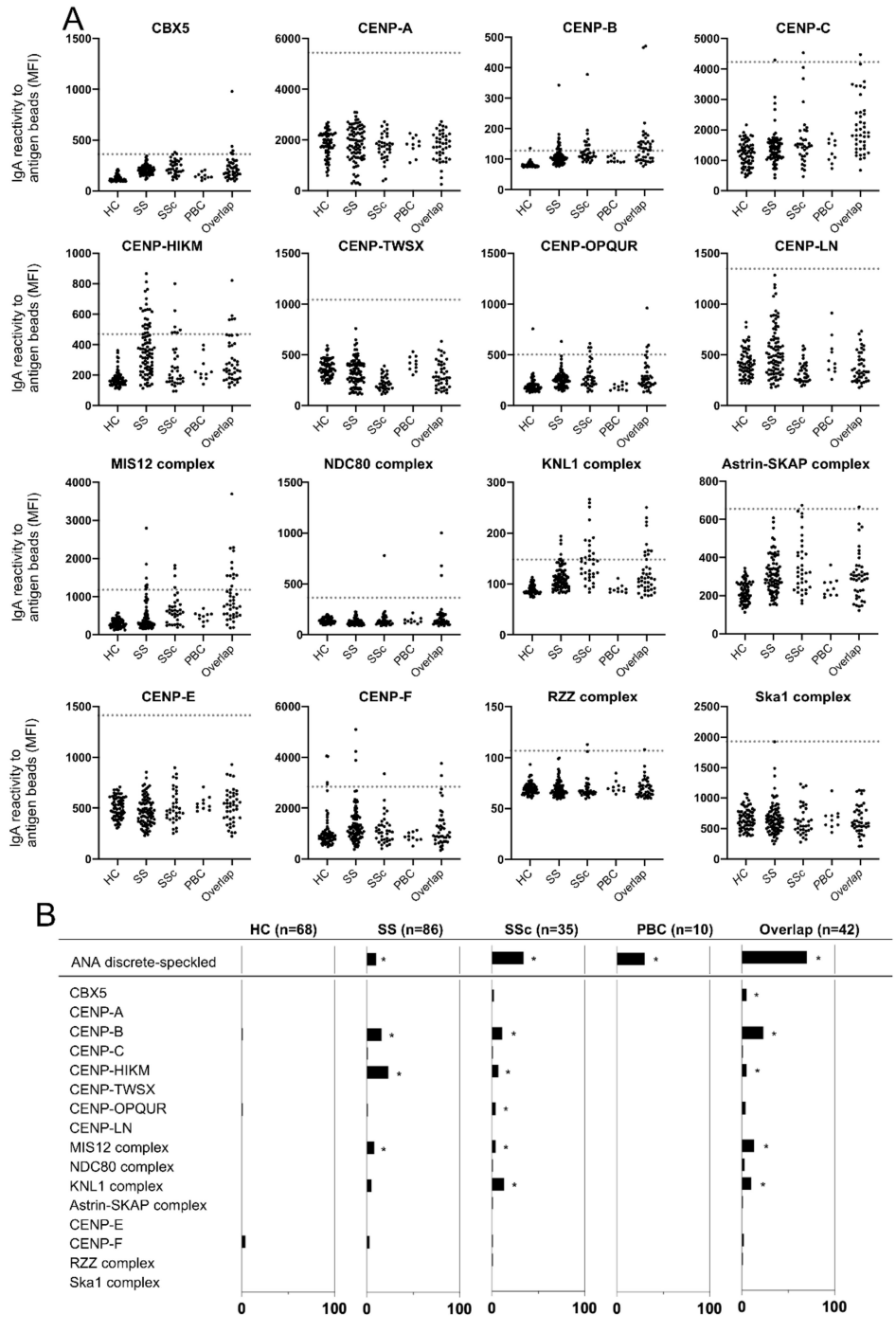
Supplementary Table S5. Lesion antibody specificity against centromere antigens

Patient ID	LB32	LB02	LB01	LB23	LB11	LB25	LB12	LB24	LB20
Serum ANA discrete speckled pattern	+	-	-	-	-	-	-	-	-
Serum anti-CENP-B antibody	+	-	-	-	-	-	-	-	-
Antibody produced, n	39	29	16	16	30	37	21	19	54
Previously identified anti-centromere antibodies ∗ , n	15	8	1	0	0	0	0	0	0
Anti-CENP-HIKM, n	2	0	0	0	0	0	0	0	0
Anti-CENP-TWSX, n	0	0	0	0	0	0	0	0	0
Anti-CENP-OPQUR, n	0	1	0	0	0	0	0	0	0
Anti-CENP-LN, n	0	0	0	0	0	0	0	0	0
Anti-NDC80 complex, n	0	1	0	0	0	0	0	0	0
Anti-KNL1 complex, n	0	0	0	0	0	0	0	0	0
Anti-Astrin-SKAP complex, n	0	0	0	0	0	0	0	0	0
Anti-CENP-E, n	0	0	0	0	0	0	0	0	0
Anti-CENP-F, n	0	0	0	0	0	0	0	0	0
Anti-RZZ complex, n	0	0	0	0	0	0	0	0	0
Anti-Ska1 complex, n	0	0	0	0	0	0	0	0	0
Total anti-centromere antibodies, n (%)	17 (44)	10 (34)	1 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

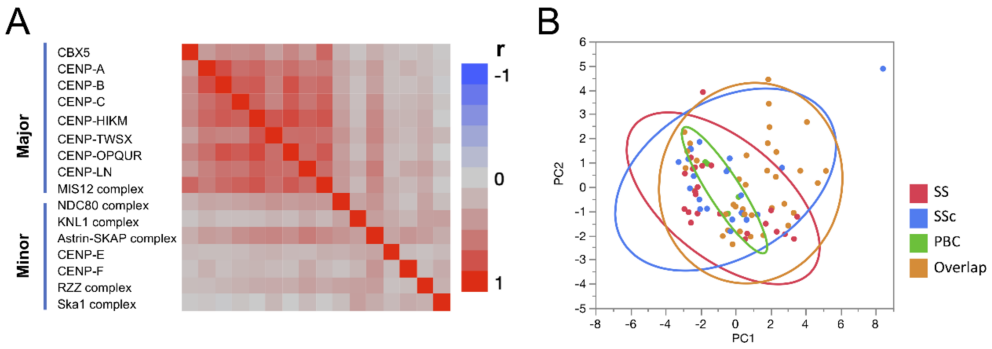
∗ Anti-CBX5, anti-CENP-A, anti-CENP-B, anti-CENP-C, and anti-MIS12 complex antibodies



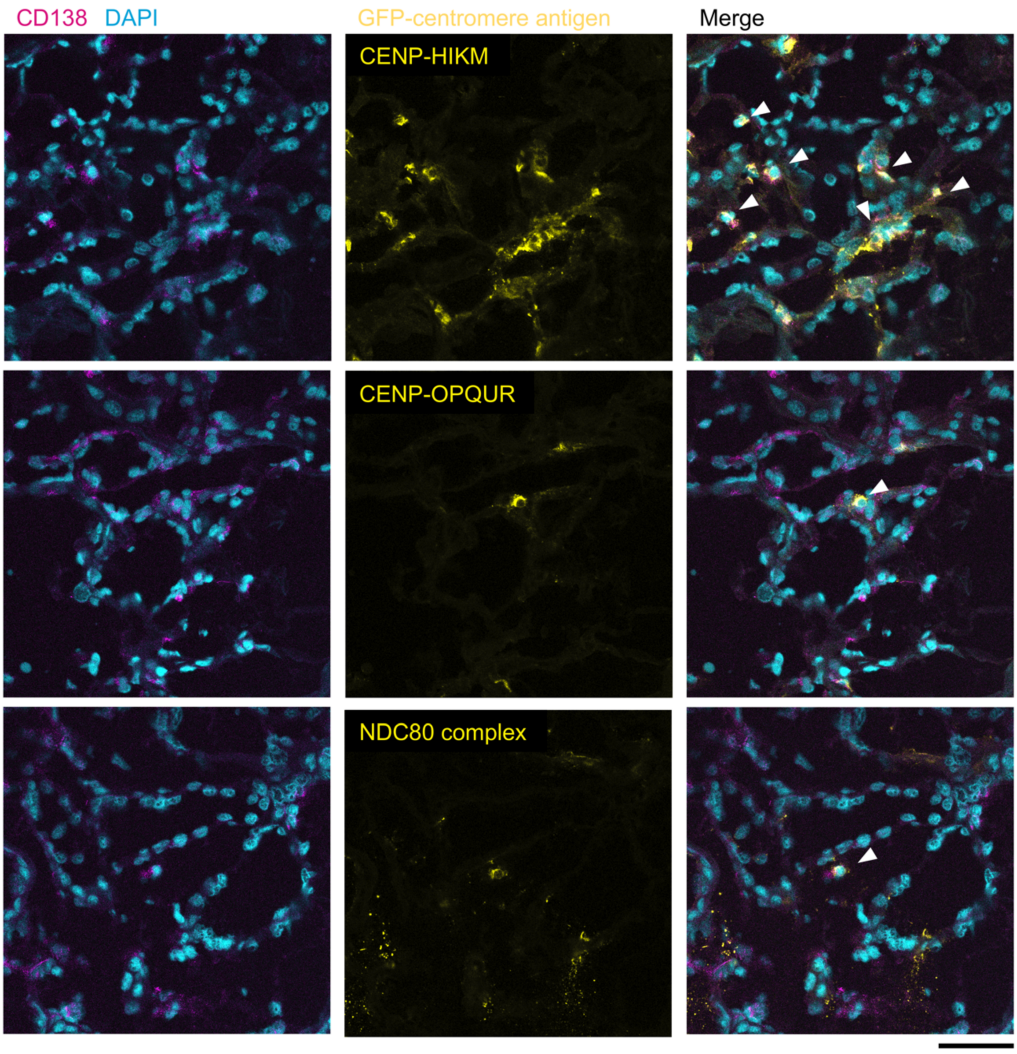
Supplementary figure S1. Protein expression of the centromere antigen library. SBP-GFP-tagged antigens were purified using streptavidin beads and electrophoresed followed by silver staining. Component proteins of each antigen were electrophoresed and blotted with anti-GFP antibody.


A
CBX5
CENP-A
CENP-B
CENP-C
CENP-HIKM
CENP-TWSX
CENP-OPQUR
CENP-LN
MIS12 complex
NDC80 complex
KNL1 complex
Astrin-SKAP complex
CENP-E
CENP-F
RZZ complex
Ska1 complex
IgA reactivity to antigen beads (MFI)
HC
SS
SSc
PBC
Overlap
B
HC (n=68)
SS (n=86)
SSc (n=35)
PBC (n=10)
Overlap (n=42)
ANA discrete-speckled
CBX5
CENP-A
CENP-B
CENP-C
CENP-HIKM
CENP-TWSX
CENP-OPQUR
CENP-LN
MIS12 complex
NDC80 complex
KNL1 complex
Astrin-SKAP complex
CENP-E
CENP-F
RZZ complex
Ska1 complex
0
100

Supplementary Figure S2. Profiling of serum IgA ACAs. Serum IgG autoantibody titers against each centromere antigen were analysed by the antigen-binding bead assay with the sera of patients with SS (n=86), SSc (n=35), PBC (n=10), or overlap disease (n=42), and healthy controls (HC; n=68). (A) Each symbol represents an antibody level in an individual's serum, and the dotted line indicates cut-off value, which was determined by the median plus 5IQR of MFI in HC. (B) Bar graphs show the prevalence of autoantibodies against centromere antigens, measured as the MFI in each disease group. The prevalence of anti-CENP-B antibody by ELISA is shown at the top. *$p<0.05$ between each disease group and the HC group; † Novel autoantigen as a form of a complex, at least 1 component molecule is known as an autoantigen; ‡ novel autoantigen identified in this assay; ANA, anti-nuclear antibody; ELISA, enzyme-linked immunosorbent assay; MFI, mean fluorescence intensity. The data of CBX5, CENP-A, CENP-B, CENP-C, and MIS12 complex were obtained from our previous study. [17]



Supplementary figure S3. Quantitative analysis of the association of serum anti-centromere IgG antibodies. (A) The heatmap shows the correlation between the titers of serum IgG antibodies against each centromere autoantigen in patients with SS, SSc, PBC, or overlap disease, and HC. (B) Principle component analysis of the titers of serum IgG antibodies against 16 antigens in patients with 2 or more ACAs. Each dot represents an individual with SS (n=23), SSc (n=21), PBC (n=5), or overlap (n=36). The colored circles show the 95% confidence ellipse of each disease group, demonstrating overlapped distribution.



Supplementary figure S4. Representative confocal images of anti-centromere antibody-secreting cells (ASCs) in the salivary glands of patients with SS (magnification, x400). Sections of salivary gland samples obtained from a serum ACA-positive patient (S3) were stained with GFP-conjugated centromere antigens (yellow), CD138 (magenta), as a marker of plasma cells, and DAPI (cyan). Arrowheads indicate ASCs against centromere antigens; CENP-HIKM, CENP-OPQUR, and NDC80 complex. Scale bar: 50 μm.