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Supplementary methods

Clinical samples

Labial salivary grands were collected from patients who were suspected of having SS and underwent biopsy for diagnosis. Serum samples were collected from patients who were suspected of having SS, or who were diagnosed as SSc or PBC at Keio University Hospital. The classification of SS, SSc, and PBC was conducted according to the classification criteria for primary SS established in 2016[4], classification criteria for SSc established in 2013[8], and criteria established in 2017[37], respectively. The following parameters were collected from medical charts: anti-nuclear antibody (ANA) titer and type; serum positivity for anti-SSA, anti-SSB, anti-CENP-B antibody (determined by ELISA, MBL, Nagoya, Japan), and rheumatoid factor (RF, measured by a latex agglutination test); Greenspan grade of the local lymphocytic sialadenitis of a labial salivary gland biopsy; and medication history. This study was approved by the Institutional Review Board of our University School of Medicine and conducted in compliance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participating individuals.

Preparation of single-cell cDNA library of ASCs from the labial salivary grands

A single-cell suspension was prepared according to previously published methods[24] with minor modifications. In brief, labial salivary gland tissue was mechanically and enzymatically digested in Dulbecco's modified Eagle's medium (DMEM) containing 1 mg/ml collagenase type 2 (Worthington, NJ, USA) at 37 °C in 5% CO_2 for 30 min. After filtering through a 40 μm cell strainer, the cells were stained with fluorochrome-conjugated antibody against CD3, CD4, CD8, CD19, CD38, and CD326 and with 7-aminoactinomycin D (7AAD). The live ASCs ($7AAD^-CD3^-CD4^-CD8^-CD326^-CD19^+CD38^{high}$) were bulk sorted using a FACSAria III flow cytometer (BD Biosciences), and then single-cell cDNA libraries were created using a C1 system (Fluidigm, CA, USA) or Smart-seq2. For the C1 system, the cells were processed in Single-cell Auto Prep Array IFC (5-10 μm) according to the manufacturer's instructions. Smart-seq2 was performed as previously described[32].

Production of an antibody expression vector

IgH, Igλ and Igκ gene transcripts were amplified by PCR according to previously published methods[20] with some modifications. The overall strategy is shown in online supplementary figure S1. Single-cell cDNA libraries amplified by C1 at a 1:10 dilution or by Smart-seq2 without dilution were used as templates. First, PCR was performed with a total volume of 20 μl containing 1 μl of cDNA library, 300 nM of each primer, and 10 μl of KAPA HiFi HS ReadyMix (KAPA Biosystems, MA, USA). The cycling parameters were as follows: 95 °C for 3 min; 30 cycles at 98 °C for 20 s, 65 °C for 15 s, and 72 °C for 30 s; and 72 °C for 1 min. To clone the IgH chain, PCR products were electrophoresed on a 0.8% agarose gel, and targeted bands were extracted from the gel using the Nucleospin Gel and PCR clean-up Kit (Takara Bio, Shiga, Japan) and sequenced (Eurofins, Tokyo, Japan). SHM status and gene usage were analyzed by IMGT/V-

QUEST (http://www.imgt.org/IMGT_vquest/vquest). A second PCR was performed using a forward primer mixture and reverse primer that was created based on its junction sequence in a total volume of 20 µl containing 1 µl of 1st PCR product, 300 nM of each primer, and 10 µl of KAPA HiFi HS ReadyMix. The cycling parameters were as follows: 95 °C for 3 min; 15 cycles at 98 °C for 20 s, 65 °C for 15 s, and 72 °C for 30 s; and 72 °C for 1 min. When the 2nd PCR failed to generate the appropriate band of the IgH-V region, the forward primer was individually designed for each cell based on the sequence information. To clone an Igλ or Igκ chain, the 2nd PCR was performed using a forward primer mixture and a reverse primer mixture (for Igλ) or a reverse primer (for Igκ) in a total volume of 20 µl containing 1 µl of cDNA, 300 nM each primer, and 10 µl of KAPA HiFi HS ReadyMix. The products of the second PCR were electrophoresed, purified and inserted into the expression vector for IgH or IgL using NEBuilder HiFi DNA Assembly Master Mix (New England Biolabs, MA, USA) according to the manufacturer's instructions. The expression vectors were transformed into DH5α cells (TOYOBO, Osaka, Japan) and isolated using the PureYield Plasmid Miniprep System (Promega, WI, USA). The expression vectors were sequenced to confirm identity with the original PCR products. List of the primer used are shown in online supplementary table S1. List of the lesion antibody panel is shown in online supplementary table S2.

Antibody production and purification

Antibody were produced by transient cotransfection of IgH and IgL vectors using the Expi293 Expression System (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions. Supernatants were collected after 7 days of culture, and antibody were purified using Ab Capcher Mag (Protenova, Kagawa, Japan). Purities and concentrations of recombinant antibody were determined by SDS-PAGE and CBB staining using a 12.5% Supersep precast gel and Quick CBB Plus (FUJIFILM Wako, Osaka, Japan); recombinant human IgG1 was used as a quantitative control.

ELISAs

The polyreactivity of each antibody was tested by polyreactivity ELISA as previously described[22] with minor modifications. Briefly, lipopolysaccharides (LPS, Sigma-Aldrich, MO, USA, 10 µg/ml), insulin (Thermo Fisher Scientific, 5 µg/ml), or dsDNA (plasmid, 10 µg/ml) was coated on a microtiter plate overnight at room temperature (RT). The plates were washed three times with phosphate-buffered saline (PBS) with 0.05% Tween-20 (PBS-T) and blocked with ELISA Blocking Buffer (Bethyl Laboratories, TX, USA) for 2 hours. After washing, the plates were reacted with antibody diluted in ELISA Blocking Buffer with 0.05% Tween-20 for 2 hours. After washing, the plates were reacted with an HRP-conjugated anti-human IgG antibody (0.66 µg/ml, Jackson ImmunoResearch, PA, USA) for 1 hour, washed, and reacted with the TMB Substrate Set (BioLegend, CA, USA) for 10 min. Reactions were stopped with H_2SO_4, and the optical density at 450 nm (OD 450) was measured. We considered antibody binding to two or more of the three antigens to be polyreactive.

mAb reactivity with SSA52, SSA60, and SSB was tested using anti-SSA52, anti-SSA60, and anti-SSB ELISAs (ORGENTEC, Mainz, Germany). mAb reactivity with CENP-B was tested using an anti-

CENP-B ELISA (ORGENTEC) and MESACUP-2 test CENP-B (MBL). These ELISAs were performed at a concentration of 2 μg/ml for each mAb, and cutoffs were determined as 80% quantile + 3 × (80% quantile - 20% quantile).

Western blot analysis

Fragmented SSB cDNAs (1-107 AA, 108-242 AA, and 243-408 AA) were cloned into the pcDNA3.4 vector (Thermo Fisher Scientific) combined with green fluorescent protein (GFP) and a linker (GGGGS) at the N-terminus. These vectors were transfected into 293T cells using polyethyleneimine (Polysciences, PA, USA). Two days after transfection, the cells were lysed in Tris-buffered saline containing 1% Triton X-100 (TBSTx) with a protease inhibitor cocktail (FUJIFILM Wako). Cell lysates were cleared by centrifugation at 16,000 g for 15 min at 4 °C, and the supernatant was aliquoted and frozen at -80 °C until used.

The cell lysates were electrophoresed by SDS-PAGE using a 12.5% gel and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with PVDF Blocking Reagent for Can Get Signal (TOYOBO) for 1 hour, followed by incubation with an anti-GFP antibody or SSB-reactive mAbs. After washing, the membranes were incubated with an HRP-conjugated anti-mouse IgG or anti-human IgG antibody for 1 hour. After extensive washing, SSB fragment-binding antibody were visualized using ImmunoStar LD (FUJIFILM Wako).

Antigen-binding beads assay

The antigens, MIS12, PMF1, DSN1, NSL1, CBX5, CENP-A, CENP-B, and CENP-C were cloned into the pEF vector[38] (kindly gifted by Dr. A. Yamashita) combined with the SBP tag and GFP at the N-terminus of the antigens. Point mutant (D355A) of SSA52 was generated by PrimeSTAR Mutagenesis Basal Kit (Takara Bio). SSA52, SSA52-D355A, and SSB were cloned into the pcDNA3.4 vector (Thermo Fisher Scientific) combined with the SBP tag and GFP at the N-terminus of the antigens. The lysates of antigen-expressing 293T cells were prepared as previously described. SSA60, which is known to be an extremely difficult recombinant protein to produce, was purchased from Diarect[39], a proven manufacturer.

To create antigen-binding beads, Dynabeads M-280 Streptavidin (Thermo Fisher Scientific) was washed once with TBSTx, added to the cell lysate, and incubated for 30 min at 4 °C with shaking at 1,400 rpm. The beads were washed extensively 3 times using TBSTx with 1 M NaCl for 1 min, once using TBSTx with 0.5 M NaCl for 10 min at 4°C with shaking at 1,400 rpm, and once with PBS.

The antigen-binding beads were used for determining the specificity and affinity of mAbs or measuring serum antibody titers by flow cytometry. Briefly, the beads were incubated with a mAb at the indicated concentration in staining buffer (PBS with 2 mM EDTA and 0.5% BSA) or serum at a 1:300 dilution in staining buffer with 0.1% Tween-20 for 20 min at 4 °C. After washing once (for a mAb) or twice (for serum) with staining buffer, the beads were stained with anti-human IgGFc antibody and anti-human IgAFc antibody. After washing once, the beads were analyzed by a FACSVerse and FlowJo software (BD Biosciences, CA, USA).

Immunoprecipitation (IP)

Lysates of K562 cells were prepared by same procedure as 293T cells. For immunoprecipitation, 1 μg of mAb was incubated with K562 cell lysate (equivalent to 2.5 × 10^6 cells) or TBSTx at 4 °C overnight. Then, 5 μl of Dynabeads protein G (Thermo Fisher Scientific) was added and incubated for 30 min with shaking. After extensive washing, the proteins bound to the beads were eluted by boiling in 1x Laemmli Sample Buffer (Bio-Rad, CA, USA), electrophoresed, and visualized by silver staining (Aproscience, Tokushima, Japan). The visualized bands were excised, and the proteins were identified by mass spectrometry. The immunoprecipitation results were confirmed by western blot analysis using antibody against DSN1, MIS12, the MIS12C, and human IgG.

Mass spectrometry

Immunoprecipitated products were analyzed using a liquid chromatography-tandem mass spectrometer (LC-MS/MS, LTQ Orbitrap, Thermo Fisher Scientific) after tryptic digestion. All MS/MS spectra data were analyzed using Mascot software (Matrix Science, London, UK).

ANA test

ANA test was performed at a concentration of 50 μg/ml for each antibody using the Fluoro HEp-2 ANA test (MBL) according to the manufacturer's instructions. Slides were analyzed using an LSM710 and ZEN 2.3 SP1 software (ZEISS, Oberkochen, Germany).

Detection of autoantibody-producing cells in the salivary grands

Lysates of SBP-GFP-antigen-expressing 293T cells were prepared as previously described. To purify the SBP-GFP-antigens, Streptavidin Sepharose High Performance (GE Healthcare Japan, Tokyo, Japan) was washed once with TBSTx, added to the antigen-expressing supernatant, and incubated for 2 hours at 4 °C with shaking. The beads were washed extensively as previously described, and then the SBP-GFP-antigens were eluted by incubating with 1x Buffer BXT (IBA, Göttingen, Germany) for 30 min at 4 °C at 1,400 rpm. The purities of the SBP-GFP-antigens were checked by CBB staining, and their concentrations were measured by a BCA protein assay (Thermo Fisher Scientific).

Immunohistochemical staining was performed according to previously published methods[15] with modifications. In brief, 4-μm sections of LSG were cut with a cryostat, fixed in acetone for 10 min, blocked with 5% BSA and 10% goat serum in PBS for 10 min at RT, and incubated with SBP-GFP-antigen and antibody against CD4, CD8, CD20, and CD138, each at a concentration of 5 μg/ml for 60 min at RT. After washing, the slides were incubated with an Alexa Fluor 594-conjugated anti-mouse or rat IgG antibody (1:500 dilution) for 30 min at RT. After washing, the slides were mounted with VECTASHIELD Mounting Medium with DAPI (Vector Laboratories, CA, USA). Autoantibody-producing cells were semiquantified using an LSM710 and ZEN 2.3 SP1 software.

Antibody

The following antibody were used: recombinant human IgG1 (QA16A12), anti-CD3 (APC/Cy7, UCHT1), anti-CD4 (PE/Cy7, SK3), anti-CD8 (BV510, RPA-T8), anti-CD19 (BV421, HIB19), anti-CD38 (FITC, HIT2), anti-CD138 (APC, MI15), and anti-CD326 (PE, 9C4) from BioLegend; anti-human IgG (HRP), anti-human IgG-Fc (APC or HRP, goat-F(ab')$_2$ fragment), and anti-human IgA-Fc (BV421, goat-F(ab')$_2$ fragment) from Jackson ImmunoResearch (PA, USA); anti-mouse IgG (Alexa Fluor 594, goat), anti-mouse IgG1 (Alexa Fluor 594, goat), anti-mouse IgG2a (Alexa Fluor 488, goat), and anti-rat IgG (Alexa Fluor 594, goat) from Thermo Fisher Scientific; anti-mouse IgG (HRP) from GE; anti-DSN1 (70-101) from BioAcademia (Osaka, Japan); anti-MIS12 (A300-776A) from Bethyl Laboratories; and anti-MIS12C (ABE2585) from Merck Millipore (Darmstadt, Germany). 7-Aminoactinomycin D was purchased from Bay Bioscience (Kobe, Japan).

Patient and public involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Statistics

Continuous data are presented as the median and interquartile range (IQR) or as a number with the percentage value, as appropriate. The cutoff for the titer of each antibody specific for a centromere-related protein was determined by receiver operating characteristic (ROC) analysis, which was performed as the patients with ACA detected by ANA test were positive. Correlations between two continuous variables were analyzed using Spearman's rank correlation coefficient. Comparisons of continuous variables were performed by a two-sided Wilcoxon's test. Comparisons of categorical variables were performed by using the Chi-square test. A *P*-value < 0.05 was considered significant. All statistical analyses were performed with JMP 13 (SAS Institute, NC, USA).

Supplementary tables

Supplementary table S1. Primers

chain	PCR	Forward primer	5' - 3' sequence
H,K,L	1st	SMARTer 2A-F	AAGCAGTGGTATCAACGCAGAGTAC
H	2nd	pME-NEB-VH1	TGAGCTACGGACTCGAGCAGGTGCAGCTGGTGCAG
H	2nd	pME-NEB-VH1/5	TGAGCTACGGACTCGAGGAGGTGCAGCTGGTGCAG
H	2nd	pME-NEB-VH3	TGAGCTACGGACTCGAGGAGGTGCAGCTGGTGGAG
H	2nd	pME-NEB-VH3-23	TGAGCTACGGACTCGAGGAGGTGCAGCTGTTGGAG
H	2nd	pME-NEB-VH4	TGAGCTACGGACTCGAGCAGGTGCAGCTGCAGGAG
H	2nd	pME-NEB-VH4-34	TGAGCTACGGACTCGAGCAGGTGCAGCTACAGCAGTG
H	2nd	pME-NEB-VH1-18	TGAGCTACGGACTCGAGCAGGTTCAGCTGGTGCAG
H	2nd	pME-NEB-VH1-24	TGAGCTACGGACTCGAGCAGGTCCAGCTGGTACAG
H	2nd	pME-NEB-VH3-33	TGAGCTACGGACTCGAGCAGGTGCAGCTGGTGGAG
H	2nd	pME-NEB-VH3-9	TGAGCTACGGACTCGAGGAAGTGCAGCTGGTGGAG
H	2nd	pME-NEB-VH4-39	TGAGCTACGGACTCGAGCAGCTGCAGCTGCAGGAG
H	2nd	pME-NEB-VH6-1	TGAGCTACGGACTCGAGCAGGTACAGCTGCAGCAG
K	2nd	pME-NEB-Vk1-5	TGAGCTACGGACTCGAGGACATCCAGATGACCCAGTC
K	2nd	pME-NEB-Vk1-9	TGAGCTACGGACTCGAGGACATCCAGTTGACCCAGTCT
K	2nd	pME-NEB-Vk1D-43	TGAGCTACGGACTCGAGGCCATCCGGATGACCCA
K	2nd	pME-NEB-Vk2-24	TGAGCTACGGACTCGAGGATATTGTGATGACCCAGACTCC
K	2nd	pME-NEB-Vk2-28	TGAGCTACGGACTCGAGGATATTGTGATGACTCAGTCTCC
K	2nd	pME-NEB-Vk2-30	TGAGCTACGGACTCGAGGATGTTGTGATGACTCAGTCTCC
K	2nd	pME-NEB-Vk3-11	TGAGCTACGGACTCGAGGAAATTGTGTTGACACAGTCTCC
K	2nd	pME-NEB-Vk3-15	TGAGCTACGGACTCGAGGAAATAGTGATGACGCAGTCTCC
K	2nd	pME-NEB-Vk3-20	TGAGCTACGGACTCGAGGAAATTGTGTTGACGCAGTCT
K	2nd	pME-NEB-Vk4-1	TGAGCTACGGACTCGAGGACATCGTGATGACCCAGTC
L	2nd	pME-NEB-Vl1	TGAGCTACGGACTCGAGCAGTCTGTGCTGACKCAGC
L	2nd	pME-NEB-Vl2	TGAGCTACGGACTCGAGCAGTCTGCCCTGACTCAGC
L	2nd	pME-NEB-Vl3	TGAGCTACGGACTCGAGTCCTATGAGCTGACWCAGCC
L	2nd	pME-NEB-Vl4/5	TGAGCTACGGACTCGAGCAGCYTGTGCTGACTCAGTC
L	2nd	pME-NEB-Vl6	TGAGCTACGGACTCGAGAATTTTATGCTGACTCAGCCG
L	2nd	pME-NEB-Vl7/8	TGAGCTACGGACTCGAGCAGRCTGTGGTGACYCAG
H,K,L	3rd	cell specific primer	TGAGCTACGGACTCGAG-(depend on variable region sequence)

chain	PCR	Reverse primer	5' - 3' sequence
H(IgG)	1st	IgG-1R	TGAGTTCCACGACACCGTCAC
H(IgA)	1st	IgA-1R	TTCGCTCCAGGTCACACTGAG

H(IgM)	1st	IgM-1R	ACAAAGTGATGGAGTCGGGAAG
H(IgD)	1st	IgD-1R	CCAGGACCACAGGGCTGTTATC
K	1st	IgK-1R	TCCGAGCTCGGTACCAAGCTAACACTCTCCCCTGTTGAAGCTC
L	1st	IgLs-1R	TCCGAGCTCGGTACCAAGCTATGAACATTCTGTAGGGGCCACT
L	1st	IgL3-1R	TCCGAGCTCGGTACCAAGCTATGAACATTCCGTAGGGGCAAC
L	1st	IgL6-1R	TCCGAGCTCGGTACCAAGCTATGAACATTCTGCAGGGGCC
H(IgG)	2nd	pME-NEB-IgG-R	GATGGGCCCTTGGTGGA
H(IgA,M,D)	2nd	cell specific primer	TGGGCCCTTGGTGGATG-(depend on junction sequence)
K	2nd	pME-NEB-IgK-R	AGACTAGTCTAGCGGCCTAACACTCTCCCCTGTTGAAGCTC
L	2nd	pME-NEB-IgLs-R	AGACTAGTCTAGCGGCCTATGAACATTCTGTAGGGGCCACT
L	2nd	pME-NEB-IgL3-R	AGACTAGTCTAGCGGCCTATGAACATTCCGTAGGGGCAAC
L	2nd	pME-NEB-IgL6-R	AGACTAGTCTAGCGGCCTATGAACATTCTGCAGGGGCC

chain	Sequencing primer	5' - 3' sequence
H(IgG)-band	IgG1234-R	GAAGTAGTCCTTGACCAGGCAGC
H(IgA)-band	IgA12-R	TCACACTGAGTGGCTCCTGG
H(IgM)-band	IgM-R	TCGTATCCGACGGGGAATTC
H(IgD)-band	IgD-R	GGCTGTTATCCTTTGGGTGTCTG
K-band	IgK-R	CTCTCTGGGATAGAAGTTATTCAGCAGG
L-band	IgL-R	GGCCGCGTACTTGTTGTTG
Vector	pME18S-seqF	CGGAAGTGTTACTTCTGCTCTAA

Supplementary table S2. Lesion antibody panel list

ID	Note	Heavy chain							Light chain				Reactivity		
		Iso	VH	D	JH	CDR3	Length	Iso	VL	JL	CDR3	Length	Polyreactivity	Main target	Other target
LB01-1H		A	3-7	1	4	VRGDNPEY	8	κ	2-24	1	MQATQFPRT	9	-	-	
LB01-2H		A	5-51	3	6	ARHGGIVNYYMDV	13	κ	1-5	2	QQYDTYPYT	9	-	SSA60	
LB01-3H		A	1-18	5	4	AKEWDSGFVGRFEV	14	λ	3-25	2	QSADISGPSVV	11	-	SSA60	
LB01-4H		A	3-74	3	4	VREYGSGVYPIDY	13	κ	6-21	3	HQSRTLPLT	9	-	SSA52	
LB01-5H		A	3-23	2	4	AKEASLNIPVVTVVVGGAWFDS	22	λ	4-69	3	QTWGPGFRV	9	-	-	
LB01-6H		A	7-4	2	6	ARDSDCSSDSCLLGNNYYYYMDV	23	λ	3-19	3	NSRDSSGHHLCV	12	-	-	
LB01-7H		M	3-30	6	4	AKNRGYNSGWIFDY	14	κ	1-33	4	QQYDNLPPLT	10	-	-	
LB01-8H		A	5-51	3	6	ARLGGIVNYYMDV	13	κ	1-5	2	QQYDSSPYT	9	-	SSA60	
LB01-9H		A	4-30	3	6	AKLSGSGTWRDYGADV	16	λ	3-27	3	YSSSPENIGV	10	-	-	
LB01-11H		A	3-13	2	3	TRAITGDDRINALDI	15	κ	3-15	1	QHFSNWPPWT	10	-	SSA60	
LB01-12H		A	1-3	2	4	ATDYMGVTSTDY	12	κ	3-11	4	QQRYGWPPH	9	-	-	
LB01-13H		A	3-15	6	1	ATRTSRASSWHAEYFQH	17	λ	4-69	2	QTWGTGIVL	9	-	-	
LB01-15H		G	3-9	6	3	AKDIGRQWLPNECGSDV	17	κ	1-39	1	QQSYSMSRT	9	-	SSA60	
LB01-17H		A	4-31	2	5	ARGAPLSEADIVEVPIKVLDS	21	κ	4-1	4	HQYYSLLT	8	-	SSA60	
LB01-18H		A	3-23	2	4	VKSGGIATTLQWMSGGGH	18	κ	1-39	4	QQCYSHPLT	9	-	-	
LB01-19H		G	4-30	3	3	ARVWASGRELGRAVFDI	17	λ	1-40	2	QTYDAGLSGSV	11	-	CBX5	
LB02-1H		A	3-23	6	3	AKGLKQQLVLSPFHT	15	λ	2-14	2	SSYAGSSTPSVV	12	-	CBX5	MIS12C
LB02-2H		A	1-18	6	5	VRSGALAGRGGLSH	14	κ	2-30	2	MQGTHWPPT	9	-	-	
LB02-3H		A	4-31	2	4	AREGMISYLFDS	12	λ	1-40	2	QSYDSSLTGYVV	12	-	-	

LB02-4H		A	3-15	2	3	TTGPPLDN	8	λ	1-51	1	GTWDNSRRVHYV	12	-	-	
LB02-5H		A	3-30	5	6	AKEDVDIVRAPAPVGAGHMDV	21	λ	3-21	3	QVWESRSYQWV	11	-	CENP-C	
LB02-6H		M	4-59	5	4	ARRWSGYDPEDY	12	λ	1-44	3	AAWDDSLNGLWV	12	-	-	
LB02-7H		A	3-48	3	6	ARGGSIKVGFYYYIDV	16	λ	3-1	2	LVWDSDNMV	9	-	-	
LB02-9H		A	3-23	6	4	AQEERRGPSPYDS	13	κ	3-15	1	QQYKAAPVA	9	-	-	
LB02-10H		A	3-21	3	4	ASGLDYDILTGYYSFDF	17	κ	1-NL1	4	QQFYSLPLT	9	-	CENP-C	
LB02-12H		A	4-31	5	4	AREGMATSILDS	12	λ	1-40	2	QSYDTGLSGFVV	12	-	-	
LB02-13H		A	4-39	6	4	ARRSPREDGRSFDS	14	κ	2-28	5	MQAQQTPTT	9	-	-	
LB02-15H		A	5-51	3	4	ARVNSTSSGDALDY	14	λ	3-25	2	QSADSSGNFVV	11	-	CENP-C	
LB02-16H		A	1-69	6	4	ARYRPASGTSLFDF	14	κ	1-39	1	QQSYSTPLT	9	-	-	
LB02-18H		A	3-30	2	3	TTSTSNLPGTFDI	13	κ	1-39	1	QQSHGVPWT	9	-	MIS12C	
LB02-19H		A	4-39	6	4	SRLKGSAGTLAY	12	κ	3-11	4	QQRSNWPPRLT	11	-	-	
LB02-21H		A	3-23	6	4	ATSESISVAQYLY	13	λ	3-21	2	QVWDSSSEHVV	11	-	-	
LB02-22H		A	3-48	6	1	AIAGSTAWAPGIPPRPDYFQH	21	κ	4-1	4	QQYYGVPLT	9	-	-	
LB02-25H		A	3-15	1	4	TTALGTN	7	κ	4-1	4	QQYYGVPLT	9	-	-	
LB02-26H		A	4-30	2	5	ARFRSAAADGDGSWFDP	17	κ	1-6	4	VQTNAYPFT	9	-	-	
LB02-27H		A	3-21	3	4	ARREGFGFSDY	11	κ	1-9	5	QQVNNYLGT	9	-	CENP-C	
LB02-28H		G	3-43	6	3	AKDIKFTNSADAFDL	15	λ	2-23	2	GSYAGTHTSVF	11	-	-	
LB02-29H		A	3-23	3	3	AAARYDILTDHPNAFDI	17	λ	3-21	2	QVWDGNSDHVV	11	-	CENP-C	
LB02-30H		A	3-33	6	4	ARETDIASPHSGPLDY	16	κ	4-1	2	QQYYSTPPYT	10	-	CENP-A	
LB02-31H		A	3-7	6	4	VGQEVSGTLPDY	12	κ	4-1	4	QQYYGVPLT	9	-	-	
LB11-1H		A	3-21	1	4	ARDLSEHSGSYYGSIFYFDF	20	κ	3-15	4	QQYDKWPPLT	10	-	-	

LB11-2H		A	3-73	1	6	TRVTKATPKSNYMDV	15	κ	1D-16	4	QQYNTFPIT	9	-	-	
LB11-3H		A	3-48	3	4	VRELLDFYSGSTFDS	15	κ	1-12	4	QQTNSFPT	8	-	-	
LB11-4H		A	1-2	2	3	AKEMGTCGGDCSGDAFDL	18	λ	1-51	1	GIWDSRLTTGV	11	-	-	
LB11-5H		A	4-59	4	4	ARGTRLRPFDS	11	κ	1-16	3	LQYNVYPLT	9	-	-	
LB11-6H		A	1-18	3	4	ATDRNSVIMYY	11	κ	1-33	2	QQSDNVPYT	9	-	-	
LB11-7H		A	4-39	4	4	VKPYSNYGFDY	11	κ	1-39	4	QQSYSSPLALT	11	-	-	
LB11-8H		A	3-23	2	4	AKGSAIDS	8	κ	2-30	1	MQAIHWPRT	9	-	-	
LB11-9H		A	3-72	3	3	ARVVDYYDGAGYSVDAFDV	19	λ	1-51	2	GTWDGTLNEVL	11	-	-	
LB11-10H		A	3-49	6	6	SRVPYIATGNNIYYSYIDV	19	λ	2-14	2	SSFTAVTNLLV	11	-	-	
LB11-11H		A	3-21	6	6	AREGQNYANSGYYYYYMDV	19	λ	3-19	1	NSRDGSGNYV	10	-	-	
LB11-12H		A	3-13	2	5	VRGLRGGLDP	10	λ	1-51	2	ATWDNSLSAVL	11	-	-	
LB11-13H		A	4-4	3	4	ARGGGAMFDF	10	κ	3-20	2	QQYDHSVIYT	10	-	-	
LB11-14H		A	3-23	2	4	AKGSAIDS	8	κ	1-39	4	QQSSRPPLT	9	-	-	
LB11-15H		A	4-59	3	3	ARSDNGDYSDALDI	14	λ	3-25	1	QSGDGSGSYF	10	-	-	
LB11-16H		A	3-30	3	6	ARDRRVYGSGSWQYYFYGMDV	21	λ	6-57	3	QSSHVSNRWV	10	-	-	
LB11-17H		A	2-5	2	4	VHASMTKY	8	κ	2-30	1	MQVTYLWT	8	-	-	
LB11-18H		A	3-15	5	1	STDVPGSGDGYNLYFQR	17	κ	3-15	4	QQYTNWPLT	9	-	-	
LB11-19H		A	3-48	2	3	VRVGSGGAFDL	11	κ	3-15	3	HQYNNWPFT	9	-	-	
LB11-20H		A	4-38	3	6	VRQDLLFYAMDV	12	λ	1-44	1	AAWDDRMNEYV	11	-	-	
LB11-21H		A	4-39	5	5	AKVSPYTTSQIEMERADWFGS	21	λ	1-44	3	EVWDDNVSGPV	11	-	-	
LB11-22H		A	4-34	5	6	RLSSRREYSYGYNYYSYVDV	20	λ	1-47	1	ATWDDSRTGPYL	12	-	-	
LB11-23H		A	4-39	3	5	VRDRAVGRLLNKGPPIRTQKDL	22	λ	1-51	1	ATWDSGLPAYL	11	-	-	

LB11-24H		A	1-45	4	5	AMTSVDGAGDYVLGL	15	κ	3-15	1	HQYNDSPPT	9	-	-	
LB11-25H		A	1-2	2	3	AKEMGTCGGDCSGDAFDL	18	λ	1-51	1	GIWDSRLTTGV	11	-	-	
LB11-26H		A	3-43	2	4	AKAVYGGDWISLEY	14	κ	2-28	2	MQGLQIPYT	9	-	-	
LB11-28H		A	4-59	3	5	ARRILSERPTSRYNWLDP	18	κ	3-11	4	QQRSVWPLT	9	-	-	
LB11-30H		A	1-46	1	4	AQESHDNWNYVAY	13	κ	1-39	2	QQSYSTPYT	9	-	-	
LB11-31H		A	3-21	3	4	AKSSQDFKGYIDF	13	κ	1-6	2	LQNYFYPFT	9	-	-	
LB11-32H		A	4-34	2	5	IRGCGGDCYEGFDV	14	λ	3-25	3	QSVDSTNIFWV	11	-	-	
LB12-1H		A	5-51	4	5	ARKRIQWNTVDP	12	κ	2-28	2	MQALQTPYI	9	-	-	
LB12-2H		A	3-23	3	5	ARGGISKGMST	11	κ	1-16	4	QQYNNYPLT	9	-	-	
LB12-3H		A	3-23	3	4	AKDGSSSLYDY	11	λ	3-19	3	HSRDSSGYRV	10	-	SSA60	
LB12-4H		A	3-9	4	4	TKDAMTSVTKGGRYFFDS	18	κ	4-1	1	QQYFDTPPT	9	-	-	
LB12-6H		A	4-34	6	3	ARTPRGSLQTRLGASDM	17	κ	1-5	2	QHYNSYPYT	9	-	-	
LB12-7H		A	3-13	7	4	ARANWPGYFDY	11	κ	4-1	1	QQYYNTPRT	9	-	SSA60	
LB12-8H		A	1-18	5	4	ARDWGASGKGFEY	13	κ	3-15	1	QQYNTWPRT	9	-	SSA60	
LB12-9H		A	3-23	3	4	AKGGQLQFDS	10	λ	2-11	3	CSYAGNYTWV	10	-	SSA60	
LB12-10H		A	3-23	6	4	TKGGQLQFDS	10	λ	2-11	3	CSYVGNYTWV	10	-	SSA60	
LB12-11H		A	1-69	2	4	GRVPIGGDTYFDY	13	λ	3-19	3	NSRDSSGNHWV	11	-	SSB	
LB12-12H		A	2-5	3	6	AHSLLWFGDYYGLDV	15	κ	2-28	1	MQALQSQT	8	-	-	
LB12-13H		A	3-23	6	4	AKGGQLQFDS	10	λ	2-11	3	CSYAGNYTWV	10	-	SSA60	
LB12-14H		A	3-15	1	4	TTYSGNFKLDY	11	λ	2-8	2	SSYVYGNTLK	10	-	-	
LB12-15H		A	1-69	2	4	ASVPIAGEKSFDH	13	λ	3-19	3	NSRDNSGDHWV	11	-	SSB	
LB12-16H		A	3-9	3	4	AKVGGYYDSSGYFFDC	16	λ	2-8	2	SSYVGTTSVV	10	-	-	

LB12-17H		A	4-4	3	5	VRSLIGGIMASFEFDP	16	λ	2-11	2	CSYAGSYTFV	10	-	-	
LB12-18H		A	5-51	1	5	ARHQGLGAWFDP	12	λ	1-40	3	QSYDTRLSGPV	11	-	-	
LB12-19H		A	3-74	6	4	ARGGYTTGRQPFDY	14	λ	4-69	2	QTWGTGIVI	9	-	-	
LB12-24H		A	3-53	2	4	AVKLRVVTATQGDVGLY	17	κ	1-5	1	QQYNSHSWT	9	-	-	
LB12-30H		A	5-51	1	5	ARHQGLGAWFDP	12	λ	1-40	3	QSYDTRLSGPV	11	-	-	
LB12-31H		A	1-18	3	4	ARDQGPITMKVVANPHY	17	κ	1-33	4	QQYDNLPPT	9	-	-	
LB20-1H		G	3-30	7	6	AKGGLSGASLGLDV	14	κ	2-28	2	MQALQPTYT	9	-	SSB	SSA52
LB20-3H		D	3-30	2	6	ARDRIQFWLSYSDMDV	16	κ	3-15	4	QQYNNGPPLT	10	-	-	
LB20-4H		G	1-18	3	4	ARDDGRGYFDH	11	κ	3-11	1	QQRANWPRT	9	-	SSB	SSA52
LB20-7H		A	3-13	2	2	ARTHYDTGGFAAGFDL	16	κ	3-20	4	QQDDASPLT	9	-	-	
LB20-8H		G	5-51	6	5	ATHMVTVPGTDSF	13	κ	2-40	3	MQRIEFPFT	9	-	SSB	
LB20-14H		G	3-15	3	6	TTEGSGSPPYYFYGVDV	17	κ	1-39	2	QQSYSTPFT	9	-	SSB	
LB20-18H		G	4-4	4	6	AKLSGSTVTTWMYGMDV	17	λ	7-46	3	LLSYSGARV	9	-	-	
LB20-21H		A	3-15	6	6	TTAAGTYYYYYGMDV	15	κ	3-20	1	QQYGSSTGT	9	-	-	
LB20-22H		G	1-3	6	4	AREGIGAAGHFDY	13	κ	1-33	4	QQYEDFLS	8	-	SSB	
LB20-25H		D	2-5	2	4	TRRRHFSYDFDY	12	λ	2-18	2	SSYTTTGADLI	11	-	-	
LB20-26H		A	7-4	6	4	ARDTSDSSSWYFDY	14	λ	2-14	2	SSYTSSNTRL	10	-	-	
LB20-28H		A	3-30	3	3	GRNERPTTGSLGAFDI	16	κ	3-15	1	QQSDNWPPYT	10	-	-	
LB20-32H		D	2-5	2	4	ARRRQESSDFDY	12	λ	2-18	2	SSYSSTNNYVI	11	-	-	
LB20-34H		G	4-4	5	6	VRMSVPEIYYFHGLDV	16	κ	1-27	1	QKYNSAPRT	9	+	SSA60	CENP-A, CENP-B, CENP-C,

															SSA52, SSA60
LB20-36H		G	3-33	1	4	AKDRGSYYLDS	11	κ	3-15	4	QHYNNWPR	8	-	SSB	
LB20-38H		G	3-23	2	4	VRHRPSGGWRSVFDF	15	κ	3-20	3	HQYGSSPLFT	10	-	SSB	
LB20-39H		A	4-31	3	3	ATYGSGRGAFDI	12	κ	2-28	2	MQALQTLYT	9	-	-	
LB20-40H		A	3-23	5	4	AKVPPQYTYGPLDY	14	κ	1-39	2	QQSYTTPPS	9	-	-	
LB20-41H		D	2-5	3	4	ARRRHQSYDFDS	12	λ	2-18	2	SSYTTSGTLV	10	-	-	
LB20-43H		G	3-21	6	6	AKDLGGAAAGGMDV	14	κ	4-1	1	QQYYSTPKT	9	-	SSB	
LB20-44H		G	3-21	6	6	AKDLGGAAAGGMDV	14	λ	2-14	2	SSYTRTSNVV	10	-	-	
LB20-45H		G	1-46	3	4	ATSFSRMIRGVIAN	14	λ	2-14	2	SSYTGGTTLGHVV	13	-	SSA60	
LB20-46H		G	3-74	4	5	VRDASINKMDH	11	κ	3-20	4	QQYGSFPLT	9	-	-	
LB20-47H		M	4-61	3	4	ARADYEDMGSFDY	13	κ	3-11	4	QQRSNWPLT	9	-	-	
LB20-48H		A	4-61	3	4	ARGGAHYDILTAYASYSFDY	20	κ	1-39	1	QQSSSTPWT	9	-	-	
LB20-49H		A	4-39	3	6	ARSPSRTDYYSYYGMDI	17	κ	3-20	5	QSYSGSPRVS	10	-	-	
LB20-50H		A	3-15	6	3	TTEMDSDYAFDI	12	λ	1-44	1	AAWDDSLNAYYV	12	-	-	
LB20-51H		A	7-4	7	4	ARHSTGEAFFDY	12	λ	3-19	2	NSRDSSGKHVL	11	-	-	
LB20-53H		G	3-30	6	4	AREKSGIVASGIDY	14	κ	1-6	1	LQDYDYPWT	9	-	SSA60	SSB
LB20-55H		G	4-39	3	4	ARLGGDTSVDYFDS	14	λ	1-40	3	QSYDSSLSALV	11	-	-	
LB20-57H		G	3-7	1	4	ARGREWDLPNQFFDC	15	κ	4-1	1	QQYYSTPWT	9	-	-	
LB20-58H		A	3-15	3	4	FYKNRAVGY	9	λ	1-40	3	QSYDNTLSGSWV	12	-	-	
LB20-59H		A	7-4	3	5	ARDVTSSSGSGSYFWFDP	18	λ	1-51	3	GTWDSSLSAWV	11	-	-	
LB20-61H		A	4-39	6	6	ARDNSGWTTYSYQYGMDV	18	λ	3-1	1	QAWDSSTHV	9	-	-	
LB20-62H		A	3-33	4	4	VKDVYGDYGAGDY	13	λ	2-14	2	ASYTRNNAL	9	-	SSA60	

LB20-63H		A	4-39	3	4	GRMEGVWFGEGPFDN	15	λ	1-40	2	QSYDTDLTNVV	11	-	-	
LB20-66H		G	7-4	3	5	ARGDRDGSGPDHANWFDP	18	λ	3-25	3	LSADSRATFWV	11	-	-	
LB20-67H		A	1-69	5	5	TRDRHVVPQGIAWFDP	16	λ	2-14	3	SSYTSRRTPWV	11	-	-	
LB20-72H		M	7-4	3	6	ARDRFGREGMDV	12	κ	2-28	3	MQALQTSFT	9	-	-	
LB20-73H		G	3-23	4	4	GKERWSKVTPRGYFDD	16	λ	2-11	2	CSYAGNNTLI	10	-	SSB	
LB20-76H		A	3-15	6	5	TAFGSGWT	8	λ	1-47	2	AAWDDSLSVVL	11	-	-	
LB20-77H		A	4-39	2	5	ARHPRGGYGGWFDP	14	κ	3-11	2	QQRSNWLVT	9	-	-	
LB20-78H		A	3-23	1	4	AKFDSGSFWWGVDYFDY	17	κ	3-20	4	QQYGSSPQVT	10	-	-	
LB20-79H		G	1-2	6	6	AKGDGTLIYGLDA	13	λ	1-51	2	GTWDSSLTAGV	11	-	SSB	
LB20-80H		G	3-30	2	4	ARGNGAYQVPSALQY	15	κ	3-15	2	QQFNKWPYT	9	+	SSB	CENP-B, SSA52, SSA60
LB20-83H		A	4-59	1	5	ARHLGDASTWFDP	13	λ	3-1	2	QAWDSTYAV	9	-	-	
LB20-84H		G	4-31	3	3	ARDSRFGGNAFDI	13	κ	1-17	1	LQHNSYPWT	9	-	-	
LB20-85H		G	3-48	3	4	TRFPVGDVLSSVYFDY	16	λ	3-1	2	QVWDSNSVV	9	-	SSA60	
LB20-86H		A	7-4	1	4	ARDFNWNDGPYYFDS	15	λ	6-57	3	QSYDSSNQGV	10	-	-	
LB20-89H		A	6-1	3	4	ARDGSSPYLITDYLDH	16	λ	1-51	2	ATWDRTLDAMV	11	-	-	
LB20-91H		G	7-4	2	4	TRDQGSYLRIDY	12	λ	2-14	2	TSCTVSSTVV	10	-	-	
LB20-92H		A	3-7	5	4	AREGLDTAFDY	11	λ	1-40	3	QSYDNTLSGSWV	12	-	-	
LB20-93H		G	3-7	3	4	ARESSGRHTTKFDY	14	κ	1-33	2	QQYDNLPYT	9	-	-	
LB20-94H		A	3-30	3	4	AKDIYGSGSYYNGPLDY	17	κ	3-15	1	QQYNNWPRT	9	-	-	
LB23-1H		A	4-31	5	3	TREETAQLWERKGALDV	17	κ	2-24	1	SQGLQFPRT	9	-	-	
LB23-2H		A	1-46	6	4	ATAIAAAPPEGRDY	14	λ	2-14	1	RSYTASSTYV	10	-	-	

LB23-3H		A	3-21	2	4	ARDAPLAYCGGDCYSLFDY	19	κ	1-12	5	QQANSFPIA	9	-	-	
LB23-4H		A	1-18	1	6	ARISGTYAPVYGLDV	15	λ	2-8	1	SSYSGSNNYV	10	-	-	
LB23-5H		A	6-1	2	4	ARGRNSAFDY	10	κ	4-1	3	QQYYTTPEFT	10	-	-	
LB23-6H		A	5-51	6	1	ARQGKSWPSSAPFFEI	16	κ	4-1	1	QQYYDFPRT	9	-	-	
LB23-8H		A	3-15	2	4	TTGNMPFYFDF	11	λ	2-14	1	SAYTGSITPVFL	12	-	-	
LB23-9H		A	3-11	3	3	ARERFGSGTYDAFDI	15	λ	2-8	2	SSFAGTSGFV	10	-	-	
LB23-10H		A	4-31	1	4	ARGYGGTTGYFDF	13	λ	1-51	3	ATWDSSLSARV	11	-	-	
LB23-11H		A	3-48	3	4	ARGLTGGYYGSGNFGY	16	λ	3-21	1	QVWDTTTNLGV	11	-	-	
LB23-12H		A	4-39	4	4	ARITMMVDY	9	κ	1-8	4	QQYYSYPLT	9	-	-	
LB23-13H		A	4-59	7	3	ARPNRGEAWGAFDI	14	κ	2-28	2	MQALEDPYT	9	-	-	
LB23-14H		A	1-46	3	4	ARDGKSLTGFYGLLPDF	17	κ	4-1	1	QQYFSTVASS	10	-	-	
LB23-15H		A	5-51	6	3	AKQLIPTKSEDDLTHPFDV	19	κ	4-1	1	QQYYNSPGT	9	-	-	
LB23-17H		A	1-69	5	6	ARGPGYSGYERDYPYYYGMDV	21	κ	1-NL1	1	QQYYTIPWT	9	-	-	
LB23-18H		A	2-5	4	4	GHVDPDLGDFHFDF	14	λ	2-14	3	ASYRTGRDLDDWV	13	-	-	
LB24-1H		A	1-3	3	4	ARATLPRYWDY	11	κ	1-NL1	3	QHYAGSPFA	9	-	-	
LB24-2H		A	3-73	3	6	SRQKVGFYPYGLDV	14	λ	3-10	3	YSADSGGNYKV	11	-	-	
LB24-3H		G	4-59	2	4	ARGVGPTSRQGRLDY	15	κ	1-5	1	QQYNSYPWT	9	-	-	
LB24-4H		M	4-39	5	4	AREKMGTFSLDS	12	κ	3-20	3	QQYGNSPPTFT	11	-	-	
LB24-5H		G	5-51	4	4	AKLRRGIVLSTGAFDS	16	κ	1-9	1	QQVNGYPRT	9	-	-	
LB24-6H		G	4-61	6	4	ARESGIGSSTWLNYFDY	17	κ	1-5	1	QQYSSFPWT	9	-	-	
LB24-7H		G	4-61	6	4	ARESGIGSSTWLNYFDY	17	λ	2-14	1	NSYTTTGTYV	10	-	-	
LB24-8H		A	3-66	4	4	ARVTVVTREFDS	12	λ	2-14	3	SSFTSSSTWV	10	-	-	

LB24-9H		G	4-59	2	6	ARPNSYFSYSMDV	13	λ	1-47	3	AAWDDSLSGPV	11	-	SSA60	
LB24-10H		G	3-23	6	4	VKGLWGILAADPFDS	15	κ	3-15	1	QQYNNGWT	8	-	SSA60	
LB24-11H		A	3-15	1	4	TTGTWALFDY	10	κ	2-30	2	MQGTRWPYT	9	-	-	
LB24-12H		A	1-18	6	5	ARVVSRADNWLDP	13	λ	1-40	3	QSYDISLSSWV	11	-	SSA60	
LB24-13H		A	4-39	3	5	ATPIAASGWFDP	12	κ	3-20	4	QQYGSSPLT	9	-	-	
LB24-14H		G	3-30	3	4	ARLMGPWLPTGPYSS	15	κ	3-15	2	QQYNKWYT	8	+	SSB	
LB24-15H		A	3-64	1	4	VKQGWAAYSGNLFDF	15	λ	7-46	2	LLSFGAARV	9	-	-	
LB24-16H		G	3-74	3	4	ARGDFYDSVGYQPPRH	16	λ	1-47	2	ATWDYSLSGPV	11	-	SSA52	
LB24-18H		G	3-30	4	4	ARSWPYGDYFDF	12	κ	1-17	1	LQHNNYPRT	9	-	-	
LB24-19H		A	1-18	6	5	ALVLSRTDNWLDP	13	λ	1-40	3	QSSDSSLSTWV	11	-	-	
LB24-21H		M	3-30	1	4	ARDGNPEIIVGAVYYFDY	18	κ	3-20	4	QQYGSSPPLT	10	-	-	
LB25-1H		G	3-30	1	4	AKDPSRYNWIYADH	14	λ	3-19	3	NSRDSSNNPSWV	12	-	-	
LB25-5H		M	3-11	2	6	AREACSGATCYRPEVYSNYGMDV	23	κ	3-15	1	QHYNNWPPWT	10	-	-	
LB25-7H		G	3-30	1	4	AREMKQGATGSRFFDF	16	λ	3-1	1	QAWAFSAAV	9	-	SSA52	
LB25-8H	=LB25-1H	G	3-30	1	4	AKDPSRYNWIYADH	14	λ	3-19	3	NSRDSSNNPSWV	12	-	-	
LB25-9H	=LB25-7H	G	3-30	1	4	AREMKQGATGSRFFDF	16	λ	3-1	1	QAWAFSAAV	9	-	SSA52	
LB25-10H		A	3-23	6	5	AKDRLATPGTGWFDP	15	κ	1-39	2	QQTYNTLNT	9	-	SSA52	
LB25-11H	=LB25-7H	G	3-30	1	4	AREMKQGATGSRFFDF	16	λ	3-1	1	QAWAFSAAV	9	-	SSA52	
LB25-14H		A	5-51	2	1	ARAQYCGGDCGSLFQY	16	κ	1-5*	1	QQYYTYPWT	9	-	-	
LB25-15H		A	4-39	5	5	ARVGYSYGRRFDP	13	λ	1-51	2	EKWDPRVSSVA	11	-	-	
LB25-17H		A	1-46	3	3	ARYPGDSSGYIDSFDV	16	λ	1-40	3	QSYDNTLSGSWV	12	-	-	
LB25-18H		A	4-39	3	3	ARLLGYNGSGSYPGVVSGFDI	21	λ	2-23	2	CSFAGSSTWVV	11	-	-	

LB25-19H		A	1-8	4	4	VRVRGIGRTPFDY	13	λ	10-5	3	SAWDSSLSARL	11	-	-	
LB25-21H		A	3-66	3	6	ARGGDVGARFGMDV	14	κ	1-39	1	QQSYSAPWT	9	-	-	
LB25-22H	=LB25-15H	A	4-39	5	5	ARVGYSYGRRFDP	13	λ	1-51	2	EKWDPRVSSVA	11	-	-	
LB25-23H	=LB25-23H	A	3-66	3	6	ARGGDVGARFGMDV	14	κ	1-39	1	QQSYSAPWT	9	-	-	
LB25-24H		A	3-23	2	3	AKGPPTTMAFDM	12	λ	2-11	1	CSYGGQYRFV	10	-	SSA60	
LB25-25H		G	1-18	3	4	AGGRSTYTYFDY	12	κ	1-12	3	QQANTFPFS	9	-	-	
LB25-26H	=LB25-7H	G	3-30	1	4	AREMKQGATGSRFFDF	16	λ	3-1	1	QAWAFSAAV	9	-	SSA52	
LB25-30H	=LB25-15H	A	4-39	5	5	ARVGYSYGRRFDP	13	λ	1-51	2	EKWDPRVSSVA	11	-	-	
LB25-35H		A	3-66	6	4	ATKRQPVPYTFDN	13	λ	2-23	2	CSYSGSFTLEV	11	-	-	
LB25-36H	=LB25-1H	G	3-30	1	4	AKDPSRYNWIYADH	14	λ	3-19	3	NSRDSSNNPSWV	12	-	-	
LB25-37H		G	3-30	6	4	ARDIMATGVTPRYCFDH	17	κ	3-15	4	QHYTNWPLT	9	-	-	
LB25-38H		A	3-15	6	4	TTWGSAAGKD	10	κ	1-17	2	LQHYSFPHT	9	-	-	
LB25-39H		A	3-7	2	4	ARDLVEAIGAFDY	13	κ	3-15	1	QQYNNWPPWT	10	-	-	
LB25-40H		G	7-4	1	4	ARDTWKVIY	9	λ	1-40	3	QSYDNRHSGWV	11	-	-	
LB25-43H		G	3-30	3	1	ARDAGDDSDRSGPKFVKHFDL	21	κ	1D-12	5	QQADVFPIS	9	-	SSA60	
LB25-45H		A	1-8	4	4	VRVRGIGRTPFDY	13	λ	3-1	2	QAWDSQTAF	9	-	-	
LB25-48H		A	3-21	1	2	ARRRERGDWFLDL	13	λ	1-51	2	GAWDSNLSDGPV	12	-	SSA60	
LB25-49H	=LB25-5H	M	3-11	2	6	AREACSGATCYRPEVYSNYGMDV	23	κ	3-15	1	QHYNNWPPWT	10	-	-	
LB25-50H		A	1-18	1	6	ARDLRRSATGNLYYHGMDV	19	κ	3-15	4	QQYNNWPQA	9	-	-	
LB25-51H		A	3-11	6	3	ARDAGGGWPGALDV	14	κ	4-1	1	QQYLGTWT	8	-	-	
LB25-52H		A	3-23	1	4	AKEWADELD	9	κ	3D-15	1	HQYHNWPRWT	10	-	-	
LB25-53H		A	3-33	6	4	ARDSITSFDY	10	κ	1-9	2	QQLISYPRT	9	-	SSA60	

LB25-55H	=LB25-1H	G	3-30	1	4	AKDPSRYNWIYADH	14	λ	3-19	3	NSRDSSNNPSWV	12	-	-	
LB25-56H		A	1-18	3	4	AREAGGLGTLPDH	13	κ	3-20	1	QHYGYSPER	9	-	-	
LB25-59H		A	3-7	3	5	ARPAKHYHDTSGYSNWFDP	19	κ	4-1	1	QQYYTSPPT	9	-	-	
LB25-60H	=LB25-1H	G	3-30	1	4	AKDPSRYNWIYADH	14	λ	3-19	3	NSRDSSNNPSWV	12	-	-	
LB32-2H		A	7-4	3	4	ARGATDDTSGYYYFDF	16	κ	4-1	5	QQYFSTLAIT	10	-	CENP-C	
LB32-3H		A	3-11	2	4	ARGLVYCAGDCYIPEY	16	λ	1-47	2	ATWDDRVSGHVV	12	-	-	
LB32-4H		A	3-74	6	4	ARGRAAGGDY	10	κ	2-30	1	MQGTHLWT	8	-	-	
LB32-5H		A	5-51	3	4	ATYRPYFYGPDDRPAGYFFDN	21	λ	6-57	3	QSYDRDNLWV	10	-	-	
LB32-7H		A	3-23	3	4	AKDYDRNEYDFPFDY	15	κ	2-24	1	MQATQFPRT	9	-	-	
LB32-8H		A	4-31	5	6	ARDQGDNPTPPYYYFYGLDV	20	λ	1-44	3	SSWDDKLNGRV	11	-	MIS12C	
LB32-9H		A	3-21	5	4	ARDLSGYDYSPFDY	14	κ	1-5	2	QQCNSYPYT	9	-	MIS12C	
LB32-10H		A	3-11	4	4	AREGPRGTVTLKRHPFDS	18	λ	2-14	3	CSYTFDTTWV	10	-	MIS12C	
LB32-11H		A	3-74	2	6	AGARDCGGGSCHQGHYYSGMDV	22	κ	2-28	2	MQGLQPPYT	9	-	-	
LB32-12H		A	3-73	4	4	ARRDGGNSGSLFDN	14	λ	4-69	1	QTWGKGIV	8	-	-	
LB32-13H		A	1-8	2	4	ARGLYRSCTGGSCYLKY	17	λ	1-40	3	LSYDSNLDDWV	11	-	-	
LB32-14H		A	3-9	3	3	ARALRGVISGFDV	13	λ	3-19	2	SCRDSTTNRLRVV	13	-	MIS12C	
LB32-15H		A	3-9	1	6	AKDMGAVGSQDYFYYALDV	19	λ	3-19	2	NSRDNTGTYLL	11	-	-	
LB32-16H		A	1-18	1	4	ARDSRLGAGSYLDN	14	κ	1-5	2	QQYKSFSYT	9	-	-	
LB32-17H		A	4-59	3	4	ASGDFGMGEIEN	12	κ	3-20	4	QQYGTPPLT	9	-	MIS12C	
LB32-19H		A	3-21	3	5	ARVLTPLLFGPTDA	14	κ	3-15	5	QQYSNWLMT	9	-	MIS12C	
LB32-20H		A	1-69	5	4	ARGKRYSGYDFDY	13	κ	1-33	2	QQYDNFPRT	9	-	MIS12C	
LB32-21H		A	3-9	3	6	AKDGEQFPYFYVLDV	15	λ	1-44	3	AAWDETLSAGV	11	-	MIS12C	

LB32-22H		A	4-59	3	4	ARDLDYFGSGITSDF	15	κ	3-15	3	QQHTYWPRT	9	-	-	
LB32-23H		A	4-61	6	3	VRNARPGIPAAGI	13	λ	3-25	2	QSVDSSGSYVV	11	-	-	
LB32-24H		A	5-51	4	4	ATLYGDLDF	9	λ	1-40	3	QSFDSSLGAWV	11	-	-	
LB32-25H		A	3-11	1	6	ARDKPGSYYSGGSYYYYGMDV	21	λ	1-44	2	AACDDSLNGHVL	12	-	-	
LB32-26H		A	3-30	4	6	ARDATTATTDHGLDV	15	λ	3-25	1	QSSDNSGTYLV	11	-	CENP-C	
LB32-27H		A	4-31	4	4	ARDRTGGGFES	11	κ	1-39	2	HQGYRTPYT	9	-	CENP-C	
LB32-28H		A	3-21	5	4	ARDLSGYDYSPFDY	14	κ	1-NL1	1	HQYNAFPWT	9	-	-	
LB32-29H		A	3-7	4	4	ARLPRQLDY	9	κ	2-30	2	MQGTHWPYT	9	-	-	
LB32-30H		A	3-74	1	6	ARVKTYYSYGMDV	13	κ	3-20	1	QQSGSSRWT	9	-	-	
LB32-31H		A	3-33	1	4	ATVGTTHSFFDW	12	κ	1-5	4	QHYDSYPLS	9	-	-	
LB32-32H		A	4-59	7	6	ARDRPLTGDVYGMDV	15	κ	1-NL1	1	QQFHNFPWT	9	-	MIS12C	
LB32-34H		A	3-9	2	4	VRDAGWVTAASVY	13	λ	1-44	3	APWDDSLKGWV	11	-	-	
LB32-35H		A	5-51	5	4	ARRGYSYYHPTEDFDL	16	κ	2-30	1	MQGTHWPLWT	10	-	-	
LB32-37H		A	5-51	5	4	ARRGYSYYHPTEDFDL	16	λ	2-11	1	CSYTGSYNYV	10	-	-	
LB32-40H		A	5-51	6	4	ARFLGASPEKFDY	13	κ	3-15	4	QQYNDGLT	8	-	-	
LB32-41H		A	3-64	2	6	AREIDCSTATCYSYYYHGLDV	21	κ	2-28	2	MQPLQTLYT	9	-	CENP-C	
LB32-42H		A	4-30	3	5	ARGGTHHDILTGHTTNWFDP	20	κ	6-21	4	HQSSYSPLT	9	-	-	
LB32-43H		A	3-74	2	1	GRDLGGFGSV	10	κ	2-28	2	MQALQTPYT	9	-	-	
LB32-49H		A	3-23	3	5	AREKLWFGEFGDSL	14	κ	1-33	2	QQYAILPT	8	-	-	
LB32-50H		A	3-9	3	6	ANDRGFQGLLRGMAV	15	λ	2-23	3	CSYAGRSSWV	10	-	CENP-C	
LB32-51H		A	1-24	2	4	TTGPGRSCIGDRCHSNDY	18	κ	1-39	4	QQSYSGLT	8	-	MIS12C	

Iso; isotype

Supplementary table S3. Clinical characteristics of the patients used for the lesion antibody panel

Patients ID	LB11	LB02	LB23	LB32	LB01	LB25	LB12	LB24	LB20
Sex	Female	Male	Female	Female	Female	Female	Female	Female	Female
Age	47	51	60	31	19	86	67	36	54
Disease	sicca	pSS	pSS	pSS	sicca	pSS	pSS	pSS	sSS,RA
Disease duration (months)	10	18	8	168	8	120	12	1	10
Greenspan grade	1	3	4	4	1	4	2	4	3
Medication	None	None	None	None	None	None	None	None	MTX
Serum antibody									
ANA titer	<1:40	<1:40	<1:40	1:640	>1:2560	1:80	1:40	1:640	>1:2560
ANA type*				h, d, n	s	h, s	s, c	s	s
anti-SSA52/60 antibody	-	-	-	-	+	+	+	+	+
anti-SSB antibody	-	-	-	-	-	+	+	+	+
anti-CENP-B antibody	-	NA	-	+	-	-	-	-	-
rheumatoid factor	-	NA	NA	-	+	-	+	+	+
Sorted cell, n	32	29	18	48	19	59	32	21	94
Antibody produced, n (%)	30 (93.8)	24 (82.7)	16 (88.9)	39 (81.3)	16 (84.2)	37 (62.7)	21 (65.6)	19 (90.5)	54 (57.4)

MTX; methotrexate, h; homogenous, d; discrete-speckled, n; nucleolar, s; speckled, c; cytosol, NA; not assessed

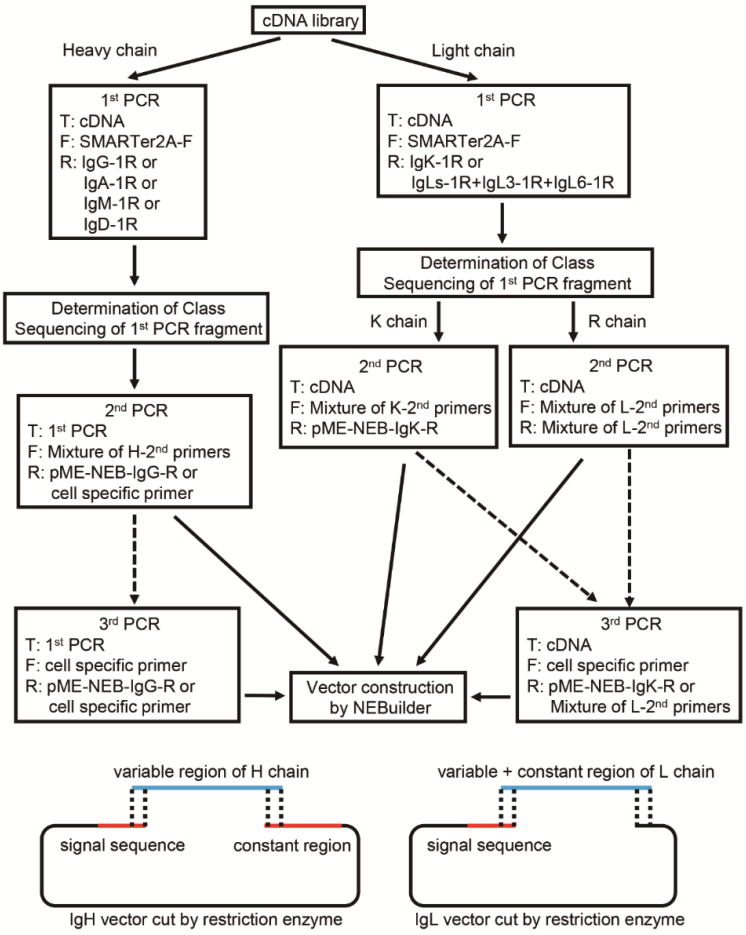
Supplementary table S4. Anti-SSA/SSB profile of serum and lesion antibody panel

Patient ID	LB11	LB02	LB23	LB32	LB01	LB25	LB12	LB24	LB20
Serum anti-SSA52/60 antibody	-	-	-	-	+	+	+	+	+
Serum anti-SSB antibody	-	-	-	-	-	+	+	+	+
Antibody produced, n	30	29	16	39	16	37	21	19	54
Anti-SSA52 antibody, n	0	0	0	0	1	5	0	1	0
Anti-SSA60 antibody, n	0	0	0	0	6	4	6	3	4
Anti-SSB antibody, n	0	0	0	0	0	0	2	1	12
Anti-SSA/SSB antibody, n	0	0	0	0	7	9	8	5	16
(%)	(0)	(0)	(0)	(0)	(43.8)	(24.3)	(38.1)	(26.3)	(29.6)

Supplementary table S5. ACA profile of serum and lesion antibody panel

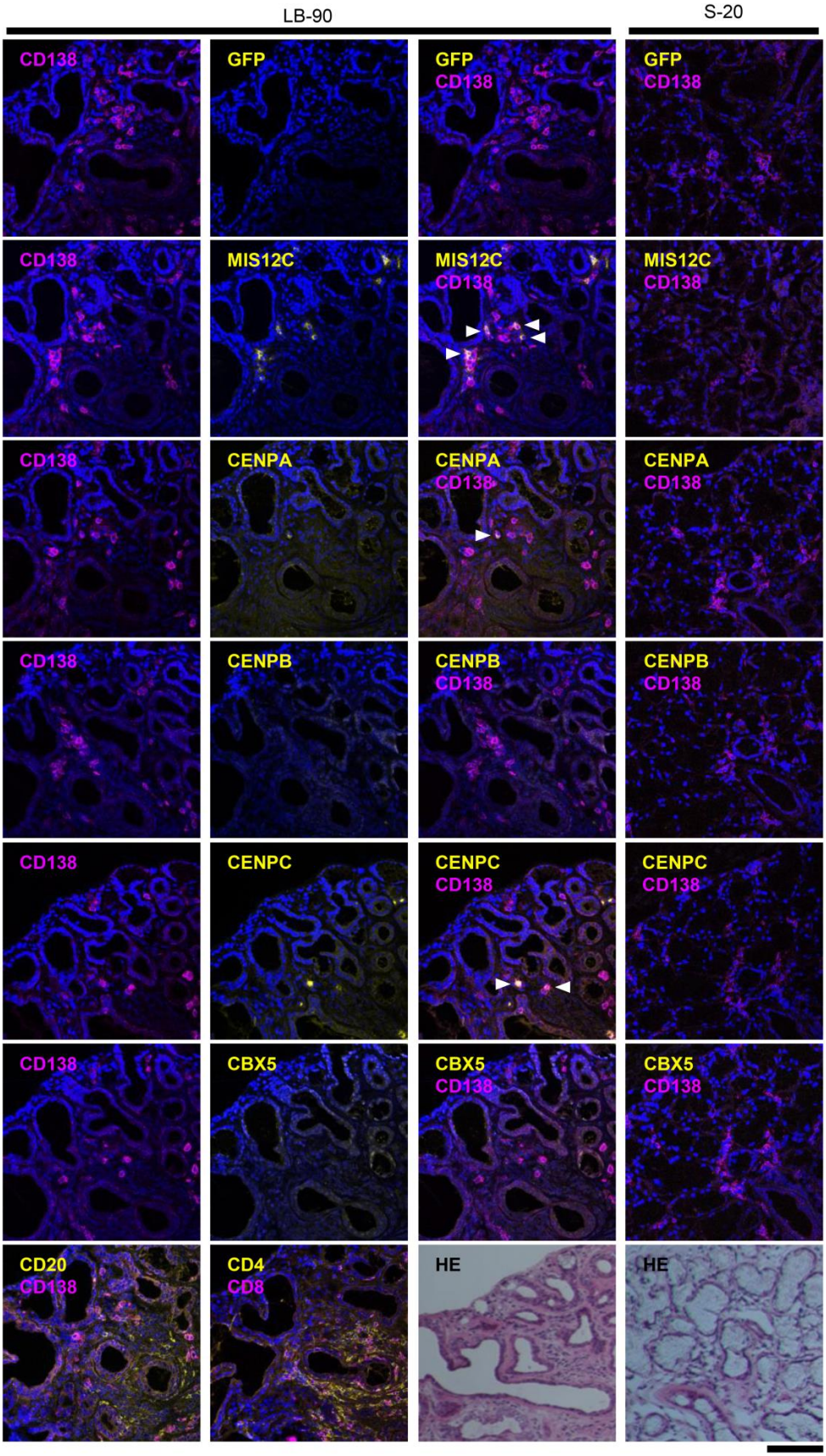
Patient ID	LB11	LB02	LB23	LB32	LB01	LB25	LB12	LB24	LB20
Serum ANA discrete speckled	-	-	-	+	-	-	-	-	-
Serum anti-CENP-B antibody	-	-	-	+	-	-	-	-	-
Antibody produced, n	30	29	16	39	16	37	21	19	54
Anti-MIS12C antibody, n	0	1	0	10	0	0	0	0	0
Anti-CBX5 antibody, n	0	1	0	0	1	0	0	0	0
Anti-CENP-A antibody, n	0	1	0	0	0	0	0	0	0
Anti-CENP-B antibody, n	0	0	0	0	0	0	0	0	0
Anti-CENP-C antibody, n	0	5	0	5	0	0	0	0	0
Centromere-related antibody, n	0	8	0	15	1	0	0	0	0
(%)	(0)	(27.5)	(0)	(38.5)	(6.3)	(0)	(0)	(0)	(0)

Supplementary figures



Supplementary figure S1. Cloning strategy of immunoglobulin sequences from a single-cell cDNA library

The variable regions of the immunoglobulin sequences for the heavy chain and light chain were cloned separately. For the heavy chain, PCR was performed from single-cell cDNA using common F primers and an isotype-specific R primer. The resulting band was cut, extracted, and sequenced. A second PCR was performed with the products from the first PCR using a mixture of major F primer and an IgG-specific R primer (for IgG) or a cell-specific R primer (other isotypes). If the band was not obtained by the second PCR, a third PCR was performed using a cell-specific F primer instead of a mixture of major F primers. For the light chain, PCR was performed from single-cell cDNA using common F primers and an isotype-specific R primer. The resulting band was cut, extracted, and sequenced. A second PCR was performed from the cDNA library using a mixture of major F primers and an isotype-specific R primer. A third PCR was performed using a cell-specific F primer and isotype-specific R primer. The obtained bands were cloned into chain-specific plasmids by using NEBuilder HiFi DNA Assembly Master Mix, and their sequences were checked.


LB-90
S-20
CD138
GFP
GFP
CD138
GFP
CD138
CD138
MIS12C
MIS12C
CD138
MIS12C
CD138
CD138
CENPA
CENPA
CD138
CENPA
CD138
CD138
CENPB
CENPB
CD138
CENPB
CD138
CD138
CENPC
CENPC
CD138
CENPC
CD138
CD138
CBX5
CBX5
CD138
CBX5
CD138
CD20
CD138
CD4
CD8
HE
HE

Supplementary figure S2. Identification of ACA-secreting cells in the salivary glands

Fresh-frozen salivary gland sections were stained with purified GFP-autoantigen fusion proteins, antibody against the ASC marker, CD138, CD4, CD8, CD20, and hematoxylin and eosin. Representative images of the salivary glands from a serum ACA-positive patient (LB90) and serum ACA-negative patient (S20) are shown (magnification, 200×). Generally, CD4 and CD20 cells were present at the most crowded part of the lymphocyte infiltration, CD138 cells were present around that area, and CD8 cells were scattered throughout the infiltration. Among them, ACA-secreted cells were present at the margin of infiltrates regardless of size, and this is consistent with the localization of anti-SSA52 antibody secreted cells[16]. White arrowheads indicate ACA-secreting cells existing in the same area of serial section. Scale bar indicates 100 μm.

Supplementary data

Supplementary data S1. Mass spectrometry results

This data is provided as excel file. Centromere-related proteins are shown in red.

Supplementary references

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38. Arias-Palomo E, Yamashita A, Fernandez I S*, et al.* The nonsense-mediated mRNA decay SMG-1 kinase is regulated by large-scale conformational changes controlled by SMG-8. *Genes & development* 2011;25:153-164.

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