

The reactivity pattern of HMGB1 autoantibodies in systemic lupus erythematosus is associated with longer disease duration with the linker region between the DNA-binding B- box and the C-terminal tail being widely recognised

Harini Pechiappan

harini.pechiappan@uib.no

University of Bergen

Cecilia Aulin

Karolinska Institutet

Richard Davies

University of Bergen

Rebecka Heinbäck

Karolinska Institutet

Marianne Eidsheim

University of Bergen

Edvard Wigren

Karolinska Institutet

Lina Wirestam

Linköping University

Christopher Sjöwall

Linköping University

Helena Erlandsson Harris

Karolinska Institutet

Research Article

Keywords: HMGB1, alarmin, autoantibodies, systemic lupus erythematosus, epitope mapping

Posted Date: October 20th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11273167/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

The reactivity pattern of HMGB1 autoantibodies in systemic lupus erythematosus is associated with longer disease duration with the linker region between the DNA-binding B-box and the C-terminal tail being widely recognised

Harini Pechiappan¹, Cecilia Aulin², Richard Davies¹, Rebecka Heinbäck², Marianne Eidsheim¹, Edvard Wigren², Lina Wirestam³, Christopher Sjöwall³, Helena Erlandsson Harris^{1,2}

1. Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, The Laboratory Building, 5th floor, Haukeland University Hospital, Jonas Lies vei 87, N-5021, Bergen, Norway.

2. Division of Rheumatology, Department of Medicine Solna, Karolinska Institutet, and Center for Molecular Medicine, Karolinska University Hospital, SE-171 76 Stockholm, Sweden.

3. Division of Inflammation and Infection/Rheumatology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

†Corresponding authors

Primary corresponding author: Harini Pechiappan (harini.pechiappan@uib.no)

Co-corresponding author: Helena Erlandsson Harris (helena.harris@ki.se)

ABSTRACT

Background: High mobility group box 1 (HMGB1) is a nuclear protein released during cell stress and cell death, where it acts as an alarmin driving inflammatory signaling. HMGB1 and HMGB1-targeting autoantibodies are elevated in SLE sera and have been linked to disease activity. Yet, the epitope specificity of these autoantibodies remains unexplored. This is a notable gap, as the region an antibody targets may determine its functional effect by, for example, blocking receptor engagement or contributing to immune-complex formation.

Aim: First, to determine the presence of HMGB1 autoantibodies and their epitope specificities in sera from patients with systemic lupus erythematosus (SLE) and for comparison in sera from healthy controls (HC). Secondly, to correlate defined specificities with different SLE features.

Methods: Sera from patients with SLE (n=210) and healthy controls (n=100) were screened by ELISA for HMGB1 autoantibodies using four recombinant HMGB1 variants: full-length, box A+B, A box, and B box. Top reactive samples were selected for high-resolution epitope mapping using a peptide microarray incorporating cyclic epitopes spanning the full HMGB1 sequence (36 SLE, 10 HC). Clinical data for SLE patients were retrieved from the clinical lupus register KLURING.

Results: Sera from SLE patients demonstrated higher anti-HMGB1 autoantibody reactivity compared with HC across all HMGB1 variants. Reactivity correlated weakly with disease duration but was not associated with age, SLEDAI-2K, SDI, complement levels, ANA status, or autoantibody profiles. 80% of patients reacted to multiple domains (168/210) as assessed by ELISA. Among selected high-reactivity sera, peptide mapping identified reactivity against Linker 2 together with B box/or C-terminal epitopes. No A box peptide met the positivity criteria. Linker 2 was recognised by 8/10 selected high-reactivity healthy controls.

Conclusions: Our findings demonstrate increased and broad anti-HMGB1 reactivity in SLE and identify Linker 2 as an underappreciated immunodominant region for HMGB1 autoantibodies in SLE and healthy controls. Epitope heterogeneity of HMGB1 autoantibodies may indicate the potential for pathogenic and protective autoantibody responses. However, functional studies are required to determine the biological consequences of autoantibody binding and their clinical relevance.

KEYWORDS: HMGB1, alarmin, autoantibodies, systemic lupus erythematosus, epitope mapping

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune rheumatic disease (SARD) driven by a loss of tolerance to self-antigens, leading to a broad repertoire of autoantibodies against nuclear, cytoplasmic, and extracellular targets (1, 2). A recurring feature of this pathology is the release of damage-associated molecular patterns (DAMPs) from dying or activated cells, which sustain innate immune activation and perpetuate the inflammatory cycle (3). Among the DAMPs implicated in SLE, HMGB1 has attracted particular interest because of its reported role as an antigen (4, 5).

HMGB1 is a conserved, ubiquitously expressed chromatin protein. In the nucleus, it stabilises nucleosomes and supports transcription, but during cellular stress such as hypoxia or oxidative stress, necrosis, or active secretion, it can enter the extracellular space. Extracellular HMGB1 acts as a potent pro-inflammatory mediator through RAGE, TLR2, and TLR4 (6-8). The biological functions of HMGB1 are closely linked to its molecular structure. HMGB1 has two DNA-binding domains, the A box and the B box, joined by a short linker. A second linker sits between the B box and the acidic C-terminal tail. These regions have different functions: the B box mediates pro-inflammatory activity, while the A box can antagonise these effects. HMGB1 activity is further regulated by redox, with the disulfide form (S-S, SH) associated with the extracellular, inflammatory signaling most relevant to SLE (9).

HMGB1 also connects directly to the broader autoantibody landscape of SLE. Anti-HMGB1 autoantibodies were first reported in SLE and other autoimmune diseases, initiating interest in HMGB1 as an autoantigen (10, 11). Through its associations with nucleosomes on apoptotic cells and its ability to promote anti-dsDNA responses through TLR2, extracellular HMGB1 may play a role in the development of autoreactive B-cell responses and autoantibody generation that characterize SLE (11, 12). Further, circulating HMGB1 is elevated in SLE and is positively associated with disease activity, marking it as a possible mediator and a candidate biomarker (5). HMGB1 has also been shown to circulate in association with immunoglobulins, as IgG depletion sharply reduced measurable HMGB1 in serum (4), further supporting a relationship between extracellular HMGB1 and the humoral immune response.

In a prior study from our group, comparing 188 patients with SLE with age- and sex-matched controls from the general population, anti-HMGB1 positivity was found in 23% of patients with SLE compared with approximately 5% of controls, demonstrating that these

autoantibodies are more prevalent in SLE but can also occur in controls (13). Clinical associations of anti-HMGB1 autoantibodies, however, remain less clear. Levels of anti-HMGB1 autoantibodies in SLE patient sera have been reported to correlate with SLEDAI (14), whereas associations with disease activity have not been consistent across cohorts.

Given the distinct biological functions of the HMGB1 domains, the region targeted by an anti-HMGB1 antibody may have functional consequences. Antibodies targeting either the A or B box could modulate HMGB1-mediated inflammatory signaling. Epitope location, in other words, may carry functional consequences (15, 16). This makes the specificity of the anti-HMGB1 response, rather than its presence alone, particularly relevant.

Structured epitope mapping identified A box as the dominant target of anti-HMGB1 autoantibodies found in sera from SLE patients (15). Additionally, reactivity of sera toward the linker region distinguished SLE from polymyositis/dermatomyositis (14). Another investigation confirmed and extended the A box pattern, linking A box reactivity to active disease and to anti-dsDNA autoantibody levels (17). The A box was not the only target, however. Antibodies to the B box were detectable in a substantial minority of patients and correlated with A box reactivity (16). Additional epitopes, including the linker, were recognized alongside the A box (15). The dominance of the A box is therefore relative, not exclusive. The acidic C-terminal tail was not examined in neither study.

Taken together, these studies established anti-HMGB1 antibodies as a recurring feature of SLE serology. But their presence alone is not disease-defining, since low-level reactivity also occurs in healthy individuals. This shifts the relevant question away from mere presence, toward how much antibody is produced and, above all, where it binds. Despite previous evidence of A box and linker-region reactivity, the fine epitope specificity of the anti-HMGB1 response remains poorly defined. In particular, the linker-region reactivity reported by Hayashi et al., which may correspond to the linker between the B box and the acidic tail, was never followed up (14).

Previous studies have predominantly examined reactivity using whole HMGB1 or larger protein regions, leaving short linear epitopes unresolved. Peptide-level mapping of anti-HMGB1 reactivity in SLE therefore remains limited. This gap is timely: the ongoing DYSALARM-322 trial (NCT05193591) is investigating the functional impact of anti-HMGB1 antibodies purified from SLE sera, yet their epitope targets are unknown. As functional work increasingly depends on purified autoantibodies, resolving where they bind becomes essential.

This study aimed to characterise anti-HMGB1 autoantibody responses in SLE at both domain and peptide-level resolution. Using an expanded cohort from the *Clinical Lupus Register in Northeastern Gothia* (Swedish acronym ‘KLURING’), we combined recombinant HMGB1 variants comprising full-length HMGB1, the A+B box, the A box and the B box with high-resolution peptide microarray mapping. We then examined how reactivity patterns relate to clinical parameters.

METHODS

Sample cohorts

Peripheral blood was collected and allowed to clot, after which serum was separated and stored at -80°C until further analysis. Serum samples were obtained from SLE patients (n = 210) recruited through the prospective follow-up program “KLURING” (Clinical Lupus Register in Northeastern Gothia) at Linköping University Hospital, Sweden (18), and from Healthy donor controls (HC, n = 100) collected from the blood bank at Haukeland University Hospital, Bergen, Norway. All SLE patients fulfilled the 1982 American College of Rheumatology (ACR-82) (19) and /or the 2012 Systemic Lupus International Collaborating Clinics classification criteria (SLICC-22) (20). Disease activity and organ damage were evaluated using the SLE Disease Activity Index 2000 (SLEDAI-2K) and the SLICC/ACR Damage Index (SDI) (21, 22). Active disease was defined as SLEDAI-2K $\geq$ 5, in line with widely accepted criteria (23). The SLE cohort was further stratified by disease duration into early/recent-onset ($\leq$ 2 years; n = 96) and established ($\geq$ 8 years; n = 114) disease. Characteristics of all participants (SLE and HC) are summarized in Table 1.

Table 1. Demographic, clinical, and treatment characteristics of the SLE cohort and healthy controls. Data are shown for 210 patients with SLE and 100 HC. Continuous variables are presented as median (interquartile range) [range]; categorical variables as number (percentage). Percentages are calculated within each group. Disease characteristics, laboratory markers, and treatments were assessed in the SLE cohort only and are not applicable to healthy controls (blank cells). Active disease was defined as SLEDAI-2K $\geq$ 5. ANA, antinuclear antibodies determined by indirect immunofluorescence (IF); C3 and C4, complement components 3 and 4; CRP, C-reactive protein. Patients could receive more than one treatment, so treatment categories are not mutually exclusive.

	SLE (n =210)	HC (n =100)
Demographics		
Female/Male, n (%)	179 (85.2%) / 31(14.8%)	92 (92.0%) / 8(8.0%)
Age, median (IQR), [range] years	49 (36-66), [18-90]	64 (58-68), [25-78]
Disease Characteristics/variables		

SLE duration (years), median (IQR), [range]	10 (1-18), [0-54]	
SLEDAI-2K, score, median (IQR), [range]	2 (0-4) [0-24]	
Active disease (SLEDAI-2K $\geq$ 5), n (%)	45 (21.4%)	
Positive/negative IF-ANA at sampling, n (%)	180 (85.7%) / 30 (14.3%)	
SLICC/ACR damage index (SDI), score	0 (0-2) [0-7]	
Laboratory markers/ variables		
C3, median (IQR) [range], g/L	0.97 (0.85-1.20) [0.38-2.40]	
C4, median (IQR) [range], g/L	0.17 (0.12-0.24) [0.02-0.83]	
CRP, median (IQR) [range], mg/L	9.96 (5.5-19.0) [0.17-63.0]	
Clinical phenotypes (ACR-82 definitions)		
1. Malar rash, n (%)	81 (38.6%)	
2. Discoid rash, n (%)	34 (16.2%)	
3. Photosensitivity, n (%)	98 (46.7%)	
4. Oral ulcers, n (%)	24 (11.4%)	
5. Arthritis, n (%)	153 (72.9%)	
6. Serositis, n (%)	76 (36.2%)	
7. Renal disorder, n (%)	60 (28.6%)	
8. Neurological disorder, n (%)	14 (6.7%)	
9. Hematological disorder, n (%)	133 (63.3%)	
10. Immunological disorder, n (%)	130 (61.9%)	
11. ANA, n (%)	205 (97.6%)	
Treatments		
Hydroxychloroquine (HCQ), n (%)	140 (66.7%)	
Corticosteroids, n (%)	138 (65.7%)	
Mycophenolate mofetil (MMF), n (%)	23 (11.0%)	
Rituximab, n (%)	10 (4.8%)	

Methotrexate, n (%)	13 (6.2%)	
Azathioprine, n (%)	6 (2.9%)	
Sirolimus, n (%)	4 (1.9%)	
Belimumab, n (%)	3 (1.4%)	
Cyclophosphamide, n (%)	2 (1.0%)	
Untreated, n (%)	12 (5.7%)	

Other treatments reported in single patients included tacrolimus, cyclosporine, and chloroquine.

Production of recombinant human HMGB1 variants

Recombinant human HMGB1 (rhHMGB1) variants were produced to map serum antibody reactivity to defined regions of the protein. Four variants were generated: full-length HMGB1 (M1 - E215), the A and B boxes without the acidic C-tail (M1 - R163), the A box alone (M1 - I79), and the B box alone (P95 - R163).

Each variant was expressed in *Escherichia coli* BL21(DE3) R3 pRARE2 from a pNIC-Bio3 expression vector, carrying an N-terminal His tag and C-terminal Avi tag, and was co-expressed with BirA for site-specific biotinylation (24). Expression was induced by adding 0.5 mM IPTG and 150 μ M Biotin at 18°C for 16 hours. The protein was purified from soluble cell extracts by immobilised metal affinity chromatography (IMAC) followed by size-exclusion chromatography (SEC) (25). The reducing agent TCEP 0.5 mM was present throughout the purification to maintain the variants in their reduced form. Purity was confirmed by SDS-PAGE, and absence of DNA contamination in the recombinant protein preparations was verified by agarose gel electrophoresis (Fig. S1). The protein concentration was determined by absorbance at 280 nm (NanoDrop).

In-house ELISA protocol

In-house ELISAs using Full-Length HMGB1 (FL) (M1 - E215), the A+B box (M1 - R163), the A box (M1 - I79), and the B box (P95 - R163) were performed to detect the presence of anti-HMGB1 antibodies in serum and assess reactivity against the different HMGB1 variants. In brief, 50 μ l per well of 1 μ g/ml of each variant in PBS was incubated at 4°C overnight in Maxisorp polystyrene 96-well plates (Merck, #M9410-ICS). The plates were then washed with PBS-0.05%Tween and blocked with 100 μ l/well for 1 hour of casein blocker in PBS (Thermo, #37582, 1% casein) 100 μ l/well for 1 hour, then washed with PBS-Tween.

Serum samples were diluted 1:50 in casein blocker in PBS with 0.05% Tween and added in duplicate (50 µl/well). A humanised anti-HMGB1 monoclonal antibody (clone 2G7) was used as a standard on the full-length HMGB1-coated plate, starting at 50 ng/ml and serially diluted two-fold in casein blocker in PBS with 0.05% Tween (50 µl/well, in duplicate) (26). Incubated for 2 hours at room temperature. The wells were washed 3 times with PBS-Tween and incubated with an HRP-conjugated rabbit anti-human IgG (Dako P0214, 1/6000 dilution) antibody for 30 min at room temperature. Plates were then washed 4 times with PBS-Tween before bound antibodies were detected using 3,3',5,5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H₂O₂). Incubated for 30 minutes in the dark. The reaction was stopped with 1 M sulphuric acid, 50 µl/well and the absorbance was measured at 450 nm. SLE samples were analysed at Karolinska University Hospital using a SpectraMax microplate reader (Molecular Devices, USA), and HC samples were analysed at the University of Bergen using a Synergy H1 (BioTek, USA). To correct for inter-plate variability, OD values were normalized across plates using a reference standard concentration of 6.25 ng/ml. The plate whose standard OD value was closest to the median of all the plates was selected as the reference. A normalization factor (NF) was calculated for each plate by dividing the reference plate OD by the respective plate OD at 6.25 ng/ml ($NF = OD_{6.25 \text{ reference plate}} / OD_{6.25 \text{ respective plate}}$). Final normalized OD values for both SLE and HC samples were obtained by multiplying each sample's OD by the corresponding plate NF.

Identifying antibody epitopes

For conformational epitope mapping of antibodies in the selected high-positive serum samples from SLE (n = 36) and HC (n = 10), a custom cyclic constrained peptide microarray was designed using the full-length hHMGB1 sequence (UniProtID: P09429) (27) and produced by PEPperCHIP® Custom Peptide Microarray (PEPperPRINT GmbH, Heidelberg, Germany). The library consisted of 110 cyclic constrained peptides generated by N-to C-terminal thioether cyclization to mimic natural conformations. Peptides were 12 amino acids in length with a 10-amino-acid overlap (2-amino-acid shift), covering the full protein sequence, and were printed in duplicate (220 spots total) directly on the chip using PEPperCHIP® laser printing technology.

The assay to identify serum IgG reactivity against individual HMGB1 peptides was performed at SciLife Labs, Stockholm, Sweden. Each serum sample was incubated on a peptide microarray containing the overlapping HMGB1 peptides described above. Prior to sample

incubation, the microarray was pre-stained with the goat anti-human IgG Fc DyLight 650 secondary antibody alone to identify and subtract background interactions resulting from direct binding of the secondary antibody to peptides on the array. Serum samples were diluted 1:1000 in staining buffer, and the microarray was blocked with Rockland blocking buffer (MB-070) for 30 minutes at room temperature. Samples were incubated overnight at 4°C on an orbital shaker. Following washing with PBS-Tween (0.05%), the microarray was incubated with a goat anti-human IgG Fc DyLight650 secondary antibody (1:1000) for 45 minutes at room temperature in the dark to detect serum-derived human IgG bound to the peptides. Influenza hemagglutinin (HA) and polio control peptides were included as positive controls on the microarray. HA control peptides were detected using a monoclonal mouse anti-HA (clone 12CA5, Cy3, 1:2000), while polio control peptides served as controls for serum IgG binding and were detected by the goat anti-human IgG Fc DyLight650 secondary antibody. The microarray was washed, rinsed with 1 mM Tris buffer, and air-dried before scanning with an InnoScan 1100 microarray scanner (Innopsys). Sample signal (DyLight650) was detected using the 635 nm laser channel, with fluorescence intensities recorded as foreground median minus background median (F635 – B635). The HA positive control (Cy3) was detected using the 532nm laser channel. Non-binding scrambled peptides and control peptides were included to assess background signal and non-specific binding.

Microarray analysis

PEPperCHIP® microarray images were analyzed with MAPIX Software. Spots indicating peptide-antibody binding were detected based on spot diameter, background ring size, and signal threshold. For each serum sample, the fluorescence intensities were averaged for each HMGB1 peptide duplicate. A sample-specific positivity threshold was calculated as the mean fluorescence intensity of all HMGB1 peptides plus two standard deviations (mean + 2SD). Peptides with averaged fluorescence intensities above this threshold were considered reactive, and a minimum of two consecutive overlapping reactive peptides was required to define a reactive epitope region. Binding sites were categorized based on known HMGB1 receptor, nuclear localization signal (NLS), and DNA binding locations, and HMGB1 structural domains (A box, linker 1, B box, linker 2, and C-terminal acidic tail).

Statistical analysis

All analyses were performed in R (version 4.5.2, R Foundation for Statistical Computing, Vienna, Austria) (28). Anti-HMGB1 reactivity was expressed as normalised OD. As the reactivity values were non-normally distributed, non-parametric tests were used throughout, and continuous data were summarised as median and interquartile range (IQR). All tests were two-sided, and $p < 0.05$ was considered significant.

Reactivity was compared between two groups (SLE versus healthy controls; ANA-positive versus ANA-negative) using the Mann-Whitney U test, and across more than two groups (serological subgroups and reactivity-pattern groups) using the Kruskal-Wallis test. Associations between reactivity and continuous clinical variables (age, disease duration, SLEDAI-2K, SDI, C3, C4) were assessed by Spearman's rank correlation.

To define reactivity of the SLE samples, cut-off values for each variant were defined using a healthy control- derived cut-off, set at the 75th percentile (full-length > 0.215 , A+B box > 0.104 , A box > 0.072 , B box > 0.047). A sample was scored positive for a variant when its normalised OD exceeded the corresponding cut-off. Patients were classified into five mutually exclusive groups based on reactivity to the isolated A box and B box domains: multiple domains (positive for both A box and B box), A box only, B box only, full-length only (negative for both isolated domains but positive for the full-length protein), and no reactivity (negative for all variants). Group assignment was based solely on reactivity to the isolated domains; therefore, patients classified as A box only or B box only could also be positive for the full-length protein. As several groups were small ($n \leq 4$), only the overall Kruskal-Wallis test is reported for group comparisons, and no pairwise post-hoc tests were performed.

For the epitope mapping, high-reactivity samples were selected. Samples above the 75th percentile of the SLE cohort and samples above the 95th percentile of the control cohort were selected, yielding 36 SLE and 10 control samples. This selection restricts the analysis to samples that were positive for at least one antigen variant and therefore characterises epitope specificity rather than reactivity prevalence.

RESULTS

Anti-HMGB1 reactivity is elevated in SLE across full-length and domain-specific variants

The domain organization of HMGB1 and the four recombinant variants used in this study are shown in Fig.1A and 1B. To determine whether concentrations and specificity of anti-HMGB1 antibodies in serum from SLE patients and healthy donors differ, we measured the serum reactivity against four recombinant HMGB1 variants by ELISA: full-length HMGB1 (M1 - E215), the A+B box (M1 - R163), the isolated A box (M1 - I79), and B box (P95 - R163) using ELISA.

Reactivity was significantly higher in serum from SLE patients ($n = 210$) than in HC ($n = 100$) for every variant (Mann-Whitney, all $p < 0.0001$). Median normalized OD values in SLE versus HC were 0.240 vs 0.077 (FL), 0.201 vs 0.030 (A+B box), 0.172 vs 0.017 (A box) and 0.200 vs 0.007 (B box) (Fig. 1C-F). Healthy controls showed low but detectable reactivity across all variants, indicating a low baseline level of anti-HMGB1 antibodies. SLE samples displayed higher reactivity than HC, and similarly, reactivity was not confined to a single region, with sera reacting to the full-length protein and to each isolated domain, showing a broad, multi-domain response rather than a single-domain specificity. The per-patient distribution of this reactivity, ordered by disease duration (for SLE) and by age (for HC), is shown in Fig. S2. Heatmaps display a subset of SLE patients showing elevated reactivity to all four HMGB1 variants, which was more frequent among those with longer disease duration. No comparable gradient towards age was seen in controls.

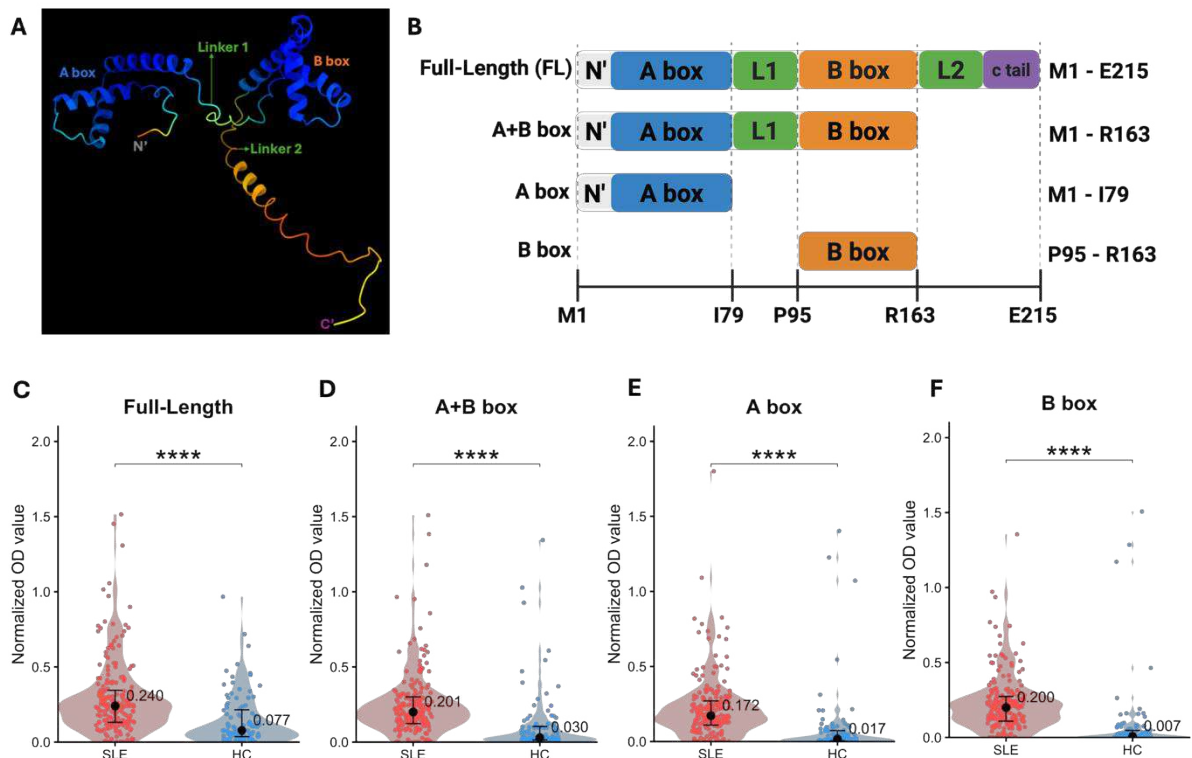


Figure 1. Serum reactivity is elevated in SLE patients across all HMGB1 variants. (A) Three-dimensional structure of full-length HMGB1 predicted by AlphaFold (UniProtID: P09429; model AF-P09429-F1-v6) and rendered in UCSF ChimeraX (v1.12), showing the A box, B box, Linker 1, and Linker 2. Colour indicates per-residue model confidence (predicted Local Distance Difference Test - pLDDT): dark blue - very high, light blue - high, yellow - low, orange - very low. (B) Schematic representation of the four recombinant HMGB1 variants: full-length HMGB1 (M1 - E215), A+B box (M1 - R163), A box (M1 - I79), and B box (P95 - R163), showing the A box, Linker 1 (L1), B box, Linker 2 (L2), and C-terminal acidic tail. (C – F) Anti-HMGB1 reactivity (normalized OD) in SLE patients (n = 210, red) and healthy controls (HC) (n = 100, blue), measured by in-house ELISA against four recombinant HMGB1 variants. (C) full-length, (D) the A+B box, (E) A box, and (F) B box. Violin plots show the distribution of values; black points and error bars indicate the median and interquartile range, with median values annotated. Reactivity was significantly higher in SLE than in HC for all four variants (Mann-Whitney U test, **** $p < 0.0001$).

Anti-HMGB1 reactivity is associated with disease duration but is independent of age, disease activity, and complement proteins

To determine whether the magnitude of anti-HMGB1 reactivity related to clinical characteristics, we correlated normalized OD values for each variant with age, disease duration, SLEDAI-2K, SDI, and complement protein levels (C3, C4) using Spearman's rank correlation.

As indicated in the reactivity heatmaps, disease duration correlated with the reactivity (Fig. 2A). A weak but significant positive correlation was present for all four variants (FL: $\rho = 0.167$, $p = 0.016$; A+B box: $\rho = 0.183$, $p = 0.008$; A box: $\rho = 0.204$, $p = 0.003$; B box: $\rho = 0.216$, $p = 0.002$), indicating that anti-HMGB1 reactivity tends to increase with longer disease duration in accordance with the gradient seen in the duration-ordered heatmap (Fig. S2).

As SLE and control cohorts were not age-matched, the association between age and HMGB1 reactivity was examined. No significant correlations with age were observed for any HMGB1 variant in either SLE patients (Fig. 2B) or in healthy controls (Fig. 2C).

In contrast to disease duration, reactivity was independent of disease activity and organ damage. No variant correlated with SLEDAI-2K or SDI and was not associated with C3 or C4 (Fig. S3). The full correlation structure is summarized in Fig. 2D, in which disease duration is the sole variable to reach significance across variants.

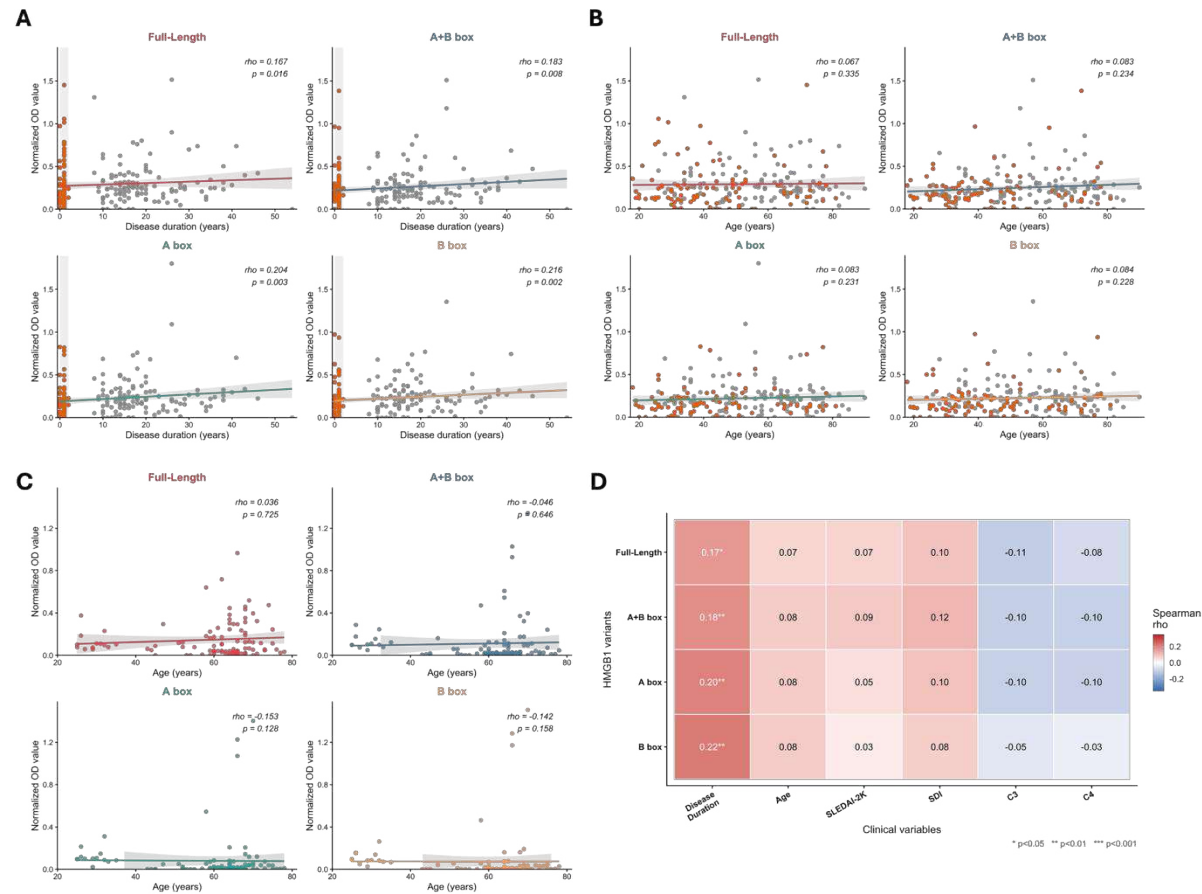


Figure 2. Disease duration, but not age, correlates with anti-HMGB1 reactivity. Spearman rank correlations between anti-HMGB1 reactivity (normalized OD) and clinical variables, for each of the four HMGB1 variants (full-length, A+B, A box and B box). Each point represents one individual; the line shows the linear fit with a 95% confidence band; ρ and p values are given per variant. In A and B, points are coloured by disease duration group (SLE) [orange - early disease ≤ 2 years, grey - established disease ≥ 8 years]. Correlation plot of SLE patients ($n = 210$): OD values/reactivity vs (A) disease duration and (B) age. (C) Healthy controls ($n = 100$): OD values/reactivity vs age, coloured by variant. (D) Spearman correlation matrix summarizing associations between the four variants and all clinical variables in SLE (disease duration, age, SLEDAI-2K, SDI, C3, C4); colour indicates the correlation coefficient (ρ) and asterisks denote significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Anti-HMGB1 reactivity is independent of the autoantibody profile

Having found that anti-HMGB1 reactivity was independent of disease activity, we next examined whether it related to the patients' autoantibody profile. We compared reactivity by ANA status and across different serological subgroups. Anti-HMGB1 reactivity did not differ between ANA-positive ($n = 180$) and ANA-negative ($n = 30$) patients for any variant (Mann-Whitney, all $p \geq 0.26$) (Fig. 3A). To determine whether reactivity was associated with any specificity, we stratified patients into six mutually exclusive groups (anti-dsDNA, ANA-positive without defined specificity, seronegative, anti-SSA/Ro, anti-RNP/Sm and other). The reactivity was again comparable across all groups for every variant (Kruskal-Wallis, all $p \geq 0.37$) (Fig. 3B). We further examined reactivity according to ANA staining pattern and observed no significant differences between the different patterns for any of the four variants (Kruskal-Wallis, all $p \geq 0.55$) (Fig. 3C).

Notably, seronegative patients showed anti-HMGB1 reactivity comparable to that of antibody-defined patients, indicating that anti-HMGB1 antibodies are not restricted to any particular autoantibody specificity.

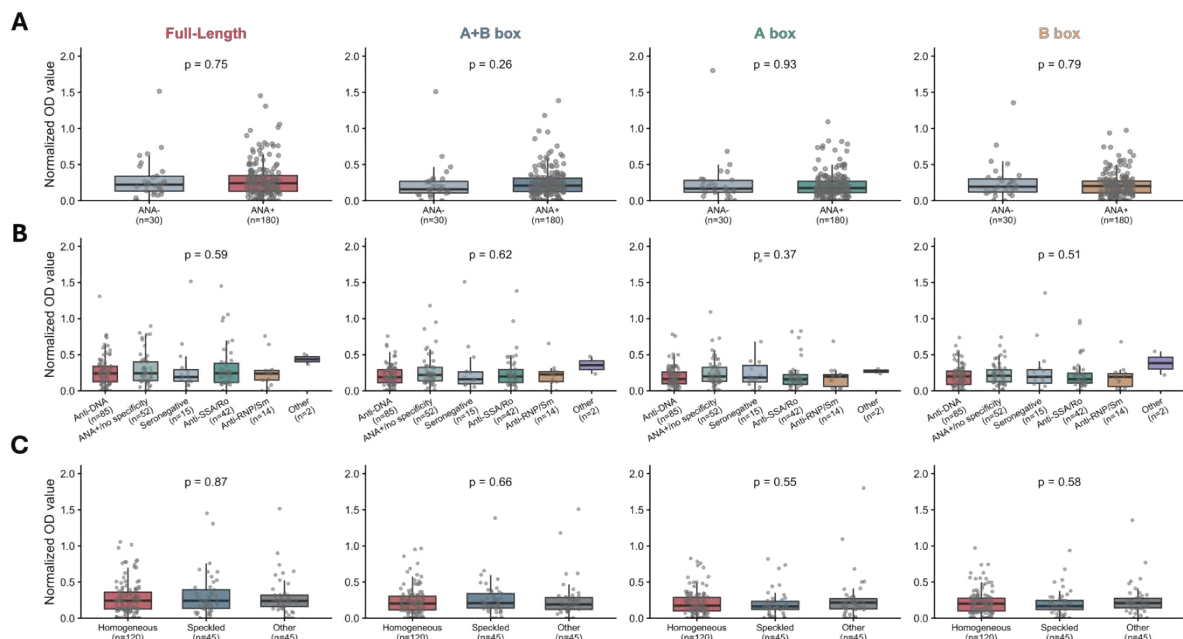


Figure 3. Anti-HMGB1 reactivity is independent of ANA status and autoantibody specificity in SLE. Anti-HMGB1 reactivity (normalized OD) in SLE patients, shown for each of the four HMGB1 variants (full-length, A+B box, A box, B box). Data shown as box plots (median, interquartile range (IQR), 1.5×IQR whiskers) with individual patients overlaid as points. **(A)** Reactivity by ANA status: ANA- ($n = 30$) vs ANA+ ($n = 180$). Reactivity did not differ between groups for any variant (Mann-Whitney U test; p values shown per panel). **(B)** Reactivity across six mutually exclusive serological subgroups: anti-dsDNA ($n = 85$), ANA-positive without defined specificity ($n = 52$), seronegative ($n = 15$), anti-SSA/Ro ($n = 42$), anti-RNP/Sm ($n = 14$), and other ($n = 2$). Reactivity did not differ across subgroups for any variant (Kruskal-Wallis test; p values shown per panel). **(C)** Reactivity across ANA staining patterns: Homogeneous ($n = 120$), Speckled ($n = 45$), and Other ($n = 45$).

Reactivity did not differ across ANA staining patterns for any variant (Kruskal-Wallis test; p values shown per panel).

Anti-HMGB1 reactivity spans multiple domains and broadens with disease duration

Having found that HMGB1 serum reactivity was not associated with clinical disease activity or autoantibody profile, we next characterised the reactivity itself: its breadth across the HMGB1 domains and its relationship to disease duration.

Each SLE patient was classified by comparing their normalized OD for each variant with the 75th percentile of HC reactivity for that variant (full-length 0.215, A+B box 0.104, A box 0.072, B box 0.047). Based on reactivity to the isolated A and B boxes, patients were classified into five mutually exclusive reactivity patterns: multi-domain (positive for both A box and B box, irrespective of full-length or A+B box reactivity), A box only, B box only, full-length only, and no reactivity.

Most patients reacted to multiple domains (168/210, 80%). Single-domain reactivity was uncommon (B box only 9.0%, full-length only 1.9%, A box only 0.5%), and 8.6% showed no reactivity (Fig. 4A). The response therefore engaged more than one region of HMGB1 in most patients. Isolated A box reactivity was found in a single patient, which argues against the A box as a domain target in this cohort.

Multi-domain and full-length-only reactors had longer disease duration (median ~11-12 years) than single-domain reactors (~1 year), though the difference across the five groups were not significant (Kruskal-Wallis, $p = 0.105$) (Fig. 4B). Disease activity showed no such relationship: SLEDAI-2K and C3 did not differ across groups ($p = 0.847$ and 0.342), whereas multi-domain reactors had higher SDI ($p = 0.034$) and lower C4 ($p = 0.020$) (Fig. S4). Together, these findings suggest that broader anti-HMGB1 reactivity may relate more closely to disease duration and accumulated damage than to current disease activity.

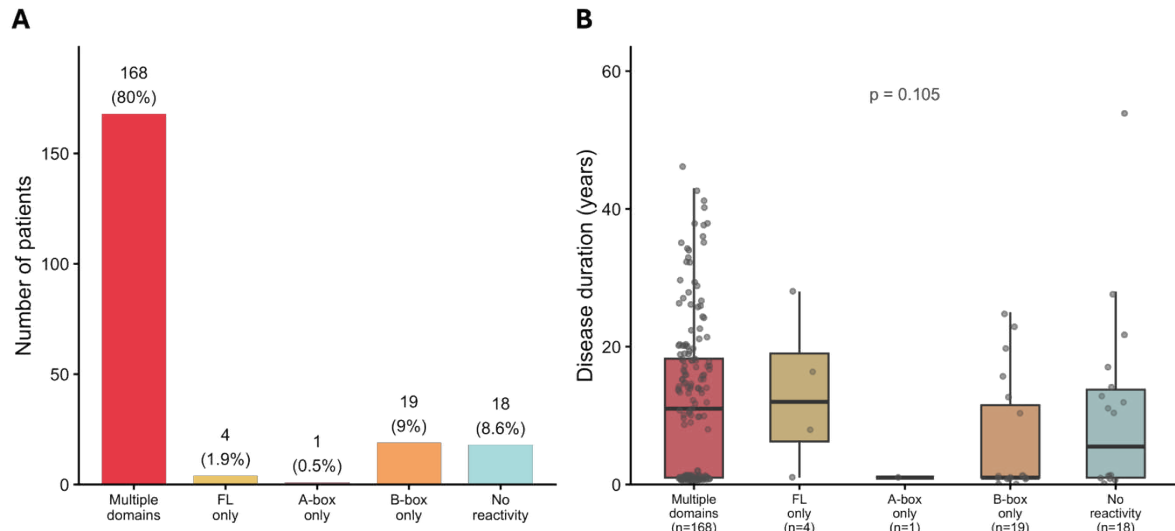


Figure 4. Most SLE patients target multiple HMGB1 domains, linked to disease duration. SLE patients (n = 210) were classified into five groups by comparing normalized OD for each variant against the 75th percentile HC cut-off for that variant (see Methods). **(A)** Number of patients in each reactivity group: multiple domains (n = 168), full-length only (n = 4), A box only (n = 1), B box only (n = 19), and no reactivity (n = 18). Values above bars show the percentage of the cohort. **(B)** Disease duration across reactivity groups. No significant difference between groups was observed (Kruskal-Wallis, $p = 0.105$). Data shown as box plots (median, interquartile range (IQR), $1.5 \times \text{IQR}$ whiskers) with individual patients overlaid as points.

Anti-HMGB1 antibodies target the Linker 2 region of HMGB1

The variant-based ELISA analysis showed that most patients mount a broad, multi-domain response. To map the response at peptide resolution, we used a cyclic constrained peptide microarray spanning the full HMGB1 sequence, tested on 36 SLE patients and 10 healthy controls selected for high reactivity (SLE above the 75th percentile of SLE reactivity, controls above the 95th percentile of control reactivity) (Fig. S5). The three-dimensional structure of the full-length HMGB1 is shown for reference (Fig. 1A).

Reactivity converged on a single region, with Linker 2 (~aa 163-185) was the dominant epitope, recognized by 86% of SLE sera (31/36) and far exceeded every other region (Fig. 5A). Mapping along the full HMGB1 sequence showed that reactivity clustered predominantly within Linker 2, with the flanking A box, B box, and Linker 1 was rarely or never recognised (Fig. 5B). At higher resolution, reactive peptides clustered within aa 172 -185 (Fig. 5C). Peptides within the A box was not targeted in any SLE serum (0/36), despite frequent reactivity to A-box variants detected by ELISA. The peptide mapping localised the response to Linker 2 rather than the folded HMG boxes.

Beyond Linker 2, a subset of sera extended to neighbouring regions (Fig. 5C). B box reactivity was detected in 19% of SLE sera (7/36) and C-tail reactivity in 22% (8/36); the C tail was recognised only in SLE and by no control (0/10), marking it as a disease-associated target.

Reactivity in healthy controls was largely restricted to Linker 2 (80%, 8/10), while broader, multi-region recognition and C-tail targeting are features of the SLE response.

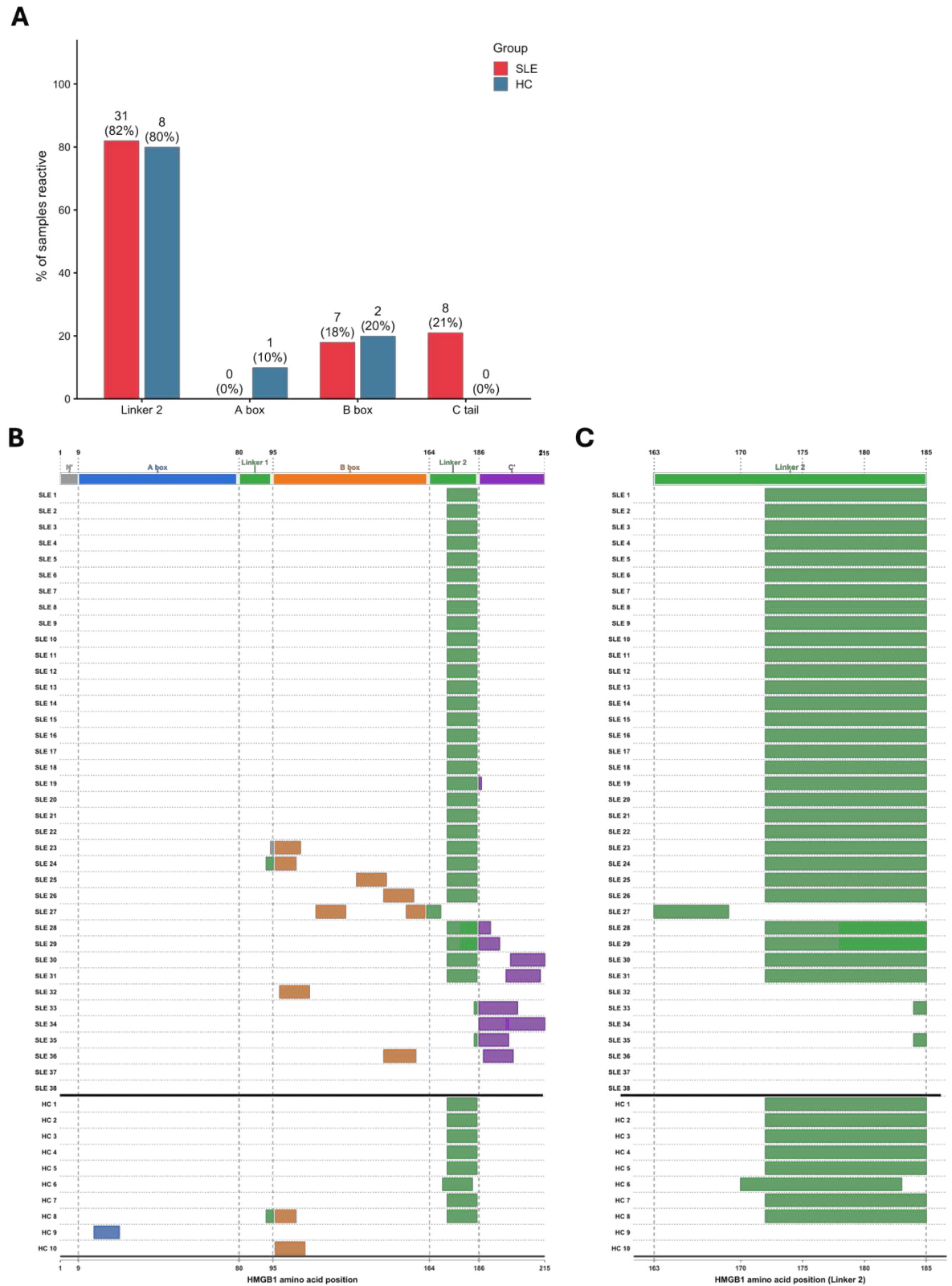


Figure 5. Epitope mapping localises the anti-HMGB1 response to Linker 2. (A) Frequency of reactivity to each HMGB1 region in SLE (red, n = 36) and healthy controls (blue, n = 10), expressed as the percentage of samples reactive; the number of reactive samples is shown above each bar. (B) Per-sample epitope map across the full HMGB1 sequence (amino acid position, x-axis) for each SLE patient (top) and control (below the black

line); coloured bars mark reactive peptides, shaded by region (A box - blue, B box - orange, Linker 1 and 2 - green, C-tail - purple). (C) Expanded view of the Linker 2 region (aa 163- 185), showing the reactive peptides per sample. Samples were selected for high reactivity and mapped cyclic constrained peptide microarray (see Methods).

Broader epitope recognition is observed in patients with longer disease duration

As Linker 2 was the most frequently recognised region among SLE sera, we next examined whether recognition beyond Linker 2 was associated with clinical characteristics. Mapped SLE patients were grouped according to reactivity pattern: Linker 2 region only (L2 only, $n = 22$), Linker 2 plus additional regions in the B box or C-tail (L2 + other, $n = 9$), or regions outside Linker 2 only (other only, $n = 5$).

Sera with reactivity to Linker 2 and additional epitopes were from patients with the longest disease duration (median 16 years), compared with a median of 1 year in both the L2-only and others-only groups (Fig. 6). A similar trend was observed for accumulated organ damage, with the L2+others showing a higher SDI (median 2) than the L2-only group (median 1) (Fig. S6). No differences were observed in disease activity or complement levels (SLEDAI-2K, C3 and C4). None of these comparisons reached statistical significance (Kruskal-Wallis test, all $p > 0.05$, disease duration $p = 0.277$, SDI $p = 0.058$).

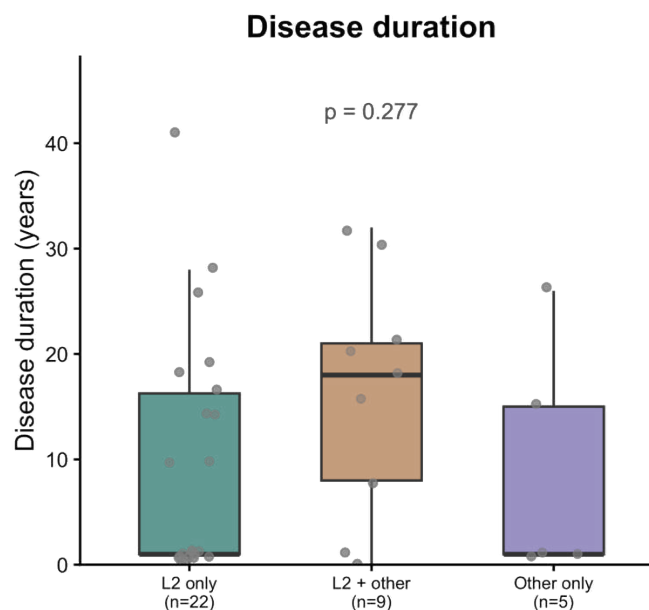


Figure 6. Disease duration by epitope-reactivity group in mapped SLE patients. Disease duration across SLE patient groups by epitope reactivity: Linker 2 alone (L2 only, $n = 22$), Linker 2 with other regions (L2 + other, $n = 9$), and others only ($n = 5$). Boxes show the median and interquartile range, whiskers extend to $1.5 \times \text{IQR}$, and points represent individual patients. Groups were compared by the Kruskal-Wallis test (p value shown in plot).

DISCUSSION

High mobility group box 1 (HMGB1) is a chromatin-associated protein that, once released, acts as a DAMP and promotes inflammation through receptors including RAGE, TLR2, and TLR4 (6). Autoantibodies against HMGB1 have been reported in SLE, although their clinical associations and relative epitope specificities remain incompletely resolved (4, 13, 14, 17). In this study, we characterised anti-HMGB1 reactivity against the full-length protein and individual domain variants in a large SLE cohort and mapped the response at peptide resolution. Anti-HMGB1 reactivity was higher in SLE than in healthy controls across all domain variants and showed a weak association with disease duration, but not with current disease activity, accumulated damage, complement levels, ANA status, or autoantibody subgroup. Peptide-mapping of high-reactivity sera identified Linker 2 as the dominant region of antibody recognition.

The increased anti-HMGB1 reactivity in SLE is consistent with previous studies identifying HMGB1 as a recurrent autoantigen (4, 13, 14, 17). However, reported associations with clinical disease activity have been inconsistent. Anti-HMGB1 antibodies have been associated with SLEDAI scores, anti-dsDNA antibodies, complement consumption, active disease, and disease exacerbations in some cohorts (4, 13, 14, 17, 29). Conversely, longitudinal comparisons found no significant difference between samples obtained at the highest and lowest recorded disease activity, and no relationship was observed with cutaneous manifestations (13, 30). In the present cohort, disease duration was the only clinical variable consistently related to reactivity across all four variants, although the correlations were weak. These results do not support anti-HMGB1 reactivity as a marker of current disease activity in this cohort. Differences in cohort composition, treatment, antigen preparation, assay thresholds, and the HMGB1 variants examined may contribute to the variability between studies.

At the protein domain level, most SLE patients showed reactivity to both the A and B boxes rather than to a single isolated domain, indicating broad recognition of HMGB1 by ELISA. At peptide resolution, recognition was concentrated predominantly within Linker 2 (aa 163-185), which was recognized in 31/36 selected SLE sera, whereas no A box peptide met the positivity criteria. Previous epitope mapping have reported A box as the major target but also reported preferential recognition of a “joiner” region by SLE sera (14). This joiner, situated between the B box and acidic C-terminal tail, largely overlaps with the Linker 2 region identified in the present study. Our findings therefore extend, rather than completely contradict, the earlier study

by identifying Linker 2 as the dominant region of linear antibody recognition in the present cohort. Differences in the relative prominence of the A box may partly reflect methodology. Recombinant-domain ELISA can detect conformational epitopes that may not be represented by short peptides, whereas the peptide array directly resolves short sequence-defined epitopes. The absence of A box peptide recognition therefore does not exclude A box antibodies that recognize conformational epitopes.

Linker 2 was also recognized by 8/10 in the healthy control group. This indicates that the recognition of the region is not itself specific to SLE. However, these controls were selected for high reactivity and are therefore not suited to estimate the prevalence of Linker 2 antibodies in the general healthy population. SLE sera showed greater overall HMGB1 variant reactivity and more frequent recognition of additional regions, including the B box and acidic C-terminal tail. These observations suggest that the magnitude and breadth of the response may distinguish SLE more clearly than recognition of Linker 2 alone, although this requires confirmation in unselected and appropriately matched groups.

The location of the dominant epitope is nevertheless biologically notable. Linker 2 lies between the B box and acidic C-terminal tail, contains the second nuclear localization sequence, and overlaps a reported RAGE-interacting region spanning aa 150 – 183 (31, 32). The adjacent acidic tail forms dynamic interactions with the A and B boxes and contributes to the regulation of HMGB1 structure and DNA binding (33, 34). Antibody binding within Linker 2 could therefore influence extracellular HMGB1 activity, although this was not addressed in the present study. The functional relevance of this region is supported by the monoclonal antibody DPH1.1, which targets HMGB1 aa 165 – 181 and consequently overlaps Linker 2 and part of the core epitope identified here. DPH1.1 inhibited HMGB1-mediated cell migration *in vitro* and reduced inflammatory-cell recruitment in experimental models (35, 36). These findings demonstrate that antibody binding within Linker 2 can modify selected HMGB1 functions experimentally, but they do not establish that naturally occurring SLE autoantibodies have the same effects. Direct functional studies using affinity-purified Linker 2 antibodies or monoclonal antibodies derived from patients will be required to determine whether they alter HMGB1-RAGE interactions, cell migration, DNA binding, or other HMGB1 activities. More broadly, epitope location may also carry therapeutic relevance: the recombinant A box acts as an HMGB1 antagonist, making it a candidate template for HMGB1-directed inhibitors (16).

Broader reactivity extending beyond Linker 2 was observed in patients with longer disease duration and greater accumulated damage (than those recognizing Linker 2 alone or epitopes outside Linker 2). However, these differences did not reach statistical significance. The findings warrant further investigation into whether epitope breadth changes over the disease course, as the present cross-sectional data does not demonstrate progressive epitope spreading.

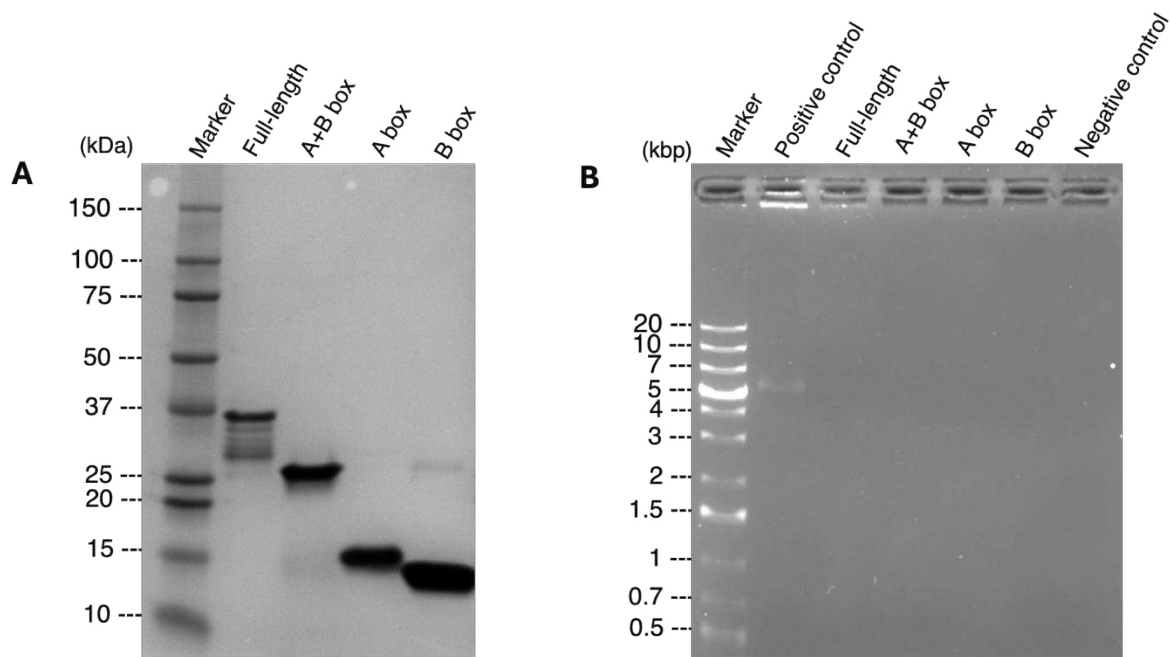
This study has some limitations. The peptide-mapped subset was small and selected for high reactivity, meaning that the mapping describes epitope specificity among selected reactive individuals rather than epitope prevalence in the wider SLE population. The SLE and control mapping subsets were also selected using different enrichment thresholds, limiting direct comparisons between them. Short peptides cannot reproduce all conformational epitopes, and the bacterially expressed recombinant variants lack mammalian post-translational modifications that may affect HMGB1 antigenicity. Finally, the cross-sectional design does not establish how anti-HMGB1 reactivity or epitope breadth changes over time. Validation in larger, longitudinal cohorts using unselected matched controls and complementary competition assays will be needed to confirm the epitope specificity and determine whether epitope breadth changes over time. Despite these limitations, combining domain-resolved ELISA in a large cohort, clinically characterized SLE cohort with peptide-resolution mapping provided a comprehensive characterization of anti-HMGB1 reactivity and identified Linker 2 as an important target for future functional studies.

CONCLUSION

We report our findings that HMGB1 autoantibody reactivity is increased in a cohort of SLE patients and is detectable towards multiple regions of HMGB1 but was largely independent of current clinical disease activity or conventional serological markers. Peptide-mapping identified Linker 2 as the dominant region of linear antibody recognition in both SLE and healthy controls, whereas recognition extending beyond Linker 2 was more characteristic of SLE. This broader epitope recognition was observed in patients with longer-standing disease and greater accumulated damage, although these associations were not statistically significant. Whether anti-HMGB1 autoantibodies have pathogenic or protective effects remains to be determined.

535 SUPPLEMENTARY DATA

536 Supplementary figures



537

538 **Figure S1. Purified recombinant HMGB1 variants used for ELISA.** (A) SDS-PAGE showing the purified
539 recombinant HMGB1 variants and their relative molecular sizes, with a molecular-weight marker (kDa). (B)
540 Agarose gel electrophoresis of the purified HMGB1 variants and positive/negative controls to assess residual
541 DNA contamination, with a size marker size marker (kbp).

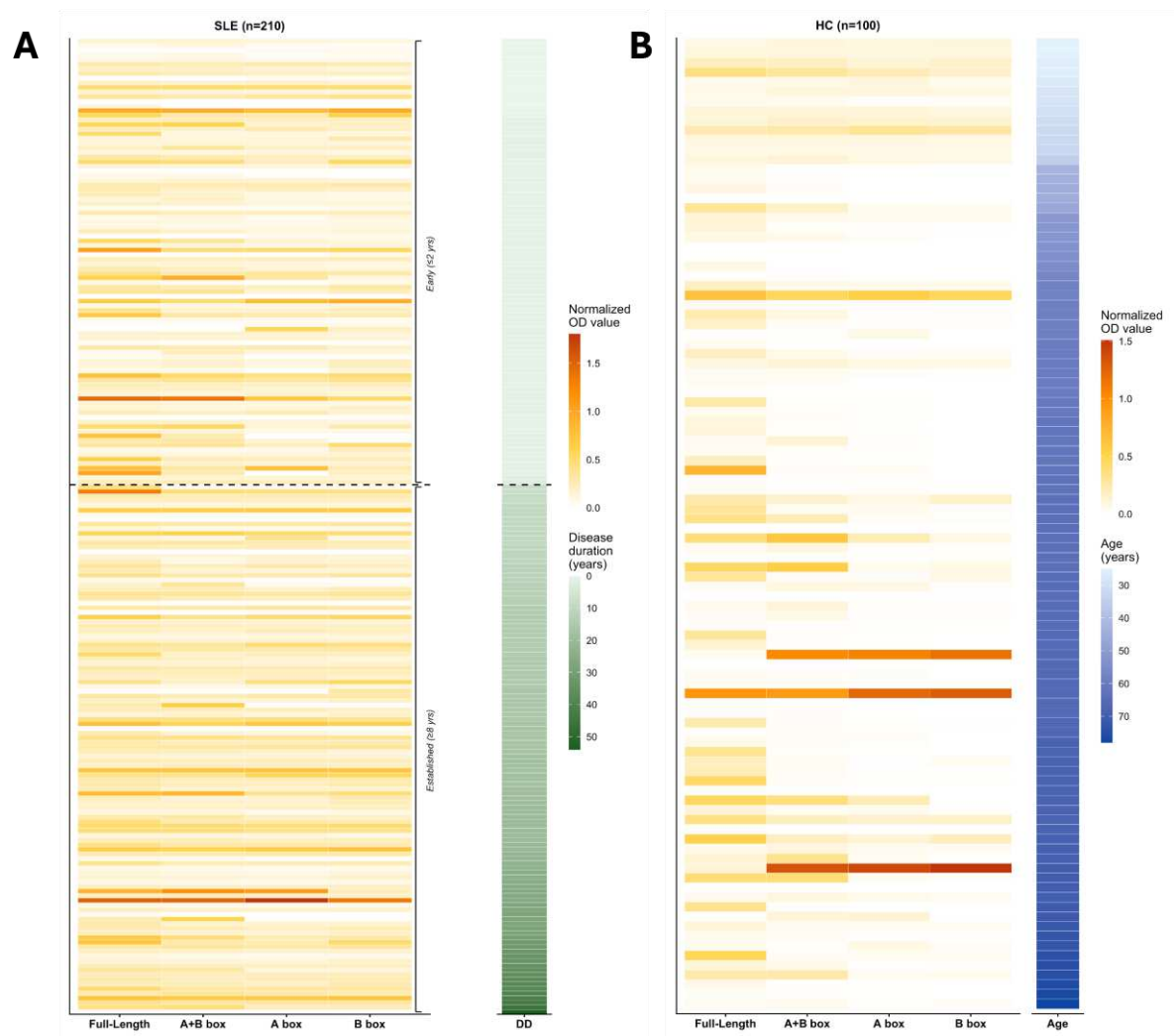


Figure S2. Sample-level anti-HMGB1 reactivity across different HMGB1 variants in SLE and healthy controls. Heatmaps of anti-HMGB1 reactivity (normalized OD) for each individual against the four recombinant variants (full-length HMGB1, A+B box, A box, and B box – as columns), measured by in-house ELISA. Each row represents one participant, and colour intensity reflects reactivity (orange scale, right). **(A)** SLE patients (n = 210) ordered top to bottom by disease duration (DD) (green scale). The dashed line separates recently diagnosed patients (≤ 2 years) from those with established disease (≥ 8 years). **(B)** Healthy controls (n = 100), ordered by age (blue scale). Reactivity in SLE is elevated across all four variants relative to the uniformly low signal in controls and appears more pronounced in patients with longer disease duration, whereas no comparable gradient is seen with age in controls.

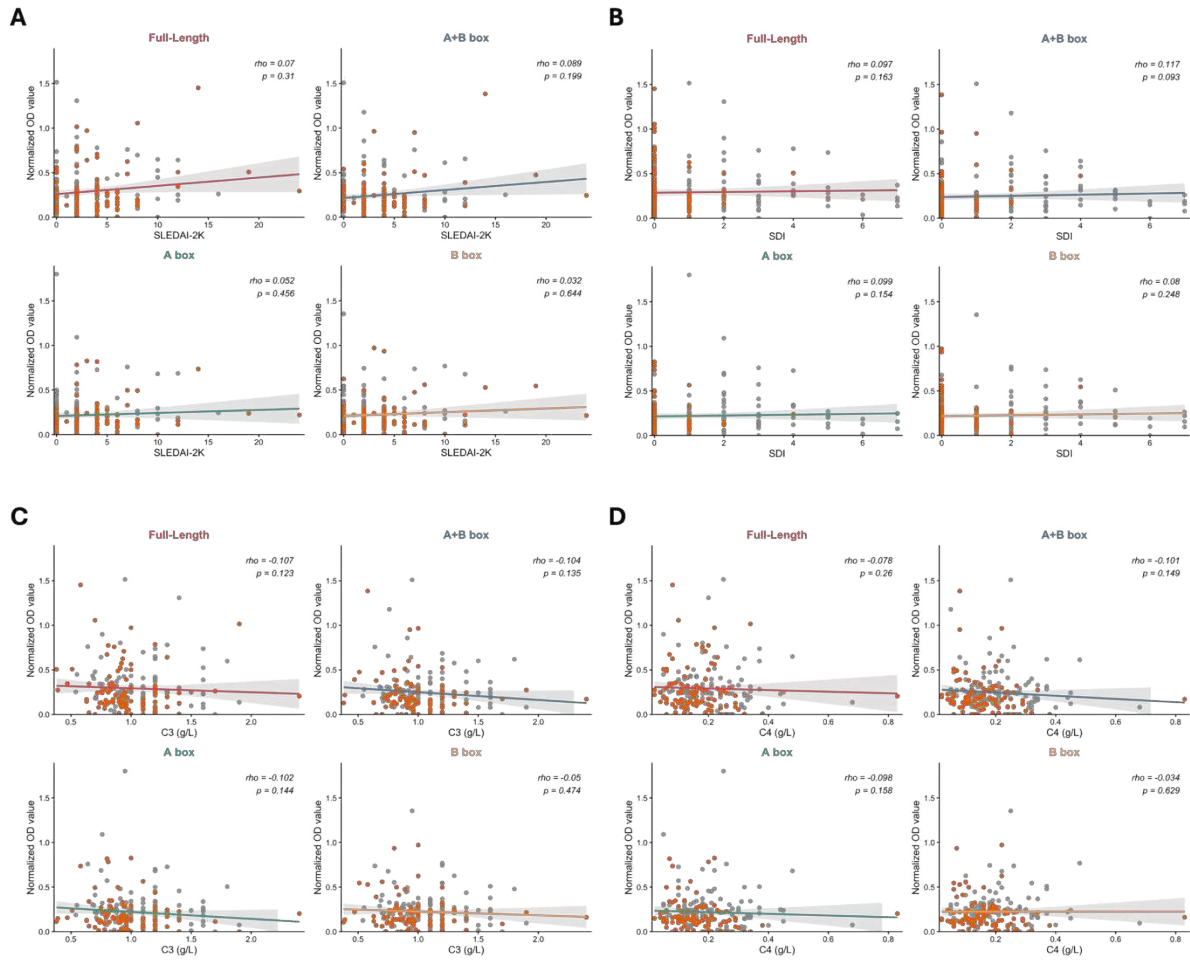


Figure S3. Anti-HMGB1 reactivity is independent of disease activity, damage, and complement. Spearman rank correlations between anti-HMGB1 reactivity (normalized OD) and clinical measures in SLE patients ($n = 210$), for each of the four HMGB1 variants. Each point represents one patient; the line shows the linear fit with a 95% confidence band; ρ and p values are given per variant. Points are coloured by disease duration group [orange - early disease ≤ 2 years, grey - established disease ≥ 8 years]. **(A)** SLEDAI-2K, **(B)** SDI, **(C)** C3, **(D)** C4. No variant correlated significantly with any variable (all $p > 0.05$).

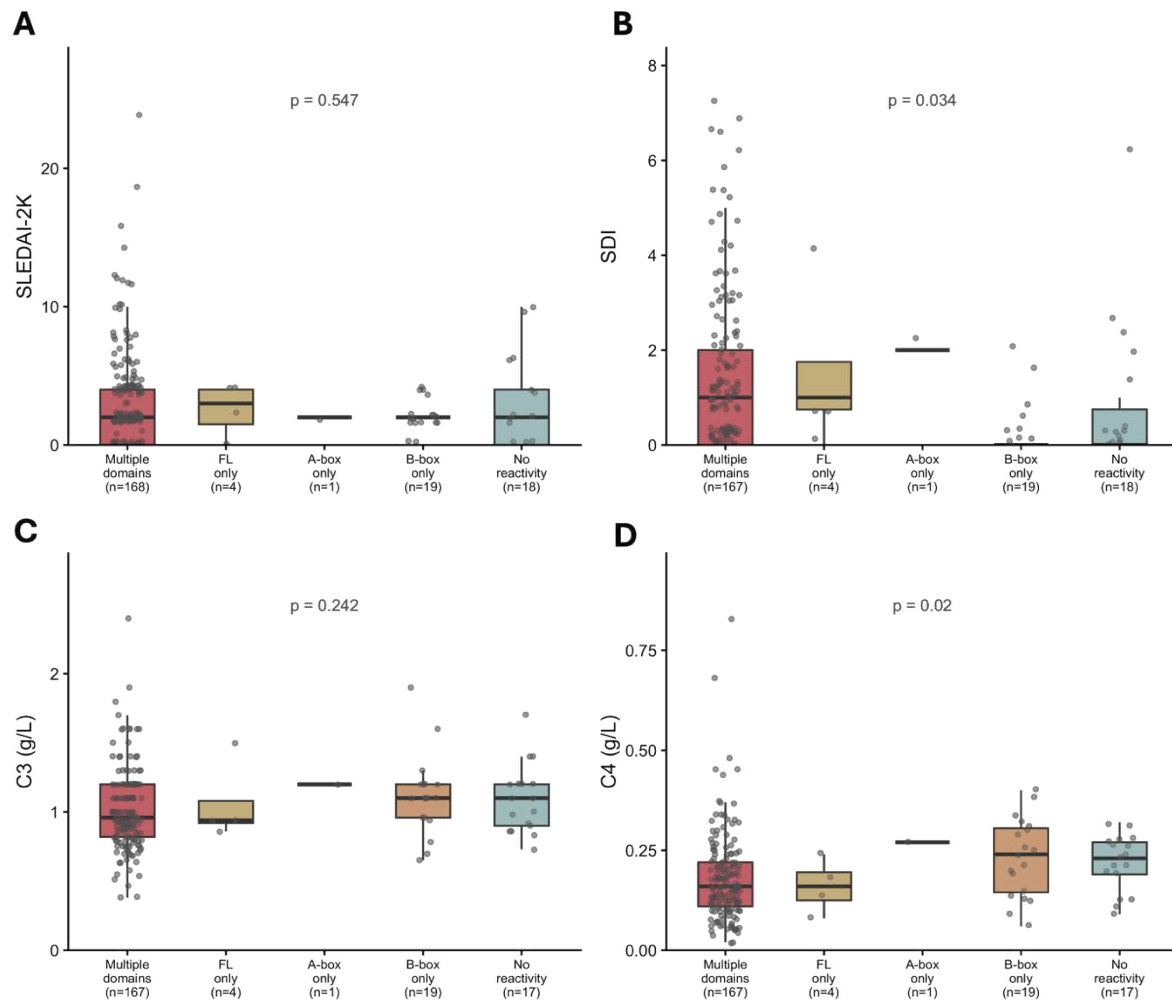


Figure S4. Clinical characteristics across anti-HMGB1 reactivity groups. Clinical measures across the five reactivity groups defined in Figure 4 (multiple domains, B box only, no reactivity, full-length only, A-box only) in SLE patients (n = 210): **(A)** SLEDAI-2K, **(B)** SDI, **(C)** C3, and **(D)** C4. Box plots show the median and interquartile range; whiskers extend to 1.5×IQR, and points represent individual patients; group sizes (n) are given under each label. SLEDAI-2K was available for all patients; SDI was missing for 1 patient and C3 and C4 for 2 patients each, who were excluded from the respective panels. Groups were compared by the Kruskal-Wallis test (p shown per panel). SLEDAI-2K and C3 did not differ across groups, whereas SDI and C4 differed significantly ($p = 0.034$ and $p = 0.020$). Because several groups were small ($n \leq 4$), only the overall test is reported, and no pairwise post-hoc comparisons were performed.

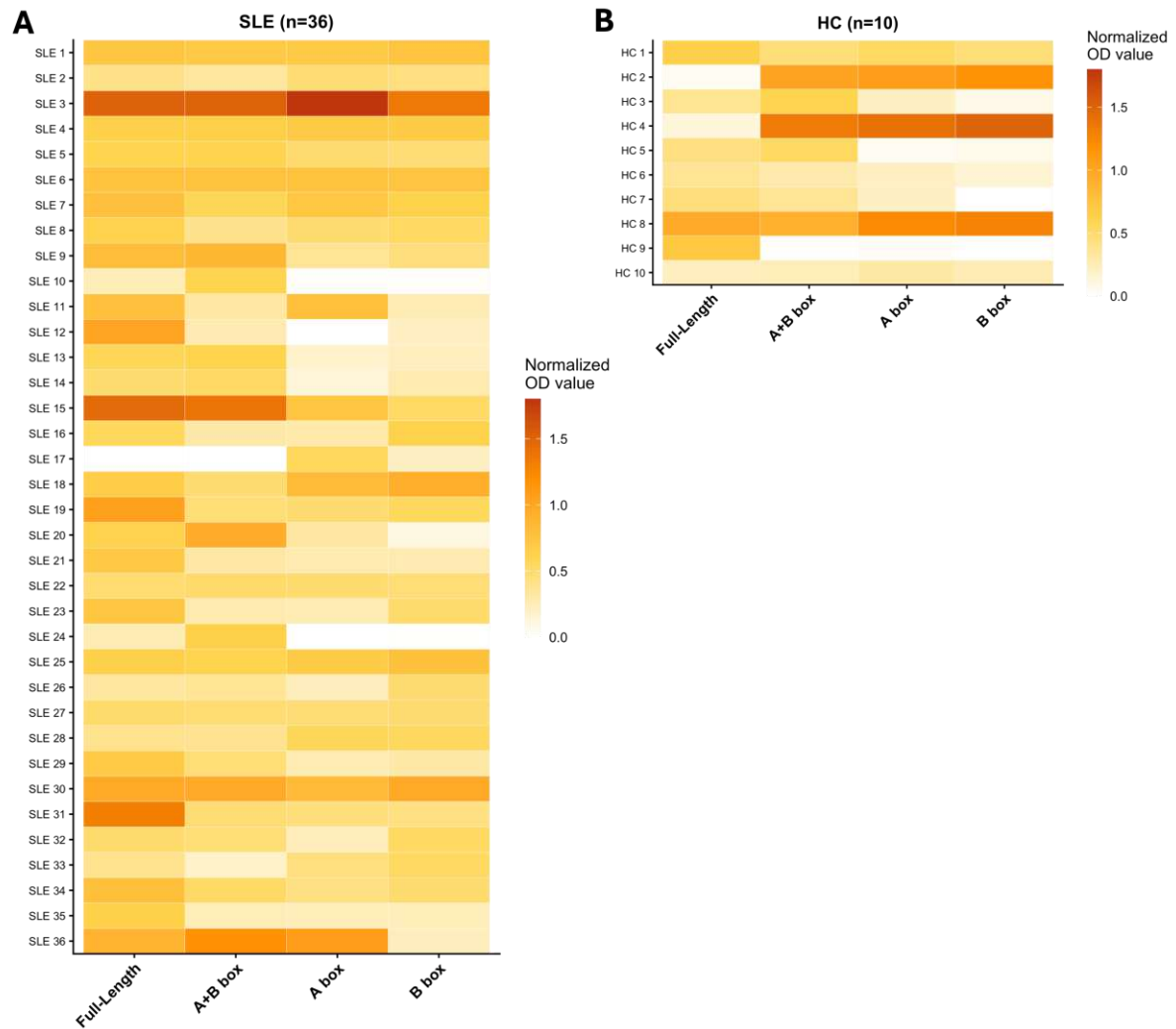


Figure S5. Selection of high-reactivity samples for epitope mapping. Normalised OD values across the four HMGB1 variants (full-length, A+B box, A box and B box) for the samples selected for peptide microarray analysis. **(A)** SLE patients (n = 36), selected as those with reactivity above the 75th percentile of the SLE cohort. **(B)** Healthy controls (n = 10), selected as those above the 95th percentile of the control group. Colour intensity reflects normalized OD (scale, right). These reactive samples were mapped at peptide resolution in Figure 5; the selection restricts the array to reactive individuals, so it characterises epitope specificity within reactors rather than reactivity prevalence.

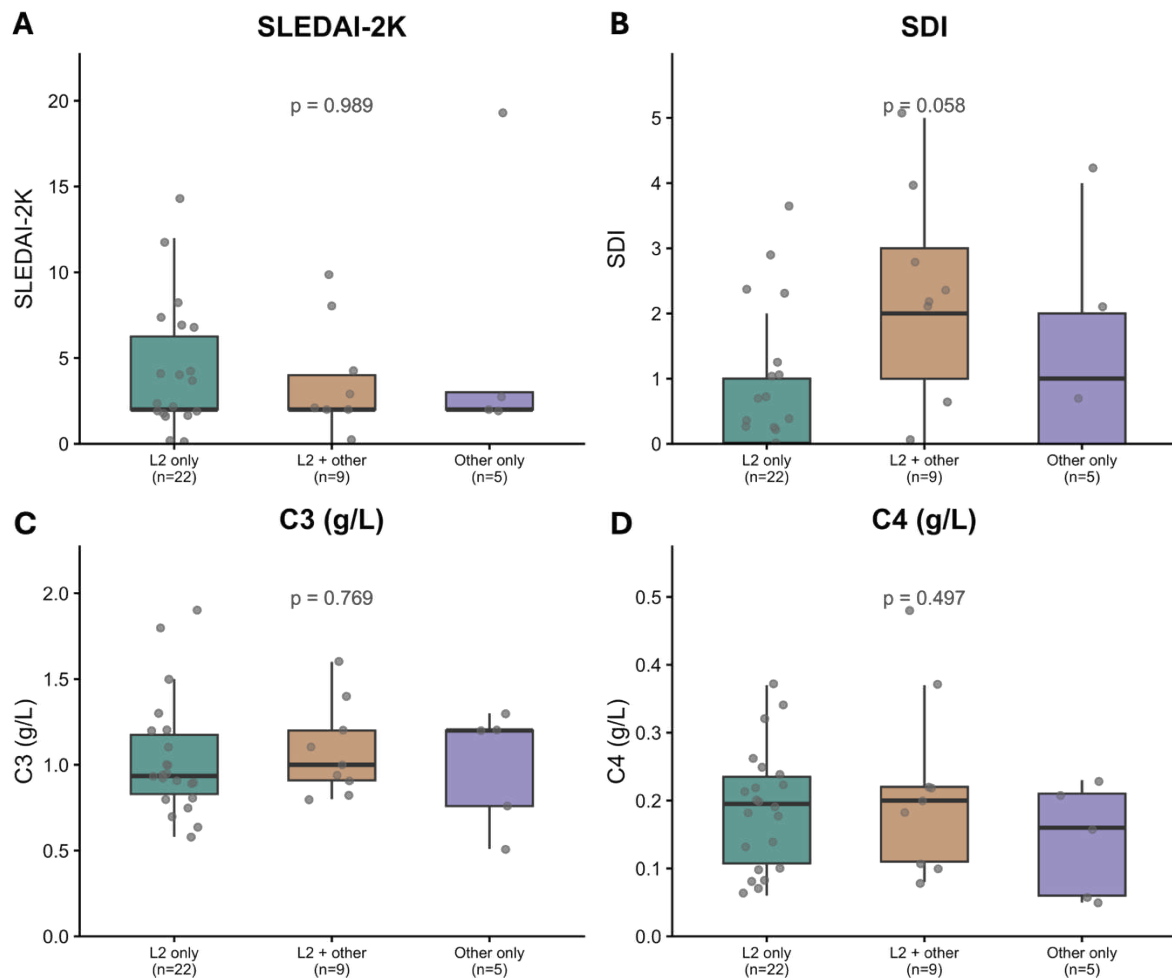


Figure S6. Clinical parameters by epitope-reactivity group in mapped SLE patients. (A) SLEDAI-2K, (B) SDI, (C) C3 and (D) C4 across the three epitope-reactive groups (L2 only, n = 22; L2 + other, n = 9; Other only, n = 5). Box plots show the median and interquartile range, whiskers extend to 1.5×IQR, and points represent individual patients. Groups were compared by the Kruskal-Wallis test (*p* value shown per panel).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Oral and written informed consent were obtained from all patients with SLE included in the KLURING registry. The study protocol was approved by the Regional Ethics Review Board in Linköping (Decision no. M75-08, May 21, 2008, Linköping, Sweden). The healthy control cohort was approved by the Regional Committee for Medical and Health Research Ethics (REK), Norway (approval no. 2009/686). The study was conducted in accordance with applicable ethical guidelines and regulations, and informed consent was obtained from all participants.

AUTHOR STATEMENT

H. Harris and C. Aulin conceptualized the study and designed the experimental framework. M. Eidsheim established and optimized the ELISA protocol and performed the initial screening of

the healthy control samples. H. Pechiappan performed the screening of SLE samples, peptide microarray experiments, data collection and processing, and statistical analyses. C. Sjöwall and L. Wirestam provided the SLE cohort samples and associated clinical and serological data. E. Wigren purified recombinant HMGB1 proteins. H. Harris, C. Aulin, R. Davies, R. Heinbäck, C. Sjöwall, and L. Wirestam contributed to the study design, data analysis, and interpretation throughout the study. H. Pechiappan wrote the initial draft of the manuscript. All authors interpreted the results, reviewed and edited the manuscript, and approved the final version.

DECLARATION OF COMPETING INTERESTS

The authors report no potential conflicts of interest.

ACKNOWLEDGEMENTS

We thank the patients and clinical staff at the Rheumatology Unit in Linköping for their participation and assistance with sample collection. We thank Opher Gileadi for the preparation and purification of the recombinant HMGB1 variants used in the study. We are grateful to the team at PEPperPRINT GmbH, particularly Sarah Schott, for their valuable support with the peptide microarray. We acknowledge the Division of Affinity Proteomics at SciLife, Stockholm, Sweden, for access to the facilities used for peptide microarray scanning and thank Ronald Sjöberg for assistance with microarray scanning and advice on data analysis.

FUNDING

This work was supported by the Broegelmann Foundation, the Swedish Research Council (grant nos. 2021-02723 and 2023-02256), the Swedish Rheumatism Association (grant no. R-993724) and Region Östergötland ALF Grants (grant no. RÖ-1919889). Open access funding was provided by the University of Bergen.

DATA AVAILABILITY

The data supporting the findings of this study are included within the article and its supplementary information. Additional data, including raw peptide microarray fluorescence data, are available from the corresponding author upon reasonable request.

- 628 1. Hoi A, Igel T, Mok CC, Arnaud L. Systemic lupus erythematosus. *Lancet*.
629 2024;403(10441):2326-38.
- 630 2. Zhu H, Luo H, Yan M, Zuo X, Li Q-Z. Autoantigen Microarray for High-Throughput
631 Autoantibody Profiling in Systemic Lupus Erythematosus. *Genomics, Proteomics &*
632 *Bioinformatics*. 2015;13(4):210-8.
- 633 3. Roh JS, Sohn DH. Damage-Associated Molecular Patterns in Inflammatory Diseases.
634 *Immune Netw*. 2018;18(4):e27.
- 635 4. Abdulahad DA, Westra J, Bijzet J, Limburg PC, Kallenberg CG, Bijl M. High mobility
636 group box 1 (HMGB1) and anti-HMGB1 antibodies and their relation to disease characteristics
637 in systemic lupus erythematosus. *Arthritis Res Ther*. 2011;13(3):R71.
- 638 5. Urbonaviciute V, Voll RE. High-mobility group box 1 represents a potential marker of
639 disease activity and novel therapeutic target in systemic lupus erythematosus. *J Intern Med*.
640 2011;270(4):309-18.
- 641 6. Yang H, Antoine DJ, Andersson U, Tracey KJ. The many faces of HMGB1: molecular
642 structure-functional activity in inflammation, apoptosis, and chemotaxis. *J Leukoc Biol*.
643 2013;93(6):865-73.
- 644 7. Yang H, Wang H, Ju Z, Ragab AA, Lundbäck P, Long W, et al. MD-2 is required for
645 disulfide HMGB1-dependent TLR4 signaling. *J Exp Med*. 2015;212(1):5-14.
- 646 8. Bianchi ME, Agresti A. HMG proteins: dynamic players in gene regulation and
647 differentiation. *Curr Opin Genet Dev*. 2005;15(5):496-506.
- 648 9. Yang H, Lundbäck P, Ottosson L, Erlandsson-Harris H, Venereau E, Bianchi ME, et
649 al. Redox modifications of cysteine residues regulate the cytokine activity of HMGB1.
650 *Molecular Medicine*. 2021;27(1):58.
- 651 10. Pisetsky DS, Erlandsson-Harris H, Andersson U. High-mobility group box protein 1
652 (HMGB1): an alarmin mediating the pathogenesis of rheumatic disease. *Arthritis Research &*
653 *Therapy*. 2008;10(3):209.
- 654 11. Pisetsky DS. The complex role of DNA, histones and HMGB1 in the pathogenesis of
655 SLE. *Autoimmunity*. 2014;47(8):487-93.
- 656 12. Taniguchi N, Kawahara K, Yone K, Hashiguchi T, Yamakuchi M, Goto M, et al. High
657 mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid
658 arthritis as a novel cytokine. *Arthritis Rheum*. 2003;48(4):971-81.
- 659 13. Wirestam L, Schierbeck H, Skogh T, Gunnarsson I, Ottosson L, Erlandsson-Harris H,
660 et al. Antibodies against High Mobility Group Box protein-1 (HMGB1) versus other anti-
661 nuclear antibody fine-specificities and disease activity in systemic lupus erythematosus.
662 *Arthritis Res Ther*. 2015;17:338.
- 663 14. Hayashi A, Nagafuchi H, Ito I, Hirota K, Yoshida M, Ozaki S. Lupus antibodies to the
664 HMGB1 chromosomal protein: epitope mapping and association with disease activity. *Mod*
665 *Rheumatol*. 2009;19(3):283-92.
- 666 15. Schaper F, Westra J, Bijl M. Recent developments in the role of high-mobility group
667 box 1 in systemic lupus erythematosus. *Mol Med*. 2014;20(1):72-9.
- 668 16. Li J, Kokkola R, Tabibzadeh S, Yang R, Ochani M, Qiang X, et al. Structural basis for
669 the proinflammatory cytokine activity of high mobility group box 1. *Mol Med*. 2003;9(1-2):37-
670 45.
- 671 17. Schaper F, de Leeuw K, Horst G, Maas F, Bootsma H, Heeringa P, et al. Autoantibodies
672 to box A of high mobility group box 1 in systemic lupus erythematosus. *Clinical and*
673 *Experimental Immunology*. 2017;188(3):412-9.

18. Arkema EV, Saleh M, Simard JF, Sjöwall C. Epidemiology and Damage Accrual of Systemic Lupus Erythematosus in Central Sweden: A Single-Center Population-Based Cohort Study Over 14 Years From Östergötland County. *ACR Open Rheumatol*. 2023;5(8):426-32.
19. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1982;25(11):1271-7.
20. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum*. 2012;64(8):2677-86.
21. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol*. 2002;29(2):288-91.
22. Gladman D, Ginzler E, Goldsmith C, Fortin P, Liang M, Urowitz M, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum*. 1996;39(3):363-9.
23. Yee CS, Farewell VT, Isenberg DA, Griffiths B, Teh LS, Bruce IN, et al. The use of Systemic Lupus Erythematosus Disease Activity Index-2000 to define active disease and minimal clinically meaningful change based on data from a large cohort of systemic lupus erythematosus patients. *Rheumatology (Oxford)*. 2011;50(5):982-8.
24. Keates T, Cooper CD, Savitsky P, Allerston CK, Phillips C, Hammarström M, et al. Expressing the human proteome for affinity proteomics: optimising expression of soluble protein domains and in vivo biotinylation. *N Biotechnol*. 2012;29(5):515-25.
25. Savitsky P, Bray J, Cooper CD, Marsden BD, Mahajan P, Burgess-Brown NA, et al. High-throughput production of human proteins for crystallization: the SGC experience. *J Struct Biol*. 2010;172(1):3-13.
26. Schierbeck H, Lundbäck P, Palmblad K, Klevenvall L, Erlandsson-Harris H, Andersson U, et al. Monoclonal Anti-HMGB1 (High Mobility Group Box Chromosomal Protein 1) Antibody Protection in Two Experimental Arthritis Models. *Molecular Medicine*. 2011;17(9):1039-44.
27. UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Res*. 2025;53(D1):D609-d17.
28. Team RC. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2025. <https://www.R-project.org/>
29. Syahidatulamali CS, Wan Syamimee WG, Azwany YN, Wong KK, Che Maraina CH. Association of anti-CLIC2 and anti-HMGB1 autoantibodies with higher disease activity in systemic lupus erythematosus patients. *J Postgrad Med*. 2017;63(4):257-61.
30. Tan G, Zhu N, Shi Z, Meng Z, Yu M, Li K, et al. Anti-high mobility group box 1 (anti-HMGB1) antibodies are not related to the occurrence of cutaneous lesions in systemic lupus erythematosus. *Scand J Rheumatol*. 2015;44(2):150-6.
31. Huttunen HJ, Fages C, Kuja-Panula J, Ridley AJ, Rauvala H. Receptor for advanced glycation end products-binding COOH-terminal motif of amphotericin inhibits invasive migration and metastasis. *Cancer Res*. 2002;62(16):4805-11.
32. Bonaldi T, Talamo F, Scaffidi P, Ferrera D, Porto A, Bachi A, et al. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *Embo j*. 2003;22(20):5551-60.
33. Knapp S, Müller S, Digilio G, Bonaldi T, Bianchi ME, Musco G. The long acidic tail of high mobility group box 1 (HMGB1) protein forms an extended and flexible structure that interacts with specific residues within and between the HMG boxes. *Biochemistry*. 2004;43(38):11992-7.

- 723 34. Stott K, Watson M, Howe FS, Grossmann JG, Thomas JO. Tail-mediated collapse of
724 HMGB1 is dynamic and occurs via differential binding of the acidic tail to the A and B
725 domains. *J Mol Biol.* 2010;403(5):706-22.
- 726 35. Sitia G, Iannacone M, Aiolfi R, Isogawa M, van Rooijen N, Scozzesi C, et al. Kupffer
727 cells hasten resolution of liver immunopathology in mouse models of viral hepatitis. *PLoS*
728 *Pathog.* 2011;7(6):e1002061.
- 729 36. Venereau E, Casalgrandi M, Schiraldi M, Antoine DJ, Cattaneo A, De Marchis F, et al.
730 Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory
731 cytokine release. *Journal of Experimental Medicine.* 2012;209(9):1519-28.

732