

1 **The DNA sensors AIM2 and IFI16 are NET-binding SLE autoantigens**

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30 **Abstract**

31 Nucleic acid binding proteins are frequently targeted as autoantigens in systemic lupus erythematosus
32 (SLE) and other interferon (IFN)-linked rheumatic diseases. The AIM-like receptors (ALRs) are IFN-
33 inducible innate sensors that form supramolecular assemblies along double-stranded DNA of various
34 origins. Here, we identify the ALR Absent in melanoma 2 (AIM2) as a novel autoantigen in SLE, with
35 similar properties to the established ALR autoantigen interferon-inducible protein 16 (IFI16). Our SLE
36 cohort revealed a frequent co-occurrence of anti-AIM2, anti-IFI16 and anti-DNA antibodies, and higher
37 clinical measures of disease activity in patients positive for antibodies against these ALRs. We examined
38 neutrophil extracellular traps (NETs) as DNA scaffolds on which these antigens might interact in a pro-
39 immune context, finding that both ALRs bind NETs in vitro and in SLE renal tissues. We demonstrate that
40 ALR binding causes NETs to resist degradation by DNase I, suggesting a mechanism whereby
41 extracellular ALR-NET interactions may promote sustained IFN signaling. Our work suggests that
42 extracellular ALRs bind NETs, leading to DNase resistant nucleoprotein fibers that are targeted as
43 autoantigens in SLE.

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45 Keywords: Systemic Lupus Erythematosus, Neutrophil Extracellular Traps, Autoantibodies,

46 Autoimmunity

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49 **Introduction**

50 Systemic lupus erythematosus (SLE) is a rheumatic disease characterized by upregulated interferon (IFN)
51 expression and autoantibody production (1). Autoantibodies inform the identification of specific disease
52 phenotypes and also provide insight into the mechanisms operative in rheumatic diseases (2). Many SLE
53 autoantigens are nucleic acid binding proteins, and nucleic acid containing immune complexes are
54 implicated in aspects of pathogenesis (3).

55 The AIM2-like receptors (ALRs) are a group of IFN-induced innate sensors of double-stranded (ds) DNA.
56 AIM2 and IFI16 are the most studied members of the ALR family, which also includes IFIX and MNDNA.
57 The ALRs bind to dsDNA in a sequence-independent manner via electrostatic interactions with the
58 dsDNA backbone, and form an oligomerized filament along areas of accessible dsDNA of any origin (4,
59 5). These innate sensors equip the cell with a means of identifying harmful stimuli, including viral
60 genomes, mislocalized mitochondrial DNA, and chromosomal DNA from tumor cells. Once activated, the
61 ALRs activate downstream innate immune signaling by type I IFN and inflammasome (IL-1/IL-18)
62 pathways (6, 7).

63 Anti-IFI16 antibodies occur in both SLE and Sjogren's Syndrome (SS), but we have previously reported
64 that the targeted epitopes differ in these diseases (8, 9). IFI16 oligomers appear to be recognized by SS
65 sera, suggesting that dsDNA binding may enhance its antigenicity. While AIM2 assembles similar
66 filamentous structures on dsDNA, its status as an autoantigen has not been reported. Here, we identify
67 AIM2 as an autoantigen in SLE (targeted in 31.3% of patients), with antibodies against AIM2, IFI16 and
68 dsDNA being highly associated with one another. To understand why anti-ALR and anti-dsDNA
69 antibodies might be closely co-targeted in SLE, we considered the possibility that ALRs bind to
70 neutrophil extracellular traps (NETs) in the extracellular space. NETs are microbicidal structures

71 consisting of protein-laden chromatin fibers generated by neutrophils in response to various stimuli
72 (10). The NET dsDNA scaffold is a structure on which a variety of molecules interact (11), representing a
73 platform for antigenic materials (including SLE autoantigens) to be presented to the adaptive immune
74 system (12). We find that both AIM2 and IFI16 bind NETs in vitro and in tissues, with their binding
75 yielding polymeric structures that confer resistance to DNase I. Together, our findings demonstrate that
76 AIM2 and IFI16 are NET-bound autoantigens in SLE.

77 **Methods**

78 **Patients:** Plasma from 131 SLE patients (defined by the SLICC criteria(13)) in the Hopkins Lupus Cohort
79 was studied for autoantibodies. Sera from 49 healthy controls was analyzed to establish a threshold for
80 assay positivity. 133 primary Sjögren's Syndrome (SS) patients (defined by ACR/EULAR criteria(14)) were
81 included as disease controls. All patients and healthy controls gave informed consent for blood used in
82 research and all work involving human subjects was approved by the Johns Hopkins Institutional Review
83 Board. Paraffin sections from SLE renal biopsies were obtained for immunostaining and are detailed in
84 Supplemental Table 3.

85 **AIM2 autoantibody assay:** Full length AIM2 cDNA was subcloned into the pET28 vector (Novagen) and
86 used to generate ³⁵S-methionine labelled AIM2 protein by *in vitro* transcription and translation (IVTT)
87 (Promega). Immunoprecipitations (IP) were performed using IVTT product diluted in Lysis Buffer (20 mM
88 Tris pH 7.4, 150 mM NaCl, 1mM EDTA pH 7.4, 1% NP40) and 1 microliter of serum (90 minutes, 4°C). 20
89 microliters of Protein G Dynabeads (Thermo Fisher) were then added to each IP, and incubated for 60
90 minutes. Beads were magnetically isolated, washed, and boiled in gel application buffer. IP products
91 were electrophoresed on SDS-polyacrylamide gels and visualized by fluorography. Films were scanned
92 and AIM2 bands quantified using Quantity One software (Bio Rad). IP products were normalized to the
93 same positive reference serum included on each gel. The cutoff for antibody positivity was set at 2

94 standard deviations above the mean control serum value. IFI16 antibodies were assayed by ELISA as
95 described (15).

96 **NET assays:** Neutrophils were isolated from healthy control PBMCs using Ficoll-Paque density gradient
97 followed by RBC lysis using ACK buffer (Quality Biological). NET formation was induced using PMA at 100
98 nM for 3 hours. For immunofluorescence studies, neutrophils were plated on glass coverslips for 15
99 minutes prior to PMA treatment. For quantitative DNase protection assays, NETs were induced with
100 PMA in 96 well plates, incubated with or without purified ALRs, then treated with DNase I at room
101 temperature (RT) prior to incubation with 5 μ M Sytox Green (Thermo Fisher) and quantification via
102 fluorimetry using a Perkin Elmer plate reader. Experiments were performed twice.

103 **Immunofluorescence:** Neutrophil samples were stained with anti-MPO-FITC antibody and mounted in
104 DAPI-containing ProLong Gold Antifade Mountant (Thermo Fisher Scientific). AIM2 and IFI16 proteins
105 were expressed, purified and fluorescently labeled as previously described (4, 5). SLE renal biopsies were
106 stained as previously described (8) using anti-MPO rabbit polyclonal (ThermoFisher), anti-MPO mouse
107 monoclonal (ThermoFisher), anti-IFI16 mouse monoclonal (Sigma), anti-AIM2 rabbit polyclonal (Sigma)
108 and Hoechst 33342 (ThermoFisher). Confocal imaging was performed with a Zeiss AxioObserver with
109 780-Quasar confocal module.

110 **Statistics:** Features of patients with and without AIM2 antibodies were compared using Fisher's exact
111 test for categorical variables and the Mann-Whitney test for continuous variables. Multivariable logistic
112 regression was utilized to determine associations between variables. P values less than 0.05 were
113 considered statistically significant.

114

115 **Results**

116 **AIM2 autoantibodies are present in SLE, and frequently co-occur with anti-IFI16 and anti-dsDNA**
117 **antibodies.**

118 To determine whether AIM2 was a target of the humoral immune response in SLE, we developed an IP
119 assay to screen for anti-AIM2 antibodies. 41/131 (31.3%) of SLE versus 2/49 (4.1%) of healthy controls
120 were anti-AIM2-positive ($p < 0.001$) (Figure 1A). Interestingly, anti-AIM2 antibodies were strongly
121 associated with both anti-IFI16 and anti-DNA antibodies in the SLE samples measured on the day of visit
122 (Figure 1B and Table 1). We found that anti-AIM2 antibodies were associated with higher measures of
123 SLEDAI (2.29 ± 2.3 versus 1.05 ± 1.61 , $p = 0.0026$, Table 1), which was largely driven by the immunology
124 component. Anti-AIM2 antibodies were associated with the presence of disease activity in the skin
125 domain of the SLEDAI index at the date of blood draw: 11/41 (26.8%) of anti-AIM2 positive patients had
126 scores > 0 in this domain, compared to 11/90 (12.2%) of anti-AIM2 negative patients ($p = 0.0463$). Anti-
127 AIM2 antibodies were also associated with a small but significant increase (0.63 ± 0.55 versus $0.43 \pm$
128 0.51 , $p = 0.0333$) in the SLE Physician Global Disease Activity score, which is based solely on clinical
129 estimation of SLE activity, rather than serologic indices. A multivariable analysis correcting for SLEDAI,
130 anti-dsDNA, and C4 results demonstrated that anti-AIM2 antibodies were significantly associated with
131 anti-IFI16 antibodies with an OR of 3.7 ($p = 0.007$, 95%CI 1.44-9.7). A subset of SLE patients ($n = 9$)
132 demonstrated particularly high levels of anti-AIM2 antibodies with normalized OD > 20 (Figure 1A).
133 These patients had higher SLEDAI values than both lower level anti-AIM2-positive and anti-AIM2-
134 negative patients (Supplemental Table 1). Among all anti-AIM2 positive patients, we found a higher
135 prevalence of positivity for anti-Ro (18/41, 44% vs 19/90, 21%, $p = 0.0114$) and anti-La (10/41, 24% vs
136 7/90, 8%, $p = 0.0125$) antibodies (Supplemental Table 2).

137 SS shares several phenotypic features with SLE, including the presence of an IFN signature and B cell
138 dysregulation (15), but anti-DNA antibodies are not characteristic of SS. We therefore analyzed SS sera

139 for the presence of anti-AIM2 antibodies, and found 46/133 (34.6%) of SS sera were positive. In contrast
140 to SLE, anti-IFI16 was not enriched in patients with anti-AIM2 antibodies in SS (35% anti-AIM2-positive
141 and anti-IFI16-positive versus 28% anti-AIM2-negative and anti-IFI16-positive in SS, $p=0.4324$), showing
142 that the association between anti-IFI16 and anti-AIM2 antibodies is specific to SLE, where these immune
143 responses are also associated with anti-dsDNA antibodies.

144

145 **AIM2 and IFI16 bind to Neutrophil Extracellular Traps and inhibit their degradation by DNase I.**

146 The close relationship between anti-AIM2, anti-IFI16 and anti-dsDNA antibodies in the SLE cohort led us
147 to consider scenarios in which ALR-DNA complexes could be generated and promote the development
148 of autoantibodies against these three antigens. Neutrophil extracellular traps (NETs) have been
149 implicated as important sources of extracellular DNA in SLE, and are linked to the IFN signature as well
150 as autoantibody generation in this disease (16). ALRs are IFN-induced, bind to dsDNA of many origins in
151 a sequence-independent manner, and AIM2 has been identified as a protein constituent of SLE NETs in a
152 proteomics analysis (17). IFI16 is released from epithelial cells undergoing apoptosis (8, 18), and
153 extracellular IFI16 is quantifiable in the sera of SLE patients (11). Considering these observations, we
154 reasoned that when ALRs are generated in the setting of IFN exposure and subsequently released from
155 cells, they might encounter and bind to extracellular NETs, accumulating on this extracellular platform
156 and creating a hub for amplification similar to that observed in the complement and coagulation
157 pathways (19).

158 To test this hypothesis, we used NETs as a DNA substrate for ALR binding: neutrophils were stimulated
159 to undergo NETosis with PMA, and then incubated with fluorescently labeled IFI16 and AIM2 proteins.
160 We found that both ALRs bind readily to NETs (Figure 2A-B). Co-localization of AIM2 and IFI16 along NET

161 chromatin fibers was visible by confocal microscopy in this analysis, suggesting that both ALRs assemble
162 into filaments on NET DNA.

163 IFI16 and AIM2 nucleoprotein filaments are highly stable and persist even after the dsDNA template has
164 been degraded by nucleases (8, 20). We therefore hypothesized that ALR-bound NETs might resist
165 nuclease exposure, potentially enhancing antigenicity. We used DNase I to explore this question, as
166 DNase I is the nuclease responsible for effective clearance of NETs (21), and DNase I deficiency has been
167 associated with SLE in both human subjects and animal models (22) . After exposure to 20 U/ml DNase I
168 for 1 hour at RT, both myeloperoxidase (MPO) and DNA signals were completely degraded, leaving no
169 observable fluorescence in any channel (Figure 2C). When NETs were first incubated with ALRs,
170 however, we observed incomplete ALR-NET degradation by DNase I – in some areas, IFI16 and AIM2
171 remained present and co-localized with MPO (Figure 2D). In addition, there was observable DNA
172 remaining in these areas of persistent ALR structures, implying that the ALRs had partially shielded NET
173 DNA from degradation. This finding suggested that both the protein and DNA components of the ALR-
174 NET structure are resistant to DNase-mediated clearance. To better quantify this, we employed a plate-
175 based Sytox Green assay to measure the dsDNA content of NETs following exposure to DNase I (Figure
176 2E). This assay confirmed that ALR-bound NETs are resistant to DNase I, leaving more DNA present
177 following nuclease treatment (Figure 2E). Together these experiments demonstrate that ALRs bind to
178 NETs, generating a protein-DNA structure with enhanced resistance to DNase-mediated clearance.

179 **IFI16-NETs are present in lupus nephritis.**

180 Prior studies have presented evidence of in vivo NET formation within the renal tissues of SLE patients,
181 supporting the notion that dysregulated neutrophil function contributes to immune pathology in this
182 disease (23). We therefore sought to determine whether ALR-NET interactions could be identified
183 among NETs present in lupus nephritis biopsies. Considering that patients with diffuse proliferative

184 lupus nephritis are known to harbor netting neutrophils in renal tissue (23), we identified patients with
185 diffuse proliferative lupus nephritis, then selected 5 samples whose biopsies demonstrated neutrophilic
186 infiltrates or karyorrhectic debris (Supplemental Table 3). We found that AIM2 was highly expressed in
187 MPO-positive infiltrating cells (Figure 3A), while IFI16 was expressed more broadly throughout renal cell
188 types (Figure 3B). We detected NETs containing both AIM2 and IFI16 in glomerular and interstitial
189 infiltrates (Figure 3C and D). High magnification, z-stack imaging (Supplemental Figure 1) confirmed that
190 these structures represented extracellular DNA that co-stained for MPO and AIM2 or IFI16, consistent
191 with ALR-bound NETs, rather than adjacent or overlapping cell nuclei. In summary, our immunostaining
192 experiments provide evidence that AIM2 and IFI16 bind NETs in the setting of diffuse proliferative lupus
193 nephritis, establishing AIM2 and IFI16 as NET-bound SLE autoantigens.

194

195 **Discussion**

196 SLE features autoantibodies that bind nucleic acids and nucleic acid-binding proteins, and extracellular
197 nucleic acids contribute to SLE pathogenesis (24). Here, we identify the dsDNA sensor AIM2 as a novel
198 autoantigen in SLE, and demonstrate that anti-AIM2 antibodies are associated with SLE disease activity
199 markers. Furthermore, we find that NETs provide a scaffold for ALR oligomerization, which in turn
200 confers resistance to nuclease degradation.

201 NETosis is a process whereby dsDNA is expelled into the extracellular space at sites of tissue damage,
202 and is of mechanistic relevance in SLE (16). The NET dsDNA scaffold is a structure on which a variety of
203 molecules can interact, and is a source of antigenic proteins in SLE and other inflammatory diseases (12,
204 25). We found that both IFI16 and AIM2 readily assemble into filaments along the length of NET dsDNA.
205 Unexpectedly, we found this ALR-NET structure resists DNase-mediated degradation. NETs promote IFN
206 signaling at sites of their generation when engulfed by immune cells (26, 27), and may have additional
207 disease-amplifying functions (28). By prolonging the stability of interferogenic NETs, extracellular ALRs
208 may enhance IFN signaling at sites of neutrophil activation, which could be further amplified by IFN-
209 induced expression of the ALRs themselves.

210 Impairment of NET removal has been specifically linked to the presence of lupus nephritis (21) - a
211 manifestation of SLE with significant associated morbidity (29). Neutrophilic infiltration of the kidney is a
212 feature of more severe forms of glomerulonephritis, and NET formation in this organ may contribute to
213 renal damage through the propagation of IFN signaling, immune cell activation and thrombosis (28).
214 Confocal microscopy has been utilized to demonstrate the presence of NET structures in renal lesions of
215 patients with SLE (23, 30) and also ANCA associated vasculitis (31), supporting the notion that NETs play
216 a pathogenic role in the immune dysregulation and tissue damage that occur in glomerulonephritis.

217 Here, we demonstrate for the first time that the DNA sensors AIM2 and IFI16 bind to NETs in vivo,
218 through imaging studies of proliferative lupus nephritis specimens. Our data include z-stack images at
219 high magnification, clearly demonstrating the presence of extracellular DNA-MPO-ALR complexes in this
220 site. This finding supports previous data (11, 32, 33) suggesting that the ALRs may have important
221 functions not just intracellularly, but also in the extracellular environment. The large chromatin fibers
222 generated through NETosis represent sizeable dsDNA templates upon which IFI16 and AIM2 monomers
223 oligomerize in the extracellular space, and are expected to result in durable immunostimulatory
224 structures at sites of IFN-induced protein expression. ALR-bound NETs therefore may promote not only
225 local immune activation, but the targeting of ALRs (and DNA) by antibodies in SLE.

226 Our data indicate that AIM2 is targeted not only in SLE but also in SS – a condition in which NETosis has
227 not been linked to disease pathology. In contrast to the relationship seen in SLE, we found no
228 association between AIM2 and IFI16 antibodies in SS, and anti-dsDNA antibodies are absent in SS. This
229 difference highlights the important role of disease-specific tissue processes in the development of
230 unique autoantibody profiles against shared antigens. In the setting of lupus nephritis, we observed ALR
231 expression by both neutrophils and resident renal cells, and suspect that these antigens may be released
232 by a variety of cell types in the kidney, leading to the observed extracellular interaction with NET DNA.
233 Contrastingly, neutrophil infiltration in target salivary tissues is not a common feature of SS, and the
234 absence of the NET DNA scaffold may explain the differing autoantibody profile observed in that
235 condition.

236 In summary, we have identified the ALRs AIM2 and IFI16 as NET-binding autoantigens in SLE. The ALR-
237 NET interaction may increase NET longevity and perpetuate NET-mediated inflammatory signaling in
238 lupus nephritis and other sites of NET generation and IFN expression. This work supports a role for the
239 ALRs in extracellular immune processes as NET-binding antigens in SLE.

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334 **Table 1. Day of visit phenotypic characteristics of SLE patients related to AIM2 autoantibody status.**

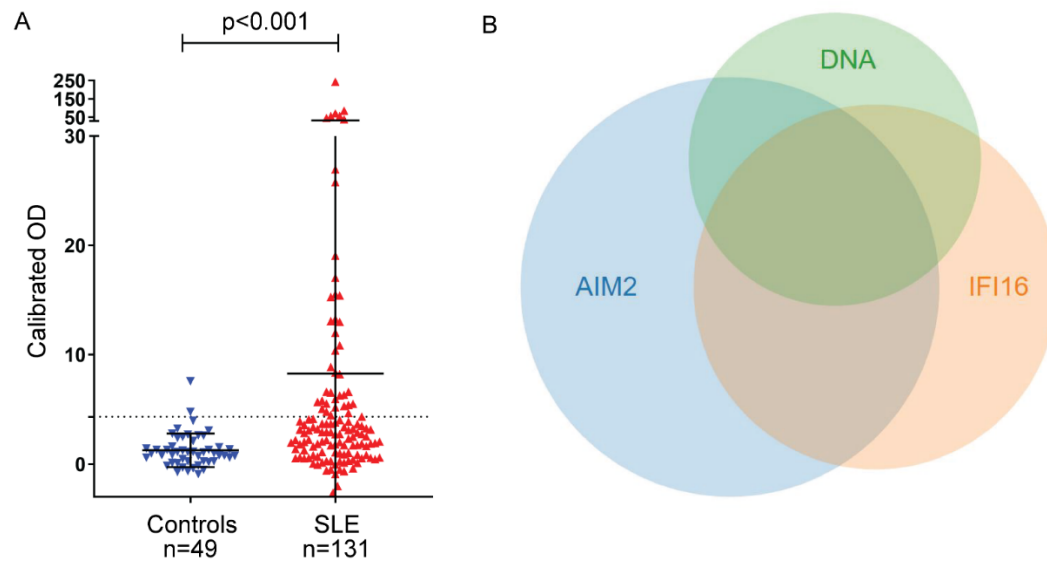
Autoantibody	Anti-AIM2+ n=41	Anti-AIM2- n=90	p value
IFI16 Positive	19/41 (46%)	12/90 (13%)	<0.0001
DNA Positive	12/41 (29%)	7/89 (8%)	0.0026
<hr/>			
Disease Activity Feature			
Physician Global Disease Activity	0.63 ± 0.55	0.43 ± 0.51	0.0333
SLEDAI	2.29 ± 2.3	1.05 ± 1.61	0.0026
C3	114.7 ± 36.9	121.4 ± 29.0	0.1352
C4	19.5 ± 8.2	25 ± 9.3	0.0005
Urine Protein/Creatinine ratio	0.134 ± 0.15	0.107 ± 0.11	0.4372

335 Numerators correspond to number of patients with indicated feature positive and denominators to total number
336 of patients with indicated feature recorded in the cohort, followed by percent (%) positive.

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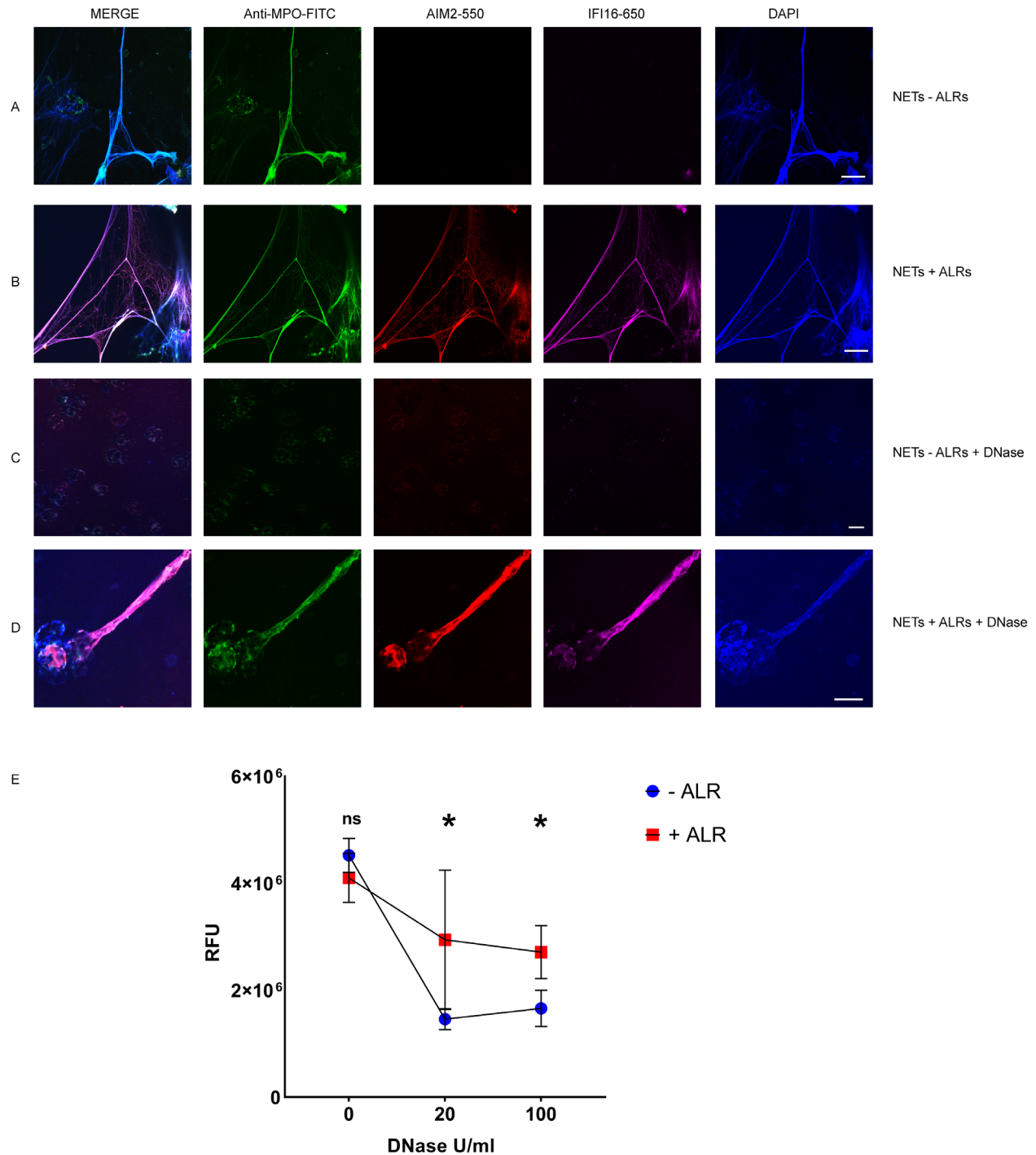
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341 **Figure 1: Anti-AIM2 antibodies are associated with anti-IFI16 and anti-DNA antibodies in SLE.**

342 AIM2 antibodies were detected using immunoprecipitation of ^{35}S -methionine labelled, *in vitro*
343 transcribed and translated protein. Data are presented as OD units calibrated to a known positive
344 reference serum. Dotted line indicates positive threshold value determined as the mean + 2 standard
345 deviations of control serum samples. AIM2 autoantibodies were identified in 2/49 controls and 41/131
346 SLE patients. Statistical significance was determined using the Mann-Whitney test for nonparametric
347 values (A). Relationship between anti-AIM2, -IFI16 and -DNA antibodies in the SLE cohort (B).



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349 **Figure 2: IFI16 and AIM2 bind NETs and prevent NET degradation by DNase I.**

350 NETs were induced in neutrophils using PMA 100 nM for 3 hours, then left untreated (A) or incubated

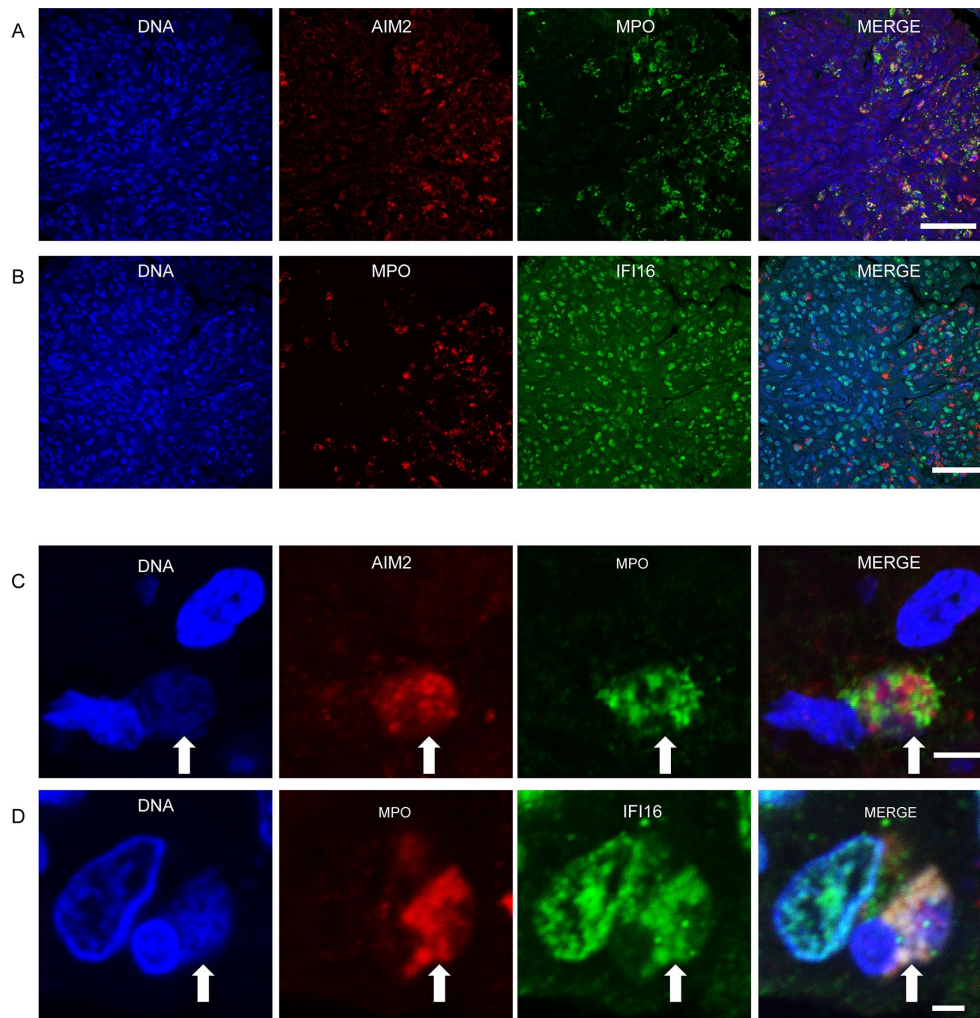
351 with fluorescently labeled AIM2 (pink) and IFI16 (red) at 200 nM at RT for 1 H (B). Following ALR

352 incubation, samples were stained with anti-MPO-FITC antibody (green) and DAPI (blue), then imaged by
353 confocal microscopy. NETs were treated with DNase I at 20 U/mL at RT for 1 hr (C). NETs incubated with
354 ALRs as in (B) were then treated with 20 U/mL DNase I for 1 hr (D). Scale bars = 20 μ m. NETs in 96 well
355 plates were incubated with ALRs at 200 nM (or buffer only) for 1 hr at RT, then treated with DNase I at 0,
356 20, and 100 U/mL for 30 minutes at RT. NETs were then stained with Sytox-Green 5 μ M, and samples
357 analyzed by fluorimetry (E). RFU = fluorescence units. Mean and standard deviation of 4 replicate wells
358 are indicated. Mann-Whitney test was used to compare groups. $p > 0.05$ = not significant (ns). $p < 0.05$ =
359 significant (*).

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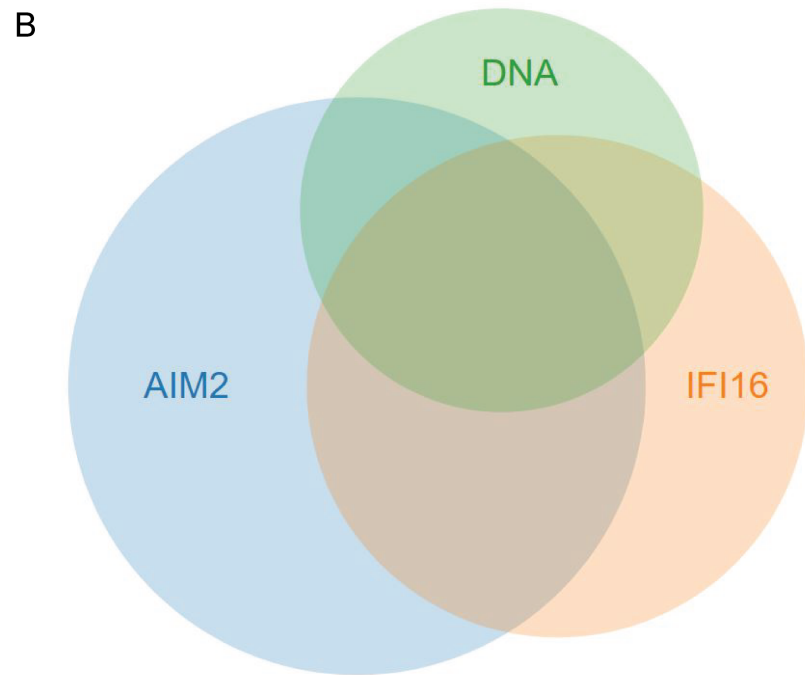
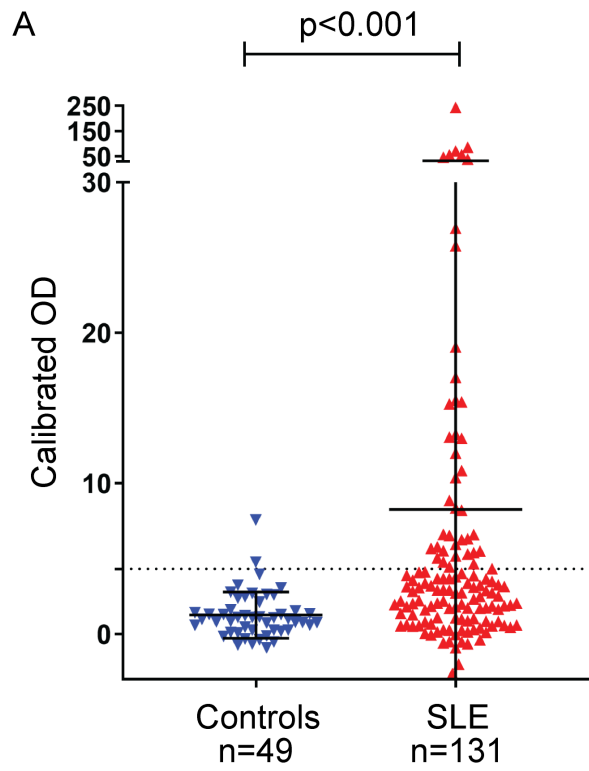
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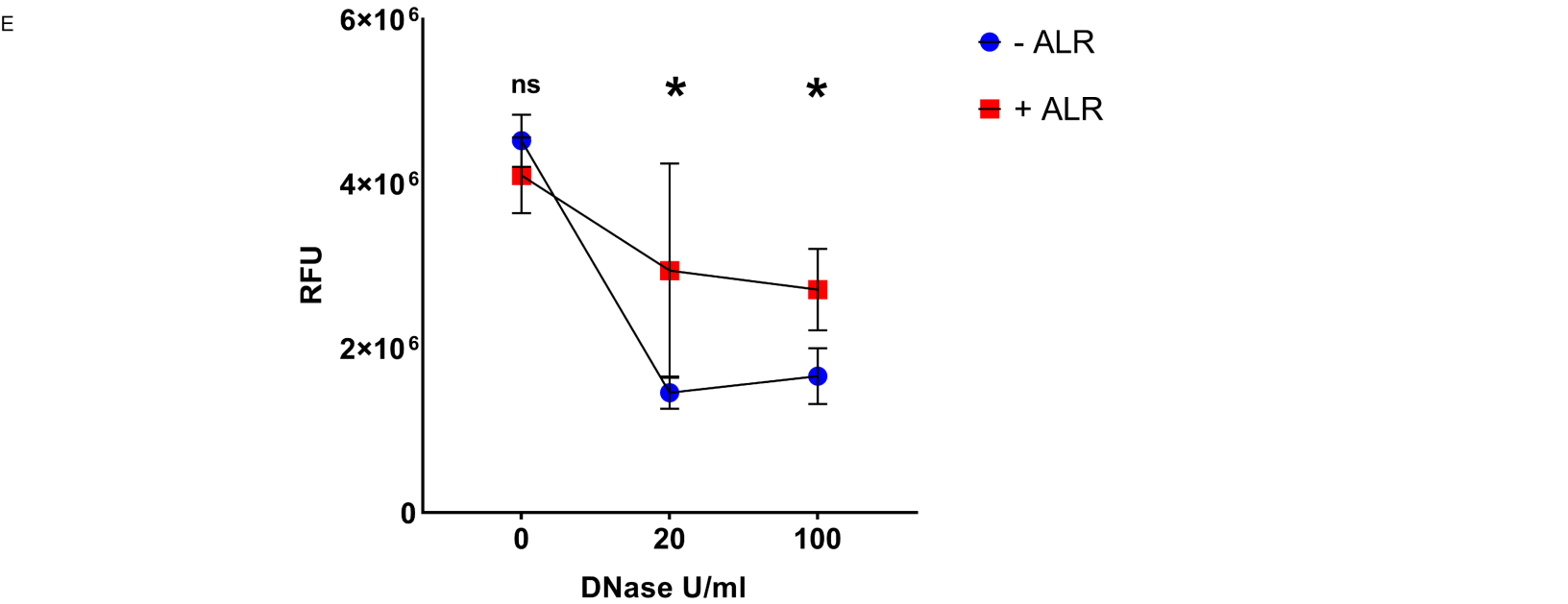
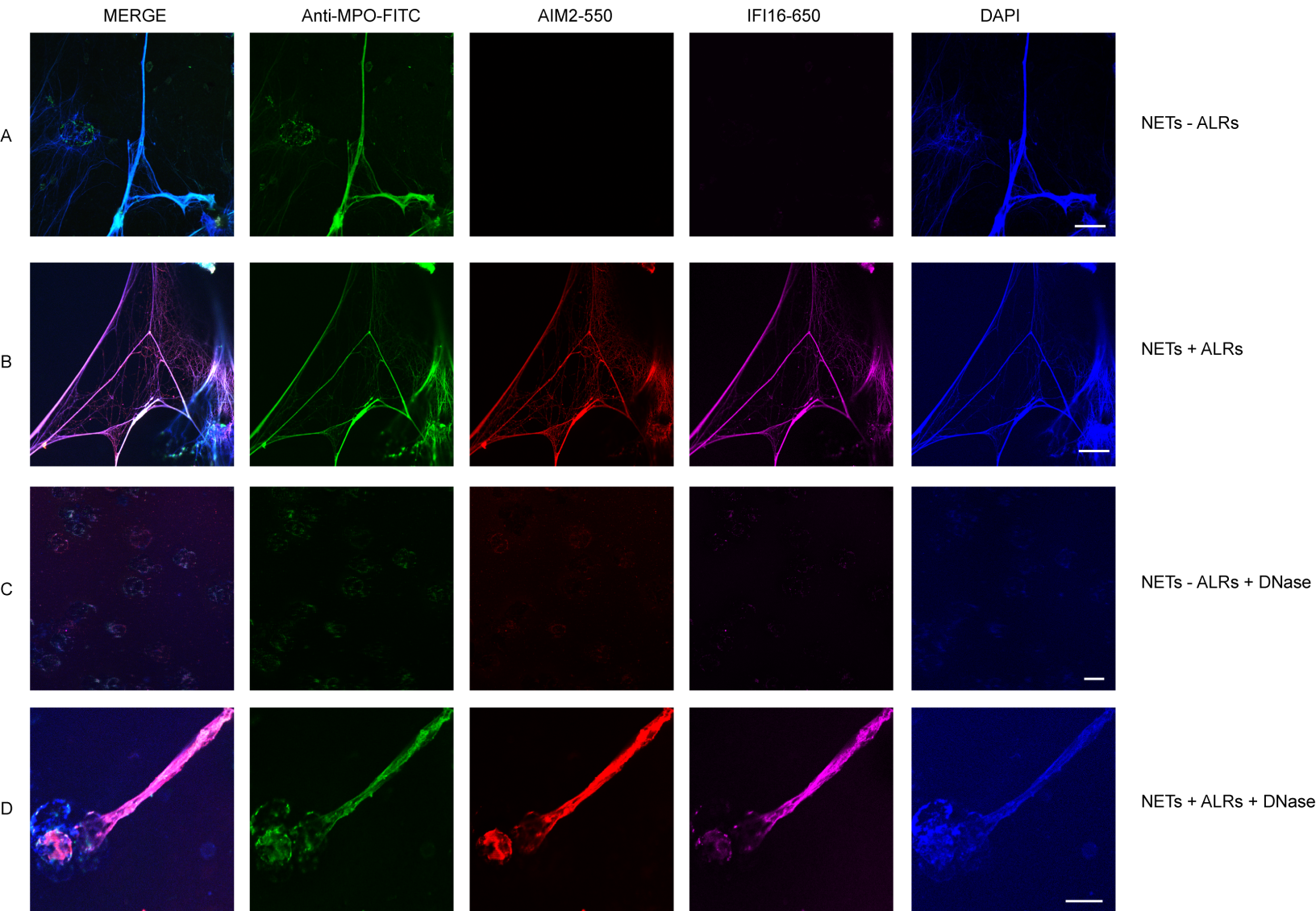
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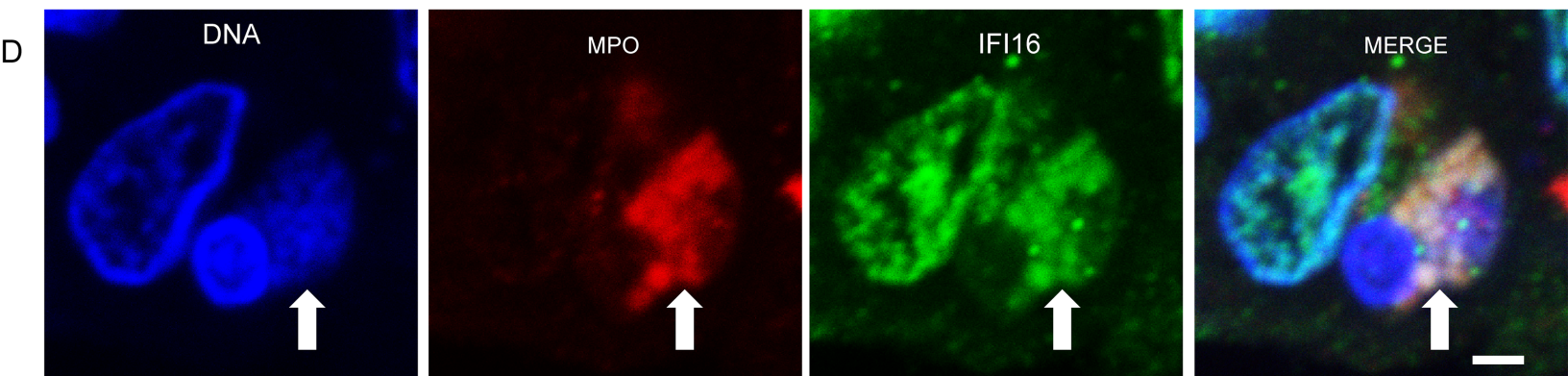
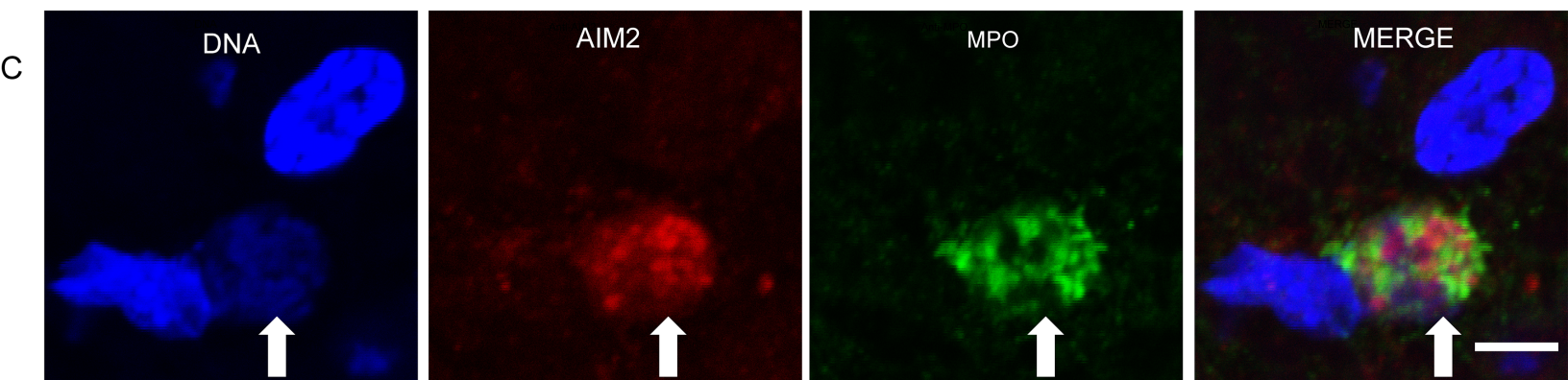
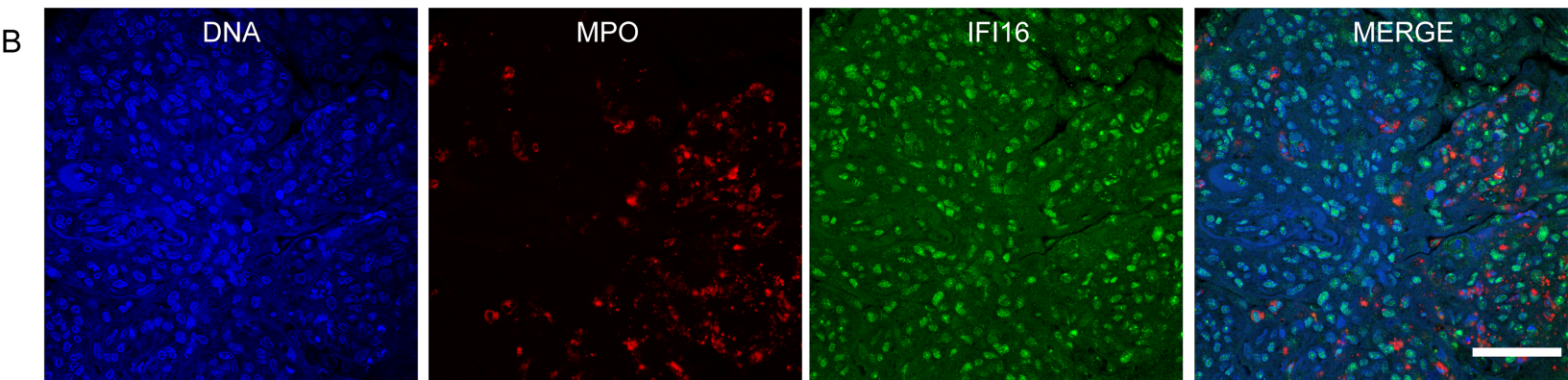
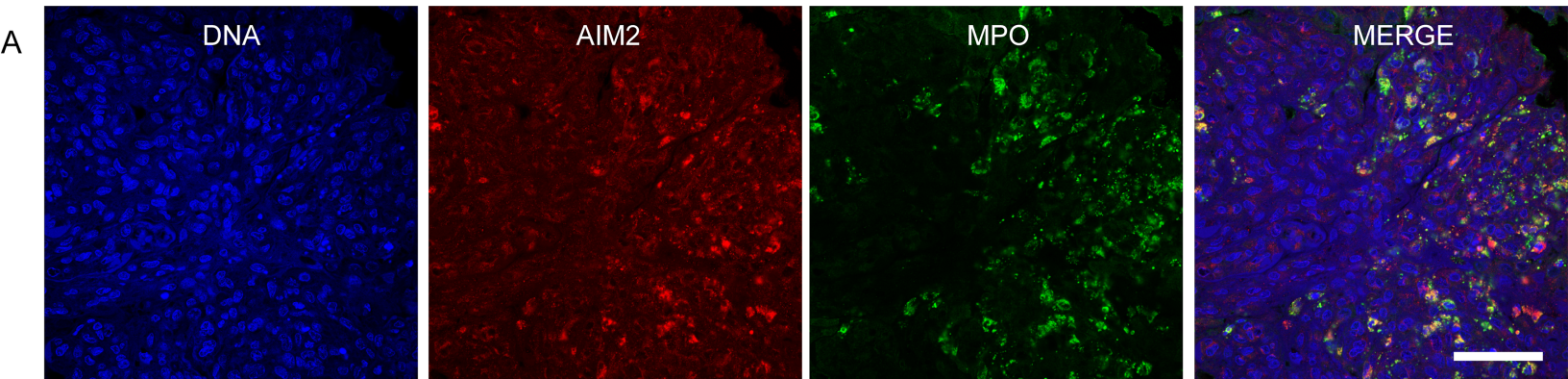
365 **Figure 3: IFI16 and AIM2 bind NETs in diffuse proliferative lupus nephritis.**

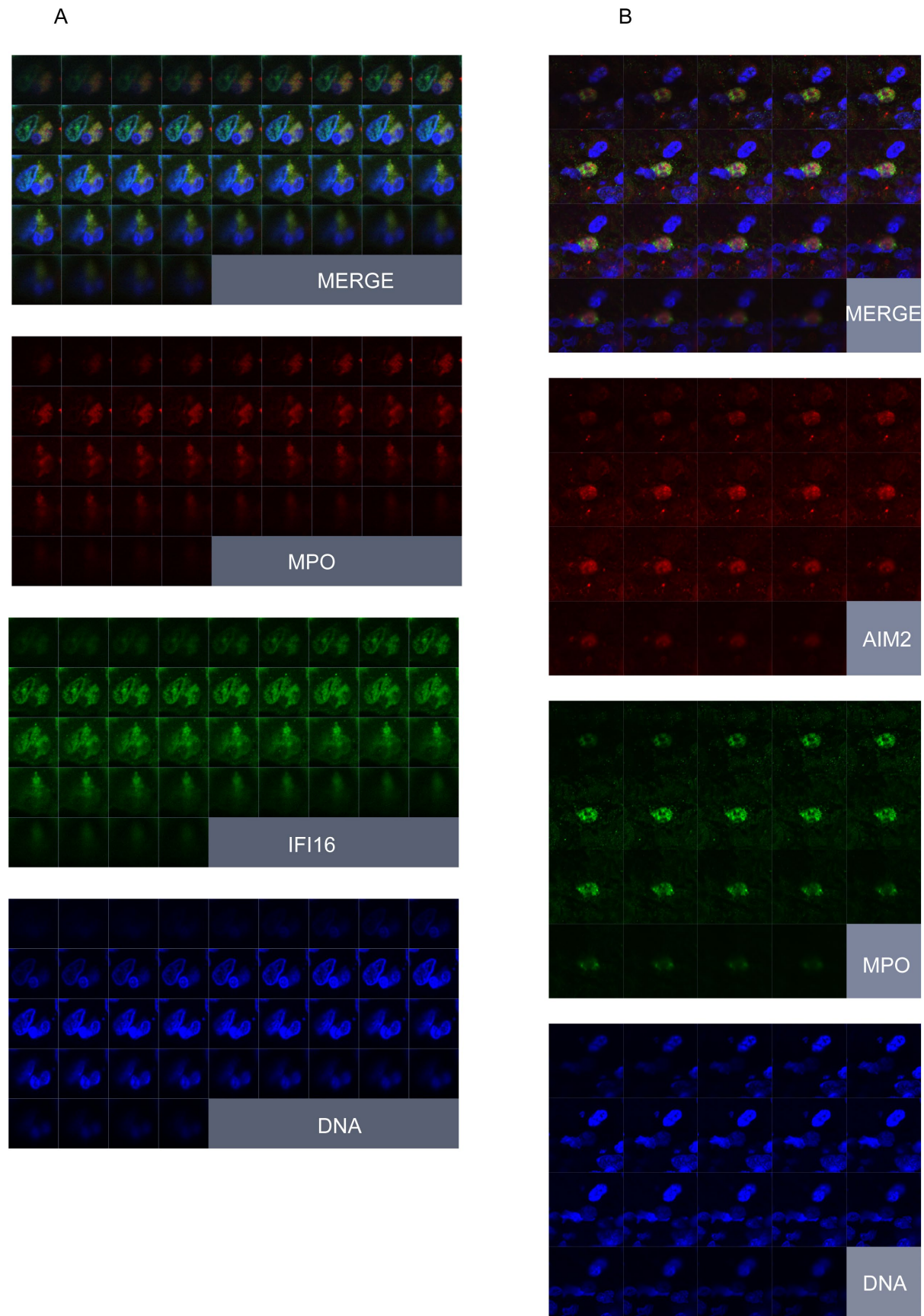
366 Representative images of ALR expression and ALR-NETs identified in patients with class IV lupus
367 nephritis. AIM2 (A) expression was largely detected in MPO expressing neutrophils, while IFI16 (B) was
368 more broadly distributed. NETs (arrows) demonstrating co-localizing staining for DNA, MPO, and AIM2
369 (C) or IFI16 (D) visualized by confocal microscopy. Scale bars: 50 μm (A, B) 5 μm (C), 2 μm (D).

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Supplemental Figure 1: Z-stack imaging of AIM2/IFI16-NETs in lupus nephritis.

Renal biopsy paraffin section stained for DNA, MPO and IFI16/AIM2 and imaged using z-stacking to identify extracellular DNA-containing structures containing MPO and IFI16 or AIM2. Individual squares within each panel represent adjacent focal planes, proceeding sequentially from top left to bottom right in each area imaged.

Supplemental Table 1. Phenotypic Characteristics of SLE Patients Related to AIM2 Autoantibody Level.

Feature	(1) Anti-AIM2+ OD > 20 n=9	(2) Anti-AIM2+ 4.3 < OD < 20 n=32	(3) Anti-AIM2- OD < 4.3 n=90	p value 1 vs 2	p value 1 vs 3	p value 2 vs 3
Age (years) at blood draw, mean ± SD	46.7 ± 13.2	52.8 ± 11.4	52.1 ± 13.8	0.1431	0.2338	0.7120
Physician Global Disease Activity	0.73 ± 0.28	0.61 ± 0.60	0.44 ± 0.51	0.2735	0.0156	0.2014
SLEDAI	3.77 ± 2.11	1.88 ± 2.21	1.05 ± 1.61	0.0249	0.0001	0.0742
IFI16 Positive	7/9 (78%)	12/32 (38%)	12/90 (13%)	0.0570	<0.0001	0.0080
DNA positive	6/9 (67%)	6/32 (19%)	8/90 (9%)	0.0106	0.0002	0.1929
C3 (mg/dL)	104.1 ± 29.0	117.7 ± 38.7	121.4 ± 29.0	0.2204	0.1069	0.3291
C4 (mg/dL)	14.9 ± 7.7	20.8 ± 8.0	25.0 ± 9.3	0.0378	0.0019	0.0084

Numerators correspond to number of patients with indicated feature positive and denominators to total number of patients with indicated feature recorded in the cohort, followed by percent (%) positive.

Supplemental Table 2. Immunologic phenotype of SLE patients related to AIM2 autoantibody status.

Feature	Anti-AIM2+ n=41	Anti-AIM2- n=90	p value
Age (years) at blood draw, mean \pm SD	51.4 \pm 11.9	52.1 \pm 13.8	0.8636
Female	38/41 (93%)	72/90 (80%)	0.0764
Anti-Smith Positive	6/41 (15%)	12/89 (13%)	>0.999
Anti-Ro Positive	18/41 (44%)	19/90 (21%)	0.0114
Anti-La Positive	10/41 (24%)	7/90 (8%)	0.0125
Anti-RNP Positive	6/41 (15%)	13/90 (14%)	>0.999
Anti-Cardiolipin Positive	27/40 (68%)	50/90 (56%)	0.2474
Anti-B2 Glycoprotein	15/39 (38%)	24/90 (27%)	0.2124
Lupus anti-coagulant	14/41 (34%)	31/90 (34%)	>0.999

Numerators correspond to number of patients with indicated feature positive and denominators to total number of patients with indicated feature, followed by percent (%) positive.

Supplemental Table 3: Lupus nephritis renal biopsies used in confocal imaging.

<u>Age</u>	<u>Sex</u>	<u>Diagnosis</u>
36.9	F	Diffuse proliferative lupus nephritis, ISN/RPA Class IV-A/C (S) with early segmental consolidation.
36.7	F	Diffuse proliferative and membranous lupus nephritis with focal segmental glomerulosclerosis (ISN/RPS CLASS IV A/C-G + V)
19.9	F	Diffuse proliferative and focally necrotizing lupus glomerulonephritis, ISN/RPS class IV-G (A) and tubulointerstitial inflammation with scattered interstitial immune complex deposits.
12.9	F	Diffuse proliferative lupus nephritis, ISN/RPS class IV-G (A/C)
28.6	F	Diffuse proliferative lupus nephritis; ISN/RPS class IV-G (A)