

1 **Identification of mitofusin 1 and complement component 1q**
2 **subcomponent-binding protein as mitochondrial targets of**
3 **autoantibodies in systemic lupus erythematosus**

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32

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53

54 **Data availability:** All datasets are available from the corresponding author upon reasonable
55 request.

56

57 **Conflict of Interest:** YB, PRF and EB have filed a provisional patent related to the work presented
58 herein.

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ABSTRACT

Objective: Mitochondria are organelles that possess several bacterial features such as a double-stranded genome with hypomethylated CpG islets, formylated proteins, and cardiolipin-containing membrane. In systemic lupus erythematosus (SLE), mitochondria and their inner components are released into the extracellular space, potentially eliciting a pro-inflammatory response by the immune system. While cardiolipin and mitochondrial DNA and RNA are confirmed targets of autoantibodies, other antigenic mitochondrial proteins in SLE remain to be identified. Herein, we aim to characterize the protein repertoire recognized by anti-mitochondrial antibodies (AMA) in SLE patients.

Methods: Using shotgun proteomic profiling, we identified 1345 proteins, 431 of which were associated with the mitochondrial proteome. Immunoreactivities to several of these candidates were assessed by direct ELISA in serum sample from a local cohort (healthy: n=30, SLE: n=87) and associated with demographic and disease characteristics.

Results: We determined that IgGs to the C1q-binding protein (C1qBP) are significantly elevated in SLE patients included in our cohort ($p=0.049$) and are associated with positivity for lupus anticoagulant ($p=0.049$). IgG against the mitochondrial protein mitofusin 1 (Mfn1) displayed promising performances in the prediction of SLE diagnoses (aOR: 2.99, 95%CI: 1.39–6.43, $p=0.0044$) in our cohort. Moreover, anti-Mfn1 were associated with positivity to anti-phospholipids ($p=0.011$) and anti-dsDNA ($p=0.0005$).

Conclusion: This study presents the mitochondrial repertoire targeted in SLE, indicating that autoantibodies can recognize secreted and/or surface proteins of mitochondrial origin. Profiling of the AMA repertoire in large prospective cohorts may improve our knowledge on mitochondrial biomarkers and their usefulness for patient stratification.

83

INTRODUCTION

84 The mitochondrion, a peculiar organelle in charge of numerous cellular pathways, is derived from
85 the endosymbiosis between an α -proteobacterium and a primitive eukaryotic cell (1,2). Despite
86 their intracellular nature, whole mitochondria and/or mitochondrial components may be released
87 into the extracellular milieu in conditions of necrosis, tissue damage (3–6), or cellular activation
88 (7–10). The release of whole mitochondria and/or mitochondrial components [i.e. molecular
89 patterns (mtDAMPs)] skews innate immunity toward a pro-inflammatory response (11–13).
90 Mitochondrial antigens may also be targeted by the adaptive immune system as indicated by the
91 presence of a humoral response, comprised of various types of anti-mitochondrial autoantibodies
92 (AMA) (14–17), in various inflammatory and autoimmune conditions. However, both the
93 pathophysiological pathway leading to the production of AMA and several of the antigens targeted
94 by several AMA remain to be characterized (14,16).

95 Systemic lupus erythematosus (SLE) is a complex autoimmune disease in which the immune
96 system generates autoantibodies recognizing self-epitopes. Antibodies directed against DNA and
97 nuclear components are hallmarks of SLE (18). Autoantibodies against the mitochondrial
98 phospholipid cardiolipin (aCL) are associated with both thrombotic and obstetric events in SLE
99 and the antiphospholipid syndrome (APS) (19). Simultaneous presence of aCL and antibodies to
100 the 60-kDa heat-shock protein (HSP60) were associated with arterial thrombosis (20). Studies have
101 also revealed that the lupus autoantibody repertoire comprises immunoglobulins against mtDNA
102 and mtRNA (15–17)

103 Despite the description of antibodies targeting mitochondrial antigens, the mitochondrial epitopes
104 expressed on the outer membrane remains to be identified (14,15). Herein, we aim to characterize
105 the antigenic protein repertoire recognized by AMA in SLE patients.

106 **MATERIAL AND METHODS**

107 *The CHU de Québec – Université Laval Systemic Autoimmune Rheumatic Disease Biobank and*
108 *DataBase repository (SARD-BDB).*

109 Serum samples from healthy donors and lupus patients were gathered, in accordance with the
110 Declaration of Helsinki, as previously reported (16). Additional information provided in
111 **Supplementary methods.**

112

113 *Mitochondrial samples:*

114 Mitochondria were isolated, following previously published procedures (15). and subsequently
115 lyzed. Additional information provided in **Supplementary methods.**

116

117 *Sample preparation for mass spectrometry identification of mitochondrial antigens targeted by AMA in*
118 *SLE:*

119 **Immunoprecipitations:** All incubations and washings used gentle rotary mixing, and were
120 performed at ambient temperature (about 21°C) – unless indicated. In all experiments, 3mg of
121 Dynabeads®-ProteinG were used. Before use, beads were washed three times with 1ml of 1X PBS
122 (Wisent, Montréal, Qc, Canada). For retention of the beads in the tubes, a DynaMag™-2 (Thermo
123 Fisher Scientific, Waltham, MA, USA) magnetic strip was used.

124

125 **Total mitochondrial antigens:** Dynabeads®-Protein G were incubated for 2hrs with 1mL pooled
126 sera (Additional information provided in **Supplementary methods**) diluted to an IgG
127 concentration of 24µg/ml in protease inhibitors-containing lysis buffer (see above). Dynabeads®-
128 bound total IgG were then washed three times in 1.5mL PBS and once in the same volume of lysis
129 buffer. Samples were incubated overnight at 4 °C with 1.5ml of mitochondrial lysate brought to a
130 protein concentration of 3mg/mL. Washing steps in 1.5mL total volume were then performed as
131 as follows: thrice in lysis buffer, containing a protease inhibitor cocktail, and twice in PBS. Beads
132 were then resuspended in 100µL of 75mM ammonium bicarbonate at pH8.0 and stored at -80°C
133 until used.

134

135 ***Enrichment in mitochondrial outer membrane (MOM) antigens by panning:*** Intact
136 mitochondria (0.5mg as quantitated by BCA assay) were incubated overnight at 4°C with 1mL of
137 diluted pooled sera (10%, in PBS with a protease inhibitor cocktail) from pooled samples of
138 healthy individuals or lupus patients. Unbound serum components were removed by three 7,000g
139 centrifugal washing steps, each performed for 10min at 4°C, with 1.5ml PBS. AwMA, bound to
140 their outer membrane mitochondrial antigens were released by incubation in 1mL lysis buffer
141 containing a protease inhibitor cocktail, overnight at 4°C to ensure the capture of IgGs by
142 Dynabeads®-Protein G. Three washing steps were performed in PBS containing a protease
143 inhibitor cocktail, followed by two final washes with PBS devoid of protease inhibitors. Samples
144 were resuspended in 100µL ammonium bicarbonate buffer, pH8.0 and stored at -80°C.

145

146 ***Negative controls:*** The same procedure as for total mitochondrial antigens was performed, using
147 an irrelevant monoclonal IgG (Clone IV.3, i.e. targeting FcγRIIa) instead of serum-containing IgG
148 in order to mitigate non-specific capture of proteins caused by either polyreactive or natural
149 antibodies that may target mitochondrial antigens.

150

151 *Identification of mitochondrial antigens recognized by AMA:*

152 ***On-bead proteolysis:*** isolated samples were resuspended in 200µL of 75mM ammonium
153 bicarbonate pH8.0 and supplemented with 2µg of a trypsin/Lys-C mix (Promega, Madison, WI,
154 USA). Proteins in the samples were digested overnight at 37°C on a rotating mixer. The digested
155 products were acidified with trifluoroacetic acid and the peptides generated from the proteolytic
156 digestion were isolated and washed on C18 tips (Thermo Fisher Scientific) according to the
157 manufacturer's instructions. The purified peptides were then dried by vacuum centrifugation and
158 stored at -80 °C before analysis by liquid chromatography with tandem mass spectrometry
159 (LC-MS/MS. Additional information provided in **Supplementary methods**).

160

161 *Detection of autoantibodies targeting selected mitochondrial antigens*

162 The assessment of autoantibody levels against several mitochondrial antigens was performed
163 either by direct ELISA, using diluted sera (1:100) from healthy individuals or patients with either
164 primary biliary cirrhosis (PBC), SLE or APS – as previously described (15–17). To assess
165 reactivity patterns in routine clinical testing, indirect immunofluorescence (IIF) assays were
166 performed using HEp-2 cells (Bio-Rad laboratories, Hercules, CA, USA). Patient serum samples
167 were tested at 1:80 and 1:160 dilutions using a PhD 1x workstation (Bio-Rad laboratories).
168 Polyclonal antibodies against C1qBP and Mfn1 were also tested for their reactivity patterns on
169 HEp-2 cells. Reactivity patterns were assessed by a trained member of the personnel and validated
170 by the laboratory director, using a BX53 immunofluorescence microscope (Olympus Corporation,
171 Shinjuku, Japan). Pictures presented herein were subsequently acquired, **using a (MODELE)**
172 **confocal microscope**. Additional information is provided in **Supplementary methods**.

173

174

175 *Statistical analyses:*

176 Sociodemographic, clinical and laboratory values are presented as either
177 median \pm inter-quartile range (IQR), n (%), or as mean \pm SD. Groups were compared using the
178 Kruskal-Wallis test with multiple comparisons to healthy donors. Spearman correlations were
179 calculated to see associations between AMA and antibodies assessed in routine clinical serology
180 tests. Youden's index was determined to set a cut-off value for positivity to anti-C1qBP and
181 anti-Mfn1. Logistic regressions were performed to predict diagnosis, separately for C1qBP ad
182 Mfn1. Additional information on these tests provided in **Supplementary methods**. Statistical
183 analyses were performed with Prism 9 software (GraphPad Software Inc., La Jolla, CA, USA) and
184 SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), while figures were assembled with
185 Photoshop CC 2019 version 20.0.4 (Adobe Systems Inc.).

186

RESULTS

187 We adopted two complementary approaches to identify proteins targeted by AMA in SLE: 1) we
188 used mitochondrial lysates to examine the complete spectrum of mitochondrial proteins targeted
189 by AMA and identified by immunoprecipitation, and 2) whole (i.e. intact) mitochondria were used
190 to capture AMA recognizing components from the mitochondrial outer membrane (MOM). An
191 irrelevant antibody, targeting a protein absent in mitochondria, was also utilized as a control. The
192 latter strategy permitted to appreciate both the non-specific binding and potential endogenous
193 proteins interacting with IgG (e.g. the complement pathway) (**Figure 1**).

194

195 A total of 1345 proteins were identified by the combined approaches, 252 of which were
196 withdrawn as usual contaminants (**Supplementary Table 4**). When grouped for protein
197 interactions, we observed an enrichment of three networks in SLE samples: the C1q complement
198 component, the serin protease inhibitor (serpin) superfamily, and members of the pyruvate
199 dehydrogenase complex. Analysis through gene ontology highlighted the mitochondrial
200 metabolism as the functions / processes with the highest significance (**Figure 2c**).

201

202 The complement component 1q subcomponent-binding protein (C1qBP) is a mitochondrial
203 protein that is stored within the mitochondrion and can be then addressed to the surface of the
204 plasma membrane and/or shed into the extracellular space (21). Although C1qBP is not strictly a
205 mitochondrial protein, its enrichment in SLE samples and its relevance to mitochondria and
206 complement stimulated further analyses (Figure 2). When assessed by direct ELISA, IgG against
207 C1qBP was significantly ($p=0.049$) elevated in patients with SLE (**Figure 3a**) in comparison with
208 healthy individuals. In contrast, the levels of anti-C1qBP were not significantly increased in APS
209 or PBC (**Figure 3a**), two diseases in which AMA are reported (22,23).

210

211 While antibodies in PBC target proteins from the mitochondria inner membrane (MIM), the inter-
212 membrane space (IMS) or the matrix (MM) (14,24–26), previous findings determined that

213 antibodies may also recognize components on the surface of the outer membrane in SLE
214 (15,17,27). Thus, we assessed the localizations of the proteins to identify members of the
215 mitochondrial proteome. 431 mitochondrial proteins were assigned to the mitochondrion, 168 of
216 which lacked references pertaining to their mitochondrial sublocalization. Ninety-three proteins
217 were expressed within the MM, 6 in the IMS, 130 in the MIM and 35 in the MOM. Of note, 13
218 proteins were associated with more than one mitochondrial compartment
219 (**Supplementary Table 4**).

220

221 A protein from the MOM, associated to the the pyruvate dehydrogenase complex but distinct from
222 the antigenic targets of AMA-M2 and presenting an elevated score in our panning approach was
223 selected – mitofusin-1 (Mfn1). IgGs against Mfn-1 were significantly increased in SLE in
224 comparison with healthy individuals ($p=0.0044$). In contrast, they were not significantly increased
225 in PBC and APS (**Figure 3b**). For comparisons, we tested the antigenicity of other mitochondrial
226 proteins with known MIM or MM locations, but with lower prediction scores in our panning
227 approach. The MM urea cycle enzymes ornithine carbamoyltransferase, carbamoyl-phosphate
228 synthase and N-acetylglutamate synthase and the metabolic enzymes aspartate aminotransferase
229 and aldehyde dehydrogenase; and the MIM beta and alpha subunits of, respectively, ATP synthase
230 and electron transfer flavoprotein, two proteins implicated in oxidative phosphorylation and
231 electron transfer, were tested. The levels of autoantibodies against these other candidates were not
232 significantly increased in SLE patients in comparison to healthy individuals (**Supplementary**
233 **Figure 1**).

234

235 Whether the autoantibodies to either of these two components, C1qBP and Mfn 1, are associated
236 with a definable cytoplasmic immunofluorescent staining pattern on routine Hep-2 substrates, as
237 seen with antibodies to pyruvate dehydrogenase complex in PBC sera (22,28), was determined
238 next. Indirect immunofluorescence labeling of Hep2 cells with a commercial antibody against
239 C1qBP produced an intense speckled staining of the nuclear region and a lower signal in the
240 cytosol. Conversely, commercial anti-Mfn 1 displayed a reticular staining of the cytoplasm, typical
241 of anti-mitochondrial antibodies (**Figure 4A**). AwMA⁺ SLE sera displayed a wide variety of

242 patterns, but none was qualified as positive for mitochondrial staining. In contrast, PBC sera
243 utilized using the same approach revealed an obvious cytosolic pattern, with no nuclear staining
244 when observed using a regular fluorescence microscope. However, when the same slides were
245 examined using a confocal microscope, the autoantibodies from 7 out of the 9 SLE patients tested
246 displayed various intensities of cytoplasmic staining, reminiscent of those observed in PBC or
247 observed using commercial antibodies against C1qBP and Mfn 1 (**Figure 4,**
248 **Supplementary Table 5**).

249

250 Although it is acknowledged that the number of patients examined is insufficient to draw definite
251 conclusions on the clinical implications of these findings, biostatistical analyses were performed
252 to determine potential associations between anti-C1qBP or anti-Mfn1 with disease characteristics
253 in the SLE patients included in our cohort. While neither anti-C1qBP nor anti-Mfn1 displayed
254 significant association to the various clinical manifestations assessed using univariate and
255 multivariate logistic regressions (i.e. thrombosis, carotid plaque, $SDI \geq 1$, $SLEDAI-2K \geq 4$, arthritis,
256 nephropathies or dermatological disorders, *data not shown*), we assessed the ability of these two
257 AMA to efficiently discriminate SLE samples from healthy controls using Youden's index.
258 Calculated cut-off values were 0.064 for anti-C1qBP and 0.116 for anti-Mfn1. Antibodies to Mfn1
259 displayed suboptimal sensitivity, but good specificities, while anti-C1qBP had high sensitivity, but
260 lower specificity (**Supplementary Table 6**). However, both autoantibodies showed high positive
261 predictive values.

262 Moreover, SLE patients positive for the lupus anticoagulant (i.e. LA) displayed increased
263 anti-C1qBP IgGs (**Table 1**). Anti-Mfn1 IgGs were increased in patients with positivity to
264 anti-phospholipids (i.e. any, or a combination of the various anti-phospholipids assessed), as well
265 as anti-double-stranded DNA autoantibodies (i.e. anti-dsDNA). When considering individual
266 IgGs, anti-Mfn1 were increased in patients positive for anti-cardiolipin and neared significance in
267 patients with anti- β_2 glycoprotein I (i.e. anti- β_2 GPI). These results were further confirmed by the
268 correlations between $OD_{405\text{ nm}}$ measured for anti-Mfn1 and continuous variables available for
269 anti-cardiolipin, anti- β_2 GPI and anti-dsDNA (**Table 2**). When matched with levels of other AMA,
270 levels of anti-C1qBP were associated with both AwMA-IgG and AmtDNA-IgG. Anti-Mfn1

271 correlated with all the AMA assessed in our cohort (**Table 2**). Of note, levels of anti-C1qBP also
272 correlated with those of anti-Mfn1 ($r_s=0.49$; $p>0.0001$).

273

274

DISCUSSION

275 Patients with SLE display antibodies from various subclasses (e.g. IgG, IgM, IgA) against a wide
276 array of self-antigens (29). The epitopes targeted by these autoantibodies comprise, but are not
277 limited to (30), phospholipids [e.g. aPL, aCL, LA] (31), anti- β_2 GPI (32), nucleic acids
278 (e.g. anti-dsDNA, AmtDNA, AmtRNA) (15,16), transcription factors and ribonucleoproteins
279 [i.e. anti-nuclear antibodies (ANA)] (33). Furthermore, distinct autoantibodies targeting various
280 types of mitochondrial biomolecules such as phospholipids (i.e. aCL targeting cardiolipin) (34),
281 nucleic acids (i.e. mtDNA, mtRNA) (15,16) and antigens whose precise nature remains to be
282 characterized (i.e. AMA-M5) were reported in SLE (14,35).

283 While various proteins involved in the mitochondrial processing of pyruvate (e.g. PDC-E2), sulfite
284 oxidase and glycogen phosphorylase are mitochondrial proteins known to be targeted by AMA
285 (i.e. respectively by AMA-M2, -M4 and -M9) in PBC (14), limited knowledge is available
286 concerning the extent of the mitochondrial proteome targeted by AMA in SLE. Antibodies to
287 cardiolipin, a phospholipid uniquely synthesized in mitochondria in humans are known to be
288 associated with vascular and obstetrical events in SLE and APS (19,36,37). To date, the only
289 mitochondrial protein with autoantibodies associated with disease manifestations in SLE is HSP60
290 (38). Herein, we used several approaches to enrich mitochondrial antigens. Thus, we identified
291 1093 different proteins with 431 (39.43%) of which were associated with the mitochondrial
292 proteome (39). These mitochondrial proteins, targeted by circulating AMA, reveal the extent of
293 the mitochondrial antigenicity in SLE. Of note, among all the previously known mitochondrial
294 proteins targeted by AMA, each of the currently known protein antigen targeted by AMA – with
295 the exception of glycogen phosphorylase – were detected by our mass spectrometry analyses.

296

297 Samples were treated with the benzonase nuclease in order to prevent the co-isolation of nucleic
298 acid interacting proteins, such as mtRNA with bound ribonucleoproteins (mtRNP). Despite these
299 precautions, our methods indicated the presence of non-mitochondrial proteins (e.g. complement
300 proteins, ficolin-3, serpins). While we restricted the focus of our study on the proteins assigned to
301 the mitochondrial proteome, such entities should be considered with caution as, in addition to their
302 own potential antigenicities, they can be co-isolated in association with other biomolecules

303 (e.g. interactors, ligands). Anti-RNP autoantibodies such as anti-small nuclear ribonucleoproteins
304 (snRNP), Ro/SSA and La/SSB are detected in mixed connective tissue disease as well as in SLE,
305 Sjögren syndrome, scleroderma and myositis (40). Of note is that ANA of the anti-Th/To family
306 were reported to cross-react with mitochondrial RNA processing in systemic sclerosis (41). These
307 elements suggest that, while we substracted mtRNP given their recognition as common
308 contaminants in proteomic analyses, further studies are needed to appreciate their immunogenicity
309 and the overlap between AMA and ANA in various systemic autoimmune rheumatic diseases.
310 Such studies could allow improvements to patient classification in cases of difficult diagnoses or
311 overlapping syndromes. While we tested a handful of mitochondrial proteins, systematic
312 characterization of patients' immunoreactivities to large subsets of mitochondrial antigens would
313 be enhanced by the use of high-throughput screening methods such as nucleic acid programmable
314 protein array.

315

316 Two proteins with significant immunogenicities stood out from the various candidates assessed
317 due to their increase in SLE samples when compared to healthy individuals. First, C1qBP, a protein
318 stored within the mitochondrion and dispatched to the MOM and/or the cell membrane, or secreted
319 into the circulation (42,43), where it may be targeted by circulating AMA. Second, Mfn1, a protein
320 embedded in the MOM that could be recognized by AMA upon the release of whole mitochondria
321 into the extracellular space (44). Of note, neither autoantibodies to C1qBP or Mfn-1 were increased
322 in PBC, another autoimmune condition characterized by the production of various AMA by
323 patients (22). They were also not increased in APS, a disease that may be associated to SLE with
324 pathogenic antibodies against cardiolipin (45). ~~We~~ Complement molecules in circulation, thus in
325 the sera tested, may target mitochondria directly (46); we thus speculate that C1qBP potentially
326 constitutes a circulating autoantigen enabling the formation of protein-protein scaffolds with C1q
327 and/or IgGs allowing pro-inflammatory signaling (e.g. respectively through the classical
328 complement pathway and signaling by Fcγ receptors) (47). The strong correlation between
329 anti-Mfn1 and AwMA suggests that mitofusin 1 might represent one of the main mitochondrial
330 antigens of the MOM. Moreover, our previous work on AMA in SLE and the correlations of AMA
331 with circulating mtDNA or anti-cardiolipin (**Tables 1 and 2**) showcase the mitochondrion as the
332 source of various immunogenic biomolecules (10,15–17). When considering the preliminary

333 performances of the two identified AMA, both displayed high positive predictive values for the
334 likelihood of an SLE diagnosis in samples from this cohort. Anti-C1qBP displayed suboptimal
335 specificity, but high sensitivity, whereas anti-Mfn1 presented high specificity and suboptimal
336 sensitivity (**Supplementary table 6**).

337 Routine detection of AMA is performed either by indirect immunofluorescence or ELISA (48).
338 We observed that commercial antibodies against Mfn1 produce a classical mitochondrial staining,
339 whereas anti-C1qBP shows staining of both nuclear and cytoplasmic regions of Hep2 cells. These
340 observations are concordant with the fact that Mfn1 is known to be uniquely expressed at the
341 surface of the mitochondrion, while C1qBP may also be found in other organelles such as the
342 nucleus or the Golgi apparatus (49). Clinical observation of SLE sera positive for AwMA, using a
343 fluorescence microscope as routinely performed in clinical laboratories, indicated that none of the
344 9 samples assessed displayed cytoplasmic staining. However, upon further scrutiny using a
345 confocal microscope, a proportion of these patients displayed significant cytoplasmic fluorescence
346 resembling that observed in PBC, along with nuclear staining. The use of confocal microscopy,
347 rather than wide-field fluorescence imaging, may thus be more optimal to discriminate different
348 subcellular stainings in conditions where the nucleus shows dominant staining. Every patient
349 included in our cohort was positive for ANA, thus we could not assess indirect
350 immunofluorescence patterns observed in AwMA⁺ / ANA⁻ specimens. SLE being characterized
351 by a wide repertoire of autoantibodies, confocal microscopy may thus be of use in order to
352 visualize indirect immunofluorescence patterns across each cellular sublocalizations (29,30,50).
353 The lack of specific indirect immunofluorescence pattern in our preliminary study suggests that
354 direct ELISA may be more informative for the detection AMA, as this technique would provide
355 quantitative information on the levels of autoantibodies specific to this target.

356

357 Our study has limitations. Due to the nature of the patient recruitment, the blood samples used for
358 this study were collected at the time of patient inclusion into the cohort, not at time of first
359 diagnosis. Thus, the levels of autoantibodies that we measured do not necessarily reflect the
360 antibodies present at the time of diagnosis. Moreover, phlebotomies were not performed at time of
361 occurrence of the clinical events. AMA levels were thus assessed for associations with histories of

362 any past and/or active clinical events. Furthermore, the sample size, the ethnic distribution of the
363 patients and the relatively low clinical activity scores of the patients at the time of the phlebotomy
364 may have influenced the various data assessed. Our preliminary findings presented herein should
365 therefore be confirmed in large inception cohorts with a broader spectrum of autoantibody
366 repertoires. Such studies would allow to appreciate fluctuations of AMA levels around the time of
367 active disease manifestations in SLE and to assess the performances of AMA as biomarkers in
368 SLE. Considering the dynamic range of antibody levels measured in patients with PBC and APS,
369 the replication of the present study in larger cohorts of patients and the evaluation of other
370 rheumatic conditions (e.g. myositis, scleroderma) would also provide information about the
371 distribution of AMA in autoimmune diseases.

372

373 In conclusion, the autoantibodies repertoire in SLE targets multiple representatives of the
374 mitochondrial proteome, notably C1qBP and Mfn1. Further studies may reveal whether the
375 detection of AMA in SLE or other autoimmune conditions may improve diagnosis, prognosis
376 and/or patient stratification in SLE.

377

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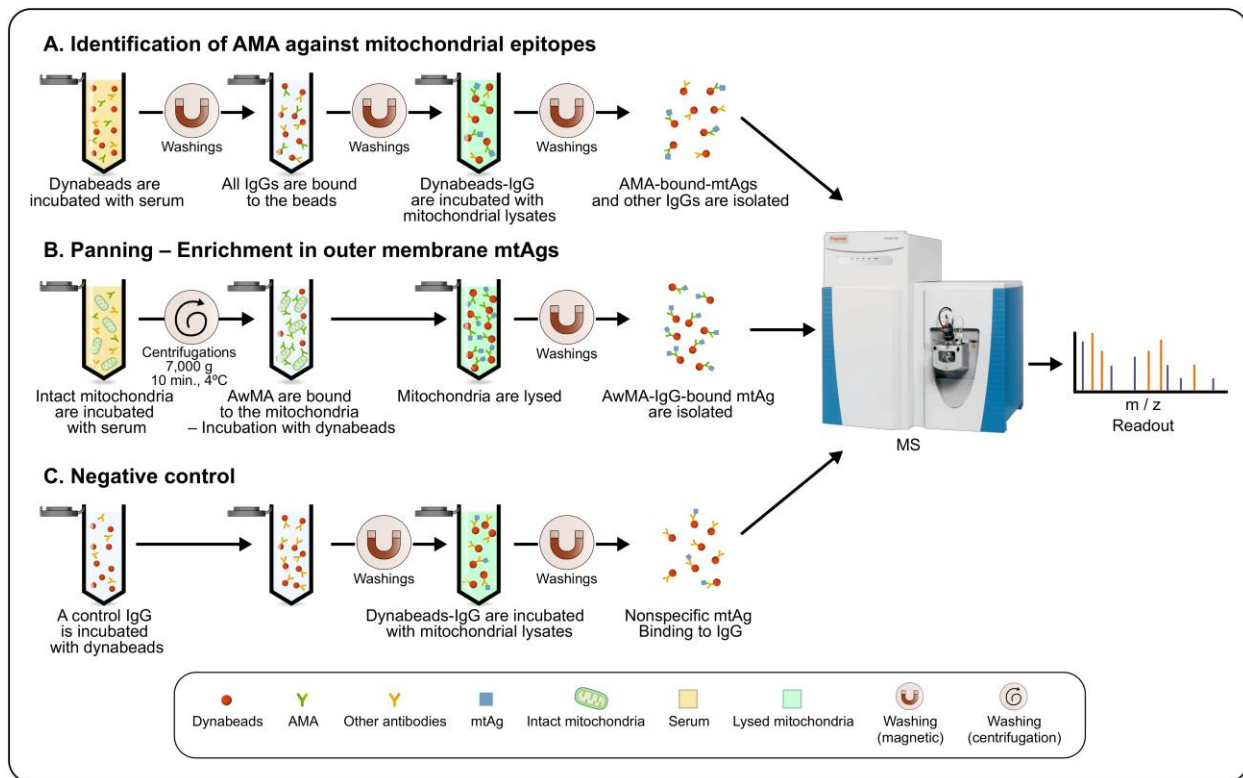
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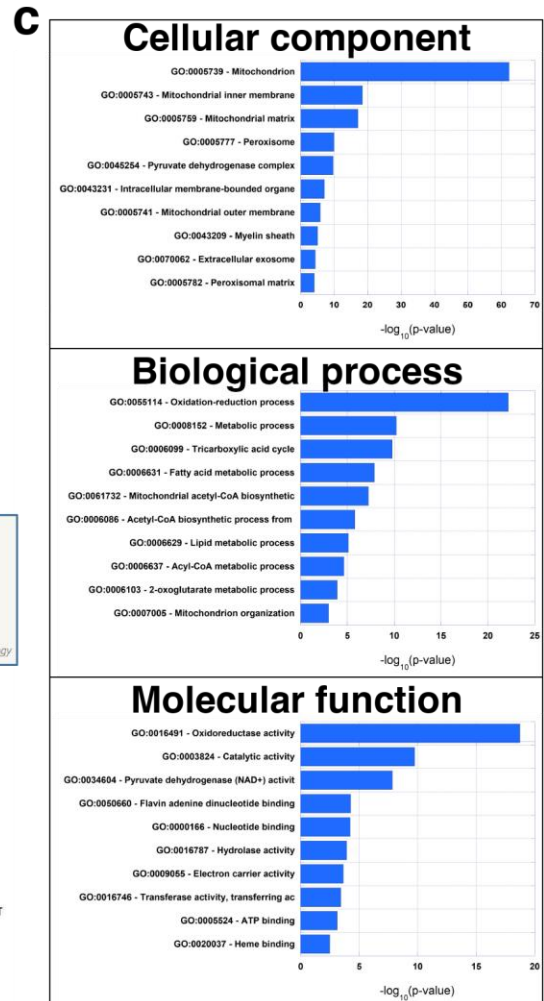
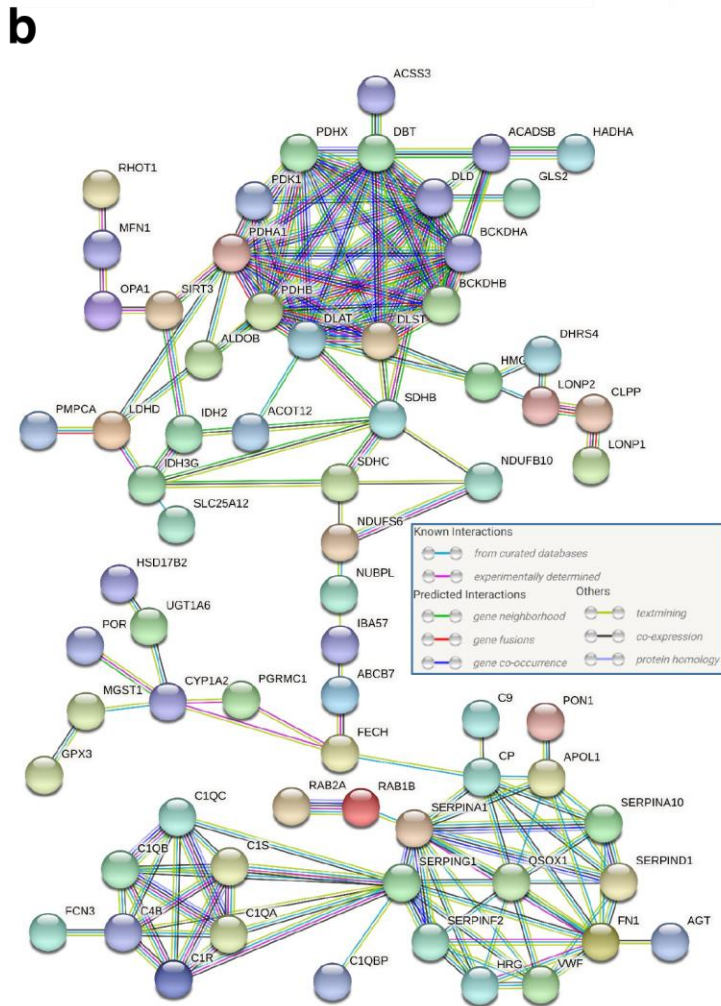
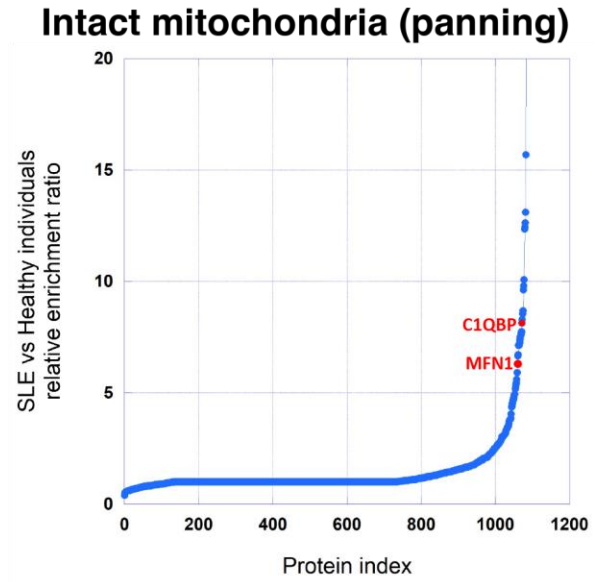
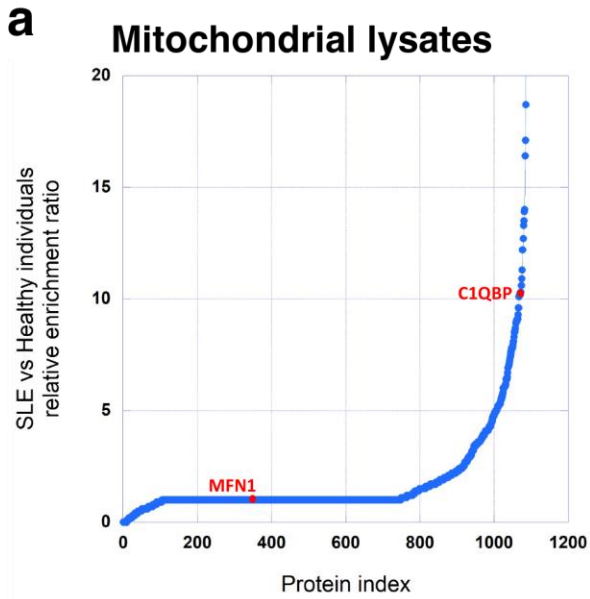
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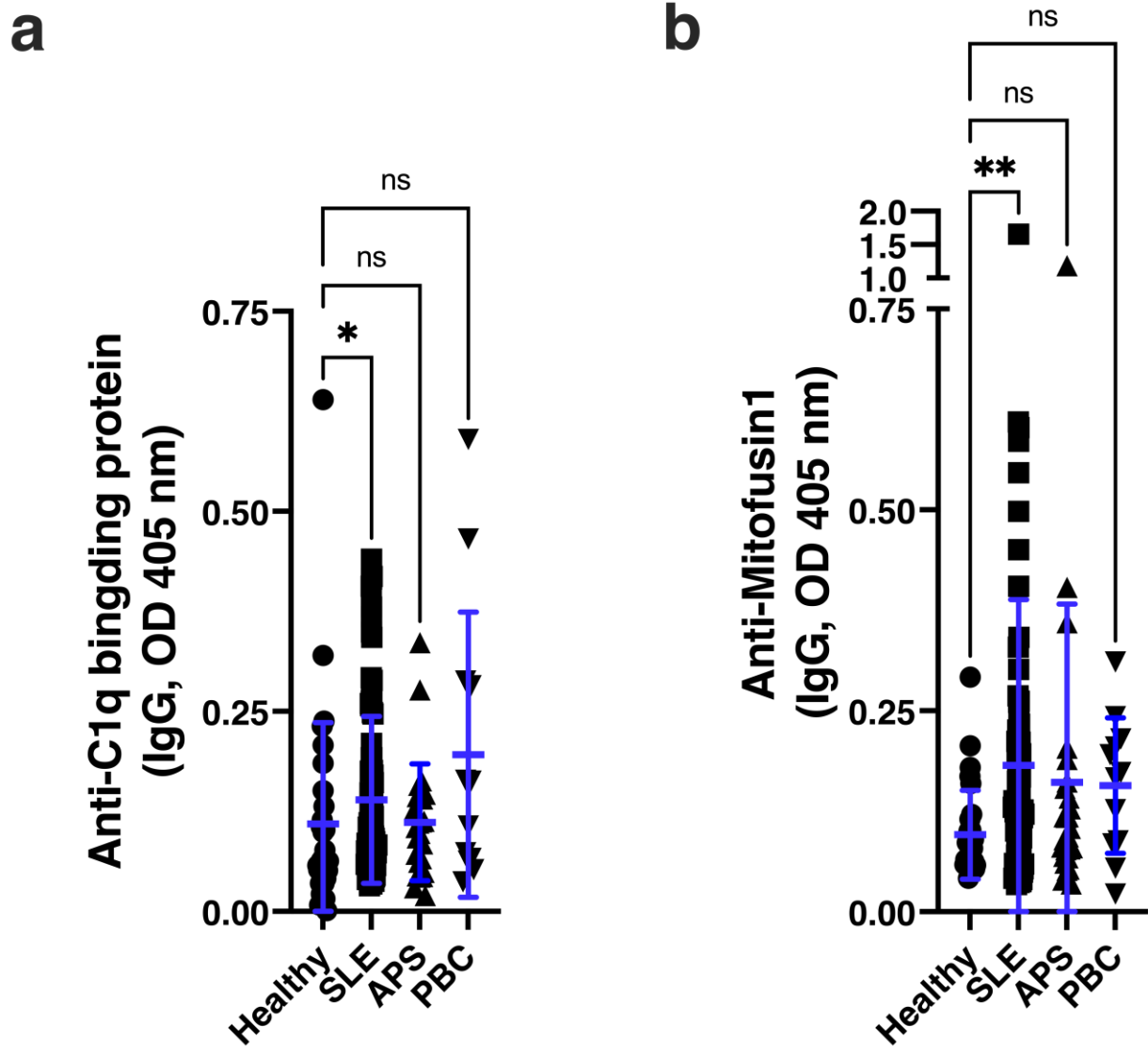
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504 **Figure 1: Workflow used for the detection of mitochondrial antigens targeted by**
 505 **anti-mitochondrial antibodies in SLE.**

506 (a) Antibodies of the IgG subclass are isolated, using Dynabeads®-Protein G, from pooled sera
 507 from either 10 healthy donors or 10 SLE patients with high levels of anti-whole mitochondrial IgG
 508 (AwMA-IgG). Dynabeads®-bound IgG were subsequently incubated with mitochondrial lysates,
 509 allowing the affinity purification of mitochondrial antigens (mtAgs) from all sub-localizations. (b)
 510 Freshly isolated intact mitochondria were incubated with pooled sera. Mitochondria incubated
 511 with AwMA were then lysed and AwMA-IgG isolated with Dynabeads™. (c) An irrelevant
 512 monoclonal IgG targeting FcγRIIa – a protein absent from mitochondria, is bound to Dynabeads™
 513 and incubated with mitochondrial lysates in order to identify non-specific binding of mtAgs. For
 514 each approach, samples were acquired in triplicate and mtAgs were identified by mass-
 515 spectrometry (MS).



518 **Figure 2: Mitochondrial hits identified and their mitochondrial sub-localization.**
519 (a) Proteins are plotted in the order of increasing ratio. A ratio > 1 suggests enrichment in SLE
520 patients relative to healthy individuals. Refer to **Supplementary Table 4** for a complete protein
521 listing and calculated ratios. (b) High-confidence protein interaction network of proteins enriched
522 in SLE patients (ratio >2). (c) Subcellular locations, processes and functions of the proteins targeted
523 by AMA in SLE.

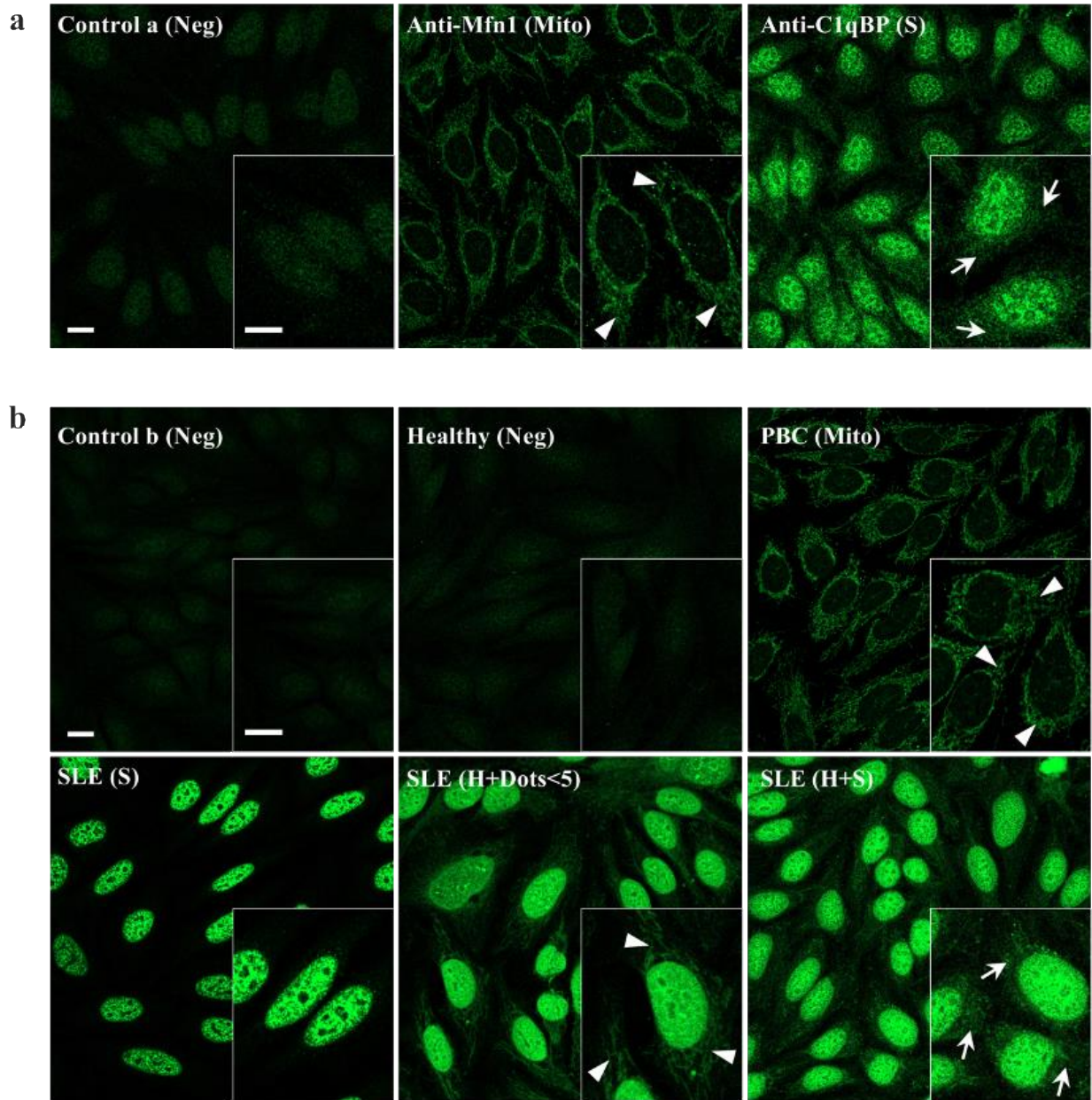


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525 **Figure 3: C1qBP and Mfn1 are two surface mitochondrial antigens with increased**
 526 **immunoreactivity in SLE.**

527 Immunoreactivity against mitochondrial proteins was assessed by direct ELISA. (a) The receptor
 528 for the complement component C1q (C1qBP) is a protein stored within the mitochondrion and
 529 subsequently dispatched to the cell surface and/or released into the extracellular space. SLE
 530 patients display increased levels of IgGs targeting C1qBP, compared with healthy
 531 individuals ($p=0.049$). (b) Mitofusin 1 (Mfn1) is a protein expressed at the surface of the
 532 mitochondrion and is responsible for the fusion of mitochondrial outer membranes;
 533 anti-Mfn-1-IgG are significantly increased in patients with SLE, compared with healthy donors
 534 ($p=0.0044$). Of note neither autoantibodies were increased in APS and PBC. Healthy: $n=30$,

535 SLE: n=87, APS: n=27, PBC: n=12. Data are mean optical densities read at 405 nm ($OD_{405\text{ nm}}$) \pm
536 standard deviation. Wilcoxon-Mann-Whitney test. Ns: $p \geq 0.05$; *: $p \leq 0.05$; **: $p < 0.01$.



537

538 **Figure 4: Indirect immunofluorescence (IIF) staining of Hep2 cells.**

539 (a) HEp2 cells were stained with an isotype-matched irrelevant antibody (Control a), or
 540 commercial antibodies (5 $\mu\text{g}/\text{mL}$ each) specific to either mitofusin 1 (Anti-Mfn1) or C1qBP (Anti-
 541 C1qBP). While anti-mfn1 displayed a reticular staining of the cytoplasm (arrowheads), typical of
 542 anti-mitochondrial antibodies (Mito), anti-C1qBP provided a speckled staining of the nucleus (S)
 543 and the cytoplasm (arrows). (b, *top row*) HEp2 cells, stained with a FITC-labelled anti-human IgG
 544 secondary antibody (Control b. (human serum-based negative control: Liquicheck negative

545 control, Bio-Rad Laboratories, Hercules, CA), or diluted sera (1:80) from healthy donors - negative
546 for AwMA, provided no signal (Neg). Sera from PBC patients showed the typical mitochondrial-
547 associated cytoplasmic reticular staining pattern provided by AMA. (b, *bottom row*) Routine
548 clinical staining of Hep2 cells with AwMA⁺ SLE sera allowed for the observation of various
549 nuclear fluorescence patterns [i.e. speckled, homogenous (H), dots] but no sample was qualified
550 as positive for AMA. However, confocal microscopy revealed that some samples may display
551 cytoplasmic patterns resembling those seen in PBC. Scale bar account for 10 μ m.

552 **Tables:**

553 **Table 1: Qualitative associations between anti-C1qBP, anti-Mitofusin 1 and autoantibodies**
 554 **routinely assessed in SLE.**

		Anti-C1qBP	Anti-Mitofusin 1
Anti-phospholipids	Negatives (n = 51)	0.09 ± 0.11	0.12 ± 0.11
	Positives (n = 23)	0.12 ± 0.21	0.18 ± 0.15
	p-value	0.097	0.011
Anti-cardiolipin	Negatives (n = 60)	0.09 ± 0.11	0.12 ± 0.10
	Positives (n = 14)	0.17 ± 0.27	0.25 ± 0.24
	p-value	0.053	0.0004
Anti-β₂ Glycoprotein I	Negatives (n = 64)	0.09 ± 0.11	0.12 ± 0.13
	Positives (n = 10)	0.11 ± 0.24	0.22 ± 0.25
	p-value	0.656	0.065
Lupus anticoagulant	Negatives (n = 67)	0.09 ± 0.10	0.12 ± 0.13
	Positives (n = 12)	0.13 ± 0.24	0.18 ± 0.12
	p-value	0.049	0.068
Anti-double-stranded DNA	Negatives (n = 62)	0.09 ± 0.10	0.12 ± 0.08
	Positives (n = 20)	0.10 ± 0.18	0.22 ± 0.23
	p-value	0.602	0.0005

555

556 Positivity to anti-phospholipids is defined here as the positivity to any, or a combination of the
 557 various anti-phospholipids assessed. Data are median ± IQR. Associations presented, herein, for
 558 anti-cardiolipin, anti-β₂-GPI and anti-dsDNA were tested for IgGs.

559 Table 2: Correlations between anti-C1qBP, anti-Mitofusin 1 and various continuous variables in
 560 SLE patients.

		Anti-C1qBP	Anti-Mitofusin 1
Clinical serology	Anti-cardiolipin antibodies (n = 80)	$r_s = 0.25$ $p = 0.02$	$r_s = 0.45$ $p < 0.0001$
	Anti- β_2 Glycoprotein I (n = 80)	$r_s = 0.12$ $p = 0.28$	$r_s = 0.26$ $p = 0.02$
	Anti-double-stranded DNA (n = 22)	$r_s = 0.11$ $p = 0.63$	$r_s = 0.44$ $p = 0.04$
Anti-mitochondrial antibodies	Anti-whole mitochondria (AwMA)	IgG $r_s = 0.32$ $p = 0.003$	$r_s = 0.65$ $p < 0.0001$
		IgM $r_s = 0.08$ $p = 0.46$	$r_s = 0.31$ $p = 0.003$
	Anti-mitochondrial DNA (AmtDNA)	IgG $r_s = 0.23$ $p = 0.03$	$r_s = 0.52$ $p < 0.0001$
		IgM $r_s = 0.07$ $p = 0.50$	$r_s = 0.24$ $p = 0.03$
	Anti-mitochondrial RNA (AmtRNA)	IgG $r_s = 0.03$ $p = 0.75$	$r_s = 0.27$ $p = 0.01$
		IgM $r_s = 0.03$ $p = 0.82$	$r_s = 0.27$ $p = 0.01$

561

562 Correlations presented, herein, for anti-cardiolipins or anti- β_2 -GPI were tested for IgGs.

Identification of mitofusin 1 and complement component 1q subcomponent-binding protein as mitochondrial targets in systemic lupus erythematosus

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Supplementary information:

Supplementary methods

Human serum samples – healthy donors, and patients with SLE or APS from the CHU de Québec – Université Laval Systemic Autoimmune Rheumatic Disease Biobank and DataBase repository (SARD-BDB).

Human serum samples from healthy donors and patients with autoimmune conditions: This study was approved by the research ethics board of the CHU de Québec–Université Laval (CHUL. #B13-06-1243 and #B14-08-2108). Healthy volunteers (i.e. with no signs or symptoms of acute infection and not on medications) were recruited at the CHUL. Serum samples were obtained from peripheral blood punctures performed at the time of inclusion. In accordance with the Declaration of Helsinki, written consent was provided by each subject, and their clinical information and biological specimen were associated with an anonymized reference number. A convenience sample was used and no sample size calculation was done. SLE patients met ACR classification criteria and each of the 11 categories was documented (1,2). APS patients met the 2006 revised Sapporo Criteria (3), and PBC patients the 2018 PBC classification criteria (4).

Clinical data collection: Information on sociodemographic variables (e.g. age, sex, ethnicity. **Supplementary Table 1**) were collected at the first visit of the patients at the SARD-BDB. Smoking history was characterized as any the consumption of tobacco, past or present. Body metrics and blood pressure were measured at the time of the visit, and co-morbidities were documented as present of absent. Information about SLE indexes was collected during the clinical visit corresponding to the blood sample

drawn. Medications presented in **Supplementary Table 1** were defined as treatments, ongoing at the time of the visit – apart from corticosteroids, that was defined as the use of prednisone in the past year.

Each of the 11 categories of the ACR classification criteria was documented (1,2). Disease activity was assessed, using the SLE Disease Activity Index–2000 (SLEDAI-2K) (5,6). SLE damages were evaluated using the Systemic Lupus International Collaborating Clinics (SLICC)/ACR damage index (SDI) (**Supplementary Table 2**) (7,8). Positivity for the various clinical outcomes assessed (e.g. lupus nephritis, arthritis, dermatological manifestations, thrombovascular events) was defined as past or present reports of one or more of these events, as defined by the SLICC Classification criteria for SLE (9).

Data from SLE patients' serology: Blood samples drawn at the time of the visit in the SARD-BDB were used to assess the various autoantibodies measured. Serological data was obtained as part of the routine care and included measurements of anti-dsDNA, anticardiolipin and anti- β_2 GPI antibodies by ELISA (i.e. laboratory cut-off of 40 UPL or above the 99th percentile of controls). Detection of LA activity was performed, following international guidelines for this assay (10). Detection of AMA (i.e. AwMA, AmtDNA, AmtRNA, IgG and IgM) was performed by our research laboratory, as previously published (11) (**Supplementary Table 3**).

Sample preparation for the identification of mitochondrial antigens targeted by AMA in SLE using mass spectrometry:

Preparation of mitochondrial lysates: Freshly isolated mitochondria were used for the panning of antibodies targeting the mitochondrial outer membrane (i.e. anti-whole mitochondria antibodies, AwMA). Dry-pelleted mitochondria were stored at -80°C until needed. Frozen mitochondria were resuspended in hypotonic lysis buffer (10mM HEPES, 2mM KCl, 0.1%

CHAPS, pH7.2) with protease inhibitors (cOmplete protease inhibitor cocktail; Roche, Basel, Switzerland) and underwent three cycles, alternating between thawing in a water bath (37°C) and freezing in an ethanol–dry ice bath, followed by a 15min sonication in an ice bath. Unbroken mitochondria were pelleted at 7,000g for 10min at 4°C and discarded. Supernatant protein (i.e. mitochondrial lysates) content was determined by the bicinchoninic acid method (BCA protein assay kit. Thermo Fisher Scientific, Waltham, MA, USA). Nucleic acids were digested by addition of benzonase nuclease (Sigma-Aldrich, St-Louis, MO, USA. 100 U/mL, 30min, 37°C). Lysates were stored at -80°C until use.

Serum selection and pooling: Optical densities at 405 nm ($OD_{405\text{ nm}}$) for 1:150 dilutions of serum samples from the SARD-BDB were previously assessed with our direct AwMA-ELISA (11) and a cut-off value for positivity to AwMA-IgG was calculated for $OD_{405\text{ nm}}$ values ≥ 0.30 . To account for interpersonal variabilities in autoantibody production, equal volumes of sera from either ten healthy donors ($OD_{405\text{ nm}}$ values ranging from 0.08 to 0.17), or from ten SLE patients positive for AwMA ($OD_{405\text{ nm}}$, 0.68 to 3.00) were pooled and their IgG concentrations were determined by Human IgG total uncoated ELISA kit (Thermo Fisher Scientific). Experiments were performed in triplicate.

Identification of mitochondrial antigens recognized by AMA:

LC-MS/MS analysis: Trypsin/Lys-C-digested peptides were separated on a Dionex Ultimate 3000 nanoHPLC system coupled to a Q Exactive™ OrbiTrap mass spectrometer (Thermo Fisher). 10µl of sample (a total of 1.5µg) resuspended in 1% (vol/vol) formic acid were loaded with a constant

flow of 4 $\mu\text{l}/\text{min}$ onto an Acclaim PepMap100 C18 column (0.3mm id \times 5mm; Dionex Corporation, Sunnyvale, CA, USA). After trap enrichment, peptides were eluted onto an EasySpray PepMap C18 nano column (75 μm \times 50cm; Dionex Corporation) with a linear gradient of 8–40% solvent B (80% acetonitrile with 0.1% formic acid) over 240min with a constant flow of 200nl/min. The HPLC system was coupled to the mass spectrometer via an EasySpray source. The spray voltage was set to 2.0kV and the temperature of the column set to 40°C. Full scan MS survey spectra (m/z 350–1600) in profile mode were acquired in the Orbitrap with a resolution of 70,000 after accumulation of 1,000,000 ions. The ten most intense peptide ions from the preview scan in the Orbitrap were fragmented by collision-induced dissociation (normalized collision energy 25% and resolution of 17,500) after the accumulation of 50,000 ions. Maximal filling times were 250ms for the full scans and 60ms for the MS/MS scans. Precursor ion charge state screening was enabled and all unassigned charge states as well as single, 7 and 8 charged species were rejected. The dynamic exclusion list was restricted to a maximum of 500 entries with a maximum retention period of 40 seconds and a relative mass window of 10ppm. The lock mass option was enabled for survey scans to improve mass accuracy. Data were acquired using the XCalibur software (Thermo Fisher Scientific).

Mass spectrometry data analysis: Mass spectra data (.RAW files) were loaded into MaxQuant version 1.6.17.0 and searched against a protein database generated by merging the *Homo sapiens* and *Mus musculus* reference proteomes (Uniprot, versions 01-29-2021: 77 027 human and 55 470 mouse proteins) complemented with a list of common contaminants maintained by MaxQuant and concatenated with the reversed version of all sequences (decoy mode). The minimum peptide length was set to 7 amino acids and trypsin was specified as the protease allowing up to two missed

cleavages. The mass tolerance was set to 7ppm for the precursor ions and 20ppm for the fragment ions. The following parameters were used: fixed carbamidomethylation of cysteine (+57.0214 Da), oxidation of methionine (+15.9949 Da) as a variable modification and a peptide-spectrum and protein match false-discovery rate of 0.01.

Intensity-based label-free quantification (LFQ) values were used to estimate the relative abundance of proteins in each replicate groups, which correspond to the sum of all peak intensities over all tandem mass spectra assigned to a particular protein. Missing LFQ data imputation was estimated by using a noise value corresponding to the lowest 1% percentile of the LFQ distribution. This noise value was imputed for each sample when the intensity value is missing (i.e. undetected proteins) and selected as the minimum LFQ intensity for low abundance protein identification for which LFQ values fall below the 1% percentile background.

Filtering and data analysis of proteomic data: Matches to reverse proteins, proteins with zero intensity values in all datasets, proteins with negative MaxQuant scores and common contaminants were removed from the final protein repertoire. Identified proteins were cross-referenced using their Uniprot ID for their cellular localization and, when the information was available, for their mitochondrial sub-localization. Pathways involving the mitochondrial proteins identified were analyzed, using the Reactome pathway knowledge base <https://reactome.org/> and the Database for Annotation, Visualization and Integrated Discovery (DAVID).

Detection of autoantibodies targeting selected mitochondrial antigens

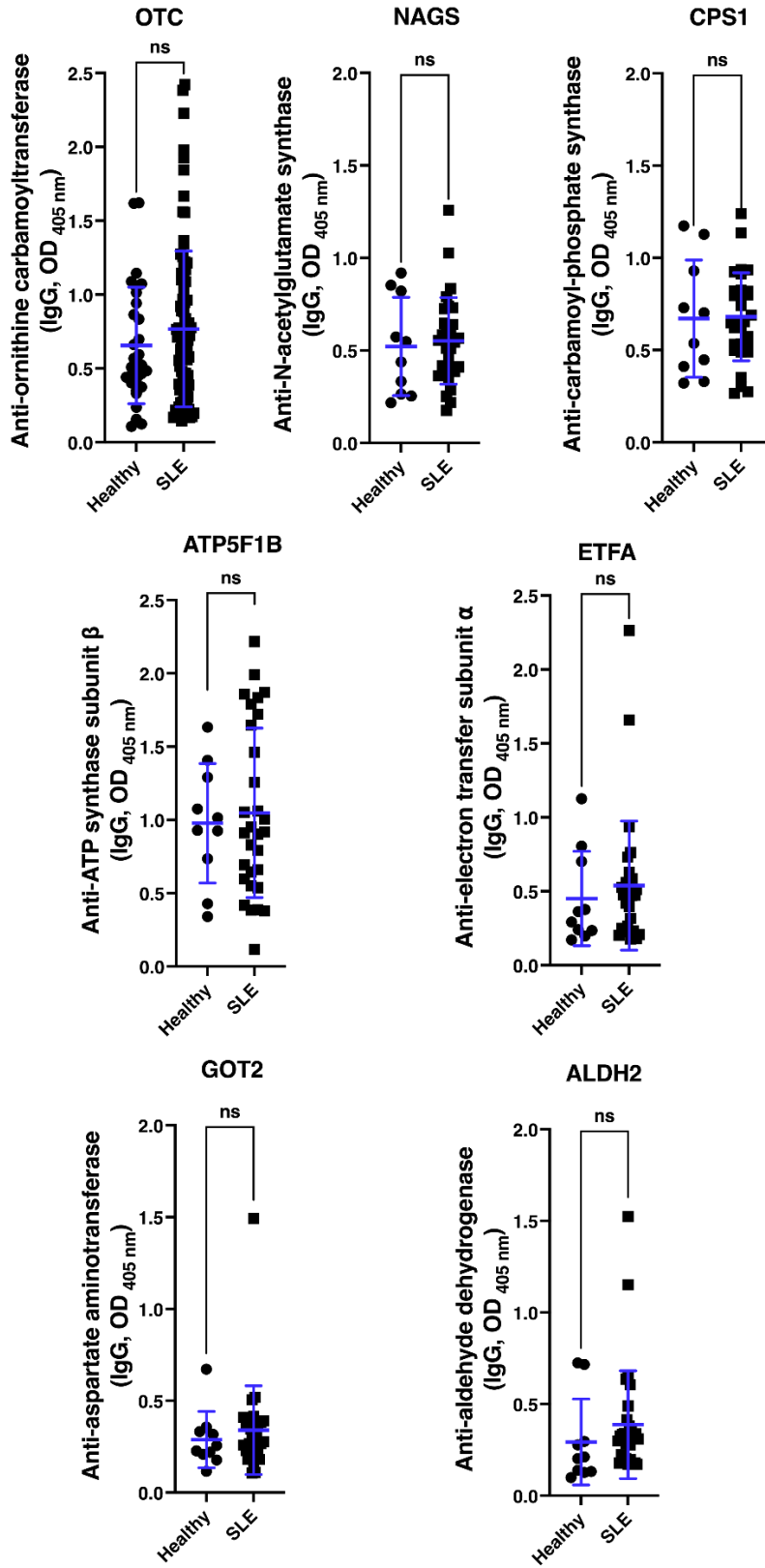
Enzyme-Linked Immunoassays (ELISAs): Detection of IgG, using various sources of mtAgs (i.e. whole/intact mitochondria, mtDNA, mtRNA) were performed as previously described (11–13). Immunoreactivity against other mitochondrial proteins were assessed. These proteins included the complement component 1 Q subcomponent-binding protein (C1qBP. Novus Biologicals, Centennial, CO, USA), mitofusin 1 (Mfn 1. Origene Technologies, Rockville, MD, USA) (Healthy: n=30, SLE: n=87, APS: n=27, PBC: n=12). Other mitochondrial proteins available, with MIM or MM locations identified by our proteomic approaches were assessed (Healthy: n=10; SLE n=30). These miscellaneous proteins were three representatives of the urea pathways [i.e. ornithine carbamoyltransferase (OTC), carbamoyl-phosphate synthase (CPS1) and N-acetylglutamate synthase (NAGS)] and two proteins implicated in electron transfer and oxidative phosphorylation [i.e. the beta subunit of the MIM complex ATP synthase (ATP5F1B. Cusabio, Wuhan, China) and electron transfer flavoprotein subunit alpha (ETF A. Origene Technologies)] as well as two proteins involved in metabolic processes [i.e. aspartate aminotransferase (GOT2. BioVision Inc., Milpitas, California, USA) and aldehyde dehydrogenase (ALDH2. RayBiotech Life, Peachtree Corners, GA, USA)]. OTC, CPS 1 and NAGS were prepared as published (14,15). Immunoreactivities against recombinant mitochondrial proteins were assessed by coating plates overnight at 4°C with 225ng protein in 25µL PBS 1X (i.e. 9ng/µL).

Statistical analyses:

The logistic regression models to predict diagnosis were adjusted for sex and age, and are expressed as their adjusted odds ratio (aOR), 95% confidence interval (95% CI) and p-value. Univariable and multivariable logistic and linear regressions were performed to predict clinical outcomes (thrombosis, presence of carotid plaque, presence of damage according to SLICC

Damage Index, presence of disease activity [SLEDAI-2k > 4], presence of nephropathies, presence of dermatological disorder and presence of arthritis, as well as continuous variables carotid intima-media thickness and Lupus Severity Index Score), separately for C1qBP and Mfn1. These models were adjusted for disease duration, age, BMI, sex, antimalarial drugs, and prednisone use.

Supplementary figure:



Supplementary Figure 1: Immunoreactivity of several mitochondrial proteins in SLE.

Autoantibodies against mitochondrial proteins implicated in the urea cycle (i.e. OTC, NAGS, CPS1), OxPhos (i.e. ATP5F1B, ETFA) and mitochondrial metabolism (i.e. GOT2, ALDH2) were assessed in serum samples from the SARD-BDB (Anti-OTC-IgG: Healthy n=30, SLE: n=87. Other autoantibodies: Healthy n=10, SLE: n=30). No differences were observed between the two groups for any of the antigens assessed. Data are mean optical densities read at 405 nm ($OD_{405\text{ nm}}$) \pm standard deviation. Wilcoxon-Mann Whitney test. Ns: $p > 0.05$.

Abbreviations: ALDH2: aldehyde dehydrogenase. ATP5F1B: ATP synthase – β subunit.
CPS1: carbamoyl-phosphate synthase. ETFA: electron transfer flavoprotein subunit α .
GOT2: Aspartate aminotransferase. NAGS: N-acetylglutamate synthase.
OTC: ornithine carbamoyltransferase.

Supplementary tables

Supplementary Table 1: Sociodemographic and clinical characteristics of the SLE patients included in the SARD-BDB.

Variable		Sample size	Value [n (%) or median \pm IQR]
Sex (%)	Female	87	72 (82.8)
	Male		15 (17.2)
	Age, in years	86	49.58 \pm 20.65
Ethnicity (%)	Caucasian		84 (97.7)
	Black	86	1 (1.2)
	Other		1 (1.2)
	Disease duration, in years	87	5.00 \pm 19
Body metrics	Waist, in cm	61	87 \pm 17
	Hip, in cm	59	98 \pm 10
	Waist / Hip ratio	59	0.86 \pm 0.11
Blood pressure (mmHg)	Systolic	69	124 \pm 15
	Diastolic	69	73 \pm 12
Comorbidities (%)	Menopause	65	38 (58.5)
	Tobacco, past or present	83	35 (42.2)
	Obesity (i.e. BMI \geq 30)	86	13 (15.1)
	Hypertension	87	11 (12.6)
	Diabetes	86	2 (2.3)
	Dyslipidaemia	87	7 (8.0)
	Corticosteroids	86	17 (19.8)
	Hydroxychloroquine	87	65 (74.7)
	Chloroquine	87	6 (6.9)
	Azathioprine	87	15 (17.2)
Medications (%)	Methotrexate	87	14 (16.1)
	Leflunomide	87	1 (1.1)
	Mycophenolate mofetil	87	10 (11.5)
	Mycophenolic acid	87	1 (1.1)
	Cyclophosphamide	87	2 (2.3)
	Anti-platelet / anticoagulants	86	13 (15.1)
	Hormones replacement (estrogen/progesterone)	87	2 (2.3)
	Contraception	87	5 (5.7)
	Diabetes medication	86	2 (2.3)
	Lipid lowering drug	86	14 (16.3)

Supplementary table 2: SLE disease characteristics of the patients included in the SARD-BDB.

Variable	Sample	Value	
	size	[n(%) or median \pm IQR]	
Clinical Scores	ACR	86	5.00 \pm 2.00
	LSI	86	5.62 \pm 2.92
	SDI	86	0.00 \pm 1.00
	SLEDAI - 2K	86	2.00 \pm 6.00
	Malar rash	86	20 (23.3)
	Discoid rash	86	13 (15.1)
	Photosensitivity	86	37 (43.0)
	Oral ulcers	86	27 (31.4)
	Arthritis	86	70 (81.4)
	Serositis	86	23 (26.7)
	<i>Pleuritis</i>	86	17 (19.8)
	<i>Pericarditis</i>	86	13 (15.1)
American College of Rheumatology Classification criteria	Renal disorders	86	24 (27.9)
	<i>Proteinuria</i>	86	24 (27.9)
	<i>Cellular casts</i>	86	9 (10.5)
	Neurological disorders	86	4 (4.7)
	<i>Seizure</i>	86	4 (4.7)
	<i>Psychosis</i>	86	1 (1.2)
	Haematological disorders	86	69 (80.2)
	<i>Haemolytic anemia</i>	86	5 (5.8)
	<i>Leucopenia</i>	86	33 (38.4)
	<i>Lymphopenia</i>	86	51 (58.6)
	<i>Thrombocytopenia</i>	86	24 (59.3)
	Anti-nuclear antibodies	86	86 (100)
	Immunological disorders	86	63 (73.3)
	<i>Anti-double-stranded DNA</i>	86	49 (57.0)
	<i>Anti-Smith</i>	86	12 (14.0)
	<i>Anti-phospholipids</i>	86	42 (48.8)
	<i>Anti-cardiolipin</i>	86	29 (33.7)
	<i>Lupus anticoagulant</i>	86	23 (26.7)
	<i>Venereal Disease Research Laboratory</i>	86	2 (2.3)
	Miscellaneous clinical information	Platelets	87
Red blood cells		87	5.60 \pm 2.70
Carotid-intima media thickness		34	0.61 \pm 0.12
Creatinine clearance		26	98.50 \pm 22.00
Thromboses		87	10 (11.5)
Carotid plaque		64	24 (37.5)
Dermatological manifestations		86	71 (82.6)

Supplementary table 3: Continuous variables acquired in blood samples acquired from SLE patients drawn at the time of their inclusion in the SARD-BDB.

	Variable	Sample size	Value (median ± IQR)
Clinical serology	Anti-cardiolipin - IgG	80	2.70 ± 6.30
	- IgM	80	6.95 ± 9.50
	Anti-β₂ Glycoprotein I - IgG	80	1.30 ± 0.80
	- IgM	80	2.15 ± 2.10
	Anti-double-stranded DNA	22	6.00 ± 21
Anti-mitochondrial antibodies	Anti-whole mitochondria - IgG	87	0.25 ± 0.19
	- IgM	87	0.41 ± 0.43
	Anti-mitochondrial DNA - IgG	87	0.35 ± 0.25
	- IgM	87	0.37 ± 0.49
	Anti-mitochondrial RNA - IgG	87	0.28 ± 0.47
	- IgM	87	0.37 ± 0.44
	Anti-C1qBP - IgG	87	0.13 ± 0.13
	Anti-Mitofusine 1 - IgG	87	0.09 ± 0.11

Supplementary table 4: Mass spectrometry identification of proteins associated with anti-mitochondrial autoantibodies in SLE.

See attached appendix

Supplementary table 5: Confocal microscopy observation in indirect immunofluorescence allows the detection of cytoplasmic patterns that are undetected using an epifluorescence microscope.

	Type of microscope	
	Epifluorescence	Confocal
Homogenous	7 (77.78 %)	7 (77.78 %)
Speckled	1 (11.11 %)	1 (11.11 %)
Nudeolar	1 (11.11 %)	1 (11.11 %)
Cytoplasmic/mitochondrial	0 (0 %)	7 (77.78 %)
Negative	1 (11.11 %)	1 (11.11 %)

Supplementary table 6: Performance of cut-off values for anti-C1qBP and anti-Mitofusin 1.

	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	p-value
Anti-C1qBP	0.064 (0.038 - 0.419)	0.79 (0.693 - 0.873)	0.53 (0.343 - 0.717)	0.83 (0.733 - 0.905)	0.47 (0.298 - 0.649)	0.65 (0.52 - 0.78)	0.021
Anti-Mitofusin 1	0.116 (0.088 - 0.125)	0.61 (0.499 - 0.712)	0.80 (0.614 - 0.923)	0.90 (0.792 - 0.962)	0.41 (0.2860 - 0.551)	0.69 (0.59 - 0.79)	< 0.001

Data are proportions (95% exact confidence interval), except for the cut-off with 95% bootstrap confidence interval. PPV=positive predictive value. NPV=negative predictive value. AUC=Area under the curve. P-value for the test $H_0: AUC=0.50$, $H_1: AUC \neq 0.50$.

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