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1 **Mitochondrial analysis of a Byzantine population reveals the**  
2 **differential impact of multiple historical events in South Anatolia**

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16

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21

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24

25 **ABSTRACT**

26 The archaeological site of Sagalassos is located in Southwest Turkey, in the western  
27 part of the Taurus mountain range. Human occupation of its territory is attested from the  
28 late 12<sup>th</sup> millennium BP up to the 13<sup>th</sup> century AD. By analysing the mtDNA variation  
29 in 85 skeletons from Sagalassos dated to the 11<sup>th</sup>-13<sup>th</sup> century AD, this study attempts to  
30 reconstruct the genetic signature potentially left in this region of Anatolia by the many  
31 civilizations which succeeded one another over the centuries until the mid-Byzantine  
32 period (13<sup>th</sup> century BC).

33 Authentic ancient DNA data were determined from the control region and some SNPs  
34 in the coding region of the mtDNA in 53 individuals. Comparative analyses with up to  
35 157 modern populations allowed us to reconstruct the origin of the mid-Byzantine  
36 people still dwelling in dispersed hamlets in Sagalassos, and to detect the maternal  
37 contribution of their potential ancestors.

38 By integrating the genetic data with historical and archaeological information we were  
39 able to attest in Sagalassos a significant maternal genetic signature of Balkan/Greek  
40 populations, as well as ancient Persians and populations from the Italian peninsula.  
41 Some contribution from the Levant has been also detected, whereas no contribution  
42 from Central Asian population could be ascertained.

43

## 44 **INTRODUCTION**

45 Anatolia, the Asian part of Turkey (Asia Minor) is at the crossroad between the  
46 Balkans, the Near East and the Caucasus, and has acted during major population  
47 expansions as a bridge between Europe, Africa and Asia. Presence of modern humans in  
48 Anatolia is attested since the upper Palaeolithic<sup>1</sup>. This region was also suggested to be a  
49 refuge during the Last Glacial Maximum<sup>2</sup>, at the end of which an improving climate  
50 allowed the coastal hunter-gatherers to venture inland, where they established  
51 temporary camps in search of game and flint<sup>3</sup>. Anatolia is also considered as an area  
52 through which the ‘Neolithic revolution’ spread into Europe<sup>4-8</sup>. However, the major  
53 cultural changes in Anatolia occurred from the Bronze Age, when various civilization  
54 and empires succeeded one another over time and increasing interactions in the eastern  
55 Mediterranean resulted in the development of trading networks, military campaigns and  
56 human colonizations, particularly during the Mycenaean, the Greco-Hellenistic, Roman  
57 Republican and Imperial, and later the Islamic periods<sup>9-13</sup>.

58 From the early Middle Bronze Age onwards city-states and federations of the Hittites  
59 and the Luwians, two originally ethnically and linguistically related groups from  
60 Transcaucasia, started to infiltrate Central to Eastern and Western to Southern Anatolia  
61 respectively<sup>14-15</sup>. Shortly afterward, from the 16<sup>th</sup> millennium BC, Minoan and  
62 Mycenaean colonization took place on the Anatolian west and southwest coast.

63 The Early Iron Age in Central and West Anatolia is characterized by the kingdom of the  
64 Phrygians (10<sup>th</sup> to early 7<sup>th</sup> century BC), followed after its collapse by the Lydians.  
65 From 546 until 334/333 BC Anatolia was incorporated into the Achaemenid Persian  
66 Empire, and later into that of Alexander the Great. Then, from the early 2<sup>nd</sup> century BC  
67 onwards Pisidia was part of the realm of the Romans<sup>16-17</sup>. It has been estimated that in

68 the Late Roman Period, the size of the Anatolian population reached 12 million  
69 individuals<sup>18</sup>.

70 In the mid of the 6<sup>th</sup> century AD the late Roman civilisation and Empire, to which  
71 Anatolia belonged, became part of the Byzantine culture and Empire, Greek in language  
72 and ideology, but Christian in religion (6<sup>th</sup>-15<sup>th</sup> century AD). Under Byzantine rule the  
73 intermittent Arabic invasions took place, before the Seljuk Turks conquered Anatolia in  
74 the late 11<sup>th</sup> century AD. After their crushing by the Mongols, followed by the Beylik  
75 period (Turkish principalities), the Anatolian peninsula was unified again into the  
76 Ottoman Empire, from the 14<sup>th</sup> to the early 20<sup>th</sup> century AD, when it became the major  
77 part of the current Republic of Turkey<sup>9-10,19</sup>.

78 Evidently, the Anatolian population history is the result of complex processes involving  
79 different population groups, the impact and contribution of which to the genetic pool of  
80 the Anatolian population is still relatively unknown. Molecular genetic analyses of  
81 modern human populations provided important clues to reconstruct the past migratory  
82 trajectories in the eastern Mediterranean and Anatolia<sup>2,20-24</sup>. Nevertheless, these results  
83 are limited by the fact that various demographic events could have obscured over time  
84 the evolutionary history inferred from modern populations. In this context, genetic  
85 analysis of ancient populations appears to be a promising way to investigate the origin  
86 of human populations and to validate hypotheses based on modern genetic data and  
87 other fields of study (e.g. historical and archaeological data).

88 In the present study, we describe the mitochondrial DNA (mtDNA) variation of a  
89 Byzantine population (11<sup>th</sup>-13<sup>th</sup> century) from the archaeological site of Sagalassos, in  
90 Southwestern Anatolia. The importance of this population is due to both (i) a  
91 continuous human occupation of its territory from the later 6<sup>th</sup> millennium BP up to the

92 13<sup>th</sup> century AD<sup>25</sup>, and (ii) its very well known historical and archaeological background  
93 resulting from 25 years of research (<http://www.sagalassos.be>) which revealed the  
94 different influences that Sagalassos had undergone since the Bronze Age through its  
95 affiliation to successive empires/civilizations in this region (see Ricaut & Waelkens<sup>26</sup>  
96 for a more detailed review of the historical context of Sagalassos).  
97 The purpose of this study is to estimate the maternal genetic signature potentially left in  
98 the Byzantine population from Sagalassos by the historic events impacting the  
99 Sagalassos region and South-western Anatolia since the Bronze Age, and whether these  
100 events may have been associated to large population movements, to more restricted elite  
101 dominance, or only to cultural diffusion. More particularly, on the basis of the available  
102 historical and archaeological records, our objective is to test whether differential  
103 contributions to Sagalassos population were made by populations from the  
104 Mediterranean area (Balkan and Italian peninsula), the Caucasus, the Near/Middle East  
105 and Central Asia.

106

## 107 **MATERIALS AND METHODS**

### 108 **Samples**

109 The Classical to early Byzantine archaeological site of Sagalassos is located in the  
110 Taurus Mountains of Southwestern Turkey at an altitude of around 1,500 m (Figure 1).  
111 The excavations of this site revealed a city with monumental architecture and several  
112 *necropoles*, which were given up during the 13<sup>th</sup> century AD<sup>11</sup>.

113 The present work analyzed 85 individuals excavated from two different locations: 57  
114 skeletons were recovered from pits in the context of a slope composed of earthquake  
115 debris caused by a seismic event in the late 6<sup>th</sup> century AD, flanking the area of the

116 'Lower Agora', while 28 individuals were found in a neighbouring churchyard, within  
117 the adjoining 'Apollo Klarios Shrine'<sup>11</sup>. The archaeological context pointed to low-  
118 social status burials which followed the Christian tradition (see Supplementary text).

119 All graves have been dated to the 11<sup>th</sup>-13<sup>th</sup> century cal AD by AMS carbon-14 dating of  
120 human bones (Analytic Inc. <http://www.radiocarbon.com>) and/or from the  
121 stratigraphical sequencing coupled with the general historical reconstruction of the site.  
122 DNA extractions were made from more than 169 bone and tooth samples from the 85  
123 skeletons.

124

#### 125 **Contamination avoidance**

126 Genetic analyses were performed in the ancient DNA (aDNA) facilities of the  
127 Laboratory of Forensic Genetics and Molecular Archaeology in Leuven. Standard  
128 contamination precautions as previously described in literature were employed for the  
129 aDNA analysis<sup>27-29</sup>, amongst which: pre- and post-PCR procedures carried out in  
130 physically separated laboratories; restricted and controlled access to the pre-PCR  
131 laboratory; mock-extraction controls in the DNA extractions and amplification  
132 reactions; independent replication of data; analysis of associated fauna remains. More  
133 details about contamination avoidance procedures are reported as Supplementary text.

134

#### 135 **DNA extraction and PCR amplifications**

136 After bone and tooth samples decontamination and pulverization, aliquots of 0.5-1 g  
137 powder were digested in a Proteinase-K lysis buffer and DNA was extracted through  
138 silica-based spin columns<sup>30</sup>. Analysis of the first and the second hypervariable segments  
139 (HVS-I and HVS-II) of the mtDNA control region was accomplished by amplifying and

140 direct sequencing respectively five and two overlapping fragments (ranging from 109 to  
141 166 bp in size), in order to read respectively 357 bp, from nucleotide position (np)  
142 16009 to 16365, and 217 bp, from np 49 to 265. Furthermore, a selection of samples  
143 was used for cloning of the PCR products. This allowed us to confirm the results  
144 obtained by direct sequencing and to have an indication about *post-mortem* DNA  
145 damage in the samples (Supplementary text). Eight diagnostic markers in the mtDNA  
146 coding region were selected and analysed by means of multiplex amplification and  
147 minisequencing reactions in order to confirm and in some instances to resolve the  
148 haplogroup classification. List of primers used and details about the amplification,  
149 sequencing and minisequencing reactions are reported in Supplementary table 1 and  
150 Supplementary text.

151

## 152 **Data analysis**

153 On the basis of the HVS-I/HVS-II haplotype and the SNPs in the mtDNA coding region  
154 each individual was assigned to a haplogroup (hg) according to the latest mtDNA  
155 phylogeny<sup>31</sup> (<http://www.phylotree.org/>). Comparative surveys with mtDNA data of up  
156 to 157 African and Eurasian populations reported in literature (Supplementary tables  
157 2,3,4, Supplementary text) were done in order to detect the genetic signature left by the  
158 populations considered descendant of those which, from historical documents and  
159 archaeological evidence, may have contributed culturally and/or demographically to the  
160 ethnogenesis of the Sagalassos population since the Bronze Age<sup>11,16,32</sup>. Search of  
161 haplotypes shared and analysis of Fst genetic distances based on the HVS-I and HVS-II  
162 variation were carried out in order to infer relationship between Sagalassos and the  
163 comparative populations (ARLEQUIN 3.11 software). The statistical significance of Fst



164 values was estimated by permutation analysis, using 10,000 permutations. The pattern  
165 of genetic differentiation was visualized by MDS (XLstat v.7.5.2) and geographical  
166 interpolation method (Surfer v.8.0, *Golden Software Inc* 2002). Furthermore, a Principal  
167 Component Analysis (PCA) was carried out on the basis of haplogroup frequencies  
168 from 80 Eurasian populations present in literature (Statistica v.8, *StatSoft Inc*). See  
169 Supplementary text for detailed method.

170

## 171 **RESULTS**

172 Even when applying strict precautions, contamination from modern human DNA cannot  
173 yet be totally ruled out<sup>29</sup>, and a strong logical chain of evidence is required to  
174 authenticate aDNA results<sup>33</sup>. In the present study, on the basis of the procedures  
175 followed and the criteria used to assess the reliability of results, we could exclude  
176 contamination with a high level of confidence and attest to the authenticity of our  
177 aDNA data (Supplementary text).

178

### 179 **Genetic diversity and kinship**

180 HVSI and HVSII sequences were determined in 53 out of the total 85 initial  
181 individuals (62%). Analysis of SNPs in the coding region of the mtDNA was done in a  
182 selection of samples and haplogroup affiliation could be ascertained in all the  
183 individuals (Supplementary table 5). Ten haplotypes were shared between two or more  
184 individuals. On the basis of the burial location in the cemetery we assumed a putative  
185 maternal relationship in two couples of individuals buried in relatively close graves  
186 (SA2005 AK41a, SA2005 AK41b2 and SA2005 AK41b, SA2005 AK41c; see  
187 Supplementary text).

188 After excluding the related individuals, the final Sagalassos dataset encompassed 51  
189 individuals, revealing 39 different haplotypes. This accounts for the fairly high  
190 haplotype diversity observed in Sagalassos ( $H=0.989 \pm 0.006$ ).

191 All Sagalassos sequences fall into a set of haplogroups within macrohaplogroups N (X,  
192 W and N1b) and R (R0a, H, V, HV, U, K, J, T) that are characteristic of West  
193 Eurasians. None of the sequences from Sagalassos belong to the East/South Asian  
194 macrohaplogroup M, and sub-Saharan haplogroups (L1, L2, and L3A).

195

### 196 **Population comparisons**

197 To infer phylogenetic relationships between the Sagalassos population and 76 Eurasian  
198 and African populations we performed a non-metric multidimensional scaling (MDS)  
199 analysis based on the  $F_{st}$  distance matrix calculated from the HVS-I/HVS-II haplotypes  
200 (Supplementary table 6). The results of the MDS (figure 2) suggest that geography can  
201 explain the grouping of populations on the two-dimensional plot. Sagalassos is located  
202 very close to the samples from Turkey, Crimea and Iran<sup>34-36</sup> which present a non-  
203 significant differentiation with Sagalassos ( $F_{st}<0.01$ ) (Supplementary table 8) and their  
204 position in the middle of the plot reflects a geographic bridge between Asia and Europe.  
205 Regarding Asian populations, a genetic proximity in the MDS plot of some Uzbek  
206 samples ( $F_{st}<0,01$ ) to Sagalassos has been observed.

207 The maternal gene pool of Sagalassos was then compared to a higher number of  
208 populations (i.e. 157 populations) but from a shorter mtDNA fragment (HVS-I), and the  
209  $F_{st}$  values obtained (Supplementary table 7) have been graphically plotted on a map of  
210 Eurasia and North Africa (Figure 3). Results are consistent with those from the HVS-  
211 I/HVS-II comparisons, showing the genetic proximity of Sagalassos with its

212 neighbouring populations, particularly some Eastern Mediterranean groups  
213 (Turkey/Anatolia), the Balkans (mainland Greece, Bulgaria, Croatia) and Crimea<sup>20,34,37-</sup>  
214 <sup>39</sup>.

215 Overall, it is remarkable that the Fst values point to a proximity of Sagalassos with the  
216 samples from Turkey, Crimea, Iran, Italy (Campania, Puglia), Cyprus and the Balkans  
217 (Bulgaria, Croatia, Greece), which show the lowest non-significant Fst values  
218 (Fst<0.005, Supplementary Table 8).

219 Haplogroup composition in Sagalassos was investigated by means of the PCA  
220 (Supplementary table 9). In the two-dimensional plot (Figure S1), which explains 32%  
221 of the total variation, Sagalassos is located in a marginal position, close to a cluster of  
222 populations from the Caucasus (the Georgian sample being the closest to Sagalassos),  
223 the East Mediterranean (Cyprus and Rhodes), and the samples from the Near/Middle  
224 East. In this pool of populations, contribution of haplogroups U3, N1, U1, and HV to  
225 the first and second PC was found to be particularly high (Figure S2).

226 Twenty HVS-I/HVS-II haplotypes from Sagalassos were found to match modern  
227 sequences (Supplementary table 10 and Supplementary text). In agreement with the  
228 results of the Fst based analyses, we detected relevant haplotype matches with the  
229 populations that historically may have contributed to the make-up of the Sagalassos  
230 mtDNA pool, particularly the Balkan area (e.g. Northern Greece, Macedonia, Bosnia  
231 and Bulgaria), Italy and Iran. Of particular note is the presence of haplotype LA35  
232 exclusively in Macedonia (frequency 0.5%), and AK41a and LA20 exclusively in Italy  
233 (both at 0.25%), as well as the high frequency of AK15a in Iran (15%). Shared  
234 haplotypes were also found in the Near/Middle East (e.g. AK62 and AK56-69\_2, 2.33%  
235 in Saudi Arabia), in particular LA54 found only in Jordan (1%). Finally, two matches

236 with Central Asian samples, one with Tajiks (the haplotype AK15a, assigned to  
237 haplogroup N1b) and one with Uygurs (AK60, assigned to haplogroup T2b) were  
238 detected.

239

## 240 **DISCUSSION**

241 The high rate of successful genetic analyses (i.e. 62% of the total individuals sampled)  
242 indicates the good state of preservation of the aDNA in the Byzantine samples from  
243 Sagalassos. This is likely related to the high altitude of the archaeological site (i.e.,  
244 approximately 1,500 m), which provides suitable thermal conditions for the  
245 preservation of DNA even at the low latitudes of South-West Turkey. Among the  
246 samples resulting in unsuccessful analyses, a large fraction (91%) is from the Lower  
247 Agora site. This site was excavated 10 to 15 years ago, and differently from Apollo  
248 Klarios, skeletons from the Lower Agora were unearthed without the necessary  
249 precautions for avoiding contamination, this confirming how crucial the post-  
250 excavations procedures are for aDNA investigations<sup>40</sup>.

251 Within the context described in the introduction, the mtDNA pool of the Byzantine  
252 population of Sagalassos brings new elements to estimate the genetic signature  
253 associated to the historic/demographic events which took place in this region since the  
254 Bronze Age. The results of our study point to a West-Eurasian composition of the  
255 mtDNA gene pool of the 11<sup>th</sup>-13<sup>th</sup>-century Byzantine population. This genetic pattern is  
256 in agreement with results from previous studies investigating Anatolian/Turkish  
257 populations history and obtained from different biological markers, e.g. proteins, Alu  
258 sequences, mtDNA, Y chromosome, and morphological data<sup>2,20-21,26,34,41-42</sup>.

259 More particularly, a genetic affinity of Sagalassos with Anatolian and Balkan  
260 populations has been revealed by our study (Figure 2, Figure 3, Supplementary tables 8  
261 and 10), suggesting a relatively similar background of the maternal gene pool in these  
262 populations. The affinity with current Anatolian populations, suggests that the same  
263 historic and demographic events that shaped the mtDNA pool of Sagalassos, might have  
264 left a genetic signature in the modern Turkish populations.

265 The genetic proximity with Balkan populations may probably be the result of the long  
266 term interactions between Anatolia and Balkan regions since at the latest the Early Iron  
267 Age, as attested by linguistic, archaeological, mythological and biological studies<sup>9-</sup>  
268 <sup>10,26,32</sup>. This might be a genetic signature of the Phrygians originating from the Balkans,  
269 who filled the political hiatus in Central Anatolia left by the Hittites from the 10<sup>th</sup>  
270 century BC onwards. Yet, it is uncertain whether domination of the Phrygians ever  
271 extended as far as Sagalassos, although Phrygian influences are present in its territory.  
272 Alternatively, the Balkan genetic signature might date back to the 3<sup>rd</sup>-2<sup>nd</sup> century BC,  
273 when Macedonian Seleucid dynasty ruling over Pisidia enlisted many mercenaries in  
274 their armies, this leading to mixed marriages. Particularly in Sagalassos a whole series  
275 of Seleucid colonies populated by Macedonian veterans and Jews were created<sup>43</sup>.

276 Our analyses also detected an affinity between Sagalassos and populations from Iran  
277 and the Italian peninsula. Within the historical context described above, this pattern may  
278 result from historical events which facilitated the installation of Persian and Roman  
279 settlers. When ruling over Sagalassos, the Persian Achaemenids (546-333/332 BC)  
280 enlisted many Pisidians in their armies, but also acquired estates near the city of  
281 Sagalassos and even must have had settlers in the city itself. As for the Italian affinity,  
282 this can easily be explained by the fact that Augustus, after his confiscation of the

283 Kingdom of Galatia, to which Sagalassos had belonged from 39 to 25 BC, established at  
284 least eight colonies of Roman veterans from Italy within a radius of a 100 km around  
285 Sagalassos<sup>16</sup>.

286 A Levantine component has been also detected in Sagalassos, which is likely related to  
287 the high frequencies of haplogroups U3 (5.66%) and N1 (11.32%) (Supplementary table  
288 9, Figures S1 and S2). This result may be explained by an intense trade system that was  
289 established with the Levantine populations from Archaic, over Hellenistic and Roman  
290 Imperial to Early Byzantine time<sup>44</sup>, when Southwest Anatolia formed a kind of cultural  
291 *koinè* in its material culture with Western Cyprus, Pamphylia and the Levant itself. This  
292 is further supported by the proximity of the sample from Cyprus in the PCA (figure S1).

293 As an alternative scenario, it should be mentioned that the introduction of Levantine  
294 mtDNAs may result from the allocation of Pisidian mercenaries by the Macedonian  
295 dynasty of the Ptolemies in the Levantine coasts, where they may have married local  
296 women with whom they eventually returned to their home cities<sup>45</sup>.

297 It is worth noting that as far as the U3 lineages are concerned, the hypothesis of a  
298 Caucasian origin cannot be totally ruled out<sup>6,46</sup>. The presence of this haplogroup in the  
299 Sagalassos territory might represent an ancient genetic legacy of the Indo-European  
300 tribes from Trans-Caucasus which occupied Anatolia at the latest around the beginning  
301 of the 2<sup>nd</sup> millennium BC. In particular, the Hittites and the Luwians settled in Central  
302 and Southern Anatolia, whereby the territory of Sagalassos became part of a Luwian  
303 state<sup>14</sup>.

304 Overall, the analyses that we performed point to the absence of a significant Central  
305 Asian genetic signature in Sagalassos, at least at the maternal side. With this regard, the  
306 apparent affinity detected with some Central Asian populations (e.g. the Uzbeks in

307 figure 2, Turkmen and Kurds in figure S1, Tajiks and Uygurs in Supplementary table  
308 10) likely result from the high frequencies of West Eurasian specific haplogroup in  
309 these samples (up to 42% in Uygurs and Uzbeks<sup>47</sup>). As far as evidenced by the  
310 archaeological data, presence of the Seljuk Turks, a nomadic tribe from Central Asia  
311 which invaded Anatolia in the late 11<sup>th</sup> century AD, is not attested in Sagalassos before  
312 the first half of the 13<sup>th</sup> century AD, when they built a *hamam* and according to Arabic  
313 sources also a caravansary in the neighbour site of Ağlasun<sup>48</sup>. Even if it is not excluded  
314 that they had already occupied part of the former territory of Sagalassos before, the 11<sup>th</sup>  
315 to 13<sup>th</sup> century AD cemetery which is analyzed here shows a clearly homogeneous  
316 Christian population. Remarkably, a 12<sup>th</sup>-13<sup>th</sup> century AD fortified site on the southern  
317 part of Sagalassos was destroyed and erased by the Seljuks in the course of the 13<sup>th</sup>  
318 century AD, making a definitive end to the occupation of the site and showing that the  
319 relations between the two population groups seem to have been hostile.

320 In conclusion, despite the limitations of investigating a single maternally inherited  
321 locus, and the impossibility to perform statistical methods to test genealogical  
322 relationships between past and present populations (within the framework of the  
323 Sagalassos project, research is restricted to the period preceding the capture of  
324 Constantinople by the Ottoman Turks in 1453 AD), our study provides new clues to  
325 pinpoint the impact of historical events, and correspondingly of the related population  
326 groups, on the maternal gene pool of the Byzantine population from Sagalassos.

327 The analysis of the *Fst* genetic distances and the shared haplotypes highlighted a genetic  
328 proximity of Sagalassos with populations from the Balkan and Italian peninsulas, and  
329 the Levant. Notably, no signature from Central Asia has been detected, while an ancient  
330 genetic legacy of the Indo-European tribes from Trans-Caucasus which occupied

331 Anatolia at the beginning of the 2<sup>nd</sup> millennium BC cannot be excluded. Whether this  
332 relatively good agreement between female history (mtDNA) and  
333 historical/archaeological records is also found in the paternal lineages, will have to be  
334 explored through investigation of Y chromosome markers.

335

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343 The mtDNA sequences reported in this paper have been deposited in GenBank  
344 (Accession Numbers: HQ007946–HQ008051).

345

## 346 **CONFLICT OF INTEREST**

347 The authors declare no conflict of interest.

348

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478

479 Supplementary Information accompanies the paper on European Journal of Human  
480 Genetics website (<http://www.nature.com/ejhg>)

481

482 **TITLES AND LEGENDS TO FIGURES**

483 **Figure 1.** Geographic location of Sagalassos.

484 **Figure 2.** Two-dimensional MDS plot of pairwise  $F_{st}$  values from HVS-I/HVS-II  
485 showing relationships among the 77 populations from Eurasia and Africa. The stress  
486 value for the MDS plot is 0.204. Yellow diamond represents the Byzantine Sagalassos  
487 population. Population codes and the matrix of  $F_{st}$  values are reported in Supplementary  
488 tables 3 and 6.

489 **Figure 3.** Spatial distribution of the  $F_{st}$  values calculated from the pairwise comparison  
490 of the Sagalassos mtDNAs (HVS-I) with those of 157 populations from Eurasia and  
491 North Africa (Supplementary table 2).  $F_{st}$  values as reported in the Supplementary table  
492 7 follow the red colour scale. Black crosses indicate the geographic location of the  
493 populations surveyed.

494

495 **SUPPLEMENTARY INFORMATION**

496 **Supplementary tables** (Ottoni\_et\_al\_Supplementary\_tables.xls):

- 497 - Supplementary table 1. List of primers used in this study and product size.  
498 - Supplementary table 2. References of the populations compiled in the HVS-I database.  
499 - Supplementary table 3. References of the populations compiled in the HVS-I/HVS-II  
500 database.  
501 - Supplementary table 4. References of the populations used in the PCA analysis  
502 - Supplementary table 5. Polymorphic positions observed in the HVS-I, HVS-II and the  
503 coding region of the mtDNA, and haplogroups affiliation in 53 ancient individuals from  
504 Sagalassos (11th - 13th centuries AD)

505 - Supplementary table 6. Matrix of Fst genetic distances between the 77 populations  
506 included in the HVS-I/HVS-II database.

507 - Supplementary table 7. Matrix of Fst genetic distances between the 158 populations  
508 included in the HVS-I database.

509 - Supplementary table 8. Pairwise Fst genetic distances between Sagalassos and the  
510 populations included in the HVS-I/HVS-II (a) HVS-I (b) databases.

511 - Supplementary table 9. Relative frequencies (%) of mtDNA haplogroups and  
512 subhaplogroups in the 80 populations included in the PCA.

513 - Supplementary table 10. Frequency (%) of 20 HVS-I/HVS-II mtDNAs from  
514 Sagalassos within modern populations.

515 - Supplementary table 11. Variable positions in the cloned HVS-I/HVS-II sequences  
516 from five Sagalassos individuals.

517 - Supplementary table 12. MtDNA HVS-I (16009-16365) and HVS-II (49-265)  
518 haplotypes of all persons involved in processing samples.

519

520 **Supplementary text** (Ottoni\_et\_al\_Supplementary\_text.doc): detailed description of  
521 experimental procedures and data analyses carried out in this study.

522

523 **Supplementary figure 1** (Ottoni\_et\_al\_Supplementary\_figure\_S1.tif): PCA based on  
524 haplogroup frequencies from 80 Eurasian populations, two-dimensional plot. Population  
525 codes and the haplogroup frequencies are reported in Supplementary table 4 and 9.

526



527 **Supplementary figure 2** (Ottoni\_et\_al\_Supplementary\_figure\_S2.tif): PCA based on  
528 haplogroup frequencies from 80 Eurasian populations, contribution of each haplogroup  
529 to the first and the second PC.  
530



stress : 0,206

- △ Asia
- ◆ East Mediterranean
- Middle East
- North Africa
- ◇ North Europe
- South Europe



