Supporting Information

Haak *et al.* 10.1073/pnas.0807592105

SI Text

Palaeopathological Evidence of Traumatic Injuries on the Skeletons. The osteological assessment and interpretation of trauma evident in the bones was made according to established guidelines (1, 2). All recordings of traumatic lesions were initially made by the same observer and confirmed by another observer. The examination of the bones was carried out, with preservation of the overall burial arrangement being given priority. Selected elements were lifted from their resting place while restoration work was in progress, thereby minimizing potential damage to the specimens. These bones have been replaced in their original position.

Grave 90. ***Individual 5.*** A flint arrowhead is firmly lodged in the ventral part of the fourth lumbar vertebra of this woman (Fig. 4). On CT-scans and in the dry bone, no bony reaction is evident in the area surrounding the stone tip, indicating a wound that had been received *perimortem*. A second arrowhead was found between the left ribs and might be evidence of another wound. No bone damage has been recognized, but poor preservation of the bones in this area precludes any further assessment.

Grave 93. ***Individual 11.*** Several injuries could be detected in this man. He has healed fractures of the left radius and left scaphoid; the latter suffered a transverse fracture of the proximal part. Injuries received *perimortem* are found on the right forearm and hand. Radius, ulna. and several metacarpals suffered oblique fractures.

Grave 98. ***Individual 7.*** This woman received two *perimortem* blows to the upper right portion of the cranial vault (Fig. S4). The first wound is located on the right parietal, radiating fractures follow the contours of the vault, and internal beveling is present. The second wound is located anterior to the first, also displaying characteristic internal beveling. A radiating fracture of this injury terminates in the area of the first wound; therefore, the blow to the posterior part of the parietal fell earlier.

Grave 99. ***Individual 2.*** This child has a small and healed depressed fracture of the frontal bone.
Individual 3. This man displays several traumatic injuries along the left arm, some *perimortem*, others clearly healed. The humerus shows signs of *myositis ossificans* (3); the distal radius bears traces of an old fracture with slight disalignment. The third and final healed injury involves the proximal interphalangeal joint of the fourth digit, where bony ankylosis has fused the two bones. All these injuries show a similar degree of healing; possibly they were received at the same occasion. *Perimortem* trauma involves both hands, which suffered oblique and spiral fractures to the metacarpals and phalanges. Damage to the anterior part of the skull might also be because of violent injuries, but this could not be securely separated from taphonomic changes resulting from soil pressure, which is clearly evident in the skulls of the children.
Individual 4. This child suffered a penetrating and *perimortem* wound to the back of his skull. Concentric and bending fractures are evident in the area surrounding the impact site. Small bone fragments, projecting into the interior of the skull are still visible.

Detailed Description and Assessment of Molecular Genetic Results

Mitochondrial DNA Haplogroup Assignments. We reproducibly obtained HVS I sequences for 9 out of 12 individuals investigated (75% success rate). These nine mtDNA sequences could be unambiguously assigned to haplogroups H, I, K, U, and X common in populations of Europe and West Asia (Table S4). Six different haplotype sequences were found among these nine individuals, whereby three individuals of grave 99 shared one distinct haplotype and another haplotype was found identical in two children of grave 98. No haplotype was found in more than one grave. None of the nine haplotypes could be found among workers involved in sampling and preparation procedures at the museum, as well as among people involved in laboratory work at the University of Mainz. The remaining three individuals revealed either no successful amplifications of the minimum number of all four products per extraction (ind. 6) or no satisfying replication of results according to our authentication criteria, and therefore no unambiguous haplotype sequence results (inds. 11 and 12).

Autosomal and Y Chromosome STR Analysis. The analysis of autosomal and Y chromosomal STRs revealed a pattern typical of ancient (nuclear) DNA. This is characterized on the one hand by a decreasing rate of successful amplification inversely correlated with single product lengths of considered loci: that is, STR systems as D13S317, FGA, D7S820, and D18S51, with product lengths of 206 bp and up to 341 bp could not be amplified at all in the samples we studied. On the other hand, we observed a considerable amount of allelic as well as loci dropout among the shorter STR systems increasing in length. The results of every single multiplex PCR are listed in Table S1; genotypes of workers involved in this study are shown in Table S5. No matching genotypes were found between both sample sets. Concerning degraded samples, consensus allele calling was carried out when an allele could be observed in at least three independent PCR from two independent extractions. A varying completion of consensus genotypes of four to five loci could be retrieved from individuals 1, 3, and 9, all of which were adult and comparatively better preserved individuals. The remaining individuals showed a lesser rate of successful amplifications, mostly restricted to PCR from extraction A. Thus, we could observe a considerable bias in successful multiplex amplification toward extraction A (no treatment of the sample with chemical agents) in all individuals. The same is true for Y chromosomal STR data carried out solely on the putatively male individuals of grave 99, because of their most promising DNA preservation and the indication of familiar kinship resulting from preceding mtDNA and autosomal STR data. Again, Y chromosome data could only be gained from extractions A of all three individuals, with a comparable pattern of variable completion of their Y chromosome haplotypes with a clear bias toward shorter loci (Table S2).

Despite the limited preservation of nuclear DNA, at least a molecular sex determination was successful for 9 of the 12 individuals. The gonosomal locus Amelogenin could be reproducibly amplified because of its short fragment length of 107/113 bp (see Table S1). The results of morphological, archaeological, and molecular genetic sex typing were in almost complete concordance which each other (Table 1), except for individual 2, who was oriented facing his mother. Taken together, we were able to determine the sex of four skeletons of children and juveniles (inds. 2, 4, 9, and 10) for which morphological indicators of sex were absent.

Combined mtDNA and Nuclear Typing Results. ***Grave 99.*** The four individuals of this grave (Fig. 2) revealed two different mtDNA

haplotype sequences. Individual 1, who is according to the combined sex determination and anthropological data an adult woman, and the two children (inds. 2 and 4) correspond in four substitutions of their HVS I sequences (see Table S4). The combination of polymorphisms 16093C, 16224C, 16311C, and 16319A is characteristic of mtDNA haplotype K1b (4). So far this haplotype has uniquely been reported in two modern Shugnans of Tadzhikistan (5). Individual 3, a 40- to 60-year-old man, shows a differing mtDNA haplotype consisting of polymorphisms 16189C, 16192T, and 16270T, characteristic of haplogroup U5b* (6, 7). For both adult individuals (inds. 1 and 3), we were also able to reproducibly obtain consensus genotypes of 4 out of 10 STR loci plus unambiguous results from the Amelogenin locus. The children (inds. 2 and 4) revealed alleles of four and five loci, respectively, but were almost completely restricted to data resulting from several PCR of extraction A. Although these results could not be replicated from an independent extraction, the retrieved alleles of both children provide strong hints toward a familiar relationship, as a comparison of the children's alleles with their putative mother and father shows unambiguous matches among their alleles (see Table S1). In contrast, no allele was detected among the children that would rule out the adults' parenthood. Because of this fact and to confirm or authenticate our results via different marker systems rather than an additional extraction (which was not possible due to the advanced state of museum preparation work), we carried out further analysis on the male individuals of this grave (inds. 2, 3, and 4) using the AmpF*l*STR Yfiler kit. We were able to retrieve consensus haplotypes from a minimum of 3 (ind. 4), 6 (ind. 2), and 14 loci (ind. 3) included in the Y-STR kit (see Table S2). Although highly incomplete, all individuals showed identical alleles concerning their comparable loci, further raising the probability of fatherhood of individual 3. Under the assumption that the latter is likely, we carried out a Web-based search in the YHRD-Y Chromosome Haplotype Reference Database (8) for the consensus haplotype derived from all nine independent typings. The consensus haplotype of the three individuals (based on most complete profile) gave two exact matches in in an European population sample of 11,213 haplotypes in a set of 100 populations (as of July 2008, Release "23" from 2008–01-15 14:44:25): one individual from Poland (1/939 from Gdansk) and one from Russia (1/48 from Tambov). The haplotype frequency in a Eurasian/European/Eastern European metapopulation calculated with the minimum amount of loci (i.e., DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS393, and DYS385; excluding DYS392) was 1.020494×10^{-3}. Because of the locus dropout of DYS392, this estimate is considered as most conservative. The Y haplotype was predicted using the Web-based program Haplotype Predictor (9). The three individuals of grave 99 belong to haplotype R1a, with a probability of 100% based on the Y-STR profile of individual 3 (10). To confirm haplogroup status, we further amplified an 85-bp fragment covering the Y-SNP marker SRY10831.2 characteristic for R1a (11). Primer sequences are given in Table S6. Sequences and sequenced clones from independent extract of all three individuals show the specific G>A transition identifying R1a (Fig. S5). Haplotype R1a is common in modern-day Eurasian populations, with frequencies reaching up to 68% in Central Asia (12). To summarize the genetic analysis, the combination of mtDNA, autosomal and Y chromosome data—although the nuclear STR DNA results of both children are not reproducible from a second extraction (because of obvious technical and chemical reasons, see below)—clearly points toward a family grave with a high probability.

Grave 98. For three out of four individuals (Fig. 3) of this grave, two unambiguous mtDNA haplotype sequences could be retrieved (ind. 8 has been omitted from the analysis). Both juvenile individuals 9 and 10 shared the same haplotype sequence at HVS I (16189C, 16223T, and 16278T) characteristic of haplogroup X (6, 13). We further analyzed np 35–396 bracketing HVS II of both younger individuals 9 and 10, as haplogroup X is split in two subhaplogroups (X1 and X2), which can be assigned by polymorphisms on HVS II. Both individuals were found to share another six identical transitions in and around the HVS II (00073G, 00153G, 00195C, 00225A, 00226C, 00263G). Polymorphisms at nucleotide position 00153, 16189, and 16278 were reported to be characteristic of haplogroup X in general, whereas position 00195C is capable of distinguishing X2 from X1 (13). At present, three exact matches were found among individuals from Iran, Syria, and Estonia showing HVS I and HVS II patterns identical with the two Eulau individuals, but differing from each other by further coding region polymorphisms. By contrast, individual 7—an adult women and according to the constellation of bodies in grave 98, the putative mother of the children—was clearly identified to carry a different mtDNA lineage by showing polymorphisms 16093C and 16221T, common in one of the emerging branches of haplogroup H (14). The results of several AmpF*l*STR Profiler Plus attempts demonstrate that nuclear DNA is not preserved well enough to provide information beyond the included sex determination. Reduced to the latter, the molecular sexing showed adult individual 7 and individual 10 to be female, and individual 9 to be male. These results are in concordance with anthropological data, which is available for the adult female (ind. 7) and archaeological data concerning the putative sex determined by grave goods or orientation. Based solely on the limited molecular data, we conclude that both subadult individuals 9 and 10 share a maternal lineage, which, because of its rareness, points to a closer rather than a wider familiar relationship (i.e., could indeed have been siblings, cousins, or other such close relations). Furthermore, we state on the basis of unambiguous and reproducible mtDNA data (although not confirmed by nuclear DNA data) that adult individual 7 is not directly maternally related to both children: that is, is to a high degree of probability not their biological mother.

Grave 93. Among the three individuals of this grave (Fig. S1), only one showed reproducible mtDNA results. The HVS I sequence of 4- to 5-year-old individual 13 revealed positions 16145A, 16224C, and 16311C that could be assigned to a branch of haplogroup K, named K1a2 (4). No nuclear data could be retrieved for this individual. Based on the limited DNA results of this grave, no further assumptions on genetic relationships can be made, except that this individual does not share its mtDNA type with any other individual from the remaining multiple burials.

Grave 90. This grave contained a double burial of an adult woman (ind. 5) and a child (ind. 6) (Fig. S2). Because of the state of skeletal preservation, DNA results could only be retrieved from samples of the adult skeleton. HVS I sequence showed polymorphic sites 16129A, 16223T, and 16391A characteristic of haplogroup I. This haplotype sequence displays the ancestral node of haplogroup I, which is present in Europe at a frequency of ≈2% (6, 15). STR profiling of individual 5 revealed sporadic amplification of shorter STR loci, but not sufficient for further analysis, so that only molecular sex via Amelogenin could be reproducibly typed, which is in concordance with morphological results. Further information based on aDNA data cannot be provided because of the limited DNA preservation.

Authenticity of aDNA Results. The key issue in studying ancient human DNA is the potential risk of contamination with modern human DNA molecules. Thus, recommended criteria for authenticity of aDNA data in general were established (16–24). Truly, not all of these criteria were introduced to address the specific issues of ancient human DNA work, as several publications already noted (25–27). Moreover, under strict scientific laws, authenticity of human aDNA cannot be proven at all (17).

Therefore, results of human aDNA work require a strong logical support of several independent (and not necessarily experimental) observations that, as a chain of evidence, allow assessing the probability of authenticity (24). This is especially true for interpreting genealogies or reconstructing familiar relationships from ancient human DNA results. Here, the situation is even more difficult, as related individuals do not differ in their uniparental markers. Therefore, any obvious kinship based on the identical mitochondrial or Y chromosome haplotype data could also be interpreted as the result of cross-contamination of the samples under study. Because of the fact that in the majority of cases nuclear DNA is not detectable anymore, so far only very few studies have addressed kinship (28–32). Consequently, being aware of the fact that human contamination cannot be ruled out entirely, and based on the aDNA criteria given above, we provide and discuss the following points, which in sum make us believe to have received authentic aDNA:

(*i*) Because of the archeological situation of the finds, a genetic relationship was reasonable to assume *a priori*. Therefore, all samples were analyzed anonymously and in random order to exclude any predetermination of results.

(*ii*) All mtDNA sequences were reconstructed from at least eight cloned PCR products of two independent extracts from two anatomically remote regions. Up to six clones were sequenced per PCR products.

(*iii*) To monitor intra-laboratory contamination, empty controls were carried out at a ratio of 1:10 throughout each extraction and 1:5 in each PCR.

(*iv*) Four overlapping amplicons enable us to track down contiguous sequences, which make phylogenetic sense. Additionally, it also enables us to exclude mosaics patterns (33, 34).

(*v*) Sequences of all PCR products show *postmortem* DNA damage-derived substitutions, as it is characteristic for ancient DNA (35–37). The majority of these were observed in positions not characteristic of human haplotypes and none of these substitutions were either reproducible or consistent.

(*vi*) The occurrence of many distinctively damaged sequences can serve as semiquantifying indicator of DNA content of the samples, suggesting that every single sequence variation stems from a single target molecule (disregarding Taq polymerase errors). Because of the samples postexcavational history, any putative contamination should be rather recent and is not expected to have gathered DNA-damaged sites (38). Under this assumption, we believe that the amount of preserved DNA molecules of most samples was sufficient to outnumber any putative contaminant ones during amplification.

(*vii*) Among few of the Eulau individuals, some cloned sequences were observed that differed from the haplotype and exhibited characteristic mutations of human mtDNA diversity. These clones were interpreted as background contamination, as they appeared randomly as single or few sequences, which occurred in one PCR from one extraction and could never be replicated. In total, only 12.5% of all cloned sequences ($n = 518$) could be identified as contaminating sequences, and 24% thereof could be ascribed to a putative source and were therefore omitted. We admit that it is impossible to completely rule out the systematic contamination of a single skeleton. But in this case, the probability can be considered as rather theoretical in the frame of the experimental design, which is based on a scheme of several independent replications.

(*viii*) Any systematic contamination of one or more skeletons could be excluded with a very high level of confidence, given the fact that all workers involved in excavation and further preparation of the samples are known to us, and were also sampled and typed after having received their informed consent. None of the haplo- and genotypes of the Eulau individuals were identical to one of the analyzed archaeologists, anthropologists, and laboratory personnel involved in the investigations (see Tables S1, S4, and S5). Furthermore, the results did not correspond with other samples that had been analyzed in earlier projects or persons who had worked in the laboratory in the past. Therefore, systematic (as well as sporadic) intra-laboratory contamination can be ruled out.

(*ix*) The detection of mtDNA haplotypes X2 and K1b, both being very rare in modern-day European populations, is unlikely to be based on the occurrence of independent contamination events.

(*x*) The results of morphological and genetic sex typing were in accordance with each other (see Table 1).

(*xi*) The combined results of molecular genetic analyses presented revealed a consistent pattern, which reasonably reflects archaeological and anthropological data. Moreover, the analysis of aDNA followed the expected patterns of appropriate molecular behavior, albeit on a high level. This means that little or no results (mtDNA) could be achieved from the fragile bones of the newborns (inds. 6, 12, and 13), whereas the bones and teeth of the best-preserved bones even allowed analysis of nuclear DNA. Although the assessment is subjective, the correlation of the macroscopic state of preservation and the potential of DNA amplification is evident. The different treatment of the bone material is considered to be a plausible explanation for the differential behavior of DNA from extraction samples A, B, and C, respectively. All samples A were taken directly at the excavation: at this stage, all remains were untreated. Samples B and C were taken some months after the excavation. Because of a planned museum exhibition, the graves were recovered *en bloc* and prepared for presentation by using a consolidant (Archaeofix) and Acetone. It seems reasonable to assume that these chemical solvents reduced the DNA content or inhibited the PCR. Nevertheless, the amount and quality of DNA extracted from samples B and C was still sufficient for successful amplification of mitochondrial DNA.

The Assessment of Biological Kinship. The verification of biological kinship based on mitochondrial data are not sufficient by forensic standards. Usually, a complete autosomal genotyping profile of both children and both putative parents is needed as a minimum requirement for a valid determination of biological siblings, especially with children of the same sex. Therefore, the confirmation of any brother-sister relationship solely based on mtDNA data are not evident *per se*. Nevertheless, depending on specific haplotype and given a certain degree of haplotyping resolution, some suggestion on biological kinship based on mtDNA can be made. In Europe, haplogroup X reaches a frequency of only around 1% (6, 39). In a more detailed study by Reidla *et al.,* further subhaplogroups of X were defined (13). In a sample-set of 21,682 European, Asian, and North-African haplotypes, 175 individuals exhibited a haplotype of X (0.8%). As mentioned above, only 3 out of 175 X-haplotype sequences were observed identical to the HVS I and HVS II substitutions of individuals 9 and 10 (grave 98). The latter corresponds to a haplotype frequency of 0.014% in modern samples. As a result of this, we postulate that it would be highly unlikely observing two individuals in one grave sharing the identical rare haplotype and not being closely related.

Further indications for genetic relationships were found in grave 99. By typing the mtDNA HVS I region, we observed the same haplotype (K1b) for the woman (ind. 1) and both infants (inds. 2 and 4). Individual 3, the putative father, showed a different maternal lineage (U5b*). Therefore, the mtDNA haplotype constellation itself is suggesting a family, confirming the hypothesis from archaeological and anthropological examinations. Today, haplogroup K has a frequency of around 6% in Europe (6, 39). The identical sequence haplotype of individuals 1, 2, and 4 has previously been observed (4, 5). In the study of Behar *et al.* (4), a global dataset of 13,359 samples were used for

the reconstruction of a phylogenetic tree of haplogroup K, including the dataset of Quintana-Murci *et al.* (5). Of these samples, 789 showed a K haplotype (5.9%) and only 2 of them were identical to the mtDNA sequences of the individuals from grave 99. This corresponds to a frequency of 0.015%. Again, this specific haplotype is very rare within the haplogroup K.

However, we are aware of the fact that the modern distribution might not reflect the haplotype distribution during the Late Neolithic. The mitochondrial haplogroup distribution of a population is likely to have changed throughout time by factors like genetic drift or events like migration or genetic palimpsest (40). As we recently showed, the frequencies of haplogroups could have been significantly different between Neolithic and present populations (41), although a similar study (from a different region in Europe) provides evidence of genetic continuity throughout the millennia (42). In any case, the calculated frequencies of the Eulau haplotypes should be treated with caution based of the comparison with modern mtDNA data. Nevertheless, we conclude that the multiple occurrences of haplogroup X2 and K1b in the Eulau burials can be interpreted as biological relationship rather than occurring by chance.

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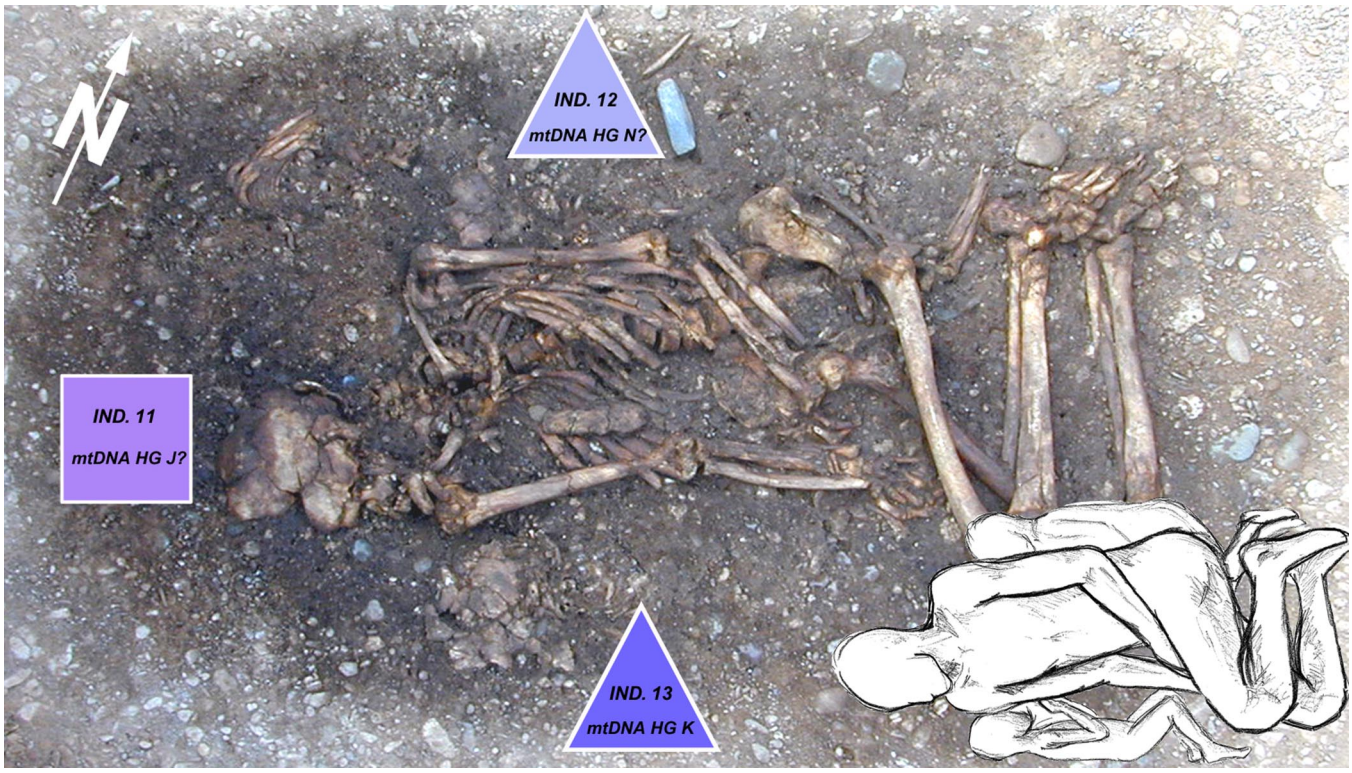


Fig. S1. Grave 93. Mitochondrial (maternal) lineages are shown as different color fillings of the symbols, with squares denoting male and triangles indifferent individuals. When no mtDNA results could be received, the symbol is shown in gray (HG, Haplogroup; Ind., Individual).

Fig. S2. Grave 90. Mitochondrial (maternal) lineages are shown as different color fillings of the symbols, with a circle denoting a female and a triangle an indifferent individual. When no genetic results could be received, the symbol is shown in gray (HG, Haplogroup; Ind., Individual).

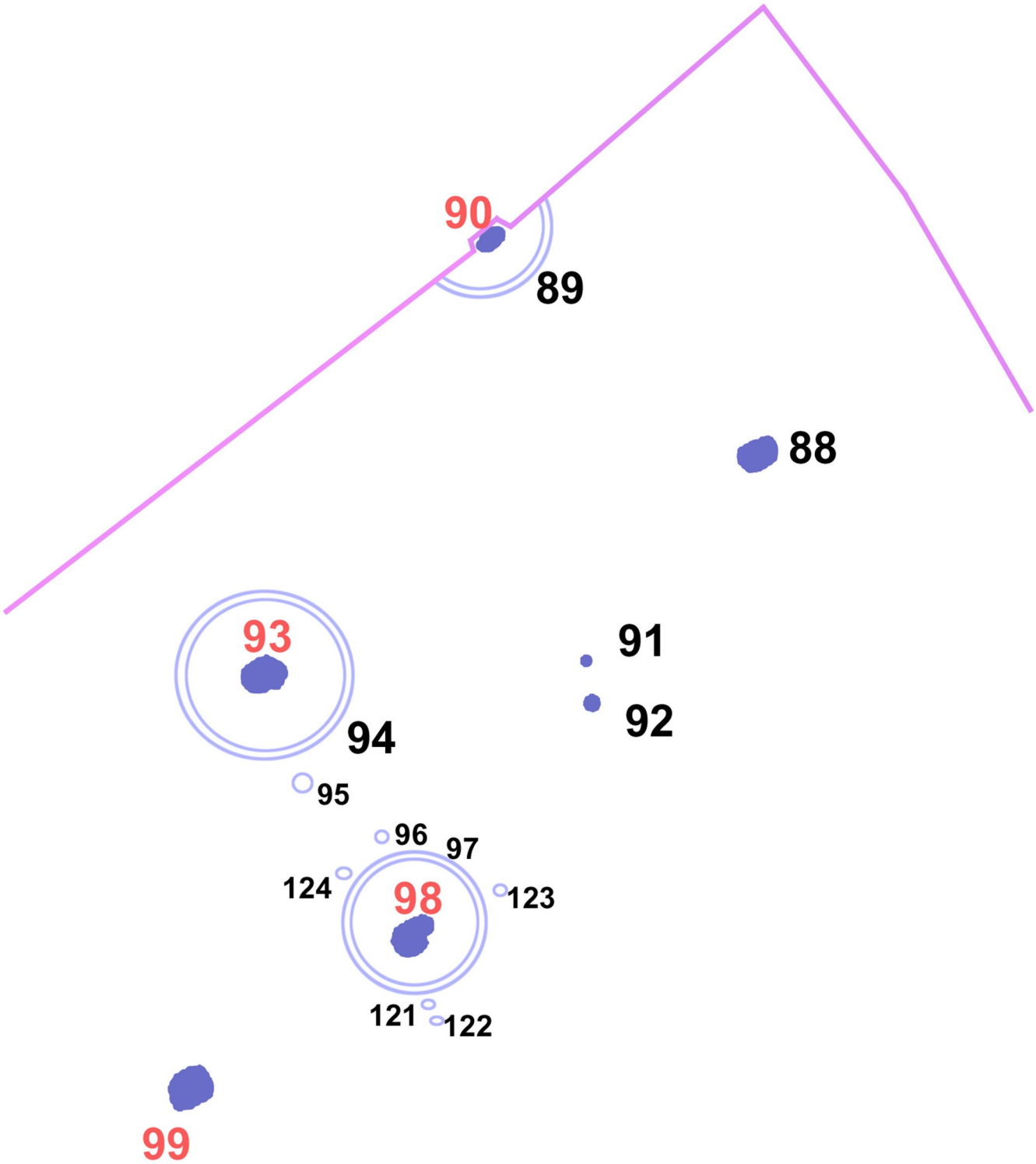
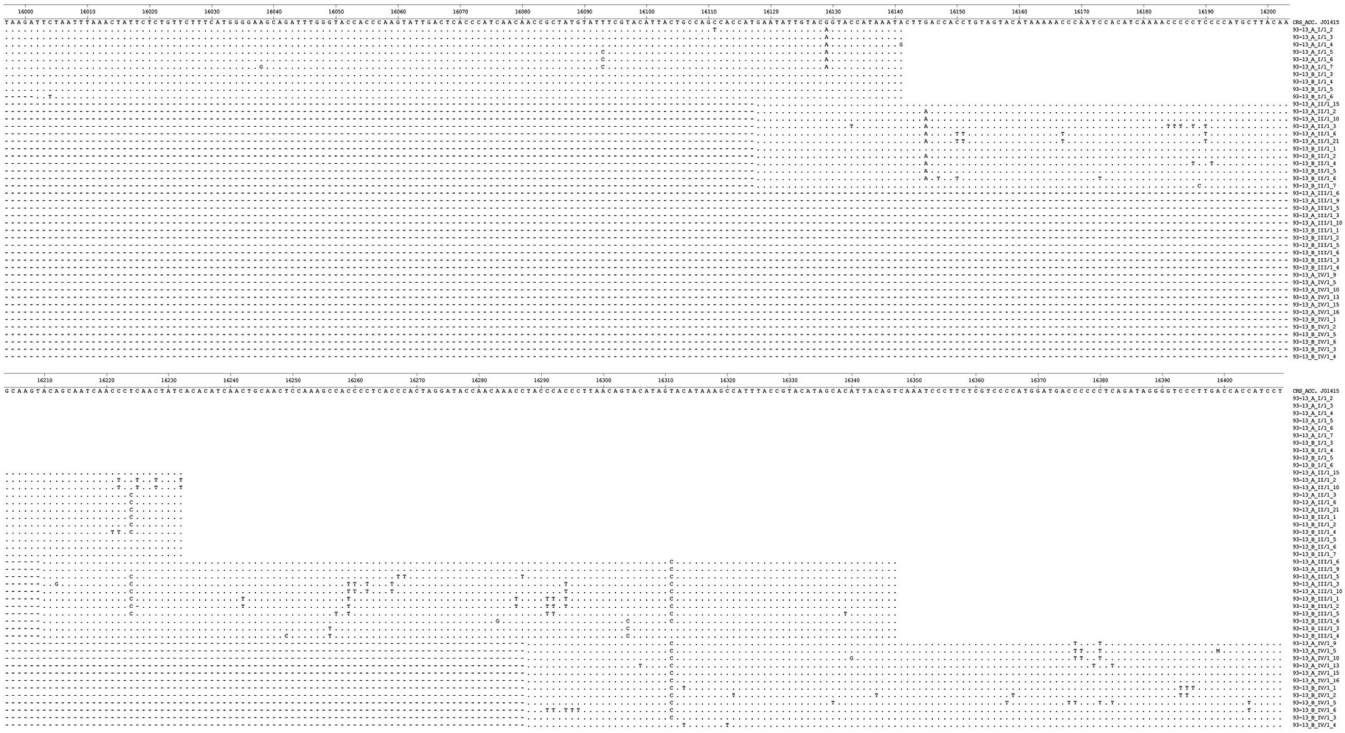


Fig. S3. Alignment of the four multiple burials from Eulau (*red numbers*) in immediate vicinity. Graves 90, 93, and 98 had been surrounded by ring ditches.

Fig. S4. Two blows on the cranial vault of individual 7 of grave 98.

Fig. S5. Sequence alignments for HVS I, HVS II, and SRY10831.2 of the Eulau samples. Names for the sequenced clones are given as follows: SAMPLENAME_Extraction as letters_primer pair as Roman numbers/PCR number_number of clone.



HVS I Ind. 93-13

2

Fig. S5. (continued)

HVS I Ind. 98-7

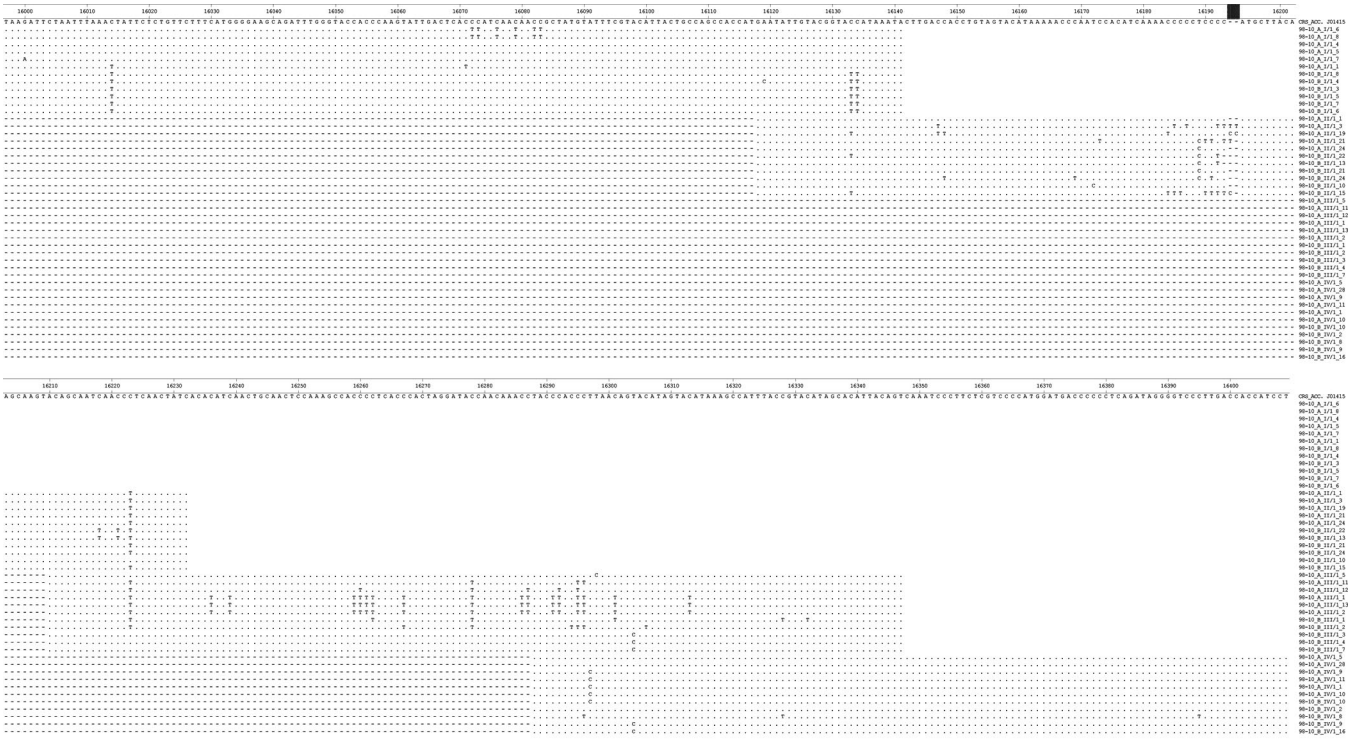
3

Fig. S5. (continued)

HVS I Ind. 98-9

4

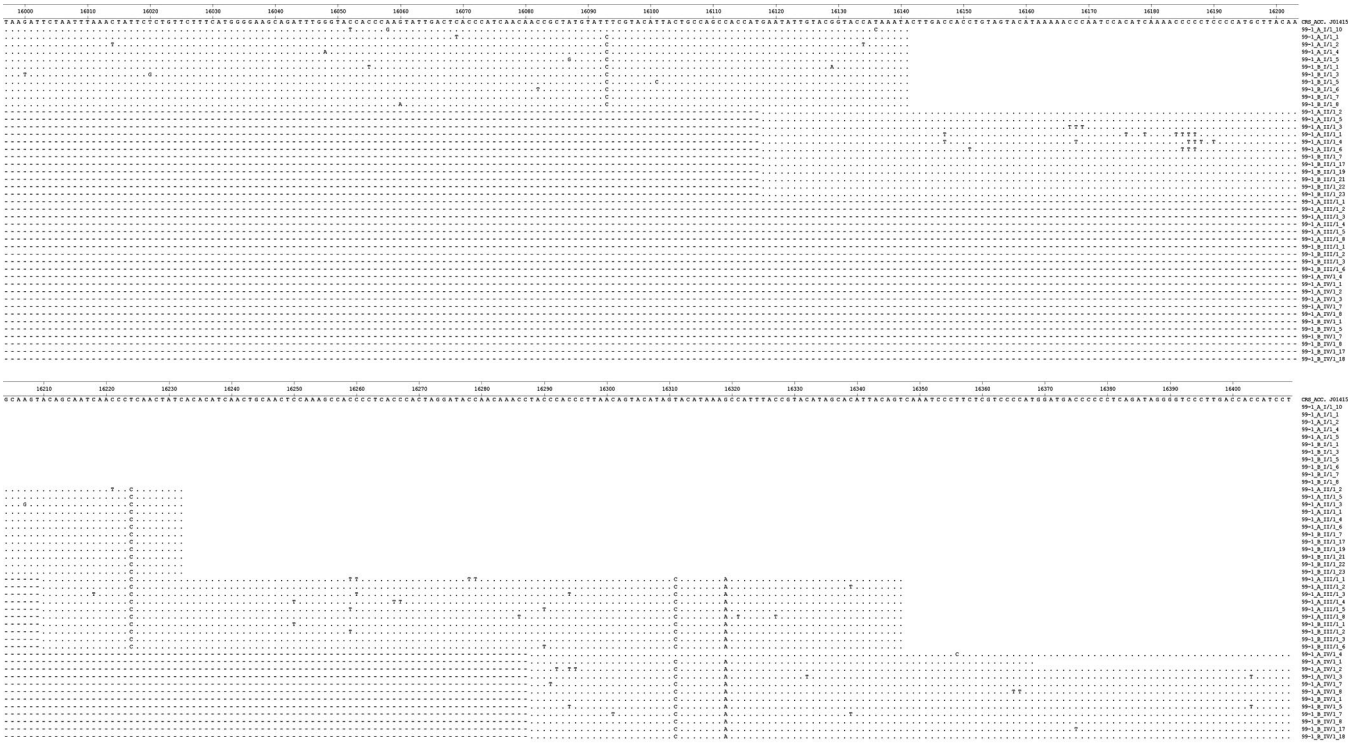
Fig. S5. (continued)



HVS I Ind. 98-10

5

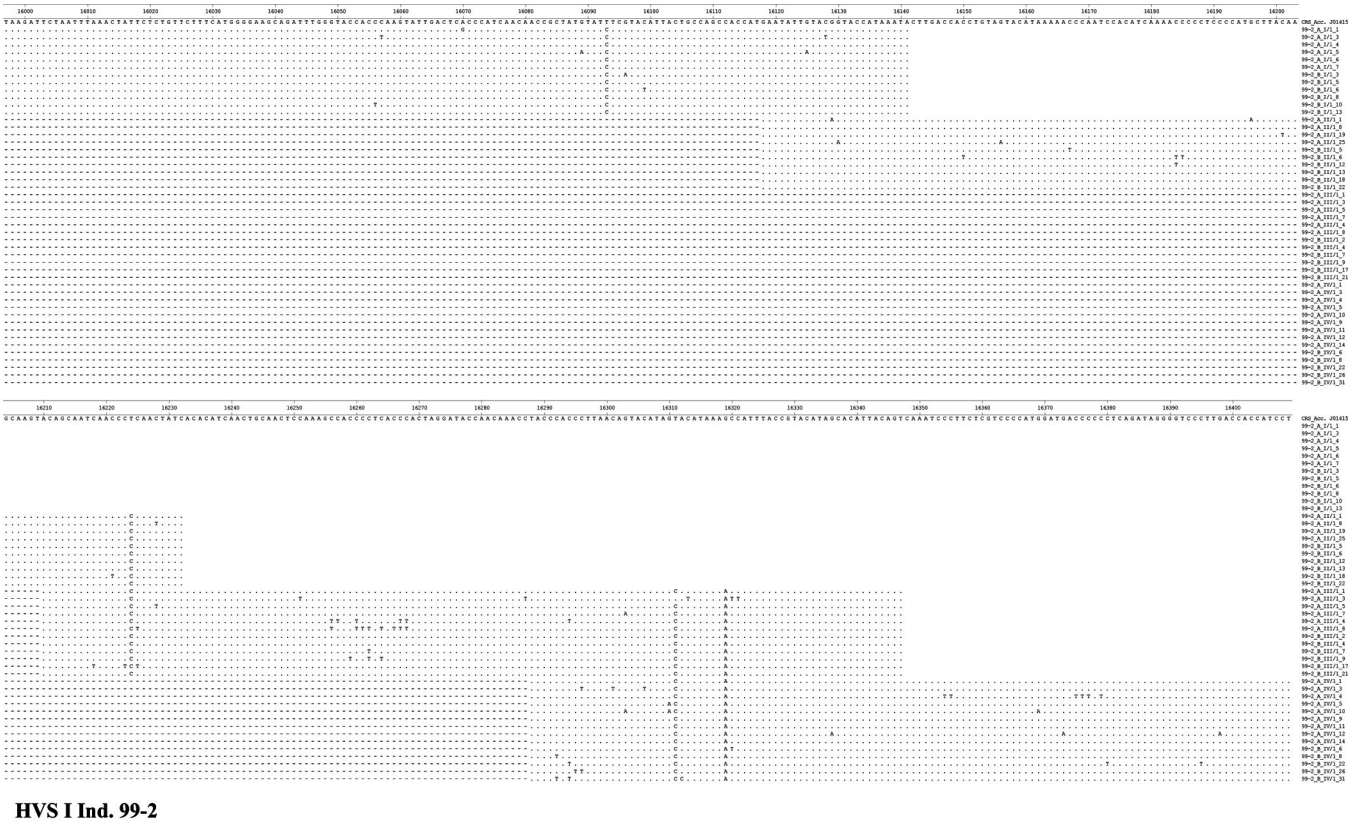
Fig. S5. (continued)



HVS I Ind. 99-1

6

Fig. S5. (continued)



7

Fig. S5. (continued)

HVS I Ind. 99-3

8

Fig. S5. (continued)

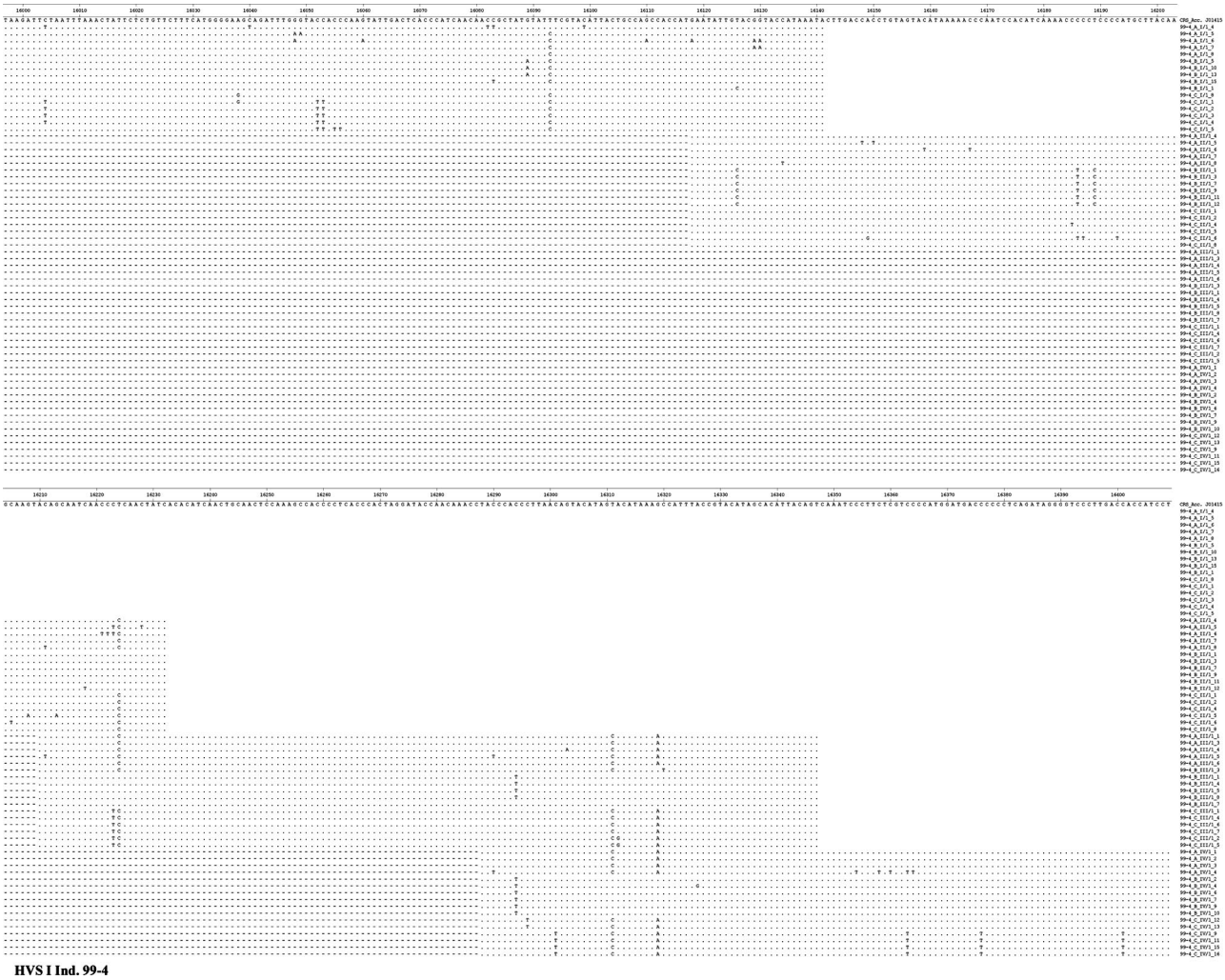
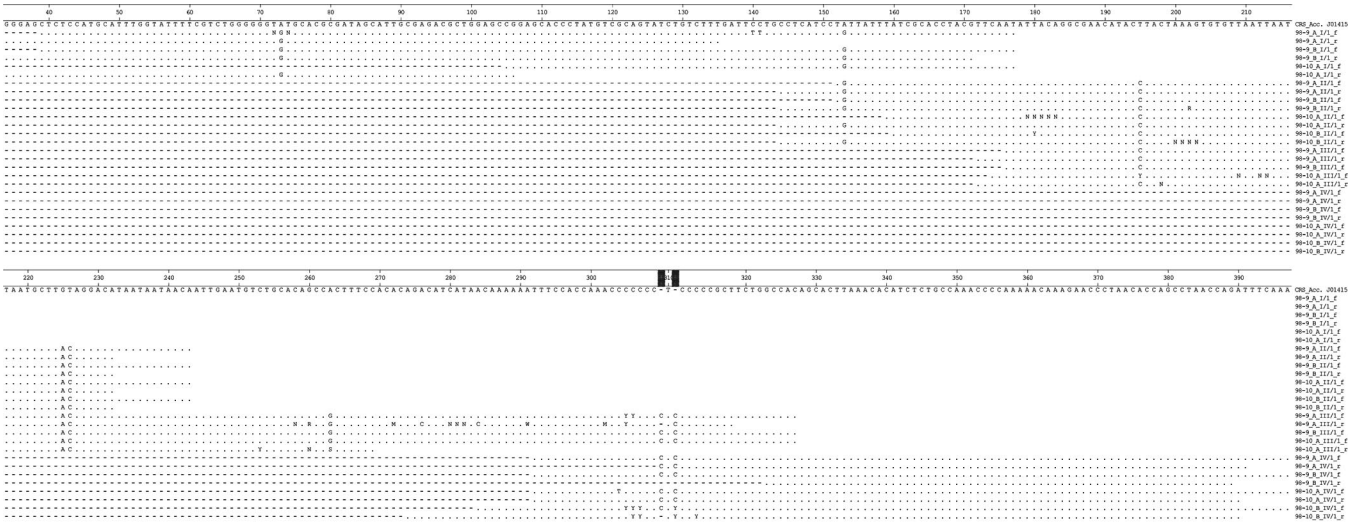


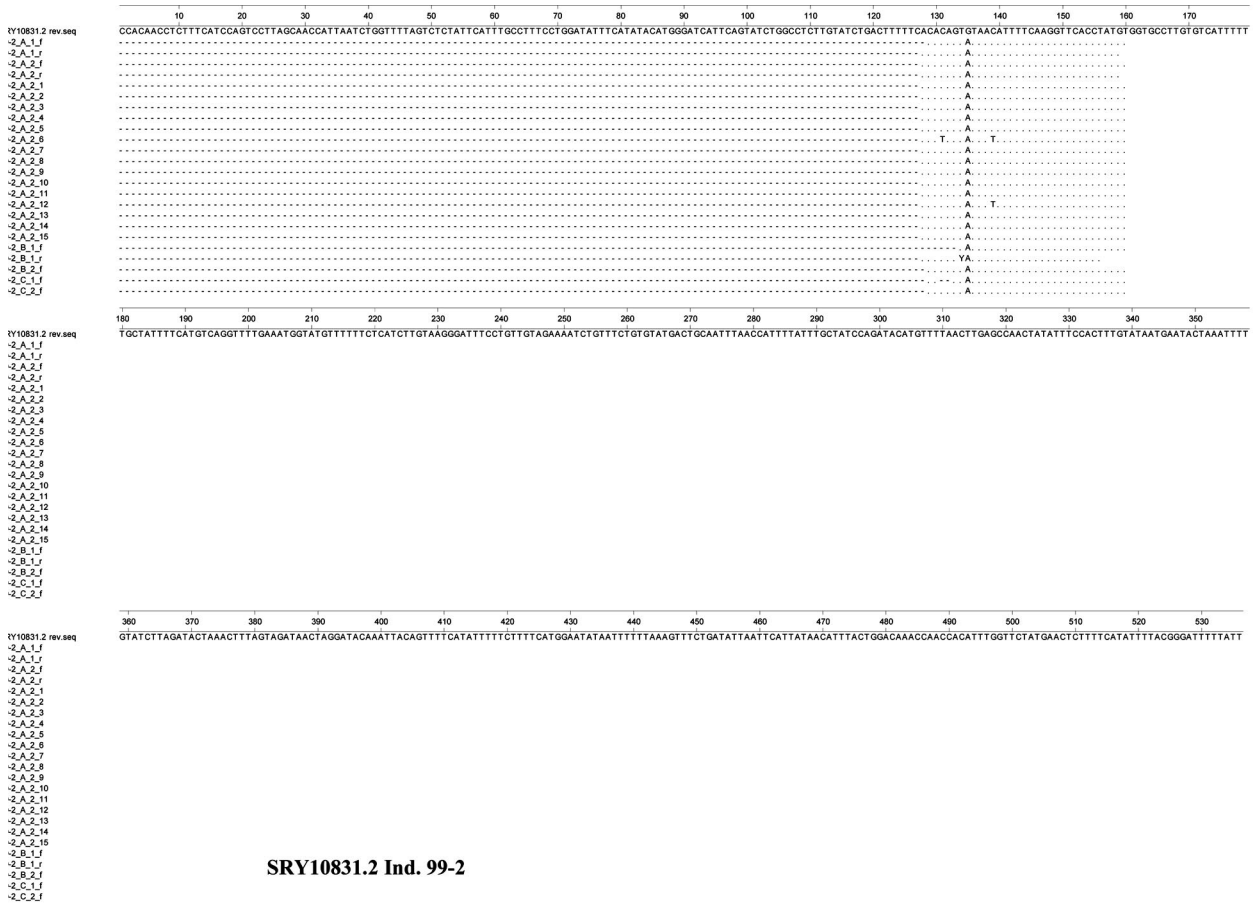
Fig. S5. (continued)



HVS II Ind. 98-9 & 98-10

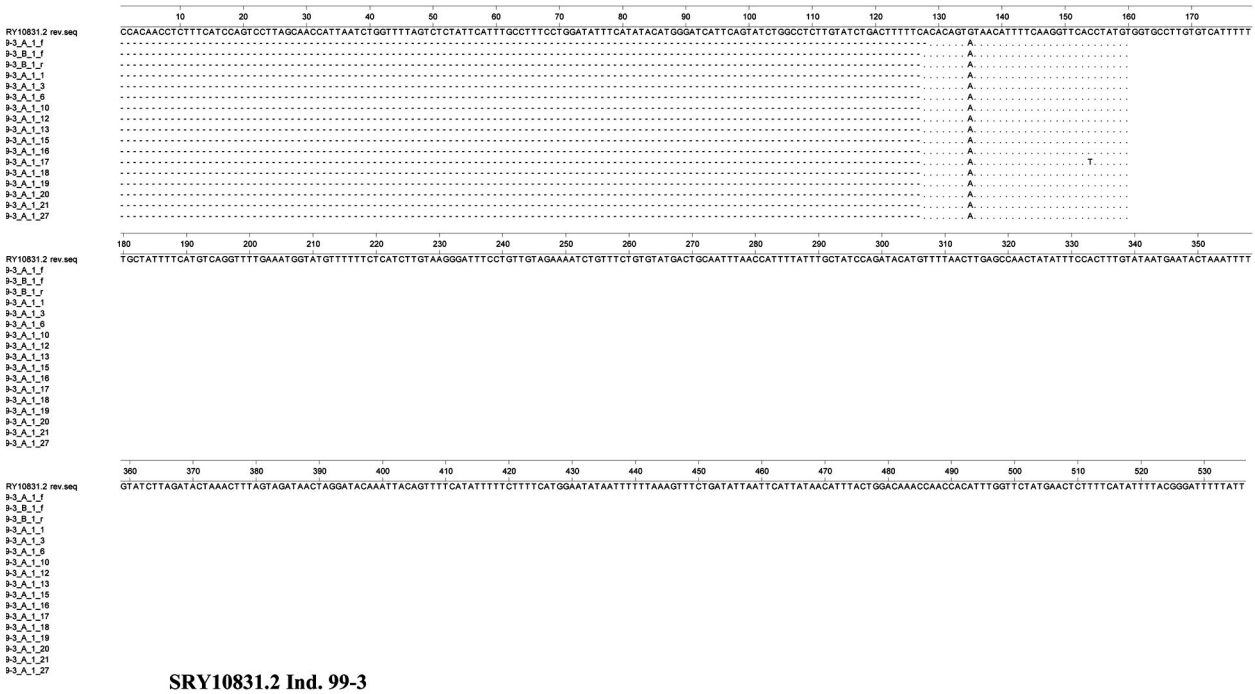
10

Fig. S5. (continued)



11

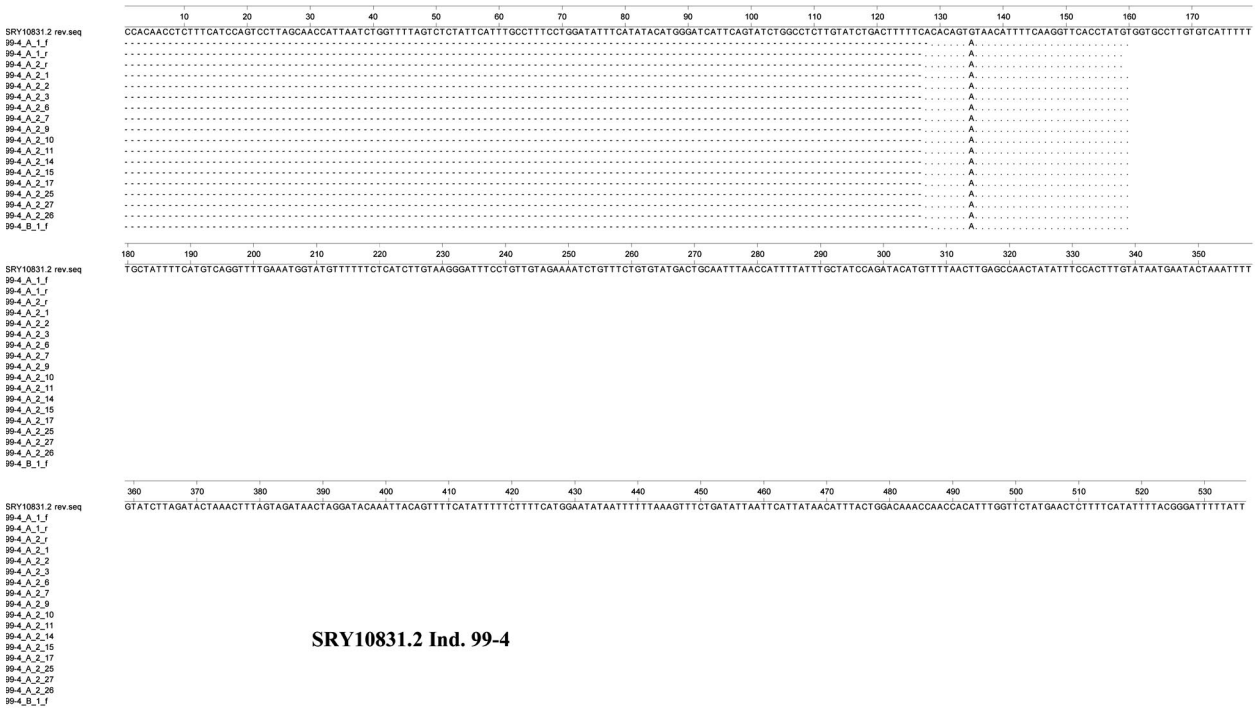
Fig. S5. (continued)



SRY10831.2 Ind. 99-3

12

Fig. S5. (continued)



13

Fig. S5. (continued)

Table S1. Results of AmpF/STR® Profiler Plus™ typings

Grave	Ind.	Ex	PCR	Amel	D3S1358	D8S1179	D5S818	vWA	D21S11	D13S317	FGA	D7S820	D18S51
99	1	A	I	X	(16)	11/13	(11)/(12)						
		A	II	X	16/18	(10)/11/13	11/12	17/18					
		A	III	X	16/18	11/13	11/12	17					
		B	I	X		11/13							
		B	II	(X)									
		B	III	X		11/13							
		B	IV	X	(16)/18	(10)/11/(12)/13	(11)/(12)	17/18					
		C	I	X	(15)/16								
		C	II	X	(17)/18	11	(10)/11						
				X	**16/18**	**11/13**	**11/12**	**17/18**					
	2	A	I	X/Y		11/(12)/13	12	(17)					
		A	II	X/Y	14	(10)/11/(12)/13	12			(12)			
		A	III	X/Y	(14)/(18)	(12)/13	12	14/17					
		B	I	Y		11/(13)							
		B	II	X/Y		11/(13)							
		B	III	X/Y		(12)/13							
		C	I	X/Y									
				X/Y		**11/13**							
	3	A	I	X/Y	14/16	(11)/12/13	11/12	14/18	(27)/28				
		A	II	X/Y	14/16	(11)/12/13	11/12	14/18	27/28				
		A	III	X/Y	14/16	(11)/12/13	11/12	14/18	27/28				
		B	I	Y	(16)	(11)/12/13	12	14					
		B	II	X									
		B	III	X			(10)/11						
				X/Y	**16**	**12/13**	**11/12**	**14**					
	4	A	I	X/Y		(9)/(10)/11							
		A	II	X/Y	14	(10)/11/(12)/13		14					
		A	III	X/Y		(10)/11/(12)/13		(16)/17					
		C	I	X									
				X/Y									
90	5	A	I	X	15				(30.2)/31.2				
		A	II	X									
		B	I	X	(14)/15		(11)/12						
		B	II	X	(14)/15	(8)							
				X	**15**								
98	7	A	I	X		(13)/14							
		B	I	X			12						
		B	II	X			12						
				X									
	9	A	I	X/Y	-	(14)/15	(11)/12						
		A	II	X/Y	(15)		(12)						
		B	I	X/Y		(12)/13							
		B	II	X/Y	15	(14)/15	12		(30)				
				X/Y	**15**	**15**	**12**						
	10	A	I	(X)									
		B	I	(X)	15/16								
		B	II	X									
				X									
93	11	A	I	X/(Y)	(14)/15	(9)/10		14					
		A	II	X/Y		10/13							
		B	I	Y									
				X/Y									

For each individual, a consensus genotype is given (boldfacegrey), when an allele has been confirmed at least once from an independent PCR (I, II, III, etc.) from an independent extraction (A, B, C). STR loci are sorted by length. Stuttering alleles with a peak height lower than 10% of the neighboring allele or alleles with low signal intensity in general are parenthesized.

Table S2. Results of AmpFlSTR® Yfiler™ typings

Ind.	Ex.	PCR	DYS393	DYS456	Y-GATA	DYS458	DYS389 I	DYS391	DYS19	DYS437	DYS390	DYS439	DYS438	DYS385 a/b	DYS635	DYS389 II	DYS448	DYS392
2	A	I	(13)/14	16	13	(14)/15	(12)/13	11	16		(24)/25	10		11/14				
	A	II	14	16			(12)/13	11		(13)/14				(14)				
	A	III	(13)/14	(15)/16	13	15	(12)/13											
			14	**16**	**13**	**15**	**13**	**11**						**-/14**				
3	A	I	14	(15)/16	13	(14)/15	(12)/13	(10)/11	16	14	25	10	11	11	23	(30)	19	
	A	II	(12)/(13)/14	(15)/16	13	(14)/15	(12)/13	11	16	14	25	-	(11)	11/14		(30)	19	
	A	III	14	16	13	(14)/15	(12)/13	(8)/11	(10)	14		10						
			14	**16**	**13**	**15**	**13**	**11**	**16**	**14**	**25**	**10**	**11**	**11/-**		**30**	**19**	
4	A	I	(12)/(13)/14	16	13		(12)/13	(10)/11										
	A	II	14	16	13	(14)/15				14								
	A	III		(15)/16							25							
			14	**16**	**13**													

Consensus genotypes were summarized for each individual (boldface grey), when an allele could be confirmed at least once from an independent PCR (note that successful Yfiler typings could only be received from extraction A: that is, samples, which were taken prior to applying museum's preserving methods). Y-STR loci are sorted by length. Stuttering alleles with a peak height lower than 10% of the neighboring allele or alleles with low signal intensity in general are parenthesized.

Table S3. $^{87}Sr/^{86}Sr$ isotope ratios from teeth and local proxies

Grave	Individual	Tooth	Mineralization	Enamel $^{87}Sr/^{86}Sr$	Dentine $^{87}Sr/^{86}Sr$
99	1	lM3	9 to 14y	0.712658 ± 0.000009	n/a
99	2	udM1	−4 to 6m	0.709905 ± 0.000004	n/a
99	3	lM2	2.5 to 7.5y	0.711525 ± 0.000011	0.709948 ± 0.00012
99	4	udM2	−3 to 12m	0.710920 ± 0.000009	n/a
90	5	uM2	2.5 to 7.5y	0.712807 ± 0.000007	0.710018 ± 0.00012
90	6	ldM2	−3 to 12m	0.710117 ± 0.000013	0.709825 ± 0.00009
98	7	uM2	2.5 to 7.5y	0.713783 ± 0.000014	0.709852 ± 0.00012
98	8	Not sampled			
98	9	ldM1	−4 to 6m	0.710694 ± 0.000005	n/a
98	10	ldM2	−3 to 12m	0.710189 ± 0.000005	0.709358 ± 0.00009
93	11	M	Between 0 and 14y	0.710600 ± 0.000009	0.709626 ± 0.00008
93	12	dM	Between −5 and 12m	0.709915 ± 0.000006	n/a
93	13	dM	Between −5 and 12m	0.710310 ± 0.000007	n/a
Sediment lechate A				0.711423 ± 0.00014	
Sediment lechate B				0.709819 ± 0.00012	
Rodent				0.709589 ± 0.00019	

Tooth position denoted by (u) maxilla, (l) mandibular, (d) deciduous, (M) molar. The age at which mineralization of the enamel commences and ceases are given in years, or months after birth (1). Negative values indicate *in utero* mineralization. Exact positions of teeth from grave 93 were uncertain so the range of possible mineralization is given.

1. Hilson S (1986) *Teeth* (Cambridge University Press, Cambridge).

Table S4. Mitochondrial DNA haplotype sequences of unambiguously typed individuals and workers involved in sampling and lab work

Ind.	HVS I (np 15997–16409)	HVS II (np 35–396)	HG
CWC individuals from Eulau			
99–1	16093C, 16224C, 16311C, 16319A	n.a.	K1b
99–2	16093C, 16224C, 16311C, 16319A	n.a.	K1b
99–3	16189C, 16192T, 16270T	n.a.	U5b
99–4	16093C, 16224C, 16311C, 16319A	n.a.	K1b
90–5	16129A, 16223T, 16391A	n.a.	I
98–7	16093C, 16221T	n.a.	H
98–9	16189C, 16223T, 16278T	73G, 153G, 195C, 225A, 226C, 263G	X2
98–10	16189C, 16223T, 16278T	73G, 153G, 195C, 225A, 226C, 263G	X2
93–13	16145A, 16224C, 16311C	n.a.	K1a2
Workers involved			
1	16221T	n.a.	H
2	16343G, 16390A	n.a.	U3
3	16129A, 16223T	n.a.	I
4	16129A, 16172T, 16223T, 16260T, 16311C, 16391A	n.a.	I
5	16145A, 16189C, 16256T, 16270T	n.a.	U5
6	16291T, 16390A	n.a.	H
7	16166G, 16311C	263G, 315.1C	H
8	16189C, 16263C	263G. 315.1C	H
9	16189C, 16311C	263G, 309.1C, 309.2C, 315.1C, 327C	H
10	16051G, 16162G, 16259T	73G, 263G, 315.1C	H

HG, Haplogroup; n.a., not analyzed. Sequences from hypervariable segment I and II (HVS) are presented as variant nucleotide positions relative to the revised Cambridge Reference Sequence (1).

1. Andrews RM et al. (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* 23:147.

Table S5. Results of AmpFlSTR® Profiler Plus™ typings of workers and authors involved

Ind.	Amelogenin	D3S1358	D8S1179	D5S818	vWA	D21S11	D13S317	FGA	D7S820	D18S51
1	X	16	14	11/12	15/18	28/30	9/11	18/21	8/10	16/17
2	X	16	16	11/12	17/18	28/29	13	21/27	9/11	12/14
3	XY	16/18	12/13	11/12	14	27/32.2	11	24	14	14/16
4	X	14/17	12/13	12/13	18/19	32.2	10/12	20/26	9/11	19/20
5	X	15/18	12	11	17/19	32.2	8/9	20/23	9/12	13/14
6	X	16	10/11	12/14	16/18	30.2/31.2	8/11	23/24	12/14	12/13
7	XY	17	12/14	11	17/20	28/31	12	22/26	9/11	12/17
8	XY	15/16	13	11/13	15/17	29/30	9/10	19/24	11	16/21
9	XY	14/18	13/14	11/13	16/17	29/30	12	22/26	8/11	12/14
10	XY	14/15	13/14	12/13	16/19	29/31.2	8	21/23	7/8	15/16

Table S6. Sequences of the overlapping primer pairs of HVS I and HVS II and the Y-SNP SRY10831.2 region

Region	Name	Sequence 5′′– 3′′	Reference
HVS I	L15996	CTCCACCATTAGCACCCAAAGC	(1)
	H16142	ATGTACTACAGGTGGTCAAG	(2)
	L16117	TACATTACTGCCAGCCACCAT	(3)
	H16233	GCTTTGGAGTTGCAGTTGATGTGT	(3)
	L16209	CCCCATGCTTACAAGCAAGT	(4)
	H16348	ATGGGGACGAGAAGGGATTTG	(3)
	L16287	CACTAGGATACCAACAAACC	(4)
	H16410	GCGGGATATTGATTTCACGG	(4)
HVS II	L00034	TCTATCACCCTATTAACCACTCAC	Present study
	H00177	TTAGTAAGTATGTTCGCCTGTAAT	Present study
	L00144	CGCAGTATCTGTCTTTGATTCCTG	Present study
	H00243	AAAGTGGCTGTGCAGACATTCAAT	Present study
	L00172	ATCCTATTATTTATCGCACCTACG	Present study
	H00327	TTGGCAGAGATGTGTTTAAGTGCT	Present study
	L00274	TGTCTGCACAGCCACTTTCCACAC	Present study
	H00397	AGTGCATACCGCCAAAAGATAAAA	Present study
SRY10831.2	Forward	GTATCTGGCCTCTTGTATCTGACTTTTTC	Present study
	Reverse	AGCAAAAAATGACACAAGGCACC	Present study

All primers were used at 58°C annealing temperature, except for SRY10831.2 (57°C).

1. Endicott P, *et al*. (2003) The genetic origins of the Andaman Islanders. *Am J Hum Genet* 72:178–184.
2. Stone AC, Stoneking M (1998) mtDNA analysis of a prehistoric Oneota population: implications for the peopling of the New World. *Am J Hum Genet* 62:1153–1170.
3. Haak W, *et al*. (2005) Ancient DNA from the first European farmers in 7500-year-old Neolithic sites. *Science* 310:1016–1018.
4. Handt O, Krings M, Ward RH, Pääbo S (1996) The retrieval of ancient human DNA sequences. *Am J Hum Genet* 59:368–376.