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Early cave art and ancient DNA record the origin of European bison

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The two living species of bison (European and American) are among the few terrestrial megafauna to have survived the late Pleistocene extinctions. Despite the extensive bovid fossil record in Eurasia, the evolutionary history of the European bison (or wisent, *Bison bonasus*) before the Holocene (<11.7 thousand years ago (kya)) remains a mystery. We use complete ancient mitochondrial genomes and genome-wide nuclear DNA surveys to reveal that the wisent is the product of hybridization between the extinct steppe bison (*Bison priscus*) and ancestors of modern cattle (aurochs, *Bos primigenius*) before 120 kya, and contains up to 10% aurochs genomic ancestry. Although undetected within the fossil record, ancestors of the wisent have alternated ecological dominance with steppe bison in association with major environmental shifts since at least 55 kya. Early cave artists recorded distinct morphological forms consistent with these replacement events, around the Last Glacial Maximum (LGM, ~21-18 kya).

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he extensive Late Pleistocene fossil record of bovids in Europe consists of two recognized forms: the aurochs (Bos primigenius), ancestor of modern cattle, and the mid/late Pleistocene 'steppe bison' (Bison priscus), which also ranged across Beringia as far as western Canada^{1,2}. The European bison, or wisent (Bison bonasus), has no recognized Pleistocene fossil record and seems to suddenly appear in the early Holocene $(< 11.7 \text{ kya})^{3,4}$, shortly after the disappearance of the steppe bison during the megafaunal extinctions of the Late $Pleistocene^{5-7}$. The Holocene range of wisent included all lowlands of Europe, and several highland areas of eastern Europe (where it was termed the Caucasian form B. bonasus caucasicus) but range reduction and hunting by humans brought the species close to extinction, with modern populations descending from just 12 mostly Polish individuals that lived in the 1920s (refs 8,9). Nuclear DNA sequences and the morphology of the wisent show close similarities to American bison (B. bison), but wisent mitochondrial DNA (mtDNA) indicates a closer relationship with cattle. This suggests some form of introgression from cattle or a related *Bos* species^{10–12}, potentially associated with the recent extreme bottleneck event.

Both aurochs and bison feature heavily in Palaeolithic cave art, with 820 depictions displaying bison individuals (\sim 21% of known cave ornamentation¹³). The diversity of bison representations has been explained as putative cultural and individual variations of style through time, since the steppe bison was assumed to be the only bison present in Late Paleolithic Europe^{14–16}. However, two distinct morphological forms of bison (Fig. 1, Supplementary Information section) are clearly apparent in cave art: a long-horned form similar to modern American bison (which are thought to be descended from steppe bison), with very robust forequarters and oblique dorsal line, and a second form with thinner double-curved horns, smaller hump and more balanced body proportions, similar to wisent. The former is abundant in art older than the Last Glacial Maximum (LGM, \sim 22–18 kya), while the latter dominates Magdalenian art (\sim 17–12 kya, see Supplementary Information section). Similarly, two distinct morphological forms of Late Pleistocene bison have been reported from North Sea sediments¹⁷.

To further examine the potential existence of a previously unrecognized fossil bison species within Europe, we sequenced ancient mtDNA and nuclear DNA from bones and teeth of 64 Late Pleistocene/Holocene bison specimens.

We reveal that the wisent lineage originated from hybridization between the aurochs and steppe bison, and this new form alternated ecologically with steppe bison throughout the Late Pleistocene and appears to have been recorded by early cave artists.

Results

New group of ancient European bison. The mtDNA sequences of 38 specimens, dated from >50 to 14 kya and ranging from the Caucasus, Urals, North Sea, France and Italy, formed a previously unrecognized genetic clade, hereafter referred to as CladeX, related to modern and historical wisent (including the Caucasian form; Fig. 2a,b). By using the radiocarbon-dated specimens to calibrate our phylogenetic estimate of the timescale, we inferred that the divergence between CladeX and modern wisent lineages occurred ~120 (92–152) kya, likely during the last (Eemian) interglacial. Both these mitochondrial clades are more closely related to cattle than to bison, suggesting that they are descended from an ancient hybridization event that took place >120 kya (presumably between steppe bison and an ancestral form of aurochs, from which the mitochondrial lineage was acquired).



Figure 1 | Cave painting example of steppe bison-like and wisent-like morphs. (a) Reproduction from Lascaux cave (France), from the Solutrean or early Magdalenian period (~20,000 kya—picture adapted from ref. 53). (b) Reproduction from the Pergouset cave (France), from the Magdalenian period (~17,000 kya—picture adapted from ref. 54).

Hybrid origin of wisent and ancient European bison. To investigate the potential hybrid origins of wisent and CladeX, we used target enrichment and high-throughput methods to sequence \sim 10,000 genome-wide bovine single-nucleotide polymorphisms (SNPs) from nine members of CladeX, an ancient (>55 kyr) and a historical (1911 AD) wisent specimen and two steppe bison (30 and >50 kyr). Principal Component Analysis (PCA) and phylogenetic analysis (Fig. 3 and Supplementary Fig. 10) of the nuclear data demonstrate that members of CladeX are closely related to the steppe bison. D-statistic¹⁸ analyses confirm a closer affinity of both CladeX and the ancient wisent to steppe bison than to modern wisent (Fig. 3b), which is explicable because of rapid genetic drift during the severe bottleneck leading to modern wisent. Concordantly, our historical wisent sample (Caucasian, from 1911) displays a signal intermediate between modern wisent and both CladeX and steppe bison (Fig. 3b(3-5),c).

The nuclear and mitochondrial analyses together suggest that the common ancestor of the wisent and CladeX mitochondrial lineages originated from asymmetrical hybridization (or sustained introgression) between male steppe bison and female aurochs (see Supplementary Fig. 20). This scenario is consistent with the heavily polygynous mating system of most large bovids¹⁹, and the observation that hybridization between either extant bison species and cattle usually results in F1 male infertility, consistent with Haldane's Rule of heterogametic crosses^{20–22}. However, it is unclear whether hybridization took place only once or multiple times, and how and at what point after the initial hybridization event(s) the wisent–CladeX forms became distinct from the steppe bison.

To examine the extent of genetic isolation maintained through time by the hybrid forms (wisent and CladeX) from steppe bison, we characterized the genomic signals originating from either steppe bison or aurochs in the wisent and CladeX lineages.



Figure 2 | Identification of CladeX. (a) Phylogenetic tree inferred from bovine mitochondrial control region sequences, showing the new clade of bison individuals. The positions of the newly sequenced individuals are marked in red for CladeX. (b) Bovine phylogeny estimated from whole-mitochondrial genome sequences, showing strong support for the grouping of wisent and CladeX with cattle (cow) and zebu. For both trees (a,b) numbers above branches represent the posterior probabilities from Bayesian inference, numbers below branches represent approximate likelihood ratio test support values from maximum-likelihood analysis and scale bars represent nucleotide substitutions per site from the Bayesian analysis. (c) Maximum-clade-credibility tree of CladeX and wisent estimated using Bayesian analysis and calibrated with radiocarbon dates associated with the sequenced bones. Dates of samples older than 50 kyr were estimated in the phylogenetic reconstruction. (d) Map showing all sampling locations, using the same colour code (red for CladeX, orange for wisent and blue for steppe bison).

Calculations of f_4 ratios²³ show the same high proportion of nuclear signal from steppe bison ($\geq 89.1\%$) and low proportion from aurochs (\leq 10.9%) in both wisent and CladeX (Fig. 3d and Supplementary Table 6). Independent calculation of hybridization levels from ABC comparisons with simulated data also shows clear evidence of hybridization, with similar proportions of nuclear signal (97.2% probability that there is at least 1% aurochs ancestry and a 87.6% probability that there is at least 5% aurochs ancestry; see Supplementary Note 2 and Supplementary Tables 10 and 11). The agreement between these two methods is compelling evidence of hybridization. In addition, a greater number of derived alleles are common to both wisent and CladeX lineages (either from the imprint of steppe bison ancestry, aurochs ancestry, or from post-hybridization drift) than expected from multiple hybridization events (see Supplementary Note 2 and Supplementary Tables 8 and 9), implying that CladeX represents part of the Late Pleistocene wisent diversity. The age of the oldest genotyped specimens of CladeX (23 kyr) and wisent (>55 kyr) confirm that the initial hybridization event (or ultimate significant introgression of steppe bison) occurred before 55 kya. Together, the long-term stability of the nuclear and mitochondrial signal in wisent and CladeX indicates that the hybrid bison lineage maintained a marked degree of genetic isolation throughout the Late Pleistocene, consistent with the different morphologies observed in the North Sea specimens¹⁷.

Hybrid and steppe Bison represent different ecological forms. The temporal distribution of genotyped individuals reveals that wisent mitochondrial lineages (including CladeX) are only observed before 50 kya and after 34 kya, when steppe bison appears to be largely absent from the European landscape (Fig. 4). The detailed records of the southern Ural sites allow the timing of the population replacements between steppe bison and wisent to be correlated with major palaeoenvironmental shifts, revealing that the wisent was associated with colder, more tundra-like landscapes and absence of a warm summer (Supplementary Fig. 22). Stable isotope data (∂^{13} C/ ∂^{15} N; Supplementary Fig. 23) and environment reconstructions show that wisent were present in a more diverse environment than steppe bison, with a more variable diet, suggesting that these two taxa occupied separate ecological niches.

Discussions

Contrary to previous palaeontological interpretations, the ancestors of modern wisent were present in Europe throughout the Late Pleistocene, and the two different bison morphs depicted in Paleolithic art suggest that early artists recorded the replacement of the steppe bison by the hybrid form (including CladeX) in Western Europe around the LGM. Two bison individuals have been genotyped from European caves during this period: a 19-kyr-old steppe bison from Southern France²⁴ and a



Figure 3 | **Genome-wide data comparison of bison. (a)** Maximum-likelihood phylogeny of modern and ancient bison from ~10,000 genome-wide nuclear sites, showing the close relationship between CladeX and steppe bison. However, a bifurcating phylogeny is not capable of displaying the complex relationships between these taxa (see Supplementary Fig. 8). Numbers above branches represent bootstrap values. (b) D-statistics from the same ~10,000 nuclear sites, using sheep as outgroup. For three bison populations, assuming two bifurcations and no hybridizations, three possible phylogenetic topologies can be evaluated using D-statistics, with the value closest to 0, indicating which topology is the most parsimonious. The topology being tested is shown on the vertical axis. Error bars are three s.e.'s (from block jackknife) either side of the data point. Data points that are significantly different from zero are shown in grey. The data point representing the topology in **a**, among a set of three possible topologies, is shown with a black outline. (**c**) Principal Component Analysis of ~10,000 genome-wide nuclear sites (ancient wisent not included due to the sensitivity of PCA to missing data, see Supplementary Fig. 10). (**d**) Proportion of steppe bison and aurochs ancestry in both wisent and CladeX lineages, calculated with f_4 ratios.

16-kyr-old wisent (CladeX) from Northern Italy (present study), corresponding to the timing of the morphological transition from steppe bison-like to wisent-like morphotypes apparent in cave art.

Combined evidence from genomic data, paleoenvironmental reconstructions and cave paintings strongly suggest that the hybridization of steppe bison with an ancient aurochs lineage during the late Pleistocene led to a morphologically and ecologically distinct form, which maintained its integrity and survived environmental changes on the European landscape until modern times. Although further analyses of deeper ancient genome sequencing will be necessary to characterize the phenotypic consequences of such hybridization, this adds to recent evidence of the importance of hybridization as a mechanism for speciation and adaptation of mammals^{25–29} as is already accepted for plants. Lastly, the paraphyly of *Bos* with respect to *Bison*, and the evidence of meaningful hybridization between aurochs and bison, support the argument that both groups should be combined under the genus $Bos^{12,19,30}$.

Methods

Ancient DNA samples description and processing. Samples from a total of 87 putative bison bones were collected from three regions across Europe: Urals, Caucasus and Western Europe (Supplementary Data 1).

Dating of 45 samples that yielded DNA was performed at the Oxford Radiocarbon Accelerator Unit of the University of Oxford (OxA numbers), and the Ångström Laboratory of the University of Uppsala, Sweden, for the Swiss sample (Ua-42583). The calibration of radiocarbon dates was performed using OxCal v4.1 with the IntCal13 curve³¹ (Supplementary Data 1).



Figure 4 | Temporal and geographical distribution of bison in Europe. Individual calibrated AMS dates from the present study and published data are plotted on top of the NGRIP δ^{18} O record⁵⁵. Age ranges for infinite AMS dates are from molecular clock estimates (Fig. 2c). Greenland interstadials (GIs) are numbered in black and marine isotope stages (MIS) in grey.

All ancient DNA work was conducted in clean-room facilities at the University of Adelaide's Australian Centre for Ancient DNA, Australia (ACAD), and at the University of Tuebingen, Germany (UT) following the published guidelines³². Samples were extracted using either phenol-chloroform³³ or silica-based

methods^{34,35} (see Supplementary Data 1).

Mitochondrial control region sequences (>400 bp) were successfully amplified from 65 out of 87 analysed samples in one or up to four overlapping fragments, depending on DNA preservation³³. To provide deeper phylogenetic resolution and further examine the apparent close relationship between *Bos* and wisent mitochondria, whole-mitogenome sequences of 13 CladeX specimens, as well as one ancient wisent, one historical wisent and one steppe bison were generated using hybridization capture with either custom-made^{36,37} (see Supplementary Note 1 for details).

In addition, genome-wide nuclear locus capture was attempted on DNA extracts from 13 bison samples (see Supplementary Table 2), using either an $\sim 40,000$ or an $\sim 10,000$ set of probes (as described in Supplementary Note 1). All targeted loci were part of the BovineSNP50 v2 BeadChip (Illumina) bovine SNP loci used in a previous phylogenetic study³⁸. Ultimately, only the 9,908 loci common to both sets were used for comparative analysis.

Genetic data analysis. *Data processing.* Next-generation sequencing data were obtained from enriched libraries using paired-end reactions on Illumina HiSeq or MiSeq machines, and processed using the pipeline Paleomix v1.0.1 (ref. 39). AdapterRemoval v2 (ref. 40) was used to trim adapter sequences, merge the paired reads and eliminate all reads shorter than 25 bp. BWA v0.6.2 was then used to map the processed reads to either the reference mitochondrial genome of the wisent (NC_014044), American bison (NC_012346—only for the steppe bison A3133) or the *Bos taurus* genome reference UMD 3.1 (ref. 41). Minimum mapping quality was set at 25, seeding was disabled and the maximum number of gap opens was set to 2 (see Supplementary Tables 2 and 3).

MapDamage v2 (ref. 42) was used to check that the expected contextual mapping and damage patterns were observed for each library, depending on the enzymatic treatment used during library preparation (see Supplementary Table 3 and Supplementary Figs 1–3 for examples), and to rescale base qualities accordingly.

Phylogenetic analyses. The 60 newly sequenced bovine mitochondrial regions (Supplementary Data 1) were aligned with 302 published sequences (Supplementary Table 4), and a phylogenetic tree was inferred using both maximum-likelihood (PhyML v3 (ref. 43)) and Bayesian (MrBayes v3.2.3 (ref. 44)) methods (Fig. 2a and Supplementary Fig. 4). The same methods were used to obtain the whole-mitogenome phylogeny of 16 newly sequenced bison (Supplementary Data 1) aligned with 31 published sequences (Fig. 2b and Supplementary Fig. 5). To estimate the evolutionary timescale, we used the programme BEAST v1.8.1 (ref. 45) to conduct a Bayesian phylogenetic analysis of all radiocarbon-dated samples from CladeX and wisent (Fig. 1c), using the mean calibrated radiocarbon dates as calibration points. All parameters showed sufficient sampling after 5,000,000 steps, and a date-randomization test supported that the temporal signal from the radiocarbon dates associated with the ancient sequences was sufficient to calibrate the analysis⁴⁶ (Supplementary Fig. 6).

Finally, phylogenetic trees were inferred from nuclear loci data using RAxML v8.1.21 (ref. 47), first from published data of modern bovine representatives³⁸ (using sheep as an outgroup; Supplementary Fig. 7) and then including five ancient samples (two ancient steppe bison, an ancient wisent, a historical wisent and a CladeX bison; Fig. 2a), which had the highest number of nuclear loci successfully called among the ~10k nuclear bovine SNPs targeted with hybridization capture (see Supplementary Fig. 8).

Principal Component Analysis. PCA (Fig. 3a and Supplementary Fig. 10) was performed using EIGENSOFT version 6.0.1 (ref. 48). In Fig. 3a, CladeX sample A006 was used as the representative of CladeX, as this sample contained the most complete set of nuclear loci called at the bovine SNP loci (see Supplementary Table 2). Other CladeX individuals, as well as ancient wisent, cluster towards coordinates 0.0, 0.0 (see Supplementary Fig. 10), because of missing data.

D and f statistics. Support for the bifurcating nuclear tree (Fig. 2a) was further tested using D-statistics calculated using ADMIXTOOLS version 3.0, git \sim 3065acc5 (ref. 23). Sensitivity to factors like sampling bias, depth of coverage, choice of outgroup, heterozygosity (by haploidization) and missing data did not have notable influences on the outcome (Supplementary Figs 12–15).

The proportion of the wisent's ancestry differentially attributable to the steppe bison, and the aurochs was estimated with AdmixTools using an f_4 ratio²³ with sheep (*Ovis aries*) as the outgroup (Supplementary Figs S16, S17 and 3D). Again, the test was shown to be robust to haploidization.

Finally, to test whether the wisent lineages (including CladeX) have a common hybrid ancestry, or whether multiple independent hybridization events gave rise to distinct wisent lineages (Supplementary Fig. 18), we identify nuclear loci that have an ancestral state in the aurochs lineage, but a derived state in the steppe bison lineage (see Supplementary Note 2 section 'Identification of Derived Alleles'). Hypergeometric tests (Supplementary Tables 8 and 9) showed strong support for an

rypergeometric tests (Supperficiently Tables o and 9) showed strong support for an ancestral hybridization event occurring before the divergence of the wisent lineages. *Testing admixture using ABC and simulated data*. Admixture proportions were

Testing admixture using ABC and simulated and. Admixture proportions were also independently tested using simulated data and an ABC approach. Nuclear genetic count data were simulated for two species trees (as described in Supplementary Fig. 19 and Supplementary Note 2 section) by drawing samples from two Multinomial distributions, where for tree topology X_1 , $n^{X_1} \sim \text{Mult}(N, p^{T,X_1})$, and for tree topology $X_2, n^{X_2} \sim \text{Mult}(N, p^{T,X_2})$. The linear combination of these counts was then considered.

ABC was performed using the R package 'abc', with a ridge regression correction for comparison of the simulated and observed data using the 'abc' function⁴⁹. The distance between the observed and simulated data sets is calculated as the Euclidean distance in a three-dimensional space, corrected for the within dimension variability. A tolerance $\epsilon = 0.005$ was chosen so that the closest $\ell \times \epsilon$ simulated data sets are retained. For each analysis we had $\ell = 100,000$, resulting in 500 posterior samples.

We performed leave-one-out cross-validation using the function 'cv4abc' on $\ell'=250$ randomly selected simulations, and report the prediction error, calculated as

$$E_{\text{pred}} = \frac{\sum_{i=1}^{\ell'} (\hat{\gamma}_i - \gamma_i)^2}{\text{Var}(\gamma_i)}$$

for each analysis. At most, the prediction error was 0.5111 s.d.'s away from zero, and so we observe that the analysis has performed well (see Supplementary Table 10).

Palaeoenvironment reconstruction and stable isotope analyses. The Urals material has the most complete sampling through time (Fig. 4 and Supplementary Fig. 22), allowing us to contrast reconstructed paleoenvironmental proxies for the region (see Supplementary Note 3). Paleovegetation types were inferred for a convex hull of the Ural study region based on geo-referenced site locations for all genotyped ancient samples (Supplementary Fig. 21). Global maps of BIOME4 plant functional types⁵⁰ were accessed for 2,000-year time steps throughout the period from 70,000 years ago to the present day, with a 1° × 1° latitude/longitude grid cell resolution. We also generated estimates of the annual mean daily temperature and Köppen–Geiger climate classification⁵¹ using the Hadley Centre Climate model (HadCM3)⁵². Finally, stable isotope values (δ 13C and δ 15N) obtained for all the genotyped bison individuals from the Ural region were compared between steppe bison and wisent (Supplementary Fig. 23).

Cave paintings. Two consistent morphological types can be distinguished within the diversity of bison representations (see Fig. 1 and Supplementary Figs 24–27). The first type, abundant before the LGM, is characterized by long horns (with one curve), a very oblique dorsal line and a very robust front part of the body (solid shoulders versus hindquarters), all traits similar to the modern American bison. The second type, dominating the more recent paintings between 18 and 15 kya, displays thinner sinuous horns (often with a double curve), a smaller hump and more balanced dimensions between the front and rear of the body, similar to modern wisent and to some extent aurochsen (see also Supplementary Note 4). The coincident morphological and genetic replacement indicate that variation in bison representations in Paleolithic art does not simply represent stylistic evolution, but actually reflects the different forms of bison genotyped in this study (that is, pre and post-hybridization) through time.

Data Availability. All newly sequenced mitochondrial control regions are deposited at the European Nucleotide Archive under the following accession numbers (LT599586–645) and all complete mitochondrial genomes at GenBank (KX592174–89). The BEAST input file (XML) is available as Supplementary Data set 2, the MrBayes input file (Nexus), including all whole-mitochondrial genomes, as Supplementary Data set 3 and the nuclear SNPs as Supplementary Data set 4 (VCF format). All other data are included in the Supplementary Material or available upon request to the corresponding authors.

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Author contributions

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Additional information

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1 Supplementary Figures





3

Supplementary Fig 1. Example of damage profile (sample LE257) obtained after sequencing of the whole mitochondrial genome using no treatment for the library preparation. As expected, there is an excess of purines found at the genomic position preceding the mapped reads, and an excess of C>T transitions at the first few positions of the reads.



Supplementary Fig 2. Example of damage profile (sample A4093) obtained after sequencing of the
 whole mitochondrial genome using UDG-half treatment for the library preparation. As expected, there
 is an excess of cytosine found at the genomic position preceding the mapped reads, and an excess of
 C>T (and complementary G>A) transitions at the first (last) position of the reads.









22 23 24 25 Supplementary Fig 4. Phylogenetic trees of mitochondrial control region sequences from 362 bovid samples. A. Majority-rule consensus tree from MrBayes. B. Maximum-likelihood tree from PhyML. The 60 newly sequenced individuals are in red font, with the Caucasian bison (B. bonasus caucasicus)

in orange. Scale bars are given in substitutions per site.



Supplementary Fig 5. Phylogenetic trees inferred from whole mitochondrial genomes. A. Majority-

- rule consensus tree from MrBayes. B. Maximum-likelihood tree from PhyML. CladeX bison
- individuals are colored in red. Scale bars are given in substitutions per site.



Iterations

Supplementary Fig 6. Date-randomization test. The red circle and dotted line represent the mean
 estimate of the molecular rate obtained in the phylogenetic analysis of wisent and CladeX, calibrated
 using the radiocarbon dates associated with the ancient sequences. The grey lines represent the 95%
 HPD intervals of rates estimated with randomized dates. None of these margins overlap with the mean
 rate estimate from the original data set, demonstrating that the radiocarbon dates used for this study
 contain sufficient temporal information for calibrating the molecular clock.



- **Supplementary Fig 7.** Maximum-likelihood phylogeny of modern bovid species (and sheep as outgroup) from ~40k nuclear loci.





45 Supplementary Fig 8. Maximum-likelihood phylogenies of modern and ancient bison (and yak as 46 outgroup), from ~10k nuclear loci. A. Phylogeny including the two ancient steppe bison. B. Phylogeny

including the three pre-modern wisent. C. Phylogeny including the two steppe bison and three pre-47

48 modern wisent (ancient, historical and CladX). D. Replicate of C. but only using transversions for the 49 non-modern samples.

50



51 52 53 Supplementary Fig 9. Pedigree of wisent from the Białowieża Forest (Poland), from which seven genotyped individuals (in red) were included in the present study.





Supplementary Fig 10: A) Principal Component Analysis for nine CladeX individuals (including
sample A006), one historical wisent, one ancient wisent, two steppe bison, seven modern wisent and 20
American bison. The numbers on the plot report the number of loci called for the individuals clustering
towards zero coordinates (from Supplementary Table 2). Eigenvector 1 explains 9.58% of the variation,
while eigenvector 2 explains 7.96% of the variation. B) Same Principal Component Analysis as Figure
3C with cattle individuals from Decker et al. (2009) projected onto original components.



63 Supplementary Fig 11: Topology testing using D statistics, with sheep as outgroup. The topology

being tested is shown on the vertical axis, with the most parsimonious of three possible topologies written in black. Data points that are significantly different (more than three standard errors) from zero

written in black. Data points that are significantly different (more than three standard errors) from zero are shown in red. The data point representing the topology closest to zero, amongst a set of three

67 possible topologies, is shown with a black outline. Error bars are three standard errors either side of the

be not possible topologies, is shown with a black outline. Effor bars are three standard effors efficient side of

68 data point, where the standard error was calculated using a block jackknife.



- 70 **Supplementary Fig 12:** Topology testing using D statistics, with sheep as outgroup. As in Supplementary Figure 11, except that sample A006 has been omitted from the CladeX group.



- 71 72 73 **Supplementary Fig 13:** Topology testing using D statistics, with sheep as outgroup. As in Supplementary Figure 11, except that genotypes called from read depths <2 have been omitted for extinct individuals.



Supplementary Fig 14: Topology testing using D statistics, with Asian water buffalo as outgroup. As
 in Supplementary Figure 11, except the outgroup has been changed.



- Supplementary Fig 15: Topology testing using D statistics, with sheep as outgroup. As in
- 77 78 Supplementary Figure 11, except in extinct individuals, alleles have been randomly sampled from sites called as heterozygotes to simulate haploid sampling.





Supplementary Fig 18: Admixture graphs representing (A) a single hybridisation event prior to the
 divergence of the wisent, and (B) two independent hybridisation events leading to a wisent clade and a

- 96 CladeX.
- 97



100 Supplementary Fig 19: A hybrid species tree (e), where individual B is a hybrid of A and C lineages,

has two contributing species trees, (a) topology X_1 , and (b) topology X_2 , with proportion γ from

topology X_1 and proportion $1 - \gamma$ from topology X_2 . The unrooted gene trees are shown for (c) topology X_1 , and (d) topology X_2 . Branch lengths T_a, T_b, T_c and T_m have units $2N_e\mu$ generations.



106 **Supplementary Fig 20.** Schematic representation of asymmetrical hybridisation between female

107 aurochs and male steppe bison, and its genetic imprint on both nuclear and mitochondrial genomes

108 after a few generations. The coloured double helix represents the nuclear genome, while the circles

109 represent the strictly maternally inherited mitochondrial genome.



Supplementary Fig 21. Location of all cave sites from which bison samples have been genotyped in the Ural region.





117 Supplementary Fig 22. Chronology of the Urals samples showing a series of replacement patterns 118 that correlate with climate events. Individual calibrated AMS dates are plotted on top of the NGRIP 119 δO^{18} record ¹. Greenland Interstatials (GI) are numbered in black, and Marine Isotope Stages (MIS) in 120 grey. Inferred average temperature, biome reconstruction and proportion of the area for different 121 Koppen climate classes are shown for the exact region where bison were sampled in southern Urals 122 (Koppen classes: D for 'snow', f for 'fully humid', then a=hot summer; b=warm summer; c=cool 123 summer; d=extremely continental). The most recent population replacement between wisent and steppe 124 bison occurs around 32-33 ky, when major environmental transitions are also observed: 1) Globally, as 125 shown on the NGRIP record with the last major interglacial event (GI 5) before a long period of cold 126 climate; but also 2) Locally, as shown on both the average temperature and biome reconstructions. In 127 this situation, wisent are associated with a cooler climate and the presence of tundra-like vegetation. 128 Although dating resolution is degrading for deeper time, a similar shift is apparent around 50-52 kya. 129 Steppe bison occupied this environment in MIS 3, but have not been detected after this stage and 130 indeed were in a severe population decline by GI 1^2 .





133 Supplementary Fig 23. Stable δ13C and δ15N isotope values for all genotyped bison sampled

134 from the Ural region.



137 Supplementary Fig 24. Steppe-like morphologies. In European Palaeolithic art, some bison

138 depictions show morphological traits and anatomical details compatible with the morphology of steppe

- 139 bison (or American bison ancestry). Dates are given as indication based on archaeological occupation
- determined for each site, or, in the absence of such dating, based on stylistic comparison with other
- 141 depictions:
- 142 1. Grotte Chauvet-Pont d'Arc (Ardèche, France). Blurred black charcoal drawing. Aurignacian period
 143 (~35,100 ± 175 calBP. (from C. Fritz and G. Tosello)
- 144 2. Grotte de Lascaux (Dordogne, France). Carving. Solutrean (~22,200 ± 380 calBP) or early
- 145 Magdalenian period (between $\sim 19,300 \pm 561$ and $\sim 20,597 \pm 375$ calBP). (adapted from A. Glory³)

- 146 3. Grotte de Lascaux (Dordogne, France). Carving. Solutrean (~22,200 ± 380 calBP) or early
- 147 Magdalenian period (between $\sim 19,300 \pm 561$ and $\sim 20,597 \pm 375$ calBP). (adapted from A. Glory³)
- 148 4. Grotte de Lascaux (Dordogne, France). Carving. Solutrean (~22,200 ± 380 calBP) or early
- 149 Magdalenian period (between \sim 19,300 ± 561 and \sim 20,597 ± 375 calBP). (adapted from A. Glory³)
- 150 5. Grotte du Gabillou (Dordogne, France). Carving. Early Magdalenian period (~20,597 ± 375 calBP).
 (adapted from J. Gaussen)
- 6. Grotte des Trois Frères (Ariège, France). Carving. Gravettian period (dating estimated based on stylistic analysis). (adapted from H. Breuil⁴)
- 154 7. Grotte du Pech Merle (Lot, France). Painting (manganese). Gravettian period (~29,447 ± 443 calBP).
 (adapted from M. Lorblanchet⁵)
- 8. Grotte du Pech Merle (Lot, France). Painting (manganese). Gravettian period (~29,447 ± 443 calBP).
 (adapted from M. Lorblanchet⁵)
- 9. Grotte de La Pasiega (Cantabria, Spain). Black and red painting. Gravettian or Solutrean period
 (dating estimated based on stylistic analysis). (adapted from H. Breuil⁴)
- 160 10. Abri du Roc de Sers (Charente, France). Carving on limestone. Solutrean period ($< 20,442 \pm 409$
- 161 calBP). (adapted from L. Henri-Martin)



- 163
- 164 Supplementary Fig 25. Wisent-like morphologies. In European Palaeolithic art, some bison
- depictions show morphological traits and anatomical details compatible with identification of wisent
 ancestry. Dates are given as indication based on archaeological occupation determined for each site, or,
 in the absence of such dating, based on stylistic comparison with other depictions:
- 168 1. Grotte de Pergouset (Ardèche, France). Carving. Magdalenian period (dating estimated based on stylistic analysis). (adapted from M. Lorblanchet⁵)
- 1702. Grotte du Portel (Ariège, France). Painting. Magdalenian period (~14,250 \pm 295 calBP). (adapted171from H. Breuil⁴)
- 172 3. Grotte de Niaux (Ariège, France). Painting. Magdalenian period (~17,000 ± 260 calBP). (adapted
 173 from H. Breuil⁴)
- 4. Grotte de Niaux (Ariège, France). Painting. Magdalenian period (~17,000 ± 260 calBP). (adapted from H. Breuil⁴)
- 1765. Grotte de Fontanet (Ariège, France). Carving. Magdalenian period (between ~14250 \pm 295 calBP177and ~16,600 \pm 1000 calBP). (adapted from A. Glory³)
- 6. Grotte de Rouffignac (Dordogne, France). Painting. Magdalenian period (dating estimated based on stylistic analysis). (adapted from C. Barrière⁶)

- 7. Grotte des Combarelles (Dordogne, France). Carving. Magdalenian period (between ~17,000 and
- ~14,300 calBP). (adapted from H. Breuil⁴)
- 8. Grotte de Marsoulas (Haute-Garonne, France). Carving. Magdalenian period (dating estimated based on stylistic analysis). (from C. Fritz et G. Tosello)





Supplementary Fig 27. Undetermined morphologies. In European Palaeolithic art, some bison 190 depictions show morphological traits and anatomical details that could be compatible with either bison 191 form. These pictures illustrate the limits of cave art analyses for morphological assessment of bison 192 forms, due to varying graphical conventions between cultures. Dates are given as indication based on 193 archaeological occupation determined for each site, or, in the absence of such dating, based on stylistic 194 comparison with other depictions:

- 195 1 Grotte de Font-de-Gaume (Dordogne, France). Black and red painting, and carving. Magdalenian 196 period (dating estimated based on stylistic analysis). (adapted from H. Breuil⁴)
- 197 2 Grotte de Niaux (Ariège, France). Painting. Magdalenian period (~17,000 ± 260 calBP). (adapted 198 from H. Breuil⁴)
- 199 3 Grotte des Trois Frères (Ariège, France). Carving. Magdalenian period (dating estimated based on 200 stylistic analysis). (adapted from H. Breuil⁴)
- 201 4 Grotte des Trois Frères (Ariège, France). Carving. Magdalenian period (dating estimated based on 202 stylistic analysis). (adapted from H. Breuil⁴)

- 5 Grotte des Trois Frères (Ariège, France). Carving. Gravettian period (dating estimated based on stylistic analysis). (adapted from H. Breuil⁴)
- 6 Grotte de La Grèze (Dordogne, France). Carving. Gravettian period (dating estimated based on stylistic analysis) (adapted from N. Aujoulat)
- 7 Grotte Chauvet-Pont d'Arc (Ardèche, France). Blured black charcoal drawing. Aurignacian period
 (~35100 ± 175 calBP). (from C. Fritz-G. Tosello)
- 209 8 Grotte Chauvet-Pont d'Arc (Ardèche, France). Blured black charcoal drawing. Aurignacian period
- 210 (~35100 \pm 175 calBP). (from C. Fritz-G. Tosello)
- 211
- 212

- 213 Supplementary Tables
- 214

215 **Supplementary Table 1.** Primers and adapters used in this study 216

	Primer	Primer Sequence (5' - 3')	Length				
Set	BovCR-16351F	CAACCCCCAAAGCTGAAG					
A1	BovCR-16457R	TGGTTRGGGTACAAAGTCTGTG	~96bp				
Set_ B1	BovCR-16420F	CCATAAATGCAAAGAGCCTCAYCAG					
	BovCR-16642R	TGCATGGGGCATATAATTTAATGTA	~1/20p				
Set_ A2	BovCR-16507F	AATGCATTACCCAAACRGGG					
	BovCR-16755R	ATTAAGCTCGTGATCTARTGG	~1840p				
Set_ B2	BovCR- 16633F ^(b)	GCCCCATGCATATAAGCAAG	~132hn				
	BovCR- 16810R ^(b)	GCCTAGCGGGTTGCTGGTTTCACGC	~1320p				
Set_ A3	BovCR- 16765F ^(b)	GAGCTTAAYTACCATGCCG	~125bp				
	BovCR-16998R	98R CGAGATGTCTTATTTAAGAGGAAAGAATGG					
Set_ B3	BovCR-16960F	CATCTGGTTCTTTCTTCAGGGCC					
	BovCR-80R ^(b)	CAAGCATCCCCCAAAATAAA					
Frag1	BovCR_16738M F ^(c,d)	CACGACGTTGTAAAACGACATYGTACATAGYACATTATGTCAA	67hn				
	BovCR_16810T R ^(c,d)	<i>TACGACTCACTATAGGGCGA</i> GCCTAGCGGGTTGCTGGTTTCACG C	~070p				
Frag2	Mamm_12SE ^(d)	CTATAATCGATAAACCCCGATA	06hn				
	Mamm_12SH ^(d)	GCTACACCTTGACCTAAC	~900p				
	GAII_Indexing_	CAAGCAGAAGACGGCATACGAGATNNNNNNGAGTGACTGGA	n/a				
	X IS4 indDCD D5 ^{(e}						
)	CGCTCTT	n/a				
	IS7_short_amp.P 5 ^(e)	ACACTCTTTCCCTACACGAC	n/a				
	IS8_short_amp.P 7 ^(e)	GTGACTGGAGTTCAGACGTGT	n/a				
	P5_short_RNAbl ock	ACACUCUUUCCCUACACGAC	n/a				
	P7_short_RNAbl ock	GUGACUGGAGUUCAGACGUGU	n/a				
	Bison_mt1_forw ard ^(f)	ACCGCGGTCATACGATTAAC					
	Bison_mt1_rever se ^(f)	AATTGCGAAGTGGATTTTGG					
	Bison_mt2_forw ard ^(f)	ATGAGCCAAAATCCACTTCG					
	Bison_mt2_rever se ^(f)	TGTATTTGCGTCTGCTCGTC					
	Bison_mt3_forw ard ^(f)	CGAATCCACAGCCGAACTAT					
	Bison_mt3_rever se ^(f)	TATAAAGCACCGCCAAGTCC					

- 217 (a): Primers are excluded from the length of PCR amplicon.
- $\begin{array}{ccc} 218 & (b):^2.\\ 219 & (c): M \end{array}$

- sequences were used as tags for primers BovCR_16738F and BovCR_16810R, respectively. This
 was done to obtain good quality Sanger sequences from short amplicons.
- (d): One-step simplex PCRs.
- (d): One step simpler i Orts.
 (e): (Meyer and Kircher, "Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing.")
- 225 (f): Primer pairs for use to generate DNA baits for mitochondrial DNA capture.

⁽c): M13 (CAC GAC GTT GTA AAA CGA C) and T7 (TAC GAC TCA CTA TAG GGC GA)

Supplementary Table 2. Summary of nuclear alleles detected at bovine SNP loci: NGS results and locus counts for ancient samples; locus counts for modern samples

	Mapping results for the 9908 SNP positions						Number of SNP called out of the 9908 targeted for each ancient individuals								
Sample ID	Method	Potoinad roads	hite norr	hita unique	e hits_raw_frac	e hits_clonality	Mean coverage	Coverage depth >=1				Coverage depth >=2			
Sample ID		Ketaineu_reaus int	mts_raw	mts_umque				Total	REF/REF	REF/ALT	ALT/ALT	Total	REF/REF	REF/ALT	ALT/ALT
A15526		7045	1821	99	0.26	0.95	0.01	49	49	0	0	1	1	0	0
A017		1280556	3893	1289	0.00	0.67	0.13	630	591	0	39	88	49	0	39
A018		967346	3116	538	0.00	0.83	0.05	253	241	0	12	28	16	0	12
A001		656008	392937	3486	0.60	0.99	0.35	1484	1268	2	214	523	307	2	214
A003		1706985	12957	3423	0.01	0.74	0.35	1569	1363	5	201	470	264	5	201
A004	10k capture	240370	132883	645	0.55	1.00	0.07	315	287	0	28	64	36	0	28
A005		1736500	25788	3519	0.01	0.86	0.35	1643	1438	7	198	464	259	7	198
A006		10413909	99392	22312	0.01	0.78	2.25	5690	3468	104	2118	4755	2533	104	2118
A007		3583539	23832	2841	0.01	0.88	0.29	1307	1084	1	222	509	286	1	222
A15654		1700840	1227601	220913	0.72	0.82	22.28	8738	4532	230	3976	8488	4282	230	3976
A4093		9400283	62631	4478	0.01	0.93	0.45	1946	1480	2	464	1031	565	2	464
A3133	Shotgun / 10k	299829433	9812523	465082	0.03	0.95	46.87	8898	4579	321	3998	8680	4361	321	3998
A875	and 40k capture	3908972	291640	234493	0.07	0.20	23.65	8433	4341	342	3750	8144	4052	342	3750
CPC98_Aurochs From published genome							8882	4770	1808	2304	8810	4698	1808	2304	
Sample ID	Retained_reads	hits_raw	hits_unique	hits_raw_frac	hits_clonality	AVG_Depth	STD_Depth	AVE_Length	STD_Length	5pC>T	3pG>A	Library repair			
-----------	-----------------------	----------	-------------	---------------	----------------	-----------	-----------	------------	------------	-------	-------	----------------			
A001	4822143	1618364	86944	0.34	0.95	432.09	224.83	80.82	37.60	0.03	0.02				
A004	5150804	2314449	220697	0.45	0.90	1152.17	541.88	84.88	36.11	0.02	0.02				
A018	3790161	1021750	24699	0.27	0.98	130.53	60.04	85.32	34.05	0.03	0.03	USER			
A4089	8618722	5380606	44044	0.62	0.99	237.83	155.46	87.18	33.56	0.02	0.02				
A3133	66864927	1958	1949	0.00	0.00	11.41	6.77	93.92	29.66	0.00	0.01				
A003	985033	371605	64372	0.38	0.83	334.44	112.68	84.31	34.07	0.08	0.07				
A005	521428	262622	39121	0.50	0.85	196.95	65.76	81.59	30.96	0.05	0.09				
A006	456078	120668	44541	0.26	0.63	208.39	93.86	75.86	25.87	0.13	0.17				
A007	431113	175432	43269	0.41	0.75	192.35	85.93	71.74	24.13	0.11	0.08	Doution LUDC			
A4093	212315	106221	16923	0.50	0.84	73.23	31.26	70.48	24.60	0.07	0.09	Partial UDG			
A15637	469884	4401	2621	0.01	0.40	8.85	7.22	50.41	12.17	0.41	0.35				
A15654	294965	29628	28329	0.10	0.04	170.48	89.68	98.23	34.91	0.05	0.02				
A15668	230709	3603	2842	0.02	0.21	11.07	7.80	59.61	15.06	0.07	0.06				
LE237	507023	4271	2677	0.01	0.37	9.84	5.70	58.98	23.99	0.55	0.51				
LE242	6912671	48793	35418	0.01	0.27	120.46	67.86	55.09	18.68	0.61	0.60	None			
LE257	4156307	184236	28788	0.04	0.84	94.38	38.34	53.17	20.00	0.52	0.50				

Supplementary Table 3. Summary statistics for NGS of whole mitochondrial genomes

229

230 Supplementary Table 4. List of published mitochondrial control region sequences used for

231 phylogenetic analysis. The Urals steppe bison are highlighted in red.

> Bison_bison AF083357 H1 0 0 Bison_bison_AF083358_H2_0_0 Bison_bison_AF083359_H3_0_0 Bison_bison_AF083360_H4_0_0 Bison_bison_AF083361_H5_0_0 Bison_bison_AF083362_H6_0_0 Bison_bison_AF083363_H7_0_0 Bison_bison_AF083364_H8_0_0 Bison_bison_BS100_29_5 Bison_bison_BS102_22_ Bison bison BS129 0 2000 Bison_bison_BS162_AK_170_30 Bison bison BS173 NTC 3220 45 Bison_bison_BS175_ICE_186_30 Bison_bison_BS177_NTC_3155_36 Bison_bison_BS200_AB_145_37 Bison bison BS342 CHL 10340 40 Bison_bison_BS348_CHL_10505_45 Bison bison BS368 0 2000 Bison_bison_BS417_AB_909_29 Bison_bison_BS419_AB_7475_45 Bison_bison_BS421_AB_8145_45 Bison bison BS422 AB 908 31 Bison_bison_BS423_AB_4660_38 Bison bison BS424 AB 202 32 Bison_bison_BS426_AB_7060_45 Bison bison BS428 AB 7105 45 Bison_bison_BS429_AB_6775_40 Bison bison BS430 9270 50 Bison_bison_BS432_AB_7310_45 Bison_bison_BS433_AB_10450_55 Bison_bison_BS434_AB_809_32 Bison bison BS439 AB 5845 45 Bison_bison_BS441_AB_1273_32 Bison_bison_BS444_AB_636_29 Bison_bison_BS445_AB_378_30 Bison_bison_BS449_6195_45 Bison_bison_BS454_AB_287_29 Bison_bison_BS456_AB_125_30 Bison_bison_BS460_AB_10425_50 Bison_bison_BS464_AB_5205_45 Bison_bison_BS465_AB_7115_50 Bison_bison_BS466_AB_3298_37 Bison_bison_BS503_BIR_2776_36 Bison_bison_BS560_AB_2807_28 Bison_bison_BS569_AB_3600_70 Bison_bison_BS570_AB_11300_290 Bison_bison_BS99_26_5 Bison_bison_U12935_0_0 Bison_bison_U12936_0_0 Bison bison U12941 0 0 Bison_bison_U12943_0_0 Bison_bison_U12944_0_0 Bison_bison_U12945_0_0 Bison_bison_U12946_0_0 Bison_bison_U12947_0_0 Bison_bison_U12948_0_0 Bison_bison_U12955_0_0 Bison_bison_U12956_0_0 Bison_bison_U12957_0_0 Bison_bison_U12958_0_0 Bison_bison_U12959_0_0 Steppe bison Bison_priscus_A3133_Yukon_26360_220 Bison_priscus_BS105_F_23380_460 Bison_priscus_BS107_F_19570_290 Bison_priscus_BS108_F_21020_360 Bison_priscus_BS109_F_20730_350 Bison_priscus_BS111_F_21580_370 Bison_priscus_BS121_F_19360_280 Bison_priscus_BS123_BIR_1730_60 Bison_priscus_BS124_BIR_11900_70 Bison_priscus_BS125_F_27440_790 Bison_priscus_BS126_F_19150_280 Bison_priscus_BS130_BIR_9000_250 Bison priscus BS133 F 33800 1900 Bison_priscus_BS145_NS_12270_50

son_priscus_BS146_NS_11810_50 Bison_priscus_BS147_NS_28120_290 Bison_priscus_BS148_NS_6400_50 Bison_priscus_BS149_NS_46100_2200 Bison_priscus_BS150_NS_10510_50 Bison_priscus_BS151_NS_21530_130 Bison_priscus_BS161_NS_21040_120 Bison_priscus_BS163_LC_13240_75 Bison_priscus_BS164_LC_19540_120 Bison_priscus_BS165_LC_26460_160 ison_priscus_BS170_YT_13040_70 Bison_priscus_BS172_LC_12525_70 Bison_priscus_BS176_LC_12380_60 Bison_priscus_BS178_LC_17960_90 Bison_priscus_BS192_F_26300_300 Bison_priscus_BS193_NS_49600_4000 Bison_priscus_BS195_NS_29040_340 Bison_priscus_BS196_NS_19420_100 Bison_priscus_BS198_Y_2460_40 Bison_priscus_BS201_Y_12960_60 Bison_priscus_BS202_AB_10460_65 Bison_priscus_BS206_Sibh_23780_140 Bison_priscus_BS211_Sibh_43800_1100 Bison_priscus_BS216_NS_47000_2900 Bison_priscus_BS218_Si_14605_75 Bison_priscus_BS222_NWT_6110_45 Bison_priscus_BS223_Si_53300_1900 Bison_priscus_BS224_AK_13125_75 Bison_priscus_BS233_SW_16685_80 Bison priscus BS235 BIR 43400 900 Bison_priscus_BS236_SW_19420_100 Bison_priscus_BS237_AB_11240_70 Bison_priscus_BS243_SW_37550_400 Bison_priscus_BS244_LC_26210_170 Bison_priscus_BS248_OCr_12350_70 Bison_priscus_BS249_F_39200_550 Bison_priscus_BS253_LC_12665_65 Bison_priscus_BS254_CHL_10230_55 Bison_priscus_BS258_F_22120_130 Bison_priscus_BS260_D_30750_290 Bison_priscus_BS261_LC_12915_70 Bison_priscus_BS262_D_29150_500 Bison_priscus_BS281_BIR_40800_600 Bison_priscus_BS282_Si_56700_3200 Bison_priscus_BS284_Y_13135_65 Bison_priscus_BS286_Sim_49500_1300 Bison_priscus_BS287_BIR_49100_1700 Bison_priscus_BS289_BIR_2172_37 Bison_priscus_BS291_NS_49700_1400 Bison_priscus_BS292_NS_35710_730 Bison_priscus_BS294_BIR_58200_3900 Bison_priscus_BS297_NS_10990_50 Bison_priscus_BS311_BIR_12425_45 Bison_priscus_BS316_SI_57700_3000 Bison_priscus_BS318_NS_12410_50 Bison_priscus_BS320_SI_49600_1500 Bison_priscus_BS321_AK_9506_38 Bison_priscus_BS323_SI_37810_380 Bison_priscus_BS327_D_31530_230 Bison_priscus_BS328_SIdy_31690_180 Bison_priscus_BS329_D_27060_190 Bison_priscus_BS337_CHL_10378_36 Bison_priscus_BS340_NS_24500_180 Bison_priscus_BS345_NS_39800_1200 Bison_priscus_BS350_NS_38700_1000 Bison_priscus_BS351_BIR_57700_3200 Bison_priscus_BS359_NTC_20020_150 Bison_priscus_BS364_NS_38800_1100 Bison_priscus_BS365_NS_47000_2900 Bison_priscus_BS387_NS_33320_540 Bison_priscus_BS388_NS_27590_280 Bison_priscus_BS389_NS_17160_80 Bison_priscus_BS390_NS_31630_440 Bison_priscus_BS392_NS_36320_780 Bison_priscus_BS393_NS_39850_1200 Bison_priscus_BS394_NS_37460_890 Bos_indicus_AB268578_0_0 Bison priscus BS395 NS 40700 1300 Bos indicus AB268580 0 0 Bison_priscus_BS396_NS_23680_170 Bos_indicus_AY378134_0_0

BS397 NS 32370 47 Bison_priscus_BS398_NS_27400_260 Bison_priscus_BS400_NS_46100_2600 Bison_priscus_BS405_SI_23040_120 Bison_priscus_BS407_NWT_55500_3100 Bison_priscus_BS412_Y_30500_250 Bison_priscus_BS414_BIR_4495_60 Bison_priscus_BS415_D_30810_975 Bison_priscus_BS418_China_26560_670 Bison_priscus_BS438_AB_53800_2200 Bison_priscus_BS440_AB_60400_2900 Bison_priscus_BS443_AB_34050_450 Bison_priscus_BS459_China_47700_1000 Bison_priscus_BS469_AB_305_24 ison_priscus_BS472_F_13235_65 Bison_priscus_BS473_AB_56300_3100 Bison_priscus_BS477_D_33710_240 Bison_priscus_BS478_D_34470_200 Bison_priscus_BS490_BIR_2415_25 Bison_priscus_BS493_NS_50000_4200 Bison_priscus_BS494_NS_44800_2200 Bison priscus BS495 NS 29570 340 Bison_priscus_BS497_NS_30000_540 Bison_priscus_BS498_NS_25980_230 Bison_priscus_BS499_NS_31410_420 Bison priscus BS500 NS 35580 550 Bison_priscus_BS517_BIR_2526_26 Bison_priscus_BS564_Si_24570_90 Bison_priscus_BS571_SIdy_32910_170 Bison_priscus_BS605_NTC_20380_90 140 Bison_priscus_BS662_SI_20000_0 Bison_priscus_IB179_LC_12465_75 European bison Bison_bonasus_AF083356_0_0 Bison_bonasus_AY428860_0_0 Bison_bonasus_EF693811_0_0 Bison_bonasus_EU272053_0_0 Bison_bonasus_EU272054_0_0 Bison_bonasus_EU272055_0_0 Bison_bonasus_U12953_0_0 Bison_bonasus_U12954_0_0 Bison_bonasus_U34294_0_0 Yak Bos_grunniens_AY521140_0 0 Bos_grunniens_AY521149_0_0 Bos_grunniens_AY521150_0_0 Bos_grunniens_AY521151_0_0 Bos_grunniens_AY521152_0_0 Bos_grunniens_AY521154_0_0 Bos_grunniens_AY521155_0_0 Bos_grunniens_AY521156_0_0 Bos_grunniens_AY521160_0_0 Bos_grunniens_AY521161_0_0 Bos_grunniens_DQ007210_0_0 Bos_grunniens_DQ007221_0_0 Bos_grunniens_DQ007222_0_0 Bos_grunniens_DQ856594_0_0 Bos_grunniens_DQ856599_0_0 Bos_grunniens_DQ856600_0_0 Bos_grunniens_DQ856603_0_0 Bos_grunniens_DQ856604_0_0 Bos_grunniens_EF494177_0_0 Bos_grunniens_EF494178_0_0 Zebu Bos_indicus_AB085923_0_0 Bos_indicus_AB268563_0_0 Bos_indicus_AB268564_0_0 Bos_indicus_AB268566_0_0 Bos_indicus_AB268571_0_0 Bos_indicus_AB268574_0_0

Bos_indicus_EF417979_0_0 Bos_indicus_EF417981_0_0 Bos_indicus_EF417983_0_0 Bos_indicus_EF417985_0_0 Bos_indicus_EF524120_0_0 Bos_indicus_EF524125_0_0 Bos_indicus_EF524126_0_0 Bos indicus EF524128 0 0 Bos_indicus_EF524130_0_0 Bos_indicus_EF524132_0_0 Bos_indicus_EF524135_0_0 Bos indicus EF524141 0 0 Bos_indicus_EF524152_0_0 Bos indicus EF524156 0 0 Bos_indicus_EF524160_0_0 Bos_indicus_EF524166_0_0 Bos_indicus_EF524167_0_0 Bos indicus EF524170 0 0 Bos_indicus_EF524177_0_0 Bos_indicus_EF524180_0_0 Bos_indicus_EF524183_0_0 Bos indicus EF524185 0 0 Bos_indicus_L27732_0_0 Bos indicus L27736 0 0 Aurochs Bos primigenius DO915522 ALL1 12030 52 Bos_primigenius_DQ915523_CAT1_5650_0 Bos_primigenius_DQ915524_CHWF_3905_185 Bos_primigenius_DQ915537_CPC98_5936_34 Bos_primigenius_DQ915542_EIL06_5830_29 Bos_primigenius_DQ915543_EIL14_5830_29 Bos_primigenius_DQ915544_LJU3_8020_50 Bos_primigenius_DQ915558_NORF_3370_30 Bos_primigenius_EF187280_PVL04_3204_56 Cattle Bos_taurus_DQ124372_T4_0_0 Bos_taurus_DQ124375_T4_0_0 Bos_taurus_DQ124381_T3_0_0 Bos_taurus_DQ124383_T2_0_0 Bos_taurus_DQ124388_T3_0_0 Bos_taurus_DQ124394_T3_0_0 Bos_taurus_DQ124398_T3_0_0 Bos_taurus_DQ124400_T4_0_0 Bos_taurus_DQ124401_T4_0_0

Bos indicus AY378135 0 0

Bos_indicus_DQ887765_0_0 Bos_indicus_EF417971_0_0

Bos_indicus_EF417974_0_0 Bos_indicus_EF417976_0_0

Bos indicus EF417977 0 0

Bos_taurus_DQ124412_T4_0_0 Bos_taurus_EU177822_T3_0_0 Bos_taurus_EU177841_T1_0_0 Bos_taurus_EU177842_T1_0_0 Bos_taurus_EU177845_T1_0_0 Bos_taurus_EU177847_T1_0_0 Bos taurus EU177848 T1 0 0 Bos_taurus_EU177853_T2_0_0 Bos_taurus_EU177854_T2_0_0 Bos_taurus_EU177860_T2_0_0 Bos_taurus_EU177861_T2_0_0 Bos_taurus_EU177862_T5_0_0 Bos_taurus_EU177863_T5_0_0 Bos_taurus_EU177864_T5_0_0 Bos_taurus_EU177865_T5_0_0 Buffalo Bubalus bubalis AF197208 0 0 Bubalus_bubalis_AF475212_0_0

Bubalus_bubalis_AF475256_0_0 Bubalus_bubalis_AF475259_0_0 Bubalus_bubalis_AF475278_0_0 Bubalus_bubalis_AF475278_0_0 Bubalus_bubalis_AY488491_0_0 Bubalus_bubalis_EF536327_0_0 Bubalus_bubalis_EF536328_0_0 Bubalus bubalis EU268899 0 0 Bubalus_bubalis_EU268909_0_0

235 236 Supplementary Table 5. List of published whole mitochondrial genome sequences used for

236	phylogenetic analysis.		
	American bison	Cattle	Yak
	GU947000_Bison_bison_Plains_Nebraska_0	FJ971080_Bos_Q_Italy_Romagnola_0	KJ704989_Bos_grunniens_ChinaGansu_Gannan_0
	GU946976_Bison_bison_Plains_Montana_0	FJ971085_Bos_R_Italy_Cinisara_0	KR011113_Bos_grunniens_ChinaTibet_QinghaiPlateau_0
	GU947004_Bison_bison_Plains_Wyoming_0	EU177841_Bos_T1_Italy_chianina_0	KR052524_Bos_grunniens_ChinaTibet_Pali_0
	GU947006_Bison_bison_Wood_ElkIsland_0	DQ124383_Bos_T2_Korea_0	KJ463418_Bos_grunniens_ChinaQinghai_Dantong_0
	GU946987_Bison_bison_Plains_Montana_0	EU177815_Bos_T3_Italy_piemontese_0	KM233417_Bos_mutus_ChinaTibet_Yakow_0
	GU947005_Bison_bison_Wood_ElkIsland_0	DQ124372_Bos_T4_Korea_0	Buffalo
	GU947002_Bison_bison_Plains_Texas_0	EU177862_Bos_T5_Italy_valdostana_0	GU947003_Bison_bison_Plains_Texas_0
	GU947003_Bison_bison_Plains_Texas_0	Aurochs	AY488491_Bubalus_bubalis
	Wisent	GU985279_Bos_P_England_6760	AY702618_Bubalus_bubalis
	JN632602_Bison_bonasus_0	JQ437479_Bos_P_Poland_1500	AF547270_Bubalus_bubalis
	HQ223450_Bison_bonasus_0	Zebu	
	HM045017_Bison_bonasus_Poland_0	FJ971088_Bos_I1_Mongolia_0	
	Steppe bison	EU177870_Bos_I2_Iran_0	
237	KM593920_Bison_priscus_SGE2_France_TroisFreres_19151		
238			
239	Supplementary Table 6 f4 ratio estim	stes $f4(A \cap X C)$ is the number of the state of the sta	$f_{A}(A \cap B \cap C)$ is the
237	Supprementary Table 0. 14 Table estim	1000, 14(10, 0.00, 0.00) is the int	interation, 14(11,0,D,C) 15 the
240	denominator.		

Supplementary Table 6. f4 ratio estimates, f4(A,O,X,C) is the numerator, f4(A,O,B,C) is the

241 **S6-A.** Including heterozygotes

	<u> </u>										
Α	0	Х	С	:	Α	0	В	С	alpha	std.err	Z
AmericanBison	Ovis_aries	AllWisent+CladeX	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.890988	0.025788	34.551
AmericanBison	Ovis_aries	AllWisent + CladeX	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.109012	0.025788	4.227
AmericanBison	Ovis_aries	AllWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.884257	0.02918	30.304
AmericanBison	Ovis_aries	AllWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.115743	0.02918	3.967
AmericanBison	Ovis_aries	CladeX	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.893978	0.022763	39.273
AmericanBison	Ovis_aries	CladeX	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.106022	0.022763	4.658
AmericanBison	Ovis_aries	AncientWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.812638	0.054701	14.856
AmericanBison	Ovis_aries	AncientWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.187362	0.054701	3.425
AmericanBison	Ovis_aries	HistoricalWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.773802	0.032319	23.943
AmericanBison	Ovis_aries	HistoricalWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.226198	0.032319	6.999
AmericanBison	Ovis_aries	ModernWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.899149	0.031184	28.834
AmericanBison	Ovis_aries	ModernWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.100851	0.031184	3.234

242 243 244

S6-B. Haploidisation by randomly sampling an allele at heterozygous sites

-				-							
Α	0	X	С	:	Α	0	В	С	alpha	std.err	Z
AmericanBison	Ovis_aries	AllWisent+CladeX	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.894329	0.027147	32.944
AmericanBison	Ovis_aries	AllWisent+CladeX	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.105671	0.027147	3.893
AmericanBison	Ovis_aries	AllWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.88342	0.030518	28.947
AmericanBison	Ovis_aries	AllWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.11658	0.030518	3.82
AmericanBison	Ovis_aries	CladeX	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.912424	0.025204	36.202
AmericanBison	Ovis_aries	CladeX	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.087576	0.025204	3.475
AmericanBison	Ovis_aries	AncientWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.813521	0.059078	13.77
AmericanBison	Ovis_aries	AncientWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.186479	0.059078	3.156
AmericanBison	Ovis_aries	HistoricalWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.786183	0.035363	22.232
AmericanBison	Ovis_aries	HistoricalWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.213817	0.035363	6.046
AmericanBison	Ovis_aries	ModernWisent	Aurochs	:	AmericanBison	Ovis_aries	Steppe	Aurochs	0.899281	0.032252	27.883
AmericanBison	Ovis_aries	ModernWisent	Steppe	:	AmericanBison	Ovis_aries	Aurochs	Steppe	0.100719	0.032252	3.123

245 246

247

Supplementary Table 7: Bootstrap resampling of genotypes for testing topologies using D statistics.

The table shows the fraction of bootstrap replicates for which the original result was not recapitulated,

247 248 249 250 from 10000 bootstraps, for 10%, 20%, etc. subsets of the genotypes. A topology is considered to be

simple if it either has a non-significant D statistic (see Supplementary Figure 11), or has a D statistic

251 closest to zero with confidence intervals that do not overlap the D statistic for the other two topologies.

Most parsimonious topology	Simple topology	10%	20%	30%	40%	50%	60%	70%	80%	90%
((CladeX, Steppe), ModernWisent)	True	0.0067	0.0001	0.0	0.0	0.0	0.0	0.0	0.0	0.0
((Steppe, HistoricalWisent), ModernWisent)	False	0.0575	0.0573	0.0284	0.0036	0.0005	0.0	0.0	0.0	0.0
((ModernWisent, CladeX), HistoricalWisent)	False	0.1753	0.371	0.485	0.4427	0.3039	0.1564	0.0549	0.0072	0.0

denominator.

((CladeX, Steppe), HistoricalWisent)	True	0.0182	0.0174	0.0154	0.016	0.0113	0.0072	0.0022	0.0004	0.0
((AncientWisent, HistoricalWisent), ModernWisent)	True	0.0565	0.0152	0.0042	0.0012	0.0	0.0	0.0	0.0	0.0
((Steppe, HistoricalWisent), AncientWisent)	False	0.0151	0.0039	0.0001	0.0002	0.0	0.0	0.0	0.0	0.0
((AncientWisent, Steppe), ModernWisent)	True	0.0484	0.0086	0.0014	0.0002	0.0	0.0	0.0	0.0	0.0
((CladeX, Steppe), AncientWisent)	False	0.0304	0.0142	0.0086	0.0063	0.0033	0.0025	0.0015	0.0001	0.0
((AncientWisent, CladeX), ModernWisent)	True	0.0703	0.0213	0.0062	0.0015	0.0007	0.0	0.0	0.0	0.0
((HistoricalWisent, CladeX), AncientWisent)	False	0.0184	0.0053	0.001	0.0005	0.0	0.0	0.0	0.0	0.0
((ModernWisent, HistoricalWisent), Aurochs)	False	0.0591	0.0031	0.0005	0.0	0.0	0.0	0.0	0.0	0.0
((Aurochs, ModernWisent), CladeX)	False	0.2229	0.2476	0.0824	0.0115	0.0009	0.0	0.0	0.0	0.0
((HistoricalWisent, CladeX), Aurochs)	True	0.0061	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
((Steppe, CladeX), Aurochs)	True	0.0001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
((Steppe, HistoricalWisent), Aurochs)	True	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
((Steppe, ModernWisent), Aurochs)	False	0.1362	0.0535	0.0048	0.0007	0.0002	0.0	0.0001	0.0	0.0
((Steppe, AncientWisent), Aurochs)	True	0.0441	0.0082	0.0001	0.0001	0.0	0.0	0.0	0.0	0.0
((AncientWisent, CladeX), Aurochs)	True	0.0276	0.0058	0.0004	0.0001	0.0	0.0	0.0	0.0	0.0

254 255 **Supplementary Table 8:** Hypergeometric test for shared derived steppe alleles. Steppe derived sites were filtered for coverage depth in the wisent lineages 1 and 2, for which the test was performed. In the last row, wisent represents all wisent other than CladeX.

1	2	Steppe	Derived 1	Derived 2	Common	Р
Ancient Wisent	CladeX	161	111	133	108	1.72E-12
Ancient Wisent	Historical Wisent	174	115	119	108	1.37E-24
Ancient Wisent	Modern Wisent	178	124	108	95	5.12E-11
CladeX	Historical Wisent	529	448	385	370	3.09E-29
CladeX	Modern Wisent	556	469	350	326	2.79E-13
Historical Wisent	Modern Wisent	618	436	372	342	5.50E-48
Wisent	CladeX	557	357	468	332	4.18E-14

Supplementary Table 9: Hypergeometric test for shared derived aurochs alleles. Aurochs derived

257 258 259 sites were filtered for coverage depth in the wisent lineages 1 and 2, for which the test was performed. In the last row, wisent represents all wisent other than CladeX.

1	2	Aurochs	Derived 1	Derived 2	Common	Р
Ancient Wisent	CladeX	758	20	9	4	4.11E-05
Ancient Wisent	Historical Wisent	822	22	11	8	1.01E-11
Ancient Wisent	Modern Wisent	826	25	22	12	1.49E-14
CladeX	Historical Wisent	2517	36	47	16	7.34E-20
CladeX	Modern Wisent	2580	39	73	15	1.99E-14
Historical Wisent	Modern Wisent	2845	58	83	39	2.66E-50
Wisent	CladeX	2634	93	41	15	1.58E-12

260

261 262 263 Supplementary Table 10: The weighted sample median M, the weighted sample mode Mo, and the

prediction error

 E_{pred} , for each ABC analysis.

Trio	^ M	Mo	E _{pred}
A875, 6A, Aurochs	0.8660	0.9204	0.4534
A3133, 6A, Aurochs	0.8480	0.9172	0.4881
A875, Historical Wisent, Aurochs	0.8636	0.9323	0.4187
A3133, Historical Wisent, Aurochs	0.8646	0.9384	0.4921
All	0.8250	0.9034	0.5111

264

265 Supplementary Table 11: Empirical posterior probabilities for levels of hybridisation 1%-5%, for each trio.

л. •	10/	20/	20/	40/	50/
1 rio	1%	Ζ%	3 %	4%	5%
A875, 6A, Aurochs	0.9620	0.9340	0.8720	0.8400	0.8120
A3133, 6A, Aurochs	0.9600	0.9600	0.8840	0.8440	0.7980
A875, Historical Wisent, Aurochs	0.9660	0.9340	0.8860	0.8520	0.7940
A3133, Historical Wisent, Aurochs	0.9580	0.9100	0.8580	0.8080	0.7640
All	0.9720	0.9440	0.9140	0.8760	0.8760

269 Supplementary Note 1:

270 Samples, DNA extraction and sequencing

271

272 Samples and radiocarbon dating

- For clarity purposes we kept the most commonly used taxonomic nomenclature of
- bovine throughout the study. Although not yet widely accepted, it has been proposed
- to sink the genus *Bison* into *Bos* based on the shallow time depth of their evolutionary
- 276 history ⁷. The validity of such genetic separation is further tested in this study.
- 277 Samples from a total of 87 putative bison bones were collected from 3 regions across
- 278 Europe: Urals, Caucasus, and Western Europe (Supplementary Data 1). As shown in
- the Supplementary Data 1, most of the samples were from bones identified as bison or
- 280 bovid post-cranial samples, because cranial material is rare for this time period.
- 281 The main set of samples, from northeastern Europe, represents isolated bones
- 282 excavated from a wide variety of cave deposits throughout the Ural Mountains and
- surrounding areas. These samples are housed at the Zoological Museum of the
- 284 Institute of Plant and Animal Ecology (ZMIPAE) in Ekaterinburg, Russia.
- 285 In southeastern Europe, bovid bone fragments were excavated in Mezmaiskaya Cave
- 286 in the Caucasus Mountains. Samples were obtained from the Laboratory of Prehistory
- 287 in St Petersburg. Additional six samples from the Caucasus are identified as
- 288 Caucasian bison (B. bonasus caucasicus, hereafter referred to as historical wisent):
- 289 two of them are from the National History Museum (NHM) in London, and four come
- from hunts in the Kuban Oblast in the early 20th century (one collected by scientist
- 291 Viktor Iwanovich Worobjew in 1906 and three hunted during the Kuban Hunt under
- the Grand Duke Sergei Mikhailovich of Russia), currently held at the Zoological
- 293 Institute of the Russian Academy of Sciences (ZIRAS Saint Petersburg, Russia).
- Four additional bones from the Caucasus region comes from the eastern border with
- 295 Ukraine and are held at the Institute of Archeology (IAKiev), Ukrainian Academy of
- 296 Sciences, Kiev.
- 297 Most western European bones come from late Pleistocene deposits on the North Sea
- bed. These specimens, now curated by the North Sea Network (NSN) in the
- 299 Netherlands, were recovered by trawling operations and as such have little
- 300 stratigraphic information. Specimens were selected on the basis of their
- 301 morphological similarities with the 'small form' described by Drees and Post⁸.
- 302 Three bones held in the collections of the Vienna Natural History Museum (VNHM),
- and three bones held in the Museum National d'Histoire Naturelle (Paris) come from
 central European Holocene sites.
- 305 Finally, one bone comes from the Monti Lessini rock-shelter site Riparo Tagliente in
- the North of Italy, one bone comes from the Swiss site of Le Gouffre de la combe de
- 307 la racine in the Jura mountains (Swiss Institute for Speleology and Karst Studies,
- 308 ISSKA), and one bone comes from l'Aven de l'Arquet in the Gard region of France
- 309 (Musée de Préhistoire d'Orgnac).
- 310 In addition, two samples from the Beringian region were used: one sample, a steppe
- bison astragalus from the Yukon territory (Canada), has previously been used in a
- 312 study of cytosine methylation in ancient DNA ⁹; and another steppe bison from
- 313 Alyoshkina Zaimka in Siberia.
- 314

- 315 All non-contemporaneous samples from which bison mitochondrial control region
- 316 sequences were successfully amplified were sent for accelerator mass spectrometry
- 317 (AMS) radiocarbon dating (except for seven samples from level 3 of the
- 318 Mezmaiskaya cave, which were expected to be older than AMS dating capabilities
- ^{10,11}). The dating was performed by the AMS facility at the Oxford Radiocarbon
- 320 Accelerator Unit at the University of Oxford (OxA numbers), the Eidgenössische
- 321 Technische Hochschule in Zürich for a Ukrainian sample (ETH number), and the
- Angström Laboratory of the University of Uppsala, Sweden, for the Swiss sample (Ua
- number). The results are shown in Supplementary Data 1, with all dates reported in
- kcal yr BP unless otherwise stated. The calibration of radiocarbon dates was performed using OxCal v4.1 with the IntCal13 curve 12 .
- 326 In addition, two bones identified as bison were previously dated at the Centre for
- 327 Isotope Research, Radiocarbon Laboratory, University of Groningen, Netherlands,
- 328 with infinite radiocarbon age, consistently with the dating performed at Oxford
- 329 (A2808-JGAC26=GrA-34533; A2809-JGAC27=GrA-34524).

330

331 Ancient DNA extraction

- All ancient DNA work was conducted in clean-room facilities at the University of
- 333 Adelaide's Australian Centre for Ancient DNA, Australia (ACAD), and at the
- 334 University of Tuebingen, Germany (UT) following published guidelines ¹³.
- 335 <u>University of Adelaide:</u>
- 336 Samples were UV irradiated (260 nm) on all surfaces for 30 min. Sample surface was
- 337 wiped with 3% bleach, then ~1 mm was removed using a Dremel tool and
- 338 carborundum cutting disks. Each sample was ground to a fine powder using a Mikro-
- 339 Dismembrator (Sartorius). Two DNA extraction methods were used during the course
- of the project (see Supplementary Data 1 for the method used for specific samples):
- 341 Phenol-chloroform method: Ancient DNA was extracted from 0.2-0.5g powdered
- bone using phenol-chloroform and centrifugal filtration methods according to a previously published method 2 .
- *In solution silica based method*: Ancient DNA was extracted from 0.2-0.3g
- powdered bone according to a previously published method 14 .
- 346 <u>University of Tuebingen:</u>
- 347 Samples were UV-irradiated overnight to remove surface contamination. DNA
- 348 extraction was performed following a guanidinium-silica based extraction method ¹⁵
- 349 using 50mg of bone powder. A DNA library was prepared using 20µl of extract for
- 350 each sample according to ¹⁶. Sample-specific indexes were added to both library
- adapters to differentiate between individual samples after pooling and multiplex
- 352 sequencing ¹⁷. Indexed libraries were amplified in 100µl reactions, followed by
- 353 purification over Qiagen MinElute spin columns (Quiagen, Hilden, Germany).

355 Sequencing of the mitochondrial control region

356 A ~600 bp fragment of the mitochondrial control region was amplified in one or up to 357 four overlapping fragments, depending on DNA preservation. PCR amplifications 358 were performed using primers designed for the bovid mitochondrial control region, 359 following the method described in 2 .

360 One-step simplex PCR amplifications using Platinum *Taq* Hi-Fidelity polymerase

361 were performed on a heated lid thermal cycler in a final volume of 25 µl containing 1

362 µl of aDNA extract, 1mg/ml rabbit serum albumin fraction V (RSA; Sigma-Aldrich,

363 Sydeny, NSW), 2 mM MgSO₄ (Thermo Fisher, Scoresby VIC), 0.6 μM of each

primer (Supplementary Table 1), 250 μM of each dNTP (Thermo Fisher), 1.25 U

Platinum *Taq* Hi-Fidelity and $1 \times$ Hi-Fidelity PCR buffer (Thermo Fisher). The

366 conditions for PCR amplification were initial denaturation at 95°C for 2 min,

followed by 50 cycles of 94°C for 20 sec, 55°C for 20 sec and 68°C for 30 sec, and a
final extension at 68°C for 10 min at the end of the 50 cycles.

369 Multiplex primer sets A and B were set up separately (Supplementary Table 1).

370 Multiplex PCR was performed in a final volume of $25 \,\mu$ l containing 2 μ l of aDNA

371 extract, 1 mg/ml RSA, 6 mM MgSO₄, 0.2 μM of each primer (Supplementary Table

1), 500 μM of each dNTP, 2 U Platinum Taq Hi-Fidelity and 1 × Hi-Fidelity PCR

373 buffer. Multiplex PCR conditions were initial denaturation at 95°C for 2 min,

followed by 35 cycles of 94°C for 15 sec, 55°C for 20 sec and 68°C for 30 sec, and a

final extension at 68°C for 10 min at the end of the 35 cycles. Multiplex PCR

products were then diluted to 1:10 as template for the second step of simplex PCR.

377 The simplex PCR, using Amplitaq Gold (Thermo Fisher) or HotmasterTM Taq DNA

polymerase (5Prime, Milton, Qld), was conducted in a final volume of 25 μl

379 containing 1 μ l of diluted multiplex PCR product, 2.5 mM MgCl₂, 0.4 μ M of each

380 primer (Supplementary Table 1), 200 μ M of each dNTP, 1 U Amplitaq

381 Gold/Hotmaster *Taq* polymerase and $1 \times PCR$ buffer. The PCR conditions were initial

denaturation at 95°C for 2 min, followed by 35 cycles of 94°C for 20 sec, 55°C for 15

383 sec and 72°C for 30 sec, and a final extension at 72°C for 10 min at the end of the 35

cycles. Multiple PCR fragments were cloned to evaluate the extent of DNA damageand within-PCR template diversity.

386 PCR products were then checked by electrophoresis on 3.5-4.0% agarose TBE gels,

and visualized after ethidium bromide staining on a UV transilluminator. PCR

388 amplicons were purified using Agencourt[®] AMPure magnetic beads (Beckman

389 Coulter, Lane Cove, NSW) according to the manufacturer's instructions. Negative

390 extraction controls and non-template PCR controls were used in all experiments.

391 All purified PCR products were bi-directionally sequenced with the ABI Prism[®]

392 BigDyeTM Terminator Cycle Sequencing Kit version 3.1 (Thermo Fisher). The

393 sequencing reactions were performed in a final volume of 10 µl containing 3.2 pmol

394 of primer (Supplementary Table 1), 0.25 µl Bigdye terminator premixture, and 1.875

 μ of 5 × sequencing buffer. The reaction conditions included initial denaturation at

396 95°C for 2 min, 25 cycles with 95°C for 10 sec, 55°C for 15 sec, and 60°C for 2 min

397 30 sec. Sequencing products were purified using Agencourt[®] Cleanseq magnetic

398beads (Beckman Coulter) according to the manufacturer's protocol. All sequencing

399 reactions were analysed on an ABI 3130 DNA capillary sequencer (Thermo Fisher).

400 Mitochondrial control region sequences (>400bp) were successfully amplified from
401 65 out of 87 analysed samples. Three samples produced a mixture of cattle and bison

- 402 amplification products; these were identified as contaminated and removed from all
- 403 analyses. Sequences from two individuals did not match bovid haplotypes and were
- 404 identified as brown bear and elk in BLAST searches (see Supplementary Data 1). This
- 405 is presumably due to the source postcranial elements being morphologically
- 406 ambiguous and misidentified.
- 407

408 Sequencing of the whole mitochondrial genome

- 409 To provide deeper phylogenetic resolution and further examine the apparent close
- 410 relationship between *Bos* and wisent mitochondria, full mitogenome sequences of 13
- 411 CladeX specimens, as well as one ancient wisent, one historical wisent, and one
- 412 steppe bison were generated using hybridisation capture with RNA probes.
- 413

414 Samples A001, A004, A018, A4089 (CladeX)

- 415 DNA library preparation
- 416 DNA repair and polishing were performed in a reaction that contained 20 µl DNA
- 417 extract, 1x NEB Buffer 2 (New England Biolabs, Ipswich, MA), 3U USER enzyme
- 418 cocktail (New England Biolabs), 20U T4 polynucleotide kinase (New England
- 419 Biolabs), 1mM ATP, 0.1 mM dNTPs (New England Biolabs), 8 μ g RSA, and H₂O to
- 420 38.5 μ l. The reaction was incubated at 37°C for 3 hours then 4.5U of T4 DNA
- 421 polymerase (New England Biolabs) was added and the reaction incubated at 25°C for
- 422 a further 30 min. Double-stranded libraries were then built with truncated Illumina
- 423 adapters containing dual 5-mer internal barcodes as in 16 .
- 424

425 Amplification of Bos taurus mitochondrial in vitro transcription (IVT) templates

- 426 RNA probes were generated from long-range PCR products of *Bos taurus*
- 427 mitochondrial DNA. The NCBI Primer-Blast program
- 428 (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to design primers to
- 429 amplify the *Bos taurus* mitochondrial genome (NC_006853.1) in three overlapping
- 430 sections: mito-1 (6568 bp), mito-2 (6467 bp), and mito-3 (5390 bp). Primer pairs
- 431 were designed with a high melting temperature to permit amplification with 2-stage
- 432 PCR and the T7 RNA promoter was attached to the 5' end of one primer from each 433 pair 18 (Supplementary Table 1). Amplification of each mitochondrial section was
- pair ¹⁸(Supplementary Table 1). Amplification of each mitochondrial section was
 performed using a heated lid thermal cycler in multiple PCRs containing 1x Phire
- 434 performed using a neared nd mermai cycler in multiple PCRs containing 1x Prine
 435 Buffer (Thermo Fisher), 25 ng calf thymus DNA (Affymetrix, Santa Clara, CA), 200
- 436 μ M dNTPs , 500 nM forward and reverse primers, 0.5 μ l Phire Hot Start II DNA
- 437 polymerase (Thermo Fisher), and H_2O to 25 µl. The mito-1 and mito-2 sections were
- 438 amplified with a thermal cycler program of 1 cycle: 98°C for 30 sec; 26 cycles: 98°C
- 439 for 10 sec and 72°C for 70 sec; and 1 cycle: 72°C for 180 sec whilst the program for
- 440 mito-3 was 1 cycle: 98°C for 30 sec, 28 cycles: 98°C for 10 sec and 72°C for 60 sec,
 441 and 1 cycle: 72°C for 180 sec. After amplification, 2 1 of each PCR was agarose ge
- and 1 cycle: 72°C for 180 sec. After amplification, 2 l of each PCR was agarose gel
 electrophoresed and the product visualized with Gel-Red (Biotium, Hayward, CA)
- staining and UV illumination. Amplification of mito-1 and mito-2 produced a single
- band and the PCRs for these mitochondrial sections were separately pooled and then
- 445 purified with QiaQuick columns (Qiagen, Chadstone Centre, VIC) following the
- 446 provided PCR cleanup protocol. Amplification of mito-3 produced unwanted
- 447 products and the correct size amplicon was size selected using gel excision followed

448 by purification with QiaQuick columns using the gel extraction protocol. Purified

449 amplicons from each mitochondrial section were quantified using a NanoDrop 2000

450 Spectrophotometer (Thermo Fisher).

451

452 Transcription of Bos taurus mitochondrial IVT templates

453 Each of the three mitochondrial IVT templates were transcribed using a T7 High 454 Yield RNA Synthesis Kit (New England Biolabs) in multiple reactions containing 455 150-200 ng purified amplicon, 1x Reaction Buffer, 10 mM rNTPs, 2 µl T7 enzyme 456 mix, and H_2O to 20 µl. The IVT reactions were incubated for 16 hours at 37°C and 457 then the DNA template was destroyed by incubating for an additional 15 min at 37°C 458 with 2U Turbo Dnase (Thermo Fisher). IVT reactions for each mitochondrial section 459 were separately pooled and purified with Megaclear spin columns (Thermo Fisher) 460 except that H₂O was used to elute the RNA instead of the provided elution buffer. The 461 elution buffer provided with the Megaclear kit was found to inhibit fragmentation in 462 the next step. Integrity of the RNA was verified on an acrylamide gel and the mass 463 quantified with a Nanodrop 2000 Spectrophotometer.

464

465 Fragmentation of mitochondrial IVT RNA

466 RNAs from the IVT transcription were fragmented with a NEBNext Magnesium

467 RNA Fragmentation Module (New England Biolabs) in reactions that contained 1x

468 Fragmentation buffer, 45 μ g RNA, and H₂O to 20 μ l. Reactions were incubated at

469 94°C for 10 min and fragmentation stopped with the addition of 2 μ l Stop Buffer.

470 After fragmentation, each reaction was purified with a RNeasy MinElute spin column

471 (Qiagen) by following the provided cleanup protocol except for the final elution. To

472 elute, $20 \,\mu L \,H_2O$ was pipetted into the column and the column was heated at 65°C for

5 min and then centrifuged at 15,000 g for 1 min. The flow-through was transferred

to a 1.5 ml tube and stored at -80°C. The fragmented RNA was quantified on a

475 NanoDrop 2000 Spectrophotometer and 100 ng was visualized on an acrylamide gel476 producing a smear in the range of 80-300 bases.

477

478 Biotinylation of fragmented RNA

479 Biotinylation was performed in several reactions containing 6.7 µg each of mito-1,

480 mito-2, and mito-3 fragmented RNA, 40 µl Photoprobe Long Arm (Vector

481 Laboratories, Burlingame, CA), and H_2O to 80 μ l in 200 μ l PCR tubes. The tubes

482 were placed in a 4°C gel cooling rack and then incubated under the bulb of a UV

483 sterilization cabinet for 30 min. Organic extractions were performed on the labelling

- reactions by adding $64 \ \mu l H_2 O$, $16 \ \mu l l M$ Tris buffer, and $160 \ \mu l$ sec-butanol to each
- 485 tube and shaking vigorously for 30 sec followed by centrifugation for 1 minute at 486 1000 g. The upper organic layers were discarded and the extraction repeated with an

486 1000 g. The upper organic layers were discarded and the extraction repeated with an 487 additional 160 ul sec-butanol. After the second organic layers were discarded, the

additional 160 µl sec-butanol. After the second organic layers were discarded, the
 remaining aqueous phases were purified with RNeasy MinElute spin columns

- following the provided reaction cleanup protocol but with a modified elution
- 439 procedure described in the previous step. Elutions with similar RNA were pooled and
- then quantified with a NanoDrop Spectrophotometer 2000 and the RNA, which will
- 492 now be called probe, was stored at -80°C in 5 μ l aliquots at 100 ng/ μ l.
- 493

494 Repetitive sequence blocking RNA

495 RNA to block repetitive sequences in bison aDNA was transcribed from Bovine HyBlockTM DNA (i.e. Cot-1 DNA, Applied Genetics Laboratories Inc., Melbourne, 496 FL) using a published linear amplification protocol¹⁹. Briefly, the HyBlock DNA 497 498 was polished in a reaction containing T4 polynucleotide kinase and T4 DNA 499 polymerase and purified with MinElute spin columns following the PCR cleanup 500 protocol provided. Tailing was performed on the polished DNA with terminal 501 transferase and a tailing solution containing 92 µM dTTP (Thermo Fisher) and 8 µM 502 ddCTP (Affymetrix). After tailing, the Hybloc DNA was purified with MinElute spin 503 columns as before. The HyBlock DNA was then heat denatured and the T7-A18B 504 primer (Supplementary Table 1), containing the T7 RNA polymerase promoter, was 505 allowed to anneal to the poly-T tail with slow cooling. A second-strand synthesis 506 reaction was then performed on the HyBlock DNA using DNA polymerase I Klenow 507 fragment (New England Biolabs) and the product was purified with MinElute spin 508 columns. The double stranded HyBlock DNA was transcribed using a T7 High Yield 509 RNA Synthesis Kit in multiple reactions containing 75 ng DNA, 1x Reaction Buffer, 510 10 mM rNTPs, 2 μ l T7 enzyme mix, and H₂O to 20 μ l. IVT reactions were incubated 511 for 16 hours at 37°C and then the DNA template was destroyed by adding 2U Turbo 512 Dnase and incubating for an additional 15 min at 37°C. The RNA was purified with 513 RNeasy MinElute spin columns as above. Purified RNA was quantified on a 514 NanoDrop 2000 and 100 ng visualized on an acrylamide gel, which produced a smear 515 80 to 500 bp in length.

516

517 Primary mitochondrial hybridisation capture

518 Truncated versions of the Illumina adapters were used for hybridisation capture

519 because full-length adapters reduce enrichment efficiency 20 . For the primary

520 hybridisation capture, three Reagent Tubes were prepared for each bison library with

521 the following materials: Reagent Tube #1- 3.5 µl of 35-55 ng/µl DNA library;

522 Reagent Tube #2- 5 µl probes, 1 µl HyBlock RNA, and 0.5 µl of 50 µM P5/P7 RNA

523 blocking oligonucleotides (Supplementary Table 1); Reagent Tube #3- 30 μl

- 524 Hybridisation Buffer ²¹: 75% formamide (Thermo Fisher), 75 mM HEPES, pH 7.3, 3
- 525 mM EDTA (Thermo Fisher), 0.3% SDS (Thermo Fisher), and 1.2 M NaCl (Thermo

526 Fisher). Hybridisation capture was performed in a heated lid thermal cycler

- 527 programmed as follows: Step 1- 94°C for 2 min, Step 2- 65°C for 3 min, Step 3- 42°C
- 528 for 2 min, Hold 4- 42°C hold. To start hybridisation capture, Reagent Tubes were
- 529 placed in the thermal cycler at the start of each program Step in the following order:

530 Step 1- Reagent Tube #1; Step 2- Reagent Tube #2; Step 3- Reagent Tube #3. For

each library, once the Hold cycle started 20 μl of hybridisation buffer from Reagent

532 Tube #3 was mixed with the RNA in Reagent Tube #2. The entire content of Reagent

533 Tube #2 was then pipetted into Reagent Tube #1 and mixed with the bison library to 534 begin the hybridisation capture. Hybridisation capture was carried out at 42°C for 48

535 hours.

536 Magnetic streptavidin beads (New England Biolabs) were washed just prior to the end

537 of the hybridisation capture incubation. For each library, 50 µl of beads were washed

twice using 0.5 ml Wash Buffer 1(2X SSC+0.05% Tween-20, all reagents Thermo

539 Fisher) and a magnetic rack. We also saturated all magnetic bead sites that could

540 potentially bind nucleic acid in a non-specific fashion using yeast tRNA, to optimise

541 the expected and specific streptavidin-biotin binding. Briefly, the beads were blocked

- 542 by incubation in 0.5 ml Wash Buffer 1+ 100 μg yeast tRNA (Thermo Fisher) for 30
- 543 min on a rotor. Blocked beads were washed once as before and then suspended in 0.5
- ml Wash Buffer. At the end of the hybridisation capture, each reaction was added to a
- 545 tube of blocked beads and incubated at room temperature for 30 min on a rotor. The 546 beads were then taken through a series of stringency washes as follows: Wash 1 - 0.5
- ml Wash Buffer 1 at room temperature for 10 min; Wash 2 0.5 ml Wash Buffer 2
- 548 (0.75X SSC + 0.05% Tween-20) at 50°C for 10 min; Wash 3 0.5 ml Wash Buffer 2
- 549 at 50°C for 10 min; Wash 4 0.5 ml Wash Buffer 3 (0.2X SSC + 0.05% Tween-20) at
- 550 50°C for 10 min. After the last wash, the captured libraries were released from the
- 551 probe by suspending the beads in 50 µl of Release buffer (0.1 M NaOH, Sigma
- Aldrich) and incubating at room temperature for 10 min. The Release buffer was then
- neutralized with the addition of 70 μ l Neutralization buffer (1 M Tris-HCl pH 7.5,
- 554 Thermo Fisher). Captured libraries were then purified with MinElute columns by first
- adding 650 μ l PB buffer and 10 μ l 3 M sodium acetate to adjust the pH for efficient
- 556 DNA binding. Libraries were purified using the provided PCR cleanup protocol and
- 557 eluting with 35 μ l EB+0.05% Tween-20.
- 558

559 Primary hybridisation capture amplification

- 560 Amplification of each primary hybridisation capture was performed in five PCRs 561 containing 5 ul of primary captured library, 1X Phusion HF buffer (Thermo Fisher)
- containing 5 µl of primary captured library, 1X Phusion HF buffer (Thermo Fisher),
 200 µM dNTPs, 200 µM each of primers IS7_short_amp.P5 and IS8_short_amp.P7
- 562 200 µM div Fy, 200 µM each of primers is 7_short_amp.P3 and iss_short_amp.P7 563 (Supplementary Table 1), 0.25 U Phusion Hot Start II DNA polymerase (Thermo
- 564 (Supplementary Fable 1), 0.25 C Fluston Hot Start II DNA polymerase (Thermo 564 Fisher), and H_2O to 25 µl. The five PCR products were pooled and DNA was purified
- 565 using AMPure magnetic beads.
- 566

567 Secondary mitochondrial hybridisation capture

- Amplified primary libraries were taken through a second round of hybridisation
 capture using the same procedure as describe in *Primary mitochondrial hybridisation capture* step.
- 570
- 571

572 Secondary hybridisation capture amplification

- 573 Indexed primers were used to convert the DNA from the secondary hybridisation
- 574 capture to full length Illumina sequencing libraries. Each library was amplified in
- 575 three PCRs containing 5 µl secondary hybridisation capture library, 1X Phusion HF
- 576 buffer, 200 μ M dNTPs, 200 μ M each of primers GAII_Indexing_x (library specific
- 577 index) and IS4 (Supplementary Table 1), 0.25 U Phusion Hot Start II DNA
- 578 polymerase, and H_2O to 25 μ l. Amplification was performed in a heated lid thermal
- 579 cycler programmed as follows 1 cycle: 98°C for 30 sec; 10 cycles: 98°C for 10 sec,
- 580 60°C for 20 sec, 72°C for 20 sec; and 1 cycle: 72°C for 180 sec. The five PCR
- 581 products were pooled and DNA was purified using AMPure magnetic beads.

582

583 Samples A003, A005, A006, A007, A017, A15526, A15637, A15668 (CladeX),

- 584 A4093 (ancient wisent) and A15654 (historical wisent)
- 585 DNA library preparation
- 586 Double-stranded Illumina libraries were built from 20 µl of each DNA extract using

587 partial UDG treatment 22 and truncated Illumina adapters with dual 7-mer internal

- 588 barcodes, following the protocol from 23 .
- 589

590 Hybridisation capture

- 591 Commercially synthesised biotinylated 80-mer RNA baits (MYcroarray, MI, USA)
- 592 were used to enrich the target library for mitochondrial DNA. Baits were designed as
- 593 part of the commercial service using published mitochondrial sequences from 24
- 594 placental mammals, including *Bison bison* and *Bos taurus*.
- 595 One round of hybridisation capture was performed according to the manufacturer's
- 596 protocol (MYbaits v2 manual) with modifications. We used P5/P7 RNA blocking
- 597 oligonucleotides (Supplementary Table 1) instead of the blocking oligonucleotides
- 598 provided with the kit. We also incubated the magnetic beads with yeast tRNA to
- 599 saturate all potential non-specific sites on the magnetic beads that could bind nucleic 600 acids and increase the recovery of non-specific DNA and therefore decrease the final
- 601 DNA yield.
- 602 Indexed primers were used to convert the capture DNA to full length Illumina
- sequencing libraries. Each library was amplified in eight PCRs containing 5 μ l
- 604 hybridisation capture library, 1x Gold Buffer II, 2.5mM MgCl₂, 200 μM dNTPs, 200
- μ M each of primers GAII_Indexing_x (library specific index) and IS4
- 606 (Supplementary Table 1), 1.25 U Amplitaq Gold DNA polymerase, and H_2O to 25 µl.
- Amplification was performed in a heated lid thermal cycler programed as follows 1
- 608 cycle: 94° C for 6 min; 15 cycles: 98° C for 30 sec, 60° C for 30 sec, 72° C for 40 sec;
- and 1 cycle: 72°C for 180 sec. The PCR products were pooled and DNA was purified
- 610 using AMPure magnetic beads (Agencourt[®], Beckman Coulter).
- 611

612 Samples LE237, LE242 and LE257 (CladeX)

- 613 Target DNA enrichment was performed by capture of the pooled libraries using DNA
- baits generated from bison (*Bison bison*) mitochondrial DNA 24 . The baits were
- 615 generated using three primer sets (Supplementary Table 1, f) designed with the
- 616 Primer3Plus software package ²⁵. All extractions and pre-amplification steps of the
- 617 library preparation were performed in clean room facilities and negative controls were
- 618 included for each reaction.
- 619

620 <u>Sample A3133 (steppe bison)</u>

- 621 DNA repair and polishing were performed in a reaction that contained 20 μl bison
- 622 A3133 extract, 1x NEB Buffer 2, 3U USER enzyme cocktail, 20U T4 polynucleotide
- 623 kinase, 1mM ATP, 0.1 mM dNTPs, 8 μ g RSA, and H₂O to 38.5 μ l. The reaction was
- 624 incubated at 37°C for 3 hours then 4.5U of T4 DNA polymerase was added and the
- reaction incubated at 25° C for a further 30 min. Double-stranded libraries were then
- built with truncated Illumina adapters containing dual 5-mer internal barcodes as in 16
- 627 with the final amplification with indexed primers using Phusion Hot Start II DNA 628 polymetrize to obtain full length Illuming sequencing libraries
- 628 polymerase to obtain full length Illumina sequencing libraries.
- 629

630 Nuclear locus capture

631 Genome-wide nuclear locus capture was attempted on DNA repaired libraries of 13

bison samples (as described above - see Supplementary Supplementary Table 2). Two

633 different sets of probe were used (as described below), but ultimately, only the 9908

634 loci common to both sets were used for comparative analysis (see nuclear locus

635 analysis section).

636

- 637 <u>Probe sets</u>
- 638 40k SNP probe set

639 This probe set was originally designed to enrich 39,294 of the 54,609 BovineSNP50 640 v2 BeadChip (Illumina) bovine single nucleotide polymorphism (SNP) loci used in a previous phylogenetic study ²⁶, allowing for a direct comparison of the newly 641 642 generated data to published genotypes. The discrepancy in the number of surveyed 643 targets was due to manufacturing constraints, as the flanking sequences surrounding 644 certain bovine SNP were too degenerate for synthesis with the MyBaits technology. 645 Probes (MYcroarray, Ann Arbor, MI) were 121-mer long, centred on the targeted 646 bovine SNP and with no tiling, as per the original design of the BovineSNP50 v2 BeadChip²⁷. 647

648 The BovineSNP50 v2 BeadChip assay targets SNPs that are variable in Bos taurus in 649 order to genotype members of cattle breeds. Consequently, SNPs are heavily 650 ascertained to be common in cattle, and their use in phylogenetic studies of other 651 bovid species results in levels of heterozygosity that decrease rapidly with increased 652 genetic distance between cattle and the species of interest. Decker et al. (2009) found 653 the average minor allele frequency in plains bison and wood bison for the 40,843 654 bovine SNPs used in the phylogenetic analysis was 0.014 and 0.009, respectively. 655 Average minor allele frequencies ranged from 0.139 to 0.229 in breeds of taurine 656 cattle.

657

658 10k SNP probe set

659 A second set of probes was ordered from MyBaits that targeted a 9,908 locus subset 660 of the previous 39,294 bovine SNPs selected for enrichment. This smaller subset was 661 chosen to minimise ascertainment bias during phylogenetic and population analyses 662 based on their polymorphism within the diversity of available modern genotypes of 663 bison (American and European), Yak, Gaur and Banteng (total of 72 individuals). All 664 of these taxa belong to a monophyletic clade, outside of the cattle diversity, and are 665 consequently all equidistant from the cattle breeds that were used to ascertain the SNP 666 ²⁷, therefore reducing the impact of ascertainment bias when conducting comparisons 667 within the clade. The exclusion of monomorphic sites across specie allows focusing 668 the capture on loci that are more likely to be phylogenetically informative within the 669 bison diversity. Furthermore, singleton sites (only variable for one modern individual, 670 and therefore not informative for the modern phylogeny) were retained on the 671 principle that they might capture some of the unknown ancient diversity of bison 672 when genotyping ancient individuals.

673 We designed 70-mer probes, and this short length, as well as the limited number of

targets, allowed for a tiling of 4 different probes for each targeted locus, within the

same MYcroarray custom kit of 40,000 unique probes. Among all potential 70-mer

676 sequences within the original 121-mer probe sequence set, only those containing the

- 677 targeted bovine SNP no fewer than 10 nucleotides from either end were retained as
- 678 potential probes. Four probes were then designed using the following criteria: i)
- 679 Estimated melting temperature closest to the average from the 40k SNP probe set; ii)
- 680 Optimum proportion of guanine based on the efficiency of the 40k SNP probe set; iii) 681
- No two probes can be closer than 7 nucleotides from one another; iv) All 'GGGG' 682 and 'CTGGAG' motifs were modified to 'GTGT' and 'CTGTAG', respectively. The
- 683 former change was incorporated on the recommendation from MyBaits to avoid poly
- 684 G stretches because their synthesis technology has difficulty with this type of motif
- 685 and the latter variation was included to remove a restriction site that will be used in a
- future protocol to produce these probes from an immortalized DNA oligo library ²⁸. 686
- 687

688 DNA library preparation

689 All DNA libraries were used for capture of both the mitochondrial genome and

- 690 genome-wide nuclear loci. See Supplementary Information "Whole mitochondrial 691 genome sequencing" for protocols.
- 692
- 693 Hybridisation capture

694 One round of hybridisation capture was performed according to the manufacturer's 695 protocol (MYbaits v2 manual) with modifications. We used P5/P7 RNA blocking

- 696 oligonucleotides (Supplementary Table 1) instead of the blocking oligonucleotides
- 697 provided with the kit. We also incubated the magnetic beads with yeast tRNA (see
- 698 above) to saturate all potential non-specific sites on the magnetic beads that could
- 699 bind nucleic acids and increase the recovery of non-specific DNA.
- 700 Indexed primers were used to convert the capture DNA to full length Illumina
- 701 sequencing libraries. Each library was amplified in eight PCRs containing 5 µl
- 702 hybridisation capture library, 1C Gold Buffer II, 2.5mM MgCl₂, 200 µM dNTPs, 200
- 703 μ M each of primers GAII_Indexing_x (library specific index) and IS4
- 704 (Supplementary Table 1), 1.25 U Amplitaq Gold DNA polymerase, and H_2O to 25 µl.
- 705 Amplification was performed in a heated lid thermal cycler programed as follows 1
- 706 cycle: 94°C for 6 min; 15 cycles: 98°C for 30 sec, 60°C for 30 sec, 72°C for 40 sec;
- 707 and 1 cycle: 72°C for 180 sec. The PCR products were pooled and DNA was purified 708
- using AMPure magnetic beads.
- 709

710 NGS and data processing

711 Whole mitochondrial genomes

712 All libraries enriched for the mitochondrial genome were sequenced in paired-end

- 713 reactions on Illumina machines (HiSeq 2500 for LE237A, LE242B and LE247B –
- 714 MiSeq for the rest), except for A017 and A15526 from which the final concentration
- 715 of DNA obtained after capture was insufficient for sequencing. The mitochondrial
- 716 genome of the steppe bison A3133 was recovered from shotgun sequencing on an
- 717 Illumina HiSeq, performed in the context of another study (see Supplementary Table
- 718 3).
- All NGS reads were processed using the pipeline Paleomix v1.0.1²⁹. AdapterRemoval 719
- $v2^{30}$ was used to trim adapter sequences, merge the paired reads, and eliminate all 720

- reads shorter than 25 bp. BWA v $0.6.2^{31}$ was then used to map the processed reads to
- the reference mitochondrial genome of the wisent (NC_014044) or the American
- bison (NC_012346, only for the steppe bison A3133). Minimum mapping quality was
- set at 25, seeding was disabled and the maximum number or fraction of gap opens
- 725 was set to 2.
- 726
- MapDamage $v2^{32}$ was used to check that the expected contextual mapping and
- damage patterns were observed for each library, depending on the enzymatic
- treatment used during library preparation (see Supplementary Table 3 and Figures S1-
- 730 3 for examples), and re-scale base qualities for the non-repaired libraries.
- Finally nucleotides at the position of the bovine SNP were called using samtools and
- beftools, setting the minimum base quality at 30 and the minimum depth of coverage
- at 2. Consensus sequences were then generated using the Paleomix script
- 734 vcf_to_fasta.
- 735
- 736 Nuclear
- 737 Nuclear DNA from historical (historical wisent: A15654) and ancient (ancient wisent:
- 738 A4093; CladeX: A15526, A001, A003, A004, A005, A006, A007, A017, A018;
- steppe: A3133, A875) samples, containing HiSeq data (A3133 and A875) and MiSeq
- 740 data (all samples), was processed using Paleomix $v1.0.1^{29}$ to map reads against the
- 741 Bos taurus reference UMD 3.1^{33} . Paleomix was configured to use BWA v0.6.2³¹ for
- mapping, with seeding disabled and -n 0.01 -o 2 (see Supplementary Table 2).
- 743 MapDamage $v2^{32}$ was used to check that the expected contextual mapping and
- damage patterns were observed for each library, and empirically re-scale base
- 745 qualities at the end of the fragments.
- 746 Variants were called using the consensus caller of samtools/bcftools v1.2³⁴ limiting
- 747 calls to the 9908 capture sites. Variant calls with a QUAL value lower than 25 were
- removed. The genotypes for historical and ancient samples were merged with
- previously published extant bovid 40k capture data²⁶, and *Bos primigenius* (aurochs)
- sample $CPC98^{35}$. Only genotypes for the 9908 loci common among all data were
- retained.
- 752

753 Supplementary Note 2:

754 **DNA analyses**

755

756 Phylogenetic analysis

757 Mitochondrial control region phylogeny

The 60 newly sequenced bovid mitochondrial regions (Supplementary Data 1) were
 manually aligned, using SeaView v4.3.5³⁶. These sequences were aligned with 302
 published sequences (Supplementary Table 4) representing the following bovid

761 mitochondrial lineages: European bison or wisent (*Bison bonasus*), American bison

762 (*Bison bison*), steppe bison (*Bison priscus*), zebu (*Bos indicus*), and cattle (*Bos*

763 *taurus*). Among these published sequences, 5 were from steppe bison collected in the

764 Urals (Shapiro et al. 2004, Supplementary Data 1).

765 The TN93+G6 model of nucleotide substitution was selected by comparison of

766 Bayesian information criterion (BIC) scores in ModelGenerator $v0.85^{37}$. A

767 phylogenetic tree was then inferred using both maximum-likelihood and Bayesian

768 methods (Figure 2A). Bayesian analyses were performed using the program MrBayes

v3.2.3³⁸. Posterior estimates of parameters were obtained by Markov chain Monte

Carlo sampling with samples drawn every 1000 steps. We used 2 runs, each of four

771 Markov chains, comprising one cold and three heated chains, each of 10 million steps.

The first 50% of samples were discarded as burn-in before the majority-rule

773 consensus tree was calculated. A maximum-likelihood analysis was performed with

the program PhyML $v3^{39}$, using both NNI and SPR rearrangements to search for the

tree topology and using approximate likelihood-ratio tests to establish the statistical

support of internal branches. Complete phylogenies inferred using both methods areshown in Supplementary Figure 4.

778 Whole mitochondrial genome phylogeny

The 16 newly sequenced bison whole mitochondrial genomes (Supplementary Data 1)

780 were aligned with 31 published sequences (Supplementary Table 5) representing the

following bovid mitochondrial lineages: 3 wisent (Bison bonasus), 8 American bison

782 (Bison bison), 1 steppe bison (Bison priscus), 5 yaks (Bos grunniens – Bos mutus), 2

783 zebus (Bos indicus), 7 cattle (Bos taurus), 2 aurochsen (Bos primigenius), and 4

784 buffalo (*Bubalus bubalis*).

785 We used the same methods as described above for the control region to align and

estimate the phylogeny. The HKY+G6 model of nucleotide substitution was selected through comparison of BIC scores (Figures 2B and S5).

788 Estimation of evolutionary timescale

To estimate the evolutionary timescale, we used the program BEAST v1.8.1 40 to

conduct a Bayesian phylogenetic analysis of all radiocarbon-dated samples from

791 CladeX and wisent (Figure 1C). The GMRF skyride model⁴¹ was used to account for

the complex population history, and a strict clock was assumed. We found support for

a strict molecular clock based on replicate analyses using a relaxed uncorrelated

 794 lognormal clock⁴², which could not reject the strict clock assumption.

795 Mean calibrated radiocarbon dates associated with the sequences were used as

calibration points. Some samples appear to be older than 55 ky: one from the Urals,

four from the North Sea and five from the Caucasus (Supplementary Data 1). Because

798 these dates have effectively infinite radiocarbon error margins, we allowed them to 799 vary in the analysis by treating them as distinct parameters to be estimated in the model⁴³. The dated samples from Mezmaiskava Cave are from stratigraphic layers 800 801 2B4 and 2B3, which lie atop of layer 3. All these lower Middle Palaeolithic layers at 802 Mezmaiskaya have 14C results beyond the radiocarbon limit, reflected in the predominance of greater-than or near-background limit ages¹¹, and therefore are 803 consistent with the electron spin resonance (ESR) chronology for these levels¹⁰, which 804 805 suggests mean ages in the range from 53 to 73 ky BP (including error margins). 806 Consequently, for each Caucasian sample, we specified a lognormal prior age 807 distribution (mean=8,000) with an offset of 50 ky and with 95% of the prior 808 probability less than 80 ky. A similar prior distribution (mean=26,000) was used for 809 the five remaining samples that had infinite radiocarbon dates, with a 95% prior 810 probability less than 150 ky. Based on the results of all four phylogenetic analyses 811 described above, which showed strong support for the reciprocal monophyly of 812 CladeX and wisent when outgroups were included, this monophyly was constrained 813 for the BEAST runs. 814 All parameters showed sufficient sampling (indicated by effective sample sizes above 815 200) after 5,000,000 steps, with the first 10% of samples discarded as burn-in. In 816 addition, a date-randomization test was conducted to check whether the temporal 817 signal from the radiocarbon dates associated with the ancient sequences was sufficient to calibrate the analysis⁴⁴. This test randomizes all dates and determines whether the 818 819 95% high posterior density (HPD) intervals of the rates estimated from the date-820 randomized data sets include the mean rate estimated from the original data set 821 (Supplementary Figure 6).

822

823

The time to the most recent common ancestor (tMRCA) between wisent and 824 825 CladeX mitochondrial lineages was estimated at 121.6 kyr (92.1 – 152.3) (Figure 2C). 826 The tMRCAs for the two lineages was inferred to be 69.3 kyr (53.4 - 89.4) for wisent 827 and 114.9 kyr (89.2 – 143.1) for CladeX. Furthermore, there is some 828 phylogeographical structure within CladeX, with all individuals from the North Sea 829 forming a basal group, which existed before the population replacement with steppe 830 bison, but complete mixture of genetic diversity between all locations after re-831 colonization. In addition, the tMRCA of the MIS 3 diversity of CladeX was estimated 832 to be about 53.1 kyr (41.5 - 67.5). This date closely matches the ages of the last 833 observed MIS 4 CladeX individuals across all sampled locations, supporting the idea 834 of a population movement and contraction of wisent individuals towards a refugium

835 during the warmer period of MIS 3 in Europe.

836

837 Nuclear phylogeny from bovine SNP locus data

838 Phylogenetic trees were inferred from nuclear locus data (see next section for

839 information about the data sets). First, a phylogenetic tree of modern representatives

of bovid species, and with sheep as an outgroup, was inferred from published 40,843

841 data²⁶ (Supplementary Figure 7). Using $RAxML v8.1.21^{45}$, the three characters

842 (genotype states AA, AB and BB) from the BovineSNP50 chip were considered as

different states in an explicit analogue of the General Time Reversible (GTR)

substitution model, with separate substitution parameters for the three possible

transformations. For all analyses, 20 maximum likelihood searches were conducted to

846 find the best tree, and branch support was estimated with 500 bootstrap replicates

847 using the rapid bootstrapping algorithm⁴⁶.

848 This species tree, estimated from genome-wide nuclear locus data, shows that the

- 849 extant bison species (wisent and American bison) are sister taxa, contrary to the
- 850 phylogenetic signal from the maternally inherited mitochondrial genome. This
- 851 topology also clearly shows the paraphyletic status of the genus *Bos* (banteng, gaur,
- 852 yak, zebu and cattle), as it also includes the genus *Bison* (wisent and American bison).
- 853

854 Using the same method, we reconstructed the phylogeny of bison with the inclusion

855 of five pre-modern samples (for which the highest number of nuclear loci were called

- 856 amongst the ~ 10 k nuclear bovine SNPs). When only the two steppe bison specimens
- 857 are included they form a sister-lineage to modern American bison (Supplementary 858
- Figure 8A). Similarly, when the steppe bison and pre-modern wisent (including 859 ancient, historical and CladeX) are included, all five pre-modern specimens form a
- 860 clade most closely related to American bison (Supplementary Figure 8C). However,
- 861 when only the pre-modern wisent is included, the three specimens (ancient, historical
- 862 and CladeX) form a clade that is most closely related to modern wisent

863 (Supplementary Figure 8B). These conflicting results reflect the complex non-tree

864 like relationships among the modern and pre-modern taxa, and are consistent with the

865 hybridisation origin of wisent/CladeX and the severe bottleneck in the recent history

866 of the wisent. Hence, we used population genomics statistics to study this nuclear

- 867 locus dataset (see next section). Finally, these topologies are robust to the removal of 868 transitions (see Supplementary Figure 8D), a minimum depth of 2 for variant calling,
- 869 and haploidisation (data not shown).

870

871 Genome wide nuclear locus analysis

872 Captured nuclear loci corresponding to bovine SNPs for ancient samples were

873 analysed with published genotypes from modern populations: 20 American bison

874 were selected on the criterion that they do not display any detectable signal of recent

875 introgression from cattle (unpublished data); 2 Yak (Bos gruniens); 10 water buffalo

876 (Bubalus bubalis); and 10 Sheep (Ovis aries). Additionally, 7 modern wisent were

selected (among 50 sequenced $-\frac{47}{10}$) as non-related individuals on a known five-877 878 generation pedigree (as shown in Supplementary Figure 9).

879

880 Principal Component Analysis

881

882 PCA (Figures 3A and S10) was performed using EIGENSOFT version 6.0.1⁴⁸. In

883 Figure 3A, CladeX sample A006 was used as the representative of CladeX, as this

884 sample contained the most complete set of nuclear loci called at the bovine SNP loci

885 (see Supplementary Table 2). Other CladeX individuals, as well as ancient wisent,

- cluster towards coordinates 0.0, 0.0 (see Supplementary Figure 10), most likely due to 886
- 887 missing data.

- 889 Topology testing with the D statistic
- 890

- 891 For three bison populations, assuming two bifurcations and no hybridisations, there
- are three possible phylogenetic topologies. For this simple case, the D statistic is
- 893 expected to be significantly different from zero for exactly two of the three topologies,
- and not significantly different from zero for the most parsimonious topology. We
- therefore calculate a D statistic ⁴⁹ for each of these three topologies, using the sheep
- 896 (*Ovis aries*) as an outgroup.
- When D statistics for the set of three topologies do not indicate zero for one topology
- and non-zero for the other two, the true phylogeny is not treelike. However, the most parsimonious topology may still be apparent when considering only small amounts of
- 899 parsimonious topology may still be apparent when considering only small amounts of 900 introgression from populations of similar size. The interpretation of a most
- 900 introgression from populations of similar size. The interpretation of a most 901 parsimonious tree topology is not valid where confidence intervals around the D
- 901 parsimonious tree topology is not valid where confidence intervals around the r 902 statistic closest to zero, contain one or more of the other D statistics.
- yoz statistic closest to zero, contain one of more of the other D statistics.
- In this manner, the D statistic was used to indicate the most parsimonious topology
 for phylogenies including CladeX, ancient wisent, historical wisent, modern wisent,
- steppe bison and aurochs (Supplementary Figure 11). D statistics were calculated
- 906 using ADMIXTOOLS version 3.0, git~3065acc5⁵⁰.
- 907 Following concern over the limited amount of data for CladeX, particularly in
- samples other than 6A, we calculated the D statistics with sample 6A omitted from
 the analysis (Supplementary Figure 12). The most parsimonious topologies match in
 both cases.
- 911 Sensitivity to other factors were also investigated, such as setting a bovine SNP site
- 912 coverage depth threshold of two (Supplementary Figure 13), changing the outgroup to
- 913 Bubalus bubalis (Asian water buffalo, Supplementary Figure 14), and haploidisation
- 914 by randomly sampling an allele at heterozygous sites (Supplementary Figure 15).
- 915 None of these factors had notable influences on the outcome.
- 916 We also considered that the obtained topologies may have been caused by the small
- 917 number of observed loci. To determine how sensitive the topology testing was
- 918 missing data, we performed bootstrap resampling of the locus calls on decreasingly
- sized subsets of the data (Supplementary Table 7). For 10,000 bootstraps, we counted
- how often we obtained a result other than shown in Supplementary Figure 11.
- 921 For this bootstrap, a topology is considered to be simple if: (1) It has a D statistic
- which, uniquely amongst the set of three, is not significantly different from zero, or (2)
- All three are significantly different from zero but one has a D statistic closest to zero,
- with confidence intervals that do not overlap the D statistic for the other twotopologies.
- 926 For simple topologies, we counted how often the bootstrap replicate suggested a
- simple topology that did not match the most parsimonious topology in Supplementary
- 928 Figure 11. For non-simple topologies, we counted how often the result suggested any
- simple topology. In both cases, a lack of support for any simple topology (such as
- multiple topologies having a D statistic not significantly different from zero) was notcounted.
- 932 This bootstrapping shows that the D statistics are robust to the small number of933 observed genotypes.
- 934
- 935

- 936 Admixture proportion determination using an f4 ratio
- 937
- 938 The proportion of the wisent's ancestry differentially attributable to the steppe bison
- 939 and the aurochs, was estimated with AdmixTools using an f4 ratio, as described in ⁵⁰
- 940 with sheep (Ovis aries) as the outgroup. For the admixture graph shown in
- 941 Supplementary Figure 16, the admixture proportion, α , is the ratio of two f4 statistics.

$$\alpha y = F4(A, 0; X, C)$$
$$y = F4(A, 0; B, C)$$
$$\alpha = \frac{\alpha y}{y} = \frac{F4(A, 0; X, C)}{F4(A, 0; B, C)}$$

- 942 For the estimation of admixture proportions using an f4 ratio, it is intended that the
- 943 ingroup A, while closely related to B, has diverged from B prior to the admixture
- 944 event. However, in the context of steppe ancestry for wisent, no such population
- 945 matching ingroup A was available. The admixture graph for wisent is shown in
- 946 Supplementary Figure 17.

$$\begin{split} &\alpha y = F_4(AmericanBison, 0; Wisent, Aurochs) \\ &x + y = F_4(AmericanBison, 0; Steppe, Aurochs) \\ &\alpha \approx \frac{\alpha y}{x + y} = \frac{F_4(AmericanBison, 0; Wisent, Aurochs)}{F_4(AmericanBison, 0; Steppe, Aurochs)} \end{split}$$

947 Where α in Supplementary Figure 17 is approximately determined by the f4 ratio for

948 small branch lengths x. The f4 ratio we calculate therefore represents a lower bound

949 on the proportion of steppe bison present in the wisent populations. The steppe

- 950 ancestry was found to be at least 0.891, with a standard error of 0.026 (Supplementary 951 Table 6-A).

952 Sensitivity to haploidisation was checked by randomly sampling an allele at

953 heterozygous sites (Supplementary Table 6-B), which had no notable influence on the 954 outcome.

955

956 Hypergeometric test for shared derived alleles

957

958 To test whether the wisent lineages (including CladeX) have a common hybrid 959 ancestry (Supplementary Figure 18A), or whether multiple independent hybridisation 960 events gave rise to distinct wisent lineages (Supplementary Figure 18B), we identify 961 nuclear loci which have an ancestral state in the aurochs lineage, but a derived state in 962 the steppe lineage (see next section 'identification of derived alleles'). Under the 963 assumption of a single hybrid origin, we expect a common subset of derived steppe 964 alleles to be present in the various wisent lineages. In contrast, multiple hybridisation 965 events would result in different subsets of derived steppe alleles being present in 966 different wisent lineages. Likewise, we expect the subset of derived aurochs alleles to 967 indicate either one, or multiple hybridisation events. 968 If the total number of derived steppe alleles is s, the number of derived steppe alleles

969 observed in one wisent lineage is a, and the number in a second wisent lineage is b,

970 then under model B, the number of sites which are found to be in common is a

971 random variable X~HGeom(a, s-a, b). Where HGeom is the hypergeometric 972 distribution, having probability mass function:

$$P(X = k) = \frac{\binom{a}{k}\binom{s-a}{b-k}}{\binom{s}{b}}$$

973For the number of derived steppe alleles in common between two wisent lineages, c,974we calculate $P(X \ge c)$. This indicates the likelihood of having observed c or more975derived steppe alleles in common, if independent hybridisation events gave rise to

976 both wisent and CladeX lineages.

Likelihoods were calculated for steppe derived alleles on all pairwise combinations of
wisent lineages (Supplementary Table 8), and then repeated for derived aurochs
alleles (Supplementary Table 9). This provides strong support for an ancestral

980 hybridisation event occurring prior to the divergence of the wisent lineages.

We note that parallel genetic drift may also result in a pattern of alleles observed to be
derived in the steppe lineage and the wisent lineages, however this is only a
confounding factor where the parallel drift occurred in the post hybridisation lineage
common to wisent and CladeX in Supplementary Figure 18A. Therefore, this only
confounds the determination of genomic positions from a specific parent population,

986 not that the wisent and CladeX lineages have shared ancestry post hybridisation.

987 Alleles under strong selection following distinct hybridisation events would also be

shared between lineages more often than if they were randomly distributed. We

989 consider this situation unlikely, as it would require that the same alleles were

990 randomly introgressed repeatedly, and then a strong selective advantage of the alleles 991 at all times and in all environments.

Although we cannot reject the hypothesis that the modern European bison morph may

be recent, and only appeared after the LGM as an adaptation to the Holocene

994 environment in Europe, it would mean that the *Bos* mitochondrial lineage has been

maintained in the steppe bison diversity throughout the late Pleistocene, and that only

996 individuals carrying this mitochondrial lineage survived in Europe. Therefore, a

hybrid origin of the European morph prior to 120 kyr, and maintained during the late

998 Pleistocene, is more parsimonious with the current data.

999

1000 Identification of derived alleles

1001

The identification of a derived allele in the B lineage of Supplementary Figure 16, for the above analysis, can be performed in a simple way. If the ancestral allele is fixed in both C and the outgroup O, and the derived allele is fixed within B, then the site may be readily identified as derived. However, such fixed alleles are likely to be rare, especially in large populations, and therefore in limited number in our 10K SNP

1007 subset. Furthermore, a steppe bison derived allele observed in a wisent population

1008 may not be fixed in the wisent, as the population may also contain the ancestral allele 1009 from the aurochs lineage.

1010 Relaxing the criterion of allele fixation in any lineage, we identify differential

1011 ancestry using the difference in allele frequencies between populations. An ancestral

1012 site is one in which the allele frequency closely matches that of the outgroup and a

1013 derived site has an allele frequency differing from the outgroup.

- 1014 For the admixture graph in Supplementary Figure 16, where population X has
- 1015 ancestry from both B and C lineages, with outgroup O, we define an allele frequency
- 1016 shift in B, analogous to a derived state, if
- 1017 $f_2(C, 0) < f_2(X, C)$ and $f_2(C, 0) < f_2(X, 0)$,
- 1018 where $\hat{F}_2(M, N)$ is an unbiased estimate of $(m n)^2$, for populations M and N with
- 1019 population allele frequencies m and n at a single locus, as in Appendix A of ⁵⁰.
- 1020 Similarly, we define the allele frequency shift in B to have the same shift in X if, in 1021 addition to the shift in B:
- 1022 $\hat{F}_2(B,X) < \hat{F}_2(B,C)$ and $\hat{F}_2(B,X) < \hat{F}_2(B,O)$ and

1023
$$f_2(B,X) < f_2(X,C)$$
 and $f_2(B,X) < f_2(X,O)$ and

1024
$$\hat{F}_2(C, 0) < \hat{F}_2(B, C)$$
 and $\hat{F}_2(C, 0) < \hat{F}_2(B, 0)$.

1025 By observing a shared allele frequency shift instead of shared fixed alleles, we obtain 1026 greater sensitivity to the phylogenetic signal that is specific to one ancestral lineage.

1027 As for fixed derived alleles, the specific sites showing an allele frequency shift are

- 1027 As for fixed derived ancies, the specific sites showing an ancie frequency shift are
- 1028 identified, and can then be compared between multiple daughter populations.

1029

1030 Admixture proportion determination using ABC and simulated data

1031 As the f4 ratio test is giving an upper limit to the amount of aurochs introgression

1032 (due to the branch length uncertainty shown in Supplementary Figure 17), we

- independently test the admixture proportions using simulated data and an ABCapproach.
- 1035 Approximate Bayesian Computation (ABC) is a likelihood-free methodology
- 1036 employed when calculating likelihood functions is either impossible or
- 1037 computationally expensive 5^{51} . The methodology relies on being able to efficiently

1038 simulate data, and then compare simulated data to observed data. When simulated

1039 data is sufficiently close to the observed data, the parameters used to simulate the data 1040 are retained in a posterior distribution.

- 1041 Consider a single locus, which for three individuals A, B, and C, two different
- 1042 genotypes are observed. The three possible patterns that can be observed are AB, BC,
- 1043 and AC, denoted by the tree tips with shared state. The observed pattern results from a
- 1044 single mutation somewhere on the gene tree, where the position of the mutation
- 1045 relative to the internal node defines which pattern is observed. For example, from the

1046 un-rooted gene tree in Supplementary Figure 19c, if a mutation occurs on the branch

- 1047 between C and the internal node, the pattern AB is observed. We assume the relevant 1048 time scales are short enough that multiple mutations at a single locus are rare (infinite
- 1049 sites model⁵²).
- 1050 Under the assumption of neutral and independent mutations, the number of fixed mu-1051 tations accumulating on a branch is Poisson distributed with mean $\mu \times t$, where μ is 1052 mutations per locus per generation, and time t is in units of $2N_e$ generations^{53,54}. The 1053 counts $\mathbf{n} = (n_{ab}, n_{bc}, n_{ac})$, of observed site patterns AB, BC, and AC, are random
- 1054 variables, which for topology X_1 (Supplementary Figure 19c),

$$n_{ab} \sim Pois(T_m + T_c)$$

 $n_{bc} \sim Pois(T_a),$

 $n_{ac} \sim Pois(T_b)$,

1055 and topology X_2 (Supplementary Figure 19d),

$$n_{ab} \sim Pois(T_c),$$

 $n_{bc} \sim Pois(T_m + T_a),$
 $n_{ac} \sim Pois(T_b),$

1056 where $\mathbf{T} = (T_a, T_b, T_c, T_m)$ are branch lengths in units of evolutionary time of $2N_e\mu$

1057 generations, and the total number of observed patterns is $N = n_{ab} + n_{bc} + n_{ac}$. Thus

1058 for a locus where two genotypes are observed, the probability of patterns AB, BC,

1059 AC, is given by $\mathbf{p}^T = (p_{ab}^T, p_{bc}^T, p_{ac}^T)$, where for topology X_1 (Supplementary Figure 1060 19c),

$$P(AB|\mathbf{T}, X_1) = p_{ab}^{T, X_1} = (T_m + T_c)/(T_m + T_c + T_a + T_b)$$

$$P(BC|\mathbf{T}, X_1) = p_{bc}^{T, X_1} = T_a/(T_m + T_c + T_a + T_b)$$

$$P(AC|\mathbf{T}, X_1) = p_{ac}^{T, X_1} = T_b/(T_m + T_c + T_a + T_b)$$

1061 and for topology X_2 (Supplementary Figure 19d),

$$P(AB|\mathbf{T}, X_2) = p_{ab}^{\mathbf{T}, X_2} = T_c / (T_m + T_c + T_a + T_b)$$

$$P(BC|\mathbf{T}, X_2) = p_{bc}^{\mathbf{T}, X_2} = (T_a + T_m) / (T_m + T_c + T_a + T_b)$$

$$P(AC|\mathbf{T}, X_2) = p_{ac}^{\mathbf{T}, X_2} = T_b / (T_m + T_c + T_a + T_b).$$

1062 We simulate site pattern counts for each of the two species trees in Supplementary

1063 Figure 19 by drawing from a Multinomial distribution, where for tree topology X_1 , 1064 $n^{X_1} \sim \text{Mult}(N, p^{T,X_1})$, and for tree topology $X_2, n^{X_2} \sim \text{Mult}(N, p^{T,X_2})$.

1065 Given a collection of site pattern counts from a hybrid tree with hybridisation

1066 parameter $\gamma \in [0,1]$ (Figure S19e), we expect that the combined site pattern counts

1067 will be a linear combination of the counts for the different topologies X_1 and X_2 . This

- 1068 assumption is reasonable for a large number of total observations N. The simulated
- 1069 counts, \boldsymbol{n}^{γ} , of site patterns for the hybridised tree is then given by

$$\boldsymbol{n}^{\gamma} = \gamma \boldsymbol{n}^{X_1} + (1-\gamma) \boldsymbol{n}^{X_2} \\ = (n^{\gamma}_{ab}, n^{\gamma}_{bc}, n^{\gamma}_{ac}).$$

1070 As branch lengths are not known (μ , N_e and number of generations are all unknown),

1071 we use uninformative priors for the branch lengths. Furthermore, we only require

1072 relative branch lengths, so branch lengths **T** used for simulation were scaled such that

1073 $T_b = 1$. Hence we can meaningfully simulate counts of site patterns \mathbf{n}^{γ} under

1074 hybridisation, for comparison to observed site pattern counts.

1075 We perform ABC using the R package 'abc', with a ridge regression correction for

- 1076 comparison of the simulated and observed data using the "abc" function⁵⁵. The
- 1077 distance between the observed and simulated data sets is calculated as the Euclidean
- 1078 distance in three-dimensional space. A tolerance $\epsilon = 0.005$ was chosen so that the
- 1079 closest $\ell \times \epsilon$ simulated data sets are retained. For each analysis we had $\ell = 100000$, 1080 resulting in 500 posterior samples.
- resulting in 200 posterior samples.
- 1081 We performed leave-one-out cross-validation using the function "cv4abc" on
- 1082 $\ell' = 250$ randomly selected simulations, and report the prediction error, calculated as

$$E_{\text{pred}} = \frac{\sum_{i=1}^{\ell'} (\hat{\gamma}_i - \gamma_i)^2}{\text{Var}(\gamma_i)}$$

1083 for each analysis. At most the prediction error was 0.5111 standard deviations away

1084 from zero, and so we observe that the ridge regression has performed well (see 1085

Supplementary Table 11).

1086 Similarly, on inspection of the cross-validation plots, we observe that the ridge 1087 regression performs well for γ , as the true simulated values of γ are well estimated by 1088 the ridge regression correction. Hence the correction has strengthened the parameter 1089 inference methodology when compared to a simple rejection algorithm.

1090 We avoid reporting sample means due to the heavy negative skew in the posterior dis-1091 tributions of γ , and hence report the median (the most central ordered observed value) 1092 and mode of each distribution. The mode is estimated using a kernel density estimate 1093 of the posterior distribution. Not all simulated data is equally 'close' to the observed 1094 data, and the median and mode are weighted according to these distances 56 .

1095 The weighted posterior median was between 0.8250 and 0.8660, and the weighted 1096 posterior mode was between 0.9034 and 0.9384. These measures of centre indicate 1097 evidence for some non-zero level of hybridisation from the Aurochs genome. 1098 Evidence against hybridsation must be indicated by overwhelming support for either 1099 $\gamma = 0$ or $\gamma = 1$ (no mixing of the tree topologies). However, these values lie on either 1100 end of the support for the prior distribution of γ , and hence any resulting posterior 1101 distribution for γ . There- fore, classical highest probability density (HPD) intervals 1102 cannot be used to indicate uncertainty in the estimates of these measures of centre, as 1103 any interval of density less than 100% will result in zero and one being artificially 1104 omitted by construction. This is not evidence for or against hybridisation, but rather a 1105 consequence of the way in which we calculate HPD intervals. 1106

Supplementary Table 11 gives empirical posterior probabilities for different levels of

1107 hybridisation. For example, the first column gives the empirical posterior probability

1108 of observing at least 1% hybridisation. This is found for each trio by calculating the 1109

- total proportion of posterior samples where $0.01 \le \gamma \le 0.99$. In general, for some
- 1110 percentage of hybridisation α , Supplementary Table 11 reports

$$[P(\frac{\alpha}{100} \le \gamma \le 1 - \frac{\alpha}{100})]$$

1111 for $\alpha = 1\%$, 2%, 3%, 4% and 5%, from the posterior distribution of γ .

1112 As there is no accepted value of γ for which we can claim that significant

1113 hybridisation has occurred, we leave it to the reader to consider what they consider to

1114 be a significant level of hybridisation, and to find the appropriate probability.

- 1115 However, if one considers 1% hybridisation to be significant, then the observed data
- 1116 indicates that the data has between a 95.80% and 97.20% chance of being from a

1117 hybridised topology. Similarly, if one considers 5% hybridisation to be significant,

1118 then the observed data has between a 76.40% and 85.00% chance of being from a

hybridised topology. 1119

1121 Asymmetrical hybridisation

1122 In this study, we show that wisent and CladeX are of hybrid origin, certainly between

ancient aurochs and steppe bison forms. This is consistent with the population

1124 structure of most bovids, where a single bull usually breeds with different females of

1125 multiple generations. As explained in^{57} , this usually results in asymmetrical

1126 hybridization when males of one species (steppe bison here) dominate males of the

1127 other species (aurochs here), therefore preferentially mating with female aurochs, as

1128 well as their offspring, potentially over several generations. In addition, male F_1

1129 hybrids are usually sterile or sub-fertile, increasing the amount of steppe bison

1130 genomic contribution to the offspring. As illustrated in Supplementary Figure 20,

after just a few generations, this mating process results in individuals that are

essentially steppe bison for their nuclear genome, but with an aurochs mitochondrial

1133 genome (strictly maternally inherited), which is the result that we obtained from the

1134 genotyping of historical and ancient wisent individuals (including CladeX).

1136 Supplementary Note 3:

1137 Paleoenvironment reconstruction and stable isotope analyses in the Ural region

1138

1139 The Urals are a well sampled region, with the highest number of genotyped bones

- 1140 through time (Figure 5 and S22). We generated a convex hull based on geo-referenced
- 1141 site locations for all genotyped ancient samples collected from the Urals
- 1142 (Supplementary Figure 21). We used the HadCM3 global circulation model and
- 1143 BIOME4 model to reconstruct paleoclimate and environmental conditions for the Ural
- region throughout the period from 70,000 years ago to the present day.
- 1145

We used the HadCM3 global circulation model to reconstructed paleoclimate proxies
for the Ural region. The HadCM3 consists of linked atmospheric, ocean and sea ice
models at a spatial resolution of 2.5° latitude and 3.75° longitude, resampled at a 1° x
1° latitude/longitude grid cell resolution ⁵⁸. The temporal resolution of the raw data is
1,000 year slices back to 22,000BP and 2,000 year slices from 22,000 to 80,000BP ⁵⁸
We used these palaeo-climate simulations to derive estimates of annual mean daily

temperature and Köppen-Geiger climate classifications ⁵⁹ throughout the period from

1153 70,000 years ago to the present day. We intersected each grid cell in the Ural study 1154 region (n = 51) with the derived climate estimates, at each point in time, using

1154 region (n = 51) with the derived climate estimates, at each point in time, using 1155 ArcGIS 10. We calculated the mean temperature for the region and change in the

1156 proportion of the study region represented by four Köppen climate classes, each

1157 differing temperature: Dfa (hot summers), Dfb (warm summers), Dfc (cool summers),

1158 Dfd (continental temperatures). These are shown in Supplementary Figure 22.

1159 Interestingly, our reconstructions for the Urals show a decrease in area with hot and

1160 warm summer conditions (Dfa and Dfb) after 35kya.

1161

1162 BIOME4 was used to infer paleovegetation types. BIOME4 is a coupled biogeographical and biogeochemical model that simulates the distribution of 28 plant 1163 functional types (PFT) at a global scale ⁶⁰. Model inputs for each grid cell are monthly 1164 1165 climate (mean annual temperature, mean annual precipitation and mean annual 1166 sunshine hours), atmospheric [CO₂], and soil texture class. Ecophysiological 1167 constraints determine which PFT is likely to occur in each grid cell. A coupled carbon and water flux model calculates the leaf area index that maximizes net primary 1168 1169 production (in gC m^{-2} year⁻¹) for each PFT. Competition between PFTs was 1170 simulated by using the optimal net primary production of each PFT as an index of 1171 competitiveness. Global maps of BIOME4 PFTs were accessed at the same spatial 1172 and temporal resolution as the paleoclimate data (http://www.bridge.bris.ac.uk/ 1173 resources/simulations/). We grouped PFTs into three categories: Grassland (PFT 1174 identify numbers = 18-20; Tundra (ID = 22-26); and Forest (ID = 7-11). For each 1175 grid cell in the Ural study region, at each point in time, we determined whether the 1176 dominant PFT was grassland, tundra or forest. Interestingly the vegetation shift 1177 between an all forest-like landscape to a landscape represented by a large proportion 1178 of tundra and grassland-like vegetation occurred after 35kya, which coincides with a 1179 decrease in hot and warm summer conditions (see above). 1180 These results from the paleovegetation and climate inferences agree with previous

1181 landscape reconstructions of the region: In the Middle Urals, where almost all the

1182 samplings sites were located, the areas covered with arboreal vegetation underwent

- 1183 changes during MIS3. Spruce and birch open forests were widespread during
- 1184 coolings, and spruce and birch forest-steppe with occurrence of pine formed during
- 1185 warmings. Mesophilic meadows dominated by forbs and grasses were also prevalent
- 1186 during warm climatic events (Lapteva, 2008; 2009; Pisareva and Faustova, 2008). In
- the south, where one of the sites (Gofmana) is situated, steppe landscapes dominated
- 1188 by Asteraceae, Artemisia, and Poaceae were widespread. Spruce, birch and pine
- 1189 forests covered the areas along the rivers (Smirnov, Bolshakov, Kosintsev et al.,
- 1190 1990). The following was reconstructed for the territory of the Irtysh River: forest-
- 1191 steppe landscapes with pine (Pinus s/g Haploxylon) and spruce forests, as well as
- 1192 meadows with a predominance of Cyperaceae and Poaceae and small quantities of
- 1193 Artemisia and Chenopodiaceae (Araslanov et al. 2009).
- 1194 During MIS2, periglacial forest-steppes dominated by herbaceous communities were
- 1195 typical of the Last Glacial Maximum. Larch, pine and birch covered the river-valleys.
- 1196 Herbaceous vegetation was dominated by goosefoot, sagebrush and grass (Grichuk
- 1197 2002). Periglacial forest-steppes with arboreal vegetation, including pine-birch forests
- and small quantities of spruce have been reconstructed for the Last Glacial
- 1199 Termination. Areas covered with sagebrush-goosefoot steppes with small quantities of 1200 grass were widespread (Lapteva, 2007).
- 1201 At later stages of MIS2, periglacial forb-grass forest-steppes with pine, birch and
- small quantities of spruce have been reconstructed for the Sur'ya 5 and Rasik 1 sites
- ⁶¹. Periglacial steppes dominated by Artemisia, Rosaceae, Chenopodiaceae,
- 1204 Cichorioideae and Poaceae have been reconstructed for the Voronovka site. Pinus
- 1205 sylvestris and Betula pubescens with occurrence of spruce (Picea), oak (Quercus) and $\frac{62}{2}$
- 1206 teil (Tilia) covered the river-valleys 62 .
- 1207 The palynological analyses and landscape reconstruction suggest that both bison
- 1208 forms inhabited semi-open landscapes of forest-steppe type, where arboreal
- 1209 vegetation was represented by birch, spruce, pine and sometimes larch, while steppe
- 1210 and meadow herbaceous communities were observed. However, only CladeX
- 1211 (specifically from the Gofmana site, during MIS 3, Rasik 1 and Sur'ya 5, and
- 1212 Voronovka sites, during MIS2) also inhabited steppe-like landscapes, showing a more
- 1213 diverse ecological niche than steppe in this region.
- 1214 In addition to the paleo-climate and -vegetation reconstructions, stable isotope values
- 1215 (δ 13C and δ 15N) obtained for all the genotyped bison individuals from the Ural
- 1216 region were compared between steppe bison and wisent (Supplementary Figure 23).
- 1217 Wisent individuals displayed more diverse stable isotope ratios than the steppe bison
- 1218 individuals. This observation is consistent with feeding in more diverse vegetations
- 1219 communities, which correlates well with the reconstructed paleo-environments for the
- 1220 region in the time periods they are found.
- 1221
- 1222 Modelled paleo-climate and -vegetation reconstruction at the sampling locations in 1223 the southern Urals suggest drastic shifts, which coincide in time with the observed 1224 population replacements between steppe bison and wisent. More specifically, between 1225 14 and 31 kya wisent were likely to exist in environmental condition characterised by 1226 relatively cold average temperatures, open landscapes with tundra-like flora, and the 1227 absence of warm summers. Although modern wisent are found today in wood-like 1228 habitats, it has been suggested that they are living in sub-optimal habitat, and 1229 paleodiet reconstructions have placed ancient wisent in tundra-like environments, in 1230 agreement with our observations ⁶³.

- Interestingly, the steppe bison was only recorded when forest vegetation was inferred
- to dominate the landscape, adding to the evidence that this form of bison might not have been exclusively steppe-adapted ^{63,64}.

1236 Supplementary Note 4:

1237 Cave painting

1238 The present survey, placing wisent across Europe (from the Urals/Caucasus to 1239 Ukraine/Italy) during MIS2 and late MIS3, suggests that depictions of bison in 1240 European Palaeolithic art, such as cave painting, carving and sculptures, are likely to 1241 include representations of wisent. Paleolithic art representations have often been used 1242 to infer the morphological appearance of steppe bison, sometimes in great detail ^{64,4,65–67}. And until now, the steppe bison (i.e., direct ancestor of modern American 1243 1244 bison) has always been assumed to be the unique model present at the time of cave 1245 painting, and therefore, the diversity within the representations of bison was mainly explained by putative cultural and individual variations of style through time $^{68-70}$. 1246 However, in the vast diversity of bison representations (820 pictures representing 1247 20.6% of all known cave ornamentation, according to ⁷¹), two consistent 1248 morphological types can be distinguished (see Fig 1 and Fig S24-27). The first type, 1249 1250 abundant prior to the last glacial maximum, is characterized by long horns (with one 1251 curve), a very oblique dorsal line and a very robust front part of the body (solid 1252 shoulders versus hindquarters), all these traits being similar to the modern American 1253 bison. The second type, dominating the more recent paintings between 18 and 15 kya, 1254 displays thinner sinuous horns (often with double curve), a smaller hump and more 1255 balanced dimensions between the front and the rear of the body, similar to the modern 1256 wisent lineage, and to some extant the *Bos* lineage. The imposing figure of the steppe 1257 bison, with its high hump and long horns stepping out the head profile, certainly was a 1258 very strong influence on the artists painting in the cave in Europe before the last 1259 glacial maximum. However, later generations thoroughly depicted the slender shape 1260 of the more recent form of bison. Considering the geographical and temporal 1261 distribution of genotyped steppe bison and wisent presented here, particularly the 1262 ~16,000 years old wisent B individual from Northern Italy, it is likely that the variety 1263 of bison representations in Paleolithic art does not just come from stylistic evolution, 1264 but actually represents different forms of bison (i.e., pre and post-hybridisation) 1265 through time. 1266

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