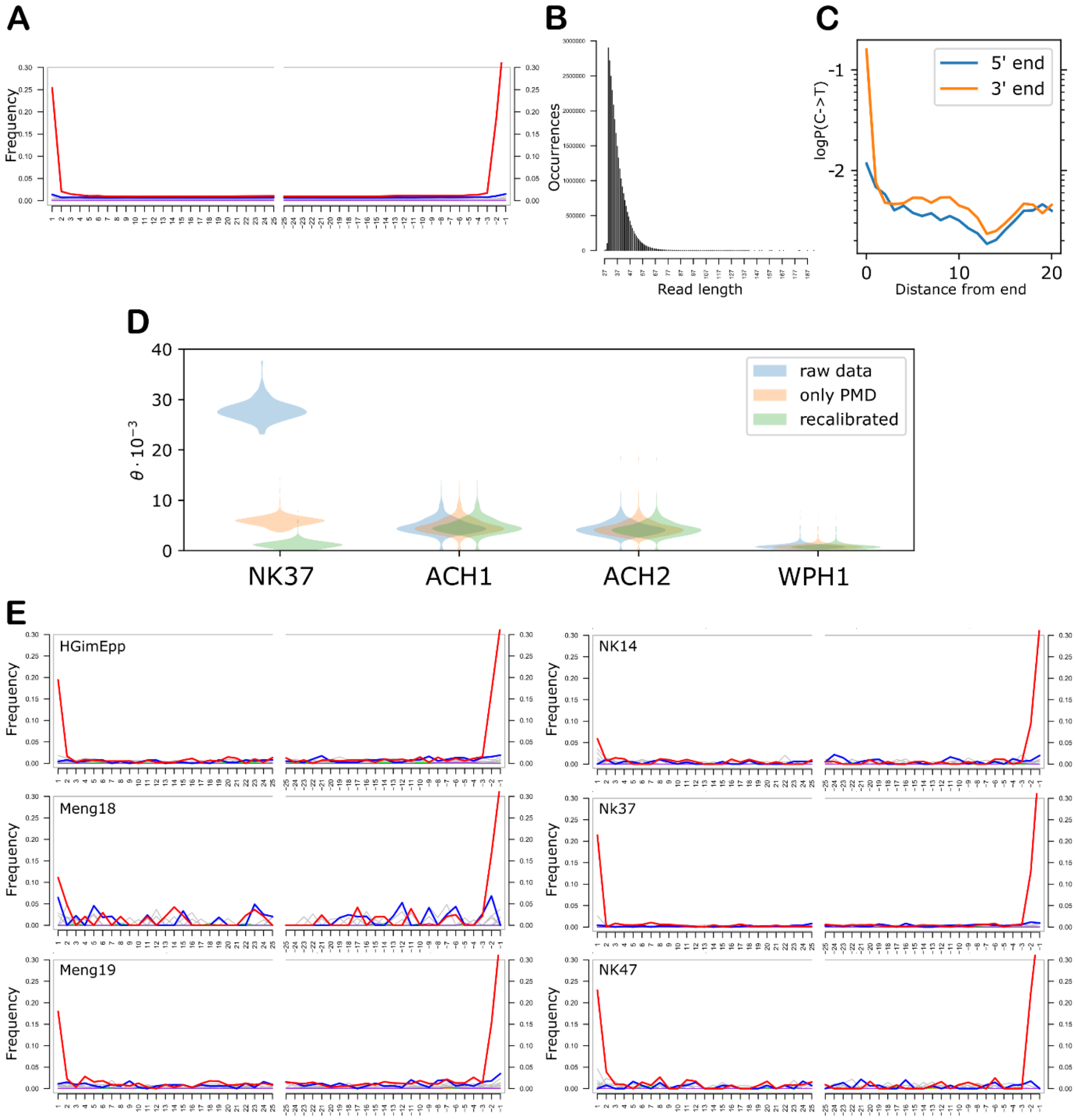

Figure S1. Ancient DNA authentication and post-mortem damage pattern. Related to Figure 1 and Figure 3 and STAR methods. (A) Frequency of C to T misincorporations (red) from the 5’ (left) and 3’ (right) end in NK37 nuclear data. (B) Read length distribution of NK37 reads mapping to the *Choeropsis liberiensis* reference genome (GenBank: GCA_023065765.1) as computed by mapDamage2. (C) Frequency of C to T misincorporations of NK37 reads mapping to the *Hippopotamus amphibius* reference genome (GenBank: GCA_023065835.1) as computed by ATLAS. (D) Effect of post-mortem damage correction and X chromosome-based recalibration using Atlas software. NK37: Late Pleistocene hippo from the Upper Rhine Graben; ACH: extant African common hippos; WPH: extant West African pygmy hippo. (E) Frequency of C to T misincorporations (red) from the 5’ (left) and 3’ (right) end for reads mapping to the *Hippopotamus amphibius* mitochondrial reference genome (GenBank: NC_000889.1) after enrichment capture.

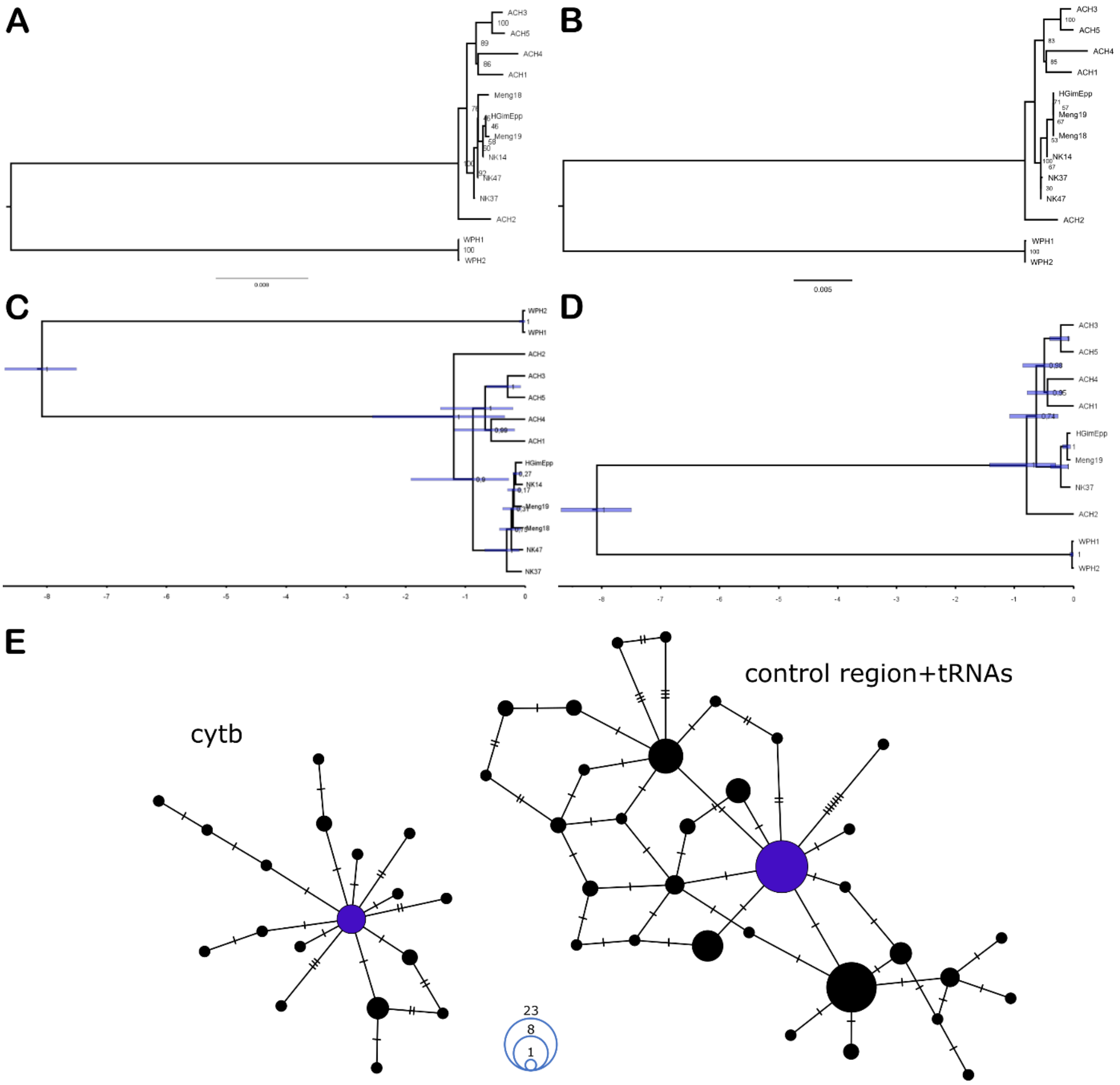

Figure S2. Phylogenetic analyses of mitochondrial data. Related to Figure 1 and STAR methods. (A) Maximum likelihood phylogeny of mitochondrial genomes using a minimum depth of 2 for consensus calling. (B) Maximum likelihood phylogeny of mitochondrial genomes using a minimum depth of 3 for consensus calling. (C) Calibrated Bayesian phylogeny of mitochondrial genomes using a Birth-Death tree prior. (D) Calibrated Bayesian phylogeny including only the three most complete (>60%) mitochondrial genomes from Late Pleistocene Upper Rhine Graben hippos and a Bayesian skyline coalescence tree prior. Numbers next to the nodes indicate ultrafast bootstrap values in (A) and (B) or posterior proability in (C) and (D) of the respective branch. Blue bars in (C) and (D) represent 98% highest posterior density of node age estimates. Numbers on axis in (C) and (D) indicate time in million years. ACH: extant African common hippos; WPH: extant West African pygmy hippos. (E) Haplotype networks of mitochondrial fragments from a larger comparative sample covering the whole geographic range of the extant African common hippo based on an 854 bp fragment of cytochrome (left) and a 929 bp fragment of tRNA-Pro, the control region and tRNA-Phe (right). Haplotypes including sequences from Late Pleistocene Upper Rhine Graben hippos are colored in blue.

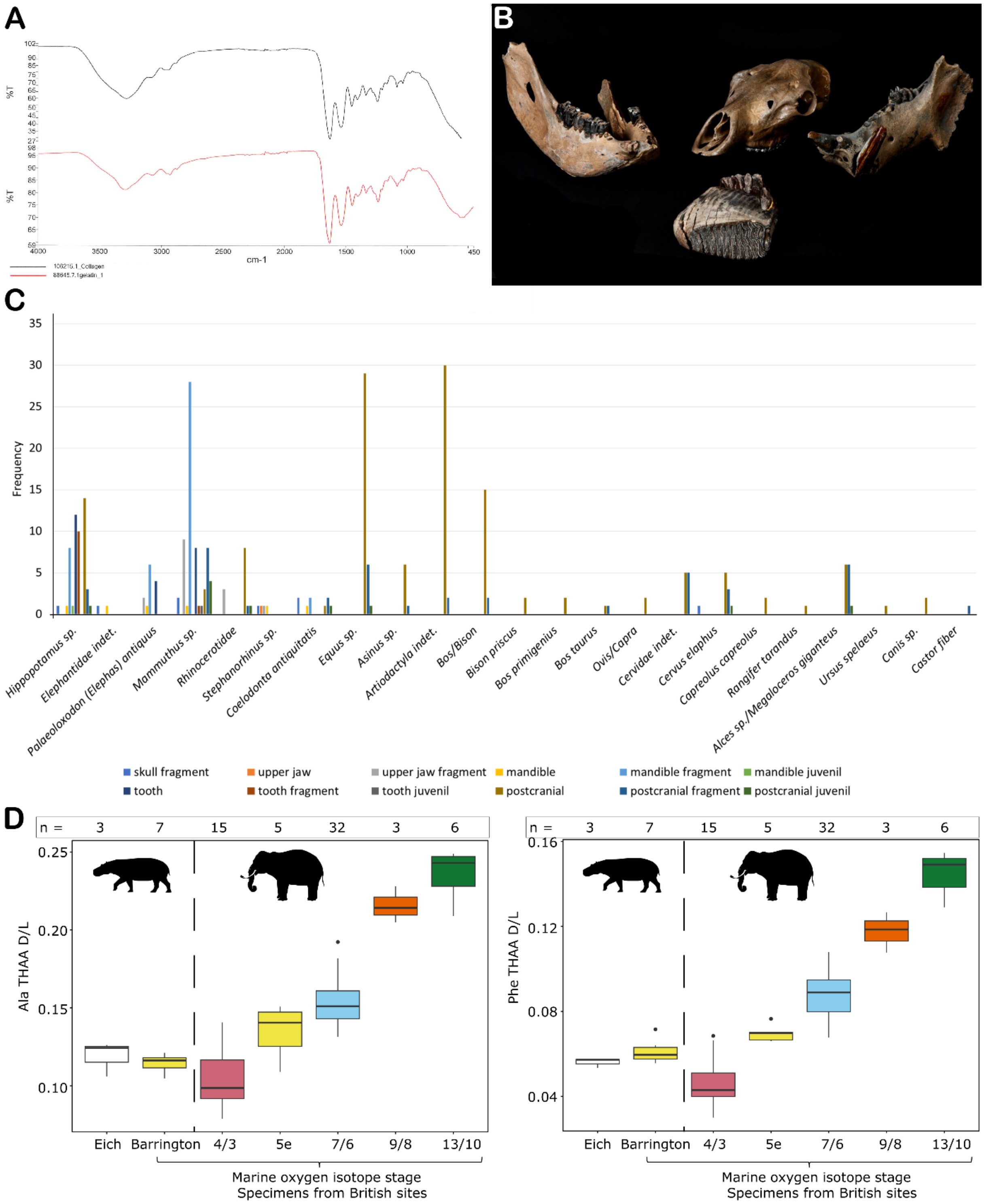

Figure S3. Preservation of fossil specimens and amino acid racemization chronology. Related to STAR methods. (A) Results of Fourier Transform Infra-Red (FTIR) spectral analysis on purified gelatin separated from the hippo sample ETH-106215 (fossil specimen NK24; black line) compared to the FTIR spectrum of gelatin separated from modern chicken bone (ETH-88647; red line). (B) Comparison of taphonomic preservation of fossils from the Upper Rhine Graben: mandible of *Coelodonta antiquus* (woolly rhino, NK962, left), skull of *Megaloceros giganteus* (giant deer, NK2, top center), isolated molar of *Mammuthus primigeminus* (woolly mammoth, NK1891, bottom center), mandible of *Hippopotamus amphibius* (hippo, NK24, right). Note the similarity in preservation of mandibles from 'glacial' (woolly rhino) as well as 'interglacial' species (hippo) in the Upper Rhine Graben that indicate limited temporal difference between them. (C) Degree of fragmentation of fossils from the Upper Rhine Graben gravel pit Eich in the Reis Collection. (D) Box plots illustrating amino acid racemization (D/L values) for Ala (left) and Phe (right) in the *H. amphibius* enamel from Eich (Upper Rhine Graben) and Barrington, UK (left). For comparison (right), enamel IcPD from British elephantids of varying ages, grouped by marine oxygen isotope stage (MIS), are also included. The plots display the median, interquartile range (IQR), and outliers, illustrating the relationship between racemization and sample age. In the box plots, the box represents the interquartile range (middle 50% of the data), with the horizontal line indicating the median. The whiskers extend to the smallest and largest values within 1.5 times the IQR, while points beyond this range are plotted as outliers.