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## Lake-centred sedentary lifestyle of early Tibetan Plateau Indigenous populations at high elevation 4,400 years ago

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The onset of sedentism on the Tibetan Plateau is often presumed to be associated with the dispersal of agriculture or farmers from archaeological sites located in the low elevation margins of the plateau. Previous studies of the plateau assumed that all foragers were probably mobile, but few systematic excavations at forager sites have been conducted to inform us about their settlement patterns. Here we report the world's highest elevation sedentary way of living exhibited by the Mabu Co site at 4,446 metres above sea level, deep in the interior of the Tibetan Plateau 4,400– 4,000 years ago. Our interdisciplinary study indicates that the site was occupied by Indigenous inhabitants of the plateau, representing the earliest known DNA evidence of foragers who predominantly harbour the southern plateau ancestry. The evidence shows that they had a sedentary lifestyle primarily supported by fishing at nearby lakes, supplemented by mammal and bird hunting, as well as small-scale exchanges of millet and rice crops.

The adoption of a sedentary lifestyle in the high-altitude regions of the Tibetan Plateau (>3,500 metres above sea level (MASL)) represents both a cultural and genetic adaptation to an environment characterized by low primary productivity, extreme weather conditions and hypoxia<sup>1-3</sup>. Archaeological and linguistic models for the onset of and pathways to sedentism on the Tibetan Plateau see its origins as being intertwined with the dispersal of agriculture or farmers from elevations at less than 2,500 MASL<sup>4-8</sup>. To date, very few systematic excavations have been carried out at sites occupied by foragers; thus, we know very little about the types of settlement and mobility patterns that foragers used. For the most part, sites that have been targeted by small-scale excavations

are consistent with small logistical campsites that are consistent with patterns of high mobility between high and low altitudes<sup>2,9-13</sup>. Genomic analysis hypothesizes that early inhabitants, who can be traced back to Late Palaeolithic foragers, originally lived on the Tibetan Plateau<sup>14</sup>, contacted farmers at an altitude of about 3,000 MASL on the eastern margins of the Tibetan Plateau around 5,000 years ago<sup>15</sup>, and then shifted from patterns of high mobility to increased sedentism, represented by the Zongri (2,800 MASL) and Karuo (3,200 MASL) sites, with a diverse subsistence strategy, including millet cultivation and hunting<sup>5,16,17</sup>. Agropastoral settlements emerged on the interior Tibetan Plateau by 3,500 calendar years before the present (cal BP), represented

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by the Qugong site (3,600 MASL) in Lhasa City, where millets followed by wheat, barley and buckwheat were grown and yak and sheep were herded  $^{\rm 18,19}$ .

Although the core of the Tibetan Plateau, with elevations well above 4,000 MASL, is considerably more environmentally challenging than these lower-altitude river valleys, at present the number of global permanent residents above this altitude is over 6.4 million people<sup>20</sup>. Who the earliest permanent residents were and when and how processes of sedentism began in this area has been unclear because of the paucity of archaeological research. In this study, we report on findings from the Mabu Co site (Co is the Tibetan word for lake) at 4,446 MASL on the Tibetan Plateau. Our excavations and interdisciplinary research reveal that the early Indigenous inhabitants used ways of living that were much more sedentary than previously thought. Their lifestyle, 4,400–4,000 years ago, revolved around fishing, hunting and the exchange of crops cultivated in lower-altitude areas. Genetic analysis indicates that individuals at the site were ancestral to the current populations who live on the southern plateau.

#### Site description

The Mabu Cosite (28.31° N, 89.43° E) is situated at the headwater region of the Nyang Qu River, one of the main tributaries in the middle reaches of the Yarlung Tsangpo River (Fig. 1a). Today, the landscape of this region is predominantly desert steppe with cold and dry climatic conditions. The mean annual temperature is 1 °C and the average annual precipitation is 277 mm, with rainfall concentrated in the summer<sup>21</sup>. Current livelihoods near the site focus primarily on herding yak, sheep, goat and horse, and planting cold-tolerant naked barley.

The site is situated on a 2-km-long sand ridge that lies between Mabu Co and Gala Co, stretching southward from the foot of a hill to the north (Fig. 1b,c). The site was discovered in 2019 and the entire area measures approximately 14 ha. During the excavations between 2020 and 2023, an area of 1,100 m<sup>2</sup> was uncovered (Extended Data Fig. 1). Burials, post mould structures, pits, stone features and living surfaces were distributed across the surface of the ridge (Extended Data Fig. 2). Refuse deposits were concentrated in the south end of the ridge, sandwiched between the topsoil and sandy gravel sediments of the lakeshore (Fig. 2a,b). Thousands of artefacts were uncovered, especially from the refuse deposits, including pottery sherds, crystal microliths, ground stone tools, bone tools and ornaments, among others (Extended Data Fig. 3).

#### Chronology

Abundant archaeobotanical and faunal remains were also recovered from the refuse deposits; 33 samples, including charred crop seeds and bone remains, were dated using accelerator mass spectrometry (AMS) (Methods and Supplementary Table 1). The ages of fish, bird and human bones were all anomalously older than the carbonized seeds and terrestrial mammal bones found from the same layer, indicating that they were probably affected by the radiocarbon reservoir effect of the lake<sup>22</sup>. They were thus excluded from consideration (Extended Data Fig. 4). Dates from the mammal bones and carbonized seeds place the occupation at 4,400–4,000 cal BP (Extended Data Fig. 4).

#### **Indigenous Tibetan foragers**

Nearly 100 burials were found at Mabu Co (Extended Data Fig. 1). We extracted DNA from seven human bones (from burials) and two teeth (from strata of refuse deposits) excavated during the 2020 and 2021 field seasons (Supplementary Table 2) according to a previously described protocol; the genomic data of these individuals were analysed alongside those of previously published individuals from ancient and present-day populations (Methods).

We found that the inhabitants of Mabu Co mainly possessed a southern plateau ancestry that spans the southern and southwestern Tibetan Plateau along the Yarlung Tsangpo River valley. Similarities were noted between the human remains at Mabu Co and 3.400-2.600-year-old individuals from the Shannan. Shigatse and Himalayan arc<sup>15</sup>. Their overall affinities to plateau populations are supported by the clustering of Mabu Co individuals with other ancient and present-day Tibetan individuals in principal component analysis (PCA), as well as the similar genetic profile shared by the Mabu Co individuals and both ancient and present-day Tibetans (Fig. 3a,b and Extended Data Figs. 5 and 6). Specifically, Mabu Co individuals are most closely related to ancient populations from Shannan and Nepal<sup>23</sup>, as a more shared drift (f3 outgroup analysis (X, Y; Mbuti), where Mbuti (a present-day population from Central Africa) serves as an outgroup, X and Y are ancient Tibetan Plateau populations, listed symmetrically in the rows and columns) was observed between these groups (Fig. 3c, Extended Data Fig. 7. Supplementary Fig. 1 and Supplementary Data 1). The southern plateau ancestry of Mabu Co individuals is further supported by the f4 analysis, in which Mabu Co shows more connection with ancient southern plateau populations, including Shannan3k and Lubrak (Supplementary Table 3 and Supplementary Data 2). This affiliation with southern plateau lineage exhibits subtle differences within the Mabu Co E1 and Mabu Co E2 subgroups (Extended Data Figs. 8 and 9, Supplementary Text, Supplementary Figs. 2 and 3 and Supplementary Data 2); it was further confirmed by the fact that Mabu Co E2 can be used as a one-way source for Shanan3k and Lubrak in qpAdm (Fig. 3d and Supplementary Table 4). Mabu Co also showed features of a deep lineage gene flow that were similar with previous Shannan3k and Lubrak (Supplementary Data 3 and 4) and exhibit characteristic of genes adapted to the plateau. The homozygous EPAS1 genotype, which confers adaptation to high altitude<sup>24</sup>, is found in Mabu Co 1 individual, with two EPAS1 heterozygotes, suggesting that this adaptive genetic trait may be undergoing selection in this population (Supplementary Tables 5 and 6).

Previous studies indicated that Tibetan Plateau ancestry is derived in large part from a single source related to a 9,500-year-old individual from the Yellow River region that diverges to three main lineages<sup>15</sup>. The northeastern lineage represented by the Zongri site can be traced back to 5,100 cal BP, but the more important southern lineage (which mainly contributes to present-day Tibetans) could only be traced back to 3,400 cal BP<sup>23</sup>. Our study revealed that the inhabitants of Mabu Co and Zongri had genetically diverged by 4,400 cal BP (Fig. 3c and Extended Data Fig. 7). The genetic profile of Mabu Co individuals shows that southern plateau ancestry, one of the major genetic components for present-day Tibetans, occurred on the southern plateau by at least approximately 4,400 cal BP. This sheds light on the genetic profile of the oldest Indigenous foragers on the southern plateau, pushing back the emergence of the southern plateau ancestry by 1,000 years earlier than previous estimates.

#### Sedentary life based on fishing

We collected thousands of bones, both fragmented and complete, from excavation trenches in the refuse deposits. These were then identified by both osteomorphological and ancient DNA analyses (Methods). The identified animals include fish, birds and mammals, indicating diverse resources for meat consumption at the site. Fish are endemic Tibetan species, including *Gymnocypris* and *Schizopygopsis* spp. Birds include coot (*Fulica atra*), great cormorant (*Phalacrocorax carbo*), common merganser (*Mergus merganser*) and pochard (*Netta* and *Aythya* spp.). The remains of mammals included endemic species, such as the Himalayan marmot (*Marmota himalayana*), Przewalski gazelle (*Procapra przewalskii*), argali (*Ovis ammon*) and red deer (*Cervus elaphus*), as well as woolly hare (*Lepus oiostolus*), lynx (*Lynx lynx*) and wolf (*Canis lupus*) (Fig. 4a and Extended Data Fig. 10).

Fish bones dominate the faunal assemblage (Supplementary Table 7), consisting of 81.5% of the total number of recovered remains. Fishing tools like gorge hooks were recovered in large numbers (Fig. 2c). We identified the materials used to make the fish gorge hooks using





members and burials are visible on the site. **c**, Evolution of the paleolake extents of Mabu Co and Gala Co before and during the period of human occupation of the site, based on data from ref. 27. The base map was prepared using ArcGIS 'World Imagery'.



**Fig. 2** | **Typical profile of the Mabu Co site, fishing tools and charred crop plant remains. a**, Photograph of the east wall of the trial excavation trench TN06E03. **b**, Schematic drawing of the east wall of TN06E03. Description of stratigraphic layers: (1) top sandy soil with abundant grass roots and few charcoal fragments, bones and artefacts; (2) yellowish brown silt with abundant charcoal fragments, animal bones, pottery sherds and a small number of human teeth, crystal artefacts, bone artefacts and ornaments; (3) light yellow silt with white

carbonate fragments and artefact assemblages similar to the upper layer; (4) dark brown sandy gravels with a few charcoal fragments, lithic artefacts and animal bones; (5) light yellow alluvial sandy gravels without artefacts; (6) natural deposits of lakeshore sandy gravels. **c**, Fish gorge hooks. **d**, Foxtail millet (*S. italica*). **e**, Broomcorn millet (*P. miliaceum*). **f**, Rice (*O. sativa* sp.*japonica*). **g**, Rice spikelet base. Scale bars, 2 cm (**c**) and 1 mm (**d**–**g**).

ancient DNA analysis (Methods); this type of tool was made using bird bones belonging to either cormorant or crane.

The stable carbon and nitrogen isotope analyses of charred millet grains and bone collagen from both human and animal remains provide evidence that the diet of humans was dominated by fish consumption (Fig. 5). We measured carbon and nitrogen stable isotopes from 41 samples, including 10 humans, 15 fish, 9 terrestrial animals, 6 birds and 1 crop plant (Methods). The human remains yielded the highest  $\delta^{15}$ N values, which are approximately 5% higher than those of fish (Fig. 5 and Supplementary Table 8), which is consistent with the isotopic enrichment at each trophic level that is usually thought to range between 3‰ and 6‰ (refs. 25,26), indicating that fish was the dominant food for the site's inhabitants. The importance of fish in the diet is also reflected by the similar  $\delta^{13}$ C values of the human remains and fish bones, which are markedly different from those of birds, terrestrial animals and crop plants (Fig. 5). This further bolsters our argument that the fish-dominated diet of humans resulted in a radiocarbon reservoir effect on the ages of the human bones (Extended Data Fig. 4). A recent study showed that Mabu Co and Gala Co spanned a total area of more than 100 km<sup>2</sup>, much larger than their modern areas (8.8 km<sup>2</sup> in total), around 4,400-4,000 cal BP<sup>27</sup>. It was this massive paleolake that provided a reliable source of fish for site residents (Fig.1c).

The seasonal analysis of bone remains from hunted fish, mammals and birds showed that approximately two-thirds of fish were caught from May to October and about one-third from November to April (Fig. 4b); mammals and birds were hunted year-round (Fig. 4a,c and Supplementary Table 9). When fishing decreased from December to February during winter, supplementary resources were available because of wintering waterfowl, including great cormorants and pochards, which inhabited the study area in abundance<sup>28</sup> (Fig. 4a), and ungulates such as red deer (Fig. 4c), which provided meat to feed the site inhabitants. In addition, ethnographic evidence suggested that fish were dried and stored for later consumption<sup>29</sup>; fish from nearby lakes was dried and traded to Bhutan decades ago. Similar practices of drying and trading fish are documented at Qinghai and Yambrok lakes, where tens of thousands of tonnes of fish were harvested annually during the 1950s and 1960s<sup>20-31</sup>.

We identified a very small proportion of cereals, including foxtail millet (*Setaria italica*), broomcorn millet (*Panicum miliaceum*) and rice (*Oryza sativa* sp. *japonica*) (Fig. 2d–g) from plant remains (Methods), indicating that residents also had a carbohydrate intake. However, neither millet nor rice can grow at Mabu Co at an altitude of 4,446 MASL because of the low temperatures around the site<sup>32,33</sup>. Therefore, the cereal grains found at Mabu Co were most probably exchanged from lower-elevation areas where millet and rice could be cultivated. Although these domesticates make up less than 10% of the entire archaeobotanical assemblage (Supplementary Table 10), they were recovered from the bottom to the top of the refuse deposits, indicating an interaction network between the inhabitants of Mabu Co and farmers at lower elevations<sup>5,34</sup>. In fact, to the east of the Tibetan Plateau, the mixed farming of millet and rice had been practised since 5,000 cal BP<sup>34</sup>.

Finally, the presence of many burials at Mabu Co (Extended Data Fig. 1) suggests that the site held notable ritual importance for its inhabitants. By choosing to bury their ancestors at this location, they symbolically and spiritually claimed the land around the site. Furthermore, the extensive scale of refuse deposits (Extended Data Fig. 1) indicates that people spent considerable periods of time at the site.

In summary, our datasets indicate that the inhabitants of the Mabu Co site fished year-round and fish were the primary food for providing sustainable, stable protein sources that supported daily dietary needs. Inhabitants also obtained carbohydrate-rich cereals by exchange.



**Fig. 3** | **Genetic characterization of Mabu Co human bones. a**, Ancient Mabu Co individuals (purple) were projected on principal components calculated from 315 present-day East Asian individuals, located in the enlarged Tibetan branch (black dotted box). The grey points represent the projected background of modern East Asian populations, forming three main branches and a Tibetan branch. The orange points represent ancient southern East Asian populations; the brown points represent ancient northern East Asian populations; the yellow points represent ancient Yellow River-related populations; and the purple, green, red and dark blue points represent ancient Tibetan Plateau individuals from the northeastern plateau, central plateau, southern plateau and Nepal, respectively. The PCA details with coordinate axis and population categories can be found in Extended Data Fig. 5. **b**, ADMIXTURE plot for early ancient Tibetans (*k* = 5): blue, Tibetan ancestry; brown, Northern East Asian ancestry; orange, ancient southern East Asian ancestry. Details can be found in Extended Data Fig. 6. **c**, f3 outgroup analysis for pattern f3(X, Y; Mbuti). The Mabu Co group cluster with ancient southern plateau populations; darker blue indicates higher genetic similarity. **d**, Genetic ancestry estimated with qpAdm. Mabu Co E2 could be a one-way source for later Shannan3k and Lubrak southern lineage populations, while Mabu Co E1 represents southern plateau ancestry (Mabu Co E2/Shannan3k) admixtured with lowland Northern East Asia-related ancestry (Supplementary Table 4).





#### Lake-centred ways of living

While the transition to sedentism in some regions is closely intertwined with farming, the adoption of sedentary ways of living by groups who did not practise farming is an equally important global phenomenon<sup>35</sup>. In other parts of the world, such as high-latitude environments with low terrestrial primary productivity, the concentrated stability and year-round availability of fish in lacustrine or maritime environments enabled increased degrees of sedentism. For instance, in Alaska and Northeast Asia, the high productivity of aquatic ecosystems allowed foragers to reduce mobility and invest in the production of pottery, which was used to process and cook these aquatic resources<sup>36-39</sup>. Studies on 89 societies along the North American Pacific coast also suggest that freshwater and marine fish resources allowed communities to have higher population densities and greater sedentism compared to subsistence focused on hunting large terrestrial animals, which requires a higher degree of mobility<sup>40</sup>. Scholars argued that we should expect

displayed on each bar indicates the quantity of fish second vertebrae used for identification. c, Thin sections of animal teeth at Mabu Co showing the death seasons: (1) thin section located at the tooth cervix; (2) gazelle (Procapra sp.) from TG1(3) c that died in the summer (clear dark-light-dark band); (3) red deer (C. elaphus) from TG1(2) b that died in the summer or autumn (a light band with a little darkness at the edge); (4) gazelle (Procapra sp.) from TG1(2) b that died in the winter (light-dark-light bands). Scale bars, 200 µm.

reduced mobility in foragers around environmental resources that are concentrated, highly productive and predictable<sup>41</sup>. Our study provides another model for pre-agricultural sedentism at very high altitudes (analogous to other high-latitude and high-altitude areas around the world), with ways of living reliant on stable food resources sourced from a massive paleolake system. Fish, waterfowl and large mammals that used the lakes as both water and food supply were reliable local resources, while crops were obtained via exchange with lower-altitude areas. This strategy contrasts with to that on the lower-elevation eastern Tibetan Plateau where crops were cultivated locally by sedentary populations.

The Mabu Co inhabitants exhibited genetic consistency with other populations in the broader southern highlands during later periods. This new DNA evidence allowed us to gain new insights into the ancestral components of the southern highlands, which revealed a different genetic profile from the populations of the northeastern plateau. This



Fig. 5 |  $\delta^{13}$ C and  $\delta^{15}$ N results for human, animal and crop remains from the **Mabu Co site.** Isotopic data are presented as scatter plots along with the means and s.d. The squares and bars represent the means and s.d., respectively. VPDB, Vienna Pee Dee Belemnite.

suggests that the southern plateau ancestry might have earlier origins than 4,400 years ago and a widespread presence in the foothills of the Himalayas, both north and south, and along the Yarlung Tsangpo River valley. Our research overturns previous assumptions about the origins and nature of sedentism on the plateau, which assumed that it was associated with the dispersal of agriculture or farmers<sup>4,6–8</sup>, largely writing Indigenous foragers out of the history of the plateau. We demonstrated that Indigenous foraging populations who were genetically adapted to high altitudes practised sedentism by relying on year-round fishing at a highly productive lacustrine environment and hunting, and engaging in limited exchange for domestic crops. Overall, this study shows that foragers had an important role on the plateau throughout the Holocene and that they are genetic ancestors of present-day inhabitants of the plateau.

The Tibetan Plateau contains lakes covering a very large area of 50,000 km<sup>2</sup> (ref. 42), which are potential aquatic food resource providers. Future research may help us understand if this lake-centred lifestyle was a key component of the process of sedentism in other high-altitude areas with similar resource availability. A regional systematic survey at the high-altitude interior of the plateau should also be conducted to clarify the role that sites like Mabu Co had in regional settlement pattern systems, and to reveal the types of connections the foragers of the site held to those who grew crops at lower altitudes, in particular to clarify if they represented members of the same or different communities.

#### Methods

#### AMS<sup>14</sup>C radiocarbon dating

Fourteen and 19 samples were AMS<sup>14</sup>C-dated at Peking University and Beta Analytic, respectively (Supplementary Table 1). The IntCal20 curve and Libby half-life of 5,568 years were used in the tree-ring calculation of all dates<sup>43</sup>. Calibration was carried out using the OxCal 4.4 program<sup>44</sup>. All ages are reported as cal BP relative to 1,950 CE.

#### Ancient human DNA

Samples of human remains were collected during the 2020 and 2021 Mabu Co archaeological excavations using sterile protocols. Ancient DNA wet laboratory work and population genetics analysis were performed in the dedicated ancient DNA laboratory, using the data server platform at the Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing, China.

**DNA extraction and in-solution capture.** For all 12 samples, DNA was extracted from less than 100 mg of bone powder using an optimized silica-based extraction protocol<sup>45</sup>. DNA libraries were prepared using either double-stranded (DS) or single-stranded (SS) protocols<sup>46,47</sup>; all DS libraries were subjected to half uracil-DNA-glycosylase (UDG) treatment<sup>48</sup> (DS-half) (Supplementary Table 2). For the nine successful library samples, solution hybridization oligonucleotide probes were used to enrich endogenous DNA for a complete mitochondrial genome<sup>49</sup> and 1.2 million nuclear SNPs (1,240,000 SNPs)<sup>50</sup>.

**Sequencing and read alignment.** For the enriched mitochondrial DNA libraries, Illumina HiSeq X Ten sequencing was used to generate 2 × 76 base pairs (bp) paired-end reads, while for the enriched nuclear DNA libraries, Illumina HiSeq 2500 sequencing was used to generate 2 × 150 bp paired-end reads. Adaptors were trimmed and sequences with a minimum overlap of 11 base pairs were merged into one sequence using leeHom (https://github.com/grenaud/leeHom)<sup>51</sup>. Merged reads with a length of at least 30 bp from the mitochondrial DNA libraries and the nuclear DNA libraries were respectively aligned to the revised Cambridge Reference Sequence and the human nuclear reference (hg19) using the Burrows–Wheeler Aligner (v.0.7.17)<sup>52</sup>. For the mapped. bam file, duplicated reads with the same orientation, start and end positions were removed; fragments with a mapping quality score below 30 were removed.

Test for contamination and genotyping. To ensure the authenticity of ancient DNA, we first identified the terminal base C-T deamination ancient DNA damage signatures, then estimated the contemporary human contamination rates for each sample with a threshold of 3% contamination using ContamMix<sup>49</sup>. All samples showed a low contamination level (0–1.6%). We then assessed pseudo-haploid genotypes by randomly sampling one fragment per position to determine the corresponding allele in that individual. The 2-bp ends of the sequencing reads for UDG-treated samples and 5-bp ends of the sequencing reads for the sample not treated with UDG were masked when determining the pseudo-haploid genotypes. We were able to obtain nine new '1,240,000' capture genome datasets with ×0.53–×5.29 coverage.

**Dataset for population analysis.** Mabu Co populations were analysed using a worldwide reference genetic dataset containing 35 present-day populations, including populations from the Human Origin SNP panel<sup>53</sup>, the Simons Genome Diversity Panel<sup>54</sup>, the Human Genome Diversity Project<sup>55</sup> and the Tibetan, Sherpa and Han populations from previous Tibetan-related publications<sup>14,15,23</sup>. All the ancient populations used for the analysis were based on already existing ancient plateau population datasets<sup>15</sup> curated in the IVPP population genetics analysis platform.

**Kinship.** Genotyped data for the nine Mabu Co individuals were transformed from genotypic to .tped files using PLINK<sup>56</sup> (v.1.9) with the parameters --alleleACGT --recode transpose, after which the READ software<sup>57</sup> was used to calculate the average pairwise PO used to infer kinship.

**PCA.** We used the smartpca program (v.16000) in EIGENSOFT (v.7.2.1) to perform the PCA, with the following parameters: numoutlierevec: 2; outliersigmathresh: 12; lsqproject: YES; and autoshrink: YES. Forty-three present-day East Asian populations on or near the Tibetan Plateau genotyped in the Human Origins dataset (597,573 SNPs) were used to construct the principal component space on which we projected all ancient individuals.

Admixture. Admixture is a method for fast model-based estimation of ancestry in unrelated individuals<sup>58</sup>. We first pruned the dataset to account for linkage disequilibrium using PLINK (v.1.90) with the parameters '--indep-pairwise 200 25 0.4'. We then used ADMIXTURE (v.1.30) to estimate individual ancestries and determine the population structure parameter '-j10', running the software ten times using different seeds to estimate the lowest cross-validation error for the 'best' k. The results were plotted using the AdmixturePlotter-master R script.

**f3 analysis.** The *f* statistic, which was introduced in ref. **53**, quantified the population genetic drift on different population trees and tested the occurrence of admixture. The f3 outgroup, f3(outgroup; X, Y), is the average of  $(P_{out} - P_X)(P_{out} - P_Y)$  across sites, where  $P_X$  and  $P_Y$  are the frequencies of a diallelic SNP in X and Y. qp3Pop (v.412) from the ADMIX-TOOLS package was used to calculate the f3 statistics. We used the Indigenous Mbuti population as the f3 outgroup (X, Y; Mbuti). Higher f3 values can be interpreted as having high genetic similarity with each other; the heatmap based on the f3 value matrix of pairs of people related to each other was used to cluster the related populations.

**f4 analysis.** qpDstat (v.712) was used to calculate the f4 statistics by turning on the 'f4mode: YES' option. Autosomal SNPs from the 1,240,000 dataset were used unless mentioned otherwise. The general form of the f4 statistic we used is f4(Mbuti, P2; P3, P4), where the Mbuti are again used as an outgroup to the individuals or populations represented by P2, P3 and P4. An f4 > 0 (Z > 3) indicates that P2 shares more alleles with P4 than P3, an f4 < 0 (Z < -3) indicates that P2 shares more alleles with P3 than P4, and f4 = 0 (Z < 3) indicates that P2 shares a similar number of alleles with both P3 and P4.

**MDS.** Multidimensional scaling demonstrates the differences (or similarities) between objects by graph. Based on the genetic relation f3 outgroup value, we used multidimensional scaling to convert the f3 outgroup matrix into two-dimensional coordinate vectors using the cmdscale function in R (v.4.2.0).

**qpAdm.** qpAdm is a method based on the f4 statistic that models the mixture of ancestry for a target population. We used qpAdm (v.634) in the ADMIXTOOLS package with the parameters 'details: YES' and 'allsnps: YES' to model ancient Mabu Co populations using genetic libraries from both ancient Tibetans and ancient lowland East Asian populations.

**EPAS1** haplotype. The 30 most differential SNPs were selected to determine the *EPAS1* haplotype<sup>24</sup>. We used SAMtools<sup>59</sup> (v.1.5) mpileup to summarize the *EPAS1* SNPs from the BAM files. The generated results were then co-analysed with previously published ancient genomic data. Changes in the *EPAS1* frequency of southern plateau populations was plotted using a bar chart.

#### Animal species identification

Animal remains were identified according to morphology and using bulk bone shotgun metagenomics (BBSM). The results from both methods agreed with each other. BBSM was used to compile the animal species while morphological analysis was performed to identify every fragment of the animal remains and calculate the relative proportions of the species.

**Morphology and osteostructure.** The animal remains from the trial excavation trench TG2 were analysed by layers. The assemblage from each layer was categorized into mammal, bird, fish and mollusc according to morphology and micromorphological structure. Each category was further sorted, identified and recorded. Identification procedures were undertaken with the aid of the reference collection in the Institute of Tibetan Plateau Research and zooarchaeological atlases<sup>60–63</sup>. An ivory

bangle was identified by observing the Schreger pattern with both the naked eye and a Dino-Lite Digital Microscope<sup>64</sup>.

BBSM. Three samples of mammals, fish and birds, respectively, were collected from each layer. Fifteen samples collected from five layers of the trial excavation trench TG6 included 1,890 bone fragments (Supplementary Table 11). These cultural layers are consistent with those in the excavation trench TG2 in terms of relative chronology and artefact styles. In addition, 12 fragmented fish gorge hooks, originally constructed from skeletal remains in TG2, were collected and combined into a single sample for material identification. The sampler conducted the sampling wearing a face mask, full-body suit, nitrile gloves and using sterile metal forceps. DNA extraction and library preparation were performed in a dedicated ancient DNA laboratory at the China University of Geosciences at Wuhan. Total genomic DNA was extracted using a modified protocol<sup>65</sup>. A doubled quantity of bone powder, EDTA and proteinase K was used. A volume of 20 µl DNA extract was used for dsDNA library construction according to the standard protocol<sup>46</sup>. DNA sequencing was conducted on an Illumina HiSeq 2500 platform. Sequencing reads were trimmed using the 'Holi' pipeline to remove low-complexity reads, duplicates and reads below 30 bp in size. These reads were thereafter aligned against the NCBI database of full mitochondrial genomes within Metazoa (downloaded November 2021). The alignments were eventually parsed through ngsLCA (https://github.com/miwipe/ngsLCA); reads with 100% similarity to the reference were retained for taxonomic assignment<sup>66</sup>. The identified taxa for each sample were profiled at the species and genus level by merging all reads below the taxon. In addition, reads were only considered as genuine if the corresponding taxa could be found in the study area (Tibetan Plateau) (Supplementary Table 12).

#### Seasonal animal death determination

Annulus increment analysis. The second vertebra of cyprinids has a distinctive shape that helped us identify the specimen to the species, and were thus selected for the annulus increment analysis. Only fish remains recovered from cultural layers were sampled for reconstruction of fishing seasonality. Vertebrae were cleaned using ultrasonication to reveal the annuli on the anterior and posterior articular surfaces. Both articular surfaces of each vertebra were examined and photographed using an M165 FC microscope (Leica Microsystems) with a DFC450C digital camera (Leica Microsystems). Unlike modern specimens on which the summer and winter growth rings can be discerned by their opaqueness and transparency, the summer and winter growth rings on the archaeological specimens were distinguished by the regular change of hues: the darker rings were winter growth rings, while the lighter rings were summer growth rings (Fig. 4b). The outermost ring type indicated the death season of the fish.

Cementum increment analysis. Seventeen specimens, including two isolated teeth and 15 teeth removed from jaws, each representing one individual, were selected for the cementum increment analysis (Supplementary Table 9). Although research suggested that molars are better samples for such analysis than premolars, canines and incisors, because of the limited number of teeth, premolars and incisors were also examined in our study. The samples were cleaned using ultrasonication and embedded in polyester resin. The embedded specimens were cross-sectioned at the tooth cervix and polished to obtain microscopic thin sections in thicknesses between 40 and 100  $\mu$ m. The thin sections were observed with a transmitted light polarized light microscope (DM3000, Leica Microsystems). The warm and cold season growth rings on the archaeological specimens were also distinguished by the regular change of hues: the light rings were winter growth rings and the dark rings were summer growth rings under transmitted light. Eight samples displayed clear cementum increment layers (Fig. 4c).

#### Stable isotope analysis

**Bone collagen.** The stable isotope values of bone and teeth reflected the isotopic composition of dietary components with known fraction offsets and were used to investigate the paleodiet of ancient people<sup>67,68</sup>. Stable nitrogen isotope values show a 3% to 6% stepwise enrichment in each trophic level from plants to herbivores to carnivores in aquatic and terrestrial ecosystems<sup>25,26</sup>. Hence, nitrogen isotope values could potentially tell us about the intake of animal versus plant proteins and aquatic versus terrestrial proteins in the past<sup>69</sup>. There is a small stepwise increase in  $\delta^{13}$ C values of about 1% between trophic levels in both aquatic and terrestrial ecosystems, thus the  $\delta^{13}$ C values of humans are close to those of their animal-based food<sup>70,71</sup>. Relying on bone and teeth collagen  $\delta^{15}$ N values, we reconstructed the paleodiet of the Mabu Co site.

Stable carbon and nitrogen isotope data were recovered from 40 bone collagen samples of the Mabu Co site (Supplementary Table 8). Sixteen samples were measured by Beta Analytic (Supplementary Data 5), after direct radiocarbon dating; four samples were measured at the Joint International Research Laboratory of Environmental and Social Archaeology, Shandong University (Supplementary Data 6) while the other 20 samples were measured at the Environmental Stable Isotope Laboratory (ESIL), Institute of Environment and Sustainable Development of Agriculture, Chinese Academy of Agricultural Sciences (Supplementary Data 7). At Beta Analytic, after removing surface contamination, bone collagen was extracted using their protocol<sup>72</sup>. A subsample of each sample was routinely analysed with isotope ratio mass spectrometry (IRMS), using the DELTA V Advantage (50-40,000 mV, Thermo Fisher Scientific), wrapped in a standard IRMS tin boat and placed into an auto-dispenser on a Costech 4010 Elemental Analyzer. The %N, %C and C:N ratio were determined using the ECS Clarity software. Measurement errors were less than  $\pm 0.3\%$  for  $\delta^{13}C$  and  $\pm 0.5\%$ for the  $\delta^{15}$ N values.

At ESIL, after collagen was extracted at ITPCAS, samples were measured using an IsoPrime 100 IRMS (Elementar) coupled with an Elementar Vario (Elementar), and calibrated with USGS 40 ( $\delta^{13}C = -26.39 \pm 0.04\%_0$ ,  $\delta^{15}N = -4.52 \pm 0.06\%_0$ ) and USGS 41a ( $\delta^{13}C = +36.55 \pm 0.08\%_0$ ,  $\delta^{15}N = +47.55 \pm 0.15\%_0$ ) as reference materials. For every 12 samples, a laboratory reference – gelatine from bovine skin ( $\delta^{13}C = -14.7 \pm 0.2\%_0$ ;  $\delta^{15}N = +7.1 \pm 0.2\%_0$ ) – was inserted for calibration and to monitor stability. Measurement errors were less than  $\pm 0.2\%$  for both  $\delta^{13}C$  and  $\delta^{15}N$  values. The %N and %C often of the fish samples were previously measured on a Vario EL III Elemental Analyzer (Elementar) at the Analysis and Testing Center, College of Chemistry and Chemical Engineering, Lanzhou University.

At the Joint International Research Laboratory of Environmental and Social Archaeology, both collagen extraction and isotopic measurement of those four samples were done in the laboratory. Samples were extracted according to the protocol published in ref. 69 and then measured using an DELTA V Advantage IRMS (Thermo Fisher Scientific) coupled with a Flash 2000 HT Elemental Analyzer (Thermo Fisher Scientific), and calibrated with USGS 40 ( $\delta^{13}C = -26.39 \pm 0.04\%$ ;  $\delta^{15}N = -4.52 \pm 0.10\%$ ), USGS 62 ( $\delta^{13}C = -14.79 \pm 0.04\%$ ;  $\delta^{15}N = \pm 20.17 \pm 0.06\%$ ) and USGS 41a ( $\delta^{13}C = \pm 36.55 \pm 0.08\%$ ;  $\delta^{15}$ N = + 47.55 ± 0.15‰), with the internal standard alanine peptide  $(\delta^{13}C = -17.94 \pm 0.12; \delta^{15}N = -0.98 \pm 0.21\%)$  as reference materials. For every ten samples, a laboratory reference-EMA B2155 (casein)  $(\delta^{13}C = -27.21 \pm 0.08\%; \delta^{15}N = +6.00 \pm 0.10\%)$ -was inserted; for every 20 samples, the internal standard alanine peptide was inserted for calibration and to monitor stability. Measurement errors were less than  $\pm 0.1\%$  for  $\delta^{13}$ C and  $\pm 0.2\%$  for  $\delta^{15}$ N.

Collagen quality was assessed after considering several preservation criteria: collagen yield greater than 1%, &C = 30-44%, &N = 11-16% and C:N ratio = 2.9-3.6 (refs. 69,73-75). According to the &C, &N and C:N ratio, all samples were well-preserved and yielded good-quality collagen for carbon and nitrogen extraction, indicating that  $\delta^{\rm 13}C$  and  $\delta^{\rm 15}N$  could be used to reconstruct the paleodiet in these samples.

**Charred millet grains.** One charred millet sample (totalling 21 individual grains) floated from deposits of TG3 during the 2020 excavation of the Mabu Co site were analysed. First, millet grains were soaked with 0.5 M HCl at 80 °C for 60 min. Second, we rinsed the millet grains with ultra-pure water six times. Third, we freeze-dried the grains and crushed them into a homogeneous powder. Finally, a 3.1-mg sample was weighed into tin containers for isotopic measurement. The  $\delta^{13}$ C and  $\delta^{15}$ N values of the powder samples were measured using an IsoPrime 100 IRMS (Elementar) at ESIL, Institute of Environment and Sustainable Development of Agriculture, Chinese Academy of Agricultural Sciences. For every 12 samples, a laboratory reference gelatine from bovine skin ( $\delta^{13}$ C = -14.7 ± 0.2‰;  $\delta^{15}$ N = + 7.1 ± 0.2‰) was inserted for calibration and to monitor stability. Measurement errors were less than ±0.2‰ for both  $\delta^{13}$ C and  $\delta^{15}$ N.

#### Flotation and identification of macroscopic plant remains

For the analysis of macroscopic plant remains, blanket flotation samples were collected during excavation along with opportunistic sampling targeting daily refuse deposits from the cultural layers. The sediment samples were processed using the bucket flotation method in the field<sup>76</sup>. Macroscopic plant remains were collected in 0.45 mm mesh bags and then air-dried in the shade. These remains were sorted and identified under a  $\times 6.8 - \times 47$  binocular stereomicroscope in the ITPCAS laboratory, using our collections and the atlas of modern seeds in China for identification<sup>77–80</sup>; 126 litres of sediments from six cultural layers of trench TG2 were examined (Supplementary Table 10). All the specimens were sorted and photographed using an M165FC microscope (Leica Microsystems) with a DFC450C digital camera (Leica Microsystems).

#### **Ethics and inclusion statement**

Our excavation and research work on the Mabu Co site were approved and supported by relevant government departments at the national, provincial and county levels: National Cultural Heritage Administration; Tibetan Cultural Heritage Administration; and Cultural Heritage Administration of Kangmar County, Tibet Autonomous Region. Before the start of the excavation, three major participating institutes, Lanzhou University, the Institute of Tibetan Plateau Research of the Chinese Academy of Sciences and the Tibetan Institute of the Preservation of Cultural Relics, signed mutually beneficial cooperation agreements. The main terms of the agreements include that the Tibetan Institute of the Preservation of Cultural Relics leads the excavation work and has the ownership of archaeological samples for the purposes of preservation and study. Lanzhou University and the Institute of Tibetan Plateau Research of the Chinese Academy of Sciences provide financial support for the excavations, participate in the excavations, carry out multidisciplinary scientific analysis of archaeological samples and provide training opportunities for the personnel of the Tibetan Institute of the Preservation of Cultural Relics. This research article is the result of collaboration among these three institutions. The researchers, including S.W. (Tibetan ancestry), Y.T. and Y. Li, from the local research institute, have appeared as co-authors. Among them, S.W. is one of the corresponding authors. S.W. worked with the Gala Town community at Mabu Co, which consented to and participated in the excavations. The involvement of the local community was ensured through community meetings, consultations and ongoing engagement. Local community members who participated in the excavations are listed in the Acknowledgements.

#### **Reporting summary**

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

#### Data availability

Key data used in this study are available in the main text. Extended Data Figs. 1-10 and the accompanying Supplementary Information. Other datasets generated in this study are available from the corresponding authors upon reasonable request. The raw sequencing reads, aligned BAM files and the mitochondria FASTA files are available through the Genome Sequence Archive and the Genbase datasets (https://bigd. big.ac.cn/gsa-human and https://ngdc.cncb.ac.cn/genbase; accession no. PRJCA024667; https://ngdc.cncb.ac.cn/gsa-human/s/LZ2QzIVY; https://ngdc.cncb.ac.cn/genbase/review/4969bfb8c3f4). The pseudo-diploid genotype calls are available through the OMIX database, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (accession no. OMIX006078; https://ngdc.cncb.ac.cn/omix/preview/g7zYGt1X: https://share.cncb. ac.cn/9eac48fa9254). Ancient human and archaeological samples have been stored at the Tibetan Institute of the Preservation of Cultural Relics.

#### **Code availability**

The software used for the population genetics analysis (smartpca, f3, f4, qpAdm) was based on the ADMIXTOOLS (https://github.com/ DReichLab/AdmixTools) and EIG (https://github.com/DReichLab/EIG) tools developed by the David Reich lab. All PAR files containing the parameters used in the analysis and the scripts used for plotting and table creation are available at https://github.com/RickRan/MabuCo.

#### References

- 1. Zhang, P. et al. Denisovans and *Homo sapiens* on the Tibetan Plateau: dispersals and adaptations. *Trends Ecol. Evol.* **37**, 257–267 (2022).
- Zhao, Y., Obie, M. & Stewart, B. A. The archaeology of human permanency on the Tibetan plateau: a critical review and assessment of current models. *Quat. Sci. Rev.* **313**, 108211 (2023).
- 3. d'Alpoim Guedes, J. & Aldenderfer, M. The archaeology of the early Tibetan Plateau: new research on the initial peopling through the Early Bronze Age. *J. Archaeol. Res.* **28**, 339–392 (2020).
- Chen, F. H. et al. Agriculture facilitated permanent human occupation of the Tibetan Plateau after 3600 B.P. Science 347, 248–250 (2015).
- 5. Song, J. et al. Farming and multi-resource subsistence in the third and second millennium BC: archaeobotanical evidence from Karuo.*Archaeol. Anthropol. Sci.* **13**, 47 (2021).
- 6. Shi, S. Tibetan-Yi Corridor: The Origin of Civilization and the Evolution of Ethnicity (Sichuan People's Publishing House, 2009).
- Sagart, L. et al. Dated language phylogenies shed light on the ancestry of Sino-Tibetan. Proc. Natl Acad. Sci. USA 116, 10317– 10322 (2019).
- Zhang, M. H., Yan, S., Pan, W. Y. & Jin, L. Phylogenetic evidence for Sino-Tibetan origin in northern China in the Late Neolithic. *Nature* 569, 112–115 (2019).
- Madsen, D. B. et al. The Late Upper Paleolithic occupation of the northern Tibetan Plateau margin. J. Archaeol. Sci. 33, 1433–1444 (2006).
- 10. Madsen, D. B. et al. Early foraging settlement of the Tibetan Plateau highlands. *Archaeol. Res. Asia* **11**, 15–26 (2017).
- 11. Rhode, D. et al. Epipaleolithic/Early Neolithic settlements at Qinghai Lake, western China. J. Archaeol. Sci. **34**, 600–612 (2007).
- Han, F., He, Y., Du, W., Cai, L. & Lu, H. Technological strategy and mobility of Middle Holocene hunter-gatherers in the high-altitude Qinghai-Tibetan Plateau: a case study from Tshem gzhung kha thog. Archaeol. Anthropol. Sci. **12**, 242 (2020).
- Jin, S. et al. Prehistoric human occupation and adaptation on the hinterland of the Tibetan Plateau in the Early Holocene. *Prog. Phys. Geogr.* 47, 931–949 (2023).

- 14. Lu, D. et al. Ancestral origins and genetic history of Tibetan Highlanders. *Am. J. Hum. Genet.* **99**, 580–594 (2016).
- 15. Wang, H. et al. Human genetic history on the Tibetan Plateau in the past 5100 years. *Sci. Adv.* **9**, eadd5582 (2023).
- Cultural Relics Management Committee of Tibet Autonomous Region & Department of History of Sichuan University. *Chamdo Karuo: A Neolithic Site in Tibet* (Cultural Relics Publishing House, 1985).
- 17. Cui, Y. et al. Stable isotopic analysis on human bones from Zongri site. *Quat. Sci.* **26**, 604–611 (2006).
- Institute of Archaeology of Chinese Academy of Social Sciences & Bureau of Cultural Relics. *Lhasa Qugong: Excavations of An Ancient Site and Tombs* (Encyclopedia of China Publishing House, 1999).
- 19. Gao, Y., Yang, J., Ma, Z., Tong, Y. & Yang, X. New evidence from the Qugong site in the central Tibetan Plateau for the prehistoric Highland Silk Road. *The Holocene* **31**, 230–239 (2021).
- Tremblay, J. C. & Ainslie, P. N. Global and country-level estimates of human population at high altitude. *Proc. Natl Acad. Sci. USA* 118, e2102463118 (2021).
- Jones, P. & Harris, I. Climatic Research Unit (CRU): Time-series (TS) Datasets of Variations in Climate with Variations in Other Phenomena v4 (CRU, 2019).
- Mischke, S., Weynell, M., Zhang, C. & Wiechert, U. Spatial variability of C-14 reservoir effects in Tibetan Plateau lakes. *Quatern. Int.* **313–314**, 147–155 (2013).
- 23. Liu, C.-C. et al. Ancient genomes from the Himalayas illuminate the genetic history of Tibetans and their Tibeto-Burman speaking neighbors. *Nat. Commun.* **13**, 1203 (2022).
- Huerta-Sánchez, E. et al. Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. *Nature* 512, 194–197 (2014).
- Bocherens, H. & Drucker, D. G. Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. *Int. J. Osteoarchaeol.* 13, 46–53 (2003).
- 26. O'Connell, T. C., Kneale, C. J., Tasevska, N. & Kuhnle, G. G. The diet-body offset in human nitrogen isotopic values: a controlled dietary study. *Am. J. Phys. Anthropol.* **149**, 426–434 (2012).
- 27. Zhang, S. et al. Late quaternary lake level variations of Mabu Co-Gala Co, southern Tibetan plateau, modulated by glacial meltwater, spillover processes and the Indian summer monsoon. *Quat. Sci. Rev.* **334**, 108743 (2024).
- Zhang, G. et al. The water bird community and home range of the bar-headed goose at Yamdrok Lake in Tibet, China. *Acta Ecol. Sin.* 36, 946–952 (2015).
- 29. Wu, Y. & Wu, C. *The Fishes of the Tibetan Plateau* (Sichuan Science and Technology Publishing House, 1992).
- 30. Tibet Autonomous Region Fisheries Bureau. Fishes and Fish Resources in Tibet, China (China Agriculture Press, 1995).
- 31. Liu, J. Resources, livelihood and ethnic groups: the fishing, transportation and marketing of *Gymnocypris przewalskiiin* Qinghai Lake from Qing Dynasty to the Republic of China. J. Chin. *Hist. Geogr.* **36**, 81–88 (2021).
- 32. Wang, R. & Song, C. Investigation and research on millets in Tibet. Chin. Agric. Sci. Bull. **20**, 39–45 (1987).
- Gumma, M. K., Gauchan, D., Nelson, A., Pandey, S. & Rala, A. Temporal changes in rice-growing area and their impact on livelihood over a decade: a case study of Nepal. *Agric. Ecosyst. Environ.* 142, 382–392 (2011).
- 34. Huan, X. The emergence of rice and millet farming in the Zang-Yi corridor of southwest China dates back to 5000 years ago.*Front. Earth Sci.* **10**, 874649 (2022).
- 35. Marshall, Y. Introduction: adopting a sedentary lifeway. *World Archaeol.* **38**, 153–163 (2006).

- Admiraal, M. et al. The role of salmon fishing in the adoption of pottery technology in subarctic Alaska. J. Archaeol. Sci. 157, 105824 (2023).
- Yanshina, O. V. & Kovalenko, S. V. New data and insights into how pottery appeared along the Amur River. *Quat. Int.* 608, 154–177 (2022).
- Jordan, P. et al. in *Maritime Prehistory of Northeast Asia* (eds Cassidy, J. et al.) 315–345 (Springer, 2022).
- Gibbs, K. et al. Exploring the emergence of an 'Aquatic' Neolithic in the Russian Far East: organic residue analysis of early hunter-gatherer pottery from Sakhalin Island. Antiquity 91, 1484–1500 (2017).
- Smith, E. A. & Codding, B. F. Ecological variation and institutionalized inequality in hunter-gatherer societies. *Proc. Natl Acad. Sci. USA* **118**, e2016134118 (2021).
- 41. Fitzhugh, B. in *Maritime Prehistory of Northeast Asia* (eds Cassidy, J. et al.) 379–422 (Springer, 2022).
- 42. Zhang, G., Luo, W., Chen, W. & Zheng, G. A robust but variable lake expansion on the Tibetan Plateau. *Sci. Bull.* **64**, 1306–1309 (2019).
- Reimer, P. J. et al. The IntCal20 northern hemisphere radiocarbon age calibration curve (0–55 calkBP). *Radiocarbon* 62, 725–757 (2020).
- 44. Ramsey, C. B. & Lee, S. Recent and planned developments of the program OxCal. *Radiocarbon* **55**, 720–730 (2013).
- 45. Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl Acad. Sci. USA* **110**, 15758–15763 (2013).
- Meyer, M. & Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* 2010, pdb.prot5448 (2010).
- Gansauge, M.-T. & Meyer, M. Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. *Nat. Protoc.* 8, 737-748 (2013).
- Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
- Fu, Q. et al. DNA analysis of an early modern human from Tianyuan Cave, China. Proc. Natl Acad. Sci. USA 110, 2223–2227 (2013).
- 50. Fu, Q. et al. An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
- Renaud, G., Stenzel, U. & Kelso, J. leeHom: adaptor trimming and merging for Illumina sequencing reads. *Nucleic Acids Res.* 42, e141 (2014).
- 52. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
- Patterson, N. et al. Ancient admixture in human history. *Genetics* 192, 1065–1093 (2012).
- Mallick, S. et al. The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* 538, 201–206 (2016).
- 55. Bergström, A. et al. Insights into human genetic variation and population history from 929 diverse genomes. *Science* **367**, eaay5012 (2020).
- Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575 (2007).
- Monroy Kuhn, J. M., Jakobsson, M. & Günther, T. Estimating genetic kin relationships in prehistoric populations. *PLoS ONE* 13, e0195491 (2018).
- Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19, 1655–1664 (2009).
- 59. Danecek, P. et al. Twelve years of SAMtools and BCFtools. *Gigascience* **19**, giabO08 (2021).

- Cohen, A. & Serjeantson, D. A Manual for the Identification of Bird Bones from Archaeological Sites (Archetype Publications, 1996).
- 61. France, D. L. Human and Nonhuman Bone Identification: a Color Atlas (CRC, 2008).
- 62. Hillson, S. Mammal Bones and Teeth: an Introductory Guide to Methods of Identification (Routledge, 2016).
- 63. Schmid, E. Atlas of Animal Bones. For Prehistorians, Archaeologists and Quaternary Geologists (Elsevier, 1972).
- Nocete, F., Vargas, J. M., Schuhmacher, T. X., Banerjee, A. & Dindorf, W. The ivory workshop of Valencina de la Concepción (Seville, Spain) and the identification of ivory from Asian elephant on the Iberian Peninsula in the first half of the 3rd millennium BC. J. Archaeol. Sci. 40, 1579–1592 (2013).
- 65. Hu, J. et al. Ancient mitochondrial genomes from Chinese cave hyenas provide insights into the evolutionary history of the genus *Crocuta. Proc. Biol. Sci.* **288**, 20202934 (2021).
- 66. Wang, Y. et al. Late Quaternary dynamics of Arctic biota from ancient environmental genomics. *Nature* **600**, 86–92 (2021).
- 67. Roberts, P. et al. Calling all archaeologists: guidelines for terminology, methodology, data handling, and reporting when undertaking and reviewing stable isotope applications in archaeology. *Rapid Commun. Mass Spectrom.* **32**, 361–372 (2018).
- van der Merwe, N. J. & Vogel, J. C. <sup>13</sup>C content of human collagen as a measure of prehistoric diet in woodland North America. *Nature* 276, 815–816 (1978).
- 69. Ambrose, S. H. Preparation and characterization of bone and tooth collagen for isotopic analysis. *J. Archaeol. Sci.* **17**, 431–451 (1990).
- 70. Fry, B. & Sherr, E. B. in *Stable Isotopes in Ecological Research* (eds Rundel, P. W. et al.) 196–229 (Springer, 1989).
- Schoeninger, M. J. Trophic level effects on <sup>15</sup>N/<sup>14</sup>N and <sup>13</sup>C/<sup>12</sup>C ratios in bone collagen and strontium levels in bone mineral. *J. Hum. Evol.* 14, 515–525 (1985).
- 72. Longin, R. New method of collagen extraction for radiocarbon dating. *Nature* **230**, 241–242 (1971).
- DeNiro, M. J. Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* **317**, 806–809 (1985).
- Harbeck, M. & Grupe, G. Experimental chemical degradation compared to natural diagenetic alteration of collagen: implications for collagen quality indicators for stable isotope analysis. Archaeol. Anthropol. Sci. 1, 43–57 (2009).
- van Klinken, G. J. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. J. Archaeol. Sci. 26, 687–695 (1999).
- 76. Pearsall, D. M. Paleoethnobotany: a Handbook of Procedures (Routledge, 2016).
- 77. Guo, Q. The Illustrated Seeds of Chinese Medicinal Plants (Agricultural Publishing House, 2009).
- 78. Liu, C., Jin, G. & Kong, Z. Archaeobotany: Research on Seeds and *Fruits* (Science Press, 2008).
- 79. Ma, X., Li, M., Liu, Y. & Liu, C. Seed Images of China National Herbarium (PE) (Henan Science and Technology Press, 2022).
- 80. Wang, Z. Farmland Weeds in China: a Collection of Coloured Illustrative Plates (Agricultural Publishing House, 1990).
- Yunnan Provincial Institute of Cultural Relics and Archaeology, Dali Prefectural CPAM, Yongping County CPAM. Excavation of the Xinguang site in Yongping, Yunnan. *Acta Archaeol. Sin.* 2, 203–234 (2002).
- Hao, X., Zhou, Z., Liu, L. & Tian, J. The Neolithic occupants in the Yanyuan Basin: excavation of the burials at the Guijiabao site, Sichuan Province, Southwest China. *Archaeol. Res. Asia* 29, 100341 (2022).

#### Article

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#### **Author contributions**

F.C., X.Y. and S.W. designed the project. S.W., X.Y., Y.G. and Songtao Chen directed the excavation. Y. Tong., N.J., Q.W., Y. Li, J.Y., Ying Zhang, Q.Y., Y.W., Y.H., S.H., Y. Tian, J.G.J. X.L., T.Y., J.R., Z.D., L.Q. and Y. Zhuang participated in the excavation. Q.F., J.R., T.W. and Y.L. performed the human ancient DNA research. Ying Zhang and Q.W. performed the zooarchaeological research. Y.G. identified the archaeobotanical remains. Songtao Chen, Y.H. and J.Y. carried out the stable isotope analysis. Yunan Zhang, Y.G. and X.W. processed the dating samples. Z.G., Shungang Chen and Q.W. performed the animal ancient DNA analysis. X.Y., F.C., S.W., Y.G., J.Y., Q.F., J.R., T.W., Y. Liu, Songtao Chen, Ying Zhang and Q.W. analysed the data. J.Y., J.R. and Q.W. performed the data visualization. X.Y., Y.G., J.R., J.Y., Songtao Chen, L.P., J.D.G., Ying Zhang, Q.W., Y. Liu, Q.F. and F.C. wrote the paper with contributions from all authors.

#### **Competing interests**

The authors declare no competing interests.

#### **Additional information**

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Extended Data Fig. 1 | Aerial view of the Mabu Co site. Plan view map of excavation units and burials referred in this study at the Mabu Co site.



**Extended Data Fig. 2** | **Post molds and typical burial from the Mabu Co site. a**, Overhead view of the trench (TN32E25) where the post molds are located. **b**, Post molds. Yellow dotted circles outline the locations of post molds. Postholes were found in an irregular aligngment which may correspond to a living structure. While the upper portion of this living structure was not found, it is possible that this structure was finished using organic material such as animal hide that was reused and moved from place to place as is common in other areas of the world. c, Close-up view of post molds. d, Illustration image of the burial NM3. Grave of women about 25-year-old, southwest-northeast orientation. A unique, local mortuary practice. The typical stone coffin burial, characterized by stone walls surrounding the occupants who were placed in an extended prone position. The positioning of the heads and feet of the human remains on a flat stone with the head facing front.



**Extended Data Fig. 3** | **Selected artifacts from the Mabu Co site. a** and **b**, Pottery sherds. The pottery styles point to a connection to the East. The shapes of the pottery objects and their characteristic decorative patterns with engraving, stamping, embossing, and stacking techniques are similar to contemporaneous pottery assemblages from the southeastern margin of the Tibetan Plateau more than 1000 km from Mabu Co<sup>81,82</sup>. **c**, Stone axe. **d**, Crystal microliths. **e**, Bone bead. **f**, Bone needle. **g**, Fish gorge hooks.



**Extended Data Fig. 4** | **Age of the Mabu Co site.** The calibrated dates have been produced using OxCal v.4.4. The colored shadows represent the 95.4% probability density distribution of the calibrated ages. The white circles and bar ranges indicate the modeled mean ages and age ranges, with 68.2% probability intervals.



**Extended Data Fig. 5** | **Principal component analysis of East Asia populations.** PCA of ancient East Asian populations projected on present-day East Asia populations (including Tibetans).



 $\label{eq:components} \textbf{Extended Data Fig. 6} | \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{Extended Data Fig. 6} | \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{Extended Data Fig. 6} | \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for selected surrounding populations for K=2 to K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results for K=12.} \\ \textbf{ADMIXTURE displaying ancestry components}, \textbf{ADMIXTURE results}, \textbf{ADMIX$ 



Extended Data Fig. 7 | Multidimensional scaling (MDS) of ancient Tibetans populations based on the genetic relation f3-outgroup value. Here, the south, central, and northeast subgroups of the plateau are primarily distinguished by blue, orange, and black. The Mabu Co population clustered with the ancient South-southwest population lineage, represented by the color blue, mainly including ancient Shannan, Nepal populations.



**Extended Data Fig. 8** | **Pair-wised individuals f4 (Mbuti, X; Mabuco\_ind, Mabuco\_ind) for grouping Mabu Co ancient samples.** We used world-wide 30 outgroup (X) to test the differences between each pair individuals, and the sum of the number of outgroups with significant differences (|Z|>3) was shown in heatmap.



**f4(Mbuti, X; MabucoE1, MabucoE2).** Certain degree of variation between MabucoE1 and MabucoE2 two groups is further verified, based on the degree of their connections with lowland East Asians (for example Qihe, Bianbian,  $DevilsCave_N), f4(Mbuti, X; MabucoE1, MabucoE2) < 0, where solid dots represent the |Z|>3, Z-scores are calculated with 5 cM jackknifing standard error (SE), and the horizontal lines mark ±3 SEs ranges.$ 



Extended Data Fig. 10 | Selected animal fossils from the Mabu Co site. a, Schizothoracids (*Gymnocypris* sp.). (1) Pharyngeal; (2) Cleithrum; (3) Basioccipital; (4) Vertebra I; (5) Vertebra II; (6) Other vertebrae. b, Red deer (*Cervus elaphus*) left metatarsal. c, Argali (*Ovis ammon*) right scapula. d, Lynx (*Lynx lynx*) left premaxilla and maxilla. e, Gazelle (*Procapra przewalskii*) right

maxilla. **f**, Woolly hare (*Lepus oiostolus*) right mandible. **g**, Himalayan marmot (*Marmota himalayana*) left mandible. **h**, Pochard (*Netta* spp. & *Aythya* spp.) left coracoid. **i**, Coot (*Fulica atra*) left coracoid. **j**, Great cormorant (*Phalacrocorax carbo*) right carpometacarpal. Scale bars, 2 cm.

# nature portfolio

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## **Reporting Summary**

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#### **Statistics**

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.	
n/a	Confirmed		
	$\boxtimes$	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement	
$\boxtimes$		A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly	
$\boxtimes$		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.	
$\boxtimes$		A description of all covariates tested	
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	$\boxtimes$	For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.	
$\boxtimes$		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings	
$\boxtimes$		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes	
$\boxtimes$		Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated	
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.	

#### Software and code

Policy information about availability of computer code		
Data collection	No software was used for data collection.	
Data analysis	The C14 dates were calibrated with OxCal v.4.4 using the IntCal20, fastqc (0.11.4), sequence data processing using EAGER (1.92.59), AdapterRemoval v2.3.1, BWA (v0.7.12), population genetics analysis (smartpca, f3, f4, qpAdm) based on AdmixTools (https://github.com/ DReichl.ab/AdmixTools). FIG (https://github.com/DReichl.ab/FIG) and ADMIXTURE.	

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Key data used in this study are available in the main text, extended data and the accompanying supplementary information. Other datasets generated in this study are available from the corresponding authors upon request. The raw sequencing reads, aligned BAM files, and pseudo-diploid genotype calls are available through

the Genome Sequence Archive (https://bigd.big.ac.cn/gsa-human; accession number: PRJCA024667), and OMIX database, China National Center for Bioinformation/ Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix, accession number: OMIX006078).

#### Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	The sex of all 9 ancient human individuals was genetically determined by comparing the amount of sequence data that map to the Y chromosome to the amount of sequence data that map to the autosomes or the X chromosome.
Reporting on race, ethnicity, or other socially relevant groupings	Ancient human bone samples only. No modern human participants.
Population characteristics	None
Recruitment	None
Ethics oversight	Permission to sampled human ancient DNA from the associated human specimens was granted by the provincial archaeological institutes that manage and care for the specimens.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

	Life sciences	

Behavioural & social sciences 🛛 🔀 Ecological, evolutionary & environmental sciences

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## Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Archaeological and paleogenomic study that aims to reveal what populations were and how they lived in lake basins in the ultra-high altitude areas of the Tibetan Plateau about 4000 years ago. This study includes paleogenomic analysis of ancient human bones, bulk bone metagenomics analysis of ancient animal bones, morphological analysis of plant and animal remains, stable isotope analysis of human, animal and plant remains.
Research sample	Research samples includes human, animal and plant remains from an archaeological site.
Sampling strategy	Archaeological sample size was not pre-determined as we sampled the maximum number of human, animal and plants remains have definite provenience informations.
Data collection	The radiocarbon dating of archaeological remains are carried out in Peking University and Beta Analytic, respectively. Ancient DNA wet lab work and population genetics analysis were performed in the dedicated ancient DNA laboratory and data server platform at the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Chinese Academy of Sciences. The isotope data are measured in Beta Analytic and the Environmental Stable Isotope Laboratory (ESIL), Institute of Environment and Sustainable Development of Agriculture, Chinese Academy of Agricultural Sciences. The morphological analysis of plant and animal remains was in the State Key Laboratory of Tibetan Plateau Earth System, Resources and Environment (TPESRE), Institute of Tibetan Plateau Research, Chinese Academy of Sciences, and Key Scientific Research Base of Bioarchaeology in Cold and Arid Regions (National Cultural Heritage Administration), Lanzhou University.
Timing and spatial scale	Archaeological samples were obtained during the survey and excavation of the archaeological site of Mabu Co from 2019-2021. All samples were collected at and around the Mabu Co site.
Data exclusions	No data were excluded from the analyses.
Reproducibility	Almost all of the samples involved in our study were left over for reproducible testing.
Randomization	No randomization was performed.
Blinding	No blinding was performed.
Did the study involve field	d work? 🗙 Yes 🗌 No

### Field work, collection and transport

Field conditions	The mean annual temperature is 1°C, and the average annual precipitation is 277 mm.
Location	28.31°N, 89.43°E, 4446 m.a.s.l.
Access & import/export	We collected the archaeological samples with permission from local institutes.
Disturbance	N/A

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

#### Materials & experimental systems

n/a	Involved in the study
$\boxtimes$	Antibodies
$\boxtimes$	Eukaryotic cell lines
	Palaeontology and archaeology
$\boxtimes$	Animals and other organisms
$\boxtimes$	Clinical data
$\boxtimes$	Dual use research of concern
$\boxtimes$	Plants

#### Methods

n/a	Involved in the study
$\boxtimes$	ChIP-seq
$\boxtimes$	Flow cytometry
$\boxtimes$	MRI-based neuroimaging

#### Palaeontology and Archaeology

Specimen provenance	We collected the archaeological samples with permission from relevant authorities, such as National Cultural Heritage Administration, Tibetan Cultural Heritage Administration, and Cultural Heritage Administration of Kangmar County.
Specimen deposition	Archaeological samples are curated in Tibetan Institute of the Preservation of Cultural Relics and Lanzhou University.
Dating methods	14C dates were obtained from Peking University and Beta Analytic. The IntCal20 curve and the Libby half-life of 5568 yr were used in the tree-ring calculation of all dates, with the calibration performed using the OxCal 4.4 program. OSL dates were measured in Lanzhou University.
🔀 Tick this box to confi	rm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight Our archaeological and environmental samples were collected with the permission of local institutes.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

#### Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A